PROGRAMME DE COOPÉRATION TRANSFRONTALIÈRE
GRENSOVERSCHRIJDEND SAMENWERKINGSPROGRAMMA

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Valorization of microalgae by-products in biorefinery

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AVEC LE SOUTIEN DU FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL
MET STEUN VAN HET EUROPEES FONDS VOOR REGIONALE ONTWIKKELING

I. Biorefinery:

A. What is it?

- **OECD (2018)** : “The biorefinery also fits the **circular economy** concept, particularly ‘bio-waste’ biorefineries that **use wastes or residues as the feedstocks**. The nature of this fit may be subtle however. Using such materials is clearly not classical recycling, reuse or remanufacturing as biorefining is **making value-added ‘virgin’ materials from waste sources.** This value creation distinguishes waste biorefining from standard waste management practices, and thus placing it within the waste management hierarchy is difficult. It highlights a need for re-defining such materials as, perhaps, ‘secondary raw materials’ to avoid a clash with waste management regulation.”

- **The International Energy Agency (2014)** (International Energy Agency Bioenergy Task 42 Biorefinery, 2012) described a biorefinery as “the **sustainable processing of biomass** into a spectrum of **marketable products** (food, feed, materials, chemicals) and **energy** (fuels, power, heat)”.

I. Biorefinery:

B. Classification

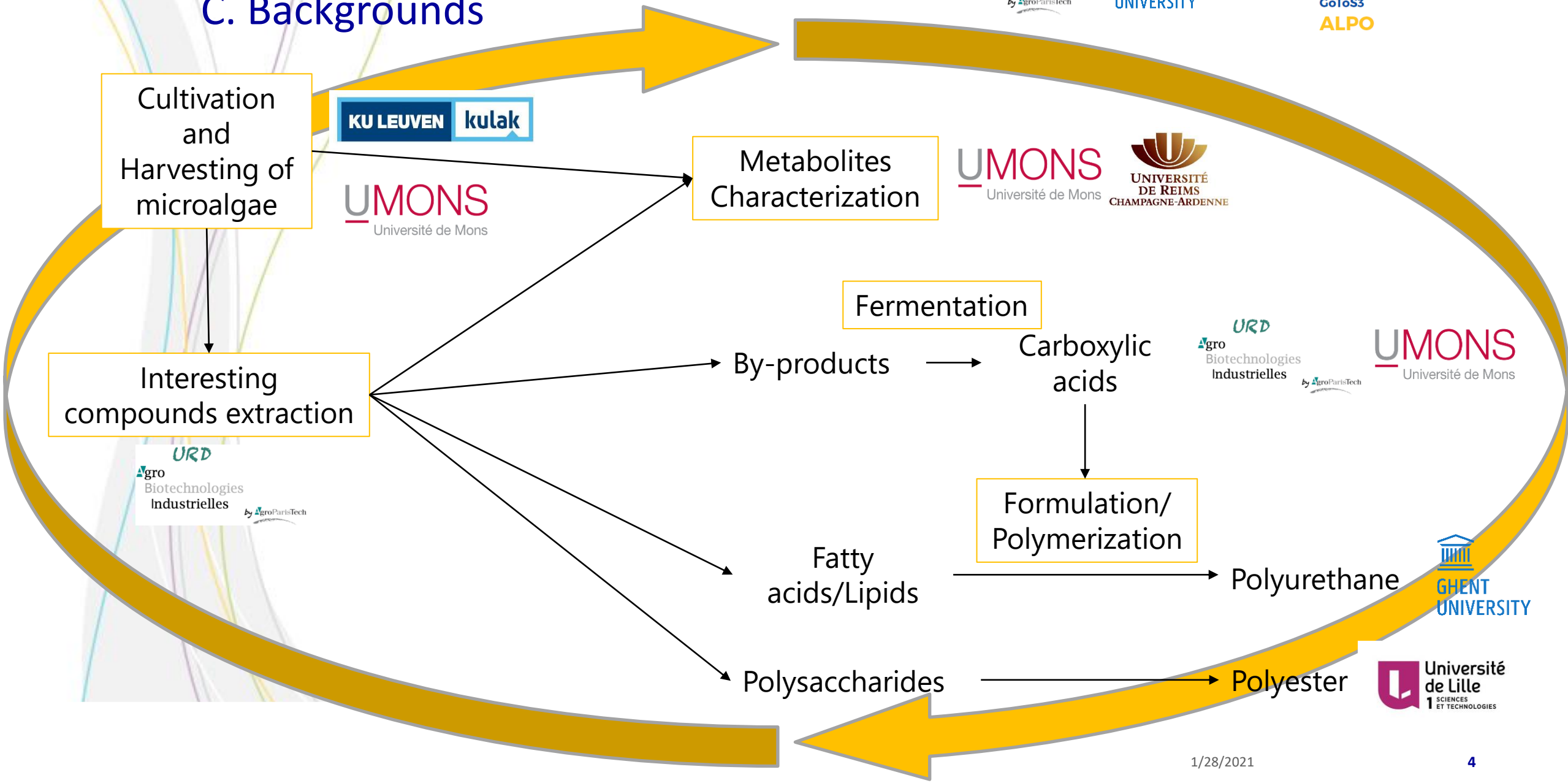
- 1srt Generation: Use of edible parts of crops

