

## Culturing *Galdieria sulphuraria* (ALG\_App004)

### Background to *G. sulphuraria*

*G. sulphuraria* is an extremophilic, spherical, spore-forming red alga. It is commonly found in hot acid springs. It is an acidophilic and thermophilic alga which grows phototrophically and mixotrophically, and is capable of heterotrophic growth on sugars, alcohols and amino acids (Gross and Schnarrenberger, 1995; Oesterhelt and Gross, 2002; Barbier et al., 2005). *G. sulphuraria* has commercial potential for wastewater remediation (Schönknecht et al., 2013; Selvaratnem et al., 2014) and the mass production of the phycobiliprotein phycocyanin (Schmidt et al., 2005).

### Aim

To confirm whether *G. sulphuraria* can tolerate high temperatures (50°C) and acidic (pH 4) conditions, and to observe how light intensity and photoperiod affect growth.

### Experimental Design

An exponentially growing culture of *G. sulphuraria* in late-log was harvested and inoculated at  $1 \times 10^6$  cells/ml into 1 L flasks with 400 ml Cyanidium medium (SAG) + 150 mM glucose (pH 4) with soil extract replaced by 1 ml Special K trace elements/L according to Kropat and Malasarn (2010). Growth comparisons were obtained for different photoperiods (12:12 photoperiod and continuous light) and light intensities (100 and 200  $\mu\text{mol photons/m}^2/\text{s}$  with red and blue light spectra as shown in Figure 1). Preliminary experiments revealed that red and blue light combined at the ratios stated resulted in better growth than white light. Temperature was maintained at 50°C with mixing at 90 rpm. OD740nm was monitored hourly until stationary was reached.

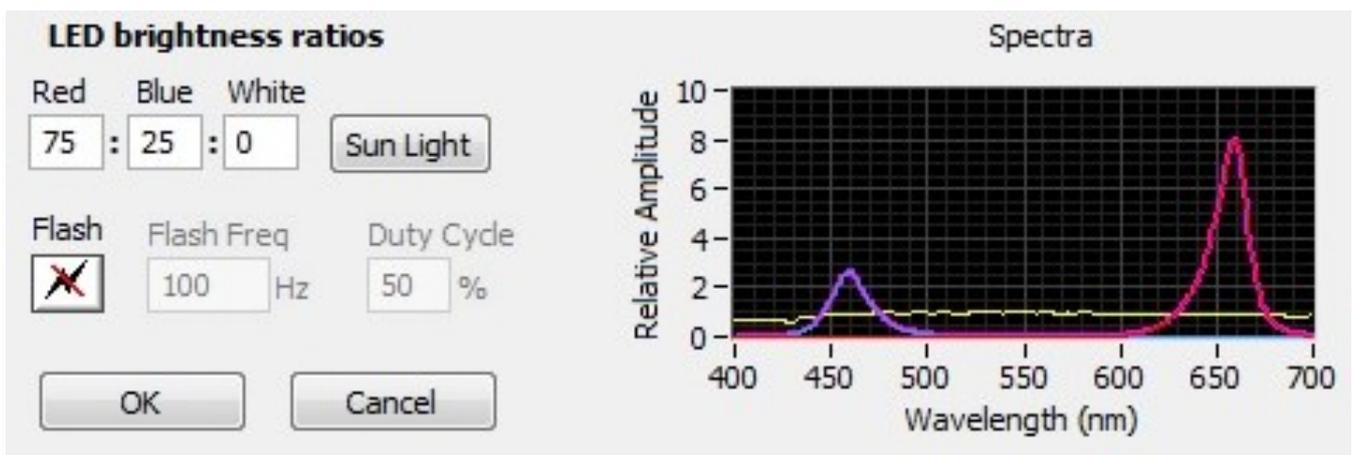


Figure 1 - LED spectra for *G. sulphuraria* cultivation with peaks included in the blue (450-500 nm) and red regions (610-700)



