Optimization of Volatile Fatty Acids Production for PHAs Synthesis from Food Wastes

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Food wastage is an ethical and environmental wrong practice. Considering a circular economy optic, food waste is a carbon rich substrate, suited for anaerobic fermentation to produce not only biogas, but also high-value chemical compounds, such as Volatile Fatty Acids (VFAs), that can be recovered in order to synthetize polyhydroxyalkanoates (PHAs). This work represented a step in a larger biorefinery process for food waste treatment, having the aim to explore the production and the profile of VFAs from of three different food waste substrates: Stillage, Condensate and Spent Coffee Ground. The substrates were considered at three different pH conditions (uncontrolled, 7, 12), anaerobically digested in batch reactors for VFAs accumulation. The best VFAs yields belonged to pH 7 tests for all the substrates. The best one on terms of VFAs productivity was Food Stillage with 36.17 gCOD/L of VFAs concentration, corresponding to a VFAs yield of 49.48 % w/w.

1. Introduction

A recent report from Food and Agriculture Organization (FAO) estimates a global food wastage at around 1.3 billion tons per year (FAO, 2019). In the European Union, 88 million tons of food waste are generated annually; more than half of them (47 million tons) derives from household, food service and retail. Considering 3 billion people cannot afford a healthy diet, food wastage is an ethically wrong practice. It is also correlated to the depletion of resources and is responsible of 8-10 % of greenhouse gas emissions worldwide (FAO, 2021). For these reasons, the valorization of food waste into biomaterials and biofuels is becoming one of the main priorities of the new environmental policies promoted by the European Commission (Battista et al., 2020b). Food waste represents a good substrate for anaerobic fermentation, due to the high content of simple carbon compounds, which can be easily converted into biofuels (Zhang et al., 2007). In particular, in the last decades food waste was mainly adopted for biogas production by anaerobic digestion (AD) (Dutta et al., 2021), leading to an under-exploitation of his potential, that is represented by the production of high-value chemical compounds, i.e., essential oils (Battista et al., 2020b) and Volatile Fatty Acids (VFAs) (Strazzera et al., 2021a). The scientific community started to be interested in the acidogenic fermentation of food waste to produce VFAs (Strazzera et al., 2018). VFAs are a highly useful compounds that can be used as precursors for the productions of innovative bio-based products with applications in chemical, pharmaceutical, food and cosmetic industries (Rizzioli et al., 2021). Previous studies showed that high VFAs production (10 and 50 gCOD/L) is favored by acidogenic fermentations of food wastes conducted at controlled pH in the neutral range (6.0–7.0), short HRT (lower than 10 days), thermophilic temperatures and an organic loading rate of about 10 kgVS/m^{3}d (Ramos-Suarez et al., 2021; Strazzera et al., 2021b). However, the VFAs production and profile strongly depends on the operational conditions of the fermentation and chemical characterization of the substrates.

An interesting application of VFAs are the polyhydroxyalkanoates (PHAs) production in pure and Microbial Mixed Cultures (MMC) systems. PHAs are a family of polyesters that are primarily synthetized as intracellular carbon and energy sources by numerous species of Gram-positive and/or Gram-negative microorganisms but also by anaerobic photosynthetic bacteria and archaea. PHAs are completely biodegradable, and, depending on their monomer composition, they can have a large spectrum of properties mostly related to the thermoplastic material.

The use of MMC technology for their production is attracting a great deal of attention because it allows to produce PHAs through a low-cost and eco-friendly approach that responds to changing environmental frameworks (Battista et al., 2020a). Previous studies by Colombo et al. (Colombo et al., 2017) used the Organic Fraction of Municipal Solid Waste (OFMSW) for VFAs production and PHAs accumulation, obtaining the following yields: 151 g VFAs/kg OFMSW and 223 ± 28 g PHAs/kg VFAs, respectively. Another study by Valentino et al. (2019b, 2019a) on OFMSW fermentation and PHAs accumulation found VFAs production yields from 0.24 to 0.45 gCOD/gTVS and PHAs overall accumulation yield of 162.5 g PHAs/kg VFAs.

This work studied how three different restaurant food wastes (RFW), having different chemical compositions, influenced the VFAs yield and profile through a batch-mode acidogenic fermentation. Results of this work will help to develop and optimize the PHAs production from VFAs derived by the RFW.

