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REVIEW ARTICLE

Anaerobic digestion of slaughterhouse waste in batch and anaerobic sequential batch reactors

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Abstract
This work focuses on the design of an effective treatment process for slaughterhouse waste management. Four different treatment sequences were proposed, based on aerobic and anaerobic technologies, as well as thermal and centrifugation pre-treatments. Biochemical methane potential tests were carried out to assess the viability in terms of biodegradability and biogas production of the anaerobic digestion units, which involved different substrates for each proposed process (raw slaughterhouse wastewater, thermal pre-treated slaughterhouse activated sludge, supernatant of thermal pre-treated slaughterhouse sludge, and co-digestion mixture of slaughterhouse wastewater and supernatant of thermal pre-treated slaughterhouse sludge). The obtained results showed that thermal pre-treatment is not effective by itself. However, if it is followed by centrifugation, organic matter removal is importantly improved. In addition, removal efficiency reached 76.0% when employing a co-digestion mixture. Kinetic analyses showed that the specific constant rate of the mixture was 1.5 times higher than with the sole supernatant. Afterwards, the co-digestion mixture was employed as a substrate for an anaerobic sequencing batch reactor working under a semi-continuous operational mode. The influence of organic load rate (OLR) on organic matter removal and biogas production was studied. The best operational OLR range was 1.16–2.16 kg/m3•d, achieving 87.8% of chemical oxygen demand removal and 0.23 $L_{CH4}/L_{digester}$·d of methane production rate. A faster organic load rate than 2.88 kg/m3•d led to bioreactor destabilisation. The obtained results were competitive against published studies that employed different anaerobic technologies and made progress towards the industrial implementation of effective technology in slaughterhouse facilities.

Keywords Anaerobic co-digestion · Slaughterhouse wastewater · Supernatant activated sludge · Kinetic modelling · Anaerobic sequential batch reactor

Highlights
- Application of circular economy principles in slaughterhouse industries
- Supernatant of treated slaughterhouse sludge as co-substrate of anaerobic digestion
- Biomethane productivity and specific rate were improved by co-digestion
- AnSBR as an effective technology to manage slaughterhouse wastes
- 80% of depuration grade and 0.21 L/g of methane productivity were achieved

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Nomenclature

AD	Anaerobic digestion
ACoD	Anaerobic co-digestion
AS	Active sludge
AnSBR	Anaerobic sequencing batch reactor
COD	Chemical oxygen demand (kg/m^3)
CODs	Soluble chemical oxygen demand (kg/m^3)
CODt	Total chemical oxygen demand (kg/m^3)
HRT	Hydraulic retention time (d)
K	Specific constant rate from the modified Gompertz model (NLCH4/kg_{SV0}·d)

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SWW	Slaughterhouse wastewater
TS	Total solids (kg/m3)
TSS	Thermal pre-treated slaughterhouse sludge
STSS	Supernatant of thermal pre-treated slaughterhouse sludge
VFA	Volatile fatty acids
VS	Volatile solids (kg/m^3)
XCH4	Percentage of methane in the biogas (%)
YCH4MAX	Maximum methane yield from the modified Gompertz model (NLCH4/kg$_{SV0}$)
λ	Lag-phase parameter from the modified Gompertz model (d

Subscript

CH4	Relating to methane
COD	Relating to chemical oxygen demand
Exp	Relating to experimental data
Digester	Relating to the operating volume
Removal	Relating to degradation of organic matter
VS	Relating to volatile solids
VS0	Relating to initial volatile solids

1 Introduction

In recent years, waste and wastewater produced in slaughterhouses and meat processing plants have been increased, due to the higher worldwide demand for meat and slaughterhouse products. The meat processing industry consumes around 25% of the overall water requirements linked to the food and beverage sector [1]. Consequently, large volumes of polluted slaughterhouse wastewater (SWW) are produced, being considered the most pressing environmental issue associated with slaughterhouse facilities [2]. According to Eurostat [3], a volume of 6.8·10^7 m^3 of SWW is generated per year. SWW usually contains high levels of organic matter, proteins and lipids, due to paunch, faecal matter, fat, undigested food, blood, urine and other suspended materials and soluble biomolecules [4].

SWW treatments are similar to municipal wastewater ones. After a preliminary physical treatment, flocculation and coagulation operations are required to separate light solids, fat and low-density molecules [1]. A biological treatment, aerobic or anaerobic, is employed afterwards, this being the main degradation step. In this sense, aerobic process by activated sludge (AS) has been the most common wastewater technology used until now, mainly due to its simple and low-cost implementation. The AS process aims to remove soluble and insoluble organic matter contained in these wastes [5]. Over recent years, the management of the waste generated in AS has been focused mainly on agricultural reuse (44%), incineration (22%), composting (15%) and landfilling (11%) [6]. However, these management methods imply environmental and potential health risks, derived mainly from first or ultimate disposal of the waste. For this reason, an increase in public pressure has been built up to persuade the meat industry to develop alternative treatments to be implemented in modern slaughterhouses [7]. In pursuit of this aim, the combination of aerobic and anaerobic treatments has been studied in order to improve the safety of the processes [1]. Anaerobic digestion (AD) has been proposed as an effective treatment in the meat industry, due to the possibility of removing organic matter and producing bioenergy at the same time, with minimal sludge production in comparison to aerobic treatment technologies [8, 9]. The self-sustainability of the slaughterhouse industry may be achieved by using renewable energy derived from the waste matter resulting from the process [10].

Over the last decade (particularly in Europe), commercial biofuel production has grown as a consequence of using residues from wastewater treatment plants as AD substrates [11]. In this sense, it is well-known among the scientific community that the production of biogas by AD can be improved by employing different wastes as co-substrates for co-digestion processes [12, 13]. Specifically, for rich-protein wastes, such as SWW and AS coming from SWW biological treatment, anaerobic co-digestion (ACoD) is recommendable on an industrial scale. Co-digestion advantages include (*i*) increasing biogas production by overcoming inhibition problems derived from mono-digestion (such as volatile fatty acid accumulation); (*ii*) macro- and micronutrient supply; (*iii*) a balanced C/N ratio; and (*iv*) an enhancement of buffer capacity [14].

