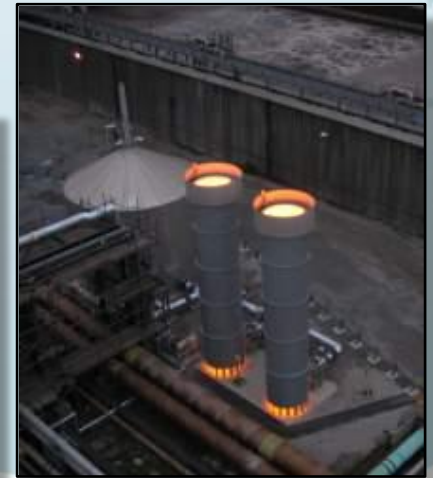
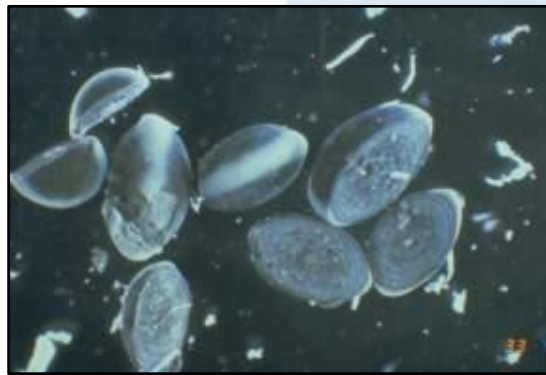
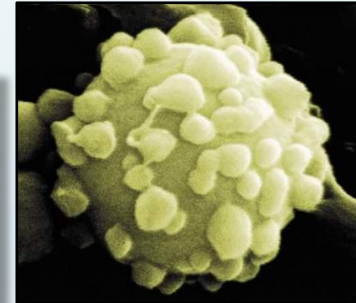


Harnessing the wonders of the microbial world to solve environmental problems

René Rozendal



***Understanding Microbial Communities - Developing the Potential
Isaac Newton Institute Open for Business Event
4th of December 2014 - Isaac Newton Institute, Cambridge***

revitalizing resources

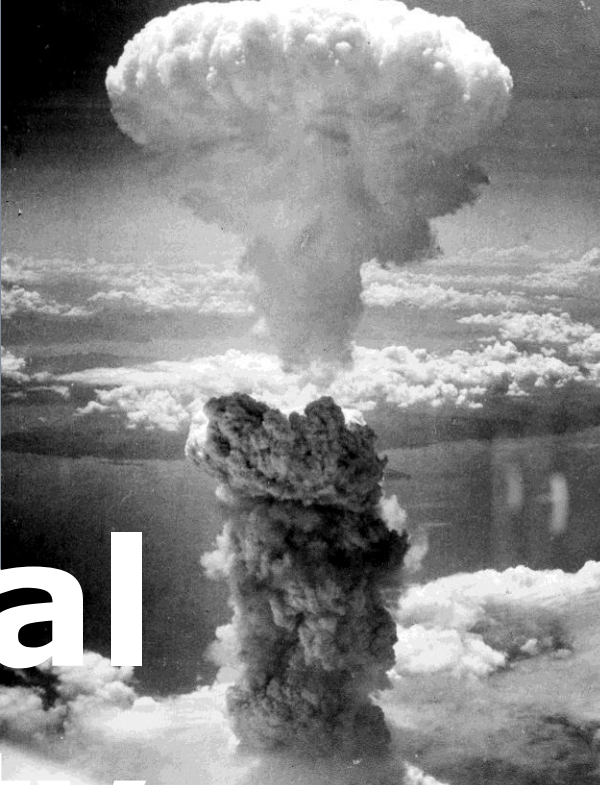
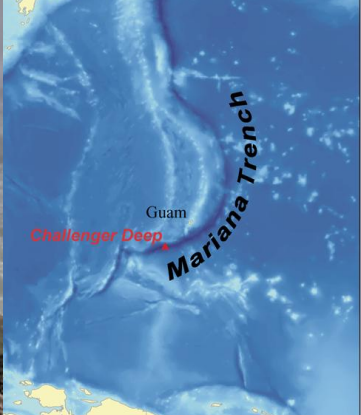


About Paques



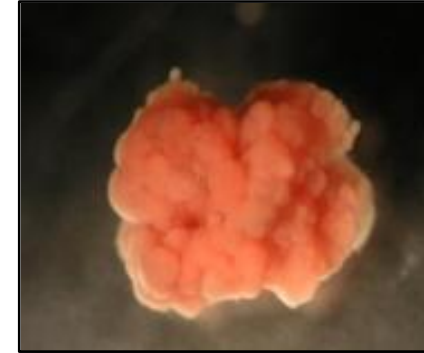
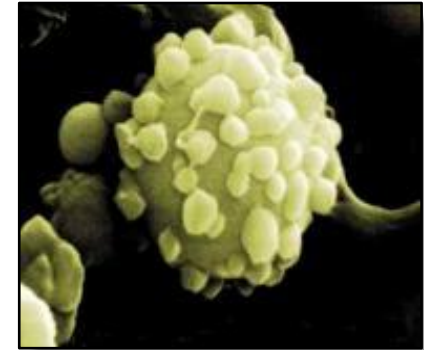
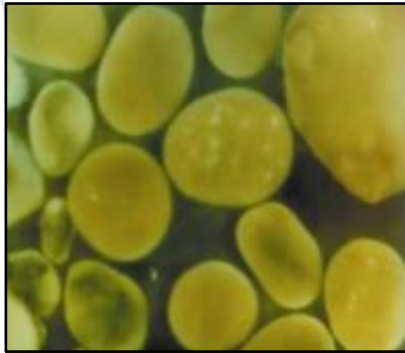
- Family owned business, founded in 1960
- Number of employees: ~400
- Number of Business Units: 4 (Europe, China, South America & India)
- Develop and commercialize novel biological solutions for wastewater and gas treatment
- More than 1,800 installations worldwide

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Microbial Diversity

Our approach



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“Application of useful microorganisms from nature in our high rate bioreactors”

In strong collaboration with universities!

Environmental Biotechnology

- **Pure culture processes not feasible on waste**
 - “Dirty” environments, already full of microbes
 - Large flows, so sterilisation not economically feasible
- **Only option:**
Understand the role that certain target microbes have in nature and recreate their preferred environment inside a bioreactor.
 - *Community engineering vs Genetic engineering*

Paques' product portfolio

Know how area	Application	Products
Organics	ANAEROBIC AND AEROBIC WATER TREATMENT	BIOPAQ [®] IC BIOPAQ [®] UASB / UASB+ BIOPAQ [®] AFR CIRCOX [®] BIOPAQ [®] UBOX BIOPAQ[®] CIRCOX[®]
H ₂ S / SO ₂	(BIOGAS) DESULFURISATION	THIOPAQ [®] BIODESOX [®] THIOPAQ[®] BIODESOX[®]
Nitrogen & Phosphate	(DE)NITRIFICATION	ANAMMOX [®] PHOSPAQ [®] ASTRASAND [®] ANAMMOX[®] PHOSPAQ[®]
Metals and sulphate	METAL RECOVERY SULPHATE REDUCTION	THIOTEQ [®] SULFATEQ [®] BIOMETEQ [®] THIOTEQ[®] SULFATEQ[®] BIOMETEQ[®]
Separation	FILTRATION AND SOLID REMOVAL	ASTRASAND [®] ASTRASEPARATOR [®] ASTRASAND ASTRASEPARATOR

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Anaerobic Treatment

- World leader in anaerobic wastewater treatment
- Removal of organic contaminants
- Biogas production
- >1000 Biopaq references in more than 60 countries

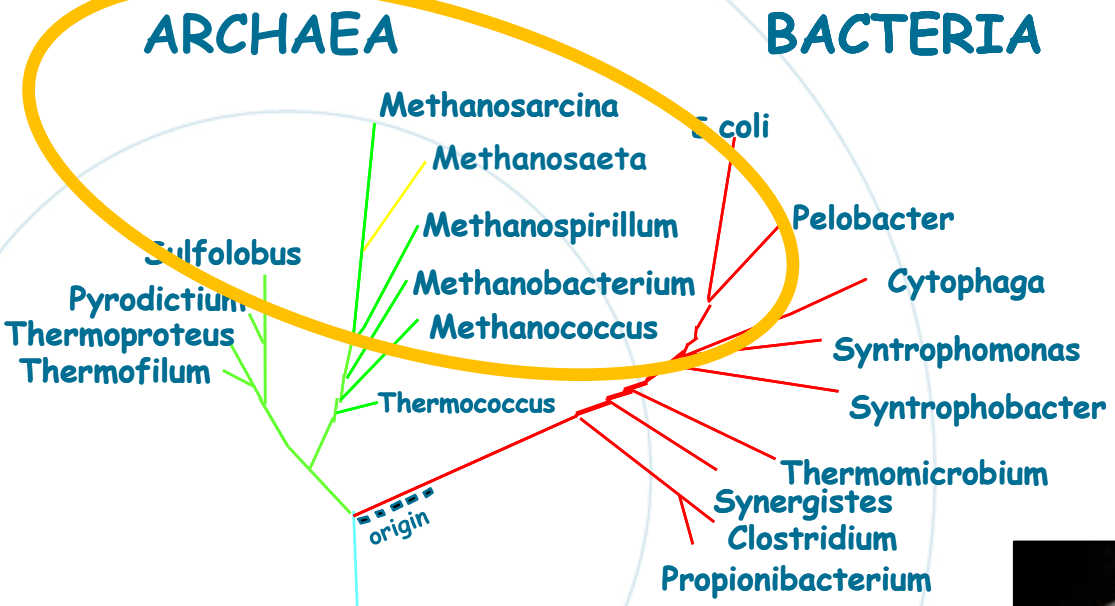
BIOPAQ®



revitalizing resources

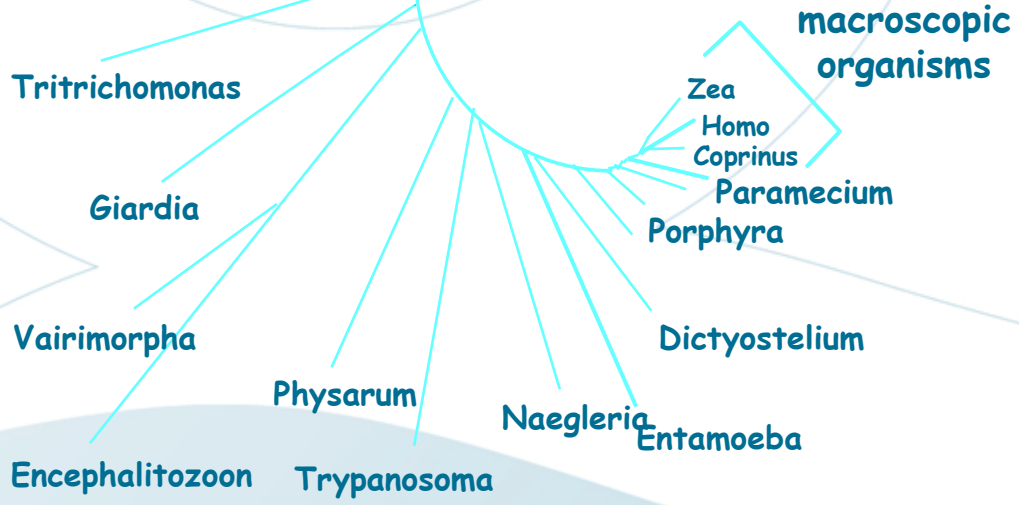
✦ DALTON COLLECTING MARSH FIRE GAS ✦





0.1 changes per nt

EUCARYA

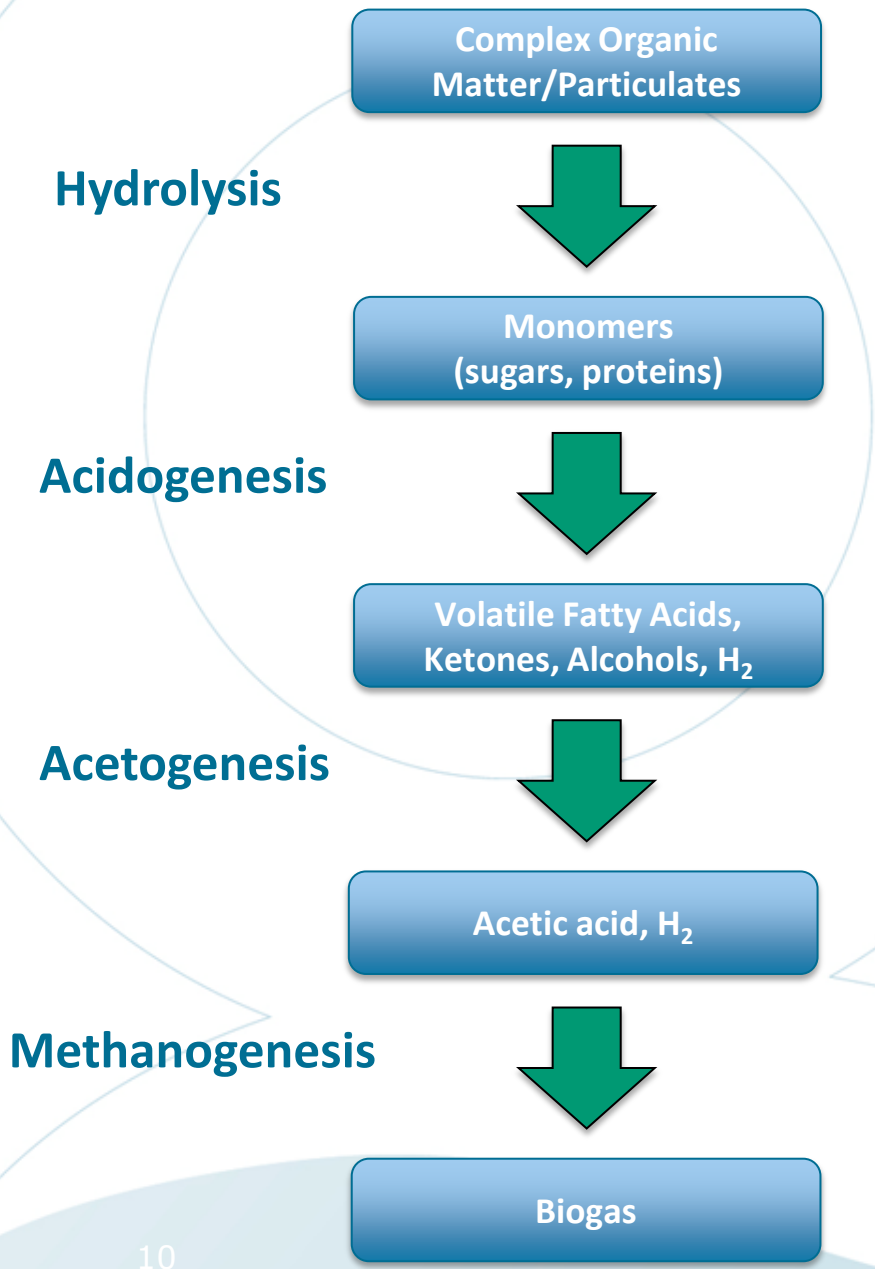


Methanosarcina
(Image by: Broad institute)

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4 stages



- Products of one stage are the substrates of the next stage.

