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New concept water purification module combined with renewable energy production

Jung-Yeol Lee, Kyung-Sok Min*

Department of Environmental Engineering, Kyungpook National University, 1370 Sankyuk-dong, Buk-gu, Daegu 702-701, Korea
Tel. +82 53 950 4787; Fax: +82 53 959 7734; email: ksmin@knu.ac.kr

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ABSTRACT

The co-culture process of *Clostridium butyricum* (dark fermentation bacteria) and *Rhodobacter sphaeroides* (photo fermentation bacteria) with a single or dual reactor was evaluated for hydrogen production. A dual reactor with optional actions of pH and illumination (first reactor: pH 5.5 and no light, second reactor: pH 7.0 and 5000 lux illumination) was used to fulfill the optimized conditions of each dark and photo fermentation bacteria. In comparison, the sole pH was applied to a single reactor. A higher rate of hydrogen production was obtained from the co-culture system with the dual module (dual system: 26.2 ml-H_2/l·h, single system: 12.4 ml-H_2/l·h). In addition, the VFA concentrations in the fermented liquid of the dual reactor system were lower than that of the single reactor system. The superiority of this operating system was proven by a repeated fed-batch run with the hydrogen production rate of 25.2 ml-H_2/l·h. The fermentation of organic waste consisting of food-wastewater and sewage sludge was also attempted in a two-phase fermentation system. A stable hydrogen production rate of 60 ml-H_2/g-COD/d demonstrated the way to differentiate this operation access from the conventional one-phase fermentation system. This system was a practicable alternative treatment process for problematic organic wastewater producing stable hydrogen as an energy source.

Keywords: Bio-hydrogen; Co-culture; Dual reactor; Organic wastes; Two-phase fermentation; Water purification

1. Introduction

Energy can be extracted from wastewater during treatment, providing biogas that can offset the treatment cost. Hydrogen is considered a valuable and renewable energy since it can be generated from natural, infinite resources such as water and organics. Fermentation of organic wastewater to produce hydrogen might be considered very feasible due to advantages among various optional techniques [1,2]. There are two types of fermentation bacteria capable of producing hydrogen from organic compounds. One of type is the dark-fermentation bacteria, the *Clostridium* species that convert organics such as glucose and xylose into volatile fatty acids (VFAs) [3,4]. The other type is the photo fermentation bacteria, such as *Rhodobacter* and *Rhodopseudomonas* species, which produce energy through photosynthesis. In addition, they also can utilize VFAs besides glucose as a carbon source [5–7]. This implies that photo fermentation after the dark fermentation system can induce an increase in hydrogen production.

There have been some studies on the increase in hydrogen yield using a co-culture process with dark

*Corresponding author.

and photo fermentation bacteria [8,9]. To the best of our knowledge, most studies on the co-culture process have focused on the co-culture process using a single reactor with an operating pH fixed at one value despite the different optimum pH for hydrogen production and cell growth for each bacterium. While dark-fermentation bacteria have been known to have enhanced hydrogen productivity under slight acidic pH condition [10], photo-fermentation bacteria have been able to maximize hydrogen production under neutral conditions [8].

An alternative way for advanced hydrogen production is a two-phase process, dark fermentation followed by photo fermentation. [11–13]. The application of phase separation to the digestion of organic wastes has been extended to the field of hydrogen production. The two-phase separation system has an advantage in the separation of solids, therefore the efficiency of light utilization by photo fermentative bacteria is increased.

Some research on two-phase fermentation from organic wastewater such as municipal solid wastes or potato starch residue for hydrogen production has been done [5,11,13]. However, some complicated pre-treatment procedures, and the addition of nutrients and microelements into the medium after centrifugation of the dark fermented wastes is required by these previous methods. Moreover, rarely has research been conducted on hydrogen production from food-wastewater and primary sewage sludge. The primary sewage sludge plays an important role in the supplement of nitrogen source as well as alkalinity, which may a decrease in pH during acid fermentation of food-wastewater.

