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An Experimental Study of the Growth and Hydrogen Production of *C. Reinhardtii*

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Abstract

Some unicellular green algae, such as *C. reinhardtii*, have the ability to photosynthetically produce molecular hydrogen under anaerobic conditions. They offer a biological route to renewable, carbon-neutral hydrogen production from two of nature's most plentiful resources – sunlight and water. This process provides the additional benefit of carbon dioxide sequestration and the option of deriving valuable products from algal biomass.

The growth of dense and healthy algal biomass is a prerequisite for efficient hydrogen production. This study investigates the growth of *C. reinhardtii* under different cyclic light regimes and at various continuous light intensities. Algal growth is characterised in terms of the cell count, chlorophyll content and optical density of the culture. The consumption of critical nutrients such as acetate and sulphate is measured by chromatography techniques.

C. reinhardtii wild-type CC-124 strain is analysed in a 3 litre tubular flow photobioreactor featuring a large surface-to-volume ratio and excellent light penetration through the culture. Key parameters of the hydrogen production process are continuously monitored and controlled; these include pH, pO_2, optical density, temperature, agitation and light intensity. Gas phase hydrogen production is determined by mass spectrometry.

1 C. Reinhardtii Growth under Different Light Regimes

1.1 Strains and growth conditions

C.reinhardtii wild-type strain CC-124 (*Chlamydomonas* stock centre) was grown in Tris Acetate Phosphate (TAP) media [1] at 25^{o}C. Cultures were agitated by magnetic stirrer and illuminated with 28 Wm^{-2} (140 $\mu Em^{-2}s^{-1}$) cool fluorescent light or grown in the dark.

1.2 Calculation of cell number and chlorophyll content

Cell samples were stained with iodine and counted using an improved neubauer hemocytometer[1]. Chlorophyll *a* and *b* content was determined spectrophotometrically in 100% Methanol according to Porra *et al* [2].

1.3 Acetate analysis

Cells were pelleted and 100 µl of supernatant analysed on an Aminex HPX-87H anion exchange column according to Mus *et al* [3].

