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Assessment of two-stage anaerobic digestion of blackwater and kitchen waste for reducing environmental impact of residential buildings

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ABSTRACT

In recent years, much attention has been directed towards municipal waste management since these residues has become a global challenge with increasing public health, environmental, social, and economic costs. Therefore, there is an urgent need to adopt widely accepted sustainable alternatives and circular economy approaches. In that sense, the decentralized waste management approach has become a feasible and sustainable strategy to valorise and minimize wastes at source. Among the available technologies, anaerobic digestion is widely used as it allows to recover energy in form of biogas from organic waste. This study proposes a decentralized system based on a two-stage anaerobic digestion for the joint valorisation of organic wastes -blackwater and kitchen wastes-in residential buildings. In the first stage (dark fermentation), different ratios of blackwater:kitchen waste were assessed and a maximum volatile fatty acids production of 5000–7000 mg L^{-1} was reached when 5 kg of black water and 0.30 kg of kitchen waste were added to the system. Then, during second stage, the volatile fatty acids rich stream was valorised in an upflow anaerobic sludge blanket reactor resulting in a biogas production of 5.45 ± 1.34 L d^{-1}, containing 75.6 ± 7.3% of methane and 22.8 ± 5.7% of carbon dioxide while reporting a low content of impurities - 0.02% of hydrogen sulphide and 0.002% of hydrogen -.

1. Introduction

Nowadays, the pressure that anthropogenic activities exercise on the environment by means of wastes-especially municipal solid waste-generation is producing an unsustainable situation in both economic and environmental framework (Brás et al., 2022; Srivastava et al., 2023). According to data provided by World Bank, waste generated per person per day ranges from 0.11 to 4.54 kg between 40% and 50% of organic fraction. More than 75% of organic municipal solid waste (OMSW) are disposed into landfills and incineration plants resulting in environmental problems such as groundwater pollution by leachate or the emission of pollutant and greenhouse gases (Muzioreva et al., 2022), while less than 25% is recycled or energetically valorised though sanitary landfill (Slipa Kaza et al., 2018).

Anaerobic digestion (AD) has been used for OMSW as it contains high volatile solid (VS) and moisture content. However, since OMSW reported complex and inconstant composition of food wastes (rapid degradation) and lignocellulosic wastes (slow

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degradation), it is difficult to maintain the AD process stable and effective (Jiang et al., 2022). Although the implementation of two-stage AD improved the methane production yield and process stability, the high operational time and complex operation have limited its deployment (Fu et al., 2020; Srisowmeya et al., 2020).

If focused on kitchen wastes (KW), AD is also the most common valorisation route reporting some challenges such as composition variability or unbalanced C:N ratio (Chatterjee and Mazumder, 2020). Besides, in recent years KW have been studied as secondary raw material for obtaining bio-based products using enzymes in waste biorefinery approaches (Varjani et al., 2023), reporting promising results at laboratory scale. However, the need of KW collection, transportation, pre-treatment, treatment and product sale difficult its implementation (Deng et al., 2023).

On the other hand, wastewater infrastructure requirements for rural and expanding urban areas lead to significant budget needs for replacing and repairing failing infrastructure as well as for expanding the existing sewage network (Thomas et al., 2022). In fact, it is estimated that in centralized wastewater management systems, less than 20% of the total cost is attributed to the treatment process while transportation reported the most part of the total cost (Jung et al., 2018). With the purpose of reducing the side cost of the wastewater treatment, a decentralized approach for wastewater management has been assessed in the last years (Muzioreva et al., 2022; Thomas et al., 2022; R. Zhang et al., 2023).

However, there are few studies aimed to face segregated blackwater (BW) treatment. Some of the treatment strategies that have obtained promising results (>80% COD, VS and *E. coli* removal) includes ectopic fermentation (Li et al., 2023), hydroponic vertical greening systems (Li et al., 2022), composting (Oarga-Mulec et al., 2019) or activated carbon amended AD (Pan et al., 2023; Zhang et al., 2022). These treatment systems reported some limitations such as the need of bulking material in the case of fermentation and composting; need of pre-treatment in the case of vertical greening systems or the requirement of activated carbon in the case of anaerobic processes.

Furthermore, a circular economy approach of wastes -both solid wastes and wastewater-management based has been spread out in the recent years, by promoting the recovery of valuable resources from waste streams (Hartley et al., 2020; W. Zhang et al., 2023). In this framework, a decentralized management at building level of BW and organic KW could contribute to valorise these waste streams into biogas and reclaimed water for non-potable uses (Cesaro and Belgiorno, 2021). There are few studies related to BW and KW co-digestion by using biological methane potential (BMP) studies (Zhang et al., 2019) and in single-stage AD (Gao et al., 2020) reporting promising results regarding organic loading rate (OLR) and methane recovery efficiency with hydraulic retention time (HRT) between 25 and 30 days.

Biological treatment, especially AD, is widely used for biogas recovery from high Chemical Oxygen Demand (COD) wastewater. AD is a very complex and sensitive process involving numerous microorganisms with ultimate operational environmental conditions. The decomposition process of organic matter can be divided into four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Hagos et al., 2017).

Hydrolysis and acidogenesis stage are significantly influenced by environmental factors such as substrate concentration, inoculums, pH, temperature, or HRT, among others (He et al., 2012). According to Zhang et al. (2019), BW reports relatively low organic loadings rates. Therefore, co-digestion with other substrates such as KW, is a good way to balance the C:N ratio and have synergistic effects in an anaerobic system.

In conventional applications (single-stage AD), the acid-forming and the methane-forming microorganisms are kept together in a single reactor system. Some benefits of this system are low cost, ease of design, ease of temperature control or reduced technical failures, among others (Kothari et al., 2014). However stability problems were encountered in these systems because both groups of microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics and sensibility to environmental conditions (Demirel and Yenigün, 2002). In addition, under high OLR conditions, in single-stage AD systems are produced and accumulated long chain fatty acids, ammonia and hydrogen gas, which are toxic to methanogens and can inhibit biogas production (Nabaterega et al., 2021). Therefore, it is beneficial to separate the acidogenesis and methanogenesis phases through two-stage AD.

Numerous researchers are working in the development of specialised systems by introducing a physical separation of the two different types of microorganisms as proposed by Pohland and Ghosh (1971) for providing optimal conditions both for acidogenesis and methanogenesis steps (Ren et al., 2018). The two-stage AD system consists of a hydrolysis-acidogenesis stage also called dark fermentation (DF) followed by the methanogenesis stage.

