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Production of biohythane from food waste via an integrated system of continuously stirred tank and anaerobic fixed bed reactors

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ABSTRACT

The continuous production of biohythane (mixture of biohydrogen and methane) from food waste using an integrated system of a continuously stirred tank reactor (CSTR) and anaerobic fixed bed reactor (AFBR) was carried out in this study. The system performance was evaluated for an operation period of 200 days, by stepwise shortening the hydraulic retention time (HRT). An increasing trend of biohydrogen in the CSTR and methane production rate in the AFBR was observed regardless of the HRT shortening. The highest biohydrogen yield in the CSTR and methane yield in the AFBR were 115.2 (±5.3) L H_2/kgVS_{added} and 334.7 (±18.6) L CH_4/kgCOD_{added}, respectively. The AFBR presented a stable operation and excellent performance, indicated by the increased methane production rate at each shortened HRT. Besides, recirculation of the AFBR effluent to the CSTR was effective in providing alkalinity, maintaining the pH in optimal ranges (5.0–5.3) for the hydrogen producing bacteria.

1. Introduction

Hythane® refers to a mixture of hydrogen and methane that was originally trademarked by the Hydrogen Components, Inc. (HCI) company (Eden, 2010). As hydrogen and methane have an independent broad commercial interest, hythane is recognized as a highly valued fuel mainly for vehicles and combustion engines (Dahiya et al., 2015; Sen et al., 2016). Several studies have reported that the application of hythane would be paramount in a positive shift of the future society towards a green economy, owing to its clean nature, high fuel efficiency, improved heat efficiency, and capability to make engines easy to ignite with less input energy (Roy and Das, 2015; Mamimin et al., 2015; Liu et al., 2013). Hythane does not only support electrical energy, but it has also been commercialized as vehicle fuel by some countries including USA and India (Cavinato et al., 2016) and received much attention from various individual companies such as Volvo and Fiat (Liu et al., 2013).

In recent years, biologically derived hythane, i.e. biohythane, has been a renewed research focus, since it involves the anaerobic digestion (AD) process and abundant organic rich wastes (Sen et al., 2016; Mamimin et al., 2015; Lee et al., 2010). Biohythane production is mainly achieved via a two-stage AD process, through simultaneous production of biohydrogen and methane with a gaseous composition between 10 and 15% and 60 and 70%, respectively (Liu et al., 2013). The first stage process is commonly known as the acidogenic or dark fermentation (DF) process, mediated by fermentative bacteria that break down organic matter into primarily H_2, CO_2 and soluble metabolic products (Ghimire et al., 2015a,b). In the second stage, archaea groups (acetogens and methanogens) convert the spent organic rich supernatant from the first stage into methane and CO_2 gas (Ariunbaatar et al., 2015; Aydin et al., 2015). Such a two-stage system enables to maintain specific environmental conditions for each microbial group in physically separated reactors (Mamimin et al., 2015; Ariunbaatar et al., 2015).

The disposal of food waste (FW) is one of the major global concerns because of its constant increasing generation rate and causing severe environmental problems (Jiang et al., 2013). According to Food and Agricultural Organization (FAO), about 1.3 billion tons per year, one third of the food produced globally for human consumption, are lost or wasted along with the supply chain, from production to consumption (FAO, 2012). Nonetheless, FW has been considered as an economical source for biofuel production due to its fundamental characteristics such as wide availability, high carbohydrate content and being a renewable source (Cavinato et al., 2016; Lee et al., 2010). Hence, utilization of the large amount of FW produced globally for biohythane production has become a promising approach to valorize the waste, solving the disposal problems and helping in the reduction of greenhouse gas emissions whilst replacing the fossil-based fuels (Sen et al., 2016). Several studies have focused on biohythane production from FW in recent years (Chinellato et al., 2013; Cavinato et al., 2012; Kobayashi et al., 2012; Chu et al., 2012; Lee et al., 2010).

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Technologically, different types of reactor configurations have been applied for the two-stage AD system, aiming at increased biomass retention, operational simplicity and reduced energy consumption (Van Lier et al., 2015; Carrillo-Reyes et al., 2016). The DF stage proceeds quicker than the methanogenic step, with relative higher bacterial growth rates and hence less environmental sensitivity (Ma et al., 2013; Dahiya et al., 2015). Consequently, continuous DF processes have been carried out mostly using a CSTR system (Ghimire et al., 2015b; Kobayashi et al., 2012; Angeriz-Campoy et al., 2015). Moreover, the CSTR has also the advantage of a simple design and select the fermentative bacteria from mixed cultures by washing out the other AD biomass, such as methanogens (Cavinato et al., 2016). The second stage is characterized by the presence of methanogenic archaea that are slow growing and vulnerable microbial groups, which are responsible for catalyzing the key and final stage of the AD process (Aydin et al., 2015). Accordingly, a number of challenges in operating the methanogenic reactor have been widely documented, foremost of which were instability and lower methane yields (Khemkhao et al., 2016; Sen et al., 2016; Parawira et al., 2006). Several studies have associated this poor performance to the difficulty of maintaining a sufficiently high concentration of methanogenic archaea in the reactor due to their slow-growth rate at the one hand and wash-out on the other hand (Schmidt et al., 2014; Ziganshin et al., 2016; Khemkhao et al., 2016; Wang et al., 2010; Ma et al., 2013).

Anaerobic fixed bed reactors (AFBRs) are practical alternatives for retaining the slow growing methanogenic archaea through facilitating their immobilization on an inert solid carrier material by providing a larger surface area (Van Lier et al., 2015). The AFBR has been applied successfully to treat various industrial and municipal wastewaters during the last two decades (Carrillo-Reyes et al., 2016; Karadag et al., 2015; Barca et al., 2015; Van Lier et al., 2015). The AFBR configuration is attractive with its high loading capacity, concentrated biomass, resistance to hydraulic or organic shocks, higher treatment efficiency and no requirement of mechanical mixing (Karadag et al., 2015). As a result, the necessitated reactor size and concomitant capital costs of the AD process are distinctly reduced (Van Lier et al., 2015). However, the system is sensitive for high solid content wastewaters that create clogging of carrier materials and reduce the stability of the process (Karadag et al., 2015).

To date, even though studies concentrated on biohythane production have shown progress, the common practice is still on individual biohydrogen or methane production in separate reactor systems. Besides, the majority of the experiments on two-stage AD systems for biohythane production have been performed on a CSTR configuration for both stages, thus resulting in a lower biohydrogen and methane production and unstable process operation. For instance, Cavinato et al. (2012) reported that the biohydrogen and methane production in two-stage AD of FW has a lack of process stability after 70 days of operation caused by a high ammonia concentration. Therefore, the optimal reactor configuration for higher biohythane production and stable process operation needs to be determined during prolonged operation experiments.

The objectives of this work were, therefore, i) to demonstrate the prolonged continuous biohythane production from FW using an integrated CSTR and AFBR two-stage system, (ii) to evaluate the performance of a biofilm-based methanogenic AFBR when shortening the HRT and (iii) to evaluate the overall system performance.

