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Volatile fatty acid production from saline cooked mussel processing wastewater at low pH

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ABSTRACT

The production of VFA using as substrate the wastewater produced in a cooked mussel processing factory, containing large COD (13.7 ± 3.2 g COD/L) and salt concentrations (21.8 ± 2.8 g NaCl/L) and characterized by low pH (4.6 ± 0.6) was evaluated. This wastewater was fed to a 5-L completely stirred tank reactor operated in continuous mode. The conversion efficiency of its COD content into volatile fatty acids (VFA) was evaluated. The maximum acidification of 43 % (total VFA on soluble COD basis) was obtained when an organic loading rate of 2.5 ± 0.4 g COD/(L·d) was applied to the reactor and corresponded to a VFA volumetric productivity of 0.72 ± 0.07 g COD_{VFA}/(L·d). Under steady-state conditions, the obtained mixture of VFA was composed by 80:18:2 as acetic:propionic:butyric acids (percentage of VFA on soluble COD basis). Carbohydrates were degraded up to 96 % while protein fermentation did not take place, probably due to the low pH value, limiting the maximum acidification of the wastewater. Batch experiments showed that the increase of the pH from 4.2 to 4.9 by the addition of $NaHCO_3$ resulted in the improvement of the acidification and changed the VFA mixture composition. Thus, this study demonstrates the opportunity of using complex substrates, as cooked mussel processing wastewater, to produce rich-VFA streams under unfavorable operational conditions, such as high salinity and low pH.

Keywords: Anaerobic fermentation; Biorefinery; Industrial wastewater; Protein degradation; Salinity; VFA.

1. INTRODUCTION

The fish and seafood canning industry is a crucial economic sector in Galicia (North-West of Spain) which nowadays amounts to 67 % and 80 % of the European and Spanish production, respectively (FAO, 2019). Indeed, Galicia is the third producer worldwide just after Thailand and China. More specifically, mussels are one of the most popularly consumed seafood, and Galicia represents 50 % of the worldwide production (OPMEGA, 2020). This industrial sector consumes an enormous amount of water, either freshwater and/or seawater, which on average is above 10 m^3/tonne of raw mussel processed. As a consequence similar large volumes of highly polluted wastewater are generated (Bello Bugallo et al., 2012). The main environmental problem associated with this produced wastewater relates to the high organic matter (up to 42 g/L), comprising proteins (15 - 20 % of wet weight) (Tay et al., 2005), and salt concentrations that could reach values over 20 g NaCl/L (Méndez et al., 1992). The discharge to the environment of these streams without appropriate treatment could provoke continual oxygen depletion, due to the contained organic matter, which causes the death of the aquatic life. Furthermore, the discharge of nitrogen from proteins favours algae overgrowth leading to the eutrophication of the receiving water body. In addition, if salty wastewater is not withdrawn directly into the sea but to freshwater water bodies is responsible for the increase of salinity of these ecosystems, similarly if it is treated in municipal wastewater treatment plants that discharge in interior areas.

The treatment of the fish and seafood processing wastewater is particularly challenging due to its complex characteristics (high organic matter and salt concentrations). In addition, the seasonal activity of the factories and the fact that they commonly process different products within one single week involves the generation of wastewater streams with different composition in the same facility. The wastewater characteristics depend on the processing step where it was generated: preliminary operations (reception, washing, brining, cutting…), processing (cooking, canning and trimming), final operations (sealing and sterilization) or auxiliary operations such as steam generation (Carrera et al., 2019; Cristóvão et al., 2016; Méndez et al., 1992). For example, a high volume of diluted washing wastewater is generated while the volume of cooking process wastewater is highly polluted is low. Nevertheless, the different generated wastewater types are usually treated together after being homogenized in a tank (Cristóvão et al., 2016). The most common technologies applied for the treatment of fish and seafood processing wastewater are based on physical-chemical (membrane separation, chemical destabilization and electrochemical methods) and biological (anaerobic and aerobic) processes (Carrera et al., 2019; Cristóvão et al., 2012). Biological processes enable the recovery of resources from wastewater especially when the valorized stream contains large concentrations of organic matter, as it is the case of the fish and seafood canning processing wastewater attracting great interest.

Anaerobic digestion is suggested as a suitable treatment for seafood wastewater due to its high organic matter removal capacity, low energy consumption, low sludge production and energy production as biogas (mainly CH_4 and CO_2) (Chowdhury et al., 2010). Anaerobic processes with high removal efficiencies (55 - 97 %) and treating organic loads of 1 - 4 kg $COD/(m^3 \cdot d)$ have been applied to treat these effluents (Méndez et al., 1992; Panpong et al., 2014; Prasertsan et al., 1994; Sillapacharoenkul and Sinbuathong, 2020). In the frame of the circular economy, the waste conversion into volatile fatty acids (VFA), which are short-chain fatty acids obtained as metabolic intermediates in the anaerobic digestion, has recently gained attention due to their wide variety of applications (Kleerebezem et al., 2015). VFA are intermediate products of the anaerobic digestion process. Thus, VFA-rich streams are produced in fermentation processes where the methanogenic step is suppressed (Wainaina et al., 2019). Application alternatives of the waste-derived VFA are the generation of biofuels, bulk chemicals, the biological removal of nutrients from wastewater and the production of bioplastics or food additives. For example, VFA can be used as a carbon source during the denitrification or the biological phosphorus removal processes. VFA act also as substrate in the production of polyhydroxyalkanoates (PHA), a type of bioplastic, by mixed microbial cultures (Atasoy et al., 2018; Wainaina et al., 2019).