However, the AD process must be tested first at the laboratory scale, by using a batch operational mode. In this sense, biochemical methane potential (BMP) tests have been extensively used in the literature as a tool for determination of the biomethane potential and biodegradability of the new co-digestion mixtures, such as slaughterhouse waste [15]. For these reasons, a great number of BMP tests have been applied to optimise the anaerobic co-digestion of slaughterhouse waste [7, 16–18]. In order to predict methane evolution in AD processes, some authors have described the kinetic modelling of Gompertz as the most suitable mathematical model for AD of organic residues, including those from slaughterhouse facilities [19–21].

This paper aims to design a treatment process sequence in the slaughterhouse industry to optimise biogas production reached in AD steps. In order to reach that goal, different treatment sequences have been proposed based on aerobic and anaerobic technologies, as well as thermal and centrifugation pre-treatments. Figure 1 shows proposed flow diagrams, which were designed based on previous results [22]. The AD unit shown at each process is fed with different substrates related to the proposed sequence. For this purpose, BMP tests employing each substrate of AD steps

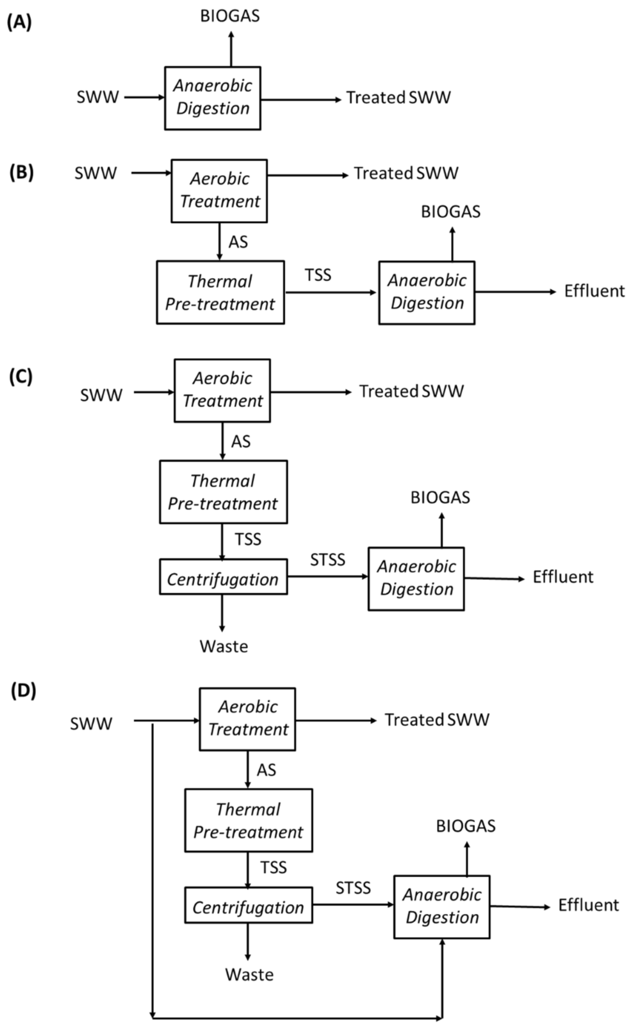

Fig. 1 Flow diagram of proposed treatment sequences based on **A** anaerobic digestion; **B** aerobic treatment, thermal pre-treatment and anaerobic digestion; **C** aerobic treatment, thermal pre-treatment, centrifugation and anaerobic digestion; and **D** aerobic treatment, thermal pre-treatment, centrifugation and anaerobic co-digestion

in the proposed sequences (raw SWW, thermal pre-treated slaughterhouse activated sludge (TSS), the supernatant of TSS (STSS) and the co-digestion of SWW:STSS) were carried out. Kinetic modelling of methane production, employing a modified Gompertz equation, was developed in order to choose the best treatment sequence for slaughterhouse waste treatment.

After BMP tests, the selection of technology operated at a semi-continuous mode is crucial for an optimal scaling up of the anaerobic process. In this sense, high-rate technologies present some advantages in comparison to low-rate technologies, including (*i*) holding a high-volume biomass inside the reactor; (*ii*) operating at high organic load rates; (*iii*) treating solid and liquid wastes; and (*iv*) a higher biogas production [23].

Several authors have previously published information about the advances in anaerobic treatment of SWW and solid slaughterhouse wastes employing high-rate technologies, such as fluidised bed reactors [24], complete stirred digesters [25–28], anaerobic filter membrane bioreactors [29, 30] and anaerobic filters [31].

The anaerobic sequential batch reactor (AnSBR) is considered an efficient high-rate technology for wastewater treatment. AnSBR was invented by Richard R. Dague [32] at Iowa State University (USA) in 1993 as a modification of anaerobic contact and anaerobic-activated sludge processes. Feed, react, settle and decant, all takes place sequentially in a single batch reactor. This system has different advantages: (*i*) the post-clarifier requirement is eliminated; (*ii*) the process is simple; (*iii*) there is a low operating and maintenance cost compared to other anaerobic digesters, such as membrane bioreactors or thermal phase reactor digesters; and (*iv*) biomass retention can be achieved by settling within the same reactor [33–35].

However, the performance of AnSBR for treating slaughterhouse wastes has not been deeply studied. Sequential aerobic and anaerobic treatment of SWW in a single unit was developed by Kundu et al. in order to remove organic carbon and nitrogen compounds [36]. In the early 2000, extensive research was conducted by Massé and Massé [37] on AnSBRs for SWW treatment. Reactors were operating at 30 °C and varying organic loading rates (OLR) (1.1–11.5 kg CODt/$m^{3}\cdot$d) achieved COD removal efficiencies between 79 and 97%.

In the present work, AnSBR as a high-rate unit in semi-continuous operational mode was tested, employing the previously selected optimal sequence treatment. The influence of increasing organic load rate (OLR) on biogas production and organic matter removal has been studied.

2 Materials and methods

2.1 Inoculum and substrates

The inoculum seed used in this work was collected from a wastewater storage tank located in a slaughterhouse (Matadero del Sur, Sevilla, Spain). Then, inoculum acclimatisation took place in a single-phase mesophilic reactor at laboratory scale, fed with slaughterhouse wastewater operating at stable conditions: 35 °C and HRT = 20 d. The inoculum digester showed constant physicochemical characteristics in terms of chemical oxygen demand (COD) and total and volatile solids (TS and VS), which are shown in Table 1.

