



# A Three-Step Microbial Hydrogen-Producing System - First Results -

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## Introduction

The photobiological way of hydrogen production has been the subject of research at the Department of Bionics and Evolutionstechnik at the Technical University Berlin for 15 years. The aim of the study is to produce hydrogen symbiotically, with green algae for carbohydrate production and the well-known purple nonsulfur bacteria for hydrogen production. For an increased system efficiency a third organism - lactic acid bacteria - is used to transform the carbohydrates produced by the green algae into lactic acid - a substance which can easily be metabolised and used as a substrate for hydrogen production by means of purple bacteria.

## Material and Methods

The green algae (*Chlamydomonas segnis*, No. 52.72, Culture Collection of Algae at the University of Göttingen) were cultivated at 30°C on a growing medium described by Koch-Schwessinger<sup>1,1</sup>, illuminated by neon light (300W/m<sup>2</sup>).

The lactic acid bacteria (*Lactobacillus amylophilus*, No. 20533 German Collection of Microorganisms) were cultivated at 30°C on a standard MRS growing medium in the dark (see also [2]).

The purple bacteria (isolated wild strain - not classified) were cultivated on a modified Ormerod growing medium described by Koch-Schwessinger<sup>1,1</sup>, illuminated by tungsten lamps (850W/m<sup>2</sup>).

250ml of the green algae suspension was mixed with 250ml of lactic acid bacteria medium to transform the carbohydrates produced into lactic acid. After sixteen days half of the suspension mixture was mixed with 250ml of purple bacteria suspension. The evolving gas was stored in an upended glass cylinder, with a linear scale for volume measurement and distilled water as sealing liquid. The dry mass was determined directly and by determining the extinction at 660nm. The extinction of the sample was measured by means of the LKB Biochrom Ultrospec 4051 spectral photometer.

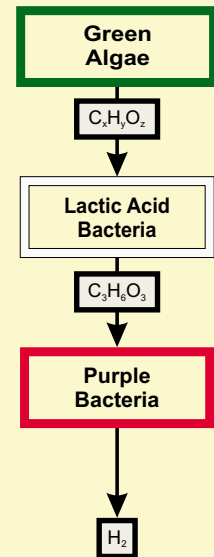


Fig. 1 Flow chart of the three-step microbial hydrogen producing system

## Results & Discussion

After the addition of the lactic acid bacteria no significant increase of the dry mass was found (Fig. 2), whereas the admixing of the purple bacteria

resulted in a considerable increase of the total dry mass.

A tangly slime mould had grown on the inner surface of the reactor bottle. In fact the slime mould peeled off after several days. This resulted in an enormous agglutination of bacterial cells in the suspension. The hydrogen yield of 150ml is certainly increaseable by optimising the growing media used. Especially the production of carbohydrates by green algae can be increased by using a higher nitrogen start concentration<sup>1,1</sup>.

The envisaged concept of a three-step photobioreactor has turned out to be workable. The implementation of the intermediate stage (lactic-acid-bacteria reactor chamber) offers the possibility to transform carbohydrates/organic matter into lactic acid. In addition, it increases the system efficiency and hydrogen yield in organic waste-based hydrogen bioreactors.

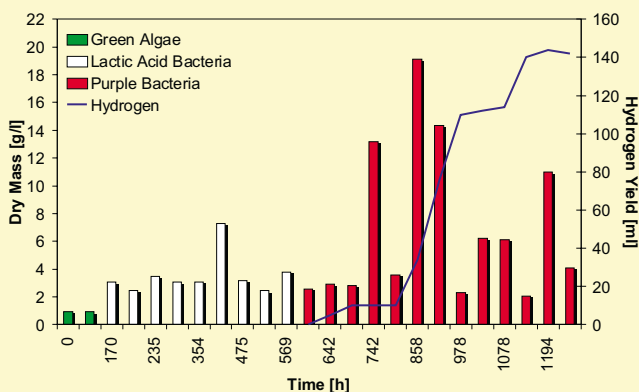


Fig. 2 Dry Mass and Hydrogen Yield - three-step bioreactor

To get more detailed knowledge, the chemical composition of the green algae, lactic acid bacteria, and purple bacteria-suspension was subject to High Performance Liquid Chromatography studies. The results have not been evaluated yet.

We hope in this way to get additional information about an improved metabolic efficiency of the reactor concept below (Fig. 3). Future researches will focus on that. The primary aim is to improve the three-step reactor concept for continuous bench scale application and to come to a workable outdoor photobioreactor for continuous or semi-continuous hydrogen production.

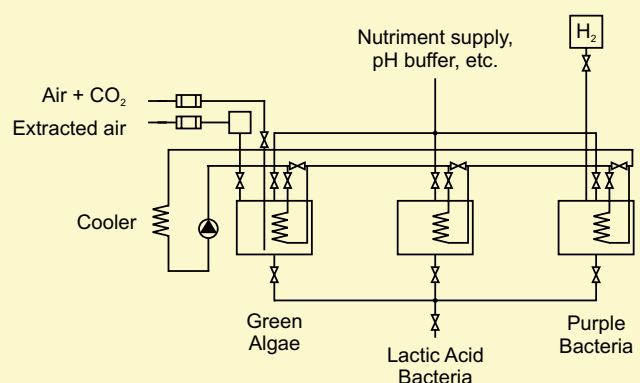


Fig. 3 Concept of a three-step bioreactor for continuous hydrogen production using green algae, lactic acid bacteria and purple bacteria

Literature: [1] Koch-Schwessinger, G., "Wasserstoffproduktion mit Purpurbakterien, frommann-holzboog, 1996

[2] Ike, A., Toda, N., Murakawa, T., Hirata, K., Miyamoto, K., "Hydrogen photoproduction from starch in CO<sub>2</sub>-fixing microalgal biomass by a halotolerant bacterial community", A paper delivered at the International Conference on Biological Hydrogen Production, Wikoia, Hawaii, 1997