Optimization of Methane Production Based on Mixture Content of Sewage Sludge, Food Waste, and Glycerol

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Abstract: This paper presents bench scale experiments related to anaerobic co-digestion of aerobic sewage sludge from a pilot WWTP (waste water treatment plants), raw glycerol from a biodiesel industry and food waste. Assays were conducted in 100 mL non-stirring penicillin vessels, at 30 ℃ and planned according to three optimization phases: (1) binary mixture of sewage sludge and FW (food waste); (2) binary mixture of sewage sludge and glycerol; and (3) ternary mixture of sewage sludge, FW, and glycerol. In the first and second phases, the highest SMP (specific methane production) was achieved by 10% (v/v) FW and 0.5% (v/v) glycerol mixtures. The optimization of the ternary mixture during the third phase was reached by the combination of 10% (v/v) FW and 0.4% (v/v) glycerol. Despite the low SMP value, the addition of glycerol and FW contributed to doubling the SMP value of the sludge sample control.

Key words: Anaerobic digestion, co-digestion, sludge, organic fraction municipal solid waste, glycerol.

Symbols and Abbreviations

CESA	Environmental Sanitation Experimental Center (CESA-UFRJ)
CH_4	Methane gas
CHP	Combined heat and power unit
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
FW	Food waste
OFMSW	Organic fraction of municipal solid waste
RG	Raw glycerol
SCOD	Soluble chemical oxygen demand
SMP	Specific methane production
TA	Total alkalinity
TVA	Total volatile acids
VS	Volatile solids (= volatile total solids)
VSS	Volatile suspended solids
VSSD	Volatile suspended solids destruction
VTS	Volatile total solids
WWTP	Waste water treatment plant

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1. Introduction

The gradual expansion of municipal sewerage and sewage treatment systems in Brazil increases the importance of knowledge about processes and technological routes that may improve sludge management efficiency at WWTP (waste water treatment plants). These processes include lower generation of sludge mass, higher generation of biomethane, and higher efficiency of methane use, and are as well as processes applied for material recovery and biosolid production.

Recovery of biomethane stands out as an attractive route to produce decentralized renewable energy, and its use in a WWTP will find different possible paths. The optimization of recovered energy use will depend on energy and sludge mass balances related to a specific route and on capital and expenditures. Recovered energy may be used for digester heating to impose thermophilic conditions and higher biogas

production. As a fuel, methane may also be used for sludge volume reduction in thermal drying unit or for heat and power generation on the CHP unit. Of course, commercialized prices of the electric energy market as well as opportunities and costs related to final disposal, material recovery, and beneficial use of sludge mass are driving forces for process optimization and better management of WWTP.

Brazilian national solid waste policy establishes that before final disposal in a sanitary landfill, municipalities must practice residue recovery and residue treatment. To fulfill this requirement, a possible viable technology route may be to combine treatment of OFMSW (organic fraction of municipal solid waste) and sewage sludge in anaerobic digester units at the WWTP.

The co-digestion of sewage sludge with other high-carbon residues and different binary and ternary mixtures have been tested for anaerobic process optimization and higher methane generation. The combination of different organic substrates increases the diversity of carbon molecules structure, which improves anaerobic bacteria metabolism, destruction of volatile solids, and generation of biogas and methane [1-3]. In this context, OFMSW has been largely studied as a potential residue for co-digestion of sewage sludge [4, 5].

As a product with limited recovery options, Brazil faces the challenge of managing the significant generation (594 kt/yr) of residual glycerol produced by the biodiesel industry [6]. Due to its large amount of easily biodegradable carbon content, Athanasoulia et al. [7] and Jensen et al. [8] state that glycerol could be an appropriate residue for sewage sludge co-digestion.

Based on different reactor models, VTS (volatile total solids) and COD (chemical oxygen demand) volumetric loads, temperature conditions and hydraulic detention time, anaerobic co-digestion of binary and ternary mixtures containing glycerol and organic residues from different sources have been evaluated for the past 10 years. Although the results have shown that

the addition of glycerol may favorably contribute for anaerobic process co-digestion, authors generally agree that different substrates must be combined under strict control due to initial organic load increase and mixture content of inhibitory as well as elements toxic to microorganism metabolism [9-11]. Carmona et al. [12] and Viana et al. [13] report that glycerol may present up to 46 g chloride/L, while a concentration between 5 and 9 g chloride/L is considered inhibitory for methanogenesis [14-17].

According to a review of literature, in a binary combination with sewage sludge, process optimization is limited to a concentration of glycerol up to 1% [19-25] or 2-3% [7, 8]. In most of these studies, authors observed similar results with regard to raw glycerol limit concentration of 1% (v/v), as it led to an organic overloading and a marked decrease in biogas production after a few days of operation. A rapid formation and accumulation of volatile acids and a consequent drop in pH values below those recommended for a good methanogenic activity have been suggested as the main causes. On the other hand, the same studies observed that concentrations equal to or less than 1% (v/v) were able to impose a higher biogas production rate.

In combination with other organic wastes, the concentration of glycerol can be much larger. Physico-chemical structures of other substrates than sewage sludge probably offer better conditions to create resilient efforts on the final mixture and to support occasional effects due to organic load increase and mixture content of inhibitory and toxic elements, in special salinity.

Working with substrates other than sewage sludge with a ternary mixture of swine manure, corn silage, and 6% (v/v) glycerol, Amon et al. [9] obtained an increase of 34% in specific methane production when compared to the binary mixture without glycerol. The research also indicated that a higher glycerol concent ration was able to decrease the biogas production.

Ma et al. [26] reported that methane production

increased six times and achieved 57% biogas composition, due to 85% of COD removal achieved during the co-digestion of potato industry effluent and glycerol. Working with swine manure and 20% (v/v) of glycerol mixture, Astals et al. [27], indicated an increase of 125% in specific methane production when compared to just swine manure anaerobic digestion.

Under mesophilic conditions, Robra et al. [28], tested the co-digestion of bovine manure and 5%, 10%, and 15% (v/v) glycerol, and they concluded that the results obtained under 10% and 15% were almost the same, with an increase of 14% in methane production. Heaven et al. [29] reported biodiesel industry efforts to produce biogas from the co-digestion of a ternary mixture of oleaginous, glycerol, and animal manure.

