



Experimental design technique is useful tool to compare anaerobic systems

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Abstract

The present work shows the results obtained in the anaerobic digestion of coffee wet wastewater processing. The anaerobic reactors were operated with two configurations, single-stage and two-stage in mesophilic-controlled conditions. The effect of both organic loading rate (OLR) and reactor configuration in the anaerobic digestion of coffee wet wastewater was investigated. The OLR values considered in the single-stage system varied in arrange of 3.6-4.1 kgCOD m⁻³ d⁻¹. The acidogenic reactor of the two-stage system was fed at a rate of 11 kgCOD m⁻³ d⁻¹, whereas the methanogenic reactor load varied in the range of 2.6-4.7 kgCOD m⁻³ d⁻¹. For the same HRT and OLR global conditions, the two-stage system showed the best results in the treatment of this type of wastewater. The present study suggests that the experimental design technique is a suitable tool to the investigation of the wastes anaerobic treatment technologies.

Keywords: Single-stage and two-stage anaerobic digestion, coffee wet wastewater, UASB reactor, acidogenic reactor, methanogenic reactor, experimental design technique

Introduction

World coffee production is major economic activity in several tropical countries. The coffee bean, which is the portion of the cherry useful for human consumption, represents 20% of the total volume of the cherry. The bean extraction process is called in Latin America "Beneficio", and generates waste accounting for 80% of total raw volume processed. There are two types of processing: dry and wet. Wet processing is the most widely used treatment method in coffee producing countries. The method emerged as an alternative to solve the problem of rapid and excessive fermentation of the cherries in tropical regions. After the harvest, the external components of the cherry are removed and the beans are placed in fermentation tanks to release the mucilage by hydrolysis. The process consumes large amounts of water that are sometimes poured without any adequate treatment to the surface waters. This situation causes a significant environmental impact since these wastewaters have high organic contamination ranging from 2400 to 21900 mgCOD l⁻¹, large amount of suspended solids, and their turbidity results in unpleasant odors and in a loss of visual quality [1,2]. Since coffee wastewaters have high carbohydrate concentration, biological processes, either aerobic or anaerobic digestion, are

suitable for their treatment.

Anaerobic treatment has some advantages over conventional aerobic treatment such as greater removal efficiency of the chemical oxygen demand (COD), reduced sludge production, low power consumption, reduced space requirements, a relatively simple construction, low nutrient requirements and generation of a gas with a high calorific power (methane). However, some other aspects like long start-up, low nutrient and pathogen removal, possible generation of odors and the need for a post-treatment have had a negative impact on the implementation of the anaerobic process [3].

High-rate anaerobic reactors have the ability to handle high organic loading rates (OLR), high up-flow velocities, and low hydraulic retention times (HRT). Therefore, a reactor of smaller volume is required even to produce large amounts of biogas. Upflow anaerobic sludge blanket (UASB) reactor and the upflow anaerobic filter (UAF) reactor are examples of high-rate reactors that have been used in the treatment of several types of wastewater. A hybrid anaerobic reactor is a combination of both a UASB and a UAF reactor, which is arranged in two different sections: an unpacked volume and a packed volume that enhances the active biomass retention in the reactor

during overload conditions.

Some experiments conducted with several types of coffee wastewaters have faced difficulties in obtaining a stable performance of the anaerobic digestion due to the acidity and low alkalinity of these wastewaters, and the presence in the latter of the inhibitory compounds of the process [4,5]. Furthermore, the coffee wet wastewaters have large amounts of organic matter of easy hydrolysis that causes a high VFA production. An accumulation of VFA in the reactor affects negatively the methanogenic bacteria due to a pH drop [6].

One possible method for increasing the efficiency of the anaerobic digestion process is the use of two-stage anaerobic digestion, with a first reactor for the hydrolysis-acidogenesis stage and a second reactor for the acetogenesis-methanogenesis stage, because both groups of acidogenic and methanogenic microorganisms are different with respect to their nutritional requirements, physiology, pH optima, growth and uptake kinetics. In each reactor, the optimal environmental conditions are created for each group of anaerobic microorganisms, thus generating a better stability and control of the anaerobic digestion. This treatment scheme provides the following advantages: an increase on the COD removal efficiency, a greater stability to the OLR increases, a greater resistance to the inhibitory compounds, and the first stage allows a pH buffering for the methanogenic bacteria of the second reactor whenever a pH drop occurs in the first reactor [7]. Therefore, the two-stage anaerobic digestion could offer significant benefits for the anaerobic treatment of the coffee wet wastewaters.

The two-stage anaerobic digestion allows the separation of acidogenic and methanogenic stages to optimize the OLR and HRT values in each of them [8]. The acidogenic microorganisms grow relatively faster and are less sensitive to pH drops than the methanogenic microorganisms; for this reason the first reactor can handle high OLRs and low THRs, with a pH interval between 5.5-6.0 that is considered optimum for this stage [9-11].

Experimental design technique can be regarded as a process by which certain factors are selected and deliberately varied in a controlled manner to obtain their effects on a response of interest, followed by the analysis of the experimental results. According the number of the factors to be investigated at a time, the experimental design can be classified into two categories: single-factor design and factorial design. Single factor design is a traditional design, which investigates the effect of one factor, while keeping the levels of other factors constant. The level of the factor to be investigated is then changed over a desired range to study its effects on one or several responses. For this purpose, experimental design have been widely reported for the process characterization, optimization and modeling in recent years [12]. Although the experimental design technique has been widely studied by many researches as an established and promising method for optimization and formulation of various types of processes. However, there are no systematic

papers in the literature specifically devoted to a study of the comparison of anaerobic systems using an experimental design technique. The literature suggests that the anaerobic digestion of the coffee wet wastewaters is possible. Nevertheless, the scheme of experimental design technique can be considered as a particular field of investigation to develop a suitable and continuous system to achieve an optimum control system to treat these wastewaters.

Based on the above-mentioned facts, the specific objectives of this study were: (1) to apply a single factor design to evaluate the potential of two anaerobic systems: single-stage and two-stage, treating coffee wet wastewaters; (2) to examine the effect of OLR on two blocks (anaerobic systems) to achieve of majors values of COD removal.

Methods

Reactors

(Figure 1) shows a scheme of the configurations of the laboratory scale anaerobic systems that were used, which consisted of two UASB reactors and a hybrid reactor. One of these two reactors constituted the single-stage system and the other UASB reactor and the hybrid reactor completed the two-stage system. The reactors were kept at mesophilic temperature ($37\pm 1^\circ\text{C}$) in a constant-temperature room. Single-stage system consisted on a glass cylindrical reactor of 0.40 m of height and 0.09 m of diameter, with a nominal volume of 2.5 L. It was equipped with a Masterflex® L/S® variable-speed modular drive (model HV-07553-75, 6-600 rpm), which provided a variable flow for the residual income and the effluent recycle. Two-stage anaerobic digestion system consisted of a first UASB reactor for hydrolysis-acidification stage and a second hybrid reactor for acetogenesis-methanogenesis stage. In this system, the first reactor was a glass cylindrical reactor, of 0.35 m of height and 0.076 m of diameter and nominal volume of 2.13 L. This reactor was equipped with a Masterflex® L/S® variable-speed modular drive (model HV-07553-75, 6-600 rpm). The second reactor was based on another glass cylinder of 0.43 m of height and 0.076 m of diameter and nominal volume of 2.0 L. This other reactor was fed with a Masterflex® L/S® variable-speed modular drive (model HV-07553-80, 1-100 rpm). In the upper third of the methanogenic reactor of the two-stage system were placed 0.67 L of crushed and sieved zeolite, with a particles diameter between 2.0-4.76 mm and filter height of 0.14 m.

