Resource recovery as a prerequisite for long term Space missions

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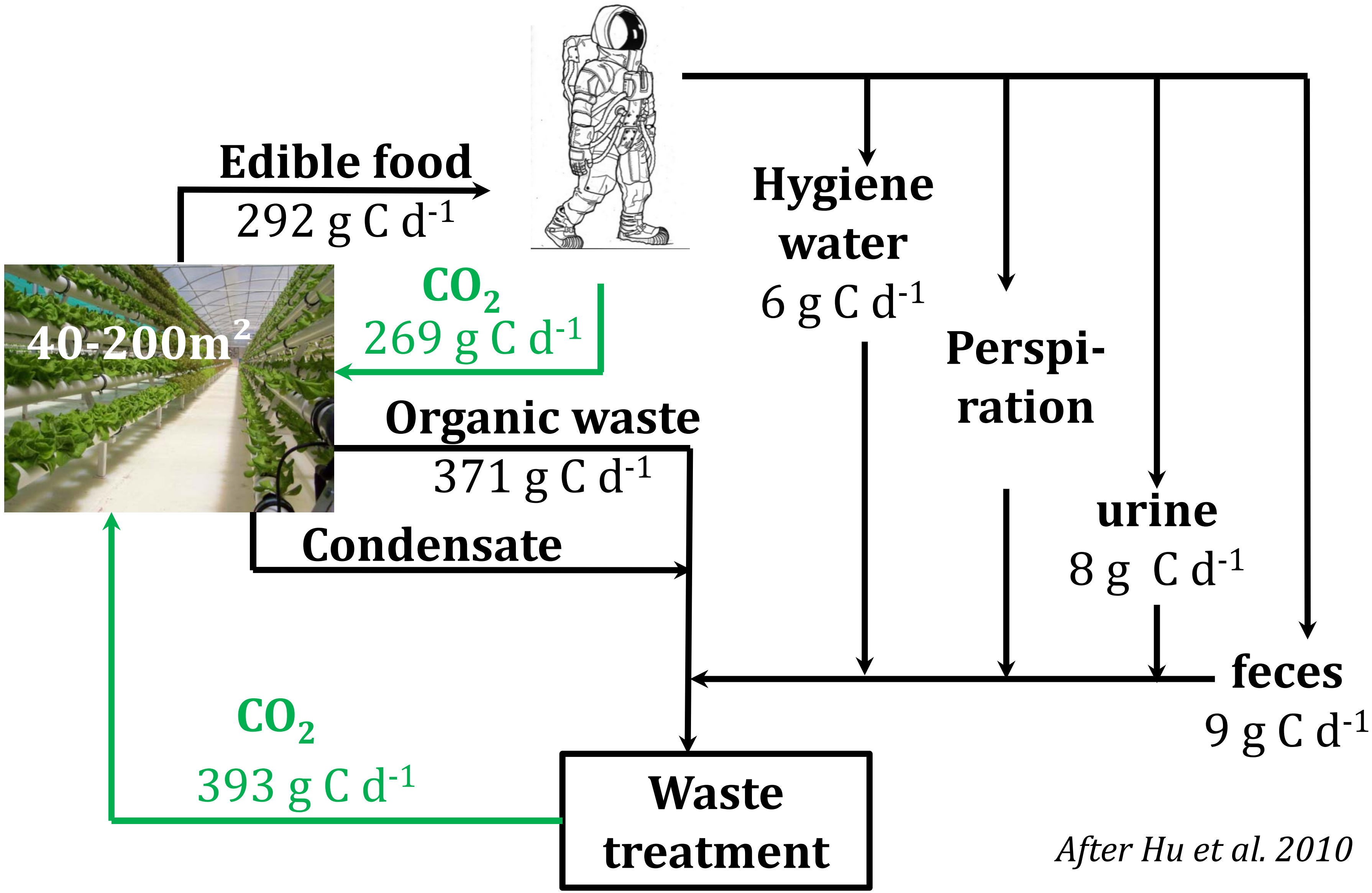
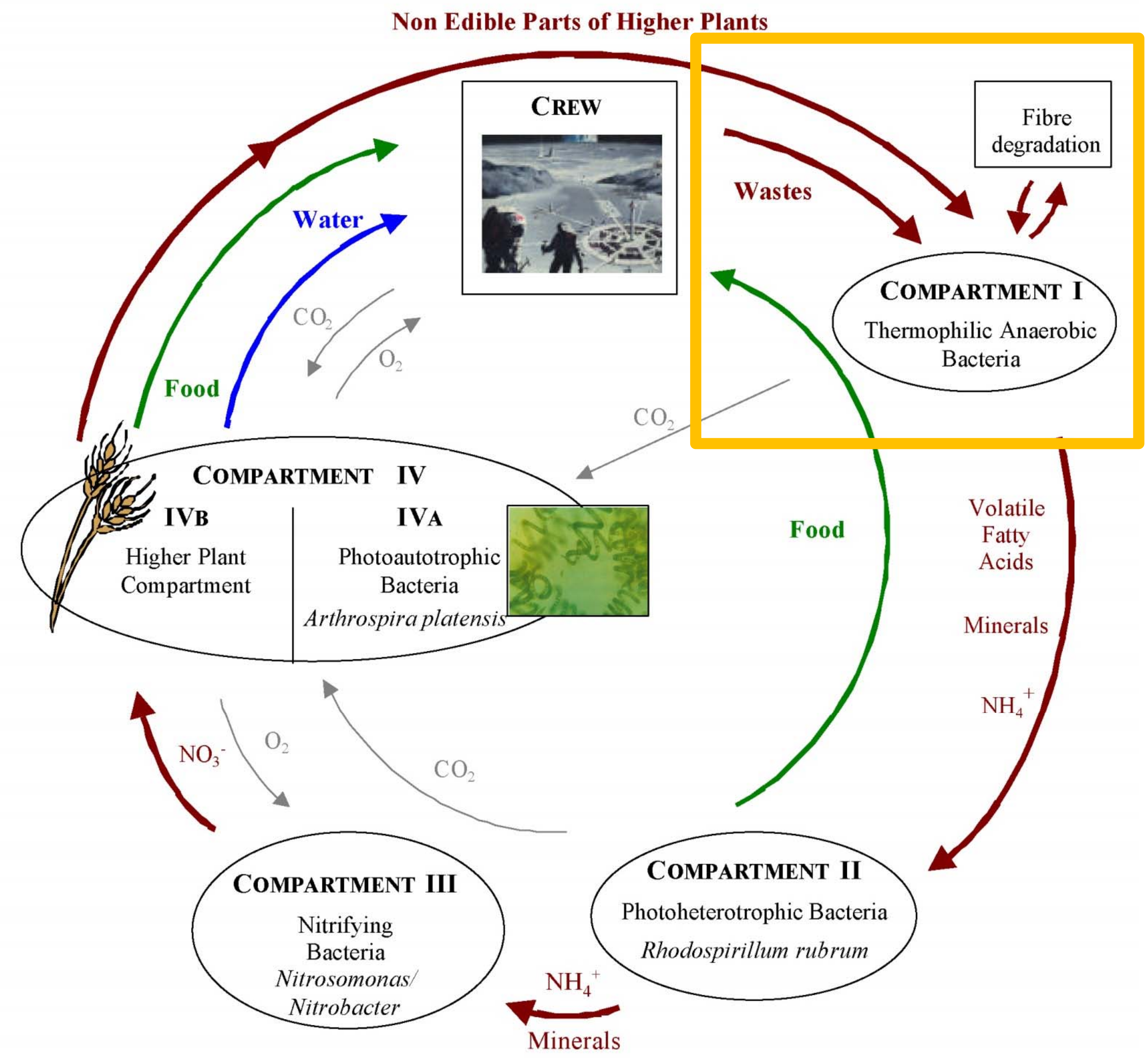
Lausanne, 2016-06-08
MELiSSA workshop

Carbon balance: order of magnitude



After Hu et al. 2010



The MELiSSA loop: compartment I

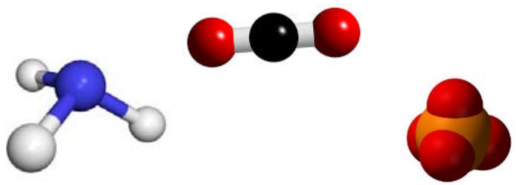
I. The MELiSSA waste compartment (C I)

Liquefaction and conversion of waste …

- Non edible parts of higher plants
- Faecal material
- Toilet paper
- (Urine)

… into useful molecules for the MELiSSA loop

- CO_2
- Nutrients (N, P, …)
- Volatile fatty acids



<u>**Requirements and needs**</u>

- **Efficient** and **effective** conversion of the organic waste for further use in the MELiSSA cycle:
  → CO_2, NH_4^+, PO_4^{3-}, (VFA), ...