The agro-industrial wastes employed in this research were SWW and slaughterhouse AS, resulting from the aerobic treatment of SWW from Matadero del Sur S.L., a slaughterhouse located in Salteras (Seville, Spain). The aerobic treatment was operated in a sequential batch reactor working under HRT = 24 d, with a purification efficiency of 80%. Depending

Table 1 Inoculum and raw substrate characteristics

Parameters	Inoculum	SWW	TSS	STSS	SWW:STSS 50:50%(v/v)
pH	7.6 ± 0.2	6.7 ± 0.2	7.3 ± 0.2	6.9 ± 0.1	7.1 ± 0.2
CODt (kg/m^3)	7.8 ± 1.6	12.9 ± 0.2	136.8 ± 0.4	26.5 ± 0.6	19.9 ± 0.2
CODs (kg/m^3)	1.5 ± 0.7	9.1 ± 0.2	36.6 ± 0.4	20.9 ± 2.2	13.8 ± 0.2
TS (kg/m^3)	7.4 ± 0.1	6.6 ± 0.1	129.0 ± 0.3	10.9 ± 0.1	8.4 ± 0.1
VS (kg/m^3)	2.8 ± 0.1	5.6 ± 0.1	108.6 ± 0.6	7.7 ± 0.1	6.5 ± 0.1
VS/VT (%)	37.8	84.8	84.2	70.6	77.4

on the specific tasks being performed in the slaughterhouse, the wastewater influent composition could vary greatly over the week. The main characteristics of slaughterhouse AS were CODt = 5.0 ± 3.0 kg/m^3, TS (%w/w) = 85.2 ± 0.2, TN (%w/w) = 2.46 ± 0.21 and pH = 6.5 ± 0.3.

Thermal pre-treatment was applied to AS by means of heating up at 120 °C during 30 min in an autoclave type Raypa steam steriliser. Efficiency of thermal treatment for AD of AS was previously described by Caballero et al. [22]. Through this thermal pre-treatment, organic matter content in the feedstock was increased significantly.

Centrifugation (1000 g, 5 min, 4 °C) after thermal pre-treatment was focused on obtaining a rich biodegradable feedstock. Whereas the final sludge dry matter was mostly insoluble (98.67 ± 0.25%w/w), supernatant of thermal pre-treated slaughterhouse sludge (STSS) contained mainly soluble organic matter, with 13 ± 0.5%w/v of dry weight. For this reason, it was selected as the substrate for AD.

All substrates were held in a tank stored in a refrigerator at 4 °C to prevent biodegradation. Table 1 shows the main characteristics of raw substrates and the mixture SWW:STSS (50:50%v/v).

2.2 Methodology

2.2.1 Biomethane potential (BMP) tests

BMP tests were carried out in order to compare biodegradability and biogas production that can be potentially reached in an AD unit at each proposed treatment sequence. As is shown in Fig. 1, each AD unit is fed by employing different raw slaughterhouse wastes (SWW, TSS, STSS) and a mixture of SWW:STSS (50:50%v/v). Therefore, four biomethane potential tests employing each substrate (SWW, TSS, STSS and SWW:STSS (50:50%v/v)) were carried out to assess the viability of each proposed treatment.

BMP tests were performed in 250-mL serum bottles with a working volume of 120 mL, using an orbital shaker at 85 rpm under mesophilic conditions (35 ± 1 °C). The digesters were loaded with a mixture of substrates and inoculum at a final ratio of 3:2 (v/v), which corresponds to 40% (v/v) of inoculum, as it has been successfully considered the optimum measure for biogas production for slaughterhouse waste digestion [38, 39].

The bottles were flushed with N_2 gas for 30 s prior to sealing, to ensure all the bottles were under anoxic conditions. All BMP bottles, including blank bottles (inoculum with distilled water), were incubated in an optic IVYMEN system orbital shaking incubator from COMECTA, Model D-2102 (at 85 rpm), at mesophilic temperature. The BMP experiments were done in triplicate. Analytical determinations were performed before and after BMP tests, and biogas volume and composition were measured daily during 25 d (until the biomethane production was < 1% of accumulated biomethane).

2.2.2 Data analysis in BMP tests

Cumulative methane volume and substrate biodegradability in BMP assays were determined, as was previously described [40]. Methane productivity (Y_{CH4}) in the base of initial VS was calculated as V_{CH4}^{t} per kg of initial VS (NL_{CH4}/kg_{VS}) in order to develop the kinetic modelling. Experimental biomethane potential (BMP_{exp}) was calculated as the asymptote of the methane productivity curve.

Evolution of productivity was predicted by employing OriginPro® software and by using a modified Gompertz model (Eq. 1). In this sense, three kinetic parameters were obtained: the maximum yield reached at an infinite digestion time (Y_{CH4}^{MAX}, NL_{CH4}/kg_{VS0}), the specific constant rate (K, $NL_{CH4}/kg_{SV0}\cdot d$) and the lag phase time constant (λ, d). Statistical validation of the fitting was developed based on the coefficient of determination (r^2) and the residual sum of squares (RSS).

$$Y_{CH4}\left({NL_{CH4}}/{kg_{SV0}}\right) = Y_{CH4}^{MAX} \cdot \exp\left[-\exp\left(-\frac{K \cdot e^{1}}{Y_{CH4}^{MAX}} \cdot (\lambda - t) + 1\right)\right] \quad (1)$$

2.3 Semi-continuous anaerobic digestion

The experimental set-up was designed to evaluate the behaviour of a high-rate AnSBR unit working in semi-continuous operational mode and employing the best feedstock evaluated in BMP tests. The influence of OLR by means of decreasing hydraulic retention time (HRT) on the organic matter removal (in terms of CODt and VS), biogas production and digester stability in co-digestion was studied.

The digesters were made in stainless steel (MiniReactor, Trallero and Schee®), with an electric heating system. The working volume was 1600 mL and headspace volume was 400 mL. Biogas was collected daily in a 5-L bag (Tedlar®), provided by a set-up for gas sampling.