Feed and seed

The inoculum used was granular sludge coming from an industrial scale UASB reactor that processed canned juice wastewaters, having a volatile suspended solid (VSS) concentration of 73.5 g l^{-1} and specific methanogenic activity (SMA) of $0.13\text{ gCH}_4\text{-COD gVTS}^{-1}\text{ d}^{-1}$. The characteristics and elements of the granular sludge are shown in Table 1. The laboratory reactors were fed with coffee wet processing wastewater, located in Ixhuatlán community, Veracruz, Mexico. This coffee

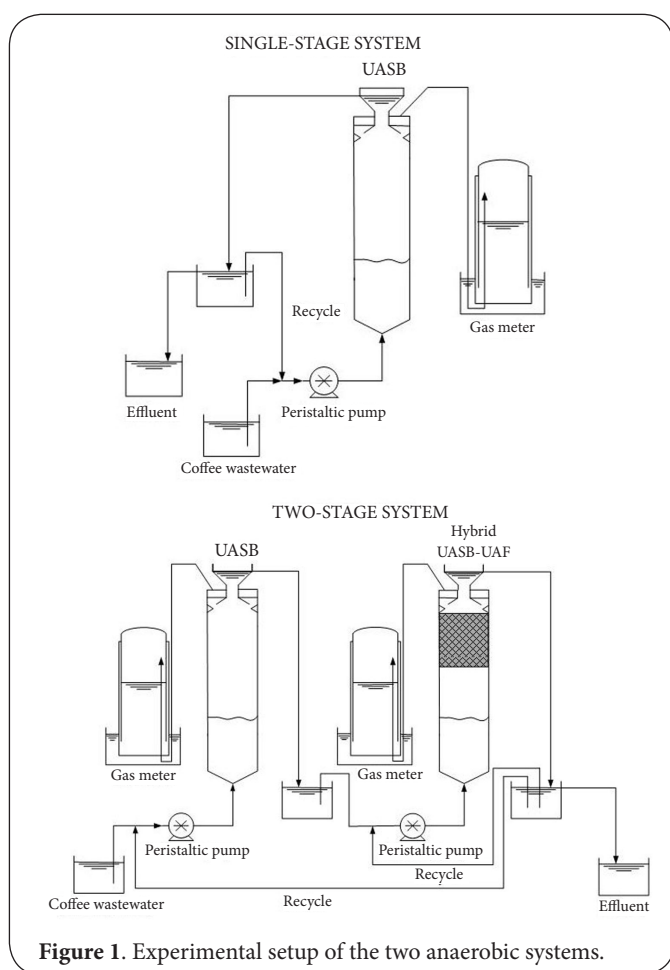


Figure 1. Experimental setup of the two anaerobic systems.

Table 1. Characteristics of granular sludge.

Parameters	Granular sludge
Specific methanogenic activity ($\text{gCH}_4\text{-COD gVTS}^{-1} \text{d}^{-1}$)	0.13
Volatile total solids (g l^{-1})	73.5
Sedimentation rate (m h^{-1})	70
Color	Coffee to black
Form	Spherical to oval
Median particle size (diameter) (mm)	3

wet processing uses a technology called “green” because it requires less water consumption, hence the wastewater had high values of pollutant concentration. The composition of the coffee wet wastewater is shown in Table 2. As the coffee wet processing wastewater was acid, its pH had to be adjusted using sodium bicarbonate (NaHCO_3).

Experimental procedure

UASB reactor of the single-stage system was inoculated with 0.4 L of the granular sludge already mentioned, whereas the hydrolytic-acidogenic stage reactor and the acetogenic-

Table 2. Characteristics of the coffee wet wastewater.

Parameters	Wastewater
Total solids (mg l^{-1})	1228.5
Total volatile solids (mg l^{-1})	1141.6
Total suspended solids (mg l^{-1})	315.7
Volatile suspended solids (mg l^{-1})	271.2
Total COD (mg l^{-1})	2545 ± 142
Soluble COD (mg l^{-1})	2302 ± 175
pH	3.79 ± 0.21
Total carbohydrates (mg l^{-1})	830
Sugars (mg l^{-1})	940
Tannins (mg l^{-1})	0.16
Phenols (mg l^{-1})	80
Caffeine (mg l^{-1})	23
VFA (mg l^{-1})	696
Alkalinity ($\text{mg CaCO}_3 \text{ l}^{-1}$)	190
Nitrogen (mg l^{-1})	195.6
Phosphorus (mg l^{-1})	5.1
Potassium (mg l^{-1})	234

methanogenic stage reactor of the two-stage system were inoculated, respectively, with 0.64 and 0.4 L of the same granular sludge. Once the inoculation was completed, the start-up stage took place. In order to determinate the proper flow rate at each moment, the systems was fed continuously by means of a variable speed pumps. During the start-up, the applied OLR to the anaerobic systems was increased gradually until the evaluation conditions desired was achieved, and all systems were fed with the same COD concentration. Each reactor had one inlet point of the bottom. In the two-stage system, the leachate flow was introduced to the methanogenic reactor after passing the acidogenic reactor. The wastewater treated was considered the effluent of methanogenic reactor. When concluding the fourth week it was considered that the systems had quasi-stationary state conditions, so the evaluation of each system began considering three increasing OLR, that were denominated Run1, Run2 and Run3. The same OLR and HRT were considered in both systems and having a definite, HRT for each for the three OLR compared. The characteristics of the different evaluation conditions are detailed in Table 3. Each OLR evaluation was carried out in a period of 3 weeks. During the whole evaluation period of the single-stage system a recycle rate of 1.0 (ratio 1:1 of the inflow wastewater to treat and the recycled flow) was applied.

Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), pH and alkalinity were determined according to the Standard Methods for the Examination of Water and Wastewater [13]. Alpha index is defined like the alkalinity contributed by the

Table 3. Operating parameters of the two systems compared.

