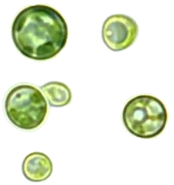
Hemicellulose valorization from three microalgal species grown in heterotrophy for biofuel production



Galdieria sulphuraria

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Auxenochlorella protothecoides

Euglena gracilis

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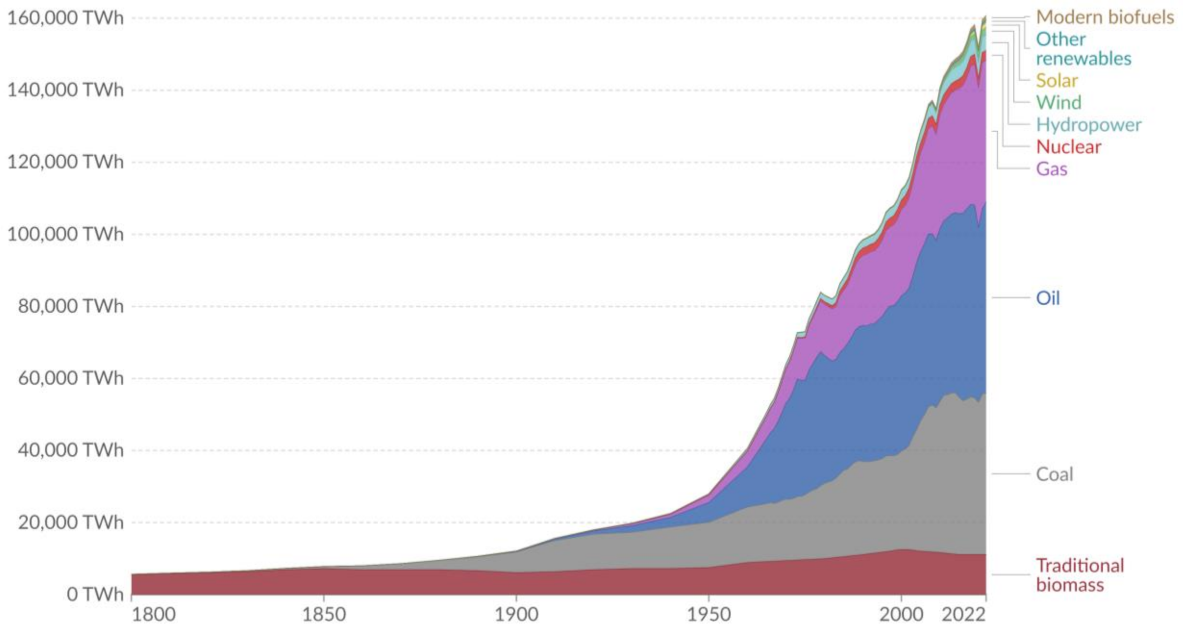
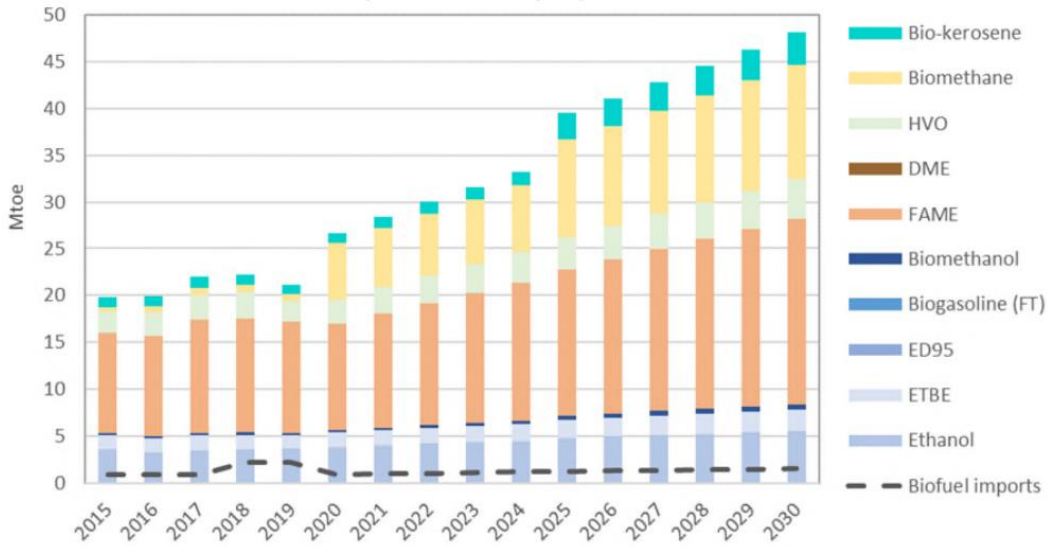
Context: fossil fuels and biofuels demand



