Modelling of the anaerobic semi-continuous co-digestion of sewage sludge and wine distillery wastewater.

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ABSTRACT

The semi-continuous mesophilic anaerobic co-digestion of sewage sludge and wine distillery wastewater was investigated. In this sense, the effects of reducing hydraulic retention time (HRT, from 20 d to 8 d) on the degradation of organic matter and methane production were determined. The experimental results showed that anaerobic co-digestion enhanced the biodegradability of the mixture in terms of VS and CODt (7-8% higher) for HRT = 8 d. The methane productivity at HRT = 8 d (18.0 $L_{CH4}/kg_{VS}·d$), was also enhanced (30% higher) than those obtained in the control experiment. In addition, a mathematical kinetic model was proposed to determine the rate-limiting step of the process. Stoichiometric parameters obtained for SS:WDW (0.115 kg_{CH4}/kg_{COD}) was higher than SS (0.094 kg_{CH4}/kg_{COD}), which means that co-digestion increased the rate of consumption because more amount of biodegradable compounds. Kinetic study showed that anaerobic co-digestion favoured methane production rate enhancing the acetate consumption step and making methanogenesis the rate-limiting step in this process.

Keywords: *anaerobic co-digestion; wine distillery wastewater; sewage sludge; organic loading rate; kinetic modelling.*

1 Introduction

The cost of sewage sludge management in urban wastewater treatments plants (WWTP) is over 50% of the overall operating costs taking into account the land value, the transportation, landfilling operation and leachate treatment, and maintenance for a correct pollution control[1]. Therefore, anaerobic digestion (AD) has been widely implemented as an effective technology at an industrial scale in WWTP[2]. AD is a renewable energy technology in which microorganisms break down biodegradable material in an oxygen-free environment to produce a solid digestate along with biogas. Biogas production has social advantages, such as the economical production of electricity or heat. In addition, AD process achieves the reduction and stabilization of rotting organic matter and partial inactivation of pathogenic agents[3-5]. However, sewage sludge (SS) organic load values undergo high variations and are often not sufficient for an economically effective operation[1]. Given that many of the anaerobic digesters installed in WWTP are oversized, the scientific community is paying close attention to simultaneous anaerobic co-digestion (ACoD) of SS and other types of waste aimed to promote the biodegradability of the feedstock and hence to enhance biogas production[6-7]. The main advantages of ACoD include: shared treatment facilities, reduced investment and operating costs, buffering of the variations in the composition of the waste over time and the diluting of toxic compounds and cytotoxic inhibitors. Several published studies have already focused on the employment of agro-industrial wastes, which contain easily degradable substrates[8-11]. Among different residues, wine distillery wastewater (WDW) is one of the main types of waste generated in the viticulture industry and its disposal constitutes an environmental concern. This waste has a strongly acidic pH and contains a high organic pollutant load (around 40 gCOD/L) including various phenolic compounds such as gallic acid, p-coumaric acid and gentisic acid[12]. In this sense, the AD of sole WDW at semi-continuos mode has been previously studied (including kinetic evaluation) as a successful biological treatment for producing biomethane: in fixed-film reactors[13]; thermophilic high rate reactors [14] and after different pre-treatments[15]. However, semi-continuos ACoD of SS:WDW only has been previously studied to produce biohydrogen at thermophilic conditions in continuous stirred tank reactor technology (CSTR)[16]. In addition, the ACoD of SS:WDW has been previously studied at batch mode without any pretreatment[17]. So, in the present work it was studied its biomethane potential as co-substrate for continuous biomethane production in CSTR technology and the kinetic parameters, complementing the recently research published about ACoD of SS:WDW at batch mode. This information will be useful for determining operational conditions in scaling-up process in regions with high volume of WDW production in order to use them in WWTP in wine-producing areas [17].

In this sense, process modelling is a useful tool for describing and predicting the performance of anaerobic digestion systems. Monod type kinetic models have been widely used to describe the process kinetics of anaerobic digesters[18]. Although there has been some success in applying Monod type kinetics to the anaerobic process, some research workers found it difficult to apply them for their systems[19-20]. In the equation proposed by Contois (1959)[21], the specific growth rate was considered as a function of the growth-limiting nutrient in both input and effluent substrate concentration by using an empirical constant, which was related to microbial concentration. On this basis, Chen and Hashimoto (1980)[22] developed kinetic models for substrate utilisation and methane production and suggested that the Contois type kinetic models would be more suitable than the Monod type kinetic models to predict digester performance. Both consider that the AD takes place throughout a single stage of biological reaction that combines a complex biological reaction network. In this paper, a novel kinetic model is proposed based on the methane production results to describe the step rates involved in the anaerobic process. An unstructured non-segregated kinetic model is proposed to describe the anaerobic digestion considering two steps: the hydrolysis of the particulate substrate (COD_P) to obtain accessible or soluble substrate (COD_S) for the microorganisms and the consumption of this accessible substrate to produce methane.

Moreover, there is not much published information available for the process kinetics of anaerobic co-digestion treating sewage sludge and wine distillery wastewater.

The aim of this paper was focused, therefore, on the experimental and kinetic description of the anaerobic semi-continuous co-digestion of SS and WDW within the mesophilic temperature range, studying the influence of the organic loading rate (OLR) on the overall process: the efficiency of organic matter removal, biogas production and kinetic analysis. In order to achieve this goal, several hydraulic retention times (HRT) were employed ranging from 20 to 8 d, corresponding to different OLR ranges (2.26 – 5.38 kgCOD/m^3·d). An anaerobic digestion trial using only sewage sludge as feedstock was also carried out as a control experiment to determine whether adding WDW to the WWTP anaerobic digester enhances the degradation of organic matter and/or methane production. The semi-continuous control experiment was carried out under the same HRT conditions (from 20 to 8 d), involving an OLR range from 3.43 to 6.99 kgCOD/m^3·d. Finally, a comparison of different kinetic parameters obtained after modelling was made to determine the rate-limiting step in each case.