- Hydrolysis, acidogenesis, and acetogenesis done by bacteria

- Methanogenesis done by specialised archaea

Before the 80's, flocs were used



**LOW BIOMASS
DENSITY**

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Then anaerobic granules were discovered

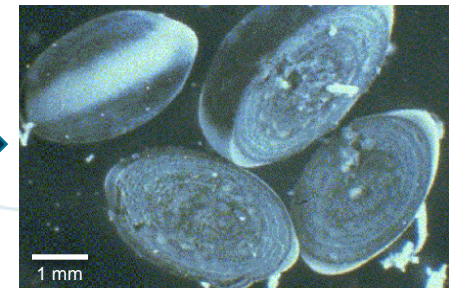
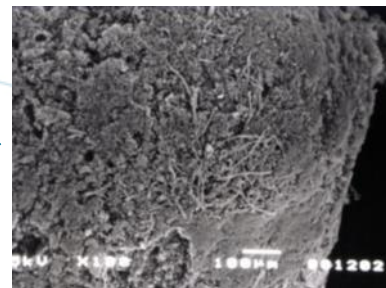
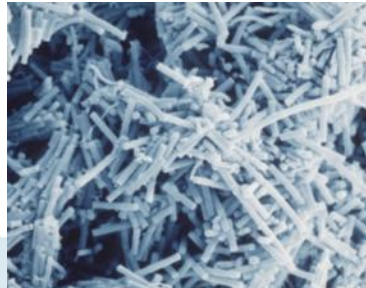
- During the 70's development of anaerobic granular sludge technology (Lettinga)
- Much more microbial biomass per m³ reactor, so much higher conversion rates



GRANULAR BIOMASS

(SIZE: 0.5-3 mm)

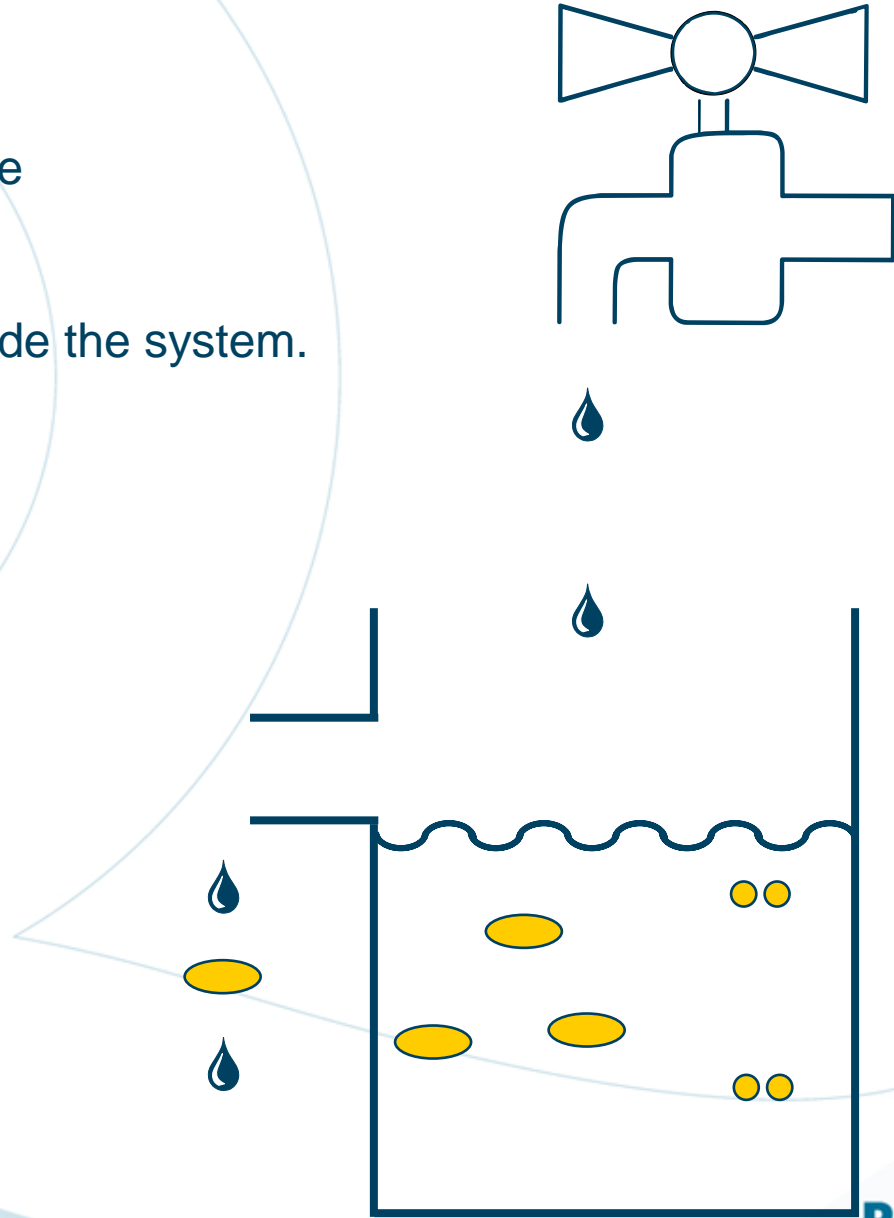
Survival strategy Biofilm & Granule formation



Photo's: *G. Gonzales-Gil, A. Alphenaar*

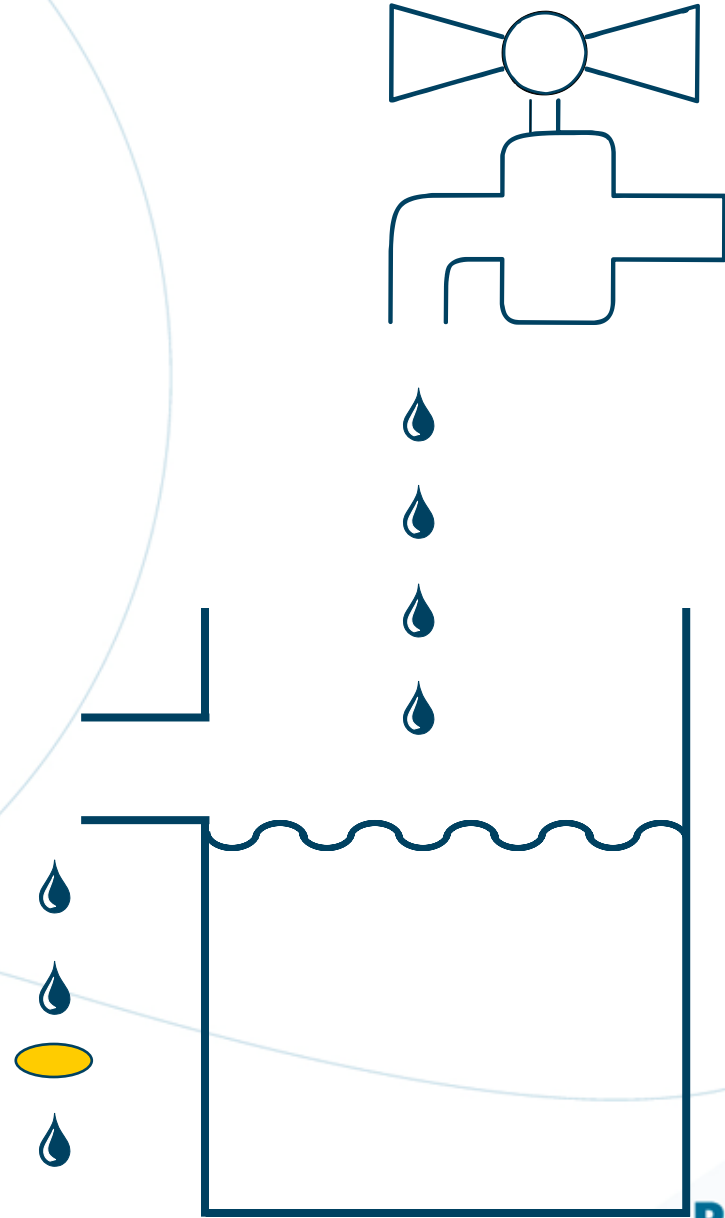
Dilution rate < growth rate

Microorganisms stay inside the system.



Dilution rate > growth rate

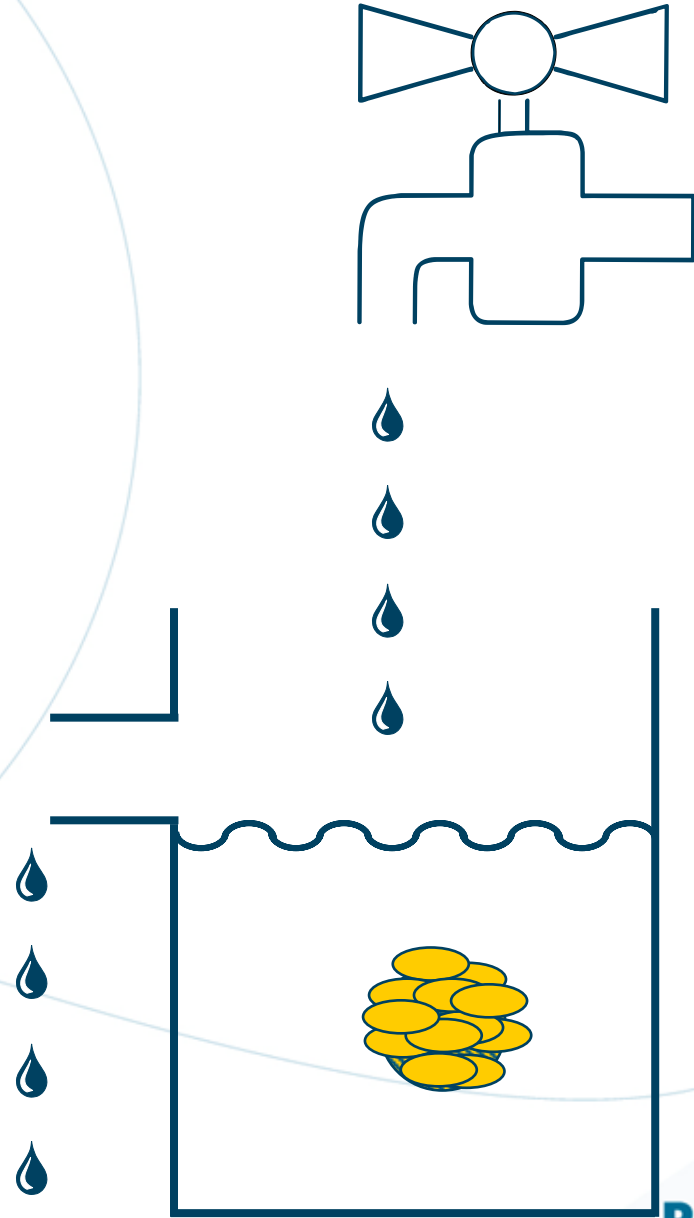
Wash out of microorganisms!



Dilution rate $>$ growth rate

However, microbes are smarter than that!

Under the right circumstances microorganisms can form granules to stay in reactor!