- 2nd Generation: Use of residues, non-edible crops and agroindustrial by-products

- 3rd Generation: Use of Algae

I. Biorefinery:

C. Backgrounds



I. Biorefinery:

D. Valorization of microalgae by-products

- Feed or food: source of proteins (Spiegel et al, 2013)
- Anaerobic digestion: biomethane (Uggetti et al, 2014)
- Fermentation:
  - Bioethanol production (Velazquez-Lucio et al, 2018; Lee et al, 2011; Phwan et al, 2018; Mirsiaghi et al, 2015)
  - 2-pyrone 4,6-dicarboxylic acid (Htet et al, 2018)
  - Lipids by oleaginous yeasts (Younes et al, 2020)

II. Fermentation of lipid-extracted *C. vulgaris* residue

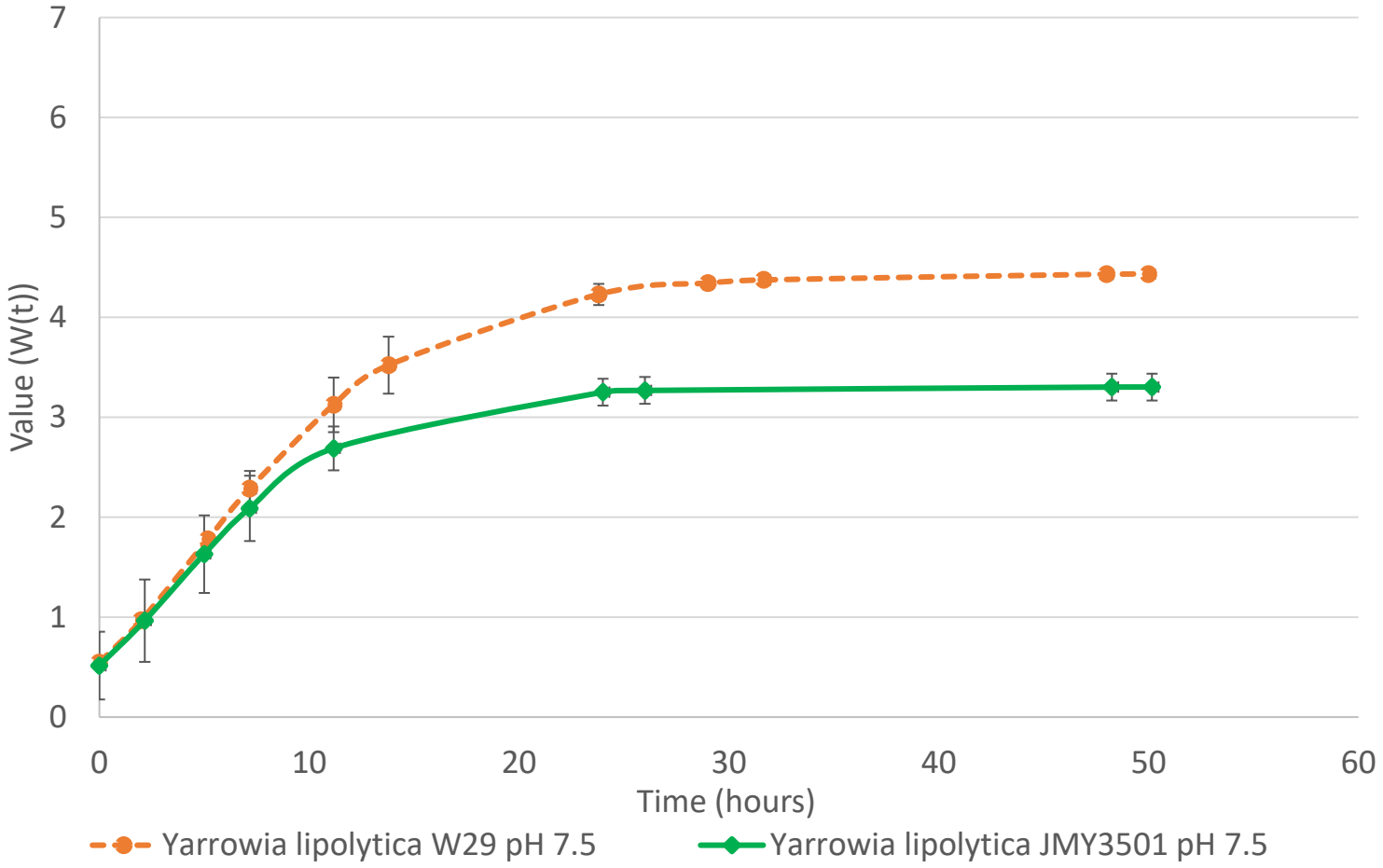
A. Culture conditions

- Substrate: 30 $g.L^{-1}$ *C. vulgaris* residue