The operational temperature was set at mesophilic range (35 °C) and stirring speed was fixed at 45 rpm. AnSBR works following four phases (reacting, settling, drawing and feeding) that are repeated daily. The operational times of each phase during AnSBR operation were feeding phase (1 min); reacting phase (23.5 h), settling phase (28 min) and drawing phase (1 min). AnSBR assays were carried out in duplicate at a semi-continuous operational mode, following phases shown in Fig. 2. The phases were run sequentially and implied digester changes along the time span. Therefore, each phase was considered as a batch step. However, the overall process took place under a semi-continuous operational mode, being fed daily according to each HRT.

Decreasing HRT between 15 and 3 d were tested, which implied progressive increases in the feed flow rate (from 0.64 $kg_{CODt}/m^3 \cdot d$ to 4.27 $kg_{CODt}/m^3 \cdot d$, respectively). Experiments were conducted under each operational condition at least three times each HRT in order to ensure a steady state (constant physicochemical characteristics and composition of effluent, as well as biogas streams). The main characteristics of the different stages of the operation are shown in Table 2. As can be seen, there was a slight difference in CODt and in solids content in SWW, due to the daily operation programme in the slaughterhouse, as was previously commented.

Analytical determinations (CODt, CODs, TS and VS) were performed to monitor and control the stability of the process in the substrate and the effluent two times a week. The volume and composition of biogas (mainly CH_4 y CO_2) were measured daily. The characterisation of the substrates was initially determined.

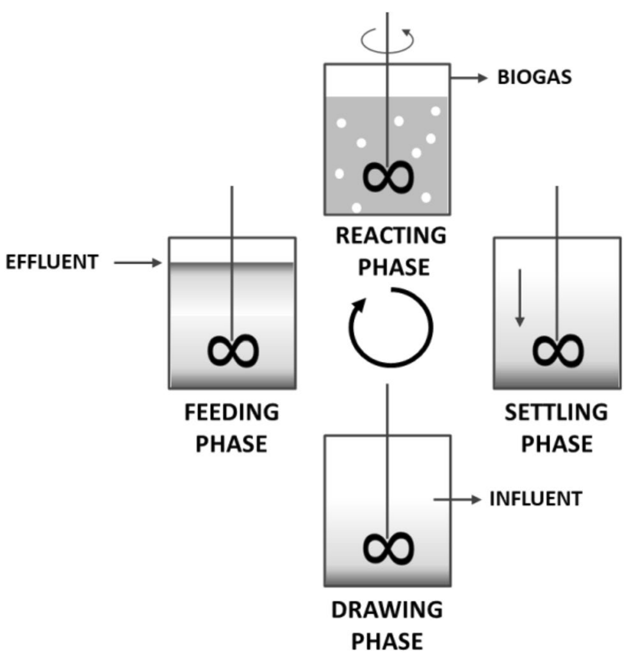

Fig. 2 Diagram of experimental set-up

3 Analytical methods

The parameters determined were pH, total solids (TS), volatile solids (VS) and chemical oxygen demand (total CODt and soluble CODs). All the measurements were performed according to the standard methods [41].

The equipment used for pH determination was a Crison MicropH 2001 bench-top pH meter. Alkalinity was measured by an automatic titrator (Crison®, Compact Titrator S +) and sulphuric acid (0.2 N) from Merck®. TS and VS determination were made using a gravimetric technique, using a Cobos auto calibrating balance with an accuracy of 0.001 g and a furnace model ELF14 of Carbolite®. Two types of COD were determined: soluble COD (CODs) from the filtered samples (0.22-µm pore) and total COD (CODt) from the samples (not filtered). After adding the reagents, the closed tubes were shaken in a vortex, and after that they were heated in a Merck® thermoreactor. The measurement of absorbance was developed using a Thermo (Electron Corporation) model Helios α spectrophotometer.

Table 2 Main characteristics of the feedstock (SWW:STSS (50:50%v/v)) in semi-continuous AnSBR

HRT (d)	CODt (kg/m^3)	CODs (kg/m^3)	OLR_{COD} ($kg/m^3 \cdot d$)	TS (kg/m^3)	VS (kg/m^3)	OLR_{VS} ($kg/m^3 \cdot d$)	pH -
15	9.6 ± 0.4	8.0 ± 0.1	0.64 ± 0.05	6.7 ± 0.4	5.8 ± 0.1	0.39 ± 0.05	7.1 ± 0.1
10	11.6 ± 0.9	8.5 ± 0.4	1.16 ± 0.05	5.9 ± 0.6	4.9 ± 0.6	0.49 ± 0.05	7.1 ± 0.1
5	10.8 ± 0.5	8.0 ± 0.6	2.16 ± 0.05	6.6 ± 0.3	5.6 ± 0.2	1.12 ± 0.04	6.8 ± 0.1
4	11.5 ± 1.5	7.5 ± 0.8	2.88 ± 0.29	6.8 ± 0.2	5.7 ± 0.2	1.43 ± 0.05	6.9 ± 0.1
3	12.8 ± 0.4	8.1 ± 0.3	4.27 ± 0.30	6.6 ± 0.1	5.4 ± 0.1	1.80 ± 0.03	6.9 ± 0.1

The volume of gas produced in the reactor was measured directly, using a high-precision gas flow meter (Ritter drum-type TG-01-Series) and gas suction pump (KNF laboport). The biogas composition (H_2, O_2, CH_4 and CO_2) was determined by employing a gas chromatographic technique (GC-2010 Shimadzu). The analysed gases' concentration was determined by means of a thermal conductivity detector (TCD) at 250 °C, using a Supelco Carboxen 1010 PLOT column. The oven temperature was programmed between 35 and 200 °C. Manual injection was carried out using a sample volume of 250 µL. The carrier gas employed was nitrogen, at a pressure of 35 kPa.

4 Results and discussion

4.1 Biochemical methane potential tests

For each BMP, composition of initial wastes composed of raw substrates and inoculum and final effluent are described in Table 3, including TS, VS, CODt, CODs and pH. Comparison between initial and final conditions were determined for each substrate (SWW, TSS, STSS) and co-digestion of SWW:STSS (50:50%v/v) in order to determine biodegradability of the waste based on each parameter (CODt removal, CODs removal, TS removal and VS removal).