Regueiro et al. [1] compared co-digestion process of a ternary mixture of 84% swine manure, 5% fish processing waste, and 11% glycerol (m/m) with 100% swine manure. Biogas and methane production from the ternary mixture co-digestion reached values 50% and 10% higher, respectively, than those obtained from just the swine manure digestion.

According to Astals et al. [30], from the co-digestion of a binary mixture of swine manure and 4% (v/v) glycerol, 62% removal of COD was obtained, which resulted in 64% increase of biogas production and 235 mL CH_4/g $VTS_{applied}$ of specific methane production. Martin et al. [31] reported a specific methane production of 385 mL CH_4/g $VTS_{applied}$ derived from the co-digestion of orange peel and glycerol.

Based on two different studies, Serrano et al. [32], compared the specific methane production obtained during the co-digestion of a binary mixture of strawberry waste and fish processing waste, and a ternary mixture, in which one glycerol was added. The result indicated that 230 mL CH_4/g $VTS_{applied}$ was achieved during the first case, while the second case was able to produce 308 mL CH_4/g $VTS_{applied}$.

Maranón et al. [33] suggested the co-digestion of sewage sludge, glycerol, and OFMSW as an interesting strategy for higher methane generation in anaerobic

digesters, but they also observed that process optimization depends on mixture content and nutrients load.

To find key elements around the proposal of Maranón et al. [33], and in order to contribute to national issues related to municipal solid waste and glycerol residue management, this paper present results are obtained from a bench scale experiment of which main objective was to optimize methane production during anaerobic co-digestion of sewage sludge, glycerol, and food waste.

2. Material and Methods

Considering the proposal to optimize ternary mixture co-digestion, the experimental design was planned according to three phases. The first was dedicated to the co-digestion optimization of a binary mixture of sewage sludge and FW (food waste); the second was based on sewage sludge and glycerol mixture; and according to those results, the third evaluated the co-digestion of the ternary mixture itself.

Excess activated sludge from a municipal WWTP located in Rio de Janeiro (WWTP Sludge) and disintegrated and homogenized food waste from a university restaurant was used during the first experimental phase. The FW was liquefied by the addition of distilled water in order to achieve similar water content as the sludge mass. This first phase was planned according to two different experiments, which varied the content of sludge and FW (v/v) in the mixture. The first focused on a higher FW content, based on (% v/v) 100:0, 80:20, 60:40, 40:60, and 20:80 ratios, while the second focused on a lower content of FW and based on (% v/v) 90:10; 85:15; 80:20, and 75:25 ratios.

Excess activated sludge from a municipal WWTP located in Rio de Janeiro (WWTP Sludge) and residual glycerol from a biodiesel industry were used during the second experimental phase. Bovine fat and soybean oil were the basic sources of the biodiesel production

process. Residual glycerol was collected after particle separation and alcohol (methanol) recovery phases. According to literature review, 1% glycerol content was the reference value for the second phase experiments. Preliminary results indicated that 1% (v/v) raw glycerol compromised the overall performance of co-digestion process and further experiments were based on 0.8, 0.5, and 0.3% (v/v) of raw glycerol content.

Based on the results of the first two phases, and to better evaluate the interaction between all variables involved in the co-digestion of sewage sludge, FW and glycerol, a complete factorial experiment 3^2 (with 3 central points, totaling 11 assays with 5 replicates each) was planned for the third phase, considering 2 variables (glycerol and FW concentration) and 3 levels (-1, 0 and +1). From the first experimental phase, 0% (v/v), 10% (v/v), and 20% (v/v) of FW were selected as -1, 0, and +1 level, while 0% (v/v), 0.4% (v/v), and 0.8% (v/v) of glycerol were selected as -1, 0, and +1 level for the second phase. Statistica 8 software (StatSoft) was applied for variance analysis with 95% of confidence level ($p < 0.05$) and VSSD (volatile suspended solids destruction) and SMP (specific methane production) were selected as response variables.

Bench scale experiments were planned in order to support and guide future pilot scale experiments at the Environmental Sanitation Experimental Center of the Federal University of Rio de Janeiro, and excess activated sludge from its pilot plant (CESA Sludge) was used in the third experimental phase. FW belonged

to the same food waste source and was prepared according to the same conditions as the first experimental phase. The same glycerol used in the second phase was used during the third phase.

Assays were conducted in 100 mL non-stirring penicillin vessels, at 30 ℃. After the mixture was introduced, vessels were closed with rubber bumpers and aluminium seal and connected to a 60 mL plastic syringe. Biogas volume production was measured daily according to syringe plunger displacement (measured volumes are presented at 30 ℃ and 1 atm). After stabilization, biogas samples were extracted and analyzed by gas chromatography to quantify methane content; vessels were also opened for sampling and analysis of digested content. Initial and final values of pH and VS concentration were measured during the 3 phases.

Before sewage sludge addition, glycerol was diluted by distilled water according to 1:10 ratio and pH was corrected to 7.0 and 7.5 values by the addition of sodium bicarbonate. During all the phases, at least 4 replicates of experiments were conducted and mean values are presented as final values.

Carbohydrates and proteins were determined according to Dubois et al. [34] and Lowry et al. [35]. TVA (total volatile acids) and TA (total alkalinity) were determined according to DiLallo and Albertson [36] and Ripley et al. [37]. Glycerol content was measured according to Bondiolli and Bella [38]. Oil and fat, soluble COD (SCOD), and VS concentrations were measured according to standard procedures [39].

Table 1   Characteristics of the sludge and FW used during the experimental phases.

Parameter	1st phase		3rd phase		
	WWTP sludge	FW	WWTP sludge	CESA sludge	FW
pH	6.9	5.2	6.8	6.9	5.5
Humidity (%)	97.8	73.2	96.5	93.5	78.5
Carbon (%)	34.7	51.6	34.7	-	53.5
Nitrogen (%)	5.4	8.0	5.4	-	8.5
Phosphorus (%)	-	0.03	-	-	0.05
TS (mg/g)[a]	19.7	267.7	14.1	12.2	236.6
VTS (mg/g)[a]	11.8	251.4	8.8	8.2	212.5

[a] wet content.

3. Results and Discussion

3.1 Substrate Composition

Table 1 presents characteristics of the sludge and FW used during the experimental phases. In general, pH values presented by each substrate were an indication that binary and ternary mixtures should be corrected in order to achieve pH value closed to neutrality. FW presented higher content of dry solids than sludge, and the addition of distilled water was required for better disintegration and homogenization.

