



Assessment of energy efficiency and performance in a two-phase anaerobic process for organic matter removal

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ABSTRACT

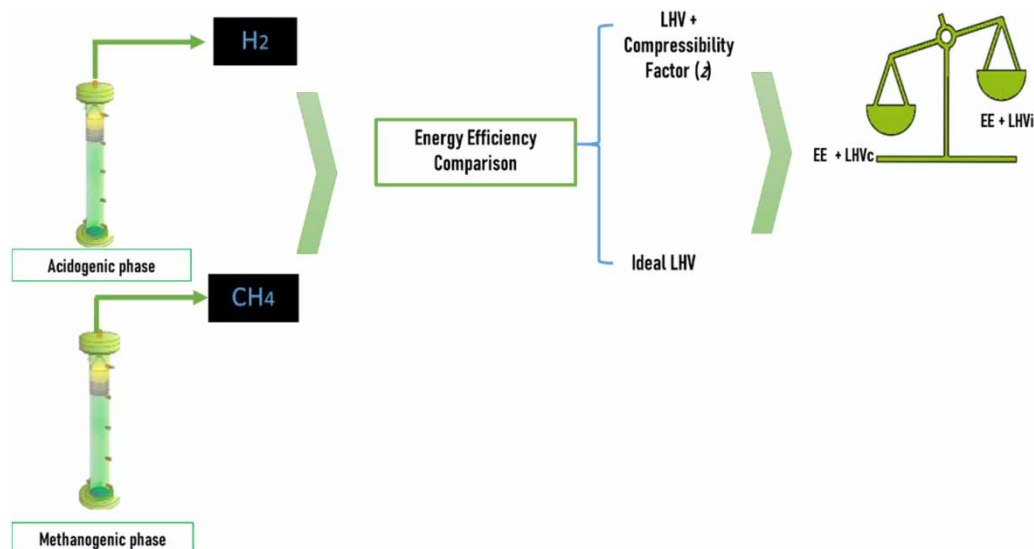
Energy efficiency (EE) depends mainly on the lower heating values (LHVs) of hydrogen and methane selected from the thermodynamics tables under ideal conditions. However, for practical applications, the heating value should be calculated by considering some environmental factors under real conditions. Accordingly, this study compared EE using the ideal LHV with the EE using the real LHV in a two-phase anaerobic digestion reactor treating synthetic wastewater. Additionally, the process performance and the stability were studied. The results showed that the EE value calculated using LHV_{ideal} was, on average, 35% higher than that evaluated using LHV_{real} ; these differences are relevant to the estimation of real energy and also for practical applications. At the same time, the index buffer intermediate alkalinity/partial alkalinity was shown to be more accurate than the pH value to analyze the stability of the process. With regards to chemical oxygen demand, the removal efficiency in the methanogenic phase decreased drastically when utilizing 100% of the acidogenic phase. Future considerations for the optimization of each phase are highlighted.

Key words: acidogenic phase, anaerobic digestion, energy gain, energy recovery, heating value, methanogenic phase

HIGHLIGHTS

- Lower heating value should be adjusted to estimate the energy efficiency.
- Different approaches are considered to evaluate the energy gain and the energetic efficiency in a anaerobic digestion process in two phases.
- The IA/PA ratio is recommended as a process indicator if compared only with the pH value.

GRAPHICAL ABSTRACT



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LIST OF ABBREVIATIONS

ASBR	Anaerobic sequencing batch reactor
COD	Chemical oxygen demand
CSTR	Continuous stirred-tank reactors
DF	Dark fermentation
EE	Energy efficiency
EEL	Total electric energy
HHV _c	Higher heating value
HRT	Hydraulic retention time
HY	Hydrogen yield
IA/PA	Intermediate alkalinity/artial alkalinity
LAB	Lactic acid bacteria
LHV	Low heating value
NA	Not available
OLR	Organic loading rate
SWW	Synthetic wastewater
TVA	Total volatile acids
TVS	Total volatile solids
VFA	Volatile fatty acids
VHPR	Volumetric hydrogen-production rate.

1. INTRODUCTION

Energy and wastewater management forms a crucial part of sustainable development goals, which are challenging to realize for developing countries. This has significantly hindered the achievement of sustainable development goals, thereby affecting the well-being and development of rural and urban communities in the long term. A sustainable economic model called the circular economy has highlighted the opportunities to use the anaerobic digestion (AD) process as a tool for efficiently treating wastewater with the potential to produce bioenergy and value-added bio-products. [Urbinati *et al.* \(2017\)](#), [Kalmykova *et al.* \(2018\)](#), and [Korhonen *et al.* \(2018\)](#) recognized the correlation between a circular economy and AD, which has emerged as a sustainable industrial strategy focused on the recovery of products that can replace fossil fuels.

According to [Intanoo *et al.* \(2016\)](#), AD will very shortly be the critical piece to obtain products with a high energy value (i.e., food waste, wastewater, municipal solid waste, and lignocellulosic biomass). Other authors like [Vergine *et al.* \(2015\)](#) have ratified this vision and add that, despite this technology having been developed to stabilize the organic matter present in wastewater, in recent years, this process has been adopted for the production of bioenergy. They cite, for example, that in Europe about 14,000 plants, 8,000 of them in Germany, already use the biogas they produce to generate heat and power (combined heat and power – CHP).

Compared with anaerobic digestion, acidogenic phase/dark fermentation does not allow the complete degradation of organic matter, which would lead to effluent rich in AGVs and with a high presence of carbon residues, approximately 70%, making it necessary to complete the conversion to CO₂. This residue must be taken to a second treatment in a methanogenic phase to close the cycle.

[Perera *et al.* \(2010\)](#) ([Kong *et al.* \(2019\)](#)), in a comprehensive review on the production of methane and hydrogen through anaerobic processes, indicated the significant advantages that systems have in two sequential phases, once the adjustment of control parameters in each of the reactors is facilitated, so such that H₂ production is optimized and degradation of the substrate to methane is promoted. These authors present the results of various studies, including on a real scale, being that in most cases, methane generation values greater than 70% and hydrogen values between 15 to 60% have been reached. It is worth mentioning that one of the most significant advantages of two-phase systems is the possibility of producing energy and other intermediaries with a high energy value with wastewater whose substrate is complicated, that is, with low pH or high content of lignocellulosic compounds.

[Fuess & Garcia \(2015\)](#) showed how the phase separation in an anaerobic process could increase bioenergy recovery in a sugar cane industry. Additionally, they emphasized the significant benefits of improving the biodegradability of specific substrate types that are recalcitrant to some compounds in the methanogenic phase. [Perera *et al.* \(2012\)](#) recommend anaerobic reactors in two phases to achieve the maximum efficiency of the treatment for every phase process: the acidogenic step offers

operative benefits for the system, such as the increment of hydrolysis rate, the efficiency of volatile fatty acids (VFA) production, and biohydrogen production.

Subsequently, the energy efficiency (EE) assessment of unconventional renewable sources is fundamental to identifying the true potential of producing biomass energy using wastewater as a raw material. EE denotes the relationship between the useful output power and input power of the system (i.e., $\text{efficiency} = \text{useful power output} / \text{power input}$) estimated basically with the heating value of the gases.

The lower heating value (LHV) is the net calorific value calculated by subtracting the heat of vaporization of water (generated during fuel combustion) from the higher heating value (Meriçboyu *et al.* 1998). Because in AD, different types of fuel gases (i.e., hydrogen, methane, and biohythane) are released as by-products, the adequate estimation of LHV becomes critical to determining the actual energy potential of the AD process.

