Start-Up Strategy and Process Performance of Semi-Continuous Anaerobic Digestion of Raw Sugarcane Vinasse

Hélène Caillet, Laetitia Adelard

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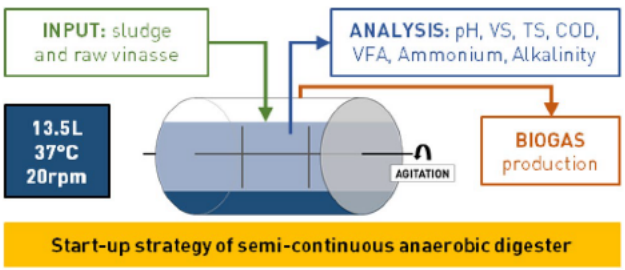
Abstract

The sugarcane distillery waste water is generated throughout the sugarcane molasses fermentation and distillation. In Reun-ion Island, a part of the vinasse production is treated by methanisation process. However, the remaining part is diluted then discharged into the sea. The aim of this work is to study the anaerobic treatment of sugar cane vinasse, with energy recovery. Nonetheless, vinasse pollutant load is difficult to treat. Regarding the experimentations, the biochemical potential (BMP) test is used for the determination of the methanogen potential. The BMP is then modelled with the modified Gompertz and the first order kinetic models. Furthermore, a laboratory study is carried out for studying the methane production of vinasse in semi-industrial scale over a period of 130 days. The start-up strategy of the 16 L pilot is proposed, in particular the gradual increase of organic load. The physico-chemical analysis of the medium is needed to prevent and explain the failure of the process. Indeed, the biogas production and physico-chemical measurements during the digestion are presented and

discussed. The maximum methane yield of the BMP is 185 $NL_{CH_4}\ kg^{-1}_{COD}$, obtained with I/S ratio in terms of volatile solids of 0.7. The outcomes showed that the first-order kinetic and modified Gompertz models fit well with the BMP test curves. Concerning the pilot, the start-up period lasted 45 days the maximum specific production was 151.00 $NL_{CH_4}\ kg^{-1}_{COD}$ (232.31 $NL_{biogas}\ kg^{-1}_{COD}$). In further studies, different mixing strategies will be studied.

Graphic Abstract



Keywords Anaerobic digestion · Sugar cane vinasse · Pilot scale · BMP · Process performance

Statement of Novelty

The anaerobic digestion of vinasse at the laboratory scale allows to study its biogas yield under conditions closed to reality. The first step, which is a key step, is the start-up of the experimental setup. Indeed, the choices made will have an impact on the yields observed over the rest of the study. Moreover, very few studies deal with the anaerobic digestion of raw vinasse, which is either diluted or studied in co-digestion, because of inhibitions. For this reason, we present in this paper the start-up phase of an anaerobic digester of raw vinasse with a volume of 16 L. Subsequently, the impact of mechanical agitation on biogas yields will be studied from these initial results.

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Introduction

Anaerobic digestion is a widely used process for waste treatment and energy production with the biogas. This natural process consists in the degradation of organic materials by microorganisms in absence of oxygen, unlike aerobic digestion. Anaerobic digestion respects the natural cycle of carbon. The biogas produced is mainly composed of methane and carbon dioxide, with traces of hydrogen sulphide and water vapour. The five modes of biogas valorisation are [1]:

- *Heat production* energy efficiency is interesting if the heat requirement of the outlets is high enough to allow the maximum use of the available energy to be exploited. In addition, nearby outlets are needed to limit the costly transport of heat or biogas.
- *Electricity production* lower energy efficiency due to the energy yield of the electricity, ranging for motors from around 33%.
- *The combined production of electricity and heat, also called cogeneration* this is the most common biogas recovery system. Besides the electricity produced by a generator, heat is recovered, mainly from the cooling system. The valorisation of heat requires a nearby outlet.
- *Fuel for vehicles* The biogas follows a series of purification/compression steps to be used as a vehicle fuel.
- *The injection of clean biogas into the natural gas network* in some European countries, the injection of biomethane into dedicated or non-dedicated networks are more common: Sweden, Germany, Switzerland, Netherlands, etc. The injection of purified biogas into the natural gas network is the most efficient method of valorisation.

Anaerobic digestion is very interesting for an insular territory such as Reunion Island, which is dependent on imported fossil energies. The ambition to achieve energy self-sufficiency by 2025–2030 was initiated by the Region in 2000 through its Regional Plan for the Development of Renewable Energies and Rational Use of Energy (PRERURE) [2]. Following the environment Grenelle, this ambition was relayed by the government with the GIP Project GERRI and it also materialised in 2009 with the program STARTER (Strategy of Energy Autonomy for the Recovery and Transition of the Reunionese econ-omy) [2]. The PETREL report, prepared by the ARER (the Regional Energy Agency of Reunion Island), presents an initial assessment of the Reunion energy mix in the hori-zons of 2020 and 2030 [2]. The demand and the electric-ity production are evaluated according to two scenarios, one of them following STARTER [2]. The valorisation of biomass is preponderant in the latter scenario, especially as it provides a so-called "base" energy because it is permanently available [2]. Different ways of biomass valorisation exist, such as the extraction of nanocrystalline cellulose [3–5]. The methanisation biomass is composed by all putrescible organic matter, animal manure, wastewater, wood waste and green waste. According to a study conducted by ADEME in 2010, the methanisation of waste in Reunion Island corresponds to the production of 34.3 million m^3 of biogas per year from 853,000 tons of waste, of which: 44% of effluents from farming, 21% of agro-industrial effluents, 21% of sewage sludge, 11% of bio-waste and 3% of wet green waste [2]. The assumptions made are that 25% of the livestock effluents produced to date on the island will be valorised by anaerobic digestion by 2020 and 50% by 2030 [2]. The sewage sludge would be 91% mobilised, 44% wet green waste and 100% agro-industrial and biowaste effluents [2].

In addition, the waste in Reunion are currently landfilled, however, this method is not sustainable because it is land consuming, not adapted to insular territories. The vinasses (sugarcane distillery waste water generated throughout alcohol production) are treated and then discarded on the high seas via an emissary at 80 m depth. The sugarcane vinasse is treated by anaerobic digestion process in many countries on an industrial scale especially in Brazil, India and South Africa by adapted processes and a simple acclimation of the microbial flora to the conditions of the vinasse. In Reunion Island, the first major biogas unit was built at the Rivière du Mât distillery in 2011. Its implementation was directly linked to the drastic increase in energy costs over the last 5 years [2], and the need of vinasse treatment. The distillery produces 8000 m^3 of pure alcohol per year and the production of vinasse is around 600 $m^3\ day^{-1}$ during the sugarcane crop season [2]. Experimental results showed particular difficulties for anaerobic digestion process, given high salinity and high organic matter content (80–120 $kg_{COD}\ m^{-3}$) [2]. The first phase of the project of anaerobic digestion of the vinasse (2011) concerned only half of the vinasse, which is treated in a 5800 m^3 digester. The organic load is reduced by 80% and the biogas produced is used in a steam boiler, ensuring the energy autonomy of the process distillation [2].

Vinasse is characterised as an highly polluting effluent, containing high levels of organic compounds and nutrients (mainly potassium but also nitrogen and phosphorous) [6, 7]. Furthermore, vinasses are recalcitrant effluents with high pollutant content. The direct discard of vinasse to environment leads to severe environmental impact like salinity, sodicity, phytotoxicity, anoxia, eutrophication, death of aquatic life, and many severe health problems [6, 7]. Moreover, the Rivière du Mât distillery in Reunion Island is constrained to treat a diluted vinasse to guarantee its

anaerobic digestion while avoiding inhibitions of the process. The drawback is therefore the massive use of water for the anaerobic digestion of the vinasse. This is why this study focuses on the anaerobic digestion of raw vinasse on a laboratory scale.