2. Material and methods

2.1. Substrate and inoculum

FW was synthetically prepared based on its characteristics of most European Countries (Ariunbaatar et al., 2015). The composition was 79% vegetables and fruits; 5% cooked pasta and rice; 6.0% bread and bakery; 8.0% meat and fish; and 2.0% dairy products (on wet basis). The FW was crushed with an electronic blender and stored in a refrigerator at −20 °C until use. The mixed culture of digested sludge was obtained from a full scale AD plant located in Salerno (Italy), treating buffalo manure and dairy wastewater at mesophilic conditions. The mixed culture was used to inoculate both biohydrogen and methane production processes. The main physico-chemical characteristics of the FW and inoculum are given in Table 1.

2.2. Reactor configuration

The reactor configurations were a CSTR and AFBR for the first and second stage, respectively (Supplementary Fig. S1). The CSTR was made up of a borosilicate serum glass bottle (SIMAX/VWR brand), having a working volume of 1.6 L. Whereas the AFBR was a cylindrical glass-made column with a working volume of 1.3 L, with an internal diameter of 24 mm and a height of 35 cm). Anox-Kaldness-K1 (Veolia, Sweden) was used as a biofilm carrier material for the AFBR. The Kaldness-K1 has a specific weight of 145 kg/m^3 and a specific surface area of 500 m^2/m^3. The temperature of the reactor was kept constant at 55 (±2) °C and 37 (±2) °C for the CSTR and AFBR, respectively, using a thermal water bath allowing hot water recirculation from a thermostatically controlled reservoir. The CSTR was continuously mixed at 300 rpm using a magnetic stirrer (Stuart™ stirrer-SB162, Sigma-Alderic®), while the AFBR was intermittently mixed in up-flow mode at a liquid flow velocity of 125 mL/min using a peristaltic pump (505S, Watson and Marlow, Falmouth, England), as described by Karadag et al. (2015). Both reactors were wrapped with a black plastic cover to maintain dark conditions.

The reactors were equipped with a feeding-effluent withdrawing port, and a gas line connected to a gas measuring system working on the water displacement technique (Esposito et al., 2012). The biogas produced from the CSTR was led to pass through acidic water (2.0% HCl) to reduce the CO_2 gas solubility as described by Ghimire et al. (2015b), while the biogas produced by the AFBR passed through a NaOH solution (12%) in order to scrub the CO_2 gas as described by Esposito et al. (2012). In both reactors, the gas production was monitored daily. The produced biohydrogen and methane were normalized to standard temperature and pressure (STP).

Table 1
Main physico-chemical characteristics of FW and inoculum.

Parameter	Unit	FW	Inoculum
pH	–	5.0 ± 0.4	7.9 ± 0.04
TS	% wet basis	22.2 ± 2.3	3.18 ± 1.1
TVS	% dry basis	23 ± 1.6	2.1 ± 1.7
TCOD	g/kg	397.4 ± 6.1	51.3 ± 4.8
TKN	g/kg	6.1 ± 1.4	5.6 ± 1.7
Total Alkalinity	mg/L as $CaCO_3$	–	2466.1 ± 5.7
N-NH_4^+	mg/L	–	306 ± 2.9
Total carbohydrate	g/kg	134 ± 1.9	1.1 ± 0.9
Total protein	g/kg	31 ± 2.5	12.9 ± 1.5
C:N ratio	–	65.0 ± 4.4	9.2 ± 2.8

2.3. Reactor start-up and operational conditions

The start-up of the AFBR was previously performed using synthetic carbohydrate rich wastewater for 150 days (data not shown). After finalizing the start-up process of the AFBR, the CSTR was inoculated and started in batch mode for 48 hours with a substrate to inoculum (S/I) ratio of 1.0 (gVS/gVS). This ratio has been shown to suppress the methanogenic activity in mixed cultures, while stimulating hydrogen producing bacteria (Chinellato et al., 2013). No inoculum pretreatment was applied in this study. Following the start-up of the CSTR, the batch feeding mode was switched to a semi-continuous mode as in the AFBR, where feeding and effluent withdrawal were performed manually once a day.

Raw FW diluted to the designated organic loading rate (OLR) was fed to the CSTR reactor. Subsequently the effluent from the CSTR was fed to the AFBR to be converted to methane. The detailed operational conditions of both reactors are presented in Table 2. No chemical reagent for pH adjustment for the reactors was used during the whole operation. The effluent from the AFBR was collected and recycled manually to the CSTR, i.e. used to prepare the raw FW at the desired OLR. The recirculation rate of the AFBR effluent to the CSTR, calculated as ratio of the returned volume of AFBR effluent to the volume of the CSTR influent, was based on the volume of the AFBR effluent at each period (Table 2). Hence, it was between 0.24–0.48, 0.5–0.8 and 0.6–1.0 in Periods I, II and III, respectively. To reduce the risk of carrier material clogging in the AFBR (Parawira et al., 2006), the CSTR effluent was mildly separated from the solid fraction through centrifugation at 4000 rpm for 10 min and the supernatant was used as a feed for the AFBR. It should be noted that due to the basic difference of the fed substrate, the organic matter of the raw FW, fed to the CSTR, was described in terms of its VS concentration (Cavinato et al., 2012), while the COD value was used to quantify the organic matter content of the AFBR influent, i.e. the CSTR effluent (Ghimire et al., 2015b). The semi-continuous operation of the CSTR was started at a HRT of 6 days and an OLR of 2.0 kg VS/m^3.day, while the AFBR started at a HRT of 20 day and OLR of 0.1 of kg COD/m^3.day. Afterwards, the HRT was decreased gradually based on the reactor performance (Table 2).

2.4. Analytical methods

Total solids (TS), total volatile solids (TVS), total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) were measured according to standard methods (APHA, 1998). The pH was determined using a pH meter having a temperature compensation electrode (pH/ION, 340i model, Germany), calibrated daily using standard buffer solutions (Hamilton DuraCal buffer, Switzerland). Total alkalinity, total organic acids and ammonium nitrogen (N-NH_4^+) concentrations were determined as described by Pontoni et al. (2015).

The concentration of lactic acid, ethanol and individual volatile fatty acids (VFAs), i.e. acetic, propionic, butyric, iso-valeric and valeric acids, were analyzed using a high performance liquid chromatograph (HPLC) equipped with a REZEX-ROA-Organic Acid H^+ column and an ultraviolet (UV) and refractive index detector. The column was operated at 60 °C and 5 mM H_2SO_4 was used as the eluent at a flow rate of 0.6 ml/minute. Prior to analysis, samples were centrifuged at 5000 rpm for 10 min and filtered through a 0.20 μm Puradisc syringe filter. The HPLC sample injection was 20 μL using an autosampler (900 Triathlon, Spark, The Netherlands).

The biogas composition (H_2, CH_4 and CO_2) was determined using a gas chromatograph (GC, Varian Star model 3400) equipped with a thermal conductivity detector (TCD) and a stainless-steel column (2 × 2 mm) packed with ShinCarbon ST (80/100 mesh, Restek®) as described by Ghimire et al. (2015b).

Table 2
Operational conditions of the CSTR and AFBR operated in this study.