System	Parameters	Run1	Run2	Run3	
Single-stage system	HRT total(h)	21.5	18.5	15.5	
	Flow(Lh ⁻¹)	0.15	0.16	0.17	
	OLR(kg COD m ⁻³ d ⁻¹)	3.6±0.1(15)	3.8±0.2(15)	4.1±0.1(15)	
	Recyclerate	1.0	1.0	1.0	
Two-stage system	Acidogenic reactor	HRT(h)	5.5	5.5	5.5
		Flow(Lh ⁻¹)	0.39	0.39	0.39
		OLR(kg COD m ⁻³ d ⁻¹)	11.4±0.5(15)	11.2±0.8(15)	10.9±0.5(15)
	Methanogenic reactor	HRT(h)	16	13	10
		Flow(Lh ⁻¹)	0.16	0.19	0.25
		OLR(kg COD m ⁻³ d ⁻¹)	2.6±0.2(15)	3.2±0.2(15)	4.7±0.3(15)
	Overall conditions	Global HRT(h)	21.5	18.5	15.5
Global OLR(kg COD m ⁻³ d ⁻¹)		3.7±0.2(15)	3.8±0.3(15)	4.1±0.2(15)	

The results are shown as “mean value ± standard deviation (number of observation)”.

ions bicarbonates and the total alkalinity (contributed for the bicarbonates, carbonates and hydrogenate carbonates ions) used to absorb the pH drop in an anaerobic reactor. The alpha index was calculated as the quotient of partial alkalinity at pH 5.75 and total alkalinity at pH 4.30. Total and soluble chemical oxygen demand (COD) analyses were carried out using a HACH COD reactor (digestion at 150°C for 2 h) according to the closed reflux colorimetric method described in Standard Methods for the Examination of Water and Wastewater [13].

Volatile Fatty Acids (VFA) were analysed with a gas chromatograph (Chromatograph SRI 8610 model, with a flame detector, Zebron column, and Helium gas carrier to 30 psi). Two millilitres samples were taken from the reactor with a syringe and deposited in the Eppendorf tube, and two drops of hydrochlorate acid were added (solution 1:1). The samples were centrifuged by half an hour at a 3500 rpm in a micro-centrifuge Eppendorf. The supernatant were filtered through Wathman paper (0.22 µm), and conserved at 4°C until being used.

Biogas was collected in gas-collection tubes connected to the digesters. The amount of biogas produced was monitored every day. The methane concentration in biogas was measured by gas chromatography (Chromatograph Fisher Gas Partitioner Model 1200, equipped with a detector of thermal conductivity, double column Porapack Q and mesh molecular SA, with Helium gas carrier flow of 25 ml min⁻¹). Molar fractions of methane from analyzed samples were determined by comparing the peak areas of the component with pure methane.

Statistical analysis

The analysis of variance was done using the ANOVA procedure of STATISTICA software (version 6.0, StatSoft Inc., USA). The response of the two anaerobic systems compared was assessed

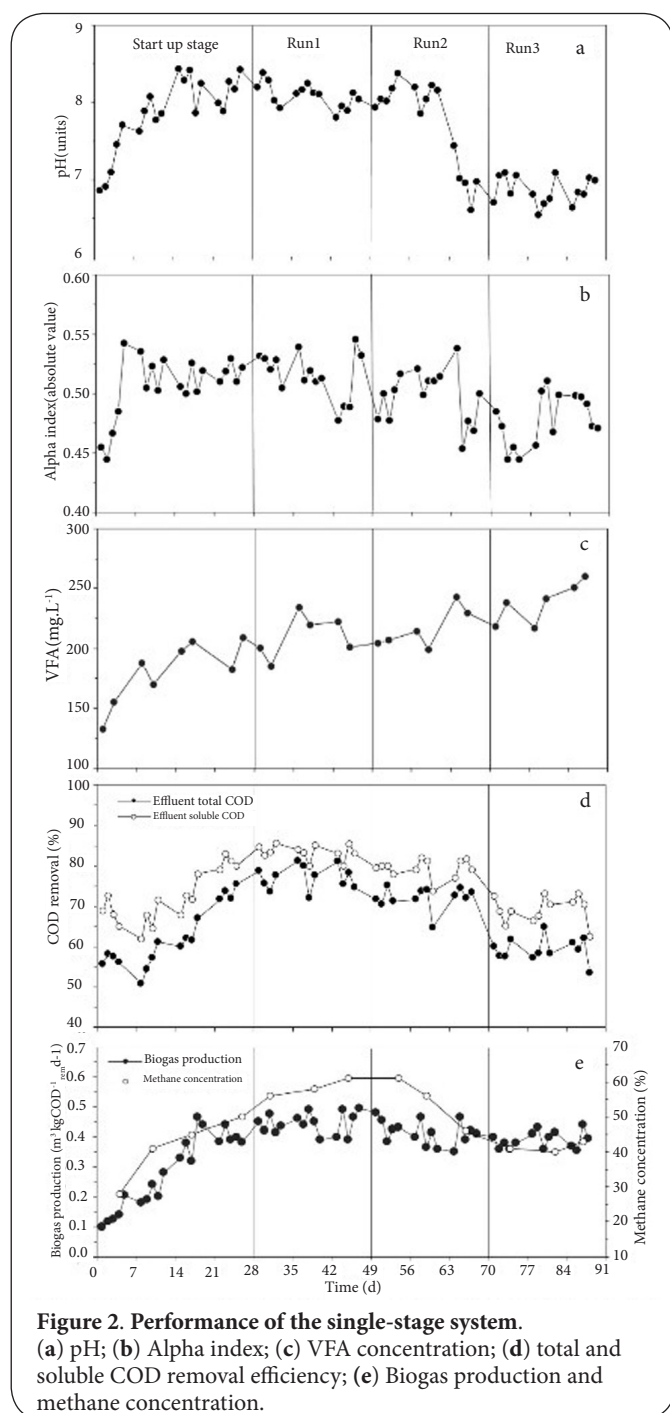
considering five variables (total and soluble COD removal efficiency, VFA concentration, biogas production and methane concentration). Treatments were assigned at random in each anaerobic system. Whenever statistically significant differences between treatments were detected, differences between means were assessed by a multiple range analysis test (Duncan) ($p < 0.05$).

Results and discussion

Performance of single-stage anaerobic digestion system

The pH behavior observed during the evaluation period of the single-stage system can be seen in **Figure 2a**. When the inflow wastewater pH was adjusted at 7.0 in start-up stage, the UASB reactor showed a stable performance, with a pH interval of 7.8-8.4 and an average value of 8.1±0.15. Occurrence of the anaerobic digestion requires a pH interval of 6.5-8.2 [14]. Values of this parameter below 6.5 favor acidification, especially in this type of wastewater with pH values < 4.0. This fact inhibits the methanogenic population and, therefore, the efficiency of the anaerobic treatment is reduced. Considering the alkaline values of the effluent and the cost incurred in the neutralization of the wastewater with sodium bicarbonate, a pH adjustment to a value of 6.5 was done in the wastewater to treat from day 33 onwards (i.e., in the course of Run2). These new conditions favored pH values of the effluent in the interval 6.5-7.1, with an average value of 6.9±0.2, until the end of the experiment. Given the characteristics of this coffee wastewater, and even though the pH values stayed within in the appropriate interval, it is recommendable, in order to prevent possible destabilizations in the reactor, to reach values of the pH near to those measured in Run1, which were obtained after adjusting to pH 7.0 the wastewater to treat.