Operational conditions of the anaerobic systems significantly influence the concentration, yield and composition of the VFA produced from wastes. Organic acid production is strongly affected by the pH of the reaction media since it has a great influence on the growth rate of the microorganisms involved in the anaerobic digestion (Wainaina et al., 2019). Indeed, methane production is barely observed out of its optimal pH range (6.5 - 8.5). Nevertheless, hydrolytic and acidogenic microorganisms operate at an optimal pH range of 5 - 11 and cannot survive in extremely acidic (pH 3) or alkaline (pH 12) conditions (Jankowska et al., 2015; Wainaina et al., 2019). The optimal pH to maximize the acidification efficiency varies according to the waste characteristics and the operational conditions. Jankowska et al. (2015) observed that, in unbuffered systems, acidic pH promoted the VFA production at short retention time (5 days) while alkaline pH (10 - 11) maximized VFA accumulation at longer retention times (15 days). Wainaina et al. (2019) stated that acidic pH is suitable to produce VFA from a variety of easily degradable wastes while alkaline pH values are recommended when complex substrates are used. For example, different optimal pH values to obtain VFA were reported: from cheese whey is 5.2 - 5.5 (Bengtsson et al., 2008), from food waste and the organic fraction of municipal solid waste is 9.0 (Cheah et al., 2019; Moretto et al., 2019), from kitchen waste is 7.0 (Zhang et al., 2005) and from wasted activated sludge ranges from 9.5 to 11.0 (Chen et al., 2007; Liu et al., 2020).

Since most cooked mussel processing factories use seawater in their processes, another primary concern with the produced wastewater is its high salinity (Xiao and Roberts, 2010). Significant salt concentrations can inhibit the anaerobic processes, especially the methanogenesis step at concentrations above 10 g NaCl/L (Panpong et al., 2015). However, the adaptation of the anaerobic biomass to high salt concentrations (Artiga et al., 2008; Sudmalis et al., 2018; Zhang et al., 2017), or the use of halophilic inoculum (Aspé et al., 1997; Scoma et al., 2017; Tan et al., 2019) are suitable strategies to develop an anaerobic treatment process for saline wastewater.

The purpose of this study was to evaluate the suitability of the wastewater generated in a cooked mussel processing factory as feedstock to produce a VFA-rich effluent, with the novelty of operating the continuous acidifying reactor at very low pH and high salt concentration. Batch experiments were also performed to investigate the effect of the pH on the productivity and composition of the produced VFA.

2. MATERIALS AND METHODS

2.1 Cooked mussel processing wastewater characterization

The wastewater used in the present study was taken directly from the cookers of a mussel processing factory (Cocedero Suárez, Vilanova de Arousa, Spain). The pH of the mussel cooking wastewater at the time of the collection was approximately 7 but it dropped to 4 - 5 (Table 1) after a couple of days stored at 4 ºC. Wastewater was stored at low temperature to prevent the degradation of the organic matter. Carbohydrates were the predominant organic compounds (50 % of the soluble COD), followed by proteins (30 % of the soluble COD). The concentration of proteins and carbohydrates as chemical oxygen demand (COD) was calculated using the following factors: 1.5 g $COD_{protein}$/g protein and 1.1 g $COD_{carbohydrates}$/g carbohydrate (Mahmoud et al., 2004). The lipid concentration was not significant. The wastewater composition fluctuated due to changes in the factory process, and its variability defined the three operational stages carried out in the acidification reactor, as indicated in Table 1.

Table 1. Average values of the main characteristic parameters of the wastewater treated and reactor operational conditions.

Parameters	Units	Stage I	Stage II	Stage III
		0 - 59 days	60 – 279 days	280 - 400 days
OLR	g COD/(L·d)	7.3 ± 0.5	2.6 ± 0.4	2.2 ± 0.2
HRT	d	3.1	6.3	6.3
pH	--	4.7 ± 0.4	4.4 ± 0.5	5.1 ± 0.7
sCOD	g/L	18.3 ± 1.3	13.1 ± 0.4	11.1 ± 1.1
Carbohydrates	g/L	ND	5.5 ± 1.6	5.3 ± 1.3
Proteins	g/L	ND	2.8 ± 0.4	1.7 ± 0.2
VFA	g COD_{VFA}/L	0.7 ± 0.3	2.2 ± 1.3	1.7 ± 0.7
Ammonium	g NH_4^+-N/L	0.09 ± 0.02	0.19 ± 0.06	0.19 ± 0.05
NaCl	g/L	19.1 ± 2.1	22.7 ± 2.4	22.1 ± 3.0

OLR: organic loading rate; HRT: hydraulic retention time; COD: chemical oxygen demand; VFA: volatile fatty acids; ND: Not determined.

2.2 Experimental set-up

2.2.1 Continuous reactor for VFA production

A continuous stirred tank reactor with a working volume of 5 L was used to produce VFA. It was directly fed with raw cooked mussel processing wastewater (Table 1). The temperature was maintained in the mesophilic range (37 ± 1 °C) using a thermostatic bath (Techne Inc., USA). The reactor was inoculated with anaerobic granular sludge from a pilot-scale up-flow anaerobic sludge blanket (UASB) reactor that treated mimicked municipal wastewater (Silva-Teira et al., 2017). Short solid retention times (SRT) were imposed to washout the methanogenic microorganisms from the anaerobic mixed culture as they present growth rates lower than the acidogenic bacteria (Khan et al., 2016). The gas-phase composition was measured during the first days of Stage I to check the absence of methane production due to the inhibition of methanogenic microorganisms.

The operation of the reactor lasted 400 days, divided into three different stages (Table 1). During Stage I (the first 59 days) an organic loading rate (OLR) of 7.3 ± 0.5 g COD/(L·d) was applied, with a hydraulic retention time (HRT) of 3.1 days. Then in Stage II (days 60 to 279), the OLR was diminished to 2.6 ± 0.4 g COD/(L·d) by increasing the HRT to 6.3 days. Finally, the OLR was further decreased in Stage III (days 280-400) to 2.2 ± 0.2 g COD/(L·d) while HRT was maintained. The SBR operated under complete mixing conditions by means of the action of a mechanical stirrer at 120 rpm (Heidolph, Germany); therefore, the SRT was equal to the HRT. The pH of the media was not controlled.

