Glycerol and glucose are efficient carbon sources for heterotrophic growth and influence pigment composition of the extremophilic red microalga *Galdieria sulphuraria*

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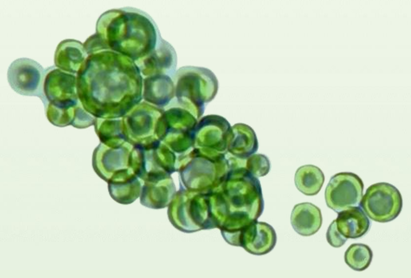
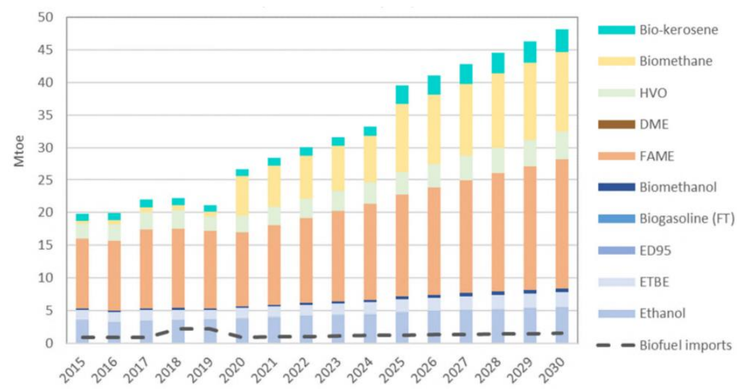
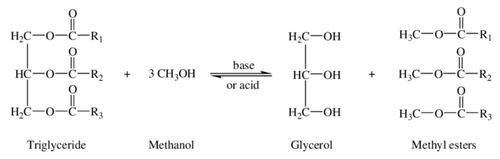


INTRODUCTION

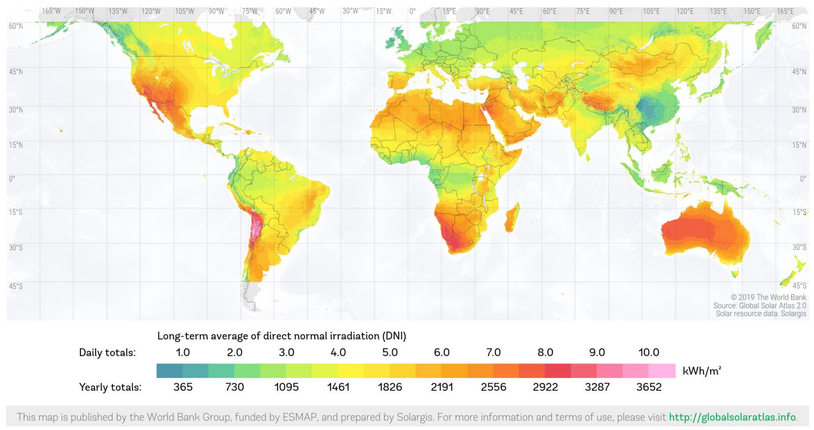


Biofuel availability in Europe from 2015 to 2030 – *© Concawe, 2021*

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 transports and industries. The type of biofuel mainly produced in Europe are Fatty Acid Methyl Esters (FAMEs), that have physical properties similar to conventional diesel. FAMEs production is generally preceded by a transesterification process of the green biomass triacylglycerols (TAGs) that generates large amounts of glycerol as a by-product.

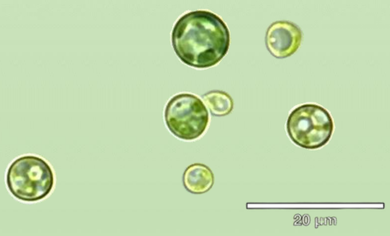


Microalgae have the ability to produce great amounts of TAGs that can be then converted into FAMEs. To minimize the cost of the microalgal biomass production for biofuel production, the commonly applied strategy is based on open ponds cultures where the algae are grown in phototrophy. Nevertheless in our latitudes this strategy is not conceivable because of the weakness and scarcity of sunshine. An original approach is therefore to grow the microalgae in heterotrophy with a organic substrate supplied in the media. In this study we grew a microalgal species in the dark, and compared its growing parameters and biomass composition in the presence of glucose or glycerol. We also performed transcriptomic analyses to compare the differentially expressed genes between heterotrophic and phototrophic growth.



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

Which microalga was studied and why ?



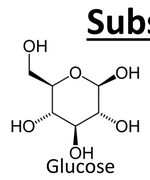
Galdieria sulphuraria

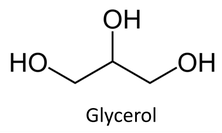
- Extremophilic red microalga
- Able to grow at high T° up to 56°C and low pH (0-4)
- Low contamination risk
- Resistant to high heavy metals and high salt concentrations
- Phototroph or heterotroph (with more than 50 carbon sources)
- Studied for high-added value biocompounds or wastewater bio-remediation

Galdieria sulphuraria heterotrophic growth in the presence of glucose (left) or glycerol (right)

METHODS

Substrates





25mM	50mM
4,50g.L^{-1}	4,60g.L^{-1}

Galdieria sulphuraria

- Heterotrophically (in the dark) with glucose or glycerol
- 2xGS Modified Allen culture medium
- T° = 42°C
- pH = 2
- Constant shaking