The BS ISO 6976:2016 (BSI 2016) details the methodology of calculating the gross and net calorific values of any natural gas or other gaseous fuel of known molar composition. The calculation mainly includes the compressibility factor (Z) in LHV estimation. The compressibility factor considers many aspects, including gas pressure and temperature. This type of evaluation allows a realistic assessment of the calorific value of biogas, as it is not an ideal gas.

Accordingly, to enhance the knowledge of the energy assessment in a two-phase AD, this study compares the EE calculated using the LHV estimated according to BSI (2016) with the EE calculated using the LHV taken directly from thermodynamics tables. Additionally, we evaluated the process stability and removal efficiency of the organic matter in the acidogenic and methanogenic phases. Our main finding is that the EE calculated using LHV_{real} is lower than that estimated using LHV_{deal} , thereby indicating that the heating value taken directly from thermodynamics tables can overestimate the calculated EE value.

2. MATERIAL AND METHODS

2.1. Experimental setup

The scheme of the experimental setup is shown in Figure 1. The reactors were constructed in rigid polyvinyl chloride (PVC) with an internal diameter of 74 mm and a net volume of 4.10 L at a laboratory scale. The reactor configuration is

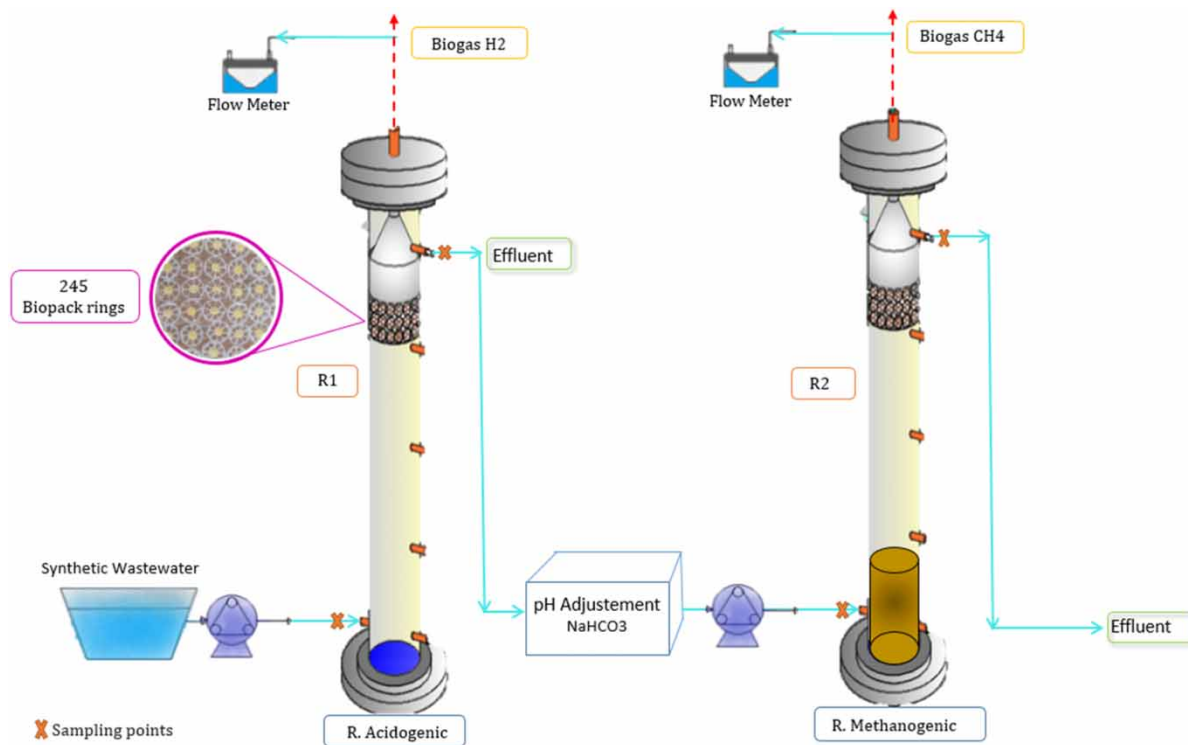


Figure 1 | Schematic diagram of the two-phase anaerobic reactors: acidogenic reactor (R_1) and methanogenic reactor (R_2).

characterized by the addition of a 200-mm-thick suspended bed composed of high-density polyethylene rings ($\text{Ø} = 19 \text{ mm}$ and height = 10 mm); the bed contains a polyurethane foam core to avoid washing of the biomass and improve the biogas-production stability (Karadag *et al.* 2015). The specific surface area of each ring was $950 \text{ m}^2 \text{ m}^{-3}$. The reactors were named: Reactor 1 (R_1) for the acidogenic phase and Reactor 2 (R_2) for the methanogenic phase.

2.2. Experimental procedures

In both reactors, HRT of 8 h was controlled by a Masterflex L/S brand peristaltic pump with the capacity to regulate flow rates from 0.0006 to 3,400 ml/min. This condition was chosen based on previous results (Mendez-Revollo *et al.* 2017). The reactors were kept in an acclimatized chamber set at $30 \pm 5 \text{ °C}$.

Reactor R_1 was supplied with synthetic wastewater (SWW) with a pH of 5.5 and organic loading rate (OLR) of $11.26 \text{ kgCOD m}^{-3} \text{ d}^{-1}$ as proposed by Hernández *et al.* (2018). Reactor R_2 was operated in five steps to avoid acid shock.

As suggested by Buzzini & Pires (2007), the first phase comprised feeding the reactor with SWW (yeast extract: 120 mg L^{-1} , ammonium chloride: 70 mg L^{-1} , monobasic sodium phosphate: 35 mg L^{-1} , and ethanol: 0.025% v/v) with an initial OLR of $4.23 \text{ kgCOD m}^{-3} \text{ d}^{-1}$. During steps, 2, 3, 4, and 5, the feed was set as a mixture of SWW and the effluent from Reactor R_1 (i.e., E_{fR_1}) in the '(SWW: E_{fR_1})' ratio of 75:25, 50:50, 25:75, and 0:100, with the OLR values of 7.75, 9.40, 10.7, and $11.2 \text{ kgCOD m}^{-3} \text{ d}^{-1}$, respectively. The pH of the effluent from R_1 was adjusted to be between 7.2 and 7.4 before the operation of Reactor R_2 . During the operation of Reactor R_2 , the alkalinity was maintained above $1,500 \text{ mg L}^{-1}$ by keeping the concentration of sodium bicarbonate (NaHCO_3) in the mixture inside the reactor between 2 and 22 g L^{-1} .

For reactor R_1 , the inoculum was obtained via the natural fermentation mechanism. The advantage of this kind of inoculation is that no pretreatment is required to enrich the acidogenic biomass. This method involved setting the temperature of the wastewater under study to ambient temperature for three days and then recirculating it for 72 h to guarantee satisfactory adherence to the support medium. Fernandes *et al.* (2013) demonstrated that natural fermentation, caused by the combination of the microorganisms in the air and tap water, is beneficial in maintaining acidogenic conditions. The methanogenic reactor (R_2) was inoculated with granulated sludge from a UASB (Up-flow Anaerobic Sludge Blanket) reactor treating effluent from a beverage industry (Bogotá, Colombia). The sludge volume was a third of a useful volume (i.e., 0.883 L), and the TVS was $4,140.29 \text{ mgTVS/L}$.