Global direct primary energy consumption by fuel – *Energy Institute - Statistical Review of World Energy (2023); Smil (2017)*

- Fuel demand is rising continuously



Biofuel availability in Europe from 2015 to 2030 – © Concawe, 2021

- Biofuel availability in Europe should increase in the coming years
- FAMEs are predicted to be the most important source of biofuels

Introduction: microalgal biomass for biofuel production

Microalgae are good candidates for biofuel production

Lipids

Transesterification
Hydrothermal liquefaction

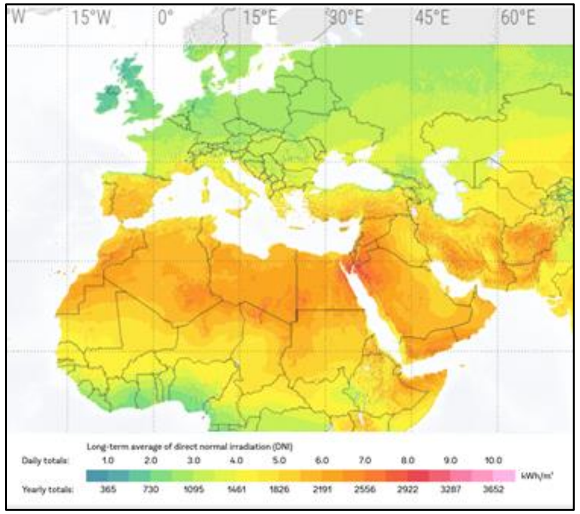
- Kerosene
- Diesel
- Gasoline

Carbohydrates

Fermentation

- Ethanol
- Butanol
- Hydrogen

Weak global irradiation in Belgium



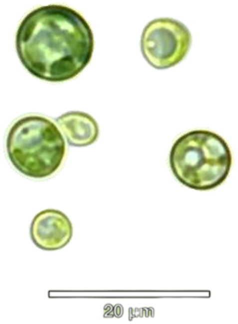
Global Solar Atlas 2.0, 2019

Phototrophic algal cultivation strategies are not economically efficient

Open pond cultivation

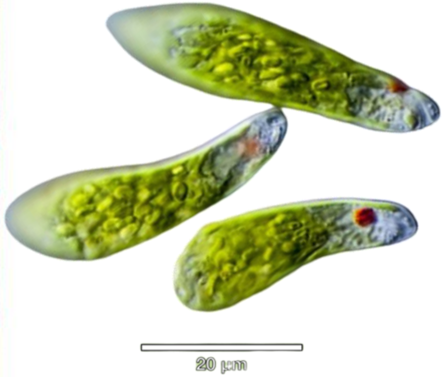
Is heterotrophy a solution ?

Introduction: strain selection for biofuel production



Galdieria sulphuraria

- Extremophilic red microalga (resistant)
- Optimum pH = 2 and T° = 42°C (low contamination)
- Able to metabolize many different carbon sources

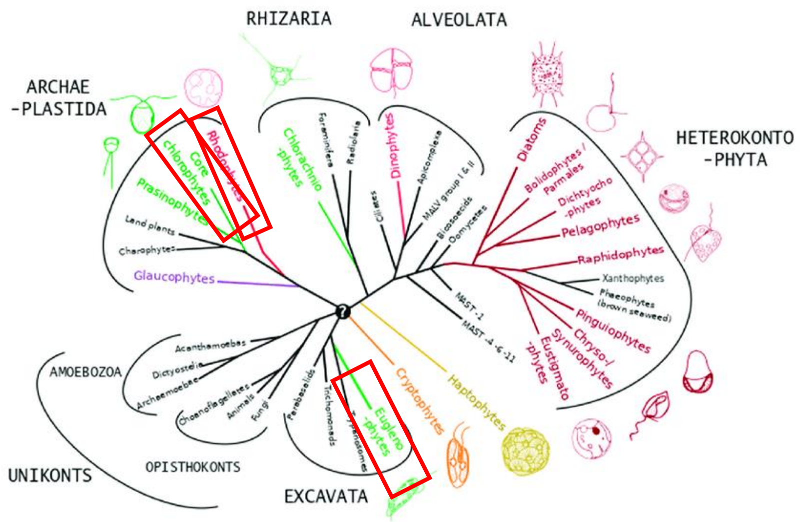


Euglena gracilis

- Cell wall-less (easy access to cellular content)
- Paramylon production in aerobic conditions
- Converted into wax esters in anaerobic conditions

