INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2013) 1–9

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/he

Simultaneous biohydrogen production and wastewater treatment based on the selective enrichment of the fermentation ecosystem

Iulian Zoltan Boboescu [a], Vasile Daniel Gherman [a], Ion Mirel [a], Bernadett Pap [b], Roland Tengölics [c], Gábor Rákhely [c,d], Kornél L. Kovács [c,d], Éva Kondorosi [d], Gergely Maróti [a,d,*]

[a] "Politehnica" University of Timisoara, Hydrotechnical Engineering Dept., Timisoara, Romania
[b] Seqomics Biotechnology Ltd., Szeged, Hungary
[c] University of Szeged, Dept. of Biotechnology, Szeged, Hungary
[d] Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary

ARTICLE INFO

Article history:
Received 30 January 2013
Accepted 30 August 2013
Available online xxx

Keywords:
Biohydrogen
Pretreatment
Microbial consortia
Synthetic wastewater

ABSTRACT

Biohydrogen production from synthetic wastewater as substrate was studied in anaerobic small scale batch reactors. Enriched anaerobic mixed consortia sampled from various environments were used as parent inocula to start the bioreactors. Selective enrichments were achieved by various physical and chemical pretreatments and changes in the microbial communities were monitored by metagenomic and molecular diagnostics approaches. Experimental data showed the feasibility of biohydrogen production using synthetic wastewater as substrate. The hydrogen generation capability of the different mixed consortia is clearly dependent on the pretreatment methods. The described approach opens the possibility for an alternative way towards simultaneous wastewater treatment and renewable energy generation.

1. Introduction

Energy is indispensable in all fields of life. The demand for the energy is permanently growing, but the reserves of our primary energy-carriers will be depleted within a few decades [1,2]. Novel safe energy carriers have to be introduced. Hydrogen satisfies all the requirements for a clean, alternative fuel producing only water as by-product upon combustion. It has the highest energy content per unit weight of any known fuel (142 kJ/g or 61,000 Btu/lb) and can be transported for domestic/industrial consumption through conventional means [3–5]. In addition to this, H_2 gas is safer to handle than domestic natural gas. It can be used directly in the internal combustion engines or in fuel cells to generate electricity. Its use in fuel cells is inherently more efficient than the combustion currently required for the conversion of other potential fuels to mechanical energy [6–8].

* Corresponding author. Hungarian Academy of Sciences, Biological Research Centre, Temesvari krt. 62., Szeged 6726, Hungary. Tel.: +36 308270455.
E-mail addresses: iulian@yahoo.com (I.Z. Boboescu), vasile.gherman@hidro.upt.ro (V.D. Gherman), ionmirel@bell.net (I. Mirel), bernadett.pap@gmail.com (B. Pap), toki_epidot@freemail.hu (R. Tengölics), rakhely@brc.hu (G. Rákhely), kornel@brc.hu (K.L. Kovács), eva.kondorosi@gmail.com (É. Kondorosi), maroti.gergely@brc.mta.hu (G. Maróti).
0360-3199/$ – see front matter Copyright © 2013, Hydrogen Energy Publications, LLC. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijhydene.2013.08.139

Among various hydrogen production processes, biological ways are known to be the least energy intensive (direct photolysis, indirect photolysis, photofermentation and dark fermentation). Dark fermentation process can utilize various organic wastes as substrate for fermentative hydrogen production, thus it is considered a viable biohydrogen evolution method driven by the anaerobic metabolism of the key intermediate, pyruvate. The complete oxidation of glucose would yield a stoichiometry of 12 moles H_2 per mole of glucose but in this case no energy is utilized to support growth and metabolism of the producing organism [9]. Dark H_2 production has the advantages of rapid hydrogen production rate and can be operated at ambient temperature (30–40 °C) and pressure [10,11], although under carefully chosen conditions thermophiles produce up to 60–80% of the theoretical maximum demonstrating that higher hydrogen yields can be reached by extremophiles rather than using mesophilic anaerobes [12].

Reducing the cost of wastewater treatment and finding ways to produce useful products from wastewater has been gaining importance in view of environmental sustainability. One way to reduce the cost of wastewater treatment is to simultaneously generate bioenergy by utilizing the organic matter present in wastewater. Wastewaters generated by various industrial processes are considered to be the ideal substrates because they contain high levels of easily degradable organic material. In the processes established so far, organic pollutants and wastes are converted into methane. Recently, emphasis started to shift to the development of novel anaerobic processes aiming the conversion of organic pollutants into hydrogen, instead of methane [13]. Thus, H_2 production using wastewater as fermentative substrate with simultaneous treatment of wastewater might be an effective way of tapping clean energy from renewable source in a sustainable approach [14].