## Results

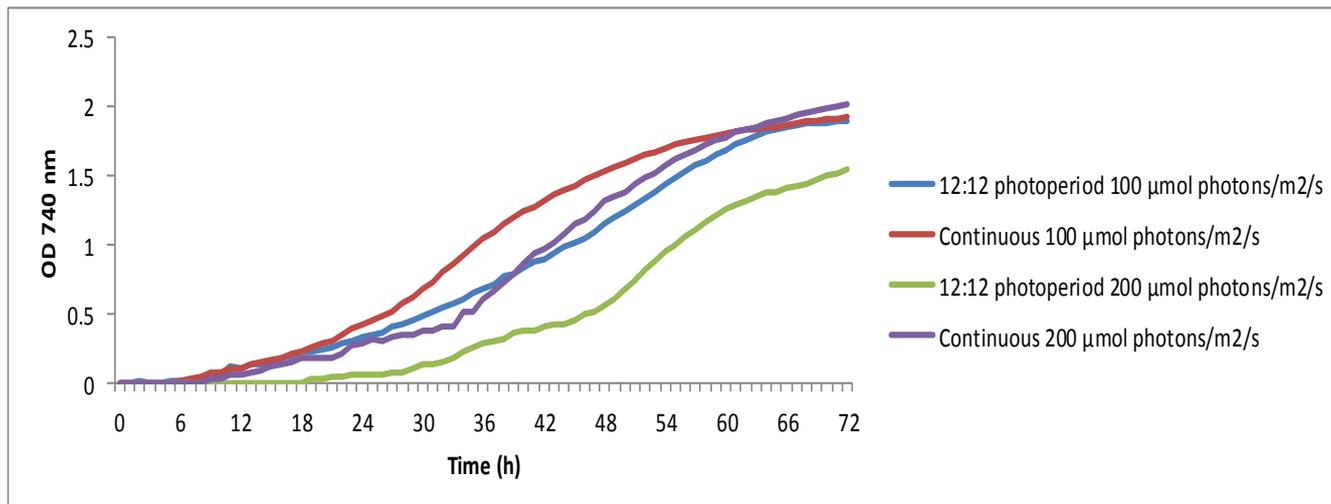


Figure 2 - Growth profile of *G. sulphuraria* cultured mixotrophically in *Cyanidium* medium with glucose at 50 °C under different photoperiods (12:12 photoperiod and continuous) and light intensities (100 µmol photons/m<sup>2</sup>/s and 200 µmol photons with red: blue light at 3:1)

## Notes

*G. sulphuraria* was able to thrive under the temperature and pH tested. *G. sulphuraria* grew better under the lower light intensity of 100 µmol photons/m<sup>2</sup>/s with best performance observed under continuous light under the conditions tested (Figure 2). A 12:12 photoperiod under 200 µmol photons/m<sup>2</sup>/s resulted in the poorest growth. For future experiments, different temperatures could be tested with strict control. Heterotrophic experiments could also be conducted where *G. sulphuraria* has been observed to have a higher doubling time (Graziani et al., 2013).

## References

- Barbier, G., Oesterhelt, C., Larson, M.D., Halgren, R.G., Wilkerson, C., Garavito, R.M., Benning, C. and Weber, A.P. (2005) Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. *Plant physiology*, 137 (2), pp. 460-474.
- Graziani, G., Schiavo, S., Nicolai, M.A., Buono, S., Fogliano, V., Pinto, G. and Pollio, A. (2013) Microalgae as human food: chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. *Food and Function*, 4 (1), pp.144-152.
- Gross, W. and Schnarrenberger, C. (1995) Heterotrophic growth of two strains of the acido-thermophilic red alga *Galdieria sulphuraria*. *Plant and Cell Physiology*, 36 (4), pp. 633-638.
- Kropat, J and Malasarn, D (2010) Merchant Lab Research Resources: Special K Traces. Available at: <http://www.chem.ucla.edu/dept/Faculty/merchant/pdf/SpecialKTraceElementsJune2.pdf>. Accessed: 21/04/16.
- Oesterhelt, C. and Gross, W. (2002) Different Sugar Kinases Are Involved in the Sugar Sensing of *Galdieria sulphuraria*. *Plant physiology*, 128 (1), pp. 291-299.
- Schmidt, R.A., Wiebe, M.G. and Eriksen, N.T. (2005) Heterotrophic high cell-density fed-batch cultures of the phycocyanin-producing red alga *Galdieria sulphuraria*. *Biotechnology and bioengineering*, 90(1), pp.77-84.
- Schönknecht, G., Chen, W.H., Ternes, C.M., Barbier, G.G., Shrestha, R.P., Stanke, M., Bräutigam, A., Baker, B.J., Banfield, J.F., Garavito, R.M. and Carr, K. (2013) Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. *Science*, 339(6124), pp.1207-1210.
- Selvaratnam, T., Pegallapati, A.K., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Van Voorhies, W. and Lammers, P.J. (2014) Evaluation of a thermo-tolerant acidophilic alga, *Galdieria sulphuraria*, for nutrient removal from urban wastewaters. *Bioresource Technology*, 156, pp.395-399.