- **With minimal** weight, energy consumption, (no) oxygen consumption, consumables (base), no (excessive) sludge production

- **Controllable - predictable**

- No/minimal **losses**

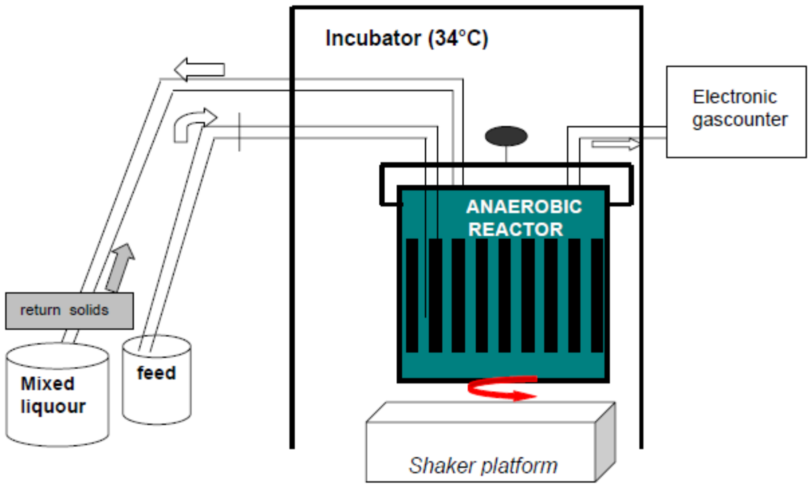
- **Biosafety**: no transfer of microorganisms to the next compartment

MELiSSA Compartment I:

History and technological *state-of-the-art*

Until 2004: mesophilic anaerobic digestion

- 5.5 L lab-scale CSTR
- 0.8% DM in the bioreactor
- residence time: 15-20 days
- Temperature: 34°C
- pH: 7.3

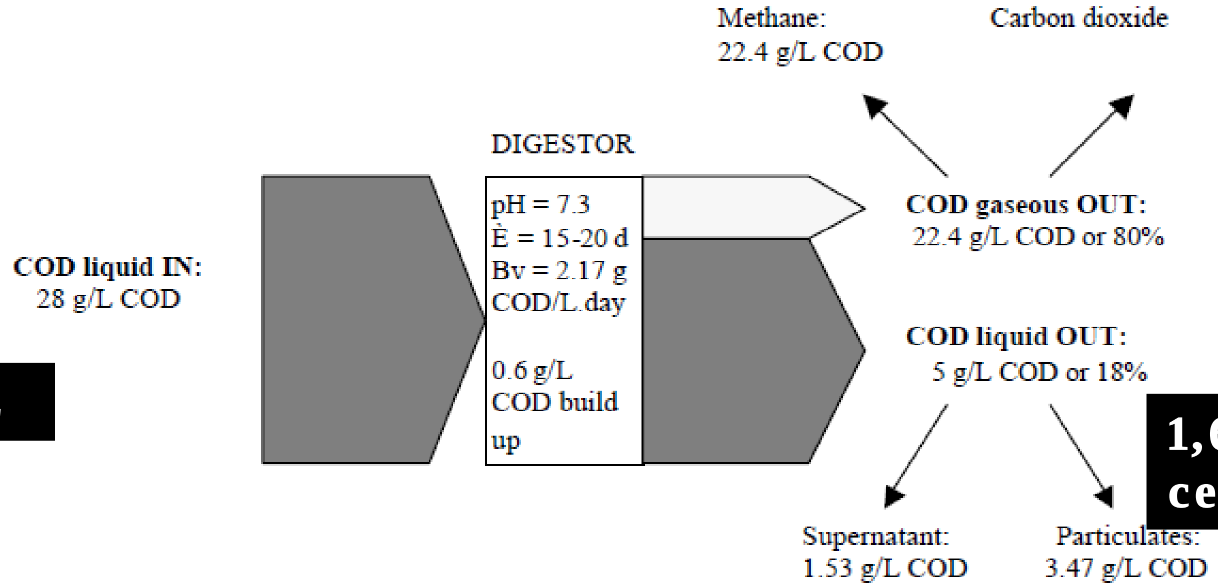


Ref: MELiSSA TN 86.1.x

Mesophilic AD: mass balance for COD and N

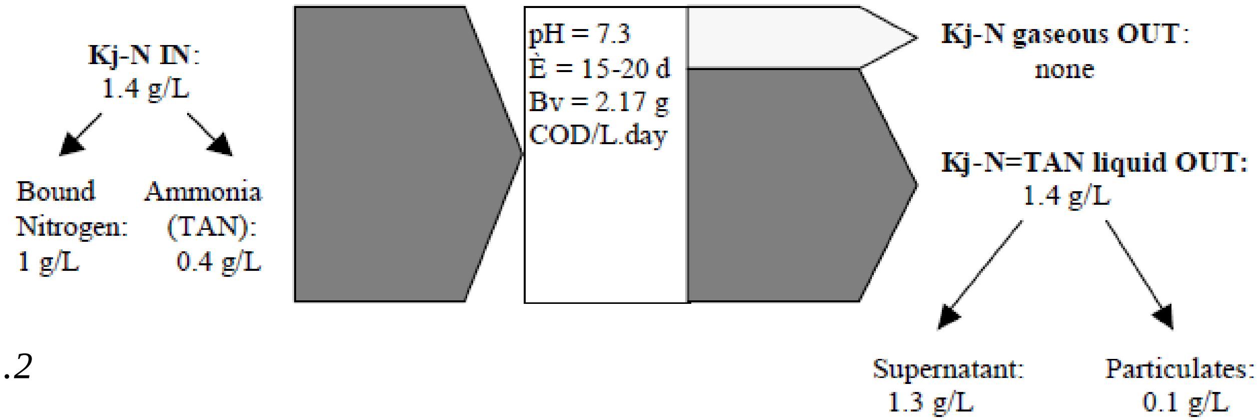
COD

5,9 g cellulose/L



1,6 g cellulose/L

N



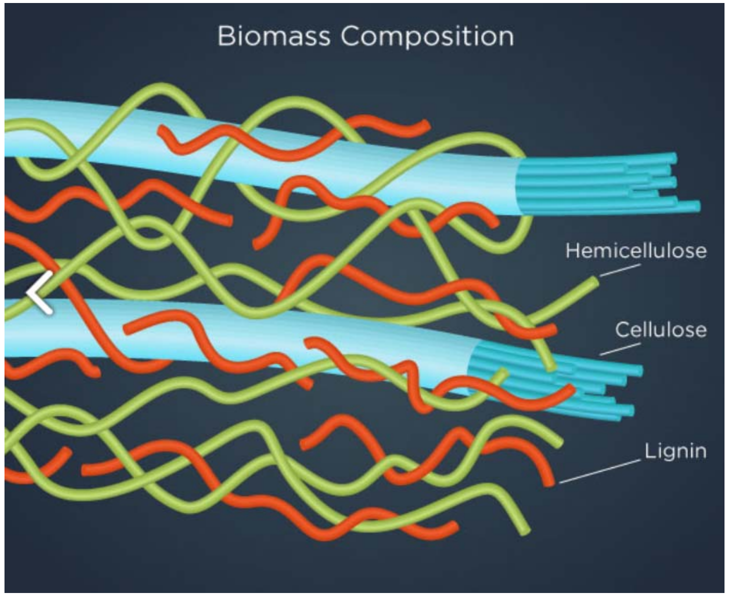
Ref: MELiSSA TN 86.1.2

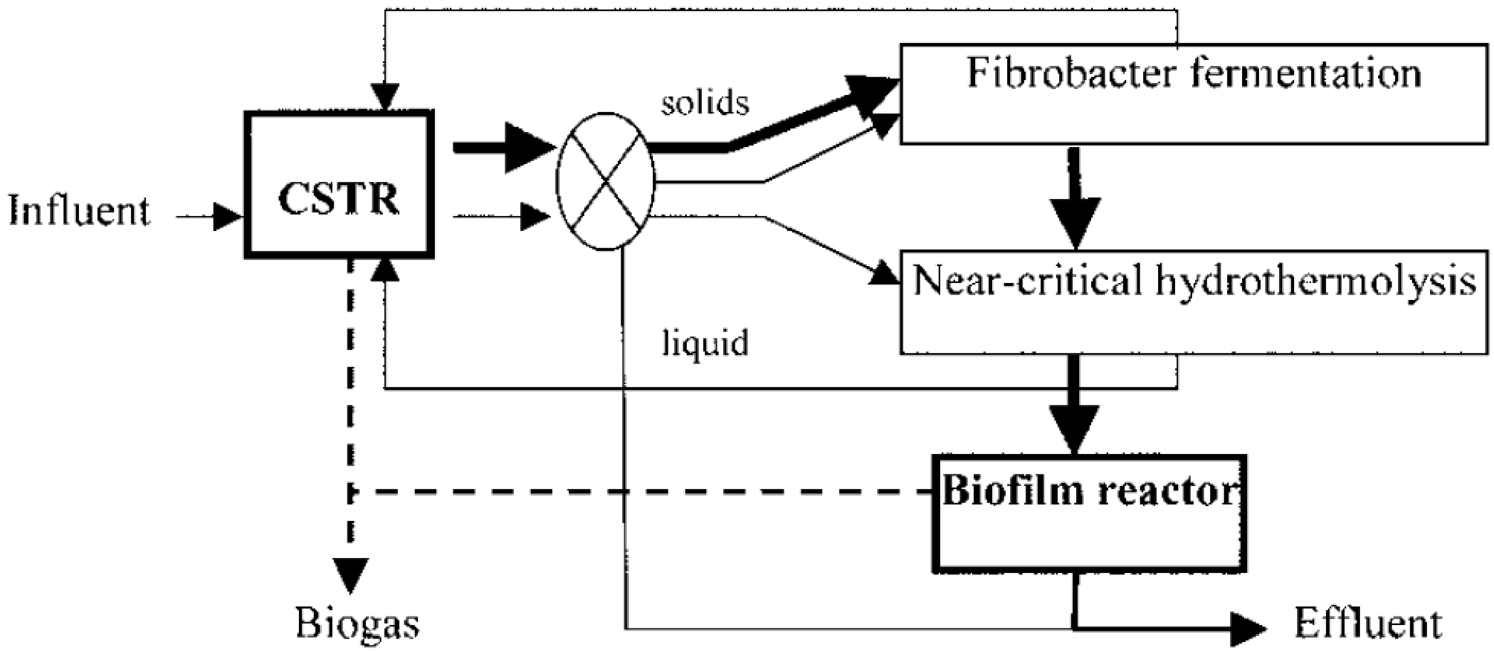
Fiber Degradation

Degradation of fibrous material (**lignin, cellulose, hemicellulose**) remains challenging