Carbon and nitrogen content of WWTP and CESA sludges were lower than in FW, which indicates that the addition of a higher carbon content source may improve the performance of the anaerobic digestion process.

During the second and third experimental phases, FW presented 69.8 mg/g and 50.1 mg/g of oil and fats, respectively, values that represented 59% and 48% of the chemical composition of the food waste. Therefore, lipid molecules are more complex to biodegrade than carbohydrates and proteins, and this should influence biogas and methane production.

Substrate analysis showed that total solid content of FW was mostly due to organic particles, while VTS:TS ratio in sewage sludge varied from just 0.5 in WWTP sludge to 0.67 in CESA Sludge. VTS concentration in FW confirmed its high carbon content.

Even CESA sludge presented carbon and nitrogen ratio higher than that presented by WWTP sludge and FW. The values were much lower than those generally suggested for anaerobic digestion and comprised from 20 to 30 [40]. This fact may also influence the performance of anaerobic co-digestion process as well as production of biogas and methane.

From a biodiesel production process that was based on bovine fat and soybean oil sources and after particle separation and alcohol recovery phases, residual glycerol used during the second and third phases presented the following characteristics: 74% (m/m) glycerol; 37,100 mg Cl⁻/L; 19 mg methanol/L; 1,119 mg COD/L, and 411 g total Carbon /L. Salinity value was closed to 46 g Cl⁻/L, which corresponded to that indicated by Carmona et al. [12] and Viana et al. [13]. The option to use raw glycerol containing high salinity content was justified by the fact that previous assays indicated that even 1% treated glycerol was able to compromise the overall performance of the co-digestion process.

3.2 First Experimental Phase: Sludge and FW Co-digestion

The first experimental phase was comprised of two different assays. One focused on a higher food waste content and was based on 100:0, 80:20, 60:40, 40:60, and 20:80 sludge to FW ratios. Fig. 1 shows pH drop and VS removal (%) at the end of the first co-digestion assay.

The results indicated that addition of FW increased biogas volume, probably due to the presence of rapidly biodegradable organic content on the binary mixture. During the first 2 days of the assay, for every (%) of FW content, a higher biogas volume production compared to sewage sludge sample control was observed. However, from the third day of experiment, for every FW content, excess accumulation of volatile acid, lower buffer capacity, and decreased pH up to 3.3 (80% of FW) strongly inhibited biogas production. Even after initial correction of pH value to 7.0, the higher the content of food waste is, the lower the pH values of the digested mixture are after stabilization. While the sewage sludge sample control presented final pH values equal to 6.7, the 60:40, 40:60, and 20:80 ratios presented final pH values equal or lower than 5.1. The pH drop influenced the stabilization of the process, resulting at the end of the experiment in VS destruction values lower than 30%.

Kim and Kang [41], also verified inhibition of specific methane production after the third day of co-digestion process due to the increase of food waste leachate content (F) in the ternary mixture (1S:4F:1A) with raw sludge (S), and algal biomass (A). According
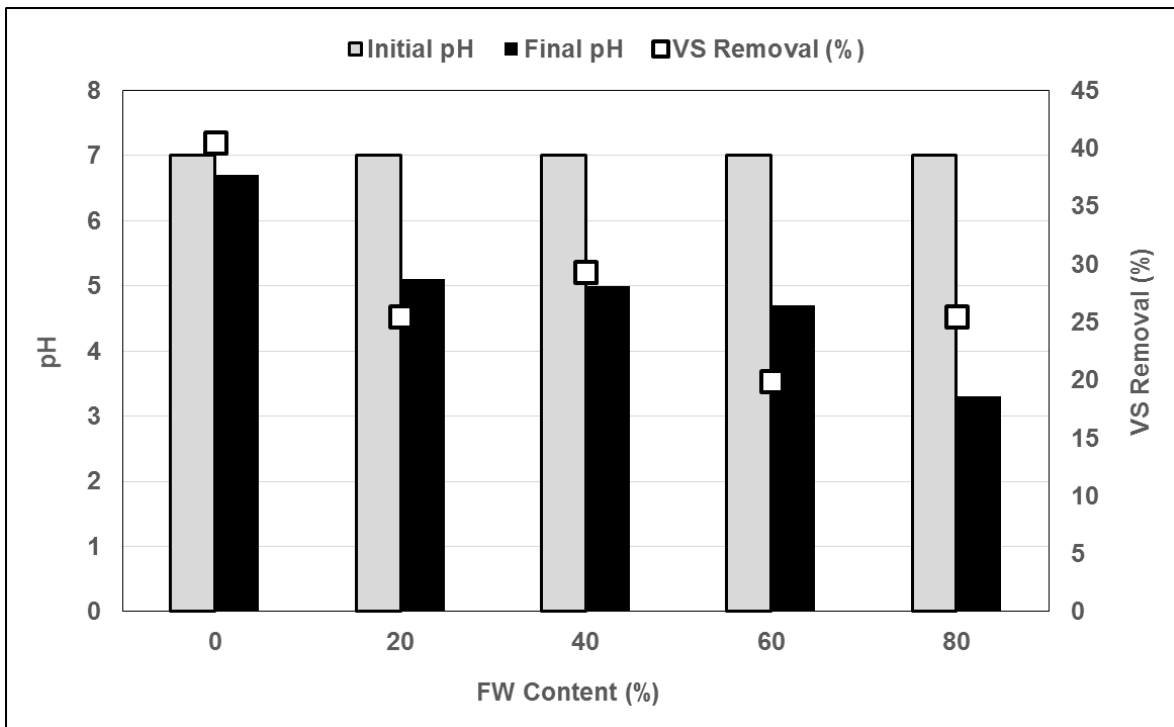
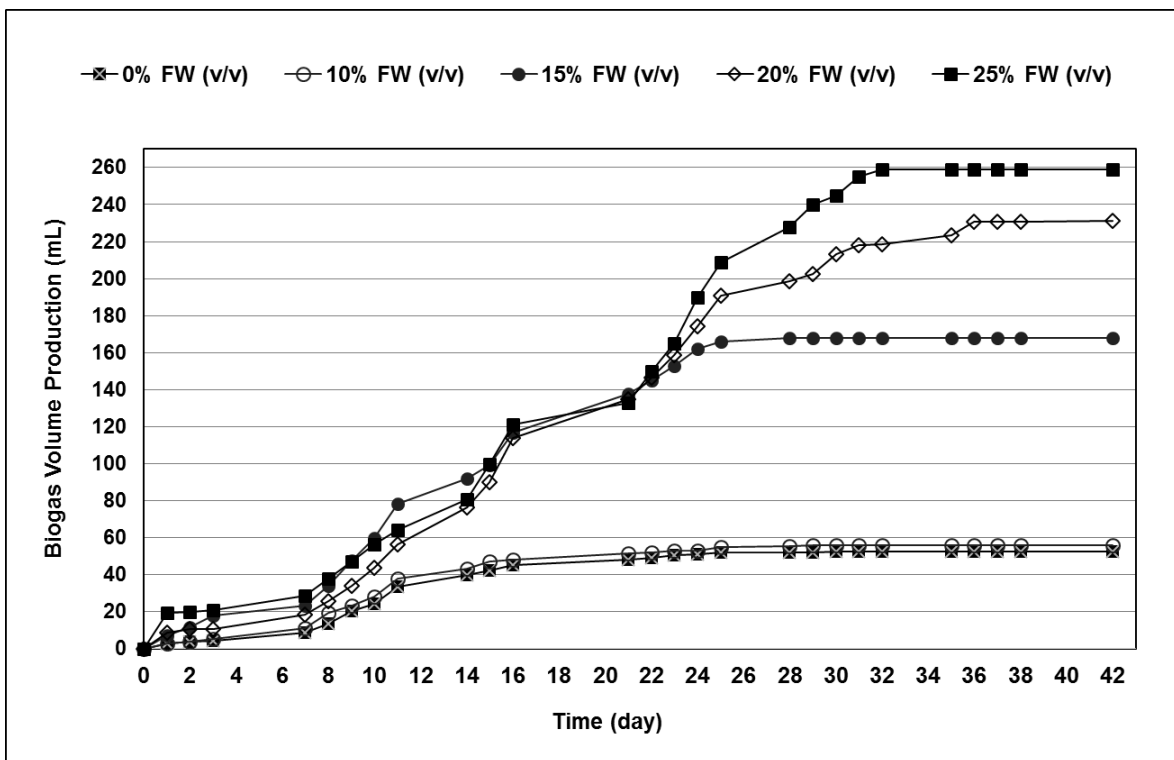Fig. 1   Initial and final pH and VS removal values achieved by first co-digestion assay.

