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Physiological and Genetic Studies towards Biofuel Production in Cyanobacteria

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Summary of the thesis:

Physiological and genetic studies towards biofuel production in cyanobacteria

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The main aim of this thesis was to contribute to the optimization of the cyanobacterial cell factory and to increase the production of cellulose as a biofuel (precursor) via a physiological and a transgenic approach. The research described in the different chapters focusses on studies on photosynthesis for the purpose of improving photosynthetic efficiency and on cellulose production by natural or transgenic strains. This resulted in a versatile thesis with one main goal: optimizing solar biofuel production with cyanobacteria.

Chapter 1 provides an overview of the current state of cyanobacterial biofuel research with particular focus on the photanol approach and the production of cellulose as a biofuel. It also contains a detailed description of bacterial cellulose synthesis and regulation.

Chapter 2 introduces a novel technique to determine the *in vivo* redox state of the plastoquinone (PQ) pool. Several aspects of the light reactions of photosynthesis, including the redox state of the PQ pool, are then analyzed. Analysis occurred under different light- and carbon-limitation conditions and under the influence of several chemicals that inhibit photosynthesis at different points in the electron transport chain. Results show that, counterintuitively and in contrast with current theory, reducing conditions, like low carbon availability and illumination with PSII light, lead to an oxidized PQ pool. Concomitantly oxidizing conditions such as PSI illumination and high carbon conditions result in a more reduced PQ pool.

Current theories on PQ pool dynamics are largely based on data acquired with PAM fluorimetry, which measures PSII bound chl *a* fluorescence.

In **chapter 3** a comparative analysis of the PAM fluorimetry parameters and the photosynthetic efficiency based on biomass production and oxygen exchange rates of the cyanobacterium *Synechocystis* sp. PCC 6803 and the green alga *Chlorella sorokiniana* is given. This analysis revealed a large discrepancy between chl *a* fluorescence parameters and photosynthetic efficiency calculations for the cyanobacteria. The strong influence of respiratory electron flow and phycobilisome fluorescence on the chl *a* fluorescence signal of cyanobacteria, which is at the root of this discrepancy, is highlighted by the use of *Synechocystis* respiratory mutants and a mutant which lacks the phycobilisome harvesting antenna entirely.

Chapter 4 describes the different growth phases of a typical cyanobacterial batch culture. These are: A lag phase, an exponential growth phase, the often overlooked light limited linear growth phase and the nutrient limited stationary phase. Analysis of photosynthetic parameters, such as chl *a* fluorescence, PQ pool redox state and the energy transfer distribution from the phycobilisomes, show marked changes in the photo-physiology of *Synechocystis* sp. PCC 6803. These changes include a clear reduction of the PQ pool in the linear growth phase, compared to the exponential phase. The importance and advantages of proper growth phase determination to both fundamental and applied research are discussed.

In **chapter 5** a study is conducted on the optimization of physiological conditions for cellulose production in the natural cellulose producing cyanobacteria *Crinalium epipsammum*. A maximal cellulose content of 40 % was achieved, using a combination of stress conditions. This is twice as high as the cellulose content previously reported for this strain. Additionally, growth of *C. epipsammum* was scaled up in a 300 L photobioreactor. The strain grew well in this device, proving that it is suitable for large scale biofuel production. Pros and cons for using natural strains like *C. epipsammum,* in particular for large scale biofuel production, are discussed.

In **chapter 6** genes for cellulose synthesis were introduced into *Synechocystis* sp. PCC 6803 with the aim of generating a cellulose overproducing strain. Although no cellulose was detected in the transgenic strains using a quantitative enzymatic assay, a change in phenotype indicative of the production of cellulose and its pre-cursor UDP-glucose is described. Also, analysis of fluorescence microscopy images of the different strains stained with the cellulose binding fluorescent dye Calcofluor White further implied low levels of cellulose production. Reasons why high-level cellulose overproduction in *Synechocystis* was not achieved are discussed.

Chapter 7 discusses and combines conclusions and implications raised in the different chapters and places them in a broader context of both each other and of current scientific literature. Chapters 2 and 4 strongly indicate that the redox state of the PQ pool is not responsible for the regulation of energy transfer from the phycobilisomes to the different photosystems, in the processes also known as state transitions. While chapters 5 and 6 raise questions on how best to produce cellulose as a biofuel and whether to use transgenic strains when high-level producing natural strains are available.