Evaluation of optimal fermentation conditions for volatile fatty acids production from artisanal fish waste

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Research

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Abstract

In this research, operational parameters were assessed to optimise the production of volatile fatty acids (VFA) from artisanal fish waste (FW) using mixed-culture fermentation. The experiments were performed using a randomised block design with pH and Inoculum/Substrate (I/S) ratio as factors with three pH levels (5, 7 and 9) and five I/S ratios levels (0.20, 0.15, 0.10, 0.05, 0.00) with VFA production, total ammonia nitrogen (TAN), biogas and soluble chemical oxygen demand (sCOD) as response variables. The results indicated that pH and I/S ratio significantly influenced VFA production and the other variables evaluated. The highest VFA concentration (54.5 g/L) was obtained at pH 7 and with an I/S ratio of 0.05. These conditions were also optimal for TAN and sCOD. External pH control was proved as unnecessary as the highest VFA production (70.3 g VFA/L) was obtained without pH control. In these conditions, the fermentation broth was able to maintain its pH between 6.6 and 7.7. The VFA profile had acetic (35%) and butyric (32%) acids as the dominant species, however, propionic, iso-valeric and iso-butyric were also produced in small amounts (< 10%). These results evidence the potential of artisanal FW as a raw material for producing high concentrations of VFA and smooth the path for the establishment of a VFA and biogas biorefinery from artisanal FW.

Introduction

In Colombia, artisanal fishing supplies around 12,000 tons of fish every year to the national market (De la Hoz-M et al. 2017), accounting for 8% of the total capture fishing in the country. This activity takes place in continental and coastal waters from the Pacific and Atlantic oceans. Artisanal fishing is responsible for generating employment, income and food for several families in rural areas with precarious economic conditions (OCDE 2016). However, artisanal fisher communities are affected economic and environmentally by fish waste (FW). The economic issues are associated with the high percentage of the total fish that become waste, It is estimated that 45% of the total capture by artisanal fishery become waste (Rai et al. 2010) producing serious economic losses to this sector. Additionally, environmental issues are associated with inadequate FW disposal, in Colombia, FW are thrown away directly into the oceans or taken to landfill (Rodríguez-Salcedo et al. 2011), these improper disposal methods generate greenhouse gases, bad odours and health issues to humans and farm animals.

FW has a high content of potentially biodegradable components such as proteins and fats (Cadavid-Rodríguez et al. 2019); therefore, FW is susceptible to be transformed into by-products such as low-value animal feed, biogas and organic acids. Anaerobic digestion (AD) for producing biogas from FW has been reported from commercial FW such as tuna, seabream and sturgeon (Eiroa et al. 2012; Hadiyarto et al. 2015; Greggio et al. 2018). Recently, artisanal FW was proved as a suitable substrate to produce biogas. In Fact, if the whole artisanal FW produced in Tumaco (Colombia) were used as substrate for AD, the biogas produced can satisfy the electric energy consumption or cooking energy demand of 230 fisherman families (Cadavid-Rodríguez et al. 2019). Although, biogas production from artisanal FW was significant, the initial Total solids (TS) required for producing biogas without observing biogas inhibition was 1%. Higher TS concentrations (1.5, 2 and 2.5% TS) produced diauxic growth and a significant increment in the concentration of volatile fatty acids (VFA). In fact, the 2.5% TS treatment reached a VFA concentration above 10 g L^{-1}, which is a high VFA concentration for the low TS utilised. Similar to other high protein wastes (Plácido and Zhang 2018a, b); artisanal FW has the potential for being used as a raw material to produce high concentrations of VFA.

VFA are the carboxylic acids with a carbon chain between 2 and 7 carbons (acetic (2), propionic (3), iso-butyric (4), butyric (4), iso-valeric (5), valeric (5), hexanoic (6), and heptanoic (7)). Traditionally, VFA are produced via a petroleum-based process, however, they can be generated during the AD acidogenic stage or using other types of fermentative processes. VFA have a high potential as a renewable source of carbon with a wide applicability in the biological removal of nutrients, production of biodiesel, generation of electricity and synthesis of complex polymers, among others (Atasoy et al. 2018; Jankowska et al. 2018). Biological VFA production had utilised principally carbohydrate-based residues such as lignocellulosic material, municipal solid waste, or agro-industrial waste. However, high protein wastes have been also effective for generating significant amounts of VFA. In fact, AD of Tuna FW has been able to produce approximately 30 g VFA L-1 (Bermúdez-Penabad et al. 2017) and AD of slaughterhouse blood was able to produce 100 g VFA L^{-1} (Plácido and Zhang 2018b). Therefore, to utilise FW for VFA production and to reduce the difference between petroleum and biological based VFA production it is necessary to optimise the fermentation parameters used for this process.

The strategies for optimising VFA fermentation have focused mainly into: 1) improvement of hydrolytic stage, 2) removal of inhibitory compounds and 3) enhancement of the acidogenesis process (Zhou et al. 2018). Regarding the latter, the effect of operational conditions such as temperature, retention time, pH, substrate and organic loading rate of the substrate has been studied (Khan et al. 2016; Atasoy et al. 2018). However, these studies have been performed principally in carbohydrate-based substrates and to the best of authors' knowledge the optimisation of the fermentation conditions for high protein wastes for VFA production have not been reported in the literature.

Inoculum-Substrate (I/S) ratio and pH are significant process variables in biogas and VFA production process using mixed-culture fermentation. However, AD processes normally employ a greater I/S ratio and mildly alkaline conditions than that of VFA production. As VFA are produced during the acidogenic phase, I/S ratio utilised for biogas production are not optimal for VFA production and its optimisation can improve VFA production. Similar to I/S ratio, pH is a critical factor in the production of VFA, since it influences, not only the acidogenesis but also the proteins hydrolysis (Zhou et al. 2018). The optimum pH for VFA production is substrate dependent and with a wide optimal range between 5 and 11 (Lee et al. 2014; Wang et al. 2014; Zhou et al. 2018). To the best of the author's knowledge, optimisation studies about I/S ratio and pH on VFA from protein-rich waste are not available on the current literature.

To improve the potential establishment of a VFA Biorefinery from artisanal FW, it is necessary to optimise the fermentation operating conditions for VFA production from artisanal FW. Therefore, the aim of this research was to determine the best pH and I/S ratio conditions for VFA production from artisanal FW. The experimental setting included the evaluation of 5 different I/S ratios and 3 different pH using a randomised block design. VFA concentration, VFA profile and ammonia concentration were used as response variables. The effect of uncontrolled pH conditions on VFA production were also tested.

Materials And Methods

Inoculum and Substrate

The inoculum was a methanogenic sludge (MS) obtained from an upflow anaerobic sludge blanket (UASB) reactor treating wastewater from a slaughterhouse plant located in the municipality of Candelaria (Colombia). The MS was acclimatised adding 1 g/L of FW daily for four weeks, at room temperature, and 5 days before the experiment the feeding was stopped. The FW was collected from artisanal fishers from the port of Tumaco (Nariño, Colombia) and comprised principally in guts, digestive tracts and viscera from a mixture of different types of fish species. After collection, FW was transported to the laboratory in a 4 °C refrigerated container. The transportation process between collection and cold storage in the laboratory had a duration of 4 hours. In the laboratory, the samples were homogenised to 5 mm particle size using a food processor (Black & Decker FP1336) and then frozen until use. Table 1 describes the characterisation of Artisanal FW and MS.