Biodegradability of SWW was around 70% of CODt and 37% of TS. These results are higher than the published results for similar systems: Córdoba et al. [42] obtained 18.4 ± 6.0% for AD of SWW with inoculum of the rumen in SWW. The COD removal percentages values in the BMP test of SWW can also be enhanced until higher values around 50% are achieved, using other strategies such as higher inoculum adapted to slaughterhouse residues previously [25], or supply with nutrients such as iron [43], or using other co-substrates with a higher carbon content, such as organic fraction of municipal solid waste [26].

Despite the high organic content of this TSS (117.3 gCODt/L), the organic matter removal was quite poor, due to the majority of that matter being insoluble (around 96%). In AD, the insoluble matter must be hydrolysed prior to being consumed by the bacteria. Therefore, COD increased during the BMP test. As a consequence, the negative COD removal was reached. This result showed that AD was not effective enough to treat this substrate. Pre-treatment of centrifugation increased soluble organic matter (around 56%), and COD and VS removal was significantly improved (76.8% and 67.7%, respectively).

Based on these results, anaerobic co-digestion of SWW:STSS (50:50%v/v) was proposed to increase the biodegradability of the feedstock. Anaerobic co-digestion enhanced COD removal to 76% and VS removal to 82%.

For each assay, the modified Gompertz model was fitted to an experimental methane yield, as is shown in Fig. 3. The estimated parameters and their statistical errors are shown in Table 4. The proposed model describes properly observed experimental data, being r^2 higher than 0.98 in all the cases.

The feedstock composition plays an important role in methane productivity. While only 19.4 NL_{CH4}/kg_{SV0} was produced employing TSS as a substrate, 201.2 NL_{CH4}/kg_{SV0} was reached employing STSS. As has been previously

Table 3 Initial and final characteristics of substrates in the serum bottle

Parameters	SWW	TSS	STSS	SWW:STSS (50:50%v/v)
CODt_initial (kg/m^3)	11.4 ± 0.4	117.3 ± 2.0	16.4 ± 0.9	12.9 ± 0.6
CODt _final (kg/m^3)	3.6 ± 0.1	90.9 ± 0.4	3.8 ± 0.1	3.1 ± 0.2
$CODt_{removal}$ (%)	67.9	22.5	76.8	76.0
CODs_initial (kg/m^3)	6.2 ± 0.1	12.6 ± 0.2	9.2 ± 0.7	9.1 ± 0.5
CODs_final (kg/m^3)	1.2 ± 0.1	20.2 ± 0.1	1.6 ± 0.1	1.2 ± 0.1
$CODs_{removal}$ (%)	80.4	− 60.3	82.6	86.8
TS_initial (kg/m^3)	6.8 ± 0.2	80.3 ± 0.5	13.5 ± 0.1	11.4 ± 0.4
TS_final (kg/m^3)	4.3 ± 0.1	65.9 ± 0.1	6.3 ± 0.1	4.3 ± 0.1
$TS_{removal}$ (%)	36.8	21.4	53.3	62.5
VS_initial (kg/m^3)	5.1 ± 0.1	66.9 ± 0.1	9.5 ± 0.1	6.2 ± 0.1
VS_final (kg/m^3)	2.5 ± 0.1	51.4 ± 0.1	3.1 ± 0.1	1.1 ± 0.1
$VS_{removal}$ (%)	51.8	23.1	67.7	81.7
pH_initial	6.7 ± 0.2	7.3 ± 0.2	6.9 ± 0.1	7.1 ± 0.2
pH_final	6.8 ± 0.2	7.1 ± 0.1	7.6 ± 0.2	7.3 ± 0.1

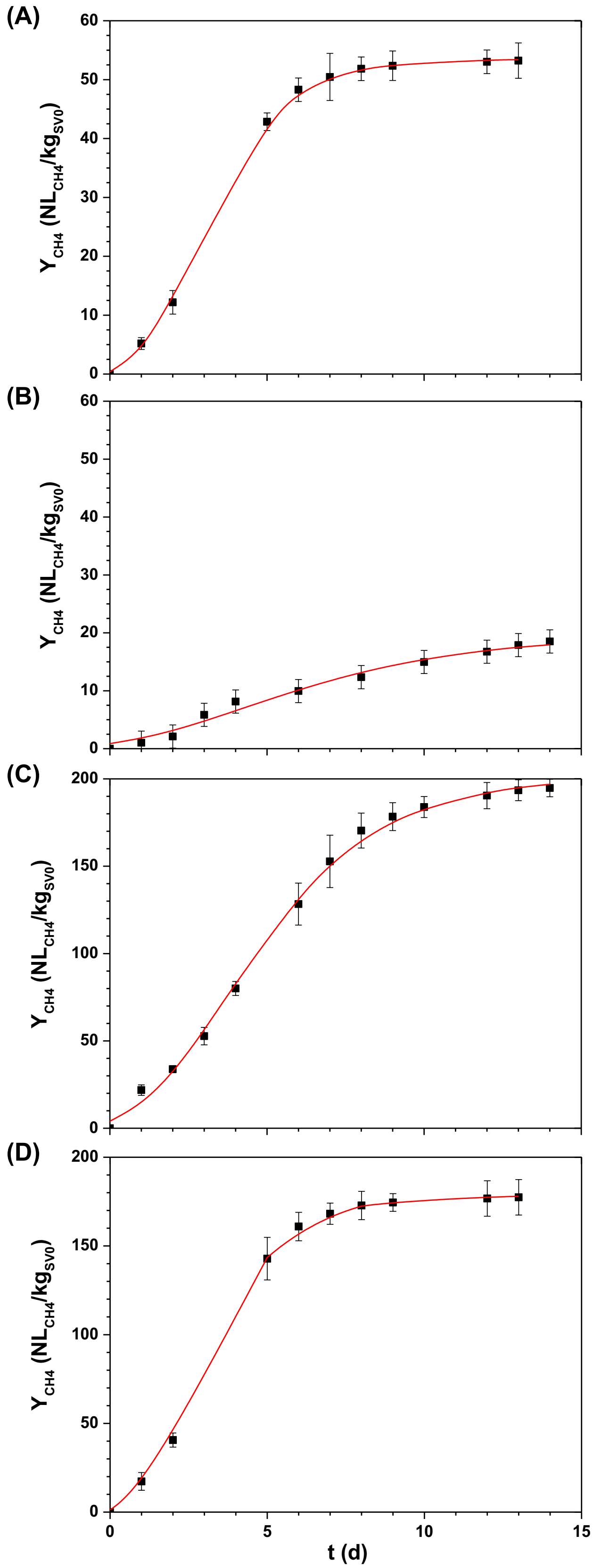

Fig. 3 Evolution of methane production in a BMP test employing as a substrate **A** SWW, **B** TSS, **C** STSS, **D** SWW:STSS (50:50%v/v). Key: Methane yield, square; kinetic Gompertz model prediction, line

commented, hydrolysis is the rate-limiting step in TSS digestion, and it could limit subsequent methanogenesis steps. These results show that centrifugation is an effective pre-treatment for methane production in slaughterhouses, improving the maximum yield as well as the specific constant rate.