2.2.2 Acidification batch tests

The acidification batch assays were carried out in 500 mL Pirex-glass bottles (400 mL of working volume), following the methodology described by Silva et al. (2013). The bottles were filled in with the corresponding volumes of substrate, macro- and micro-nutrients solutions and acidifying biomass from the continuous acidification reactor (Table 2). The substrate composition corresponded to Stage I of Table 1. The substrate to biomass ratio was set at 3 g COD/g VSS.

In total, 6 bottles were prepared with 3 different conditions (duplicates): two as control experiments without inoculum addition for measuring the abiotic disappearance of the substrate (E1); two without alkalinity addition (E2) and two containing $NaHCO_3$ in a ratio of 1:1 with respect to VSS (E3). After the addition of the substrate, biomass and medium, the headspace of each vial was bubbled with N_2 and the bottles were sealed with rubber stoppers and capped with plastic seals. Then, bottles were incubated in a shaker (120 rpm) at 37 °C. VFA production was monitored throughout time by the analysis of the periodically collected samples from the liquid phase of each bottle. Before collecting these liquid samples, 1 mL-gas sample was taken and measured by gas chromatography (Hewlett Packard 5890 Series II instrument) to assess the occurrence of methane production. The evolution of the concentration of VFA (expressed as g COD_{VFA}/L) versus time was plotted. The specific acidogenic activity (g COD_{VFA}/(g VSS·d)) was estimated as the ratio between the maximum slope of the appearance of VFA (g COD_{VFA}/(L·d)) and the concentration of biomass present in the bottles (g VSS/L).

Table 2. Initial operational conditions of the acidification batch experiments.

Volumes added of different compounds	**Experiment**		
	Control (E1)	**No alkalinity (E2)**	**Alkalinity (E3)**
Acidifying sludge (mL)	0	23	23
Wastewater (mL)	61.2	61.2	61.2
Macronutrients solution (mL)*	66	66	66
Micronutrients solution (mL)*	13	13	13
10 g $NaHCO_3$/L solution (mL)	0	0	28

*Compositions of macro- and micronutrient solutions described in Silva et al. (2013).

2.3 Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), alkalinity and COD were analysed according to *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WEF, 2017). Liquid samples were filtered through a cellulose-ester filter of 0.45 µm of pore size (Advantec, Japan) for the quantification of total organic carbon (TOC), ammonium (NH_4^+), soluble chemical oxygen demand (sCOD), proteins, carbohydrates, VFA and other ions to determine salt concentration. Ammonium concentration was determined according to the Bower and Holm-Hansen method (Bower and Holm-Hansen, 1980). TOC concentration was determined by catalytic combustion (Analyser model TOC-L CSN, Shimadzu, Japan). VFA concentration was determined by gas chromatography (GC) (Hewlett Packard, USA). Protein and carbohydrate concentrations were measured according to Lowry et al. (1951) and Loewus (1952) methods, respectively. Anions (e.g. Cl^-) and cations (e.g. Na^+) were determined by ion chromatography (861 Advanced Compact IC system, Metrohm, Switzerland).

2.4 Calculations

The individual acid concentrations for acetic acid (HAc), propionic acid (HPr), butyric acid (HBu) and valeric acid (HVa) were converted to COD units by the application of corresponding coefficients: 1.07 g COD_{HAc}/g HAc, 1.51 g COD_{HPr}/g HPr, 1.82 g COD_{HBu}/g HBu and 2.04 g COD_{HVa}/g HVa. The acidification percentage was calculated as the sum of the individual VFA measured by GC, converted to COD units (g COD_{VFA}), and divided by the amount of COD at the beginning of the experiment (COD_i), as indicated in the following equation:

$$\text{Acidification (\%)} = \frac{\text{g COD}_{\text{VFA}}}{\text{g COD}_{\text{i}}} \cdot 100$$

Statistical analysis of data was carried out using the software R version 3.5.1. The normality and homogeneity of variance were evaluated by means of the Shapiro-Wilk and Levene tests, respectively. ANOVA parametric test was used when both tests could be confirmed, and if not, non-parametric Kruskal-Wallis test was applied. Differences in the experimental values of the pH, acidification percentage and VFA concentration obtained in the acidification batch tests were compared with the calculation of the area under the curve (AUC) using the package PK.

3. RESULTS AND DISCUSSION

3.1 Operation of the completely stirred acidogenic reactor

3.1.1 VFA production at low pH

The 5-L acidification reactor was operated for 400 days fed with wastewater from a cooked mussel processing factory (Table 1). Both in the raw wastewater and the effluent of the acidification reactor, the soluble COD (sCOD) corresponded approximately to 97 % of total COD (tCOD). Therefore, during the whole operation, the COD was expressed as sCOD. Although anaerobic biomass was used as inoculum, methane was not detected in the gas phase. Indeed, the mass balances of sCOD indicates a non-significant difference between influent and effluent, below 10 % that can be attributed to biomass growth and to inaccuracies in analytical determination. The acidogenic reactor was operated without pH control that, due to the low pH of the wastewater fed, was maintained below 5. Acidogenic populations are significantly less sensitive to pH than methanogenic ones. In this way, the low pH values achieved in the reactor favoured the natural selection of acidogenic over methanogenic microorganisms (Wainaina et al., 2019). Chen et al. (2007) reported the influence of pH on methane and VFA production and they observed a complete methanogenic activity inhibition at pH 4 and the acidification of 20 % of sCOD. In the present research work, the acidic conditions were caused by the accumulation of VFA in the reactor and the low buffer capacity of the wastewater (approximately 170 mg $CaCO_3$/L).

