Improvement of biomethane potential of sewage sludge anaerobic co-digestion by addition of "Sherry-wine" distillery wastewater.

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Abstract

Co-digestion of sewage sludge (SS) with other unusually treated residues has been reported as an efficient method to improve biomethane production. In this work, Sherry-wine distillery wastewater (SW-DW) has been proposed as co-substrate in order to increase biomethane production and as a breakthrough solution in the management of both types of waste. In order to achieve this goal, different SS:SW-DW mixtures were employed as substrates in Biomethane Potential (BMP) tests. The biodegradability and biomethane potential of each mixture was determined selecting the optimal co-substrate ratio. Results showed that the addition of SW-DW as a co-substrate improves the anaerobic digestion of SS in a proportionally way in terms of CODs and biomethane production The optimal co-substrates ratio was 50:50 of SS:SW-DW obtaining $\%VS_{removal}$ = 54.5%; Y_{CH4}= 225.1 L CH_4/ kgsv or 154 L CH_4/kg_{CODt} and microbial population of 5.5 times higher than sole SS. In this case, $\%VS_{removal}$ = 48.1%; Y_{CH4} =183 L CH_4/ kgsv or 135 L $CH_4/kg_{CODt.}$ The modified Gompertz equation was used for the kinetic modelling of biogas production with successful fitting results (r^2 = 0.99). In this sense, at optimal conditions, the maximum productivity reached at an infinite digestion time was (Y_{CH4}^{MAX}) = 229± 5.0 NL/kg_{SV}; the specific constant was K = 25.0 ± 2.3 NL/ kg_{SV}·d and the lag phase time constant was (λ) = 2.49 ± 0.19.

Keywords: *biochemical methane potential, anaerobic digestion and co-digestion, sewage sludge, kinetic parameters, biogas production.*

1 Introduction

Sewage sludge is produced in large quantities in urban areas all over the world. This waste is usually managed by wastewater treatment plants (WWTP) where digesters are often oversized and the cost of sludge treatment representing approximately 50% of the total running cost of WWTPs. For this reason, in the context of circular economy established in H2020 European strategy, Anaerobic Digestion (AD) process is of great importance due to that this process achieve the highest utility of the sewage sludge (SS), replacing other energy resources and limiting the associated CO_2 emissions derived from SS disposal (Gherghel et al., 2019). There have been multiple studies about how improve the production of biomethane in WWTP such as pretreatments or co-digestion (Kor-Bicakci, and Eskicioglu, 2019). In this sense, co-digestion with agro-industrial wastes has been reported as an efficient method to improve biomethane production of SS as well as to manage other unusually treated residues (Maragkaki et al., 2017). In general, the main advantages of anaerobic co-digestion (ACoD) are related to the optimization of the required ratio of nutrients, the dilution of potential toxic compounds (Sosnowski et al., 2003), as well as supplying buffering capacity and establishing the required moisture content (Mshandete et al., 2004).

In the South of Spain (Cádiz region) there were 83 WWTP according to Andalusian Ministry of Environment and Town Planning (AMET 2017). Seven 7 of them were located in the “Sherry-wine” cellar region. “Sherry-wine” (SW) is the most important wine produced in Cádiz region. The winemaking process of Sherry wine is marked by specific climatic conditions and unique industrial process (“solera” system) used exclusively in the Sherry area (Roldán et al., 2010). In this region, according to Regulatory Council of D.O "Jerez-Xérès-Sherry"-"Manzanilla-Sanlúcar de Barrameda" - "Vinagre de Jerez"; RCDO Sherry, 2017) there are 63 cellars focusing not only on wine aging but also winemaking. However, as others winemaking industries, these generate large volumes of sherry-wine distillery wastewater (SW-DW) (also called wine vinasses).

SW-DW is a mixture of produced wastewater on the bottom of the distillery unit, grape juice spills and chemical cleaning products of equipment and tanks. This waste constitutes an environmental issue due to its strongly acidic pH and high organic load (around Chemical oxygen demand (COD) = 40 g O_2/L), which includes several recalcitrant pollutants such as polyphenols (e.g tannins) (Petta et al., 2017) and other chemical compounds such as melanoidins, (Yavuz 2007) fertilizer and pesticides (rich in nitrogen and phosphorous) or chaustic soda (Ioannou et al., 2013). Consequently, wineries must manage this waste using effective technologies in order to comply with environmental policies (Siles et al., 2011). In this sense, these industrial wastes are generated in a limited production period, so ACoD with SS could be economically advantageous in terms of sharing installations, ease of handling of the wastes (avoiding disposal) and improving economic viability (Mata-Álvarez et al., 2014). In addition, the co-digestion of both substrates will avoid the disposal of SW-DW on soils/evaporation lagoon. Moreover, in the case of using SW-DW as an agro-industrial co-substrate, it could enhance the C/N ratio of SS substrate (Zeshan et al., 2012). This is a simple way of improving biomethane production of SS, avoiding other expensive and complex techniques proposed in bibliography such as pre-treatments (Siles et al., 2011).

Furthermore, a proper kinetic study is helpful for reproducing the AD process and understanding the feasible inhibitory mechanism. In addition, it is important to develop an up-to-date model taking into account the different variables involved: operational conditions, mode of operations, origin of feed, type of inoculum, etc. Continuing with this approach, several mathematical models such as Logistic, Gompertz, Sigmoid (Martín et al., 2018) or Chen-Hashimoto model (Borja et al., 2003) have been applied.

AD kinetics models have been developed mainly in sewage sludge feedstock as well as in pig and crop wastes and recently, in other ago-wastes (Martín et al., 2010). In this sense, the AD of sole SW-DW has been previously studied (including kinetic evaluation) as a successful biological treatment for controlling the pollution of this waste and to recover energy in semi-continuous mode in different technologies: fixed-film reactors (Pérez et al., 2005a); high rate reactors (Pérez et al., 2005b) and after different pre-treatments such as biological (Jiménez et al. 2006) and advanced oxidation (Siles et al., 2011). However, there are no kinetics contributions to batch mode of the co-digestion of these both residues without any pretreatment. So, it is important to study its potential, operational feasibility and kinetic in order to evaluate the possibility of scaling-up such process as method of management of these both substrates together (Chowdhary et al., 2018).