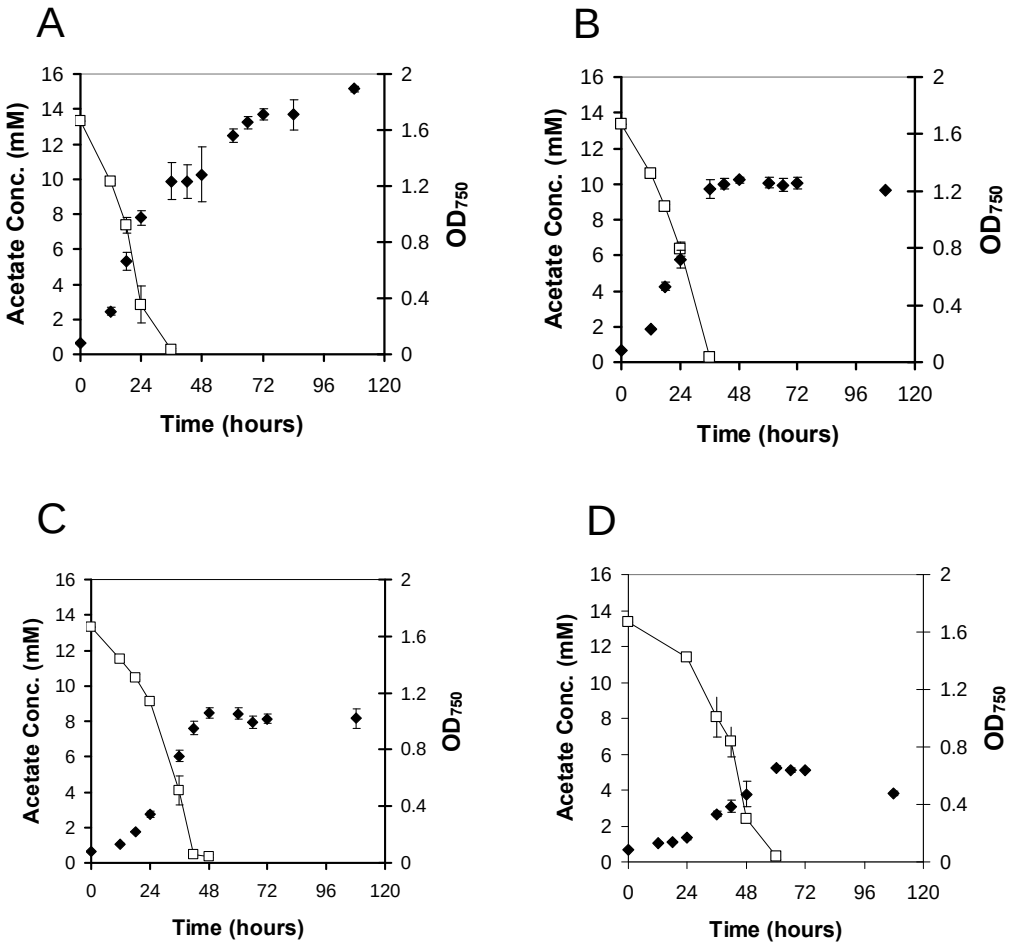

Figure 1: ***C. reinhardtii*** **CC-124 growth under varying environmental conditions; OD_{750} (♦) and acetate (□) concentration, (A) under continuous illumination bubbled with 5% CO_2 air mixture, (B) under continuous illumination, (C) during 12h:12h dark-light cycles, and (D) in the dark.**

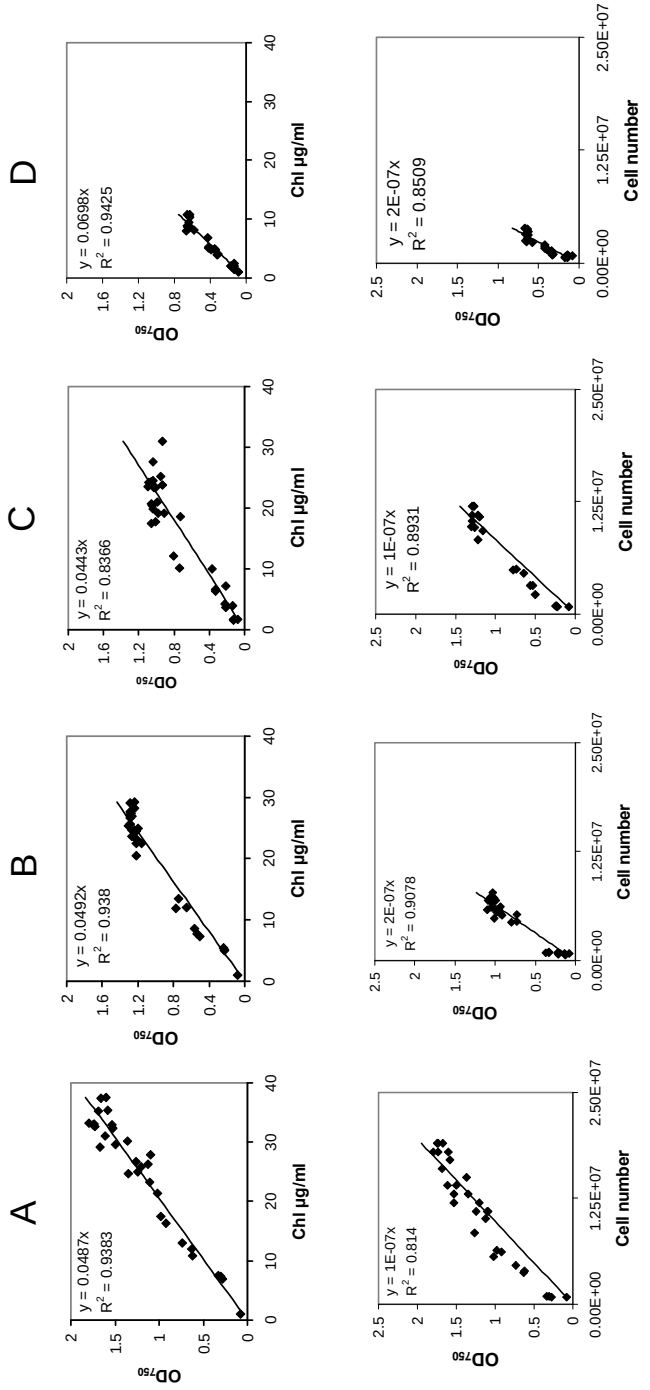

Figure 2: Analysis of the correlation between growth phase optical density (OD_{750}), chlorophyll content and cell number (cell counts were stopped upon reaching stationary phase as clumping of cells was observed) for *C. reinhardtii* CC-124 under varying environmental conditions; (A) under continuous illumination bubbled with 5% CO_2 air mixture, (B) under continuous illumination, (C) during 12h:12h dark-light cycles, and (D) in the dark; there was a good correlation between OD_{750} and Chl content or cell number independent of growth phase.

