

## SUSTAINABLE BIOREACTOR SYSTEMS FOR HYDROGEN PRODUCTION

Richard Rocheleau, Scott Turn, Yasuyuki Nemoto,  
Oskar Zaborsky and JoAnn Radway  
Hawaii Natural Energy Institute,  
School of Ocean and Earth Science and Technology,  
University of Hawaii at Manoa  
Honolulu, HI 96822

### Abstract

The overall goal of Hawaii's BioHydrogen Program is to generate hydrogen from water using solar energy and microalgae under sustainable conditions. Our process for hydrogen production (Hawaii Process) consists of two stages. Stage 1 is the growth of the microalgae; Stage 2 is the production of hydrogen. Since the cells are easily concentrated by screening, we are using filamentous cyanobacteria of the genus *Arthrospira* (*Spirulina*).

Since we examined the characteristics of a bioreactor system for stage 1 last year, this year we studied the activity of hydrogen production in the isolates of *Arthrospira*. It was found that the cells produce hydrogen under anaerobic (100% nitrogen) and dark conditions. This suggests that the hydrogen production is due to reversible hydrogenase. The activity of hydrogen production was ca. 1  $\mu$ mole hydrogen/12 hr/mg dry weight.

Next year, we will combine the data on both stages and evaluate the whole process for its sustainability.

## **Introduction**

Over the past 25 years, advances have been made in the elucidation of the complex physiological, biological, and genetic processes underlying the hydrogen evolution capabilities of microalgae and bacteria. In the area of engineering research and system integration, however, little progress has been made. In fact, no hydrogen-evolving process that showed promise has been demonstrated in the field for any length of time let alone commercialized.

In this regard, the most important is the sustainability of the whole process. We are aiming at building a bioreactor system for hydrogen production that can be operated in a sustainable manner.

## **Objective**

The objective of the work of this year is to examine the hydrogen production activity of *Arthrospira* strains. Also, we made a web page for Hawaiian Culture Collection in this year.

## **Approach**

Our approach for a sustainable bioreactor system is based on a two-stage process that uses but one single organism for producing hydrogen gas. Stage 1 is the growth of the microbe: stage 2 is the generation of the hydrogen gas. The overall process entails several steps; (1) the growth of cyanobacteria in open bioreactors to accumulate carbohydrates using solar energy; (2) the concentration of the algal biomass followed by the anaerobic adaptation in the dark to turn on the biochemical machinery that is able to produce hydrogen; (3) the generation of hydrogen from the stored carbohydrates in the adapted cyanobacteria with solar energy (the second light-dependent step); and (4) the recycling of the carbohydrate-depleted algae to the initial growth bioreactors.

## **Technical Goals**

The goal of this study is to make a sustainable bioreactor for hydrogen production based on the 2-stage process described above. Sustainability is the key point here, and a long-term efficiency should be considered.

Then, we will develop an economically feasible bioreactor for hydrogen production. To do so, it is necessary to use natural seawater with minimum supplements and run the reactor under natural conditions.

## Major Barriers to Meeting Technical Goals

The overall energy consumption for running the bioreactor should be as low as possible. So, we have to examine how much energy is consumed in circulating and maintaining the culture in the tubular bioreactor, harvesting the cells from the bioreactor for the hydrogen production in Step 2, and getting hydrogen gas from the Step 2 culture, and so on.

## Past Results

In year 1, we completed the fabrication of a tubular photobioreactor system, and evaluated effects of process operating parameters such as biomass density, recycling time, nighttime oxygen deprivation. It was found that the tubular photobioreactor is working well for the growth of the microbe at the Stage 1 of the process.

## Current Year Accomplishments and Status

This year, we isolated several strains of Hawaiian *Arthrospira*, and investigated dark H<sub>2</sub> production by them. It was found that isolate C has the highest activity.

Then, we examined the activity of hydrogen production in isolate C in more detail. To do so, the cells of *Arthrospira* isolate C were grown in complete Zarrouk's medium (Hi N; ca. 400 mg/l NO<sub>3</sub>-N) and low-N Zarrouk's medium (Lo N; ca. 5 mg/l NO<sub>3</sub>-N). They were harvested by screening, and then washed and resuspended in N-free Zarrouk's medium. Density of the cell concentrates were ca. 1.5 mg/ml. Reaction flasks contained 1.9 ml of the cell suspension, and were flushed with N<sub>2</sub>. The cell suspensions were preincubated for 2 hr under dark conditions at 32°C at 100 rpm shaking. After 3 hr of incubation, some of the flasks (MV/DT) received reduced methyl viologen (1 mM methyl viologen plus 5 mM sodium dithionite at final conc.), while others received an equal amount of N-free medium.

In either condition, the cells showed a similar activity of hydrogen production (Fig. 1), which is ca. 1 μmole H<sub>2</sub>/12 hr/mg cell dry weight. This value is similar to the data by Aoyama et al. (1997) with *Spirulina platensis*.

As for the culture collection, we continued to maintain and distribute them as well as established on-line database on the collection at <http://www.hawaii.edu/hicc/>.

## Plans for Future Work

In the next year, we will combine the results of the past two years to make a sustainable

bioreactor systems for hydrogen production, and evaluate the efficiency of the whole process of hydrogen production.

### **Acknowledgment**

This material is based on work supported by the Department of Energy, Hydrogen Program, under award no. DE-FC36-97GO10202, originally given to O. R. Zaborsky, initial principal investigator. In FY 1999, PI of this program was switched to Richard Rocheleau, HNEI, Univ. Hawaii. Any opinions, findings and conclusions expressed in this publication are those of the authors and do not necessarily reflect the views of the Department of Energy. We thank Gerald Cysewski, Cyanotech Corporation, for *Arthrospira* sp. starter cultures.

### **References**

Aoyama, K., I. Uemura, J. Miyake, and Y. Asada. 1997. "Fermentative Metabolism to Produce Hydrogen Gas and Organic Compounds in a Cyanobacterium, *Spirulina platensis*." *J. Ferment. Bioeng.*, 83:17-20.

### **Figures**

Fig.1 Dark Hydrogen Production by *Arthrospira* Isolate C. Hi N, cells cultured in complete Zarrouk's medium (ca. 400 mg/l NO<sub>3</sub>-N; Lo N, cells cultured in low-N Zarrouk's medium (ca. 5 mg/l NO<sub>3</sub>-N). MV/DT, cells given reduced methyl viologen (1 mM methyl viologen and 5 mM sodium dithionite).

Fig.1 Dark Hydrogen Production by *Arthrospira* Isolate C

