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Research article

Feasibility of thermophilic anaerobic processes for treating waste activated sludge under low HRT and intermittent mixing

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ABSTRACT

Thermophilic anaerobic digestion (AD) arises as an optimized solution for the waste activated sludge (WAS) management. However, there are few feasibility studies using low solids content typically found in the WAS, and that consider uncommon operational conditions such as intermittent mixing and low hydraulic retention time (HRT). In this investigation, a single-stage pilot reactor was used to treat WAS at low HRT (13, 9, 6 and 5 days) and intermittent mixing (withholding mixing 2 h prior feeding). Thermophilic anaerobic digestion (55 °C) was initiated from a mesophilic digester (35 °C) by the one-step startup strategy. Although instabilities on partial alkalinity (1245–3000 mgCaCO$_3$/L), volatile fatty acids (1774–6421 mg/L acetic acid) and biogas production (0.21–0.09 m^3/m$^3_{reactor}$.d) were observed, methanogenesis started to recover in 18 days. The thermophilic treatment of WAS at 13 and 9 days HRT efficiently converted VS into biogas (22 and 21%, respectively) and achieved high biogas yield (0.24 and 0.22 m^3/kgVS$_{fed}$, respectively). Intermittent mixing improved the retention of methanogens inside the reactor and reduced the washout effect even at low HRT (<9 days). The negative thermal balance found was influenced by the low solids content in the WAS (2.1% TS) and by the heat losses from the digester walls. The energy balance and economic analyses demonstrated the feasibility of thermophilic AD of WAS in a hypothetical full-scale system, when the heat energy could be recovered from methane in a scenario of higher solids concentration in the substrate (>5% TS).

1. Introduction

Anaerobic digestion (AD) is used to improve the treatment of waste activated sludge (WAS) and offers several advantages, including the stabilization of biodegradable fractions, the reduction of greenhouse gas emissions associated with sludge landfilling, and the production of renewable energy as methane. AD processes can be performed over three temperature ranges: psychrophilic (10–30 °C), mesophilic (20–50 °C) and thermophilic (35–75 °C). Several industrial AD applications designed for WAS management have utilized the mesophilic range due to economic advantages, especially in tropical regions (Foresti et al., 2006; De Baere and Mattheeuws, 2012).

Thermophilic anaerobic digestion presents a rate-advantage over the mesophilic process because kinetics is faster and the yields larger. Besides, thermophilic AD has higher loading bearing, which sounds advantageous when high organic loading rates (OLR) are used (Mata-Alvarez et al., 2000; Mao et al., 2015).

Typically, the start-up of a thermophilic process is accomplished by adapting mesophilic microbiota to higher temperatures. Two strategies have generally been used to change operational temperatures, namely, one-step, or single-step, and step-wise approaches (Bouskouva et al., 2005). This temperature change process benefits the bacteria and methanogens subpopulations selection, which are naturally present in low concentrations in mesophilic systems. However, the transition from mesophilic to thermophilic

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conditions may be slow, and long acclimation times are frequently required. Moreover, this transition may bring about instabilities, resulting in decreased alkalinity and pH, incomplete organic substrate bioconversion and low methane yields (Van Lier et al., 1993; De la Rubia et al., 2013).

In a steady state thermophilic reactor, because all metabolism kinetics are enhanced and degradation rates are increased, digester dimensions can be reduced for a given load and may support the use of low hydraulic retention times (HRTs, below the typical 15–20 days). Therefore, thermophilic AD demands lower capital and installation costs than mesophilic AD (Buhr and Andrews, 1977; Metcalf and Eddy, 2014). However, low HRTs result in significant methanogenic biomass washout, affecting methane production, substrate degradation and digestate quality. Several lab-scale thermophilic anaerobic digesters treating WAS have shown specific methane potential (SMP) values approximately 0.2 $m^3/kgVS_{fed}$ with 8–9 days HRT, despite poor correlations between methane production and organic load removal rates (Nges and Liu, 2010; Braguglia et al., 2015). Another study has presented SMP of 0.10 $m^3/kgVS_{fed}$ with 10 days HRT, but with methane levels below 53% (Gianico et al., 2015).

The aforementioned studies were conducted under continuous mixing. Alternatively, intermittent mixing provides solids stratification before feeding the digester. This can be used to retain solids within the digester longer than the operational HRT to increase the stabilization efficiency and decrease methanogen washout, especially for low HRT operations (Kaparaju and Angelidaki, 2008; Kaparaju et al., 2008). In fact, in a typical AD process, the regeneration times for Archaea organisms are much longer (10–15 days) compared with hydrolytic bacteria (24–36 h) and acid-forming microorganisms (80–90 h). These properties indicate that methanogens are more susceptible and sensitive to washout and inhibition (Deublein and Steinhauser, 2008).

Different types of mixing equipment have been studied to evaluate their applicability for each AD process. The main objectives of a proper mixing include proper nutrient distribution to cells for healthy growth and enhanced hydrolysis of particulate substrates (Trad et al., 2017). When comparing results from different AD processes carried out in lab-scale, pilot-scale and batch reactors under different mixing modes, Lindmark et al. (2014) verified that an intermittent mixing regime enhanced process performances regarding reductions in total and volatile solids (TS and VS), and chemistry oxygen demand (COD) compared with continuously mixed systems.

Moreover, energy demands of a biogas plant may be cut off by using intermittent mixing modes as it usually considers reduced mixing time and lower mixing intensity (Zhang et al., 2016; Lindmark et al., 2014). This may convey to positive power balance and the economic feasibility and applicability of anaerobic digesters.

There have been few studies of AD processes applied to the treatment of waste activated sludge (as a single substrate) under intermittent mixing. A more realistic approach would be to consider the low solid content present in WAS after static (gravity) thickening, which is commonly used in full-scale sewage treatment plants.

In this work, the influence of HRT on WAS stabilization (2.1% TS) was tested using an anaerobic digester operated under thermophilic temperature and by withholding mixing for 2 h prior to feeding. The digester was conducted at low HRT values (from 13 to 5 days) to raise the OLR by changes in the influent flow. Moreover, we depicted outcomes from temperature conversion processes and considered the feasibility of the designed AD system based on costs analysis and energy balances in a full-scale implementation scenario.