Auxenochlorella protothecoides

- Oleaginous microalga
- High saturated fatty acids distribution (oxidative stability)
- High growth rate in heterotrophy

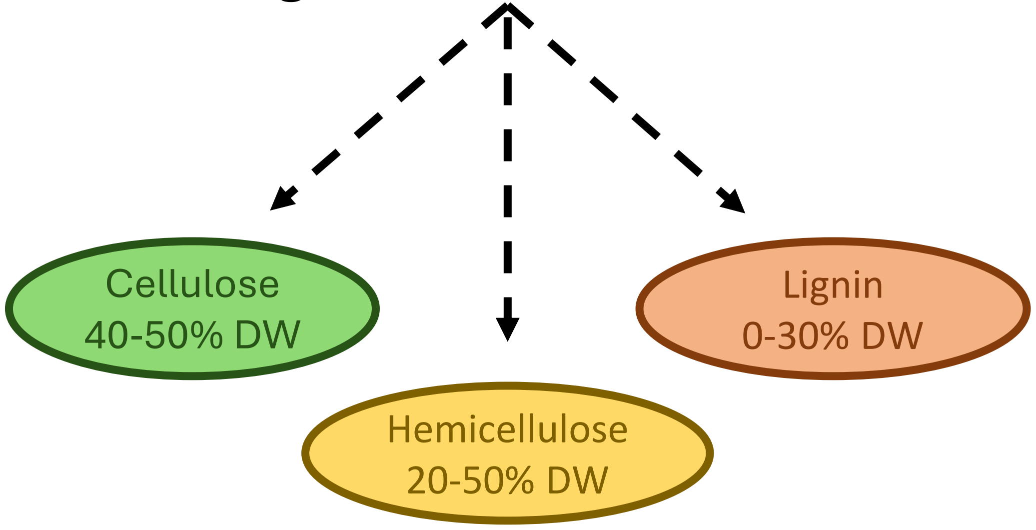


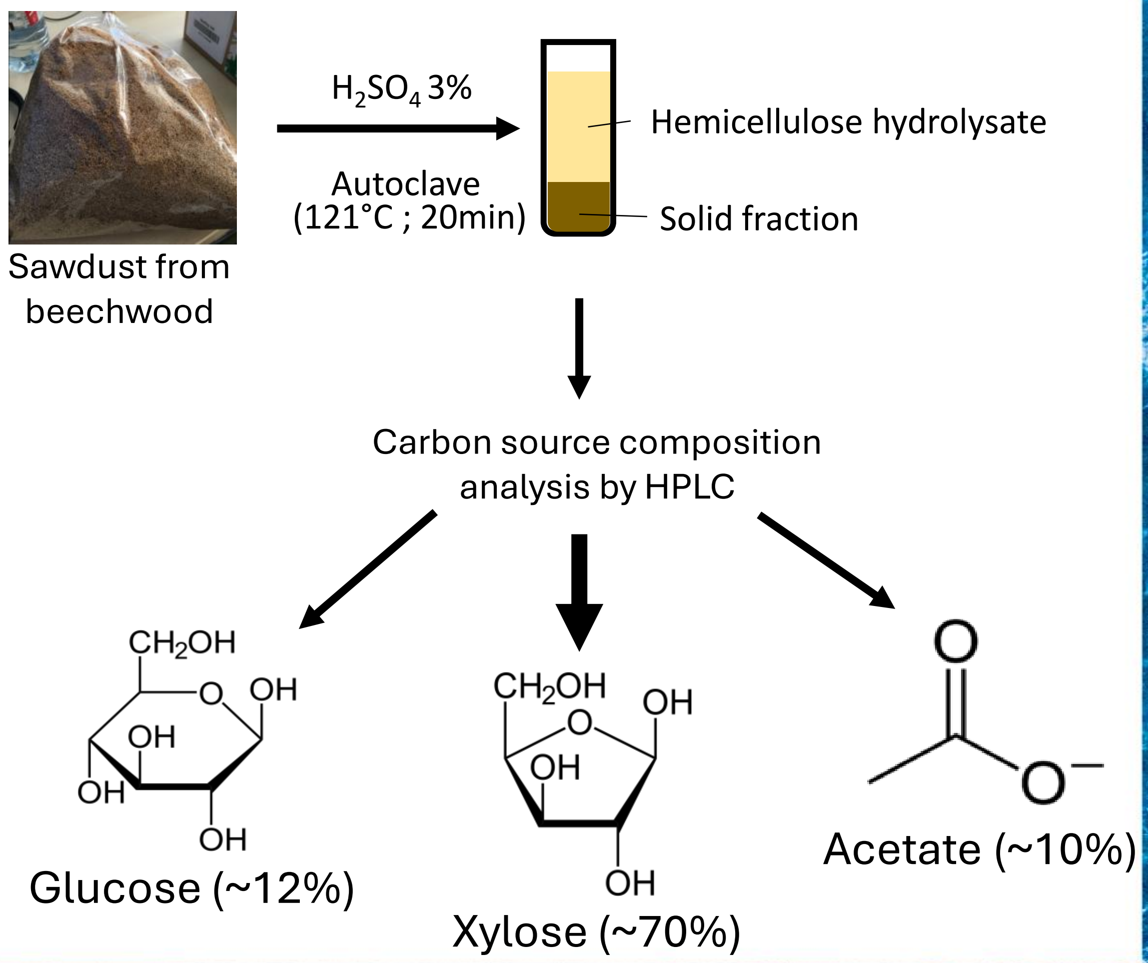
Pierre et al., 2019

Introduction: hemicellulose as feedstock for microalgal growth