2.3. Analytical determinations

The organic matter content (COD) and volatile suspended solids concentration were analyzed according to the standard methods provided in APHA (2012). The phenol method (Herbert *et al.* 1971) was used to analyze the total concentration of carbohydrates. To assess the stability and the buffer capacity of the methanogenic phase, the partial and intermediate alkalinities, total volatile acids (TVA) concentration, pH, and the ratio IA/P were evaluated according to the recommendations of Ripley *et al.* (1986) and Lahav & Morgan (2004). With regard to the acidogenic phase, the concentration of VFA and the TVAs was monitored.

All the parameters were measured twice per week, at least during ca. 167 days of work, and the results were reported with the mean and the standard deviation using the ORIGIN PRO 8.5 software. The yield and volumetric production of the biogas (i.e., H_2 and CH_4) were calculated according to Moureira *et al.* (2013).

The composition of the biogas (H_2 , CO_2 , and CH_4) was measured by the gas chromatography (Agilent Technologies) 7890A GC system, which was equipped with a thermal conductivity detector and capillary column Carboxen 1010 plot ($30 \text{ m} \times 0.25 \text{ mm} \times 25 \text{ }\mu\text{m}$). Nitrogen was used as the carrier gas, and the injection volume was 0.6 mL. The average values were calculated as the result of three measurements per sample.

The analysis of volatile fatty acids was carried out by a gas chromatograph Agilent 7890^a GC, using an FID detector and capillary column HP-INNOWAX ($30 \text{ mm} \times 0.25 \text{ mm} \times 0.025 \text{ }\mu\text{m}$). The oven temperature was maintained at 210 °C and the flow rate at 15 ml/min, the system was coupled to a Headspace brand HS 7694E for automatic injection. Nitrogen was used as the carrier gas.

The measurement of biogas's flow was conducted through the CaudalisG flowmeter, equipment of local fabrication. This device was coupled and placed at the biogas exit of each reactor. The datasets generated were sent via Bluetooth and stored in a desktop computer through the LabVIEW[®] software.

2.4. Energy assessment

The energy assessment was performed using the equations proposed by Xie *et al.* (2008), Das & Veziroö (2001), and Fuess & Garcia (2015). These equations used the ideal heating values of hydrogen and methane, i.e., 242 and 801 kJmol⁻¹, respectively. We proposed to correct these heating values using Equations (1) and (2). These two equations define the thermodynamic factors of the biogas mixture. One has the following:

- Compressibility factor

$$Z(\text{dimensionless}) = 1 - \left(\frac{P_2}{P_0}\right) \left[\sum_{j=1}^n x_j s_j(t_2, P_0) \right]^2 \quad (1)$$

- Lower heating value

$$LHV_c (MJ m^{-3}) = \frac{x_j \left(\sum_{j=1}^n LHV_{ideal j} - \sum_{j=1}^n \left(\frac{b_j}{2}\right) H \right)}{\left(\frac{ZRT_2}{P_2}\right)} \quad (2)$$

In Equation (1), the terms P_2 , P_0 , x_j , and s_j denote the local atmospheric pressure (kPa), standard pressure (101.325 kPa), mole fractions of the components (i.e., H₂, CO₂, and CH₄) in the biogas, and summation factor for a combustion temperature at ambient conditions (15 °C).

In Equation (2), the terms LHV_c , $LHV_{ideal j}$, R , and T_2 denote the calculated heating value of the biogas mixture, heating value of each component at a combustion temperature of 15 °C (kJ mol⁻¹), gas constant (J mol⁻¹ K⁻¹), and absolute temperature of operation (K), respectively. The terms b_j and H denote the number of hydrogen atoms in the molecule of each component (H₂, CO₂, and CH₄) of the biogas mixture, and vaporization enthalpy of water at a combustion temperature of 15 °C, respectively.

Table 1 presents the equations used to estimate the energy efficiency in this study. The energy assessment was performed using the ideal heating value (LHV_{ideal}) and calculated heating value (LHV_c) for each case.

The organic mass balance was calculated according to Algapani *et al.* (2018) as the Equation (3):

$$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{H_2} + Loss \quad (3)$$

where COD_{CH_4} and COD_{H_2} correspond to the COD converted to produce gas, and they are calculated using the theoretical values of 0.35 L CH₄ g⁻¹ COD and 1.4 L H₂ g⁻¹ COD, respectively.

3. RESULTS AND DISCUSSION

3.1. Bioreactor's performance

3.1.1. Acidogenic reactor

The biogas volumetric flow (Q_g), percentage of H₂ in the biogas mixture, and concentrations of TVAs in the influent and effluent of the acidogenic reactor are shown in Figure 2. The results show a continuous hydrogen production despite the low pH values of 3.30 ± 0.33 . Notably, the initial pH in Reactor R₁ was adjusted to 5.5, as the literature recommended the optimum pH to be between 4.5 and 6.5 (Castelló *et al.* 2020) and (Rao & Basak 2021). The results of Mota *et al.* (2018) indicated that hydrogen gas could be produced in highly acidic conditions. This finding is advantageous because most industrial wastewater types, including vinasse, non-alcoholic beverage wastewater, and coffee wastewater, have low pH values. Additionally, the experimental results of Mota *et al.* (2018) showed that a volumetric OLR of 25 gCOD L⁻¹ d⁻¹, coupled with an HRT of 4.6 h and pH of 2.7, achieved a volumetric hydrogen production of 175 mLH₂ L⁻¹ h⁻¹ and hydrogen yield of 3.4 molH₂ mol_{sac}⁻¹.

In this study, the acidic conditions with an HRT of 8 h did not promote lactate accumulation. Therefore, hydrogen production increased. This behavior can be deemed similar to that of the gastrointestinal tract due to the acidic conditions, which seem to be the critical factor in regulating lactate accumulation (Belenguer *et al.* 2011). Furthermore, these authors reported that the pH values depended on HRT and OLR, thereby indicating that high values of OLR promote lactate accumulation. This excess lactate resulted in a decrease in the pH value and, consequently, a decrease in hydrogen production.

Table 1 | Energy assessment for AD in a two-phase system

Authors	Definition	Equation
Xie <i>et al.</i> (2008)	Efficiency of energy cogeneration (two phases)	$EEC[\%] = \frac{HY * LHV_{idealH2} + MY * LHV_{ideal CH4}}{LHV_{Sac}}$ $EECa[\%] = \frac{LHV_c [HY + MY]}{LHV_{Sac}}$
Das and Veziroä (2001)	Final efficiency of hydrogen conversion into energy (1 phase)	$FCE_{H2}[\%] = \frac{G_{H2} * HY * \eta_{fc}^a}{LHV_{Sac}}$
Fuess <i>et al.</i> (2017), Fuess and Garcia (2015)	Total amount of electricity-EEL (two phases)	$BFR_{CH4}[m^3h^{-1}] = \frac{Q_{influent} * COD_0 * COD_e * MY}{f_{CH4}}$ $BFR_{H2}[m^3h^{-1}] = \frac{Q_{influent} * CH * CHC * HY * R(273.15 + T)}{1000 M_{sac} * f_{H2} * P_1}$ $IPC[MW] = \frac{BFR_{H2} * LHV_{idealH2} * \eta_{fc}^a + BFR_{CH4} * LHV_{ideal CH4} * \eta_{CHP}^b}{3600}$ $IPCa[MW] = \frac{LHV_c [BFR_{H2} * \eta_{fc}^a + BFR_{CH4} * \eta_{CHP}^b]}{3600}$ $EEL[MW.h] = IPC * 24$ $EELa[MW.h] = IPCa * 24$

