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VOLATILE FATTY ACIDS PRODUCTION FROM FISH WASTEWATER

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TÍTULO: Volatile fatty acids production from fish wastewater

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Abstract

In this work, two experiments were performed to investigate the production of volatile fatty acids (VFAs) through the anaerobic degradation of an effluent formed by cleaning, cooking and brine wastewaters collected from a fish processing industry. The first experiment was carried out in an anaerobic continuous reactor at pH 8. Several strategies were tested to improve the VFAs production such as different HRT (18 h, 14 h, 8 h and 6 h), different feed´s dilution (50 x, 10 x, 2 x and 1 x) and different feed´s salinity (2.8 g/L and 20 g/L). For several HRT and dilutions, the concentration of VFAs was very low and methane (CH_4) was detected in the reactor. The increase of feed´s salinity appear to be a good strategy, since the concentration of VFAs increased (401 mg COD/L with 2.8 g/L of salt to 900 mg COD/L with 20 g/L of salt) and the percentage of CH_4 in the reactor decreased. The second experiment was carried out in batches at different initial pH values (5, 6, 7, 8 and 9). Acetate and propionate were the main products obtained at the end of the experiment. The highest production of VFAs (1583 mg COD/L) was obtained at pH 6.

Keywords: anaerobic digestion, acidogenesis, VFA, methane, dilution.

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1 Introduction. Anaerobic digestion

Anaerobic digestion is the process in which a series of microorganisms decompose the biodegradable organic matter of a given substrate in the absence of oxygen (O_2). The degradation of this substrate generates diverse gases, among which carbon dioxide (CO_2) and CH_4 are the most abundant. However, depending of the microorganisms present and the type of substrate it is possible to found traces of other elements such as nitrogen (N_2), hydrogen (H_2), hydrogen sulfide (H_2S), which can complicate the application of biogas and reduce its quality (Moreno, 2011), (Corrales, , Diana Marcela Antolinez Romero , Johanna Azucena Bohórquez Macías, & Vargas, 2015).

The wastes used as a substrate in this process can be obtained from different origin (Moreno, 2011).

- Animal origin: urine and animal manure, livestock waste, fish waste.
- Vegetal origin: remains of crops, sawdust, wood.
- Human origin: feces and urine, garbage.
- Forest origin: leaves, bark of trees.
- Aquatic origin: marine algae, aquatic plants.

All these wastes must demonstrate suitable characteristics for the correct development of the microorganisms, which means, contain organic matter like carbon (C) and N_2 in an adequate quantity. All the wastes mentioned above are susceptible to being degraded into useful by-products through the anaerobic digestion process. However, it is important to mention that depending of the biochemical composition of the waste, the biogas production dynamics will be different (Moreno, 2011).

The choice of a suitable substrate is essential since, depending on the type of substrate, a different product in composition and quantity can be obtained. In this way, approximately 90 % of all available energy obtained by direct oxidation is transformed into CH_4 while the remaining 10% of the energy is used by the microorganisms for its own growth (Moreno, 2011).

In particular, the effluents from fish processing industries are formed by water and a large amount of salt and organic compounds among which are numerous proteins and oils/fat. Due to the large amount of fats, these effluents can present values of chemical oxygen demand (COD) between 300 to 93000 mg/L (Santiago & Con, 2015).

In this type of effluent, the pH is approximately between 6-7. It is also necessary to emphasize that, in general, a chemical treatment of acidification is carried out to prevent its decomposition (García-Sifuentes et al., 2009).

However, the composition of the effluent depends on the species of fish, physiological state of the animal, storage conditions, among others (Iwendiola, Achútegui, Sáncliez, & San, 1998) (García-Sifuentes et al., 2009).

1.1 Anaerobic digestion: stages

The anaerobic digestion is a slow process that has a very high complexity due to the large number of microorganisms present in the process and the high number of biochemical reactions that occur. Some of these reactions occur sequentially and another part occurs simultaneously (Ortega, 2006).