Bacteria and other microbes capable of hydrogen production widely exist in natural environments such as soil, wastewater sludge, compost, etc. [15–17]. Thus, well selected and concentrated derivatives of these sources can be used as inoculum for fermentative hydrogen production. Dark hydrogen production processes using mixed cultures are more efficient than those using pure cultures, because the formers represent more simple systems to operate and easier to control, and may accept a broader source of feedstock [18]. However, in a fermentative hydrogen production process using mixed cultures, the hydrogen produced by hydrogen-evolving bacteria can be utilized by hydrogen-consuming bacteria. Thus, restriction or termination of the methanogenic process is crucial to render H_2 to an end-product in the metabolic flow [19]. There are pretreatment possibilities to permit selective enrichment of specific groups of parent cultures by inhibiting H_2-consuming methanogenic bacteria [14,20]. Pretreatment also prevents competitive growth and co-existence of further H_2-consuming bacteria [21]. The enrichment methods reported for hydrogen-producing bacteria from mixed cultures mainly include heat-shock, acid and base treatment, aeration, freezing and thawing, chloroform, sodium-2-bromoethanesulfonate or 2-bromoethanesulfonic acid and iodopropane treatments [19,20,22].

In the present study, a two-step biohydrogen production process was investigated using different types of microbial communities as starting inocula. Prior to the inoculation, selective enrichments of the bacterial populations were achieved by various physical and chemical pretreatments. In the first experimental step, a glucose rich environment was applied, while in the second experimental step, defined synthetic wastewater was used as fermentation substrate. The model system was investigated in anaerobic small scale batch reactors. Our aim was to determine the factors involved in the desired shift from the traditional biogas forming communities to an ecosystem favoring hydrogen evolution rather than methane formation. In a more simple way, the specific goal was the elaboration of a method suitable for the selective elimination of methanogenic archaea, therefore suitable for simultaneous biohydrogen production and wastewater treatment.

2. Materials and methods

2.1. Seed inocula

Four different inocula were used during the experimental setups. Samples were taken from: Timisoreana's brewery effluent (S1), Bocsa's natural pool (S2), USAMVBT methane producing bioreactor (S3) and Timisoara's wastewater treatment plant (S4).

2.2. Identifying the optimum pretreatment methods for the inoculum, in relation to the substrate used

In order to enrich the hydrogen producing bacteria, four pretreatment methods plus a control, were used for each of the inoculum. The batch experiments were performed in triplicate. The following pretreatment methods were used: heating of the inoculum at 70 °C for one hour, acid pretreatment bringing the pH down to 3 for 24 h at room temperature using 1 N HCl, ultra-sonication of the samples for 30 min at a discontinues discharge of 24 KHz (0.5 s discharge followed by 0.5 s pause) and a combination of all of the pretreatments.

2.3. Design of synthetic wastewater

Designed synthetic wastewater (SW) [(mg/l) glucose—3700, NH_4Cl—500, KH_2PO_4—250, K_2HPO_4—250, $MgCl_2 \times 6H_2O$—300, $FeCl_3$—25, $NiSO_4$—16, $CoCl_2$—25, $ZnCl_2$—11.5, $CuCl_2$—10.5, $CaCl_2$—5 and $MnCl_2$—15] was used as substrates for H_2 production. The pH was adjusted to 6 using 1 N HCl.

2.4. Enrichment of hydrogen producing bacterial consortia

Enrichment of the sediment samples was done in DMI medium following pretreatment. One liter of the DMI medium contained 5240 mg of NH_4HCO_3, 6720 mg of $NaHCO_3$, 125 mg of K_2HPO_4, 100 mg of $MgCl_2$, 15 mg of $MnSO_4$, 500 mg of Na_2S, 10 mg of $FeSO_4$, 10 mg of resazurin and 17,800 mg of carbon source (glucose) as a substrate in 1 L distilled water. The enrichments were conducted in 30 ml serum vial with 20 ml of DMI medium and 4 ml of pretreated sediment samples as inocula. The bottles were capped with rubber septum stoppers and aluminum rings and the medium in each bottle was

Please cite this article in press as: Boboescu IZ, et al., Simultaneous biohydrogen production and wastewater treatment based on the selective enrichment of the fermentation ecosystem, International Journal of Hydrogen Energy (2013), http://dx.doi.org/10.1016/j.ijhydene.2013.08.139