Pretreatments Testing of High Biodiversity Inocula with Simultaneous Biohydrogen Production and Wastewater Treatment

VASILE DANIEL GHERMAN[1], PAUL MOLNAR[1], MARILENA MOTOC[2], ADINA NEGREA[1]*

[1]Politehnica University of Timisoara, Hydrotechnical Engineering Departmen, 2 Victoriei Sq., 300006, Timisoara, Romania

[2]Victor Babes University of Medicine and Pharmacy Timisoara, 2 Eftimie Murgu Sq., 300041 Timisoara, Romania

Hydrogen represents a renewable energy resource and it is an ideal alternative to fossil fuels since it does not contribute to the greenhouse effect. In the present study it has been proposed to develop an experimental model to test and compare fermentative capacity of waste water with biohydrogen production for two high biodiversity heterotrophic microbial inocula. All the pretreatment methods tested yielded good results, but heat and acid pretreatment had the best results. These observations open the way for the development and application of new technologies using microbial consortia specially developed to serve the dual role of biological waste water treatment and the production of a renewable energy in the form of biohydrogen.

Keywords: biohydrogen, biofuels, biodiversity, pretreatment, dark fermentation

A large number of microorganisms are metabolically capable to converting different types of biomass into various energy-rich substances that can be used by humans as alternative sources of energy. The microorganisms can converted biomass into bioethanol, biogas or biohydrogen and produced more interest to industry in recent decades.

Hydrogen generates no pollutants, unlike ethanol for example, whose large scale use is predicted to release large amounts of carcinogenic acetaldehyde with the generation of large amounts of smog. Hydrogen has the highest energy of any known fuel and can be transported for domestic/industrial consumption through conventional means [1-3]. H_2 gas is representing a renewable energy resource and it is an ideal alternative to fossil fuels since it does not contribute to the greenhouse effect [4-6]. Hydrogen combustion produces only water being one of the cleanest forms of energy.

H_2 production by biological method using bio-photolysis, photofermentation and heterotrophic dark fermentation processes or a combination of these processes. Photofermentation process transform solar energy into hydrogen by photosynthetic bacteria but this method has a low utilization because is not efficient process and are many difficulties in designing of photoreactors [7, 17]. Hydrogen production by dark fermentation process is widely used because it treat organic wastes and produce clean energy.

In natural environments rich in organic maters there are many microbes capable of hydrogen production [9-10]. In last years, it has been found that inoculum biodiversity greatly influences hydrogen production and using mixed microbial cultures as starting inocula are more efficient than pure cultures. High biodiversity of inocula is important because complex microbial communities can degrade a larger range of substrates. However, in complex microbial communities, there are also a number of microorganisms that consume the hydrogen produced (methanogens) [17-19]. For good yields in hydrogen production process, the microorganisms that consume the hydrogen must be removed by different types of inocula pretreatment (acid, heat, ultrasonication) [11].

In the present study it has been proposed to develop an experimental model to test and compare fermentative capacity of waste water with biohydrogen production for two high biodiversity heterotrophic microbial inocula.

Experimental part

Bioreactor design and operation

In order to develop a biohydrogen production model following wastewater degradation, it is necessary to overcome a major impediment to this process, namely the very low biohydrogen production rate.

The rate of biohydrogen production is governed by a range of physical, chemical and biological parameters such as: the hydrogen producing and consuming bacteria development, substrate used, inorganic nutrients, operating conditions [7]. Thus, the microorganism's inoculation and the optimization of the operating parameters, especially the nutritional and environmental ones, are a decisive aspect for the proper functioning of the process.

Our experimental model was performed using two parallel 15L bioreactors. Metabolic processes were monitored by hydrogen and *p*H sensors and gas chromatography (fig. 1.).

Fig. 1. Experimental model.

For better control of process parameters, synthetic wastewater was used in the experimental model. Synthetic wastewater composition was: (mg/L) glucose 3700, K_2HPO_4 250, $FeCl_3$ 25, NH_4Cl 500, $MgCl_2$ $6H_2O$ 300, $NiSO_4$ 16, $CoCl_2$ 25, $ZnCl_2$ 11.5, $CuCl_2$ 10.5, $CaCl_2$ 5 and $MnCl_2$ 15. The *p*H was adjusted to 6 using 1 N HCl.