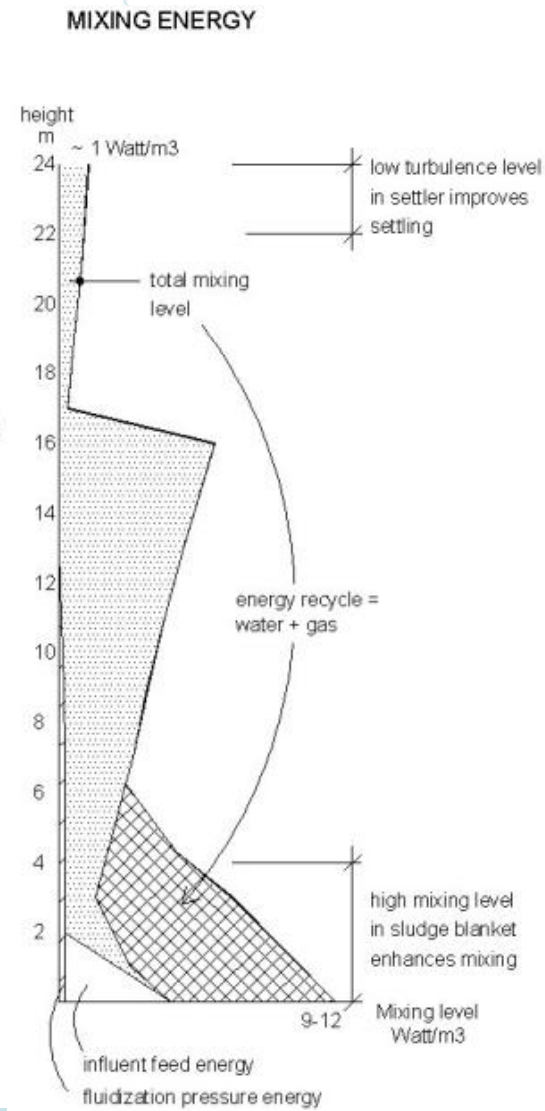
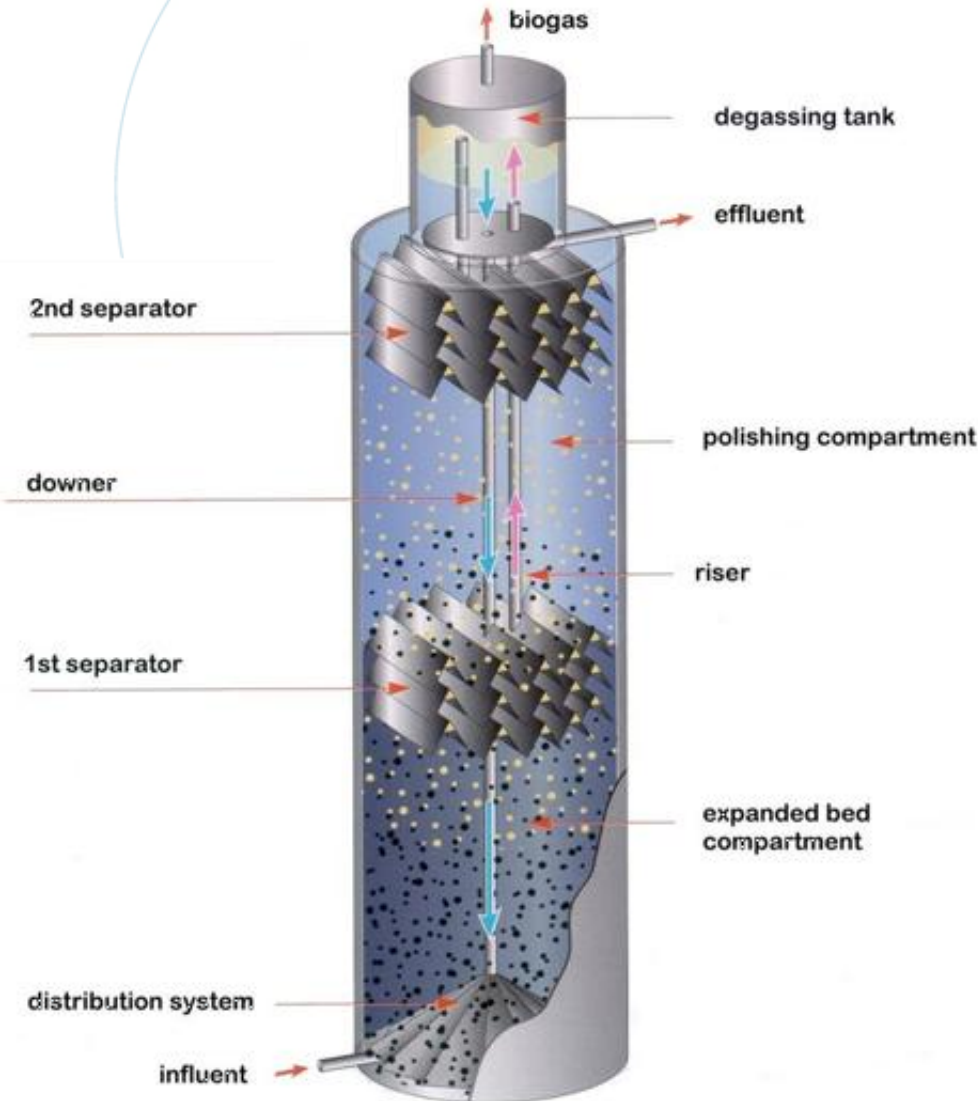


BIOPAQ[®]IC settler 8 m diameter



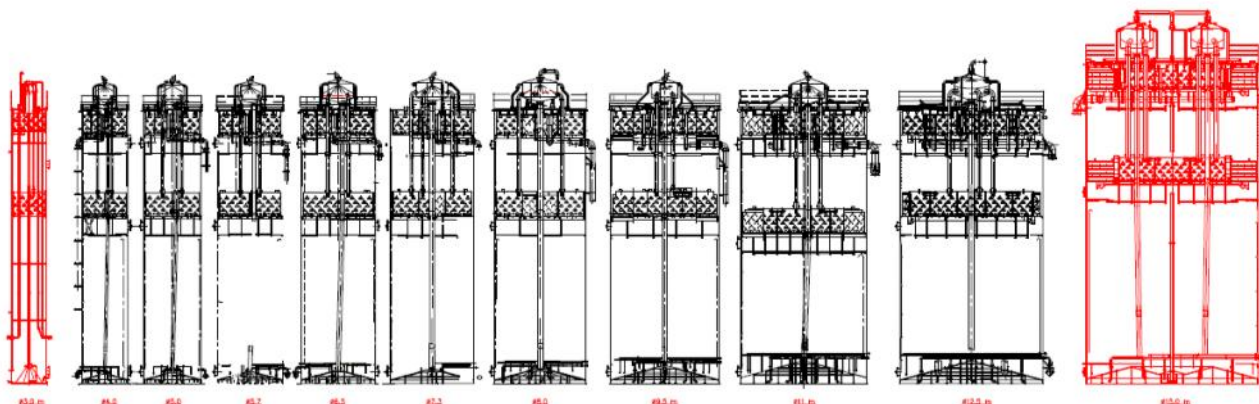
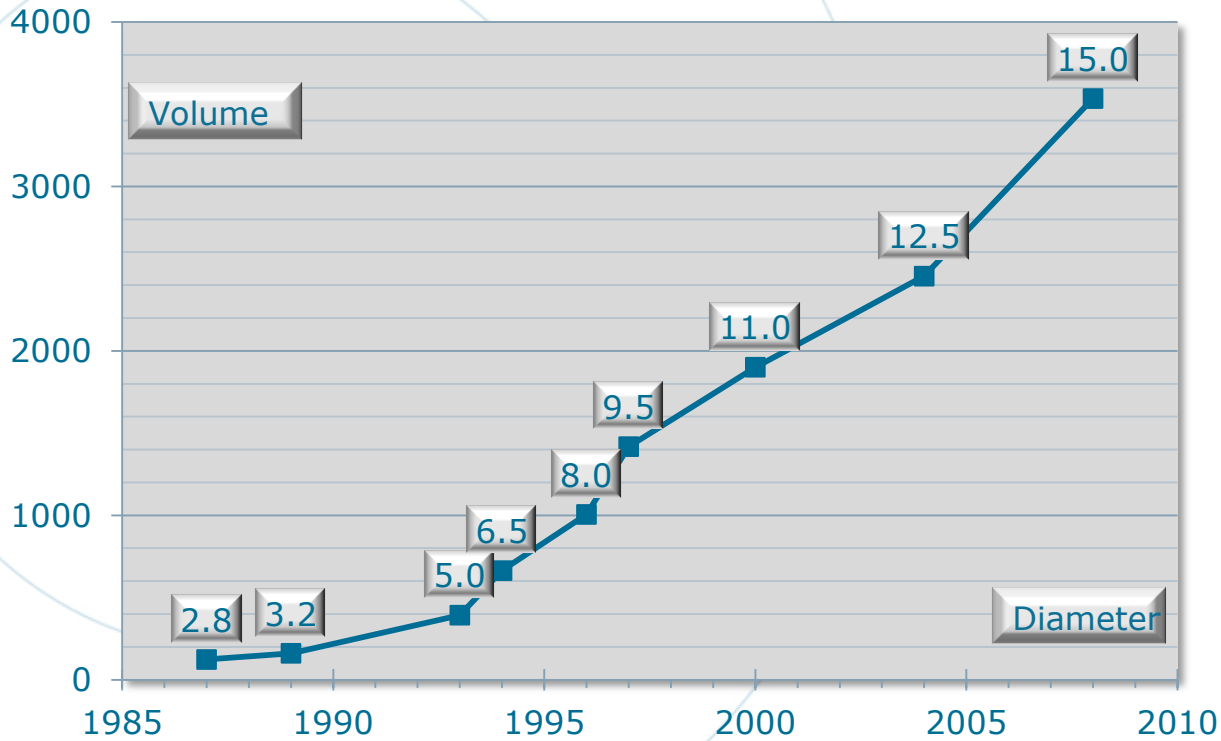
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BIOPAQ® IC Internal Circulation



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Product development - Size



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Large scale biotechnology

...up to 5000 m³ (15 m diameter and 28 m height)

Capacity:

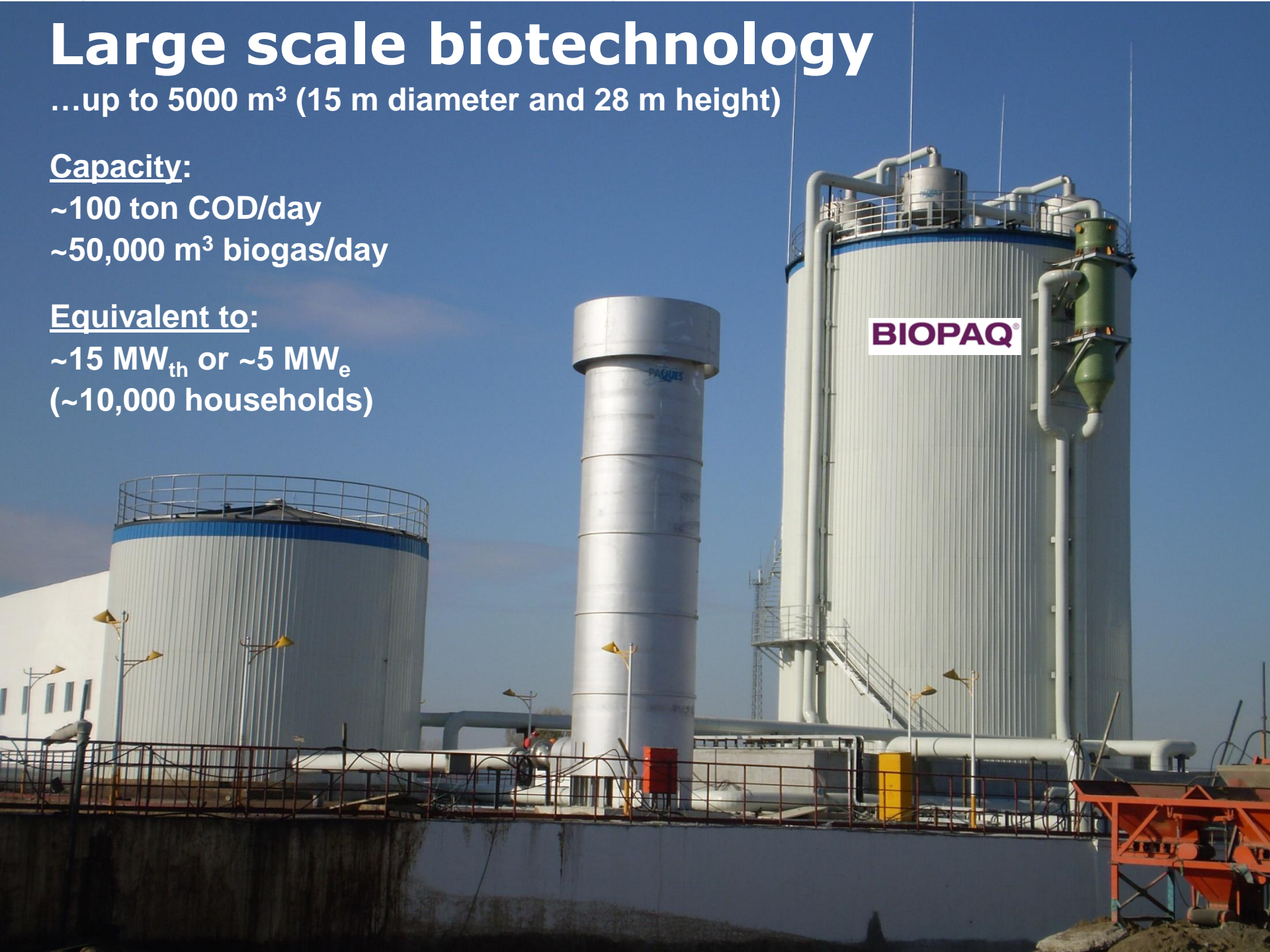
~100 ton COD/day

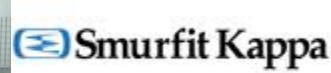
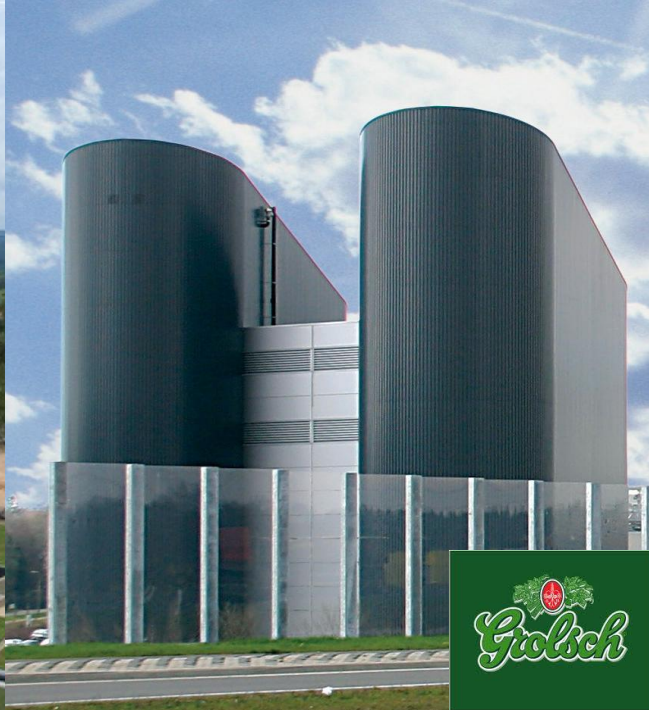
~50,000 m³ biogas/day

