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Physiological Studies to Optimize Growth of the Prototype Biosolar Cell Factory Synechocystis sp. PCC 6803

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Summary

The oxygenic photoautotroph *Synechocystis* sp. PCC6803 is a present-day cyanobacterium, a lineage with billions of years of history. Its ancestors first evolved the ability to perform oxygenic photosynthesis: using sunlight to extract electrons from water to form sugars out of CO_2. This unbounded success has seen them spread all over Earth, including in the chloroplasts of algae and plants. They are responsible for the high percentage of oxygen in the atmosphere in the so-called Great Oxygenation Event that shapes our current climate.

The cyanobacteria are extensively researched organisms, of which *Synechocystis* is a well-characterized model organism for various types of research including fundamental research of photosynthesis itself. Recently, in an effort to stop climate change and move society to a sustainable future, *Synechocystis* and other photoautotrophic microalgae are being engineered to produce fuels and other chemicals directly from CO_2. This requires a thorough understanding of the inner workings of the organism in order to meet demand, now and in the future.

The work presented in this thesis aimed to increase our understanding of metabolism and regulation thereof in *Synechocystis*. To this end, state of the art photobioreactors were used to precisely control and measure all parameters of growth.

Chapter 1 is a review of the current state of achieving a bio-based society with the use of microalgae. One of the biggest challenges remains to efficiently grow microalgae in high density mass-cultures. Wild type microalgae thrive on minimal light intensities and evolved methods of dissipating energy when it is in excess in order to deprive their competition of light. In an industrial setting, this leads to excess light energy causing damage at the outer layers while the innermost layer remains in darkness. In the chapter, we discuss the strategies proposed so far to overcome this challenge with a special emphasis on mass-culturing technology and optimizing strains for use in such large-scale facilities.

In **Chapter 2**, we explore the limitations of growth of *Synechocystis* by investigating the commonly used growth medium, BG-11, and growth conditions. We present an improved medium suited for prolonged cultivation, BG-11-PC, that dissolves the precipitation issues that plague BG-11 in continuous cultivation systems. We show that, unexpectedly, it is the sulfate concentration that limits growth in batch cultivation and causes entry into the stationary phase. Importantly, sulfate limitation was shown to cause rapid bleaching and death of *Synechocystis* which is linked to the formation of reactive oxygen species.
By applying these results, we report on high growth rates of up to 4.1 h doubling time that can be sustained in photobioreactors operated in chemostat mode. This represents a considerable increase in the maximum growth rate from the traditionally considered maximum of 7-8 h doubling time for continuous growth.

Chapter 3 and **4** show the results of a recently developed technique to analyze time-resolved fluorescence emission spectra *in vivo* in *Synechocystis*. This technique allows the total spectrum to be decomposed into the spectra and concentrations of the individual

fluorescing species. Using this technique, we show that in a mutant of *Synechocystis* that lacks photosystem I (PSI), an unknown quencher acts on photosystem II (PSII) in an oxygen-dependent way when subjected to a dark-to-light transition. Interestingly, the amount of time spent in the dark prior to the transition has a considerable effect on the timing of the quenching observed. The obtained data were used to create a minimal model to describe the system.

We expand on this in **Chapter 4** and extend the application of the method to a *Synechocystis* mutant that lacks a functioning type I NDH complex as well as the wild type itself. The data obtained in this chapter lead us to conclude that the quencher constitutes a pool of alternative electron sinks that bypasses the PQ pool. We propose that this oxygen-dependent quencher may be the flavodiiron proteins Flv4/2. Furthermore, we present evidence for the PSII-PSI-PBS megacomplex that has recently been postulated to exist.

Chapters 5, **6** and **7** delve into the circadian clock of *Synechocystis*. The clock is an essential mechanism for many organisms to keep the time in order to deal with fluctuating presence of light/nutrients and to separate mutually incompatible processes in time. In **Chapter 5**, the clock was definitively shown to be present in *Synechocystis* and is surprisingly robust. The highly controlled and stable growth conditions as a result of **Chapter 2** were used to show sustained oscillations without measurable damping for weeks. Importantly, the clock was mainly found to have an impact on growth rate, which varied periodically to much greater extent than cellular composition.

This was further explored in **Chapter 6**, which dissects proteomics, transcriptomics and metabolomics in a large investigative study. *Synechocystis* was subjected to a diel cycle of 12 h dark / 12 h light in a low-oxygen environment to characterize its physiology. We show that growth rate is a function of time in the light and completely halts in the dark. The dry weight is remarkably constant throughout the day while the composition varies. Glycogen is assimilated during the day and is slowly consumed during the night until shortly before light when it is rapidly degraded. The very slow consumption, despite fermentative conditions, indicates that the maintenance requirements are extremely low.

In **Chapter 7**, we explore the physiology of *Synechocystis* mutants lacking the core clock components. Deletion of the *kaiAB1C1* operon led to the complete absence of circadian rhythms. Interestingly, this mutant displayed a severe impaired growth phenotype in the photobioreactors, even in continuous light. However, this phenotype could be rescued by what appears to be a suppressor mutation, the identity of which remains to be elucidated.

In an effort to construct a so-called 'hourglass' clock, which requires sequential periods of light/dark to reset the clock every day, *kaiB1* was deleted without disrupting the remaining genes of the operon. This mutant displayed circadian rhythms in day/night, but not in continuous light. The growth rate of this mutant is reversibly decreased in continuous light. The putative additional *kai* genes present in *Synechocystis*, i.e. *kaiC2B2*, *kaiB3* and *kaiC3* could not rescue the wild type phenotype of either mutant, indicating they are not essential to clock function.

Finally, **Chapter 8** puts the results in a broader context and discusses the implications for future research.