Thus, hydrogen production by a co-culture of *Clostridium butyricum* and *Rhodobacter sphaeroides* using a single or dual reactor was evaluated through both a batch and repeated fed-batch run. In addition, a two-phase fermentation system with a mixture of food-wastewater and sewage sludge was performed without any additives as mentioned above. Not only the hydrogen production rate but also the loads of pollutants were investigated in terms of renewable energy production and environmental treatment for organic wastewater.

2. Materials and methods

2.1. Microorganisms and media

C. butyricum and *R. sphaeroides* were obtained from the Korea Culture Center of Microorganisms (KCCM). *C. butyricum* was cultivated in the PYG medium (pH 6.5) containing K_2HPO_4 (0.9 g/l), KH_2PO_4 (0.9 g/l), NaCl (0.9 g/l), $(NH_4)SO_4$ (0.9 g/l), $MgSO_4$ (0.09 g/l), $CaCl_2$ (0.09 g/l), peptone (10 g/l), yeast extract (5 g/l), cysteine·HCl (0.5 g/l), $Na_2CO_3 \cdot 10H_2O$ (4.0 g/l), aminobenzoic acid (100 µl/l) and glucose (10 g/l). This medium was sterilized by autoclaving at 121°C for 15 min before being inoculated with *C. butyricum*. Serum bottles with a 200 ml working volume were used for the batch cultivation. Argon was used to create an anaerobic atmosphere in the open space of the bottle.

R. sphaeroides was cultivated in a sterilized sistrom's minimal medium at 30°C for 72 h under tungsten lamps with 5000 lux. The sistrom's medium consisted of K_2HPO_4 (34.8 g/l) or KH_2PO_4 (27.2 g/l), $(NH_4)_2SO_4$ (5.0 g/l) or NH_4Cl (1.95 g/l), succinic acid (40.0 g/l), L-glutamic acid (1.0 g/l), L-aspartic acid (0.4 g/l), NaCl (5.0 g/l), nitrilotriacetic acid (2.0 g/l), $MgSO_4 \cdot 7H_2O$ (3.0 g/l) or $MgCl_2 \cdot 6H_2O$ (2.44 g/l), $CaCl_2 \cdot 2H_2O$ (0.334 g/l), $FeSO_4 \cdot 7H_2O$ (0.020 g/l), $(NH_4)_6Mo_7O_{24}$ (0.2 ml/l of a 1% solution), trace elements solution (1 ml/l) and vitamins solution (1 ml/l). The vitamins solution (100 ml) consisted of nicotinic acid 1.0 g, thiamine·HCl 0.5 g, and biotin 0.01 g. For the trace elements solution, EDTA 1.765 g, $ZnSO_4 \cdot 7H_2O$ 10.95 g, $FeSO_4 \cdot 7H_2O$ 5.0 g, $MnSO_4 \cdot H_2O$ 1.54 g, $CuSO_4 \cdot 5H_2O$ 0.392 g, $Co(NO_3)_2 \cdot 6H_2O$ 0.248 g and H_3BO_3 0.114 g were dissolved in 100 ml of distilled water. The medium was diluted ten times and the initial pH was 7.0.

After being boiled at 95°C for 15 min, dark fermentative bacteria collected from the digester of a domestic wastewater treatment plant were used in a two-phase fermentation system with a mixture of food-wastes and sewage sludge. Food-waste collected from a municipal waste treatment facility was homogenized with a mixer after being screened by a standard 2 mm sieve. Sewage sludge was also collected from the first settler of the same facility and then mixed with the food-wastewater. The mixture was diluted with distilled-water after its thermal treatment at 90°C for 15 min. Detailed characteristics of the food-wastewater, sewage sludge and diluted mixture (named as mixed waste) are presented in Table 1.

