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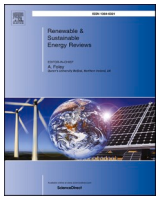

Production, purification and recovery of caproic acid, Volatile fatty acids and methane from Opuntia ficus indica

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ABSTRACT

Opuntia ficus-indica can grow in arid and semi-arid environments characterized by low water and nutrients availability. These features make it a more sustainable alternative to the common energy crops for biorefinery purposes. This work focused on the potential benefits of anaerobic processes applied to this plant. Specifically, it considered i) the substrate preparation, demonstrating the effect of the apparent viscosity on the process; ii) the evaluation of biomethane, Volatile Fatty Acids (VFAs), and caproic acid production in semi-continuous mode at different hydraulic retention times; iii) the purification of the Fatty Acids-rich output through pressure-driven membrane filtration. The rheology analysis found that a 5 %w/w water dilution of the substrate is needed to lower the apparent viscosity to 173 cP, which is below the acceptable apparent viscosity level of 200 cP for a good bioreactor mixing. Keeping this condition, the semi-continuous trials with the best biomethane performance was at HRT of 20 days, with 210 $mLCH_4/gVS$ and 232 $mLCH_4/gCOD_{in}$ of production and specific yield, respectively. The VFAs and caproic acid production reached their best at Hydraulic Retention Time 5, with 26 and 7.9 gCOD/L of VFAs and caproic acid, corresponding to specific yields of 79 and 30 % respectively. Pressure-driven filtration at 330 kDa allowed to obtain a permeate with a VFAs and caproic acid content of 96.72%w/w. Finally, the adsorption and desorption tests allowed to separate caproic acid from the permeate and to concentrate it from about 7.5 g_{COD}/L to about 26 g_{COD}/L.

1. Introduction

Opuntia ficus-indica (OFI), commonly known as prickly pear or nopal cactus, thrives in high-temperature, low-precipitation environments due to its crassulacean acid metabolism (CAM) [1].

For this reason, OFI is diffused in all the arid and semi-arid regions of the world (southern USA, southern China, India, Chile, Brazil, North Africa and the southern regions of Mediterranean Countries) [2]. In Italy, OFI cultivation is concentrated in Sicily (Italy), where it accounts for the 96 % of total Italian production [3,4]. The OFI fruits maturation is favoured by the pruning of the cladodes, which, consequently, can be considered by-products of the OFI fruit production [5].

The applications of OFI cladodes are quite limited today. OFI were tested as feed for ruminants [6], for the extraction of phenolic compounds, flavonoids and pectines [7,8], for bioethanol production [9], and for the realization of building materials as an eco-friendly and sustainable material, as a bio-composite, and as an insulator [10].

Even if these applications are very interesting, they remain isolated study cases. In the last years the OFI is receiving an increasing interest for biogas production by Anaerobic Digestion (AD).

According to Stintzing & Carle [5], fresh OFI cladodes are characterized by a high-water content (88–95 %w/w); while the dried matter analysis reported a high carbohydrate content: 48 and 21.6 %w/w of polysaccharides and cellulose, respectively. The remaining part is composed by ash (16.6 %w/w), lipids and waxes (7.2 %w/w), and lignin (3.6 %w/w). The lignin content of OFI is lower than the other energy crops feedstocks, which account for 9–30 % w/w [3]. These characteristics make OFI a good candidate for biomethane production by Anaerobic Digestion (AD) in semi-arid regions. This approach encounters the recent environmental policies of the EU, who approved the "REPowerEU" [12,13] to promote the EU energetic independence by the increasing of biomethane production from 3 bcm (billion cubic meters) of the 2021 to 30 bcm by 2030. In this context OFI can become a promising candidate for bioenergy production, especially when exploiting uncultivated semi-arid areas of southern Europe (southern regions of Italy and Spain, as well as Greece, Malta, Cyprus), where the OFI plants naturally grows [3]. In the scientific literature, there are few works regarding OFI AD as single substrate: Garcia et al. [14] reported a potential biomethane yield of 345 LCH_4 per kgVS; while lower yields