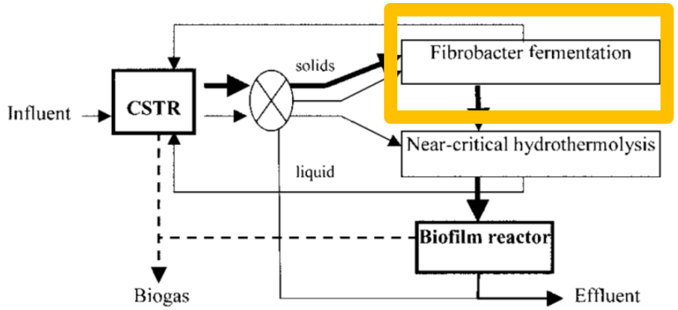
Strategies to increase the degradation efficiency:

- Sonication
- Acidification
- Fenton chemistry
- Enzymatic treatment
- Lignolytic fungi
- Rumen bacteria
- Hyperthermophilic bacteria
- Hydrothermal treatment





Ref: Lissens et al. 2004



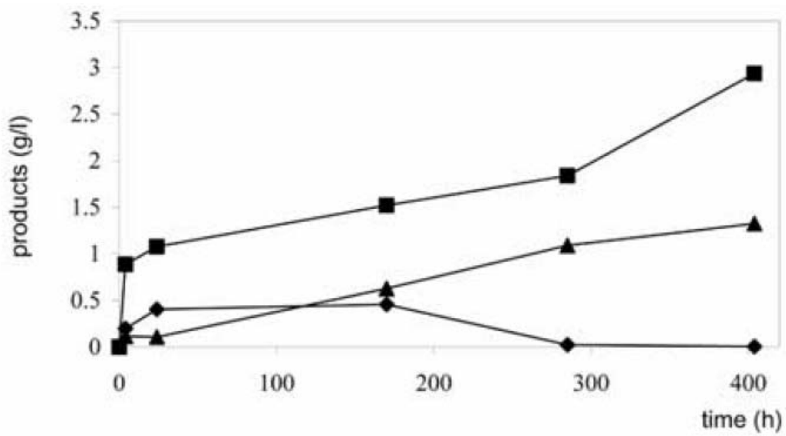


Figure 4. Production of organic acids during *Fibrobacter succinogenes* fermentation on CSTR- effluent solids at 15 g l^{-1}. Key: ■ = acetate, ▲ = propionate, ♦ = succinate.

Ref: Lissens et al. 2004

- **Additional fiber degradation by *Fibrobacter succinogenes***
- **Inhibition by fecal material**
- **Sensitive towards contamination**

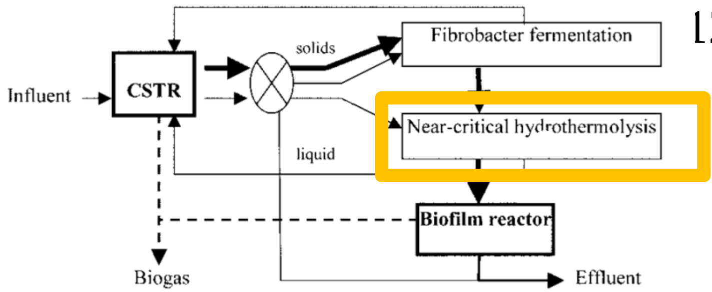
substrates	Total fibers (%DM)	Hemicellulose (%DM)	Cellulose (%DM)	Lignin (%DM)	Degradation by *F.s.* (%DM)
Wheat straw	72	26	39	7	31,7
Soya	12	5	6	1	62,6
Cabbage	16	2	13	1	78,2

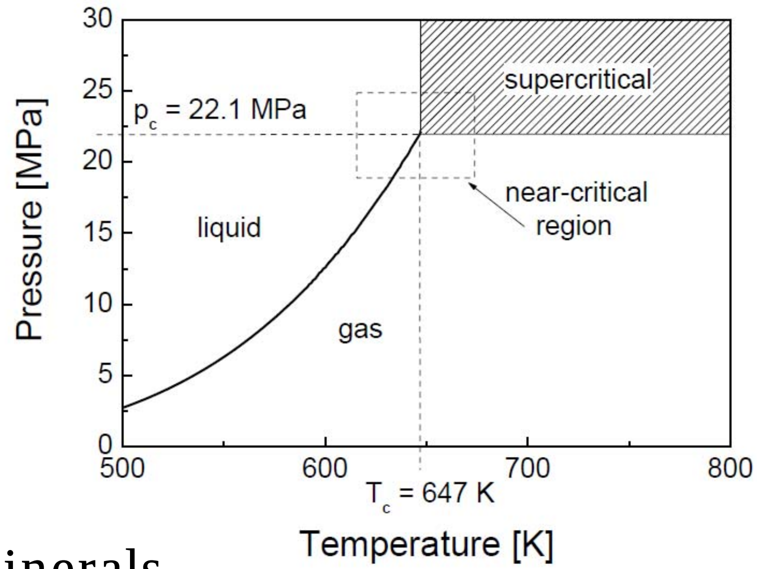
Hydrothermal treatment

Near or supercritical water oxidation:

- High temperature
- High pressure

[+ addition of oxidant, e.g. O_2 or H_2O_2]





$$\text{Organic compounds} + O_2 \xrightarrow{H_2O_{supercrit.}} CO_2 + C_{soluble} + \text{minerals}$$

Water becomes a better solvent for hydrolysis of organic compounds

I. The MELiSSA waste compartment (C I)

a) Liquefaction unit: insoluble C → soluble C + CO_2

-Cellulose (290°C; 25 MPa):
100% solubilization

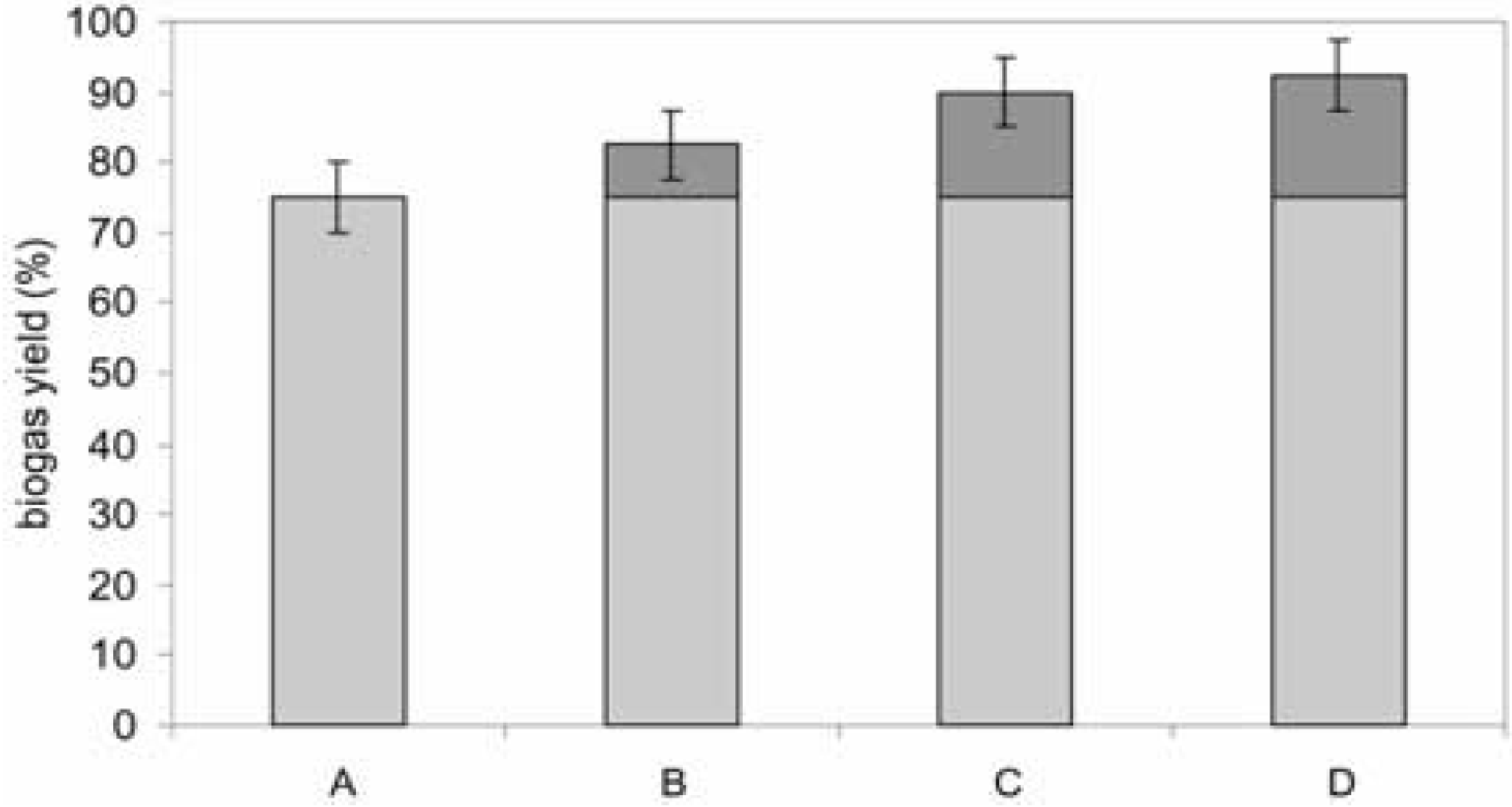
-Lignin (390°C; 25 MPa; $\mathbf{H_2O_2}$):
90% to CO_2 & 10% soluble C

