

**Report from**

Dr.phys. Janis Kleperis, MSc.  
Ilze Dimanta, BSc. Arturs  
Gruduls, MSc. Laimonis  
Jekabsons,  
Hydrogen and Gas Sensors  
Laboratory, Institute of Solid  
State Physics, University of  
Latvia

## Biological production of hydrogen by bacterial anaerobic fermentation

### Introduction

Biological production of hydrogen by bacterial anaerobic fermentation of widely available biomass is a promising and advantageous area. Microorganisms are capable to produce hydrogen via two main pathways: fermentation and photosynthesis. Bacterial hydrogen production by fermentation of carbohydrate-containing substrates is frequently preferred to photolysis, because it does not rely on the availability of light resources. Various combinations of bacteria strains and substrates were explored to obtain highest bio-hydrogen yields in our laboratory. Our scientist group in the Institute of Solid State Physics and Microbiology department of Faculty of Biology is working on bio-hydrogen and biogas production, harvesting, storage and usage technologies. Our goal is to explore possibilities for bio-hydrogen and biogas production using alternative local resources – industrial and agricultural wastes as well as byproducts of food industry, e.g. whey, glycerol, different plant oils, sludge and etc. One of the substrates that can be effectively used for microbial hydrogen production is glycerol, which is a by-product from the process of biodiesel production. Because of large quantities of available crude glycerol and the

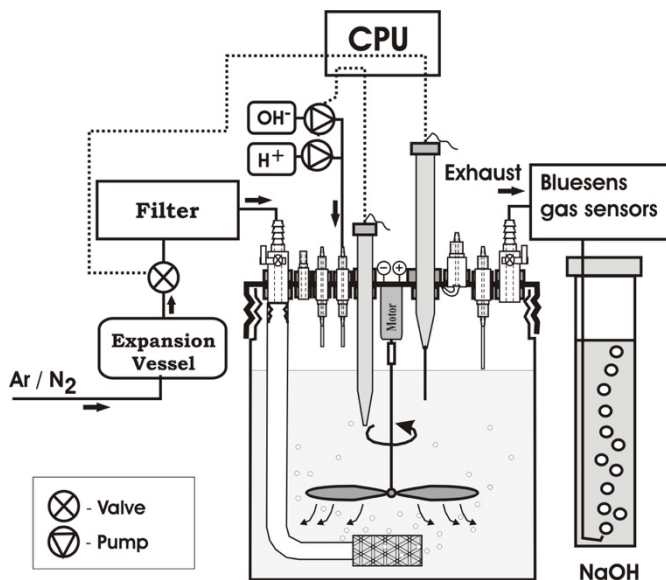
highly reduced nature of carbon in glycerol per se, microbial conversion of it seems to be economically and environmentally viable possibility. Similarly, milk whey due the abundance in dairy production and high concentration of lactose can be considered as a prospective substrate for hydrogen production in Latvia. Representatives from breweries and biodiesel plants already have inquired about opportunities to work with our team. We have tested several strains of bacteria for optimized and efficient bio-hydrogen production. We also have isolated some *wild type* hydrogen producers from nature.

### Methods and materials

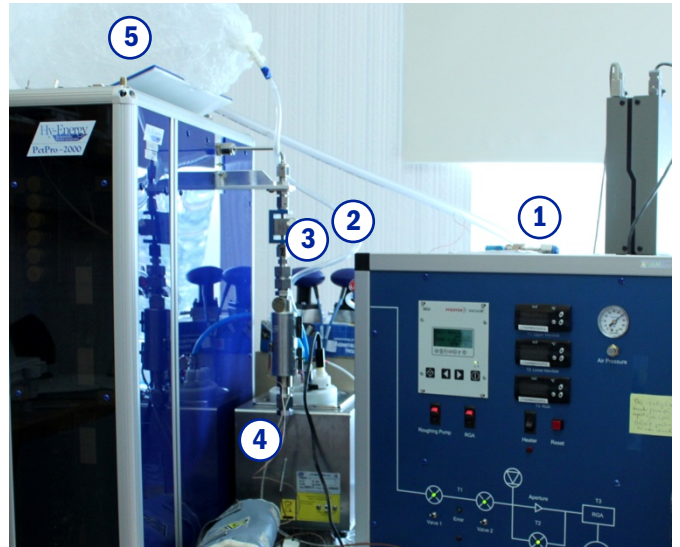
We constructed laboratory scale bioreactor prototype (figure 1.) which is easy to use and was showing good results during experiments. In the measurements we could observe and regulate medium pH, oxygen and hydrogen concentrations in liquid phase, response to environmental temperature change, as well as, it was possible to take different medium and gas samples for analyses during the fermentation process. *BlueSens* gas sensors were used for the exhaust gas analyses the bacterial test-system (CH<sub>4</sub> sensor and H<sub>2</sub> sensor).