2.2. Experimental procedure

Pre-cultured *C. butyricum* and *R. sphaeroides* were harvested with a centrifuge at 4000 rpm for 20 min. and were used in this study. A modified PYG medium consisted of 10 g/l glucose, 5 g/l peptone, 2 g/l yeast extract, 4.2 g/l $Na_2HPO_4 \cdot 12H_2O$, 1.5 g/l KH_2PO_4, 0.18 g/l $MgCl_2 \cdot 6H_2O$ and 0.1 g/l $FeSO_4 \cdot 7H_2O$. A batch co-culture of *C. butyricum* and *R. sphaeroides* with a modified PYG medium containing 10 g/l glucose was run. A co-culture system with a single reactor (1 l) was operated at pH 6.25 and under a light of intensity of 5000 lux. Pre-cultured *C. butyricum* and *R. sphaeroides* after centrifugation at 4000 rpm for 20 min was re-suspended in the single reactor. The dual reactor consisted of the former one (0.1 l and pH 5.5) followed by another one (0.9 l and pH 7.0). The harvested cells of *C. butyricum* and *R. sphaeroides*

Table 1
Characteristics of the food-wastewater, sewage sludge and mixed waste used in the two-phase fermentation system for hydrogen production

	Food-wastewater	Sewage sludge	Mixed waste*
pH	3.63	5.98	5.50
$TCOD_{Cr}$ (mg/l)	229,000	17,500	73,400
$SCOD_{Cr}$ (mg/l)	77,400	678	20,140
TS (total solids) (mg/l)	101,300	16,860	40,100
VS (volatile solids) (mg/l)	78,400	11,360	27,500
T-N (mg/l)	1599	1133	1652
T-P (mg/l)	1220	248	375
TA (total alkalinity) (mg/l)	–	775	3230

*It consisted of food-wastewater, sewage sludge and distilled water at a volume ratio of 2:1:7.

were inoculated into the partial dual reactor. And the cells were re-circulated throughout the dual system by a peristaltic pump. In the case of a repeated-fed batch run, 200 ml/d (hydraulic retention time of 5 d) of fresh medium was fed to the single or dual reactor system based on the co-culture of *C. butyricum* and *R. sphaeroides*.

The two-phase fermentation process consisted of a 1 l dark fermentation reactor with dark-fermentative microorganisms and a 1 l photo fermentation reactor containing *R. sphaeroides*. The mixed wastes of the food-wastewater and sewage sludge were fed to the former dark fermentation reactor based on a repeated fed-batch run. The hydraulic retention time, temperature and pH of the dark fermentation reactor were 0.5 d, 30°C, and 5.5, while those of the photo fermentation reactor were 4.5 d, 30°C, and 7.0, respectively.

All reactors used in this study were purged at 30°C after the replacement of the gas phase with argon. The volume of evolved gas was measured with an inverted cylinder containing 10% NaOH solution. The compositions of the gas and fermented liquid samples were analyzed periodically.

2.3. Analytical methods

The hydrogen content in the biogas was analyzed using gas chromatography (Agilent 7890, USA) equipped with a capillary column (Agilent, 30 m, 535 μm, 25 μm) and a thermal conductivity detector (TCD). The operating temperatures of the oven, injector and detector were 50, 200, and 250°C, respectively. Helium was used as a carrier gas with a flow rate of 11.15 ml/min. The VFAs in the fermentation effluent were measured by gas chromatography equipped with a capillary column (Restek, 30 m, 530 μm, 0.25 μm) and a flame ionization detector (FID). Helium gas was distributed with at a ratio of 10 to 1 as the carrier gas. The temperature of the oven increased up to 145°C at the rate of 20°C/min after it was initially maintained at 95°C for 2 min. Then it was increased again up to 200°C at a rate of 50°C/min. The injector and detector were set at 200 and 240°C, respectively. The glucose concentration was measured by the 3,5-dinitrosalicylic acid (DNS) method as described elsewhere [10]. The cell concentration in the medium was determined to be as the unit of suspended solid (SS) by filtering a 4 ml sample through a 1.0 μm Millipore filter and thereafter, drying at 105°C. Analysis of the pH, TCODcr, SCODcr, T-S, V-S, TKN, and T-P was done based on standard methods [14].

