

Bio-hydrogen production from food waste by a co-culture of *Clostridium acetobutylicum* and *Rhodobacter sphaeroides*

Fatemeh Rezaei Tavabe^{1*}, Solmaz Saadat², Nasser Talebbeydokhti³

¹Master of Science Student, Department of Civil and Environmental Engineering, Shiraz University, Iran.

²Assistant Professor, Department of Civil and Environmental Engineering, Shiraz University, Iran.

³Professor, Department of Civil and Environmental Engineering, Head of Environmental Research and Sustainable Development Center, Shiraz University, Iran.

Abstract

Bio-hydrogen production by single-step dark and combined dark and photo fermentation of food waste was investigated in batch fermentation. The combined process contained a mixture of *Clostridium acetobutylicum* and *Rhodobacter sphaeroides* with a certain light/dark (1/2) bacteria ratio. In dark fermentation, the cumulative hydrogen production was approximately 14.2 L. In subsequent combined system, the average cumulative hydrogen production was approximately 22.7 L. which was about 1.65 times compared to the amount that produced in dark fermentation only. Meanwhile, the COD decreased with a removal efficiency of 22.5% in hybrid system and 14% in dark fermentation. These results demonstrate that food waste could be ideal substrate for bio-hydrogen production and a hybrid process combining dark-photo seems ideal to achieve dual benefits to hydrogen and waste stabilization.

Keywords: Bio-hydrogen, Co-culture, Dark fermentation, Photo fermentation, Volatile Fatty Acids

1. Introduction

The global energy demand has been increasing with the advancement of new technology and population growth. The main source of energy has been formed from fossil fuels, resources of these fuels have been decreasing. In addition, the combustion of fossil fuels has serious negative effects on environment because of incomplete combustion process and CO₂ emission. To protect the environment from pollution and energy crisis issues, the discovery of new sustainable energy sources are emerging. Hydrogen as a carbon-free fuel with the highest conversion efficiency (an energy yield of 122kJ/g, which is 2.72 times greater than that of fossil fuels) [1, 2] is considered as an alternative energy carrier. Due to increasing the world's energy needs, development of cost-effective and efficient hydrogen production technologies has gained significant attention in recent years.

The most common methods for hydrogen gas production is steam reforming and coal gasification which starts from fossil fuels [3]. Compared with these chemical processes, hydrogen production by fermentation of carbohydrate-rich raw materials has significant advantages due to operation under mild conditions (30–35 °C, 1 atm), while chemical processes require higher energy [4]. In recent years sequential dark and photo fermentations were used for bio-hydrogen production from carbohydrate resources. The first step in bio-hydrogen production by sequential fermentation from carbohydrate containing organic wastes is acid or enzymatic hydrolysis to highly concentrated sugar solution. This step is then followed by dark fermentation in which the organisms belonging to genus *Clostridium* such as *C. acetobutylicum* produce volatile fatty acids (VFA), hydrogen and CO₂ [5]. Volatile fatty acids produced are further fermented by photo-heterotrophic bacteria (*Rhodobacter sp*) for H₂ and CO₂ production. Compared to sequential fermentation, combined dark–photo fermentation can be more efficient and cost effective for H₂ production.

Bio-hydrogen formation in combined fed-batch fermentation was investigated in this study. To the best of our knowledge there is no reported study on the bio-hydrogen production of food waste in a combined dark and photo fermentation system.

2. Materials and methods

2.1. Microbial inocula and media

Clostridium acetobutylicum was obtained from Persian culture collection center and was cultivated in a growth medium containing (per 1 L): glucose 10 g, peptone 3 g, yeast extract 1 g, K₂HPO₄ 2.8 g, KH₂PO₄ 3.9 g, MgSO₄.7H₂O 0.2 g, NaCl 0.1 g, CaCl₂.6H₂O 0.01 g, FeSO₄.7H₂O 0.05 g, L-cysteine 0.2 g, microelements 1 mL

* Corresponding author. Department of Civil and Environmental Engineering, Shiraz University, Iran.
Tel:+989394243173. E-mail address: Rezaei.fateme7171@gmail.com

and the initial pH value was adjusted to 7.0. The *Rhodobacter sphaeroides* culture which used as the photo-heterotrophic bacteria was isolated in our laboratory and cultivated on medium containing (per liter): KH_2PO_4 0.33 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.33 g, NaCl 0.33 g, NH_4Cl 0.5 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.05 g, yeast extract 0.02g, Sodium succinate 1.0 g, 0.02% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution 0.5 mL, trace salts solution 1 mL and distilled water filled up to 1 L in an incubator at 37 °C (pH = 7). Trace salts solution were the same in all mediums used in this study and consisted of (per 1 L): $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.003 g, H_3BO_3 0.03 g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.02 g, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 0.001 g, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.002 g, NaMoO_4 0.003 g. The organisms were grown for one week at 30 °C under 5000 lux.

