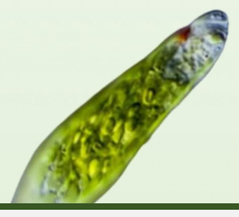
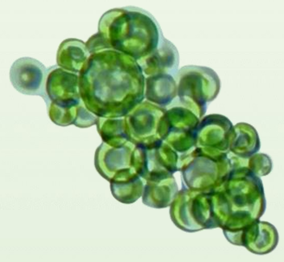
Hemicellulose valorization for biofuel production from microalgae grown in heterotrophy

Pablo Perez Saura[1,2], Pierre Cardol[1], Claire Remacle[1]

[1] Laboratory of Genetics and Physiology of Microalgae, InBios Phytosystems, University of Liège

[2] pablo.perezsaura@uliege.be

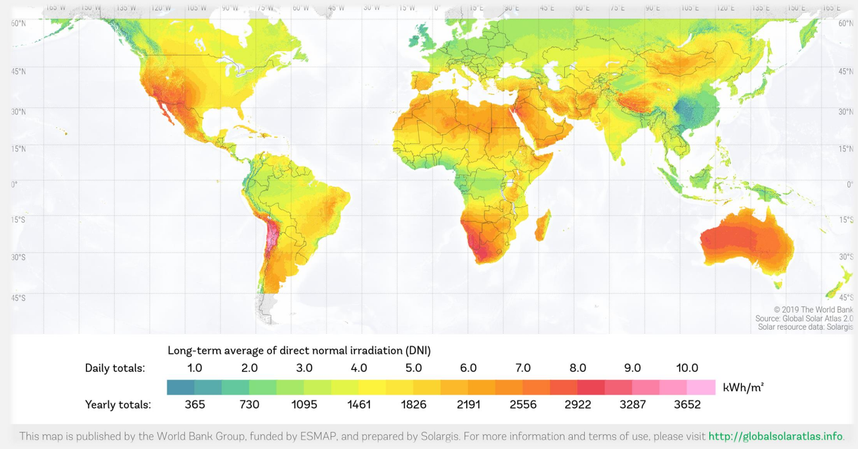




INTRODUCTION

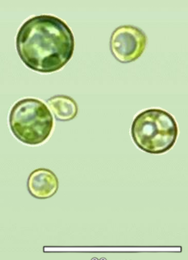
Fuel demand is rising continuously every year around the world. Global energy needs and fossil fuels' impact on climate can be partially managed by an increase in the use of **biofuels** for transport and industries. Due to their **lipid-rich** biomass content, many **microalgal** species are good candidates for biodiesel and biokerosene production. To minimize the cost of microalgal biomass production, the commonly applied strategy is based on open ponds and photobioreactor cultures where the algae are grown in phototrophy. Nevertheless, in Belgian latitudes, this strategy is not conceivable because of the weakness and scarcity of sunshine. An alternative approach is, therefore, to grow the microalgae in **heterotrophy** with an organic substrate supplied in the media.

After cellulose, **hemicellulose** is the second most abundant material found in plants. Hemicellulose hydrolysis mainly liberates **xylose**, **glucose**, and **acetate** in variable proportions depending on the lignocellulosic material and hydrolytic process. Here, we selected three microalgal species, from different phylogenetic origins, capable of growing heterotrophically and showing interesting features for biofuel production: ***Galdieria sulphuraria***, ***Euglena gracilis***, and ***Chlorella protothecoides***. We analyzed their capacity to grow in the presence of the three carbon sources mentioned above and characterized their **biomass content**.



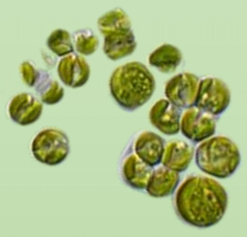
Global direct solar irradiation - *Global Solar Atlas 2.0, 2019*

Which microalgae are studied and why?



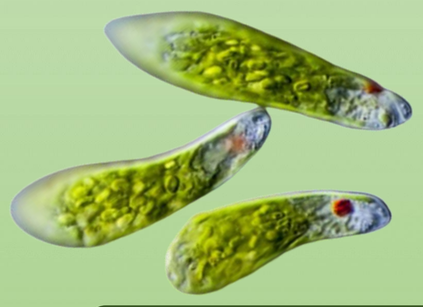
Galdieria sulphuraria

- Extremophilic red microalga
- Optimum pH = 2 and T° = 42°C (low contamination)
- Resistant to high metal and salt concentrations
- Able to grow in heterotrophy in the presence of at least 26 different carbon sources, including xylose