In the present study, ACoD of sewage sludge (SS) and SW-DW is proposed as an effective new alternative in order to improve biomethane production in WWTPs from Sherry-wine region. The main objective of this work has been to study the influence of SW-DW in anaerobic co-digestion with SS on biodegradability and biomethane production. In addition, a kinetic model as a previous step for co-digestion scaling up process has been proposed.

2 Material and methods

2.1 Substrates and co-digestion mixtures

The substrates used in the experimental stage were collected directly from two real industrial facilities. The SS came from a secondary treatment floatation unit from Guadalete WWTP in Jerez (Cádiz, Spain). The SW-DW was obtained from Gonzalez-Byass, an ethanol producing wine-distillery plant located in Jerez. Substrates were collected fresh and stored at 4 ºC for a maximum of one month. The pH values of co-digestion mixtures were in the range of 6.0-7.0 for this reason it was adjusted to 7.0-8.0 using 2 M sodium hydroxide solution prior to digestion. Different mixtures of SS:SW-DW (% v/v) were employed in the present study (75:25; 50:50; 25:75), as well as sole SS and sole SW-DW.

2.2. Inoculum characteristics

The inoculum was collected from a mesophilic 5-L laboratory-scale Continuously Stirred Tank Reactor (CSTR) available in the Research Group operating at HRT = 20 d and fed with SS coming from secondary decanter of WWTP from Jerez (Cádiz-Spain). The characteristics of the inoculum are shown in Table 1.

2.3 Experimental set-up and procedures

BMP tests were carried out according to Angelidaki et al., (2009). Serum bottles were used as reactors with total volume of 250 mL. The effective volume was 150mL and the head space was 100 ml. Reactors were placed in an orbital shaker at 85 rpm under mesophilic conditions (35 ± 1 ºC). The digesters were loaded with a mixture of inoculum and substrate, resulting in a final concentration of 40% w/w of inoculum which is considered optimum for biogas production and substrate acclimatization (Montañés et al., 2014). The wastes were then added to the reactors in different proportions to obtain the following SS:SW-DW (% v/v) ratios: 75:25, 50:50, 25:75 (Table 2) as well as only SS and SW-DW. The control reactor, containing only anaerobic inoculums and water, was also incubated in order to determine background gas production.

Due to the strong influence of the microbial activity of the inoculum on methane yield and methane production rate, pre-incubation of the inoculum was carried out at 35 ºC for 7 days before starting the BMP assays. This procedure, which is used to reduce the endogenous methane production of the inoculum, is recommended by several authors with the aim of developing a standardized method for BMP assays (Hollinger at al., 2016).

All the reactors were run in triplicate and the averages of the data collected were calculated and reported. All the reactors were subsequently purged with 100% N_2 for 3-4 min to maintain anaerobic conditions at the appropriate pH and then sealed with natural rubber stoppers and plastic screw caps. BMP tests were performed until daily methane production meant less than 1% of total (25 days)

Biogas production and biogas composition were determined daily during the digestion period. At the end of the digestion period, pH and data on total and volatile solids (TS, VS), Volatile Fatty Acids (VFA) and total and soluble chemical oxygen demand (CODt, CODs) were collected for all the reactors so as to calculate the efficiency of the biological treatment.

2.4 Analytical methods

pH, TS, VS, CODt, CODs and TN were determined according to Standard Methods (APHA et al., 2005). pH determination was taken by pHmeter type CRISON MICROPH 2001 with a temperature probe. For TS, VS and FTS, samples were weighed in ceramic boats in a laboratory balance Cobos type and drying in oven type ELF14 de CARBOLITE.

TN was determined by using a total nitrogen analyzer provided by Skalar Company, mod. FormacsHT and FormacsTN.

VFA (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and heptanoic acid) were determined by gas chromatography (GC-2010 Plus Shimadzu). Total acidity was calculated by the sum of the individual fatty acids.

Gas composition was determined employing a gas chromatography 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 employing a sample volume of 250 μL. The carrier gas was helium at 35 kPa of pressure (Montañés et al., 2014).

2.5 Microbial analysis

FISH technique was used to determine the percentage of each microbial population group in best operational condition and in sample with sole SS in order to compare them. In FISH methodology, probe(s) 16S ribosomal ribonucleic acid (rRNA)-targeted oligonucleotide were used to identify the group of microorganisms (Zahedi et al., 2018). The counting of microorganisms had been developed using an Axio Imager Upright epifluorescence microscope (Zeiss) equipped with a 100 W mercury lamp and a 100 × oil objective. Microbial groups determined were: *Eubacteria*, *Archaea*, butyrate utilising acetogens (BUA) propionate utilizing acetogens (PUA), hydrogen utilizing methanogens (HUM) and acetate utilizing methanogens (AUM). Percentages of each group were calculated taking as total the sum of the relative amounts of *Eubacteria* and *Archaea*. Acetogens were calculated as the sum of the relative amounts of PUA and BUA. Hydrolytic acidogen bacteria (HAB) were calculated as the difference in the relative amounts of *Eubacteria* and Acetogens

(Zahedi et al., 2018). The microbiological analyses were carried out in triplicate at the end of BMP test.

2.6 Data analysis

2.6.1 Methane production and methane productivity.

Biogas production was daily determined by indirect measuring of the cumulative pressure inside the bottles with pressure transducers. Pressure data were used to infer the volume of biogas at standard temperature and pressure conditions, according to the ideal law of gases, Eq. (1).

$$P \bullet V = n \bullet R \bullet T \qquad \textbf{(1)}$$

where *P* is absolute pressure (kPa), *V* is volume (m^3), n is amount of substance (moles) *T* is temperature (K), and *R* is the universal gas constant (8.3145 L·kPa/K·mol).

Cumulative methane volume production was calculated by means of the sum of the daily methane volume as indicated in Eq. (2):

$$V_{CH4}^{t}(NL) = \sum_{i=1}^{i=t}(V_{CH4}^{i} - V_{control}^{i}) \qquad \textbf{(2)}$$

Where V_{CH4}^{t} is the net volume of methane, V_{CH4}^{i} is the experimental volume of methane measured when co-substrate is used and $V_{control}^{i}$ is the volume of methane produced in the control experiment. Methane productivity (Y_{CH4}) in base of initial VS was calculated as V_{CH4}^{t} per kg of initial VS (NL_{CH4}/kg_{VS}) in order to developed the kinetic modelling. Experimental biomethane potential (BMP_{exp}) was calculated as the asymptote of the methane productivity curve. Methane productivity (Y_{CH4}) in base of initial COD was calculated as V_{CH4}^{t} per kg of initial COD (NL_{CH4}/kg_{CODt}) in order to compare the results with bibliography.