Based on the microbiological studies, the process can be divided into four main phases: 1. Hydrolysis; 2. Fermentative or acidogenic stage; 3. Acetogenic stage; 4. Methanogenic stage. These stages are represented in the following scheme.

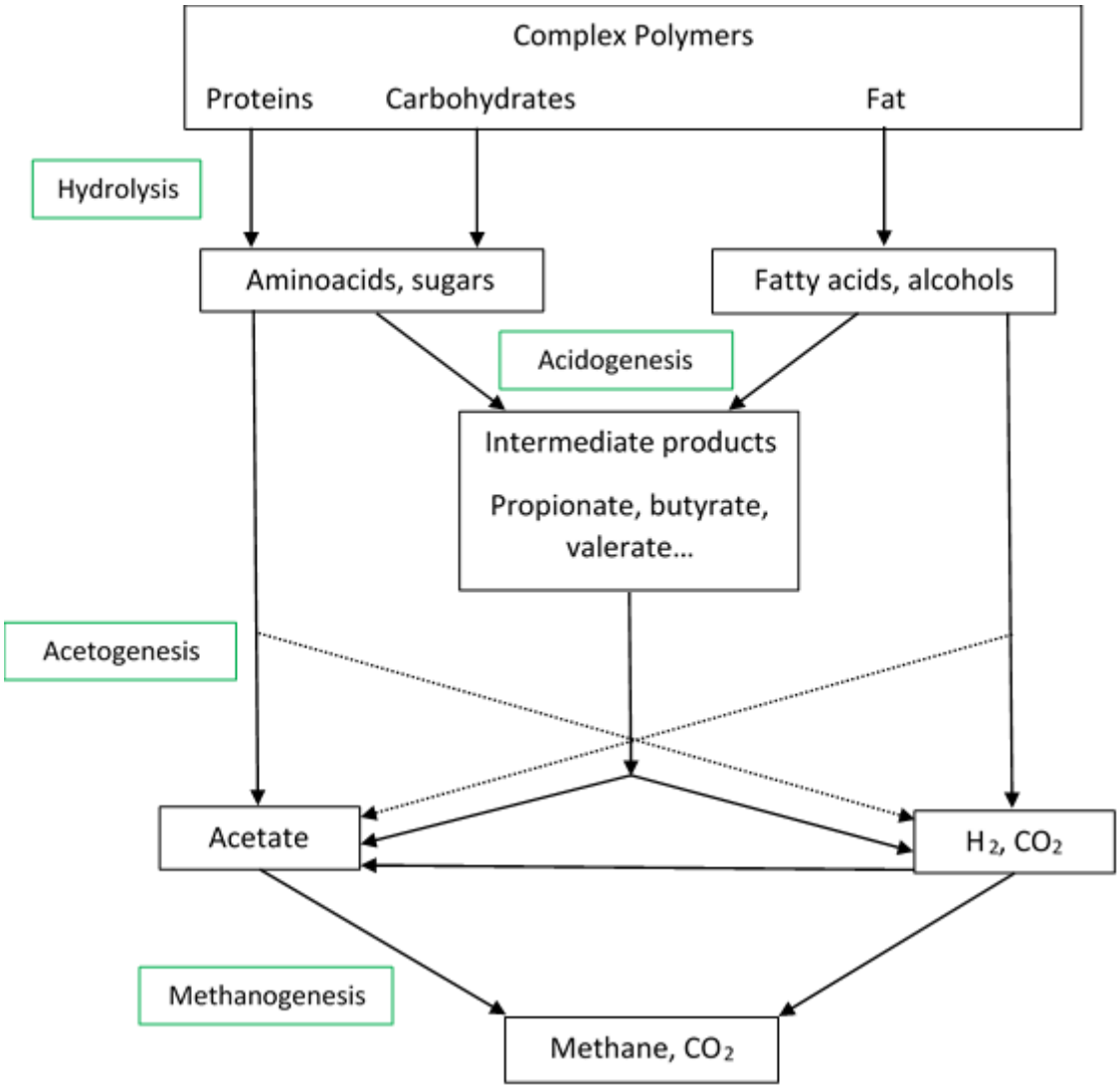

Figure 1- Schematic representation of the anaerobic digestion process (Adapted from Morales, 2001).