How to select a carbon source ?

Lignocellulosic matter

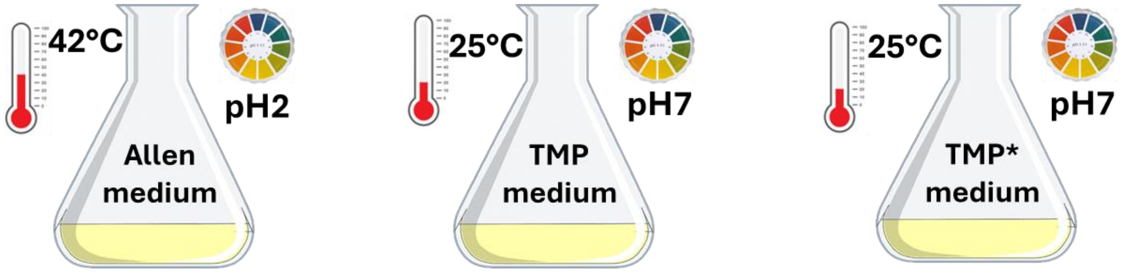




Experimental design

- All strains are grown **in the dark** under constant agitation

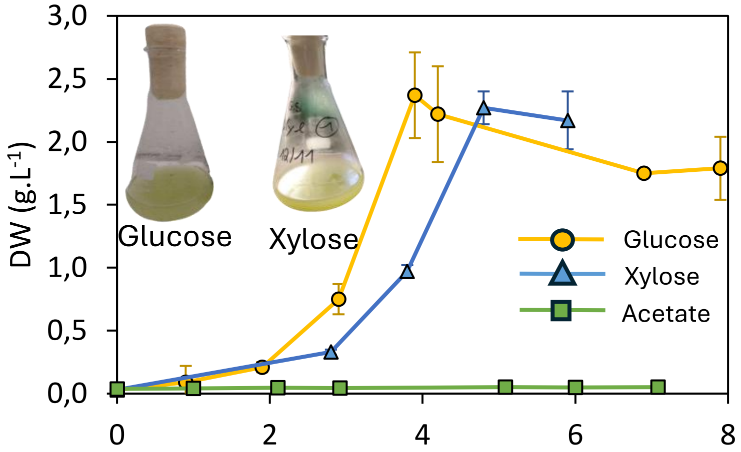
G. sulphuraria — *A. protothecoides* — *E. gracilis*