Fig. 2   Biogas volume production achieved by second co-digestion assay.

to increased OFMSW content, Gou et al. [4] varied the volumetric organic load of the binary mixture with sewage sludge, from 1 to 8 g VTS/L.d., and in a continuous process co-digestion they observed a pH drop from 7.0 to 4.9 and 43% of VTS removal.

Therefore, the second assay limited the addition of FW up to 25% (v/v). As shown in Fig. 2, for any mixture ratio, co-digestion process presented the expected pattern with regard to time for production of biogas, and stabilization was generally reached after 35 days. The correction of initial pH value to 7.5 contributed for co-digestion buffer capacity and final pH values were higher than 7.0 for any FW content.

As shown in Fig. 3, the addition of FW generally increased production of biogas. Binary composition according to 75:25 ratio achieved values up to 259 mL, a volume almost 5 times higher than that produced by 100:0 control ratio (53 mL).

Sewage sludge sample control and 10% FW content reached almost the same VTS removal (27% and 25%) and biogas volume production (53 mL and 56 mL). A

higher methane content in biogas always responded to the presence of FW, even with lower content mixture 10% (v/v) and according to values up to 84%.

Considering these results, 20% and 25% (v/v) ratios corresponded to the optimal condition for methane generation, as they presented the highest biogas volume production (231 mL and 259 mL) with high methane content (74% and 71%). The same figure shows that the highest SMP based on $VTS_{applied}$ load was related to 75:25 ratio (116 mL CH_4/g $VTS_{applied}$), a value 4.3 times higher than the sewage sludge sample control. Kim et al. [42] evaluated the co-digestion of sludge (80%) and food waste (20%) and obtained for 71% methane content, which is a SMP 1.4 times higher.

The increase in FW content in the binary mixture also corresponded to an increase of $VTS_{applied}$ load and consequently in the increase of the SMP. A better equilibrium between successive steps of anaerobic degradation process, especially a well balanced acidogenesis phase, may justify the better performance of the 75:25 ratio mixtures.
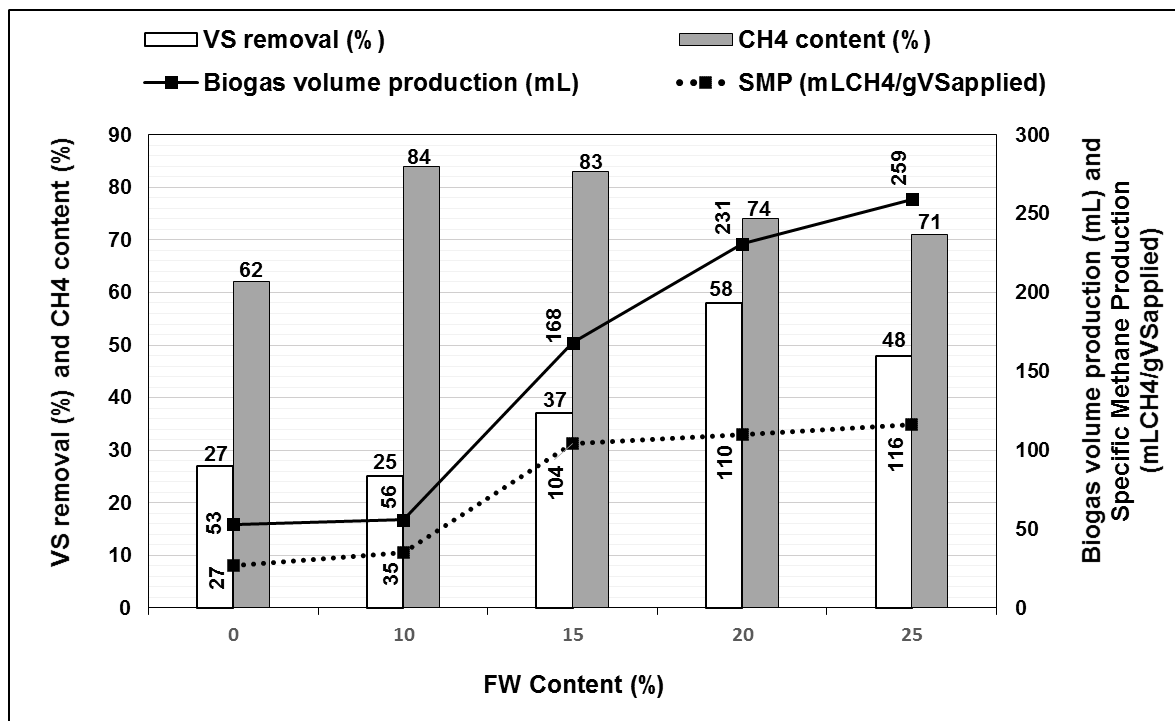