2. Materials and Methods

2.1 Substrates and Inoculum characterization

The influence of three different typologies of RFW (RFW Stillage, RFW Condensate and Spent Coffee Grounds (SCG)) on the VFAs productivity and VFAs profile was investigated. The substrates were originated from RFW collected from restaurants of the central touristic zone of Athens, Greece. The substrates were pre-treated by the School of Chemical Engineering of the National Technical University of Athens and then shipped to the Department of Biotechnology of the University of Verona. In particular, RFW Stillage was the RFW previously pretreated by a commercial amylase formulation for starch hydrolysis for 1 h at 65 °C (Salimi et al., 2021); RFW Condensate was obtained as the by-product of RFW dehydration by a rotary drum dryer for 17 h at 105 °C (Salimi et al., 2021); SCG derived from a solid/liquid extraction for the coffee oil recovery by a lab-scale Soxhlet apparatus, followed by an alkaline treatment (NaOH 1 N; 16 h) to favor the depolymerization of recalcitrant lignocellulosic materials (Barampouti et al., 2021).

The inoculum was an agricultural digestate obtained from a full-scale biogas plant near Isola Della Scala, Verona, Italy. The characterization of the substrates and inoculum are listed in Table 1.

Table 1. Chemical characterization of inoculum and substrates. "na" means "not available"

	Digestate	Condensate	Spent Coffee Grounds	Food Stillage
Total Solids (TS) (% w/w)	2.25 ± 0.31	0.02 ± 0.01	5.63 ± 0.50	18.56 ± 0.19
Volatile Solids/Total Solids (VS/TS) (%)	57.44 ± 1.05	99.99 ± 0.01	62.62 ± 1.52	86.03 ± 0.53
pH	8.83 ± 0.01	4.39 ± 0.02	13.65 ± 0.02	5.85 ± 0.01
Chemical Oxygen Demand (COD) (g/L)	30.21 ± 0.23	23.46 ± 0.41	55.20 ± 0.67	94.81 ± 0.84
Total Kjeldahl Nitrogen (TKN)(g/L)	2.65 ± 0.81	nd	1.16 ± 0.15	nd

2.2 VFAs production from substrates

In order to evaluate the VFAs production from the acidogenic fermentation of the substrates, the three substrates were tested in a CSTR reactors having a working volume of about 500 mL in batch-mode. The tests were conducted in mesophilic condition (37 °C), under three different pH conditions: uncontrolled pH, 7 and 12. pH was adjusted at the beginning of the tests by the addition of NaOH or HCl (1 M). The inoculum was added with a fixed substrate-inoculum ratio of 10:1, considering Chemical Oxygen Demand (COD). This substrate-inoculum ratio was chosen following the work from Strazzera et al. (2018), in order to guarantee carbon availability to the inoculum and promote VFAs production. Each test was conducted in triplicate in order to assure its repeatability. The end of the tests was established considering the reaching of a stable VFAs concentration from the different typologies of substrates. 1mL of samples were collected every day for VFAs determination by ion chromatography system (Dionex ICS 1100 with ICE AS1 column, Thermo Fisher Scientific, USA).

The VFAs production yields were referred to the COD amount added in the reaction medium following the formula:

$$Y(\%) = 100 \frac{m_{VFA}}{m_{COD\ added}} \tag{1}$$

Where m_{VFAs} was calculated from the final concentration of VFAs, expressed as g_{COD}/L, multiplied by the reactor's volume. m_{COD} is the COD amount from substrates at the beginning of the tests.