The higher value of the specific constant rate was achieved by employing the mixture of SWW:STSS (50:50% v/v) as a substrate. Anaerobic co-digestion of both wastes shows a synergic effect on the methane production rate, which constitutes a decisive factor to implement AD on an industrial scale. Based on these results, the proposed sequence shown in Fig. 1D, based on thermal pre-treatment of AS, centrifugation and co-digestion, is considered the optimal treatment in slaughterhouses in order to maximise the biogas production rate.

4.2 Anaerobic co-digestion using a sequential batch reactor

Having established the co-substrate SWW:STSS (50:50%v/v) as the best feedstock based on its biomethane potential and the specific constant rate, a semi-continuous ACoD in an AnSBRs was carried out. The influence of OLR on organic matter removal, methane production and digester stability has been studied. The results shown in this section correspond to average values of each parameter under steady state, reached at each HRT condition. The main characteristics of the effluents and daily methane production at the steady state are shown in Table 5.

One of the main parameters that determines the stability of the bioprocess in the operation is pH. At each condition, pH remained within the range 7.1 and 7.8 through the process, thus demonstrating the stability of the studied system. It is known that the optimal pH value varies with substrate and digestion technique [44].

It has been reported that biomethane content in biogas was increased instead of hydrogen sulphide when the pH range was 6.5–8.0 in batch anaerobic digestion of slaughterhouse wastewater sludge [45]. On the other hand, Salehiyun et al. [7] investigated the effect of an HRT increase on co-digestion of slaughterhouse waste and waste mixed sludge coming from a wastewater treatment plant. They claimed that the process inhibition occurs at pH values < 7 (6.7) caused by an OLR increase, because of volatile fatty acid (VFA) augmentation. In this assay, as HRT was increased, a gradual decrease of pH values was observed, reaching values around 7.2 when HRT = 3 d (OLR_{COD} = 4.27 kg/m^3·d; OLR_{VS} = 1.80 kg/m^3·d). In this sense, according to bibliography pH values, no destabilisation is expected at all HRT conditions reached.

Table 4 Kinetic parameter of the modified Gompertz model

Substrate	Y_{CH4}^{MAX} (NL_{CH4}/kg_{SV0})	K (NL_{CH4}/kg_{SV0}·d)	λ (d)	RSS	r^2	$Y_{CH4}{}^{MAX}{}_{exp}$ (NL_{CH4}/kg_{SV0})	Relative error (%)
SWW	53.5 ± 3.5	12.3 ± 1.7	0.95 ± 0.12	3.41	0.998	53.2 ± 3.3	0.6
TSS	19.4 ± 6.1	1.9 ± 1.1	0.50 ± 0.09	7.81	0.983	18.5 ± 4.8	4.6
STSS	201.2 ± 2.4	27.5 ± 0.9	0.97 ± 0.05	181.63	0.997	194.8 ± 5.2	3.2
SWW:STSS (50:50%v/v)	178.3 ± 4.8	40.9 ± 1.6	0.94 ± 0.05	38.11	0.999	177.4 ± 10.4	0.5

Table 5 Main characteristics of the effluents and daily methane production at the steady state

HRT (d)	CODt (kg/m^3)	CODs (kg/m^3)	TS (kg/m^3)	VS (kg/m^3)	pH -	V_{CH4} (L/d)	X_{CH4} (%)
15	1.6 ± 0.3	1.1 ± 0.1	3.4 ± 0.9	1.1 ± 0.5	7.7 ± 0.1	0.15 ± 0.03	73.3 ± 1.7
10	1.4 ± 0.2	1.2 ± 0.1	1.9 ± 0.2	0.8 ± 0.1	7.7 ± 0.1	0.24 ± 0.02	75.4 ± 2.1
5	3.1 ± 0.5	1.2 ± 0.2	2.2 ± 0.1	1.4 ± 0.1	7.5 ± 0.1	0.51 ± 0.02	76.6 ± 3.5
4	5.2 ± 0.8	1.9 ± 0.3	2.8 ± 0.1	1.8 ± 0.1	7.3 ± 0.1	0.55 ± 0.02	74.3 ± 5.9
3	5.6 ± 0.3	2.4 ± 0.2	3.7 ± 0.4	2.7 ± 0.2	7.2 ± 0.1	0.48 ± 0.02	74.5 ± 2.0

Other stabilisation parameters were analysed in order to establish the optimal OLR range and the OLR for destabilisation in the co-digestion of slaughterhouse wastewater and pre-treated sludge. Hence, the main experimental results of both COD and VS removals are shown in the Fig. 4a–b), respectively. In Fig. 4a, the evolution of %CODt$_{removal}$ results can be shown. As can be observed, when the OLR values were increased, the CODt_$_{removal}$ were reduced, obtained in order of decreasing: 84% at OLR_{COD} slower than 1.2 kg/m^3·d > 70% at ORL_{COD} = 2.16 kg/m^3·d; 60% at OLR_{COD} = 2.88 kg/m^3·d and 55% at OLR_{COD} = 4.27 kg/m^3·d.

The same behaviour was observed for %VS$_{removal}$ (Fig. 3b), where the optimal occurred at OLR = 0.39–0.49 kg/m^3·d, obtaining %VS$_{removal}$ = 82%. At OLR_{VS} = 1.12 kg/m^3·d, %VS$_{removal}$ = 78%, at OLR_{VS} = 1.43 kg/m^3·d, %VS$_{removal}$ = 70%, and at OLR_{VS} = 1.80 kg/m^3·d, %VS$_{removal}$ = 52%. Both behaviours can be explained because of the increasing in VFA when OLR was increased [7]. However, in attending to the pH values, it seems to be that the accumulation of VFA is not enough for the inhibition of the process.