The VFA production highly fluctuated during the 400 days of operation of the acidification reactor due to continuous variations in the substrate composition (Figure 1 and Table 3). During the first operational days, the VFA production was low (average 19 % of acidification), probably due to the high applied OLR (7.3 ± 0.5 g COD/(L·d)), which was then half reduced on day 60 of operation. From that day onwards, the acidification efficiency was enhanced, and the average productivity reached a value of 0.62 ± 0.19 g COD_{VFA}/(L·d) in Stage II. In Stage III, the operational conditions remained stable, with an average acidification percentage of 42.9 ± 5.6 %, which corresponded to a VFA productivity of 0.72 ± 0.07 g COD_{VFA}/(L·d). Both values were higher than in the previous operational period. This indicated that continuous operation allowed the acclimation of the acidifying microorganisms to the unfavourable operational conditions (low pH and high salinity). Statistical analysis showed no significant differences in the amount of VFA produced between Stages I and II (p = 0.08), but significant ones between Stages II and III (p = 0.0002), with 95 % confidence.

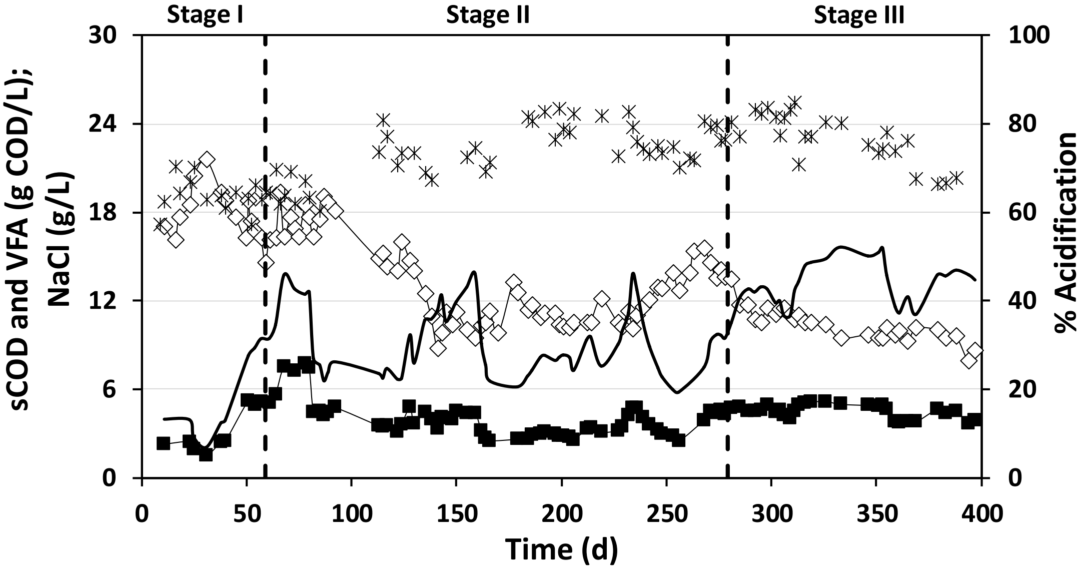

Figure 1. Evolution of sCOD (◇), VFA (■) and NaCl (✳) concentrations, and acidification percentage (-) in the effluent throughout the operation of the acidification reactor.

Table 3. Average values of the parameters measured in the effluent throughout the operation of the acidification reactor.

Parameters	**Units**	**Stage I**	**Stage II**	**Stage III**
		0 - 59 days	**60 – 279 days**	**280 - 400 days**
pH	--	4.4 ± 0.2	3.8 ± 0.2	4.2 ± 0.1
sCOD	g/L	17.7 ± 1.8	13.1 ± 2.8	10.5 ± 1.3
VFA	g COD/L	3.3 ± 1.5	3.9 ± 1.2	4.5 ± 0.4
Acidification	%	18.7 ± 9.9	30.6 ± 7.6	42.9 ± 5.6
Carbohydrates	g/L	ND	1.7 ± 1.0	0.3 ± 0.2
Proteins	g/L	ND	2.8 ± 0.4	1.8 ± 0.4
Ammonium	g N/L	0.17 ± 0.04	0.23 ± 0.06	0.20 ± 0.04
TSS	g/L	3.5 ± 0.4	3.4 ± 0.8	3.4 ± 0.3
VSS	g/L	2.5 ± 0.2	2.2 ± 0.5	2.3 ± 0.3
VSS/TSS	%	70.5 ± 7.9	64.4 ± 8.8	68.9 ± 3.9
NaCl	g/L	19.2 ± 1.1	22.2 ± 1.9	23.0 ± 1.6

ND: Not determined

Differences between Stages II and III can be explained by different factor variations such as pH, OLR, salt, ammonium, carbohydrate or protein concentration in the fed wastewater (Table 1). Among these parameters only pH, OLR and protein concentration change significantly from Stage II to III, being the pH value the one that showed the highest increase, while the OLR remained in a quite small range and protein concentration are not relevant as they are neither degraded nor causing an inhibitory effect on acidification.

The effect of pH on the acidogenesis of different substrates was researched in previous studies. Most of them focused on the improvement of the solubilisation of solid wastes at alkaline conditions, such as tuna processing waste (Bermudez-Penabad et al., 2017), food waste (Cheah et al., 2019) or waste activated sludge (Chen et al., 2007; Liu et al., 2020). Alkaline conditions favoured the organic matter solubilisation as the hydrolysis of proteins and carbohydrates increases fostering the potential VFA production (Wainaina et al., 2019).

Acidic pH values (above 5) were demonstrated to promote the growth of acidogenic bacteria, with an inhibitory effect at pH values below 3 (Khan et al., 2016). Few studies have evaluated the acidification at pH values below 5, and different results were obtained. For example, Bengtsson et al. (2008) operated a chemostat reactor using cheese whey as substrate and they reported an acidification efficiency of 30 % at pH 3.6, which increased up to 84 % when the pH value rose to 6.0 in a chemostat reactor. Gouveia et al. (2017) also treated cheese whey obtaining an average acidification value of 64 % when pH varied from 5 to 7, but the VFA production decreased by 18 % when the pH dropped to 4.