- Bioreactor: 300 mL operating volume
- Strains: *Y. lipolytica* W29 (Wild-type) and JMY3501 (GMO)
- pH: 7.5 (H_2SO_4 2M and KOH 4M)
- Dissolved oxygen: 30% saturation level

II. Fermentation of lipid-extracted *C. vulgaris* residue

B. Growth of *Y. lipolytica*

Growth curves of *Y. lipolytica* W29 and JMY3501 at pH 7.5 in *C. vulgaris* residue (30 g.L^{-1}) obtained with Gompertz model



Strains	Specific growth rate (h^{-1})	Generation time (h^{-1})	Stationary phase (h)
Y. lipolytica W29	0.26 ± 0.01	2.64 ± 0.09	25
Y. lipolytica JMY3501	0.26 ± 0.03	2.69 ± 0.30	15

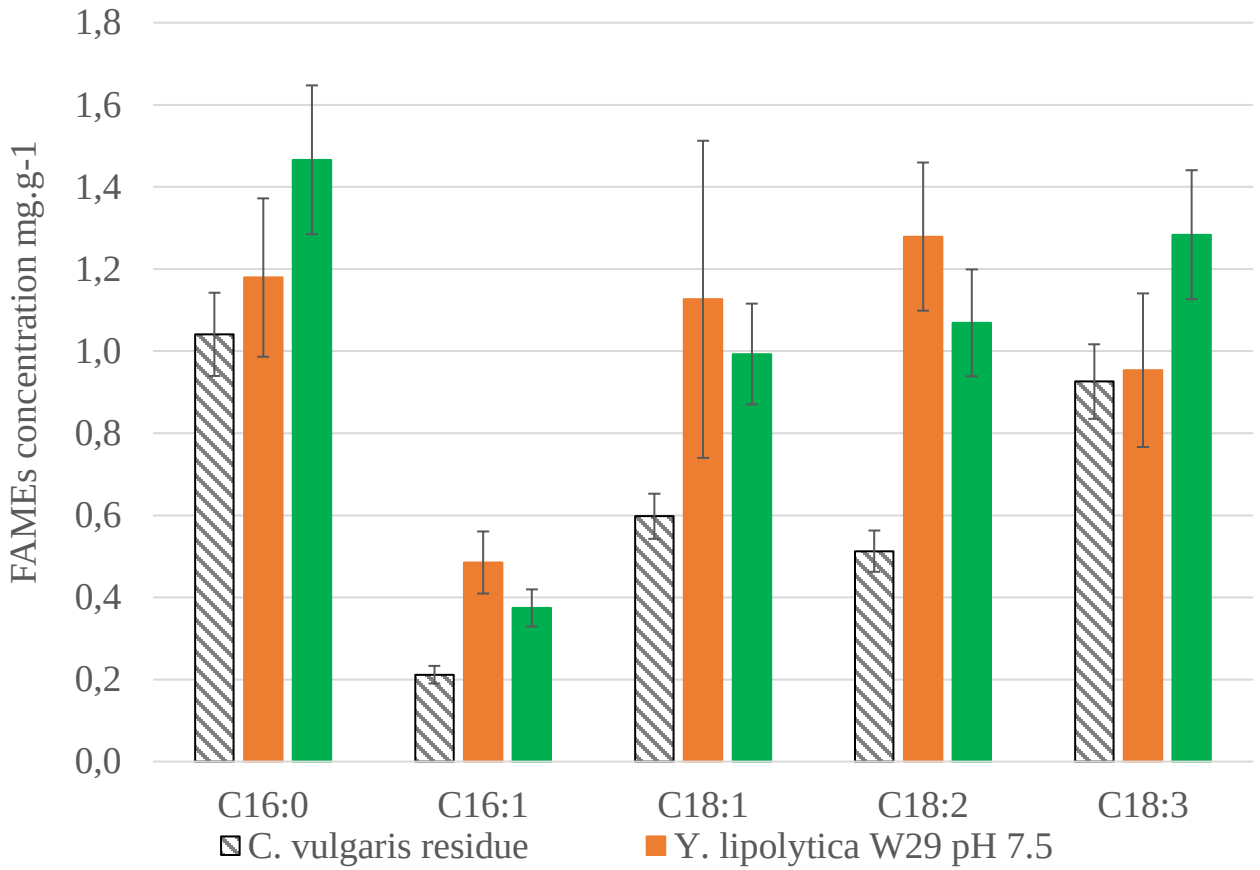
Gompertz model: $W(t) = Ae^{-e^{\left(\frac{\mu max \cdot e}{A}(\lambda - t)+1\right)}}$