Before the industrial treatment of a specific waste, the process must be studied on a laboratory and pilot scale. Indeed, biochemical potential test (BMP) are broadly used for evaluating the methanogenic potential of organic materials. The protocol of this test have been recently standardised because the outcome can vary significantly between laboratories [8]. Nevertheless, a key parameter of this test, the ratio of volatile solid (VS) from inoculum to VS from the substrate (I/S ratio) depends on the substrate [8]. The ratio should be between two and four for most applications [8]. However, for less degradable substrates, a ratio less than or equal to one can be applied, and only if two ISRs lead to the same BMP, one can assume that there was no overload or inhibition [8]. Concerning unknown substrates, the authors recommend to test several ratios [8]. Thereby, in this study, different ratios were tested in order to evaluate the methane yield of the sugarcane vinasse.

There is no standardised method for the pilot experiments. Studies have recently been carried out on pilot tests on sugarcane vinasse, mainly in co-digestion. Among these studies, we find the work led in 2015 on start-up strategies of anaerobic co-digestion of sugarcane filter cake and bagasse [9]. At the beginning of the pilot start-up phase, the load increase is done gradually and the steady conditions were obtained after 70 days [9]. The effect of total solid (TS) was studied with the addition of water [10]. The result showed that vinasse/water ratio of 1/3 (TS 7.015%) produced the maximum total biogas (37.409 mL $g_{COD}{}^{-1}$) however vinasse/water ratio of 1/2 (TS 9.310%) had the biggest chemical oxygen demand (COD) removal (23.580%) than others [10]. Moreover, in a study published in 2015, the authors said that biogas production failed when sugar beet vinasse alone was fed to the reactor [11]. For this reason, they studied the addition of cow manure during digestion, which has the consequence of increasing the C/N ratio, which is low in the case of vinasse substrate [11]. Anaerobic digestion was the most stable when cow manure was supplied to digestion of vinasse [11]. The steady conditions were obtained after 50 days [11]. Another study has been carried out on vinasse in 2016 in order to evaluate the anaerobic conversion of vinasse into biomethane with gradual increase in organic loading rate (OLR) in two up flow anaerobic sludge blanket (UASB) reactors of 21.5 L (R2) and 40.5 L (R1), in mesophilic conditions [12]. The OLR values applied in the reactors were 0.2–7.5 $g_{COD}\ L^{-1}\ day^{-1}$ in R1 and 0.2–11.5 $g_{COD}\ L^{-1}\ day^{-1}$ in R2 [12]. The average COD removal efficiencies ranged from 49 to 82% [12]. In 2017, co-digestion of sugarcane press mud with vinasse was studied in order to improve the digestion of press mud [13]. The methane yield was 64% higher in case of co-digestion compared to mono-digestion of press mud and the process was more stable [13]. All these studies show that co-digestion of vinasse improves yields and stabilises the process. Nevertheless, in this study, we will not carry out experiments in co-digestion. We present the outcomes for the case of vinasse in mono-digestion and slowly increase the OLR to avoid destabilisation of the process as [11].

Furthermore, these studies previously mentioned presented examples of pilot start-up. We retain from these articles that the pilot digester must be started with inoculum (such as manure or sludge), followed by the micro-organisms acclimation and then a gradual increase in load. In this paper, we present the methodology and outcomes for the vinasse and sludge characterisation especially the BMP test of raw vinasse, the pilot start-up and the monitoring of experimentation over a period of 130 days. The aim of this study is firstly to propose a protocol for start-up a pilot with raw vinasse as substrate. Secondly, we study the increase of OLR of the pilot while following the physico chemical properties of the medium to avoid dysfunctions of the process in case of an increase of OLR too fast. Finally, we study the pilot in steady-state conditions while following the physico chemical properties. In this work, we choose not to add chemicals to adjust the physico-chemical parameters, and not to dilute the vinasse. Indeed, we want to obtain data without interfering with the process and let the process stabilise alone. This case has not been studied yet. The data produced will allow us to create an experimental database to study the impact of mechanical agitation on the anaerobic digestion of raw vinasse in further studies.

Methodology

Substrate and Inoculum

The vinasse and the sludge come from the active mesophilic biogas plant of the sugarcane distillery Rivière du Mât (Saint-Benoit, Reunion Island). The sludge is used as the inoculum in the biochemical potential (BMP) tests and the start-up of the pilot digester. It is then stored at ambient temperature and incubated at 37 °C before the BMP tests, which is the process temperature (mesophilic conditions). Regarding the vinasse, it is stored in cold storage at 4 °C before the tests in order to avoid the degradation of the substrate before the digestion in the pilot. The vinasse is stored in the cold storage for a maximum of two months before being used. In this study, the vinasse is not frozen because the freezing has the effect of breaking up the cells, which improves the digestion of the latter.

Physicochemical Analysis and Biochemical Potential Tests (BMP)

The substrate and inoculum are homogenised with the Ultra turrax IKA T25 digital at 12,000 rpm for 10 min before the BMP tests. We carried out TS, VS, pH, COD, total organic carbon (TOC), volatile fatty acids (VFAs), ammonium (Am), alkalinity (Alk) and Kjeldahl nitrogen (Ni) measurements. The TS content is obtained after drying 20 g of the samples for 24 h at 105 °C and the VS content after burning the dried samples for 4 h at 550 °C. The chemical tests were conducted on the Hach Lange DR5000 Spectrophotometer, using the Hach Lange tests LCK 914 (COD), LCK 381 (TOC), LCK 365 (VFA), LCK 303 (Am), LCK 362 (Alk) and LCK 338 (Ni). The physico-chemical characteristics of the vinasse and the sludge are given in the Table 1.

The BMP tests are carried out using the Automatic Methane Potential Test System II (AMPTS II—Bioprocess Control). We refer to the last recommendations for the BMP test organisation [8]. The tests are carried out in mesophilic conditions in 50 days. As the substrate is unknown, we must test different inoculum to substrate ratios [14]. The ratios of COD from the substrate to VS from the inoculum tested are 1, 2, 2.5 and 3, which corresponds to a ratio of VS from the inoculum to VS from the substrate of 1.8, 0.9, 0.7 and 0.6. The total volume of the digesters is 650 mL. The operating volume is 400 mL. The digesters volume are adjusted with distilled water in order to have the same test working volumes. The experiment includes substrate tests, the positive tests and the blank tests. All the tests are carried out in triplicates. The BMP value is expressed in the volume of methane produced per gram of organic matter which is expressed in COD as the vinasse is liquid.

Table 1 Physico-chemical characteristics of vinasse and sludge

Characteristics	Sludge	Vinasse
TS (%)	1.99	6.64
VS (%)	0.72	4.04
TSS (g L−1)	–	10.0
pH	7.57	4.84
COD (g_{O_2} L^{-1})	11.60	86.70
TOC (mg L^{-1})	–	29,875
VFA (g L^{-1})	16.34	19.36
Am (mg L^{-1})	–	37.40
Alk (mg_{CaCO_3} L^{-1})	2361.9	1080.6
N (mg L^{-1})	1070	1120
Phosphorus (mg L−1)	–	190
Alk/COD	0.204	0.013
VFA/Alk	6.92	17.91
C/N ratio	–	26.67

The Biodegradability and the COD Removal

The biodegradability of the substrate in the BMP test is estimated by the following equation:

$$B = \frac{Y_{\max}}{350}, \tag{1}$$

where 350 represents the theoretical maximum biodegradability of methane expressed in liters of methane per kilogram of removal COD at normal temperature and pressure.

