**Effect of organic loading rate on dark fermentative hydrogen
production in the continuous stirred tank reactor and continuous
mixed immobilized sludge reactor from waste pastry hydrolysate**

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Abstract: Waste pastry (6%, w/v) was hydrolyzed by the produced glucoamylase and protease to obtain the glucose (19.8 g/L) and free amino nitrogen (179 mg/L) solution. Then, the effect of organic loading rate (OLR) ($8\text{--}40\text{ kgCOD}/(\text{m}^3\cdot\text{d})$) on dark fermentative hydrogen production in the continuous stirred tank reactor (CSTR) and continuous mixed immobilized sludge reactor (CMISR) from waste pastry hydrolysate was investigated and compared. The maximum hydrogen production rate of CSTR ($277.76\text{ mL}/(\text{h}\cdot\text{L})$) and CMISR ($320.2\text{ mL}/(\text{h}\cdot\text{L})$) were achieved at OLR of $24\text{ kgCOD}/(\text{m}^3\cdot\text{d})$ and $32\text{ kgCOD}/(\text{m}^3\cdot\text{d})$, respectively. Carbon recovery ranged from 75.2% to 84.1% in the CSTR and CMISR with the balance assumed to be converted to biomass. One gram waste pastry could produce 0.33 g (1.83 mmol) glucose which could be further converted to 79.24 mL (3.54 mmol) hydrogen in the CMISR or 91.66 mL (4.09 mmol) hydrogen in the CSTR. This is the first study which reports dark fermentative hydrogen production from waste pastry.

Keywords: dark fermentative hydrogen production; waste pastry hydrolysate; organic

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loading rate; continuous stirred tank reactor; continuous mixed immobilized sludge reactor

1. Introduction

Because of the shortage of fossil fuels and environmental problems, the international community has shown an increasing interest in clean and renewable energies which do not have negative effects on environment (Ma et al., 2016; Soltani et al., 2015).

Hydrogen is being focused on as a promising alternative to fossil fuels because it does not discharge contaminants (Panda et al., 2016). Besides the energy density of hydrogen is 10.9 kJ/L which is 2.75 times higher than that of conventional fossil fuels (Singh et al., 2015).

Hydrogen production can be divided into physiochemical and biological methods (Nong et al., 2015). Physiochemical hydrogen production (such as electrolysis and carbohydrate pyrolysis) requires not only enormous production costs, but also greater consumption of the fossil fuels (Clark et al., 2015). Biological hydrogen production seems to be more attractive because organic waste materials can be used as substrate and the hydrogen-producing system can be operated under low temperature and pressure conditions (Sivagurunathan et al., 2016a). Among the biological hydrogen production, dark fermentation is an effective way in terms of the absence of light and rapid hydrogen production rate (Ghanbarian et al., 2016).

A wide variety of raw materials can be used for dark fermentative hydrogen production, including organic waste or wastewater (Redondas et al., 2012). Waste pastry,

which is one of the most common bakery wastes, is reported to be 1.3 million tons per year in China (Han et al., 2016). Waste pastry could be a promising substrate for dark fermentative hydrogen production because it could not only lower the cost of the feedstock, but also deal with the waste recycling. However, the nutrients stored in the waste pastry are in the form of macromolecules (such as starch and protein) which have to be hydrolyzed into micromolecules (such as glucose and free amino nitrogen, FAN) before utilized by microorganisms for hydrogen production (Alibardi and Cossu, 2016). So, the hydrolysis is considered to be limiting step for dark fermentative hydrogen production from waste pastry (De Gioannis et al., 2013). Furthermore, the nutrients of the waste pastry are in the solid phase which inhibits the nutrient conversion efficiency for dark fermentative hydrogen production (Tawfik et al., 2011; Zhou et al., 2013). For these reasons, it is difficult to directly use the cost-effective waste pastry as substrate for dark fermentative hydrogen production. It is reported that physiochemical treatment could accelerate the liquification and hydrolysis of organic solid wastes, while the inhibitory (e.g. furfural) for the subsequent dark fermentative hydrogen production could also be generated. Enzymatic hydrolysis, which could effectively release the micromolecules from the organic solid wastes, could be a promising pretreatment for dark fermentative hydrogen production from waste pastry. However, information about dark fermentative hydrogen production from waste pastry hydrolysate was limited.