-Wheat straw (>300°C; 25 MPa; $\mathbf{H_2O_2}$):
90% to CO_2 & 10% soluble C

-MELiSSA anaerobic sludge (360°C; 25 MPa):
95% solubilization

Ref: MELiSSA TN 86.4.9

Enhanced waste conversion efficiencies AD + ...

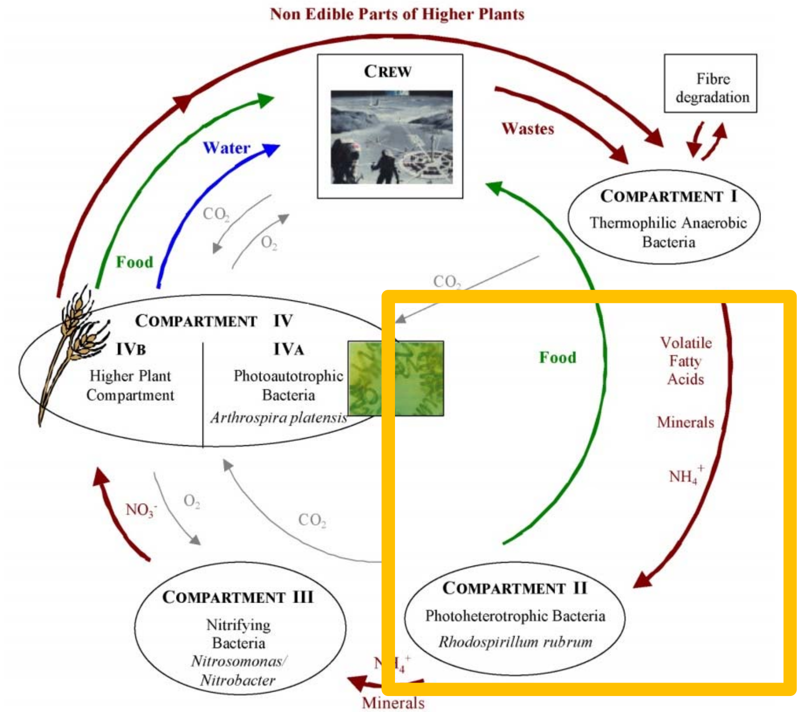


AD CSTR	AD CSTR + Fibrobacter + AD biofilm	AD CSTR + Hydrothermolysis + AD biofilm	AD CSTR + Fibrobacter + Hydrothermolysis + AD biofilm

Ref: Lissens et al. 2004

However …

- Value of methane in LSS?
- What about gas separation in microgravity?
- Production of VFA more meaningful?
- Energetic efficiency?



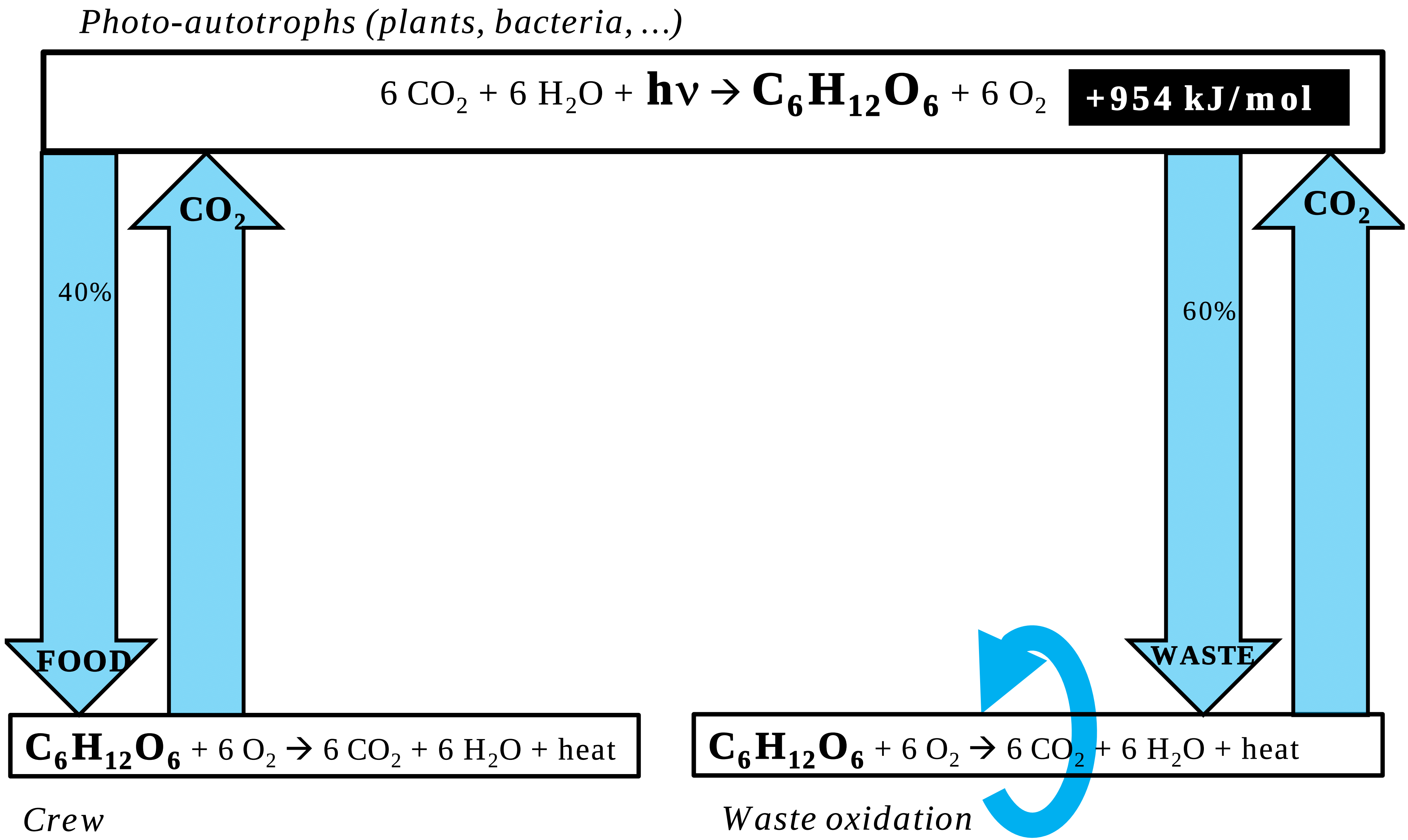


Photo-autotrophs (plants, bacteria, ...)
6 CO2 + 6 H2O + hν → C6H12O6 + 6 O2
+954 kJ/mol
CO2
40%
FOOD
CO2
60%
WASTE
C6H12O6 + 6 O2 → 6 CO2 + 6 H2O + heat
Crew
C6H12O6 + 6 O2 → 6 CO2 + 6 H2O + heat
Waste oxidation