Fig. 3   Biogas volume production, VS removal, CH4 content, and specific methane production according to different mixture ratios of sludge and FW.
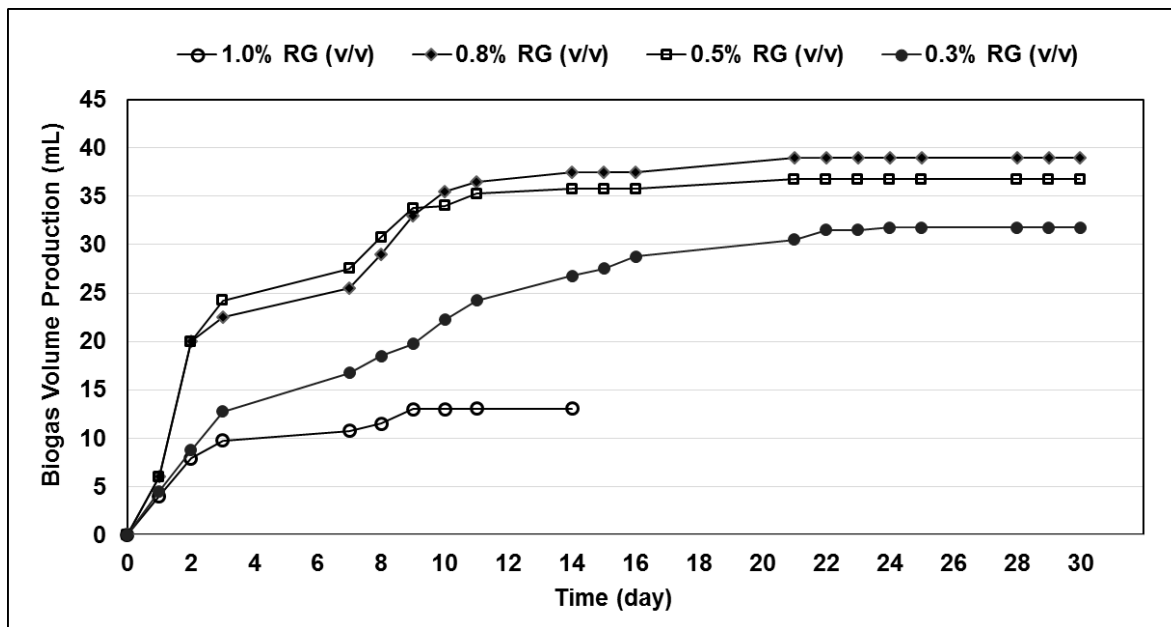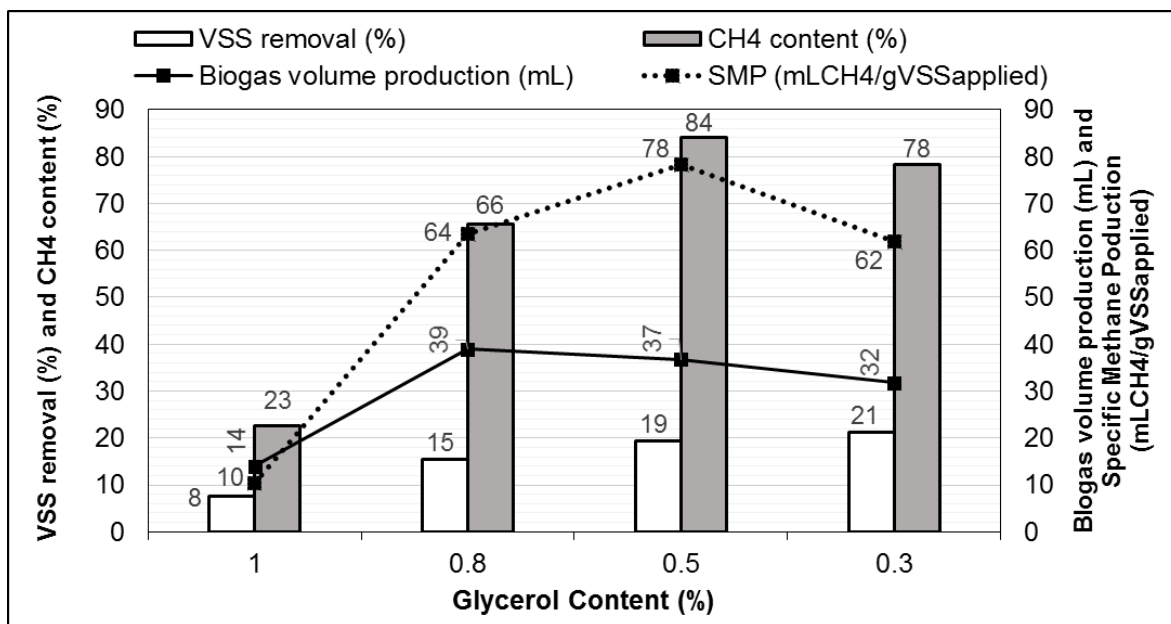
Fig. 4 Biogas volume production during sludge and glycerol co-digestion.

Fig. 5 Biogas volume production, VSS removal, CH$_4$ content, and specific methane production according to different mixture ratios of sludge and glycerol.