In the present research work, the pH in the reactor was below 4.5 during most of the operational period, which could limit the acidogenic activity. Moreover, the cooked mussel processing wastewater consisted of 50 % carbohydrates and 30 % proteins, on sCOD basis. The carbohydrate concentration in the substrate slightly varied and showed an average value of 5.5 ± 1.4 $g_{carbohydrate}$/L, but the removal efficiency varied during the reactor performance (Figure 2 and Table 3). Until day 280 the average pH value was 3.8 ± 0.2 and the degradation of carbohydrates was approximately 68 %. Then, from day 280 of operation onwards an increase of the pH of the substrate (an average value of 5.04) provoked the increase of the pH inside the reactor up to 4.2 ± 0.1, which favoured the carbohydrate removal up to 96 %.

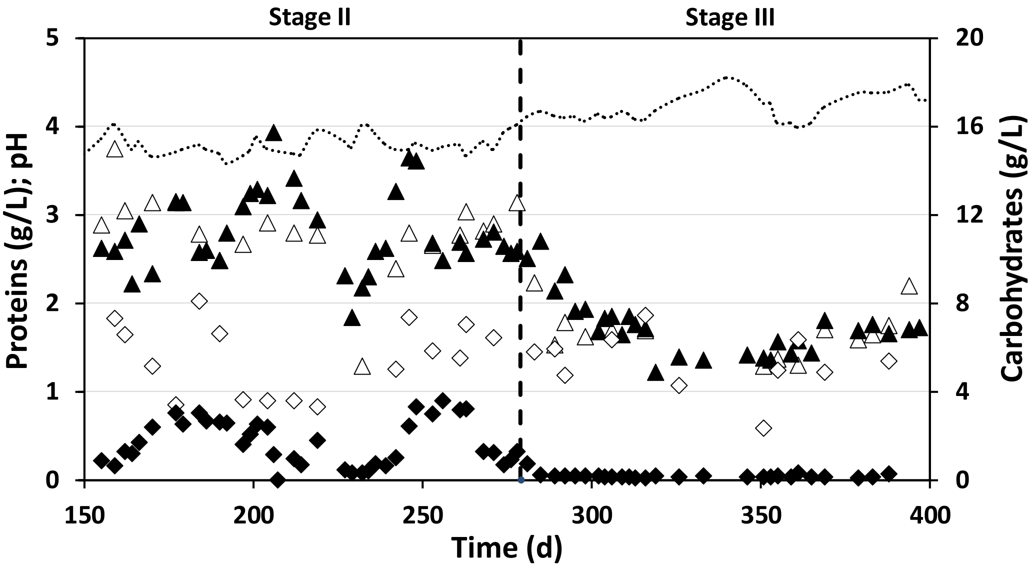

Figure 2. Evolution of the concentration of proteins in the influent (△) and effluent (▲), carbohydrates in the influent (◇) and effluent (◆), and pH in the effluent (···) of the acidification reactor throughout the operational period of 150 - 400 days.

The protein concentration in the feeding was of 2.3 ± 0.7 $g_{protein}$/L until day 280, and 1.7 ± 0.3 $g_{protein}$/L from that day onwards (Table 2). However, compared to carbohydrate removal, the protein degradation was almost negligible during the whole reactor performance (Figure 2 and Table 3). Thus, the VFA production from proteins was not considered. Since proteins are the second most important organic component of the substrate, its lack of degradation contributed to a low VFA production concerning the global sCOD in the wastewater. Previous studies demonstrated that hydrolytic and acidogenic microorganisms could degrade proteins more effectively under neutral or alkaline conditions using sewage sludge as substrate (Liu et al., 2012). Duong et al. (2019) found, using gelatine for mimicking a protein-rich stream, protein degradation inhibition when pH was shifted from 7 to 5. The low conversion of proteins under acidic conditions could be attributed to the decrease of enzymatic activity (Duong et al., 2019). Carbohydrate hydrolases are active at an optimal pH of 5, whereas the protease activity has an optimal pH at higher values (6 - 7) (Parawira et al., 2005). The degradation efficiency of carbohydrates was demonstrated to be less pH-sensitive than that of proteins at pH 4 using dairy wastewater with a high carbohydrate and protein content (Yu and Fang, 2002), as the substrate of the present study. Thus, the acidic conditions of the present study could limit the protein degradation and therefore the maximum acidification throughout the performance of the reactor.

3.1.2 The composition of the VFA mixture

Apart from the variable acidification percentage, different mixtures of VFA were generated during the operation of the acidification reactor (Figure 3). Acetic, propionic and butyric acids were the dominant compounds produced during the acidogenesis of the wastewater from mussel cookers. These short-chain fatty acids can be directly formed by degradation of carbohydrates, whereas the presence of higher molecular-weight VFA, such as valeric and caproic acids, is attributed to acidogenesis of proteins (Yu et al., 2018). In this way, the lack of protein degradation correlated with the low production of these acids. Operational conditions such as pH value, OLR or HRT, among others, not only affect the acidification degree but also the VFA composition (Atasoy et al., 2019; Wainaina et al., 2019). In the present study, HRT was only increased on day 60 (Table 3) while VFA composition varied throughout the reactor operational period. Thus, other parameters, such as the pH of the reactor medium, could be driving the VFA distribution in the following Stages.

During Stage I, the pH remained at an average value of 4.5. In terms of VFA composition, results indicated that the operational conditions promoted the production of butyric acid, which became the dominant VFA. At the end of Stage I the composition of the acids produced corresponded to 30:2:62:6 as HAc:HPr:HBu:HVa expressed as a percentage of VFA on COD basis. After the decrease of the HRT and, thus, the OLR on day 60, a shift of the VFA produced was clearly observed (Figure 3). During this stage, the production of acetic and propionic acid production increased, while the butyric acid concentration decreased. The VFA composition on day 281 of operation was of 78:12:10:0, corresponding to HAc:HPr:HBu:HVa. Results seem to indicate that the increase of the HRT from 3.1 to 6.2 days promoted a shift of the VFA distribution. Bengtsson et al. (2008) investigated the effect of the retention time on the VFA composition, and also observed a higher production of acetic and propionic acids when the retention time was increased from 11 to 24 h, using paper mill wastewater. Similary, Jankowska et al. (2015) obtained a decrease of butyrate and an increase of acetic and propionic acid production during acidification of primary and waste activated sludge, when the retention time was prolonged from 5 to 15 days and the pH was maintained at 4. Zhang et al. (2006) observed evidence of wash-out effect on propionate producing populations after the shortening of the HRT.

