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Photobiotechnological Hydrogen Production with Microalgae

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Abstract
Hydrogen derived from water has been identified as one of the most promising sources of clean fuel for the future. However, the viability of a future hydrogen economy critically depends upon the development of efficient, large-scale and sustainable hydrogen production systems. This has drawn attention to certain green algae like *C. reinhardtii*, which have evolved the ability to use solar energy to produce hydrogen from water.

1 Basics

Towards the end of the 1930s Gaffron and co-workers discovered that under certain conditions unicellular green algae are able to produce hydrogen during illumination [1, 2]. Subsequently the hydrogenase enzymes, which catalyze the recombination of protons and electrons to form molecular hydrogen, were shown to have a high specific activity [3, 4]. However, the algal hydrogenase was found to be highly sensitive to oxygen inhibition. In fact, it was not until the ground breaking work of Melis and co-workers in 2000 that this challenge was overcome through the cyclical depletion and repletion of liquid C. reinhardtii cultures with sulfur [5]. The underlying principle is that solar powered hydrogen production by green algae can be divided into two stages (see figure 1):

During the first aerobic stage, the algae cells are cultivated photoautotrophically in order to produce biomass. Thereby, water is splitted into oxygen, electrons and protons. The energy of the photosynthetic charge separation reactions is used to synthesize carbohydrates from carbon dioxide.

Finally, the initiation of a sulfur deprivation leads to anaerobiosis, which induces the subsequent hydrogen production stage. As sulfur is needed for amino acid synthesis, protein synthesis cannot function properly in sulfur depleted medium and therefore degraded proteins cannot be replaced properly. This particularly affects proteins with high turn-over rates such as the D1 protein, a key component of photosystem II (PSII) [6]. Thus in the absence of sulfur, the water splitting reaction of PSII can not be maintained at high levels. Consequently, oxygen production decreases over time. Simultaneously, oxygen consumption via oxidative respiration results in an overall reduction of cellular oxygen concentration and

ultimately leads to the onset of hydrogenase mediated H_2 production [5]. Thus, hydrogenases serve as terminal acceptors for electrons of the photosynthetic water-splitting reaction and electrons from starch degradation as well during anaerobic conditions.

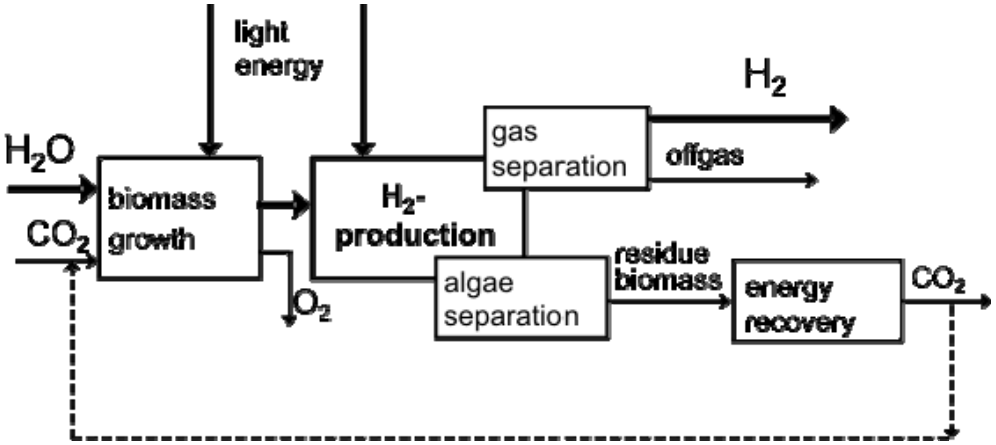

Figure 1: Microalgal hydrogen production – a two-stage process.

2 From Molecular Biology to Bioprocess Engineering

Recently, several micro algae mutant strains related to biohydrogen production have been isolated in different laboratories. One of these is *C. reinhardtii* stm6, which shows increased starch levels and a block in state transitions of the light harvesting complexes resulting in a substantial increase in H_2 production capacity. This strain was calculated to have a photon to hydrogen conversion efficiency of approximately 2% at 20 W/m² in the presence of acetate [7], which is a significant increase compared to wildtype strains. This necessitates the development of appropriate reactor systems and process management strategies to make this process viable also under an economic point of view [8]. In order to generate a data basis for substantiated profitability analyses, precise growth and hydrogen production kinetics are investigated in an ongoing research project [9].

To be able to measure precise phototrophic kinetics, a new reactor system has been developed (see figure 2). This was inevitable due to the fact that light quality and distribution within the photobioreactor is of fundamental importance, as all phototrophic microorganisms need light as an energy source. But due to absorption and shading effects, light intensity drops exponentially from the surface to the centre of the reactor. This results in an uneven light distribution inside the reactor, thus leading to imprecise kinetic data. Due to the special geometry of a new designed LED-lighting system, light is focused to the centre of a 2L-model reactor, which partly compensates the shading effects of the cells and therefore results in an almost homogenous light distribution inside the reactor at moderate cell densities.

