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Biohydrogen Fermentation of Mixed Liquid of Kitchen Waste and Napier Grass with Anaerobic Fluidized Bed Process

Sheng-Shung Cheng, Department of Environmental Engineering, Sustainable Environment Research Center(SERC), National Cheng Kung University, No.1, University Rd., East District, Tainan City 701, Taiwan (R.O.C.)
Yu-Chieh Chao, **Yi-Chieh Chen**, **Yu-Min Tien**, Department of Environmental Engineering, National Cheng Kung University, No.1, University Rd., East District, Tainan City 701, Taiwan (R.O.C.)

1 Introduction

Hydrogen is an emerging energy carrier that produces only water after combustion. Biohydrogen technology can convert organic wastes into hydrogen and simple fatty acids. Among all of the organic wastes, carbohydrates were identified as the main electron donors, which contribute to the formation of hydrogen gas. Napiergrass (NG) can provide sufficient carbohydrates, be easily grown and produce a great yield in wild mountain area of Taiwan. The content of total carbohydrates (including soluble sugars, cellulose and hemicelluloses) was 51%, which made up of 57.6% in the organic part. Soluble sugar and cellulose made up 7.5% and 20 % respectively. The percentage of hemicellulose and other sugars is estimated to be 23%, with 8.5% protein content and 29 % lignin content. Nevertheless, Napier Grass could be a substrate for biohydrogenation, like other cellulosic materials, hydrolysis is the rate-limiting step in hydrogenation. As a result, in this study, an anaerobic fluidized bed reactor was adopted with a long solid retention time, which can provide complete reaction duration in hydrolyzing and producing hydrogen gas from cellulosic fermentation.

In Taiwan, the recycling amount of kitchen waste (KW) was collected locally up to 1,900 ton/day in average. Kitchen waste contains high organic substance, such as nitrogen and abundant nutrients which are necessary for microbial propagation. Ueno (Ueno et al., 2001 [1]) noticed that microbial diversity shifted when the nitrogen source was different. Kitchen waste is easy to obtain and can be collected for various objectives. Other than pig feeding and composting, anaerobic digestion is an efficient way to treat kitchen waste. The mixed liquid of kitchen waste is not suitable for pig feed and composting because of high water content. However, it contains ample nitrogen sources and other trace elements, which was lacking in the Napier Grass. Therefore, kitchen waste seived liquid (KWSL) was used as feeding substrate and mixed with Napier Grass for biohydrogenation in this study. The water content of KW was more than 75% and with abundant nutrient, KWSL is suited for biohydrogenation as well as mixed completely in anaerobic fluidized bed (AnFB) with less suspended solid.

2 Materials and Methods

The characteristic of KWSL are as follows: Total COD : 104,000 ± 16,000 mg/L (69% soluble) , Total Carbohydrate: 26,500 ± 6,300 mg/L (85% soluble) , Total Org-N: 2,560 ± 270 mg/L (70% soluble) , lipid: 8,300 ± 3900 mg/L , suspended solid: 26,000 ± 5,330 mg/L (91 % volatile SS) , and it contained acetate (2,500 ± 340 mg/L) and high concentration of lactate (10,700~14,500 mg/L) . Napier Grass (1.1 g-COD/g- Napier Grass dregs) is composed by about 20% cellulose and other lignin cellulose which are difficult to be decomposed. This study expected that adding the Napier Grass dregs with KWSL could co-metabolize the cellulose and serve as a kind of biofilm supporter in the AnFBR.

The advantage of operation in an AnFB is to maintain suspended solid (substrate and microbes) react for a longer contact time in reactor. Fluidized bed was most adopted on industrial wastewater treatment (Holst et al., 1997 [2]), but there was few study of fluidized bed performance with solid content substrates. This study would focus on the cellulose hydrolyzing and hydrogen production process of cellulosic feedstock. To promote the microorganism better hydrogen producing activity, high organic loading was necessary (Li et al., 2008 [3]; Wang et al., 2009 [4]), however, we would also experience some hardware operating problems. Recirculation pumps provide the driving power of fluidization. However, short fibers and lipid could easily clog centrifugal pumps and cause reactor shut down. Therefore, we selected an axis seal separation pump to improve the recirculation in the fluidized bed returning flow this year. Fig. 1 illustrates the flow chart of AnFB reactor.