2. Material and methods

2.1. Reactor setup

A 0.1-m^3 single-stage, completed stirred tank reactor (CSTR) was used to perform the AD of WAS under mesophilic (35 °C) and thermophilic (55 °C) temperatures. Mixing was achieved using a triple two-blade impeller with a vertical shaft connected to a three-phase motor (380 V, WEG 0.36 kW) initiated by a moto–reducer (WEG CFW 10) set at 60 RPM.

The digester was mixed under intermittent mixing mode. This was accomplished by turning on and off the mixing system each day. Two hours prior to feeding, mixing was stopped and heavy sludge particles sank to the bottom. Because effluent extraction occurred in the middle section of the digester, a layer with low suspended solids content was automatically removed by the hydraulic pressure provided during the feeding. Moreover, the internal pressure of the anaerobic digester was kept at approximately 0.2 m of water column.

2.2. Substrate characteristics

The substrate used in this study consisted of municipal waste activated sludge originating from a 140,000 PE wastewater plant that treats 34,560 m^3/d of municipal wastewater using the Biological Nitrogen Removal Process. The plant is located in Florianópolis (south Brazil).

Table 1 summarizes the main substrate characteristics used throughout the experiment. Total solids concentration (TS) was 2.1%, wherein the volatile solids (VS) were 70%. The COD/VS ratio surrounded 1.6 instead of the typical 1.4; this difference is mainly related to the inertial solids content in the activated sludge system due the high hydraulic retention time (HRT) applied in the biological tank (20 days) (Batstone et al., 2002, 2010). The substrate presented COD:N:P ratio of 67:4:1, which is lower than the typical 300:5:1 used for anaerobic bacterial growth and biogas production (Malina Jr. and Pohland, 1992; Annachhatre, 1996).

2.3. Experimental conditions and analytical procedures

All activities were performed under three operational periods. Period 1 (P1) encompassed the mesophilic stage (35 °C) prior to temperature conversion (days 1–10). The system was conducted under 7 days HRT and an organic load of 1.9 $kgVS/m^3 \cdot d$. The feed was supplemented with 2 L 6 N $NaHCO_3$ in a 100-L feed tank to increase the pH and the partial alkalinity to strengthen environmental conditions prior to changing the temperature to prevent bacteria inhibition or digester souring (Cecchi et al., 1993; Chan et al., 2012). This feed mixture had a sodium bicarbonate

Table 1
Characteristics of the waste activated sludge used as substrate in this study.

Variable	Average	Standard deviation
pH	6.34	0.23
TS (g/L)	20.8	1.9
VS (g/L)	14.7	1.0
Chemical oxygen demand (COD) (g O_2/L)	23.4	2.0
Total phosphorus (Total P) (mg/L)	351.1	45.4
Total Kjeldahl nitrogen (TKN) (mg/L)	1278	24.7
Ammonia (mg $N-NH_4^+$/L)	130.7	17.9
Total alkalinity (mg $CaCO_3$/L)	1037	152.1
Partial alkalinity (mg $CaCO_3$/L)	667	158.7
Total coliforms (CFU/gTS)[a]	10^8	10^7
Fecal coliforms (CFU/gTS)[a]	10^6	10^5

[a] Colony forming unit on $\log_{10}$ base.

concentration of 5.14 g/L. Feeding was interrupted on the 7th day and temperature adjustment was performed on the 11th day. During Period 2 (P2) (days 11–32), the reactor was conducted under thermophilic temperature (55 °C) and required daily monitoring. Feeding was restarted after the methane content in the biogas exceeded 40%.

Throughout Period 3 (P3) (250 days), the steady-state thermophilic digester functioned as the HRT gradually decreased, i.e., from 13, 9, 6 to 5 days with organic loading rates changing from 1.3, 1.6, 2.2 to 2.9 $kgVS/m^3 \cdot d$, respectively, by means of an increasing influent flow. Feeding was performed in semi-continuous mode: once per day, seven days per week using an automatic time-controlled feeding apparatus.

Because the HRT was gradually decreased from 13 days, data mining considered all datasets obtained in each HRT over time. The elapsed time in each HRT was 69, 57, 32 and 92 days for 13, 9, 6 and 5 days HRT, respectively.

Substrate and digested sludge samples were collected twice a week for analysis. The stability of the digester was evaluated in terms of pH, partial and total alkalinity, total volatile fatty acids (VFA) and ammonia, expressed as total ammonia nitrogen (TAN). The thickened sludge and digested sludge were monitored according to chemical oxygen demand (COD), total Kjedahl nitrogen (TKN) and total phosphorus (Total P). All physicochemical determinations were performed according to Standard Methods (APHA et al., 2005). Over the periods P1 and P2, VFA were analyzed by distillation-titration method and results were expressed as acetic acid equivalents. In period 3 VFA were determined by high-performance liquid chromatography (HPLC) using a Dionex chromatograph model 3000 equipped with an Ultimate Acclaim column (5 μm, 4.0 × 150 mm) coupled to an ion conductivity detector (Leite et al., 2013). Samples were pre-filtered through 0.45-μm cellulose nitrate membrane filters.

Biogas production was measured through a fluid displacement device. CH_4 and CO_2 contents were measured using a portable infrared detector biogas analyzer (GEM Model 2000, Landtec). The biogas produced were expressed as volumes and as specific yields: gas production rate (GPR) and specific gas production (SGP). Influent sludge and digested sludge were analyzed based on total and fecal coliforms using Standard Methods (APHA et al., 2005).

2.4. Analysis of the microbial community structure

Bottom sludge samples were collected monthly along P3 for the microbiota characterization using fluorescence in situ hybridization (FISH) (Amman, 1995). Cells were hybridized with ARC 915 probe (5′ − GTGCTCCCCCGCCAATTCCT − 3′), which is specific for Archaea organisms.

2.5. Energy balance and economic considerations

Energy and economic estimations considered typical sludge production from a wastewater treatment plant (WWTP) designed for 140,000 inhabitants equivalent. The following parameters and values were assumed: a 1 kcal/kg °C sludge specific heat, a 20 °C sludge temperature, a 5500 $kcal/m^3$ biogas low calorific power (LCP), thermal ($n_{thermal}$) and electrical ($n_{electrical}$) yields from a cogeneration unit (CHP) of 50% and 40%, respectively, and a thermal calorific yield of 90% for a boiler (Zupančič and Roš, 2003; Metcalf and Eddy, 2014).