2.6.2 Substrate biodegradability.

Substrate biodegradability was related to the removal rates obtained after AD in terms of biodegradability parameters removal as shown in Eq. (3):

$$parameter(P)\ removal(\%) = \frac{P_0 - P_t}{P_0} \cdot 100 \qquad \textbf{(3)}$$

Where "P" is the biodegradability parameter analysed in this study: CODt, CODs, VS, VFA and P_0 and P_t are the initial and final value of the respective parameter.

2.6.3 Kinetic modelling.

Biogas production during AD involves a complex reactions network with many stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). Therefore, it is necessary to assume several simplifications in order to mathematically describe the macroscopic system behaviour. In the present study, the modified Gompertz model (Eq. 5) was used to predict biogas production. This model has been the most widely applied kinetic model for describing anaerobic digestion by previous studies (Awais et al., 2016; Zhen et al., 2016; Zhao et al., 2018). The modified Gompertz model assumes that biogas production is proportional to microbial activity and that gas production follows an exponential rise to reach maximum level.

$$Y_{CH4}\left({NL_{CH4}}/{kg_{SV0}}\right) = \mathrm{Y_{CH4}^{MAX}} \cdot \exp\left[-\exp\left(-\frac{\mathrm{K}\cdot e^{1}}{\mathrm{Y_{CH4}^{MAX}}} \cdot (\lambda - t) + 1\right)\right] \qquad \textbf{(5)}$$

Three kinetic parameters are required in the modified Gompertz model to predict the evolution of the methane productivity: the maximum yield reached at an infinite digestion time ($\mathrm{Y_{CH4}^{MAX}}$), the specific constant rate (K) and the lag phase time constant (λ). Kinetic modelling was performed employing OriginPro® software. Simple non-linear curve fitting was carried out to reproduce the biogas methane production for each assay.

3 Results and Discussion

The characteristics of raw co-substrates are shown in Table 1. As it can be observed the characterization values in SS are in the common range presented in bibliography

(Thorin et al., 2018). SW-DW also showed values of COD, TS, VS, and pH in the common range reported by bibliography: CODt = 0.8-182 g O_2/L, TS = 2-127 g/L, VS = 0.12-1.33 g/L and pH = 3.5-7.3 (Beltrán et al., 1999; Petrucciouli et al., 2000; Benítez et al., 2003; Eusebio et al., 2004; Pérez et al., 2006; Lucas et al., 2009). However, VFA value was lower than bibliography (VFA = 1.33-77 g/L). This fact can be explained because the type of grape that was used in the sherry-wine making process ("palomino" grape) which contains low values of total acidity and high pH values (García et al., 2009).

Moreover, SS showed a low C/N ratio (Table 2). Using only SS could affect AD by rapid consumption of nitrogen. This could affect AD operation by accumulation of VFAs (Li et al., 2011) and inhibiting methanogens leading to low biogas production. However, when SW-DW was increased, the C/N ratios were higher (Table 2) contributing to enhance AD development. In spite of C/N ratio varies with type of substrates (Li et al., 2011); it is known that the optimal C/N ratio for a proper AD is 20-30 (Zeshan et al., 2012); which is reached in this work when concentrations of SW-DW were 75 and 100%.

3.1 Substrate biodegradability

Substrate biodegradability was measured by removal of initial characteristics in serum bottle. Characterization parameters at the beginning and at the end of the BMP tests are shown in Table 2. In general, all the parameters were slightly reduced when SW-DW was increased because the lower content of organic matter. In order to compare reduction tendency, it has been calculated the removal percentage of each parameter (Figure 1). The biodegradability of SS in terms of $CODt_{removal}$ is similar than co-substrate mixtures when SS ≥ 50% obtaining values around 48.5 ± 1.11%. Whereas, the biodegradability values of co-substrates were enhanced when proportion of SS < 50% obtaining, $\%CODt_{removal}$ values of 56.3% ± 4.1 for 25:75 and 66.5 ± 8.7 % for SW-DW . The increasing in $COD_{removal}$ tendency is more remarkable regarding CODs. In this case, in order of decreasing removal of CODs: 86% for SW-DW > 76.7% for 25:75 of SS:SW-DW (v/v) > 65% for 50:50 of SS:SW-DW (v/v) > 54% for75:25 of SS:SW-DW (v/v) > 40.8% for only SS. In fact, there was a linear relationship ($\%CODs_{removal} = 0.452 \cdot \%SW\text{-}DW + 41.9$; $r^2 = 0.995$) for this parameter as it can be seen in Figure 1. So, in spite of linear augmentation of CODs elimination, CODt removal did not follow this tendency until proportion of SW-DW was > 50%. At this point, SW-DW soluble compounds were in high quantity and the contribution of CODs in the mixture with SS to CODt was higher (70%).

Attending to $\%VS_{removal}$, a similar tendency that CODt was observed. In this case, the $\%VS_{removal}$ values obtained for SS, 75:25 and for 50:50 of SS:SW-DW (%v/v) were 50.0% ± 0.8. After that, when SW-DW was 75% the values were increased to 54% ± 0.4 and when SW-DW was 100% the $VS\%_{removal}$ was 61.4% ± 2.7. So, in general the increment of SW-DW proportion in the co-substrate mixture improves the removal rate of main biodegradability parameters of SS after biological treatment, due to the higher content of dissolved organic matter provided.

Finally, in general, the analysis of VFA content at the end of BMP test showed that there was an accumulation of 8% of VFA after AD of SS as it was expected by poor C/N ratio. However, this accumulation is not enough for inhibiting the whole process of AD but reducing biomethane production as it can be seen in the next section. However, after ACoD the elimination of VFA was higher when %SW-DW was increased, being complete at concentration ≤ 75% of SW-DW where C/N ratios was between 20-30.

3.2 Biogas production in BMP tests

The evolution of the cumulative gross methane volume for each run (including the control test) can be observed in Figure 2 (A). It can be seen that the methane production was increasing with content of SS. The highest methane production was obtained for both anaerobic digestion of SS and 75:25% v/v of SS:SW-DW, and the lowest methane production was obtained when the substrate was only SW-DW. In all the cases, the maximum percentage of CH_4 in biogas was 70%. Initial characterization of the employed substrates showed that SS contains a higher organic load (in terms of VS, as well as CODt) than SW-DW (Table 2). Thus, the higher net amount of biodegradable organic matter in SS leads to a higher gross methane volume production.