An aspect related to pH is alkalinity, where the alpha index indicates the absorbing capacity of the system to any abrupt



pH change in the reactor. In this study, a quick increase in the alkalinity from 1612 and 2192 mgCaCO₃ l⁻¹ was observed in the start-up stage. A similar behavior was observed in Run1 when the pH in the inflow wastewater was kept constant and equal to 7.0, with an alkalinity average value of 1935±128 mgCaCO₃ l⁻¹. When the pH was readjusted to 6.5, the alkalinity decreased to a value of 1355±67 mgCaCO₃ l⁻¹. Whenever the pH value of the wastewater was 7.0 and its average alkalinity 1935±128 mgCaCO₃ l⁻¹, the reactor behavior was similar to that

observed in the start-up stage. Similarly, this so high value of the alkalinity favored increases of the pH above the optimal interval even though the wastewater to be treated had been neutralized. Some authors have suggested that the larger the alpha index value the better the buffering capacity of the system. Jenkins et al., [15], recommended that the alpha index values should be larger than 0.5. As a rule of thumb, this figure shows a good performance of the reactor. As it can be seen in Figure 2b, the alpha index reached an average value very close to the optimal, 0.51±0.02, after the first week of the start-up stage. However, when the pH in the inflow was adjusted to 6.5 the average alpha value decreased to 0.48±0.02. This decrease in the alpha value with the pH is associated to an accumulation of acid species in the reactor that could cause system instability. pH is not a sensitive indicator, since it could conceal the increase of the H⁺ concentration even though it could show suitable values. Therefore, to carry out a continuous monitoring of an anaerobic processes, and even to make decisions, the alpha index is a better option [16].

One of the main problems of the anaerobic biological degradation of this type of wastewater is the high content of easily fermentable organic matter. Organic matter compounds cause a fast acidification of the wastewater that results in a high production of VFA and, therefore, it is necessary the addition of an alkaline substance to increase the pH. In order to prevent the accumulation of VFA, it is advisable to recycle the treated effluent with the aim of re-use the alkalinity of the anaerobic process to reduce the consumption alkaline substances [17]. In addition, the recycle can be used to maintain a suitable hydraulic load in the anaerobic reactors when high concentrated wastewaters are being treated [18].

Total VFA concentration was considered as the sum of the acetic, propionic and butyric acids concentrations. It was observed a tendency to increase VFA concentrations in the effluent with increasing OLR values (Figure 2c). In an overloaded anaerobic system it can be observed an accumulation of VFA because the methanogenic bacteria can not remove the hydrogen and VFA produced [19]. Therefore, the VFA increase in the effluent reveals an increase in the load applied to the system. As expected, Run1 resulted in the lowest VFA concentration in the effluent, with an average value of 220±18 mg l⁻¹, although not significant differences were observed between Run1 and Run2. However, Figure 2c shows that in Run2 the concentration of these acids increased. These results indicated that the HRT did not affect the relative composition of the organic acids in the effluent, but their concentration increased when the OLR increased. Acetic acid reached the higher proportion (60%) followed by propionic acid (28%) and butyric acid (12%), with concentrations of 131±10 mg l⁻¹, 63±7 mg l⁻¹ and 26±2 mg l⁻¹, respectively. These percentages reveal an adequate proportion of these acids in the effluent that avoids the inhibition of the anaerobic digestion by VFA [14].

Total COD removal efficiency with the increase in the applied OLR can be seen in Figure 2d. Significant differences

were observed between the efficiencies in all the treatments compared, with average values of 77.2%, 72.1% and 59.2% for Run1 (584 mg l⁻¹), Run2 (688mg l⁻¹) and Run3 (1041 mg l⁻¹), respectively (p -value>0.05). These results confirm that when the load applied to the system increases, the methanogenic bacteria cannot completely degrade the VFA produced; therefore the efficiency and stability of the reactor are affected negatively [20]. For the first OLR evaluated (Run1), the soluble COD removal efficiency exhibited a high value (83.4%; 426 mg l⁻¹), indicating a successful treatment of the wastewater in study.

Biogas production and methane concentration generated in the single-stage system are shown in **Figure 2e**. The largest biogas productions were obtained in Run1. Although, significant differences (p -value<0.05) were observed between the biogas obtained in Run1 and Run2, their respective average values were similar. The total biogas production fluctuated in the range of 0.35-0.5 l d⁻¹, revealing that the effect of the HRT in the conversion of the organic residual to biogas was not significant. According to Lin et al., [21], the biogas production is independent of the HRT and the substrate concentration.

Methane concentration decreased in Run3. The highest methane concentration values were achieved in both Run1 and Run2 (61%). This circumstance indicated an indirect correlation between the VFA concentration and methane concentration because methane production decreased when the VFA concentration increased. These results coincide with those reported by Dogan et al., [22], who studied the effect of the variety and concentration of the VFA in a UASB reactor, and concluded that these factors have a significant effect on the methanogenic activity, besides their synergy with other products. Nevertheless, the main factor affecting the single-stage system was the pH, because when it was adjusted to 6.5 in the wastewater to treat (day 64) a decrease in the methane concentration values were observed. Other authors have also reported an increase in the CO₂ concentration in the biogas with a pH drop [11,23-25].

A summary of publications related to the anaerobic treatment of coffee processing wastewaters is presented in **Table 4**. In our study, a UASB reactor, operated at an OLR of 3.6 kgCOD m⁻³ d⁻¹, achieved a total and soluble COD removal efficiency of 77.2±2.9% and 83.5±1.87%, respectively, and a methane concentration of 58±2.5%. These results are comparable with those reported by other authors. Fernandez and Foster [26], operating two anaerobic filters at 37°C and 55°C, with an OLR of 4.0 kgCOD m⁻³ d⁻¹, observed a COD removal efficiency of 63%, treating a synthetic wastewater made up of coffee bean extract. Silva and Campos [27] studied the feasibility of a laboratory scale UASB reactor that was used to treat coffee wet wastewater. The wastewater pollutant load was 3250 mg l⁻¹, the system operating conditions were adjusted to an HRT of 69 hours and an OLR of 0.59 kg COD m⁻³ d⁻¹, and the COD removal efficiency achieved was 78%. Recently, Fia et al., [2] evaluated three different support materials in an AFBR

anaerobic reactor and the largest COD removal efficiency, 80%, was obtained at an OLR of 4.4 kgCOD m⁻³ d⁻¹.

Performance of the two-stage anaerobic digestion system *Assessment of the hydrolysis-acidogenesis stage*

Figure 3a shows the evolution of the pH values in the two-stage system. The start-up stage in the acidogenic reactor was accomplished adjusting the pH of the inflow wastewater to a value of 7.0. In these conditions, the effluent reached pH values close to 7.4. On day 33 from the start of the experiment the pH of the inflow wastewater was readjusted to a value of 6.5 and since then the reactor's effluent pH dropped gradually to values within the range 6.07-6.67, and an average value of 6.42±0.19, until the end of the experiment. In consequence, for wastewaters with OLR values similar to those considered in this work are to be treated it is necessary to raise their pH to a value of 6.5.

