Optimization of production of DHA from *Crypthecodinium cohnii* utilizing a dark fermentation effluent

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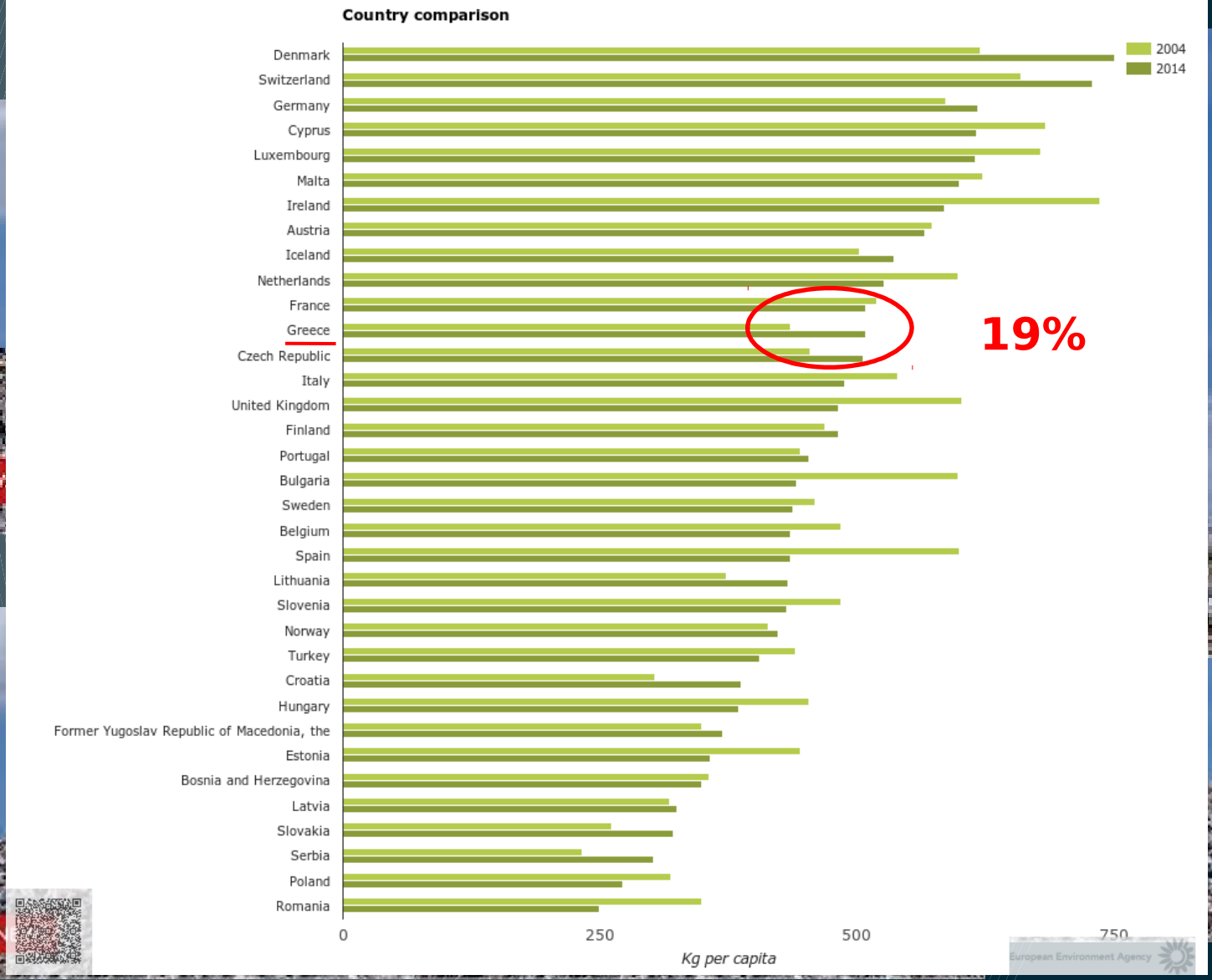
The accumulation of waste