The influent raw sludge flow (Q_{sludge}) was determined from (Eq. (1)). The digester volume (V_d) was determined according to the applied HRT (Eq. (2)) and varied between 1820 m^3 and 700 m^3 when the HRT decreased from 13 to 5 days HRT. The daily organic load (VS_{fed}) and biogas production (P_{biogas}) were determined from (Eq. (3)) and (Eq. (4)), respectively:

$$Q_{sludge}\left(\frac{m^3}{d}\right) = \left(P_{sludge} x\ PE\right) \Big/ TS \tag{1}$$

$$V_d\left(m^3\right) = Q_{sludge} x\ HRT \tag{2}$$

$$VS_{fed}\left(\frac{kgVS}{d}\right) = P_{sludge} x\ \frac{VS}{TS}\ x\ PE \tag{3}$$

$$P_{biogas}\left(m^3 \big/ d\right) = VS_{fed} x\ SGP \tag{4}$$

where P_{sludge} is the per capita sludge production; PE is the sewage plant population equivalent; TS is the total solids concentration; and VS/TS is the organic fraction ratio.

The theoretical amounts of heat (Heat) and electricity (Energy) produced in a CHP unit from biogas were determined using (Eq. 5).

$$Heat\ or\ Energy\left(\frac{Kcal}{d}\right) = P_{biogas}\ x\ LCP\ x\ n_{thermal\ or\ electrical} \tag{5}$$

Thermal energy losses were estimated based on the digester dimensions. Estimations considered the specific biogas production levels determined experimentally in each HRT. At the same operational conditions applied in the pilot-scale digester, the biogas and methane yields normalized to substrate input should present similar values regardless of digester size. The energy balance was further estimated considering different TS concentrations in the substrate to verify the influence of the sludge thickening capacity in the energy balance. A preliminary power estimation for the mixing system was carried out at a power input of 8 $W/m^3_{reactor}$ (USEPA, 1974; Karim et al., 2005; WEF, 2010; Trad et al., 2017). However, these outcomes were not considered in the energy balance so that the estimated energy requirements were only limited to heat demands and losses.

To define the economic costs for the implementation of an anaerobic digester, the payback period for the investment (capital cost) was calculated based on similar anaerobic digestion studies (Bolzonella et al., 2007; Cavinato et al., 2010; Gianico et al., 2015).

3. Results and discussion

3.1. Digester performance prior to temperature conversion (P1)

During the mesophilic phase, total and partial alkalinity (average values of 3262 ± 997 and 2618 ± 925 mg/L, respectively) were due to bicarbonate ion supplementation from the $NaHCO_3$ solution. As a consequence, the pH increased from 6.11 to 7.26 (Fig. 1). The average VFA concentration was initially 2324 ± 221 mg/L (as acetic acid equivalents), but decreased to 1774 ± 160 mg/L on the 11th day after the feeding was interrupted on day 7.

While feed flow was maintained (from the 1st to 6th days), the average daily biogas production per cubic meter of reactor volume was 0.21 ± 0.1 m^3. After the feeding interruption, the biogas yield dropped to 0.16 ± 0.1 $m^3/m^3_{reactor}$ largely due to the accumulation of VFA in the digester. However, the biogas composition was not influenced by the feeding interruption; the amounts of methane and carbon dioxide produced between the 7th and 11th days were at average concentrations of 61± 2.5% and 37± 7.7%, respectively.

3.2. Temperature conversion (P2)

The single-step temperature conversion on the 11th operational

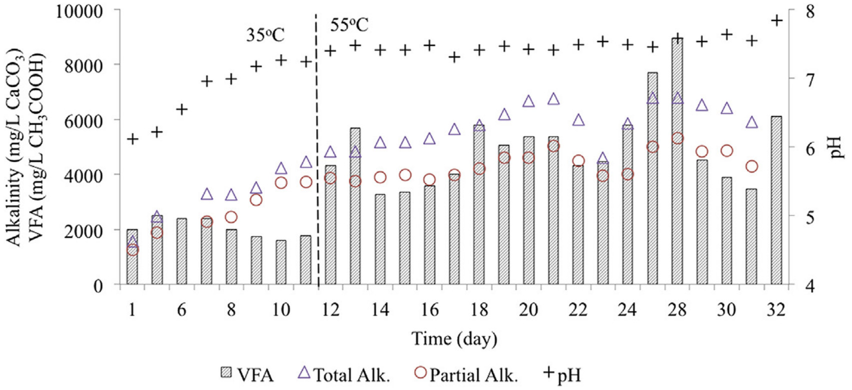


Fig. 1. Monitoring of pH, volatile fatty acids and alkalinity along P1 and P2.

day influenced the production of VFA. VFA concentration sharply increased the following 2 weeks, reaching 6421 mg/L (acetic acid equivalents; Fig. 1). As a consequence, the total alkalinity increased, despite a lack of buffering capacity at pH greater than 5.0. The partial alkalinity was maintained at approximately 3000 mgCaCO$_3$/L mostly due to alkali dosing during P1 and increasing levels of carbonic acid in the liquid phase, which remained in equilibrium with CO$_2$(g) (Batstone et al., 2002).

Fig. 2 presents the biogas yields and composition along P1 and P2. The gas production rate reached a peak of 0.23 m^3/m^3·d when substrate was fed to the digester. High biogas production (a mean of 0.20 m^3/m^3·d) was also observed from day 7 to day 9 (Fig. 2) due to VFA conversion into gas (Fig. 1). Subsequent GPR values substantially decreased due to the feeding interruption between days 10 and 11 (0.09 m^3/m^3·d). Although the initial GPR values after the temperature shift were slightly higher (0.12 m^3/m^3·d), they were primarily attributed to the reduced solubility of carbon dioxide at higher temperatures instead of VFA conversion into biogas (Gallert and Winter 1997). In fact, the biogas production during P2 was quite low, with an average of 0.05 m^3/m^3·d. These results indicated that immediately following the temperature conversion, methanogenesis was apparently inhibited in the digester due to the acclimation of anaerobic microorganism (especially methanogenic Archaea) to the new temperature condition.