- µmax: Maximal growth rate
- λ: lag time
- t: time
- $A = \frac{ln_{x_{max}}}{ln_{x_0}}$

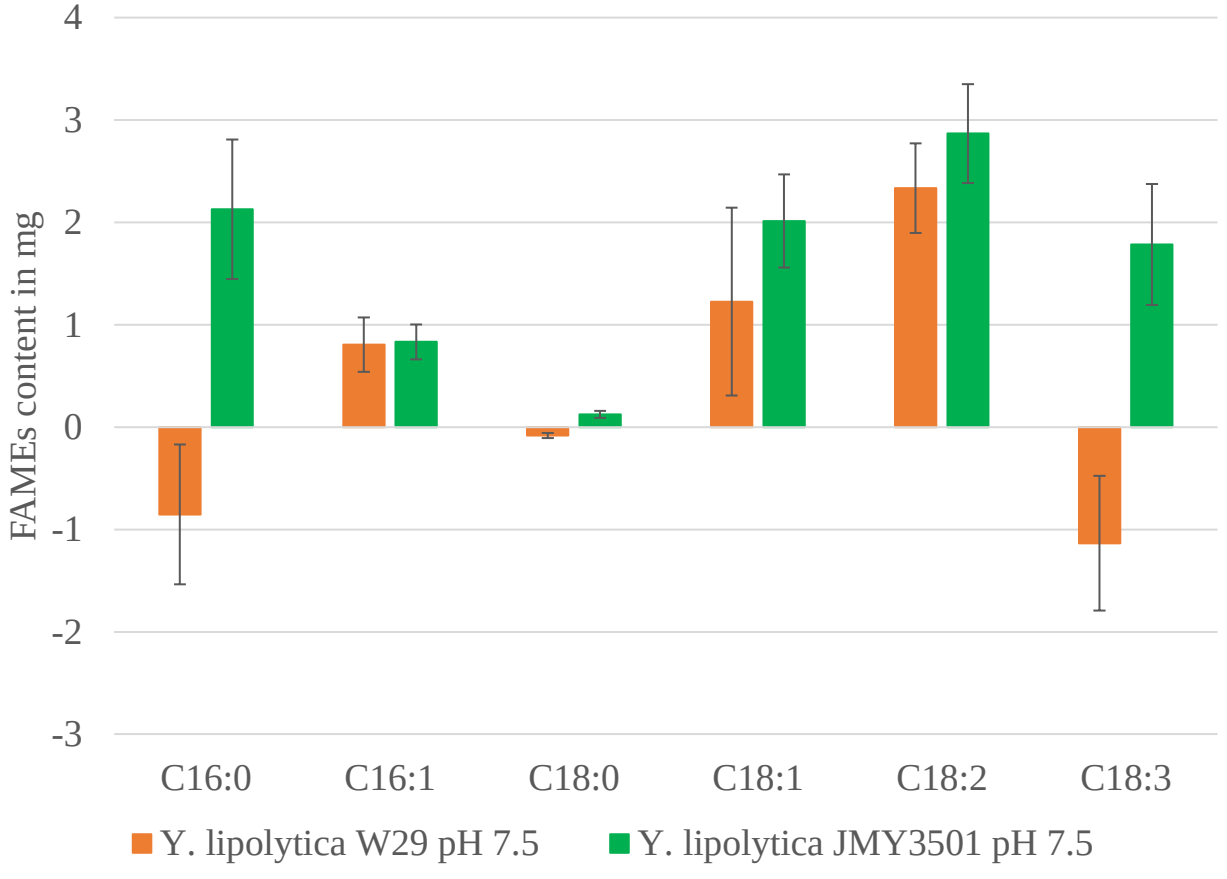
II. Fermentation of lipid-extracted *C. vulgaris* residue

C. FAMEs profiles of *Y. lipolytica*

Fatty acids content of *C. vulgaris* residue, *Y. lipolytica* W29 and JMY3501 strains cultivated on *C. vulgaris* residue for 50 h at pH 7.5



