# Enhancing starch production of the green microalga *Chlamydomonas reinhardtii* through sub-lethal temperature treatment

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The current study investigates the effect of sub-lethal temperature on biomass and starch production by the green microalga *Chlamydomonas reinhardtii* both in laboratory and pilot-scale.

In the laboratory-scale experiments, cultures of *C. reinhardtii* (21 gr) were synchronized by light/dark regime (14/10 light/dark cycle). During the experiments, the cells were grown is HS medium at control (30°C) and sub-lethal temperature (39°C) under a constant incident light intensity of 500 $\mu mol.m^{-2}.s^{-1}$.

In the control, cell division occurred at the $15^{th}$ hour of the cell cycle and was preceded by an increase and was followed by a rapid decrease in starch content. At the sub-lethal temperature, starch accumulation was faster than in the control and cell division was blocked. The cultures cultivated at 39°C exhibited significant increase in starch content (up to 90% of dry weight).

The pilot-scale experiments were performed with non-synchronous cultures of the same strain and natural sunlight as a source of light energy. A control cultivation was performed comparing the effect of temperature on starch productivity at 30°C and 39°C. Moreover, to investigate the optimal culture density, the cultures were cultivated at three different starting concentrations in flat panel bioreactors.

The control experiments confirmed the results from the laboratory-scale experiments with cells cultivated at 39°C exhibiting inhibition of cell division resulting in accumulation of high starch levels. The culture density experiments showed that starch production is proportional to the light availability per cell.

These results suggest that sub-lethal temperature affects the cell cycle in *C. reinhardtii*, leading to the complete inhibition of cell division and resulting in enhanced accumulation of starch. The pilot scale experiments showed that this process can be upscaled and used for industrial production of starch. However cultivation conditions and bioreactor design still need to be optimized.

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