3. Results and discussions

3.1. Hydrogen- and acids-producing properties of the batch co-culture systems using single or dual reactor

Fang et al. [15] reported that a maximum production of hydrogen from glucose by mixed fermentation bacteria was attained at a pH of 5.5, but its conversion efficiency did not reach 100% at that pH. Additionally,a pH range between 5 and 6 is preferred for dark-fermentation bacteria when competing with methanogenic bacteria [15,16]. According to previous studies, the optimum pH for cell growth and hydrogen production for photo-fermentation bacteria ranges between 6.5 and 7.0 [11]. In our study, therefore, pH values of 5.5 and 7.0 were chosen as the operating pHs for the single or dual reactor systems using dark- and/or photo-fermentation bacteria.

Fig. 1 illustrates the concentrations of SS, glucose and accumulated hydrogen evolved by the batch co-culture of *C. butyricum* and *R. sphaeroides* with the single or dual reactor. The pattern for hydrogen production by the co-culture system with the dual reactor was similar to the system with the single reactor until 20 h. After that, there was a slight difference between the accumulated amounts of hydrogen evolved in both systems. The accumulated hydrogen of the single reactor system reached only 1220 ml, while more evolved hydrogen was observed at 1780 ml in the dual reactor system. In the single reactor system, glucose was completely degraded in 20 h, but it took over 40 h to be degraded in the dual reactor system. This is due to from the difference in the microbial activities of the co-culture system. In the single reactor system, *R. sphaeroides* must have competed with *C. butyricum* for a substrate that was easily fermented, such as glucose resulting in its rapid depletion. However, *R. sphaeroides* could also utilize other substrates, i.e., VFAs generated from the degradation of glucose in the dual reactor system.

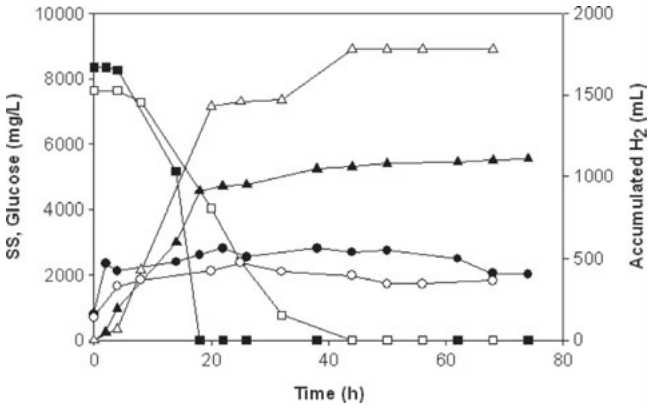

Fig. 1. Time-dependent concentrations of SS (●, ○), and glucose (■, □), as well as the accumulated amount of evolved hydrogen (▲, △) in the batch co-culture systems of *C. butyricum* and *R. sphaeroides* using a single reactor (closed symbols) or dual reactor (open symbols).

Time-dependent compositions of VFAs generated during the co-culture of *C. butyricum* and *R. sphaeroides* with the single or dual reactor are shown in Fig. 2. It shows that acetic acid and butyric acid were the two most abundant species in the fermented liquid. The order of generated VFA compositions were acetic acid, butyric acid and propionic acid in both systems. It is well known that acetic acid and butyric acid are major VFAs generated from the degradation of glucose by *C. butyricum* [15].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

The VFA concentrations of the single reactor system were higher than that of the dual reactor system. It implies that *R. sphaeroides* efficiently utilized the VFAs, which resulted in enhanced hydrogen production in the dual reactor system (Fig. 1). The ratio of butyrate/acetate (B/A) is used as an indicator to evaluate hydrogen production. The increased amount of hydrogen evolved by the dark fermentation bacteria was likely due to a high B/A ratio [17]. In addition, the amount of butyric acid decreased in the dual reactor system after an initial considerable increase in butyric acid. Thus this implies that *R. sphaeroides* used butyric acid as a carbon source after 32 h, which indicates the second lag-phase of hydrogen production in the dual reactor system (Fig. 1). It was reported that *Rhodobacter sp.* could produce hydrogen from butyric acid as a sole carbon source [18]. The hydrogen production rate of 26.2 ml-H_2/l·h detected in the dual reactor was substantially higher than the rate of 12.4 ml-H_2/l·h. observed in the single reactor system. The VFAs produced in the dual reactor system was lower than that in the single reactor system since these were further degraded by the photo fermentation bacteria under optional conditions of pH and illumination.