Although two-stage AD cannot be considered a new process, in the last years it has regained attention due to its availability to treat high organic load wastewaters while requiring relatively small volume. The operational advantages of a two-stage system over a single-stage reactor are: (i) better stability with better pH control as the optimal pH for DF is between 5.5 and 6.5, while methanogenic microorganisms prefer a pH around 7.0–8; (ii) higher loading rate in the DF reactor; (iii) higher specific activity methanogens resulting in a higher methane yield; (iv) higher COD reduction efficiencies and (v) higher potential for pathogens controlling (Ghasimi et al., 2015; Vongvichiankul et al., 2017). Besides, two-stage AD also showed potential to enhance pathogen destruction and achieve class-A biosolids requirements for digestate valorisation (Wahidunnabi and Eskicioglu, 2014).

During the acidogenic fermentation, hydrolytic bacteria can hydrolyze the organic compounds (e.g. proteins, polysaccharides and lipids) into simpler monomers (e.g. amino acids, sugars and fatty acids) while acidogenic bacteria can further convert these monomers into volatile fatty acids (VFAs), hydrogen and carbon dioxide (Zhang et al., 2020). Products of DF stage contain mainly VFAs which are used as substrates for the methanogenesis stage (Chalima et al., 2017).

For the methanogenesis stage, there are several types of reactors in use that can be operated at batch of continuous mode, including anaerobic sequencing batch reactor (ASBR), tubular reactor or continuous stirred tank reactors (CSTR), among others (Moussa, 2022; Muzioreva et al., 2022; Nasir et al., 2012). Besides, in last years, several novel reactors such as up-flow anaerobic sludge blanket (UASB) reactor, anaerobic filter (AF) or anaerobic fluidized bed (AFB) reactor have been deployed for increase the rate of reaction per unit volume of reactor (Li et al., 2021; Singh and Viraraghavan, 1998). Among them CSTR and UASB reactors are the most used ones (López-Gutiérrez et al., 2021).

CSTR system is usually used for high suspended solid streams as it reported great mixing performance, promoting the continuous contact between the substrate and the microbial population (Kaparaju et al., 2009; Tawai et al., 2020). On the other hand, UASB reactor is capable to obtain high biomass concentrations with high specific activity, resulting in high OLR and short HRT (Kongjan et al., 2011). By contrast, it is recommended for wastewater with <3% of suspended solids content.

Therefore, as most of the suspended solids content have been removed during the acidogenesis stage, it seems feasible to use a UASB reactor for the valorisation of VFA rich stream. This will result in smaller installations, which would make easier its implementation in residential buildings.

In this work, the technical feasibility of decentralized valorisation of organic wastes, namely blackwater and kitchen wastes, from residential buildings is studied. Two-stage AD was assessed for increase the system stability and shorten HRT compared with previous studies and DF stage was improved for increasing VFA production by testing different BW:KW ratios, pH, temperature and HRT. The VFA rich stream was further valorised for biogas production in an UASB reactor.

In addition, some key performance indicators were studied to assess the efficiency of the treatment process. Biogas production rate, biogas quality or organic matter removal are determined to compare the performance of different operational conditions (Preisner et al., 2022).

2. Materials & methods

2.1. Biowaste selection and blackwater collection

The reactor feedstock was prepared by mixing segregated BW with grinded KW obtained from Leitat Technological Center headquarter facilities (Terrassa, Spain). The KW was obtained from the local canteen and conditioned prior its use by (i) removing hard parts such as bones or fruit pits for facilitating the grinding process and (ii) removing excess of protein -fish and meat-for avoiding the generation of ammonium during digestion. The BW was collected twice a month and stored at 4 °C in a climatic chamber to avoid degradation of the organic matter.

2.2. Batch tests

Batch tests were conducted to optimize the operational conditions of both steps of the two-stage anaerobic digestion process. The optimum parameters obtained in the batch tests were used in the bench-scale reactor operation.

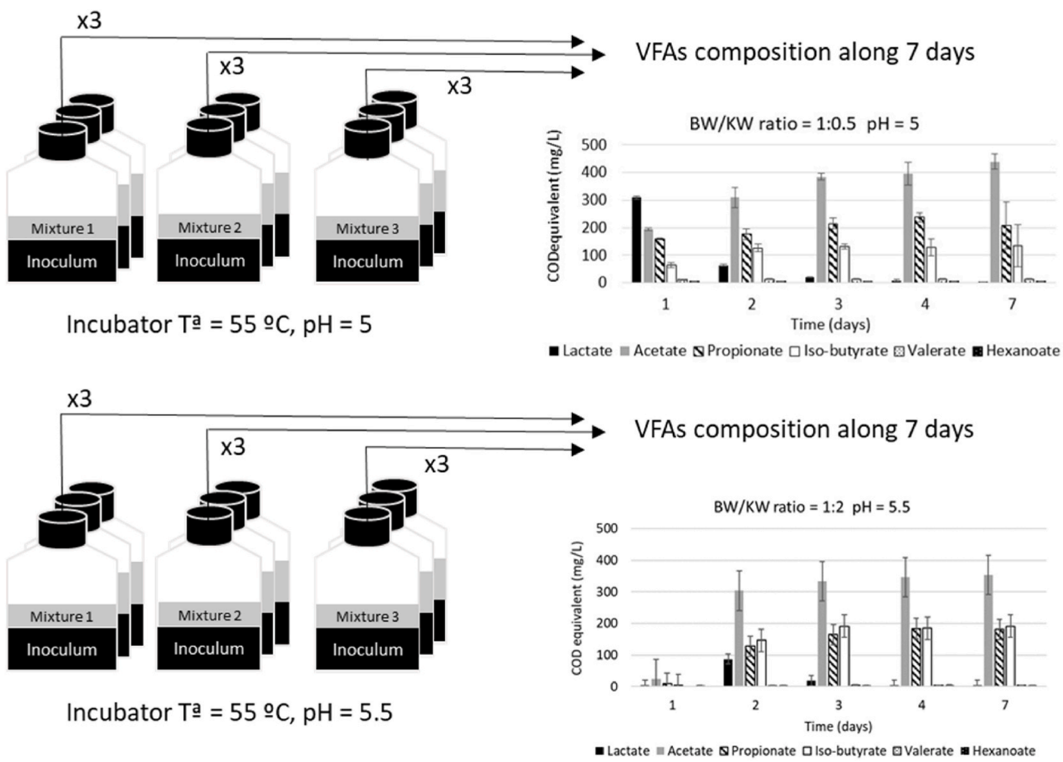

Fig. 1. Scheme of the DF tests.