Hydrolysis.

The microorganisms are not able to consume the polymeric organic matter unless this matter is degraded to soluble compounds in the hydrolysis stage. Any type of substrate consists basically of three types of macromolecules: carbohydrates, proteins and lipids. Therefore, during this stage, the action of extracellular enzymes, which are produced by hydrolytic microorganisms, realize the hydrolysis of these components and provides organic substrates for the subsequent stages like alcohols, sugar, fatty acids, among others (Moreno, 2011).

Fermentation or Acidogenesis

At the beginning of the second stage, complex organic compounds have already been degraded to simpler or more soluble organic compounds. These simpler compound are then used by the acidogenic microorganisms in the acidogenesis stage. These acidogenic microorganisms are formed by acid-forming bacteria (Moreno, 2011), (Ortega, 2006).

These bacteria degrade the soluble organic compounds in compounds that can be used directly by methanogenic microorganisms (such as acetic acid) and other organic compounds that can be used in the next stage by acetogenic microorganisms (such as propionic, butyric, valeric, lactic acid and ethanol) (Moreno, 2011).

Most of the microorganisms present in the acidogenesis stage are also present in the hydrolysis stage, such as Bacteriocides, Clostridia, Bifidobacteria, Streptococci, Enterobacteriaceae, Paenibacillus, Ruminococcus, among others (Moreno, 2011).

Acetogenesis

In this stage, acetogenic microorganisms are responsible for degrading compounds such as etanol, VFAs and some aromatic compounds to simpler products, such as acetate and H_2 (Ortega, 2006).

Subsequently, it is the next stage, the acetate and H_2 obtained will be used as food for the methanogenic microorganisms (Ortega, 2006).

Methanogenesis

In this stage, all the products obtained up to this moment are susceptible to be degraded by the methanogenic microorganisms. These microorganisms have a great importance within all the groups of anaerobic microorganisms, because they are responsible for the formation of CH_4 (Moreno, 2011).

The production of CH_4 completes the process of anaerobic digestion due to the action of methanogenic microorganisms, which degrade acetate and H_2 into CH_4 (Moreno, 2011).

As the objective of the work is to produce VFAs by minimizing the action of methanogenic microorganisms, the most important factors that affect acidogenesis stage will be in the following section.

1.2 Acidogenesis: determinant operational parameters

It is important to examine some of the important factors that govern the acidogenic step since the microorganisms are highly susceptible to changes in environmental conditions (Estevan, 2016).

For that reason, there are certain parameters that influence the performance and composition of VFAs in the process. These parameters are generally studied one by one, so the effects of each one will be explained separately in this report (Lee et al., 2014).

Nutrients

The various residues that can be used in anaerobic degradation have different origins: vegetable, animal, agro-industrial, forest, domestic, among others. The biochemical characteristics of these residues should allow the development and microbial activity of the anaerobic system. The microbiological process not only requires C and N sources, but also mineral salts (sulfur, phosphorus, potassium, calcium, magnesium, iron, manganese, molybdenum, zinc, cobalt, selenium, tungsten, nickel and other minors) should be present in a certain balance (Moreno, 2011).

For example, C/N ratio should be between 15/1 and 45/1, with an optimum value of 30/1. If you have a lower C/N ratio than 15/1, the reaction speed decrease and if you have a higher C/N than 45/1 inhibition problems appears (Moreno, 2011), (Elena Campos & Xavier Flotats, 2004).

pH and alkalinity

One of the most important factors in the production of VFAs is pH because most of the acidogenic bacteria cannot survive in very acidic (pH 1-3) or very basic environments (pH 12-14). However, there is no optimal pH value for the acidogenesis step, since the optimal pH will depend on the type of effluent used (Lee, Chua, Yeoh, & Ngoh, 2014).

The alkaline conditions favour the hydrolysis of a sludge-based effluent causing the breakdown, of the carboxylic groups in the effluent. Consequently, soluble substrates appear for the production of VFA. For this type of effluent, an alkaline environment in the digester causes a decrease in methanogenesis, so that the produced VFA will not be used for methane production (Lee et al., 2014).