2 Materials and methods

The experimental protocol was designed to study the influence of increasing OLR on the efficiency of an anaerobic digestion treatment within the mesophilic temperature range employing a 1:1 (v/v) ratio of SS:WDW, as well as SS alone (as a control experiment).

The methods and materials used are briefly described in this section. Each trial was carried out in duplicate and all the reported results correspond to the average values of the last data obtained.

2.1 Feedstock and inoculum

The substrates used in the experimental stage were collected directly from two real industrial facilities. SS corresponds to the activated sludge from the secondary treatment employed at Guadalete municipal WWTP (located in Cadiz, Spain). WDW was obtained from Gonzalez-Byass, an ethanol producing wine-distillery plant located in Jerez de la Frontera (Cadiz, Spain). All the samples were characterized on reception at the laboratory and were stored at 4 ºC for a maximum of one month before being used in the experiments in order to prevent their degradation.

The experimental work was carried out over a period of 6 months. During the experiments, waste samples had to be collected several times. Parameters related to organic material content, such as total solids (TS), volatile solids (VS) and chemical oxygen demand (COD), were determined.

The inoculum was obtained from a 5-L laboratory scale anaerobic digester operating the Research Group under stable conditions at 20 d HRT within the mesophilic temperature range (35 ºC). This reactor was fed with SS coming from secondary decanter of WWTP from Jerez (Cádiz-Spain). The characteristics of the inoculum are shown in Table 1.

Table 1. Characteristics of inoculum

Parameters	**Inoculum**
pH	7.60 ± 0.02
CODt (kg/m^3)	20.1 ± 0.5

CODs (kg/m^3)	10.1 ± 0.4
TS (g/L)	32.0 ± 2.5
VS (g/L)	23.4 ± 2.0

2.2 Experimental equipment

A 3-L semi-continuous stirred tank reactor with a working volume of 2-L was used (MiniReactor, Trallero and Schee®) in this study. The digester was sealed with a lid provided with several openings for different purposes (biogas output, pH probe, temperature probe, stirring system). The stirring speed was set at 20 rpm. The temperature was maintained within the mesophilic range (35 ± 1 ºC) by means of an electric heater. The produced biogas was collected in 5-L Tedlar® bags, employing a special syringe to sample the gases.

2.3 Operating conditions

In the present study, the influence of the OLR on the anaerobic co-digestion of a 1:1 (v/v) mixture of SS and WDW was analysed in semi-continuous operating mode within the mesophilic temperature range (35 ºC). These results were compared with the behaviour of an anaerobic digester using only SS working under similar operating conditions (OLR and temperature range). Initially, both digesters were loaded with a mixture of inoculum and substrate at a ratio of 1:2.5 (v/v), which is considered optimal for biogas production[17, 23]. The start-up of the reactors took place at 20 d HRT. The subsequent series of HRT were set at 15, 10 and 8 days, which involved progressive increases in feed flow rates (from 0.10 to 0.15, 0.2 and 0.25 L/d, respectively). These operating conditions were selected on the basis of the previous experience of our research group [8,10] and other research contributions [24-25]. At least 3 trials employing the corresponding HRT (20, 15, 10 and 8 d, respectively) were conducted under each operating condition in order to ensure the steady state. The reactors were fed once a day with SS:WDW (1:1 (v/v)) and SS without the addition of nutrients in order to establish the semi-continuous process. Each HRT was maintained at least 3 times to ensure that steady-state conditions were reached. The composition of the industrial wastes employed varied during the experimental period. Therefore, the OLR was determined for each condition in terms of feed VS (OLR_{VS}) and feed COD (OLR_{COD}). The main characteristics of the feedstocks are shown in Table 2 (a, b).

Table 2.Main characteristic of the feedstock (a) 1:1 SS:WDW and (b) SS.

HRT (d)	TS (kg_{TS}/m^3)	VS (kg_{VS}/m^3)	CODt (kg_{COD}/m^3)	CODs (kg_{COD}/m^3)	OLR ($kg_{VS}/m^3 \cdot d$)	OLR ($kg_{COD}/m^3 \cdot d$)
20	30.6 ± 1.6	23.4 ± 0.8	45.2 ± 2.4	24.9 ± 1.7	1.17 ± 0.04	2.26 ± 0.12
15	31.7 ± 1.9	25.3 ± 1.1	43.0 ± 1.9	22.4 ± 1.3	1.90 ± 0.08	3.23 ± 0.14
10	31.8 ± 0.3	24.7 ± 0.4	41.9 ± 1.9	20.6 ± 2.2	2.47 ± 0.04	4.19 ± 0.19
8	34.7 ± 0.3	25.6 ± 0.4	43.0 ± 1.9	22.4 ± 1.3	3.20 ± 0.05	5.38 ± 0.24

(a)

HRT (d)	TS (kg_{TS}/m^3)	VS (kg_{VS}/m^3)	CODt (kg_{COD}/m^3)	CODs (kg_{COD}/m^3)	OLR ($kg_{VS}/m^3 \cdot d$)	OLR ($kg_{COD}/m^3 \cdot d$)
20	52.8 ± 2.0	43.2 ± 1.7	68.5 ± 3.3	15.9 ± 3.2	2.16 ± 0.09	3.43 ± 0.17
15	52.4 ± 0.4	42.6 ± 0.3	68.5 ± 2.0	10.4 ± 2.5	3.20 ± 0.02	4.57 ± 0.15
10	54.8 ± 0.3	42.9 ±0.2	65.8 ± 3.3	9.9 ± 1.6	4.29 ± 0.02	6.58 ± 0.33
8	44.4 ± 0.5	37.8 ± 1.2	65.9 ± 0.8	10.4 ± 1.1	4.66 ± 0.15	6.99 ± 0.10