Table 1
Physicochemical characterization of inoculum and substrate

Parameter	Fishing waste	Methanogenic sludge
Moisture (%)	74.8 ± 0.41	94.3 ± 0.96
Total Solid (TS) (%)	25.2 ± 0.69	5.7 ± 0.11
Volatile Solid (VS) (%TS)	88.9 ± 1.23	84.9 ± 1.35
pH	7.4 ± 0.15	7.7 ± 0.26
COD (mg/Kg) / (mg/L)	265000.0 ± 885.12	297.5 ± 1.37
C (%ST)	50.6 ± 0.95	41.6 ± 1.26
N (%ST)	8.8 ± 0.15	8.9 ± 0.11
C/N Ratio	5.7 ± 0.11	4.7 ± 0.15
VFA (mg/Kg) / (mg/L)	1515.0 ± 3.21	454.5 ± 1.10
Ammonia (mg/Kg) / (mg/L)	627.2 ± 1.58	487.2 ± 2.30
Alkalinity (mg$CaCO_3$/Kg) / (mg$CaCO_3$/L)	650.0 ± 2.95	1150.0 ± 1.95

VFA production from artisanal FW

The acidogenic fermentation experiments were conducted in batch mode using 500 mL glass bottles with an effective volume of 300 mL and equipped with rubber stoppers provided with a valve to release biogas. The experiments were carried out for 15 days, as this is the maximum time for completing the acidogenic phase (Cadavid-Rodríguez et al. 2019), with the temperature set at 37 ± 2 °C and constant agitation of 200 rpm. The experiments were performed under a complete randomised block design with a 5 × 3 factorial arrangement. In this design, pH and I/S ratio were selected as factors with 3 (5, 7, 9) and 5 (0.20, 0.15, 0.10, 0.05, 0.00) levels, respectively (**see** Table 2). The response variables included VFA concentration, VFA profile, ammonia concentration, biogas, and soluble COD. All the experiments were performed in duplicates and were sampled during days 1, 3, 5, 7, 10 and 15. After sampling, the removed solutions were centrifuged at 4000 rpm for 20 minutes in a SL8 Centrifuge - Rotor High Conic III from Thermo Scientific, and stored at -20 °C until processing. At the beginning and during the process, the reactors' pH was adjusted to their corresponding values (5, 7 and 9) using solutions of sodium hydroxide (NaOH, 1M) or/and sulfuric acid (H_2SO_4, 1M). An additional experiment without controlling pH was performed using the optimum I/S ratio from the past experiment. The sampling times and response variables were the same as the previous experiment.

Table 2
Experimental design

Treatment	Inoculum/substrate ratio	pH
1	0.2	5
2	0.15	5
3	0.1	5
4	0.05	5
5	0.2	7
6	0.15	7
7	0.1	7
8	0.05	7
9	0.2	9
10	0.15	9
11	0.1	9
12	0.05	9
13	0	5
14	0	7
15	0	9

Analytical methods

Inoculum and substrate characterisation analyses, as well as the analysis of total VFA and ammonia, were carried out following the methods outlined in Standard Methods (APHA 2005). Individual volatile fatty acids were analysed using an Agilent 7890th gas chromatograph with CHEM STATION 32 data system, equipped with a capillary column (30 m x 25 mm x 0.25 μm), polar stationary phase of polyethylene glycol treated with nitro-terephthalic acid, automatic injector and a flame ionisation detector (GC-FID).

Statistical analysis

The variables ammonia content, VFA production, biogas and soluble COD, were analysed using the area under the curve (AUC) method. The AUC for these variables was calculated with the trapezoid's method. The total COD reduction % was not analysed using the area under the curve. The Biogas AUC % did not exhibit a normal distribution; therefore, this data sets was transformed using a natural logarithm transformation (Tabachnick et al. 2007). The experiment was analysed with a two-way ANOVA with randomised blocks for each variable using the proc ANOVA statement (SAS Software University Edition). The Duncan's multiple range test was used to evaluate the differences in the main-effects when necessary while, the proc GLM was utilised for plotting the interactions plot in the variable(s) that required that analysis.

Results

3.1 Effect of I/S and pH on the VFA production from artisanal FW

3.1.1 VFA production and VFA profile

Figure 1 describes the VFA production from artisanal FW obtained at the different I/S and pH combinations **(Table 2)**. The VFA production kinetic was similar for the different I/S ratios, in all cases, the highest production was achieved by the treatment with pH 7 followed by the treatment with pH 9 and pH 5. The treatments with an I/S ratio of 0.05 obtained the highest VFA concentrations at pH 7 (T8: 54.5 g/L), 9 (T12: 45.6 g/L) and 5 (T4: 20.1 g/L) (**Figure 1d**). Additionally, it was observed that the production of VFA increased as the I/S ratio decreased (0.2 to 0), the VFA concentration reached its maximum in treatments with an I/S ratio of 0.05 (**Figure 1d**). However, the treatments without additional inoculation (I/S=0) obtained a significant VFA production (T13: 21.15 g/L, T14: 52.55 g/L and T15: 52.02 g/L) as much as the I/S ratio of 0.05 (**Figure 1e**). Treatments with higher I/S ratios obtained similar VFA concentrations at the same pH **(Figures 1a, 1b, 1c)**. The statistical analysis of the VFA production utilising the area under the curve (AUC) (**Annex 1-Supplementary material**) revealed a similar description as depicted in **Figure 1**. The two-way ANOVA demonstrated that existed significant differences between the treatments (p value <0.0001, α=0.05). In this case, pH and I/S ratio, the main effects, were significant to the model (pH p value <0.0001 and I/S ratio p value <0.04, α=0.05), however, the interaction between pH and I/S ratio was not significant (p value <0.9737, α=0.05). These results agreed with the data presented in **Figure 1,** where the figures had a similar profile for the three pH even though they have different values. As the interaction term in ANOVA's model was not significant, the main effects can be described and optimised individually. The Duncan's multiple range test was used to evaluate the differences in the pH and I/S ratio for the AUC of the VFA production (AUC-VFA). **Figure 1f** describes the Duncan groupings for means of pH and I/S ratio for the AUC-VFA variable. The Duncan's test evidenced significant differences among the three pH and indicated that pH 7 produced the best VFA production from artisanal FW, pH 7 was followed by pH 9 and 5. The I/S ratio's Duncan's test indicated that even though the I/S ratio of 0.05 and 0 produced the highest VFA production, these I/S ratios were statistically similar to 0.1 and 0.15; whereas, the 0.2 I/S ratio produced the lowest VFA production, but it was also similar to 0.1 and 0.15 I/S ratios. The statistical analysis indicated that pH 7 and I/S ratio between 0 and 0.1 produce the highest amounts of VFA from FW. So, given that with I/S ratio of 0.05 and pH 7 it was possible to achieve the highest VFA concentration, these conditions were selected as optimal to produce VFA.