Chlorella protothecoides

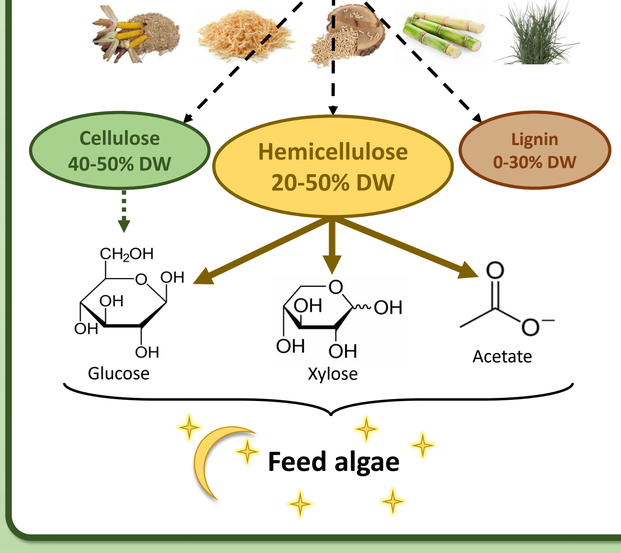
- High dry weight (DW) lipid content
- High levels of saturated fatty acids
- High oxidative stability of biodiesel



Euglena gracilis

- Wall-less (easy access to cellular content)
- Paramylon production in aerobic conditions
- Converted into wax esters in anaerobic conditions interesting for biokerosene production

METHODS



Fixed culture conditions depending on the strains

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

Strain	Medium	T°	Starting pH
G. sulphuraria	Allen	42°C	2
C. protothecoides	TMP	25°C	7
E. gracilis	TMP*	25°C	7

* Some *E. gracilis* essential vitamins were added to the medium after autoclaving (B1, B8, B12)

Variable carbon source content

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

RESULTS AND CONCLUSIONS

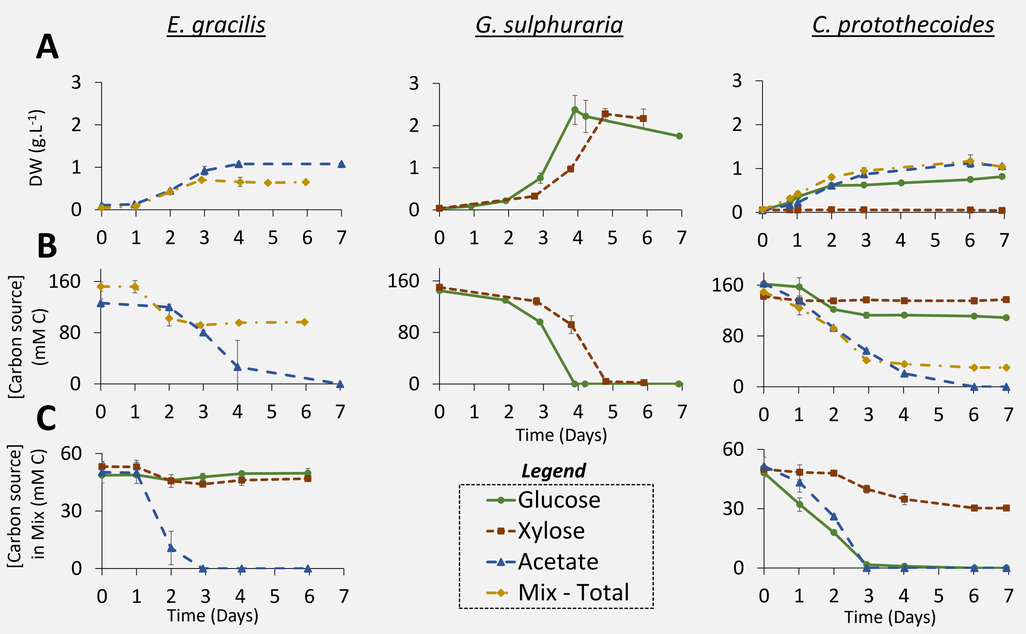


FIGURE 1: Growth curves and carbonated substrates consumption of *E. gracilis, G. sulphuraria,* and *C. protothecoides* grown in the dark in the presence of different substrates found in hemicellulose hydrolysate. Graphs **(A)** show the dry weight (DW) evolution of each strain in the presence of glucose (solid line, black dots), xylose (dashed line, light gray squares), acetate (dashed line, mid-dark gray triangles), or an equal mix of the three substrates (dashed line, dark gray diamonds) over time (days). Data are expressed in g.L^{-1} of culture. Graphs **(B)** show the carbon sources' consumption in the culture medium over time for each strain and condition. Visual code is similar to graphs (A). Data are expressed in mM of carbon atoms (mM C) in the culture. Graphs **(C)** show the carbon sources' consumption for each strain in the condition where acetate, glucose, and xylose were mixed in the culture medium. Visual code is similar to graphs (A). Data are presented as means of at least three independent biological replicates. Error bars represent the standard deviation of the mean (±SD).