The electrical energy efficiency of converters: x^a Fuel cell $\eta_{fc} = 60\%$ (Wang *et al.* 2018)

x^b Combined Heat and power module $\eta_{CHP} = 33\%$ (Pöschl *et al.* 2010)

where: *HY*, Hydrogen yield ($\frac{molH2}{mol Suc}$); *MY*, Methane yield ($\frac{molCH4}{mol Suc}$); LHV_{Sac} , LHV of sucrose ($\frac{kJ}{molSuc}$); G_{H2} , Gibbs free energy of hydrogen ($\frac{kJ}{mol H2}$); BFR_{CH4} , Normalized flow rate of methane ($\frac{Nm^3CH4}{h}$); BFR_{H2} , Normalized flow rate of hydrogen ($\frac{Nm^3H2}{h}$); $Q_{influent}$, Wastewater influent flow rate ($\frac{m^3}{h}$); COD_0 , Chemical oxygen demand concentration in R_2 influent ($\frac{gCOD}{l}$); COD_e , Chemical oxygen demand removal rate in R_2 (%); *CH*, Sucrose concentration in R_1 influent ($\frac{gC_{12}H_{22}O_{11}}{l}$); *CHC*, Sucrose conversion in R_1 (%); f_{H2} , Hydrogen content in the biogas (%); f_{CH4} , Methane content in the biogas (%); M_{sac} , Molar mass of sucrose ($\frac{gC_{12}H_{22}O_{11}}{mol}$); P_1 , Local atmospheric pressure (atm); *R*, Universal gas constant (atmL/molK); *T*, Operating temperature (°C); *IPC*, Installed power capacity of a fuel cell and CHP plant (MW); *IPCa*, installed power capacity using the adjusted LHV (MW); *EECa*, Energy efficiency cogeneration using the adjusted LHV (%); *EELa*, Total amount of electricity obtained using the adjusted LHV (MW.h).

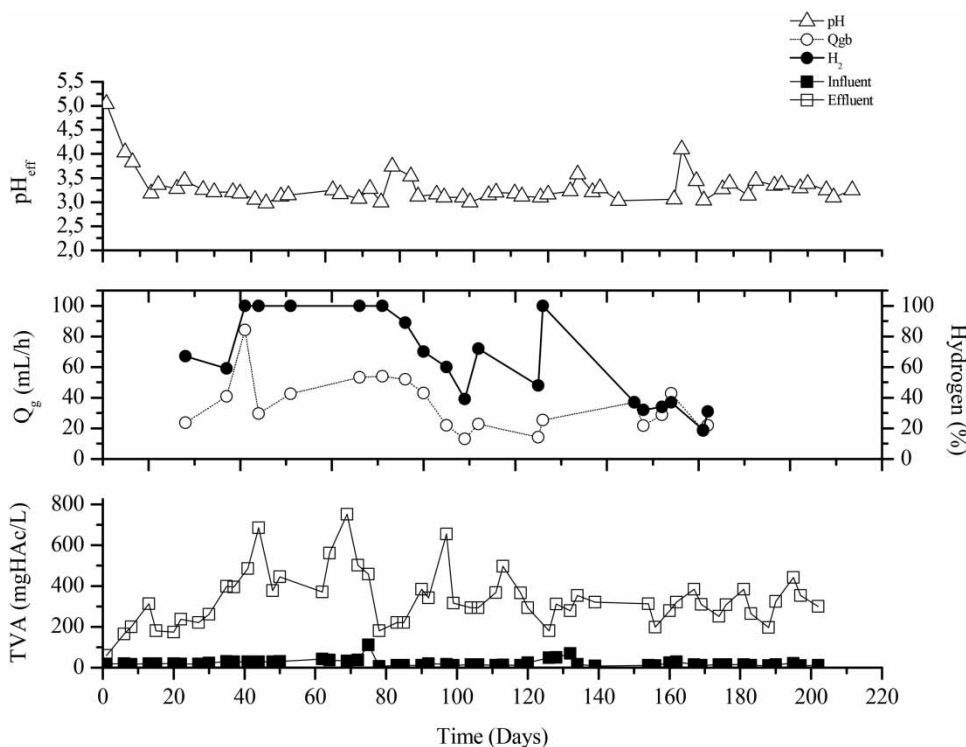


Figure 2 | Temporal variation of pH and TVAs. (-Δ-) pH_{eff}, (-●-) percentage of hydrogen, (-○-) Q_g, (-■-) TVAs_{in}, (-□-) TVAs_{eff}.

Guo *et al.* (2010) established that pH values below 4.5 facilitated change in the metabolic routes. However, these changes could not promote hydrogen production. Recently, Castelló *et al.* (2020) extensively reviewed the causes, effects, and solutions for hydrogen production via dark fermentation; also presented the operational parameters used in various other studies. Accordingly, it can be seen that the most studied pH range has been between 4.0 and 6.5.

The volumetric hydrogen-production rate (VHPR) was between 20.05 and 493.46 mLH₂ L⁻¹ d⁻¹. Remarkable VHPR results were achieved on the 41st and 66th operation days, and they were 493 and 311 mLH₂ L⁻¹ d⁻¹, respectively, both of which corresponded to a 100% hydrogen production in the biogas.

Between the 20th and 40th days of the operation, the concentration of TVAs gradually increased until reaching the maximum value of 751.8 mgHAc L⁻¹, which matched the 100% H₂ production in the biogas and values close to 500 mLH₂ L⁻¹ d⁻¹ of VHPR. Additionally, the %H₂ and VHPR production values significantly decreased by the end of the operation, obtaining 18.6% and 20.05 mLH₂ L⁻¹ d⁻¹, respectively.

In Figure 2, the temporal variation of the biogas flow is shown. The Q_g when the hydrogen percentage was 100% varied between 84.3- and 29.7-mL h⁻¹. A possible explanation for this observation can be that the flow of biogas was directly affected by the bed setup because the bubbles were trapped in the medium, thereby preventing the measurement of the gas flow in the *CaudalisG*.

Authors including Sikora *et al.* (2013) indicated that reactors with fixed beds have advantages and disadvantages. One of the main benefits of a fixed bed in a UASB is the reduction in the washing of the biomass. For instance, the accumulation of excess biomass could become a significant disadvantage. The biomass is agglutinated, either in the form of long flocs, granules, or biofilm, and it adheres to the rings (support medium), thereby decreasing the H₂ production. In this case, the main mechanisms to inhibit biomass accumulation could be homoacetogenesis or the presence of propionate-producing microorganisms. Although our study did not analyze the microbial community, the presence of both propionate and homoacetogenic microorganisms might have imbalanced the process.

Bundhoo & Mohee (2016) mentioned several inhibitors from the microbial consortium that promoted the decrease or cessation of hydrogen production, i.e., methanogenic hydrogenation-promoting bacteria, homoacetogenic bacteria, propionate producer bacteria, sulfate-reducing bacteria, nitro-reducing bacteria, and lactic acid bacteria (LAB). Some authors, namely, Hung *et al.* (2007), Noike *et al.* (2002), Sreela-or *et al.* (2011), and Yang *et al.* (2007), agreed that the presence of LAB resulted in the total cessation of hydrogen production. However, this situation did not occur in our study.