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Abbreviations

AD	Anaerobic Digestion
bcm	billion cubic meters
BMP	Bio-Methane Potential
CAM	Crassulacean Acid Metabolism
COD	Chemical Oxygen Demand
EU	European Union
HRT	Hydraulic Retention Times
K_b	saturation value constant (g_{VS}/L d)
OFI	Opuntia Ficus Indica
OLR	Organic Load Rate
PAC	Powdered Activated Carbon
PER	Permeate
RET	Retentate
R_{max}	maximum substrate removal rate
S_0	inlet concentration of the Volatile Solids
S_e	outlet concentration of the Volatile Solids
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids
Y_{CH4}	Yield of methane
Y_{VFAs}	Yield of Volatile Fatty Acids

were obtained by Krümpel et al. [15], and Danzì et al. (2020) [4], who reported a biomethane productivity of 295 and 278 LCH_4 per kgVS, respectively. Previously, similar results were obtained by Jigar et al. (2011) [16], while better performances were achieved by Comparetti et al. (2017) [17] who obtained a biomethane yield of 300–350 LCH_4/kgVS. Different pretreatments were also tested to improve OFI degradation and conversion into biogas but the results from the different works often differed: for example alkaline treatment seemed to be very promising in the work by Beshir Belay et al. [18] with a methane production from 350 to 600 L/kgVS, while no significant improvement was found in the research by Calabrò et al. [19], who obtained better results with the acidic pretreatment.

Beyond to biomethane production, AD can produce other bio-based products, such as Volatile Fatty Acids (VFAs), which are short-chain carboxylic acids used as "building blocks" in chemical, pharmaceutical, food, cosmetic and bioplastic industries. VFAs are higher-value compounds with commercial prices ranging from 600 US$/ton for acetic acid, to 4251 and 3815 US$/ton for valeric and caproic acid, respectively [20]. Currently, the 90 % of the total market demand of VFAs are produced from non-renewable sources with high environmental impact [21,22]. Consequently, VFAs bioproduction can represent an opportunity to further increase the value of the OFI cladodes according to a biorefinery scheme for the simultaneous production of biofuels and bioproducts. Regarding the VFAs production, in the last years the interest was focused on caproic acid, which can be obtained by the "reverse β oxidation" process, where shorter chain VFAs, derived from the acidogenic phase of the AD, are used to elongate the carbon chain through the presence in the reaction medium of electron donor-compounds, such as ethanol, lactate, methanol, n-propanol of biological origin [23].

This work had the ambition to express the full potential of the AD of OFI, as never done before, for the simultaneous production of different bioproducts and biofuels by the simple modulation of the operating conditions of the anaerobic process. Firstly, some batch tests were carried out to identify the OFI-water mixture able to reduce the high apparent viscosity of the minced OFI, which can negatively affect the rheology of the reactor [24]. Then, the best OFI-water mixture was used for the semi-continuous tests, evaluating the influence of different Hydraulic Retention Times (HRT) on VFAs, caproic acid and biomethane productions. Finally, the attention was focused on the caproic acid recovery, due to its high economic value. Therefore, the reaction medium from the tests with the highest caproic acid and VFAs productions was sent to sequential pressure driven membranes to increase the purity of the medium, removing all the non-VFAs compounds. Finally, the caproic acid was separated from the other VFAs by adsorption on Powdered Activated Carbon (PAC) and concentrated by the following desorption step using a lower amount of eluent.

2. Materials and methods

2.1. Substrate and inoculum characterization

The OFI cladodes substrate was sourced from the company "Assoro Biometano s. r.l." in Assoro, Enna (Sicily, Italy). The inoculum used for the tests was from a mesophilic anaerobic digester treating agricultural residues and livestock effluents nearby Verona (Italy).

Table 1 shows the characterization of the substrate and the inoculum.

2.2. Batch tests for the optimization of the rheological features of the reaction medium

OFI cladodes were minced through a professional mixer to reduce the particle dimensions and increase the specific superficial area. The apparent viscosity of the minced OFI was measured by a Viscotech Hispania S.L.U "VR3000" viscometer. It was observed that minced OFI cladodes, used as substrate, were characterized by a high apparent viscosity, due to the interaction between the protein fraction and the polysaccharide content, which forms intermolecular bonds [11]. A high apparent viscosity can lead to mixing problems inside the bioreactor, increasing the energetic output needed for the system [25], reducing the process yield. Consequently, Bio-Methane Potential (BMP) batch tests were carried out at different water dilutions of the minced cladodes. The tested water concentrations in the minced OFI-medium were of 5, 13, 24, 51 and 64 %w/w, corresponding to TS contents of 6.79, 6.32, 5.39, 3.49, 2.54 % w/w, respectively. The BMP tests were performed accordingly to Angelidaki et al. (2009) [26] and Holliger et al. (2016) [27] protocols. The tests were performed in a 1 L sealed glass bottle with an Inoculum:Substrate VS ratio of 2, at 37 °C. The apparent viscosity was measured at the beginning and at the end of the BMP trials.

2.3. VFAs, caproic acid and biomethane optimization on semi-continuous systems

Semi-continuous tests at different HRT were tested to evaluate its influence on VFAs, caproic and biomethane productions, adopting the OFI-water mixture with the best biomethane production along batch

Table 1
Substrate and inoculum characterization.