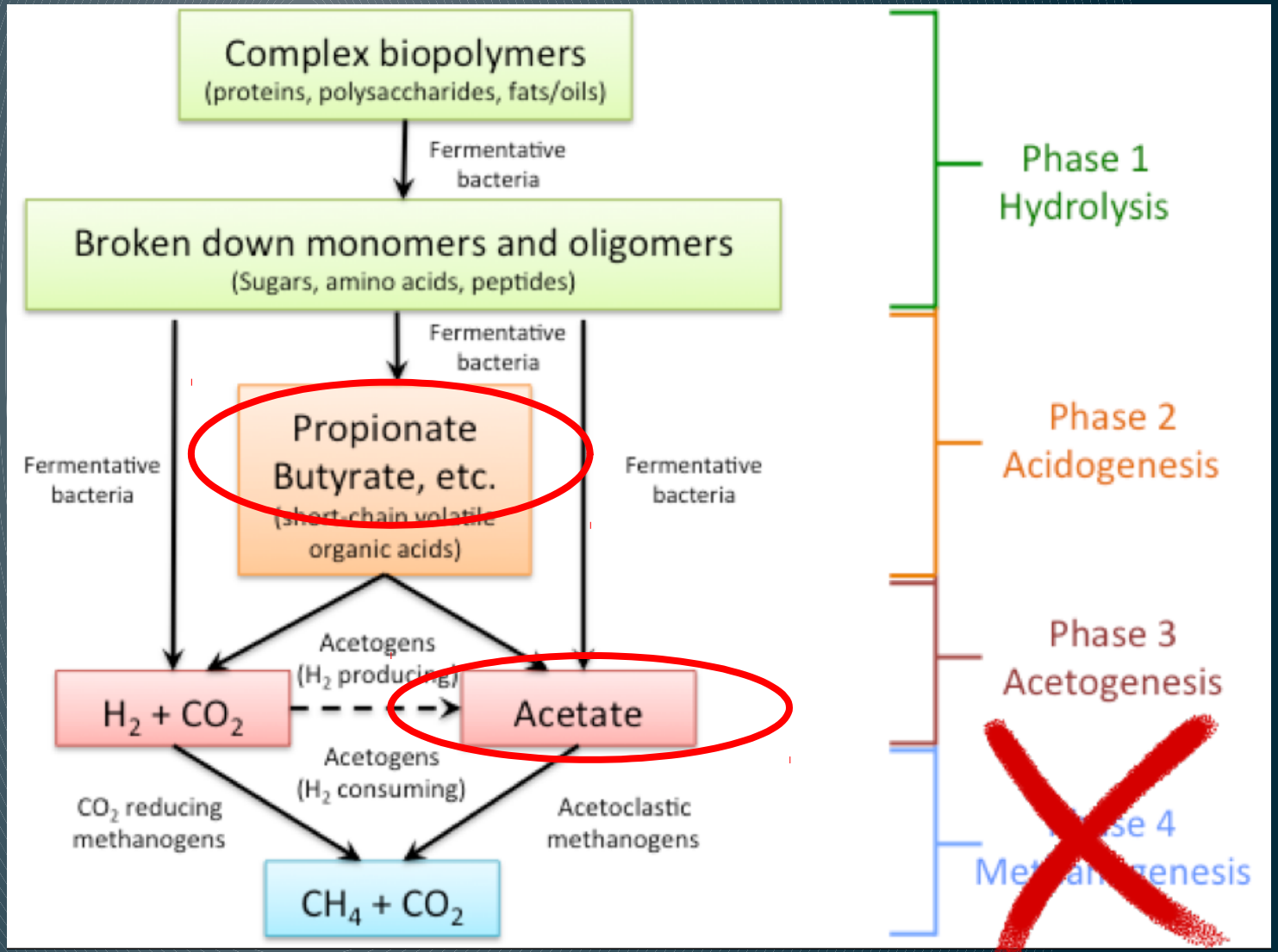
19%

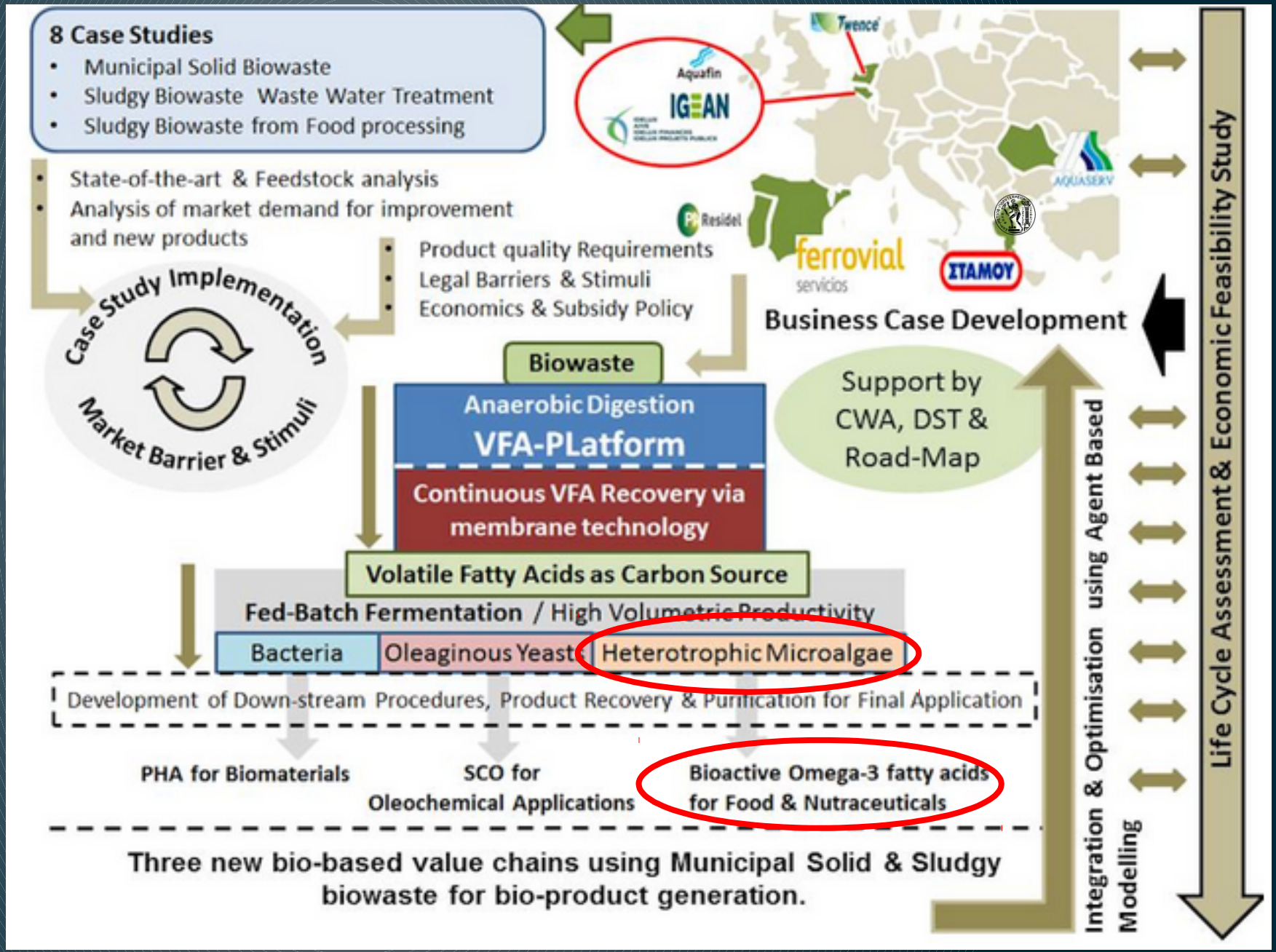
Bioconversion of biowaste to high added-value products (carotenoids, omega-3, biopolymers…)