Dark fermentative hydrogen production has been performed in a variety of bioreactors, including suspended and attached microbial growth systems (Sivagurunathan et al., 2016b). The conventional continuous stirred tank reactor (CSTR)

is the most common suspended microbial growth system and has been widely used to produce hydrogen (Fang and Liu, 2002). In a conventional CSTR, the solid retention time is the same as the hydraulic retention time (HRT) which would limit the biomass concentration in the mixed liquor and therefore reduce the substrate utilization and hydrogen production (Wu et al., 2008; Zhu et al., 2010). However, the addition of immobilized sludge in the bioreactor (attached microbial growth system) could prevent the biomass from being washed out at high dilution rate (Barros et al., 2011; Lin et al., 2006). Recently, we have successfully developed a novel continuous mixed immobilized sludge reactor (CMISR) which could significantly enhance hydrogen production rate by increasing the retention of hydrogen-producing sludge (Han et al., 2012). However, direct comparison of attached microbial growth system (CMISR) with conventional suspended microbial growth system (CSTR) for hydrogen production, especially using waste pastry hydrolysate as substrate, is not yet available in the literature.

Therefore, the objective of this study was to examine the effect of organic loading rate (OLR) on the performance of hydrogen production in a novel CMISR and a conventional CSTR from waste pastry hydrolysate. It aimed at identifying preferable operation condition for suspended and attached microbial growth systems to achieve better hydrogen production performance, thereby promoting dark fermentative hydrogen production for commercial application. The proposed enzymatic hydrolysis pretreatment could also provide a potential way for industrial hydrogen production from high-starch containing raw materials.

2. Material and methods

2.1 Microorganisms and enzymatic hydrolysis

The waste pastry used in this study was provided by the local Wumei Supermarket. Table 1 showed the composition of the waste pastry which was analyzed according to the Standard Method (APHA, 2005).

The glucoamylase and protease used in this study were produced by *Aspergillus awamori* and *Aspergillus oryzae* via solid state fermentation. An amount of 15 g waste pastry was added to a Petri dish with 1 mL of *A. awamori* (4.2×10^5 spores/mL) or *A. oryzae* (1.6×10^5 spores/mL) inoculating on the surface of the waste pastry. The mixed samples were then cultured in the incubator at 30 °C for 5 days to get the solid meshes of glucoamylase and protease.

The sludge used in this study was collected from a local municipal wastewater treatment plant and screened by a sieve (diameter: 2 mm) to eliminate large particulate materials. The sludge was heat pretreated (30 min at 100 °C) to inhibit the hydrogen consumers and harvest the spore-forming microorganisms.

The enzymatic hydrolysis of waste pastry was carried out in two 2.5 L bioreactors which were equipped with automatic temperature controller and stirrers. The produced enzymes (glucoamylase and protease) were used to hydrolyze waste pastry (6%, w/v) to generate waste pastry hydrolysate which was rich in glucose and FAN. The enzymatic hydrolysis was operated at 55 °C throughout the experiment with agitation speed of 500 rpm. Samples were taken every hour to analyze the glucose and FAN production. The

resultant broth was centrifuged at 10,000 rpm for 30 min and filtered by Whatman No. 1 filter paper to obtain the waste pastry hydrolysate which was used as substrate for subsequent dark fermentative hydrogen production.

2.2 Experimental set-up of CSTR and CMISR

Dark fermentative hydrogen productions from waste pastry hydrolysate were carried out in the CSTR and CMISR with the same working volume of 5.6 L. Nitrogen gas was purged into the bioreactors for 20 min to attain anaerobic condition. Operating temperature and agitation speed of the bioreactors were 35 °C and 250 rpm, respectively. Fermentation pH was controlled above 4 by using 5 M NaHCO₃ solution. The influent flow rate was controlled by a feed pump to regulate the HRT at 6 h. The biomass activity of the CSTR and CMISR was 73% (VSS/SS). The generated biogas was collected by the water displacement method through the port designed at the top of the reactors.