The COD removal is calculated by the following equation:

$$R = \frac{M_{COD,digested}}{M_{COD,added}} \cdot 100 = \frac{M_{COD,added} - M_{COD,pilot}}{M_{COD,added}} \cdot 100 = 100 - \frac{M_{COD,pilot}}{M_{COD,added}} \cdot 100, \tag{2}$$

where $M_{COD,added}$ is the cumulated mass added in terms of COD, $M_{COD,digested}$ is the cumulated digested mass in terms of COD and the $M_{COD,pilot}$ is the COD measured on the sample collected from the pilot.

Kinetic Models

First-Order Model

According to Kim et al. (2003), anaerobic degradation after initial lag-phase time is limited by the terms associated with substrate and kinetics, which are generally represented by a first order kinetic law [15]. According to the approach reported by Llabres-Luengo and Mata-Alvarez (1987), the first order model is expressed by [16]:

$$Y(t) = Y_{\max}\left(1 - e^{-kt}\right), \tag{3}$$

where Y is the volume of produced methane (NL_{CH_4} kg^{-1}_{COD}) at digestion time t (s), $Y_{\max}$ is the maximum volume of methane accumulated at an infinite digestion time (NL_{CH_4} kg^{-1}_{COD}) and k the kinetic constant (day^{-1}). Nielfa et al. (2015), assumed that k is the specific microorganisms growing speed [17] (day^{-1}).

Modified Gompertz Model

It has been shown that the BMP fits with the modified Gompertz equation in case of mono-digestion [15, 18]. This model assumed that the biogas production is proportional to the microbial activity [17, 18].

$$Y(t) = Y_{\max} \exp\left[-\exp\left(\frac{R_m(\lambda - t)\exp(1)}{Y_{\max}} + 1\right)\right], \tag{4}$$

where Y_{max} is the maximum volume of methane accumulated at an infinite digestion time ($NL_{CH_4}\ kg_{COD}^{-1}$), R_m is the specific rate constant ($NL_{CH_4}\ kg_{COD}^{-1}\ day^{-1}$) and λ is the lag-phase time constant (days).

Statistical Analysis

The root mean square error (RMSE) and the determination coefficient R^2 are used to evaluate the model results. The RMSE is expressed as follows:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_{m,i} - Y_{c,i}\right)^2}, \quad (5)$$

where n is the number of measurements, Σ is the sum operator, $Y_{m,i}$ is the measured cumulated methane production ($NL_{CH_4}\ kg_{COD}^{-1}$) and $Y_{c,i}$ is the calculated cumulated methane production ($NL_{CH_4}\ kg_{COD}^{-1}$).

As mentioned in the last recommendations for the BMP tests, test results must be rejected if the average standard deviation of the triplicates is upper than 10% [8]. The standard deviation is calculated with the following equation:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}, \quad (6)$$

where N is the number of assays, x_i are the maximum volume of methane produced of each assays ($NL_{CH_4}\ kg_{COD}^{-1}$) and $\bar{x}$ is the average value of the maximum volume of methane produced ($NL_{CH_4}\ kg_{COD}^{-1}$).

Pilot tests

Degradation Index

The degradation index is calculated according to the following equation [9]:

$$D_{index} = \frac{SMP}{TMP}, \quad (7)$$

where SMP is the specific methane production of the pilot ($NL_{CH_4}\ kg_{COD}^{-1}$) and TMP the theoretical methane production of the biochemical potential tests ($NL_{CH_4}\ kg_{COD}^{-1}$).

The Pilot Set-Up

The set-up of the pilot is shown in Fig. 1. The total volume of the pilot is 16 L. The operating volume is 14.5 L and the headspace is 1.5 L. The experiment is carried out in mesophilic conditions at 37 °C in order to limit energy consumption. The temperature is controlled with water recirculation in the double membrane. The water is maintained at a constant temperature with a thermostatically controlled water bath. The digester is equipped with a mechanical mixing system. Its intensity can vary from 20 to 100 rpm. The mixing mode employed in this work is detailed subsequently in the document. The biogas volume is measured with a bucket counter.

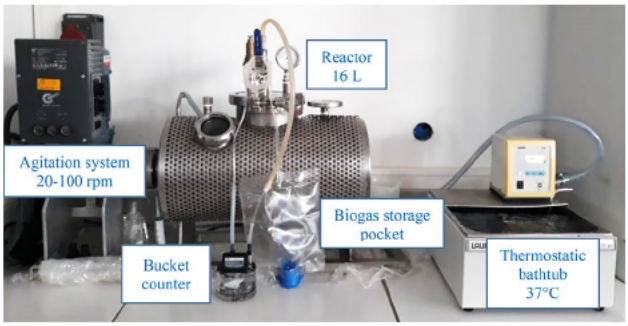

Fig. 1 Pilot 16-L set-up

Start-Up and Monitoring

For the start-up of the digester, the digester was filled with 13.5 L of sludge from the sugarcane distillery (which corresponds to 97.20 g_{VS} and 156.60 g_{COD}) for the micro-organisms input and 500 mL of vinasse (which corresponds to 24.40 g_{VS} and 43.35 g_{COD}). The physico-chemical properties of the sludge are presented in the Table 1. As the sludge comes from the same distillery of the vinasse, the sludge is already acclimatised to the vinasse and therefore, acclimatisation period is not needed. Next, the digester is fed with 500 mL of vinasse and a digestate sample of 500 mL is taken once a week during one month. Experimentally, for the digester feeding, we have to open it to recover the digestate and add the substrate. The objective of the pilot start-up phase, is to gradually replace the sludge by the vinasse. We slowly feed the digester with vinasse in order to avoid inhibitions and failure of the process. The major drawback of the feeding of the digester is the fact of allowing oxygen to enter the medium, which must stay anaerobic.

Physico-chemical tests are carried out on the digestate samples: the pH, the COD, the ammonium, the VFA concentrations, alkalinity and the TS and VS percentages. The biogas volume is measured with a bucket counter and then stocked in a 1500 mL gas storage pocket and daily analysed in terms of percentage of methane, carbon dioxide and oxygen.

As previously said, if the physico-chemical parameters are not optimal, we do not adjust them with addition of chemical, but change the OLR.

Mixing Conditions

During the start-up of the pilot, the mixing is minimal: 15 min at 20 rpm before the taking of sample in order to homogenise the medium. This choice is made in the aim to homogenise the medium before taking a sample without destabilising the process or the bacterial centers. Indeed, opening the pilot and allowing oxygen to enter necessarily disrupts the process that can cause the death of methanogenic archaea, and so the reduction of the population of micro-organisms. Moreover, as the growth of methanogenic populations is slow, we must preserve them.

Then the minimal mixing was maintained during two weeks before modification. Indeed we then tested intermittent agitation: 10 h day^{-1} at 20 rpm. As it revealed an absence of biogas production, the mixing was stopped and returned to minimal agitation for the rest of the experiment. We supposed that the continuous stirring prevented the micro-organisms from digesting the substrate. Consequently, the OLR was changed, in order to recover initial physicochemical parameters and biogas production. Indeed, according to Vavilin et Angelidaki (2005), during the start-up of the digester, the methanogenesis is the limiting-step, consequently, vigorous agitation must be avoided to prevent the dissipation of methanogenic centers [19].

Organic Load (OLR)

The Table 2 shows the OLR used in literature for vinasse in case of mono and co-digestion. The maximum OLR in terms of VSs is 3.0 and 11.5 in terms of COD. During the digestion of the sugar beet vinasse and press mud, the authors make the choice to dilute the vinasse with water. In addition, recirculation makes it possible to reduce the dilution of the vinasse [12]. Moreover, press mud is used as co-substrate for the digestion of vinasse [13], or cellulose and straw for additional carbon source [11]. Vinasse having a pH lower than the optimal pH of the anaerobic digestion, the authors alkalise the vinasse by adding NaOH solution [13].