(b)

2.4 Analytical methods

Analytical characterization of the feedstock and the digestate were carried out twice a week during the experimental stage. The main parameters (total solids (TS), volatile solids (VS) and chemical oxygen demand (COD)) were determined in accordance with the Standard Methods[26]. In order to better understand the system behaviour, the total and soluble COD (COD_T and COD_s, respectively) of both the feedstock and effluent were determined. Volatile fatty acids (VFA) (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and heptanoic acids) were determined on a gas chromatograph (GC-2010 Plus Shimadzu) employing a Nukol® capillary column and a FID detector. Total acidity was calculated by the addition of the individual fatty acids in terms of acetic acid concentration equivalent. Gas composition was determined employing a gas chromatographic technique (GC-2010 Shimadzu). The analysed gases (H_2, CH_4, CO_2, O_2 and N_2) were measured by means of a thermal conductivity detector (TCD) at 250 ºC using a Supelco Carboxen 1010 PLOT column. The oven temperature was programmed between 35 and 200 ºC. Manual injection was carried out using a sample volume of 250 μL. The carrier gas employed was nitrogen at a pressure of 35 kPa. The biogas volume was collected daily in Tedlar bags. Volumes were directly determined employing a gas flow meter (Ritter Wet Drum TG 0.1 mbar). The pH was measured using a Crison 20 BASIC pHmeter.

2.5 Process efficiency

Process efficiency was related to the percent removal efficiency obtained by anaerobic digestion in terms of VS removal and CODt removal. In order to evaluate biogas production, two parameters related to specific methane production (SMP) (Eq. 1 and 2) and methane productivity (MP) (Eq. 3 and 4) were defined.

$$\boldsymbol{SMP_{VS}}\left({}^{\boldsymbol{L_{CH4}}}/_{\boldsymbol{kg_{VS}}}\right) = \frac{\boldsymbol{V_{CH_4}}}{\boldsymbol{OLR_{VS}}} \quad \textbf{(1)}$$

$$\boldsymbol{SMP_{COD}}\left({}^{\boldsymbol{L_{CH4}}}/_{\boldsymbol{kg_{COD}}}\right) = \frac{\boldsymbol{V_{CH_4}}}{\boldsymbol{OLR_{COD}}} \quad \textbf{(2)}$$

$$\boldsymbol{MP_{VS}}\left({}^{\boldsymbol{L_{CH4}}}/_{\boldsymbol{kg_{VS}} \cdot \boldsymbol{d}}\right) = \frac{\boldsymbol{V_{CH_4}}}{\boldsymbol{V_{Digester}} \cdot \boldsymbol{VS_{INLET}}} \quad \textbf{(3)}$$

$$\boldsymbol{MP_{COD}}\left({}^{\boldsymbol{L_{CH4}}}/_{\boldsymbol{kg_{COD}} \cdot \boldsymbol{d}}\right) = \frac{\boldsymbol{V_{CH_4}}}{\boldsymbol{V_{Digester}} \cdot \boldsymbol{COD_{INLET}}} \quad \textbf{(4)}$$

3 Results and discussion

3.1 ***Process stability: pH evolution and VFA***

The evolution of pH inside the digesters throughout the experiments are shown in Figure 1-A (i, ii). The optimal pH range for the activity of methanogenic microorganisms (7.5–8.5) in mesophilic anaerobic digestion is well known[27-29].

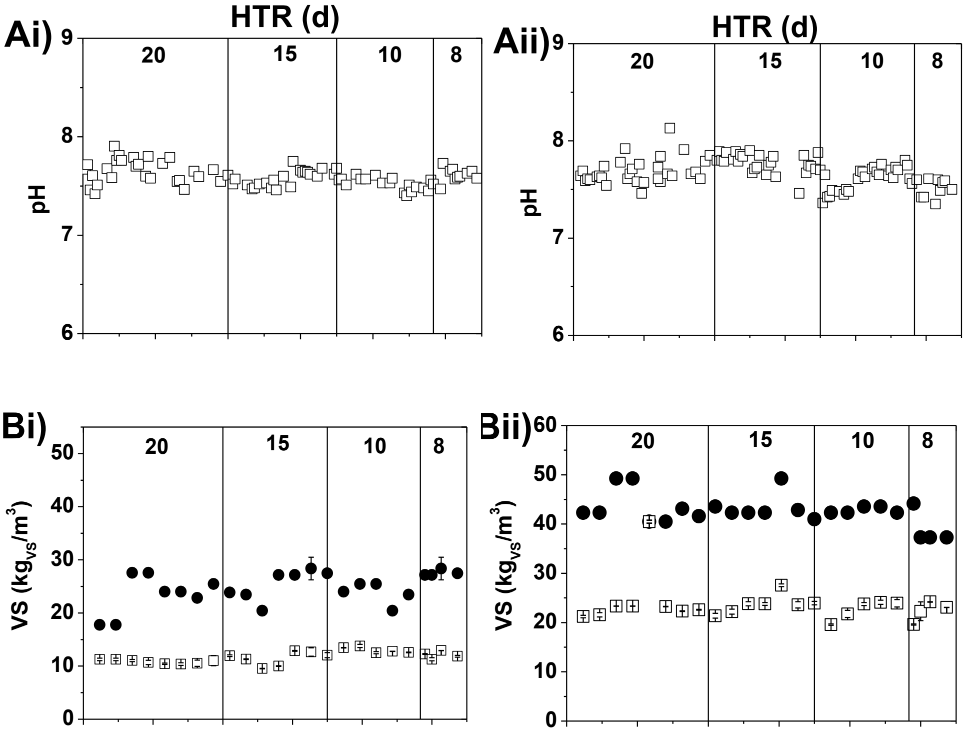
Thus, the monitoring of the physicochemical parameter provides useful information with respect to the anaerobic digestion steps that are taking place. The pH values remained within the 7.3–8.2 range in both the ACoD digester and the control digester for months. The pH was maintained constant due to two causes: (i) daily adjustment of substrate before feeding in the case of co-digestion (due to low values of pH of WDW) and (ii) the microbial consortia activity. So, the pH was maintained at optimal range and the volatile fatty acids, which are being formed during hydrolytic-acidogenesis pathways, are neutralized avoiding acidification of the reactor and allowing the normal activity of acetogenic and methanogenic bacteria.

