(04-008) - Green-chemicals and energy production from food waste and sewage sludge through two-stage anaerobic digestion.

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Sustainable urban waste valorisation is a global target to be accomplished. In this sense, integrating urban biorefineries in waste treatment sector further enhances the principles of circular economy such as zero waste production. For organic matter fraction, biorefineries mainly based on anaerobic processes represent a sustainable alternative that allows waste treatment and valorisation. This study aims at understanding the biochemical processes governing dark fermentation of the most biodegradable organic fraction of urban wastes, i.e., food waste and sewage sludge.

After a previous literature review on the production of "green" chemicals and energy through the valorisation of food waste and WWTP sludge by dark fermentation, the design of the experimental set-up (batch and laboratory scale tests) and the start-up of the process are carried out. In this sense, several batch tests will be carried out in order to establish the optimal operating conditions to favour the different dark fermentation pathways (acid and lactic). Finally, the design of two laboratory-scale AnDMBR (anaerobic dynamic membrane reactor) systems for two-stage anaerobic co-digestion will be carried out.

Keywords: Biorefinery; dark fermentation; food waste; sewage sludge; hydrogen; organic acids.

Producción de químicos "verdes" y energía a partir de residuos alimentarios y fangos EDAR mediante digestión anaerobia en dos etapas

La valorización sostenible de los residuos urbanos es un objetivo global a alcanzar. Por eso, la integración de biorrefinerías urbanas en el sector del tratamiento de residuos es la opción ideal. Las biorrefinerías basadas en procesos anaerobios representan una alternativa sostenible que permite el tratamiento y valorización de la fracción orgánica de los residuos. Este trabajo tiene como objetivo comprender los procesos bioquímicos que rigen la fermentación oscura de la fracción orgánica biodegradable de los residuos alimentarios y los fangos de EDAR.

Después de un trabajo previo de revisión bibliográfica sobre la producción de químicos "verdes" y energía mediante la valorización de residuos alimentarios y fangos de EDAR por fermentación oscura, se lleva a cabo el diseño del montaje experimental (ensayos en discontinuo y a escala de laboratorio) y la puesta en marcha del proceso. En este sentido, se llevarán a cabo varios ensayos en discontinuo, con el objetivo de establecer las condiciones de operación óptimas para favorecer las diferentes vías de fermentación oscura (ácida y láctica). Finalmente, se ejecuta el diseño de dos sistemas AnDMBR (reactor anaerobio dinámico de membrana) a escala de laboratorio para la co-digestión anaerobia en dos etapas.

Palabras clave: Biorefinería; fermentación oscura; restos alimentarios; fangos EDAR; hidrógeno; ácidos orgánicos.

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1. Introduction

The European Union defines "Circular Economy" as the model of production and consumption that encompasses the actions of sharing, reusing, repairing, renewing, and recycling existing materials and products for as long as possible. In other words, to minimise the amount of waste produced and enhance the life cycle of these products (EU News, European Parliament, 2015). In an economic model based on the circular and sustainable economy, the final aim is to produce zero waste, obtaining instead valuable products, thus closing cycles or loops in industrial systems.

Biorefinery is a way to take advantage of or revalue waste, to reintroduce it into the supply chain and generate new high value-added products. The engine of a biorefinery is to satisfy the energy and material demand from waste (Hingsamer & Jungmeier, 2018). Analogous to a conventional petrochemical refinery, the carbon sources of a biorefinery come from renewable carbon sources (biomass). It introduces new technologies to generate biofuels, as well as high value-added bioproducts (Battista et al., 2020a).

Concerning types of waste as feedstock for biorefineries, substrates such as food waste (FW) or primary sludge (sewage sludge) from urban wastewater (UWW) treatment plants are suitable due to their high organic matter and nutrient content. These wastes represent most of the biodegradable organic matter produced by municipalities. Currently, food waste from municipalities and sewage sludge from wastewater treatment plants (WWTPs) are mainly valorised in the form of biogas and stabilised biosolids for future applications as fertiliser by anaerobic processes.