For increase the contact surface area of the microbial populations a porous ceramics support material was introduced into the bioreactors. The temperature in the

* email: adina.negrea@upt.ro, Phone: +40 256 404192

bioreactors was ensured by electrical metal rods that were heated, the temperature being adjustable.

Seed inocula

In this experimental model were tested two microbial inocula taken from two ecosystems rich in organic matter. The sampling media of these microbial consortia are: Inoculum 1: -Active sludge from the wastewater denitrification stage of the Timisoara wastewater treatment plant; Innocent 2: -Sludge taken from Danube Delta, very rich in organic substances.

These ecosystems have a high biodiversity of microorganisms, capable of degrading a wide range of organic compounds.

Before inoculating the experimental energy conversion model, these microbial consortia were subjected to the pretreatment and cultivation processes on the DMI enrichment liquid medium. These pretreatments consisted of high heat (C), acid (A), ultrasonication (U) and a combination of all these pretreatments (CAU). Also, for each type of inoculum used, there were control experiments where microbial inocula were not subjected to pretreatment (M).

Results and discussions

Figures 2 and 3 show the results for hydrogen production using the two inocula with different types of pretreatments.

A similar biohydrogen production rate is observed for the two inocula used in synthetic waste water degradation processes. In the case of Inoculum I, biohydrogen production is more gradual and stabilized and the control (M), without pretreatment, keeps the hydrogen production rate over time to some extent. However, a good stabilization of the rate of biohydrogen production is observed at Day 9 for inoculum II. The best biohydrogen production was recorded using acid and heat pre-treatment for both inocula. The biohydrogen concentration in the biogas produced was about 29%.

Throughout synthetic waste water anaerobic fermentation, the average glucose concentration was recorded using high performance liquid chromatography (fig. 4, fig. 5.).

The initial glucose concentration in synthetic waste water was 3 g / L. This was the main source of energy for the microorganisms involved in the biohydrogen producing process. Acid and heat pretreatment where recorded a higher consumption of glucose and with a high biohydrogen production. As expected, the glucose consumption (organic substrate) is directly correlated with biohydrogen production rate (fig. 6). Thus, there are microbial communities capable of rapid and complete degradation of the organic substrate with simultaneous biohydrogen production.

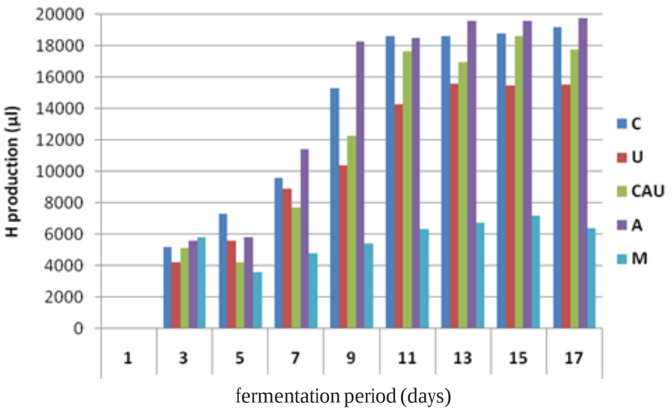

Fig. 2. Biohydrogen production after degradation of synthetic waste water using Inoculum I (sludge treatment plant Timi°oara) (M - control, A - pretreatment with acid, C - pretreatment with heat, U - pretreatment by ultrasonication and CAU - use of all pretreatments)

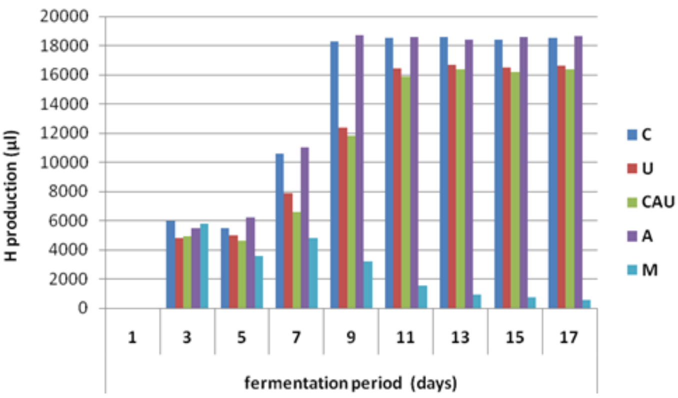

Fig. 3. Biohydrogen production after degradation of synthetic waste water using Inoculum II (Danube Delta sludge) (M - control, A - pretreatment with acid, C - pretreatment with heat, U - pretreatment by ultrasonication and CAU - use of all pretreatments)