With this pilot test, we chose to study the vinasse in mono-digestion, in that respect, no addition of carbon source was made. Moreover, we also chose to not dilute the vinasse as we want to study the anaerobic digestion without consuming water. As these actions would help to stabilise the process, the organic load was increased very gradually to avoid inhibitions of the process.

The organic load and the feeding frequency used in this study are recapitulated in the Table 3 for the whole study. The initial feeding is 13.5 L of sludge and 0.5 L of vinasse, which corresponds to 14.28 g_{COD} L^{-1}. During the first study phase, the digester is fed with 500 mL of vinasse every 2 days. The biogas is produced in two days when adding 500 mL, which corresponds to an OLR of 0.85 g_{VS} L^{-1} day^{-1} and 1.51 g_{DCO} L^{-1} day^{-1}. A low initial OLR is chosen because inhibitions due to an accumulation of VFA occur during an excessive OLR during the anaerobic digestion of the vinasse. The latter having a low C/N ratio, the inhibitions are frequent in the case of mono-digestion of vinasse.

The feeding frequency is then changed in order to open the digester less often, to reduce the oxygen input into the digester. Thus 750 mL of vinasse are added every three days, then 1000 mL every 4 days. During the next phase, the OLR is increased from 0.25 to 0.38 L day^{-1}.

In case of a drop in biogas production, 250 mL of vinasse and 250 mL of sludge are added instead of 500 mL of vinasse. Sludge is introduced to input micro-organisms.

Results and Discussions

Biochemical Potential Test Performance

The methane production of the vinasse and the blank are represented in Fig. 2. The maximum production is reached in 12 days for the vinasse and in 43 days for the inoculum. The biochemical potential of vinasse measured with the ratios in terms of VS 0.9, 0.7 and 0.6 are almost the same

Table 2 Organic load in literature

Source	Substrate	Remark	OLR range	
			g_{VS} L^{-1} day^{-1}	g_{COD} L^{-1} day^{-1}
[20]	Molasses	Mono-digestion	–	1.5–7.5
[11]	Sugar beet vinasse	Dilution of vinasse Addition of cellulose and straw	2.0–3.0	–
[9]	Sugarcane filter cake and bagasse	Co-digestion Addition of water	2.0–3.0	–
[12]	Vinasse	Dilution with water, then recirculation Addition of NaOH	–	0.2–11.5
[13]	Press mud and water Vinasse and press mud	Mono-digestion with addition of water Co-digestion	0.5–2.2	–

Table 3 Mixing strategies and organic load

Description	Period (days)	Mixing strategy	Feeding frequency	Average feeding rate		Average OLR	
				Sludge (L day^{-1})	Vinasse (L day^{-1})	g_{VS} L^{-1} day^{-1}	g_{COD} L^{-1} day^{-1}
Initial feeding	0	No mixing	Initial	13.5	0.5	8.69	14.28
Pilot start-up	1–28	Minimal mixing[a]	Once a week	–	0.07	0.25	0.45
	29–42	Minimal mixing	Every 2 days	–	0.25	0.85	1.51
Continuous mixing test	43	10 h at 20 rpm	Every 2 days	–	0.25	0.85	1.51
Resumption of minimal agitation	44–53	Minimal mixing	Every 2 days	–	0.25	0.85	1.51
Addition of sludge	54–63	Minimal mixing	Every 2 days	0.125	0.125	0.49	0.85
Modification of feeding frequency	64–68	Minimal mixing	Every 3 days	–	0.25	0.85	1.51
	69–72		Every 4 days				
Increase of OLR	73–112	Minimal mixing	Every 4 days	–	0.31	1.06	1.88
	113–130			–	0.38	1.27	2.26

[a]Minimal mixing: 15 min at 20 rpm before feeding

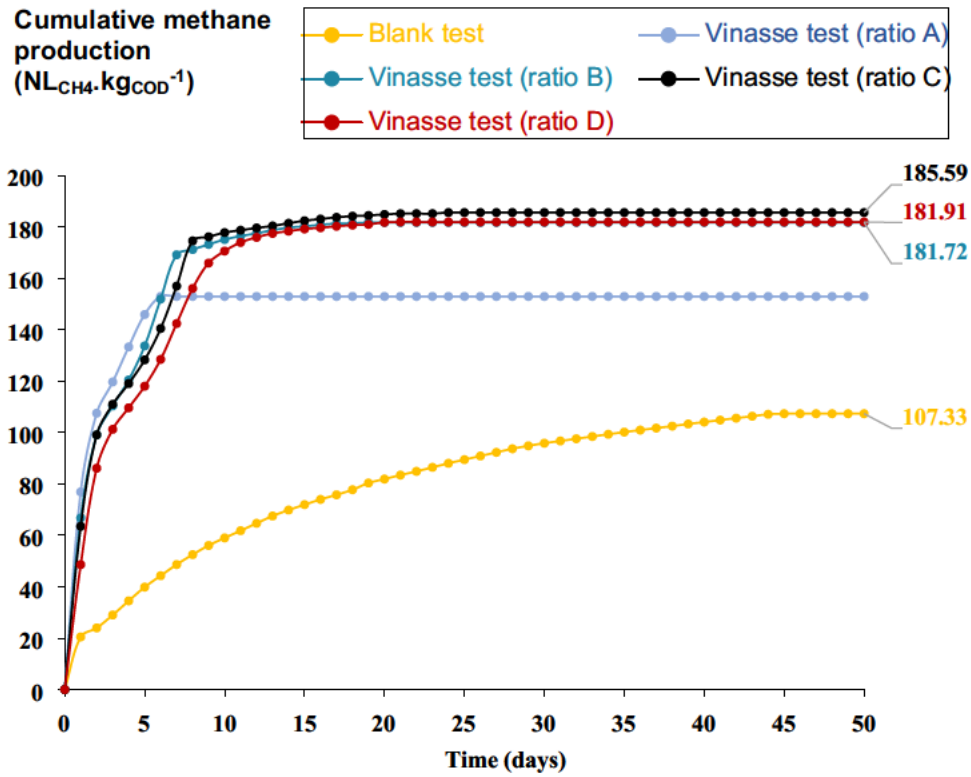

Fig. 2 Biochemical potential test results

(181.72 to 185.59 NL_{CH_4} kg_{COD}^{-1}). The maximum value is obtained with the ratio 0.7. The potential obtained with the ratio 1.8 is lower with a value of 152.95 NL_{CH_4} kg_{COD}^{-1}. As we obtained similar methane yields for different ratios, we can retain the BMP value. The biodegradability (B) for each ratio is given in the Table 4. The biodegradability is 0.44 for the S/I ratio 1, 0.52 for the ratios 2 and 2.5, and 0.53 for the ratio 3. Thus, we recommend to use an I/S ratio inferior to 1 for the vinasse BMP and we retain the BMP value of 185.59 NL_{CH_4} kg_{COD}^{-1}.