As the highest differences in VFA concentration were associated with the different pH tested, **Figure 2** describes the VFA profile for the treatments with an I/S ratio of 0.05 and the three different pH. At pH 5, the average production of the principal VFA species were acetic (58 %), butyric (18 %), propionic (9%), iso-valeric (6%), hexanoic (5%) and iso-butyric (4 %) acids. However, these percentages changed throughout the process. The most significant changes included the 10 % reduction in the acetic acid concentration between the initial days and day 15 (**Figure 2a**), and the 2-fold increment in the concentrations of hexanoic and isovaleric acids during the same period. At pH 9, the principal VFA species were similar to pH 5, however, their percentages were different as acetic (47 %), butyric (30 %), iso-valeric (9%), propionic (5%), iso-butyric (4 %) and hexanoic (3%) acids, in this pH was the only to have significant amounts of heptanoic acid in its profile (2%). At this pH, the acetic acid concentration incremented 10% between the initial and the final day of the process, whereas, the butyric acid concentration decreased 5% during the same time (**Figure 2b**). In contrast to pH 5 and 9, pH 7 evidenced the most different profile as acetic and butyric acid had the highest concentration, both with 37%, followed by propionic (10%), iso-valeric (8%), and iso-butyric (4 %). In this case, between the initial and last days of fermentation, acetic and butyric acid exhibited a concentration reduction of 7 and 6%, respectively; while, propionic and iso-valeric acids concentrations incremented 5 % (**Figure 2c**). Acetic acid was the dominant product of the fermentation at pH 5 and 9, while, acetic and butyric acids were the dominant product at pH 7. However, the butyric concentrations at pH 9 was almost the double than pH 5, indicating the effect of the pH in the production of butyric acid. In all cases, valeric acid was not found and higher chain VFA (hexanoic and heptanoic) were found at alkaline or acid concentrations.

3.1.2 TAN production

Figure 3 depicts the Total Ammonia Nitrogen (TAN) production from FW for the different I/S and pH treatments **(Table 2**). In all cases, the TAN had a high production peak around day 3, which was the highest TAN concentration in the process. After that day, TAN production decreased until day 5 and it was approximately constant from that day until the end of the process. Treatments at pH 7 exhibited the highest TAN concentrations, followed by pH 9 and pH 5. The highest TAN concentrations were observed at pH 7 with I/S ratios of 0.15 and 0.1, with values of 27.89 g/L and 28.13 g/L, respectively (**Figure 3b, 3c**), whereas, the lowest was reported in the treatment with pH 5 and 0.15 I/S ratio. Treatment at pH 9 and any I/S ratio reached the second largest TAN concentrations. The I/S ratio of 0.15 achieved the highest concentration of the treatments at pH 7; whereas, the I/S ratio of 0.2 had the highest concentrations for treatments with pH 9 (**Figure 3a**) and the I/S ratio of 0.05 achieved the highest TAN concentrations for pH 5 (**Figure 3d**). The treatments that achieved the highest VFA production, those with pH 7 and I/S ratio of 0 and 0.05, (**Figure 3d, 3e**) had a lower TAN concentration (22.58 g/L and 19.86 g/L, respectively) than the treatments with pH 7 and an I/S ratio of 0.2, 0.15 and 0.1 (24.93 g/L, 27.89 and 28.23 g/L, respectively) (**Figure 3a, 3b, 3c**). Similar to VFA production, pH 5 exhibited the lowest TAN concentration and pH 7 the highest TAN concentration. TAN statistical analysis (**Annex 1-Supplementary material**) also utilised AUC to have a more complete view of the TAN production process **Figure 3**. The two-way ANOVA demonstrated that existed significant differences between the treatments (p value <0.0001, $\alpha=0.05$). In this case, only the pH was significant to the model (pH p value <0.0001) while I/S ratio (p value <0.4427, $\alpha=0.05$) and the interaction between pH and I/S ratio (p value <0.99952, $\alpha=0.05$) were not significant. Although, the TAN figures described high peaks at different I/S ratio, the lack of significance in this variable revealed the importance of using a variable that unite all the points instead of individual maximums. As I/S ratio and the interaction were not significant, pH was the only main effect that can be utilised for optimisation. The Duncan's multiple range test was used to evaluate the differences in the pH for the AUC of the TAN production (AUC-TAN). **Figure 3F** describes the Duncan groupings for means of pH for the AUC-VFA variable. The Duncan's multiple range test revealed that pH 7 and pH 9 had statistically similar effects in TAN production, whereas, pH 5 had less effect for TAN production. The statistical analysis indicated that pH 7 and pH 9 produced the highest positive effect and that the I/S ratio was not relevant for TAN production. As pH 7 and I/S ratio of 0.05 belonged to the optimal conditions for both VFA and TAN production, these conditions were selected as optimal to produce VFA and TAN.

3.1.3 Biogas

Biogas production under the evaluated conditions is described in **Figure 4.** Different from the previous variables, biogas evidenced a higher production under acidic pH conditions. In fact, pH 5 treatments obtained the maximum biogas production for all I/S ratios evaluated. The Biogas production differences between pH 5 and the other pHs were very significant, specially at high I/S ratios (0.2, 0.15 and 0.1) (**Figure 4a, 4b, 4c**), as the pH 5 treatments produced 5-times more biogas than the other pHs. Treatments at pH 7 and pH 9 produced similar results at all I/S ratios (**Figures 4d, 4e**) indicating the strong effect the pH has over the biogas production. The treatments with high production of biogas exhibited a second production phase after a stationary phase, this can be associated with the consumption of VFA or the transformation of molecules that require more energy consumption. Biogas production was limited at both neutral and alkaline pHs and with low I/S ratios (0 and 0.05). Biogas production behaved similar to

VFA and TAN production in the importance of pH over I/S ratio; however, they were opposite in how the pH conditions affect these variables because biogas production was low at neutral and alkaline pH while VFA and TAN production thrived under those conditions. The AUC from the biogas plots (**Figure 4**) was used as variable for the statistical analysis (**Annex 1-Supplementary material**). The two-way ANOVA demonstrated that existed significant differences between the treatments (p value <0.0229, α=0.05). Similar to TAN, only the pH was significant to the model (pH p value <0.0028) while I/S ratio (p value <0.2078, α=0.05) and the interaction between pH and I/S ratio (p value <0.4071, α=0.05) were not significant. The statistical result confirmed the strong effect the pH had on biogas production as showed in **Figure 4**. As I/S ratio and the interaction between pH and I/S ratio were not significant, pH was the main effect employed for optimisation. The Duncan's multiple range test determined the differences produced by the pH on the AUC of the Biogas figures (AUC-TAN). The Duncan's multiple range test (**Figure 4f**) revealed pH 5 as the best treatment for Biogas production and significant different compared to pH 7 and pH 9, which were statistically similar. In this case, the optimal conditions include pH 5 and I/S ratios between (0.1 and 0.2). As the most important factor in this work was VFA production, the optimal conditions for biogas production are not compatible for VFA production as they contradict the VFA conditions.