There are three phases for this study and the operational parameters are showed in Table 1. The major substrates used in this study were KWSL and Napier Grass. The operational parameters of AnFB were shown as follows: with a hydraulic retention time (HRT) maintained about 7.3 days. pH was held at 6.0±0.1 and the temperature controlled at 55±0.5 ^{0}C. The AnFB was started-up in batch mode and KWSL was used as substrate. Seeding sludge was taken from the KW composting in Tainan city and was enhanced with vegetable kitchen waste (picking up the vegetables from kitchen waste as feeding substrate) in previous study (Li et al., 2008 [3]). After 37 days' operation, the influent of AnFB was changed to continuous-pulsed input for hydrogen fermentation. Continuous input for cultivating hydrogen-producing microorganisms is better than the batch culture (Herbert et al., 1956 [5]). It can also provide substrate continuously, dilute out the products from metabolism and reduce the product inhibition to the hydrogen-producing microorganisms. As a result, it can enable the hydrogen producing microorganisms to keep producing hydrogen stably.

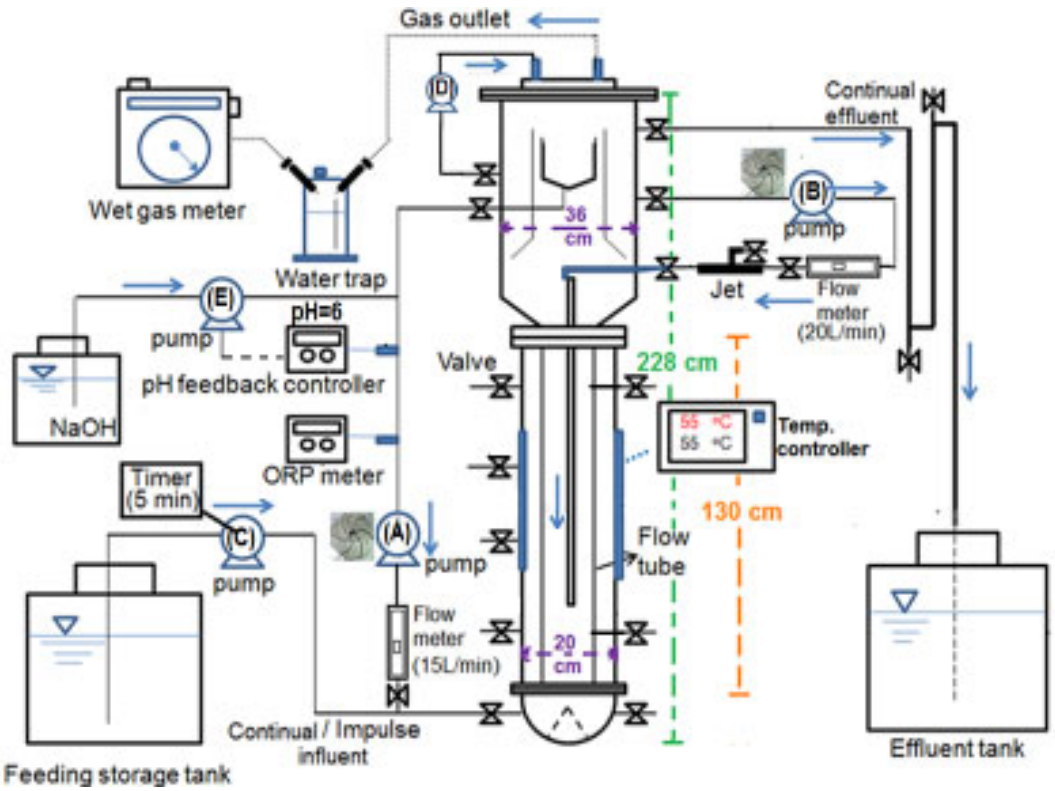

Figure 1: Schematic diagram of 110 L anaerobic fluidized bed (AnFB) hydrogen fermentor fed with Napier Grass and kitchen waste.

Table 1 illustrates the three runs conditions of AnFB process as follows.

(1) In Run 1: the input substrate was KWSL (operation days: 0~70).

In order to reduce the block-up problem of pump and tube we encountered in previous study (Li et al., 2008 [3]), and to cultivate the hydrogen-producing organisms, we controlled the concentration of the suspended solid in kitchen waste sieved liquid less than 26,000 mg/L.

(2) In Run 2: the major input substrate was KWSL and the minor substrate was 5,000 mg/L Napier Grass powder (operation days: 71~143).