	Substrate (OFI)	Inoculum (Digestate)
Total Solids (TS)	7.37 ± 0.26 % w/w	6.86 ± 0.37 % w/w
Volatile Solids (VS)	5.01 ± 0.20 % w/w	4.53 ± 0.19 % w/w
VS/TS	68.05 ± 0.38 %	66.02 ± 0.28 %
Chemical Oxygen Demand (COD)	573.64 ± 0.53 mgO_2/gTS	644.56 ± 12.03 mgO_2/gTS
Total Kjeldahl Nitrogen (TKN)	14.50 ± 0.39 mg N/gTS	6.97 ± 0.42 g N/L

tests.

The semi-continuous systems were inoculated using the same agricultural digestate used for the batch trials according to an Inoculum: Substrate VS ratio of 2:1. The semi-continuous systems were tested at different dilution rates (D), HRTs and Organic Load Rates (OLR), as listed in Table 2. Daily, a volumetric amount equal to the dilution factor of the reactor was removed and replaced with a mixture of minced OFI cladodes at 5 %w/w water dilution. The reactor pH was checked daily and corrected to 7 with 1 M NaOH. The tests were performed at laboratory-scale in 1 L glass bottles, with a working volume of 720 mL. The temperature was kept constant at 37 °C. The duration of each semi continuous test was about 3 HRT.

The performance of both biomethane and VFAs productions were evaluated by the corresponding substrates conversion yields, referred to the daily inlet COD amount (g_{CODin}) (Equations (1) and (2)):

$$Y_{CH_4} = \frac{mL_{CH_4}/d}{gCOD_{in}/d} \quad (1)$$

$$Y_{VFAs} = \frac{(gCOD_{VFAs}/d) * V_{out}}{gCOD_{in}/d} \quad (2)$$

The productivity was also determined by multiplying the specific production of biomethane and VFAs with the dilution rate (D) of the corresponding test.

Besides the specific yield and the productivity, the experimental data were used to interpolate the kinetic parameters of the process. Kinetic models are used in anaerobic processes to check the initial hypothesis and to predict the system performance. Consequently, they also represent a tool for the scale-up of the process. The modified Stover–Kincannon model is one of the most common models to evaluate the organic substance removal rate as a function of organic loading rate at steady state [28]. The model can be described as reported in Equation (3).

$$\frac{HRT}{S_0 - S_e} = \frac{k_b}{R_{max} - S_0} HRT + \frac{1}{R_{max}} \quad (3)$$

where:

S_0 and S_e are the VS concentration (g_{VS}/L) for the inlet and the outlet, respectively. R_{max} is the maximum substrate removal rate (g_{VS}/L d) and K_b is the saturation value constant (g_{VS}/L d).

According to this model a plot of the quotient $\frac{HRT}{(S_0 - S_e)}$ versus HRT should result in a straight line with slope equal to $\frac{k_b}{R_{max} - S_0}$ and intercept equal to $\frac{1}{R_{max}}$.

2.4. VFAs purification by sequential steps of pressure driven membranes

At the end of the fermentation (both acidogenic and methanogenic) and of the chain elongation processes, the reaction medium was rich in VFAs and caproic acid. But it contained also the most recalcitrant organic matter, which was not or partially converted, and the bacteria, which promoted the biological processes [21]. The reaction medium from the HRT with the highest VFAs and caproic acid yields was sent to a purification step. Membrane processes were adopted due to their physical nature, which do not require the addition of chemicals to facilitate the separation of the reaction medium into a solid fraction, also named retentate (RET), and in a liquid fraction that passes through the membrane, called permeate (PER).

The purification steps involved a first phase of centrifugation at 4000 rpm for 10 min to remove the bigger solid particles. Then, the supernatant was sent to different sequential pressure driven membranes for micro and ultrafiltration (0.45 μm, 300 kDa and 1 kDa). The RET obtained from each membrane was removed from the purification line, while the PER, rich in VFAs and caproic acid, was kept in the purification line. The 0.45 μm, 300 kDa and 1 kDa membranes were purchased by SANI membranes A/S (Denmark), which operated at 0.6 bar, 2 bar and 2.5 bar, respectively. For each filtration step the distribution of the inlet into the RET and PER streams was calculated. Moreover, the purity degree (PUR) for each stream was determined accordingly to the following equation:

$$\mathrm{PUR}(\%, \mathrm{w}/\mathrm{w}) = \frac{amount\ of\ VFAs\ and\ caproic\ acid\ (g)}{amount\ of\ the\ total\ COD\ which\ enters\ in\ the\ membrane\ (g)} \quad (4)$$

2.5. Recovery and concentration of caproic acid

The caproic acid was recovered through solid-matrix adsorption, using Powdered Activated Carbon (PAC) 7440-44-0, supplied by Merck. PAC chemical and physical properties were listed in Table 3. PAC was chosen for its higher affinity for hydrophobic compounds than hydrophilic ones [21]. Consequently, PAC are the best candidates to adsorb longer-chain and hydrophobic fatty acids, such as valeric and caproic acid, allowing their separation from smaller-chain FAs such as acetic of butyric acid.