3.2. Hydrogen- and acids-producing properties of the repeated fed-batch co-culture systems using single or dual reactor

Repeated fed-batch runs based on the co-culture systems of *C. butyricum* and *R. sphaeroides* with a single or dual reactor were conducted in order to produce hydrogen continuously (Fig. 3). The amounts of hydrogen evolved in the single or dual reactor system averaged 76.5 ml/d and 121 ml/d, respectively. The hydrogen production rate of the single reactor system was 15.9 ml-H_2/l·h, whereas the rate of the dual reactor system was 25.2 ml-H_2/l·h. Taking these figures into consideration, the dual reactor system is more likely to be used for sustainable hydrogen production.

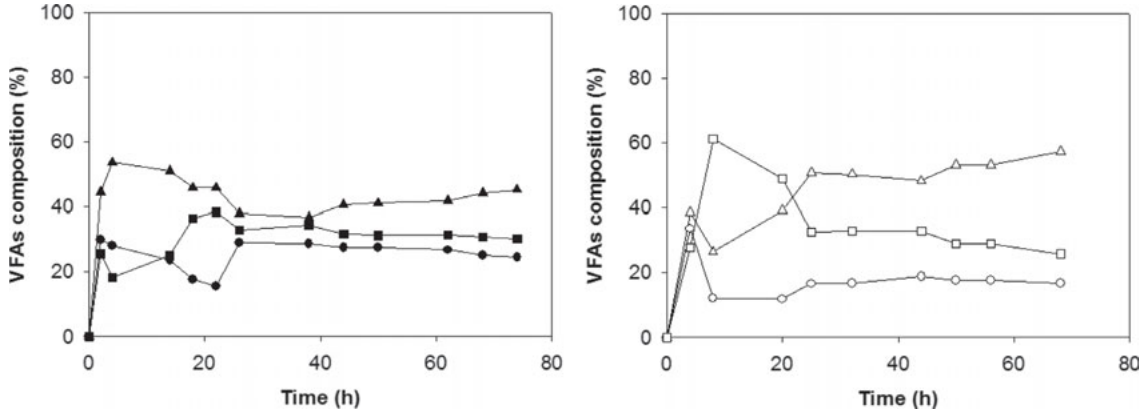

Fig. 2. Time-dependent composition of propionic acid (●, ○), butyric acid (■, □), and acetic acid (▲, △) in the batch co-culture systems of *C. butyricum* and *R. sphaeroides* using single reactor (closed symbols) or dual reactor (open symbols).

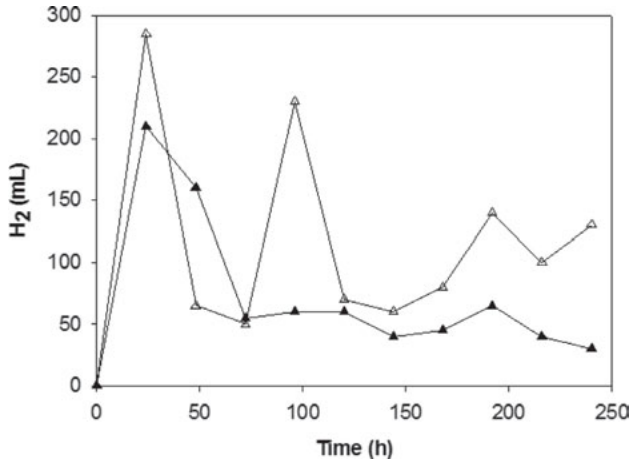

Fig. 3. Amount (▲, △) of hydrogen produced by the repeated fed-batch co-cultures of *C. butyricum* and *R. sphaeroides* using a single reactor (closed symbols) or dual reactor (open symbols).