2.2.1. Dark fermentation optimization tests

DF tests were performed using different BW-VS:KW-VS ratios (1:0.5, 1:1 and 1:2) and at two different pH values (5 and 5.5) to optimize the BW:KW ratio and the working pH. Moreover, the VFA production was assessed along 7 days to establish the optimum HRT of the DF stage. These tests were performed in 500 mL hermetic glass bottles - 400 mL of effective volume – using a substrate to inoculum (S:I) ratio of 0.4 (on VS basis) with 9 g VS·kg^{-1} of inoculum. A detailed scheme of these batch tests is shown in Fig. 1.

The inoculum was obtained from the anaerobic digester of the Terrassa wastewater treatment plant (WWTP) and was pre-incubated at 37 °C to deplete the residual biodegradable organic matter. All tests were performed by triplicate in an incubator at 55 °C and the bottles were mixed manually once a day.

2.2.2. Biochemical methane potential (BMPs) tests

The BMP assay aimed to optimize biogas production by varying BW-VS:KW-VS ratio with special focus on the effects of KW on the digestion process. The BMP method was adapted from Holliger et al. (2016) and tests were performed in 500 mL hermetic glass bottles - 400 mL of effective volume - by using an Automatic Methane Potential Test System (AMPTS) provided by Bioprocess Control (Sweden) as is shown in Fig. 2.

The used inoculum was the same than the DF optimization tests and residual biodegradable organic matter was also depleted by pre-incubation at 37 °C.

Four BW-VS:KW-VS ratios - 1:0, 1:0.5, 1:1 and 1:2 - were studied and a negative control assay with inoculum and Milli-Q water was conducted to measure inoculum background BMP to correct the result estimation. The conditions regarding volatile solids (VS) content in the BMPs tests are summarized in Table 1. All tests were performed by triplicate at 37 °C of incubation temperature using substrate to inoculum (S:I) ratio of 0.4 (on VS basis) with 9 g VS kg^{-1} of inoculum. The methane produced from BMP tests was quantified by using the flowmeters of the AMPTS apparatus.

2.3. Bench scale reactor operation

2.3.1. DF reactor

VFAs production was also tested at bench scale in a two phases experimental set-up, consisting of 15 L stirred reactors: (i) a hydrolysis tank (TK1) and (ii) a settler (TK2) where biomass was separated by gravity from the supernatant.

Under anaerobic conditions, TK1 was operated at 55 °C (thermophilic range) at pH 5.5 ± 0.1 for enhancing VFAs production while inhibiting methanogens activity (Zhang and He, 2014). The pH was maintained by adding HCl 5 M and NaOH 5 M. 10.88 kg of mesophilic anaerobic sludge from Terrassa municipal WWTP was used as inoculum. The inoculum was acclimatized progressively from 37 °C to 55 °C at a rate of 0.5 °C/day and from a pH of 7.5 to pH of 5.5 at a rate of 0.5 points of pH per day.

The settler was operated at room temperature where from the upper part the effluent rich in VFAs was collected as supernatant for further valorisation and the settled biomass, at the bottom, was recirculated to the hydrolysis tank.

Once inoculum was acclimatized, the operation consisting of 75 batch was divided into different periods according to different operational conditions regarding BW:KW ratio and HRT as is summarized in Table 2. A batch is defined as the process consisting of the fermentation stage at TK1, followed by 3 h of settling at TK2, biomass recirculation to TK1 and the introduction of fresh mixture of BW and KW, which indicates the start of a new batch.

VFAs production was assessed along different batch reporting a negligible increase of VFAs concentration from day 2 to day 3. Therefore, as can be noticed in Table 2, from batch 38 onwards the HRT was decreased from 3 to 2 days allowing to increase the treatment capacity of the reactor.

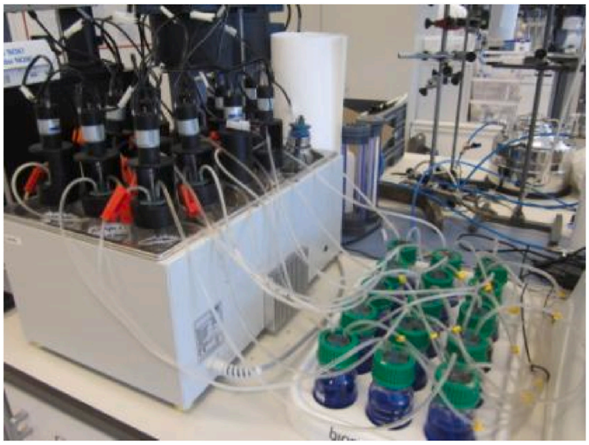

Fig. 2. Automatic Methane Potential Test System. Water bath with 15 bottle reactors each equipped with a mixer (left) and carbon dioxide fixing unit with sodium hydroxide solution that absorbs the carbon dioxide and hydrogen sulphide produced during the anaerobic digestion process (right). The gas flow measuring unit (not shown in the figure) consist of 15 parallel cells, where the gas is measured through water displacement.

Table 1
Conditions for the BMP tests.

Mixture	BW:KW VS ratio	Inoculum (g)	Mixture (g)	Inoculum (mg VS)	Mixture (mg VS)	Total VS (mg VS)	Total VS (mg kg^{-1})
1	1:0	200	200	27160	1134	28294	7074
2	1:0.5	250	150	33950	1311	35261	8815
3	1:1	270	130	36666	1524	38190	9547
4	1:2	290	110	39382	1608	40990	10248
Blank	–	400	0	54320	0	54320	13580

Table 2
Operational conditions for the partial hydrolysis reactor.

Period	Batch	BW (kg)	KW (kg)	HRT (d)
I	Batch 1 - Batch 6	4	0.1-0.2	3
II	Batch 7 - Batch 8	8	0.1-0.2	3
	Batch 9 - Batch 13	10	0.1-0.2	3
	Batch 14 - Batch 15	8	0.1-0.2	3
III	Batch 16 - Batch 29	4	0.1-0.2	3
IV	Bacth 30 - Batch 42	5	0.1-0.2	3[b]
	Batch 43 - Batch 45	6	0.1-0.2	2
	Batch 46 - Batch 52	7	0.1-0.2	2
V	Batch 53 - Batch 63	7	0.3-0.4	2
VI	Batch 64 - Batch 69	7	0.5-1.75[a]	2
VII	Batch 70 - Batch 74	7	2.0	2
	Batch 75	7	1.2	2

[a] From batch 64 to batch 69 the amount of KW was increased 0.25 kg per batch.
[b] HRT = 3 d until batch 37. From batch 38 onwards HRT = 2 days.