The biorefinery context to be assessed in the project in which this work is framed will be focused on the valorisation of urban wastes, i.e., food waste and sewage sludge from WWTP, through anaerobic processes. Particularly, the anaerobic digestion process will be assessed in two stages: dark co-fermentation to obtain added-value bio-products (a mixture of volatile fatty acids (VFAs), bio-hydrogen, or lactic acid, or other targeted acids), and further anaerobic digestion of the digestate from dark fermentation to obtain biogas and stabilised biosolids for its application in agriculture.

The different added-value bio-products to be produced will mainly depend on the specific economic, social, and environmental context of the facility location. Thus, a tailor-made biorefinery will be required to keep zero waste and maximum economic profit.

2. Objectives

The objective of this study was to evaluate the operational conditions conducive to different pathways of dark fermentation, namely the VFA/H_2 pathway and the LA pathway. This investigation aims to contribute to the optimisation of the simultaneous production of bioenergy and green chemicals in urban biorefineries. This research imperative is particularly pertinent when considering the potential for green chemical and energy generation from food waste and primary sludge. These materials are characterised by their high abundance and availability, low cost, and appropriate content of micro and macronutrients that support fermentation processes.

Specifically, this study focuses on experiments designed to assess the impact of the food waste to sewage sludge ratio on process efficiency. This examination focuses on both the quantity and quality of the products obtained, including hydrogen, mixed volatile fatty acids (VFAs), and lactic acid.

3. Methodology

FW was collected from the restaurant of the School of Engineering of the Universitat de València, whose main characteristics were: total solids (TS) content of 32.9 % wb., volatile solids (VS) content of 97.9 %TS, and total organic carbon (TOC) of 45.1 %wb. Concentrated primary sludge (PS) was collected from a full-scale WWTP with the following main characteristics were: 38.5 g TS·L^{-1}, 25.8 g VS·L^{-1}, and chemical oxygen demand (COD) of 42.4 g COD·L^{-1}. Anaerobic sludge was used as inoculum, it was taken from the anaerobic membrane bioreactor (AnMBR) pilot plant located in the full-scale WWTP.

Batch assays were conducted using 500-mL glass vessels incubated at controlled temperature with constant mixing. The initial pH was adjusted. The working volume was 300-mL and the headspace were flushed with nitrogen for displacing air from the vessel. Glass vessels were closed with a rubber cover to allow sampling gas from the headspace and sampling liquid from the mixed liquor. A given number of replicates were running in parallel when using common glass vessels. Gas samples were taken from all replicates at the same sample time to confirm that replicates ran consistently.

A Box-Behnken experimental design was conducted to optimise the conditions required for each desired metabolic pathway. Overall, the following levels were defined for each factor:

- Temperature: 35 and 55 ºC.
- Initial pH: 6, 8 and 10.
- FW:PS ratio: 20:80, 30:70, 50:50, 70:30 and 80:20 g VS:g VS.
- Substrate to inoculum (S/X) ratio: 0.25, 1 and 4 g VS·g^{-1} VS.

The substrates were extensively characterised before starting the experiments.

- The TS and VS contents were measured according to the standard methods of the American Public Health Association (APHA, 2017).
- The TOC (total organic carbon) and elemental analysis (CHONS) were analysed in the Atomic and Molecular Spectroscopy Section of the Central Research Support Services of the University of Valencia.
- The pH was measured using a benchtop multi-parameter pH and conductivity meter.
- The chemical oxygen demand (COD) was analysed using the reflux method. COD concentrations were determined using a potentiometric titration Metrohm (APHA, 2017).
- Metals concentrations were analysed in the Atomic and Molecular Spectroscopy Section of the Central Research Support Services of the University of Valencia.
- The proteomic analysis was performed in the proteomics facility of SCSIE University of Valencia. This proteomics laboratory is a member of Proteored.

