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 and propionic, iso-valeric and iso-butyric were minor species (< 10%). An economic assessment evaluating the establishment of a FW VFA biorefinery in Tumaco, Colombia was performed including capital, operational and labour cost, discounted payback period and cost curves. These results evidenced 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 FW.

1. 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 products such as low-value animal feed, biogas and organic acids. Biogas production from FW using Anaerobic digestion (AD) 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. 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 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 could 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 had 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 authors' 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 optimal 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. Finally, a preliminary economic assessment was carried out for a VFA biorefinery using artisanal FW from Tumaco Colombia.

2. Materials And Methods

2.1 Inoculum and Substrate

The inoculum was a methanogenic sludge (MS) obtained from an up flow 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.

2.2 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 5x3 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 centrifuge (SL8 Centrifuge - Rotor High Conic III, 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.

2.3 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 (30m x 25mm x 0.25μm), polar stationary phase of polyethylene glycol treated with nitro-terephthalic acid, automatic injector and a flame ionisation detector (GC-FID).

2.4 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 the 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.

2.5 Economic assessment

An initial economic assessment for a VFA biorefinery using artisanal FW from Tumaco Colombia was performed including discounted payback period, scaling up costs, and cost curves. The fixed capital investment (FCI) was assumed 1500 USD/Kg FW and it was based in Colombian prices estimates. The economic assumptions used included: 1) the plant life was 20 years, 2) the annual labour and operational costs were assumed to be 25% and 21% of the FCI (Petersen et al. 2018). 3) the FCI for scaling up the VFA biorefinery plant was defined by **Equation 1**.

$$FCI_x = \left({}^{Capacity_{initial}} / {}_{Capacity_x} \right)^C \qquad \textbf{Equation 1}$$

Where C is the capacity exponent, in this case C was equal to 0.8. 4) the digestate yield was assumed at 0.15 and its price was defined at 0.055 USD/kg (Golkowska et al. 2014; Wainaina et al. 2019), 5) the FW gate fee was assumed at 0.02 USD/kg (CEMPRE 2017), 6) The VFA purification system is an advanced method with TOA and octanol with a VFA recovery of 75% (Plácido and Zhang 2018a), 7) the price for acetic, propionic, butyric, iso-butyric, isovaleric, and hexanoic acid were 2.3, 3, 2.4, 4.3, 3, 3 USD/kg, respectively. These values were obtained from Alibaba.com and included prices from industrial grade acids. Discounted payback period was calculated using present values of the income and costs of the biorefinery plant.

3. 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 Duncan's test (**Figure 1f**) evidenced that pH 7 produced the highest 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 (**Annex 1, Supplementary material**) indicated that pH 7 and I/S ratio between 0 and 0.1 produce the highest amounts of VFA from FW. Therefore, the treatment with an I/S ratio of 0.05 and pH 7 was selected as optimal conditions 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 that of 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. **Figure 3F** describes the Duncan groupings for means of pH for the AUC-VFA variable. The statistical analysis (**Annex 1, Supplementary material**) 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 Duncan's multiple range test (**Figure 4f**) and (**Annex 1, Supplementary material**) the statistical analysis revealed the pH 5 and I/S ratios between (0.1 and 0.2) as optimal conditions. 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 Hydrolysis efficiency, acidogenesis, sCOD, COD reduction %

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 (**Figure 5F & Annex 1, Supplementary material**) 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, were selected as the optimal conditions for sCOD.

3.2 Uncontrolled pH on VFA production

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 (**Figure 6a**). 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.

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.

3.3 Economic assessment

The previous sections evidenced the potential for using artisanal FW as a raw material for VFA production. Therefore, it is important to evaluate the potential of a VFA biorefinery and economic projections in communities with significant presence of artisanal fishers. As the project associated with this article was performed in the city of Tumaco, Colombia. The economic assessment was evaluated to utilise the artisanal FW produced in Tumaco. In a year, the artisanal fish landed in Tumaco is approximately 1131 tons (De la Hoz-M et al. 2017), producing 509 tons of FW (FW = 0.45 x Fish landed) per year or 1.39 tons of FW per day (Rai et al. 2010). In this economic assessment, the yields (g Acid / g

TS FW) used for each acid were those obtained from the treatment with no pH control and an I/S ratio of 0.2. **Table 3** includes the yields and cost for the different parameters included in this assessment.