Equivalent to:

~15 MW_{th} or ~5 MW_e

(~10,000 households)





Biogas composition

Compound	Vol%
Methane (CH ₄)	50-90
Carbon dioxide (CO ₂)	10-45
Water (H ₂ O)	0-5
Hydrogen sulphide (H ₂ S)	0-3.5



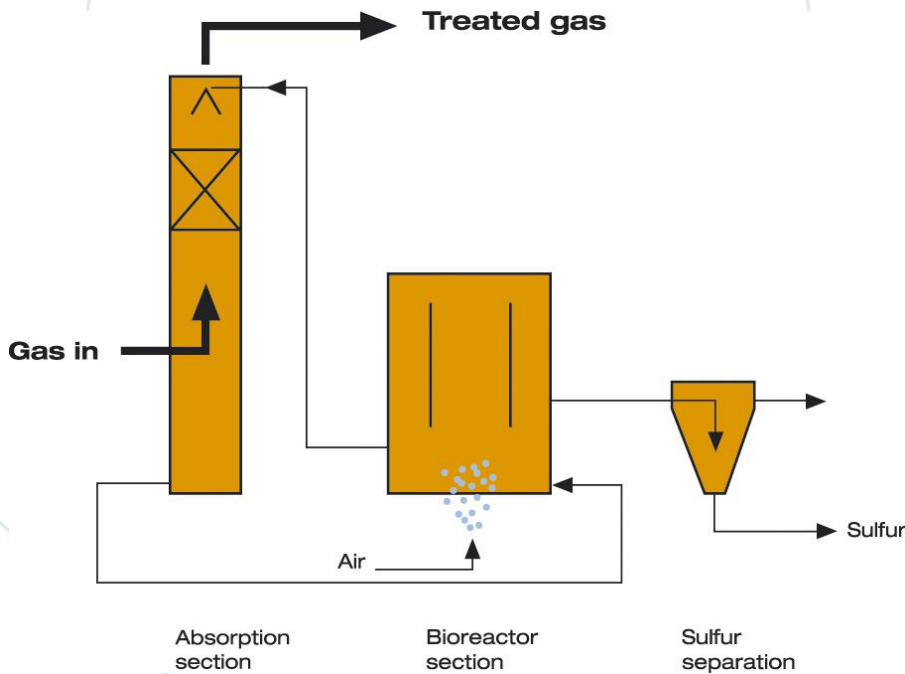
Consequences of H₂S exposure

H ₂ S conc. (ppm)	(Possible) consequences
0.02	"Rotten egg" smell
1.6	Exposure limit (average over 8 hours)
10	Evacuation limit
100	Numbing of the olfactory nerves in 3 to 15 minutes Stinging of eyes and throat Risk of pulmonary oedema
200	Acute numbing of olfactory nerves Stinging of eyes and throat Risk of pulmonary oedema
500	Impairment of mental abilities Loss of sense of balance Numbing of respiratory organs in 30 to 45 min. Lethal after 30 to 60 min.
700	Rapid loss of consciousness (max. 15 min.) Breathing stops Lethal after 12 to 15 min.
1000	Immediate loss of consciousness Acute numbing of breathing "Knock down effect" Lethal within 3 min.

**H₂S in Biogas:
>>1000 ppm!!!**

THIOPAQ[®] technology: Conversion of H₂S to elemental sulphur

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Absorber



Bioreactor



H₂S removal by biological oxidation

Two possibilities:

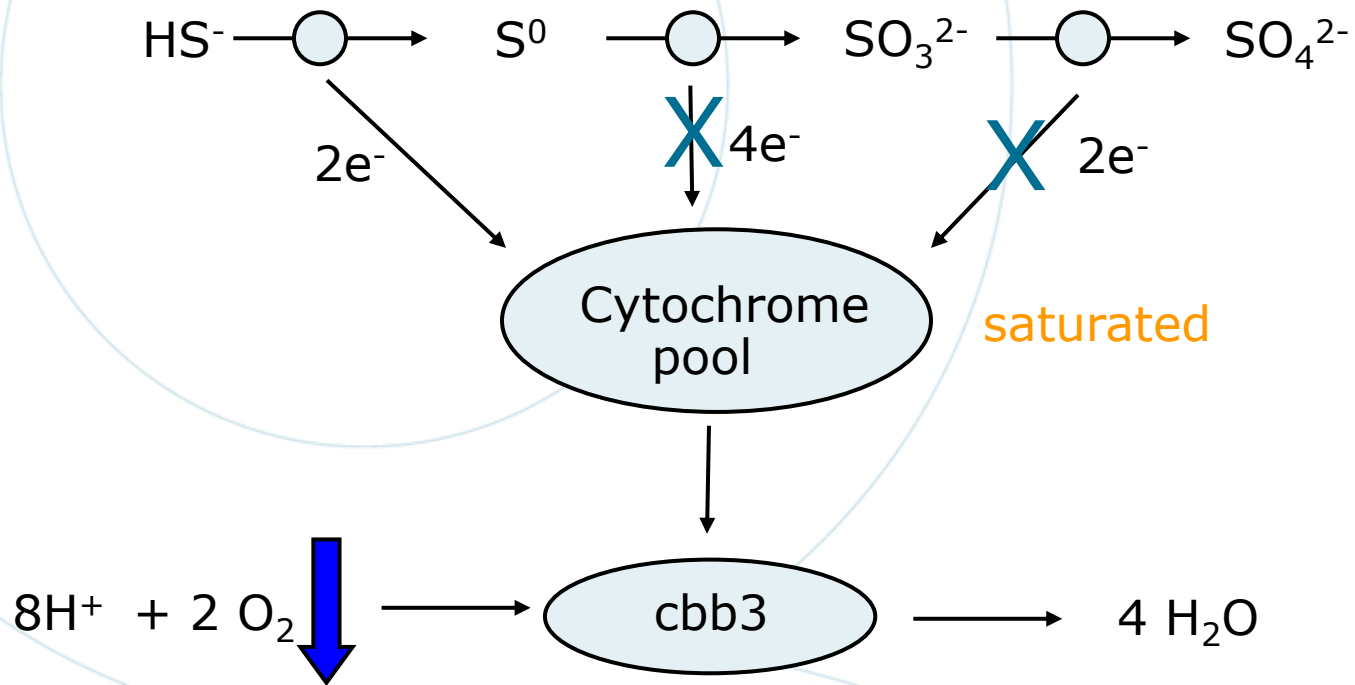
- Complete oxidation:



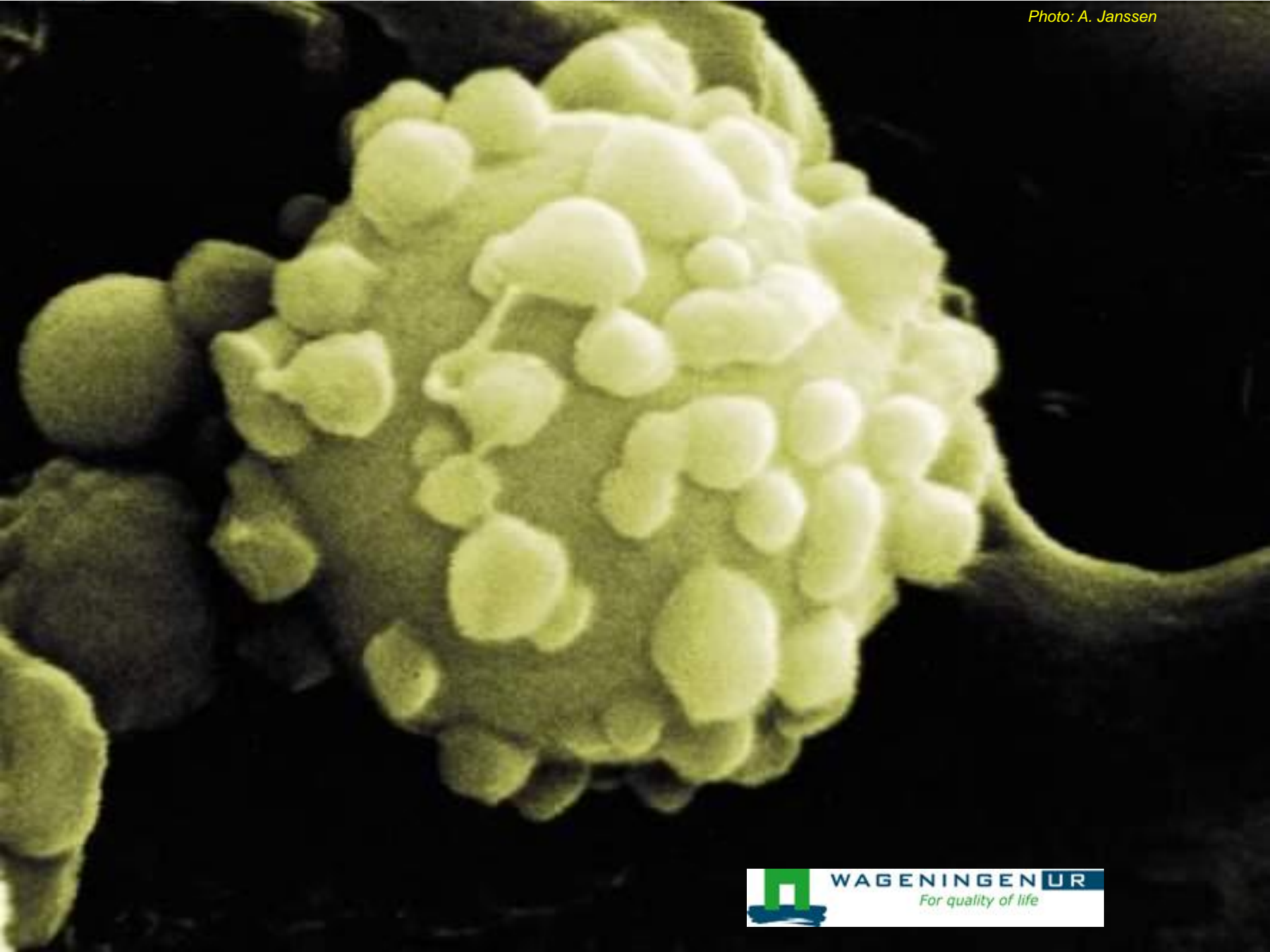
- Partial oxidation:



How to stimulate sulfur accumulation?



Oxygen supply via Redox Control (Janssen 1995)



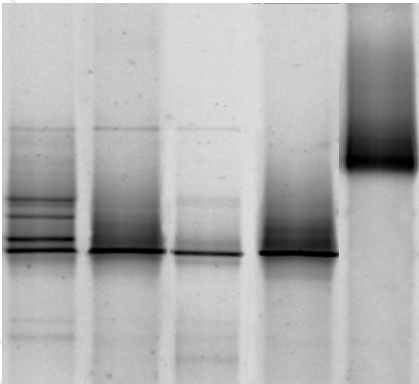
Natural Environment Thiopaq inoculum



Sodium Bicarbonate

Fig. 14.1
Typical small shallow soda lake (Kulunda steppe, Altai, Russia)

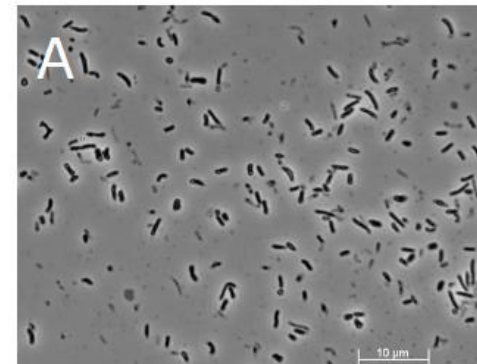
DGGE analysis 16S rRNA



2009 2005 2006
Full Scale

Halothiobacillus W5, original dominant organism

Pure culture Thioalkalivibrio sp.



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0.02 ton S per day



0.5 ton S per day



0.9 ton S per day



2.8 ton S per day



4 ton S per day



13 ton S per day



THIOPAQ®

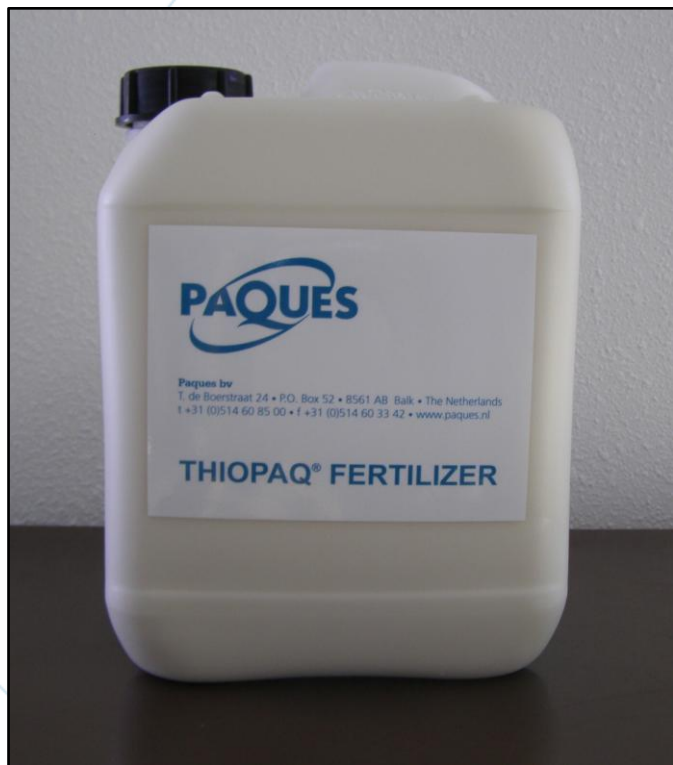


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Gas desulphurisation with recovery Paqell.