3.2 Organic matter removal

The organic matter content in the feedstock and effluent was determined by measuring VS and COD_T in both streams. The evolution of these two key parameters during AD at each tested HRT are shown in Figures 1-B (i, ii) and 1-C (i, ii) and the average of these parameters after reaching steady state is showed in Table 2. As it can be seen in Figures 1B-C, the start-up of the process (20 d HRT) showed a proper acclimation of the inoculum to the waste. The present study was conducted employing real industrial wastes as feedstock. Thus, the characteristics of the initial feedstock varied over the six months of experimental work. Nevertheless, the organic matter determined in the outlet stream remained stable (Figure 1 B-C) regardless of the changes in feedstock, as it was concluded in other previous studies employing different types of wastes[10]. On average, VS and CODT removal in all the cases was 44.6 and 49.9% for SS and 52.7 and 56.7% for SS:WDW for OLR ≤ 3.2 ($kg_{VS}/m^3 \cdot d$).

Total VFA (mgAc/L) at the steady state is also shown in Table 2. There is a slight increase between 20 and 8 d HRT of the total VFA concentration, being higher in co-digestion digesters than in those fed only with SS. Taking into account that initial VFA were very high (4424 ± 122 mg Ac/L and 6827 ± 135 mg/L Ac/L for WDW:SS and SS, respectively) the VFAs were used almost fully: between 76-90% and 90-96% for WDW:SS and SS, respectively. It should be pointed out that this parameter had no effect on pH, which remained stable during the tests. According to these results, the co-digestion system has proven to be stable under the operating conditions studied here.

In Table3, VS/TS ratios are also shown, obtaining on average 61.4% ± 2.0 and 71.2 ± 1.3% for SS:WDW and SS, respectively. Regarding energy efficiency (EE) as the energy cost of recovering usable energy from the sludge, according to Li and Feng (2018)[30] when AD is operating at VS/TS ≥ 60%, the system was more than twice efficient than the



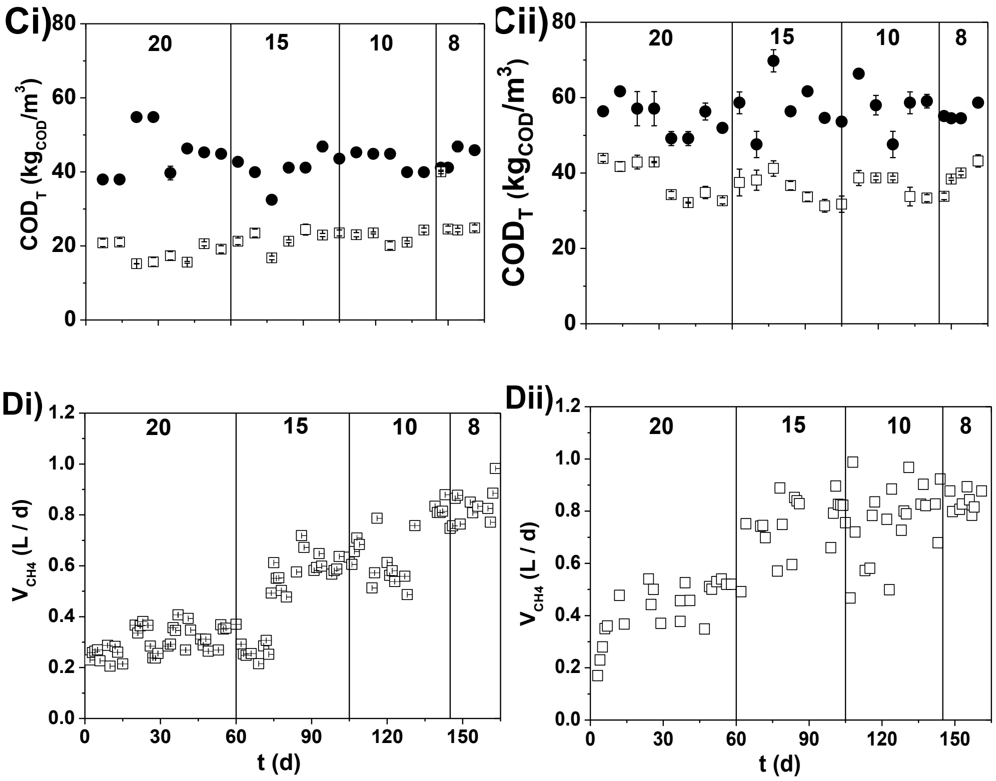


Figure 1. Evolution of **(A)** pH inside the digesters, **(B)** VS of the feedstock and the effluent, **(C)** COD of the feedstock and the effluent, and **(D)** daily methane volume in the semi-continuous anaerobic digesters of **(i)** SS:WDW (1:1 (v/v)), and **(ii)** SS. *Key* ●*feeding* □*effluent.*

system working at VS/TS = 70%. Then, the ACoD system proposed not only will be an eco-friendly way of wine making residue valorization but also an energetically efficient process.

Table 3. Main characteristic of the effluents and daily methane production at the steady state: (a) 1:1, SS:WDW and (b) SS.