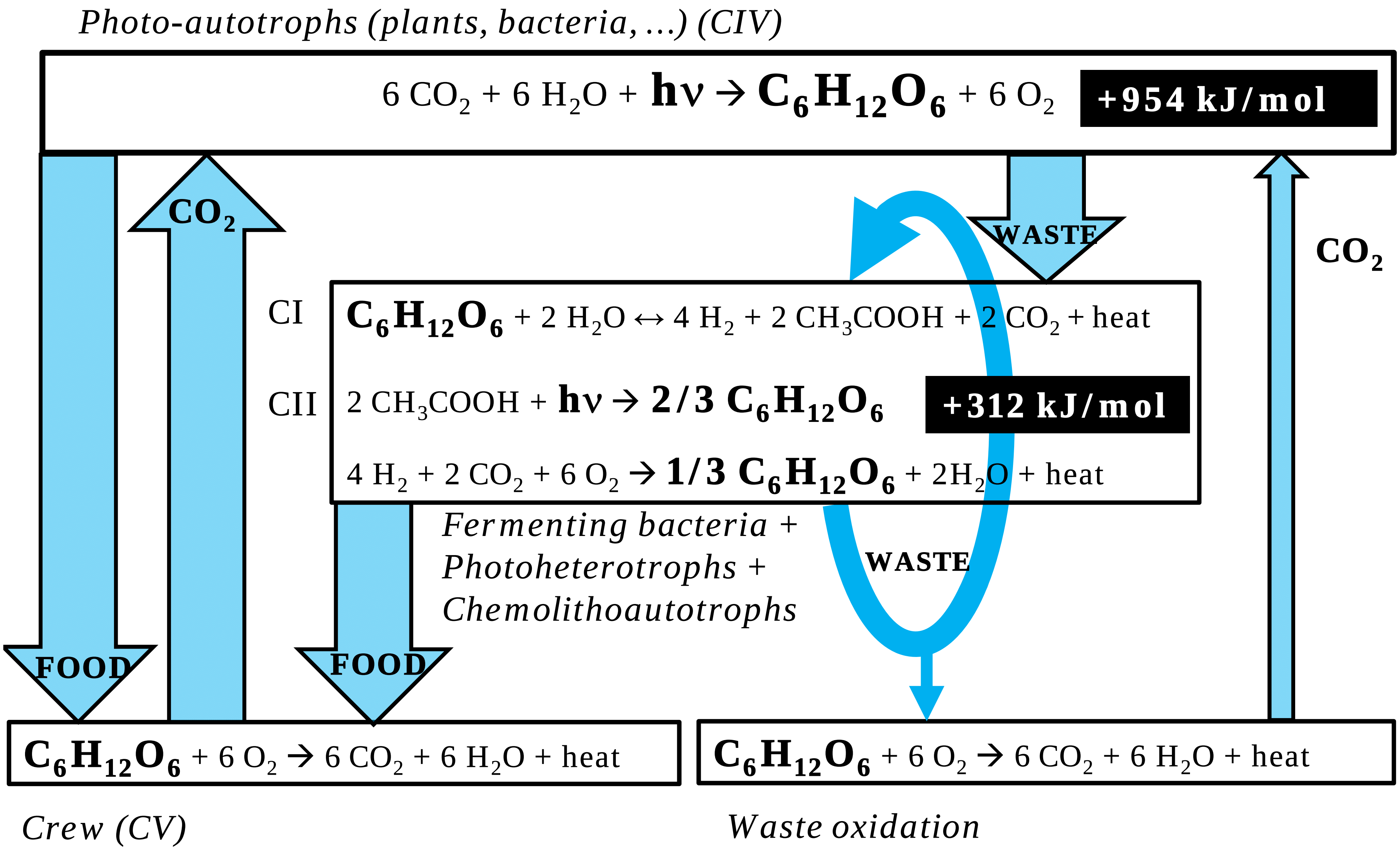
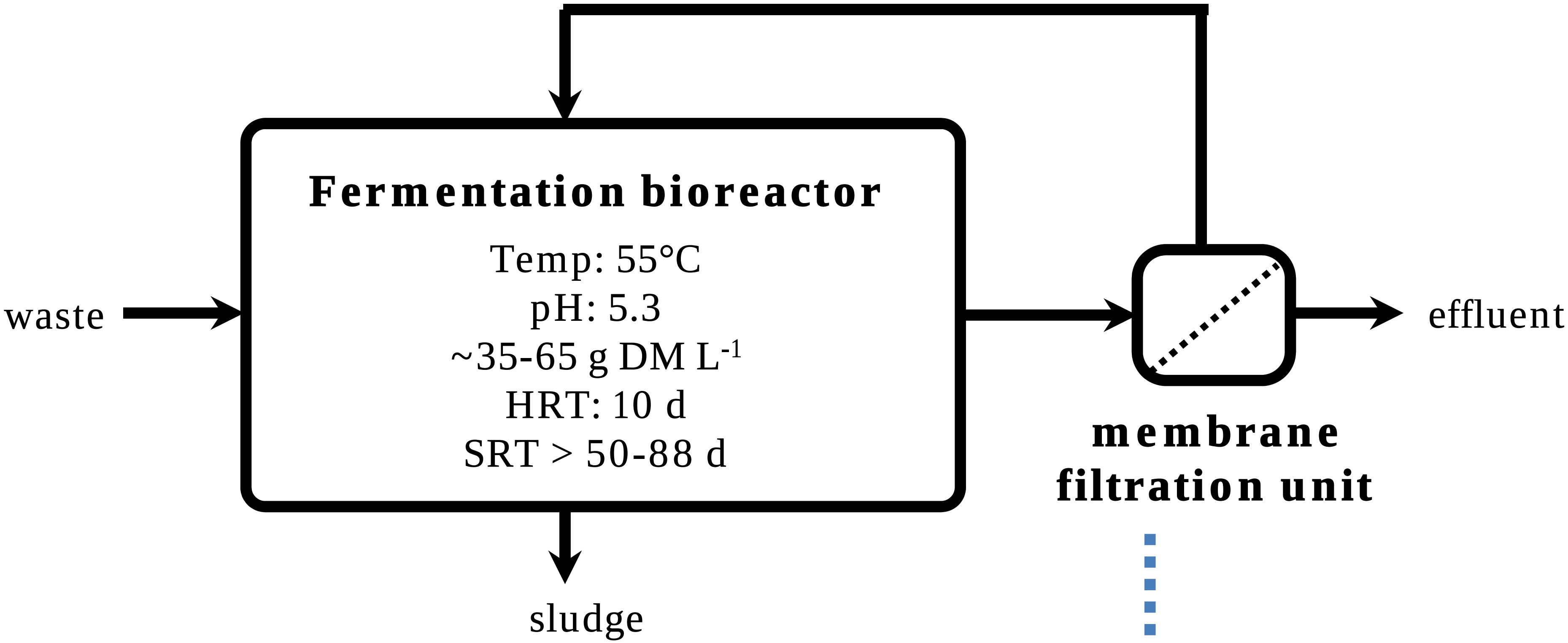


Photo-autotrophs (plants, bacteria, ...) (CIV)
$6\ CO_2 + 6\ H_2O + h\nu \rightarrow C_6H_{12}O_6 + 6\ O_2$
+954 kJ/mol
CO_2
WASTE
CO_2
CI
$C_6H_{12}O_6 + 2\ H_2O \leftrightarrow 4\ H_2 + 2\ CH_3COOH + 2\ CO_2$ + heat
CII
$2\ CH_3COOH + h\nu \rightarrow 2/3\ C_6H_{12}O_6$
+312 kJ/mol
$4\ H_2 + 2\ CO_2 + 6\ O_2 \rightarrow 1/3\ C_6H_{12}O_6 + 2H_2O$ + heat
Fermenting bacteria +
Photoheterotrophs +
Chemolithoautotrophs
WASTE
FOOD
FOOD
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + heat
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + heat
Crew (CV)
Waste oxidation

Thermophilic fermentation: operational conditions

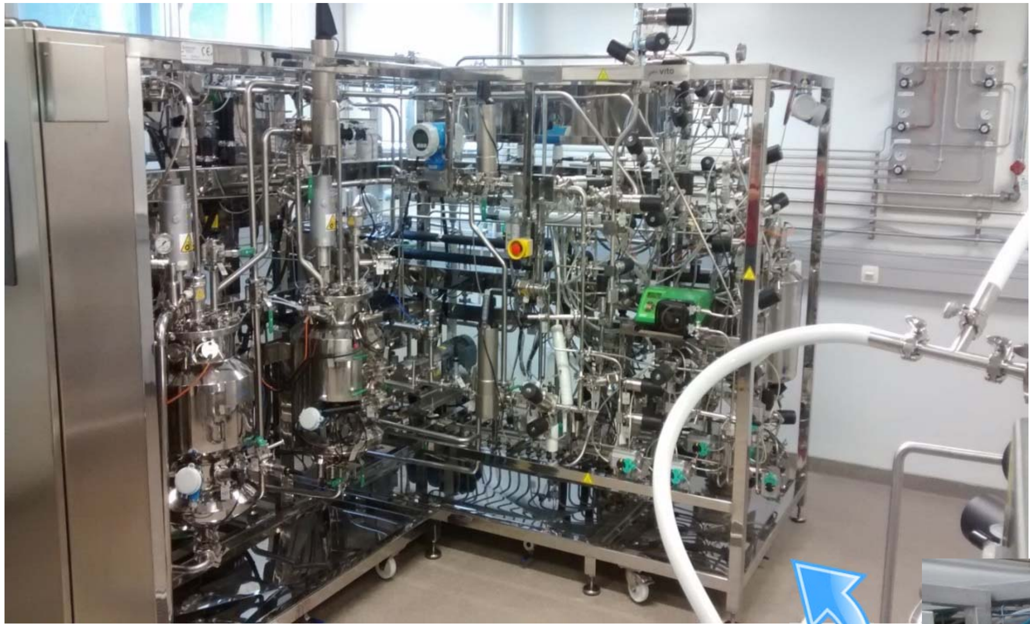


- Single channel ceramic membrane
- 0.05 µm pore size
- Pressure 0-0.4 bar
- Continuous recirculation
- Cross-flow velocity 2 m s^{-2}