3.1.4 sCOD, COD reduction % and acidogenesis

The soluble chemical oxygen demand (sCOD) for the experiments evaluating the production of VFA from FW is depicted in **Figure 5.** Between day 0 and 15, the sCOD increased in most of the treatments, except the treatments with an I/S ratio of 0, in this case, the sCOD concentration at the end of the process was lower than at the beginning. At pH 7 and I/S ratios between 0.05 and 0.2, the sCOD exhibited an increment during the initial days followed by a stationary state. At pH 9 and I/S ratios between 0.05 and 0.2, the sCOD evidenced slightly increments instead of a stationary phase and finally, pH 5 had a considerable reduction in sCOD after the initial days. In this variable was possible to observe that both VFA and I/S ratio had influence in the sCOD concentration. The statistical analysis of the AUC for sCOD (**Annex 1-Supplementary material**) corroborated the effect of pH and I/S ratio on sCOD concentration **Figure 5**. The two-way ANOVA demonstrated that existed significant differences between the treatments (p value <0.0546, α=0.1). In this variable, pH and I/S ratio were significant to the model (pH p value <0.0039 and I/S ratio p value <0.096, α=0.1), while, the interaction between pH and I/S ratio was not significant (p value <0.6187, α=0.1). As both main effects were significant, their best conditions can be selected using the Duncan's test. **Figure 5F** describes the Duncan groupings for means of pH and I/S ratio. For the pH, the Duncan's test described pH 7 and 9 as the best conditions for sCOD as they had the highest values and were statistically similar. As expected, pH 5 was different than the other pHs and with lower values. On the other hand, the Duncan's test showed the I/S ratios of 0.05, 0.1 and 0 as the better conditions for sCOD as they had the highest values and were not statistically different, whereas, 0.1, 0, 0.15 and 0.2 were not significantly different as the less effective conditions for sCOD. The statistical analysis indicated pH 9 and 7 and I/S ratio of 0.05, 0.1 and 0 as the better conditions for sCOD.

Therefore, the conditions selected for VFA and TAN, pH 7 and I/S ratio of 0.05, can be also selected as the best conditions for sCOD.

The efficiency of hydrolysis in the VFA production can be expressed as the conversion of the solid organic matter initially fed into soluble compounds, measured as sCOD/initial COD ratio (**Annex 2, supplementary material**). While the degree of acidogenesis was measured in terms of VFA (converted to COD equivalent)/sCOD ratio, which represent the amount of solubilised organic matter converted effectively into VFA (Cadavid-Rodríguez and Horan 2014; Wang et al. 2018). In this experiment, the hydrolysis occurred rapidly and within the first 3 days around 50% of the total COD was in soluble form, for all the pH conditions analysed. The hydrolysis yields increased above 60% for day 10, confirming that FW was an easily hydrolysable substrate. Regarding acidogenesis (**Annex 2, supplementary material**), the lowest acidification level was exhibited by the treatment with pH 5, at this pH, VFA/sCOD ratio reached 16% after starting at 5%. This low percentage restate that VFA production from FW was inhibited at low pH. Even though, the acidification level was relatively high in this study, our maximum VFA/sCOD ratio (45%) was lower compared with the one reported by Bermúdez-Penabad *et al.*, (2017), whose higher ratio was 81% at pH 8.

3.2 Uncontrolled pH on VFA production

Although, pH 7 and I/S ratio of 0.05 were selected as the better conditions for VFA production, uncontrolled pH conditions were not evaluated in the first set of experiments, and different authors have cited the buffer potential of high protein wastes and how they can increase VFA production. Therefore, the importance of pH control for VFA production was tested in this experiment and all the different parameters evaluated in VFA production from FW without pH control are described in **Figure 6**. VFA production increased constantly from day 0 to 10 (**Figure 6a**), from there, the VFA concentration reached a stationary phase. The highest VFA concentration was recorded during the 10th day (70 g VFA/L) and it corresponded to a 29% increment between the uncontrolled pH treatment and the selected treatment (55 g VFA/L, pH 7 and I/S ratio of 0.05). Similar to the pH-controlled treatments, the VFA profile (**Figure 6c**) included acetic (38%), butyric (35%), propionic (10%), iso-valeric (9%), iso-butyric (6%) and heptanoic (3) acids. Between the initial days and the end of the process, the VFA profile evidenced a 10% and 5% reduction in the butyric acid and acetic acid concentration, respectively; whereas, an increase of 2 and 6-times was observed in iso-valeric and heptanoic acids, respectively. Biogas production was also enhanced by the lack of pH control (**Figure 6a)**. In fact, biogas was produced during the whole process in volumes higher than the ones reported in the previous experimental phase. Biogas highest production increment was obtained during the first three days; however, biogas production did not evidence a stationary phase or a decrease in their production. During the whole experiment, the pH ranged between 6.57 and 7.67 (**Figure 6b**); even though, VFA concentration was almost 10% w/v, this neutral pH indicated the high buffer capacity of FW and the ammonia released by the amino acid's hydrolysis. TAN concentration (**Figure 6d**) increased rapidly during the initial 5 days of the process; this increment was associated with protein and amino acid hydrolysis and was the principal reason why the pH did not significant variate during the fermentation process. TAN production combined with the pH allowed the significant increment in VFA production because the ionic form of the acids did not inhibit the microorganisms. The grade of acidification started in 5% and rose to 58% by day 10, which was a significant change compared with the treatments with controlled pH. The fast increment in sCOD (**Figure 6b**) observed in the initial days accorded with the results and curves describing the other variables. The high VFA production without controlling pH simplifies the operation and reduces the costs associated with the use of external buffers. Therefore, it can be recommended no controlled pH to produce VFA from Artisanal FW.

Discussion

This is the first article evaluating the optimal fermentation conditions for producing VFA from Artisanal FW and the first study evaluating the effect of pH and I/S ratio on this process. PH and I/S ratio were significant factors in VFA production and were significant for the other variables evaluated in the process, the highest VFA production reached in this process was 70 g VFA/L and was obtained using no pH control and an I/S ratio of 0.05. This high production is 2.5-times higher than that of VFA production (27 g VFA/L) using tuna waste from a cannery industry as raw material (Bermúdez-Penabad et al. 2017) and 3.5-times higher than that of lignocellulosic material (20 g/L) and mixed food waste (20 g/L) (Forrest et al. 2012; Jankowska et al. 2017). To the authors knowledge, the VFA concentration achieved in this research is the second largest reported following the VFA production obtained from slaughterhouse blood (100 g/L) (Plácido and Zhang 2018b). The high VFA concentration in this process is associated with the high TAN concentration in the reactors; the advantage of using Artisanal FW is the simultaneous TAN and VFA production during hydrolysis and acidogenesis.

During the artisanal FW acidogenesis, acetic acid and butyric acid were the predominant acids followed by propionic, iso-butyric and iso-valeric acids. VFA profile from artisanal FW fermentation had a similar distribution as the one obtained with tuna waste (Bermúdez-Penabad et al. 2017). The acetic and butyric acid predominance has been also reported during acidogenic fermentation of food waste (Jiang et al. 2013) and casein (Bevilacqua et al. 2020). The VFA profile produced from artisanal FW varied depending of the pH; although, VFA distribution was relatively constant during the fifteen days of fermentation. Acetic acid was predominantly obtained during acidogenic phase, as result of aminoacids metabolism. Amino acids metabolism under anaerobic conditions includes different pathways and the metabolic products depend of the type and concentration of amino acids. Acetic acid is the main fermentation product from most of amino acids in FW proteins, in contrast, n-butyric acid mainly comes from amino acids as glutamate, threonine, histidine, lysine and valine (Ramsay and Pullammanappallil 2001). Furthermore, through amino acid fermentation, the cell generates 0.5 moles of ATP *per* mole of amino acid transformed (Nisman 1954). These results evidenced the importance of pH conditions over total VFA production and how the individual VFA are produced and distributed.

