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This document appeared in

Detlef Stolten, Thomas Grube (Eds.):
18th World Hydrogen Energy Conference 2010 - WHEC 2010
Parallel Sessions Book 2: Hydrogen Production Technologies – Part 1
Proceedings of the WHEC, May 16.-21. 2010, Essen
Schriften des Forschungszentrums Jülich / Energy & Environment, Vol. 78-2
Institute of Energy Research - Fuel Cells (IEF-3)
Forschungszentrum Jülich GmbH, Zentralbibliothek, Verlag, 2010
ISBN: 978-3-89336-652-1

Hydrogen Generation from Waste Glycerol in Dark Fermentation Process

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1 Introduction

Recently, the international activities in the area of sustained world concentrate basically on three topics, strictly related to the energetic problems: more efficient use of fossil fuels, increase if the renewable sources of energy and elimination of the harms caused by human activities in the field of energy. One of the proposals is based on an application of biofuels as energy carrier – generated in transesterification process of plant oils. The easy access, regenerativity, low concentration of sulfur compounds and high biodegradability favour this process. The main disadvantage can be related with high viscosity of the biodiesel, emission of nitrogen oxides and generation of tremendous amounts of contaminated glycerol as a by-product [1] during production. Glycerol contaminated with potassium, sodium hydroxides and methanol and not complexly transformed methanol, represents almost 25 vol.% of total amount of the produced fuel. Although there are many industries in which glycerol can be applied, the amount generated in transesterification is still extremely high and is a challenge both for academia and industry [2].

Among different alternative fuels which can be used in future, the biofuels and hydrogen are most frequently cited. High energetic value (33 Wh/g) of hydrogen is as twice as big as of this for methane (14.2 Wh/g) [3]. Moreover, burning of hydrogen both chemically or electrochemically always leads towards harmless water.

Hydrogen, often called the fuels of XXI century, becomes very attractive alternative to direct solar energy, wind and geothermal energy. Hydrogen can produced on different ways: microbiological belongs to one of the most promising methods. In fermentation process the anaerobic bacteria of e.g. *Clostridium, Enterobacter* or *Bacillus* transforms simple organic compounds into hydrogen, carbon dioxide and light volatile organic acids [4]. Organic wastes of different origin can be applied as the substrates for this reaction, including non-purified glycerol fraction [5,6]. The efficiency of hydrogen generation depends on different factors, among the most important are: pH [7], concentration of products [8], substrates [9] and participation of the municipal sludge. During dark fermentation hydrogen and CO_2 are the main gaseous products, whereas the main liquid metabolites are 1,3–propanediol, 2,3-butanediol, butyric acid and ethanol [10]. Isolated metabolites such as 1,3-propanediol can be applied in production of polyurethanes or polyesters [11], excellent solvents, plasticizers, artificial silk or fragrances and dyes [12].

The main goal of this paper was focused on the possibility of application of waste glycerol for hydrogen production in "dark" fermentation process,. It was established that waste glycerol is a good candidate for this purposes. Established optimization conditions: pH and the best glycerol concentration allows now for expanding this research in larger scale. It was also established the liquid metabolites, especially 1,3 –propanediol can be isolated from studied bacterial systems.

2 Materials and Methods

2.1 Inoculum

Anaerobic digested sludge received from municipal purification facility was boiled for 15 minutes and kept frozen at -20°C. In all experiments the sludge was defrosted and kept at 20°C for 4 hours a day before inoculation.

2.2 Methods and procedures

All experiments were performed in continuous stirred tank reactor L-1523 from Bioengineering A.G. (max. capacity – 7 litres, 200 rpm) with continuously controlled temperature (37°C) and pH. The nutrient solution was composed of: $NaHCO_3$ (1g), NH_4Cl (0,5g), KH_2PO_4 (0,25g), K_2HPO_4 (0,25g), $MgSO_4{\cdot}7H_2O$ (0,32g), $FeCl_3$ (50mg), $NiSO_4$ (32mg), $CaCl_2$ (50mg), $Na_2B_4O_7 \cdot H_2O$ (7mg), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (14mg) $ZnCl_2$ (23mg), $CoCl_2 \cdot 6H_2O$ (21mg), $CuCl_2 \cdot 2H_2O$ (10mg), $MnCl_2 \cdot 4H_2O$ (10mg). All given amounts are expressed per litre [13]. Working capacity was three litres but volume of inoculum was in the range of 10%. Systems with constant pH – 4,5; 5,0; 5,5; 6,0; 6,5, were stabilized and this values were kept till the end of the process. Concentration of this waste glycerol in medium was 23, 47, or 70 g/l.