THIOPAQ® Fertilizer

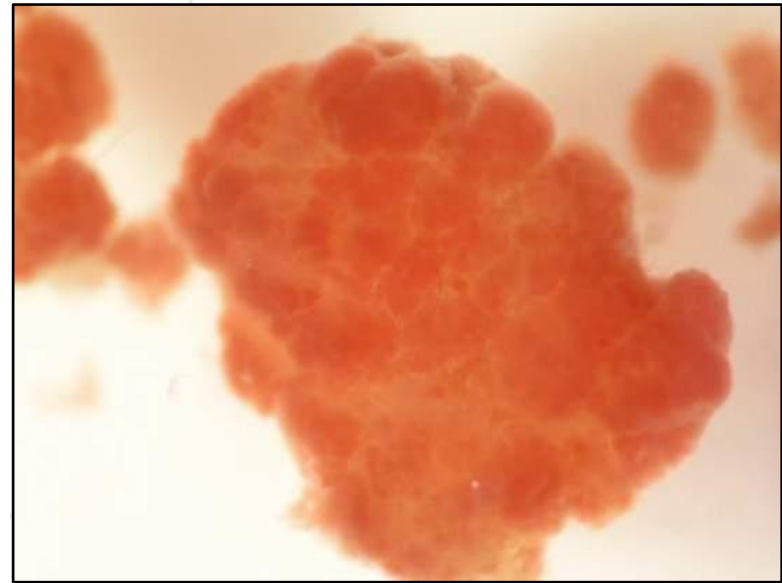
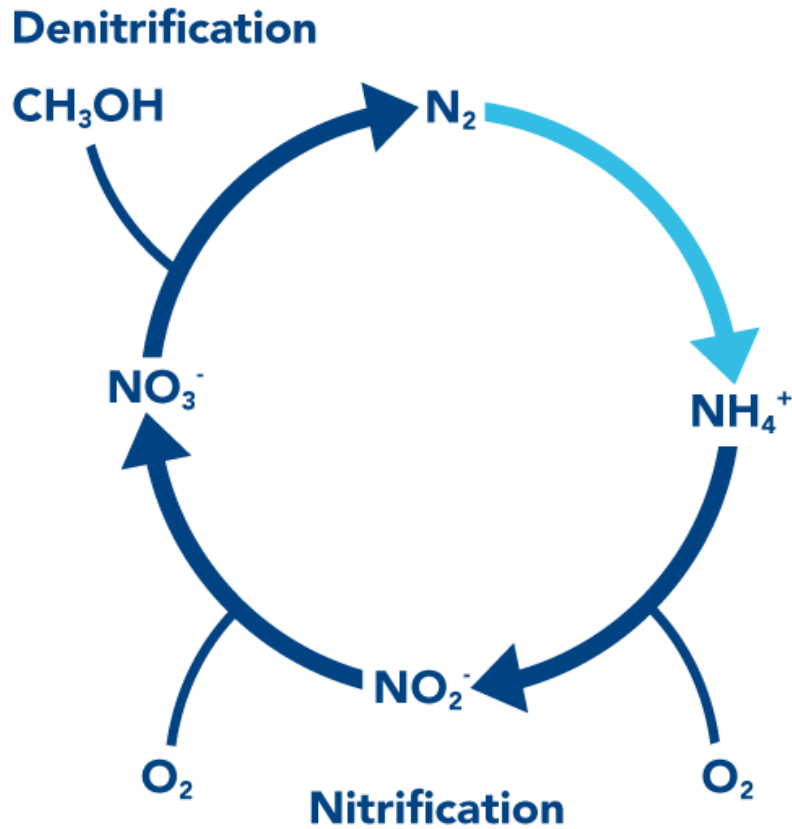


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ANAMMOX[®] TOTAL NITROGEN REMOVAL

Nitrogen Cycle



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Comparison Conventional vs. ANAMMOX®

Conventional:
nitrification + denitrification

Ammonium

aeration
(2.8 kWh/kg N)

Nitrate

COD
(>5 kg/kg N)

Nitrogen gas

>4.7 ton CO₂/ton N

ANAMMOX®

Ammonium

aeration
(1 kWh/kg N)

Ammonium

Nitrite

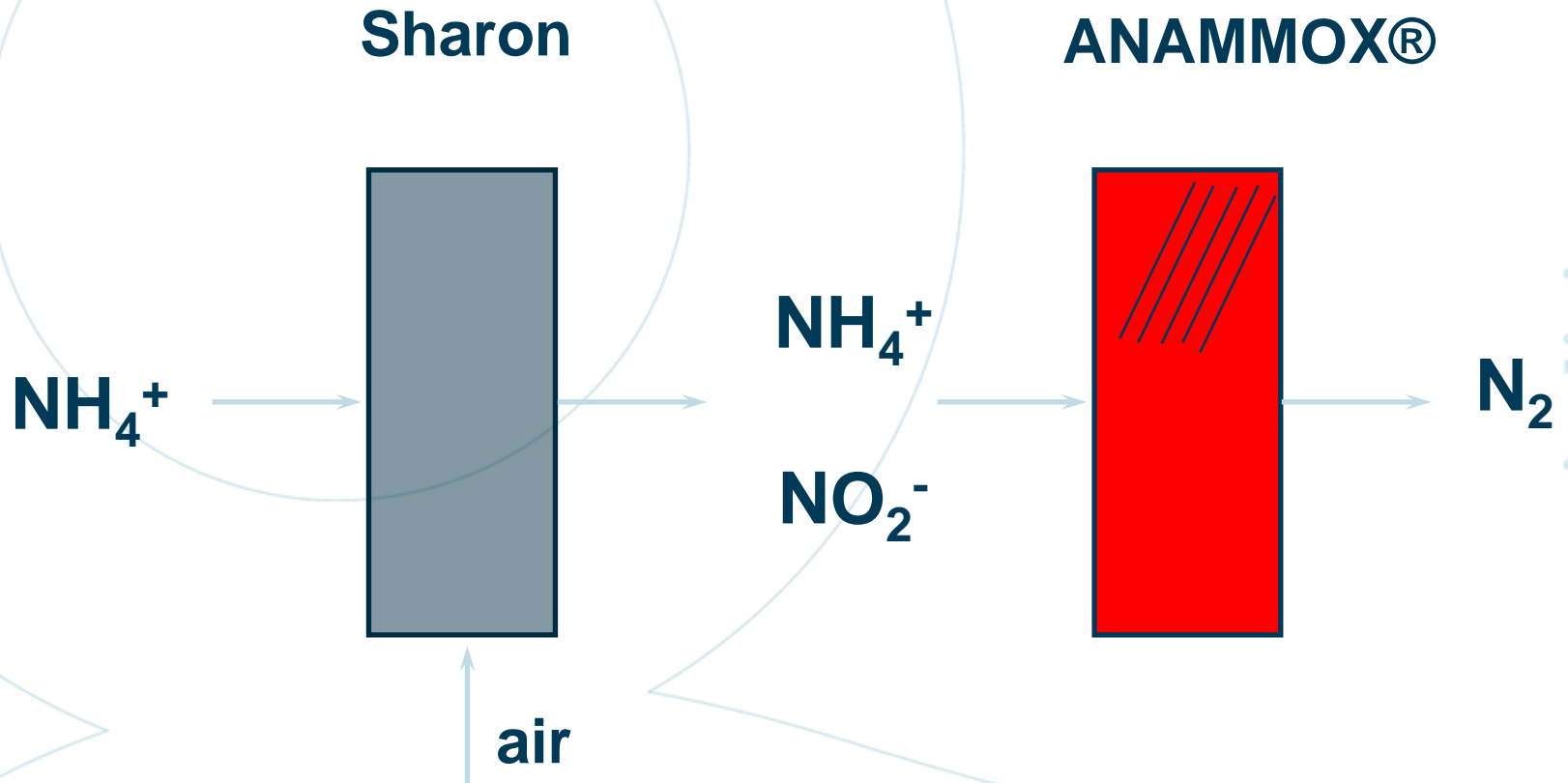
COD
(0 kg/kg N)

Nitrogen gas

0.7 ton CO₂/ton N

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Our starting point: two-step ANAMMOX

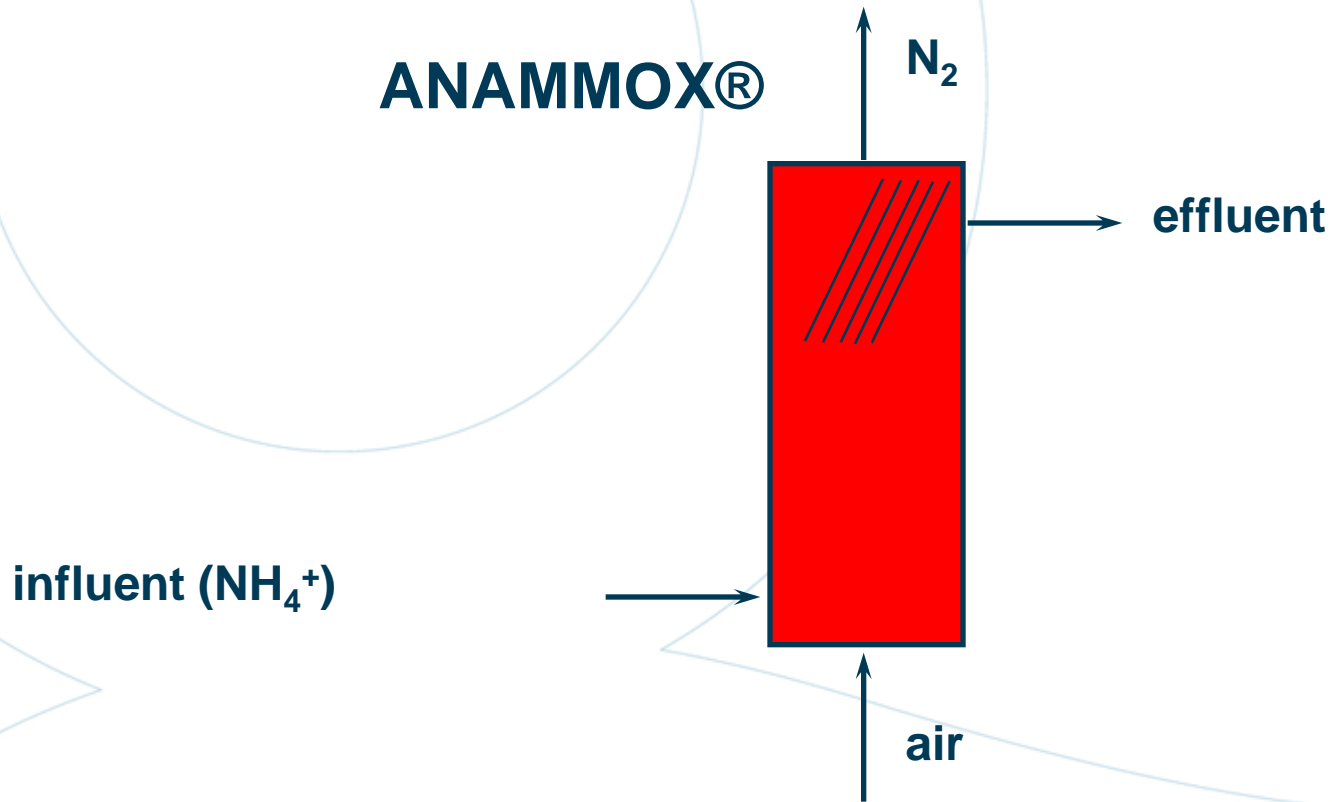


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Rejection water WWTP Rotterdam 2002

Next step: one-step reactor

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GRANULES AGAIN!