The sequence of microbial events that occurs during the digestion process (i.e., particulate organic material hydrolysis, acid formation and methanogenesis) is affected by high temperatures. Hydrolytic and fermentative bacteria have faster metabolic rates than methanogenic archaea, which enhance growth under thermophilic conditions. As a result, their metabolic byproducts, such as VFA and CO$_2$, are produced at higher rates than acetic acid, which is the main precursor for methane production (Mata-Alvarez et al., 2000). Thus, immediately after temperature conversion (days 12–15), the kinetics of acid fermentation improved (Fig. 1), and more carbon dioxide was produced (a mean value of 59%), whereas a lower methane composition in biogas was observed (a mean value of 39%, Fig. 2). Indeed, the higher partial pressure of CO$_2$ in biogas during this period contributed to maintaining a steady pH (between 7.3 and 7.6, Fig. 1) (Vindis et al., 2009).

The imbalance between VFA production and consumption after the temperature conversion resulted in accumulating acids (Fig. 1) and minimal gas production (Fig. 2). According to Van Lier et al. (1993) high concentrations of acetate and/or hydrogen may deteriorate propionate degradation, which would accumulate in the system. This is likely due to higher hydrogen partial pressures in the biogas (between 20 and 25% in volume, not quantified), which was emitted from the liquid-phase and therefore, no longer available for hydrogenotrophic methanogens (Gallert and Winter 1997). Thus, after methanogenic metabolic capacities were recovered, new substrates were added (Ahring, 1995).

The methane content in the biogas ranged from 24 to 29% over the 16 days after the temperature shift. A slight increase was registered 17 days after the temperature conversion, increasing from 29% to 35% and finally to 36% (between the 27th and 29th operational day). Although this increase was moderate, such evidence indicated that feeding should restart on the 30th day. Thus, the temperature adaptation and methanogen acclimatization lasted 18 days, which was similar to ranges reported by other authors.

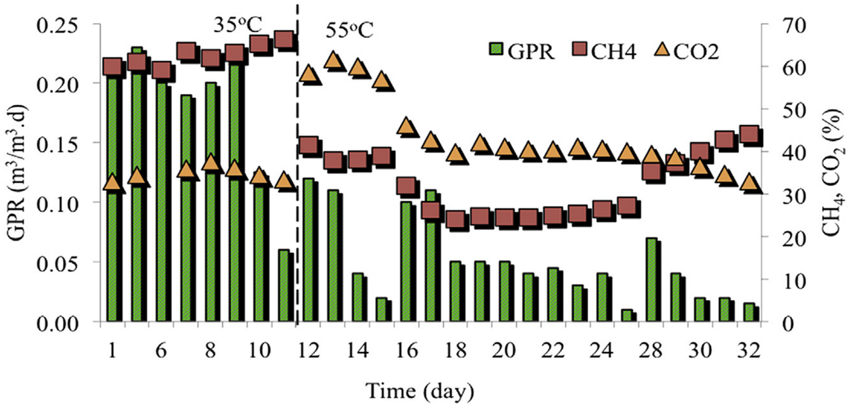


Fig. 2. Biogas production and composition along P1 and P2.

Ortega et al. (2008) achieved a lower period of 7 days, using anaerobic granular biomass as seed and food waste as substrate. However, investigations carried out under similar conditions as the present study required longer times to establish methanogenic conditions after the temperature change, e.g., 28 days (Bouskova et al., 2005) and 20 days (Tian et al., 2015).

Although the one-step temperature increase introduced disturbances, the thermophiles were initially grown under optimal temperatures. However, by following a step-wise approach, the gradual changes in conditions over an increasing temperature gradient may benefit the thermophiles and prolong the time required for a successful start-up and an efficient anaerobic digestion process (Tian et al., 2015). Therefore, the one-step temperature increase seemed to be an effective option for anaerobic digestion systems.

3.3. *Performance and yields of the thermophilic digester (P3)*

Table 2 presents the mean values obtained for the digested sludge (liquid-phase effluent). The gradual HRT reduction proportionally increased the influent mass flow rate of sludge which consequently increased the solids concentration in the digestate. The digested sludge had the lowest organic content when the digester worked at 6 days HRT (VS/TS ratio by 30%). This was due to solids degradation and solids sedimentation inside the digester (a discussion on this is provided in a subsequent section). Total ammonia nitrogen (TAN) concentration increased as the hydraulic retention time decreased, which could be due to the degradation of proteins and urea present in the sludge (Kobayashi et al., 2009). Other than the trial carried out at 5 days HRT, ammonia nitrogen concentrations reached 700 mg/L. Although free ammonia (FA) (NH_3) is toxic to anaerobic bacteria, no inhibition due to NH_3 was observed because the FA average concentrations, which are based on the values of TAN, pH (Table 2) and reactor temperature (55 °C), were low (32.2, 45.2, 28.0 and 18.2 for 13, 9, 6 and 5 days HRT, respectively) (Anthonisen et al., 1976). Total alkalinity did not significantly deviate over all HRT tested. This indicated balance between ammonia, carbonates and VFA concentrations. The pH presented low deviations throughout the experiment seemingly due to the solubility of biogas CO_2 in the liquid phase.

Concentrations of volatile fatty acids steadily increased as the HRT reduced. The highest concentration of total VFA (i.e., acetic, propionic and butyric acids) was obtained at 5 days HRT and was 2.5 times greater than that of the digester at 13 days HRT. In fact, when the retention time was reduced, the digester showed progressive increases in the organic load rate and mass transfer (1.31 ± 0.15, 1.63 ± 0.25, 2.23 ± 0.53 and 2.86 ± 0.24 $kgVS/m^3 \cdot d$ at 13, 9, 6 and 5 days HRT, respectively). Thus, the combination of excess substrate and low retention time enhanced the production kinetics of VFA, i.e., the main fermentation byproduct of organic substrates (Miron et al., 2000; Damasceno et al., 2007).

Despite high VFA concentrations typically decreasing the pH, pH variations were low inside the digester (Table 2). The VFA/alkalinity ratio remained around 0.3 to 0.4 when the process was carried out at 13 and 9 days HRT. These results indicated process stability and an ability to neutralize acids (Rincón et al., 2008). However, under lower HRTs, higher VFA/alkalinity ratios were observed (i.e., greater than 1.0 for 6 and 5 days HRT). During these periods, the digester had poor stability with substantially increased risks of acidification and souring.