The maximum concentrations of VFA under controlled pH conditions were reached at pH 7 and I/S ratio of 0.05. Similar results were reported for pineapple peels (Babel et al. 2004) and kitchen waste (Zhang et al. 2005) in those cases, pH 7 favoured VFA production compared with other alkaline and acid pHs. However, other authors have reported acidic and alkaline conditions as optimal for VFA production from other wastes. Under alkaline conditions (pH 8 and 9), the production of VFA using tuna waste (Bermúdez-Penabad et al. 2017) and activated sludge ((Zhao et al. 2018) as raw materials increased 10% and 5%, respectively. In contrast, slightly acidic conditions (pH 6) were optimal for VFA production from food waste and screenings, achieving a production of 51,3 g COD/L with food waste (Wang et al. 2014) and approximately 10 g/L with screenings (Cadavid-Rodríguez and Horan 2014). On the other hand, the inhibition of the production of VFA at pH 5, observed in this study, can be explained by the presence of non-dissociated acids in the medium (Babel et al. 2004). At low pHs the undissociated molecular forms of the acids predominate, which can easily permeate the cell membrane and, once inside the cytoplasm, the non-dissociated forms of the acids are dissociated due to the increase in intracellular pH, this phenomenon causes significant wear in the cell, which leads to impaired growth (Bermúdez-Penabad et al. 2017).

The optimal pH condition to produce VFA depends on the nature of the substrate (Lee et al. 2014). Therefore, for easily hydrolysable substrates, such as food waste, waste water and protein waste, neutral conditions lead to a greater solubilisation of carbohydrates, lipids and proteins, therefore to a greater VFA production and, on the contrary, alkaline conditions affect the metabolism of acidogenic bacteria, decreasing the VFA production (Zhang et al. 2005; Lee et al. 2014; Bermúdez-Penabad et al. 2017; Jankowska et al. 2017). While, for substrates that are complex and difficult to hydrolyse, such as activated sludge, the alkaline conditions improve hydrolysis of the compounds by the ionisation of the carboxylic groups of the proteins and carbohydrates (Wingender et al. 1999). In fact, it has been reported that alkaline pH improves the hydrolysis, while the acidogenesis is favoured at neutral pH (Jankowska et al. 2017).

The artisanal FW fermentation evidenced the importance of the I/S ratio for VFA production, which agrees with previous reports about optimisation of AD processes for biogas production (Sri Bala Kameswari et al. 2012; Xu et al. 2017), however, before this study, the effect of I/S ratio on acidogenesis has not received much attention. The decrease in the I/S ratio inhibits methane production, due to an excessive accumulation of VFA (Zhou et al. 2011). Lower I/S ratios imply a larger amount of substrate to digest *per* unit of inoculum, generating a hydrolysis and acidogenesis reaction rate greater than that of the methanogenesis. This difference in reaction rates produces the VFA accumulation in the reactor and inhibits the methanogenic microorganisms. VFA accumulation was also benefited by the high TAN concentration obtained in the transformation of FW. Ammoniacal nitrogen functions as an additional buffer in the culture medium, since ammonia combines with carbon dioxide and water to form ammonium bicarbonate which is a natural buffer (Zhang et al. 2005; Zhou et al. 2018). Thus, taking into account that the acidogenesis generally leads to a pH decrease, which can affect the viability of microorganisms, with protein-rich waste, the action of ammonium bicarbonate maintains the appropriate pH conditions without need to add an external buffer (Zhang et al. 2005). The high TAN concentration facilitates high VFA production because it inhibits methanogenic microorganisms and its buffering capacity facilitate the increment of VFA concentration without trigger inhibitory effects on the acidogenic microorganisms. These two factors associated with high protein waste such as artisanal FW, reduce operational costs and process complexity, as pH control and methanogens inhibition steps are not required. The conditions selected in this research will facilitate the evaluation of separation methods and further reactor configuration studies seeking to establish a biorefinery system for artisanal FW.

Conclusions

Artisanal fish waste is a suitable raw material to produce high concentration of VFA under different pH and I/S ratio conditions. Under pH-controlled conditions pH 7 and an I/S ratio of 0.05 were the optimal conditions for VFA production (54.5 g/L) from artisanal FW, these conditions were also optimal for TAN and sCOD. Low pH and high I/S ratio influenced negatively VFA production while enhanced biogas production. The VFA profile was composed by acetic, butyric, propionic, iso-valeric and iso-butyric acids and their proportion was dependent on the pH. The highest VFA concentration (70 g VFA/L) was obtained without pH control evidencing how the reactor was able to maintain neutral pH without been externally controlled. These results pave the way for the development of a VFA biorefinery from artisanal FW and open a new application for this type of residues.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LSCR was responsible of experiment's conception and design, acquisition of data, analysis and interpretation of data, and manuscript drafting. VECL was responsible of experiment's conception and design, acquisition of data, analysis and interpretation of data, and manuscript drafting. JP was responsible of the experiment's conception and design and final manuscript, analysis and interpretation of data, and manuscript drafting. All authors read and approved the final manuscript.