2 Growth of C. Reinhardtii

Under ambient conditions, the cell density of *C. reinhardtii* increases exponentially with a doubling time of approximately 6 h [1]. Our data (Figure 1) gives doubling times of:

3.6±0.5 h	under continuous illumination
2.8±0.2 h	under continuous illumination bubbled with 5% CO2 air mixture
5.1±0.5 h	during 12h:12h dark-light cycles
15.7±0.8 h	in the dark

In photobioreactors, such as our 3 litre tubular-flow reactor, algal cell density is principally limited by the light penetration through the culture and the availability of key nutrients (carbon, nitrogen, sulphur and phosphate) [4].

We have measured that the *C. reinhardtii* wild-type CC-124 growth rate increases significantly with an increase in light intensity (Figure 3a). Additionally, the maximum attainable optical density at very low light intensities (4 Wm^{-2}) is approximately 15% lower than at 12 Wm^{-2} or 20 Wm^{-2}. Higher light intensities ensure that more algal cells can receive sufficient illumination to facilitate photosynthetic growth, and the culture therefore grows thicker.

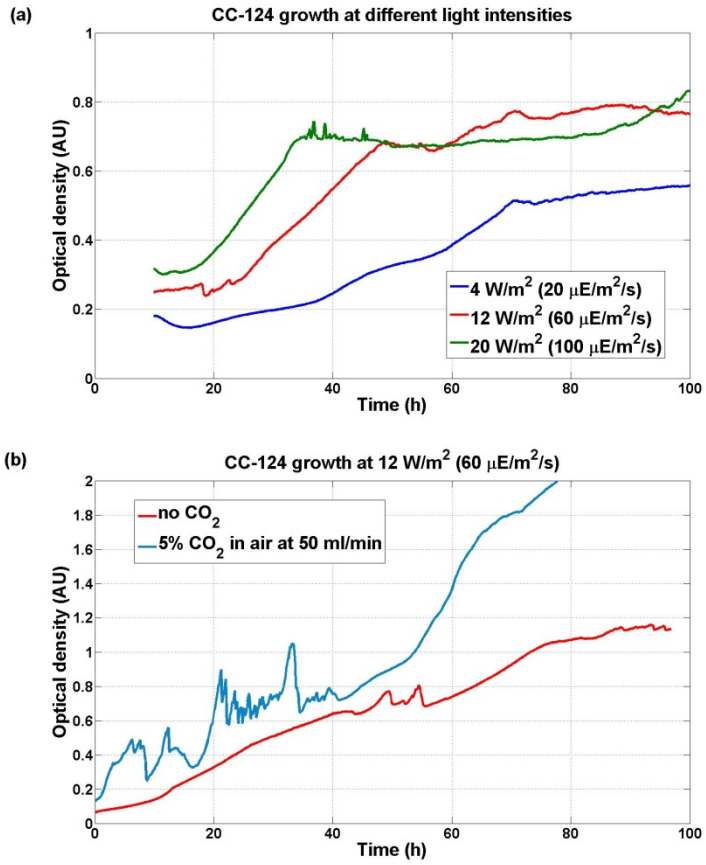

Figure 3: ***C. reinhardtii*** **wild-type strain CC-124 growth, measured in terms of the culture optical density at 875 nm, as a function of (a) light intensity, and (b) the presence of CO_2 sparging.**

C. reinhardtii may be grown using an organic source of carbon such as acetate (photoheterotrophic growth), an inorganic source such as CO_2 (photoautotrophic growth – not studied) or using a combination of acetate and CO_2 (photomixotrophic growth) [5]. Our results show that mixotrophic growth leads to an increase in CC-124 growth rate, and a near-doubling of the maximum attainable OD (Figure 3b).