In order to investigate the effect of OLR on dark fermentative hydrogen production in the CSTR and CMISR, the waste pastry hydrolysate was diluted by distilled water to certain OLR (8-40 kgCOD/(m³·d)). The amount of inorganic nutrients was proportional to the influent OLR (e.g., if the influent OLR was doubled, the nutrient concentration was also doubled).

The reactor performance (biogas production, composition in hydrogen and soluble microbial products, SMPs) was monitored daily throughout the experimental period. Complete characterization of the reactor performance took place at each steady state.

Steady-state condition was considered to be achieved when the biogas production and the proportion of SMPs were consistent within 5% for three consecutive days.

2.3 Analytical methods

The glucose and FAN, which were produced in the waste pastry hydrolysate, were measured according to our previous study (Han et al., 2015). Determinations of dissolved chemical oxygen demand, suspended solid (SS) and volatile suspended solid (VSS) were carried out according to Standard Methods (2005). The mixed liquor samples were centrifuged at $10,000 \times g$ for 15 min at 4 °C and the supernatant was filtered through a 0.45 µm membrane filter. The hydrogen production rate (HPR) could be calculated according to Eq. (1).

$$\text{HPR mL}/(\text{h} \cdot \text{L}) = \text{Hydrogen yield (mL)} / (\text{Running time (h)} \times \text{Working volume (L)}) \quad (1)$$

Biogas was quantified with a gas chromatography (SC-7, Shandong Lunan Instrument Factory) equipped with a thermal conductivity detector (TCD) and a stainless steel column (2m×5mm) filled with Porapak Q (50-80 meshes). Nitrogen was used as the carrier gas at a flow rate of 30 mL/min. A dose of injected sample was 0.5 mL each time. Biogas samples were compared to five standard levels of pure gas injections. Based on the percentage of hydrogen in biogas, the hydrogen yield could be calculated.

Detection of soluble microbial products (SMPs) in the fermentation solution was analyzed by another gas chromatograph (GC112, Shanghai Anal. Inst.Co.) using a flame ionization detector (FID). A 2-m stainless steel column was packed with the supporter GDX-103 (60-80 meshes). The temperatures of the injection port, oven and detector

were 220 °C, 190 °C and 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min.

3. Results and discussion

3.1 Enzymatic hydrolysis of waste pastry

In this study, the produced enzymes (glucoamylase and protease) were used to hydrolyze the waste pastry (6%, w/v) to produce the waste pastry hydrolysate. The yield of glucose and FAN from the waste pastry hydrolysis was shown in Fig. 1. It was observed that both glucose and FAN increased with time and reached the saturation concentrations within 24 h. The final glucose and FAN concentration derived from the waste pastry hydrolysate were 19.8 g/L and 179 mg/L, respectively. Linear regressions results show that the correlation between glucose production (y) and time (x) could be expressed as $y = 5.493\ln(x) + 1.854$ ($R^2 = 0.97$). And, the correlation between FAN production (y) and time (x) could be expressed as $y = 44.292\ln(x) + 31.423$ ($R^2 = 0.95$).

As shown in Table 2, the glucose production and glucose yield obtained from this study were comparable to the previous study which used as food waste as substrate (Han et al., 2015). This is because the starch content in the waste pastry and food waste were similar. However, it was observed that the conversion ratio of waste pastry (73.9%) was lower than that of food waste (85.6%) probably due to the smaller physical size of the food waste. The produced waste pastry hydrolysate was rich in carbon and nitrogen sources which could be a good substrate for dark fermentative hydrogen production.