CSTR reactor							
Period	I	II	III				
HRT (day)	6	5	3.7				
OLR (kgVS/m^3.day)	2.0	2.5	3.4				
Flow rate (ml/day)	266.7	320	433.3				
Duration (days)	74	73	53				
AFBR reactor							
Period	I	II	III	IV	V	VI	VII
HRT (day)	20	15	10	8	5	3	1.5
OLR (kgCOD/m^3.day)	0.1	0.4	1.01	1.26	1.8	3.5	6.0
Flow rate (ml/day)	65	86.3	130	162.5	260	433.3	866.7
Duration (days)	25	39	33	8	31	34	30

3. Results

3.1. Biohydrogen and soluble products in the CSTR

3.1.1. Biohydrogen production

Fig. 1a illustrates the evolution of the hydrogen production rate (HPR) of the CSTR. Fig. 1b shows the composition of the biogas produced from the CSTR. During the initial 15 days, a decreasing trend of HPR was observed (Fig. 1a), accompanied with a drop of pH from around 6.1–4.5 (Fig. 1c). This might be due to the higher solubilization of organic matter and acidification of FW, which lower the pH and affect the fermentative bacteria (Dahiya et al., 2015). A similar decreasing trend of biohydrogen production was reported previously during the DF of FW (Jiang et al., 2013). To overcome the observed pH drops, recirculation of the AFBR effluent was started from day 15 onwards in order to support the DF process with alkalinity. Afterwards, a stable pH ranging between 5.0 and 5.3 was observed regardless of the HRT shortening (Fig. 1c). This pH range is considered optimal for an efficient biohydrogen production using mixed cultures (Liu et al., 2006; Angeriz-Campoy et al., 2015).

From day 16 onwards, the HPR increased and was stable at each HRT operation (Fig. 1a). Hydrogen and carbon dioxide were the main components of the produced biogas (Fig. 1b) with hydrogen percentages ranging between 32 and 42%. Methane gas was not detected after 5 days initiation of the reactor operation, indicating that no methanogenic activity existed in the CSTR. The average HPR was 178.2 (±12.3), 253.5 (±17.3) and 391.7 (±19.7) L H_2/m^3.day in Periods I, II and III, respectively. The corresponding average biohydrogen yield was 89.1 (±6.1), 101.5 (±8.3) and 115.2 (±5.3) L H_2/$kgVS_{added}$ in Periods I, II and III, respectively.

3.1.2. Intermediate soluble metabolite production

The production of biohydrogen was accompanied with hydrolysis of particulate organic matter and release of soluble metabolic products, i.e. short chain alcohols and VFAs. Fig. 2a shows the soluble COD profile of the CSTR effluent as a function of operational time. The average soluble COD concentration was 6000 (±25.7), 8000 (±34.6) and 11,000 (±30.4) mg/L in Periods I, II and III, respectively. The hydrolysis of the particulate fraction of FW can be characterized by the increase of soluble COD in the reactor (Jiang et al., 2013), representing the optimal activity of hydrolytic bacteria in the CSTR (Wang et al., 2014). Fig. 2b depicts the distribution of the VFAs in the CSTR effluent in each Period during steady state operation. Acetic and butyric acids were the dominant VFAs in all tested

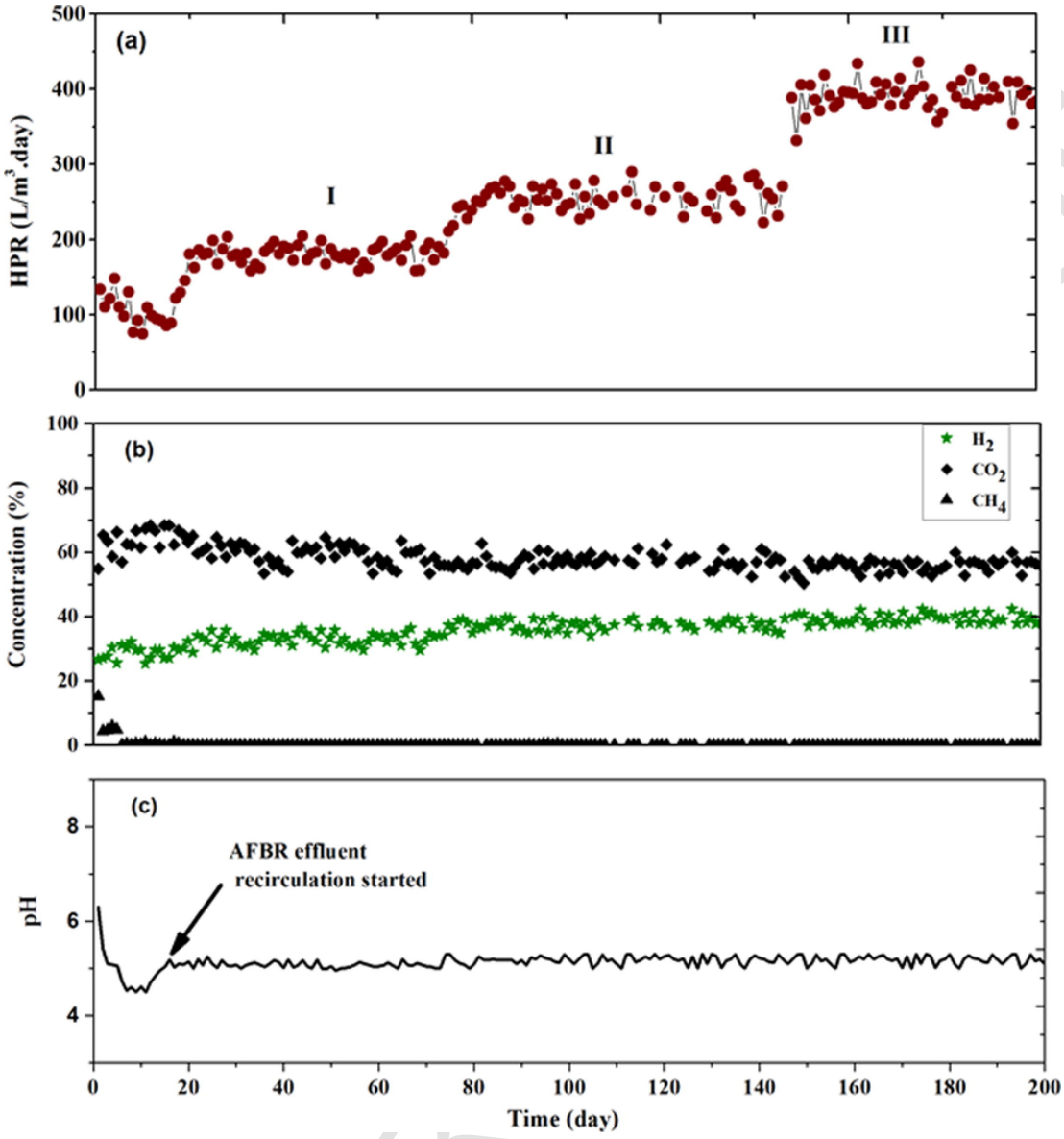

Fig. 1. Biohydrogen production rate (a), biogas composition (b) and pH (c) during the CSTR operation.