3.3 Second Experimental Phase: Sludge and Glycerol Co-digestion

The second experimental phase consisted of just one assay. Based on a literature review and on preliminary test results, the experiment was planned in order to evaluate the effects of glycerol on co-digestion process with content values lower than 1.0% (v/v). As shown in Fig. 4, 1.0% (v/v) content had limited biogas

production. Just based on biogas volume production criteria and in order to compose the ternary mixture, results indicate that biogas volume production increased according to the addition of glycerol and that it should be limited to 0.8 mg/L.

On the other hand, Fig. 5 indicates that the presence of glycerol restricted VSS (volatile suspended solids) removal ratio up to only 21%, which is a lower value

than the conventional 50% removal ratio usually reached by anaerobic digestion process. Athanasoulia et al. [7] also reported a decrease in VTS reduction from 36% to 24% by adding 3% (v/v) of glycerol to the sludge in a CSTR. VSS removal was increased according to glycerol content decrease, and it may be an indication that residual glycerol from biodiesel industry presents a chemical composition and a molecular structure that impair bacteria metabolism and anaerobic process efficiency. The lower VSS removal in the sewage sludge and glycerol mixtures may have occurred due to the higher availability of carbon from the glycerol, contributing to the maintenance of the still active biomass and reducing the stabilization of the VTS [19].

Even based on the highest VSS removal ratio, 0.3% glycerol mixture (v/v) was not able to produce the highest volume and methane content in biogas. The highest SMP (78 mL CH_4/g $VSS_{applied}$) was reached by 0.5% glycerol content (v/v), due to its highest methane biogas concentration (84%).

Several authors related an SMP increase and the high biodegradability level of glycerol (above 85%) in a binary mixture co-digestion with sewage sludge. From co-digestion process of sewage sludge and treated glycerol, Siles López et al. [20] reported a specific methane production of 0.306 m^3 CH_4/kg of treated glycerol. Fountoulakis et al. [19] verified that the addition of 1% of raw glycerol (v/v) to sludge presented the best result in terms of biogas production (2,353 ± 94 mL/d) and that the addition of higher values caused process instability and biogas production decrease. Baba et al. [18] suggested that daily glycerol load must be gradually increased in order to optimize methane production rate and indicated that 90% of COD removal and a specific biogas production of 358 mL/g of removed COD was obtained for a mixture content of 1% of glycerol (v/v) and sludge. Athanasoulia et al. [7] varied the addition of glycerol to sludge from 0 to 4% (v/v) and concluded that under 3% condition, the best biogas production equal to 150 L/d

was accomplished, a value 5 times higher than the one without glycerol. Nghiem et al. [21], evaluated the co-digestion of 0.63 up to 3.00% (v/v) of raw glycerol and sludge mixture, and they reported that the lowest raw glycerol content presented the best result in terms of biogas production, achieving 1.3 $m^3 CH_4$/L applied raw glycerol.

Razaviarani et al. [22, 23] added up to 1.8% (v/v) of raw glycerol to sludge and deduced that co-digestion optimization was related to the specific production of 880 mL CH_4/g $VTS_{applied}$, a value obtained by the addition of just 1.0% raw glycerol. Later, the same authors reported inhibitory effects to anaerobic co-digestion by the addition of 1.8% (v/v) and that 1.1% (v/v) addition was able to increase VTS destruction and biogas production [25]. Values obtained by Jensen et al. [8], under 2% of glycerol were 75% VTS removal and 490 mL CH_4/g $VTS_{applied}$. Silvestre et al. [25] reported that 1% raw glycerol addition to sludge (v/v) resulted on 58% VTS removal and a specific methane production of 325 mL CH_4/g $VTS_{applied}$.

With the addition of 2.7% (v/v) of raw glycerol to sludge, which represented an organic load of 2.1 g VTS/L.d., Nartker et al. [3] observed that methane production was increased in 82%, achieving a specific methane production equal to 920 mL CH_4/g $VTS_{removed}$. Rivero et al. [24] evaluated a mixture content of 1% (v/v) of glycerol and sludge, obtaining 89% of VTS removal and a specific methane production of 148 mL CH_4/g $VTS_{removed}$.

3.4 Third Experimental Phase: Sludge, FW, and Glycerol Co-digestion

Based on the results of the first two experimental phases, the third one focused on optimization of methane production during anaerobic co-digestion of sewage sludge, glycerol, and FW. At this phase, 11 trials were done to a complete factorial design 3^2. Fig. 6 shows the VSS removal (%) and SMP (mL CH_4/g $VSS_{applied}$) results obtained according to the ternary mixture composition (% v/v).
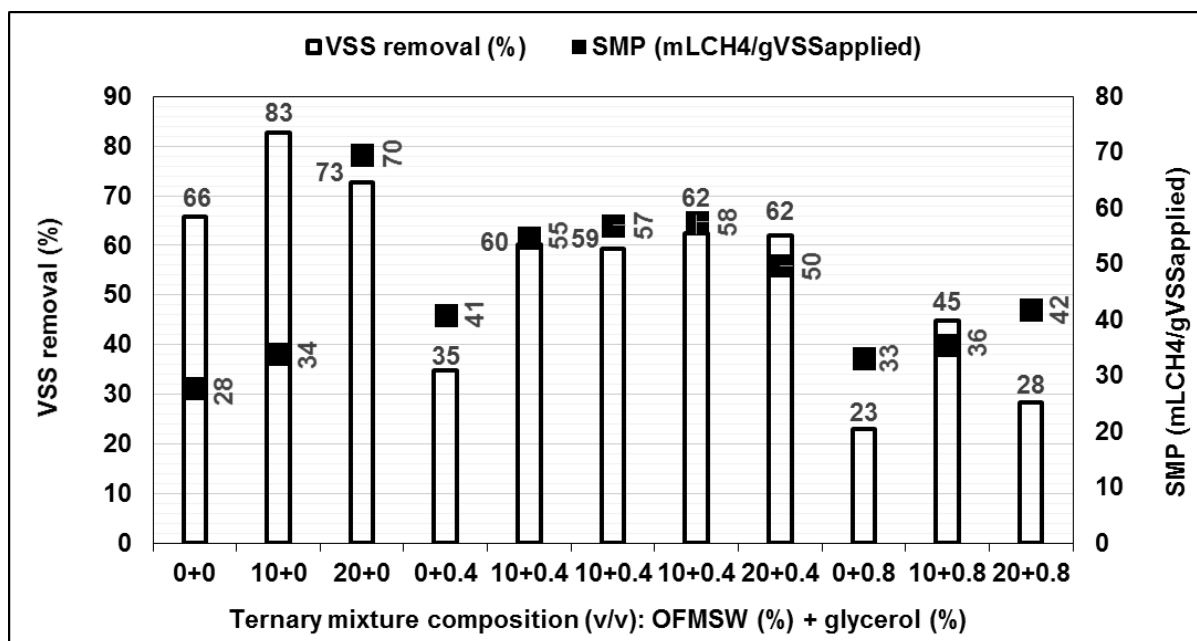
Fig. 6 VSS removal and specific methane production according to different mixture ratios of sludge, FW, and glycerol.