The methane yield in terms of COD, VS, sample and the standard deviation are given in the Table 4. We clearly see that the methane yield is becoming more important with increasing the ratio S/I. Moreover, we notice that the

Table 4 Methane yield and standard deviation

Ratio S/I	B	Methane yield			Standard deviation ($NL_{CH_4}\ kg_{COD}^{-1}$)			Standard deviation (%)		
$g_{COD}\ g_{VS}^{-1}$		$NL_{CH_4}\ kg_{VS}^{-1}$	$NL_{CH_4}\ kg_{COD}^{-1}$	$NL_{CH_4}\ L_{sample}^{-1}$	Min	Max	Average	Min	Max	Average
1	0.44	180.53	152.95	8.81	19.51	53.49	42.51	15	38	28
2	0.52	219.93	181.72	16.14	3.80	9.17	5.33	2	13	3
2.5	0.53	251.86	181.59	12.35	7.36	11.10	10.11	5	12	6
3	0.52	322.95	181.91	15.72	0.61	9.19	4.61	1	6	3

Table 5 First-order kinetic and modified Gompertz model coefficients

	Parameters	Units	Coefficients				
Ratio	S/I	$g_{COD}\ g_{VS}-1$	1	2	2.5	3	**A**[a]
	I/S	$g_{VS}\ g_{VS}-1$	1.8	0.9	0.7	0.6	
First-order kinetic model	Kinetic constant k	day^{-1}	0.61	0.32	0.29	0.25	0.29
	Maximum methane production Y_{max}	$NL_{CH_4}\ kg_{COD}^{-1}$	152.9	181.7	185.6	182.5	183.3
	Correlation factor R^2	–	0.97	0.99	0.98	0.99	0.99
	RMSE	$NL_{CH_4}\ kg_{COD}^{-1}$	4.60	4.01	4.77	3.98	4.25
Modified Gompertz model	Methane production rate Rm	$NL_{CH_4}\ kg_{COD}^{-1}\ day^{-1}$	50.39	28.54	25.44	22.01	25.33
	Lag-phase time λ	D	0.866	1.483	1.598	1.967	1.683
	Maximum methane production Y_{max}	$NL_{CH_4}\ kg_{COD}^{-1}$	150.4	181.2	184.8	181.0	182.3
	Correlation factor R^2	–	0.97	0.97	0.97	0.98	0.97
	RMSE	$NL_{CH_4}\ kg_{COD}^{-1}$	4.06	5.65	6.29	5.53	5.83

[a]Average of BMP (ratio 2, 2.5 and 3)

standard deviation is at least five times more important for ratio 1 than for the other ratios The maximum standard deviation for the ratio 1 is 53.49 $NL_{CH_4}\ kg_{COD}^{-1}$ (38%), which is upper than 10%, thus this test result must be rejected. Since the standard deviation for the ratio 1 is important, this means that the dispersion of the BMP results for this ratio is important, the average BMP results for this ratio is therefore not representative. We interpret this result as follows, for this ratio we put a sample mass too small to be representative of the substrate. Indeed, vinasse being heterogeneous, it requires a sufficiently large sample to be representative of the substrate in terms of methanogenic potential. We free ourselves from the heterogeneity of the vinasse by increasing the ratio S/I, allowing to obtain representative results with standard deviation less than 10%. A second explanation is that inhibitions occur at this ratio, which would explain that the production of some BMP is lower. Concerning the other tested ratios, the average standard deviation is lower than 6%, thus the BMP results are acceptable.

Modelling the Kinetics of Methane Production

The coefficients for the first-order kinetic and the modified Gompertz models for each ratio are given in the Table 5. The first-order kinetic model provides the kinetic constant of hydrolysis. The highest value of 0.61 day^{-1} is obtained with the S/I ratio 1. However, the kinetic constant for the ratio 1 is rejected because this BMP was rejected. Then, the kinetic constant decreases with the increasing of S/I ratio. The kinetic constant is 0.32 day^{-1} for S/I ratio 2. The lowest value is therefore obtained with the S/I ratio 3 with a value of 0.25 day^{-1}. Thereby, despite the fact that the kinetic constant is higher for the ratio S/I 1 (I/S of 1.8), the maximum methane production is the lowest. Thus, lower kinetic coefficients led to higher methane yields. Moreover, the average kinetic constant, rejecting the value of the ratio 1, is 0.29 day^{-1}. The kinetic constant values obtained are similar, with a standard deviation of 0.04 day^{-1} (12%). The kinetic constant will be used in biochemical model as the hydrolysis constant. As the hydrolysis phase is considered as the rate-limiting step, this coefficient is crucial in the anaerobic digestion modelling. The correlation factor is between 0.97 and 0.99 and the RMSE between 3.98 and 4.77 $NL_{CH_4}\ kg_{COD}^{-1}$. The modified Gompertz model provides the methane production rate and the lag-phase time. The methane production rate decreases with the increasing of S/I ratio from 56.16 to 27.43 $NL_{CH_4}\ kg_{COD}^{-1}\ day^{-1}$. The correlation factor is between 0.97 and 0.98 and the RMSE between 4.06 and 6.29 $NL_{CH_4}\ kg_{COD}^{-1}$.

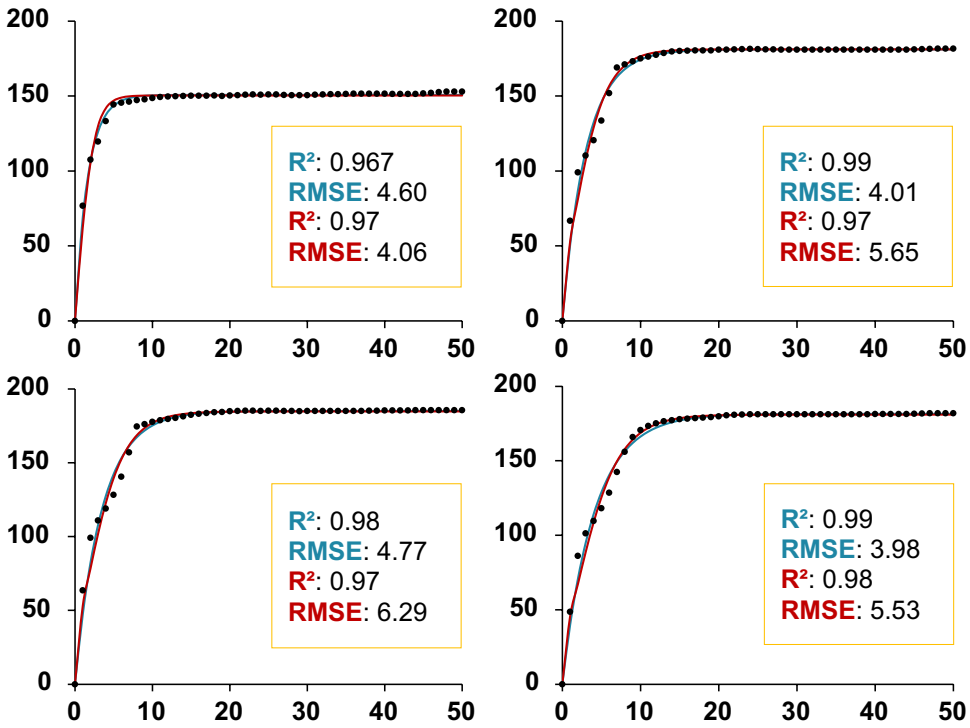

Fig. 3 Comparison between measured data (full line), calculated data with the first order kinetic (triangles) and calculated data with modified Gompertz model (points) for cumulative methane production at different S/I ratios (cumulative methane production in $NL_{CH_4}\ kg^{-1}_{COD}$ in function of time in days)

Table 6 Comparison of kinetic constant (first-order kinetic) with literature

Source	Substrate	Kinetic constant range (day−1)
This paper	Vinasse	0.25–0.32
[13]	Vinasse + water	0.30
	Press mud	0.16
	Vinasse + press mud	0.23–0.33
[21]	Vinasse + rumen	0.073–0.210
	Vinasse + rumen + urea	0.087–0.206