Fermentation process efficiency with defined substrates and bacterial strains was estimated, as well as exact concentrations of hydrogen and methane. Several strains of bacteria and combinations of substrates were tested to achieve better gas production yields. Per each experiment 400ml of inoculated medium was used, therefore detectable hydrogen and methane concentrations were achieved. Reactor unit was placed in thermostat with gas exhaust tube leading to outside. *BlueSens* gas sensors analyzed the exhaust gas.

### Experimental set-up drawing and image



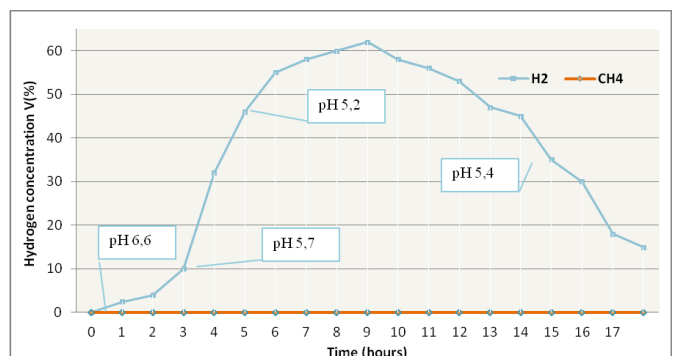
**Fig. 1.** Schematic diagram of constructed bioreactor prototype.



**Fig. 2.** Experimental setup: 1-RGAPro-100 mass-spectrometer, 2-*BlueSens* methane gas sensor, 3-*BlueSens* hydrogen gas sensor, 4-Prototype bioreactor, 5- Argon gas in balloon.

### Results and Conclusions

Measuring the bacterial hydrogen production using glucose as substrate in a prototype bioreactor system with *BlueSens* gas sensors, maximum of 62% hydrogen volume was gained in the 9<sup>th</sup> hour of fermentation. Methane gas concentration remained unchanged – there was no methane evolving in the process (Fig.3). High result of the measured hydrogen concentration has to be further compared with results from massspectrometric analysis.



**Fig. 3.** Hydrogen production measurements in a prototype bioreactor system using glucose as substrate measured with *BlueSens* gas sensors and pH meter (PE-05T Lutron electronics). Different bacterial strains hydrogen production rates were measured using glycerol as substrate and results

are shown in Table 1. *Clostridium sporogenes* had the highest hydrogen production rates and almost all hydrogen transfer from liquid to gaseous state (respectively - 1,5 mmol/L/h in liquid and 1,42 mmol/L/h in gaseous phase. *A. Aneurinilyticus*, *K.ascorbata*, *E. Limosum* didn't show high results in hydrogen production using glycerol fermentation (Table 1).

Table 1. H<sub>2</sub> production rates using different bacterial cultures and glycerol as substrate.

Substrate	Substrate concentration, mmol/L	Bacterial strain	H <sub>2</sub> production rate in liquid phase, mmol/L/h	H <sub>2</sub> production rate in gaseous phase, mmol/L/h
Glycerol	240	<i>A. aneurinilyticus</i>	0,06	0
Glycerol	240	<i>K.ascorbata</i>	0,09	0,04
Glycerol	240	<i>C.sporogenes</i>	1,5	1,42
Glycerol	240	<i>E. limosum</i>	0,07	0,18

#### ACKNOWLEDGEMENTS

We acknowledge BlueSens Company for providing the gas sensing equipment for dating research.