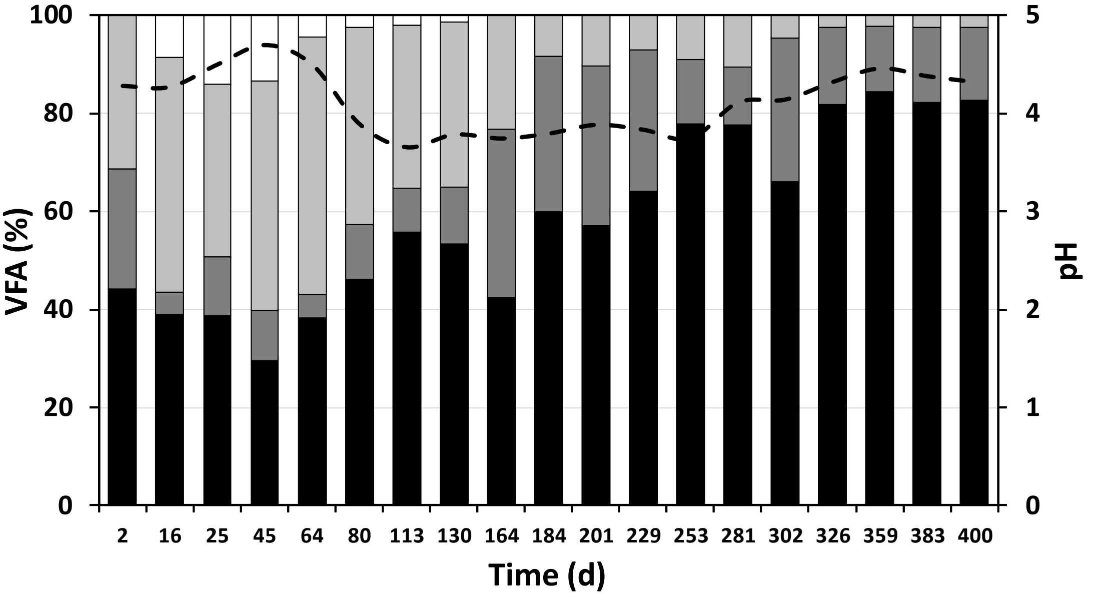

Figure 3. Evolution of the composition of the VFA produced in the acidification reactor and the pH value throughout the operational period. Percentages corresponding to HAc: acetic acid (■), HPr: propionic acid (■), HBu: butyric acid (■) and HVa: valeric acid (☐); and pH (- - -) value.

From day 281 onwards (Stage III) the VFA composition was relatively stable, which correlated with the improvement of the acidification shown in Figure 1 due to the increase of the pH value above 4. During this period, the dominant component was acetic acid with an average concentration in the effluent of 3.5 ± 0.3 g COD_{HAc}/L, followed by propionic acid (0.8 ± 0.2 g COD_{HPr}/L) and butyric acid (0.2 ± 0.1 g COD_{HBu}/L). Even though the reactor was subjected to changes in the composition of the cooked mussel processing wastewater during the whole operation, it showed more stability during the Stage III when the acidification degree and composition of the mixture of VFA remained relatively constant.

3.2 Alkalinity effect on VFA production: proteins degradation

Batch tests were performed to evaluate the influence of the pH value on the VFA production from cooked mussel processing wastewater (Figure 4 and Table S1 in Supporting Material). Acidifying biomass from the reactor was collected on day 76 and used as inoculum. An experiment without acidifying sludge or alkalinity addition was carried out as control (E1). Then, the effect of the alkalinity was studied without (E2) and with (E3) the external addition of $NaHCO_3$, in batch experiment that already contained the same inoculum and substrate concentrations. The initial VFA concentration in all bottles (E1, E2 and E3) was approximately 900 mg COD_{VFA}/L. Even though the biomass collected from the acidification reactor was washed before the experiment, the inoculum media contained a remaining amount of VFA (< 0.1 g COD_{VFA}/L). In all the bottles, no methane production was observed during the tests. In the control flasks (E1), where only substrate was added, no differences in the VFA concentrations were observed throughout the batch test. Experiments E2 and E3 with substrate and acidifying biomass showed an increase of the VFA concentration during the first days of the batch experiment (Figure 4). However, the increase of the acidification in experiment E2 was lower than in E3 and the acidification values on day 2 were 48.2 % and 61.6 %, respectively. From that day onwards the VFA concentration remained at approximately 1.4 ± 0.2 g COD_{VFA}/L in E2, whereas in E3 reached a value of 2.5 ± 0.1 g COD_{VFA}/L after 23 days of experiment. This latter value corresponded to an acidification degree of 70 % of initial COD. Statistical analysis was applied by comparing the area under the curve (AUC) described by the VFA produced throughout the batch test and showed significant differences in the acidification percentage between the flasks without (E2) and with (E3) alkalinity ($p = 0.061$), with 90 % confidence.