On the other hand, for food waste and wastewater, neutral and acidic conditions favour the production of VFAs (Lee et al., 2014).

In addition, pH can also affect the type of VFA produced, therefore, depending of the effluent used, the optimum pH for the production of a specific VFA will vary (Wang, Yin, Shen, & Li, 2014).

Temperature

Temperature is also one of the most important parameters that must be taken into account in the process of acidogenesis. This parameter greatly influences the speed of digestion and, in addition, the process can be destabilized with sudden changes in temperature. The process must be carried out at a homogeneous and constant temperature (Moreno, 2011).

There are four temperature ranges in which anaerobic microorganisms can work
- psychrophilic (between 4 - 20°C);
- mesophilic (between 20 and 50°C);
- thermophilic (between 50 and 60°C) and
- extreme/hyper-thermophilic (between 60 and 80°C)
(Lee et al., 2014).

For some authors working in the thermophilic or hyper-thermophilic range would promote a better acidogenesis than using temperatures of the mesophilic range (Mengmeng, Hong, Qingliang, Ngai, & Jie, 2009). However, for other authors it is better

to work at a lower temperature to achieve greater acidogenic activity (Zhuo, Yan, Tan, Dai, & Zhou, 2012). Furthermore, others authors demonstrated that work at temperatures between 40 – 70 °C does not influence the production of VFAs (Yu, Zheng, Tao, Zuo, & Wang, 2013). This is possibly because the acidogenic process is influenced by numerous parameters at the same time. Although working at higher temperatures would be more profitable from the point of view of the production of VFAs, the sustainability of the process should be analysed by relating the greater production of VFAs with the extra expense to maintain a high temperature (Lee et al., 2014).

Hydraulic retention time

The hydraulic retention time (HTR) is defined as a parameter that measures the ratio, expressed in hours, between the flow to be treated and the volume of the reactor used in the process, being a very important factor in the process of acidogenesis. According to (Caldera, Madue, Griborio, Gutiérrez, & Nola M Fernández, 2006) a small contact time between the biomass and the effluent can lead to the collection and accumulation of VFAs in the reaction system and to a decrease in the percentage of organic matter removal.

However, according to (Lee et al., 2014) a high HTR is advantageous for the production of VFAs since the microorganisms have more time to react with the waste. However, a very prolonged HTR could lead to stagnant VFAs production or allow the VFAs consumption by methanogenic microorganisms to form CH_4 (Lee et al., 2014).

The key to optimal HTR is that it should be high enough to produce VFAs and short enough so that the methanogenic microorganisms do not have time to act (Lee et al., 2014).

The objective of this work was to obtain VFAs from fish processing. Several strategies were investigated to inhibit the methanogenic activity to improve the VFAs production.

2 Materials and methods

In order to investigate the production of VFAs from fish wastewater two types of tests were performed.

- i) Anaerobic continuous reactor at pH 8: to study the effect of HTR and the dilution of the substrate in the production of VFAs (Experiment 1).
- ii) Anaerobic batch assays: to study the production of VFAs at different pH values (Experiment 2).

2.1 Experiment 1) Anaerobic continuous reactor at pH 8

Inoculum

The inoculum used in this assay was a granular anaerobic biomass collected from a wastewater treatment plant, located in the north of Portugal (Unicer, Matosinhos), where brewery's wastewater is treated. Before starting the test, the biomass was boiled at 100 ^{o}C during 15 minutes, with the objective of inhibit the action of the methanogenic microorganisms.

Effluent

The effluent used as substrate in this work was formed by cleaning (125 m^3/d), cooking (15 m^3/d) and brine (2m^3/d) wastewaters. This effluent was collected from a fish processing industry in the north of Portugal (Poveira, Póvoa de Varzim).