HTR (d)	**VS (kg_{VS}/ m^3)**	**VS/TS (%)**	**CODt (kg_{COD}/ m^3)**	**CODs (kg_{COD}/ m^3)**	**VFAs (mg_{ac}/L)**	**V_{CH4} (L/d)**	**X_{CH4} (%)**
20	10.6 ± 0.1	59.9	15.2 ± 0.9	8.5 ± 0.5	418 ± 87	0.47 ± 0.03	68.1 ± 1.6
15	11.3 ± 0.7	60.4	16.8 ± 1.4	8.8 ± 0.9	721 ± 60	0.63 ± 0.03	66.8 ± 1.1

10	12.7 ± 0.1	64.8	20.6 ± 0.1	9.6 ± 0.8	1018 ± 103	0.75 ± 0.06	68.0 ± 1.4
8	12.2 ± 0.5	60.4	22.4 ± 5.2	10.5 ± 2.3	1608 ± 189	0.92 ± 0.01	69.4 ± 1.6

(a)

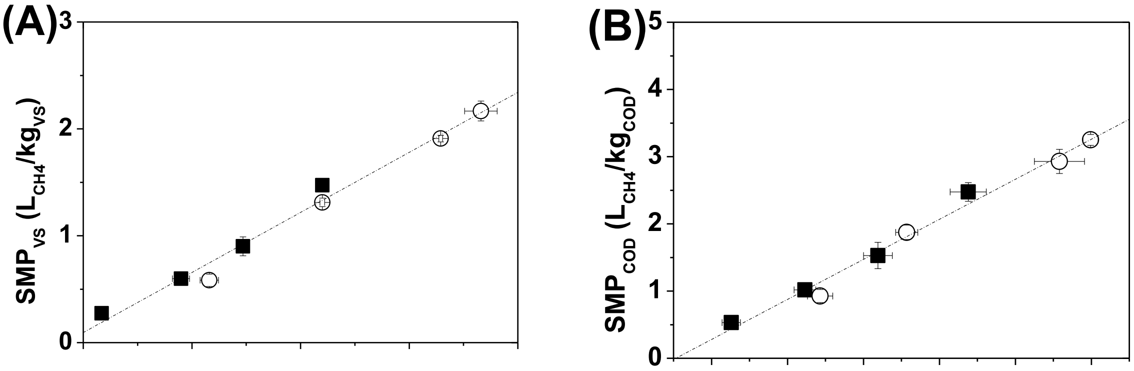
HTR (d)	**VS (kg_{VS}/ m^3)**	**VS/TS (%)**	**CODt (kg_{COD}/ m^3)**	**CODs (kg_{COD}/ m^3)**	**VFAs (mg_{ac}/L)**	**V_{CH4} (L/d)**	**X_{CH4} (%)**
20	22.7 ± 0.4	70.5	28.9 ± 1.9	17.3 ± 0.9	271 ± 69	0.54 ± 0.03	68.8 ± 2.4
15	23.1 ± 0.4	70.2	31.5 ± 1.8	14.7 ± 0.5	398 ± 90	0.82 ± 0.02	68.3 ± 1.5
10	23.2 ± 0.7	70.5	36.1 ± 1.5	15.0 ± 2.6	446 ± 76	0.89 ± 0.01	68.0 ± 2.9
8	23.3 ± 0.4	73.5	38.2 ± 1.6	14.0 ± 0.5	658 ± 102	0.93 ± 0.01	67.7 ± 1.2

(b)

3.3 Biogas production

Figure 1-D (i, ii) shows the evolution of daily methane production. In response to changing OLR conditions, an acclimation stage is observed before to reaching the steady state. The lower the HRT (which means the higher the OLR), the higher the biogas production. The average methane production under each condition is summarized in Table 2. The maximum value obtained was 0.92 ± 0.2 L/d at 8 d HRT, regardless of substrate used. Table 2 also shows the percentage of methane contained in the biogas (X_{CH4}), near of 70% in both trials, similar than previous reported by ACoD of these both substrates[17].

The influence of OLR on specific methane production (SMP) in terms of VS and COD are shown in Figure 2 (A, B). Increasing OLR lead to a linear increase in SMP, regardless of the waste employed (SS:WDW or SS). However, as it can be seen in Figure 2 (C, D) the influence of OLR on MP shows a different trend. ACoD of SS:WDW significantly enhances MP with respect to AD of SS alone in terms of both VS and COD, because initial SS had more VS and COD than SS:WDW (Table 1) . The explanation of this result lies in the favourable characteristics of WDW (high dissolved organic matter content and biodegradability) improving methane production rate. The reduced effectiveness of the system is also reflected in the higher accumulation on VFA intermediate compounds in the AD (Table 2). Based on these results, employing HRT below 8 d could entail the destabilization of the digester and the breakdown of efficiency. The best methane productivity was obtained when ACoD of SS:WDW was operated at HRT = 8d (OLR = 3.2 $kg_{VS}/m^3 \cdot d$), obtaining 18.0 $L_{CH4}/kg_{VS} \cdot d$ and 10.7 $L_{CH4}/kg_{COD} \cdot d$, being 30% higher than those observed in the AD of SS alone. Other researchers have obtained similar MP increases on mono-digestion of SS by addition of agri-food residues. Donoso-Bravo et al.,[31] studied the effect of ACoD of SS with two co-substrates: (i)beverage wastewater and (ii) thermally pretrated biological sludge. Results obtained improved values of MP in 21.4% and 16.2% by using 10% v/v of co-substrates and 90% of SS. Montañés et al.,[32] obtained 33.6% higher MP in terms of $m^3CH_4/kg\ COD_{removal}$ by using 10% v/v of leaching of sugar beep pulp as co-substrate at OLR = 3.55 $kg_{VS}/m^3 \cdot d$. Pitk et al., [33] obtained an increase of 37.7% at OLR ~ 3 $kg_{VS}/m^3 \cdot d$ using 7.5% of slaughterhouse residues.