Periods, with respective concentration of 10.1 (±1.7) and 5.4 (±0.9) mM in Period I; 13.4 (±2.3) and 7.01 (±1.1) mM in Period II; and 24.4 (±2.9) and 12.0 (±1.8) mM in Period III. Other VFAs (propionic, valeric and iso-valeric), lactic acid and ethanol were always detected in very low concentrations (between 0.02 and 2.5 mM) in all Periods of reactor operation (Fig. 2b).

3.2. AFBR operation at various HRTs

3.2.1. Continuous methane production

Methane production during the long term operation of the AFBR is illustrated in Fig. 3. Throughout the experimental period of 200 days, seven steps of HRT decrease were applied. The OLR was correspondingly increased according to Table 2. The duration of each Period was kept long (more than 20 days) in order to assess the continuous performance of the reactor. The daily methane production rate increased in accordance to the profiles of decreasing the HRT and increasing the OLR (Fig. 3). The average daily methane production rate was 34.6 (±3.42), 139.03 (±9.9), 352.6 (±12.3), 400.6 (±9.4), 636.5 (±32.6), 1190.6 (±21.4), 2041.7 (±48.2) L CH_4/m^3.day in Periods I, II, II, IV, V, VI and VII, respectively. The average methane yield in each Period was close to the theoretical methane potential of organic substrates (Table 3). In addition, an immediate and stable adaptation of the daily methane production rate was observed at each HRT change (Fig. 3). The highest methane production rate of 2041.7 (±48.2) L CH_4/m^3.day was obtained at the shortest designated HRT (1.5 day), which indicted that no hydraulic overloading had occurred in the system.

3.2.2. Characteristics of the AFBR effluent

Fig. 4 shows the total alkalinity, total organic acid, pH and COD removal efficiency of the AFBR effluent. The total alkalinity was between 1000 and 2000 mg/L and the pH ranged between 7.2 and 7.7 throughout all operational Periods, showing the optimal conditions for methanogenic activity. The total organic acid concentrations were maintained below 100 mg/L and the COD removal efficiency ex-

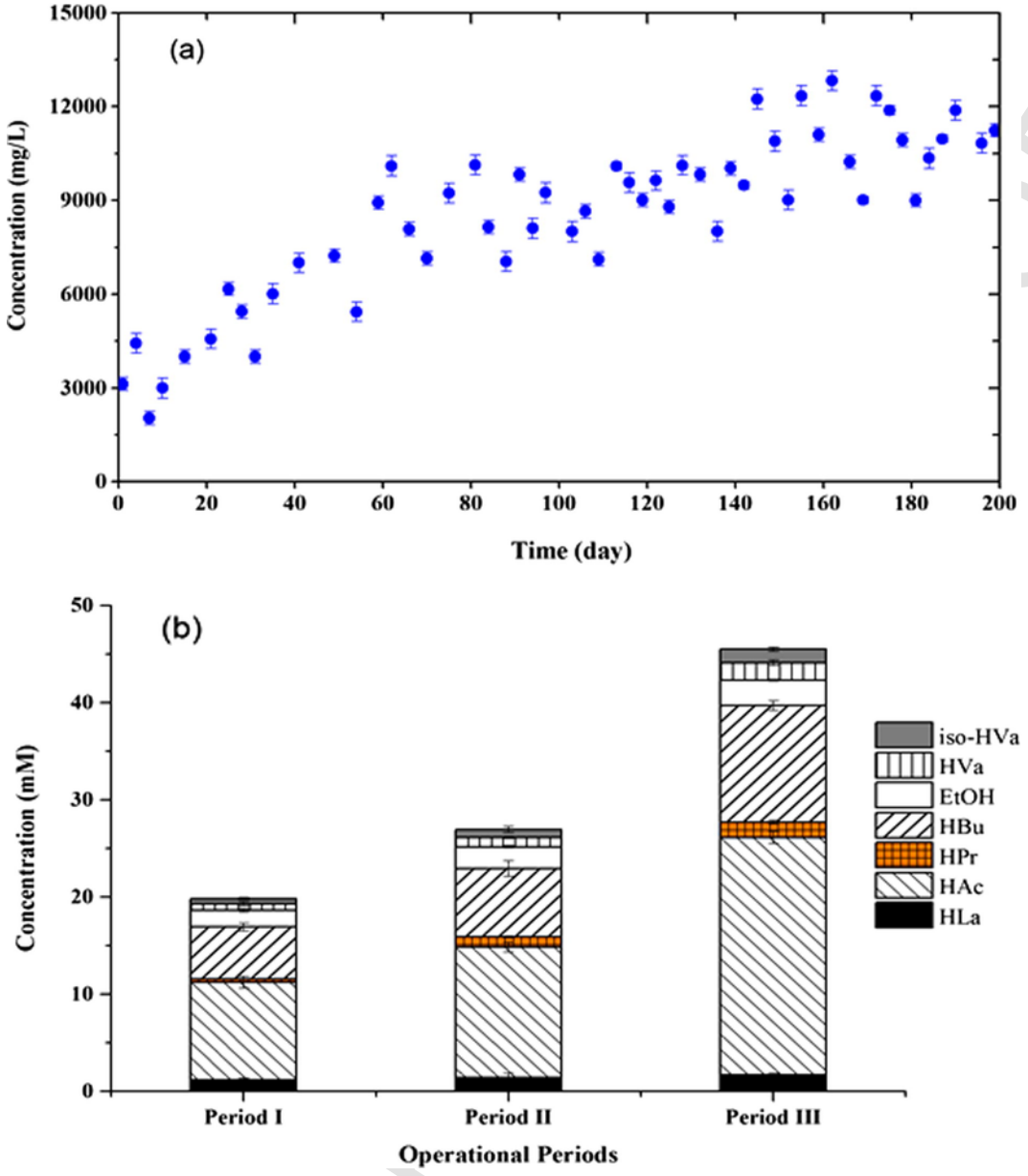

Fig. 2. CSTR effluent characteristics: soluble COD (a) and VFAs, lactic acid and ethanol distribution (b) in each Period.

ceeded 90% during the entire reactor operation (Fig. 4). Regarding the concentration of individual VFAs, negligible amounts of acetic acid were detected during Periods I, II and III. Afterwards the acetic acid concentration remained below the detection limit in all remaining Periods (Table 4), reflecting an efficient consumption of the VFAs during methane production. The TS and VS concentrations were found very low compared to other studies (Wang et al., 2010). This indicated a minimum washout of methanogenic biomass from the AFBR system, as the VS concentration represents the biomass concentration (Khemkhao et al., 2016).

The $N\text{-}NH_4^+$ concentration of the AFBR effluent during the entire operational period is shown in Fig. 5a. Unlike other studies, for example Cavinato et al. (2012), in which the two-stage AD of FW was inhibited by high $N\text{-}NH_4^+$ concentrations after 70 days, lower $N\text{-}NH_4^+$ concentrations (below 110 mg/L) were obtained in this study during the entire operational period. In order to better understand these results, further analyses of the TKN concentrations were performed on the AFBR influent and effluent (Fig. 5b). The result showed that the average TKN concentration in the AFBR influent, i.e. the effluent of the CSTR, was low (between 200 and 250 mg/L) during each operational Period (Fig. 5b).