During the first 48 days of operation (03/05/18 - 02/05/18) a fish wastewater contained high quantity of fat and, therefore, high quantity organic matter was used. Before use in the reactor, the fish wastewater was filtered (500 µm pore size) to remove large solids such as plastic and pieces of wood. The pH of this effluent was 5.5 and the COD was 16 g/L.

From 2/05/18 until the end of the test, a fish wastewater with lower fat was used. This effluent was collected after a physical pre-treatment of gradation and flotation to remove solids and grease with large dimensions. The pH of this effluent was 6.5 and the COD was 1.06 g/L. This wastewater did not contain solids of large dimensions and because of this it was not necessary to realize any previous treatment.

Experimental procedure

The material used in this experiment was a glass reactor with temperature control at 37 ^{o}C, a peristaltic pump 101U/R (Watson-Marlow, USA) for feed, a peristaltic pump 405U/R1 (Watson-Marlow, USA) for recirculation, a gas counter PMMA/PVDF (Ritter, Germany), a container with the feed and an agitator to homogenize the feed.

The reactor was operated at pH 8. To achieve that pH, the effluent was basified by adding sodium hydroxide (NaOH, 10M) and a buffer solution with potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4) was used. The work volume was 1015 mL, which was composed by 30% of inoculum (305 mL) and 70% of substrate diluted 50 x. The concentration of substrate fed to the reactor, in terms of COD, was increased by decreasing the dilution of the substrate (50 x, 10 x, 2 x and 1 x). The dilution was performed by using the buffer solution. Also, different HRT (18 h, 14 h, 8h, 6h) were tested to improve the production of VFAs.

At 57 days of operation (11/05/18), the percentage of brine wastewater in the feed was increased from 1.4 % to 10 % as a strategy to inhibit the methanogenic activity.

2.2 Experiment 2) Anaerobic batch assays

Inoculum

The inoculum used was a suspended biomass, collected from a municipal wastewater treatment plant (Parada, Maia). Before starting the test, the biomass was boiled at 100^{o}C during 15 minutes with the objective of inhibit the action of the methanogenic microorganisms.

Effluent

The effluent used in this test was a fish wastewater collected from a fish processing industry (Poveira, Póvoa de Varzim). This wastewater was composed by cleaning (125 m^3/d), cooking (15 m^3/d) and brine (2m^3/d) wastewaters. Before used in the assay, the fish wastewater was also filtered (500 µm pore size) to remove large solids. The pH of this effluent was 5.5 and the COD was 16 g/L.

Experimental procedure

In this experiment the effect of pH on the production of VFAs was studied. A set composed by 17 glass bottles of 120 mL volume was used. The bottles had a work volume of 75 mL composed by 15 mL of inoculum (anaerobic suspended sludge), 30 mL of substrate (fish wastewater) and 30 mL of buffer solution (KH_2PO_4/K_2HPO_4). A blank assay formed by 15 mL of inoculum and 60 mL of buffer at pH 7 was also performed in duplicate. The blank assays were performed to assess the concentration of VFAs produced from the substrate present in the inoculum.

To achieve the desired pH, hydrochloridic acid (HCl, 10M) was used to obtain the acidic pH values and sodium chloride (NaOH, 10M) was used to obtain the basic pH values. To maintain the initial pH of each bottle, a buffer solution of KH_2PO_4 and K_2HPO_4 was used. The quantity of each of these components is represented in the Table 1.

Table 1 - Amount of each reagent used for the preparation of the buffer solution according to the pH.

pH	**5**	**6**	**7**	**8**	**9**
KH_2PO_4 (g)	0,1014	0,0961	0,0631	0,0142	0,0016
K_2HPO_4 (g)	0,0008	0,0076	0,0498	0,1124	0,1286

The bottles were sealed and the air present in the headspace of the bottles was replaced by N_2. After that, 0.6 mL of Sodium sulfide nonahydrated ($Na_2S \cdot 9H_2O$) was added. Na_2S was used to reduce any trace of O_2 that may be present inside the bottles.

The experiments were performed in triplicate and the bottles were maintained at 37^{o}C to keep the working temperature constant.