**Anammox
Granules**

**Anaerobic
Granules**

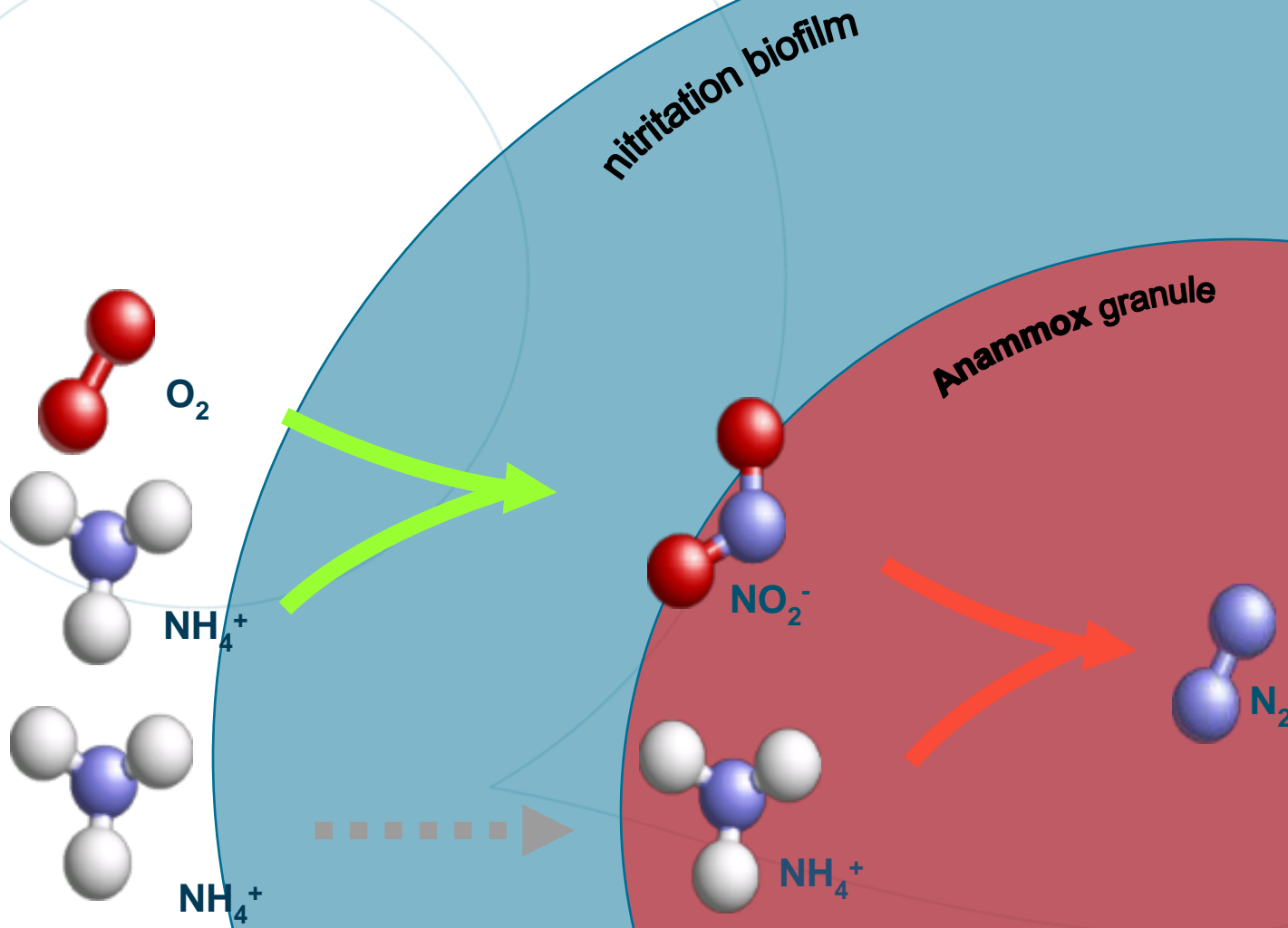
Principle One Step ANAMMOX®

nitritation biofilm

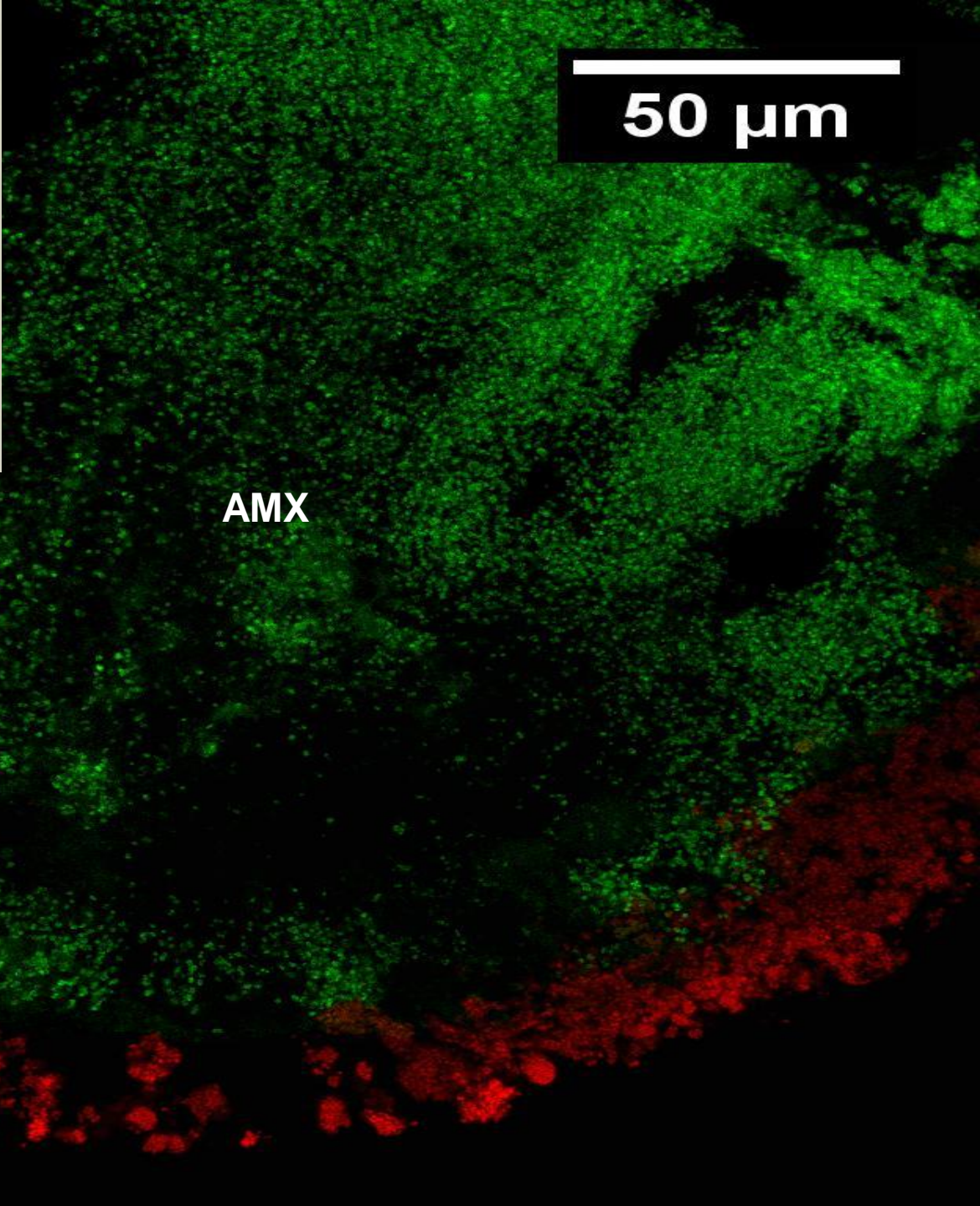
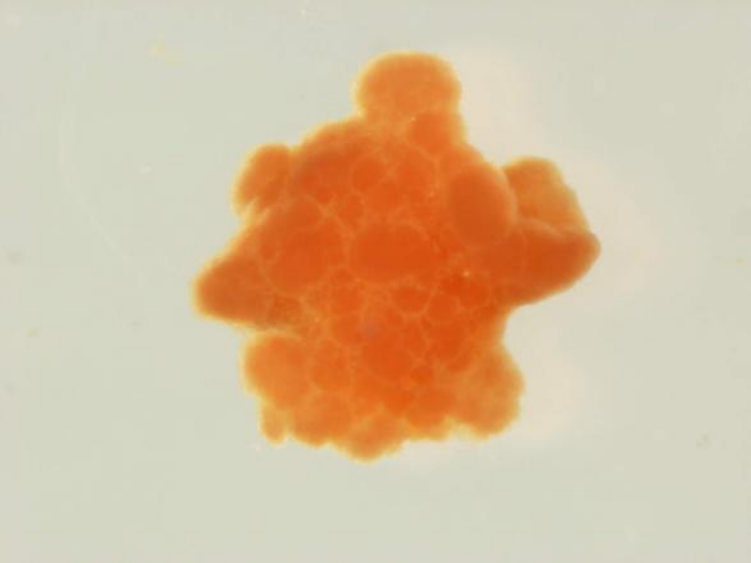
Anammox granule

resources
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Principle One Step ANAMMOX[®]



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50 μm

AMX

AOB

MEI HUA Group
Glutamate production
China
11,000 kg N/day



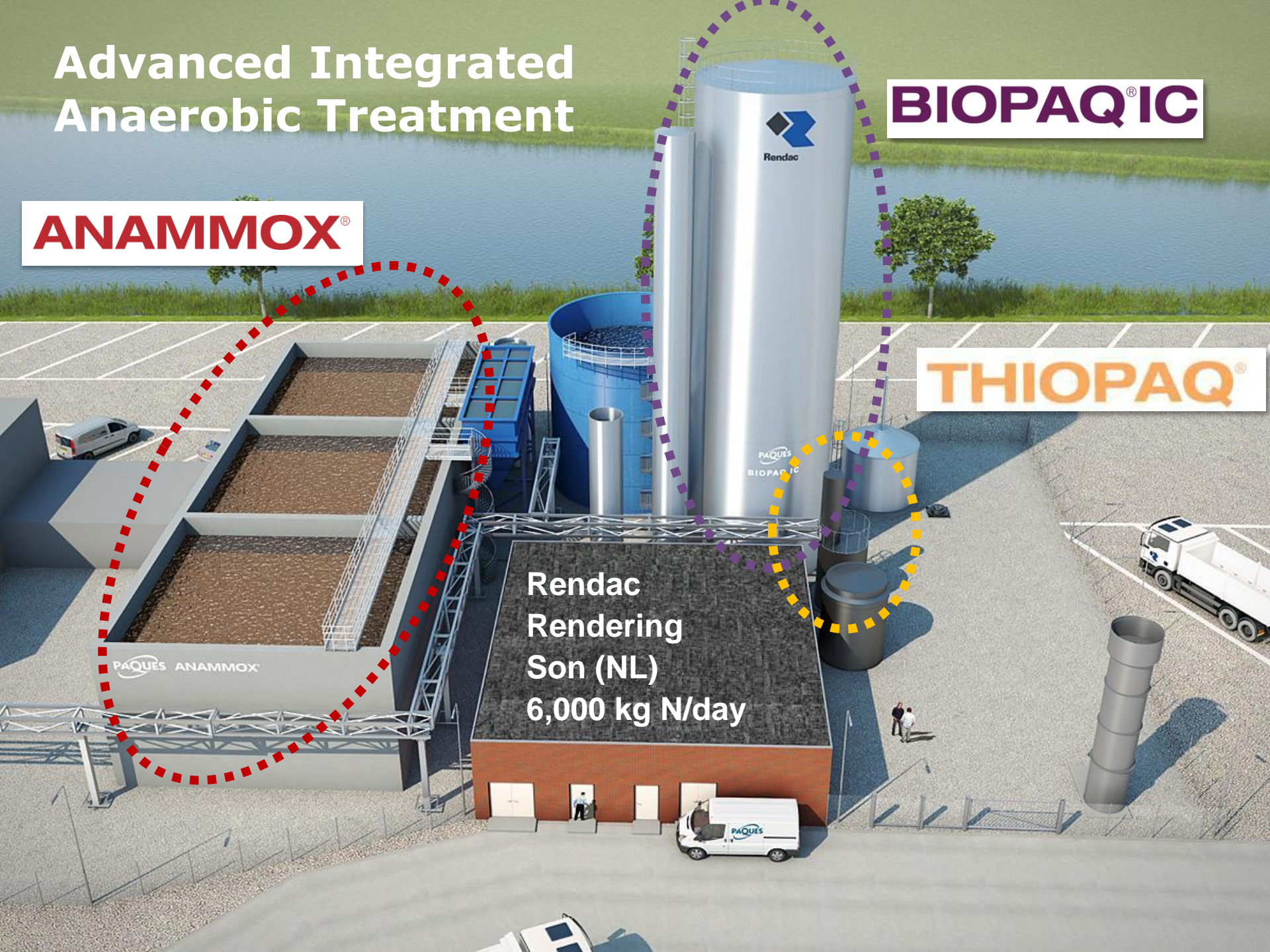
Advanced Integrated Anaerobic Treatment

ANAMMOX[®]

BIOPAQC[®] IC

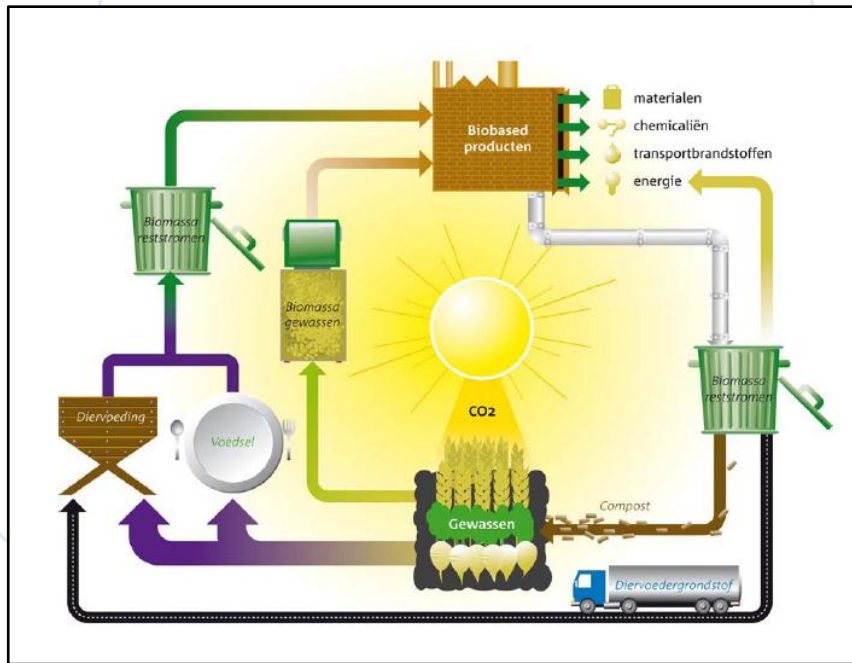
THIOPAQC[®]