3 H_2 Production by *C. Reinhardtii*

C. reinhardtii has the ability to photoproduce H_2 under the anaerobic conditions imposed by sulphur deprivation. The dilution method of sulphur deprivation developed by Laurinavichene *et al* is a procedure well suited to laboratory scale H_2 production [6]. A small volume (typically 10% v/v) of growing *C. reinhardtii* culture is diluted in sulphur-deprived TAP medium. The algae continue to photosynthesise and grow as long as they have access to sulphur. Once the sulphur runs out, the algae will use up all oxygen in the system by respiration and eventually enter the phase of anaerobic H_2 production.

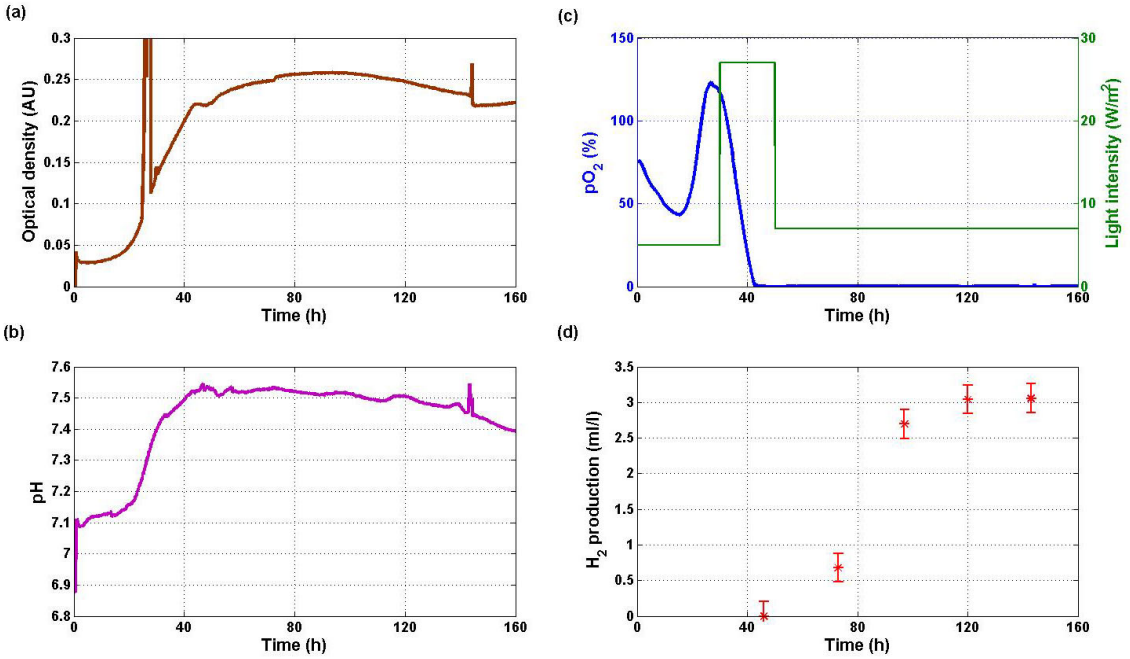

Figure 4: Analysis of the H_2 production process in CC-124, featuring continuous *in situ* measurement of the (a) optical density at 875nm, (b) pH, and (c) dissolved oxygen content (pO_2); light intensity is adjusted to the optimal values required for algal growth, sulphur-deprivation and (d) anaerobic H_2 production phases.

Key parameters of the H_2 production process, such as optical density, pH, light intensity and pO_2 were measured continuously and *in situ* (Figure 4). The optical density initially rose during the algal grow phase, but subsequently fell during the H_2 production phase because some biomass was used up by catabolic processes (Figure 4a). The pH value behaved similarly to optical density (Figure 4b). Light intensity was set at 5 Wm^{-2} during algal growth, 27 Wm^{-2} during sulphur deprivation and 7 Wm^{-2} during H_2 production, as recommended by

Kosourov *et al* [7]. Anaerobic conditions were attained approximately 42h after dilution (Figure 4c). H_2 production was maintained for a period of approximately 5 days (Figure 4d). The total H_2 yield was 3.1±0.2 ml/l of culture. This corresponds to a photochemical efficiency in the order of 0.1%.

Acknowledgements

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