From the concentration results of VFAs (see Figure 3), it can be observed that the predominant volatile acids were propionic acid, followed by butyric acid and acetic acid. The highest production of propionic acid indicated that the inhibition in the

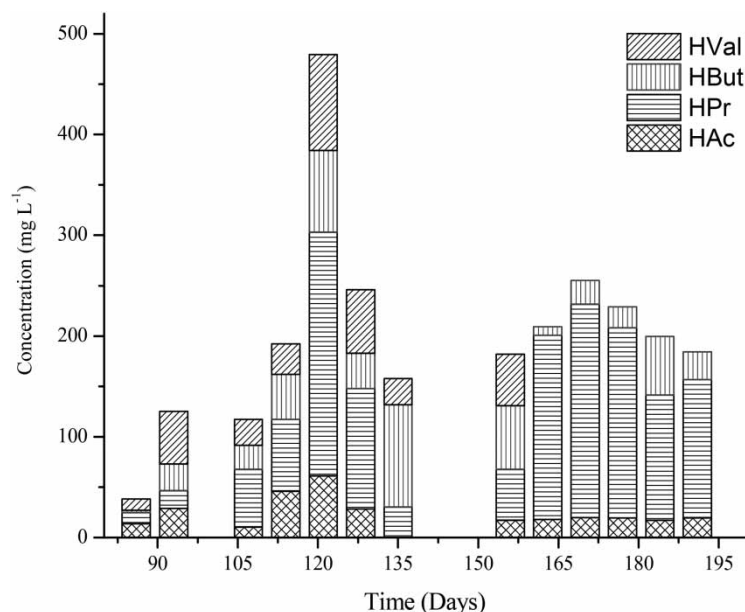


Figure 3 | Concentration of volatile fatty acids in effluent R₁ (H Val: Baleric acid, H But: butyric acid, HPr: propionic acid, and H Ac: acetic acid).

H₂ production was primarily caused by propionate-producing organisms, followed by the toxicity due to butyric acid (Bundhoo & Mohee 2016). Concerning acetate, the values ranged between 61.17 and 10.33 mg L⁻¹.

Wong *et al.* (2014) and Baghchehsaraee *et al.* (2008) stated that the molar ratio between acetate and butyrate was also an indicator of the hydrogen-production efficiency; accordingly, high values of this ratio were associated with increased production of bioH₂. In this study, the acetate: butyrate ratio was 1.29 ± 0.74 , and, at the end of the operation process, when the H₂ production decreased to 18%, the ratio was 0.40, thereby verifying the claims made by these authors.

Figure 4 shows the results of the biomass-concentration profile for different sampling points in the acidogenic reactor. Markedly, in this profile, the highest concentration of biomass was achieved in the support medium. During the operation, the highest concentration value was 1,104 mg SSV L⁻¹, which corresponded to the 100th day of the observation.

3.1.2. Methanogenic reactor

The successful operations of anaerobic treatment depend on the ability to neutralize the organic volatile acids produced and adequate bicarbonate buffering. Ripley *et al.* (1986) and Martín-González *et al.* (2013) suggested that one strategy for this control was monitoring the intermediate alkalinity/partial alkalinity (IA/PA) ratio, the concentration of total volatile acids, and the pH value.

As shown in Figure 5, the volatile acid concentration in the influent varied from 323 ± 284 to 908 ± 498 mg L⁻¹, resulting in a variation in the volumetric organic load from 4.22 to 11.23 kg m⁻³ d⁻¹. The pH was adjusted during the entire operation period, as well as the alkalinity, by adding NaHCO₃ in the range of 0.028 to 0.057 gNaHCO₃ g⁻¹ COD.

Although sodium bicarbonate would be more expensive for full-scale applications than sodium hydroxide, it offers the dual advantage of adjusting the pH and providing alkalinity, thereby resulting in pH self-balancing during the process. Ye *et al.* (2018) discussed the influence of different neutralizers on the stability of AD that treats food waste, and they indicated that both NaOH and NaHCO₃ offered advantages and disadvantages during the operation of anaerobic reactors. Additionally, they stated that the use of either NaOH or NaHCO₃ depends on each particular case. However, they also indicated that the addition of alkalizers was an unavoidable strategy for engineering applications.

Fuess *et al.* (2017) studied several alkalization strategies to optimize the operation and energy recovery of two-phase anaerobic reactors that treat vinasse. They concluded that the best approach was associated with the addition of sodium

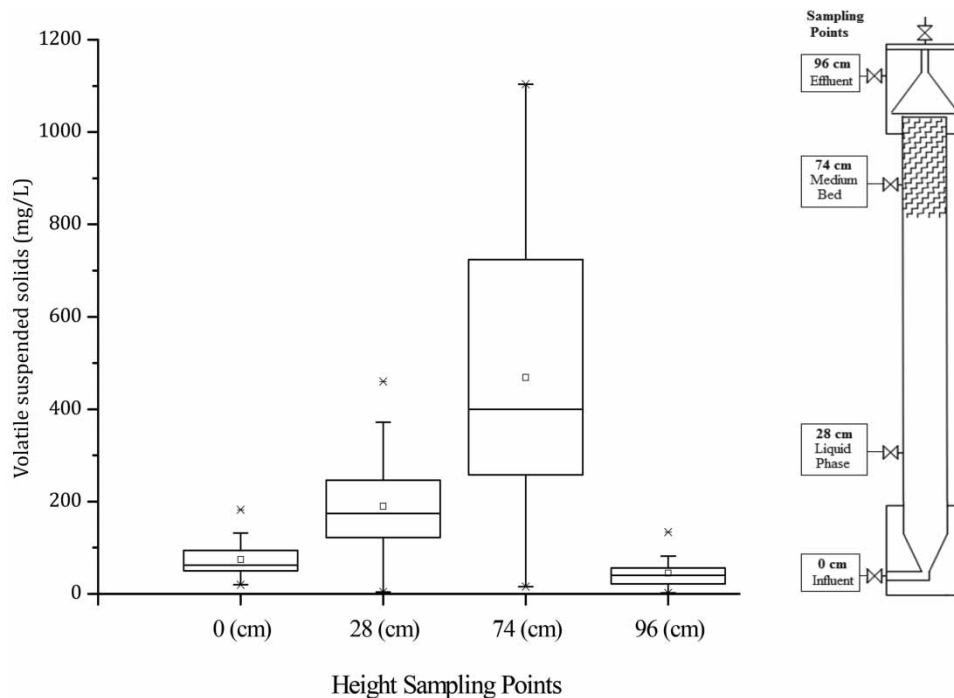


Figure 4 | Box plot of the volatile solid suspended profile in R₁. (The scheme at the right-hand side shows the points of the samples). (□, mean, ×, maximum and minimum value, $n = 52$.)