As can be seen from Fig. 4, acetic acid and butyric acid were the two major components of VFAs produced in both systems. The concentration of acetic acid observed in the dual reactor system was lower than that of the single reactor system. It was likely that *C. butyricum* produced more acetic acid and butyric acid in the dual reactor system at an optimum pH 5.5. Thus *R. sphaeroides* could produce more hydrogen by utilizing the VFAs. Therefore, the pH condition of each reactor should be optimal for each bacteria cultured within it. A similar result was reported by Fang et al. [19]. They observed that more acetic acid and butyric acid were produced and remained in the pure culture system of *C. butyricum*. Briefly, low VFA concentration might be one of the indicators that can predict the performance of hydrogen production in a co-culture system.

3.3. Hydrogen production from organic wastes by a repeated fed-batch two-phase fermentation

Organic waste consisted of food-wastewater and sewage sludge (Table 1). The mixed waste was fed to the dark fermentation reactor. The supernatant after the centrifugation of dark-fermented waste was used in the photo-fermentation reactor as a substrate for *R. sphaeroides*. Table 2 lists some of the characteristics of the mixed wastes, dark-fermented waste and photo-fermented

Table 2
Characteristics of the mixed wastes and each fermented wastes with the two-phase fermentation system for hydrogen production

	Mixed waste	Dark-fermented waste	Influent for photo fermentation*	Photo-fermented waste
pH	5.5	4.53	5.85	7.49
TCODcr (mg/l)	73,400	41,500	38,900	34,900
SCODcr (mg/l)	20,140	32,300	28,900	25,900
TS (total solids) (mg/l)	40,100	34,900	26,400	26,200
VS (volatile solids) (mg/l)	27,500	21,600	12,000	10,500
T-N (mg/l)	1470	686	580	294
T-P (mg/l)	375	300	300	245
TA (total alkalinity) (mg/l)	3230	2730	4100	8700

*Dark-fermented waste was used as an influent for photo fermentation after centrifugation.

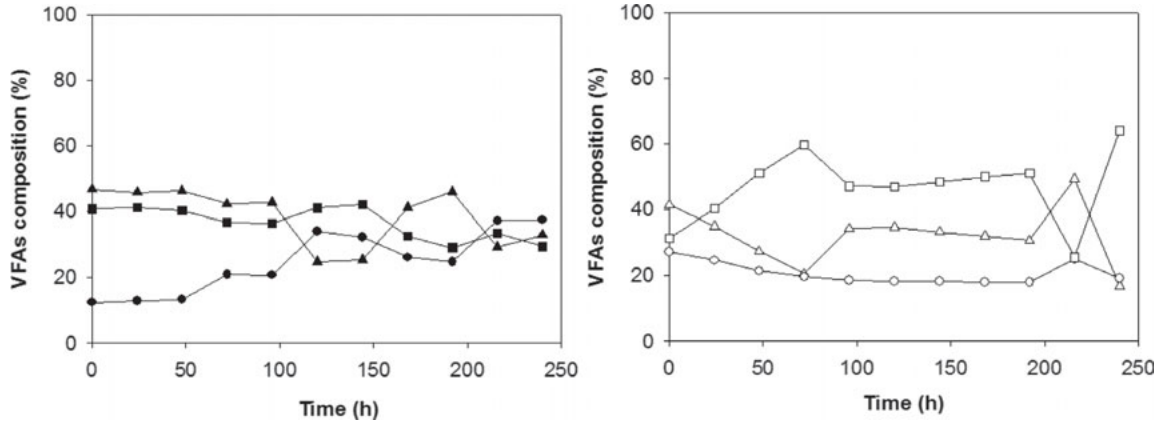

Fig. 4. Time-dependent composition of propionic acid (●, ○), butyric acid (■, □), and acetic acid (▲, △) produced by the repeated fed-batch co-cultures of *C. butyricum* and *R. sphaeroides* using single reactor (closed symbols) or dual reactor (open symbols).

waste. Total organics were significantly decomposed by the sequential dark and photo fermentation system. Total COD concentration decreased from 73,400 mg/l to 34,900 mg/l, while soluble COD concentration increased from 20,140 mg/l up to 32,300 mg/l during the dark fermentation run, in which hydrogenesis as well as hydrolysis of high molecular compound took place. The removal efficiencies of TCOD, TS, VS, T-N and T-P were determined to be 52%, 35%, 62 % 80% and 35%, respectively.