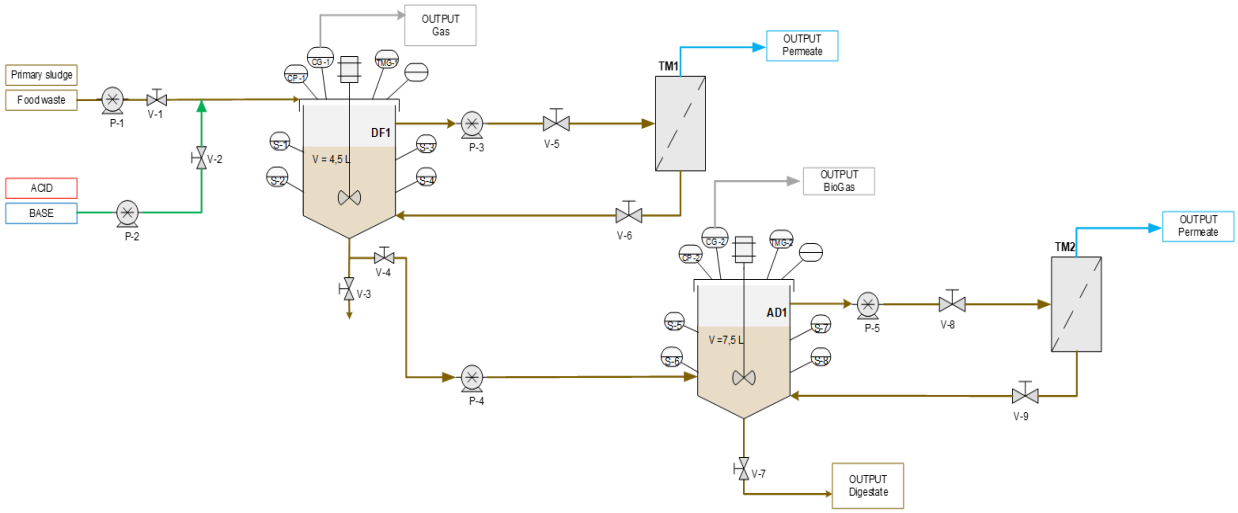
The concentration of VFAs (i.e., acetic (C2), propionic (C3), iso-butyric (iC4) butyric (C4), iso-valeric (iC5), and valeric (C5) acid) was determined by gas chromatography (GC; Agilent 6890) equipped with a flame ionization detector (FID). The GC consisted of an injector, a capillary column (FFAP) and a flame ionisation detector. The mobile phase was nitrogen gas at a flow rate of 5 ml·min^{-1}. Before analysis, the samples were mixed with a solution of one g·L^{-1} of éthyle-2-butyrique acid at proportions 1:1 volume (acting as internal standard).

The concentration of lactic acid was determined by ionic chromatography (IC) with a Metrosep C 6 – 250/4.0 column, at a flow rate of 0.9 ml·min^{-1}. 5 g of sample is dissolved in 500 mL eluent. Protein precipitation by heating to 50ºC for 5 min. injection after filtration (0.2µm). As eluent nitric acid at 4.0 mmol·L^{-1}. This column separates sodium, potassium, magnesium, and calcium by ion exchange. Both lactic acid and the cations can be determined in the same run applying direct elutes as an early positive peak. In this case, the separation mechanism is

cation exchange for Na, K, Mg and Ca. For lactic acid is ion exclusion. The polarity for the ion exclusion is positive and the recording time is 5 minutes.

After elucidating the operational conditions that favour both LA-type and VFA-type metabolic pathways, 2 bench-scale systems have been started up to assess the potential for continuously producing different bio-based products (i.e., hydrogen, biogas, mixed-VFAs, other targeted acids, stabilized bio-solids). Each bench-scale system consisted of a 2-stage anaerobic co-treatment process, formed by a 4.5-L dark fermentation reactor (1st stage) and an 8-L anaerobic digester (2nd stage), both based on AnMBR technology. Thus, two treatment lines are being fed in parallel using the same substrate (results not shown here).

Figure 1: Flow diagram of the 2-stage anaerobic co-treatment systems.



The reactors consist of cylindrical vessels made of polypropylene that are continuously mixed by mechanical agitation. The reactors have an electrical resistance with a thermostat that automatically regulates the temperature.

The 1st stage will be started up with the optimum food waste to sewage sludge ratio to be determined from the batch experiments for each metabolic pathway (mixed-VFAs and lactate-type route). The 2nd step will be fed with the wasting sludge from each fermenter jointly with sewage sludge for maximising methane production.