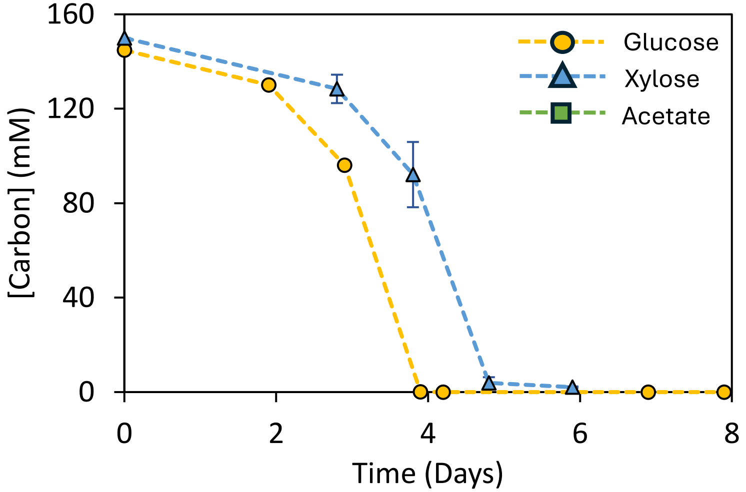


- Variable carbon source content

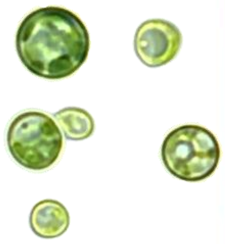
	Carbon source	Carbon atoms (mM)	Concentration (g L^{-1})	Concentration (mM)
	Glucose	**150**	4.50	25
or	**Xylose**	**150**	4.50	30
or	**Acetate**	**150**	6.15	75
or	**Mix** of glucose – xylose – acetate	**50-50-50**	1.50 – 1.50 – 2.05	8.33 – 10 – 25

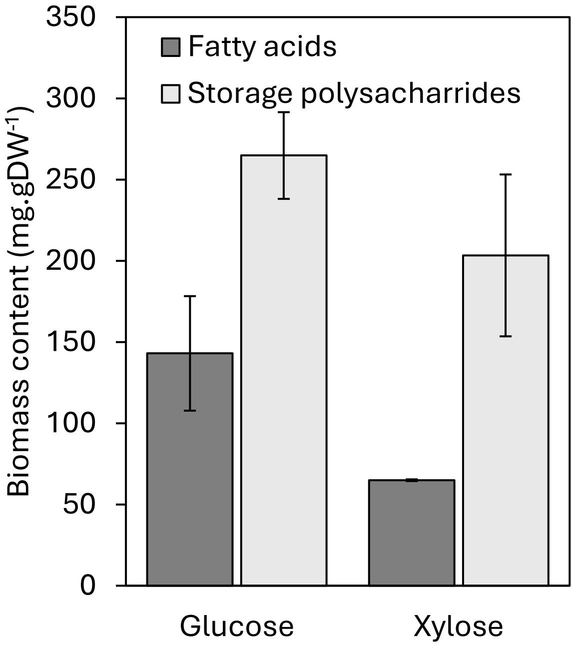
Results: *Galdieria sulphuraria* grows well on xylose



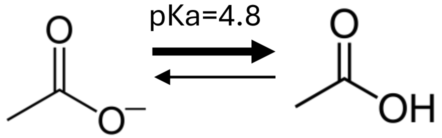


	Glucose	Xylose	Acetate
μ (d^{-1})	1.10 ± 0.03	0.97 ± 0.03	/
DW yield ($g\ g_{sugar}^{-1}$)	0.53 ± 0.08	0.51 ± 0.01	/





- Good specific growth rates and biomass yields on glucose and xylose
- Growth inhibition in the presence of acetate due to low pH condition (pH=2)



- Low FA content with high SFAs distribution
- Glycogen content up to 25%

Substrate	% SFA	% MUFA	% PUFA
Glucose	**57 ± 1**	8 ± 1	34 ± 1
Xylose	**63 ± 1**	17 ± 1	20 ± 0