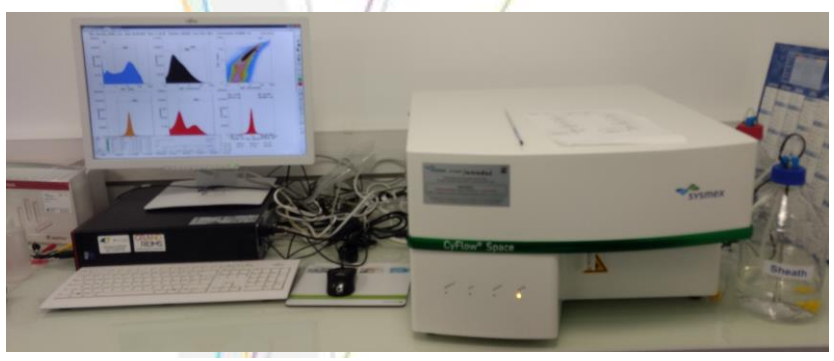
Evolution of fatty acid quantities in the fermenter after 50 h cultivation of *Y. lipolytica* W29 and JMY3501 strains in *C. vulgaris* residue



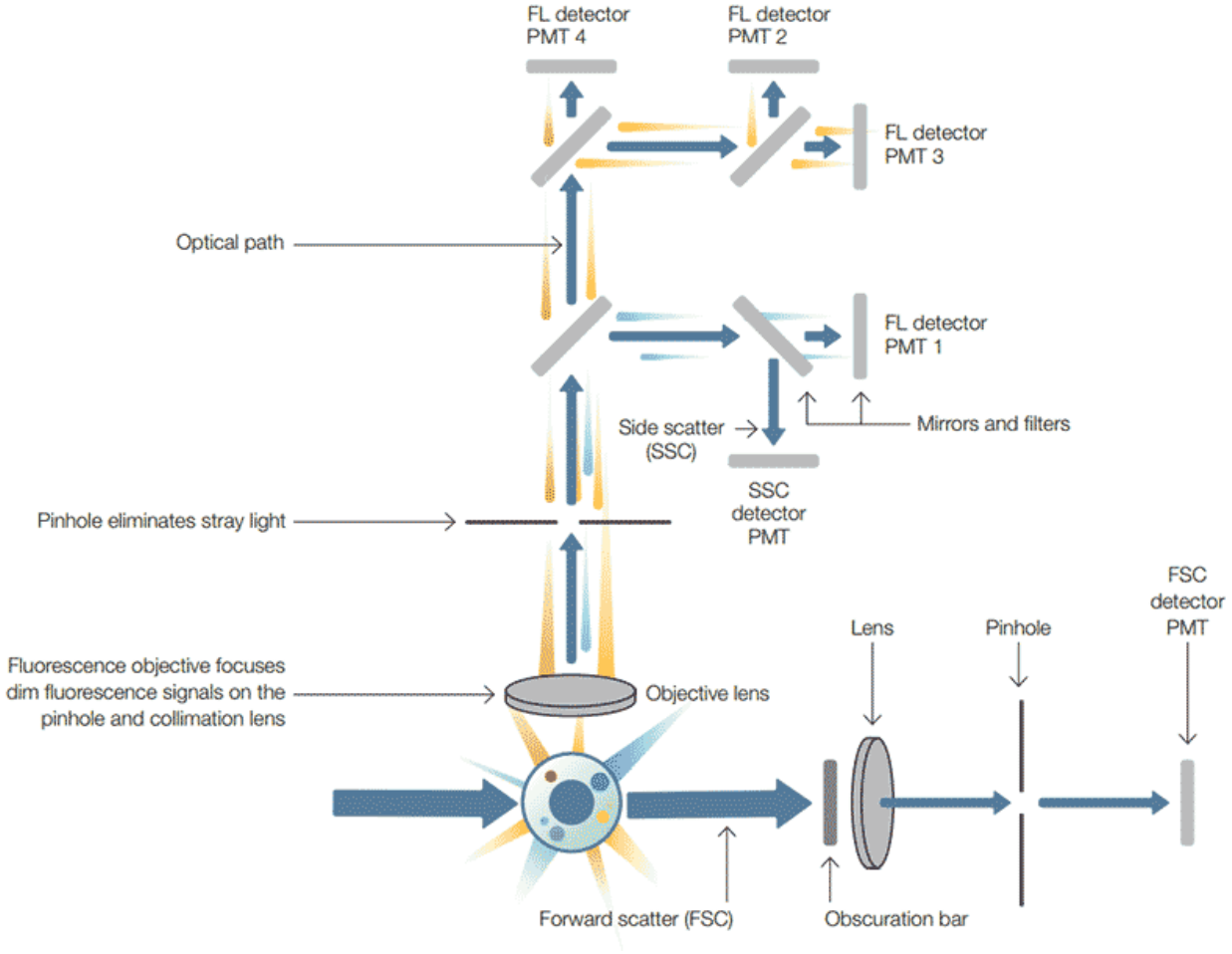
II. Fermentation of lipid-extracted *C. vulgaris* residue

- *C. vulgaris* by-product is a convenient substrate for *Y. lipolytica* growth
- Interesting production of C18:3 at pH 7.5
- Difficulties to discriminate microalgae and yeasts
- How improve monitoring of yeasts growth ?
- How monitor lipids accumulation in yeasts ?