Figure 1 shows the flow diagram of the systems. When required, acid and/or base will be dosed to control the pH. The first reactor will be operated to favour the dark fermentation of the pre-defined mixture of food waste and primary sludge. The gaseous stream consisting of a mixture of hydrogen and carbon dioxide will be produced. At the same time, the wasted sludge will be pumped to the anaerobic digester to carry out the second stage of the anaerobic digestion process, maximising methane production. A stabilised solid product will be obtained.

The impact of varying different operating conditions on dark fermentation and 2nd stage anaerobic digestion process will be assessed. Parameters to be evaluated include: SRT, HRT, OLR, T, pH, feed blending and composition, mixing intensity, etc. KIPs monitored will mainly focus on VFA distribution and concentration and syngas production and characterisation. Other parameters to be monitored will include COD and BOD concentration, VS and TS concentrations, TAN and FAN concentrations, total phosphorus concentration and fractionation, microbial identity, and dynamics, etc. To evaluate the possible use of the digestate as a fertiliser, pathogens (mainly E. coli and Salmonella) and heavy metals (Cd, Cu,

Ni, Pb, Zn, Hg and Cr) will be analysed, according to regulations in force. Viruses, other micropollutants (emerging and priority substances) and antibiotic resistant genes (ARG) will be also analysed.

For the dynamic membrane filtration assessment, the impact of varying different operating conditions on dynamic membrane filtration in a dark fermentation process will be assessed. Parameters to be evaluated include permeate flux (J), gas sparging intensity for membrane scouring (SGD), filtration cycle (FC), sludge recycling flow (SRF), etc. KIPs monitored will include cake formation time, solids and colloids retention, characterisation of membrane inflow and outflow, permeate quality, transmembrane pressure, downtime and cleaning requirements, characterisation of membrane fouling, etc. Based on experimental data, the optimum operating conditions that maximise filtration process productivity (i.e., minimising process costs) will be determined.

4. Case study

Given the wide range of variation of the key operating parameters to favour the different metabolic pathways, an experimental design has been proposed to conduct a series of experiments aimed at determining the optimal ones.

Three batch assays designed to evaluate the lactate-type metabolic pathway are shown here (see Table 1).

Table 1: Experiential conditions of the batch assays evaluated.

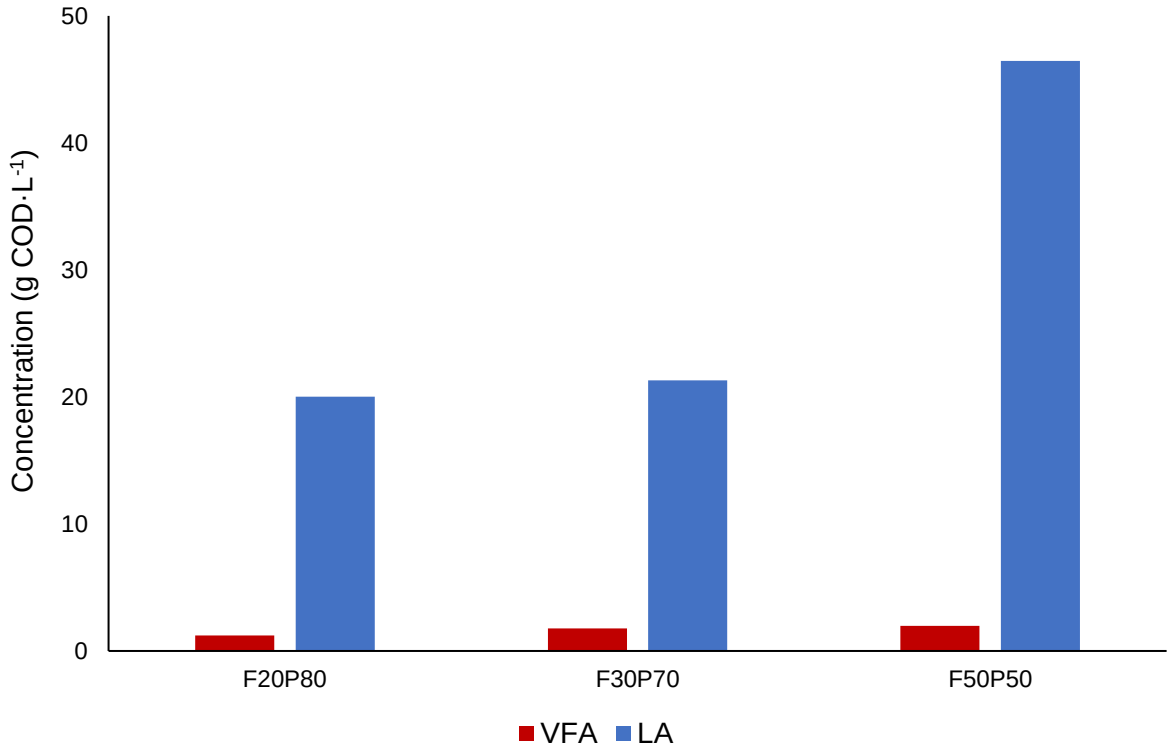
Assay ID	FW:PS ratio (g VS: g VS)	S/X (g VS ·g^{-1} VS)	Initial pH	Temperature (ºC)	Mixing velocity (rpm)
F20P80	20:80	1	6.0 ± 0.2	35	150
F30P70	30:70				
F50P50	50:50				