Horizon 2020

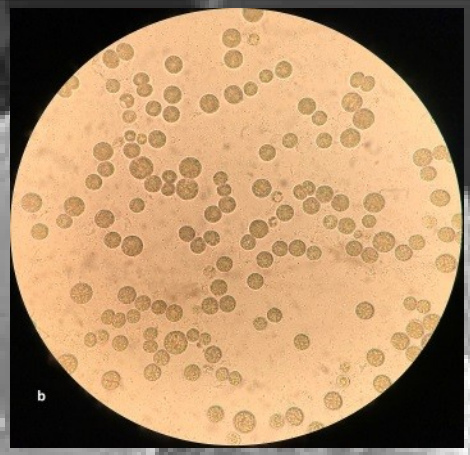
Volatile Fatty Acids Platform from Biowaste by a dark fermentation process

Dark fermentation



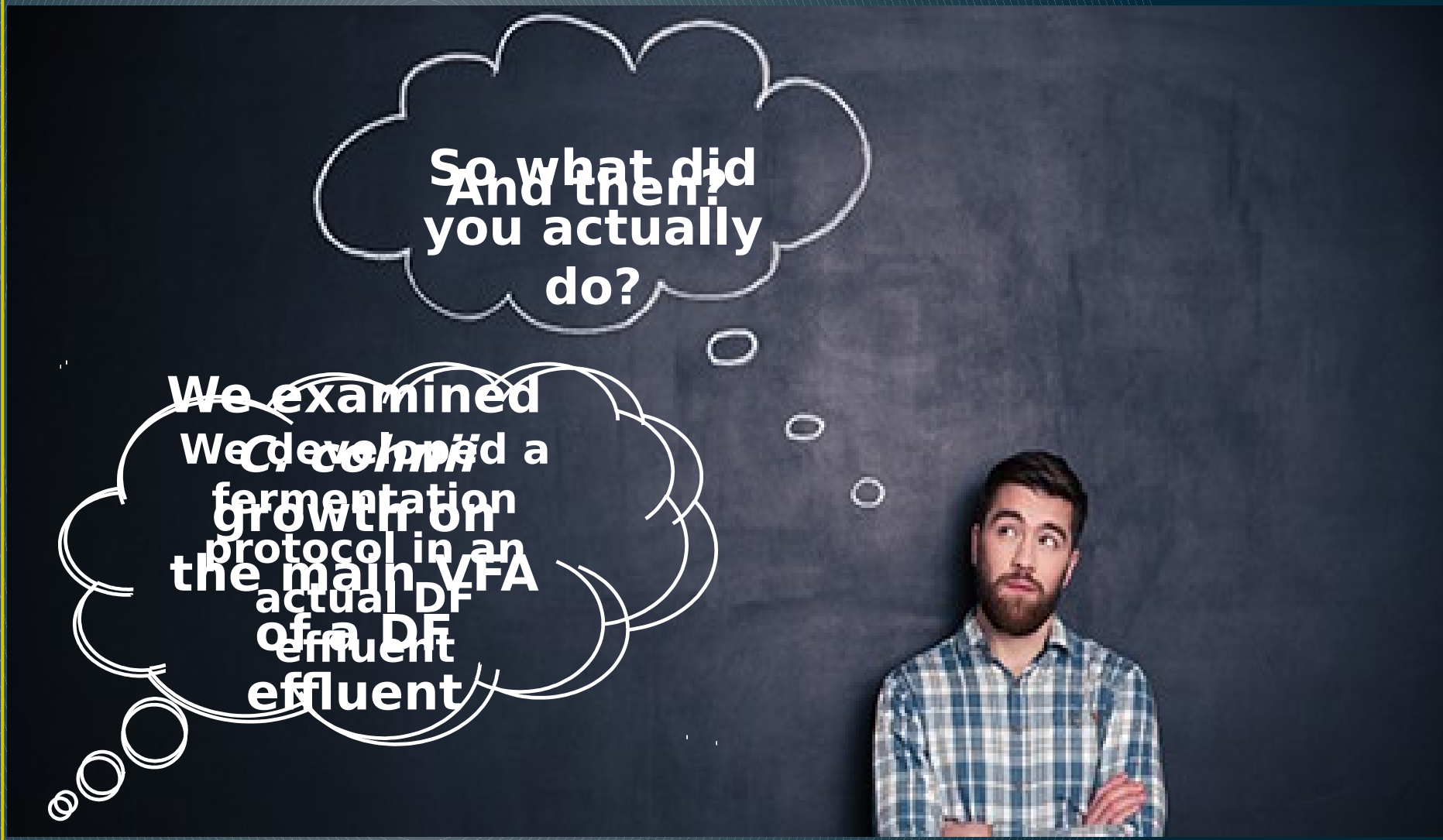

8 Case Studies
Municipal Solid Biowaste
Sludgy Biowaste Waste Water Treatment
Sludgy Biowaste from Food processing
State-of-the-art & Feedstock analysis
Analysis of market demand for improvement and new products
Product quality Requirements
Legal Barriers & Stimuli
Economics & Subsidy Policy
Case Study Implementation
Market Barrier & Stimuli
Business Case Development
Biowaste
Anaerobic Digestion
VFA-PLatform
Continuous VFA Recovery via membrane technology
Support by CWA, DST & Road-Map
Volatile Fatty Acids as Carbon Source
Fed-Batch Fermentation / High Volumetric Productivity
Bacteria
Oleaginous Yeast
Heterotrophic Microalgae
Development of Down-stream Procedures, Product Recovery & Purification for Final Application
PHA for Biomaterials
SCO for Oleochemical Applications
Bioactive Omega-3 fatty acids for Food & Nutraceuticals
Three new bio-based value chains using Municipal Solid & Sludgy biowaste for bio-product generation.
Integration & Optimisation using Agent Based Modelling
Life Cycle Assessment & Economic Feasibility Study