2.3.2. VFAs valorisation through UASB reactor for biogas production

Once the VFA production was optimised, the resulting effluent was assessed as feed for an UASB reactor consisting of a cylindrical column with an effective working volume of 4 L including the gas-liquid-solid separator. The inner diameter of the column was 0.08 m and the total reactor height to column diameter ratio was 12.5. The detailed diagram of the reactor is presented in Fig. 3. The reactor was inoculated with 1.2 L of granular sludge collected from a mesophilic UASB used for kitchen and beverage wastewater treatment (Nufri located at Mollerusa, Lleida, Spain).

The UASB was operated in continuous mode at 37 °C, and pH was maintained at 7.5 ± 0.1 by adding NaOH 1 M and HCl 1 M. It was fed with an influent flowrate of 1.4 L d^{-1} through the bottom using a dosing pump (Prisma APG 2001, Dosim, Spain) and the proper upflow velocity (V_{up}) of 0.05 m h^{-1} was reached by recirculating 2.34 L d^{-1} of the outlet stream. The V_{up} used in this study is in the

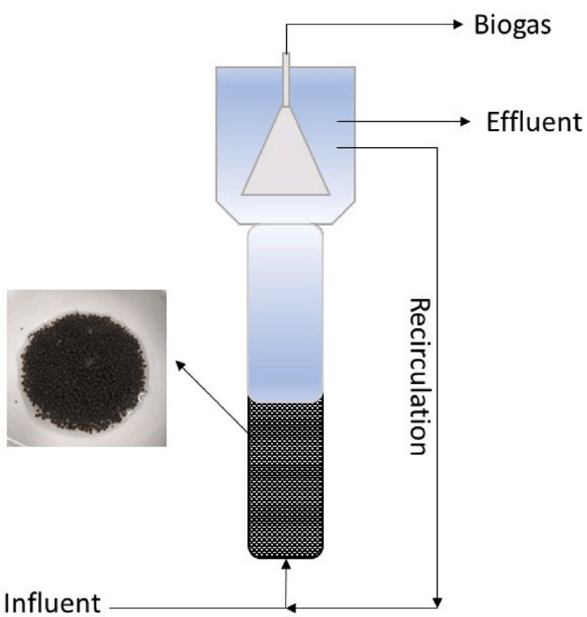

Fig. 3. Schematic diagram of the UASB reactor set-up.

range that those calculated according to Bakraoui et al. (2020). The biogas production was measured with a gas meter (Ritter®, Germany) connected to the gas outflow line.

Finally, the integration of batch operating DF with continuous operation UASB was performed by using TK2 acted as a buffer tank.

2.4. Performance indicators

Some indicators were used for comparing the system performance in terms of COD reduction, biogas production rate or biogas quality. The indicators calculation will be performed only with data from periods IV, V and VI, introduced in previous sections, as these are the periods where the two-stage AD worked at relatively stable conditions. The statistical analysis was performed through one way ANOVA test by using Minitab Statistical Software.

As one of the main objectives of this study is the reduction of organic matter from domestic wastes, the removal efficiency of organic matter (I_{OM}) plays a key role in this context. The Eq. (1) is used to obtain this indicator according to (Guo et al., 2017):

$$I_{OM} = \frac{COD_{in} - COD_{out}}{COD_{in}} \tag{1}$$

Where COD_{in} and COD_{out} are the soluble COD in the influent and effluent, respectively (g L^{-1}).

The biogas obtained comes from the decomposition of the organic matter contained in BW and KW. This process is an eco-friendly way to reduce wastes with the minimum environmental footprint. In this context, the biogas production efficiency (I_b) indicator is implemented by using Eq. (2) adapted from Salguero-Puerta et al. (2019).

$$I_b = \frac{Q_b}{m_{BW} + m_{KW}} \tag{2}$$

Where Q_b is the amount of biogas obtained from anaerobic digestion (mL day^{-1}) and m_{BW} and m_{KW} are the amount of BW and KW fed (kg day^{-1}).

Methane is the main component of biogas which is produced following the equations (3)–(5). The concentration of methane in the biogas as a quality measure is a key parameter (Ali Abd and Roslee Othman, 2022).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{3}$$

$$2C_2H_5OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \tag{4}$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{5}$$

The equation applied to calculate the biogas quality in terms of methane content is driven by Eq. (6) adapted from Güngören Madenoğlu et al. (2019):

$$I_{CH_4} = \frac{H_{CH_4}}{H_{BG}} \tag{6}$$

Where H_{CH4} and H_{BG} are the methane and the biogas production, respectively (mL).

2.5. Sample analysis

COD, Ammonium nitrogen ($N–NH_4^+$), Total Nitrogen (TN) and Chloride (Cl^-) content were analysed by using colorimetric methods and analysed by a spectrometer (HACH DR3900, Spain) as is summarized in Table 3.

Total solids (TS) and VS were analysed according to APHA standard method 2540 (Bridgewater et al., 2017). VFAs concentration was measured using ionic chromatography coupled to conductivity detector (DIONEX, USA) and the quality of the biogas was assessed through gas chromatography (GC, Agilent 490, Spain) in terms of volumetric content of methane, carbon dioxide, nitrogen, oxygen, hydrogen sulphide and hydrogen.

3. Results & discussion

3.1. Batch tests

3.1.1. Dark fermentation optimization tests

DF tests using different BW-VS:KW-VS ratios (1:0.5, 1:1 and 1:2) and at two different pH values (5 and 5.5) were performed (Fig. 4). BW-VS:KW-VS ratio of 1:0.5 at pH 5.5 did not show an increase in VFAs production along the time whereas at pH 5 the acetic acid

Table 3
Physicochemical parameters determined by spectrophotometry.