Thermophilic fermentation: waste pretreatment

Specialized straw cutter and waste mixer

Thermophilic fermentation: hardware



UGent inoculum
bioreactor (5L)

BELISSIMA hardware VITO (20L)

MELiSSA pilot plant Barcelona (100 L)

Thermophilic fermentation: mass balance for COD and C

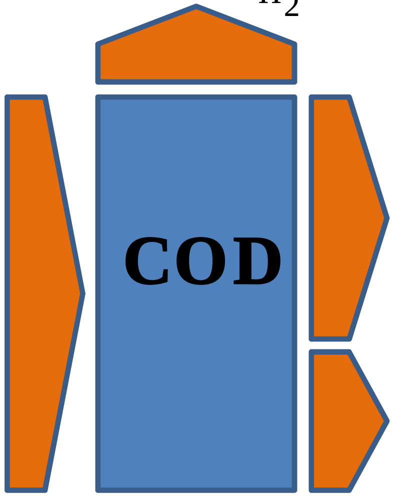
Gas: COD_{H_2}: 0-1%

IN:

~ 2.1% DM

~ 12 g fibers/L

~26 g COD/L
(B_v: 2.6 g COD/L.d)



COD

Filtrate:

COD_{VFA}: 21-26% (0.5-0.8 g COD_{VFA}/L.d)

$COD_{soluble, other}$: 15-20%

Sludge:

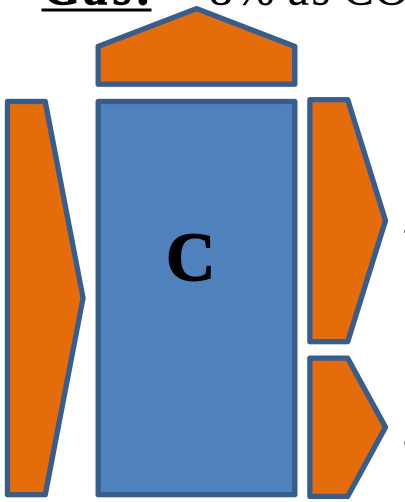
COD_{sludge}: 41-49%

8-18%
COD gap

Gas: ~ 8% as CO_2

IN:

~7,9 g C/L



C

Filtrate: 37% as soluble C

Sludge: 49%

6% C gap

Refs:
-Report on CI compartment operation (2012)
-MELiSSA TN 80.522
-MELiSSA TN 91.2 (under preparation)

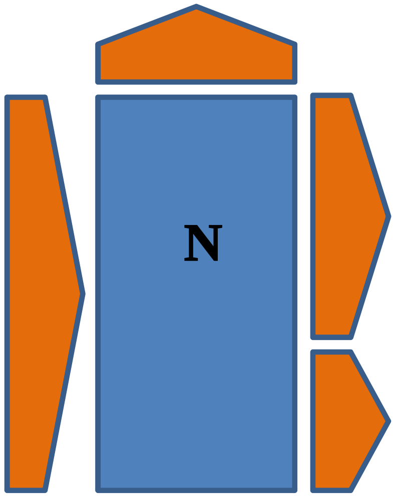
Thermophilic fermentation: mass balance for N

Gas: 0-21% as N_2

IN:

~ 0.65 g TN/L
(Load: 50-80 mg N/L.d)

*N_{org}: 73-95%
*N_{amm}: 0-6%
*$N_{NO_3^- + NO_2^-}$: 4-21%



Filtrate:

*N_{org}: 18-22%
*N_{amm}: 22-34% (15-30 mg N/L.d)
*$N_{NO_3^- + NO_2^-}$: 0%

Sludge:

N_{total}: 29-37%

N

0-13% N gap

Refs:
-Report on CI compartment operation (2012)
-MELiSSA TN 80.522
-MELiSSA TN 91.2 (under preparation)

Thermophilic fermentation: challenges

- Low volumetric conversion rates
- Low degradation of fibers
- Gas tightness
- Sludge treatment?
- Controllability/predictability?
- Consumables: NaOH
- Sterility after membrane filtration
- …

Fermentation residue: supercritical water oxidation

95% TOC removal & 90% conversion of 'CI sludge' to **CO_2** in 1 min.

However:

→ High peroxide consumption

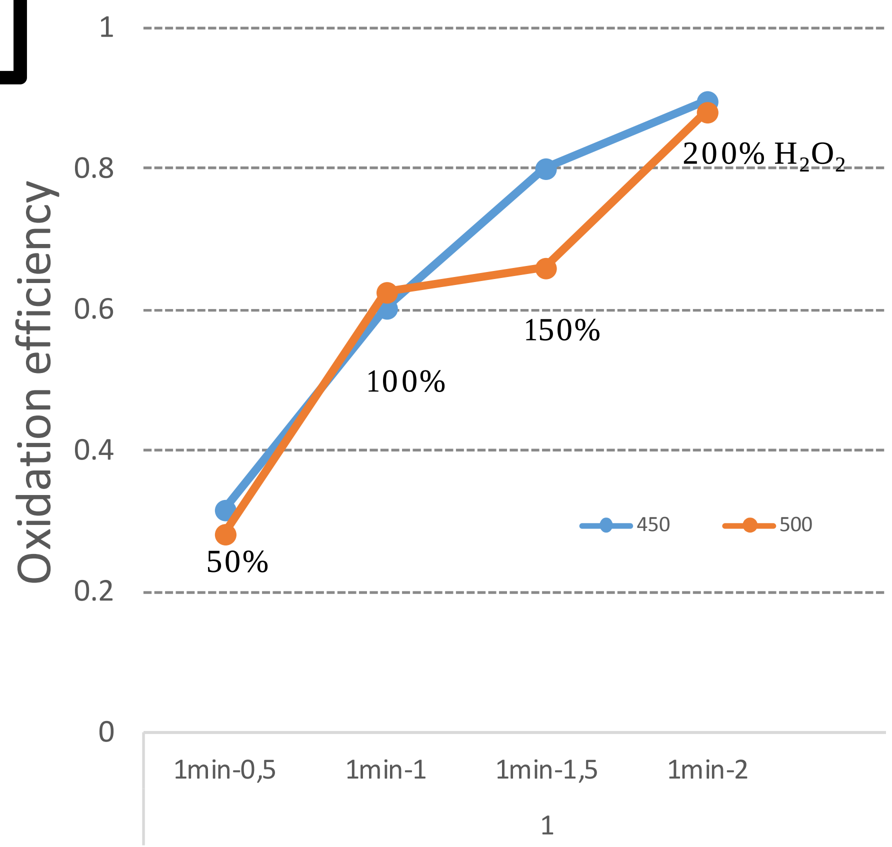
→ Production of (toxic/recalcitrant) organic compounds?

→ Leakage of Chromium (µg/L)

→ Complexity/safety?

→ Fate of N, P, …?

- Temp. > 400°C
- Corresponding P
- Varying H_2O_2 dosage



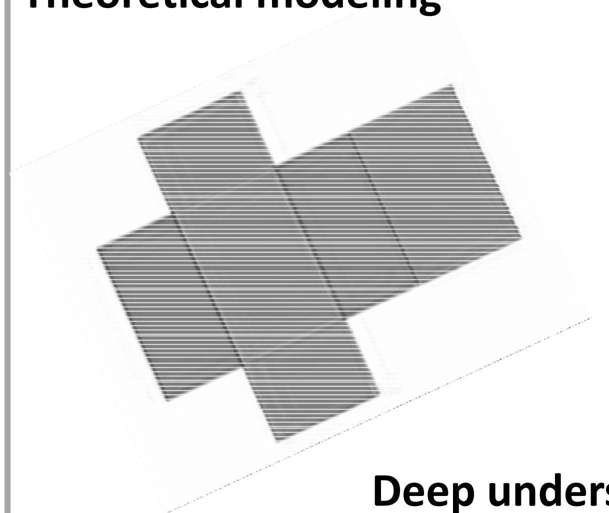
MELiSSA Compartment I:

Microbial ecology

Waste processing: the microbiome challenges

- **Waste processing is performed in non axenic conditions in the first compartment of the MELiSSA loop**
- **Environment is continuously changing**
- **Same level of control required than in axenic compartment**
- **Predictive model-based control is also needed!**

Theoretical modeling



Empirical modeling

Knowledge model

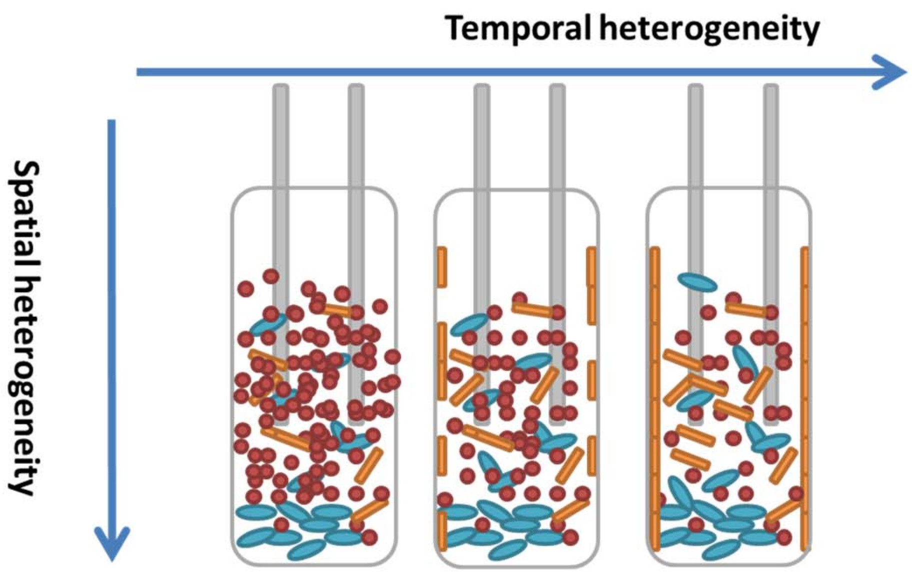
Deep understanding of the process is needed