Rendac
Rendering
Son (NL)
6,000 kg N/day

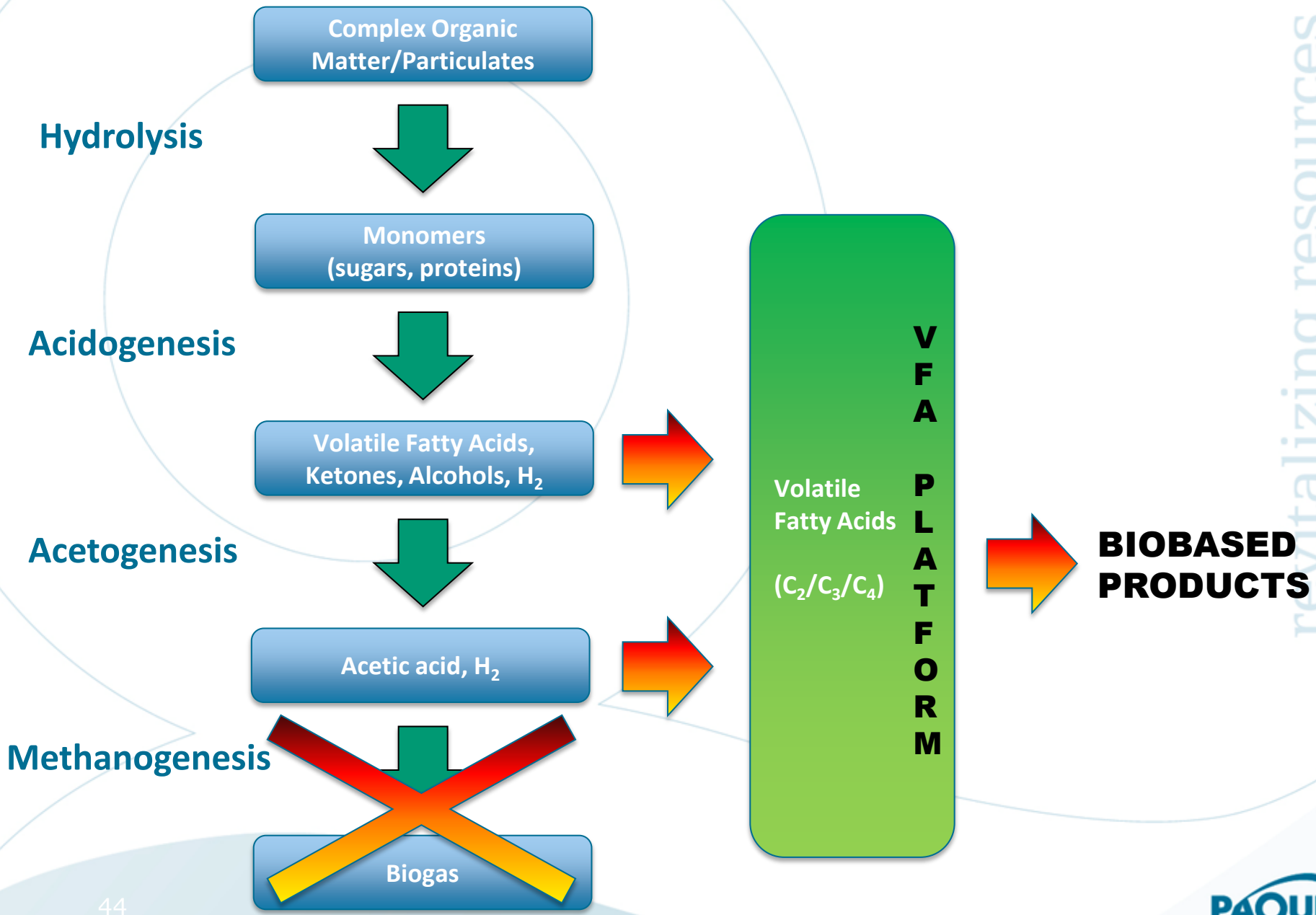


What's next?

The Biobased Economy



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High Rate VFA Production

- High rate systems for the production of VFA
- Granular biomass
- $VLR \gg 100 \text{ kg COD/m}^3/\text{day}$
- Product: $>10 \text{ g/L VFA}$
 - Mixture of acetate, propionate, butyrate, lactate, etc.



Bioplastic

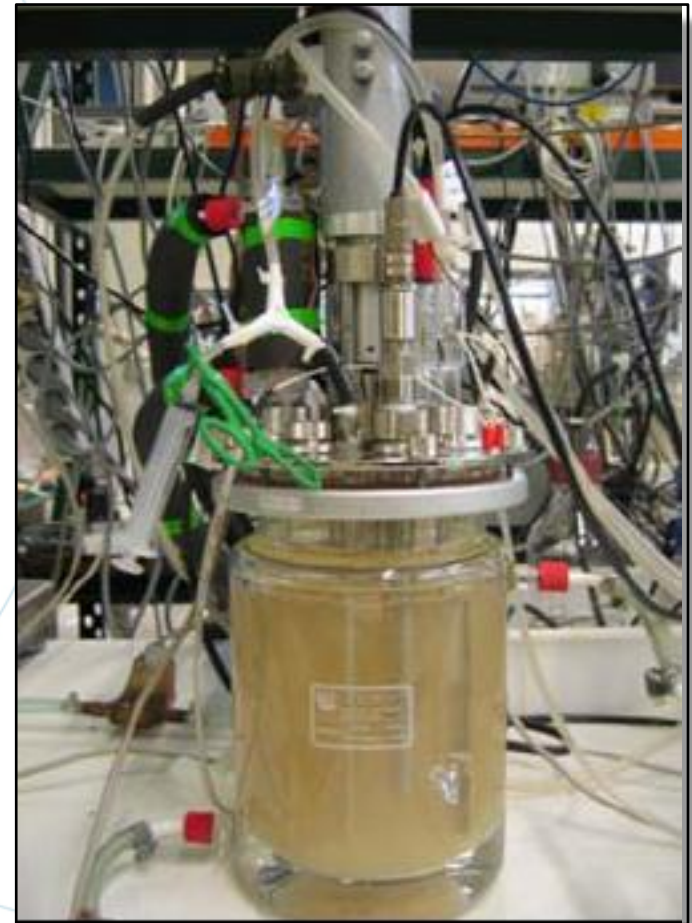
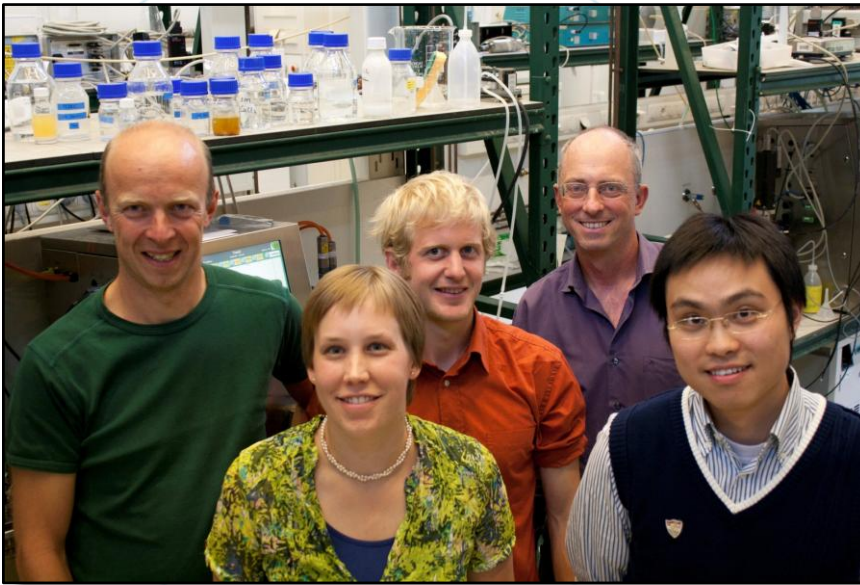


Van Loosdrecht & Kleerebezem

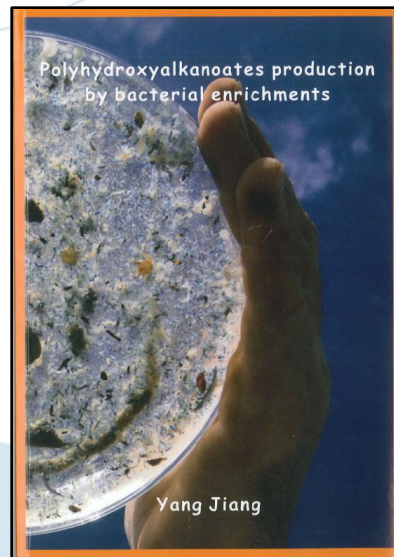
- Poly- β -hydroxyalkanoate (PHA)
 - Common and widespread storage material in microorganisms
- Storage up to 90 wt% in granules
- Properties similar to petrochemical plastics
- Biodegradable
- Made from renewable resources
- Currently made using conventional fermentation.
- Too expensive to become a commercial success.

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New process developed



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How does it compare?

Characteristics

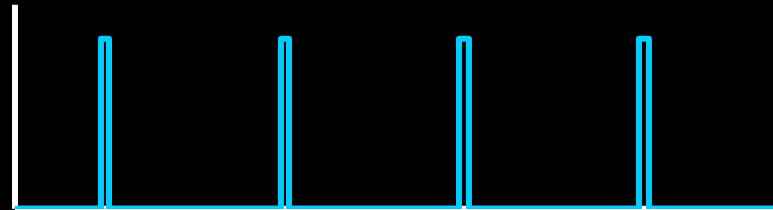
- Mixed microbial cultures, so “normal” wastewater treatment
 - **No** genetically engineered micro-organisms!
- RBCOD (i.e., VFA) from wastewater as feed
 - **No** expensive raw materials required!
- High PHA accumulation percentages (80-90% of dry weight)
 - Lower Downstream Processing costs
 - Direct use possible (i.e., without Downstream Processing)
- Result: major cost improvement compared to conventional fermentation process!

Ecological role of bioplastics

PHA = *Microbial Fat*



Feed
supply

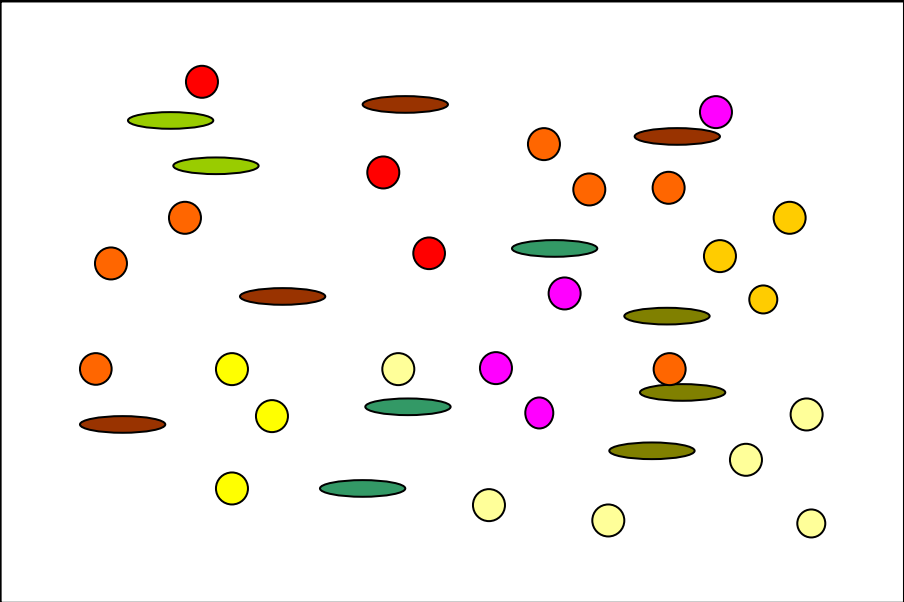
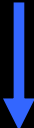


Time

Pulse feeding of substrate favors growth of bioplastic producing microorganisms: feast famine regime