The evolution of the alpha index can be seen in **Figure 3b**. By adjusting the pH of the wastewater to be treated to a value of 7.0, the alkalinity reached a mean value of 552±85 mgCaCO₃ l⁻¹. In the final stage of Run1 a sudden increase in alkalinity occurred, with values above 2000 mgCaCO₃ l⁻¹. In Run2, the pH wastewater was adjusted to 6.5 and the alkalinity decreased further to an average value of 552±98 mgCaCO₃ l⁻¹, along with an alpha index of 0.46±0.03.

In each of the three OLR levels assessed, the VFA concentration and the COD removal efficiency can be seen in **Figures 3c** and **3d**, respectively. At the start-up stage, VFA production increased when the pH value was adjusted to 7.0. This similar behaviour was also observed by Bengtsson et al., [11] with CSTR reactors. The acidification degree is a parameter that assesses to what extent the acidogenic fermentation in an anaerobic reactor succeeds. It is defined as the ratio of the concentration of the VFA produced and the concentration of the soluble COD (SCOD) present in the effluent [10,28,29]. When the pH of the wastewater to be treated in the acidogenic reactor was 6.5, the acidification degree stood in the range of 43-52%. Among the products obtained by the anaerobic digestion, the best methane precursors are acetic and butyric acids [14]. The amount produced of the latter, together with the remaining VFA formed; provide a measure of the acidogenic stage performance. According to Bouallagui et al., [6], the acidification degree is determined by the OLR, as well as the concentration and composition of the VFA present in the soluble COD. For all OLR compared, the average concentrations of acetic, propionic and butyric acids were 530±46 mg l⁻¹, 423±17 mg l⁻¹ and 168±5 mg l⁻¹, respectively. Among the VFA produced in the acidogenic stage, the acetic acid represented 46-52%, followed by propionic acid, 33-40%, and butyric acid, 11-16%. These results are similar to those obtained by Mcdougall et al., [30], who assessed the acidogenic stage of a digester processing synthetic wastewater coffee.

As it can be seen in **Figure 3e**, average biogas production in the acidogenic reactor was 0.61±0.14 l d⁻¹. Although the

Table 4. Summary of some publications related to the anaerobic digestion of the coffee wastewaters processing.

SINGLE-STAGE SYSTEM						
Reactor	OLR (kgCOD m ⁻³ d ⁻¹)	HRT (h)	Temperature (°C)	COD removal efficiency (%)	Methane concentration (%)	References
UAF	4	24	37-55	63	70	[26]
UAF	3.33	--	37	65	55	[30]
UASB	0.59	69	--	78	--	[27]
Hybrid UASB-UAF	9.55	18	--	61	58	[1]
AFBR	4.41	25.4	6.4-32.9	80	--	[2]
UASB (This study)	3.6	21.5	35	77.2	58	--
TWO-STAGE SYSTEM (ACIDOGENIC REACTOR)						
Reactor	OLR (kgCODm ⁻³ d ⁻¹)	HRT (h)	Temperature (°C)	Acidification degree (%)	pH	References
CSTR	10	24	37-55	38	5.5-6.5	[30]
CSTR	10-16	12-24	55	22-38	5-6	[36]
SBR	5	10*	35	87	6	[37]
UASB	20	6	55	--	--	[38]
UASB (This study)	11	5.5	37	43-52	6.5	--
TWO-STAGE SYSTEM (METHANOGENIC REACTOR)						
Reactor	OLR (kgCOD m ⁻³ d ⁻¹)	HRT (h)	Temperature (°C)	COD removal efficiency (%)	Methane concentration (%)	References
UAF	3.33	--	37-55	78	70	[30]
UASB	10-16	12-24	35-55	63-77	71-79	[36]
SBR	0.5	--	32	85-95	80	[37]
UASB	3.5	48	35	98	73	[38]
Hybrid UASB-UAF (This study)	2.6	16	37	84.2	57	--

* day.

presence of CO₂ in the biogas was remarkable the methane concentration reached 12±2% (Figure 3e). These results are in agree with those of Parawira et al., [31], who found that 10-30% of the methane produced was generated in the acidogenic reactor. According to Hai-Lou et al., [32], a low presence of CH₄ in the biogas produced in the hydrolysis-acidogenic stage of an anaerobic digestion process is the result of a rapid fermentation. In acid conditions, the acidogenic bacteria prevail over the methanogenic bacteria and the biogas produced has a high concentration of CO₂ and hydrogen [23,24].

In this study it was also observed that using a stage separation anaerobic system to treat wastewater with an OLR of 11.0 kgCOD m⁻³ d⁻¹, a pH of 6.5, and a mesophilic temperature of 37°C, it can be achieved an acidification degree of 47±4% in the acidogenic reactor. This latter value was larger than those obtained by other authors (Table 4).

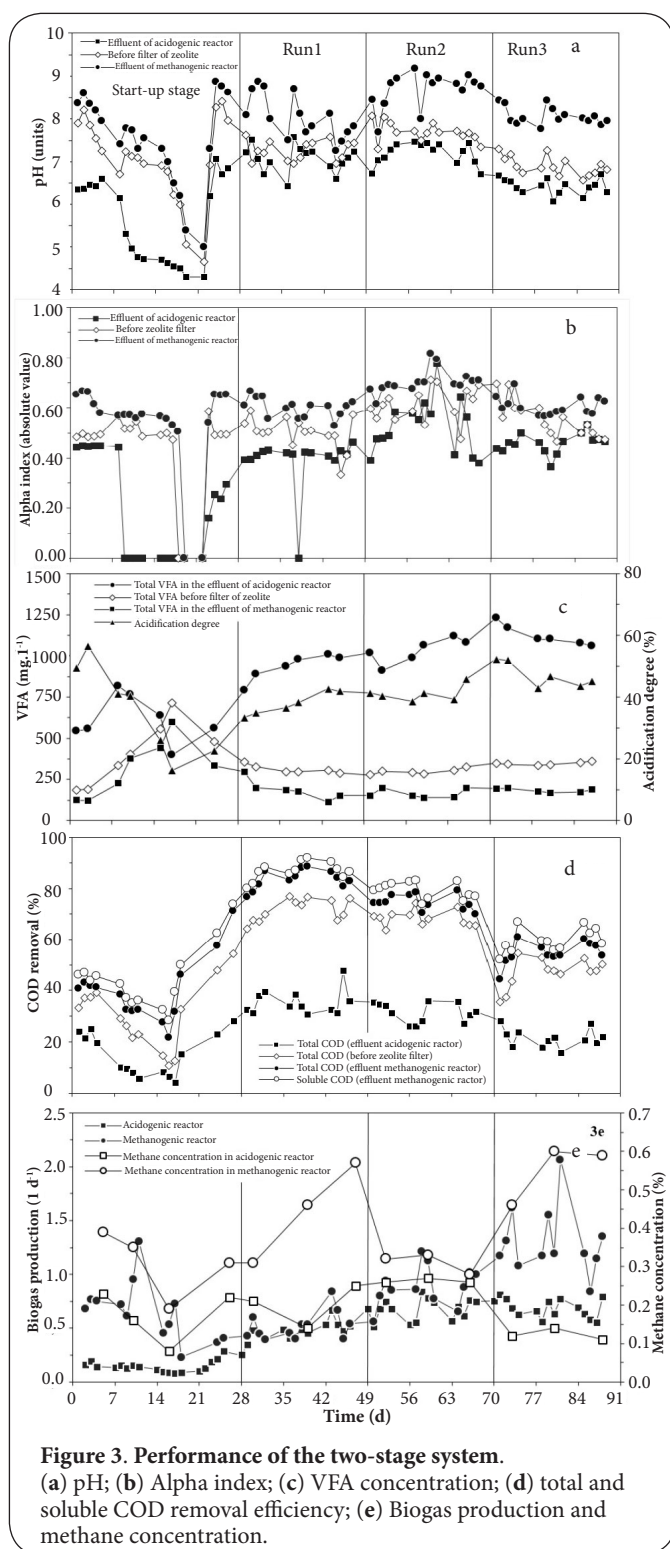
Assessment of the acetogenic-methanogenic stage