Figure 7a describes how income and cost changed depending on the amount of FW accepted in the VFA biorefinery plant every day. The VFA biorefinery income is obtained from three principal sources, VFA production, gate fees, and digestate. VFA production is responsible for 89% of the income followed by gate fees (10%) and digestate (1%). The capital costs per kg of FW decreased with the capacity increment of the VFA biorefinery plant. However, this decrease was more significant during the first ton as capital costs per kg of FW decreased 226 USD/kg of FW between 100 kg and 1 ton, whereas, from 1 to 2 tons the change is 48 USD/kg of FW and from that point on the cost for duplicating capacity (2 to 4, 4 to 8, 8 to16, 16 to 32) is between 48 and 28 USD/kg of FW. In contrast, the income increased steadily with the size increment and total income grew faster than operational and labour costs with the increment of size, as the total income line intersected both operational and labour costs lines.

VFA total income included the same acids as the VFA profile obtained from the experiment without pH control. However, The VFA income did not distribute the same as the VFA profile (acetic (38%), propionic (10%), butyric (35%), iso-butyric (6%), isovaleric (9%) and heptanoic (3%) acids). The VFA income (**Figure 7b**) had a different behaviour as acetic and butyric acid reduced their participation 5 and 2 %, respectively; while, propionic, isobutyric, isovaleric and heptanoic acids increased their participation 4, 1, 1, and 1 %, respectively. Although, the increment of these acids in VFA income is associated with their greater price, the most significant contributions to the income were made by acetic and butyric acids. **Figure 7c** describes the payback years and capital cost associated with the capacity increment. The necessary amount of years to payback the initial capital cost reduced significantly during the initial hundreds of kg and were associated with less considerable increments of capital cost. Reducing payback period below 10 years required more significant amounts of capital cost investments than the previous years.

Tumaco's artisanal FW maximum production is approximately 1.4 tons per day; therefore, that is the maximum size for a VFA biorefinery in that location. The payback time for a plant of 1400 kg is 16 years (**Figure 7c**) with an initial capital cost of 493.166 USD (**Figure 7a and 7c**) and the production of 34 tons of VFA per year. Although, the payback time and initial capital cost is not extremely high, this type of project will require the investment from local, state, or national government. Artisanal fishers communities in Tumaco did not have the income to support this type of project and a smaller plant with less capital cost will generate greater payback times as the VFA and gate fees incomes will reduce significantly. Alternatively, a larger VFA biorefinery can be installed in this place, however, this plant will require the utilisation of FW from the commercial fisheries in Tumaco. Industrial fisheries land 11.000 tons of fish every year producing 4.950 tons of FW. This amount of FW corresponds to 13.5 tons of FW per day increasing the total FW available for the VFA biorefinery to almost 15 tons per day. Using the same assumptions and calculations, a VFA biorefinery able to manage 15 tons per day will require an investment of 3.288.246 USD for capital costs and will produce 362 tons of VFA per year. The discounted payback period for this plant is 6 years with a capital cost of 219 USD/per kg of FW.

4. 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 on 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 amino acids metabolism. Amino acids metabolism under anaerobic conditions includes different pathways and the metabolic products depend on 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.

The principal restriction for an artisanal FW biorefinery located in Tumaco is the amount of FW generated. The maximum artisanal FW production, 1.4 tons per day (Table 3), restricts the capacity of the biorefinery plant, the payback time (16 years) and estimated capital cost (493.166 USD). Although, the cost and payback time is not that high, it is high compared with other options available for this resource in this place and for the fisherman communities. In a previous paper, the potential of using Tumaco's artisanal FW for biogas production was assessed (81,488m3 of biogas) and evidenced the possibility of using FW to electrify or fulfil cooking energy demand for more than 200 homes (Cadavid-Rodríguez et al. 2019). A program to supply these families with the equipment required to fulfil their cooking energy supply can cost between 115.000 and 345.000 USD and has a payback period between 3 to 8 years. The difference in total cost and payback period between biogas and VFA production and the necessities of the fisherman communities in this area to access energy for electricity and cooking makes biogas production as the better option for artisanal FW in Tumaco. In contrast, a VFA Biorefinery, it is more significant for industrial fisheries because their FW production did not represent the same level of restriction as artisanal FW. Using the current industrial FW production (14 ton/day) a VFA Biorefinery can solve the environmental issues associated with FW, create jobs, and generate a source of income for Tumaco's fishing industry. This larger plant can be financed via public-private partnership or a private only investment, in both cases, a program for artisanal FW collection can be established to incentive the artisanal fishers to collaborate with the VFA biorefinery.

5. 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. The economic assessment estimated that an artisanal FW VFA biorefinery can be established in Tumaco; however, FW production is a limiting factor as low FW production is associated with greater capital cost per kg and longer payback periods. These results pave the way for the development of a VFA biorefinery from FW and open a new application for this type of residues.