Parameter	Analytical method	Reference
COD	Potassium Dichromate Method	Bridgewater et al. (2017)
$N–NH_4^+$	Indophenol blue method	(U.S. EPA, 1993)
TN	Nitrophenol method	International Standard Organization (1986)
Cl^-	Thiocyanate ions method	(U.S. EPA, 1986)

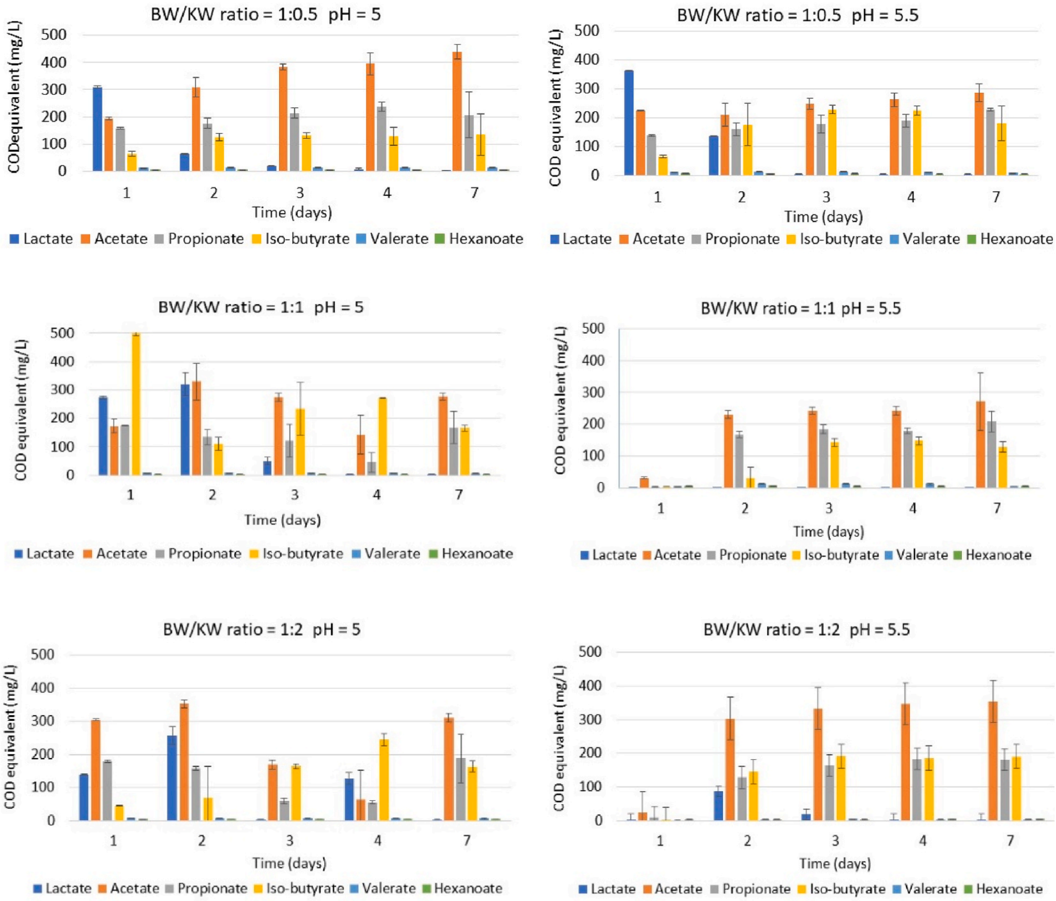

Fig. 4. VFA production during the DF optimization tests.

showed an increase from day 1 to day 3. The ratio BW-VS:KW-VS of 1:1 showed the lowest VFAs productions, so this mixture is discarded. Finally, BW-VS:KW-VS ratio of 1:2 at pH 5 showed a decrease in the acetic production from day 3 onwards and the rest of VFAs did not show any increase.

Therefore, the optimum scenario is the ratio 1:2 at a pH of 5.5, because the acetic acid showed an increase from day 1–2 and the propionate and iso-butyrate also showed an increase from day 2–3. Hence, the optimum conditions for the partial hydrolysis are a pH of 5.5 and an HRT of 3 days, since from day 3 onwards, the increase in the VFAs concentration is negligible. These results are quite similar with conclusions highlighted in previous works such as Strazzera et al. (2018), in which optimal conditions were stablished as pH 6–7 and HRT is comprised between 1 and 7 days depending on the matrix complexity, being improved by the present work. Another study also revealed that slightly acidic pH (6) shows to be better conditioned for VFA production (Lukitawesa et al., 2020). In this study, the highest VFA production was obtained for a pH of 6 and an I/S ratio of 1:3 using food waste as the only substrate. However, the VFA concentration and their distribution pattern depend on the substrate composition, operational parameters, available microbial community, type of reactor and the process design (Khan et al., 2016; Stein et al., 2017). Therefore, there is not an agreement on the optimum conditions of pH for VFAs production. Some authors reported optimum pH between 4 and 5 using mixed cultures and other researchers have reported pH 6 under varying conditions using activated sludge (Lukitawesa et al., 2020; Wang et al., 2014).

3.1.2. BMP tests

KW co-digestion with BW at BW/KW VS ratios between 1:0.5, 1:1 and 1:2 clearly improved the BW BMP as shown in Table 4. This is due to the high ammonia concentration in BW which can inhibit the methanogenesis process reducing the COD removal and methane

Table 4
Methane production from the BMP tests.

BW/KW ratio	Methane (mL)	Net methane (mL g VS^{-1} added)
1:0.5	314 ± 66	239
1:1	520 ± 61	342
1:2	718 ± 149	446
Blank	78 ± 54	–
Inoculum + BW	213 ± 83	187

production efficiencies (Gao et al., 2020; Wang et al., 2023). In fact, the recommended COD/N ratio for a stable anaerobic digestion is 40 (Zappi et al., 2019) and, in this study, the COD/N obtained for the BW was 12.8. Therefore, the BMP improvement could be attributed to the balanced C:N ratio under co-digestion conditions compared to the digestion of only blackwater.

Anaerobic co-digestion of BW and KW at VS ratios of 1:2 achieved the best results in terms of methane production. Zhang et al. (2019) obtained the same trendy identifying as the optimal BW/KW ratios 1:2 and 1:3, however, methane productions (680 mL g VS^{-1}) were higher than those obtained in this study for all the mixtures. In contrast, the methane productions reported by Wang et al. (2020) for the co-digestion of BW and KW were lower than those obtained in this study, with values 313.2 and 296.4 mL g VS^{-1} using BW-VS: KW-VS ratios of 1:1 and 3:1 respectively. The differences in methane productions could be attributed to differences in raw materials and reaction conditions.

Results in both BMPs and DF tests revealed the best results in terms of methane and VFAs production respectively for BW-VS:KW-VS ratio of 1:2. Therefore, this ratio was selected to be employed as feedstock for the DF reactor.