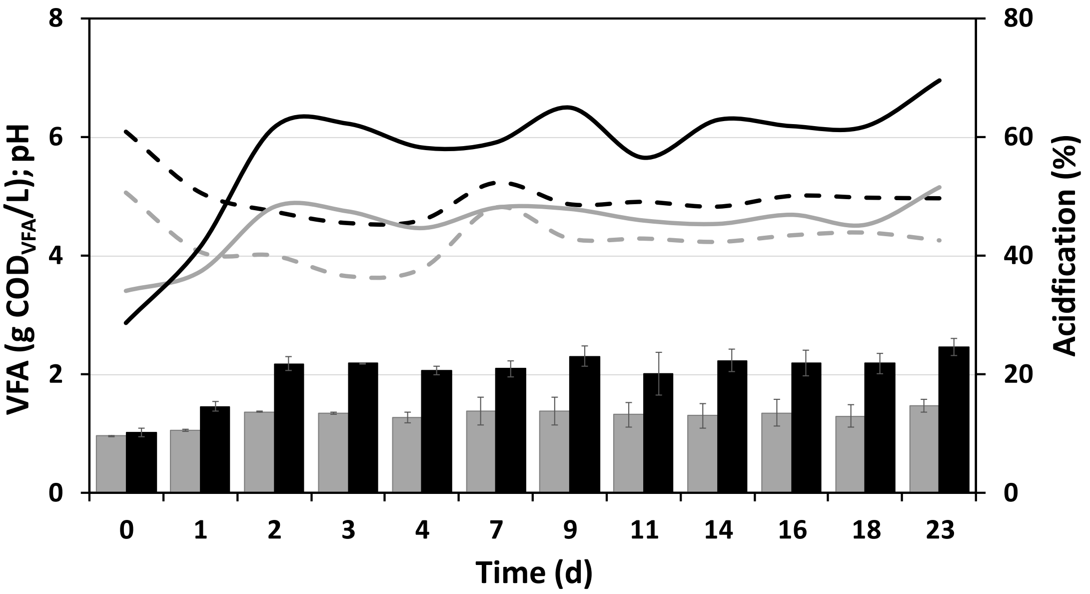

Figure 4. Evolution of the VFA concentrations (columns), percentage of acidification (continuous lines) and pH value of the liquid media (discontinuous lines) in the acidification batch experiments using cooked mussel processing wastewater. Grey colour corresponds to experiment E2 and black colour to experiment E3. The error bars of the columns represent the standard deviation of the point.

The specific acidogenic activity of 0.79 g COD_{VFA}/(g VSS·d) in E3, was almost three times higher than in E2 (0.27 g COD_{VFA}/(g VSS·d)). The main difference in both experiments was the pH value. Without alkalinity (E2) the pH value was 4.2 ± 0.3, whereas in E3 the addition of $NaHCO_3$ promoted the maintenance of higher pH (4.9 ± 0.1). These results were in accordance with the specific activities estimated for the acidifying reactor. During Stage I the acidogenic activity was 0.24 ± 0.11 g COD_{VFA}/(g VSS·d), which was very similar to the value obtained in E2. This value increased during Stage III when a higher pH was measured in the reactor and correlated with an increase of the acidogenic activity, being the average value of 0.33 ± 0.03 g COD_{VFA}/(g VSS·d). Therefore, batch results indicated that the increase in the pH, by addition of alkalinity, had a positive effect in terms of conversion of VFA from the cooked mussel processing wastewater. Yu and Fang (2002) also observed changes in the VFA production from dairy wastewater at variable pH and obtained an increase of the microbial activity from 0.146 g COD/(g VSS·d) at pH 4 to 0.320 g COD/(g VSS·d) at pH 5.5.

A shift of the VFA distribution was observed in experiments at different operational pH (Figure 5 and Table S1 in Supporting Material). During the acidification experiments without (E2) and with (E3) alkalinity, acetic acid was the dominant organic acid, whereas valeric acid was produced at the lowest concentration. However, propionic and butyric acids showed inverse behaviour in the two experimental conditions (Figure 5). In E2 (lower pH), the butyric acid concentration increased and reached the same value as acetic acid from day 7 onwards (Figure 5A). Propionic acid concentration slightly increased during the first days, and it remained stable during most part of the experiment. In E3 (higher pH), butyric acid concentration did not experience the same evolution as in E2 and approximately the same concentration was maintained until the end of the experiment. However, propionic acid production increased at the beginning of the assay and became the second most-produced acid after acetic (Figure 5B).

Previous studies have reported the influence of the pH not only on the concentration of VFA produced but also on the metabolic pathways in acidogenic fermentation and, therefore, of the product distribution. However, there are no consistent conclusions on the influence of pH on the composition of VFA (Zhou et al., 2018). In the batch experiments of the present research work, butyric acid production was improved under low pH conditions. These results agreed with previous studies that reported that the butyrate metabolic pathway was enhanced under acidic conditions (González-Cabaleiro et al., 2015; Jankowska et al., 2017; Temudo et al., 2007).

A positive effect of the acidic pH was observed in the reactor to select acidifying bacteria and wash out methanogenic microorganisms from the anaerobic mixed culture used as inoculum. However, the acidogenic activity was limited by the low pH values (below 4 during most of the operational time). Results of the batch experiments showed that the addition of alkalinity improved the VFA production and modified the obtained products, with respect to the experiments without $NaHCO_3$ addition. However, the increase of pH up to 5 was insufficient to achieve complete acidification of the substrate. Even though the protein concentration was not measured during the batch experiment, the 30 % of non-acidified COD probably corresponded mainly to the protein content of the substrate.