6. Declarations

6.1 Ethics approval and consent to participate

Not applicable

6.2 Consent for publication

Not applicable

6.3 Availability of data and materials

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

6.4 Competing interests

The authors declare that they have no competing interests

6.5 Funding

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6.6 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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Table 3

Table 3. Economic analysis summary for a VFA biorefinery using Tumaco’s artisanal FW

Tumaco’s artisanal fish production		
Tumaco’s Artisanal fish landed per year	1131 tons	
Tumaco’s Artisanal FW per year (TAFWY)	509 tons	
aco’s Artisanal FW average production per day (TAFWY)	1.39 Tons/day	
Volatile fatty acid yield and selling prices		
Acid Type	Yield (g Acid /g TS FW)	Price (USD/kg)
Acetic	0.133	2.3
Propionic	0.035	3
Butyric	0.1225	2.4
iso-butyric	0.021	4.3
Iso-Valeric	0.0315	3
Heptanoic	0.0105	4
Additional income sources		
Source	Price (USD/kg)	
FW gate fee	0.02	
Digestate	0.15	

Figures

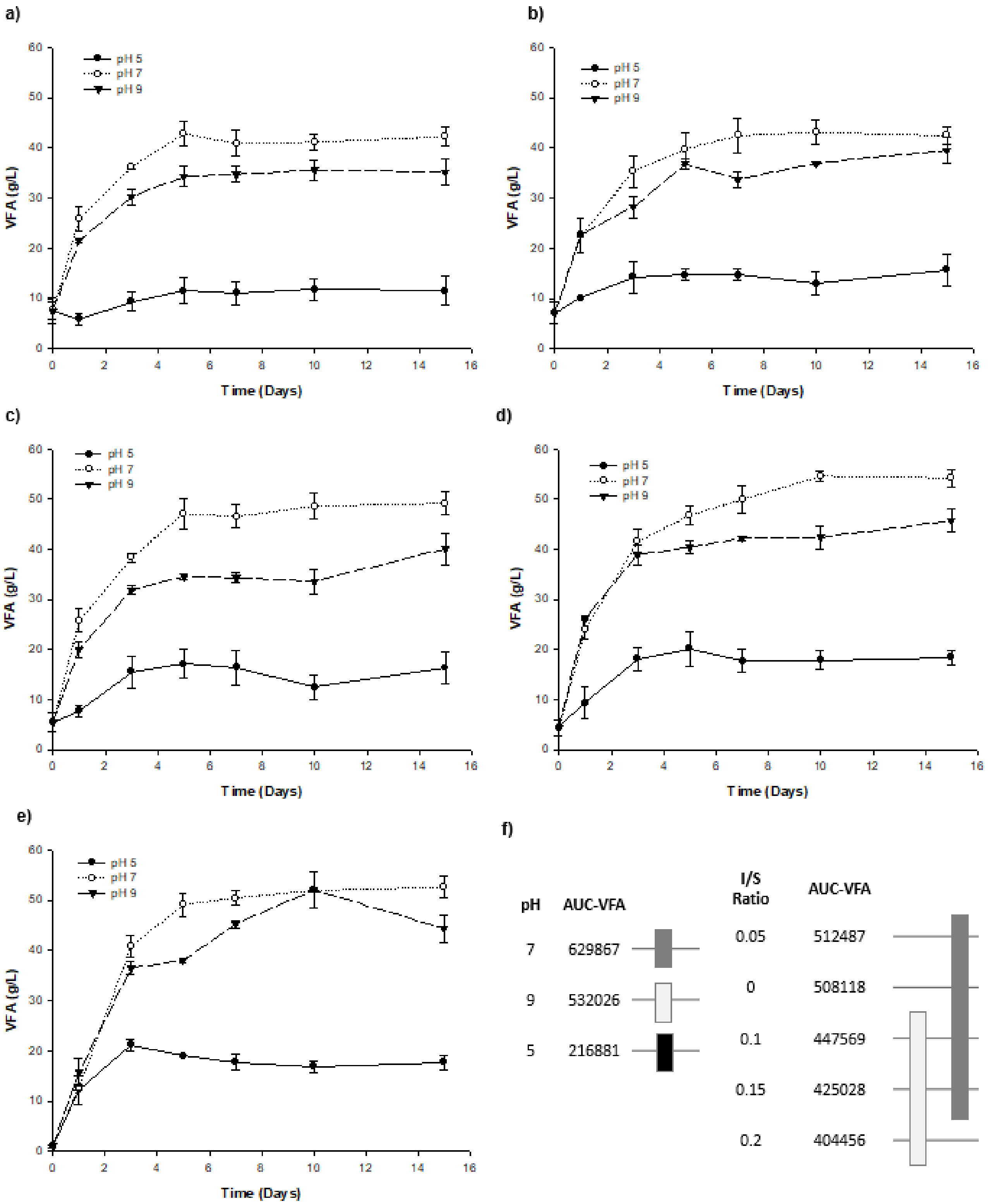

Figure 1

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

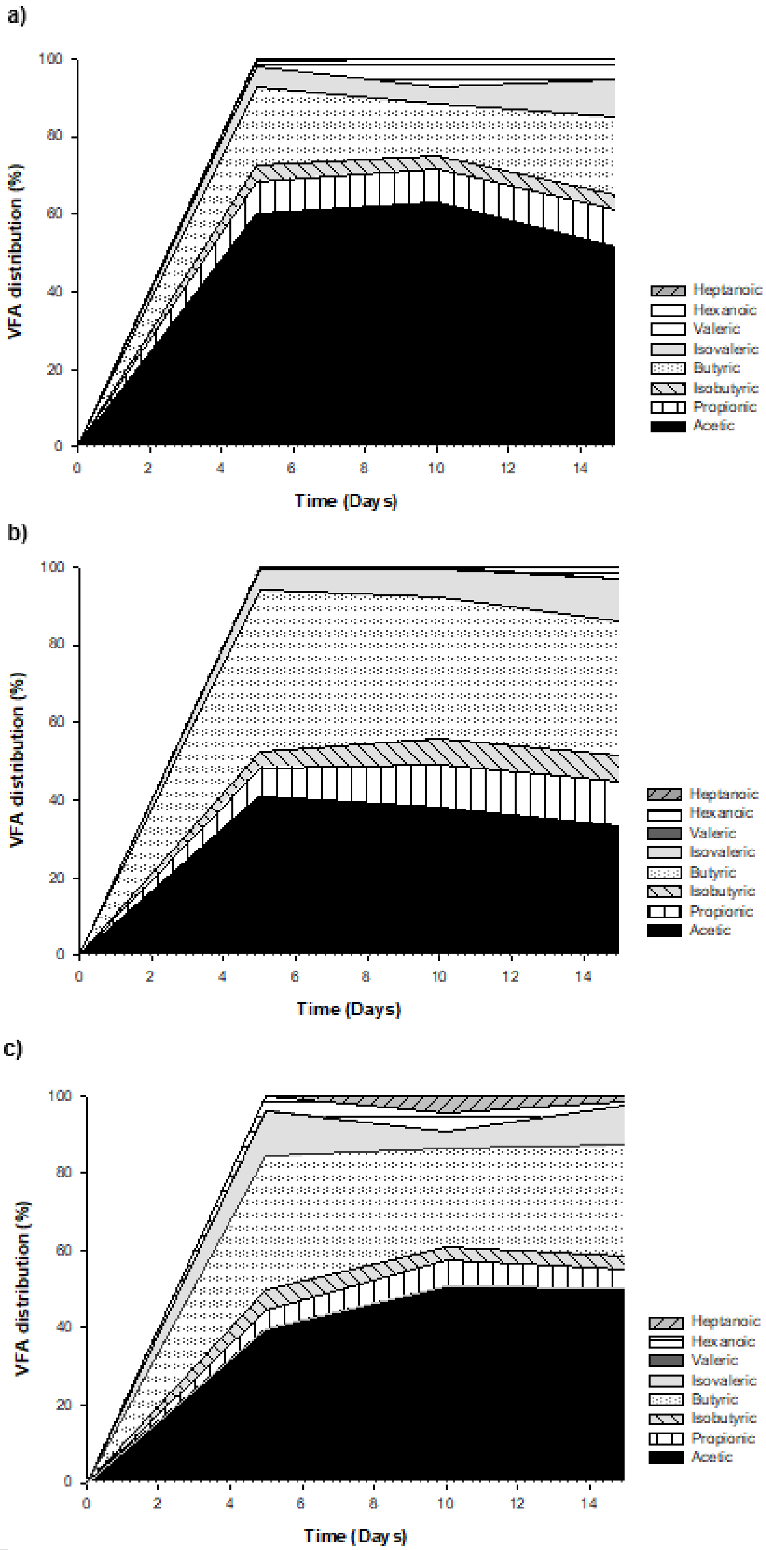

Figure 2