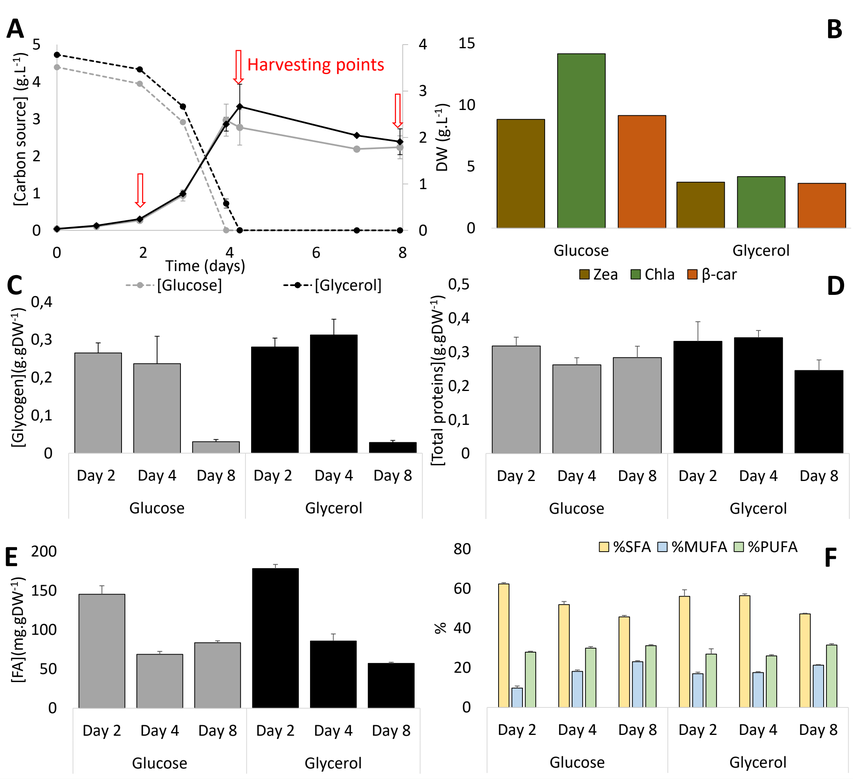
Transcriptomic

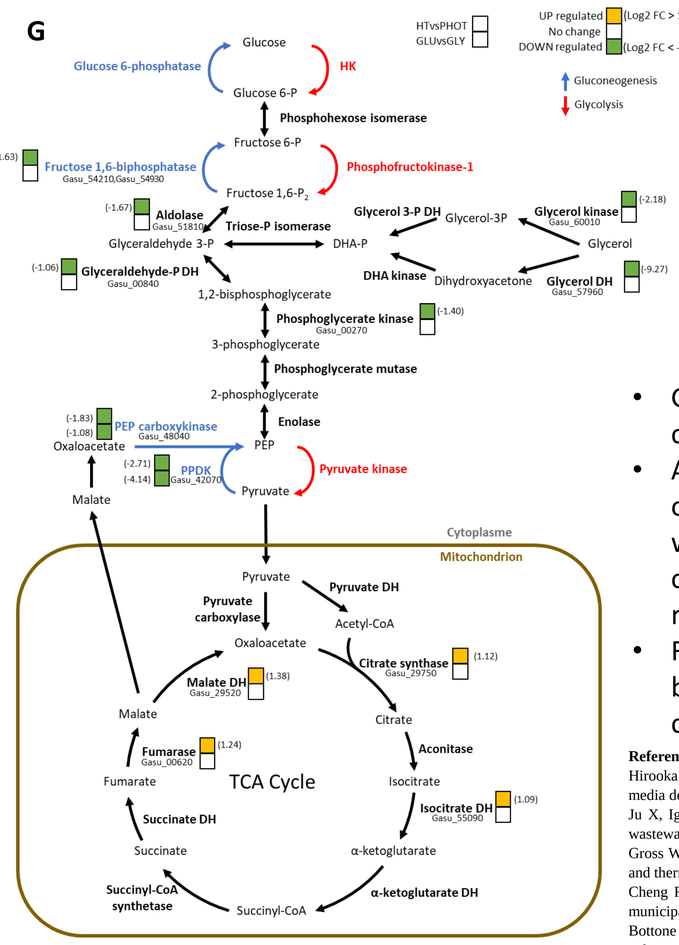
RNAs were extracted in three biological replicates from cultures at mid-exponential phase in heterotrophy with glycerol or glucose and in phototrophy.

RESULTS AND CONCLUSIONS

- Maximal biomass reached in these two conditions was not significantly different (~ 2,5 g. L^{-1}), and correspond to substrate depletion.
- Fatty acid profile, protein content, and storage carbohydrate contents were also statistically similar, irrespectively of the metabolised carbon source.
- Pigment content was decreased in heterotrophy compared to phototrophy. This diminution is more important in the presence of glucose than glycerol.
- DEGs analysis confirmed that the expression of many genes involved in photosynthesis was downregulated in heterotrophy compared to phototrophy and that this repression was stronger in the presence of glucose than glycerol (not shown).
- Phosphoenolpyruvate formation enzymes are strongly downregulated when glucose is present, suggesting gluconeogenesis repression.
- Gluconeogenesis seems activated in phototrophy when compared to heterotrophy.
- An increased expression of four genes encoding enzymes of the Tricarboxylic Acid Cycle (TCA) has been found, which would suggest a transcriptional upregulation of this cycle in heterotrophy, to sustain the respiratory chain in redox equivalents.
- Fatty acid profile and productivity are interesting for biofuel production (high SFA %) as well as low pigments content that avoid NOx generation.

Biomasse evolution and subtrate consumption **(A)**. Phototrophy to heterotrophy pigment content ratio at day 2 **(B)**. Glycogen **(C)**, protein **(D)** and total fatty acid content **(E)** heterotrophy. Saturated, unsaturated and polyunsaturated fatty acid distribution **(F)**. Glycolysis, gluconeogenesis and TCA cycle pathways and DEGs [adj. p<.05 and log2(fold change)>1] in heterotrophy compared to phototrophy and in the presence of glucose compared to glycerol **(G)**.





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