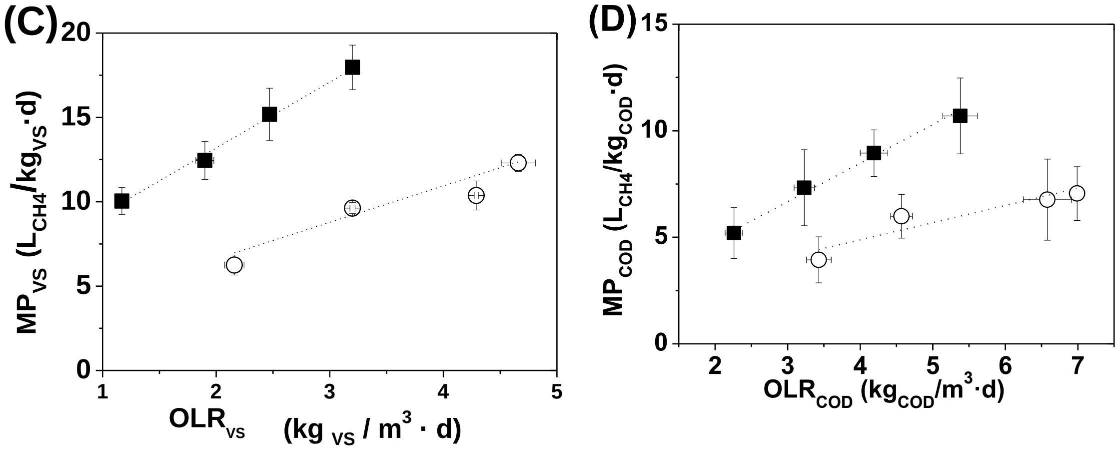


Figure 2 Influence of OLR on **(A)** SMP in terms of VS, **(B)** SMP in terms of COD, **(C)** MP in terms of VS and **(D)** MP in terms of COD. *Key:*■*SS:WDW (1:1)* ○*SS.*

3.4 Kinetic modelling

A kinetic model is proposed based on the MP results to describe the step rates involved in both the ACoD of SS:WDW and the AD of SS alone. AD takes places via a complex biological reaction network. An unstructured non-segregated kinetic model is proposed here to describe the anaerobic digestion of WDW and SS at the macroscopic scale. The two reactions considered are: hydrolysis of the solid substrate (COD$_P$) to obtain accessible substrate (COD$_S$) for the microorganisms (Eq. 5), and consumption of this accessible substrate to produce methane (Eq. 6). The Eq. 5 simplify the equations produced during the hydrolysis because the exo-enzymes activity of microbes. In this sense, the organic matter particulate (measured as CODp) is converted to organic matter soluble (CODs). On the other hand, the Eq. 6 simplify in a unique reaction the organic matter consumption reactions that produce biomethane. This reaction schematically represents all the proccesses that occur when biodegradablae organic matter (which indirectly is measured by CODs) is converted to biomethane (CH_4). It is necessary to establish a relationship between both parameters with the stoichiometric coefficient. This parameter is analogue to biomethane yield. As it is known, that this complex process does not occur in a unique step, this yield involves the stoichiometry in macroscopic terms.

Hydrolysis $$\boldsymbol{COD_P \xrightarrow{r_{hydrolysis}} COD_S} \quad (5)$$

Consumption $$\boldsymbol{COD_S \xrightarrow{r_{consumption}} Y_{CH4/CODs} \cdot CH_4} \quad (6)$$

The proposed kinetic equations depend on the substrate concentration via a first-order reaction (Eq. 7 and 8), in line with previously published papers by other authors[34-36].

Hydrolysis $$\boldsymbol{r_{hydrolysis}\left({kg_{COD}}/{m^3 \cdot d}\right) = k_{hydrolysis} \cdot COD_P} \quad (7)$$

Consumption $$\boldsymbol{r_{consumption}\left({kg_{COD}}/{m^3 \cdot d}\right) = k_{consumption} \cdot COD_S} \quad (8)$$

Considering the mass balance in the digester and assuming that the digester behaves as an ideal complete mixed tank bioreactor, the hydrolysis and consumption rates are related to properties of the feedstock and effluent at the steady state, and the HRT condition. Therefore, both rates can be calculated using inlet and outlet biodegradability parameters (Table 2) at the steady state for each HRT condition (Eq. 9 and 10). According to Eq. 9 $r_{hydrolysis}$ is calculated by difference of inlet and outlet CODp (which represent the soluble matter formed inside the reactor) referred to HRT. However, in Eq.10 the consumption rate shows the difference between CODs in the reactor (CODs that enter with feeding plus the CODs that already is inside the reactor as a consequence of hydrolysis step) and the CODs that exit from reactor.

Hydrolysis

$$\boldsymbol{r_{hydrolysis}} = \frac{\mathbf{1}}{\boldsymbol{HRT}} \cdot \left(\boldsymbol{COD_P^{Inlet}} - \boldsymbol{COD_P^{Outlet}}\right) \qquad \textbf{(9)}$$

Consumption

$$\boldsymbol{r_{consumption}} = \frac{\mathbf{1}}{\boldsymbol{HRT}} \cdot \left(\boldsymbol{CODs^{inside\ reactor}} + \boldsymbol{CODs^{inlet}}\right) - \boldsymbol{COD_s^{Outlet}} \qquad \textbf{(10)}$$

Based on these equations, the kinetic parameters of the model ($k_{hydrolysis}$ and $k_{consumption}$) and the pseudo-stoichiometric parameter ($Y_{CH4/COD}$) can be estimated (Table 3) by fitting the experimental data (Figure 3) to linear regression.

