application note

Culturing *Galdieria sulphuraria* (ALG_App004)

Background to *G. sulpharia*

G. sulpharia is an extremophilic, spherical, spore-forming red alga 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* (SAG 107.79) can tolerate high temperatures (50°C) and acidic (pH 4) conditions, and to observe how light intensity and photoperiod affect growth.

Experimental Design

Two experiments were conducted, one investigating the effects of temperature, and the other investigating photoperiod and light intensity. Growth comparisons were made based on hourly optical density (OD) measurements at 740nm.

For both experiments exponentially growing cultures of *G. sulphuraria* in late-log phase were harvested and inoculated at 5 x 10^5 cells/ml into 1 L flasks with 400 ml Cyanidium medium (SAG) + 150 mM glucose (pH 4). Soil extract was replaced by 1 ml/L Special K trace elements according to (Kropat and Malasarn, 2010). *G. sulphuraria* was cultured with red and blue light as indicated in Figure 1; preliminary experiments having revealed that red and blue light combined at the ratio stated resulted in better growth than white light. Flasks were mixed at 90rpm without aeration.

To investigate temperature cultures were incubated at 25°C, 40°C, and 50°C under continuous light at 100 µmol photons/m^2/s. Investigations into photoperiods and light intensity were conducted at 50°C under either a 12:12 photoperiod or continuous light, both at light intensities of 100 and 200 µmol photons/m^2/s.

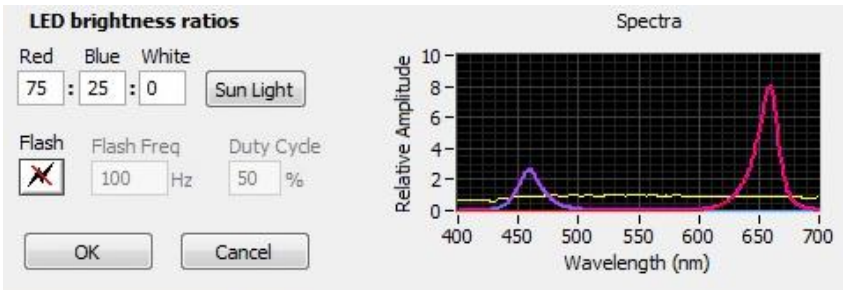

Figure 1 - LED spectra for *G. sulphuraria* cultivation with peaks included in the blue (450-500 nm) and red regions (610-700)