Increasing the proportion of FW in binary sludge mixtures raised VSS removal from 66% in pure sludge to 83% and 73% in mixtures with 10% and 20% FW, respectively. In contrast, increasing the proportion of raw glycerol in ternary mixtures with sludge and FW resulted in a lower removal of VSS, despite SMP gain over control in almost all mixtures. For mixtures containing sludge and 10% FW, VSS removal fell from 83% to 59% and 36% with 0.4% and 0.8% glycerol, respectively. While the mixtures of sludge with 20% FW showed VSS removal of 73% without glycerol and 62% and 28% with 0.4% and 0.8% of glycerol. A probable explanation for the lower VSS removal efficiency in the tests with 0.4% and 0.8% of glycerol, relative to the control and binary mixtures with sludge and FW, would be a preferential consumption of glycerol in detriment of the insoluble organic constituents (measured as VSS), consistent with the high consumption of glycerol (above 80%). Athanasoulia et al. [7], also found SSV removal (25%) is lower than in the control (36%), in the co-digestion of sewage sludge with 2% to 4% (v/v) glycerol in reactor operating with hydraulic retention time of 12 to 20 d. Fountolakis et al. [19] and Ma et al. [26] establish

that the addition of glycerol provides an extra amount of organic matter to the active biomass, which increases its growth and VSS concentration.

According to the planned factorial experiment 3^2, binary mixtures with FW and without glycerol were the ones that presented better values in terms of VSS removal (%). The increase in glycerol content, with or without the presence of FW, decreased VSS removal. Even the control assay, comprising just sludge digestion, achieved better VSS removal than glycerol binary and ternary mixture assays. With or without the addition of glycerol, the presence of food waste always improved VSS removal. For any content of glycerol, 10% food waste addition (v/v) presented higher VSS removal values. However, binary mixtures with FW and without glycerol were not the ones that presented better values in terms of SMP.

Statistica 8 software (StatSoft) generated linear regression curves with coefficient of determination equal to 0.99 for VSSD and to 0.82 for SMP, with 95% of significance level ($p < 0.05$). Student's t-test was applied in order to evaluate the significance effects on dependent variables (VSSD and SMP) and results are visualized in a Pareto Chart, as shown in Figs. 7 and 8.
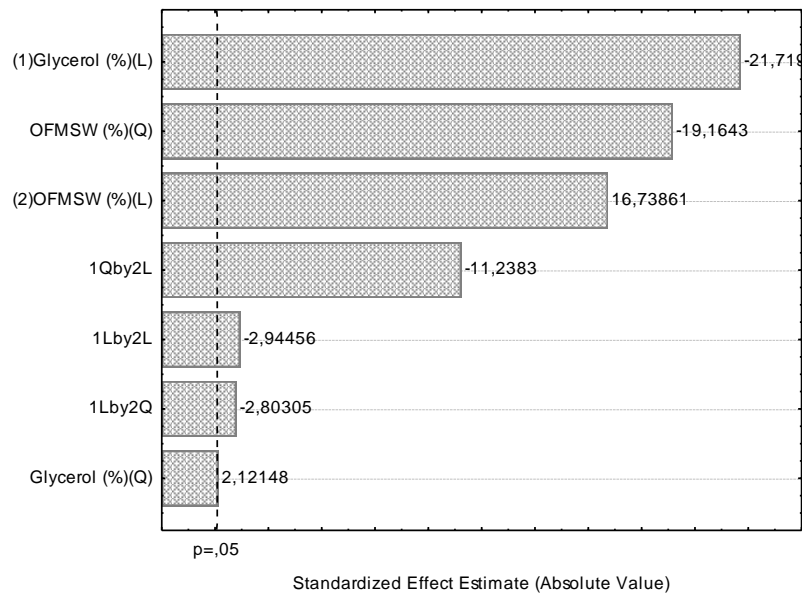
Fig. 7 Pareto Chart representing the effects of glycerol and FW content (expressed as OFMSW) on VSSD (1 = raw glycerol and 2 = FW represent factors and interactions).
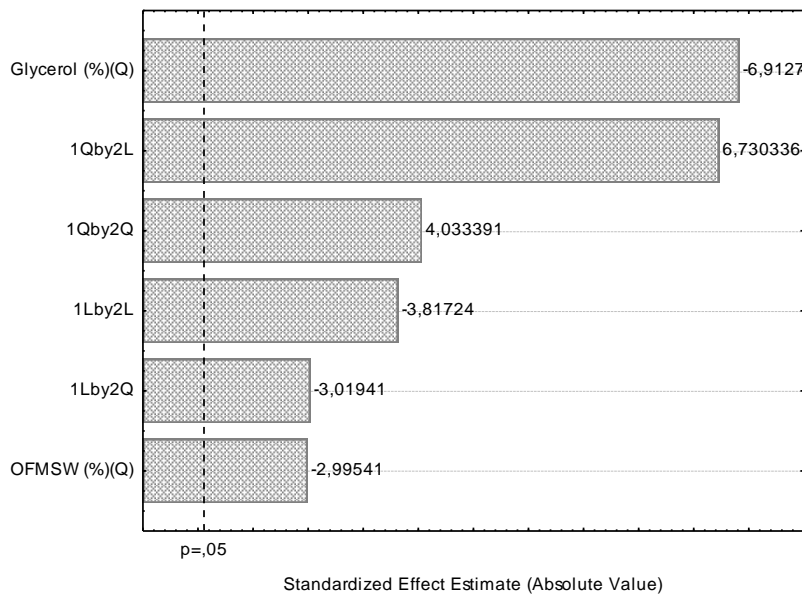

Fig. 8 Pareto's graph representing glycerol and FW content (expressed as OFMSW) effects on SMP (1 = raw glycerol and 2 = FW represent factors and interactions).