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

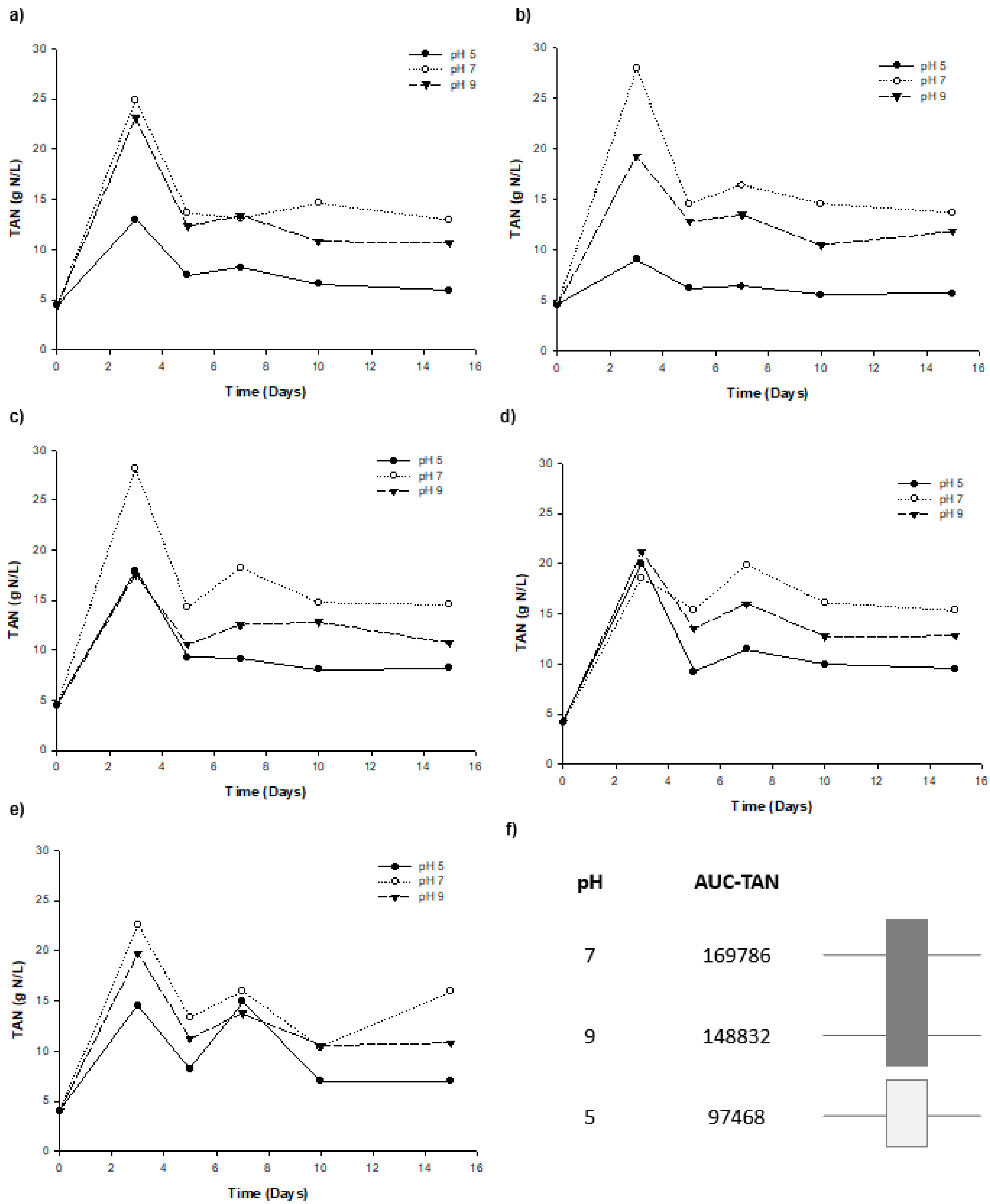

Figure 3

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

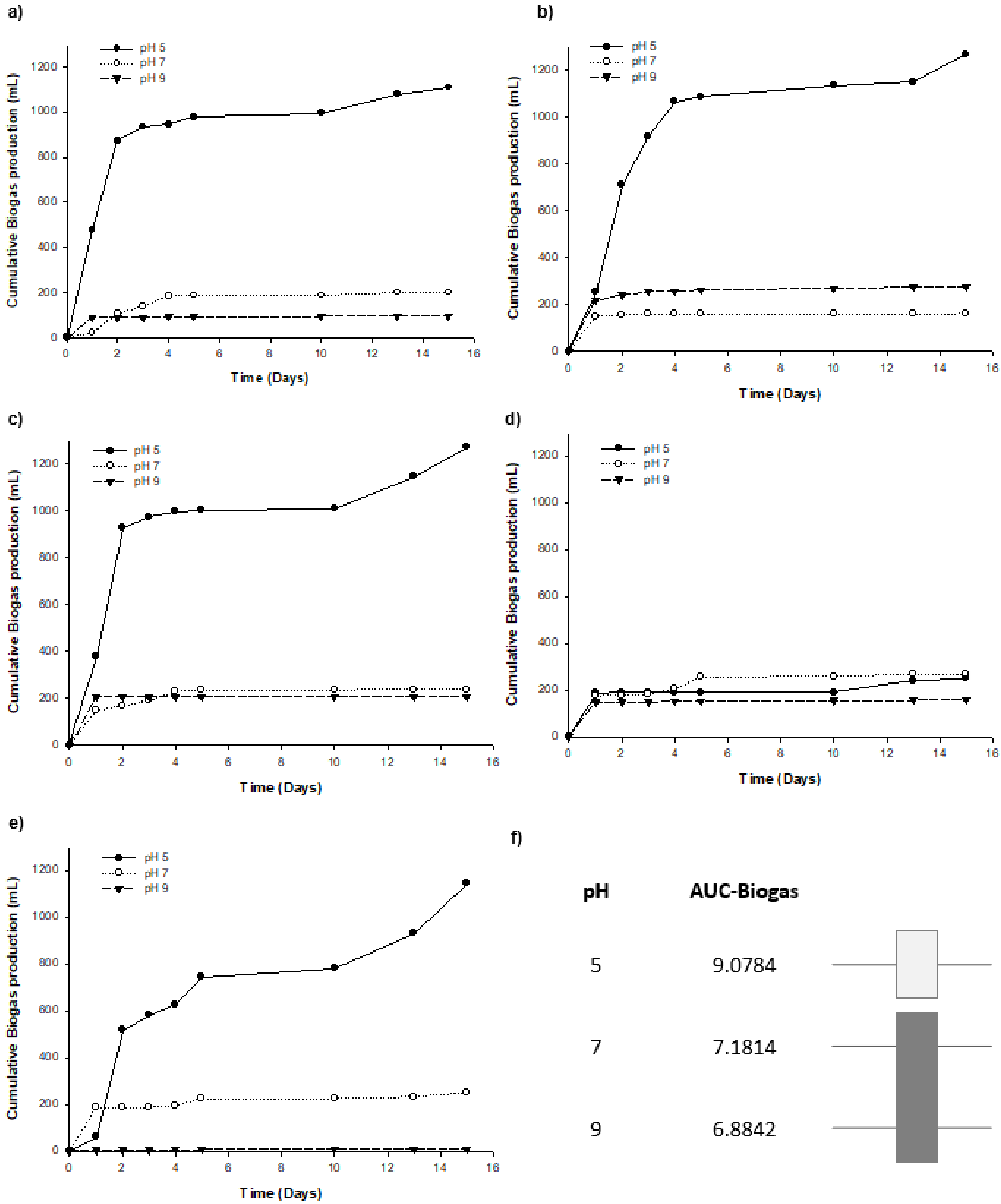

Figure 4

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

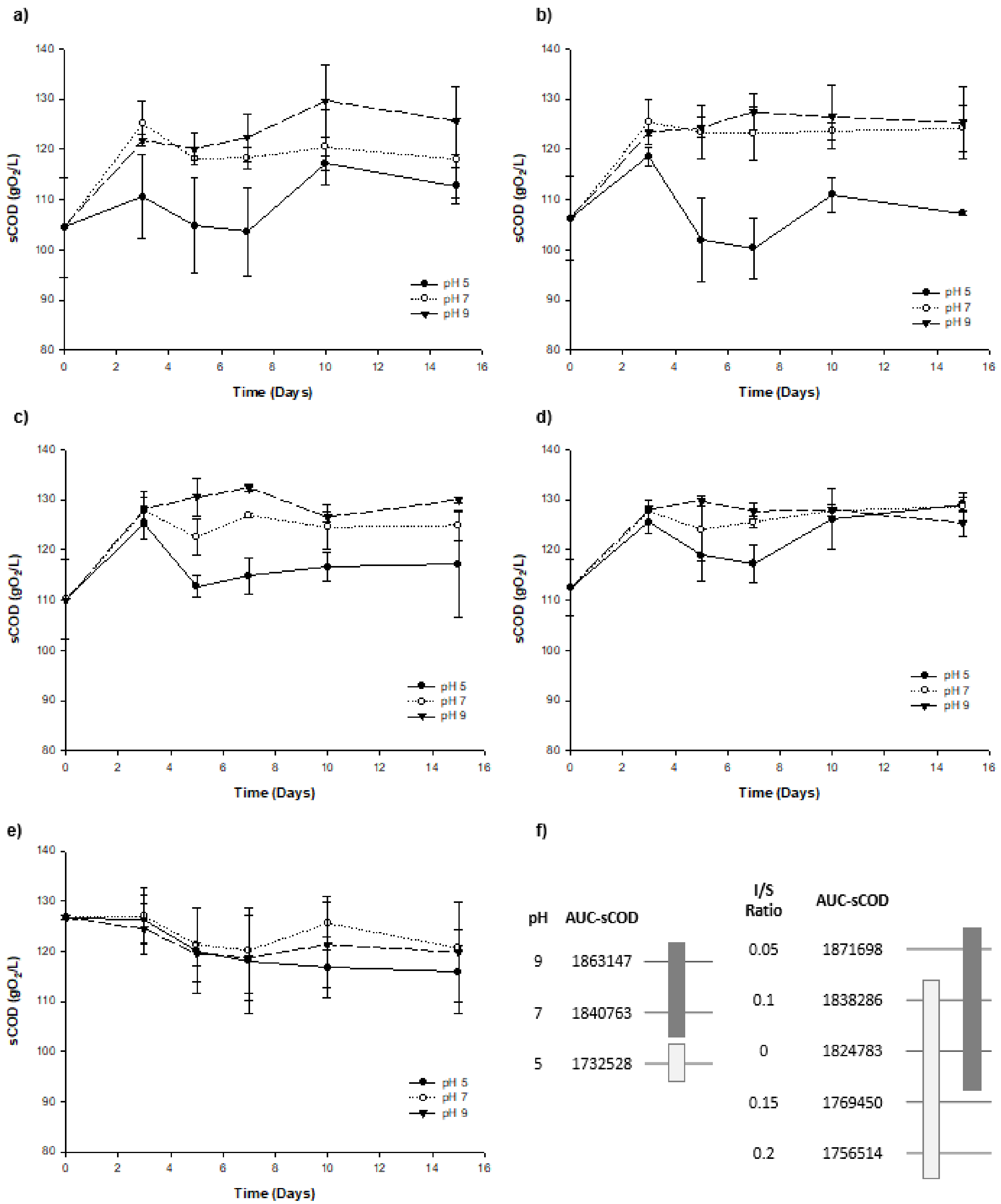

Figure 5

a) Hydrolysis efficiency for the different pHs evaluated. b) Grade of acidification for the different pHs evaluated