In order to enhance the cultivation of cellulose-hydrolyzing and hydrogen-producing microorganisms, small amount of Napier Grass powder was added with 5 g/L in KWSL feeding, which could not only increase the organic loading rate but also served as a microbial carrier, resulting in a suitable circumstance for cellulose-degrading microorganisms.

(3) In Run 3: the major input substrate was KWSL and the minor substrate was 20 g/L Napier Grass powder to enhance the hydrogen production rate and the efficiency of AnFBR. (operation days: 144~193).

The biogas produced from the reactor was measured by a gas meter continuously; biogas was collected by water displacement method then analyzed by a gas chromatograph (Model China Chromatography, GC 8900T) equipped with a thermal conductivity detector (TCD). The concentration of sucrose was determined by phenol-sulfuric method(Herbert et al., 1971 [6]). Water quality analyses were conducted according to the procedures described in the Standard Method 19th edition (APHA, 1995 [7]).

Table 1: Operational parameters of AnFB for bio-hydrogenation fed with kitchen waste sieved liquid (KWSL) and Napier Grass (NG) from Run 1 to Run 3.

Parameters	Unit	Run 1	Run 2	Run 3
Period	Days	0~70 (70 days)	71~143 (74 days)	144~193 (50 days)
Feeding	-	KMSL	KMSL + 5 g-NG/L	KMSL + 20 g-NG/L
Feeding type	-	From Batch input to Impulse input	Impulse input (5 min a time)	Impulse input (5 min a time)
Feeding volume	L	15	15	15
HRT	Days	7.3	7.3	7.3
pH controlling	-	6.0	6.0	6.0
Recirculation liquid flow rate	L/min	35	35	35
Organic loading rate	g-COD/L/day	14.2±2.5	14.7±1.8	17.7±2.4

3 Results and Discussion

3.1 Experimental results of AnFB process performance

Three phases were conducted in the operation of AnFB. In the first phase, the KWSL was used as substrate. In the second phase, 5 g/L Napier Grass was added in the influent substrate of KWSL. In the third phase, the addition of Napier Grass was increased to 20 g/L with KWSL as substrates. The biogas variation and characterization of substrates and metabolites in these three phases were shown in Figure 2, Figure 3 and Table 3. The ORP varied between -300 to -500 mV and pH controlled in phase one is more stable. Under the organic loading rate of 14.2 g-COD/L/day and using only KWSL as substrate, AnFB can achieve a hydrogen performance up to 1.41 L H_2/L/day, which means KWSL can be converted into hydrogen stably.

5 g/L Napier Grass was added to KWSL in phase two, which contributed to a slight increase in total COD of the inflow. Therefore, the OLR was increased a little to 14.7 g-COD/L/day. The AnFB can convert 6.2 % of COD into hydrogen and the average hydrogen production rate was 1.41 L H_2/L/day. The hydrogen concentration was 40 % of total biogas production and the hydrogen yield was 3.84 mmol H_2/g-COD_{in}. The hydrogen performance was almost the same as in phase one. Because only a little amount of Napier Grass was added in the inflow, the increase of OLR was limited, resulting in no improvement to hydrogen production. Besides, cellulose and hemicellulose, which are both difficult to be utilized for microbes, contribute 43 % in COD basis in the Napier Grass and only 7.5 % of soluble sugars were contained. From the comparisons between inflow and effluent of the AnFB, the cellulose removal ratio was only 7.1 %, which indicated that cellulose was not converted by the microbes. The differences of cellulose concentration between inflow and effluent in the earlier stage of phase two was due to the entrapment of Napier Grass in AnFB reactor.

Table 2: Characteristics of Napier Grass from Livestock Research Institute.