Figure 3a shows that once this latter reactor was adjusted to a pH of 7.0, the pH value of the methanogenic reactor effluent increased rapidly. During Run2, the pH of the methanogenic

reactor outflow rose to a value of 9.0, but it fell to an average value of 8.07±0.21 when the pH of the incoming wastewater from the acidogenic reactor was adjusted to a value of 6.5. According to Caramillo and Rincón [7], a pH close to 8.0 promotes the activity of the bacteria of the methanogenic stage in a two stage anaerobic system.

Since the start-up stage ended (Figure 3b), the values observed of the alpha index were within the range of 0.57-0.69, whereas the alkalinity mean value was 1038±67 mgCaCO₃ l⁻¹. These optimal values of the alpha index revealed that favourable environmental conditions were reached in the methanogenic reactor.

In Figure 3c it can be seen the VFA concentration in the methanogenic reactor effluent. When the methanogenic bacteria transform the VFA as soon as they are formed, their accumulation is prevented and this fact ensures the adequate alkalinity of the system. By contrast, if the conditions are not favourable, the VFA are not consumed at the same rate as they are produced by the acidogenic bacteria; therefore, they are accumulated in the anaerobic system, and this causes a rapid decrease of the available alkalinity that results in a



pH drop [16]. In each OLR evaluated (Table 5), no significant differences were observed between the concentration mean values of the different species that constitute the VFA. For the acetic, propionic and butyric acids, these values were 87-122

Table 5. Analysis of variance (ANOVA).

Source	SS ^a	DF ^b	MS ^c	F-ratio	F-tab ^d	p-value
VFA concentration						
OLR ^e	2493.73	2	1246.87	1.33	3.29	0.2777
System ^h	16996.30	1	16996.30	18.18	4.15	0.0002 ^e
Error	29914.30	32	934.82	--	--	--
Total	49404.40	35	--	--	--	--
Total COD removal efficiency						
OLR	6625.88	2	3312.94	240.91	3.13	0.0000 ^e
System	50.38	1	50.38	3.66	3.98	0.0598 ^f
Error	935.12	68	13.75	--	--	--
Total	7611.39	71	--	--	--	--
Soluble COD removal efficiency						
OLR	5485.19	2	2742.59	149.12	3.13	0.0000 ^e
System	86.00	1	86.00	4.68	3.98	0.0341 ^e
Error	1250.65	68	0.01	--	--	--
Total	6821.84	71	--	--	--	--
Biogas production						
OLR	0.04	2	0.02	13.95	3.13	0.0000 ^e
System	0.15	1	0.15	103.21	3.98	0.0000 ^e
Error	0.10	68	0.001	--	--	--
Total	0.29	71	--	--	--	--
Methane concentration						
OLR	238.78	2	119.39	0.97	3.74	0.4016
System	272.22	1	272.22	2.22	4.60	0.1583
Error	1715.44	14	122.53	--	--	--
Total	2226.44	17	--	--	--	--

^aSums of squares.

^bDegree of freedom.

^cMean squares.

^dF-tabulated (F_α; df OLR or CONF; df Error).

^ep-value<0.05 was considered as significant.

^fp-value<0.1 was considered as significant.

^gOLR: organic loading rate.

^hSystem: single-stage and two-stage.

mg l⁻¹, 25-86 mg l⁻¹, and 0-33 mg l⁻¹, respectively. Similarly, no significant differences were detected between the total concentration values of the organic acids that were observed in the effluent of the methanogenic reactor. These concentration values were in the range of 164-188 mg l⁻¹. However, significant differences were observed between the total COD removal efficiencies of the three OLR compared (Figure 3d). The highest efficiency, 83.5% corresponded to Run1, followed by Run2, 74.6%, and Run3, 54.7% (average total COD removal of 428 mg l⁻¹, 643 mg l⁻¹ and 1127 mg l⁻¹, respectively). Soluble COD removal efficiency was higher in Run1, 86.7% (344 mg l⁻¹), in comparison with Run2, 79.1% (528 mg l⁻¹), and Run3, 59.5% (1006 mg l⁻¹) (Figure 3d). These results agree with those

of Guardia-Puebla et al., [5], who reported COD removal efficiencies higher than 85% when treating wastewater with a concentration of organic pollutants which doubled that of the wastewater evaluated in this work.

The use of a zeolite filter in the top third of the methanogenic reactor improved clearly the solid retention (Figure 4). The solid concentration in the effluent of the single-stage system was of 170-183 mgTSS l⁻¹, but it dropped dramatically to 17-44 mgTSS l⁻¹ in the two-stage system. These results show that the system stability improved when a hybrid anaerobic reactor was used in the methanogenic stage, because it resulted in a larger solid retention even when the digester was overloaded. As the solids, retention time increases the rate of production of new bacteria decreases gradually until a stable population is reached. These conditions result in an increase of the waste degradation and in a decrease of the sludge production; because the older cells use the energy they consume to maintain their cellular activity.

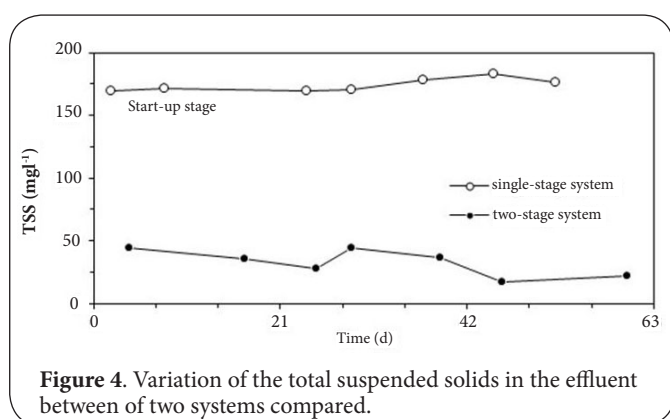


Figure 4. Variation of the total suspended solids in the effluent between of two systems compared.