Microbiome metabolism has to be characterized

Waste processing: the microbiome challenges

Weeks 1 2 3 4 5 6 7 8

- Consortium composition
- Active part of the consortium determination
- Metabolism occurring in the consortium



- **Elaborated sampling and sample preparation strategy needed**
- **Presence of large amount of biological wastes**

Omics based analysis



Genomic, transcriptomic, proteomic, metabolomic

Integrated omics analysis

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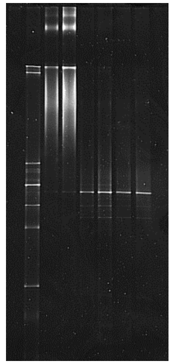
Meta-omics

Pure culture vs. consortium

Characterization of MELiSSA C1 microbiome

- **Targeted taxonomic marker analysis (DGGE - 16S)**
  - Relative abundance
  - Dynamic of consortium evolution
  - Resistance to invasion



- **Metagenomic analysis**
  - Genes catalogue
  - Potential functionalities

- **Metatranscriptomic analysis**
  - Expressed genes
  - Strain activity

- **Metaproteomic analysis**
  - Operating pathways
  - Functional abundance
- **Metabolomic analysis**
  - SIP to track nutrient fluxes

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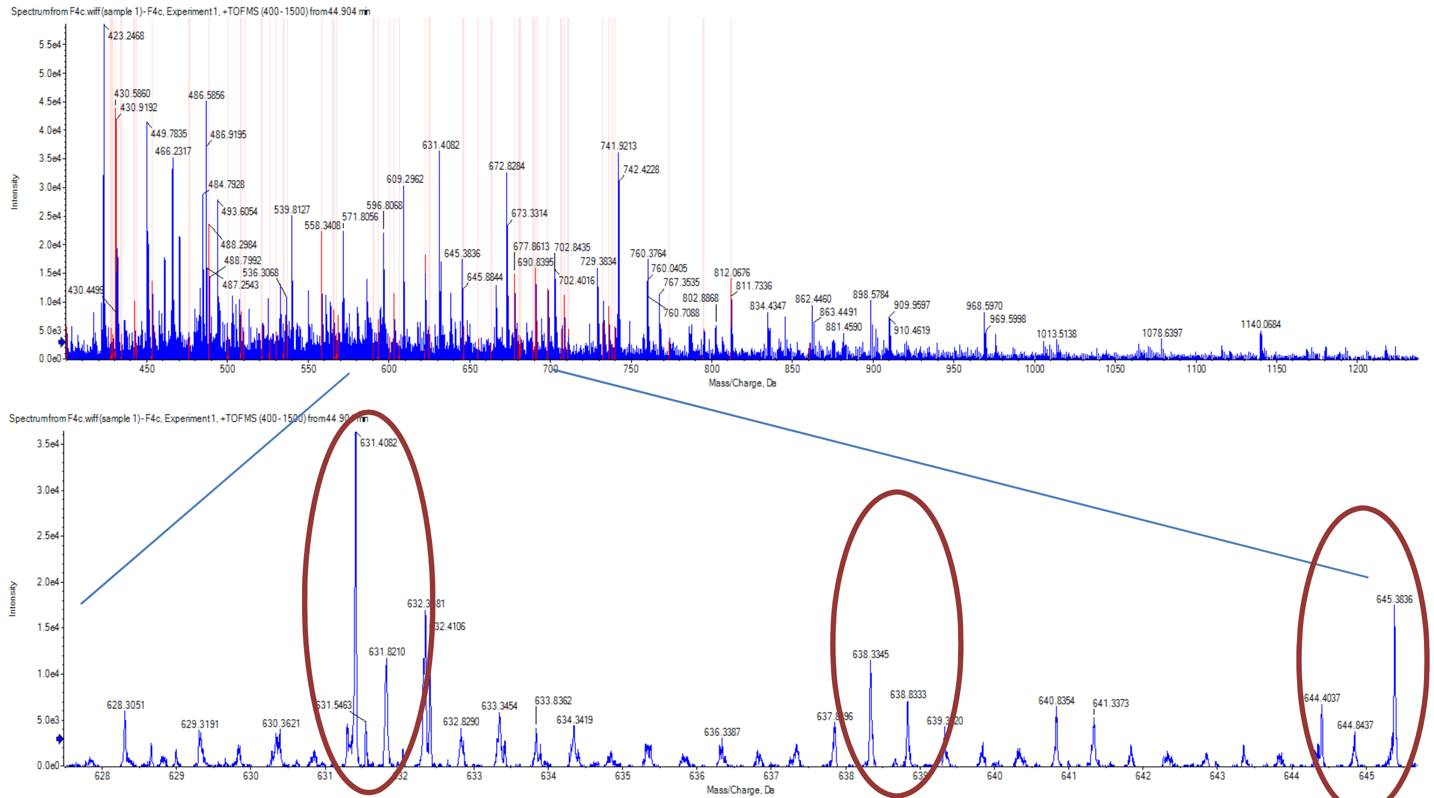
Meta-proteomics of MELiSSA C1

Consortium unevenness is a major drawback

Usual mass spectrometry data acquisition strategies favors detection of most abundant proteins

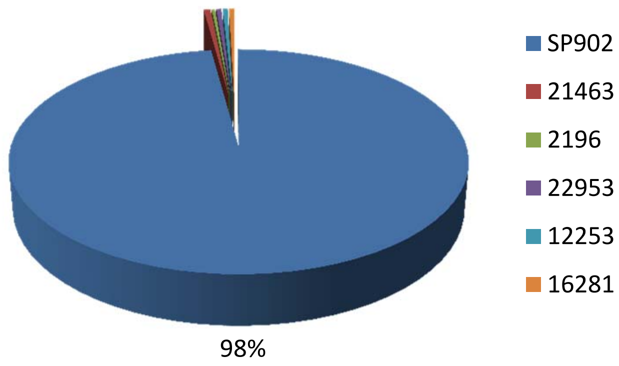


Low abundance strains poorly represented in dataset

Meta-proteomics of MELiSSA C1

Regular MS/MS

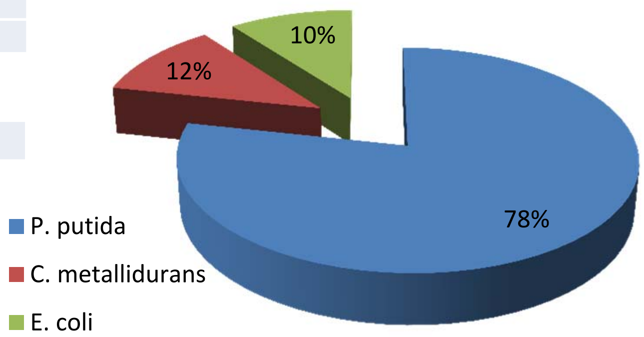
Strains	SP902	21463	2196	22953	12253	16281
F,G- 4 days	91.59%	0.86%	0.00%	6.35%	0.93%	0.06%
# PROTEINS	682	4	2	3	3	3
# PEPTIDES	2457	4	4	3	3	6



Use of Data Independent acquisition mode (SWATH) helps in decreasing effect of culture unevenness

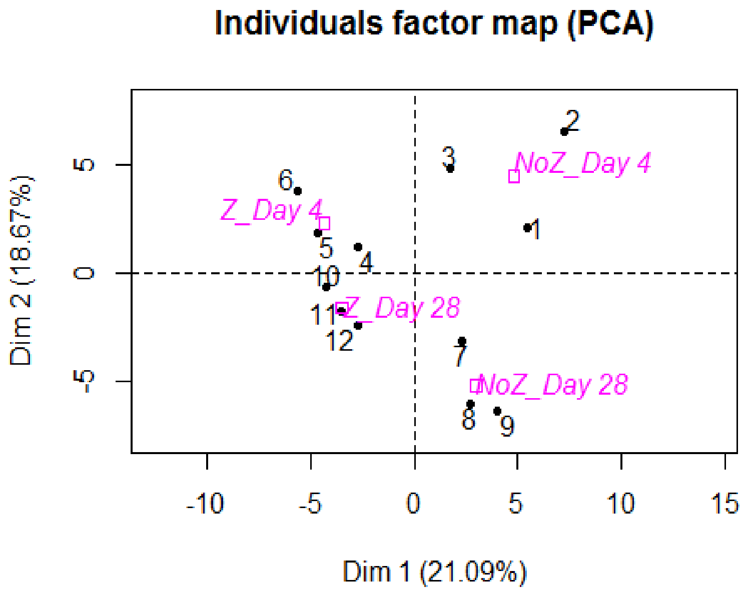
SWATH MS/MS

Strains	P. putida	C. metallidurans	E. coli
F,G- 4 days	27-90%	0.86%	0.00%
# PROTEINS	586	85	77