Therefore, the increase in OLR applied to the system causes a decrease in the removal efficiency in terms of CODt and SV. For the values of HRT 5 and 4 d, the consumption of organic matter is around 60% CODt and 70% SV. The HRT of 3d causes a marked decrease in the organic matter removal.

The optimal conditions for the biodegradability of the wastes were reached at OLR_{COD} range 0.64–1.16 kg/m^3·d, being organic matter removal similar to the BMP test (87.8% CODt_removal and 83.2% VS_removal).

Table 5 shows data about the average of volume and composition (CH_4 and CO_2) of biogas in each HRT studied. As can be seen in all the cases, the percentage of methane in biogas is higher than 73%. The volume of biogas was increased with the decreasing of HRT, obtaining the highest level at HRT = 4 d (V_{biogas} = 0.55 L/d). Then, when HRT was reduced to 3 d, the volume of biogas started to decrease to 0.48 L. In addition, no H_2S was generated, showing that experimental conditions seem to enhance the growing of methanogenic bacteria instead of sulphate reducer bacteria.

Figure 5a shows the influence of OLR on the methane production rate in semi-continuous AnSBR under steady state. Regarding the methane production rate, it rises with the increasing OLR in the range between 0.64 and 2.88 kg/m^3·d, being the methane production rate 0.23 $L_{CH4}/L_{digester}$·d. However, a subsequent increase in the organic load at OLR = 4.27 kg/m^3·d supposes a drastic reduction of biomethane production. So in spite of the fact that the rest of the parameters (pH, CODt$_{removal}$, VS$_{removal}$) show an inefficient but stable process at this operational condition, a disrupted biomethane production can be observed, derived from the high OLR used, which leads us to think about the next inhibition phase.

The influence of ORL on methane productivity in semi-continuous AnSBR under steady state conditions is shown in Fig. 5b. Methane productivity reached a maximum value (0.21 L_{CH4}/g_{VS}) at the OLR range from 0.49 to 1.13 kg/m^3·d. This value is within the range (0.09–0.38 L_{CH4}/g_{VS}) reported by the bibliography for AnSBR of different slaughterhouse

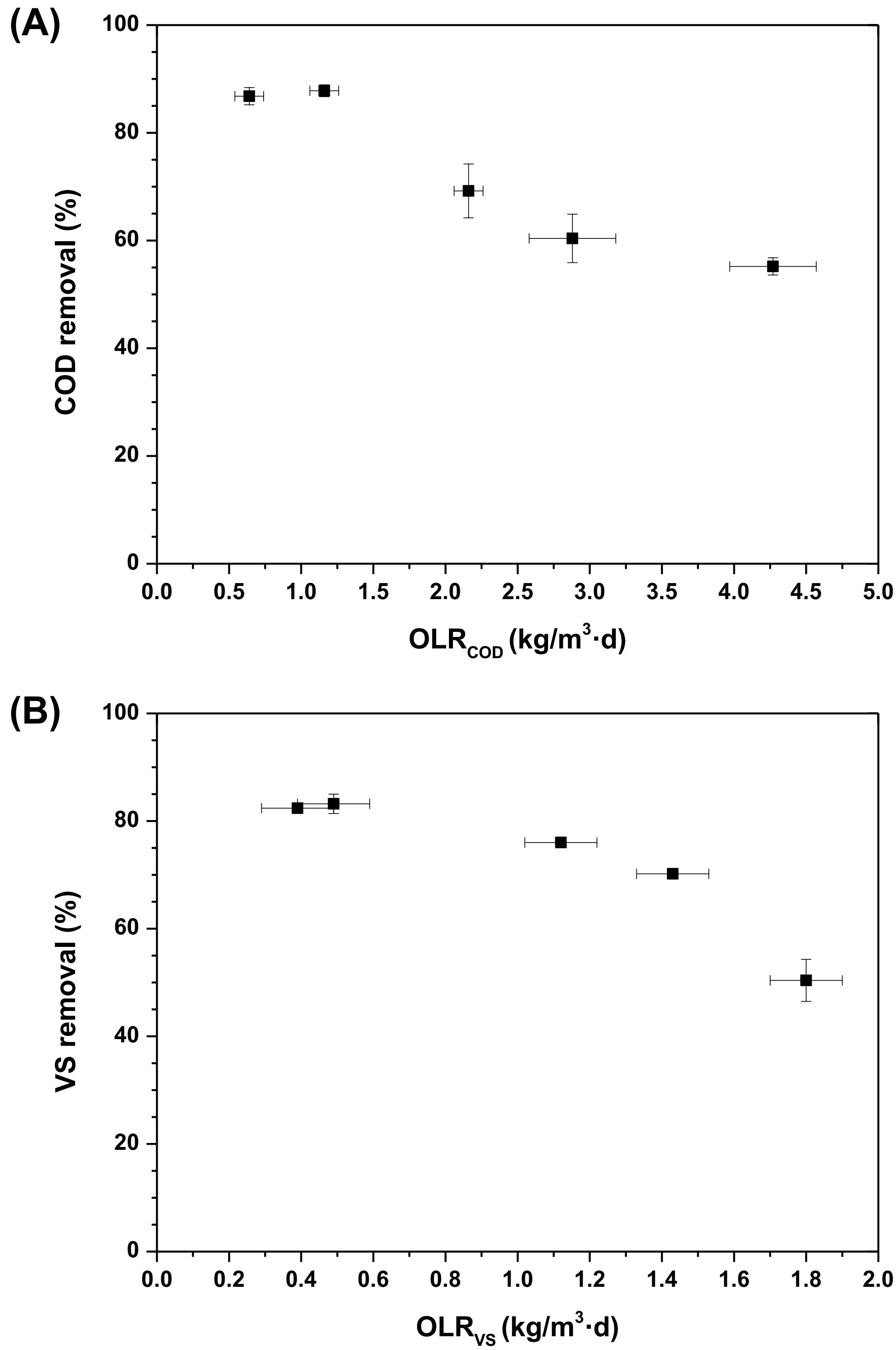

Fig. 4 Influence of OLR on **a** CODt removal and **b** VS removal in semi-continuous AnSBR under steady state

residues for HRT = 4–15 d [46–48]. A subsequent increase in OLR applied to the digester causes a sharp decrease in methane productivity, due to a decrease in biogas production. Therefore, the destabilisation of the system is observed at a faster OLR than 1.43 kg/m^3·d.