3. Results and discussion

3.1 VFAs production from SCG

As described above, this substrate derived from a combination of a solid/liquid Soxhlet extraction and an alkaline pretreatment. The solid/liquid extraction removed all the coffee oils, composed by lipids and long fatty acids, and thus the compounds easier convertible into VFAs by anaerobic fermentation, leaving only a small percentage of sugars, and the most recalcitrant compounds: cellulose and hemicellulose. These latter were partially hydrolyzed by the alkaline pretreatment. It allowed to explain the different kinetics of VFAs production from SCG. The simplest compounds derived from the alkaline pretreatments were converted into VFAs in the first week (Figure 1), while the remaining recalcitrant lignocellulosic materials required longer time. The phenomenon was more evident for neutral pH. These results are consistent with a previous work by Battista et al. (2021), which found a low production of biogas from defatted SCGs, as consequence of a low production of VFAs. For these reasons, the fermentation of defatted alkaline pretreated SCG gave very poor results in all pH conditions. VFAs production yields for uncontrolled pH, 7 and 12 were 5.55 %, 10.30 % and 1.84 %, with a final concentration of 1.63, 4.48 and 0.86 gCOD/L respectively. Regarding the uncontrolled pH test, the VFAs production was inhibited by the high initial pH (Table 1) which led to slow VFAs production. By the time, a slight increasing of VFAs concentration was probably the consequence of the progressive acidification of the reaction medium, which reached the value of 7.36 ± 0.04 at the end of the test. The VFAs profile of the pH 7 test showed that 34 %, 27 % and 39 % of the total VFAs production were composed by acetic, propionic and butyric acid respectively, consistent with previous works on acidogenic fermentation of food waste with similar substrate-inoculum ratio (Ramos-Suarez et al., 2021). Regarding the pH trend along the time, any significant variation was observed: final pH was of 6.80. Due to the poor results of pH 12 and uncontrolled pH tests, the VFAs profile was not determined for these tests.
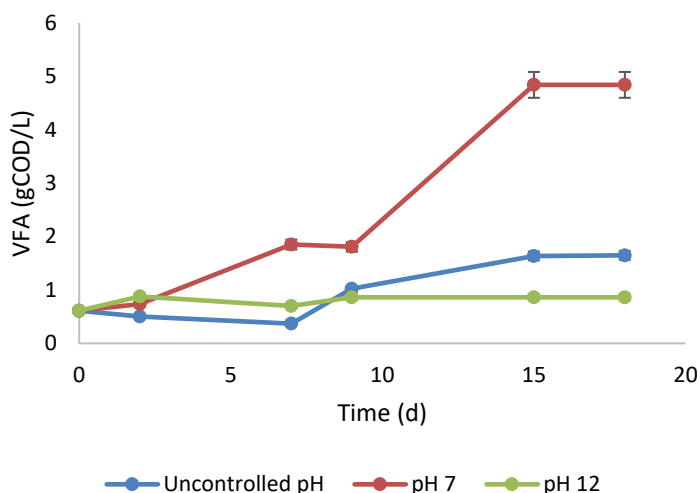

Figure 1. VFAs production for alkaline pre-treated SCGs.

3.2 VFAs production from RFW Condensate

The condensate obtained from the dehydration process of RFW was rich in acetic acid, as consequence of its low boiling point, with a starting concentration of 4.83 gCOD/L. The initial pH was of 4.39. This pH condition is considered inhibitory for acidogenic fermentation, due to the high concentration of protonated VFAs, that cause a toxic effect on the cellular membrane. (Strazzera et al., 2021a). VFAs' production yields for this substrate were of 16.66, 39.92 and 17.02 for uncontrolled pH, 7 and 12 respectively. The uncontrolled pH and pH 12 tests showed almost no production of VFAs, besides the initial concentration of acetic acid. At neutral pH conditions, a final concentration of 8.71 gCOD/L of VFAs was reached. The VFAs profile showed a prevalence of acetic acid (52 %), followed by butyric acid (29 %) and, interestingly, caproic acid (18 %). A possible explanation for the production of caproic acid could be attributed to the initial substrate's composition. As mentioned above, this substrate was obtained by a thermal dehydration of RFW, thus leading a separation from the dried RFW of low boiling point compounds, including ethanol and acetic acid (Lytras et al., 2020) that, under neutral pH and anaerobic conditions, could trigger a chain elongation process from acetic (C2) and butyric (C4) acid to caproic acid (C6) (Roghair et al., 2018; San-Valero et al., 2019; Steinbusch et al., 2011).

Due to being Middle-Chain Fatty Acid (MCFA), the caproic acid is a more desirable product than shorter chain VFAs in terms of extraction viability and economic value (San-Valero et al., 2019).
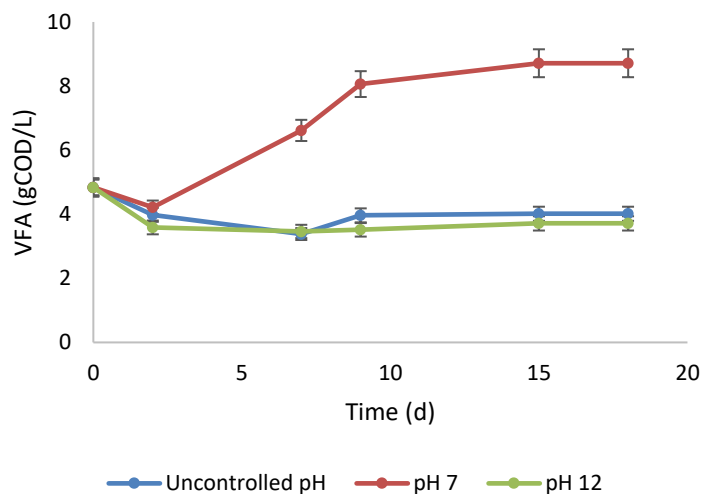