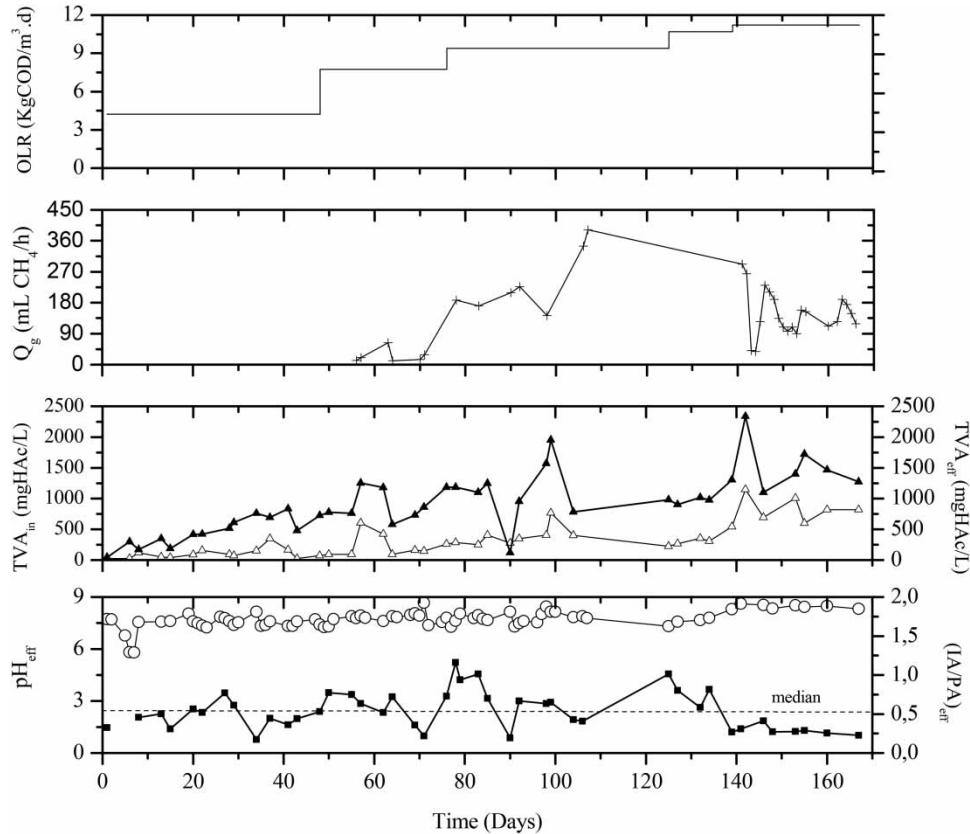


Figure 5 | Temporal variation in the operational parameters on the methanogenic reactor. (- + -) Biogas flow rate, (-▲-) TVA_{in} , (-Δ-) TVA , (-○-) pH_{eff} , and (-■-) IA/PA_{eff} .

hydroxide. However, this approach did not offer the same buffer effect as the one provided by sodium bicarbonate, and sodium hydroxide was more technically and economically favorable for AD systems on a real scale.

The IA/PA ratio (see Figure 5) showed more sensitive responses than the value of the pH in the effluent of the R_2 . During operation, several drops and oscillations were observed in the IA/PA ratio. For instance, on day 78, the IA/PA reached a value of 1.16, while five days before, it was a mean of 0.51. In contrast, the pH value did not change and remained stable at ca. 7.71. According to this observation, an IA/PA ratio can be a better indicator of stability than only the pH value. Similar recommendations were found in Boe *et al.* (2010), who also indicate that suitable indicators are necessary for proper monitoring of the anaerobic digestion.

During phase 5, the concentration of total volatile acids reached $2,339.8 \text{ mgHAc L}^{-1}$ that corresponds to the maximum value observed during the experiment. However, during this phase, a median value of 0.29 was observed in the IA/PA ratio. Such facts can be explained because, in this period, not only was the R_2 better adapted to the substrate but also the change in the OLR was not abrupt (10.7 to $11.2 \text{ kgCOD m}^{-3} \text{ d}^{-1}$) if compared with phase 2, in which the value increased from 4.23 to $7.75 \text{ kgCOD m}^{-3} \text{ d}^{-1}$. Despite all these responses, the reactor did not show accumulation of acids and acidic shock was not observed.

Additionally, our results reflect slight variations between the median and the mean value of the IA/PA ratio and concentration of TVAs, indicating that these data are homogeneous and their distribution is symmetrical. Here the median of IA/PA was 0.51, and the median for TVA was $863.76 \text{ mgHAc L}^{-1}$. The decrease in alkalinity was significantly low, and the partial alkalinities in the influent and effluent were $9,775$ and $10,700 \text{ mgCaCO}_3 \text{ L}^{-1}$, respectively.

The organic matter removal (COD removal) in the acidogenic reactor reached $6.19 \pm 3.96\%$. While, in the whole treatment ($R_1 + R_2$), removal was $26.6\% \pm 8.78\%$. This is a useful result because compared with one phase (R_1), a two-phase AD not only allows the recovery of by-products with added energy value but also improves the organic matter removal.

Figure 6 shows the COD removal in R_2 . The median value was 20.44%, reflecting that in the methanogenic phase more than 75% of the total organic matter removal of the two-phase AD process occurs. Regarding the percentage of the methane content in the biogas, it did not exceed 10% when the substrate was 100% of the acidogenic reactor effluent (Phase 5); and the maximum methane production occurred in phase 2, where it reached 39.15%.

3.2. Energy analysis and COD balance

The mass balance was based on gas production and COD balance per day during phase 3 of the methanogenic reactor (50 SWW-50 Ef R_1). Figure 7 shows that 0,22 g d^{-1} of COD was converted into hydrogen gas in Reactor R_1 and that 5.13 g d^{-1} of

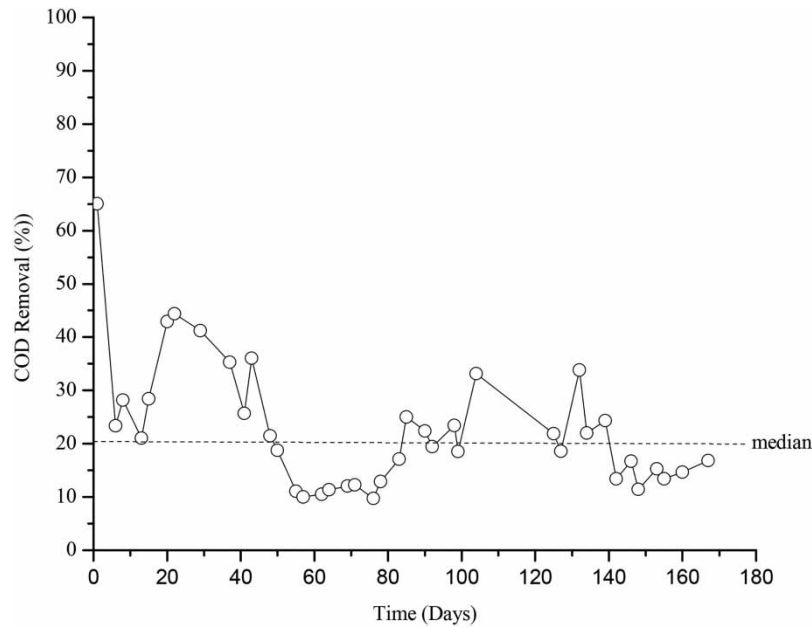


Figure 6 | Temporal variation of organic matter removal as COD in R_2 .