3.2 Effect of OLR on the performance of dark fermentation in the CSTR and CMISR

3.2.1 Hydrogen production rate with different OLRs in the CSTR and CMISR

Hydrogen production rate (HPR) is a key criterion to evaluate the performance of fermentative hydrogen-producing system. The HPRs at different OLR conditions in the CSTR and CMISR were shown in Fig. 2. For OLRs between 8 and 24 kgCOD/(m³·d), the HPR increased with increasing OLR in both systems. However, the HPRs in the CSTR were higher than the data obtained from the CMISR due to the higher mass transfer efficiency (Hwang et al., 2011). The optimal HPR in the CSTR was 277.76 mL/(h·L) occurred at OLR of 24 kgCOD/(m³·d). However, the variations of HPR showed completely different trends when the OLR further increased to 32 kgCOD/(m³·d). The HPR decreased to 161.9 mL/(h·L) in the CSTR because of the wash-out of the biomass and low biomass activity under high OLR (Fig. 3). Compared to the CSTR system, the HPR in the CMISR increased and got the maximum value of 320.2 mL/(h·L) since the CMISR system could effectively maintain high biomass concentration under high OLR (Fig. 3). When the OLR reached the maximum value of 40 kgCOD/(m³·d), the HPR further decreased to 72.3 mL/(h·L) in the CSTR due to the lowest biomass concentration and activity (Fig. 3). However, it was interesting to find that the HPR did not increase with OLR further increased to 40 kgCOD/(m³·d), although the CMISR could keep high biomass concentration and activity at high OLR. This is probably because the high OLR (40 kgCOD/(m³·d)) could also have a direct

negative effect on the hydrogen-producing microorganisms in the CMISR. There is no big difference of hydrogen contents (32.4%-37.8%) in the biogas with various OLR in the CSTR and CMISR.

Table 3 described the literature and compared the use of glucose as substrate for dark fermentative hydrogen production in the suspended and attached microbial growth systems. Fang and Liu (2002) obtained the maximum HPR of 540 mL/(h·L) at OLR of 28 kgCOD/(m³·d) in the CSTR. Shen et al. (2009) showed the maximum HPR of 170 mL/(h·L) in the AnMBR with OLR of 22 kgCOD/(m³·d). In this study, the maximum HPR in the CSTR and CMISR were 277.76 mL/(h·L) and 320.2 mL/(h·L) which were comparable to the reported studies. However, the HPRs obtained this study seemed to be more attractive because the substrate used in this study was waste pastry hydrolysate.

3.2.2 Biomass concentration and activity with different OLRs in the CSTR and CMISR

For the same OLR, the biomass concentration (g/L) and activity (VSS/SS, %) were higher in the CMISR than in the CSTR (Fig. 3). In the CSTR, biomass concentration increased from 4.55 to 5.85 g/L and biomass activity were stable at 74%-77.7% when the OLR increased from 8 to 24 kgCOD/(m³·d). However, the biomass concentration and activity decreased significantly to 1.72 g/L and 34.2% when OLR further increased to 40 kgCOD/(m³·d). Compared to the CSTR, the biomass concentration in the CMISR similarly increased when the OLR increased from 8 to 24 kgCOD/(m³·d), and increased further with OLR increasing to 32 kgCOD/(m³·d), but then slightly decreased as the OLR increased to 40 kgCOD/(m³·d). The biomass activity in the CMISR ranged from

74.6% to 79.8% with all OLR conditions (8-40 kgCOD/(m³·d)). The results obtained from this study indicated that the CMISR system could effectively reduce the wash-out of the biomass and keep stable of biomass activity compared to the CSTR system, especially at high OLRs (32-40 kgCOD/(m³·d)).