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Not applicable

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Figures

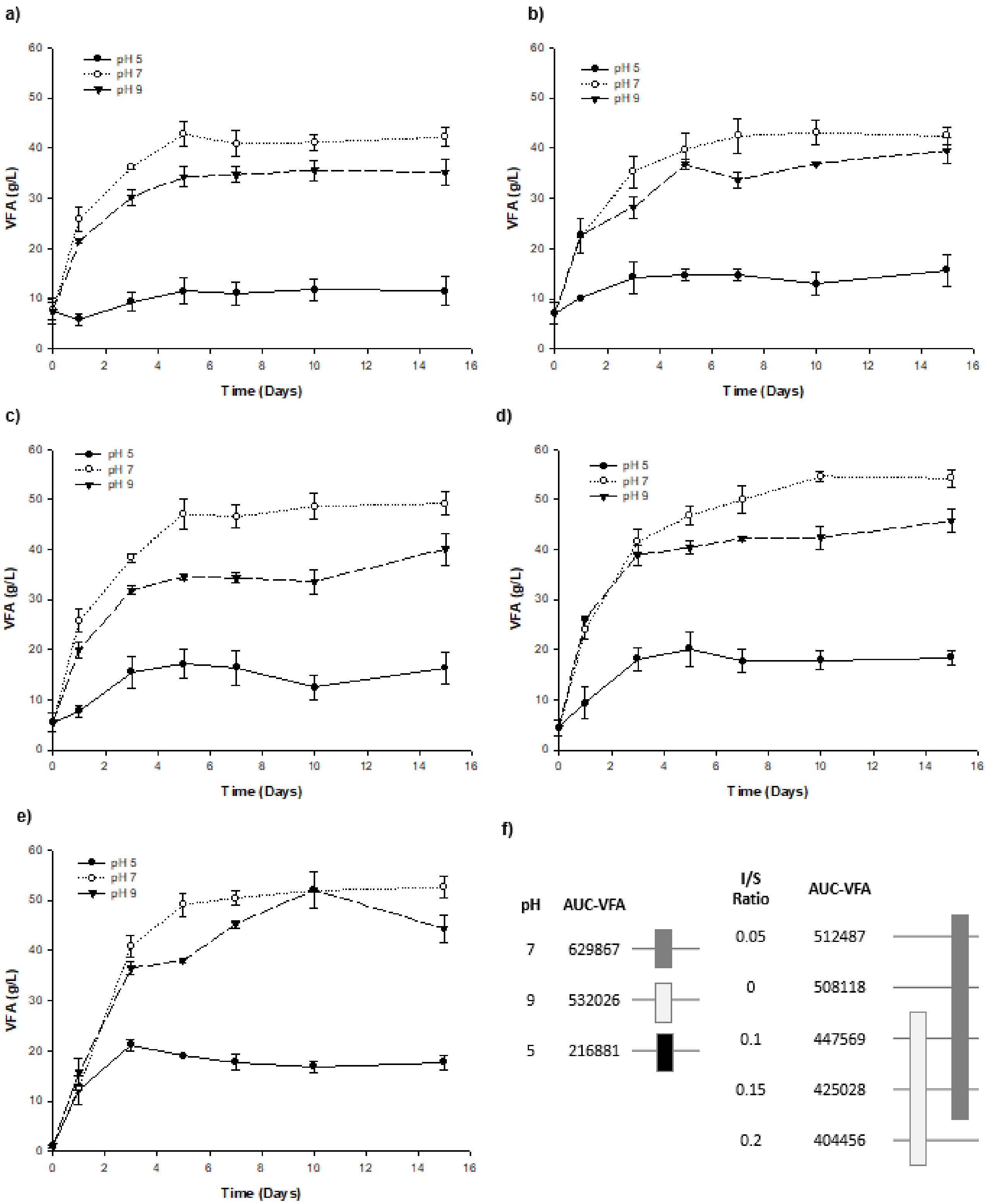

Figure 1

VFA production from FW using different pH and I/S ratios. Each figure represents a different I/S value: a. 0.20, b. 0.15, c. 0.10, d. 0.05 and e. 0.00. f. Duncan groupings for means of pH and I/S ratio for the AUC-VFA variable