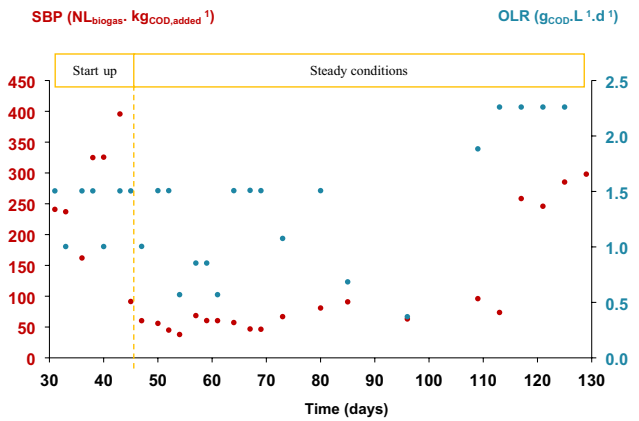

Fig. 4 Specific biogas production in NL $kg^{-1}_{COD,\ added}$ and organic loading rate (OLR)

The Figure 3 shows the comparison between measured data, calculated data with the first order kinetic and the calculated data with modified Gompertz model for cumulative methane production at different S/I ratios. The correlation factor of the first-order kinetic is between 0.97 and 0.99. It is similar to the correlation factor of the modified Gompertz model, which is between 0.97 and 0.98. Concerning the RMSE, it is between 3.98 and 4.77 for the first-order kinetic, and between 4.06 and 6.29 for the modified Gompertz model. The first-order kinetic and the modified Gompertz model fit well with the measured data.

In the Table 6, we present the comparison of kinetic constant (first-order kinetic) with literature. In this paper, we retain the range 0.25–0.32 day^{-1}. In addition, we exclude the value 0.61 day^{-1} because the methane production of this BMP has a standard deviation upper to 10%. The average value is therefore 0.29 day^{-1}. Compared to literature, the kinetic constant found in this study is similar to the kinetic constant of vinasse with a value of 0.30 day^{-1} [13] and vinasse with press mud with a range of 0.23–0.33 day^{-1} [13].

Pilot Tests Performance and Physico-chemical Analysis

The Specific Biogas Production and COD Removal

The Figure 4 shows the specific biogas production in liters per kilogram of COD added in the digester and the OLR.

The graph illustrates two phases, the start-up phase from day 0 to 45 and the steady conditions phase from day 46 to 130. The steady conditions phase begins when the production of biogas stabilises. The duration of the start-up phase is in the same order of magnitude as the duration that the study [11] which is 50 days. During the continuous agitation test (day 43), the production of biogas stopped, so we only tested this stirring for a period of 10 h and then return to minimal agitation that we maintained until the end of the experiment. Thus, this agitation was tested just before the end of the pilot start-up period. However, as the production of biogas stabilises during the recovery of minimum agitation, we still consider that the steady-conditions phase starts on day 46. We conclude from this test that continuous agitation stops the production of biogas, this may be due to the fact that there is no longer sufficient contact between the microorganisms and the substrate. Stirring the medium only during the filling of the pilot is enough in the case of the treatment of a liquid waste for the studied volume (16 L). However, it would be interesting to study other intensities of agitation.

During the period 113–130 days, the production of biogas per kilogram of COD is three times higher than in the previous period. It seems that the micro-organisms concentration is larger and the micro-organisms are better acclimated, which would explain this consequent increase in production. In fact, the TS and the VS of the sludge and the liquid phase increase over this period, which means that the biomass concentration increases within the digester. Thus, the physico-chemical conditions at this time are conducive to the anaerobic digestion of the raw vinasse.

The Table 7 shows the COD removal, the specific methane production (SMP), the specific biogas production (SBP) and the degradation index. The biogas production during the start-up phase is more important than the production during the steady conditions phase. It goes from an average of 182.69 $NL_{CH_4}\ kg_{COD}^{-1}$ during the period 29–42 days (start-up phase) to an average of 37.79 $NL_{CH_4}\ kg_{COD}^{-1}$ during the period 43–53 days (beginning of the steady-conditions phase). Indeed, during the start-up phase, the production of methane is due to the digestion of the vinasse but also to the digestion of the sludge initially introduced into the digester. This is consistent with the BMP blanks assays where only vinasse sludge is inserted into the digesters, we note that the maximum production of methane is reached after days around 45 days, the same duration as the start-up phase.

The average production of biogas during the start-up phase is 281.17 $NL_{biogas}\ kg_{COD}^{-1}$ and during the steady conditions phase is 104.93 $NL_{biogas}\ kg_{COD}^{-1}$. During the steady conditions phase, the minimum and maximum biogas production are respectively 37.90 $NL_{biogas}\ kg_{COD}^{-1}$ at day 54 (OLR is 0.85 $g_{COD}\ L^{-1}\ day^{-1}$) and 298.10 $NL_{biogas}\ kg_{COD}^{-1}$ at day 130 (OLR is 2.26 $g_{COD}\ L^{-1}\ day^{-1}$). We observe that the production of biogas per kilogram of COD added tends to increase: with an average of 58.13 $NL_{biogas}\ kg_{COD}^{-1}$ over the period 43–53 days, 61.68 $NL_{biogas}\ kg_{COD}^{-1}$ over the period 54–63 days, 66.89 $NL_{biogas}\ kg_{COD}^{-1}$ over the period 69–72 days, 82.74 $NL_{biogas}\ kg_{COD}^{-1}$ over the period 73–112 days, and 232.31 $L_{biogas}\ kg_{COD}^{-1}$ over the period 113–130 days. In the Table 7, for the calculation of the production of methane, we assume that the percentage of methane in the biogas is 65% in the results following the analysis of biogas on some samples, all samples must be analysed by chromatography to obtain the proportion of gases for the entire study. Thereby, the specific methane production has an average of 37.79 $NL_{CH_4}\ kg_{COD}^{-1}$ over the period 43–53 days and 151.00 $NL_{CH_4}\ kg_{COD}^{-1}$ over the period 113–130 days (Table 7). The maximum biogas production is obtained with the maximum OLR tested of 2.26 $g_{COD}\ L^{-1}\ day^{-1}$.

The Table 7 shows also the percentage of COD removal and the degradation index for each period delimited by a change in OLR. During the start-up phase (the first two periods of the Table 7), the COD removal has an average of 50%, and during the steady conditions phase, the COD removal gradually increases from 64 to 85% with an average of 73%. Concerning the degradability index, it varies

Table 7 COD removal, specific methane production, specific biogas production and degradation index

Period (days)	COD removal %	SMP[a] $NL_{CH_4}\ kg_{COD,r}^{-1}$	SMP $NL_{CH_4}\ kg_{COD}^{-1}$	SBP $NL_{biogas}\ kg_{COD}-1$	D_{index}
1–28	51	–	–	–	–
29–42	49	–	182.69	281.07	–
43–53	64	59.64	37.79	58.13	0.20
54–63	68	58.59	40.10	61.68	0.22
64–68	75	40.65	30.32	46.65	0.16
69–72	74	58.50	43.48	66.89	0.23
73–112	79	69.06	52.60	82.74	0.28
113–130	84.3	179.40	151.00	232.31	0.81
Steady conditions 46–130	73	87.54	68.21	104.93	0.37

[a]COD, r: removal COD

from 0.16 to 0.81 during the steady conditions phase with an average value of 0.37.