4. Discussion

4.1. Performance of the CSTR: role of methanogenic effluent recirculation

This study demonstrated the long term continuous production of biohythane, using an integrated system including a biohydrogen producing CSTR and methane producing AFBR. In the DF process, the use of a mixed culture as a fermentative seed inoculum has been generally preferred over pure cultures, since the complex microbial com-

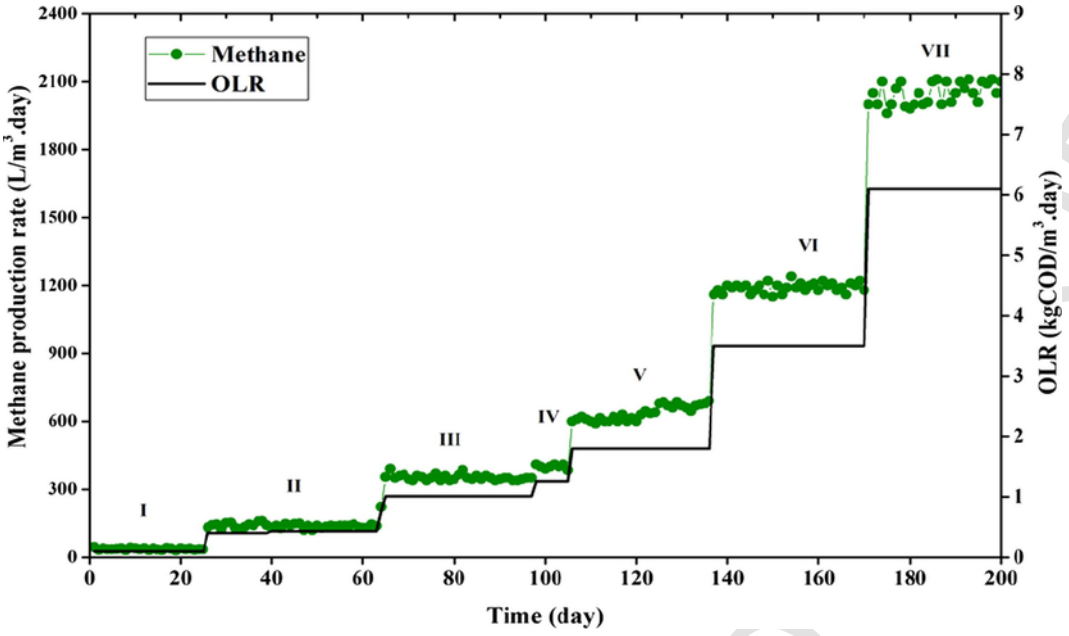

Fig. 3. Daily methane production rate of the AFBR in each Period.

Table 3
Average methane production rate and yield in each Period of the AFBR.

Period	OLR (kgCOD/m^3.day)	Average methane production rate (L CH_4/m^3.day)	Average methane yield (L CH_4/kgCOD.day)
I	0.1	34.56 ± 3.42	345.6 ± 4.7
II	0.4	139.03 ± 9.9	347.6 ± 11.9
III	1.01	352.6 ± 12.3	349.1 ± 8.5
IV	1.26	400.6 ± 9.4	317.9 ± 10.2
V	1.8	636.5 ± 32.6	353.6 ± 7.7
VI	3.5	1190.6 ± 21.4	340.2 ± 13.1
VII	6.1	2041.7 ± 48.2	334.7 ± 18.6

munities are likely to contain a suite of various microorganisms and are thus potentially more robust to face changes in operational conditions (Ghimire et al., 2015a). However, the coexistence of non-hydrogen producing and/or hydrogen-consuming microorganisms in the mixed culture often affects the DF process and reduces the net biohydrogen yields (Angeriz-Campoy et al., 2015; Kobayashi et al., 2012; Shanmugam et al., 2014; Chinellato et al., 2013). In order to optimize the conditions for the fermentative bacteria and improve biohydrogen production, a broad number of studies have revealed the strong effects of operational conditions (i.e. pH, HRT, temperature, reactor configuration and inoculum/substrate pre-treatment) on the selection of fermentative bacteria and DF enhancement (Roy and Das, 2015; Cavinato et al., 2016; Ghimire et al., 2015a).

In particular, the pH plays a major role in maintaining favourable conditions for the fermentative bacteria, thus maximizing the hydrogen production rate and metabolic end products (Wang and Zhao, 2009; Dahiya et al., 2015; Roy and Das, 2015; Cavinato et al., 2016). For very low pH values (below 4.5), the microbial pathways shift to the non-hydrogen producing pathways and result in solvent produc-

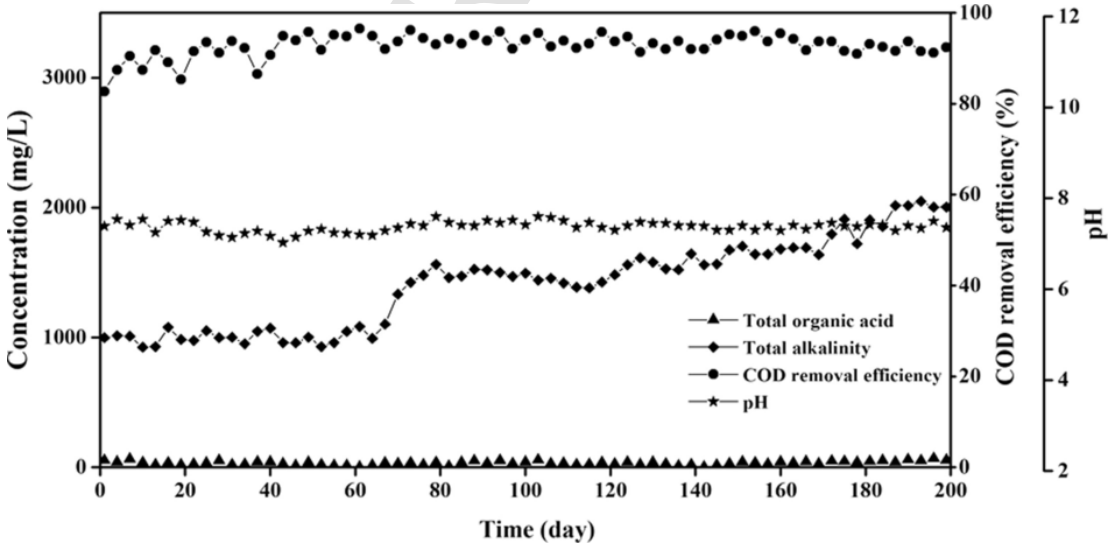

Fig. 4. AFBR effluent characteristics: total alkalinity, total organic acid, pH and COD removal efficiency.

Table 4
Average physico-chemical characteristics of the AFBR effluent.