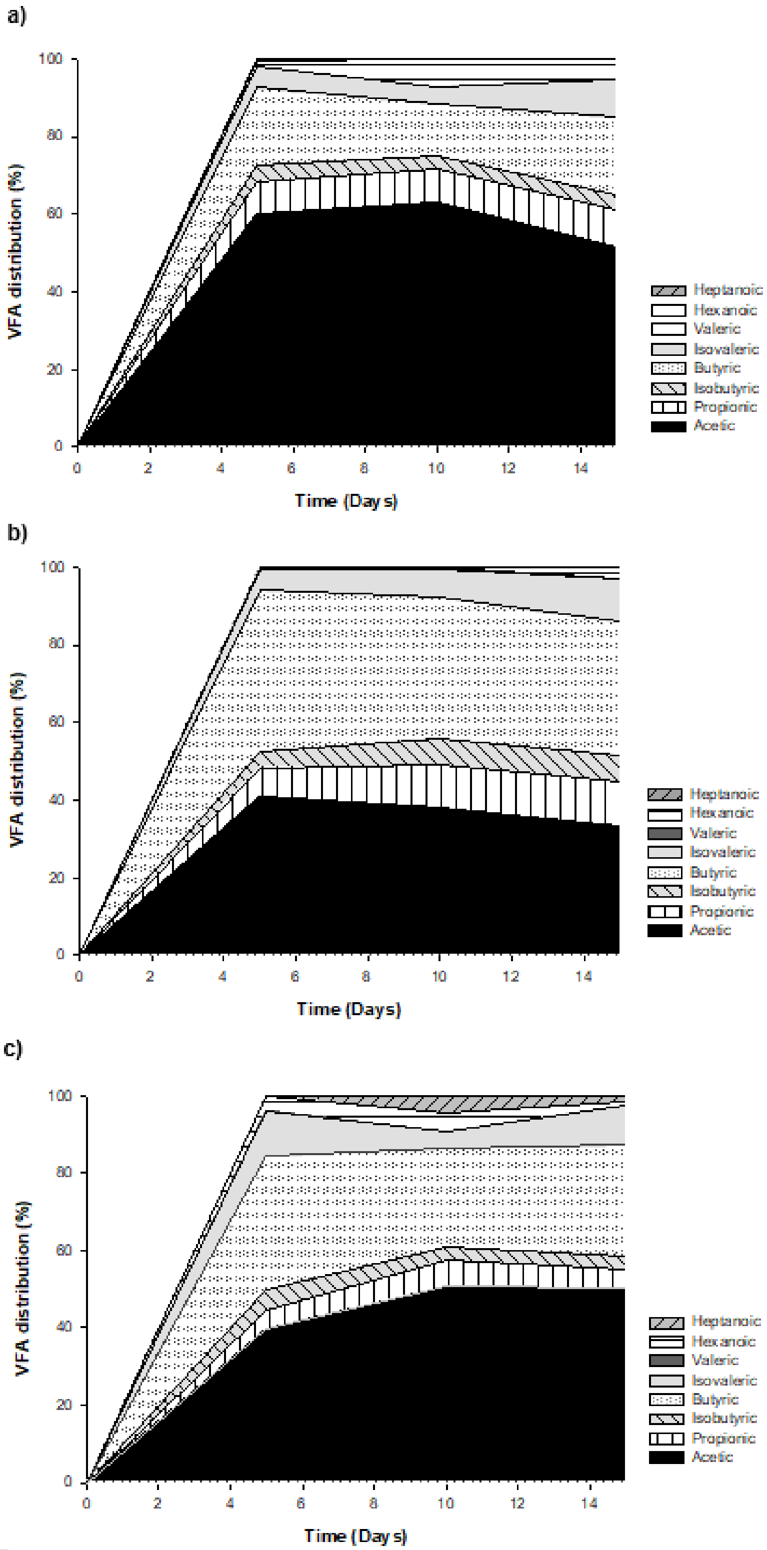

Figure 2

VFA profile for the different pHs evaluated: a. 5 b. 7 c. 9

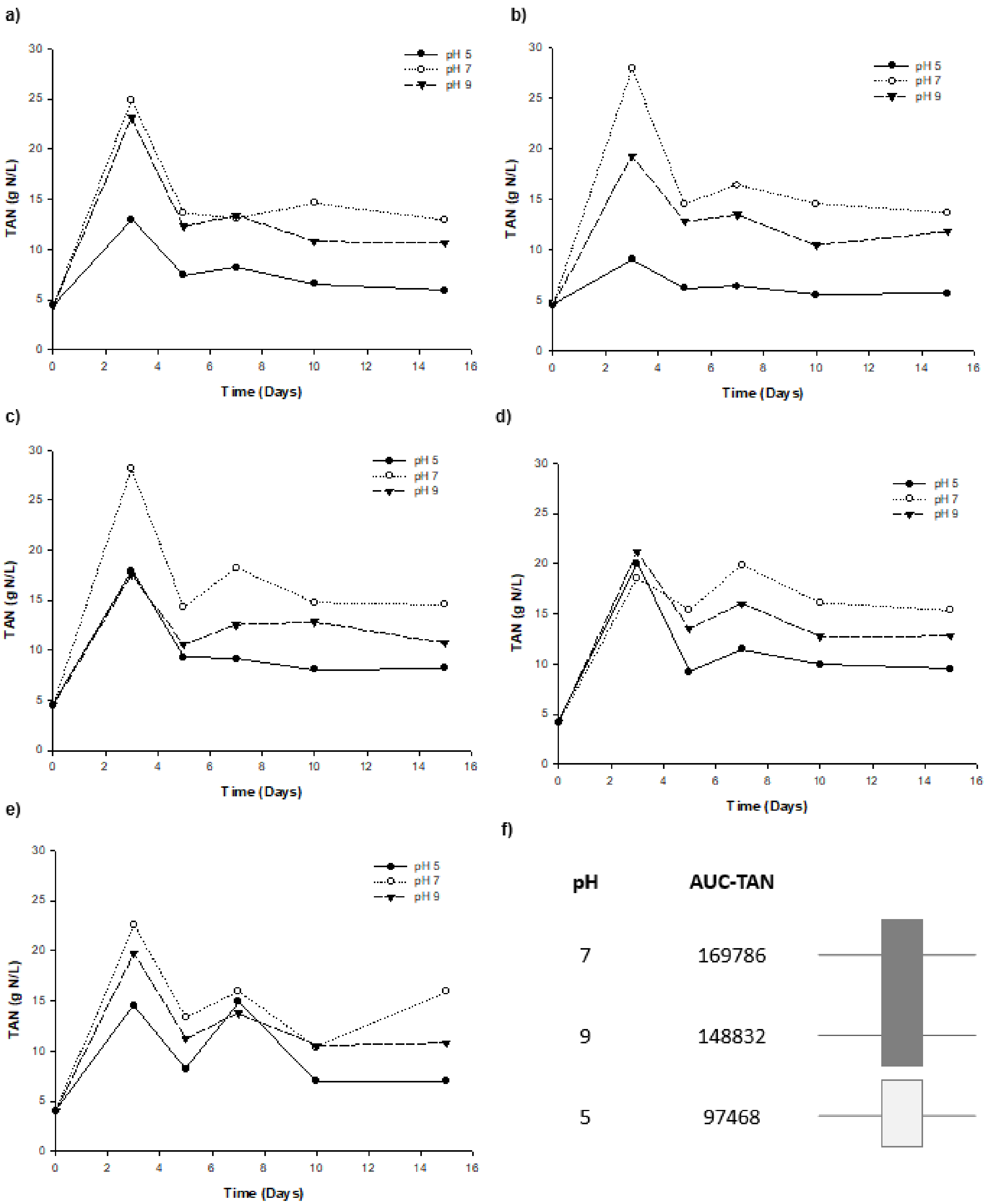

Figure 3

Ammonia production during FW transformation into VFA using different pH and I/S ratios: Each figure represents a different I/S value: a. 0.20, b. 0.15, c. 0.10, d. 0.05 and e. 0.00 f) Duncan groupings for means of pH and I/S ratio for the AUC-TAN variable

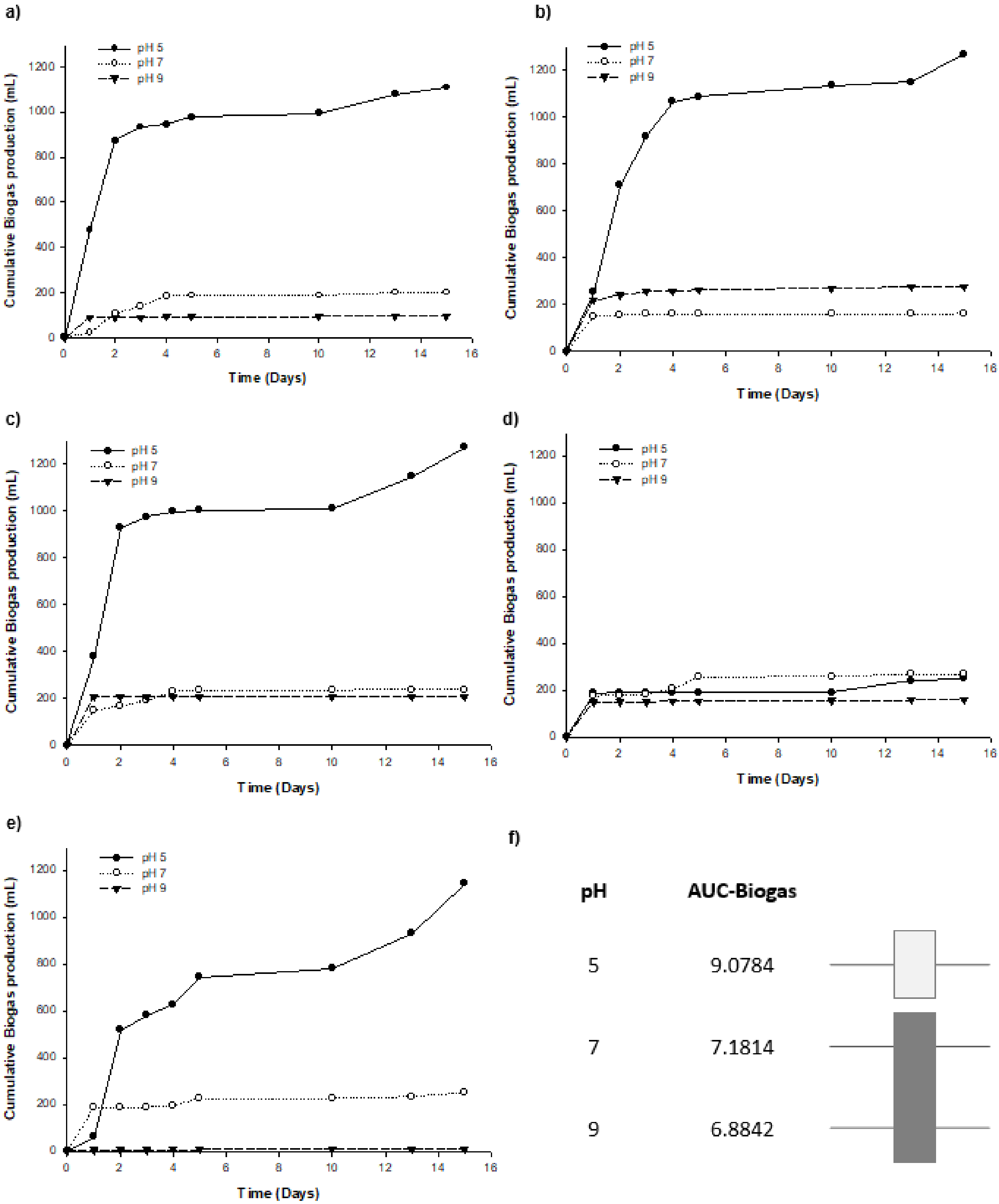

Figure 4

Cumulative Biogas production during FW transformation into VFA using different pH and I/S ratios: Each figure represents a different I/S value: a. 0.20, b. 0.15, c. 0.10, d. 0.05 and e. 0.00 f. Duncan groupings for means of pH and I/S ratio for the AUC-Biogas variable