3.2. Bench scale reactor performance

3.2.1. Volatile fatty acids production in the dark fermentation reactor

The DF reactor was operated for 75 batches under different conditions (Table 2). Fig. 5 shows the distribution of fermentation products at the start and at the end of each batch (coloured bars) moreover, the black line represents the BW loading rate for periods I to IV.

The system was initially operated with a BW:KW ratio of 1:2, which corresponds to the optimum ratio obtained in BMPs and partial anaerobic digestion tests (section 3.1). The first period (6 initial batches) showed an increase on the VFAs concentration from initial to final values. In fact, VFAs were not present at the start-up of the system (batch 1) however, some acetate and butyrate were present at the end of the same batch. VFAs concentration increased in the next batch (5 and 6) in a detriment of the soluble COD concentrations.

At batch 8 (period II), the amount of BW added was increased to 10 kg per batch maintaining the KW in 0.15 kg to increase the treatment capacity of the system and moreover, to study the response of the system to this perturbation, so a sudden change in the BW-VS:KW-VS ratio was forced. In Fig. 5, this change is represented by a sharp decrease in the soluble COD and VFAs concentrations. The change in the BW-VS:KW-VS ratio caused the decrease of the COD equivalent from 6000 mg L^{-1} to 2500 mg L^{-1}.

After those changes in the system, it was decided finally to return to the initial ratio and the system was recovered (batches 16–26, period III). Regarding the distribution of VFAs in the DF reactor, during period III, the proportion of propionate (red bar) decreased from batch 21 onwards, experimenting the butyrate (green bar) an increase. This is in accordance with the work developed by Shin et al. (2004), where under thermophilic conditions butyrate was the main produced acid. Moreover, other studies reported that butyric acid is usually predominant in food waste fermentation followed by acetic acid. The fact that the butyrate was the main produced acid is also related to the composition of the KW, which were mainly carbohydrates (fruits and vegetables) (Yin et al., 2016).

From batch 30 onwards (period IV), the amount of BW added per batch was increased progressively until 6.6 kg, maintaining once more the concentration of KW. In this case, the change was progressive and the VFAs production slightly decreased instead of experimenting a sharply decrease occasioned by the sudden change. Therefore, this revealing that the system was not able to support sudden perturbations but a progressively change in the BW-VS:KW-VS ratio had less impact to the soluble COD and VFAs concentration. However, the addition of bigger amounts of KW is needed to increase the VFAs production. The distribution of VFAs during period IV is the same observed on period III.

Table 5 presents the VFAs yield for the different operation periods. As can be observed, on period II the VFAs yield decreased which is in accordance with the decrease in the soluble COD and VFAs concentrations. Then, on period III the VFAs yield increased due to the return to the fed conditions imposed in period I. However, the yields values were lower than those obtained in period I, this could be attributed to the fact that the system was recovering after the perturbation made during period II. Finally, on period IV, the VFAs yield obtained is higher than the yield on period II indicating that the system response is better in face of a gradual change.

The obtained yields are in the range than those obtained in other studies: Feng et al. (2009) reported the maximal VFAs production 520 mg COD g VSS^{-1} for the co-fermentation of secondary sludge and rice, Ma et al. (2017) reported a value of 282 mg COD g VS^{-1} for the co-digestion of food waste and pretreated secondary sludge from a WWTP; Owusu-Agyeman et al. (2020) obtained a VFAs yield of

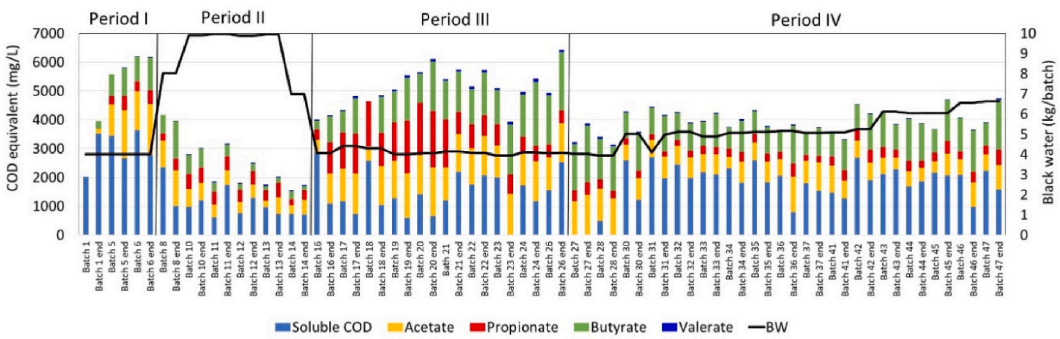

Fig. 5. Distribution of fermentation products at different blackwater loading rates per batch.

Table 5
Distribution of fermentation products along the different experimental periods on average.

Period	Batches	Soluble COD ($mg\ L^{-1}$)	VFA yield ($mg\ COD\ g\ VS^{-1}$)	Acetate ($mg\ COD\ L^{-1}$)	Propionate ($mg\ COD\ L^{-1}$)	Butyrate ($mg\ COD\ L^{-1}$)	Valerate ($mg\ COD\ L^{-1}$)
Period I	1-8	2491.41 ± 1059.64	712.35 ± 113.92	1220.01 ± 734.60	444.94 ± 42.91	926.19 ± 460.11	7.58 ± 3.20
Period II	9-15	1131.26 ± 429.56	233.21 ± 12.96	537.98 ± 57.51	455.35 ± 75.25	341.84 ± 216.26	31.16 ± 6.45
Period III	16-29	1345.12 ± 887.60	530.96 ± 106.00	1317.35 ± 288.79	976.83 ± 552.15	1522.03 ± 381.92	71.86 ± 381.92
Period IV	30-42	2684.04 ± 3122.99		851.34 ± 225.19	293.28 ± 100.41	1130.37 ± 134.63	29.86 ± 6.92
	43-45	2068.40 ± 206.09	255.29 ± 25.70	549.46 ± 186.34	313.61 ± 98.95	1203.54 ± 300.47	15.29 ± 1.43
	46-52	1818.73 ± 1058.26		1011.83 ± 725.09	299.61 ± 224.16	839.31 ± 594.17	20.06 ± 11.67
Period V	53-63	2115.22 ± 524.82	422.52 ± 54.86	829.11 ± 185.31	216.17 ± 64.97	2267.06 ± 705.44	16.76 ± 5.68
Period VI	64-69	7287.85 ± 3928.68	1237.99 ± 284.51	1674.23 ± 1776.30	1367.44 ± 1578.63	4097.43 ± 768.75	31.94 ± 14.24
Period VII	70-74	18724.18 ± 4711.64	644.72 ± 128.83	1522.39 ± 345.91	290.43 ± 228.41	2921.35 ± 1178.59	29.49 ± 27.18

301 mg COD g VS^{-1} when organic waste and primary sewage sludge were jointly treated.