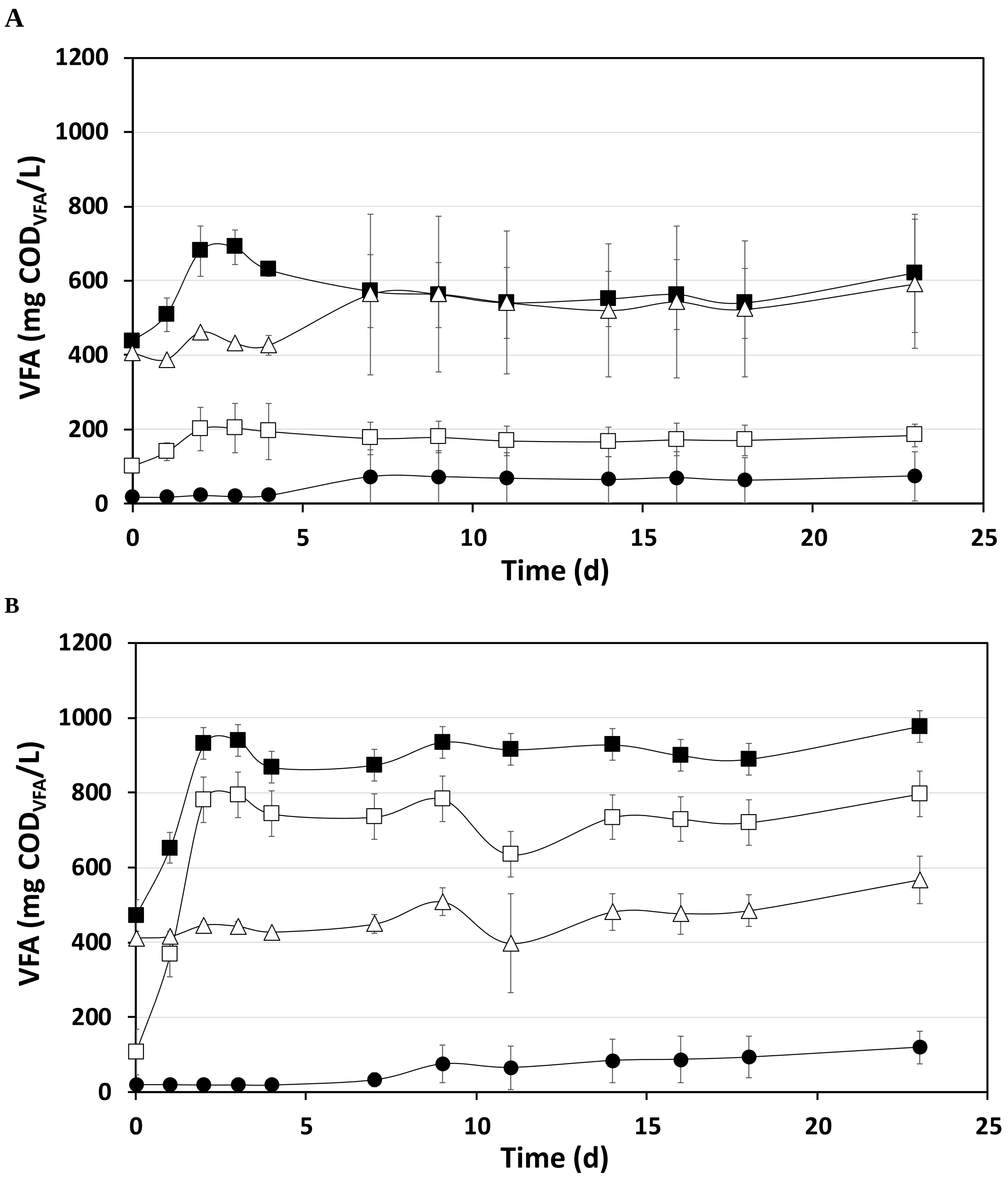

Figure 5. Concentrations of VFA produced in the batch assays without-E2 (A) and with-E3 (B) alkalinity. Acetic acid (■), propionic acid (□), butyric acid (△) and valeric acid (●). The error bars represent the standard deviation of the point.

Residual carbohydrate concentration is also expected due to kinetic and energetic or thermodynamic conversion limitations (González-Cabaleiro et al., 2015). Considering the carbohydrate affinity for the process of 1 mM (expressed as glucose) (González-Cabaleiro et al., 2015), 0.2 g COD/L would remain as carbohydrates. Thus, protein partial degradation is suggested to contribute to the achievement of the 70 % of acidification in E3. If only carbohydrate were degraded, the acidification efficiency would be limited to 64 %. A more detailed study is required to optimise the pH via the long-term addition of $NaHCO_3$ to the feeding of the reactor and to evaluate is effect on protein degradation and, eventually, on the amount of VFA produced.

To sum up, obtained results suggested that an increase in the pH of the reactor media could promote protein degradation fostering VFA production. However, a techno-economical study would be required to evaluate the process benefits in terms of acidification efficiency and increase of operational costs due to the addition of chemicals to adjust the pH value. Other factors like HRT and OLR should be considered to define the best operational strategy and set the optimal pH value. The obtained VFA-rich stream could be used to produce PHA, as carbon source for nutrient removal or purified to use the VFA as platform chemicals, among other applications. Depending on the final use, the composition of the VFA mixture will be relevant (as platform chemical or affecting the obtained PHA properties) or not (for nutrient removal) (Atasoy et al., 2018). The final application will also determine the downstream processes required to obtain the final product and a clean effluent for discharge. In the present study, it was demonstrated that mussel cooking wastewater is a good candidate to produce VFA-rich streams. Thus, this wastewater could be valorised, under uncontrolled pH conditions, instead of being just treated consuming resources like energy or chemicals. As in the present study the aim is to produce VFA subsequent treatment/processing steps are required to produce an effluent with the required composition to be discharged to the environment.

4. Conclusions

Acidogenic fermentation of cooked mussel processing wastewater resulted in a significant VFA productivity of 0.72 ± 0.07 g $COD_{VFA}/(L \cdot d)$, considering the complex composition of the substrate, mainly characterized by high organic matter content (13.8 ± 3.2 g COD/L), high salinity (21.8 ± 2.8 g NaCl/L) and low pH (4.6 ± 0.6). The maximum acidification percentage obtained was 43 % and the composition of the VFA mixture obtained was of 80:18:2 as HAc:HPr:HBu. Carbohydrate conversion reached up to 96 % and contributed to the production of VFA. However, the acidification efficiency was hindered by a deficient protein degradation, probably associated to the acidic conditions inside the reactor.

Batch experiments showed that the increase of the pH from 4.2 to 4.9 by the addition of $NaHCO_3$ resulted in a higher acidification efficiency. In addition to increasing VFA production, the composition of the mixture switched from containing mostly acetic and propionic acids to containing mostly acetic and butyric. Nevertheless, part of the COD remained as non-acidified COD even at pH 5, probably due to the slight degradation of proteins.

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