3.2.3 Soluble microbial products with different OLRs in the CSTR and CMISR

The produced soluble microbial products (SMPs), which included volatile fatty acids (VFAs) and ethanol, were described in Table 4. The SMPs increased when the OLR increased from 8 to 24 kgCOD/(m³·d) in both systems. Ethanol and acetic acid were the major SMPs. However, the SMPs decreased in the CSTR when the OLR further increased to 40 kgCOD/(m³·d) due to wash-out of biomass and low biomass activity (Fig. 3). The percentage of ethanol and acetic acid in the SMP decreased to 67.75%-69.5%. The propionic acid increased from 0.34 mmol/L to 3.08 mmol/L and HPR decreased from 277.76 mL/(h·L) to 72.35 mL/(h·L) as OLR increased from 24 kgCOD/(m³·d) to 40 kgCOD/(m³·d). This result corresponded with Wang's (2006) study that the production of propionic acid exhibited a low hydrogen production capability (Reaction 1).

However, it was observed that the SMPs in the CMISR continuously increased as OLR increasing to 32 kgCOD/(m³·d) and slightly decreased when the OLR was 40 kgCOD/(m³·d). The ethanol and acetic acid were the predominant products regardless of OLRs. The SMPs indicated that the cultural environment was favorable for hydrogen

production at different OLRs because ethanol was the predominant product in the most efficient hydrogen-producing system (Reaction 2) (Ren et al., 2007).

The correlation between hydrogen and EtOH producing pathways could also be observed from Fig. 4. The hydrogen and EtOH productions were in general proportionately correlated regardless of the different OLRs in the CSTR and CMISR. Linear regression results show that the correlation between HPR (y) and EtOH production (x) could be expressed as $y_1 = 13.427x_1 - 46.826$ ($R^2 = 0.958$) in the CSTR and $y_2 = 14.788x_2 - 79.692$ ($R^2 = 0.961$) in the CMISR, respectively.

3.2.4 Glucose utilization and carbon recovery with different OLRs in the CSTR and CMISR

The variations of glucose utilization and carbon recovery with different OLRs in the CSTR and CMISR were shown in Table 5. It was found that the glucose utilization reached 80.2-94.2% in the CSTR when the OLR increased from 8 kgCOD/(m³·d) to 24 kgCOD/(m³·d). Then, the glucose utilization decreased to 23.8-45.6% with OLR further increased to 32-40 kgCOD/(m³·d) due to the wash-out of biomass and inhibition of high substrate concentration. Compared to the CSTR, the glucose utilization in the CMISR got 73.2-88.5% when the OLR increased from 8 kgCOD/(m³·d) to 32 kgCOD/(m³·d). However, it was interesting to observe that the lowest glucose utilization of 53.7% was achieved at OLR of 40 kgCOD/(m³·d) probably due to the inhibition of hydrogen-producing microorganisms caused by the high substrate concentration.

According to the consumed carbon and carbon recovered in SMP and carbon dioxide, the carbon recovery could be calculated. In this study, it was observed that carbon recovery ranged from 75.2% to 84.1% in the CSTR and CMISR with the balance assumed to be converted to biomass.

3.2.5 Carbon and material balance with different OLRs in the CSTR and CMISR

Table 6 indicated the carbon balance of dark fermentative hydrogen production from waste pastry in the CSTR and CMISR with the optimal OLRs (CSTR: 24 kgCOD/(m³·d); CMISR: 32 kgCOD/(m³·d)). The carbon content in the waste pastry included 26.1% for undigested waste pastry and 73.9% used by microorganism for biogas and SMP production. The ethanol and carbon dioxide accounted for the largest parts of consumed carbon because they were the main soluble and gaseous products for dark fermentative hydrogen production from waste pastry in the CSTR and CMISR. Furthermore, the sludge percentage of CMISR (17.52%) was higher than CSTR (14.3%) which indicated that the CMISR could be favorable to growth of hydrogen-producing microorganisms. This is an important finding in terms of dark fermentative hydrogen production from various bioreactors.