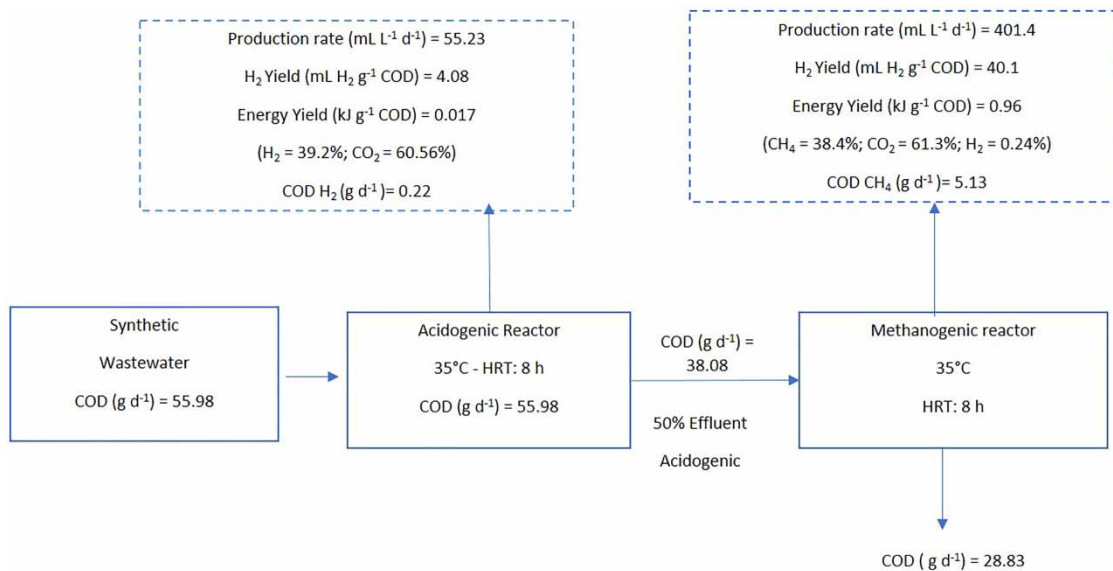


Figure 7 | COD and energy balance of a two-phase AD process.

COD was turned into methane gas in Reactor R₂, with the final COD effluent of 28.83 g d⁻¹. Accordingly, only 0.4% and 13.5% of the COD fed to the R₂ reactor were transformed into hydrogen and methane gases, respectively. The unbalanced COD from R₂ was 10.8%, a higher percentage compared with 4.85% of the unbalanced COD from R₁. These COD losses could be attributed to unmeasured biomass, undetected metabolites, or to the fact that during the period of the acidogenic phase, approximately 10% of the biodegradable organic matter was used for bacterial growth cells (Nualsri *et al.* 2016; Seengyoung *et al.* 2019).

Regarding the energy yield, from Figure 7, it can be observed that Reactor R₁ produced 17 J g⁻¹ COD_{added}, and Reactor R₂ produced 960 J g⁻¹ COD_{added}. These results were obtained by adjusting the net calorific values. The overall energy recovery of the system was 0.98 kJ g⁻¹ COD_{added}; this was a low value compared with 15.34 kJ g⁻¹ COD and 5.64 kJ g⁻¹ COD total energy yield observed in previous laboratory studies by Nualsri *et al.* (2016) and Kongjan *et al.* (2013). However, these authors used POME and desugared molasses as feedstock.

Figures 8–10 show the analyses of the EE achieved from the biogas generated in the two-phase AD reactors.

Figure 8 shows the results of the EE values of hydrogen and methane production and the EE of cogeneration, as suggested by Xie *et al.* (2008). The EE was calculated by dividing the heat value of the yields of hydrogen and methane by the heat value of the sucrose. Compared with the EE values calculated using LHV that does not include environmental factors, the EE values calculated using the LHV values of H₂ and CH₄ with the parameters indicated in the ISO 6976:2016 (BSI 2016) were lower. The EE of cogeneration calculated using the LHV taken from the thermodynamics tables was 13.74%, and the EE calculated using the adjusted LHV was 4.91%, which is 35% lower than the EE calculated with the typical value of LHV.

Also from Figure 8, it can be observed that compared with a single-phase the operation of the reactors in two phases is a better strategy to improve energy recovery. For example, the unitary energetic efficiencies of Reactors R₁ and R₂ were 2.61% and 2.29%, respectively.

Figure 9 shows the final energy conversion assuming that the fuel cell used in Reactor R₂ has an efficiency of 60% (Wang *et al.* 2018). For this study, the theoretical maximum value was 16%. However, this value could not be achieved during the operation of the system. The maximum value reached was 8.2% on day 41. The average value was 3.3%, and the minimum value was 0.2%. Despite these results, this measurement showed that continuous voltage changes could be achieved from hydrogen gas without gas storage, thereby making the technology significantly attractive from the viewpoint of practical usage.

Das & Veziroä (2001) stated that the H₂ gas must be purified before use, due to the unavoidable presence of other gases such as CO₂. Notably, in this study, the gas mixture was not purified. However, this did not directly affect the results because the fuel cell only works with the produced hydrogen gas. Humidity in the gas mixture should also be considered, as it influences the decrease in the heat value.

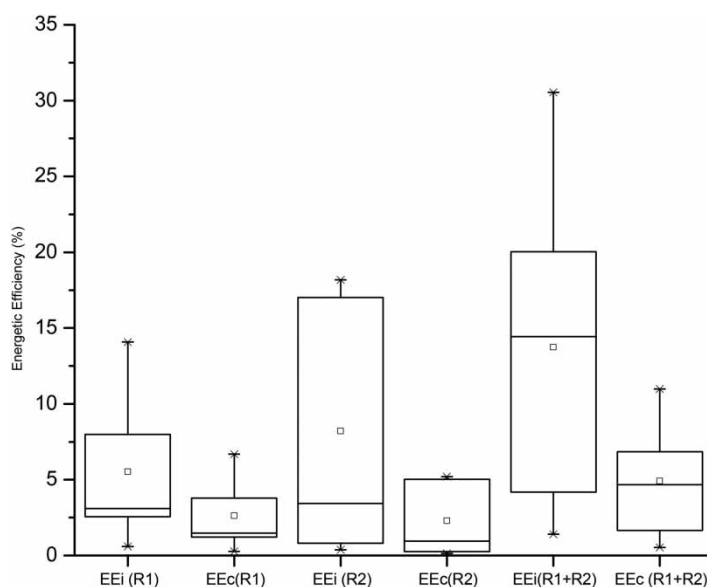


Figure 8 | Box plot of the comparison of individual EE of each reactor and cogeneration EE (R₁ + R₂) with LHV_{ideal} and LHV_{adj} (EEi: ideal EE, EEc: corrected EE, and EEC: cogeneration EE) (□, mean, ×, maximum and minimum value, n = 16).

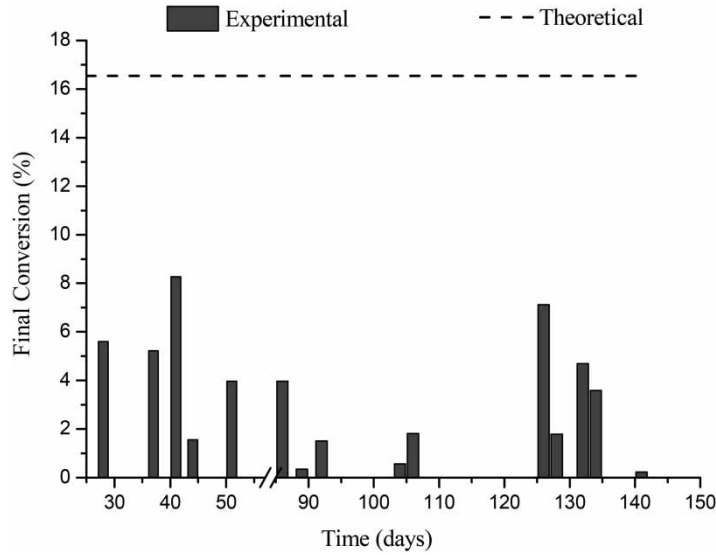


Figure 9 | Final energy conversion.