In Figure 3 it is shown the evolution of reaction rate in each step (Hydrolysis and consumption) at each condition: mono-digestion of SS and co-digestion of SS:WDW. In the case of SS condition (Figure 3-B) the hydrolysis and consumption rate are very similar. This occurs because the hydrolysis was the rate-limiting step since the SS contains a high number of complex structures that must be hydrolyzed before the rest of the process and hence the consumption step depends on hydrolysis. Taking into account the consumption rate calculation (Eq. 9), two effects occurred in mono-digestion of SS: (1) CODs inlet was low because low content of easily hydrolizable compounds in SS so $K_{hydrolysis}$ must be high for transforming the major number of complex structures to simple ones (2) acetogenesis is quicker than hydrolictic-acidogenesis[37] so: in spite of high activity of hydrolysis transforming CODp into CODs, the consumption of CODs is higher than the production. So, in the calculation of $K_{consumption}$ it is important to take into account that the majority of CODs formed (CODs inside the reactor, Eq.10) is assimilated. And hence, its concentration measured in the reactor is very low (only 0.04-1% of substrate CODs, Table 2) and the sum with the inlet CODs is lower than CODs outlet obtaining a negative $K_{consumption}$ (Table 4).

However, in the case of co-digestion of SS:WDW (Figure 3-A) , the hydrolysis rate was higher than consumption one, due to the high amount of easily biodegradable compounds supply by WDW co-substrate. So in this case, the hydrolysis was not the limiting-step (as it usually happens in AD) because the WDW contribute directly with easily hydrolizable organic matter compounds (inlet CODs, in Eq. 10). In addition, the higher amount of TVS in WDW enhance the nutrients matter transfer to microorganisms[37.] Other authors have previously identified the degradation of acetate (an intermediate compound in the metabolic pathway) as the rate-limiting step in co-digestion processes [38-39]. As acetate assimilation is one of the reactions that occur during consumption process, in this case, the rate-limiting step can be the consumption step and $K_{hydolysis}$ was higher than $K_{consumption}$ (Table 4).

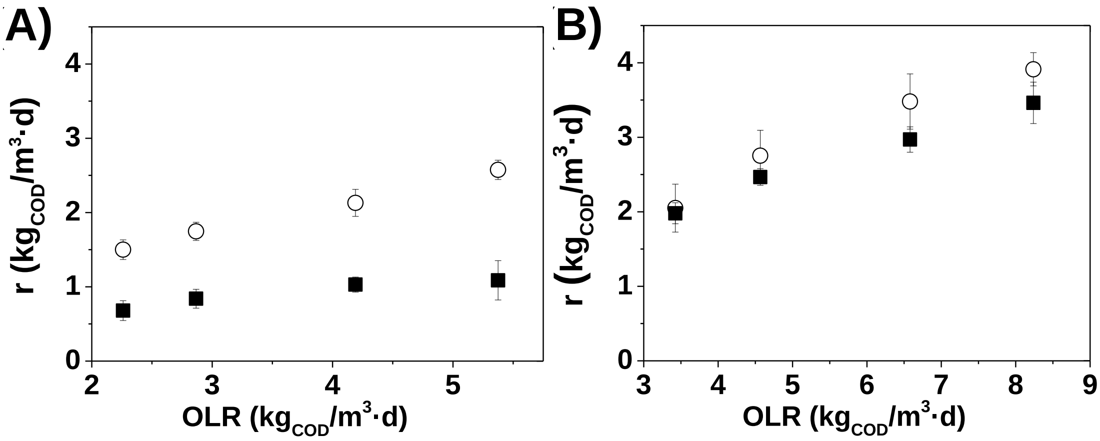


Figure 3 Influence of OLR on the hydrolysis and consumption reactions rate in the AcoD digester of SS:WDW (1:1 (v/v)) and in the control digester: **(A)** SS:WDW (1:1 (v/v)). and **(B)** SS. *Key:* ○*hydrolysis* ■co*nsumption.*

Table 4 Values of the kinetic and stoichiometric parameters obtained by regression using the first kinetic model, Eq. (5-8).

Feedstock	Parameter	Value	r^2	SSR
Co-digestion (1:1; SS:WDW)	$k_{hydrolysis}\ (d^{-1})$	$5.22\cdot10^{-1}$	0.996	0.0102
	$k_{consumption}\ (d^{-1})$	$9.57\cdot10^{-2}$	0.962	0.0044
	$Y_{CH4/COD}\ (kg_{CH4}/kg_{COD})$	$1.15\cdot10^{-1}$	0.966	0.0004
Digestion (SS)	$k_{hydrolysis}\ (d^{-1})$	$1.65\cdot10^{-1}$	0.997	0.0250
	$k_{consumption}\ (d^{-1})$	$-4.98\cdot10^{-1}$	0.833	0.0346
	$Y_{CH4/COD}\ (kg_{CH4}/kg_{COD})$	$0.95\cdot10^{-1}$	0.999	0.0020

Comparing both conditions, in spite of having a $k_{hydrolysis}$ in the same order in both conditions, the high $k_{consumption}$ in the case of co-digestion increase the global rate of the process obtaining a higher stoichiometric parameter for SS:WDW (0.165 kg_{CH4}/kg_{COD}) than that for SS (0.094 kg_{CH4}/kg_{COD}). Then, ACoD of SS:WDW improved the biomethane potential and hence also the methane production efficiency of the systems removing the limiting effect of hydrolysis step.

Finally, the available organic matter consumption (elimination of CODp) and production (production of CODs) were calculated by Eq. 11 and 12, respectively; and the methane production by Eq. 13; taking into account its roles in the net of reactions proposed in the kinetic model as well as the kinetic values of constant (k) at the steady state in the semi-continuous anaerobic digesters. The results were drawn-up in Figure 4, evaluating the influence of HRT on each of them.