At the end of Run1, the maximum methane concentration in the biogas was 57%. However, increasing the pH values of the methanogenic reactor in Run2 inhibited the methanogenic bacteria, and as a result of this, the methane concentration in the biogas produced decreased (Figure 3e). According to Sandberg and Ahring [33], an excessive alkaline pH leads to a disintegration of the granular sludge that affects the methanogenic process negatively. When Run3 was conducted, the pH of the wastewater entering to the methanogenic reactor was adjusted to a value of 6.5 and the methane content in the biogas produced rose up to 60%. This fact confirms that whenever the system reaches a suitable pH value, the efficiency of the anaerobic process is recovered again once the optimal pH levels are established [34]. The total biogas production (Figure 3e) was similar in all three trials with a mean value of 0.17±0.04 l d⁻¹.

Comparison of the performance of both systems

The behavior of the two systems compared for the treatment of wet coffee wastewaters was assessed considering five

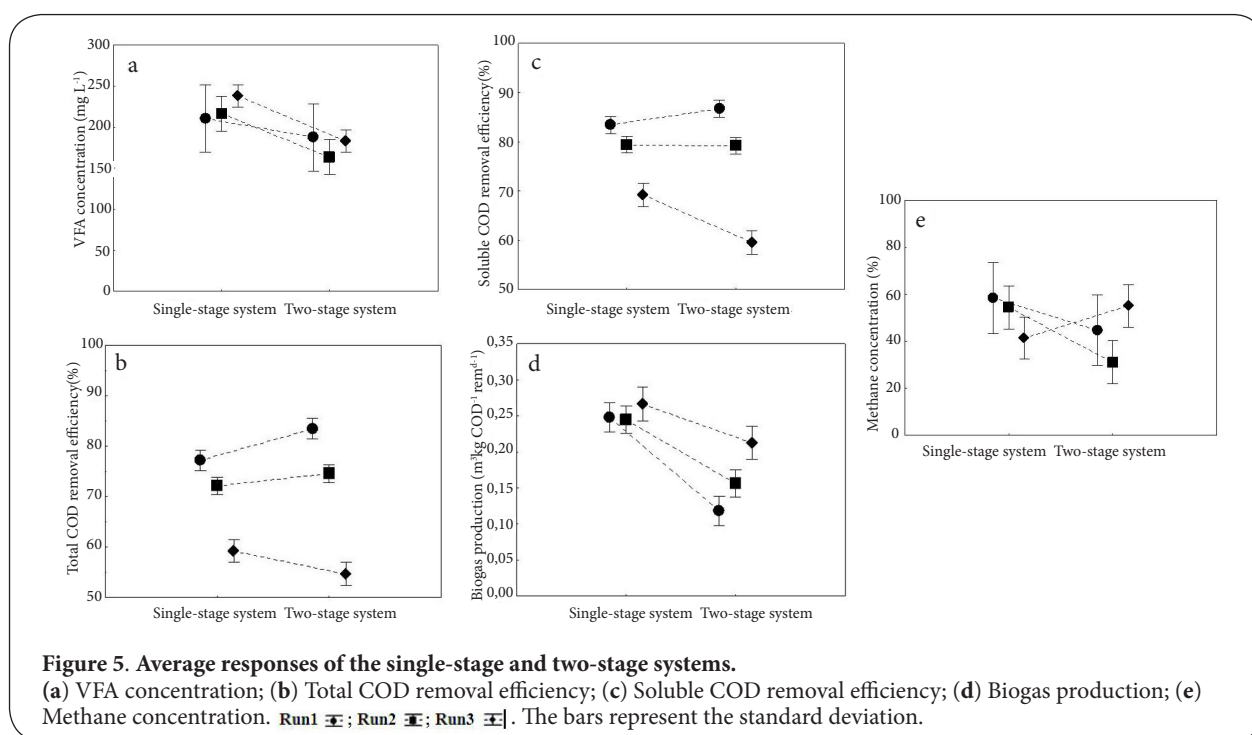
variables: total and soluble COD removal efficiencies, VFA concentration, biogas production and methane concentration. The ANOVA results listed in Table 5 showed that there were significant differences in four of these variables, but not in the case of the methane concentration. Furthermore, the ANOVA did not reflect any significant effect of the OLRs considered in the concentration of both VFA and methane.

Table 6 shows a summary of the results of multiple range analysis test (Duncan). Conversion of the VFA produced was higher in the two-stage system than in the single-stage system. The difference between the amounts of VFA converted in the two-stage system and in the single-stage system increased when the OLR applied to the anaerobic configurations increased. In the presence of overloaded organic conditions, the two-stage system exhibited a larger resistance to accumulate VFA (Figure 5a). In both systems, the VFA concentration in the treated effluent increased slightly, and not significantly, with OLR increase. In Run1, total and soluble COD removal efficiencies were higher in two-stage than in single-stage (Figures 5b and 5c), whereas in Run2 no significant differences were observed, and in Run3 the efficiencies of the single-stage system were higher than those of the two-stage. The reason of this behaviour was the changes experienced by the pH of the acidogenic reactor while the optimum value was encountered. Once the optimum pH of 6.5 was established, the methanogenic reactor was unable to recover totally despite the acidogenic reactor effluent was appropriate to be degraded by the methanogenic bacteria. Biogas production in the single-stage system was significantly higher than in the two-stage system in all OLRs compared. For this comparison, the biogas considered was that produced in the methanogenic reactor of the two-stage system. The effect of the OLR in the single-stage system was no significant in the biogas production (Figure 5d); therefore increasing the OLR did not imply any increase in the amount of biogas produced. However, significant differences were observed in the methanogenic reactor of the two-stage system. Indeed, the largest biogas production in the methanogenic reactor was achieved when the pH of the effluent from the acidogenic reactor was adjusted to a value of 6.5. For this reason, it might be concluded that the pH affects the biogas production. In the two lowest levels of OLR considered, tests Run1 and Run2, the content of methane in the biogas produced in the single-stage system was greater than in the two-stage system, but the opposite situation occurred with the highest level of OLR, Run3 (Figure 5e). Therefore, if conditions are suitable, that is, a pH of 6.5 in the effluent of the methanogenic stage, the methane production in the two-stage system is higher than in the single stage system. Demirel and Yenigün [35] have stressed the importance of pH on the performance of the two-stage anaerobic digestion. The composition of the VFA was also sensitive to pH values of the treated effluent in acidogenic stage and the results obtained in this study coincide with those of other authors [10,11]. The highest total

Table 6. Duncan 's multiple range test.