Substrate and co-substrate were added into the glass vessels, jointly with the corresponding amount of inoculum to keep a substrate to inoculum (S/X) ratio of 1 g VS·g^{-1} VS. The FW to primary sludge co-fermentation ratio was set at 20:80, 30:70 and 50:50 g VS ·g^{-1} VS). The working volume was 300 mL and the headspace were flushed with nitrogen for displacing air from the vessel. To correct the contribution to the biogas produced of any substrate present in the inoculum, a blank vessel was run in triplicate. The activity of the inoculum was previously verified using ethanol as substrate (0.5 g COD·g^{-1} VS). The reactors were incubated at controlled temperature under constant mixing. Initial pH was adjusted.

5. Results

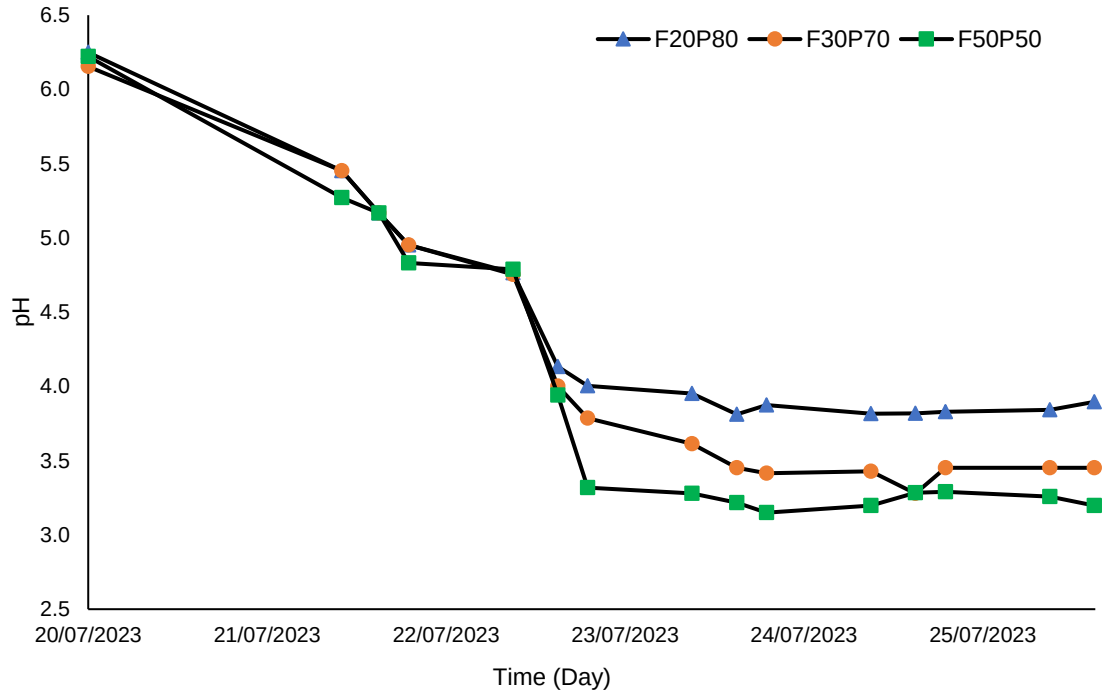
Figure 2 shows the maximum acid lactic and mixed- VFAs concentration reach in each assay. If the cumulative lactic acid concentration is compared with the cumulative total VFAs concentration, it can be concluded that the metabolic route that followed the acidogenic fermentation was the lactate-type metabolic pathway. These batch experiments have shown that lactic acid can be produced in a pH uncontrolled system, but that much process optimisation is still required.