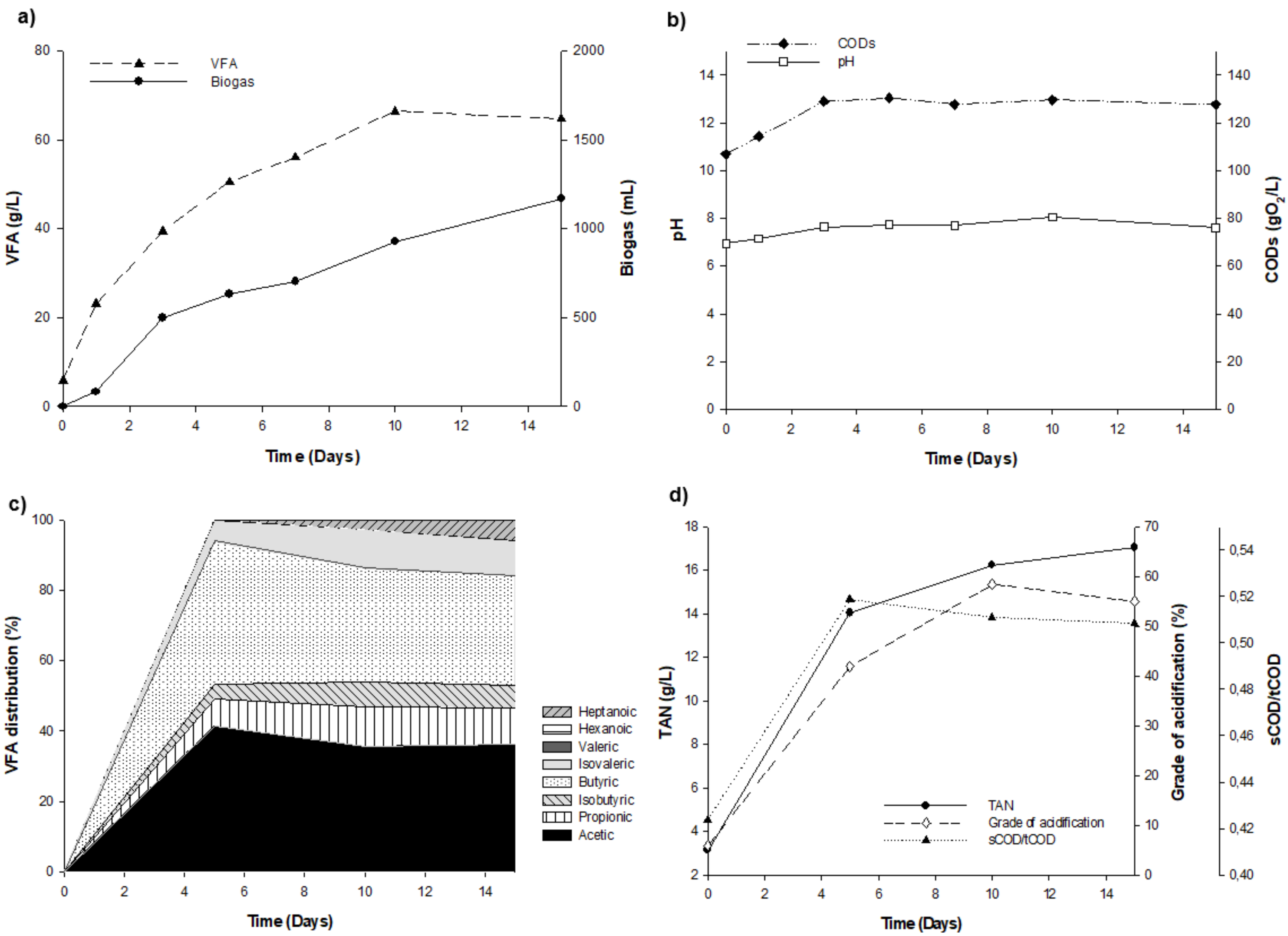

Figure 6

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

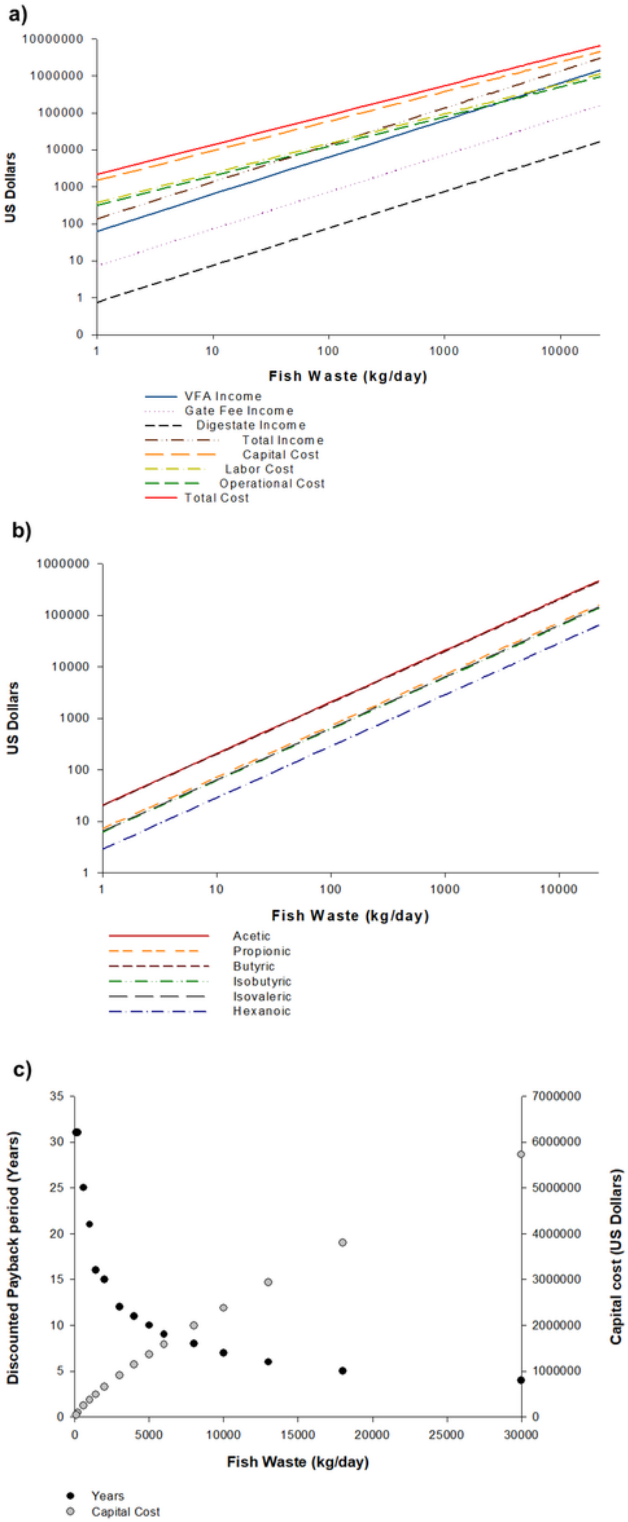

Figure 7

a) Income and costs generated by a VFA biorefinery plant vs daily FW intake, the x and y axis are in log 10 scale b). income produced by each VFA vs daily FW intake the x and y axis are in log 10 scale. c) discounted payback period and Capital cost vs Daily FW intake

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