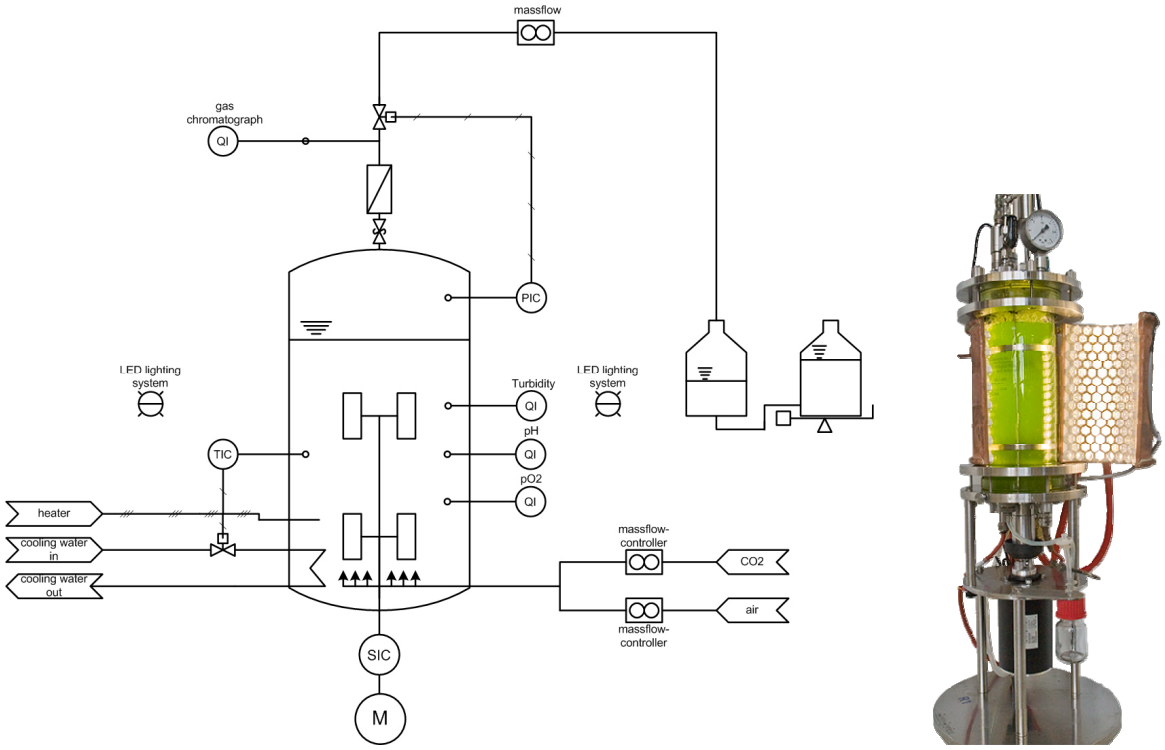

Figure 2: Process flowsheet and photo of the 2L model photobioreactor.

In technical scale, the crucial process step is the establishment of a sulfur deprivation after biomass growth. An exchange of the culture media, as done in laboratory scale, is not economically applicable for large scale. Therefore, two different approaches to initiate hydrogen production in large scale have been developed. The basic underlying principle is to add just as much sulfate as the cells need for biomass growth which leeds to a self-desulfatisation at the end of the first process stage. This can either be realized by a fed-batch or a well balanced batch approach as well. Figure 3 shows exemplarily the transition from biomass growth to the hydrogen production stage via fedbatch approach.