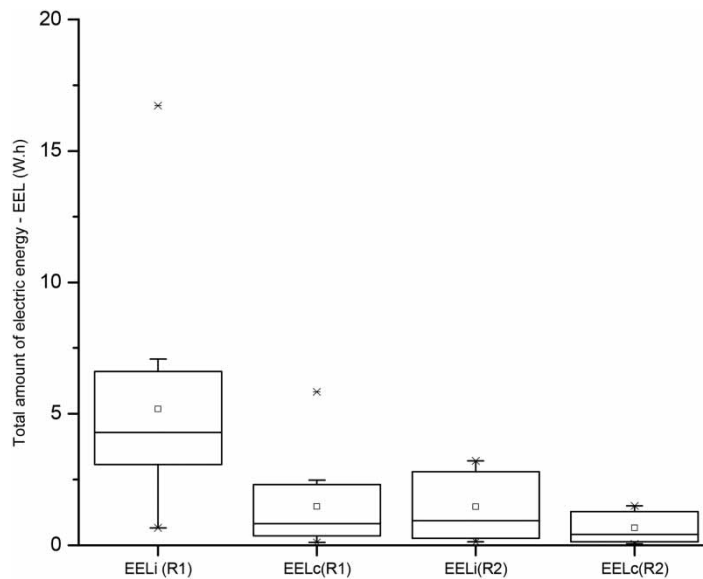


Figure 10 | Total amount of electric energy (*EELi*: ideal total electricity, *EELc*: corrected total electricity). (□, mean, ×, maximum and minimum value, $n = 16$).

Figure 10 plots the total amount of electrical energy measured based on the efficiency of the converters. Evidently, the amount of electrical energy decreases in any of the cases in which the low heat value (LHV_c) is corrected. In the methanogenic reactor (R₂), the maximum EEL value with the heating value adjusted was 1.50 W·h on the 50th operation day. While, for the same day, the EEL value with the heating value taken from the literature (LHV_{idealCH₄}) was 3.21 W·h. Additionally, in the acidogenic reactor, the maximum EEL value with LHV_{idealH₂} was 16.72 W·h on day 56; in comparison, the maximum EEL value with the heating value adjusted was 5.83 W·h.

In Table 2, the energy yields from different two-phase AD processes depending on the HV calculated are compared. The terms HHV_c and LHV_c correspond to the higher and lower heating values of the biogases, respectively, and they have been corrected according to the British Standards Institute (BSI 2016). Furthermore, an energy yield comparison was conducted by considering the calorific-value type chosen by different authors and the biogas composition. For example, Mamimin *et al.*

Table 2 | Comparison of the energy yields from different two-phase AD processes depending on the HV calculated

Reactor phase	Temperature (K)	HHV _c (kJ L ⁻¹)	LHV _c (kJ L ⁻¹)	Energy yield reported (kJ g ⁻¹ COD)	Energy yield with HV _a (kJ g ⁻¹ COD)	Δ Energy yield (%)	Reference
ASBR-H ₂	328.15	10.5	8.9	0.95	0.76	19.3	Seengenyoun <i>et al.</i> (2019)
UASB-CH ₄		34.6	31.1	13.73	11.82	13.8	
CSTR-H ₂	310.15	11.1	9.4	0.95	0.83	13.1	Nualsri <i>et al.</i> (2016)
UASB-CH ₄	303.15	37.4	33.7	9.77	9.14	6.4	Mota and Zaiat (2018)
UASB-H ₂	303.15	11.4	9.6	2.14	1.90	11.3	
UASB-CH ₄		37.4	33.7	18.55	17.45	5.9	Mamimin <i>et al.</i> (2015)
ASBR-H ₂	328.15	10.5	8.9	27.20	2.21	18.9	
UASB-CH ₄	304.15	37.3	33.6	12.65	11.75	7.1	

*Metering reference pressure = 101.1 kPa. Temperature refers to the temperature of the reactor. Combustion temperature = 15°C.

(2015) and Seengenyoun *et al.* (2019) used the higher heating value for energy yield calculations, while Nualsri *et al.* (2016) used LHV.

From Table 2, it is evident that the percentage difference in the methane energy yield ranged between 5.9% and 13.8%, whereas that for the hydrogen energy yield ranged higher between 11.3% and 19.3%. These differences are relevant for estimating the real energy yield and also performing evaluations to use this kind of system in real-life applications.

Notably, the fuel is selected according to its calorific value. Hence, fuels with higher calorific values should be selected to produce high power in the ignition engine. Fuels with less calorific value burn inefficiently, causing unwanted exhaust and air contamination. Thus, variations in heating values not only affect the energy balance but also allow reconsidering the quality and use of biofuel.

CONTRIBUTIONS

In any industry, EE is a crucial industrial term, which is complex and challenging to quantify. Practically, companies are always seeking strategies to increase productivity not only by reducing energy consumption and inputs but also by becoming increasingly independent concerning the use of fossil fuels.

Li & Tao (2017) and Boyd & Pang (2000) emphasized that for the productive sector, EE is strongly linked to trade security, environmental benefits (including greenhouse gas reduction bonuses and business competition), and national government policies. However, Henriques & Catarino (2017) indicated that it was significantly challenging to convince the senior management to implement clean practices in the processes that lead to energy benefits. This challenge is mainly due to the difficulty in quantifying the energy gain.

Li & Tao (2017) presented the three main indicators for EE evaluation, namely, thermodynamic, thermophysical, and thermo-economic indicators. The third one, according to the authors, is the most complex to quantify as it depends on time and the national economic development.

Since these three indicators are based on thermodynamics, the calorific value becomes essential for this evaluation. Thus, the proposal of this article significantly affected the industry. However, many challenges and barriers remain to be overcome, and further studies on the subject must be performed.

There is no consensus in the literature on how to evaluate the energy gain in two-phase AD. Some authors proposed to perform such energy gain evaluations based on the produced biogas (Das & Veziroä 2001; Xie *et al.* 2008), the flow of biogas (Si *et al.* 2016), moles of the gas produced (Kumari & Das 2015), energy input throughout the process (Perera *et al.* 2012), or biogas-production rate (Fuess *et al.* 2017). However, in most of these proposals, the common denominator is LHV. In this regard, the main contribution of our study is not only the revision of different points of view for this analysis but also the demonstration of how the LHV influences the final energy gain.

CONCLUSIONS

A two-phase anaerobic digestion process was studied to evaluate the performance and the energetic efficiency of treating synthetic wastewater. The IA/PA ratio was a suitable indicator of the stability even more than the value only of the pH.

Moreover, the biological process did not show accumulation of acids and an acidic shock was not observed. Also, the presence of propionic acid-induced instability in the production of H₂. Considering that the base of the calculation of the energetic efficiency (EE) was the parameter named the calorific heat value, it was observed that this parameter should be corrected by including mainly the compressibility factor. This correction directly influences the EE results of both hydrogen and methane gases, showing that if the calorific heat value is used under ideal conditions, the energy gain is overestimated. This is important for practical engineering, as this overestimation would decrease the importance of the real-life uses of hydrogen and methane. Accordingly, industries or communities that implement two-phase AD would not be able to decrease their energy consumption. Nevertheless, this observation is not conclusive, and further studies are still needed.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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