Characteristics of Napier Grass dregs (w/w, %)			
Moisture	7.2	±	0.3
TVS	88.5	±	1.5
Total carbohydrate	51	±	4.0
Soluble carbohydrate	7.5	±	0.1
Cellulose	20	±	3.0
Protein	8.5	±	0.3
Inerts	4.5	±	0.3
COD	1.1 ± 0.05 g-COD/g-Napier Grass		

Accordingly, 20 g/L Napier Grass was added to KWSL during the third phase, attempting to cultivate cellulose degrading microbes and improve the hydrogen production rate. Because of the additional supplement of Napier Grass, the OLR in phase three was raised to 17.7 g-COD/L/day. However, only 6 % of electrons were transferred to hydrogen, and hydrogen yield was 3.74 mmol H_2/g-COD_{in}, which was slightly lower than phase two. In the later stage of phase three, the cellulose concentration in the effluent was almost the same as in the inflow. The cellulose removal ratio decreased to 1.5 %, which revealed that the microbes in the AnFB reactor still cannot utilize cellulose efficiently. The addition of 20 g/L Napier Grass only contributed to organic loading rate but little was converted into hydrogen. As a result, though the hydrogen production rate was the highest (up to 1.64 L H_2/L/day) among these three phases, the hydrogen yield was the lowest (only 3.74 mmol H_2/g-COD_{in}). From the aspect of the metabolites, the main volatile fatty acids (VFA) in the effluent were butyrate (13,000~17,000 mg/L) and acetate (2,300~33,00 mg/L) production. Remarkably, the high concentration lactate in KWSL was degraded remarkably (maximum removal efficiency was 98.3% in 2nd phase),and from the batch test of lactate degradation, it showed that lactate was degraded and hydrogen was produced, which the reaction was similar to "Lactate + 0.4 Acetate +0.7 H^+ → 0.7 Butyrate + 0.6 H_2 + CO_2 + 0.4 H_2O". Regarding the high lactate concentration in KWSL, it has great advantages for biohydrogenation in reducing the feed-back of alkali.

These could be the main pathways for biohydogenation in AnFB:

1. Lactate metabolism to produce hydrogen:

   Lactate + 0.4 HAc +0.7 H^+ → 0.7 HBu + 0.6 H_2 + CO_2 + 0.4 H_2O

2. Carbohydrate metabolism to produce hydrogen:

   $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2HAc$, $C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + HBu$

The existence of actate and butyrate could interact with the lactate metabolism and carbohydrate catabolism. If the butyrate produced from pathway (1) was excluded, which contributed to lactate metabolism, the ratio of HBu/HAc(mol:mol) was close to 1, infering that the stoichiometric equation of carbohydrate metabolism was close to : $3C_6H_{12}O_6 + 2H_2O \rightarrow 8H_2 + 6CO_2 + 2$ HAc + 2 HBu.

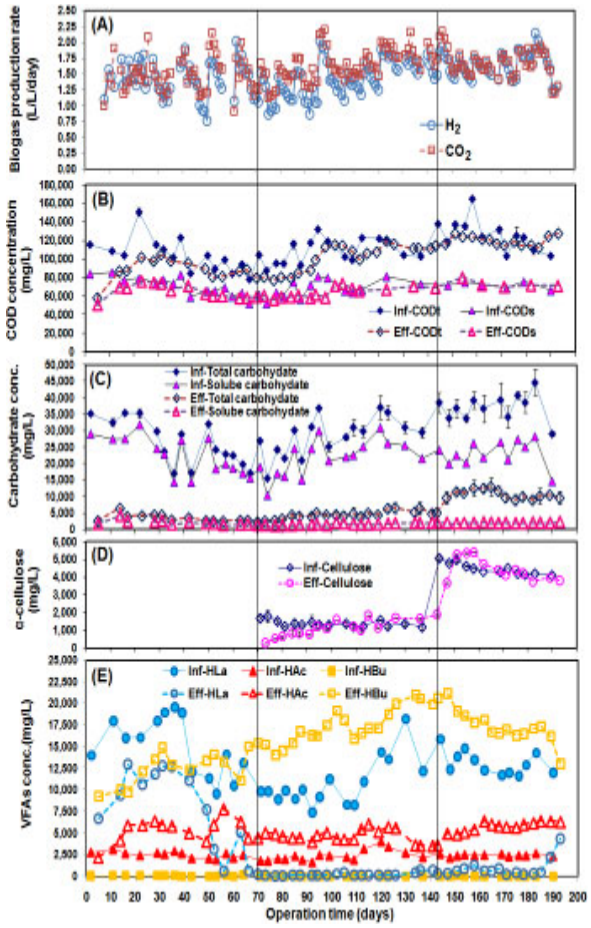

Figure 2: **Variation of (A) biogas production rate, (B) COD, (C) carbohydrate, (D) cellulose, and (E) volatile fatty acids in AnFB influent and effluent during operation time from run1 to run3.**