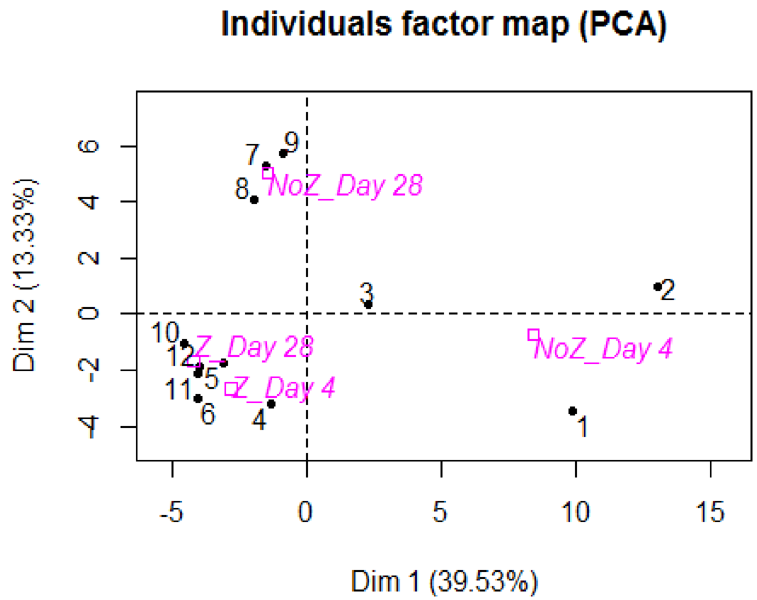


Meta-proteomics of MELiSSA C1

Use of Data Independent acquisition mode (SWATH) helps in quantifying proteome modifications



C. metallidurans



E. coli

Only useful for characterized consortium and cultivable organisms!

Meta-proteomics of MELiSSA C1

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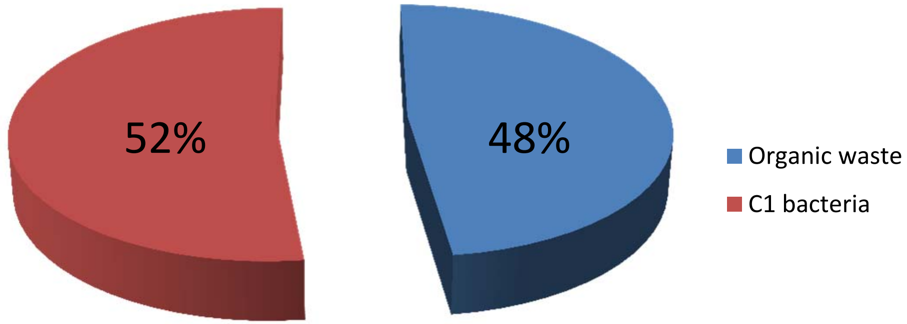
Presence of large amount of organic wastes!!

Proteomic analysis on C1 sludge



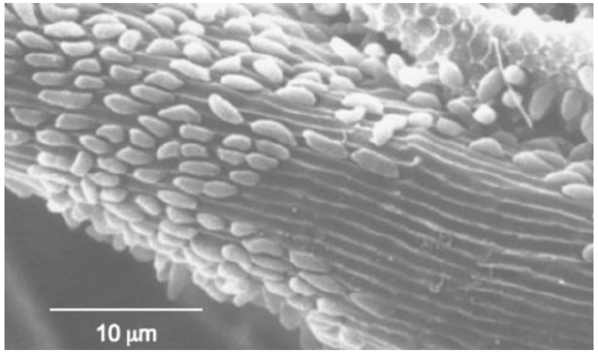
Elaborated sample preparation strategy needed!

MELiSSA thermophilic C1 is not 100% efficient

Fibers degradation: a microbial solution??

Fibrobacter succinogenes

- One of the most powerful anaerobic fiber degrading bacteria
- Proved efficient at degrading cellulose



Modified from Brumm et al. 2011

- But how to manage its poor cultivability (Feacal mater inhibition, sensitivity to contaminant…) ?
- What about lignin degradation?
- Analysis of *F. succinogenese* metabolism and (biotic) stress resistance?
- Selection of *F. succinogenese* more tolerant strains?
- Other fiber degrading strains from mammalian rumen?
- Pressure selected natural consortium?

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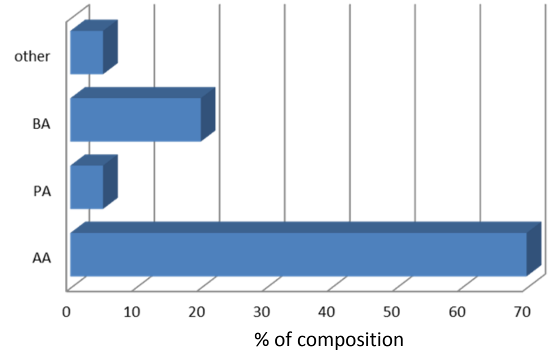
MELiSSA thermophilic C1 is not 100% efficient

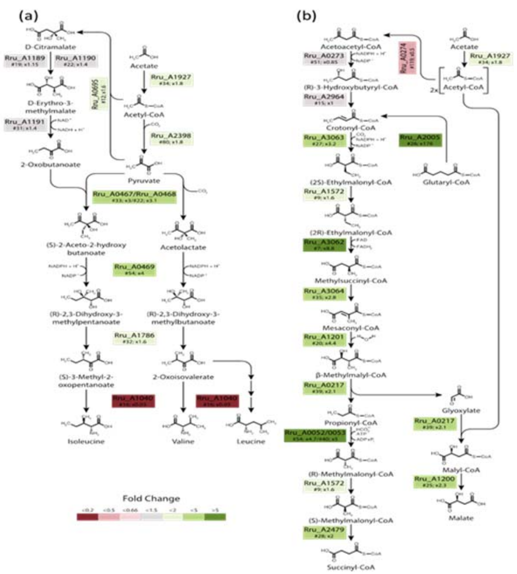
VFA produced

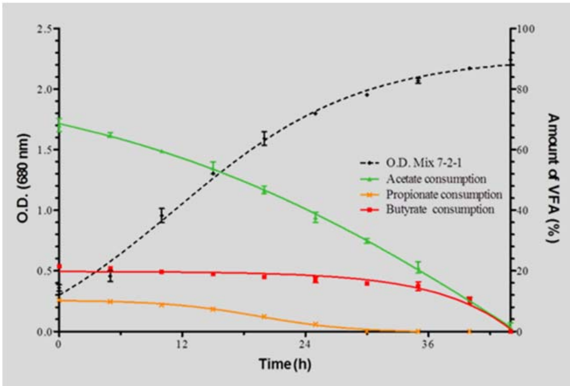
- **C1 fermentation produced large amount of VFAs**
- **Acetate, butyrate, propionate,…**

- **Photoheretrophic removal of the VFAs by *Rhodospirillum rubrum* analyzed at metabolic level**

Typical profile of C1 effluent VFAs composition
5mg/L in effluent; 300/d.g OM





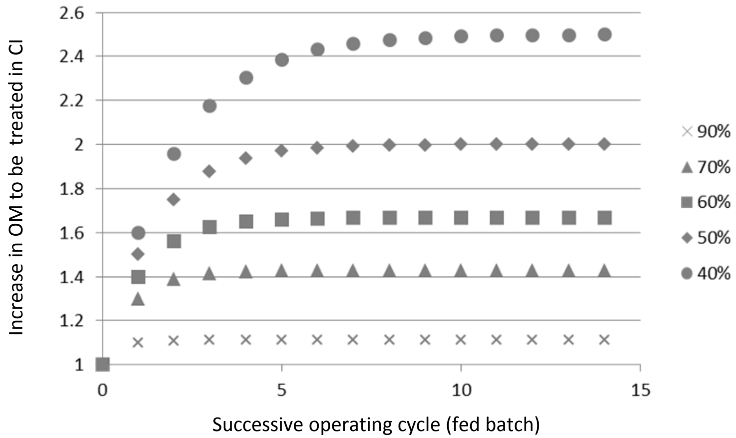


Net consumption of CO_2 for redox balancing

MELiSSA thermophilic C1 is not 100% efficient

VFA produced

- **Use of *Rh. rubrum* biomass (food source or feed source…) ?**
- **Reinjection of *Rh. rubrum* biomass (feasibility depends on C recovery yield in C1)**



- **Modification of operational conditions for removal of the VFAs by *Rh. rubrum* in order to decrease CO2 consumption ?**
- **Development of bioelectrical device based on *Rh. rubrum* excess reducing power?**

MELiSSA thermophilic C1 is not 100% efficient

Other today open question regarding MELiSSA waste?

- **Low amount of H_2 (and potentially CH_4) produced**
- **What about bacteriophages, Quorum-sensing molecules?**
- **Relevance of fecal matter treatment**
- **Accumulation/degradation of micropollutant (hormones, drugs,…)**

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esa **team**