VFA levels were maintained inside the digester by dilution with the daily feed, especially when the lowest HRT were applied. The substrates in the feed had low organic solids content and partial alkalinity useful as a buffer (Table 1). Indeed, the degradation of amino acids and proteins from the feed sludge cells may release deprotonated ammonia, which contributes toward increasing buffer capacity (Veeken et al., 2000). Hence, decreased partial alkalinity was mostly due to buffer reactions with high concentrations of soluble compounds in the digestate (SCOD and VFA, Table 1). These mechanisms simultaneously buffer the pH during the entire experiment The behavior of pH, alkalinity and VFA during Period P3 is presented in the Supplementary material 2.

According to the data presented in Table 2, the thermophilic digester showed an average biogas production above 0.20 $m^3/kgVS_{fed}$ at 9 days HRT. For lower retention times, the SGP value ranged from 0.15 to 0.10 $m^3/kgVS_{fed}$. Considering only the solids fraction was removed within the digestate, the average VS removal efficiencies were 74, 84, 85 and 48% at 13, 9, 6 and 5 days HRT, respectively. A more thorough evaluation may consider that the solids fraction settled inside the digester due to intermittent mixing (a further discussion is provided below). Lindmark et al. (2014) showed that digesters should be mixed immediately before feeding to homogenize the content and provide better mixing effects.

The lowest VFA:SCOD ratio was obtained at 9 days HRT, whereas the highest ratio was observed at 5 days HRT. Therefore, almost 49% and 75% of all soluble COD, respectively, were volatile fatty acids. Acids accumulation likely triggered instabilities in anaerobic

Table 2
Digestate characteristics and biogas yields obtained for each HRT tested.

	HRT = 13 d	HRT = 9 d	HRT = 6 d	HRT = 5 d
Digested sludge				
pH	7.2 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.0 ± 0.2
COD (gO_2/L)	6.3 ± 2.3	4.1 ± 1.6	6.6 ± 3.7	16.4 ± 6.6
SCOD[a] (mgO_2/L)	942 ± 124	991 ± 23.6	1020 ± 42.5	1994 ± 113
TS (g/L)	6.4 ± 2.5	5.0 ± 1.0	7.3 ± 3.5	12.9 ± 6.3
VS (g/L)	3.8 ± 1.7	2.4 ± 0.7	2.2 ± 6.5	7.7 ± 4.2
Total P (mg/L)	208 ± 35	164 ± 25	247 ± 61	250 ± 46.1
TKN (mg/L)	800 ± 78	780 ± 99	702 ± 63.5	840 ± 183
TAN ($mg\ N{-}NH_4^+/L$)	543 ± 209	635 ± 64	690 ± 195	475 ± 80
Total VFA[b] (mg/L)	1450 ± 59	1457 ± 114	2934 ± 180	3605 ± 218
Total alkalinity (mg $CaCO_3/L$)	2530 ± 135	2011 ± 244	2151 ± 227	2651 ± 800
Partial alkalinity (mg $CaCO_3/L$)	2215 ± 125	1811 ± 235	1811 ± 202	1686 ± 447
Biogas yields				
GPR ($m^3/m^3_{reactor}.d$)	0.31 ± 0.05	0.35 ± 0.04	0.33 ± 0.06	0.29 ± 0.07
SGP ($m^3/kgVS_{fed}$)	0.24 ± 0.03	0.22 ± 0.02	0.15 ± 0.03	0.10 ± 0.03
Methane fraction in biogas (%)	64 ± 2	64 ± 2	65 ± 1	60 ± 6

[a] Soluble COD.

[b] Individual volatile fatty acids concentrations are presented in the Supplementary material 1.

digestion mechanisms, decreasing biogas production, especially at 6 and 5 days HRT.

The decreased HRT may lead to a sudden biomass washout, especially slow growing methanogens (Siegrist et al., 2002). The average methane contents in biogas were similar at 13 and 9 days HRT (64%, $p\text{-value} > 0.05$). Furthermore, these values were similar to those obtained at lower HRTs. Thus, the methane composition in biogas was not substantially affected by the hydraulic retention time. In fact, the identification of methanogenic Archaea in samples from the bottom sludge collected during the thermophilic period (P3) suggested the occurrence of methanogenic Archaea inside the digester (Fig. 3). Hence, the biological activity that arose in the layer of solids at the bottom of the digester compensated the washout of methanogenic biomass (Kaparaju et al., 2008; Karim et al., 2005).

Although FISH analysis has limitations especially in quantifying the number of microorganisms, the outcomes shown in Fig. 3 revealed important insights regarding metabolically active cell morphology and their arrangement and spatial distribution in bottom sludge in situ samples collected during the thermophilic period (P3).

Methane yields were 0.15 and 0.14 $m^3/kgVS_{fed}$ at 13 and 9 days HRT, respectively. These results agreed with those found by Braguglia et al. (2015), who reported 0.16 $m^3/kgVS_{fed}$ during the thermophilic AD of waste activated sludge in completed stirred tank reactors at 15 days HRT and 1.0 $kgVS/m^3 \cdot d$. Furthermore, the present results were three times greater than those reported by Gianico et al. (2015) (0.05 $m^3/kgVS_{fed}$) whose operational conditions were similar to the current study (10 days HRT and 1.2 $kgVS/m^3 \cdot d$). The biogas production and composition during the thermophilic period (P3) is shown in the Supplementary material 2.

The thermophilic anaerobic process reduced 3-$\log_{10}$ of total coliforms and 1-$\log_{10}$ of fecal coliforms expressed as colony forming units (CFUs) per gram of dry digested sludge (CFU/gTS). Thus, according to Brazilian regulatory law (CONAMA, 2006), the digestate had microbiological characteristics compatible with Class B biosolids. Coliform reduction was achieved at 5 and 6 days HRT, which was far below the typical range of 10–15 days suggested for sludge stabilization under thermophilic temperatures (Appels et al., 2008). These results showed that low HRT could be applied in thermophilic AD processes to achieve coliforms within an acceptable range.

3.4. Substrate and nutrients mass balance

Mass balances for solids, COD, total nitrogen and total phosphorus were calculated using data from Tables 1 and 2. Waste activated sludge was considered as input. Digestate, organic matter conversion into biogas and bottom sludge were jointly considered as output. The characteristics of the bottom sludge were estimated as the residual mass flow to account for 100% of the input flow. Moreover, the contents of TS, VS and COD in biogas were estimated by multiplying the number of moles of biogas (methane and carbon dioxide) and the molecular weight of methane and carbon dioxide. All calculations assumed standard temperature and pressure conditions (273.15 K and 1.01325 bar).