The Follow-Up of the Physico-chemical Properties of the Pilot Medium and the Sludge Analysis

The Figure 5 regroups the graphics of the physico-chemical analysis of the effluent: the ammonium, the VFA, the alkalinity, the VFA/Alk ratio, the pH, the COD and COD removal, the TS, the VS and the VS/TS ratio. The Table 9 shows the maximum, minimum and average values of physico-chemical analysis of the effluent during the start-up period, and the Table 10 shows the values during the rest of the study. These values must be taken cautiously because of the layer of sedimentation at the bottom of the digester. Thus, the results correspond to the liquid phase of the pilot. As the test progresses, the solid particles sediment and accumulate at the bottom of the pilot, forming a mud. The mud is also present on the side walls as well as on the agitator. The physico-chemical measurements on this mud were carried out. The measurements are given in the Table 8. The COD, the VFA and the alkalinity of the sludge are higher than the medium. The pH and the ammonium concentration of the sludge are in the same range as the medium. Thus the recalcitrant COD and VFA accumulate in the bottom of the pilot, but the pH is barely affected thanks to the increasing of the alkalinity. Furthermore, the VFA concentration is two to three times that of the liquid medium, this means that the products of the acidogenesis accumulate in the mud, and that organic loading is too high compared to the kinetics of

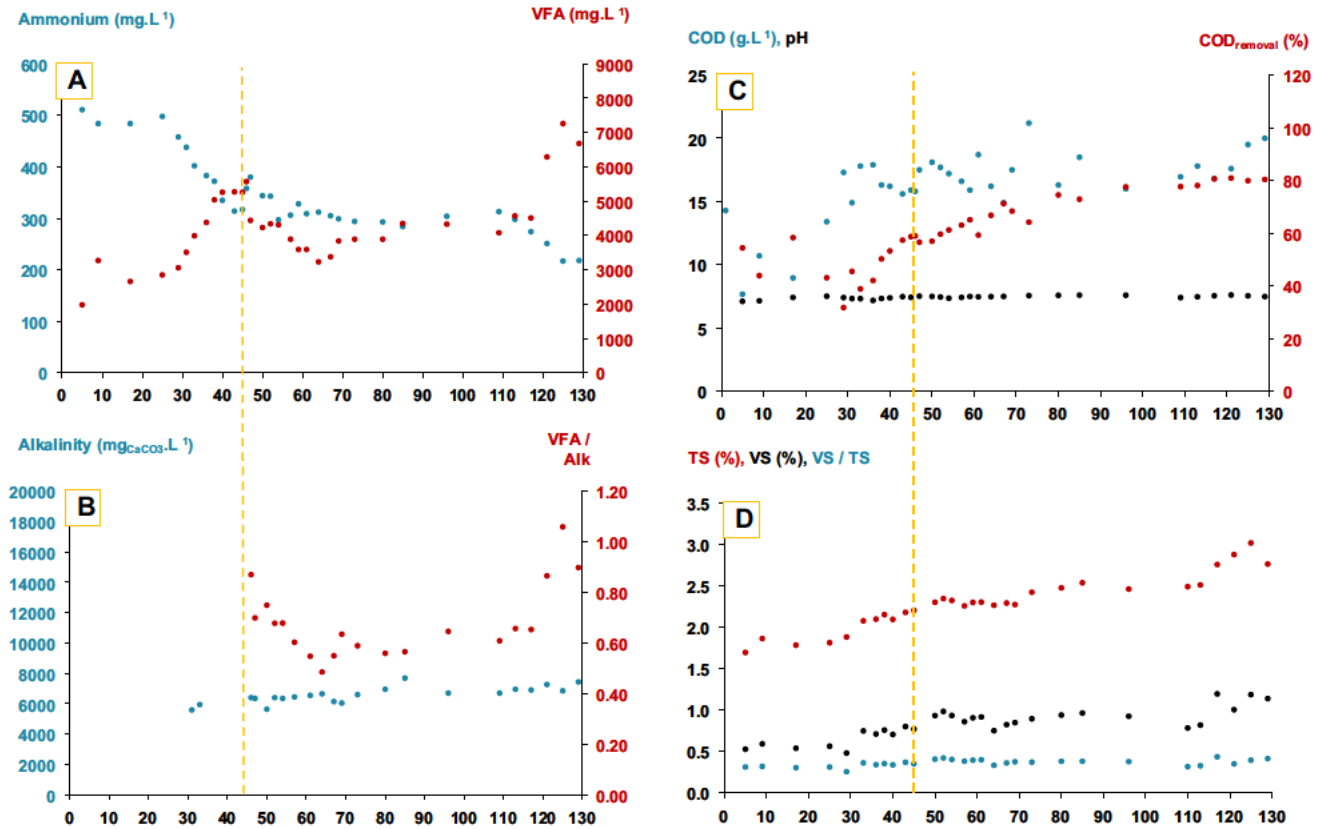

Fig. 5 Physico-chemical analysis in function of time (days) of the effluent: **a** ammonium and VFA, **b** alkalinity and VFA/Alk ratio, **c** pH, COD and COD removal, and **d** TS, VS and VS/TS

Table 8 Physico-chemical properties of the pilot sludge

Day	pH	TS (%)	VS (%)	COD (g_{O_2} L^{-1})	VFA (mg L^{-1})	Am (mg L^{-1})	Alk (mg_{CaCO_3} L^{-1})	VFA/Alk
109	7.469	5.48	2.85	53.1	10,760	312	10,685	1.01
117	7.813	5.81	3.92	47.7	18,500	226	14,868	1.24
129	7.428	–	–	41.3	14,900	224	13,507	1.10

acetogenesis reactions. Indeed, under ideal digestion conditions, AGV production rates are compensated for by the rates of consumption and thus there is no accumulation of AGV. Nevertheless, certain conditions can cause imbalances: an organic overload, the presence of organic or inorganic toxins, or temperature fluctuations [22].

The ammonium concentration (A) decreases during the start-up phase from 511 to 314 mg L^{-1}, and stabilises during the steady-conditions phase with an average value of 310.45 mg L^{-1}. The VFA (A) increases during the start-up phase from 1980 to 5270 mg L^{-1}, and stabilises during the following phase with an average value of 4279.00 mg L^{-1}. Sludge was added in the digester between days 55 and 65, we note that the VFA decreases from 4310 to 3230 mg L^{-1}. Thus, the addition of sludge has the effect of reducing the VFA concentration. The VFA and ammonium concentrations stabilised over the period 46–112 days. However, when we increase the OLR from 1.88 to 2.26 g_{COD} L^{-1} day^{-1} (113–125 days), we note an increase in VFA concentration from 4570 to 7260 mg L^{-1} and a decrease in ammonium concentration from 298 to 217 mg L^{-1}. This may lead to inhibitions of digestion, we stop the increase of the OLR until these concentrations stabilise. Despite the increase in the VFA concentration and the decrease in the ammonium concentration, the biogas production increases significantly over this period, from 73.67 to 285.29 NL_{biogas} kg_{COD}^{-1}. We conclude that these concentration variations do not lead to inhibitions of the process, but on the contrary, favour the production of biogas. However, inhibitions may occur if the VFA concentration continues to increase and ammonium to decrease. Indeed the acclimation of the microorganisms and the selection of the populations makes it possible to have a better resistance to the high contents of AGV for a stability of the processes [22]. Compared to literature, the VFA was between 3570 and 7850 mg L^{-1} in the case of the digestion of vinasse [11].

The alkalinity (B) globally increase over the test period. It varies between 5594 to 7691 mg_{CaCO_3} L^{-1}. The VFA/Alk ratio (B) varies from 0.49 to 0.87 with an average value of 0.65 over the period 46–72 days. Then, it varies from 0.56 to 0.65 with an average value of 0.59 over the period 73–112 days. Next, the ratio increases over the period 113–130 with the augmentation of the OLR, the ratio varies from 0.65 to 0.87 with an average value of 0.73. The minimum value follows the addition of sludge from day 54 to 63. The VFA/Alk ratio above 0.8 may inhibit methanogenic archaea, of 0.3–0.4 indicates an unstable system, and a ratio of 0.1–0.2 is appropriate [12, 23]. Compared to the ranges of literature, we have an unstable system. However, the digester is only started for 130 days and the ratio decreases so we could be in appropriate conditions.