Figure 2: VFAs and lactic acid accumulative concentration for each experiment.



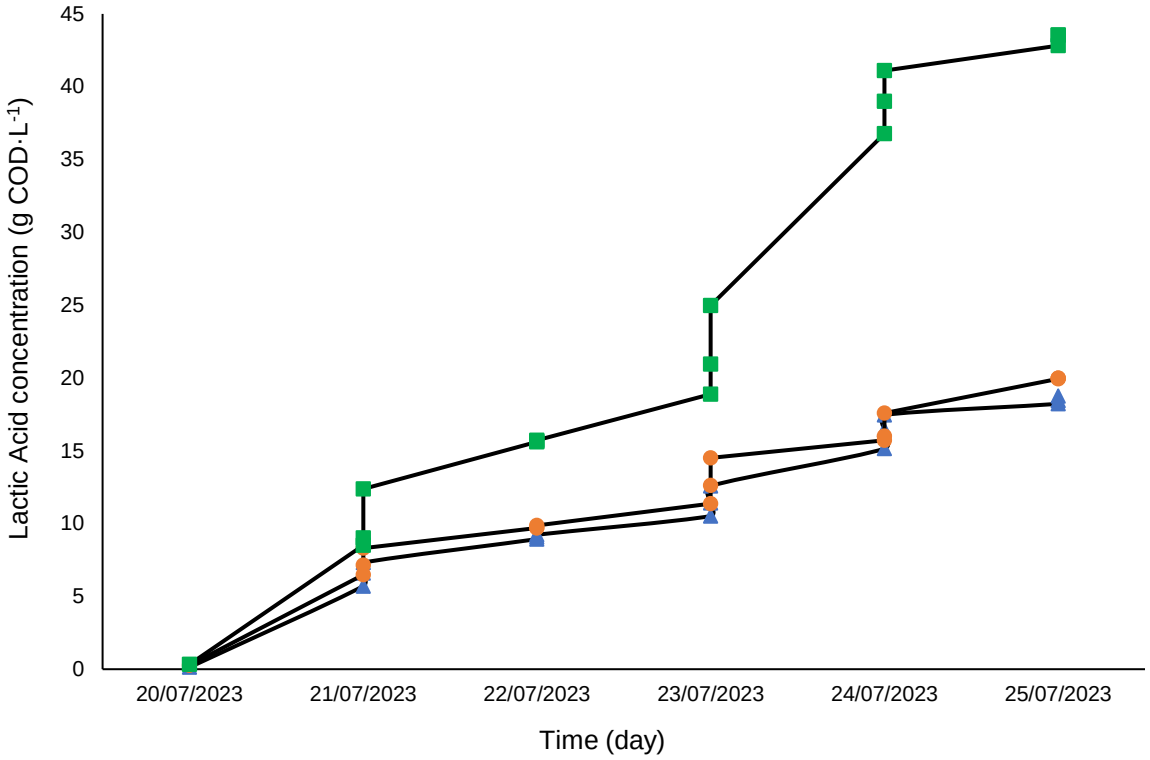
Eventually, if the pH drops low enough, lactic acid will start to accumulate due to metabolic shifts occurring in acidic environments. This acid does not generate significant amounts of hydrogen during its production and, moreover, it affects greatly pH, causing a further decrease in the pH. At this point, the addition of buffers would be necessary. pH had significant effects on hydrolysis and acidification.

Figure 3: pH variation for each experiment.



The uncontrolled pH of these experiments decreased to values from 4.0 to 3.0 in 48 hours (see Figure 3). These low values promoted the great production and accumulation of lactic acid (see Figure 4) because some types of lactic acid bacteria can produce lactic acid at pHs below 4.0 (Tang et al., 2016).