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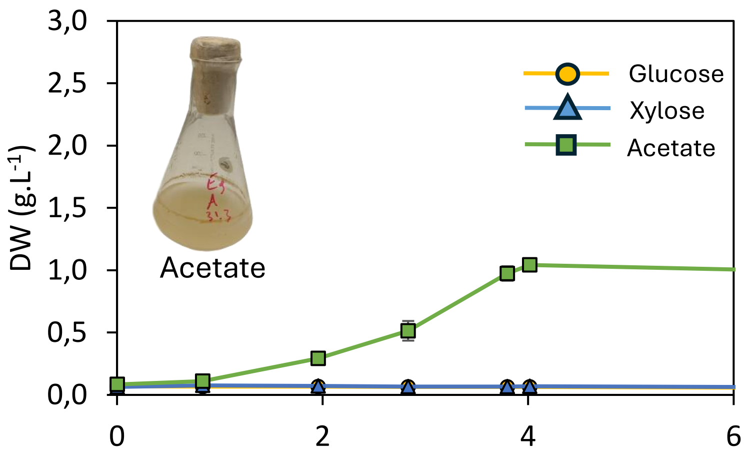
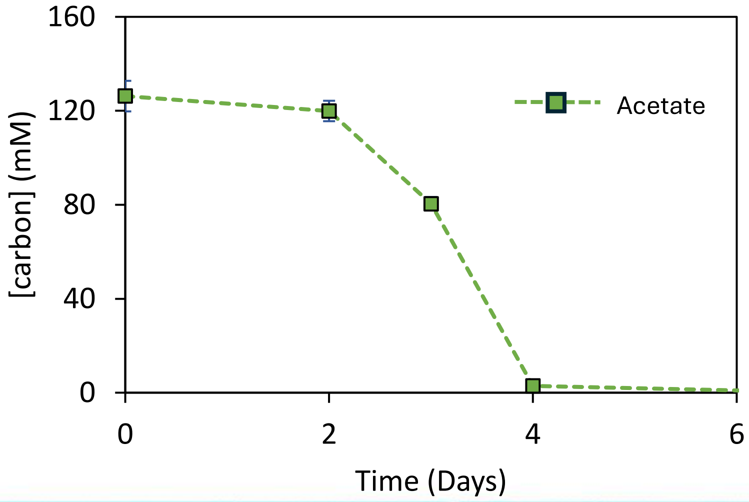
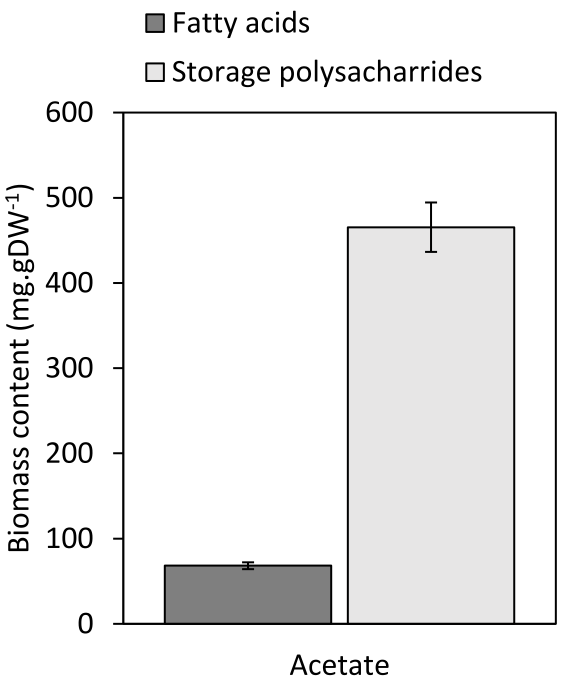
Results: *Euglena gracilis* produces paramylon on acetate



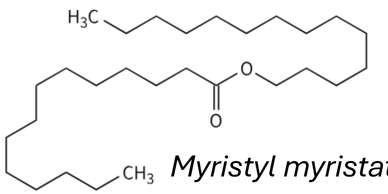


	Glucose	Xylose	Acetate
μ (d^{-1})	/	/	0.73 ± 0.02
DW yield ($g\ g_{sugar}^{-1}$)	/	/	0.26 ± 0.02



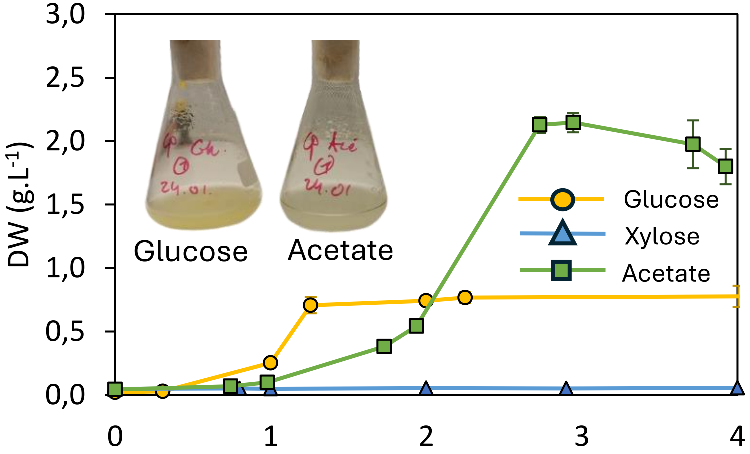
- Growth only observed in the presence of acetate
- Low FA content with high SFA distribution (~70%)
- High levels of paramylon, convertible into wax esters in anaerobic conditions