As can be seen from Fig. 5, VFAs were generated as byproducts during the dark fermentation and utilized as carbon sources for photo fermentation. The average concentration of VFAs was 24,900 mg/l in the dark fermented wastes and 16,500 mg/l in the photo-fermented waste. Thus 8400 mg/l of VFAs were utilized in the production of hydrogen by the photo fermentation bacteria, *R. sphaeroides*. The hydrogen production yield of dark fermentation was 0.02 l-H_2/g-COD, while the overall hydrogen production yield of the two-phase fermentation was 0.06 l-H_2/g-COD. Photo fermentation contributed to 67% of the total hydrogen production due to the additional hydrogen evolved from the generated VFAs by the dark fermentation bacteria. Our results were quite coincident with those of other researchers. Yokoi et al. [13] reported that *R. sphaeroides* could produce more hydrogen than the dark fermentation bacteria. Kim et al. [20] developed a model for the potential hydrogen production through the dark fermentation of food-wastewater and sewage sludge. This previous study mentioned that food-wastewater and sewage sludge were favorable to the dark fermentative bacteria as carbon and nitrogen source, respectively. It was concluded that the anticipated maximum hydrogen potentiality by the developed model was 0.12 l/g COD when the VS ratio of food wastes to sewage sludge was 87:13.

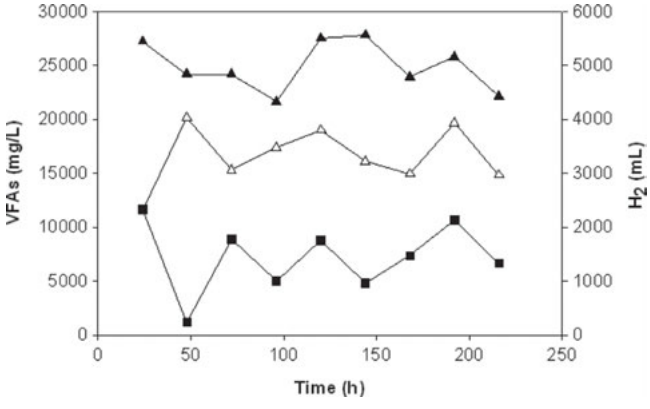

Fig. 5. Time-dependent concentrations of the total VFAs (▲, △) in the dark-fermented wastes (closed symbols) and in the photo-fermented wastes (open symbols), and daily accumulated amount of evolved hydrogen (■) in a two-phase fermentation system.

The reason why the measured hydrogen yield of this research was low could be a result of not only the different VS ratio of food wastes to sewage sludge, (14:1), but also due to the characteristics of the mixed wastes. The system of this study was efficient in the treatment of organic-polluted water with the stable hydrogen production.

4. Conclusions

A co-culture of *Clostridium butyricum* and *Rhodobacter sphaeroides* using a single or dual reactor was evaluated based on the hydrogen production rate. The optimum pH for dark and photo fermentation bacteria ranged from 5 to 6 and 6 to 7, respectively. Thus, separated reactors (dual reactor) were designed taking into consideration the different pH, hydraulic retention time and illumination while only a single pH was used with the single reactor in which a mixed-culture of *C. butyricum* and *R. sphaeroides*, was inoculated. The hydrogen production rate of the dual reactor system was 26.2 ml-H_2/l·h, whereas the hydrogen production rate was only 12.4 ml-H_2/l·h in the co-culture system with the single reactor. This implied that the photo fermentation bacteria, *R. sphaeroides* efficiently utilized the VFAs produced by the dark fermentation bacteria under the optimized operation parameters of the dual reactor system. A repeated fed-batch run of the co-culture system with the dual reactor proved to be sustainable with a stable hydrogen production rate of 25.2 ml-H_2/l·h. Finally, the fermentation of organic waste composed of food-wastewater and municipal sewage sludge was also fermented in a two-phase fermentation system. This system was a practicable alternative illustrating not only a stable hydrogen production rate of 60 ml-H_2/g-COD/d but also the efficient treatment of problematic organic wastewater.

Acknowledgements

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