According to the enzymatic hydrolysis of waste pastry (Fig. 1) and dark fermentative hydrogen production from waste pastry hydrolysate (Fig. 2), it could be calculated that 1 g waste pastry could produce 0.33 g (1.83 mmol) glucose in the waste pastry hydrolysate. Then, it could be further converted to 79.24 mL (3.54 mmol) hydrogen in the CMISR or 91.66 mL (4.09 mmol) hydrogen in the CSTR (Fig. 5). Although the

CMISR could get higher HPR at high OLR compared to the CSTR (Fig. 2), the substrate conversion to hydrogen in the CSTR is higher than the CMISR probably because the higher mass transfer efficiency could be achieved in the CSTR and more glucose was utilized for biomass production in the CMISR (Table 6). To our knowledge, this is the first study which reports dark fermentative hydrogen production from waste pastry.

4. Conclusions

Waste pastry (6%, w/v) could be hydrolyzed by glucoamylase and protease to obtain glucose (19.8 g/L) and free amino nitrogen (179 mg/L) solution with glucose yield of 0.33 g/g pastry and conversion ratio of 73.9%. At lower OLR (8-24 kgCOD/(m³·d)), the HPRs obtained in the CSTR were higher than in the CMISR due to the higher mass transfer efficiency. However, the HPRs in the CSTR were lower than the CMISR at higher OLR (32-40 kgCOD/(m³·d)) because the wash-out of biomass happened in the CSTR. The CMISR could be favorable to growth of hydrogen-producing microorganisms.

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Figure legends

Table 1 Composition of waste pastry used in this study (per 100 g).

Table 2 Comparison of theoretical glucose production and conversion rate from food waste hydrolysates.

Table 3 Comparison of hydrogen production rates by using the glucose as substrate in the literature and this experiment.

Table 4 Soluble microbial products with various organic loading rates in the CSTR and CMISR.

Table 5 Carbon recoveries with various organic loading rates in the CSTR and CMISR.

Table 6 Carbon balance of dark fermentative hydrogen production from waste pastry in the CSTR and CMISR with the optimal organic loading rates (CSTR: 24 kgCOD/(m³·d); CMISR: 32 kgCOD/(m³·d)).

Fig. 1. Glucose and FAN productions in the enzymatic hydrolysis of waste pastry.

Fig. 2. Hydrogen production rates with various organic loading rates in the CSTR and CMISR.

Fig. 3. Biomass concentration and activity with various organic loading rates in the CSTR and CMISR.

Fig. 4. The correlation between hydrogen production rate and ethanol production in the CSTR and CMISR.

Fig. 5. Material balance of dark fermentative hydrogen production from waste pastry in the CSTR and CMISR with the optimal organic loading rates (CSTR: 24 kgCOD/(m³·d); CMISR: 32 kgCOD/(m³·d)).

1 **Table 1** Composition of waste pastry used in this study (per 100 g).

Component	Value (g)	Component	Value (g)
Moisture	32.4 ± 1.6 g	Carbohydrate	30.2 ± 1.7
Starch (dry basis)	40.2 ± 2.3 g	Protein (N × 5.7)	6.8 ± 0.4 g
		(dry basis)	
Phosphorous (dry	1.3 ± 0.1 g	Ash (dry basis)	2.1 ± 0.3 g
basis)			

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Table 2 Comparison of theoretical glucose production and conversion rate from food waste hydrolysates.

Substrate (g)	Glucose production (g)	Glucose yield (g/g substrate)	Starch content (%)	Starch (g)	Theoretical glucose production (g)	Conversion ratio (%)	References
60 (Pastry)	19.8	0.33	40.2	24.12	26.8	73.9	This study
60 (Food waste)	22.89	0.382	40.6	24.36	27.06	85.6	Han et al., 2015

Table 3 Comparison of hydrogen production rates by using the glucose as substrate in the literature and this experiment.

Reactor type	Systems	HRT (h)	OLR (kgCOD/(m ³ ·d))	HPR (mL/(h·L))	References
CSTR	Suspended	6	28	540	Fang and Liu, 2002
AFBR	Immobilized	20-10	16	125	Peinter et al., 2010
AnMBR	Immobilized	8	22	170	Shen et al., 2009
UASB	Suspended	5.3	-	480	Hu and Chen, 2007
CMISR	Immobilized	6	32	320.2	This study
CSTR	Suspended	6	24	277.76	This study

1 **Table 4** Soluble microbial products with various organic loading rates in the CSTR and
2 CMISR.

