INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2016) 1–10

Available online at www.sciencedirect.com

ScienceDirect

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

Enhanced hydrogen production from palm oil mill effluent using two stage sequential dark and photo fermentation

Puranjan Mishra, Sveta Thakur, Lakhveer Singh, Zularisam Ab Wahid*, Mimi Sakinah*

Faculty of Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Kuantan, Pahang, Malaysia

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 12 July 2016
Accepted 17 July 2016
Available online xxx

Keywords:
Hydrogen
Clostridium butyricum LS2
Dark fermentation
Palm oil mill effluent
Photo-fermentation
Rhodopseudomonas palustris

ABSTRACT

The aim of this study was to investigate the maximum hydrogen yield as well as the chemical oxygen demand (COD) reduction from palm oil mill effluent (POME) by using two stage sequential dark and photo fermentation. The first stage operation was carried out using *Clostridium butyricum* LS2, which has the maximum hydrogen yield of 0.784 ml H_2/ml POME and COD removal of 57%. The dark fermentative effluent was diluted with 50% of tap water (DEPOME-50), for better penetration of light and was subsequently used as substrate to the second stage fermentation using *Rhodopseudomonas palustris* as hydrogen producer. Hydrogen production was monitored under optimized light illumination of 7 klux, in batch mode. The two-stage fermentation enhanced the total hydrogen yield from 0.784 (dark fermentation) to 3.064 ml H_2/ml POME (dark/photo-fermentation). Meanwhile, a 93% of total COD removal was also achieved.

Introduction

In today's era, the global demand for the energy sources has increased significantly and at the same time, the fossil fuel reserves are depleting at an alarming rate. In recent decades, hydrogen has attracted much attention as a potential candidate to meet the desired demand of an eco-friendly and promising energy fuel. This is because hydrogen does not emit greenhouse gases on combustion compare to the energy derived from fossil fuels. Moreover, hydrogen exhibits approximately three times higher energy density compared to hydrocarbon fuels [1]. Several physicochemical methods have been reported for the production of hydrogen from various sources. Amongst these physicochemical methods used for hydrogen production, fermentative hydrogen generation offers a remarkable option by utilizing the wastes from industries and agriculture. In particular, the hydrogen production using two stages or sequential dark/photo-fermentation is better technological route compared to the single stage dark or photo-fermentation process. The preferred choice of the sequential dark/photo-fermentation is due to its dual benefits of high yield of hydrogen production as well as mitigation of environmental pollution.

* Corresponding authors.
E-mail addresses: lakhveer@ump.edu.my (L. Singh), zularisam@ump.edu.my (Z. Ab Wahid).

http://dx.doi.org/10.1016/j.ijhydene.2016.07.138

Currently, the two-stage anaerobic dark and photo-fermentation are foreseen for high energy recovery as well as improved wastewater treatment at industrial scale. Evidently, the two-stage process achieved a maximum hydrogen yield of 28.28 ml H_2/g sucrose and chemical oxygen demand (COD) removal of nearly 90% [2]. Findings of Chong et al. [3], who utilized the *Clostridium butyricum* EB6 for hydrogen production from palm oil mill effluent (POME) using single stage dark fermentation show that, maximum yield of 31.95 ml H_2/g COD was obtained at pH 5.5, 10 g/l glucose and at 37 °C of temperature.

The performance of sequential dark-photo fermentation system can further be enhanced using specific and efficient inoculum for each stage of the fermentation. *Clostridium* sp. has proven to be the efficient mesophilic hydrogen producer during the dark fermentation [4,5] while *Rhodopseudomonas palustris* has the potential to produce hydrogen in photo-fermentation [6–8]. In dark fermentation, *Clostridium* sp. can convert the organic substrates into volatile fatty acids and alcohols, which can further be utilized by *R. palustris* in photo-fermentation stage as substrate. *C. butyricum* LS2, applied in this study, has potential to produce hydrogen with the maximum yield of 350 ml H_2/g $COD_{removed}$ in the continuous fermentation of POME in up-flow anaerobic sludge blanket reactor [4]. *R. palustris* is well known for its hydrogen production ability in photo-fermentation. The *Rhodobacter sphaeroides* NCIMB8253 has been reported to produce a maximum hydrogen yield of 4.670 ml H_2/ml substrate using 25% POME diluted with paper mill effluent [7]. In a different study, *R. palustris* WP3-5 generated a hydrogen yield of 20.24 ml H_2/g sucrose in a photo-bioreactor illuminated with side light optical fibers [2]. Teera et al. reported the maximum hydrogen yield of 0.06 ml H_2/g COD after using crude glycerol and five times diluted dark fermented effluent of glycerol as substrate for dark and photo-fermentation respectively [9]. Moreover, in another study, cassava and food wastes were used for hydrogen production in the two-stage sequential fermentation system. The results of that study showed that a maximum hydrogen yield of 810 ml H_2/g substrate and 671 ml H_2/g substrate was obtained from cassava and food waste, respectively [10]. This demonstration also achieved significant COD removal efficiency of 84.3 and 80.2% from cassava and food waste respectively in batch mode operation [10]. Therefore, the process of sequential dark fermentation and photo-fermentation could produce efficient hydrogen and also promise to be useful in waste treatment.