However, in order to compare the biomethane potential from different wastes, methane productivity in base of organic matter (VS and CODt) must be calculated to normalize the values. In this sense, the evolutions of the methane yield during the sole digestion of SS and SW-DW and the co-digestion of different mixtures are shown in Figure 2 (B). According to these results, the methane yield in base of VS of co-digested mixtures was proportional to the composition employed. In this respect, the addition of SW-DW as a co-substrate in the anaerobic digestion of SS improved the methane yield in all the studied cases. In order of decreasing it was obtained 300 NL CH_4/kg_{VS0} for SW-DW> 250 NL CH_4/kg_{VS0} for 75% of SW-DW > 225 NL CH_4/kg_{VS0} for 50% v/v of SW-DW > 210 NL CH_4/kg_{VS0} for 25% v/v of SW-DW > 175 NL CH_4/kg_{VS0} for SS (Figure 2A).

Regarding CH_4 yield with respect $CODt_0$ (data not shown), the maximum yield was 154 L CH_4/kg_{CODt} for 50:50% v/v of SS:SW-DW; following by 146 LCH_4/kg_{CODt} for 75:25% v/v of SS:SW-DW and 135 LCH_4/kg_{CODt} for the rest (sole digestions of SW-DW and SS and co-digestion at 25:75% v/v SS:SW-DW proportion). So, the maximum productivity obtained was achieved by mixing 50:50% v/v of both co-substrates. Similar CH_4 yield results were obtained from previous studies using pretreated sludge by microwave disintegration as a substrate of anaerobic digestion (Kavitha et al., 2018), being the mixture with SW-DW more economically feasible.

It should be noted that pre-incubation of the inoculum at mesophilic temperature for 7 days was found to be an appropriate treatment to reduce endogenous methane production, as it can be seen from the results of the blank assay. Some authors have previously established that inoculum production should be below 20 % of total methane production in the BMP test (Hollinger et al., 2016). In the present study, endogenous methane production did not exceed 11 % of the production from co-digestion of the studied substrates. Furthermore, the inoculum still remained metabolically active after pre-incubation, as it is assumed in initial methane production in BMP tests. Therefore, the results obtained in this work validate the experimental procedure.

3.3 Kinetic modelling

For each assay, the modified Gompertz model was fitted to experimental data as shown in Figure 3. Generally, there is an excellent overall agreement between the model prediction and the experimental data, reaching the highest regression coefficients in all cases (r^2 results above 0.99). This means that this model might explain 99% of the total variation of experimental data (Figure 3). As it can be seen in the Figure 3, when proportion of SW-DW was increased, the inflection point (K/e) appeared sooner: 7.5 d (A) > 7 d (B) > 6.5 d (C) > 5d (D) > 4d (E). So, the slope of the lineal growing from ending of lag phase to inflection point was higher when higher SW-DW was used, leading to higher growing velocity.

The values for each kinetic parameter and their statistical errors as well as those for the experimental BMP are summarized in Table 4. When proportion of SW-DW was increased, the K was augmented and the lag phase was reduced. These both facts are the consequence of more available organic matter that permit microorganisms to grow sooner (lower λ) and easily, reaching higher K values. In this sense, methanogenic population growing lead to more production of methane and hence higher Y_{CH4}^{MAX} values. Regarding this parameter, the meaning of the theoretical kinetic parameter is directly related to the experimental one. The relative error between both parameters had a difference below 7% in all runs (Table 4), showing an excellent model prediction of the studied system. It is also important to remark that the lag phase is higher when higher proportion of SS is used in the co-digestion.

Table 4 also summarizes the values of the kinetic parameter of the modified Gompertz model previously published by other authors. When SW-DW is used as co-substrate, the Y_{CH4}^{MAX} parameter is higher (218-294 NL/kg$_{VS}$) than those obtained using only SS (167 NL/kg$_{VS}$) (Cordova et al., 2017) or in co-digestion with synthetic organic fraction of municipal WWTP or microalgae (148 and 164 NL/kg$_{VS}$ respectively) (Nielfa et al., 2015 and Zhen et al., 2016).

However, when SS was used as substrate the kinetic parameters K and Y_{CH4}^{MAX} were similar than bibliography values (Table 4) supporting the repeatability and reliability of the BMP method. Only lag phase was higher when using inadapted inoculum.

In this study, when SW-DW is used alone or as co-substrate, the Y_{CH4}^{MAX} parameter was also higher than those obtained for only SW-DW in previous research (Syaichurroz et al., 2013 and Budiyono 2013-2014, Table 4) probably because the origin of the vinasses was the sugarcane production instead of sherry-wine production. This underline the availability of organic matter presents in SW-DW that is also reflected in higher K and lower λ parameters.

The influence of feedstock composition on the value of the kinetic parameters is shown in Figure 4. As previously stated, BMP depends directly on the composition of the employed substrate, being proportional to the ratio of the mixture.

The influence of substrate composition on the specific constant rate seems to be analogous to the observed trend for maximum methane production. The lowest value was obtained for anaerobic digestion of SS, while the highest value was observed for SW-DW. In the co-digestion assays, the specific constant rate is proportional to the composition of the mixture. Consequently, co-digestion of SS with SW-DW leads to a faster rate of anaerobic degradation and its associated biogas production than anaerobic digestion of SS alone.

Finally, the lag phase time constant (λ) shows the duration of the first stage of the process, during which methane production occurs at a slow rate. This macroscopic kinetic parameter is probably associated with the hydrolysis stage, which is the main rate-determining step in anaerobic digestion. In this sense, SW-DW contains many simple organic compounds that anaerobic bacteria are able to metabolize easily into biogas such as organic acids, carbohydrates and ethanol (Nayak et al., 2018). On the other hand, SS

contains a high amount of lignocellulosic compounds, which need more time to be degraded increasing the lag phase (Syaichurrozi et al., 2013). Regarding the results of this work, biogas started to be produced after a lag phase of 0.43 days during SW-DW fermentation, compared to 2.58 days in SS fermentation. It should be emphasized that co-digestion reduces lag phase time considerably, as it can be seen in Figure 4 (C).