The dotted vertical line ($p = 0.05$, the tabulated student's t value) indicates the minimum significant effect value for 95% of confidence level. Values higher than 0.05 indicate that the correspondent factor has importance for the analysis and interpretation, and results indicate that the effect of glycerol content was the most important factor for VSSD and SMP. However, while glycerol has a negative and linear

effect on VSSD, it has no linear effect but a negative quadratic effect on SMP. This result can be attributed to the higher availability of carbon in glycerol, contributing to VTS destruction and process stabilization, as already mentioned [19]. At the same time, the increase in readily biodegradable carbon availability contributed to a higher SMP.

The second significant linear effect on VSSD was

FW, with positive value, indicating increased VSSD relative to increased FW content in the ternary mixture. This conclusion is different from that one obtained during the first phase of experiments, when VS removal decreased according to the increase of FW content. However, the linear effect of FW on SMP was not significant to a 95% confidence level in student's t-distribution. Both variables FW and glycerol have negative quadratic effect on SMP. Comparison between the magnitude of the most significant effects on the dependent variables shows values 3 to 4 times higher related to VSSD, indicating limitation in the effects of glycerol and FW on SMP.

Figs. 9 and 10 show the surface charts of interactions between glycerol and FW variables. The glycerol and FW interactions also appear as significant on Pareto Charts, which indicated that for a higher SMP, the trials of co-digestion should be conducted with levels of 10% FW (v/v) and 0.4% glycerol (v/v) or 20% FW (v/v) without glycerol. On the other hand, high VSSD values were obtained in the range of 10 to 20% FW (v/v) and lower levels of glycerol. However, higher levels of VSSD, up to 60%, were observed in the ternary mixture of 10% FW (v/v) and 0.4% (v/v) glycerol. As a general conclusion, based on STATISTICA 8.0 software
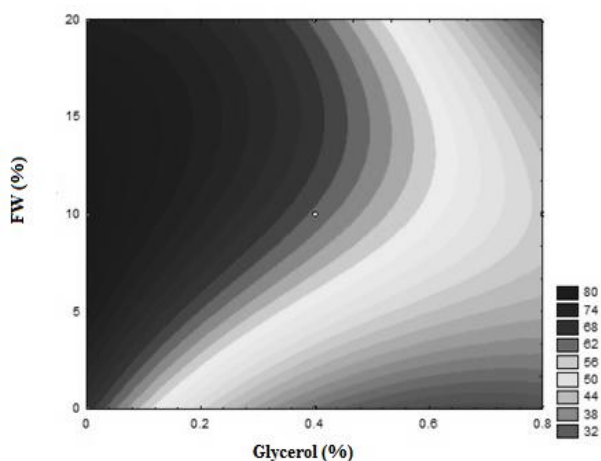
Fig. 9   Response surface describing the interaction of FW (vertical axis-y) and glycerol (horizontal axis-x). Contour lines represent VSSD values (%) obtained for each combination.
Statistical model: VSSD (%) = $61.5 - 17.8x + 1.6x^2 + 13.7y - 14y^2 - 1.7xy - 2.8xy^2 - 11.3x^2y$.
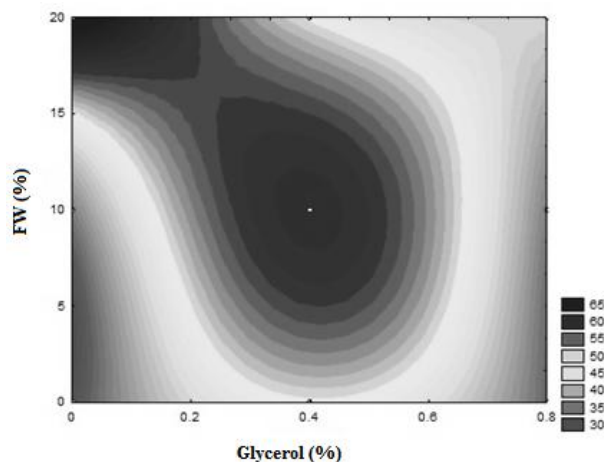
Fig. 10   Response surface describing the interaction of FW (vertical axis-y) and glycerol (horizontal axis-x). Contour lines represent SMP values (mL CH_4/g $VSS_{applied}$) obtained for each combination.
Statistical model: SMP (mL CH_4/g $VTS_{applied}$) = $58.5 - 22.9x^2 - 9.9y^2 - 6.9xy - 5.5xy^2 + 12.2x^2y + 18.4x^2y^2$.

and statistical analysis according to 95% of significance level (p-value < 0.05), high SMP and VSSD values should be obtained in the ternary mixture of sewage sludge, 10% FW (v/v) and 0.4% (v/v) glycerol or in the binary mixture of sludge with 20% FW (v/v).

4. Conclusions

(1) FW proved to be a good source of carbon and nutrients for the anaerobic co-digestion process, without any complementary external source. Due to its high percentage of VTS, biogas production and methane content were favored up to the limit of 25% FW (v/v) added to sewage sludge. Higher values resulted in TVA accumulation and pH drop.

(2) In general, the addition of lower FW content in the binary mixture with the sewage sludge (10 to 25% v/v) followed by the initial pH correction to 7.5, allowed better buffering condition, and a higher VTS removal and SMP was achieved than for the sewage sludge sample control.

(3) Co-digestion of sewage sludge and RG (raw glycerol) according to values lower than 1% (v/v) resulted in the increase of methane production and did not impair binary mixture stabilization. The best

condition was related to the addition of 0.5% of glycerol (v/v), in which an SMP was 7.8 times greater than that obtained by the sewage sludge sample control.

(4) According to the planned factorial experiment 3^2, a ternary mixture with 10% FW (v/v) and 0.4% glycerol (v/v) resulted on high VSSD (average value of 61%), equivalent to the value obtained by the sewage sludge sample control (66%), and with high SMP (average value of 56 mL CH_4/g $VSS_{applied}$), which was twice that achieved by the control.

(5) The planned factorial experiment 3^2 also showed a better condition for the co-digestion of a binary mixture of sewage sludge and 20% FW (v/v), which reached an SMP equal to 70 mL CH_4/g $VSS_{applied}$, a value 2.5 times higher than that obtained by the sewage sludge sample control.

Acknowledgements

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