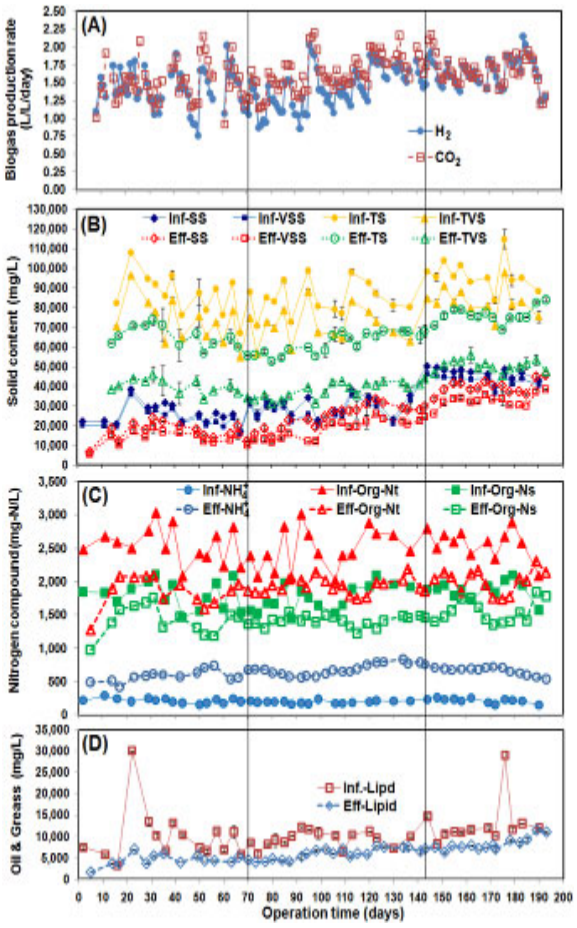

Figure 3: **Variation of (A) Biogas production rate, (B) solid contents, (C) nitrogen compounds, and (D) oil and grease in AnFB influent and effluent during operation time from run1 to run3.**

Table3: Characteristics of influent and effluent in Run 1 (n=15), Run 2 (n=16) and Run 3 (n=12).

Characteristic		Unit	Run 1 Influent	Run 1 Effluent	Run 2 Influent	Run 2 Effluent	Run 3 Influent	Run 3 Effluent
Solid content	TS	mg/L	86,800 ± 10,700	64,930 ± 8,160	84,670 ± 8,610	62,360 ± 5,390	96,760 ± 7,850	76,490 ± 4,000
	TVS	mg/L	72,900 ± 11,300	39,720 ± 6,600	70,880 ± 8,840	38,320 ± 4,050	83,220 ± 6,970	50,570 ± 2,860
	SS	mg/L	25,900 ± 5,330	17,860 ± 3,760	30,250 ± 4,770	25,250 ± 5,550	46,760 ± 2,970	39,770 ± 3,320
	VSS	mg/L	23,600 ± 5,050	14,390 ± 2,800	27,540 ± 4,520	18,660 ± 4,750	43,430 ± 2,640	32,830 ± 3,100
	VSS/SS	-	0.91 ± 0.05	0.81 ± 0.03	0.91 ± 0.05	0.78 ± 0.05	0.93 ± 0.01	0.83 ± 0.02
	TVS/TS	-	0.84 ± 0.04	0.61 ± 0.01	0.84 ± 0.03	0.61 ± 0.02	0.86 ± 0.01	0.66 ± 0.04
COD	CODt	mg/L	104,000 ± 18,000	90,400 ± 8,240	108,150 ± 13,100	101,700 ± 14,610	130,000 ± 17,270	119,710 ± 5,160
	CODs	mg/L	75,700 ± 8,240	69,000 ± 6,600	68,070 ± 9,210	64,018 ± 5,610	71,530 ± 3,620	73,020 ± 4,000
Carbohydrate	Total	mg-hexose/L	26,500 ± 6,340	3,240 ± 800	28,400 ± 5,900	4,880 ± 860	37,810 ± 3,300	10,705 ± 1,360
	Soluble	mg-hexose/L	23,500 ± 6,000	1,770 ± 470	21,900 ± 5,500	1,550 ± 340	23,860 ± 3,900	1,970 ± 110
	cellulose	mg-hexose/L	-	-	1,380 ± 180	1,280 ± 400	4,500 ± 330	4,420 ± 630
Protein	Org-N_{total}	mg-N/L	2,500 ± 270	1,890 ± 160	2,480 ± 310	1,940 ± 120	2,580 ± 220	2,010 ± 170
	Org-$N_{soluble}$	mg-N/L	1,800 ± 200	1,450 ± 180	1,760 ± 190	1,410 ± 80	1,880 ± 140	1,550 ± 170
	NH_4^+	mg/L	220 ± 40	590 ± 90	195 ± 20	690 ± 80	220 ± 40	665 ± 60
Lipid	Oil & Grease	mg/L	8,300 ± 3,060	4,710 ± 1,030	9,430 ± 1,810	6,440 ± 1,000	11,500 ± 1,630	8,300 ± 1,500
VFA	HLa	mg/L	14,500 ± 2,500	8,100 ± 4,550	10,700 ± 2,770	184 ± 190	13,100 ± 1,370	670 ± 550
	HAc	mg/L	2,500 ± 340	5,630 ± 1,070	2,360 ± 660	4,620 ± 750	2,420 ± 140	5,750 ± 540
	HPr	mg/L	205 ± 70	550 ± 170	100 ± 30	300 ± 70	100 ± 20	320 ± 40
	HBu	mg/L	54 ± 51	13,250 ± 1,230	N.D.	17,500 ± 2,110	N.D.	17,600 ± 1,400
Alcohols	EtOH	mg/L	3000 ± 800	3400 ± 720	3270 ± 1390	3450 ± 770	4120 ± 840	4220 ± 210