Crypthecodinium cohnii

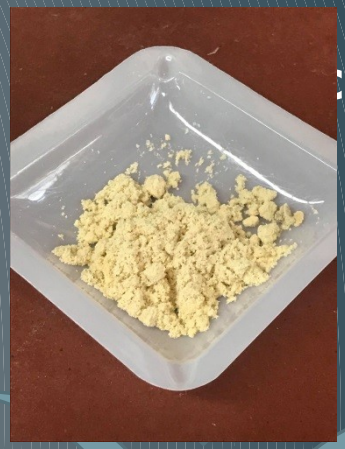


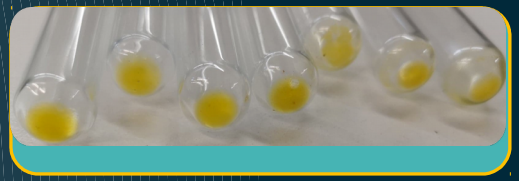
- Marine dinoflagellate
- High lipid content (<70%)
- High percentage of DHA (docosahexaenoic acid)
- Able to grow on glucose, acetate, ethanol

Used commercially for the production of DHA by Martek Bioscience

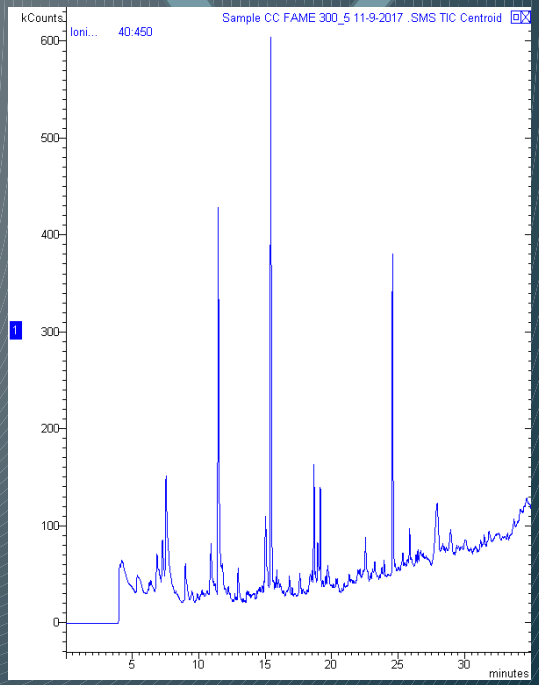

So what did you actually do?
And then?
We examined
We developed a fermentation protocol in an actual DF effluent
C. comm growth on the main VFA of a DF effluent