OLR	8		16		24		32		40	
(kgCOD/(m ³ ·d))										
Operational system	CSTR	CMISR	CSTR	CMISR	CSTR	CMISR	CSTR	CMISR	CSTR	CMISR
EtOH (mmol/L)	10.35	8.38	18.08	14.02	23.5	21.84	14.09	27.7	8.57	24.9
HAc (mmol/L)	5.9	5.6	9.8	8.28	13.85	11.78	8.6	16.12	5.25	14.81
HBu (mmol/L)	1.35	2.3	2.6	2.86	5.14	1.95	4.9	3.43	3.5	3.14
HPr (mmol/L)	0.99	0.24	0.57	0.21	0.34	0.39	5.08	0.38	3.08	0.38
TVFA (mmol/L)	8.24	8.14	12.97	11.35	19.33	14.12	18.58	19.93	11.83	18.33
SMP (mmol/L)	18.59	16.52	31.05	25.37	58.83	35.96	32.67	47.63	20.4	43.23
(EtOH + HAc)/SMP (%)	87.4	84.6	89.7	87.8	90.68	93.4	69.5	92	67.7	91.8

3 EtOH: Ethanol; HAc: Acetic acid; HBu: Butyric acid; HPr: Propionic acid; TVFA (Total

4 Volatile Fatty Acid): HAc + HBu + HPr; SMP (Soluble microbial product): TVFA +

5 EtOH

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1 **Table 5** Carbon recoveries with various organic loading rates in the CSTR and CMISR.

Operational	CSTR					CMISR				
system										
OLR	8	16	24	32	40	8	16	24	32	40
(kgCOD/(m ³ ·d))										
Glucose	94.2	87.6	80.2	45.6	23.8	88.5	83.1	76.4	73.2	53.7
utilization (%)										
Consumed	1.884	3.504	4.812	3.648	2.38	1.77	3.324	4.584	5.856	5.37
glucose, g/L	(10.47)	(19.47)	(26.73)	(20.27)	(13.24)	(9.83)	(18.46)	(25.46)	(32.53)	(29.83)
(mmol/L)										
Consumed	64.02	116.82	160.38	121.62	79.42	58.98	110.76	152.76	195.18	178.9
carbon (mmol/L)										
Carbon dioxide	12.79	25.15	33.14	15.25	10.43	11.72	19.96	30.06	44.6	41.38
(mmol/L)										
Carbon	53.39	95.2	129.42	95.47	61.31	49.6	90.8	124.7	148.9	134.5
recovered in										
SMP and carbon										
dioxide										
(mmol/L)										
Carbon recovery	83.4	81.5	80.7	78.5	77.2	84.1	80.2	78.4	76.3	75.2
(%)										

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Table 6 Carbon balance of dark fermentative hydrogen production from waste pastry in the CSTR and CMISR with the optimal organic loading rates (CSTR: 24 kgCOD/(m³·d); CMISR: 32 kgCOD/(m³·d)).

Percentage (%)	CSTR	CMISR
Ethanol	18.42	16.93
Acetic acid	10.88	9.85
Butyric acid	4.03	2.1
Propionic acid	0.27	0.24
Carbon dioxide	26	27.26
Sludge and others	14.3	17.52
Undigested solid	26.1	26.1
Total	100	100

Fig. 1. Glucose and FAN productions in the enzymatic hydrolysis of waste pastry.

Fig. 2. Hydrogen production rates with various organic loading rates in the CSTR and CMISR.

Fig. 3. Biomass concentration and activity with various organic loading rates in the CSTR and CMISR.

Fig. 4. The correlation between hydrogen production rate and ethanol production in the CSTR and CMISR.

Fig. 5. Material balance of dark fermentative hydrogen production from waste pastry in the CSTR and CMISR with the optimal organic loading rates (CSTR: 24 kgCOD/(m³·d); CMISR: 32 kgCOD/(m³·d)).