3.2 Microbial characterization with biomonitoring technology

A clone library was constructed to investigate the microbial diversity in AnFBR. There were three main groups of bacteria in this system. The most abundant microbe was similar to *Clostridium* sp. Strain Z6, which takes 37 % of total clone library. However, the similarity of the 16S rDNA fragment was only 93 %. It revealed that those bacteria enhanced by Napier Grass and KWSL were quite unique. About 18% of the clones were similar to *Thermoanaerobacterium thermosaccharolyticm* , which was reported as cellulose, starch, xylan, dextrin, xylose etc. degrading and hydrogen-producing bacteria (O-Thong et al., 2008 [8]). Finally, about 8 % of total clones were similar to *Lactobacillus plantarum* strain LMG 14188, and *Lactobacillus brevis* (2 % of total clone library). Those were isolated from Chinese sauerkraut fermentation broth and was related to lactate metabolism and cellulose hydrolysis. A diverse microbial community was revealled in this clone library, such as *Lactobacillus fermentum*, *Lactobacillus coryniformis*, *Lactobacillus crustorum*, *Lactobacillus curvatus*, *Lactobacillus sanfranciscensis*, *Lactobacillus vaccinostercus* and *Lactococcus lactis*. These diverse microbes were related to lactate metabolism and took up to 20 % of total clone library. This phenomenon in biohydrogen system is very unique; however, when kitchen waste was fed as substrate, this result was reasonable, because lactate accumulated very fast in kitchen waste. Lactate was reported to be utilized with acetate consumption by some species, such as *Clostridium beijerinckii*, *C. tyrobutyricum*, and *C. Acetobutylicum*. However, these Clostridia did not observed in this system. Accordingly, what/which microbes were responsible for lactate degrading is still unclear and further study is needed.

4 Conclusion

This study adopted an AnFB to conduct the hydrolysis of Napier Grass and co-digest with kitchen waste sieved liquid to produce hydrogen. KWSL could promote high loading to let microorganisms utilize the nutrients to produce hydrogen. In the first phase of the process, it was proved that KWSL as feeding substrate of AnFB could operate stably and was suitable for hydrogen production. In order to enhance the hydrolyzing ability of cellulosic materials, Napier Grass was added and increased to 20 g/L in the second and third phase.

During the operation period, a batch test was conducted to evaluate the cellulosic hydrolyzing potential. The result showed that microorganisms in an AnFB could not hydrolyze cellulose but could hydrolyze xylan. Lactate in the kitchen waste also contributed to the total hydrogen recovery and the stoichiometric equation of carbohydrate metabolism was close to: $3C_6H_{12}O_6 + 2H_2O \rightarrow 8H_2 + 6CO_2 + 2\ HAc + 2\ HBu$.

Three main groups of bacteria were identified by clone library construction of 16S rDNA, *Clostridium* sp., *Thermoanaerobacterium thermosaccharolyticm*, and a diverse *Lactobacillus* species.

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