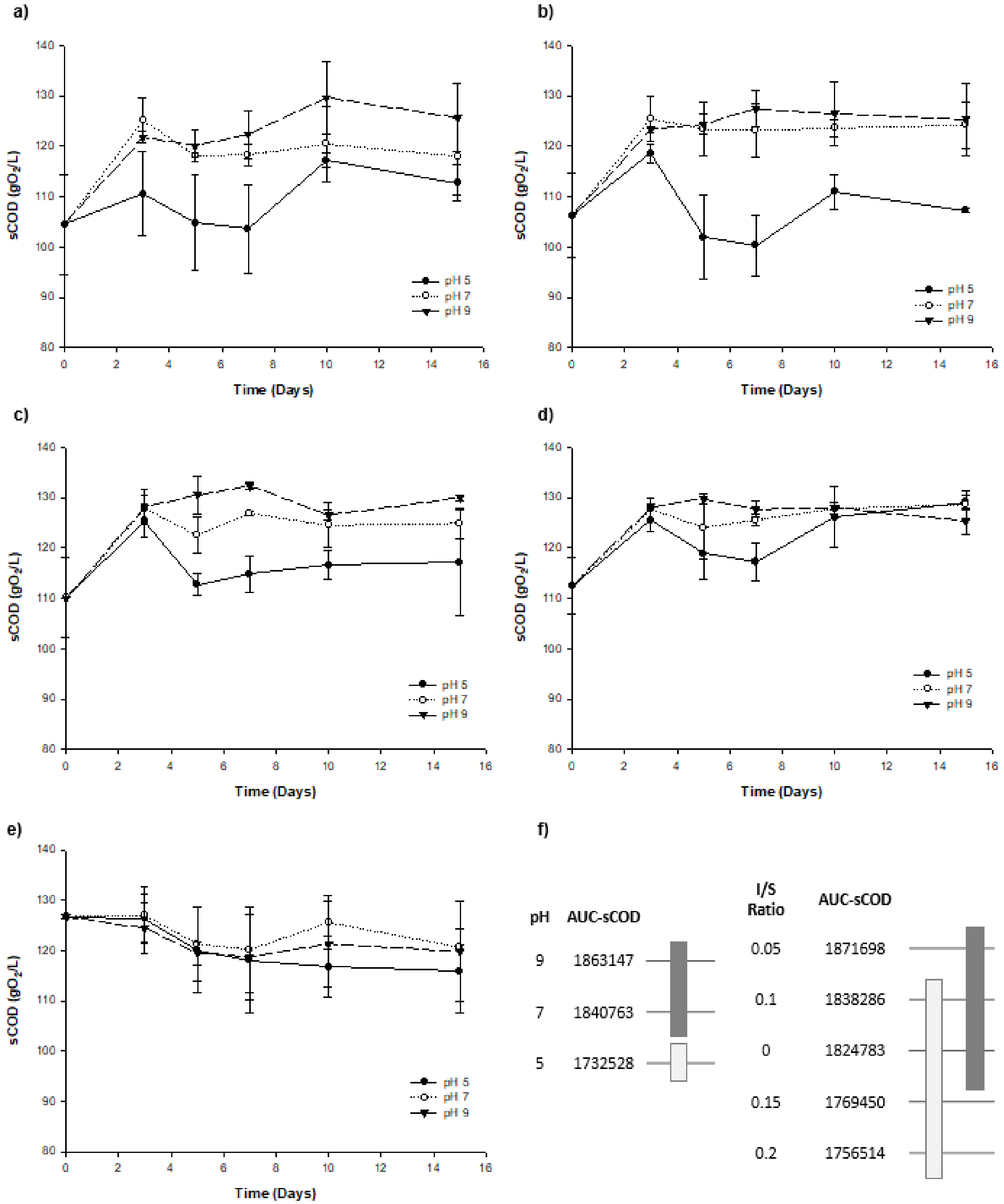

Figure 5

Soluble COD during FW transformation into VFA using different pH and I/S ratios: Each figure represents a different I/S value: a. 0.20, b. 0.15, c. 0.10, d. 0.05 and e. 0.00 f. Duncan groupings for means of pH and I/S ratio for the AUC-sCOD variable

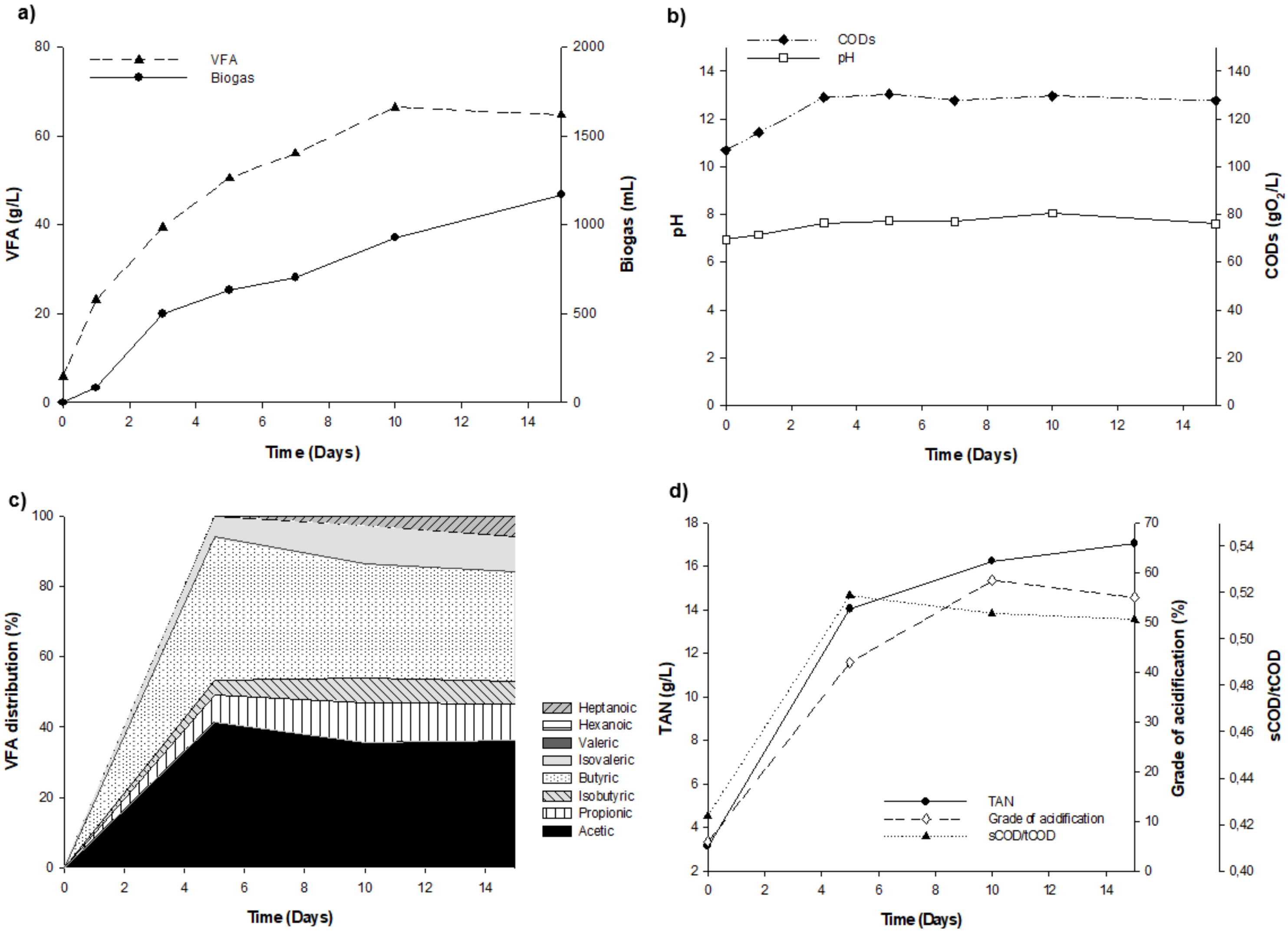

Figure 6

Uncontrolled pH treatment with I/S ratio of 0.05 a) VFA and biogas production b) pH and sCOD c) VFA profile d) TAN, grade of acidification, hydrolysis efficiency

Supplementary Files

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