Figure 4: Lactic acid concentration variation for each experiment.



Another reason that justifies these enormous lactic acid concentrations (see Figure 4) is that lactic acid bacteria are much rapid under mesophilic conditions (around 35 ºC). Figure 3 shows the trend followed by the pH along the experiments. As mentioned above, it is decreasing until it reached the minimum value of 4.8, 3.9, and 3.3 for F20P80, F30P70 and F50P50 tests, respectively. Thus, these pH values were assumed to be inhibitory for methanogens, thus explaining the negligible methane productions.

Food waste is a natural habitat for *Lactobacilius spp*. For instance, Probst et al. (2013) showed that 86% of the bacteria present in the organic fraction of municipal solid waste were *Lactobacilius spp.* and the community was stable for several days, highlighting the potential of utilising the natural occurring flora in lactic acid fermentations. Probst et al. (2013) obtained the maximum lactic acid concentrations (approximately 30 $g \cdot L^{-1}$) when operating at 37 ºC and uncontrolled pH or controlled pH of 5, without substrate pre-treatment.

In reference to VFAs production and concentration, the greatest variation can be granted to acetic acid. From the first day of experimentation, it can be observed that acetic acid concentration (and production) increases until reaching maximum values of around 1000, 1500 and 1750 mg $COD \cdot L^{-1}$ for F20P80, F30P70 and F50P50 tests, respectively. Besides acetic acid, the more abundant VFA was propionic acid, followed by butyric, isobutyric, valeric, and isovaleric acids. This trend was repeated in the three experiments conducted, i.e., F20P80, F30P70, and F50P50 tests. Since methanogens were inhibited mainly due to the pH of the medium, VFAs were not consumed. The maximum concentration for each VFA is shown in Table 2.

Table 2: maximum VFAs concentrations during the conducted experiments.

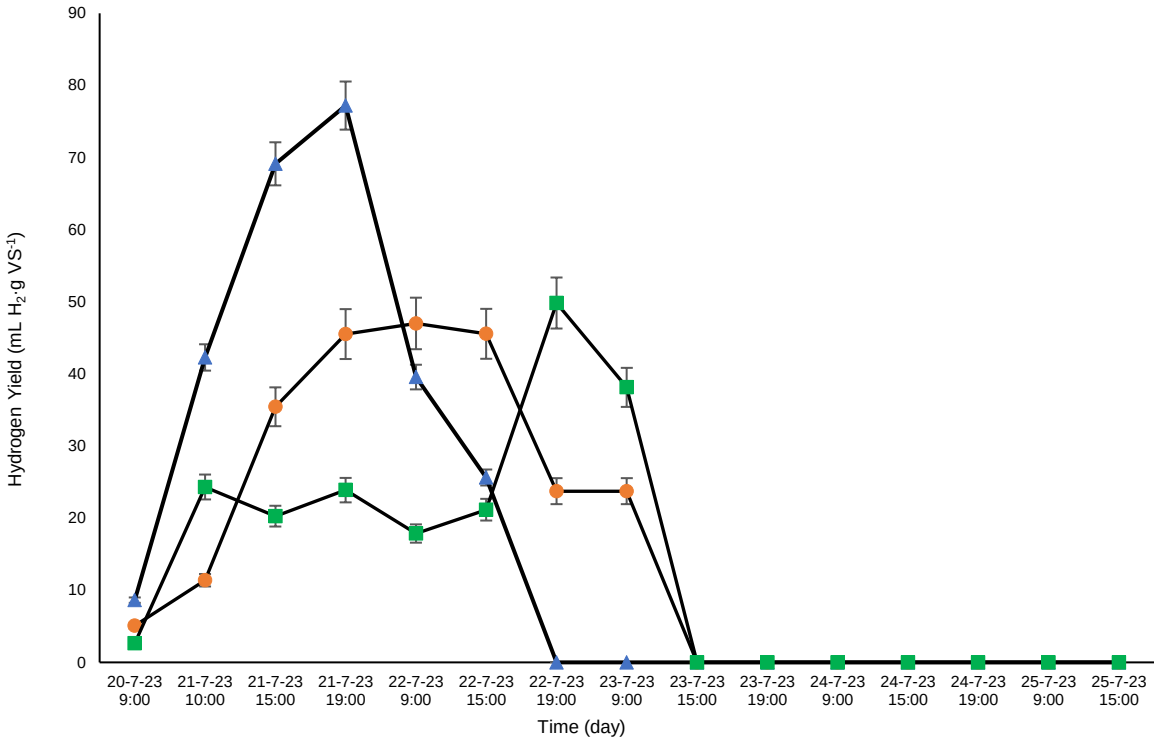
Experiment	C2	C3	iC4	C4	iC5	C5
	(mg COD·L^{-1})					
F20P80	1007.2	85.78	25.8	54.2	22.6	n.d.
F30P70	1527.1	123.6	227	60.9	35.2	n.d.
F50P50	1732.7	147.5	40.4	21.7	20.8	n.d.