Period	TS (% wet basis)	VS (% wet basis)	pH	Acetic acid (mg/L)	Butyric acid (mg/L)
I	0.367 ± 0.02	0.231 ± 0.01	7.8 ± 0.02	9.3 ± 0.01	N/D
II	0.386 ± 0.01	0.243 ± 0.03	7.2 ± 0.07	8.6 ± 0.04	N/D
III	0.394 ± 0.04	0.192 ± 0.01	7.5 ± 0.09	3.4 ± 0.03	N/D
IV	0.314 ± 0.03	0.143 ± 0.02	7.5 ± 0.06	N/D	N/D
V	0.302 ± 0.01	0.128 ± 0.03	7.46 ± 0.08	N/D	N/D
VI	0.296 ± 0.01	0.078 ± 0.04	7.4 ± 0.07	N/D	N/D
VIII	0.316 ± 0.02	0.114 ± 0.01	7.38 ± 0.05	N/D	N/D

N/D: Not detected.

tion (solventogenesis) (Ghimire et al., 2015a). In case of a pH above 6.5, the produced hydrogen could be consumed by homoacetogens and methanogens (Roy and Das, 2015; Guo et al., 2010). In both scenarios, the hydrogen yield as well as the distribution and quantities of the produced metabolites would be greatly affected (Zhu et al., 2009). The optimum pH for higher biohydrogen production has been indicated in the range of 5.0–6.0 when using mixed cultures (Cavinato et al., 2016; Zhu et al., 2011; Liu et al., 2006).

However, operating at a relatively shorter HRT and higher OLR with easily biodegradable substrates, like FW, the DF process is often associated with a rapid production and build-up of the VFAs (Angeriz-Campoy et al., 2015). The VFAs accumulation is detrimental for the fermentative metabolism, as the cell membrane integrity is destroyed, hampering the maintenance of the internal pH (Dahiya et al., 2015; Roy and Das, 2015). As a result, the non-hydrogen production pathways/solventogenesis are favoured (Ghimire et al., 2015a). In order to avoid the inhibition of the fermentative metabolism and its interactive effects, various DF studies have been conducted with controlled pH via automatic addition of external reagents such as alkali ($NaOH/Ca(OH)_2$) (Carrillo-Reyes et al., 2016; Kobayashi et al., 2012; Jiang et al., 2013) or buffer solutions (carbonate/phosphate) (Zhu et al., 2009; Lin and Lay, 2004). However, in spite of the increased biohydrogen production, these approaches have appeared to be impractical options that pose extra operational costs to the process and/or are environmentally not sustainable (Zhu et al., 2009, 2011). In addition, the cations used as alkali could eventually have an adverse impact on the fermentative bacteria during prolonged reactor operation (Lee et al., 2010). In the two-stage AD system, implementing an internal recirculation of the effluent from the methanogenic stage has been indicated as a viable strategy for pH control, improv-

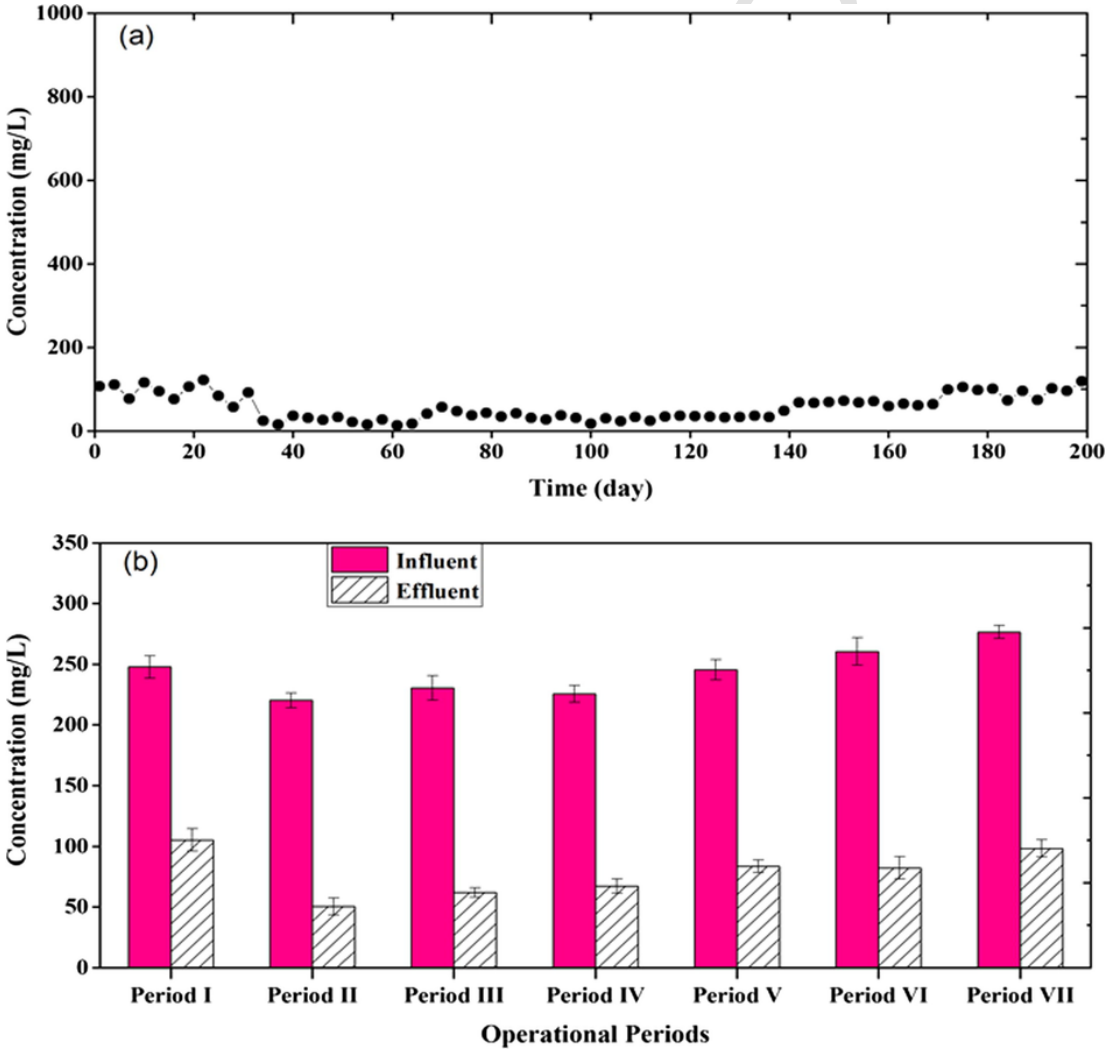

Fig. 5. Evolution of the $N\text{-}NH_4^+$ concentration in the AFBR effluent (a) and average TKN concentration in the influent and effluent of AFBR in each Period (b).

ing the efficiency and economics of the DF process (Redondas et al., 2014; Lee et al., 2010; Cavinato et al., 2016; Chu et al., 2008; Chinellato et al., 2013). It was demonstrated in this study that the recirculation of the AFBR effluent provided enough alkalinity to the DF process to reach the required pH range for optimal activity of the fermentative bacteria, i.e. between 5.0 and 5.3 (Fig. 1c). Hence, an improved and stable prolonged HPR was observed (Fig. 1a). Besides, it should be noted that the AFBR effluent was used to dilute the FW to the desired OLR for the CSTR, i.e. the AFBR effluent was partially recirculated to regulate the pH. Similar to the current study, Cavinato et al. (2012) obtained a higher and stable biohydrogen yield by recirculating the methanogenic effluent to the DF process during a two-stage AD of FW. The authors highlighted the potential use of methanogenic effluent recirculation in exploiting the residual buffer capacity, such as carbonate and ammonia to the DF process (Cavinato et al., 2012). They also indicated this application allows a balance of macro and micronutrient intake (Cavinato et al., 2016).