2.3 Analysis

The cumulated amount of generated biogas containing only CO_2 and H_2 was measured volumetrically under atmospheric pressure. The composition of evolved gases was measured by gas chromatography (GC 3800 from Varian equipped with TCD detector) applying capillary column Carboplot P7. Liquid metabolites: butyric acid (HBu), acetic acid (HAc) and 1,3-propanediol (1,3-PD) were extracted from medium applying ethyl ether and analyzed with gas chromatography (GC 3900 from Varian equipped with FID detector) applying capillary column WF-WAXms. Lactic acid content was determined with colorimetric method [14].

3 Results and Discussion

Literature data and our earlier research [15] indicated that pure glycerol can be used as the substrate in dark fermentation process. With inoculum (10 vol.%) and concentration of glycerol equal 30 g glycerol/l medium and pH= 5.5 the highest amount of fermentative hydrogen was observed (almost 2l H_2/l medium). This prompted us to study hydrogen generation from waste glycerol. In the experiments with waste glycerol, pH was 5.5 and inoculum close to 10 vol.%. The highest volume of generated hydrogen (5.7 l) was found in bioreactor (working volume 1.5 l) containing equivalent of 47 ml of pure glycerol per litre (see Figure 1). Under these conditions system indicated the shortest lag time. At higher concentration of glycerol (eg.70 ml/l) no increase of evolved hydrogen was observed. This was explained by so called substrate inhibition [16].

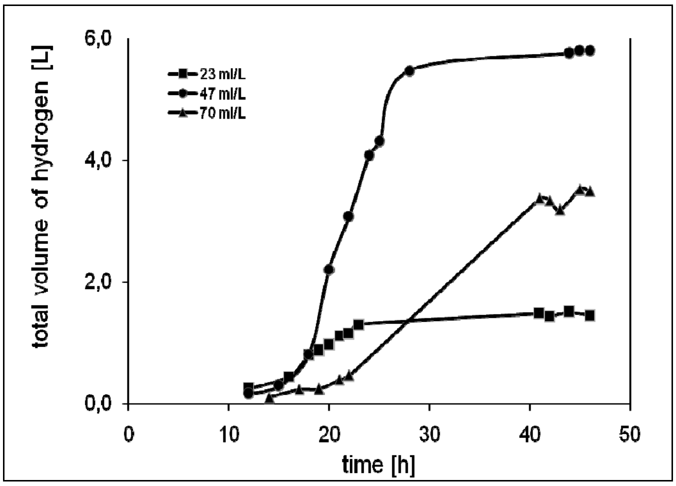

Figure 1: Hydrogen generation with medium containing different amounts of waste glycerol (pH=5.5, 10%vol. of inoculum).

The influence of initial pH (4.5-6.5) on the yield of hydrogen production was the next step of performed research. It was found the pH = 5.5 provide the best conditions for hydrogen biogeneration (see Figure 2). Our earlier studies with pure glycerol indicated that pH of 5.5 represents the best value for the fermentative generation of hydrogen [17].

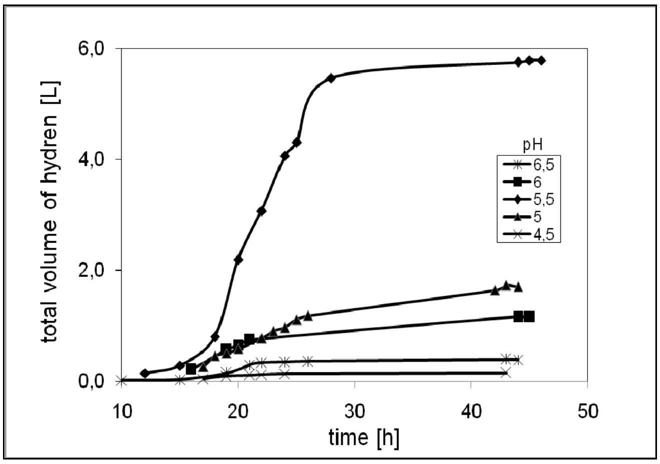

Figure 2: Hydrogen generation with medium containing waste glycerol (47ml/l, 10%vol. of inoculum) at different pH.