Periods V, VI and VII are shown on Fig. 6, the performance of the system during period V (from batch 54 to batch 63), in terms of VFAs distribution and COD concentration, was similar to the behavior observed in period III, being butyrate, the main acid produced. This could be attributed to the fact that during period V the same ratio BW-VS:KW-VS of 1:2 was imposed again.

Then, on period VI, the amount of KW was increased at a rate of 0.2 kg per batch while the amount of BW was maintained at 7 kg added per batch. At the end of this period, from batch 69 onwards (1.8 kg of KW added per batch), it can be observed the occurrence of lactate as the main VFAs produced. The proliferation of lactate is reported to be detrimental for the next methanogenesis stage (Zhang and He, 2014). Moreover, it was noticeable that, in this period the settleability of the biomass was deteriorated and it was observed the presence of more suspended solids that affected the UASB performance (see section 3.2.2). During period VII VFAs exhibited a homogeneous distribution pattern, being the lactate the dominate VFA followed by butyrate and acetate. This is in contrast with the results reported by Zhang et al. (2020) where a two-phase leachate bed bioreactor was operated obtaining a heterogeneous distribution of VFAs which the authors attributed to the poor stability of fermentation process due to the relatively high organic loading rate.

The relationship between KW and soluble COD was tested and corroborated being higher COD concentration with higher amount of KW as well as higher concentration of VFAs, reaching concentrations of 35000 mg L^{-1} when 2 kg of KW was added instead of 5000–7000 mg L^{-1} with 0.2–0.3 kg of KW.

The VFAs yield on period V (Table 5) is in the range of the yield observed during period III, because the ratio BW-VS:KW-VS is the same. On period VI, the high VFAs yield experienced a high increase, which can be attributed to the fact that the KW amount added per batch was progressively increased from 0.3 kg to 2 kg per batch, so the VFAs productions per batch increased from 1000 to 1200 mg VFAs L^{-1} to 4000–6000 mg VFAs L^{-1} respectively. Finally, on period VII the VFAs yield decreased since there was an increase in the soluble COD concentrations (blue bar) in detriment of VFAs production.

3.2.2. Biogas production in UASB reactor

The UASB reactor was operated for 105 days, treating the VFA-rich stream produced in the DF reactor. Fig. 7 shows the evolution of VFAs and solids concentration for the influent and effluent. The VFAs concentration in the influent of this system was around 2500 mg VFA L^{-1} until day 90 and the VFAs removal efficiencies during this period were around 94% being the VFAs concentration in the effluent below 100 mg L^{-1} (Fig. 7A). From day 93 onwards an increase in the VFAs concentration of the influent, with values around 12.00–13.00 mg VFAs L^{-1}, and the lactate appearance (with an average concentration of 9.000 mg L^{-1}) resulted in a system saturation and an accumulation of VFAs can be observed in the reactor. Lactate is reported to be detrimental for the methanogenesis step, as previously mentioned in section 3.2.1, so its accumulation in the system led to a drop in the pH resulting in the cease of biogas production. Considering that the supernatant rich in VFAs obtained during the DF step was the influent of the UASB reactor, the

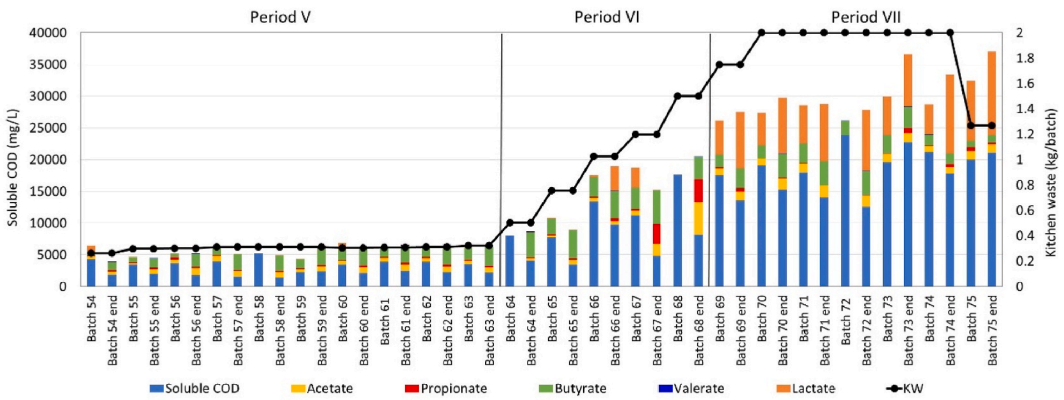

Fig. 6. Distribution of fermentation products at different KW loading rates per batch.

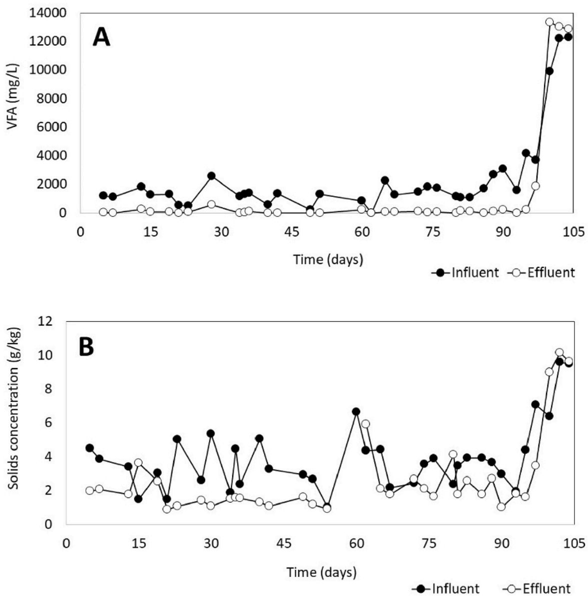

Fig. 7. Evolution of (A) VFA content and (B) solids concentration along experimentation.

increase in the influent and effluent VFAs concentrations in the UASB reactor was related to the KW amount increase during period VII of hydrolysis reactor.