On the other side, irrespective of providing alkalinity, the suspended biomass from the methanogenic reactor could be recirculated together with the methanogenic effluent. The AD biomass groups, in particular homoacetogens and hydrogenotrophic methanogens, are consumers of hydrogen for their metabolic activity (Angeriz-Campoy et al., 2015; Guo et al., 2010). Specifically, the homoacetogens can tolerate the low pH conditions (Dahiya et al., 2015). Hence, their prevalence interferes with the biohydrogen production process (Kobayashi et al., 2012; Carrillo-Reyes et al., 2016). In order to suppress their activity, different methods such as heat treatment, filtration and aeration have been previously applied to the methanogenic effluent prior to recirculation to the DF process (Cavinato et al., 2012; Zhu et al., 2011; Kobayashi et al., 2012). Such pre-treatments were not required for the AFBR effluent produced in this study, as most methanogenic biomass was attached to the Kaldness-k1 carrier material (Supplementary Fig. S2) and the VS concentration in the AFBR effluent was very low in each operational Period (Table 4), showing that the methanogenic biomass wash out was negligible. This is one of the benefits of the system developed in this study, which may encourage practical implementation of the process.

Regarding the effluent of the CSTR, the soluble COD concentration indicated the degree of hydrolysis of the particulate fractions during the DF of FW (Dahiya et al., 2015; Jiang et al., 2013). The increase of soluble COD in the CSTR effluent (Fig. 2a) suggested that high amounts of particulate fractions of FW were converted into soluble organic components via hydrolytic bacteria (Mamimin et al., 2015). The profiles of the VFAs reflect the metabolic pathway of fermentative bacteria and can be used to monitor the performance of the DF process (Ghimire et al., 2015b). In this study, acetic and butyric acids were the predominant soluble metabolites, with acetic acid the dominant in all Periods (Fig. 2b). According to the composition of the VFAs, the acetate-butyrate pathway was the dominant fermentation pathway during the biohydrogen production in the CSTR reactor (Wang and Zhao, 2009; Ghimire et al., 2015b).

The higher production of acetic and butyric acid was mainly attributed to the higher content of carbohydrate in the FW (Table 1), which is the preferred organic matter during the DF process as they are utilized faster than proteinous compounds (Wang et al., 2014; Dahiya et al., 2015). The polysaccharides present in the FW were initially degraded to glucose, then to pyruvate, which was easily converted to acetyl-CoA and readily consumed to produce hydrogen, acetic acid and butyric acid (Roy and Das, 2015). The results are in accordance with the study of Jiang et al. (2013), who obtained a higher acetic and butyric acid concentration during the DF of FW at a pH 5.0 compared to the operation at higher pH values (6.0 and 7.0).

4.2. Effect of shortening the HRT on the AFBR performance

The AFBR performance was assessed while decreasing the HRTs and increasing OLRs. The HRT was decreased in successive steps, each decrease occurring after prolonged stability was proven in terms of methane production rate in the previous step (Fig. 3). The HRT shortening resulted in a noticeable increase of the methane production rate from an average of 34.6 (±3.42) L CH_4/m^3.day at HRT 20 days (Period I) to 2041.7 (±48.2) L CH_4/m^3.day at HRT 1.5 days (Period VII) (Fig. 3). The HRT is an important parameter from an economic perspective as it has a direct relationship with capital costs, considering that shorter HRTs allow smaller reactor size (Schmidt et al., 2014). In a suspended growth AD systems such as a CSTR, shortening of the HRT often causes a dual effect: i) washout of active methanogenic biomass growing in suspension due to the increased flow rate and ii) increase of the OLR (Khemkhao et al., 2016). These effects often led to a lower methane yield, process imbalance and eventually system failure (Regueiro et al., 2015; Khemkhao et al., 2016; Kinnunen et al., 2014). Accordingly, it is widely accepted that a minimum HRT of 15–30 days is obligatory to prevent the methanogenic biomass from wash-out and to allow enough time for effective substrate degradation (Schmidt et al., 2014; Ziganshin et al., 2016; Kinnunen et al., 2014). Fixed bed reactor systems, such as AFBR, provide a solution to avoid the wash out of the active AD biomass at the desired short HRT (Yehshanew et al., 2016b; Carrillo-Reyes et al., 2016; Roy and Das, 2015).

The results in this work elucidated the presence of a well-attached and matured methanogenic biofilm in the AFBR that did not suffer any wash-out, even when reducing the HRT to 1.5 days. Besides, after each change of HRT, the system stability e.g. in terms of pH and COD removal efficiency is crucial in operation of AD reactors (Regueiro et al., 2015). The optimal pH conditions, the presence of sufficient alkalinity, the low concentration of organic acid and high COD removal efficiency (Fig. 4) were clear indicators of a stable and healthy methanogenic process. This good reactor performance along with visual inspection of the reactor (Supplementary Fig. S2) confirmed the retention of a sufficient concentration of methanogenic archaea in the AFBR. It also reflected the successful establishment of a methanogenic biofilm during the start-up process of the AFBR. A similar performance of a methanogenic suspended biofilm reactor was reported by Chu et al. (2008) during operation of a two-stage AD system treating FW, but with a longer HRT (5 days). Likewise, Lee et al. (2010) obtained a higher and stable methane production while reducing the HRT from 15.4 to 7.7 days in a thermophilic anaerobic packed bed reactor treating FW. However, the methane production and reactor efficiency were deteriorated upon further shortening the HRT to 5.13 days as indicated by the accumulation of VFAs and drop of the pH (Lee et al., 2010).

Lower concentrations of N-NH_4^+ in the AFBR effluent were observed during the operational Periods (Fig. 5a), which was probably due to the low TKN concentration of the CSTR effluent (Fig. 5b). This was ascribed to a minor protein degradation occurred during the DF of FW at a pH of around 5.0 (Jiang et al., 2013; Wang et al., 2014; Dahiya et al., 2015). The lower hydrolysis of proteins can also be seen from the smaller concentration of valeric and iso-valeric acids in the CSTR effluent (Fig. 2b). The presence of N-NH_4^+ is important for the production of new microbial cells (Fricke et al., 2007) and to increase the buffering capacity of the system, thus improving the stability of the process (Dahiya et al., 2015; Prochàzka et al., 2011). It has been reported that with a concentration up to 1000 mg/L N-NH_4^+, the ammonium stabilizes the pH value in most mesophilic AD reactors (Fricke et al., 2007). In contrast, high concentrations of N-NH_4^+ are toxic to the AD biomass (Ariunbaatar et al., 2015;

Cavinato et al., 2016). In the present study, surprisingly, no signs of process imbalance and inhibition were observed, despite the $N\text{-}NH_4^+$ concentration was smaller than the reported optimal values. Instead, methane yields closer to the theoretical value and stable process were observed in each operational Period (Figs. 3 and 4). This was possible as most active AD biomass was retained in the AFBR system (Table 4 and Supplementary Fig. S2) and hence less nitrogen was required for cell synthesis compared to the suspended growth systems (Negi et al., 2015). The buffering capacity of the system likely comes from the alkalinity produced by methanogenic archaea in the form of carbon dioxide and bicarbonate (Kumaran et al., 2016). Such a reactor configuration thus has the potential of treating organic wastes and residual biomass with low nitrogen content, such as agricultural residues.

