BlueSens

understanding bioprocesses

Application Report

Report from

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Bio-hydrogen production by fermentative bacteria using crude glycerol as substrate in experimental testsystems

Introduction

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 technology research. 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. Industrial and agricultural organic waste used as feedstock for bacteria is a perspective way for alternative energy production and it noticeably decreases the raw material cost. During the conversion of organic wastes, in anaerobic environment, hydrogen or methane gas is produced as by-products. One of the substrates that can be effectively used for microbial hydrogen and methane production is glycerol, which is a by-product from the process of biodiesel production. Because of large quantities available of 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, especially because, over the last several years, the demand and production of biodiesel has remarkably increased [1].

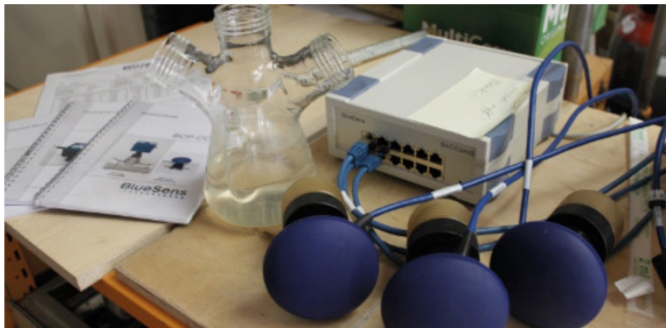


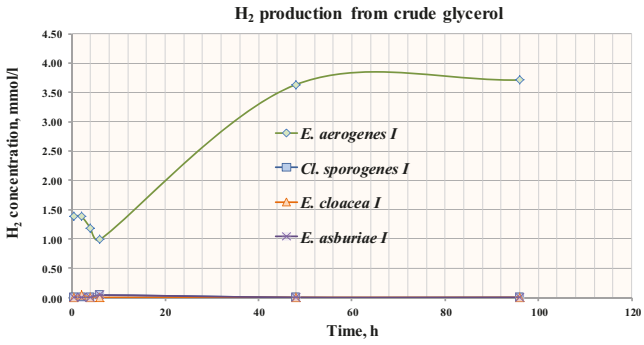
Figure 1

Methods ans materials

Growth media, cultivation and experimental set up

Different anaerobic and facultatively anaerobic bacteria were used for hydrogen production measurements from Microbial Strain Collection of Latvia (MSCL) - Clostridium sporogenes MSCL 764, Enterobacter asburiae MSCL 839, Enterobacter cloacae MSCL 778, Enterobacter aerogenes 758 MSCL. Experiments were continued with the best hydrogen producer using crude glycerol as substrate - Enterobacter aerogenes 758 MSCL. Bacterial cultures were inoculated in 200 ml flasks containing AB medium (2,5 g/L yeast extract, 1g/L tryptone, 12,5g/L glycerol), adapted from Tolvanen et al. (2011) [2]. Strains were cultivated overnight aerobically in shaken flask at 37°C for 12 hours at 150 rpm using a multi-shaker PSU-20 (BioSan, Latvia). Optical density (OD) calibration curve was used to find out number of cells in 1mL of culture [3]. The overnight culture in AB liquid medium was put in a measurement flask sterilized for measurements. The measurement flask was kept in a termostat (Precisterm 2-110, 2L), in order to maintain temperature around 37±2 ˚C. Analytical glycerol (AG) (97%) and crude glycerol (CG) (40% wt/wt, determined with HPLC analysis) from biodisel fuel production was used as substrate, final concentration of glycerol used was 135 mM. Glycerol was autoclaved for 60 min at 121 oC. Argon gas (99.99 % purity) bubbling through the media was used to sustain anaerobic environment. BlueSens gas sensors were used for the exhaust gas analyses the bacterial test-system (H_2, CO_2 and CH_4 sensors). Sensors were calibrated in AB medium using pure hydrogen, carbon dioxide and methane and air and argon gas for zero measurements. The evolved gas was also subsequently injected in the mass-spectrometer RGAPro-100 (HyEnergy, Setaram, France) for hydrogen gas measurement. All cultivation experiments were carried out in three independent repeats.

Figure 2



Substrate	Bacteria	H_2 production rate $_{max}$, mmol/L/h	H_2 production rate $_{average}$, mmol/l/h
CG	*Enterobacter aerogenes*	1.700	1.063
AG		0.831	0.736
CG	*Clostridium sporogenes*	0.019	0.014
AG		1.084	0.546
CG	*Enterobacter cloacea*	0.938	0.260
AG		1.223	0.620
CG	*Enterobacter asburiae*	0.009	0.006
AG		0.013	0.011

Table 1

Results

Representative results from gas production experiments of each type are shown in Table 1 and Figure2. There were no CH_4 gas production in the samples and CO_2 gas was measured only for determination of fermentation process and results are not displayed in the report. When comparing analytical glycerol and crude glycerol as hydrogen fermentation substrates in liquid phase with different anaerobic and facultatively anaerobic bacteria, substantial differences were observed. The highest rates were gained using crude glucose as substrate (H_2 production rate max - 1.700 mmol/L/h) with Enterobacter aerogenes.

Increased rate using crude glycerol (concentration - 40%, comparably analytic glycerol concentration was 97%) must be regarded to different impurities in crude glycerol as biodisel by-product that increase rude glycerol's energy value- 19 MJ/kg (analytical glycerol), 25,3 MJ/kg (crude glycerol) [4].

Acknowledgements

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References

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Hydrogen and Gas Sensors Laboratory:

Research and development of new materials and devices for hydrogen production via electrolysis, using direct current and short voltage pulses;

Development of technology for bio-hydrogen production in anaerobic fermentation process; elaboration and research of hydrogen separation membranes;
Development of new materials for hydrogen storage in metal hydrides and composites;
Development and research of proton exchange membranes and electrodes for fuel cells, rechargeable MH/Ni and Lithium batteries and their applications in electric – hydrogen vehicles;
Research on gas sensors and sensor arrays with application for specific tasks (searching the rapid diagnostic methods to detect lung diseases for patients and wheat diseases).