2.2. Feedstock

Synthetic food waste was prepared from typical locally produced food waste with the composition of 70% carbohydrate (rice and grains), 20% vegetable and 10% meat (w/w). The feedstock was then ground in a blender to particles of 0.5 mm diameter and used with no prior sterilization. The substrate (with concentration of 13650 mg/l) was boiled at 90 °C for 20 min then added to the batch fermented. Table 1 demonstrates the average characteristics of the mixed feedstock.

Table 1-Characteristics of the feedstock

parameter	unit	content
TCOD	mg L ⁻¹	13650
SCOD	mg L ⁻¹	9780
TS	%	35.2
VS	%	75
Ammonia	mg L ⁻¹	13
Initial pH		6.5

2.3. Batch fermentation

The experimental systems consisted of a 6 liter batch fermented were set up with a working volume of 5500 ml. Fermenters were fed with a medium containing 13650 mg/lit of food waste (FW). During the co-culture processes *R. sphaeroides* and *C. acetobutylicum* were mixed under optimal volume ratio of 2/1 (20%:10% v/v) [5]. The concentration of the microorganisms were adjusted to OD₆₆₀ 1.8-2, which were used as inoculums. Batch fermenters were sealed with nitrogen gas for 15 min, which could provide an anaerobic environment. Then incubated at 35 °C and 100 rpm under 15000-lux illumination using halogen lamp as the best light source for photo-heterotrophic bacteria [7]. In addition pH of the feed was set to 7.0. The liquid samples were analyzed for pH, SCOD and TCOD every 24 h.

2.4. Analytical methods

Gaseous products (H_2 and CO_2) were collected by gas flow meter and analyzed by a gas chromatography (GC) equipped with a thermal conductivity detector and a 1.8 m × 3.2 mm stainless-steel column. Helium used as the carrier gas. Chemical oxygen demands (COD), total solids (TS), ammonia were measured according to standard methods [8]. To measure soluble chemical oxygen demand (sCOD), samples were filtered through 0.45 μm filter paper which removes particulate COD then sCOD was determined. Cell concentration of the culture was determined measured spectrophotometrically. Cumulative hydrogen formation data was simulated with the Gompertz equation (Equ. (1)) [9].

$$\text{Equ. 1: } H(t) = p \exp\left\{-\exp\left[\frac{R_{max}}{P}(\lambda-t)+1\right]\right\}$$

Where $H(t)$ is the cumulative hydrogen production (ml) at t time (h), R_{max} is the maximum H_2 production rate (ml/h), P is the biogas production potential (ml), λ and t are the lag phase duration (h) and fermentation time (h), respectively and $\exp = 2.718$.

3. Results and discussions

3.1. Cumulative bio-hydrogen formation in dark and combined fermentation

Two batch fermentation experiments were performed with dark and combined fermentation by using food waste as the substrate. H_2 production in batch tests with pH control was conducted. The biogas generated from food waste only consists of H_2 and carbon dioxide (CO_2). Fig. 1 depicts the cumulative hydrogen production (CHP) during the hydraulic retention time in both dark and combined fermenters. It shows that combined fermentation yielded higher cumulative hydrogen production (22.7 L) than the dark fermentation (14.2 L), Because the fatty

acids existed in the effluent of dark fermentation could be utilized well in photo-fermentation to produce H_2 . Hydrogen production increased dramatically during the first 5 days, then started to decrease slightly. Hydrogen gas production was completed within 8 days. The combined hydrogen production process adopted in this study increased CHP by more than about 1.65 times compared to that by dark fermentation only. On the basis of the CHP results, combined fermentation was more suitable than the single-step dark. The highest hydrogen gas percentage in the head space of reactors was 70% in the first and second days.

3.2. Variation of Chemical Oxygen Demand (COD) in dark and combined fermentation

Fig.2. shows that COD removal in combined fermentation is higher than the dark fermentation. The total hydrogen production in the hybrid system was near 1.65 fold of the dark fermentation hydrogen production. An additional consideration is that the byproducts of fatty acids, which contribute to the high COD and low pH in the dark-fermentation effluent and emit an unpleasant odor, may be a potential threat to the environment. Our investigation demonstrated that fatty acids existed in the effluent of dark fermentation could be utilized well in photo-fermentation, and the COD during the photo fermentation process decreased with a removal efficiency of 22.5% compared with the dark-fermentation medium of food waste that the removal efficiency is 14% Fig.2. In addition, COD removal efficiency increased during the first 4 days, then started to decrease slightly. Therefore, the hybrid process combining dark and photo-fermentation can be a promising alternative to solve the problems caused by fatty acids in anaerobic fermentation effluents.