% SFA	% MUFA	% PUFA
67 ± 1	9 ± 1	28 ± 1



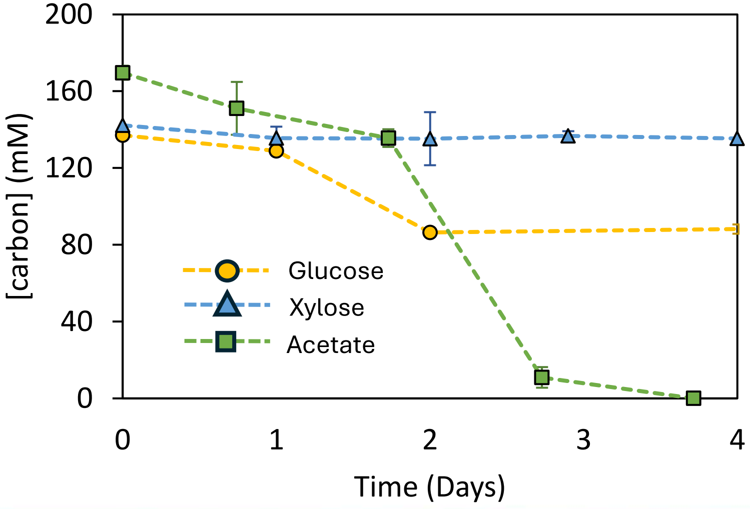
Myristyl myristate

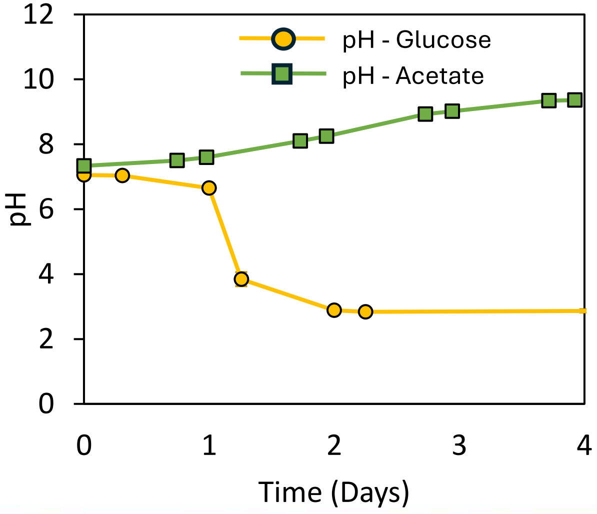
Results: *Auxenochlorella protothecoides* cannot assimilate xylose



	Glucose	Xylose	Acetate
μ (d^{-1})	3.29 ± 0.01	/	1.74 ± 0.09
DW yield ($g\ g_{sugar}^{-1}$)	0.54 ± 0.04	/	0.42 ± 0.02

- Growth observed with acetate and glucose
- No growth was observed with xylose when supplied alone





- Growth arrest observed during glucose assimilation due to rapid drop in pH
  - ➢ High respiration rate ➔ CO_2 ↗
  - ➢ NH_4^+ assimilation lowers the pH

$$NH_4^+ + \alpha - \text{keto acid} \xrightarrow{algae} \textit{proteinaceous amino acid} + H^+$$

Wang & Curtis. *J Appl Phycol*. (2016)

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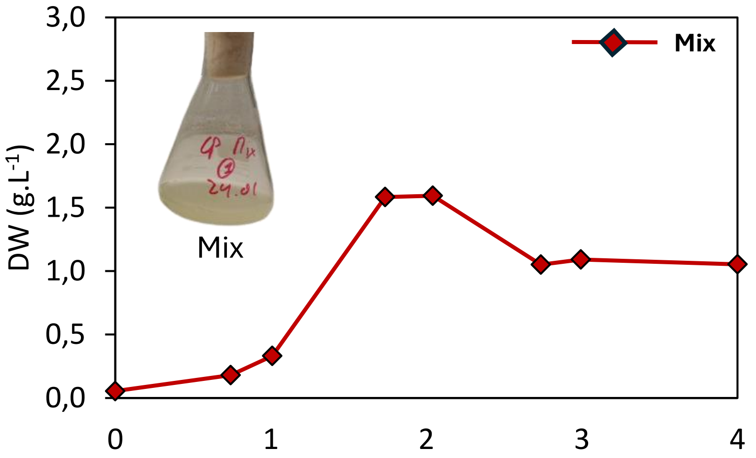
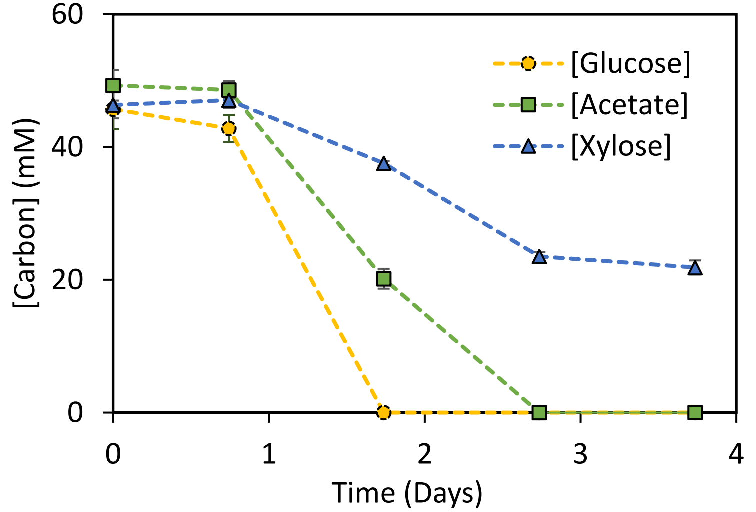
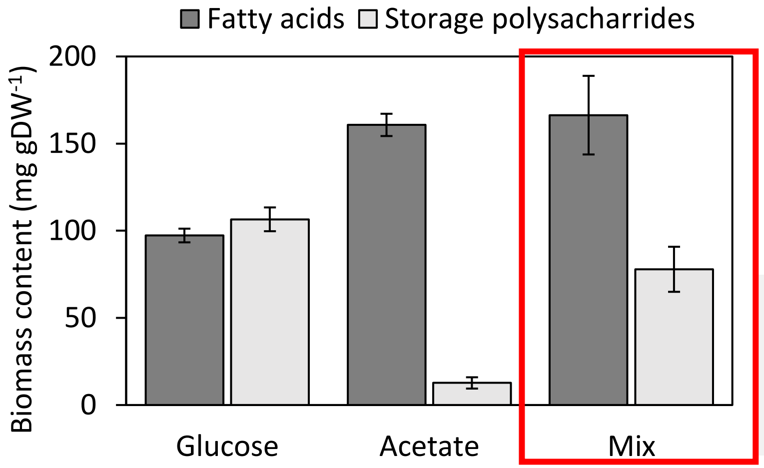
Results: *Auxenochlorella protothecoides* assimilates xylose in the carbon mix