III. Lipids tracking by flow cytometry



- Only one reading for:
  - Number of cells
  - Size → discriminate population
  - With staining:
    - Viability
    - Lipids content

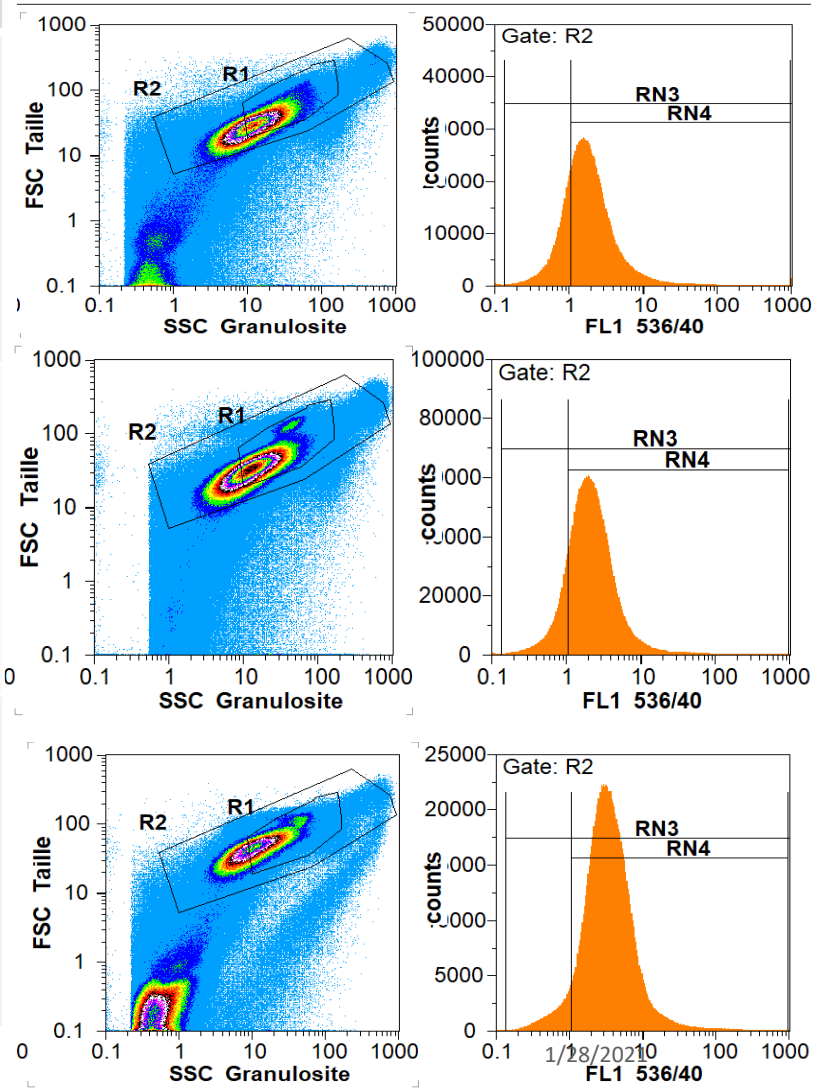


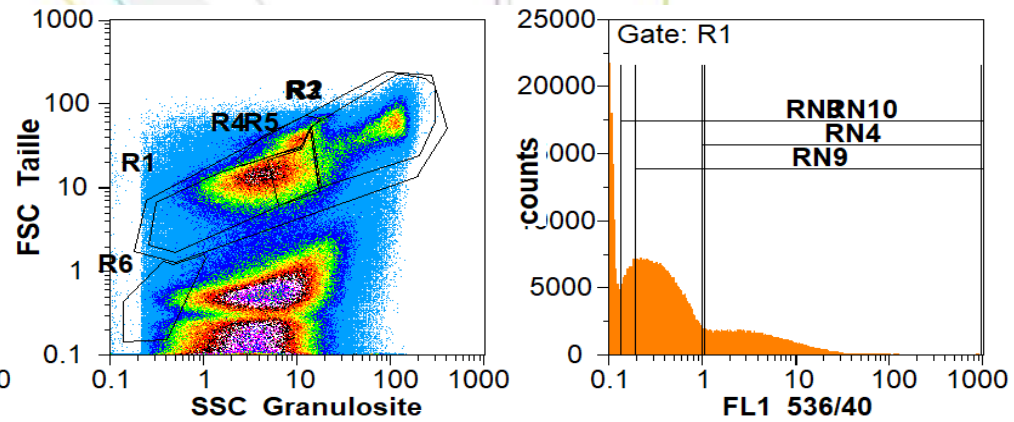
https://www.bio-rad-antibodies.com/flow-cytometry-optics-detection.html