3.4 Microbial population at optimal conditions

A summary of the main microbial groups involved in the co-digestion of SS:SW-DW % v/v 50:50 (the best conditions) and mono-digestion of SS is shown in Table 5. Figure 5 shows some photomicrograph of microbial groups in the SS:SW-DW 50:50 % BMP test. Increasing in biomethane production is mainly reflected in total microbial population augmentation. Total microbial population obtained in BMP of SS:SW-DW% v/v 50:50 was $2.46 \cdot 10^{10}$ cell/ml, 5.5 times higher than those obtained in SS BMP test ($4.49 \cdot 10^{9}$ cell/ml). Microbial population groups also showed different profiles at these both conditions. Thus, *Eubacteria* percentage was higher in the case of using only SS as substrate than in the case of 50:50% v/v of SS:SW-DW. Specifically, acetogenic bacteria was 53.4% in the case of SS and 18% in the case of 50:50% v/v SS:SW-DW. However, because higher population in the former case, it was $2.39\ 10^{9}$ cell/ml of acetogenic bacteria in SS against $4.42 \cdot 10^{9}$ cell/ml of 50:50% v/v SS:SW-DW. Attending sub-groups in acetogenic bacteria the proportion BUA/PUA were 2.23 and 3 for SS and 50:50% v/v of SS:SW-DW respectively. On the other hand, in both cases HAB was low (0-1%) due to hydrolytic stage had been concluded. In addition, when 50:50% v/v of SS:SW-DW was used, 81.9% of population was *Archaea* (being the majority AUM, 74.4%) against only 45.2% when SS is used as substrate (being the majority also AUM, 41.8%).

Hence, in general, it can be said that the different ratios *Eubacteria*:*Archaea* were observed in the SS and SS:SW-DW BMP tests: 54.8:45.2 and 18.1:81:9, respectively; making co-digestion microbial population more rich in *Archaea* (above all aceticlastic methanogens).

4 Conclusions

The addition of SW-DW, as a co-substrate, improves the anaerobic digestion of SS in a proportionally way in terms of $CODs_{removal}$ and biomethane production. Optimal conditions were 50:50% v/v SS:SW-DW with removal values of $\%VS_{removal}$ = 54.5%; BMPexp = 225 L CH_4/ kg_{VS} and productivity values of 154 L CH_4/kg_{CODt} . The experimental results indicate that, the Gompertz model can explain the final behaviour and kinetics of the process with high degree of reliability ($r^2 > 0.99$) and pointing to the best co-digestion configuration. In this sense, kinetic parameters determined at optimal conditions 50:50% v/v of SS:SW-DW were (K = 25.0 ± 2.3 NL/ kg_{VS}·d; λ = 2.49 ± 0.19 and Y_{MAX} = 229± 5.0 (NL/kg_{VS}). This results are also supported by microbial analysis where there was an enrichment of *Archaea* group in co-digestion, particularly in aceticlastic methanogens. This optimal co-digestion mixture, can be used as starting point in order to study the scaling up of the process. Controlled co-digestion of SS and SW-DW should be desirable in order to obtain higher amount of methane in WWTPs of "Sherry-wine" area by regularly addition of SW-DW collected. In this sense, because the proximity and the volume of generation of both substrates, "Sherry-wine" region can be considered as being well placed geographically for a successful management of both substrates by co-digestion without using any pre-treatment saving energy and cost.

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Nomenclature

Acet Acetogenic bacteria

Arch Archaea

AUM Acetogens utili

BMP Biomethane potential (NL_{CH4}/kg_{SV})

BUA Butyrate utilising acetogens

CODs Chemical oxygen demand (soluble)

CODt Chemical oxygen demand (total)

Eub Eubacteria

$g_{H\text{-}Ac}/L$ Acetic acid concentration (g/L)

HAB Hydrolitic acidogenic bacteria

HRT Hydraulic retention time (d)

HUM Hydrogen utilising bacteria

K Kinetic parameter from the modified Gompertz model ($NL_{CH4}/kg_{SV}\cdot d$)

PUA Butirate utilising acetogens

TS Total solids

SS Sewage sludge

Y_{CH4} Methane yield (NL_{CH4}/kg_{SV})

Y_{CH4}^{MAX} Maximum methane yield from the modified Gompertz model measured in NL_{CH4}/kg_{SV}.

V_{CH4}^{t} Net volume of methane (NL_{CH4})

VFA Volatile Fatty Acids

VS Volatile solids

SW-DW Sherry-wine distillery wastewater

WWTP Wastewater treatment plant

λ Lag-phase parameter from the modified Gompertz model (d)

Subscript

t	Relating to time t
0	Relating to the initial condition
H-Ac	Relating to acetic acid

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TABLES

Table 1 Inoculum and raw co-substrates characteristics

Table 2 Initial and final characteristics of substrates in serum bottle.

Table 3 Kinetic parameter of the Gompertz model.

Table 4 Summary of published studies on kinetic modelling of SS and WDW employing the modified Gompertz model: value of the kinetic parameter of the model.

Table 5 Percentages of groups of microbiota for sole SS and 50:50% v/v of SS:SW-DW.

FIGURES

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Figure 1 Influence of feedstock composition on the %removal of main biodegradability parameters.

Figure 2 **(A)** Evolution of gross methane volume production for each assay
(B) Evolution of methane yield for each substrate

Figure 3 Evolution of methane yield and kinetic Gompertz model prediction for each substrate and co-digestion mixtures: **(A)** SS **(B)** SS:SW-DW 75:25 (% v/v); **(C)** SS:SW-DW 50:50 (% v/v); **(D)** SS:SW-DW 25:75 (% v/v); **(E)** SW-DW.

Figure 4 Influence of feedstock composition on the kinetic parameters of the modified Gompertz model **(A)** Maximum yield obtained, **(B)** specific constant rate, and **(C)** lag phase time constant.

Figure 5 Electron Microscopy photos of microbial population from different groups of microorganisms after BMP test. Operational conditions: 50:50 SS:SW-DW, T = 35 ºC, Dilution Factor (DF) = 1:200.

Figure Captions

Figure 1 CODt: square; CODs: circle; VS: upward triangle; VFA: downward triangle.

Figure 2 Control: square; SS:circle; 75:25 (% SS:SW-DW): upward triangle; 50:50 (% SS:SW-DW): downward triangle; 25:75 (% SS:SW-DW): diamond; SW-DW: star.

Figure 3 Methane yield: square; kinetic Gompertz model prediction: line.