- Results showed that *E. gracilis* was only able to assimilate acetate, whereas ***G. sulphuraria*'s growth is inhibited by the presence of acetate** in the studied concentrations. This is probably due to the low pH environment that promotes the toxic acidic acid form of the dissociated acetate in the medium. In contrast, ***C. protothecoides*** **could grow in the presence of all carbon sources except xylose alone**.
- ***G. sulphuraria*** was the only strain able to grow in the presence of **xylose alone**. After 5 days, the maximum reached biomass, biomass productivity, and doubling time were similar to that observed in the presence of glucose and more than two times higher compared to the maximum biomasses of the other strains regardless of the studied condition.
- Even if ***C. protothecoides*** could not grow in the presence of xylose alone, we observed a **partial xylose depletion** in the medium when other carbon sources were still present in the medium. This result suggests that xylose assimilation is mediated by non-specific sugar transporters.
- Biomass content showed that ***C. protothecoides*** **has the highest fatty acids production** (±32% of its DW in the presence of acetate), which is twice higher compared to the other strains. Surprisingly, fatty acids distribution between SFAs, MUFAs, and PUFAs is homogeneous (around 33% each) contrary to the high SFA percentage reported in the literature.
- ***G. sulphuraria*** **and** ***E. gracilis*** were producing relatively low amounts of fatty acids relative to their total biomass weight (5-15%). However, they were producing **high proportions of SFAs** (around 60%).
- In addition to lipids, protein, pigment, and storage polysaccharides contents were also measured. Proteins account for about 30% of the total biomass in *E. gracilis* and *G. sulphuraria* while the total biomass of *C. protothecoides* was made up of 60% of proteins (Data not shown). Polysaccharides were mostly found in *G. sulphuraria* (25% of its DW) in the form of phytoglycogen. **Pigment content was extremely low for all strains** in every studied conditions compared to phototrophy (Data not shown).

TABLE 1: Comparison of the growth parameters, biomass productivities, biomass substrate yields, and biomass content of *G. sulphuraria, C. protothecoides,* or *E. gracilis* grown in heterotrophy in the presence of glucose, xylose, acetate, or a mix of the three substrates.

Strain	Substrate	Doubling Time (Days)	Max DW (g.L^{-1})	Max productivity (gDW.L^{-1}.d^{-1})	Sugar to biomass conversion (gDW.gsugar^{-1})	Max [Fatty acids] (mg.gDW^{-1})	% SFA	% MUFA	% PUFA	Max [Carbohydrates] (mg.gDW^{-1})
G. sulphuraria	Glucose	0.63 ± 0.02	2.37 ± 0.34	1.62 ± 0.22	0.54 ± 0.08	143,0 ± 35,0	57,4 ± 0,5	8,1 ± 0,7	34,4 ± 0,5	264,4 ± 2,7
	Xylose	0.72 ± 0.02	2.27 ± 013	1.31 ± 0.08	0.52 ± 0.01	64,9 ± 0,5	62,9 ± 0.7	16,9 ± 0,6	20,2 ± 0,1	203,3 ± 0,1
	Acetate	/	/	/	/	/	/	/	/	/
	Mix	/	/	/	/	/	/	/	/	/
C. protothecoides	Glucose	0.37 ± 0.02	0.82 ± 0.02	0.59 ± 0.08	0.17 ± 0.01	218,3 ± 18,9	33,3 ± 4,5	31,2 ± 1,1	35,6 ± 3,4	75,1 ± 17
	Xylose	/	/	/	/	/	/	/	/	/
	Acetate	0.44 ± 0.01	1.12 ± 0.02	0.39 ± 0.01	0.17 ± 0.01	318,2 ± 2,6	30,5 ± 0,4	39,6 ± 0,4	29,9 ± 0,3	23,8 ± 5,2
	Glu-xyl-ace	0.34 ± 0.01	1.17 ± 0.14	0.46 ± 0.14	0.23 ± 0.04	216,2 ± 32,0	26,4 ± 0,2	39,1 ± 0,9	34,5 ± 0,8	22,3 ± 2,4
E. gracilis	Glucose	/	/	/	/	/	/	/	/	/
	Xylose	/	/	/	/	/	/	/	/	/
	Acetate	0.72 ± 0.05	1.08 ± 0.04	0.47 ± 0.10	0.21 ± 0.02	68,2 ± 4,6	67,2 ± 1,1	8,8 ± 0,6	27,8 ± 1,3	ND
	Glu-xyl-ace	0.67 ± 0.01	0.70 ± 0.01	0.33 ± 0.02	0.14 ± 0.01	54,4 ± 6,4	75,4 ± 4,7	6,7 ± 0,6	76,5 ± 4,2	ND

Data are presented as means of at least three independent biological replicates. Error bars represent the standard deviation of the mean (±SD). /: No growth. ND: No data

In conclusion, none of the strains could assimilate all the carbonated substrates found in hemicellulose hydrolysate in heterotrophic conditions. Because of its high lipid content and substrate assimilation, *C. protothecoides* is an interesting candidate for biodiesel production in the studied condition. Nevertheless, due to its high biomass productivity in the presence of xylose and high SFAs proportions, *G. sulphuraria* shows valuable features in the context of biofuel production using hemicellulose as a substrate.

As a perspective, a two-step strategy involving acetate and glucose removal of the hemicellulose hydrolysate using *C. protohecoides,* followed by the biomass production of *G. sulphuraria* using the remaining xylose is already studied in our laboratory. With this method, we would be able to valorize all the carbon sources constituting hemicellulose, producing high-added microalgal biocompounds.

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