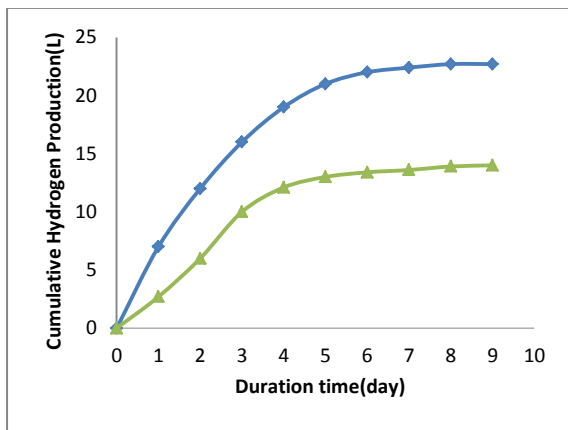


Fig.1. Cumulative Hydrogen Production (CHP) .
(▲) dark fermentation (◆) combined fermentation

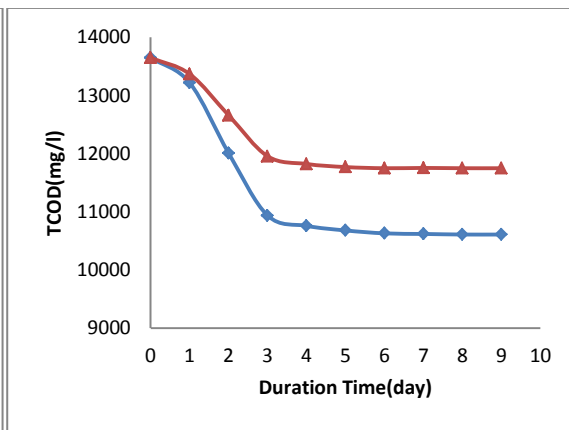


Fig.2. Variation of Chemical Oxygen Demand (COD) in dark and combined fermentation .
(▲) dark fermentation (◆) combined fermentation

4. Conclusions

Anaerobic digestion is an attractive process for generation of hydrogen, which involves complex microbial processes on decomposition of organic wastes and subsequent conversion of metabolic intermediates to hydrogen. This study successfully demonstrated that the combined system was more efficient than the single-step dark fermentation of food waste regarding the energy recovery. The CHP from hybrid system was 1.65 times compared to the one stage dark fermentation. Experimental results showed that co-culture bio-hydrogen production can be effective during the first 5 days and after that no significant change will be observed in the hydrogen production. In other words, the cumulative hydrogen production after 120 and 216 h reached 21 and 22.7 L, respectively. This work demonstrated that two stage fermentation achieved a better performance than one stage process.

Acknowledgement

The authors would like to express their gratitude for the financial support provided by the Department of Civil and Environmental Engineering, Shiraz University, Iran. Also we would like to appreciate the efforts of Mr. Vahid Hassanpur.

References

- [1] Kapdan, I.K., Kargi, F.: Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*. 38(5): p. 569-582 (2006).

- [2] Tian, X., Liao, Q., Zhu, X., Wang, Y., Zhang, P., Li, J., Wang, H.: Characteristics of a biofilm photobioreactor as applied to photo-hydrogen production. *Bioresource Technology*. 101(3): p. 977-98 (2010).
- [3] Holladay, J.D., Hu, J., King, D.L., Wang, Y.: An overview of hydrogen production technologies. *Catalysis Today*. 139(4): p. 244-260 (2009).
- [4] Argun, H., Karg, F.: Effects of light source, intensity and lighting regime on bio-hydrogen production from ground wheat starch by combined dark and photo-fermentations. *International Journal of Hydrogen Energy*. 35(4): p. 1604-1612 (2010).
- [5] Ozmihci, S and Kargi.: Effects of starch loading rate on performance of combined fed-batch fermentation of ground wheat for bio-hydrogen production. *International Journal of Hydrogen Energy*. 35:p 1106-1111(2010)
- [6] Kargi, F and Ozmihci, S.: Effects of dark/light bacteria ratio on bio-hydrogen production by combined fed-batch fermentation of ground wheat starch. *Biomass and Bioenergy*. 34(6) :p. 869-874 (2010).
- [7] Argun, H. and F. Kargi.: Photo-fermentative hydrogen gas production from dark fermentation effluent of ground wheat solution: Effects of light source and light intensity. *International Journal of Hydrogen Energy*,. 35(4): p. 1595-1603 (2010).
- [8] Association., A.P.H., Standard methods for the examination of water and wastewater. (1395), APHA: Washington DC, USA.
- [9] Lay, J.-J., Y.-J. Lee, and T. Noike.: Feasibility of biological hydrogen production from organic fraction of municipal solid waste. *Water Research*,. 33(11): p. 2579-2586 (1999).