<u>Solid substrate consumption</u>

$$(-\boldsymbol{R_{CODp}}) = \boldsymbol{r_{hydrolysis}} \qquad \textbf{(11)}$$

Soluble substrate consumption $$(-R_{CODs}) = r_{hydrolysis} - r_{consumption} \quad \textbf{(12)}$$

Methane production $$R_{CH4} = Y_{CH4/CODs} \cdot r_{consumption} \quad \textbf{(13)}$$

As it was explained before, the ACoD of SS:WDW (1:1 v/v) avoid the limiting effect of hydrolysis step and as it was expected, the solid substrate consumption rate was much higher than the soluble substrate consumption being the consumption the rate-limiting step. In short, adding WDW to the feedstock involves a switch in the rate-limiting step in the process due to the high dissolved organic matter contained in this waste. Then, for ACoD of SS:WDW (1:1 v/v) the methane production rate depend on the consumption rate mainly because acetate degradation limiting effect.

When sole SS was used as substrate, the CODp and CODs were very similar and it was reduced with the increasing of HRT (Figure 4-A). However, in this case of the methane production obtained lower values than hydrolysis or solid/soluble substrate consumption. This means that not only hydrolysis but also methanogenesis is the rate-limiting step in this process. This occur because the high sensibility of the *Archaea* microorganisms to changes in the environment as well as the diffusional limitations of biomethane in the liquid medium [40-42]. Therefore, the control of the biomethane production in SS mono-digestion was due to the hydrolysis and methanogenesis steps.

In spite of being hydrolysis step the rate-limiting step in SS mono-digestion, in both conditions, the highest rates of methane production were reached at minimum OLR (Figure 3) and at maximum HRT = 8d (Figure 4) because the augmentation of new organic compounds used for microbial population.

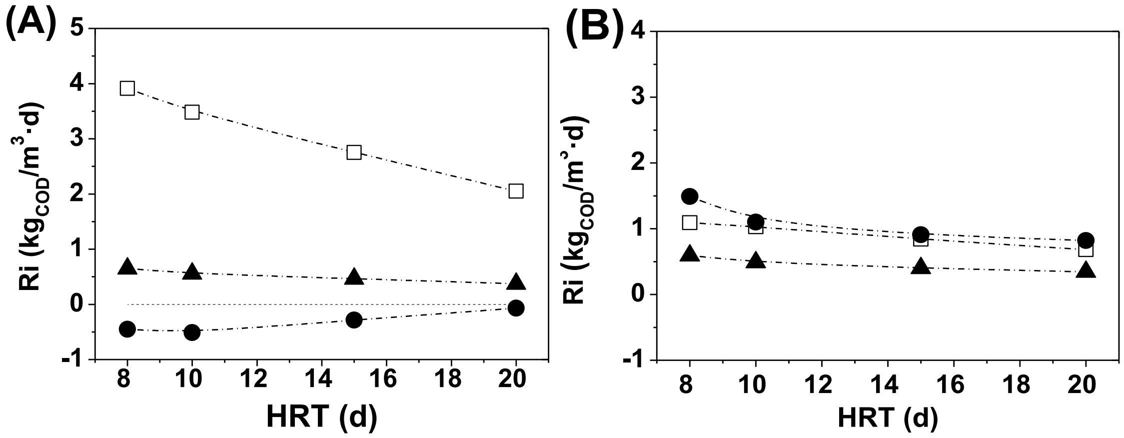


Figure 4 Influence of HRT on consumption rates in the semi-continuous anaerobic digesters of **(A)** SS:WDW (1:1 (v/v)), and **(B)** SS. *Key:* □COD_P ●COD_S ▲CH_4.

4 Conclusions

The proposed ACoD system promotes efficiently wine-making industry water sustainability by its use in WWTP as a co-substrate with sewage sludge. When SS:WDW was anaerobically digested, biodegradability of the mixture in terms of VS and CODT was 7-8% higher (52.7 and 56.7%, respectively for OLR ≤ 3.2 ($kg_{VS}/m^3 \cdot d$)) and methane production was 30% higher than when SS was used as sole substrate. It was reflected in kinetic study results that co-digestion improved biomethane potential and methane production efficiency by switching the rate-limiting step. In this sense, methane production related to the amount of organic matter was higher in the AcoD SS:WDW (0.165 kgCH4 / kgCOD) than in AD of SS alone (0.096 kgCH4 / kgCOD). These results open a new path in optimization studies of WWTP design and operation by using new agro-industrial residues in an eco-friendly way.

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Nomenclature

AD	Anaerobic digestion
COD	Chemical oxygen demand (kg/m^3)
COD_P	Particulate chemical oxygen demand (kg/m^3)
COD_S	Soluble chemical oxygen demand (kg/m^3)
COD_T	Total chemical oxygen demand (kg/m^3)
EE	Energy efficiency
HRT	Hydraulic retention time (d)
k	Kinetic constant (d^{-1})
MP	Methane productivity (L/kg·d)
OLR	Organic loading rate (kg/m^3·d)
r	Reaction rate (kg/m^3·d)
R	Production rate (kg/m^3·d)
SMP	Specific methane production (L/kg)
SS	Sewage sludge
TS	Total solids (kg/m^3)
V	Volume (L)
VFA	Volatile fatty acids (mg/L)
VS	Volatile solids (kg/m^3)
WDW	Wine distillery wastewater
WWTP	Wastewater treatment plant
X_{CH4}	Percentage of methane in the biogas (%)
Y	Stoichiometric parameter

Subscript

COD	Relating to chemical oxygen demand

Consumption	Relating to the consumption reaction
CH4	Relating to methane
Digester	Relating to the operating volume
Hydrolysis	Relating to the hydrolysis reaction
i	Relating to compound i
Inlet	Relating to the feed stream
Outlet	Relating to the effluent stream
VS	Relating to volatile solids

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Figure 4 Influence of HRT on production rates in the semi-continuous anaerobic digesters of **(A)** SS:WDW (1:1 v/v) and **(B)** SS.

Figure Captions

Figure 1 *Key* ●*feed* ■*effluent.*

Figure 2 *Key:*■*SS:WDW (1:1)* ○*SS.*

Figure 3 *Key:* ○ *hydrolysis* ■ *consumption*

Figure 4 *Key:* ☐COD_P ●COD_S ▲CH_4.