Figure 4 Kinetic parameters of the modified Gompertz model.

Figure 5. White dots : ufc.

Table 1 Inoculum and raw co-substrates characteristics

Parameters	Inoculum	SS	SW-DW
pH	7.8	7.6	6.4
CODt (kg/m^3)	19.9 ± 0.4	53.9 ± 1.2	24.6 ± 2.2
CODs (kg/m^3)	9.7 ± 0.3	19.0 ± 0.3	20.7 ± 0.6
TS (%)	2.09 ± 0.03	3.67 ± 0.01	1.47 ± 0.11
VS (%)	1.21 ± 0.01	2.69 ± 0.03	1.06 ± 0.09
VS/TS (%)	58.0 ± 1.3	73.8 ± 0.5	72.6 ± 2.9
Alkalinity (g_{CaCO3}/L)	5.81	3.53	0.019
VFAt ($g_{H\text{-}Ac}$/L)	0.41	2.85	0.75
TN (kg/m^3)	2.15	14.8	1.09
C/N	9.2	5.2	17.5

Table 2 Initial and final characteristics of substrates in serum bottle.

Parameters (kg/m^3)	**SW-DW (% v/v)**				
	0	**25**	**50**	**75**	**100**
$CODt_0$	35.5 ± 0.2	32.0 ± 1.3	26.7 ± 0.4	24.5 ± 0.4	20.6 ± 0.9
$CODt_f$	18.8 ± 0.4	16.1 ± 1.0	13.7 ± 0.6	10.7 ± 0.6	6.9 ± 0.6
$CODs_0$	15.7 ± 0.3	16.2 ± 0.2	16.3 ± 0.3	17.2 ± 0.1	16.2 ± 0.4
$CODs_f$	9.3 ± 0.4	7.4 ± 0.4	5.7 ± 0.3	5.9 ± 0.6	2.3 ± 0.1
VS_0*	1.96 ± 0.05	1.73 ± 0.04	1.52 ± 0.09	1.20 ± 0.02	0.95 ± 0.03
VS_f *	1.03 ± 0.01	0.89 ± 0.01	0.70 ± 0.01	0.54 ± 0.02	0.37 ± 0.01
$VFAt_0$ **	1.68	1.21	1.19	0.92	0.63
$VFAt_f$ **	0.12	0.05	0.0247	n. d.	n. d.
C/N_0	5.2	10.8	16.4	21.9	27.5

** Unit: %; ** unit : gH-Ac/L.*

Table 3 Kinetic parameter of the modified Gompertz model.

SS:SW-DW (% v/v)	$\mathbf{Y_{CH4}^{MAX}}$ **(NL/kg$_{VS}$)**	**K (NL/ kg$_{VS}$·d)**	**λ (d)**	**r^2**	**BMP_{exp} (NL/kg$_{VS}$)**	**Relative error (%)**
SS	195.8 ± 4.6	13.4 ± 0.9	2.58 ±0.22	0.995	183 ± 11.6	6.7
75:25	218.8 ± 5.8	19.8 ± 1.8	2.60 ±0.24	0.989	210 ± 16.2	4.0
50:50	229.8 ± 5.0	25.0 ± 2.3	2.49 ±0.19	0.990	225 ± 23.4	2.1
25:75	256.0 ± 2.0	26.2 ± 0.8	1.25 ±0.07	0.998	255 ± 13.4	0.2
SW-DW	294.6 ± 3.5	31.7 ± 1.8	0.43 ±0.12	0.995	301 ± 15.4	2.5

Table 4 Summary of published studies on kinetic modelling of SS and wine distillery wastewater employing the modified Gompertz model: value of the kinetic parameter of the model.

Feedstock	Y_{CH4}^{MAX} (NL/kg$_{VS}$)	K (NL/ kg$_{VS}$·d)	λ (d)	r^2	Reference
Sewage Sludge	148.1	31.4	0.00	0.96	Nielfa et al. (2015)
	167.0	32.4	< 0.01	0.98	Cordova et al. (2017)
	163.5	13.4	0.00	0.94	Zhen et al. (2016)
	195.8	13.4	2.58	0.99	This study
Wine Distillery Wastewater	140.1	16.1	0.21	0.97	Syaichurrozi et al. (2013)
	115.0	24.7	0.80	0.99	Budiyono et al. (2013)
	39.4	7.0	0.96	0.99	Budiyono et al. (2014)
	296.6	31.7	0.43	0.99	This study

Table 5. Percentages of groups of microbiota for sole SS and 50:50% v/v of SS:SW-DW.

% SW-DW	Microbial population							
	Eub	HAB	Acet	PUA	BUA	Arch	HUM	AUM
0%	54.8	1.5	53.4	16.2	37.2	45.2	3.4	41.8
50%	18.1	0.1	18.0	4.41	13.5	81.9	7.5	74.4

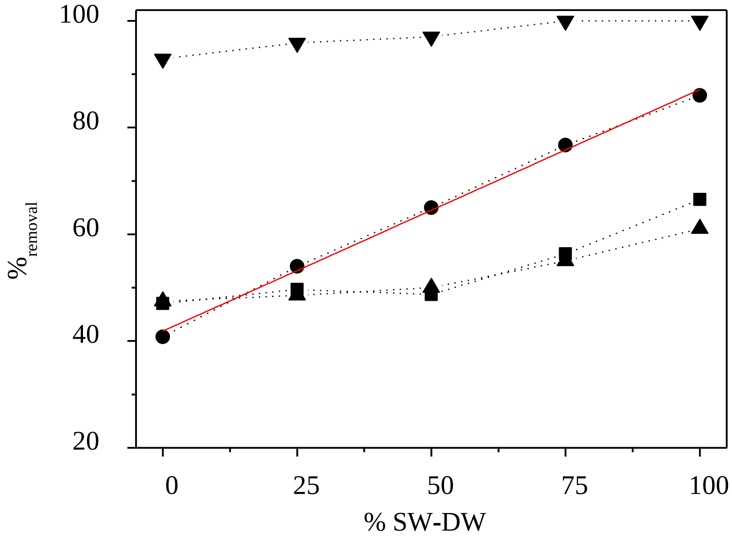

Figure 1. Influence of feedstock composition on the %removal of main biodegradability parameters. (CODt: square; CODs: circle; VS: upward triangle; VFA: downward triangle; red line: linear adjustment of data).

