INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 40 (2015) 9096–9105

Available online at www.sciencedirect.com

ScienceDirect

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

# Hydrogen production by an enriched photoheterotrophic culture using dark fermentation effluent as substrate: Effect of flushing method, bicarbonate addition, and outdoor–indoor conditions

*Virginia Montiel-Corona* [a], *Sergio Revah* [b], *Marcia Morales* [b,*]

[a] *Doctorado en Biotecnología, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, C.P. 09340, México, D.F., Mexico*

[b] *Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, Avenida Vasco de Quiroga 4871, Colonia Santa Fe, Delegación Cuajimalpa de Morelos, C.P. 05348, México, D.F., Mexico*

ARTICLE INFO

*Article history:*
Received 19 December 2014
Received in revised form
7 May 2015
Accepted 11 May 2015

*Keywords:*
Biohydrogen
Photo-fermentation
Reduced pressure
Photoheterotrophic culture
*Rhodobacter capsulatus*
Outdoor

ABSTRACT

This work compares the photo-fermentative hydrogen production by an enriched photoheterotrophic culture (IZT) and by *Rhodobacter capsulatus* under outdoor or indoor conditions using dark fermentation effluent (DFE) as substrate, and argon, $CO_2$ and reduced pressure for flushing the headspace and bicarbonate as electron sink.

The highest $H_2$ production (1478 ± 17 mL $H_2$ $L^{-1}$ with 89% COD removal) was obtained under indoor conditions by the IZT culture with reduced pressure and 250 mg $L^{-1}$ of $NaHCO_3$, followed by *R. capsulatus* (1252 ± 20 mL $H_2$ $L^{-1}$ with 65% COD removal) under the same conditions. Outdoor $H_2$ production was reduced to 883 ± 4 and 866 ± 46 mL $H_2$ $L^{-1}$ with IZT culture and *R. capsulatus*, respectively. Poly-3-hydroxybutyrate accumulation was up to 5% for IZT culture and 29% for *R. capsulatus*.

Enhancement on hydrogen production using reduced pressure and DFE along with the achieved COD removal and poly-3-hydroxybutyrate accumulation can contribute to abate the costs of hydrogen production.



## Introduction

Fermentative hydrogen production from wastes is an alternative to get renewable energy and manage organic waste in an environmentally friendly way. Bio-hydrogen production can be achieved by either dark fermentation with fermentative bacteria or photo-fermentation by purple non-sulfur bacteria (PNSB). Dark fermentation has faster metabolic rates and lower operational requirements [1] but has low $H_2$

* *Corresponding author*. Universidad Autónoma Metropolitana Cuajimalpa, Avenida Vasco de Quiroga 4871, Colonia Santa Fe, Delegación Cuajimalpa de Morelos, C.P. 05348, México, D.F., Mexico. Tel.: +52 55 58 14 65 00; fax: +52 55 58 04 64 07.
E-mail address: mmorales@correo.cua.uam.mx (M. Morales).

http://dx.doi.org/10.1016/j.ijhydene.2015.05.067

yields due to the incomplete degradation of wastes and to the accumulation of acids, limiting the yield to less than 20% of the theoretical value of 12 mol $H_2$ $mol^{-1}$ glucose. The volatile fatty acids (VFAs) accumulated during the dark-fermentation can be further converted to $H_2$ with near stoichiometric efficiency and yields up to 8 mol $H_2$ $mol^{-1}$ glucose can be obtained with two-stage process comprising both dark and photofermentation [1].

Photoheterotrophic hydrogen production by PNSB typically uses pure cultures under sterile conditions [2] with model substrates such as succinate, lactate, butyrate, malate, acetate and propionate [2–4] but recently more complex substrates such as dark fermentation effluents (DFE) of corncob, potato steam peel, cheese whey, molasses, barley straw hydrolysate and others [5–9] have been used. Enriched PNSB cultures obtained from wastewater could be interesting since they can be metabolically more versatile and more effective using complex substrates than pure cultures [2], however, enriched photoheterotrophic cultures with complex substrates have not been broadly used.