Batch cultures of the strain



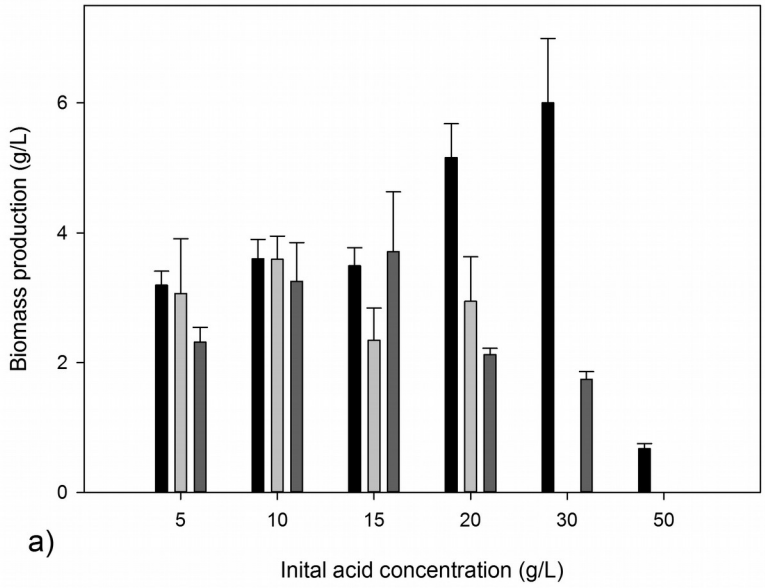


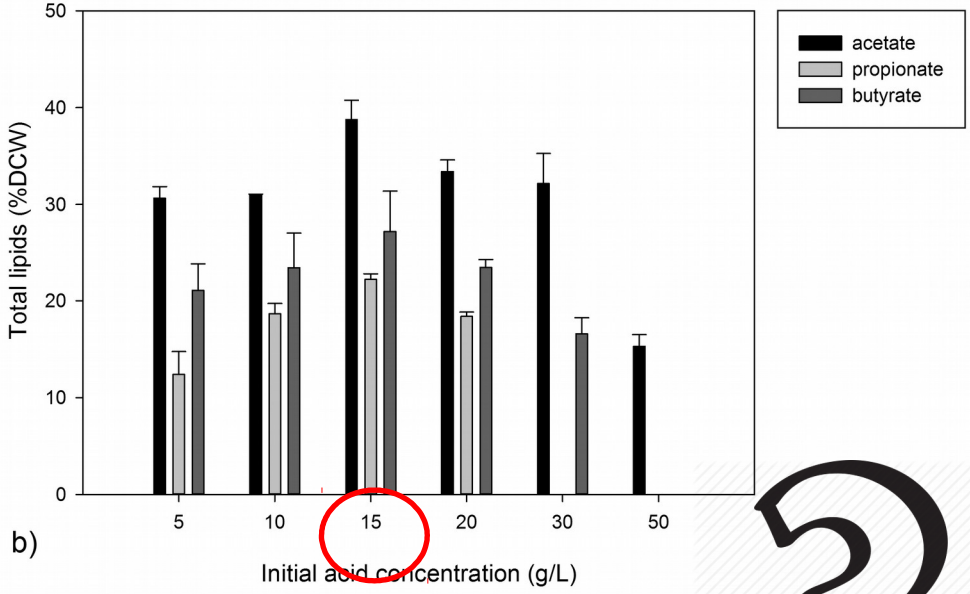
Acetic acid

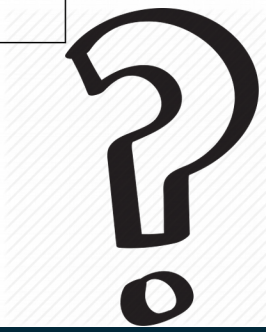


Butyric acid

Initial concentrations: 5-50 g/L



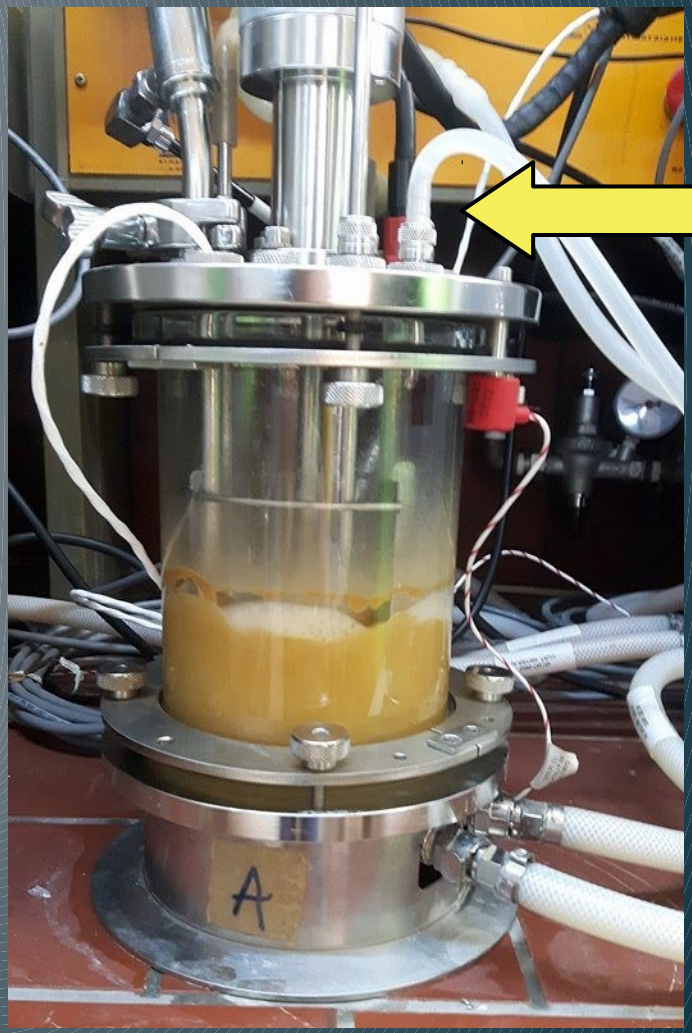




Low biomass production/ Specific tolerance to each VFA/ pH rise

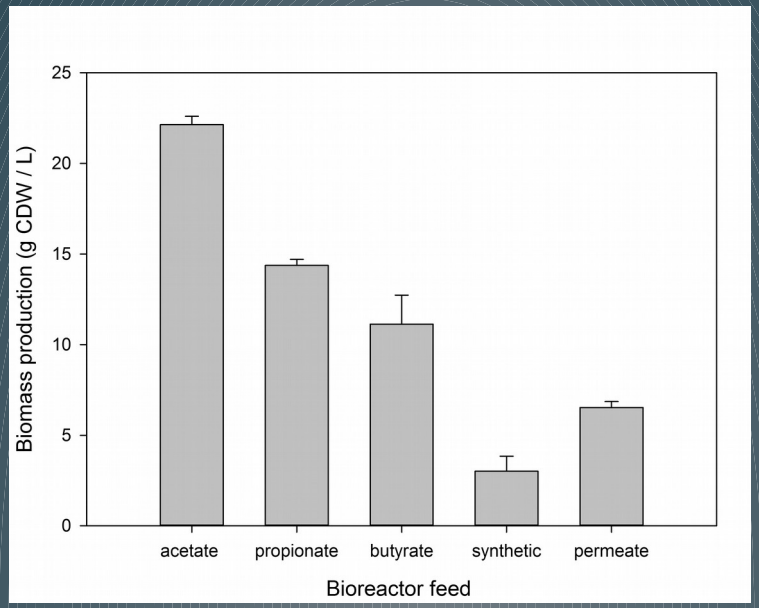
Fed-batch pH-auxostats