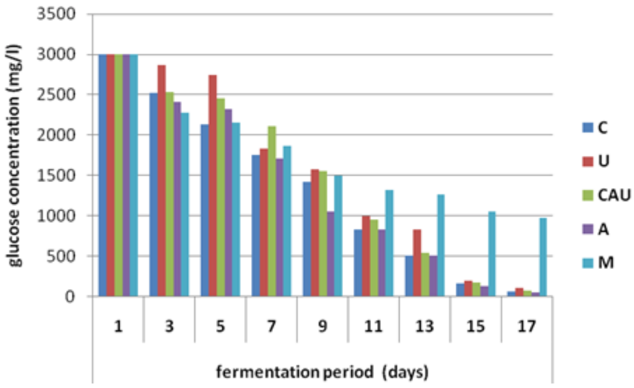

Fig. 4. Evolution of the glucose concentration (mg / L) from synthetic waste water during the fermentation period, using the Inoculum I (sludge treatment plant Timisoara) (M- control, A- pretreatment with acid, C- heat pretreatment, U - pretreatment by ultrasonication and CAU - use of all pretreatments)

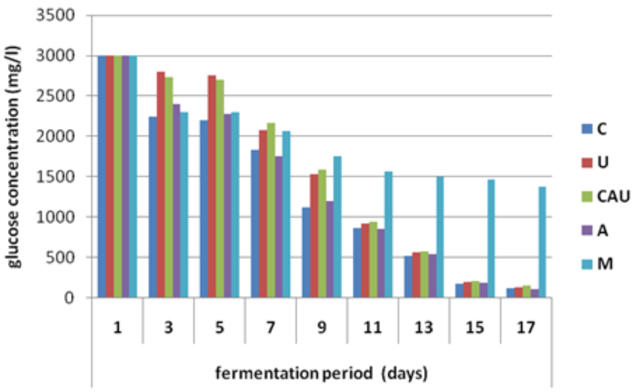

Fig. 5. Evolution of the glucose concentration (mg / L) from synthetic waste water during the fermentation period, using the Inoculum II (Danube Delta sludge) (M- control, A- pretreatment with acid, C- heat pretreatment, U - pretreatment by ultrasonication and CAU - use of all pretreatments).

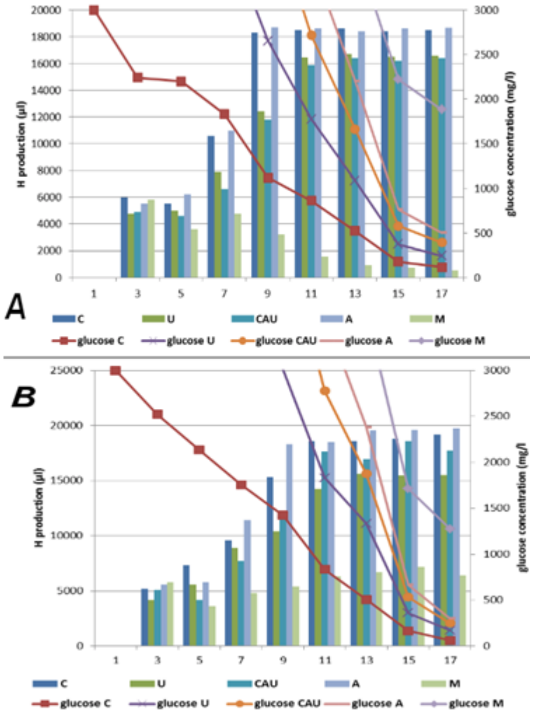

Fig. 6. Biohydrogen production rate in parallel with glucose degradation: A. Inoculum II (Danube Delta sludge); B. Inoculum I (sludge treatment plant Timisoara)

Conclusions

Following the fermentation tests in the experimental model consisting of two parallel 15 L bioreactors, both microbial inocula had a good rate of biohydrogen production. In both cases, production gradually increased in the first nine days, after which it stabilized relatively well. All the pretreatment methods tested yielded good results, but heat and acid pretreatment had the best results. These observations open the way for the development and application of new technologies using microbial consortia specially developed to serve the dual role of biological waste water treatment and the production of a renewable energy in the form of biohydrogen.

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