2.3 Analysis

During the experiment, numerous analyzes were performed. To measure Total COD and soluble COD, Hach cuvette tests (LCK 514) (Hach, Germany) and a DR 2800 spectrophotometer (Hach, Germany) were used. To measure the soluble COD, it was necessary to centrifuge (15.000 rpm for 15 minutes) and filter (0,22 µm pore size) the samples. A pH meter inoLab pH 7110 (WTW, Germany) was used to measure the pH.

To measure CH_4, a gas chromatography (GC-2014) (Shimadzu, Japan) was used equipped with a FID detector and a PoraPak Q (80/100 mesh, 2 m x 1/8 inch, 2 mm,

stainless steel) column using N_2 as carrier gas (30 mL/min). Column, injector and detector temperatures were 35, 110 and 220 °C, respectively.

VFAs were analysed by high performance liquid chromatography (HPLC) (Jasco, Japan) equipped with a UV detector and a Chrompack column (6.5 x 30mm^2) at 60 °C. Sulphuric acid (2.5 mM) at a 60 mL/min was used a mobile phase.

3 Results and discussion

3.1 Experiment 1) Anaerobic continuous reactor at pH 8

Although several strategies were tested to improve the VFAs production. During the reactor operation, different HRT were tested (18 h, 14 h, 8 h and 6 h) to improve the production of VFAs, as well as the increase of effluent concentration by decreasing the dilution (50 x, 10 x, 2 x and 1 x).

Furthermore, the CH_4 production was detected in the reactor (~ 5-10 %). This suggest, that the methanogenic microorganisms completed the anaerobic process by transforming the produced VFAs into CH_4.

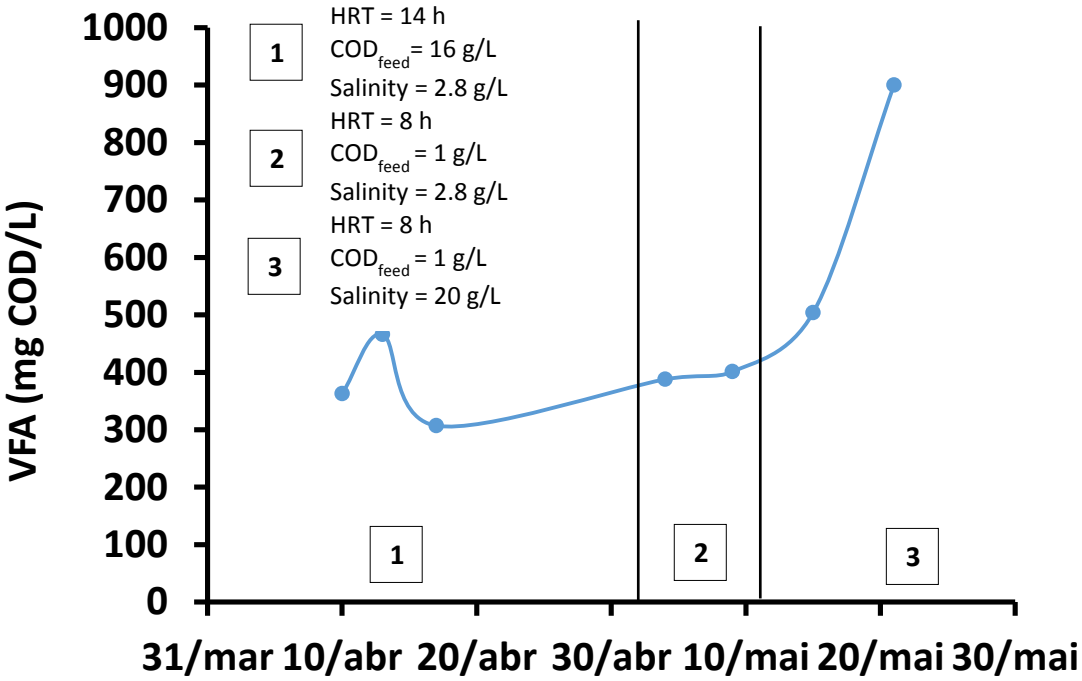

Figure 2- VFA production throughout the experiment

For undiluted feed, a VFAs concentration of approximately 400 mg COD/L was obtained, which corresponds to a very low conversion of the initial COD (16 g/L). The increase in the wastewater salinity by increasing the amount of brine wastewater in the feed was also tested as of 11/05/2018. This strategy appears to have potential, since a small increase in the production of VFAs was observed during the first days of operation (400 mg COD/L with 2.8 g/L of salt to 900 mg COD/L with 20 g/L of salt), which corresponds to a conversion of 90 % of the initial COD (1060 mg/L). With this strategy the percentage of methane decreased.