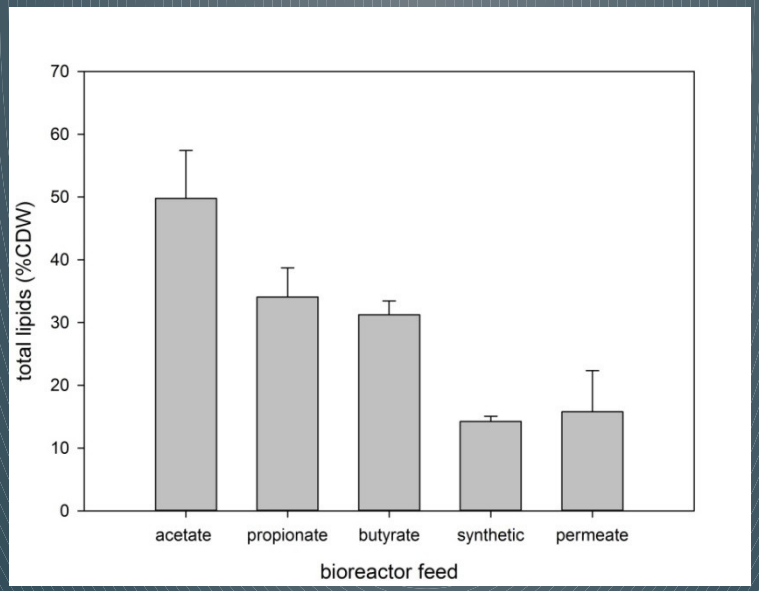
- 15 g/L sodium acetate
- 7.5 g/L yeast



Acetic acid (33% v/v)
Propionic acid (25% v/v)
Butyric acid (25% v/v)

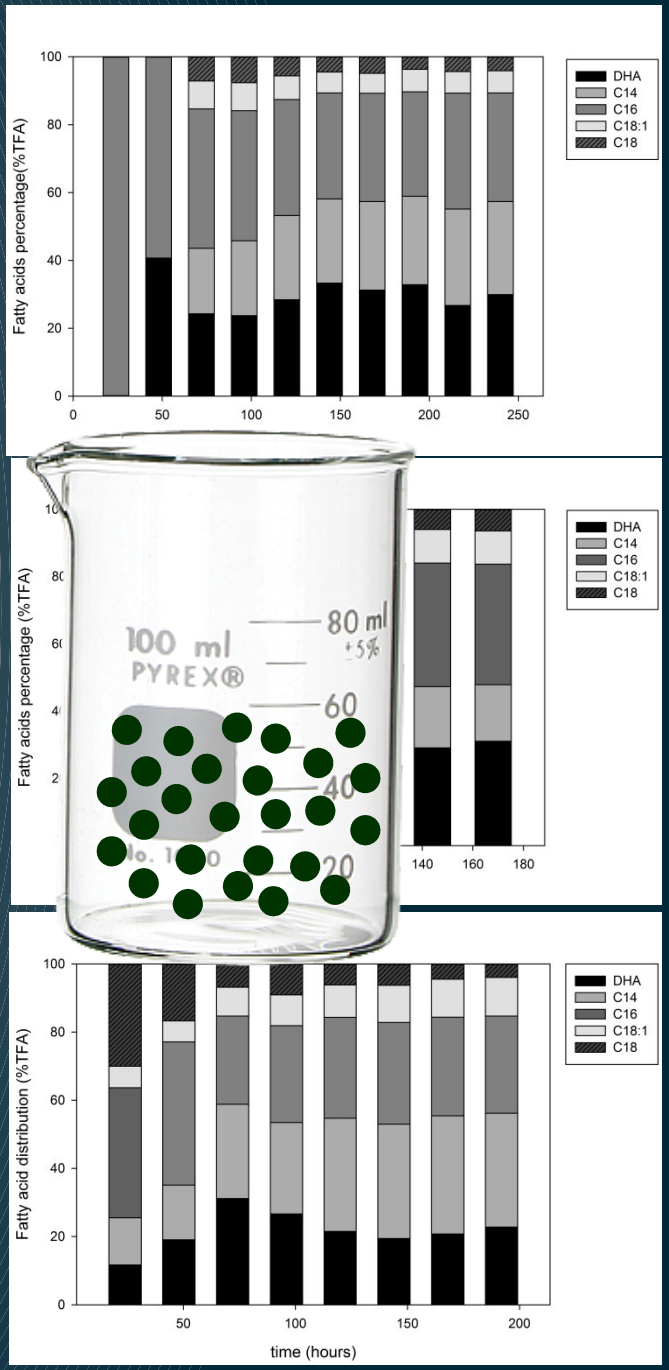
DF ultrafiltrated effluent
Synthetic medium





12 days

48 h



A. Chalima, et al. Integration of a dark fermentation effluent in a microalgal-based biorefinery Applied Energy 241 (2019) 130-138