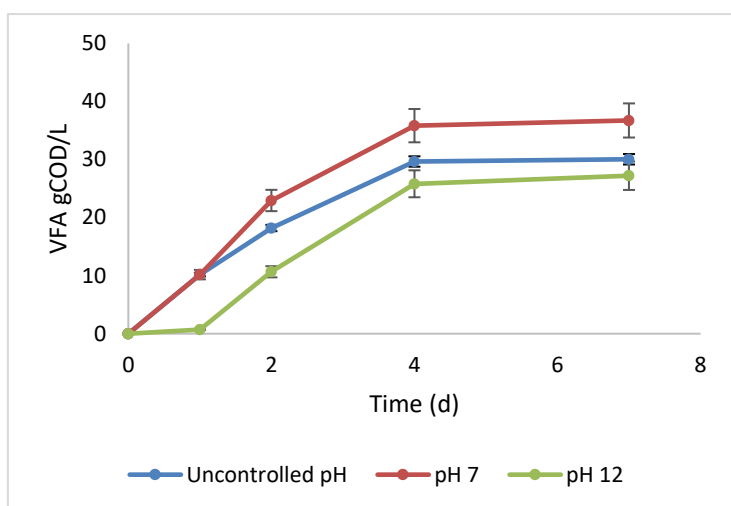*Figure 2. VFAs production for Food Waste Condensate.*

Figure 3. VFAs production for Food Waste Stillage.

3.3 VFAs production from RFW Stillage

This substrate, obtained from the enzymatic hydrolysis of RFW starch, had a high initial concentration of simple sugars of about 70 g glucose for 100g of substrate (Salimi et al., 2021). The fermentation started immediately reaching the VFAs concentration of 10 gCOD/L at the end of the first day of fermentation only for uncontrolled and pH 7 test. Then, the VFAs concentrations increased to 36.72, 30.04 and 27.21 gCOD/L, corresponding to a production yield of 49.98 %, 40.89 % and 37.03 % for the pH 7, uncontrolled and pH 12 tests, respectively. Beside to the high sugars concentration, RFW stillage had good concentration of proteins too. This avoided the decreasing of pH under the inhibiting level. The degradation of proteins led to ammonia formation, known to favour the buffer effect of the system (Strazzera et al., 2021a). The RFW stillage at pH 12 showed lower VFAs kinetics due to the initial inihbiting alkaline pH of the substrate. The VFAs production started after a 3 day lag-phase, achieving a lower final VFAs concentration. Previous studies by He et al. (2012) and Liu et al. (2018) on anaerobic digestion of untreated food waste reached a final concentration of 16 and 26 gCOD/L respectively, confirming the positive effect of enzymatic starch hydrolysis on VFAs production. The VFAs profile showed a prevalence of butyric acid followed by acetic and propionic with, respectively, 71, 25 and 4 % of the total VFAs production for uncontrolled pH.

The same trend has been found in the pH 7 test, with 67, 21 and 12 % of butyric, acetic and propionic acids, respectively. This is consistent with the work of Wang et al. (2014), which stated that at the pH range between 5.0 and 6.0, the butyric acid fermentation is favoured. For the pH 12 test, the VFAs profile was different, with 38, 27 and 35 % of acetic, propionic and butyric acid respectively.

4. Conclusions and future perspectives

This article evaluated the VFAs production performance of various food waste substrates at different conditions of pH and pretreatments. The pH 7 emerged as the best condition for VFAs production for all the substrates. At pH 12, the VFAs production is greatly inhibited, due to toxic effects of strong basic pH on microbial flora (Mengmeng et al., 2009). When the pH was not controlled, the VFAs production varied according to the initial pH conditions of the different substrates. The best substrate for VFAs production was the enzymatically treated food waste (RFW stillage), due to the high content of sugars which can be immediately converted in VFAs. This substrate had a VFAs yield of 49.98 % corresponding to a VFAs final concentration of 36 gCOD/L, making this substrate well suited for PHAs production, that will be investigated in the near future. Another interesting result was the high caproic acid concentration from RFW condensate due to the instauration of VFAs chain elongation's reactions caused by the high the initial presence of ethanol and acetic acid in this substrate. Finally, the defatted and alkaline pretreated SCG substrate was not suited for VFAs production, due to the high concentration of recalcitrant compounds, cellulose and hemicellulose. This typology of substrate could probably fit better for pyrolysis and gasification processes.

The batch tests conducted in this research work allowed to individuate the highest VFAs production of the considered substrates at different pH conditions. Continouos tests will be organized in order to find the Organic Load Rate and Hydraulic Retention Time which can optimized the VFAs productivity following the procedures of some previous works (Moretto et al., 2019; Strazzera et al., 2021b).

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