The anaerobic digestion process occurs in the pH range of 6.0 to 8.3 [24]. Most methanogens have an optimal pH

Table 9 Maximum, minimum and average values of physico-chemical analysis of the effluent during the start-up period

Parameters	Units	Start-up: 0 to 45 days		
		Min	Max	Average
Ammonium	mg L−1	314	511	425.4
VFA	mg L−1	1980	5270	3751.8
Alkalinity	mg_{CaCO_3} L^{-1}	5594.4	5947.2	5770.8
VFA/Alk	–	–	–	–
pH	–	7.10	7.50	7.32
COD	g L−1	7.66	17.90	14.38
COD removal	%	36.25	62.65	50.96
TS	%	1.69	2.18	1.96
VS	%	0.48	0.80	0.64
VS/TS	–	0.25	0.37	0.32

Table 10 Maximum, minimum and average values of physico-chemical analysis of the effluent at different OLR

Parameters	Units	46–72 days			73–112 days			113–130 days		
		1.51 g_{COD} L^{-1} day^{-1}			1.88 g_{COD} L^{-1} day^{-1}			2.26 g_{COD} L^{-1} day^{-1}		
		Min	Max	**A**[a]	Min	Max	**A**[a]	Min	Max	**A**[a]
Ammonium	mg L−1	297	380	325.6	284	313	297.6	217.0	298	260.0
VFA	mg L−1	3230	5570	4037	3890	4350	4108	4510	6290	5123
Alkalinity	mg_{CaCO3}.L−1	5645	6653	6300	6602	7691	6931	6854	7268	6996
VFA/Alk	–	0.49	0.87	0.65	0.56	0.65	0.59	0.65	0.87	0.73
pH	–	7.35	7.51	7.46	7.40	7.59	7.54	7.46	7.60	7.53
COD	g L−1	14.9	18.7	16.9	16.0	21.2	17.8	16.8	19.5	17.9
Total COD removal	%	62.5	76.5	68.5	71.3	82.3	78.8	82.7	85.0	84.3
TS	%	2.25	2.34	2.29	2.42	2.53	2.47	2.51	3.01	2.79
VS	%	0.75	0.98	0.88	0.89	0.96	0.93	0.82	1.19	1.05
VS/TS	–	0.33	0.42	0.38	0.37	0.38	0.38	0.33	0.43	0.38

[a]Average value

between 7 and 8 while acid-forming bacteria often have a lower optimum [24]. The pH (Fig. 5c) of the influents were 7.57 for the sludge and 4.84 for the vinasse. Then, the pH of the medium varies between 7.10 and 7.50 during the start-up phase with an average of 7.31. It varies between 7.35 and 7.60 during the next phase with an average of 7.49. According to the graphic, we see that the pH stabilises during the steady conditions phase. The pH remains stable over the period 113–130 days despite the increase in VFA concentration during this period. Thus, the alkalinity of the digestion medium is sufficiently important to guarantee the stability of the pH. In most digesters, a neutral condition, as indicated by an average pH of 6.8–7.2, is considered normal [23].

Concerning the COD concentration (C), the initial concentration of COD is 14.28 g L^{-1}. During the start-up phase, small amount of vinasse was added, thus, the concentration of COD in the pilot is mainly brought by the sludge. As in the BMP tests, the maximum methane production of the blanks assays (sludge only) is reached in 43 days, we make the assumption that the COD of the sludge is fully consumed after 45 days (steady conditions phase). It slowly decreases between days 1 and 17 from 14.28 to 8.95 g L^{-1}. Then, the COD concentration globally increases during the rest of the test from 8.95 to 19.5 g L^{-1} with a maximum value of 21.20 g L^{-1} at day 73. We deduce from this outcomes that the vinasse has a recalcitrant COD which will accumulate in the digestion medium, unlike the sludge. However, this accumulation of organic matter does not seem to affect the production of biogas since it increases over the test period.

In terms of the variation of TS and VS (D), both increase during the assay period. The TS of the samples varies from 1.69% (day 5) to 3.01% (day 125) and the VS from 0.52 to 1.18% (day 125). The averages of TS and VS during start-up phase are respectively 1.96% and 0.64%, and during the steady conditions phase are 2.45% and 0.92%. The ratio VS/TS (E) ranges from 0.25 to 0.42 with an average of 0.32 for the start-up period and ranges from 0.31 to 0.43 with an average of 0.38 for the steady conditions period. The TS and the VS increase on the period 113–130 days, respectively from 2.51 to 3.01% and 0.82 to 1.19%, and the VS/TS ratio remains constant. As previously said, it is the period with the maximum biogas production, which means that this augmentation of TS is due to the micro-organisms growth. Measures of biological oxygen demand could confirm this hypothesis.

As we see on the graphic (Fig. 5a), the VFA and ammonium concentrations do not stabilise on the last period. Therefore, we need to continue the test until the stabilisation of these parameters.

Perspectives

In this work we obtained the results (biogas production and physicochemical analysis) in the case of mono-digestion of vinasse without any pre-treatment and with constant stirring. Thus, we have data on the physicochemical properties of the digestion medium with the biogas yields. We now have a working pilot. We can vary a selectable parameter to assess its impact on yields and properties of the digestion medium. Thereafter, we will continue the increase of the OLR up to a value of 3 g_{VS} L^{-1} day^{-1} as did the authors cited in this article during their pilot studies. The broader perspectives will be to study a pre-treatment of vinasse to improve the biogas yields obtained without diluting the vinasse. The major interest is to limit water consumption in anaerobic digestion plants. In addition, a study will also be performed on different modes of agitation including intermittent agitation and the variation of the stirring intensity.

Conclusion

The study showed that the first-order kinetic and the modified Gompertz model fit well with the BMP test curves with a correlation coefficients respectively upper to 0.98 and 0.97. Concerning the I/S ratio, the outcomes demonstrated that a ratio in terms of VSs lower than 1 (0.9, 0.7 and 0.6 ratios) gives the maximum methane yield with a value of 185.59 NL_{CH_4} kg_{COD}^{-1} (I/S ratio of 0.7). Moreover, we retain a value of 0.29 $days^{-1}$ for the kinetic constant of the sugarcane vinasse. This parameter is useful for the modelling of the anaerobic digestion process based on reactions kinetics.

In the present study, the biogas production and physicochemical analysis are given for the pilot test over a period of 130 days. The results showed that the start-up period with vinasse sludge from the same distillery as the vinasse, lasted 45 days. Then the physicochemical parameters and the biogas production were stabilised for each OLR. That said, although the physicochemical parameters have stabilised, the production of biogas continues to increase. Indeed, with the increase of the pilot's age, the biogas yield improves. The BMP of the vinasse was 185.59 NL_{CH_4} kg_{COD}^{-1} and the average specific biogas production of the last period (OLR of 2.26 g_{COD} L^{-1} day^{-1}) of the test was 151.00 NL_{CH_4} kg_{COD}^{-1} (232.31 NL_{biogas} $kg_{COD}{}^{-1}$), which gave a degradability index of 0.81. The maximum COD removal is 84.3%, it was obtained during the steady-conditions phase (OLR of 2.26 g_{COD} L^{-1} day^{-1}). We emphasise that the experimental results show that biogas production can be optimised by varying the OLR. It would have been interesting to be able to compare the biogas yields with different pilot start-up strategies, notably by increasing the OLR more or less quickly in order to study the impact of the pilot's loading rise in the start-up phase.

As the biogas yield gradually increased in pilot test, the OLR will be further increased progressively in future studies; in order to avoid inhibitions. The objective is the creation of a database for mesophilic mono-digestion of raw

vinasse. The next step will be to test different mixing conditions and pre-treatment on vinasse.

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