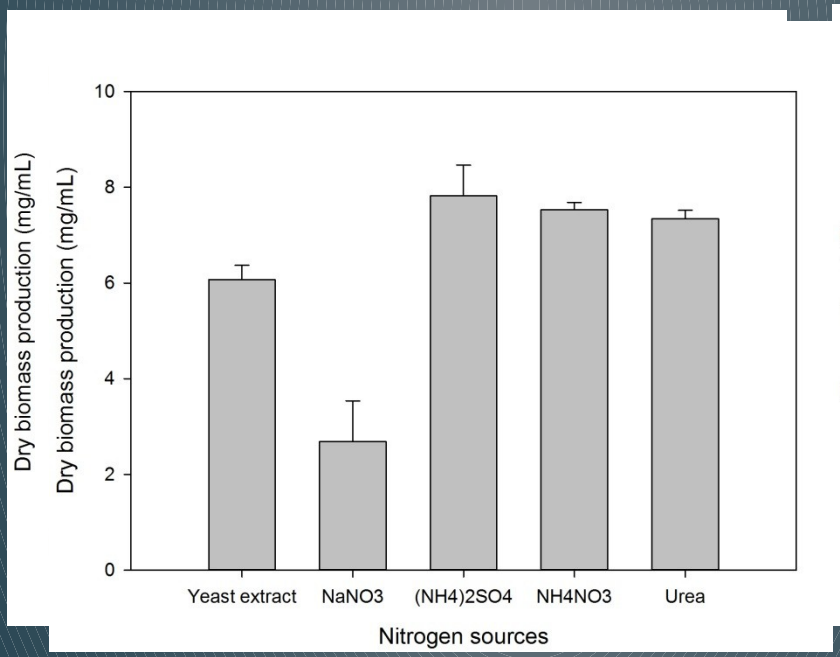
Optimization

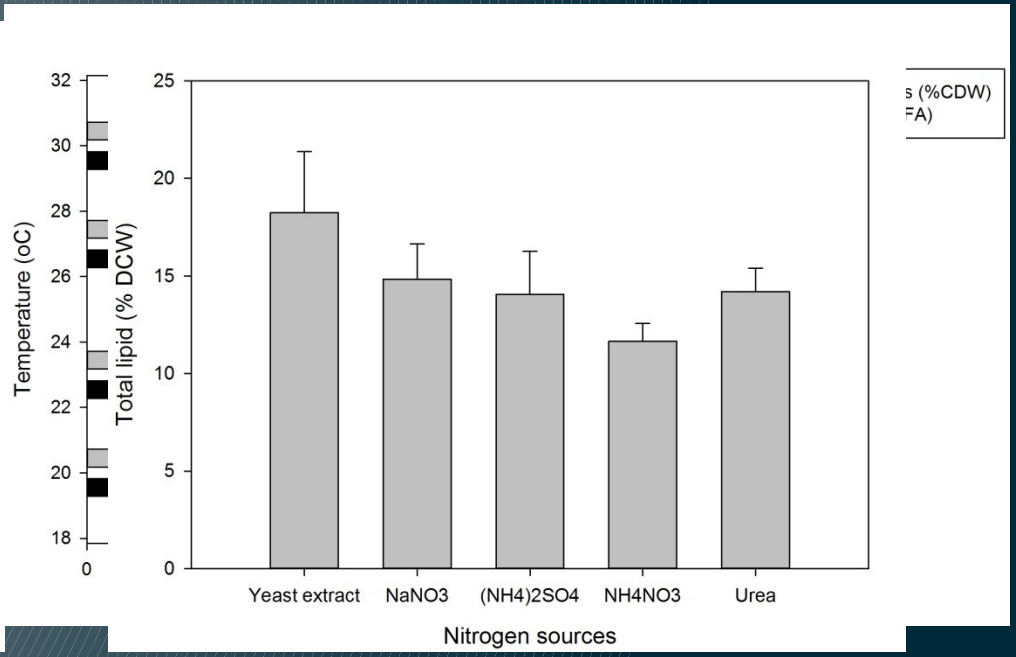
Culture conditions

Feed composition

Optimization of lipid production (1. culture conditions)

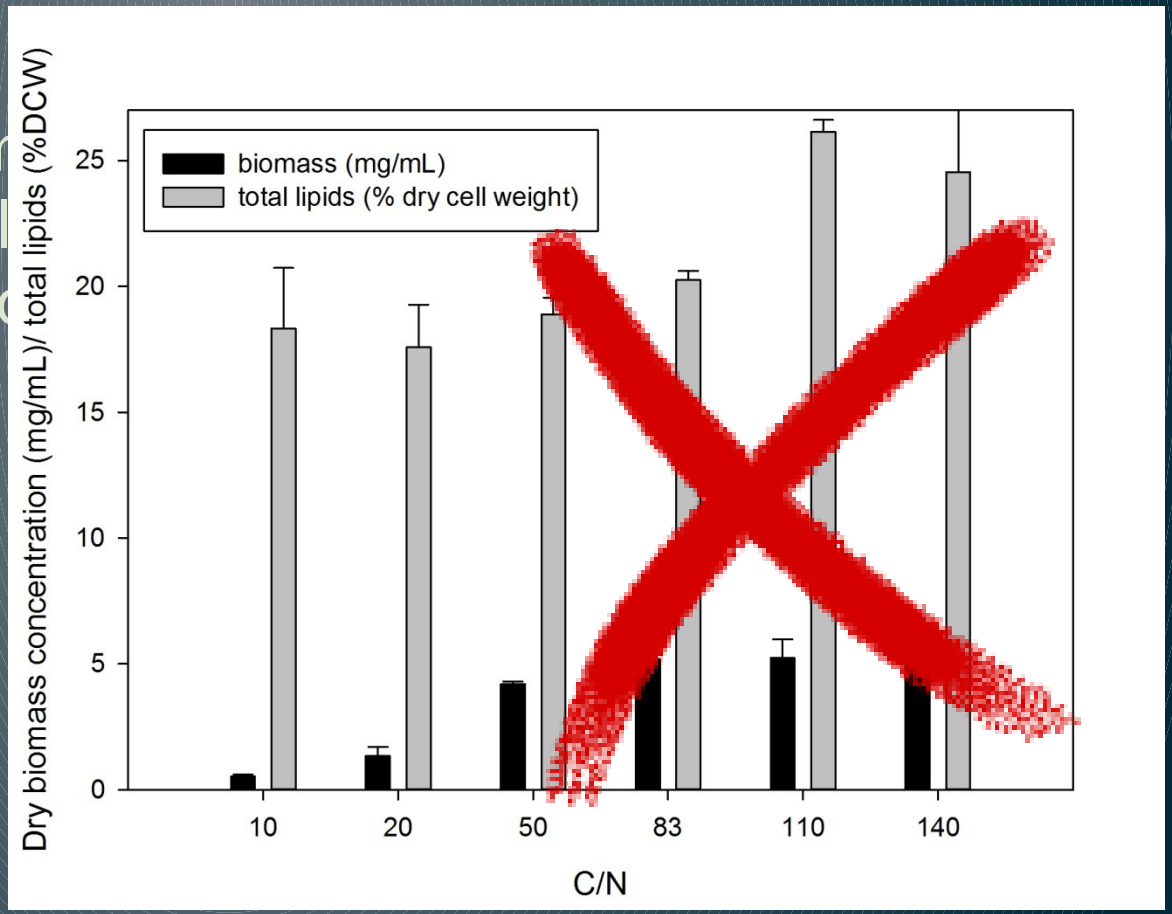
- Different Nitrogen sources
  - Ammonium- Urea- Yeast extract- Nitrate
  - 20-30oC