The DF reactor was operated in batch mode, so, three times per week a certain amount of mixed liquor of this reactor was transferred to the settler and this transfer causes agitation in the settler affecting the biomass settleability. This can explain the variability in the solid concentration of the influent (Fig. 7B). Solid concentrations in the effluent of UASB reactor were more stable than those observed in the influent, except for day 62. From day 90 onwards, a high increase in the solid concentrations of both influent and effluent were observed, being related with the increase in the KW amount added per batch to the hydrolysis reactor (period VII).

Regarding biogas production, it was detected a production rate of 5.45 ± 1.34 L d^{-1} (1–1.5 L L^{-1} d^{-1}) of biogas with an average composition of 75.60 ± 7.35% methane and 22.81 ± 5.68% of carbon dioxide being negligible the content of impurities (0.02% of hydrogen sulfide and 0.002% of hydrogen). Biogas productions rates obtained in the present study are slightly higher than those reported by Liu et al. (2019) (0.7–1 L L^{-1} d^{-1}) in a two-stage AD system feeding with food waste and waste activated sludge, in which, the first step was thermophilic and the second one mesophilic with HTRs similar to those applied in this study.

From day 90 onwards, the biogas production experienced a drop until day 100 when the biogas production ceased completely coinciding with lactate proliferation in the DF reactor, which as previously mentioned is an inhibitory component for the biogas production.

3.3. Operational conditions assessment

The performance of different operational conditions is assessed through COD removal efficiency, biogas production efficiency and biogas quality in terms of methane content during period IV – VI. The selection of the periods was performed according to the less variability of obtained results within each period for assuring the significance of the conclusions. The obtained results as well as the P-value for assessing statistical significance of the obtained data are shown in Table 6.

As can be observed in Table 6, organic matter removal efficiency and biogas quality reported low variability within each operational period, which means that the process stability was achieved within each working period despite the intrinsic variability of BW and KW composition. Therefore, by implementing the two-stage AD it has overcame one of the main challenges of DA by using KW as substrate (Chatterjee and Mazumder, 2020).

However, biogas production rate reported very variable values, resulting in higher standard deviation. This fact could be explained by the intrinsic variability of COD contained in BW and KW and the loss of some produced biogas as dissolved gas in the effluent. In fact, the solid-liquid-gas separator is a key parameter in UASB reactors but laboratory-scale designs are not well described (Pan et al., 2017). There is an absence of defined guidelines, operation rules and, specially, the start-up conditions, while full-scale industrial applications of UASB reactors are developed and described in literature (Pererva et al., 2020).

When comparing the performance of different working periods, statistical analysis showed not significant differences between periods regarding biogas quality and biogas production rate. Therefore, it can be concluded that by implementing two-stage AD, methanogenesis stage performance remained stable in terms of VFAs degradation, as was observed in previous studies (Rajendran et al., 2020).

However, organic matter removal efficiency reported significant difference which can be attributed to the performance of the DF stage. Period VI showed the highest value for the organic matter removal efficiency indicator, while also operated at the highest soluble

Table 6
Key performance indicators for period IV, V and VI and statistical analysis P-value.

	Period IV	Period V	Period VI	P-value
Organic matter removal efficiency (I_{OM})	0.89 ± 0.07	0.81 ± 0.09	0.96 ± 0.02	0.004
Biogas production efficiency (I_b) [ml kg^{-1}]	104.33 ± 86.05	92.95 ± 56.84	192.71 ± 165.06	0.366
Methane content [I_{CH4}]	0.71 ± 0.09	0.81 ± 0.04	0.79 ± 0.05	0.314

COD conditions. This fact could be explained by a better C:N balance in the feed solution or an improved VS removal rate (Rajendran et al., 2020; Wei et al., 2022). However, further research is needed for better explain the phenomena as most of two-stage AD studies aimed to report the performance of the system as a whole instead to deeply study the performance of each stage separately.

It is worth to mention that, as stated previously, the reported biogas production differed not significantly between the studied periods, which underlines the design problems of the solid-liquid-gas separator in the UASB reactor. Therefore, further investigation is needed for improving the design at laboratory scale of solid-liquid-gas separator, aimed to maximize the recovery of produced biogas (Hao and Shen, 2021).

After evaluating the obtained indicators, further experiments should be carried out using two-stage AD equipped with an improved solid-liquid-gas separator with the working conditions of period VI (BW-VS:KW-VS ratio, HRT, pH and temperature). In this way, more accurate results regarding its performance in biogas production rate can be obtained for reaching solid conclusions.

4. Conclusions

This work shows the operation of a two-stage AD for the decentralized treatment of BW and KW while obtaining biogas. The DF stage performance was assessed under different BW:KW ratios at a reduced HRT of 2 days. The BW-VS:KW-VS ratio was increased from 1:2 to 1:36 observing that VFA production is proportional to the amount of the added KW until system limit is reached. System's limit was found at BW-VS:KW-VS ratio of 1:36 due to the accumulation of lactate and the settleability problems. An excessive increase of the solids content in the DF stage would imply higher HRT in the settler to reach the required decantation level for UASB proper operation.

UASB reactor was used to valorise the VFA-rich stream to obtain biogas. The UASB reactor was operated successfully with VFA removal efficiencies above 90% and an average biogas production of 5.45 ± 1.34 L d^{-1}. Despite the changing feed mixture with different BW:KW ratios, the UASB reactor performance remained stable in terms of biogas production efficiency and biogas quality, showing the effectiveness of two-stage AD for treating feed solutions with unstable composition.

Finally, organic matter removal efficiency reported a significant difference between periods with no noticeable raise in the biogas production. This fact could be attributed to an inefficient gas – liquid separation due to unoptimized design of the solid-liquid-gas separator in the UASB reactor. Therefore, further research must be done by using an effective design of solid-liquid-gas separator for obtaining solid conclusions.

CRediT authorship contribution statement

Natalia Rey-Martínez was the person in charge of conducting all the analytical work and to write the first draft of the document: Conceptualization, formal analysis; methodology; writing, review and editing – original draft. **Rubén Rodríguez-Alegre** was in charge of the data curation, formal analysis, visualization and editing the document. **Xialei You** was in charge of conceptualization and visualization and editing & reviewing the document; **Sergio Martínez-Lozano** was in charge of funding acquisition; project administration and supervision; **Eduard Borràs** was in charge of editing & reviewing the document and **Julia García-Montaño** was in charge of review and editing and funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julia Garcia-Montano reports financial support was provided by European Commission.

Data availability

Data will be made available on request.

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