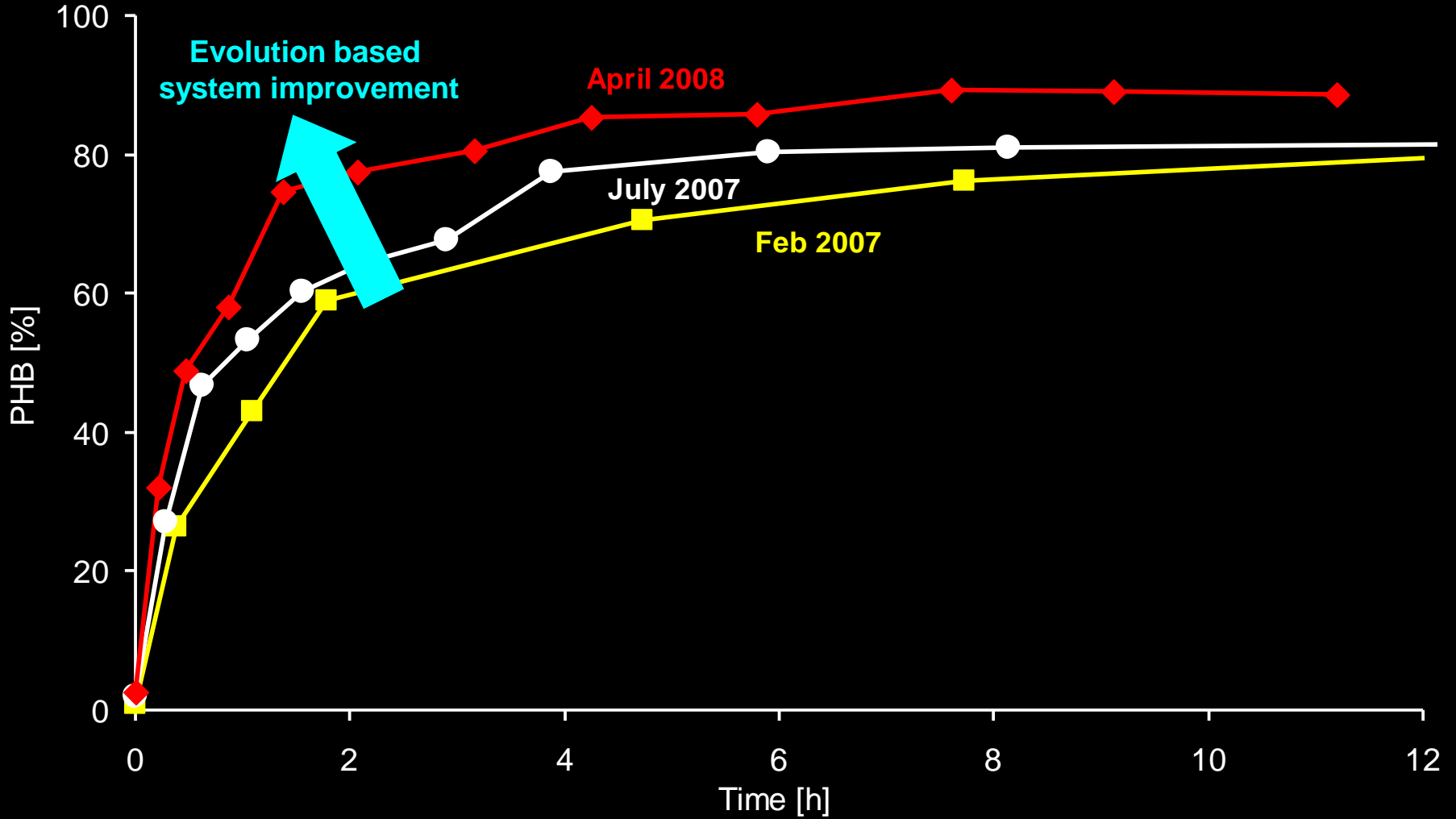
Microbial selection

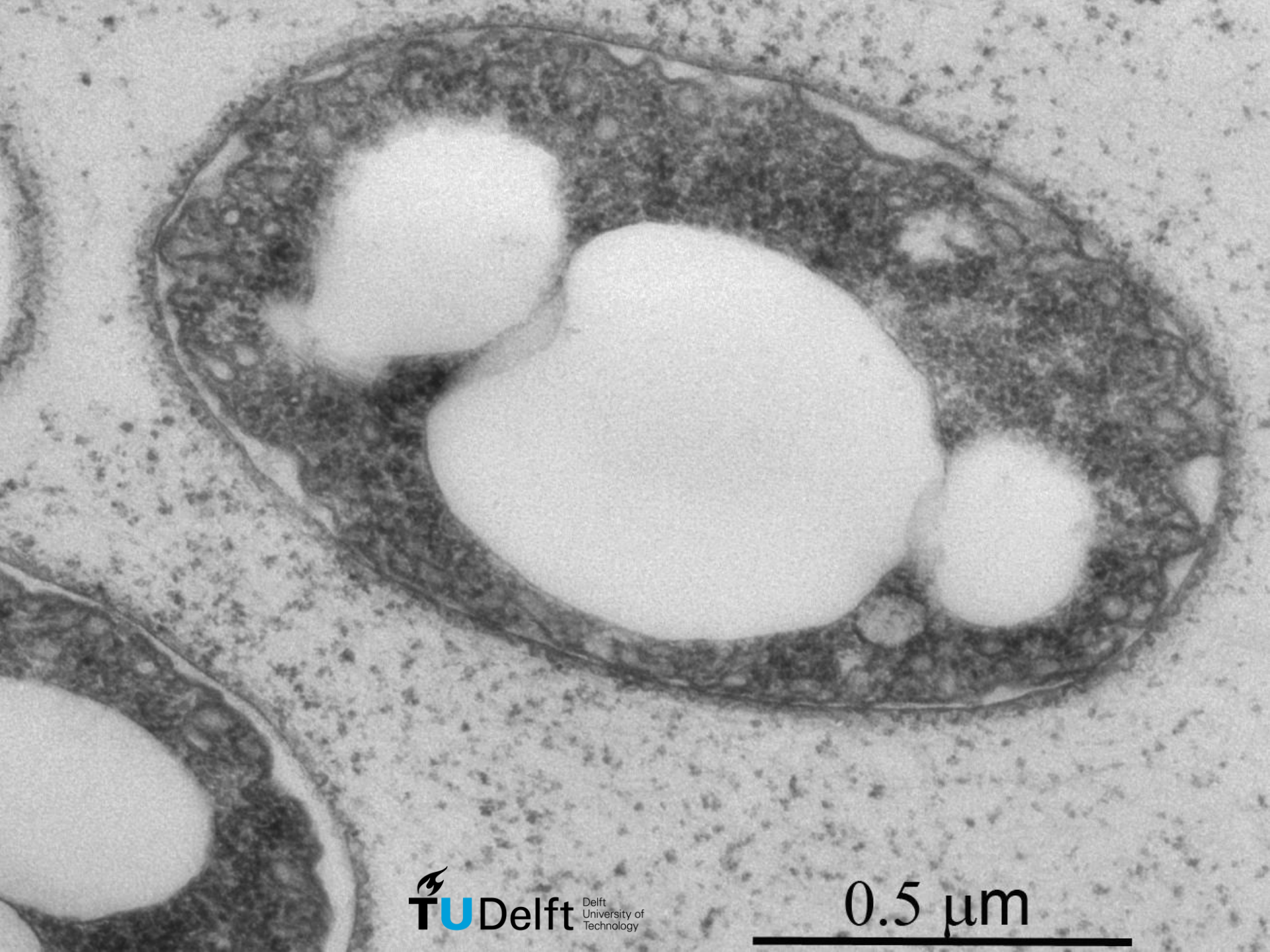
Substrate



● PHA storing bacteria

○ Non PHA storing bacteria





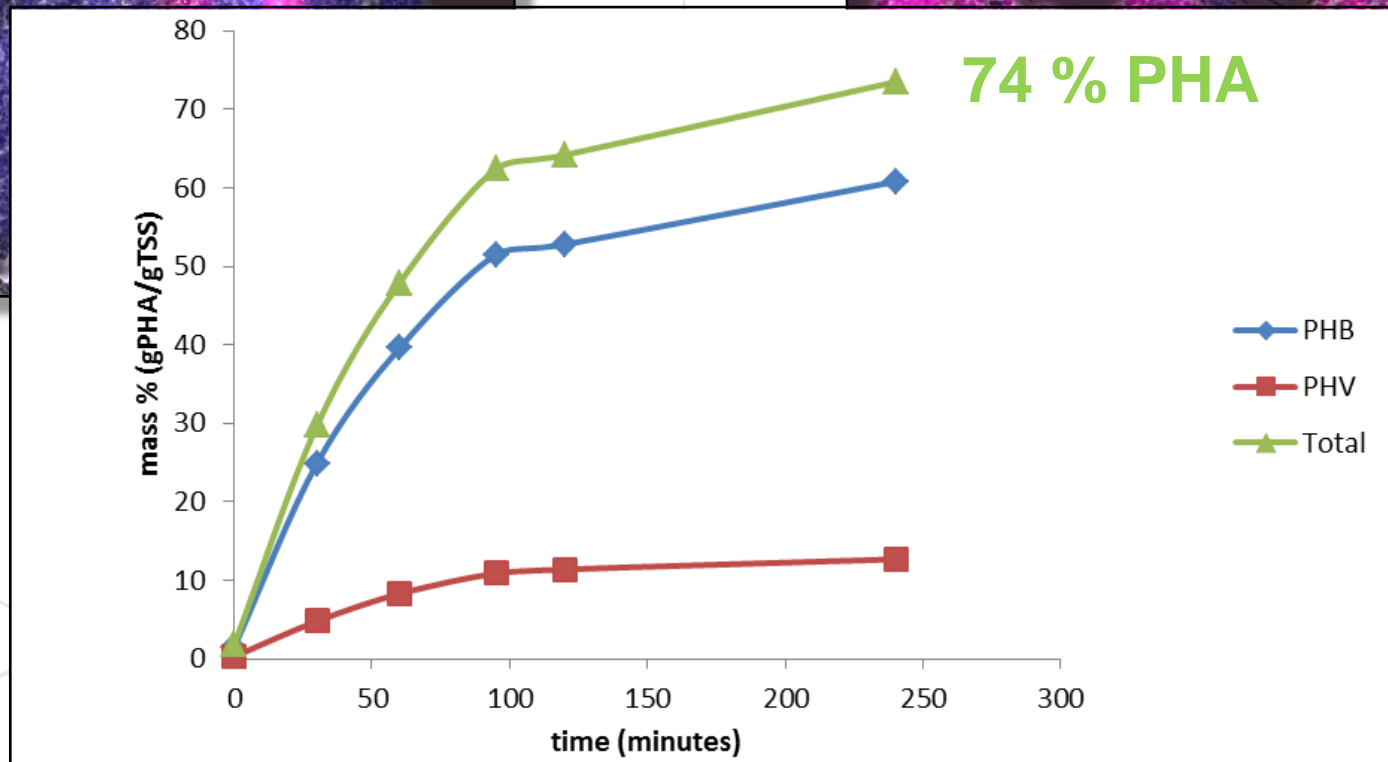
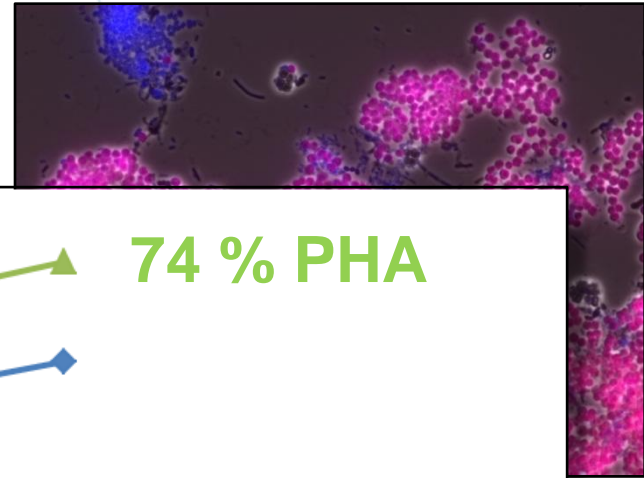
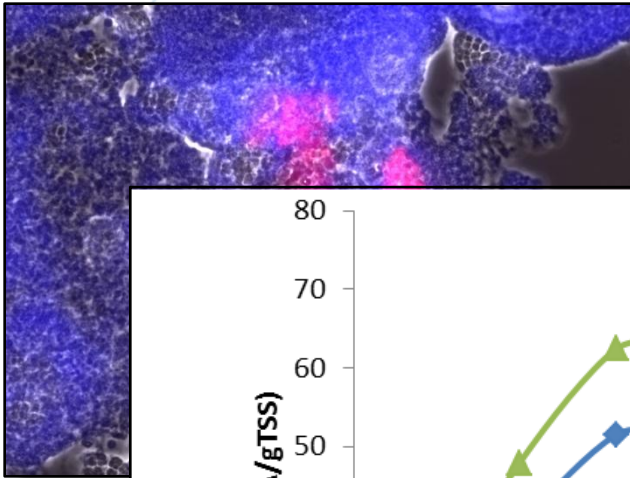
PHA Pilot Plant



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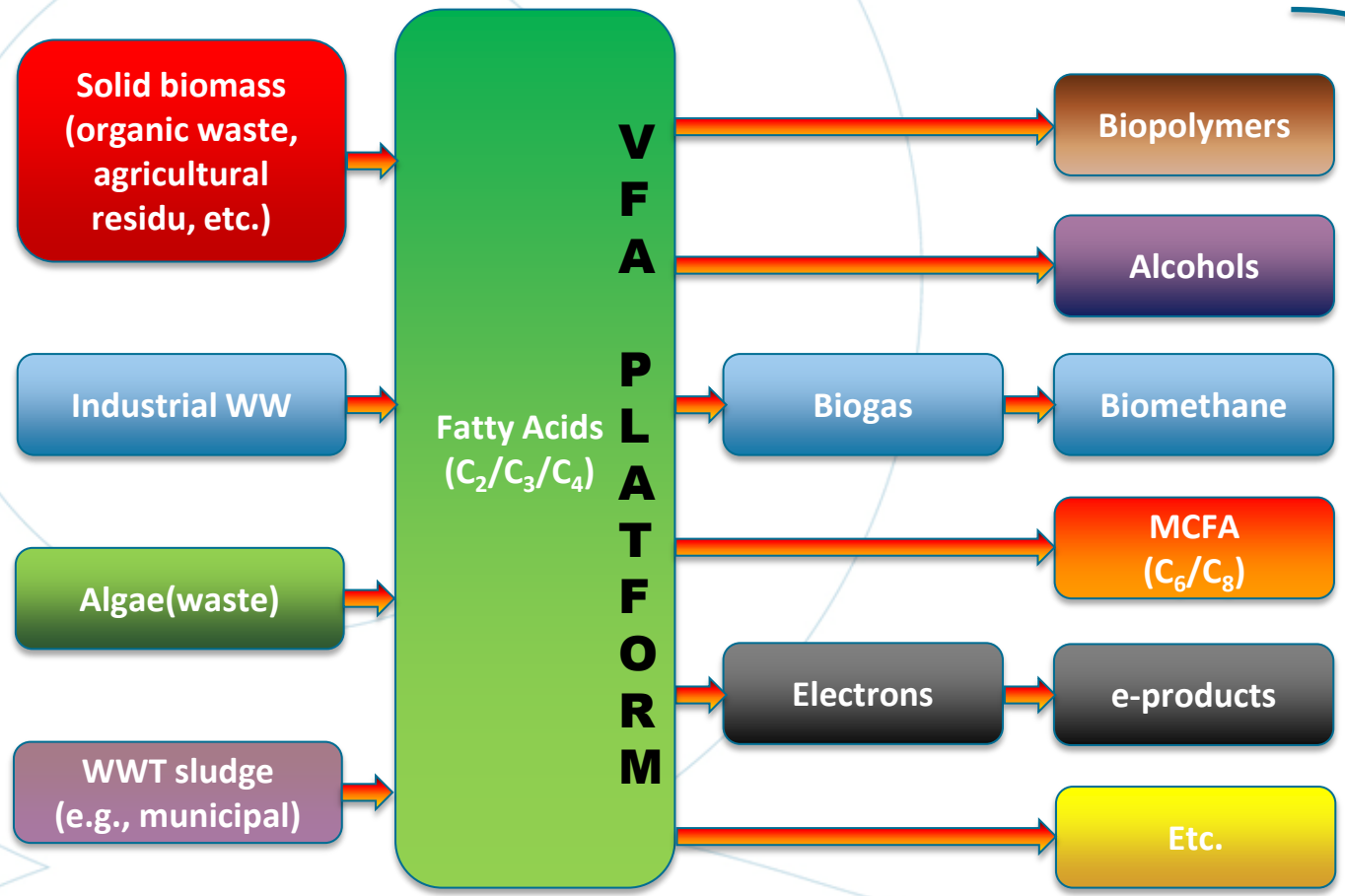
Mars production plant Veghel, Netherlands
Capacity : 1 kg/day

PHA pilot : Resultaten



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VFA or carboxylate Platform



Products / Intermediates

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Take away messages

- Environmental biotechnology is about understanding the role certain target microbes have in nature and recreating their preferred environment inside a bioreactor.
- In the past environmental biotechnology was mainly about treatment (C, N, S, etc.); the future will be about resource recovery (bioplastic etc.).
- All our technologies originated at universities, so for us a strong collaboration between universities and companies is of vital importance.



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PAQUES