	Glucose (alone)	Mix	Acetate (alone)
μ (d^{-1})	3.29 ± 0.01	**2.18 ± 0.04**	1.74 ± 0.09
DW yield ($g\ g_{sugar}^{-1}$)	0.54 ± 0.04	**0.43 ± 0.02**	0.42 ± 0.02

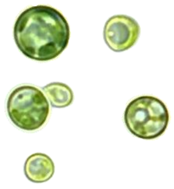
- No pH imbalance issues encountered
- Intermediate growth rate between glucose and acetate conditions
- Xylose assimilation was observed (hexose-induced transport?)



- Highest level of FAs during the study
- >70% of SFAs and MUFAs

Substrate	% SFA	% MUFA	% PUFA
Glucose	24 ± 1	39 ± 1	37 ± 0
Acetate	25 ± 1	43 ± 1	32 ± 1
Mix	35 ± 1	36 ± 1	29 ± 1

Conclusions: take-home messages



- *G. sulphuraria* shows remarkable growth rates on xylose with a carbohydrate content of 25%. Does not grow on acetate.

- *E. gracilis* has low FA content (<10%) but high SFAs distribution (~70%) and paramylon content (~50%), convertible in wax esters. Only assimilates acetate.

- *A. protothecoides* was able to assimilate all three carbon sources with a specific growth rate > 2
  - High SFAs and MUFAs content (>70%)

Acknowledgments

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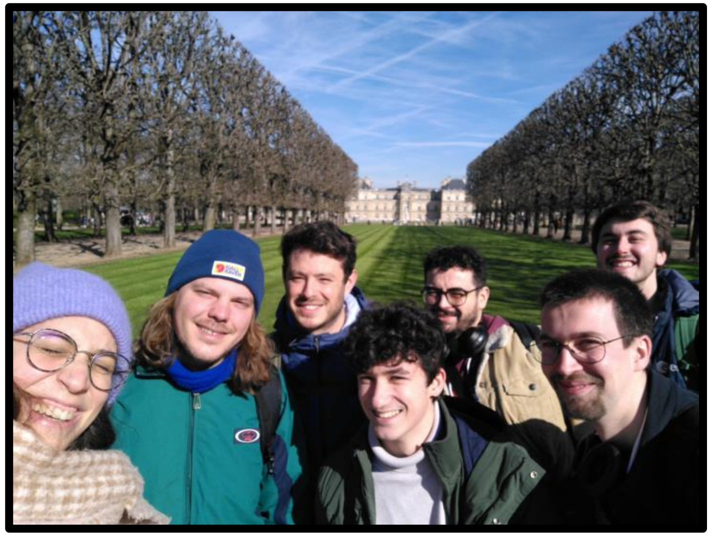

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Thanks for your attention!

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