4.3. Overall system performance

The continuous production of biohydrogen and methane from FW via an integrated system of CSTR and AFBR was successfully conducted, as shown by the stable gas production and sustained operation of both reactors (more than 6 months). The AD process is often limited by two major steps depending on the nature of the substrate, i.e. hydrolysis and methanogenesis (Esposito et al., 2011). The former step can slow down the whole AD process, particularly for complex organic substrates containing a high particulate fraction (e.g. FW), as it makes the substrate available to the microorganisms for further metabolism. The methanogenesis step can be the rate-limiting step when using soluble substrates (Ma et al., 2013). The present system seems to favour the kinetics of both steps, speeding-up the whole AD process. The raw FW was treated in a CSTR via the DF process, producing biohydrogen and an organic rich liquid fraction. The spent liquid fraction was further utilized in a methanogenic AFBR, functioning on a biofilm-based system. The optimal operation of the CSTR has a vital effect on the subsequent methanogenic step (Zhu et al., 2009).

Such an integrated system avoids clogging of the carrier material during operation of the AFBR, since the soluble fraction from the CSTR was a preferred substrate for the biofilm-based reactor configuration (Parawira et al., 2006). Clogging of carrier materials has been indicated as a technical obstacle deterring the potential application of AFBRs, particularly for substrates containing a high suspended solids concentration such as FW (Wang et al., 2010). It occurs when the particulate fractions of the FW stick onto the surface and pores of the carrier material. This coupled with the low hydrolysis rate of particulate organics leads to rapid clogging and mass transfer resistance (Fuentes et al., 2009). The integrated system proposed in this study overcomes such operational challenges. However, a post treatments of the solid residue left after the mild separation of the DF effluent is recommended in order to accomplish a full conversion and valorization of the FW (e.g. composting process).

In addition, a COD mass balance was calculated to evaluate the substrate conversion efficiency of the system (Supplementary Table S1). The COD mass balance calculation was based on the COD conversion coefficient of each of the major end products from the CSTR (H_2, liquid effluent and solid fraction) and from the AFBR (CH_4 and AFBR effluent) (Chu et al., 2008). The percentage of the input COD recovered as H_2 COD in the CSTR and CH_4 COD in AFBR was 4.9% and 79.5%, respectively, corresponding to a total energy recovery of 84.4% of the influent COD. The results are comparable with the study of Chu et al. (2008), who obtained a total of 86% energy recovery from the incoming COD during production of biohythane from FW using a two-stage AD system.

Table 5 compares various studies on biohythane production of FW at various operational conditions. The overall energy recovery obtained in this study is comparable to the other reports (Table 5), though the energy recovery depends on operational conditions such as reactor size, HRT, OLR and temperature. The proposed system can improve the energy recovery from FW by eliminating the costs needed for substrate/inoculum pretreatment, chemicals for pH control and maintenance works due to clogging. Additionally, the methanogenic AFBR was operated at a reduced HRT (1.5 days),

Table 5
Comparison of different studies producing biohythane from FW at different operational conditions.

Reactor type[a]		Temperature (°C)		HRT (day)		OLR (kgVS/m^3.day)		Stage I pH control[b]	Biogas yield (L/$kgVS_{added}$)		Total energy recovery (MJ/$kgVS_{added}$)[e]	Reference
Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II		Stage I, H_2	Stage II, CH_4		
CSTR	CSTR	52	52	3	12	20.0	ns	R	117.0	311.0	5.54	Chinellato et al. (2013)
CSTR	ASuBR	55	35	1.3	5.0	38.4	6.6	R	205.0	464.0	12.4	Chu et al. (2008)
CSTR	CSTR	55	55	3.3	12.6	16.3	4.8	R	67.0	720.0	18.8	Cavinato et al. (2012)
CSTR	CSTR	55	55	2	10	n.s	n.s	C	66.0	364.0	17.9	Chu et al. (2012)
RDR	CSTR	40	40	6.7	26	22.7	4.61	NA	65.0	546.0	13.7	Wang and Zhao (2009)
CSTR	CSTR	37	37	2	15	37.5	4.1	ns	43.0	500.0	20.2	Liu et al. (2006)
CSTR	ABR	55	55	2.87	14.4	ns	ns	R + C	147.3	383.0	18.3	Kobayashi et al. (2012)
SCR	SC-PBR	55	55	1.28	7.7	58.5[c]	8.4[c]	R	59[c,d]	250.0[c,d]	9.58[c]	Lee et al. (2010)
CSTR	AFBR	55	37	3.7	1.5	3.4	6.1[c]	R	115.0	334.0[c]	12.3	This study

n.s. not specified

[a] ASuBR: Anaerobic suspended biofilm reactor, RDR: rotating drum reactor, ABR: Anaerobic baffled reactor, SCR: semi-continuous reactor, SC-PBR: semi-continuous packed bed reactor.

[b] R: methanogenic (stage II) effluent recirculation, C: controlled automatically using chemical reagent, NA: no pH adjustment.

[c] OLR in terms of gCOD/L.day, biogas yield in terms of $L/kgCOD_{added}$ and energy recovery in terms of MJ/kgCOD.

[d] Calculated from article data.

[e] Calculated from article data at STP conditions based on the heating values of hydrogen (242 kJ/mol) and methane (801 kJ/mol) (Ghimire et al., 2015b).

showing the possibility of minimizing reactor sizes of the AD process.

5. Conclusion

The proposed integrated two-stage system, comprised of a CSTR and an AFBR, showed promising results of producing biohythane from FW for a prolonged duration. Both reactors have demonstrated an excellent performance of producing biohydrogen and methane, irrespective of lowering the HRT. Particularly, a stable methane production rate and reactor performance at each shortened HRT was observed during the AFBR operation. Besides, the AFBR effluent provided sufficient alkalinity for the DF process and maintained the pH between 5 and 5.3 throughout the CSTR operation. The maximum biohydrogen and methane production rates were 391.7 (±19.6) L H_2/m^3.day and 2021.6 (±16.5) L CH_4/m^3.day, respectively. This highest production of biohythane was obtained at the shortest designated HRTs of 3.5 and 1.5 days for the CSTR and AFBR, respectively. The AFBR effluent exhibited low $N\text{-}NH_4^+$ concentrations, in the range of 120–20 mg/L during the entire operation, without compromising the process stability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.08.078.

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