Hydrogen production by PNSB is associated with the nitrogenase that promotes the conversion of dinitrogen gas and protons to ammonia and $H_2$. However, nitrogenase is inhibited by oxygen [10,11] and therefore it is necessary to keep anaerobic conditions. Low $H_2$ partial pressure also needs to be maintained in the headspace because hydrogenases (such as NiFe-hydrogenase) may re-oxidize the produced hydrogen into protons and electrons [10]. Argon has been often used to flush both oxygen and nitrogen and to keep low $H_2$ partial pressure in the reactors but it increases production costs and hinders $H_2$ purification. Some reports have alternatively tested reduced pressure and $CO_2$ for flushing the headspace and maintaining low $H_2$ partial pressure in dark fermentation [12,13], but in photofermentation the information is scarce.

Photofermentative outdoor $H_2$ production is affected mainly by solar light energy and by temperature. These non-controlled variables regulate photosynthetic activity and therefore daily (day/night cycle), seasonal and geographical variations greatly influence growth and the amount of $H_2$ produced. Therefore it is important to evaluate the effect of such conditions on potential $H_2$ producing cultures.

It is also known that PNSB need an electron acceptor when the substrate is more reduced than cell components. It has been demonstrated that the presence of carbonate ion in PNSB growth media enhances VFAs uptake, since carbonate serves as an excess electron sink for propionic and butyric acid substrates which are more reduced than the cellular components [14,15]. Despite this evidence and that butyric acid is one of the major by-products of dark fermentation, carbonate has not been used to improve photofermentative hydrogen production from DFE rich in butyric or propionic acid.

PNSB can accumulate poly-3-hydroxybutyrate (PHB) as energy storage material when they are faced with suboptimal environments. The amount of PHB that PNSB can accumulate depends on the PNSB strains, substrate and their metabolic pathways. For example, *Rhodobacter sphaeroides* uses the Ethylmalonyl-CoA pathway to assimilate acetate. This pathway shares common elements with that used for PHB biosynthetic pathway enabling *R. sphaeroides* to accumulate high PHB levels (80% of its cell dry weight) [16]. The acetate assimilation pathway in *Rhodobacter capsulatus* is through the citramalate cycle [17]. In *Rhodopseudomonas palustris*, the acetate assimilation goes through the glyoxylate cycle, Wu et al. [18] reported that strain WP3-5 cannot utilize malate and lactate to produce PHB and only synthesizes it on acetate and propionate accumulating 10% PHB. PHB accumulation in PNSB can compete with $H_2$ production for electrons and energy distribution, so it is important to determine PHB accumulation to evaluate hydrogen production loss [18]. As PHB is a valuable biodegradable polymer [17], it may also be recovered as a by-product from the hydrogen production process.

This work explores hydrogen production by both an enriched photoheterotrophic culture and *R. capsulatus* under non-sterile conditions using a real dark fermentation effluent as substrate. Argon, reduced pressure and $CO_2$ were tested to keep anaerobic conditions and lower $H_2$ partial pressure. The use of $NaHCO_3$ was also explored to enhance $H_2$ production. The experiments were performed under indoor conditions with continuous illumination or outdoors with natural light/dark cycles. Chemical oxygen demand (COD) removal and PHB accumulation were also measured to estimate potential benefits such as production of added-value compound and waste management.

## Materials and methods

### *IZT culture and* R. capsulatus

The photoheterotrophic culture was obtained by using a Winogradsky column prepared with activated sludge collected at the wastewater treatment plant "Cerro de la Estrella" located in the San Juan Xalpa, Iztapalapa municipality in México City.

Winogradsky columns contained 800 mL of activated sludge; 12.5 g $CaSO_4$, 12.5 g $CaCO_3$ and 25 g of sawdust were added to a glass cylinder of 4 cm diameter and 1 L capacity. The column was placed next to a window at room temperature (25–28 °C) for 15 days; after that period, a 5 mL sample of the reddish band below the surface of the water column was collected and transferred to 120 mL serological bottles with 85 mL of modified Pfennig medium not containing either sulfur source nor dextrose to avoid proliferation of fermentative or purple sulfur organisms. Argon was used as gas phase to maintain anaerobic conditions during this enrichment stage. These bottles were incubated at 3 kLux in a photoregulated chamber using halogen and LEDS lamps, the temperature was maintained at 30 °C.

A total of six subsequent subcultures were done every two weeks, transferring 5 mL of the reddish culture into 85 mL of fresh mineral medium and incubating it under the above-mentioned conditions. The resulting enriched culture was the photoheterotrophic IZT culture employed in this study.

*R. capsulatus* ATCC 17015 was obtained from the ATCC Bacteriology Collection. It was also cultivated in modified Pfennig medium.