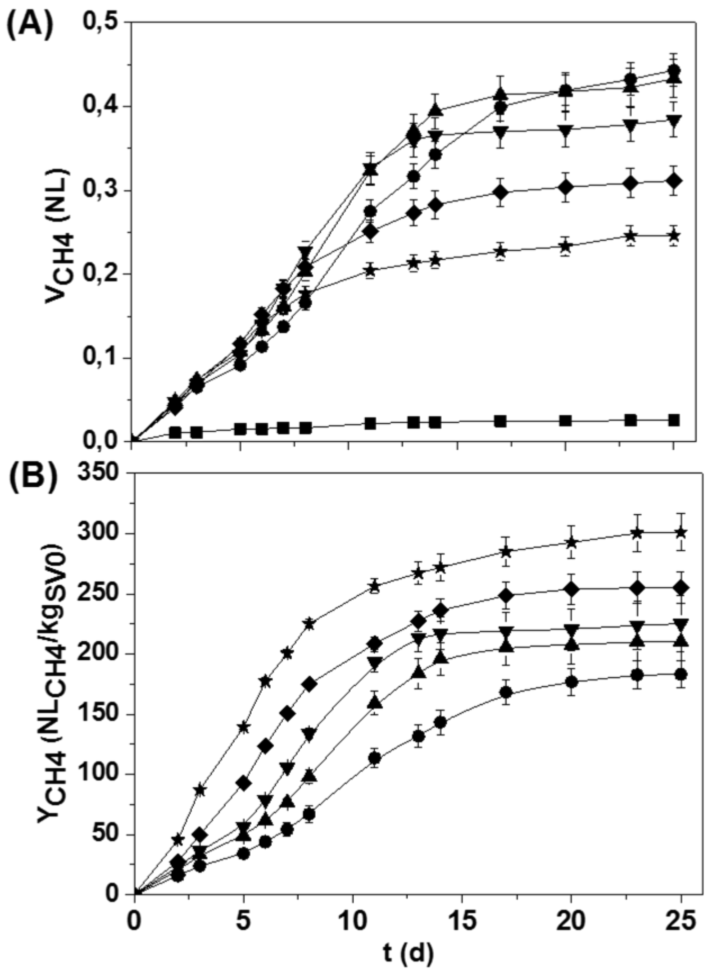

Figure 2 **(A)** Evolution of gross methane volume production for each assay **(B)** Evolution of methane yield for each substrate (control: square; SS: circle; 75:25 (% SS:SW-DW): upward triangle; 50:50 (% SS:SW-DW): downward triangle; 25:75 (% SS:SW-DW): diamond; SW-DW: star).

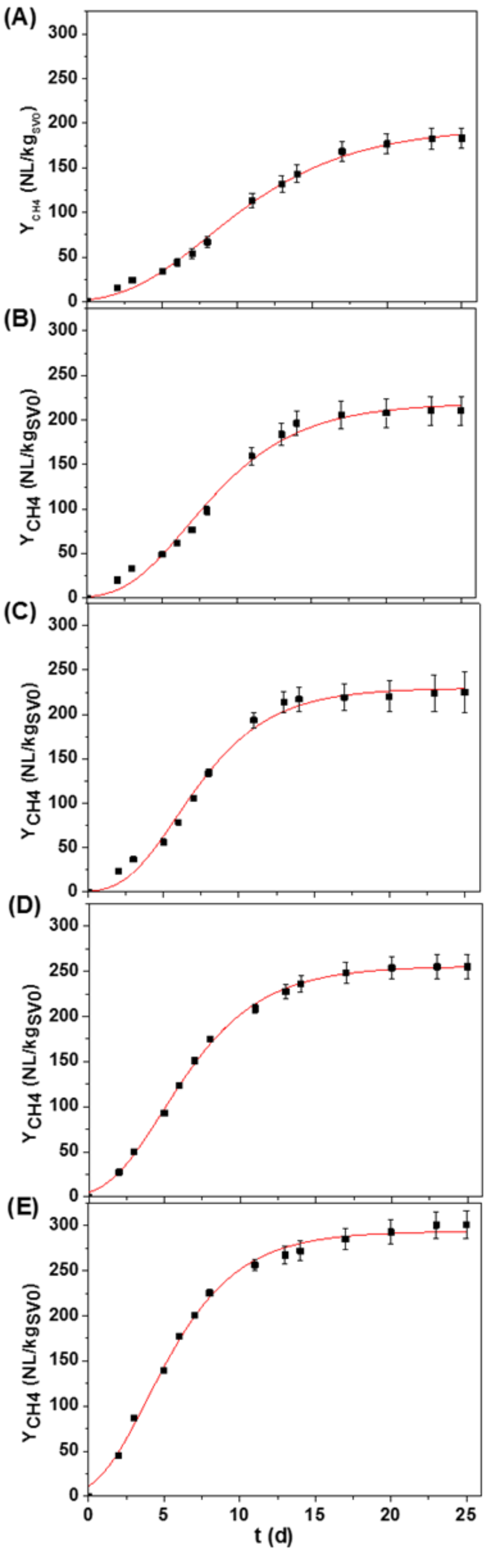

Figure 3 Evolution of methane yield (square) and kinetic Gompertz model prediction (line) for each substrate and co-digestion mixtures: **(A)** SS v/v); **(B)** SS:SW-DW 75:25 (% v/v); **(C)** SS:SW-DW 50:50 (% v/v); **(D)** SS:SW-DW 25:75 (% v/v); **(E)** SW-DW