Fig. 4 shows the estimated outcomes for the substrate and nutrients mass balances. Volatile solids and COD were partially removed through biogas formation, with average efficiencies following decreasing HRTs (22, 21, 14 and 10% for VS and 14, 13, 9 and 7% for the COD as the HRT progressively decreased from 13 to 5 days). In some cases, these results were slightly lower than those reported by Kaparaju et al. (2008) (0.22 $m^3CH_4/kgVS_{fed}$) during the anaerobic digestion of manure at 55 °C under intermittent mixing (interruption of mixing 2 h prior to extraction and feeding). Comparatively, in the present study, the HRT was greater (15 days) and the TS content in the feed ranged from 6.5 to 7.5%. In fact, there is a lack of similar AD studies that treat WAS using the proposed

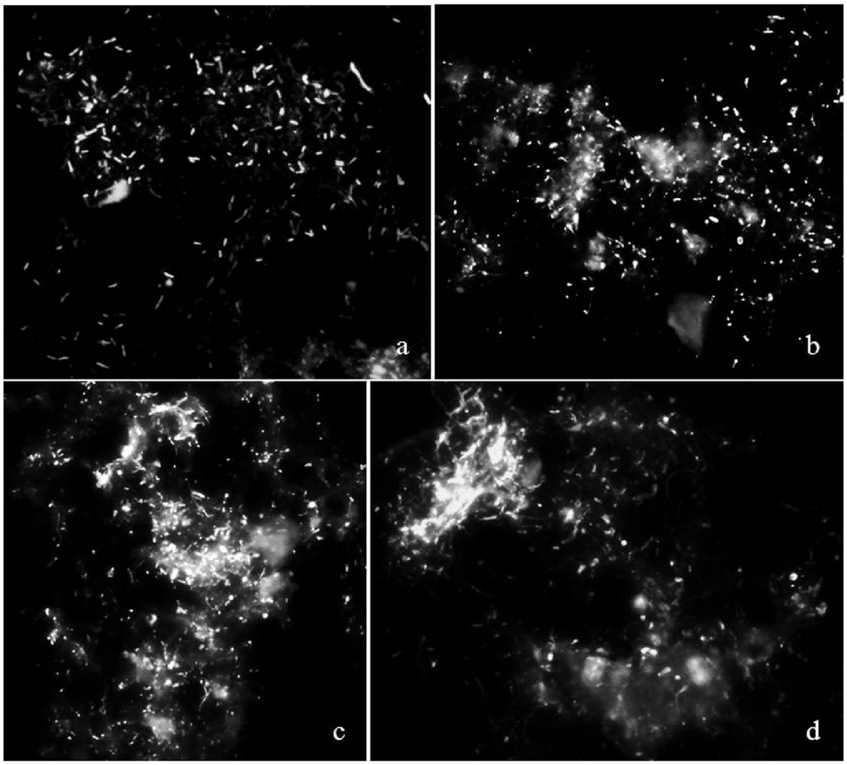
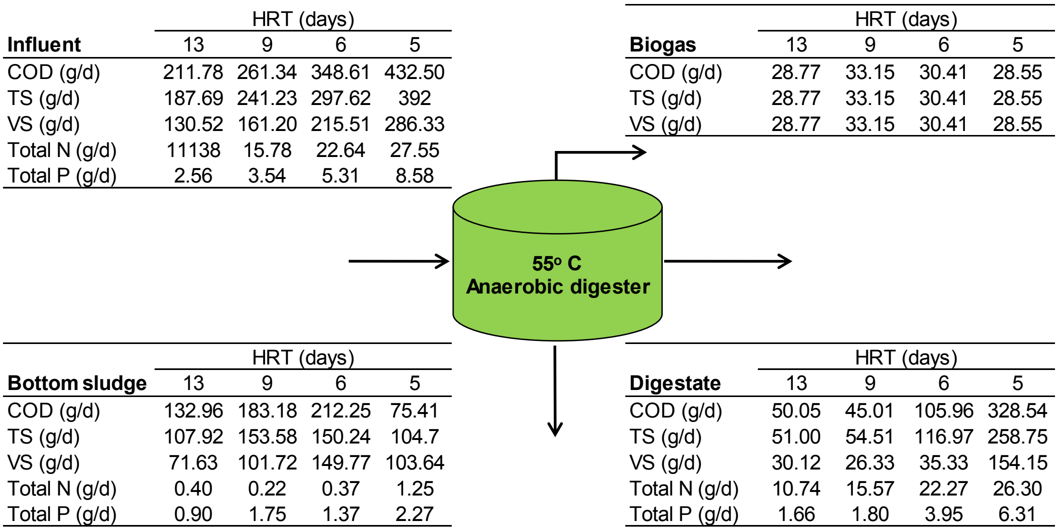


Fig. 3. Hybridized cells (White points) indicating Archaea occurrence along all HRT conditions tested: 13 days (a), 9 days (b), 6 days (c), 5 days (d).



Influent	HRT (days) 13	9	6	5
COD (g/d)	211.78	261.34	348.61	432.50
TS (g/d)	187.69	241.23	297.62	392
VS (g/d)	130.52	161.20	215.51	286.33
Total N (g/d)	11138	15.78	22.64	27.55
Total P (g/d)	2.56	3.54	5.31	8.58

Biogas	HRT (days) 13	9	6	5
COD (g/d)	28.77	33.15	30.41	28.55
TS (g/d)	28.77	33.15	30.41	28.55
VS (g/d)	28.77	33.15	30.41	28.55

Bottom sludge	HRT (days) 13	9	6	5
COD (g/d)	132.96	183.18	212.25	75.41
TS (g/d)	107.92	153.58	150.24	104.7
VS (g/d)	71.63	101.72	149.77	103.64
Total N (g/d)	0.40	0.22	0.37	1.25
Total P (g/d)	0.90	1.75	1.37	2.27

Digestate	HRT (days) 13	9	6	5
COD (g/d)	50.05	45.01	105.96	328.54
TS (g/d)	51.00	54.51	116.97	258.75
VS (g/d)	30.12	26.33	35.33	154.15
Total N (g/d)	10.74	15.57	22.27	26.30
Total P (g/d)	1.66	1.80	3.95	6.31

Fig. 4. Solids, COD and nutrients mass balances.

intermittent mixing approach (i.e., withholding mixing for 2 h prior to extraction and feeding).