3.2 Experiment 2) Anaerobic batch assays

Anaerobic batch assays were performed to study the effect of initial pH in the VFAs production from fish wastewater. The total production of VFAs (mg COD/L) according to the initial pH is shown in Figure 3.

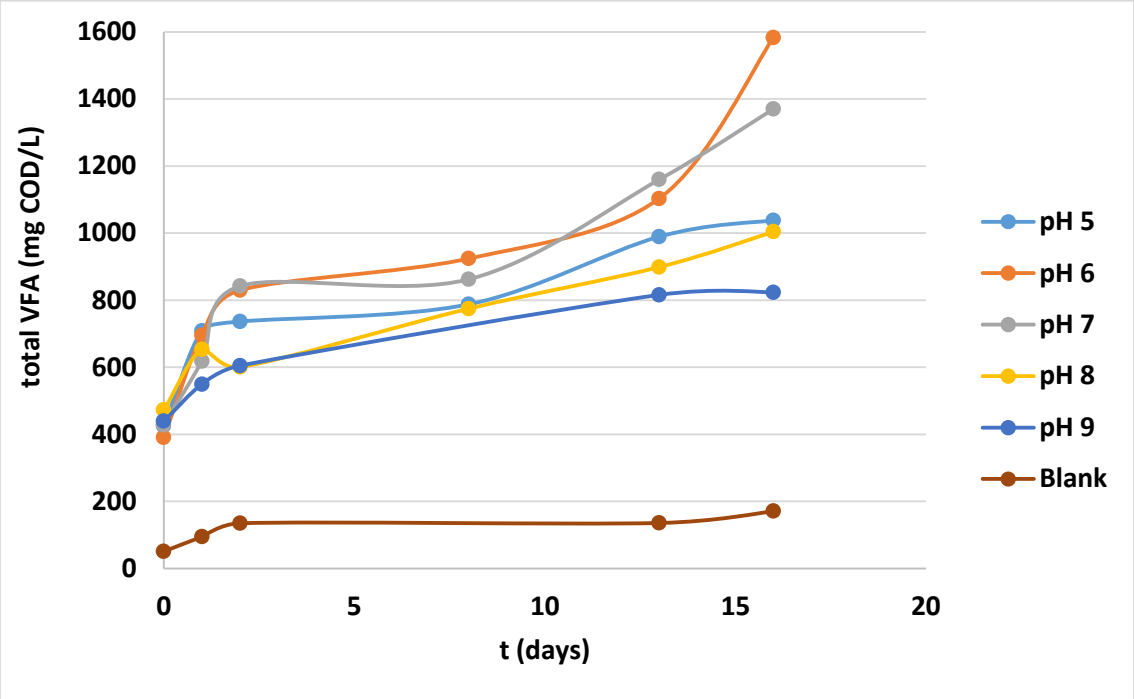

Figure 3- Production of VFAs throughout the experiment

The production of VFAs increased until the last day of the experiment. According to the obtained results, the highest concentration of VFAs was obtained at pH 6 (1583 mg COD/L), followed by pH 7 (1370 mg COD/L) and pH 5 (1038 mg COD/L).

The concentration of each individual VFA, at the end of the experiment, is represented in Figure 4.