OLR	Variables response									
	VFA concentration (mg L ⁻¹)		Total COD removal efficiency (%)		Soluble COD removal efficiency (%)		Biogas production (l d ⁻¹)		Methane concentration (%)	
	Single-stage	Two-stage	Single-stage	Two-stage	Single-stage	Two-stage	Single-stage	Two-stage	Single-stage	Two-stage
Run1	210.5±18 a ¹ A ²	187.6±61 aA	77.2±2.93 aA	83.5±3.72 bA	83.4±1.87 aA	86.7±3.59 bA	0.44±0.04 aA	0.14±0.04 bA	58.3±0.03 aA	44.7±0.13 bA
Run2	216.3±17 aA	163.7±28 bA	72.1±2.73 aB	74.6±3.04 aB	79.4±2.35 aB	79.1±3.24 aB	0.42±0.05 aA	0.17±0.02 bB	45.3±0.07 aB	31.0±0.03 bB
Run3	237.8±17 aB	182.9±12 bA	59.2±2.93 aC	54.7±4.43 bC	69.1±3.31 aC	59.5±4.65 bC	0.39±0.03 aA	0.19±0.04 bC	41.3±0.02 aC	55.0±0.07 bC

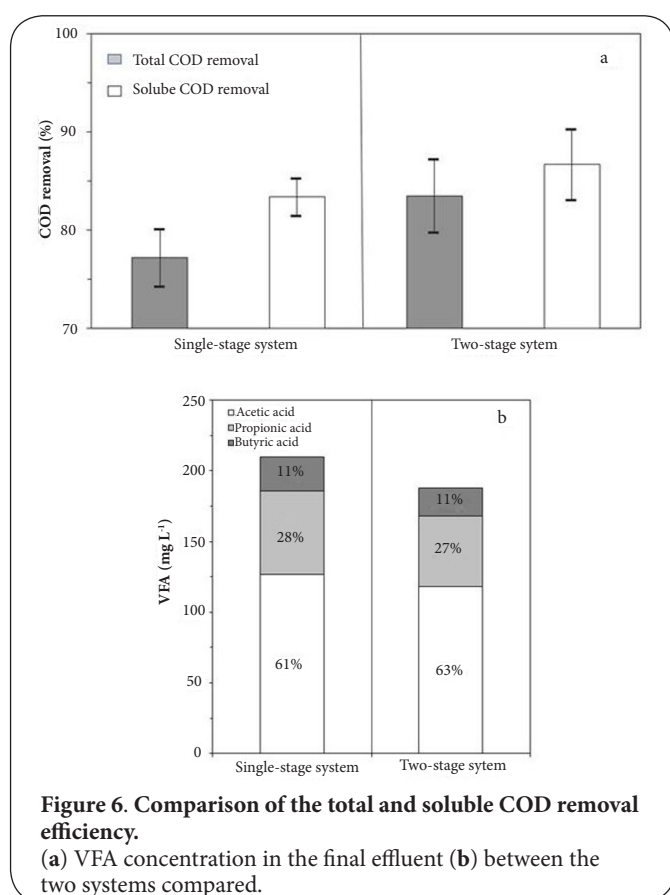
1. For each variable, means in each row followed by the same lower case letter are not significantly different between systems (p -value<0.05).
 2. For each variable, means in each column followed by the same upper case letter are not significantly different between OLR for the same system (p -value<0.05).



and soluble COD removal efficiencies were observed in Run1 conditions, but they were greater in two-stage than in single-stage. For example, both efficiencies in the two-stage system exceeded those in the single-stage system at 6.3% and 3.3%, respectively, and the VFA concentration in single-stage exceeded by 10.9% to that in two-stage (Figure 6).

These results are similar to those of Dinsdale et al., [36] (Table 4) who evaluated the effect of temperature on the performance of the methanogenic stage; they achieved pollution removal efficiencies of 68-77% in the mesophilic reactor, whereas the efficiencies dropped to 63-68% in the thermophilic reactor. The methane concentration in the mesophilic reactor was

of 71-79%. Furthermore, in a methanogenic reactor working with an OLR of 0.5 kgCOD m⁻³ d⁻¹, were observed COD removal efficiencies of 85-95% as well as a methane concentration of 80% [37]. Despite the excellent range of efficiency achieved by these authors, the low value of OLR used would involve a large volume of reactor. They recognized that to optimize the process it would be required the use of anaerobic methanogenic reactors capable to cope with high rates of wastewaters such as the UASB reactor or the hybrid UASB-UAF reactor. In a recent article, Jung et al., [38] achieved a very high COD removal efficiency (98%) and a large methane concentration (73%) using a two-stage system for treating wastewater from



a plant producing instant soluble coffee; the composition of this wastewater was mixing crude coffee, starch, lactose and sugars. However, a previous hydrolysis stage, in a CSTR reactor with a HRT of 8 days, was used before feeding the acidogenic reactor thus increasing the HRT of the system.

Stage separation is the key factor to optimize an anaerobic digestion. For coffee wet wastewater, it is necessary to improve the development of the hydrolysis-acidogenic stage to obtain the largest quantities of methane VFA precursors, namely acetic and butyric acids. Getting as many VFA as possible requires optimizing some design parameters such as OLR, type of reactor, effluent recycle, as well as some environmental factors like pH of the waste to be treated. All of them would result in obtaining a stable and appropriate substrate to feed the methanogenic reactor and to reach the greatest efficiencies of COD removal and methane production.

Conclusions

The application of a single factor design, helped in reaching an objective comparison of two anaerobic systems, and was evaluated the effect of OLR in both systems. Based on this study we reached the following conclusions:

Coffee wet wastewaters were successfully treated in a single-stage reactor. Both total and soluble COD removal efficiencies observed were higher than 75% and 80%, respectively, whereas

the methane concentration was in a range of 56-61% at an OLR of 3.6 kgCOD m⁻³ d⁻¹.

In this study it was found that in a two-stage anaerobic system, whose acidogenic reactor was fed with coffee wastewater having an OLR of 11 kgCOD m⁻³ d⁻¹ and pH 6.5, the acidification degree reached an optimum in which the acetic acid represented 46-52% of the VFA produced.

Total and soluble COD removal efficiencies above 80% were observed in the methanogenic hybrid reactor of the two-stage system when it was operated at an OLR of 2.6 kgCOD m⁻³ d⁻¹.

In the two-stage system total and soluble COD removal efficiencies were higher than in the single-stage system. Methane concentration in the biogas produced was similar in both systems, but the VFA concentration in the final effluent was lower in the two-stage system.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	YGP	SRP	JJH	VSG	JMS	AN
Research concept and design	✓	✓	--	--	✓	✓
Collection and/or assembly of data	✓	--	✓	--	--	--
Data analysis and interpretation	✓	✓	✓	✓	✓	✓
Writing the article	✓	✓	--	✓	--	--
Critical revision of the article	✓	✓	✓	✓	✓	✓
Final approval of article	✓	✓	✓	✓	✓	✓
Statistical analysis	✓	--	--	✓	--	--

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