Intermittent mixing provided a minimal solid (TS and VS) sedimentation rate of 50% at 13 and 6 days HRT. Regarding the COD, this value increased to 70% at 9 days HRT. Heavy suspended solids had a tendency to settle to the bottom after the stirring interruption (2 h before feeding). Thus, the stratification decoupled the solids retention time (SRT) from the HRT and increased the SRT. The high VS/TS ratio in the bottom sludge (greater than 60% for all HRT) emphasized the presence of anaerobes in this layer, whose metabolism supported the observed biogas yields (Kaparaju and Angelidaki, 2008).

In terms of total nitrogen, while the organic fraction was predominant in the influent sludge (Table 1), total ammonia nitrogen ($N{-}NH_4^+$) corresponded to at least 40% of the total nitrogen in the effluent produced over all tested HRT. Thus, when considering only the soluble ammonia, the ammonification efficiencies were 40, 45, 50 and 36% at 13, 9, 6 and 5 days HRT, respectively (Table 2 and Fig. 4).

The highest ammonification rates correlated with the lowest HRT since this condition benefited hydrolysis reactions and consequently, the conversion of organic nitrogen into inorganic nitrogen. This evidence suggested the solids solubilization rate was higher than expected; in fact, hydrolysis has been recognized as the main rate-limiting step in the AD of WAS (Appels et al., 2008). The low particulate solids content in the WAS (2.1% TS, Table 1) and its great inertial content (COD/VS ratio of 1.6) likely benefited enzymatic hydrolysis.

Approximately 30–50% of the total phosphorus remained in the bottom sludge, which could be used in agronomic applications.

The proper management of waste activated sludge comprises sludge dewatering prior to final disposal. Solid concentration, floc size and density can affect sludge dewaterability (Lo et al., 2001). Several recent studies have provided data on the capillary suction time (CST) and outcomes for a poor filterability of thermophilic anaerobic digested sludge with high TS content (over 5.0 g/L) (Leite et al., 2016; Wang et al., 2016). Under these conditions, and considering Fig. 4, the decreased HRT may negatively influence the CST value because the estimated solid content on the bottom sludge would significantly increase (4.6, 14.1, 25.2 and 21.1 gTS/L at 13, 9, 6 and 5 days HRT, respectively). While these figures were estimated based on a mass balance, the solids present in the liquid-phase digestate were not considered. Therefore, a more specific dewatering analysis, while considering solids from the digestate, would provide a more thorough discussion of sludge filterability and its impacts on energy requirements and economic costs.

3.5. Energy requirements for the thermophilic digester

The heat requirements and energy balance analyses considered a hypothetical WWTP 140,000 population equivalent. Table 3 presents the energy yields and the energy requirements necessary to maintain the operation of the single-stage thermophilic anaerobic digester. The highest energy yield was obtained when the digester was operated at 13 days HRT (3.0 MWh/d). The conversion of biogas into electric power was 2.5 kWh/m^3, i.e., a consistent value for an electrical yield of 40% (Deublein and Steinhauser, 2008).

The heat energy produced at 13 days HRT was 8% higher than that obtained at 9 days HRT. Moreover, the heat energy produced at 13 days HRT was 38% and 58% greater than those obtained at 6 and 5 days HRT, respectively. However, the difference between the available thermal energy and the thermal requirements (i.e., the sum of sludge heating and heat losses) was always negative regardless of the HRT tested. These results were largely due to the use of diluted sludge (2% TS) as substrate.

Sustainable energy production of such a digester configuration under thermophilic temperatures was obtained when a more concentrated sludge was used as the feed to provide a higher biogas production, thereby increasing sludge calorific power, optimizing digester size and decreasing heat losses (Bolzonella et al., 2012; Leite et al., 2016).

The additional temperature required to maintain the digester at 55 °C due to heat losses through the digester walls were estimated to be 3.3, 2.6, 2.0 and 1.8 °C at 13, 9, 6 and 5 days HRT. Clearly, at higher HRTs, larger digester volumes were required resulting in greater additional temperature to make up for natural losses through the digester walls.

The waste activated sludge flow rate was constant 350 $m^3{}_{sludge}/d$ (50 gTS/inhab·d) regardless of the applied HRT in the digester. Thus, the energy demand to heat the influent sludge was always the same (i.e., 12,250,000 kcal/d). Because the retention time defined the digester working volume (i.e., 4550, 3150, 2100 and 1750 m^3 at 13, 9, 6 and 5 days HRT, respectively) and their heat losses (moving

Table 3
Energy requirements and thermal balance in the single-stage anaerobic sludge digester under thermophilic temperature.[a]

HRT (Day)	Heat energy production (kcal/d)		Heat requirements (kcal/d)	Power capacity (kW)	Power production (MWh/d)
	CHP	Boiler			
13	3,234,000	5,821,200	13,412,692	125	3.0
9	2,964,500	5,336,100	13,159,908	115	2.8
6	2,021,250	3,638,250	12,944,390	78	1.9
5	1,347,500	2,425,500	12,864,916	52	1.3

[a] Thermal balance = (heat energy production − heat requirements).

from 1,162,692 kcal/d to 614,916 kcal/d at 13 and 5 days HRT, respectively), the differences among the heat requirements presented in Table 3 were due to heat losses through the digester walls (calculations are presented in Supplementary material 3 in Science Direct).

A preliminary estimation of the mechanical energy consumption was performed based on the power input used in other similar high rate AD processes to design proper digester mixing modes (8 $W/m^3_{reactor}$) (USEPA, 1974; Karim et al., 2005; WEF, 2010; Trad et al., 2017). Energy requirements for mixing were 288, 200, 133 and 111 MWh/year at 13, 9, 6 and 5 days HRT, respectively. Moreover, the power requirements for intermittent mixing accounted for 22–29% of the power capacity for heat requirements in hypothetical full–scale reactors (Table 3).

As discussed above, an increased digester working size would require greater power for mixing. However, there is no real consensus on how much mixing (i.e., intensity and duration) is required (Lindmark et al., 2014).