The qualitative and quantitative analysis of liquid metabolites showed that among liquid products of the reaction the highest concentration was reached for 1,3 – PD (Figure 3). This is the main metabolite in bacterial decomposition of glycerol. Other organic metabolites with certain practical use, when appropriately separated, are acetic acid (HAc), butyric acid (HBu) and lactic acid (HLac) [18]. The best concentration of 1,3 –PD (9.8 g/l) was found for the system producing the highest amount of generated hydrogen. Simultaneously, butyric acid produced in the same system reached the largest concentration (4.01 g/l). Both results are in agreement with glycerol biochemical pathway described by Papanikolau et al. [19].

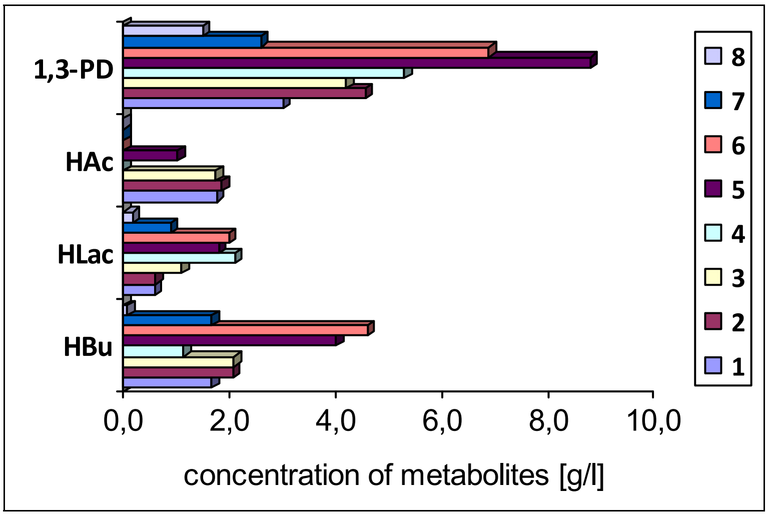

Figure 3: Concentration of 1,3-propanediol (1,3-PD), lactic(HLac), acetic (HAc) and butyric (HBu) acids in medium containing 10%vol. of inoculum, different pH and waste glycerol (1,2 - pH= 6.5; 3, - pH=6.0; 4,5,6 - pH=5.5; 7 - pH=5.0; 8 - pH=4.5; waste glycerol: 2,6 - 70ml/l; 1, 3, 5, 7,8 - 47ml/l; 4 - 23ml/l).

There are two ways of glycerol transformations: one in which mainly 1,3-PD is generated, and another one in which HBu and HAc are formed. Hydrogen generation accompanies both processes. Although both processes compete, the amount of generated hydrogen is independent from the chosen pathway. Theoretical speculations predict that large amount of HAc is strictly connected with large amount of hydrogen (1 mole of HAc is equivalent to 4 moles of H_2). Therefore, one could expect large amounts of acetic acid with high productivity of hydrogen. The lowered values for generated hydrogen show that metabolic pathway is directed to 1,3-PD rather than to HAc. The amounts of HLac are comparable with HAc.

Presented results show that waste glycerol can be used in fermentative hydrogen generation and to best conditions for large quantities of H_2 are obtained with systems operating at pH = 5.5 with 47 ml of glycerol per litre of medium. Moreover, fermentative glycerol decomposition is directed towards 1,3–PD formation. Appropriate separation technique can utilize the described processes towards microbiological production of 1,3–PD from wastewaters.

Acknowledgements

The financial support by the Ministry of Science and Higher Education (MNSiW – grant N204 031 32/0793) is gratefully acknowledged.

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