Summarising all data showed before, this build-up of acids can be attributed to the high biodegradability of FW. Therefore, it can be seen that the higher the proportion of food waste in relation to primary sludge, the higher the concentration of VFAs produced. The hypothesis behind this fact could be that the higher the proportion of food waste, the higher the proportion of biodegradable organic matter (FW has a higher proportion than primary sludge) and therefore is more easily converted into VFAs. When the acids accumulation started, a decrease on the pH is produced, affecting methanogenesis: one key rate limiting step of the anaerobic digestion process. So, at greater initial concentrations of FW, more substrate was acidified and the obtained peaks of VFAs were more pronounced, causing greater pH drops.

It's crucial to highlight that the use of mixed consortium is generally associated to numerous metabolic pathways in the fermentation process, which led to a differentiation of the products: lactic acid, syngas, and VFAs (and a diversified VFA spectrum).

Figure 5 shows the hydrogen yield monitored during the experiments. Hydrogen production was low for the three scenarios assessed. As commented above, low pH significantly affects hydrogen production due to significant impacts on the structure of the microbial community. Mesophilic dark fermentation led to low hydrogen yields and high lactic acid concentrations. The maximum hydrogen yield was achieved for F20P80, i.e., 77.2 mL H_2·g VS^{-1}. F30P70 and F50P50 resulted in maximum hydrogen yields of 46.99 and 49.84 mL H_2·g VS^{-1}, respectively.

Figure 5: hydrogen yield for each test.



As a matter of fact, the addition of sewage sludge to food waste synergistically enhanced the H_2 fermentation performance at the beginning of the experiments. To evaluate this fact, future research will include experiments without sewage sludge.

Negligible methane productions were observed, detecting maximum methane yields of 26.9, 4.7 and 3.9 mL CH_4·g VS^{-1} for F20P80, F30P70 and F50P50, respectively.

6. Conclusions

Lactate-type metabolic pathway was dominant within the operating conditions evaluated in this work. Due to the lack of pH control in these experiments, the pH drop was very important, which implies the growth of a population of microorganisms that usually occur in acidic environments, such as lactic acid bacteria. The appearance of these microorganisms and the high production of lactic acid from primary sludge and food waste resulted in an exaggerated pH drop. For each experiment we had similar results. Therefore, for the same conditions and varying the substrate/co-substrate ratio, the results are reproducible.

Experimental results showed an increasing VFA yield as increasing the food waste to primary sludge ratio. The increasing VFA yield was because of the introduction of protein and carbohydrate-rich substrate. Food waste composition can be used to drive the VFA profile to a certain extent, but the prediction of the VFA yield is not only related to food waste composition.

As far as biogas production and composition are concerned, in general, the production of H_2 and CO_2 as well as CH_4 was of interest to observe in this study. CH_4 production has been imperceptible, which means that the process was not inhibited by methanogens, probably because of the drop in pH. H_2 production was also not high, although higher than CH_4 production, which is good news but also reinforces the idea that the process must be optimised for H_2 production.

The results obtained from the batch assays are currently being used to select adequate initial operating conditions for the start-up of the bench-scale systems. Further steps will involve assessing the performance of these systems, focusing on maximizing the economic revenues of urban bio-refineries based on AnMBR systems for co-valorisation of FW and PS.

7. References

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