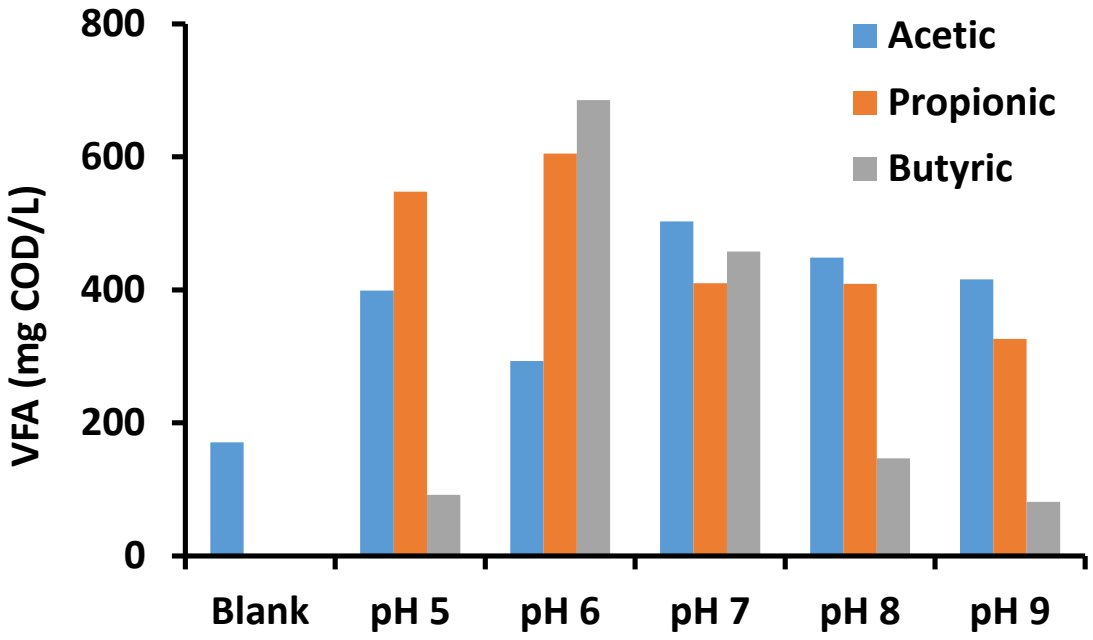

Figure 4- Concentration of each indiviual VFA for the different pH tested

Figure 4 shows the effect of pH on the production of acetic, propionic, and butyric and acids. During the batch assays, acetic, propionic, butyric were the only VFAs detected. For all pH values tested, acetic and propionic acids were found in a higher concentration, in comparison with butyric acids.

Basic pH values (8 and 9) favours the production of acetic and propionic acids whereas acid pH values favours the production of acetic and propionic acids at pH 5 and propionic and butyric acids at pH 6. For neutral pH, the acetic (503 mg COD/L), propionic (410 mg COD/L) and butyric (458 mg COD/L) acids were produced in a similar proportion. The highest production of acetic acid (503 mg COD/L) was obtained at pH 7 whereas the highest production of propionic acid (605 mg COD/L) was obtained at pH 6. The highest production of butyric acid (685 mg COD/L) was obtained at pH 6. The highest production of VFAs were obtained at pH 6 (1583 mg COD/L).

4 Conclusions

The objective of this work was to investigate the production of VFAs through the anaerobic degradation of an effluent from fish processing industry.

The experimental results obtained in the reactor showed that a large production of VFAs was not obtained since the anaerobic digestion process was completed due the presence of CH_4 in the reactor. On the other hand, the production of VFAs increased when the salinity of the effluent was increased demonstrating that this can be a strategy to use.

The experimental results obtained in the batch assays showed an efficient degradation of the effluent and a significant production of VFAs for all pH values tested. The highest production of VFAs was achieved at pH 6 (1583 mg COD/L). Acetic and propionic acids were the main products formed in all assay whereas butyric acid was found in lower concentration. For each pH, the concentration of each VFA varies differently. For propionic and butyric acids production, the optimum pH was 6 and for acetic acid production the optimum pH was 7.

To try to improve the production of VFAs in the reactor, the decrease of the HRT to reduce the contact time between the methanogenic microorganisms and the VFAs and the increase of the salinity of the feed are two aspects that could be studied in the future.

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