Fig. 5 shows the thermal energy balance required to treat the waste activated sludge used in this study (approximately 2.0% TS, Table 1). Estimations also considered higher solids content to verify the influence of TS and sludge thickening on the thermal balance.

When the digester was conducted at low HRT, higher organic loads were introduced in the system (Fig. 4), and biogas production was negatively affected (Table 2). The data in Fig. 5 suggests that a higher TS content in the thickened influent sludge resulted in a positive thermal balance.

The thermal modeling emphasized the importance of increasing sludge concentrations (approximately 5% TS) to achieve energy self-sufficiency (in this case, either at 13 or 9 days HRT). This evidence was consistent with results reported in other AD studies applied in the treatment of WAS (Mata-Alvarez et al., 2000; Leite et al., 2016). Improvements on the gravitational thickening step can promote the sludge quality and improve the thermal balance (Metcalf and Eddy, 2014). However, the data shown in Fig. 5 was also supported by differences in the digester design (sizing), which affected heat losses and energy demands as a consequence.

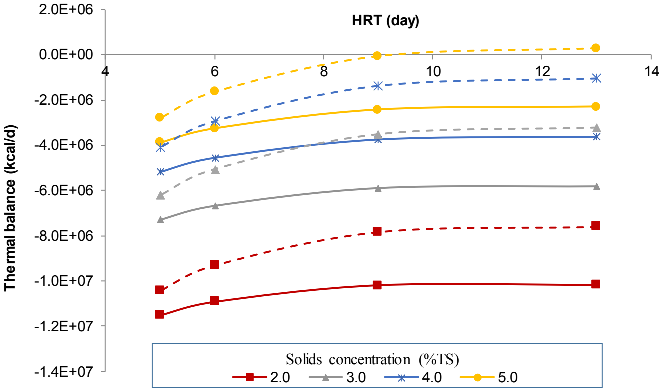


Fig. 5. Thermal balance at different hydraulic retention time and solids concentration in the substrate (CHP: solid line, Boiler: dashed line).

According to Pilli et al. (2015), when the sludge TS content is higher than 3.0%, energy yields can be limited due to lower solids degradation rates. Thus, several sludge pretreatments techniques may increase solids biodegradability and improve the energy balance (Braguglia et al., 2014, 2015; Ma et al., 2016). Alternatively, the use of two-stages or phased reactors, i.e., a hydrolytic-fermentative reactor and a methanogenic digester, could also improve the AD, especially when low HRTs or high OLRs are used (Ghosh, 1987; Demirel and Yenigun, 2002; Bolzonella et al., 2012).

3.6. Economic considerations

Economic analyses were performed considering two different scenarios. The first one (scenario 1) considered a WWTP for handling 140,000 inhabitants equivalent without a sludge stabilization unit but with a gravity thickening step (2.0% TS), a mechanical thickening step (25% TS) and sludge landfilling (€34.00/$m^3_{wet\ mass}$). The second situation (scenario 2) considered an added single stage anaerobic sludge digester prior to mechanical thickening. The digester works at 9 days HRT under thermophilic temperature (55 °C). Investments costs were estimated as 278 euros taking into account prior experiences in sanitation costs in Brazil (based on the WWTP sludge source in this study).

Annual costs regarding sludge landfilling in scenario 1 were estimated at approximately € 345,713.00 (approximately 1,279,138 million Brazilian Reais). When an anaerobic digester was considered (scenario 2), 1500 tons of sludge would not have to be landfilled, thereby reducing costs by 15%. The estimated investment for the implementation of the single stage anaerobic thermophilic digester was over € 966,860.00. Boilers and hydraulic materials costs were assumed to be 10% of the digester cost (Bolzonella et al., 2007). Based on these estimations, the payback time for implementing an anaerobic sludge digester (2%TS, 9 days HRT) would be 19 years. These estimations did not consider costs related to mechanical mixing (€ 28,873.00 per year); hence, the economic feasibility could be even worse. In any case, as shown in Table 3 and Fig. 5, the digester did not show a positive thermal energy balance with the current TS concentration in the substrate. Electrical energy from surrounding municipalities may be required.

Because the biogas yields were quite similar among the applied HRT (Fig. 4), the negative effect of working at low HRT was minimized. Indeed, the proposed mixing mode provided conditions to produce as much methane as possible even in very low HRT conditions (5 and 6 days, Table 3). This feature provided outstanding advantages in reducing mixing intensity and/or mixing times and operational and maintenance costs (Lindmark et al., 2014).

Despite non-viable economic outcomes, the applicability of such a process follows a new concept that utilizes energy recovery and represents a more sustainable way to manage sludge. The anaerobic digestion resulted in a considerable reduction in the VS fraction of the sludge to be disposed (21% for 9 days HRT). Moreover, the biogas production can provide 40% of the required heat to maintain the sludge temperature at 55 °C (Table 3). Considering the low solids contents in the sludge after the gravity thickening (as is the case in several WWTP), the combination of intermittent mixing and low HRT may result in energy and area savings in a full-scale scenario. Furthermore, the solids fraction of the digested sludge can be used as a fertilizer in agricultural applications, whereas the enriched VFA liquid fraction may be sent upstream to an activated sludge tank as a carbon source for denitrification purposes, thereby reducing associated costs.

4. Conclusions

The single-step temperature conversion supported thermophilic growth under their optimal temperature from the beginning. As a result, methanogenesis was rapidly reestablished in 18 days after temperature change. Decreasing the hydraulic retention time was not favorable to specific gas production under thermophilic conditions, which was greater than 0.22 $m^3/kgVS_{fed}$ at 9 and 13 days HRT and below 0.16 $m^3/kgVS_{fed}$ at 6 and 5 days HRT. Similarly, digesters at 6 and 5 days HRT impaired the volatile solids removal. Mixing interruptions 2 h before feeding provided good conditions to compensate for methanogen washout, even at low HRTs. However, the methane fraction in biogas was greater than 60% in all HRT tested, given the presence of methanogens in the bottom sludge. The suspended solids sedimentation rate was above 50%, and a clarified effluent was produced. Intermittent mixing could cut electrical energy costs, which accounted for nearly 30% of the energy required to support the thermophilic AD. The energy balance suggested that a self-sufficient system could be achieved with a more concentrated sludge in the feed (5% TS).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2017.06.069.

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