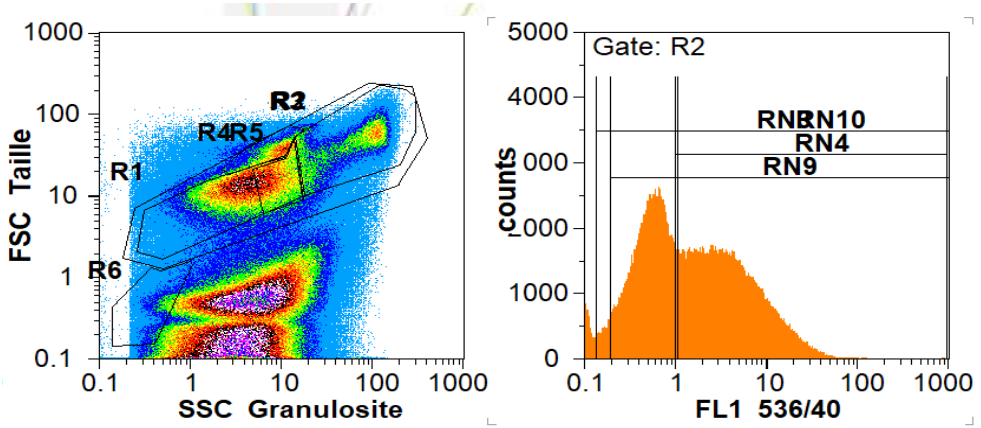
III. Lipid tracking by flow cytometer

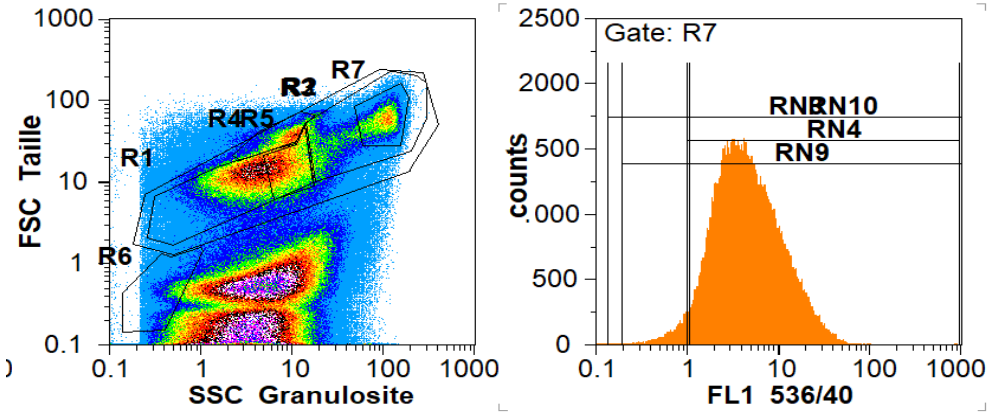
- Culture conditions:
  - 50 mL in Flask (500 mL)
  - 30 $g.L^{-1}$ *C. vulgaris* residue
  - *Y. lipolytica* JMY3501
  - 28°C, 220 rpm
- Increasing with time of culture:
  - Fluorescence Intensity
  - Background noise

Culture Time (hours)	
0h	
4h	
24h	









- After 50 h of culture of *Y. lipolytica* JMY3501 in *C. vulgaris* residue in 5L-bioreactor
- Culture conditions:
  - 30 $g.L^{-1}$ *C. vulgaris* residue
  - *Y. lipolytica* JMY3501
  - 28°C, pO_2 30% and pH 7.5
  - 2 L in 5L-Bioreactor
- Discrimination between microalgae and yeasts is possible
- Specific Fluorescence of population could be used to determine lipids content in yeasts

Conclusions and Perspectives

- Growth of *Y. lipolytica* in *C. vulgaris* residue
- pH 7.5 is the best condition for growth and lipids accumulation
- Flow cytometry to follow yeasts growth and lipids accumulation
  - Need some improvements for labelling and correlation between lipids and dye