Results

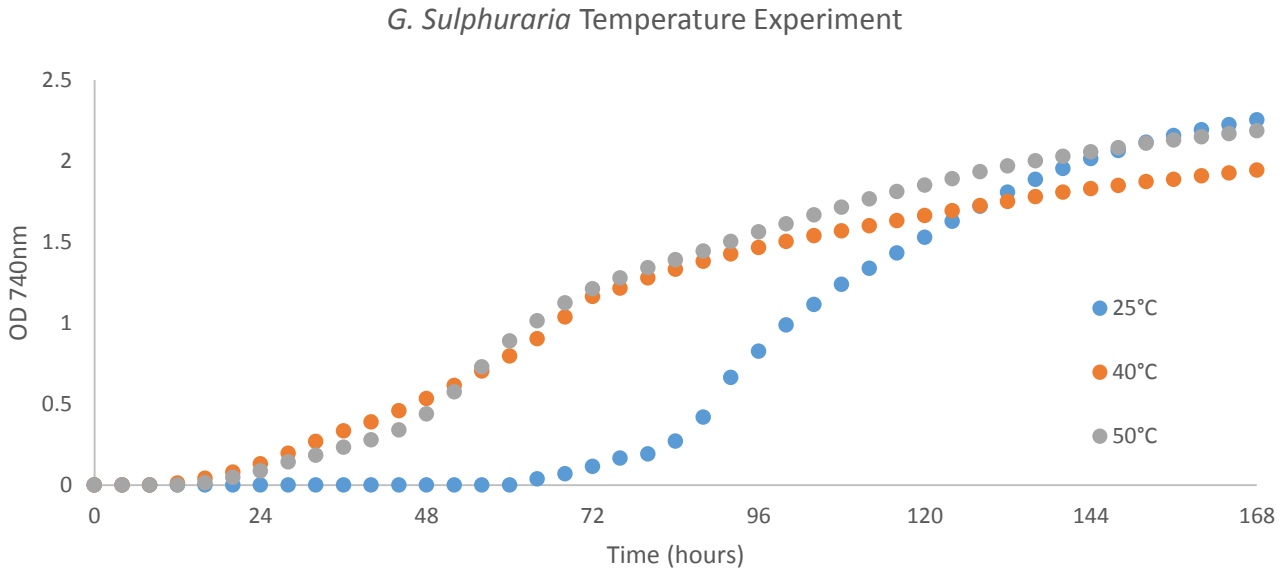

Figure 2 - Growth profile of *G. sulphuraria* cultured mixotrophically in Cyanidium medium with glucose at 100 µmol photons/m²/s continuous light at different temperatures (25, 40 and 50°C) with red: blue light at a ratio of 3:1

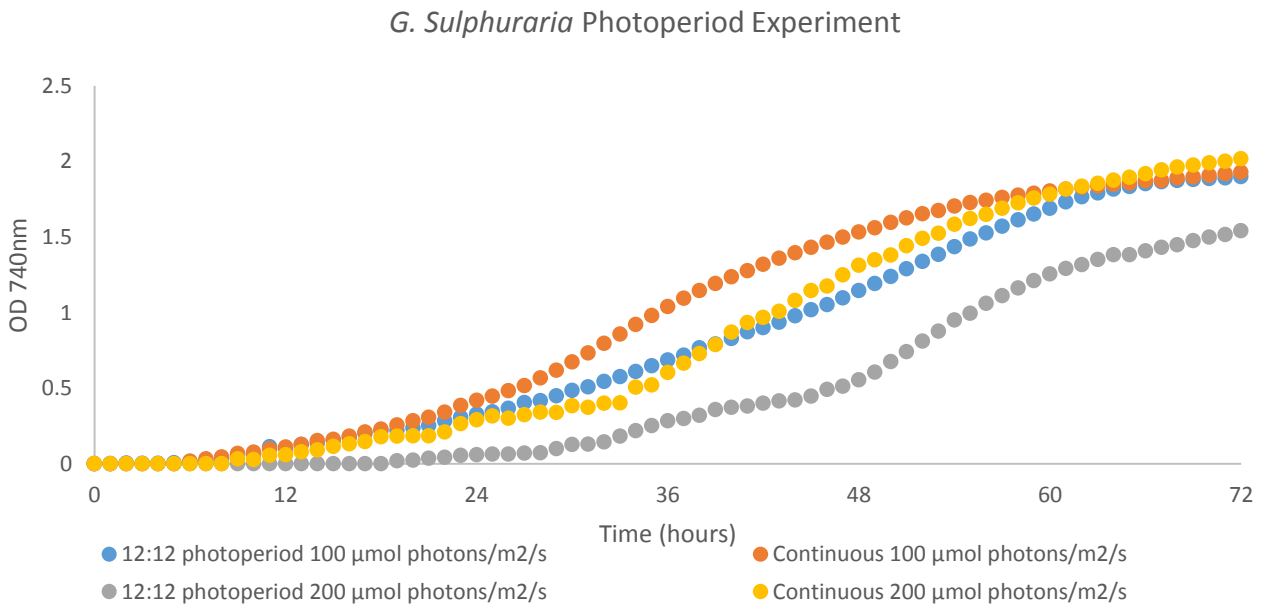

Figure 3 - Growth profile of *G. sulphuraria* cultured mixotrophically in Cyanidium medium with glucose at 50 °C under different photoperiods (12:12 photoperiod and continuous) and light intensities (100 µmol photons/m²/s and 200 µmol photons with red: blue light at 3:1)

Notes

G. sulphuraria was observed to grow optimally at 50°C with a similar growth profile at 40°C but a slightly lower final OD_{740} nm maximum (Figure 2). There was a long lag when *G. sulphuraria* was cultured at 25°C (Figure 2). *G. sulphuraria* appears to not just be thermotolerant but thermophilic and acidophilic. *G. sulphuraria* grew better under the lower light intensity of 100 µmol photons/m²/s with similar growth patterns under a 12:12 photoperiod and continuous light under the conditions tested (Figure 3). A 12:12 photoperiod under 200 µmol photons/m²/s resulted in the poorest growth. Future experiments should focus on culturing *G. sulphuraria* at high temperatures comparing photoautrophic media with heterotrophic conditions where *G. sulphuraria* has been observed to have a higher doubling time (Graziani et al., 2013).

References

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