Malaysia is one of the leading producers of palm oil in the world and, the estimated annual production of POME is about 50 million tons every year [11]. POME also contains a high level of organic acids, carbohydrate, lipids, minerals, and proteins that can serve as growth nutrients for the microorganisms, therefore, is a suitable substrate for the hydrogen production. Besides, the sequential dark-photo fermentation also allows the substantial reduction in COD, makes it suitable to be discharged to the environment. Additionally, many studies have reported that the process of sequential dark- and photo-fermentation achieved higher hydrogen yields from various substrates compared to hydrogen yields by dark fermentation or photo-fermentation alone [12,13]. Along with this, several studies have used different carbon sources for sequential dark and photo-fermentation [7,9,11] but no reports have been found on hydrogen production from POME using sequential dark-photo fermentation system yet.

The present study aims to investigate (i) Two-stage hydrogen production process using POME as substrate and *C. butyricum* LS2, in dark and *R. palustris* under the light as hydrogen producers. (ii) The removal of COD during the two-step hydrogen producing process to address how much waste could be removed through sequential two stage system. (iii) The effect of different light intensities on hydrogen productivity from dark fermented POME.

Materials and methods

Wastewater for sequential fermentation

The raw POME was collected from the final discharge point of Felda palm oil industry, Lepar Hilir, Gambang, Pahang, Malaysia. POME was preserved and refrigerated at 4 °C prior to use in the study, to decrease the biological degradation and acidification. The physiochemical characteristics of POME are given in Table 1.

Dark fermentative bacterial inoculum

C. butyricum LS2, earlier examined in our previous studies, was used as hydrogen producer for dark-hydrogen fermentation [4,11]. The *Clostridium* sp. was cultured in modified reinforced clostridial medium (RCM), its composition was (g/l): $CaCl_2 \cdot 5H_2O$, 0.000125; $CaCl_2 \cdot 2H_2O$, 0.1 Glucose, 4.81; yeast extract 0.3 g; 0.5 g of L-cysteine; $MnSO_4 \cdot 6H_2O$, 0.015; $FeSO_4 \cdot 7H_2O$, 0.025; $CuSO_4 \cdot 5H_2O$, 0.005; $NaHCO_3$, 15; NaCl, 0.717; K_2HPO_4, 0.125; $MgCl_2 \cdot 6H_2O$, 0.1. The initial pH of the medium was adjusted to 5.5 ± 0.2 by adding 1 M HCl or NaOH. The inoculum was grown at 37 ± 1 °C. The 10% of inoculum or 100 ml of active *Clostridium* culture (OD600 ~ 1) was inoculated to the 2 L continuous stirred tank reactor (CSTR) which contains 1 L of fresh POME.

Photo-fermentation bacterial inoculum

R. palustris (1.8929) was purchased from china general microbiological culture collection (CGMCC), China. Further, *R.*

Table 1 – Different characteristic of palm oil mill effluent before and after consecutive fermentation.

Parameter	Initial POME	Dark fermented POME effluent (DEPOME)	Photo fermented POME effluent
BOD	51,510	31,422	8700
COD	49,500	28,500	12,200
pH	6.4	4.0	5.5
Oil	2100	1540	1190
Total solids	36,700	17,000	9650
VSS	13,300	19,000	10,000

Note- All values are given in mg/l except pH. BOD-biological oxygen demand, COD-chemical oxygen demand, VSS-Volatile suspended solids.