Thank you for your attention

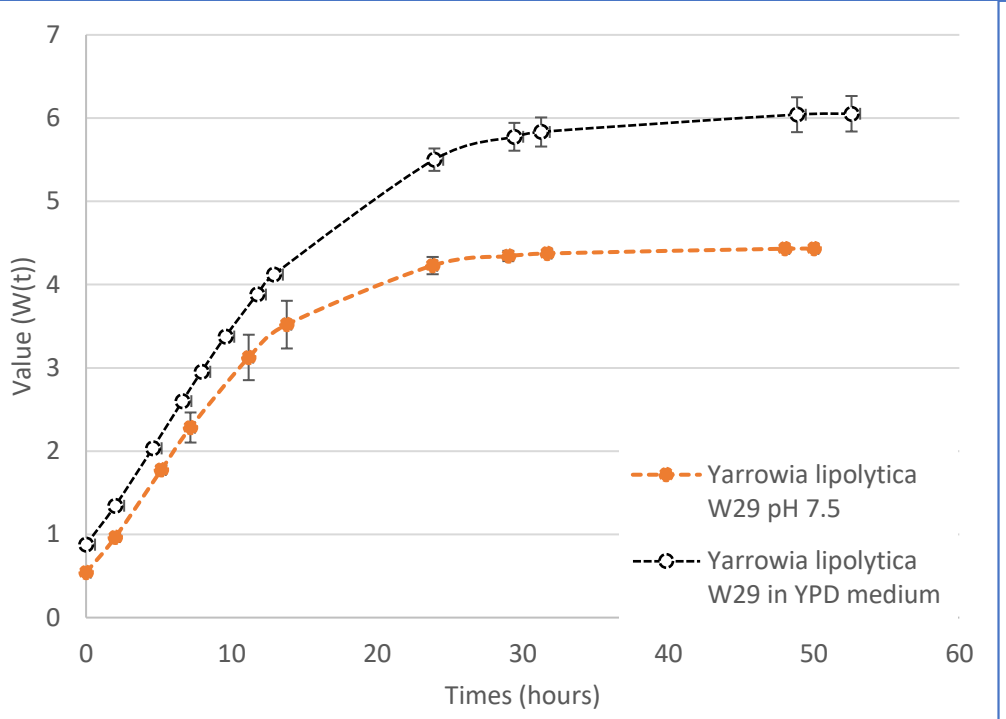
Typical composition of *Chlorella vulgaris*

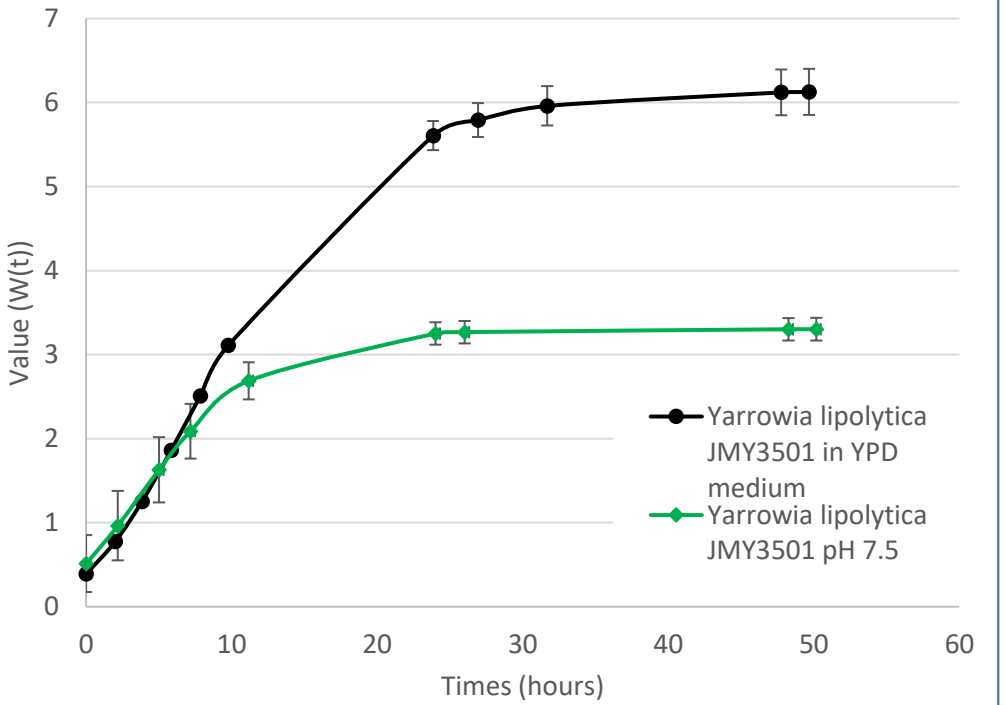
Reference	Lipids	Proteins	Carbohydrates	Ashes
(Mahdy *et al.*, 2016)	4.8 ± 1.1	63.4 ± 0.5	25.7 ± 2.9	6.2 ± 0.1
(Bernaerts *et al.*, 2018)	6.6 ± 1.5	39.4 ± 0.3	1.8 ± 0.1 storage polysaccharides 9.3 ± 0.7 cell wall polysaccharides 0.5 ± 0.1 extracellular polysaccharides	6.7 ± 0.1

C. vulgaris residue

- Simulate substrate of project
- Extraction of lipids : Soxhlet method

Proteins (% of dry biomass)	Reducing Carbohydrates (% of dry biomass)	Ashes (% of dry biomass)	Residual Fatty acids (% of dry biomass)
40.1 ± 5.3	15.6 ± 2.1	11.2 ± 0.1	0.34 ± 0,02





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