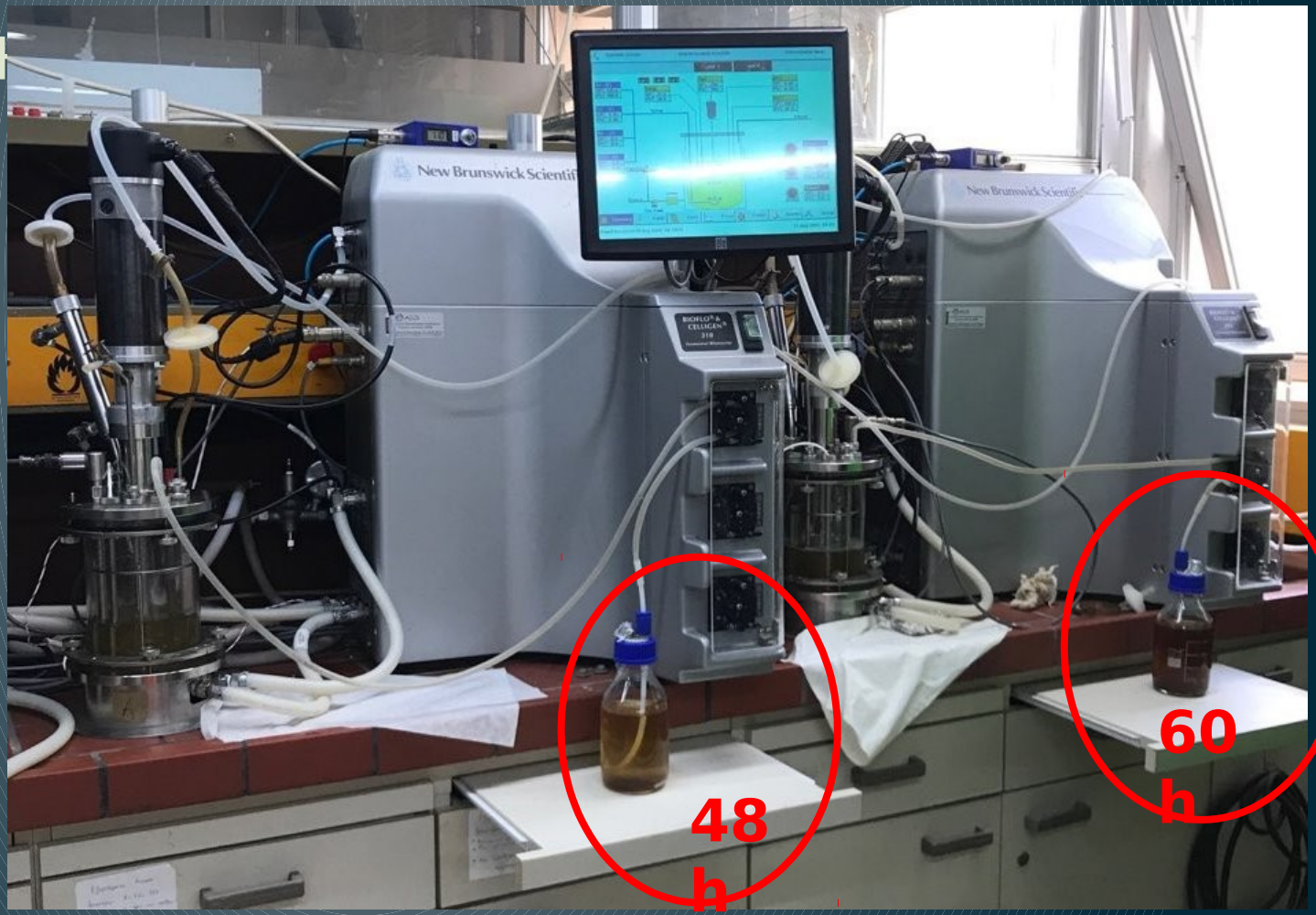
Optimization of lipid production (1. culture conditions)

- Different C/N ratio 10-140
- Chem
Ethanol
Salicylic



C/N=50
DHA=36
%

Optimization of lipid production (2. Feed)

- 

28%

Conclusions

- Acetate, butyrate, propionate ✓
- DF effluent ✓
(utilization of total organic content)

Higher VFA concentration is favorable

- Ammonium
- 27 ºC

- C/N= 110

Gazing into the future…

- Use of DF effluent as an initial medium for the bioreactors more efficient utilization (in progress…)
- Examination of utilization of lactic acid
- Application of a two-stage fermentation protocol

"The future starts today, not tomorrow…"

Thank you very much!!

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