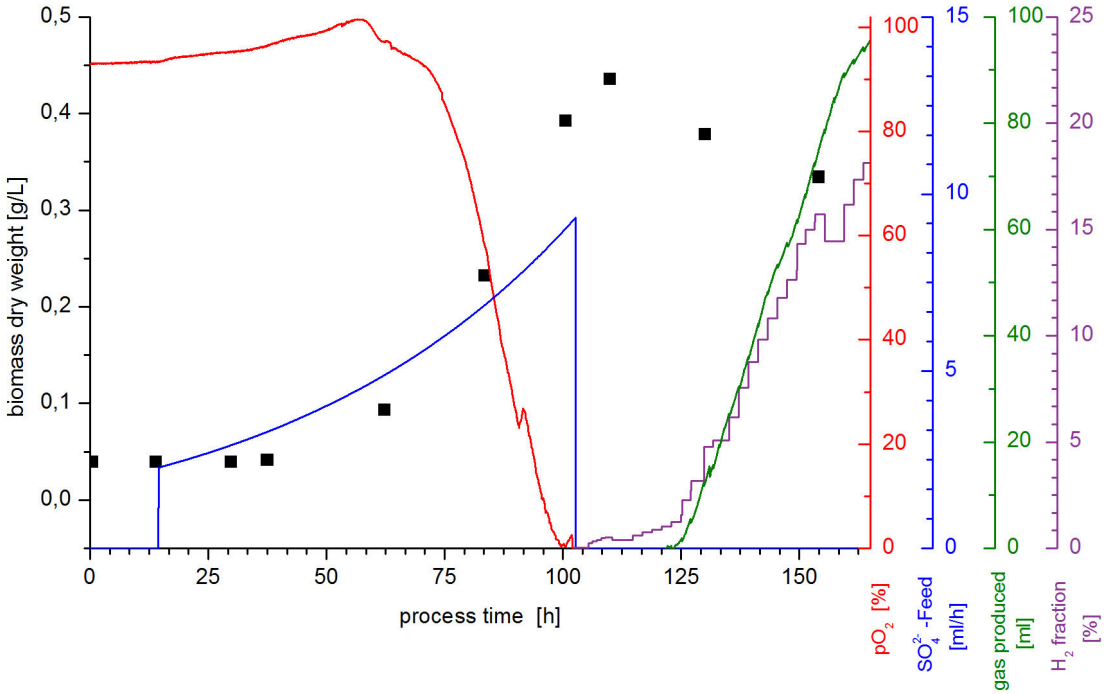

Figure 3: Transition from biomass growth to hydrogen production stage via fed-batch approach.

As can be seen in figure 3, without having sulfate in the basic nutrient solution, biomass growth is clearly controlled by the exponential sulfate-feed. Feeding parameters were adjusted to achieve minimum free sulfate concentration in the culture medium, resulting in an immediate sulfate limitation with stopping the feed flux (see approx. t=100 h, figure 3). Due to this sulfate limiting conditions, the dissolved oxygen concentration (pO_2) of the culture drops continuously and reaches zero with the ending of the sulfate feeding. After a short transition phase, gas production starts with increasing hydrogen fraction of total gas volume (produced gas plus residual gas of the reactor system). These results clearly show that hydrogen production can be induced without expensive exchange of the culture medium.

Similar results were obtained by applying a sulfate-limited batch approach, in which the sulfate amount of the nutrient solution was calculated precisely in order to yield a given biomass concentration. Thus, a self-desulfatisation during biomass growth has been achieved (see figure 4).

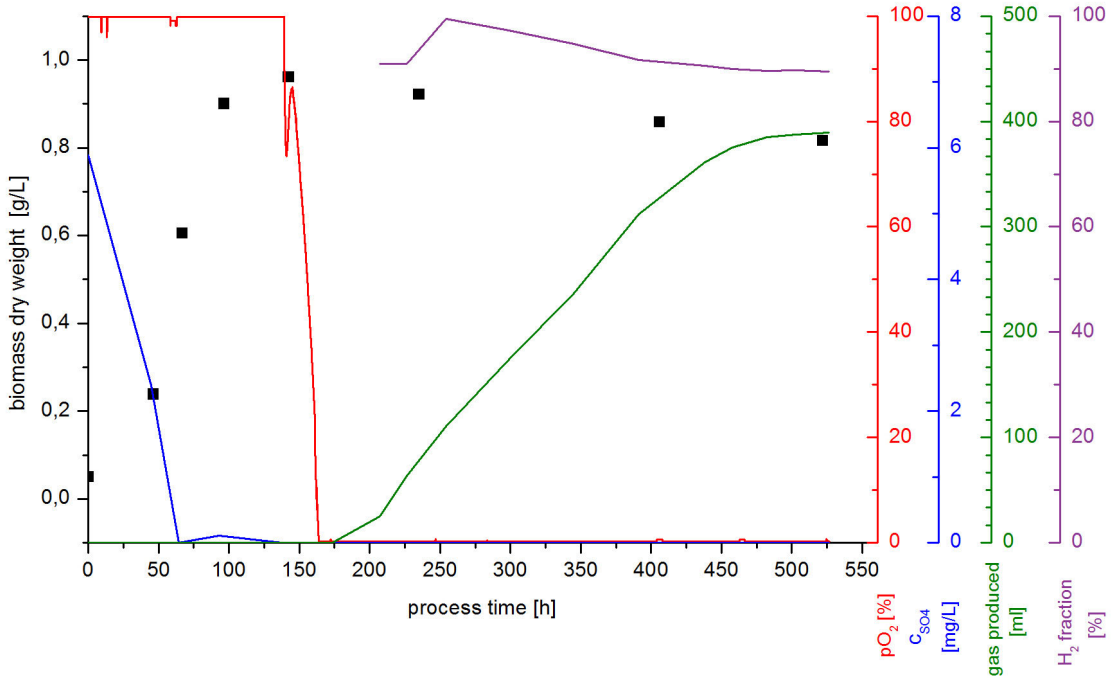

Figure 4: Sulfate-limited batch cultivation of *C. reinhardtii* with subsequent hydrogen production.

With depleting sulfate concentration, biomass growth stops and anoxia is reached. This leads to the transition to the hydrogen production stage, which lasts approximately 300 hours with a hydrogen fraction of the produced gas around 90%.

This new experimental setup is currently used to investigate the hydrogen production kinetics as a function of irradiation. Together with already measured growths kinetics (data not shown), this should lead to precise data sets for an economical assessment of the process. Therefore, these data sets can be fed into mathematical models to evaluate different geographical locations concerning biomass and hydrogen yield on the basis of solar radiation and temperature distribution.

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