In conclusion, the efficacy of AnSBR for slaughterhouse waste has been demonstrated, with OLR$_{COD}$ between 1.16 and 2.16 kg/m^3·d being the best operational conditions as agreement conditions between depurative effectiveness and biogas productivity.

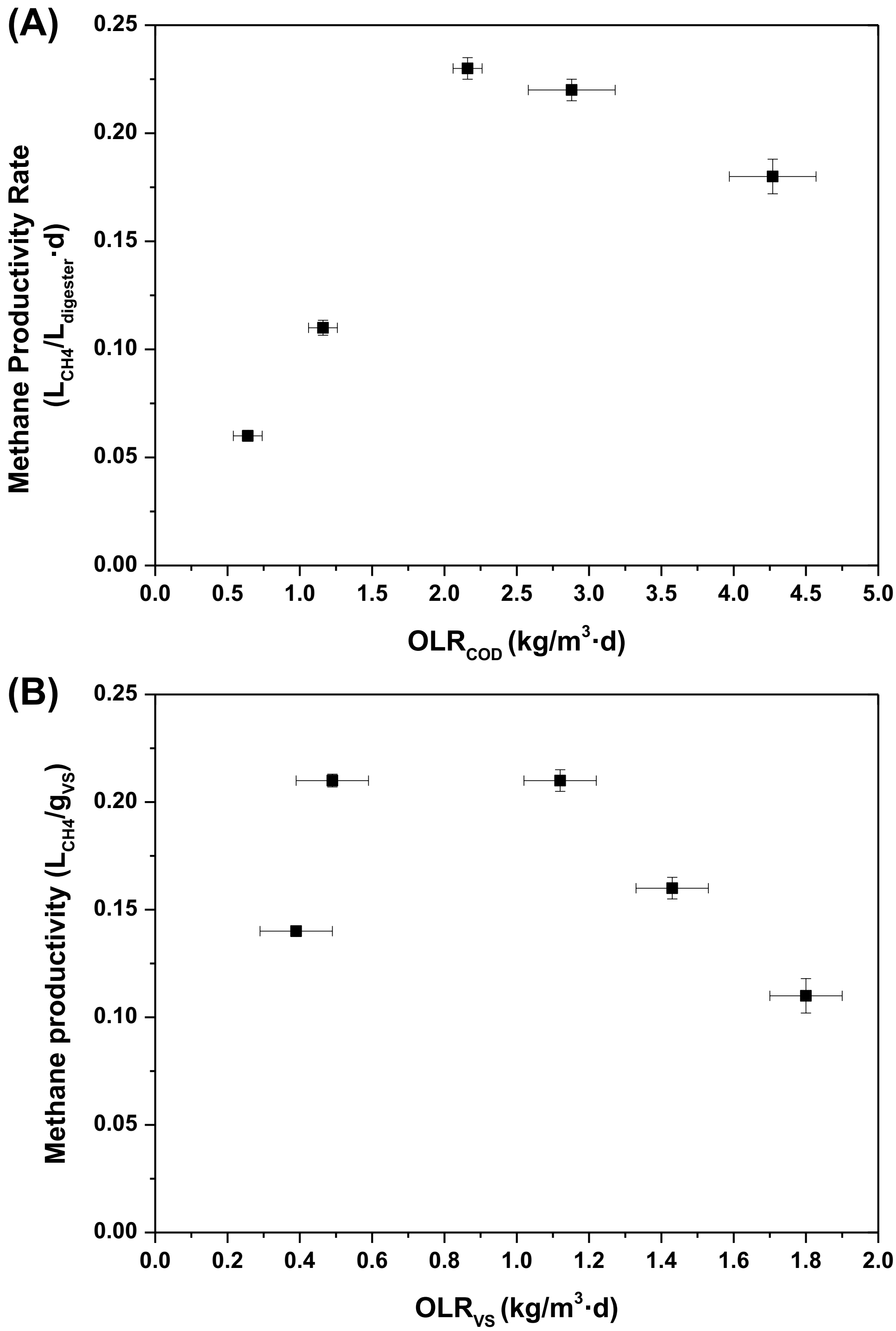

Fig. 5 Influence of HRT on **a** methane production rate and **b** methane productivity in semi-continuous AnSBR under steady state

5 Conclusions

Different treatment sequences based on anaerobic digestion have been tested in order to optimise waste management and biogas production in slaughterhouses. Batch experiments showed that co-digestion of slaughterhouse wastewater and supernatant of thermal pre-treatment slaughterhouse activated sludge enhanced organic matter removal and the biogas production rate. Biodegradability of co-digestion mixture achieved 76.0%. In addition, the specific constant rate for biomethane production was 40.9 $NL_{CH4}/kg_{VS}{\cdot}d$, this being 1.5 times higher than with the sole supernatant. Based on these results, the best design of the process entailed a co-digestion mixture, thermal pre-treatment and a centrifugation unit.

Thus, the viability of the best sequence in semi-continuous operational mode employing an AnSBR has been successfully demonstrated. Optimal OLR range was between 1.16 and 2.16 kg/m^3·d, achieving around 80% of the depuration grade and 0.23 $L_{CH4}/L_{digester}$·d of the methane production rate.

Author contribution Vanessa Ripoll: methodology, validation, formal analysis, data curation, writing (original draft), visualisation. Cristina Agabo-García: validation, formal analysis, data curation, writing (original draft), visualisation. Rosario Solera: conceptualisation; resources; data curation; writing, review and editing; supervision; project administration; funding acquisition. Montserrat Perez: conceptualisation; resources; data curation; writing, review and editing; supervision; project administration; funding acquisition.

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Declarations

Consent to participate The authors mutually agree with their participation in the present work.

Consent for publication The paper is an original work of the authors, and all of them mutually agree with its publication.

Conflict of interest The authors declare no competing interests.

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