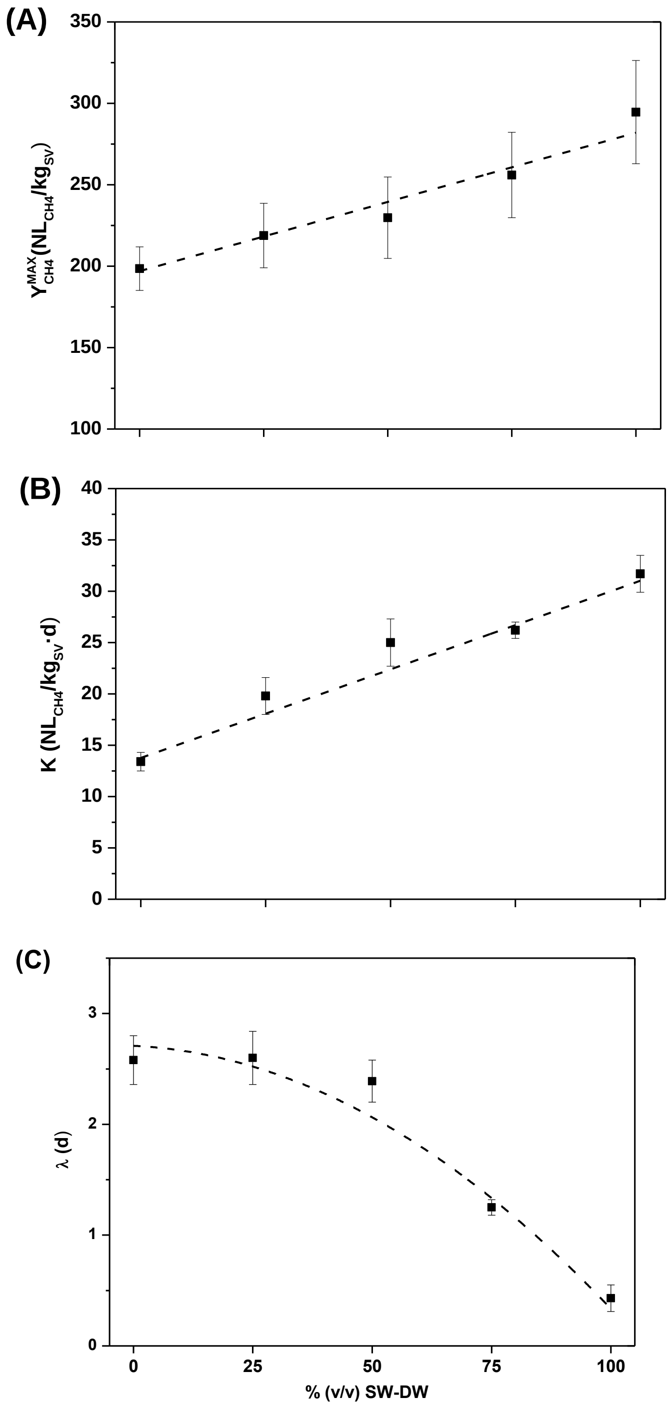

Figure 4 Influence of feedstock composition on the kinetic parameters of the modified Gompertz model **(A)** Maximum productivity obtained, **(B)** specific constant rate, and **(C)** lag phase time constant.

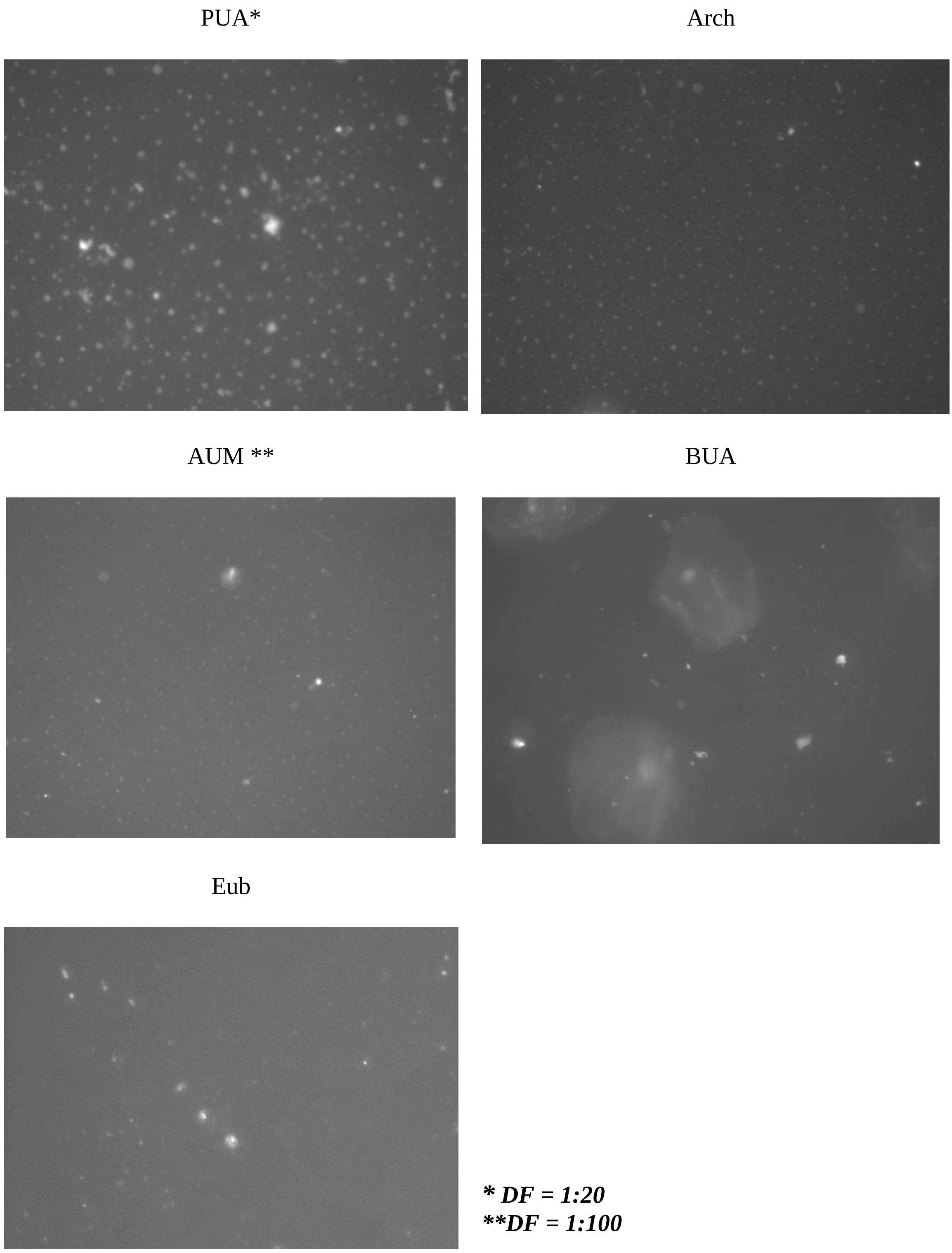

Figure 5. Electron Microscopy photos of microbial population from different groups of microorganisms after BMP test. Operational conditions: 50:50 SS:SW-DW, T^a= 35 ºC, Dilution Factor (DF) = 1:200.