The tests were made in 15 mL falcon vials, with a working volume of 10 mL of purified fatty acids from the 300 kDa PER, using different amounts of PAC: 0.10, 0.25, 0.50, and 0.75 g. The adsorption tests were performed at room temperature for 3 h; the vials were shaken in an orbital mixer for the entire adsorption process.

At the end of the adsorption step, the PAC was separated from the starting mixture through vacuum filtration. Then, the VFAs and caproic acid were desorbed from the PAC testing two different eluents: i) distilled water (1 M NaOH); ii) ethanol (1 M NaOH). The eluent volume for the desorption tests was 2 mL.

2.6. Analytical methods

For all the tests, the biogas production was analyzed through water displacement method. The biomethane concentration was obtained with a GeoTech® Biogas 5000 gas analyzer. The methane production and concentration are average values referred to the steady state period of the tests, usually the last two HRT. VFAs concentration were obtained with a ThermoFischer Dionex ICS-1100 ion chromatography system, reported as gCOD/L. Total and Volatile Solids, Chemical Oxygen Demand, and Total Kjeldahl Nitrogen determination were performed accordingly with standard methods [29]. Ethanol and lactate concentrations were determined by Megazyme kits [30,31].

3. Results and discussion

3.1. Batch tests

The aim of the first part of the experimental campaign was to evaluate the minimal minced OFI-water dilution able to reduce the system apparent viscosity in batch tests. The performances of the tests were

Table 2
Operational parameters tested during the semi-continuous trials.

D (d^{-1})	0.95	0.40	0.20	0.10	0.05	0.03	0.025
HRT (d)	1.05	2.5	5	10	20	30	40
OLR ($gVS*L^{-1}*d^{-1}$)	38.5	16.2	8.1	5.7	2.8	1.8	1.1

Table 3
PAC chemical and physical composition.

	PAC 7440-44-0
Chemical composition	Carbon
Particle size (mm)	0.001–0.150
Approx. Pore volume (cm^3/g)	0.65
Approx. Surface area (cm^2/g)	0.12

evaluated in terms of biogas production.

Minced OFI dilution was necessary due its high apparent viscosity. As reported in Fig. 1, the apparent viscosity of the undiluted minced OFI, which corresponded to a TS concentration of 7.37 %w/w, was of 932.94 ± 197.45 cP at 25 °C. These values are usually not suitable for stirred bioreactors. Even if the rheological performances of the system differ from case to case, depending on the substrates, the geometry of the reactor and of the mixing system, and the operational conditions of the reactor, previous studies showed a system inhibition caused by high apparent viscosity. As reported by Battista et al. (2016), focusing on AD biogas production from olive pomace, an apparent viscosity of 200–300 cP caused an inadequate mixing of a bioreactor using conventional impellers, for example Rushton or marine types. A further research work on enzymatic hydrolysis for fermentable sugars production from wheat straw, demonstrated that the reaction medium, having an apparent viscosity of about 450 cP, had bad rheology performances even with complex impellers, such as a double helicoidal impeller [24]. Moreover, it was observed that high apparent viscosity can cause solids sedimentation and layering of fibers in the superior part of the reaction medium [32]. This aspect prevents the biogas passage from the liquid phase towards the gaseous phase of the reactor headspace, inhibiting the fermentation processes [33,34].

As expected, it was challenging to mix undiluted minced OFI with the impellers adopted conventionally in AD plants, like Rushton and marine impellers.

Table 1 reported the percentage of added water on the minced OFI. It was observed that a dilution as small as 5 %, corresponding to a TS concentration at 6.79 %w/w, allowed to reduce the apparent viscosity down to 173.58 ± 23.33 cP (Fig. 1), under the critical range of 200–300 cP, able to guarantee an adequate mixing performance for OFI. By this way, it was possible to solve the mixing issue with very low water addition, which means with low environmental and economic impacts on the process. The apparent viscosity was also measured at the end of the AD process. It was observed that all the tests had a significant apparent viscosity reduction of the reaction medium, with values close to the water viscosity of 1 cP (Fig. 1). This can be attributed to the effect of the hydrolysis process. In this first AD process stage, microorganisms break down complex compounds like polysaccharides and cellulose, resulting in lower molecular weight compounds [35,36].

Regarding the biomethane production, the different dilutions seemed to not have a significant influence on the specific methane productivity: the biomethane specific production ranged from 180 to 220 mL/gVS for all the tests, with the maximum value observed in correspondence of the lowest dilution of 5 % w/w (Fig. 2). The dilution seemed to act on the kinetics of the tests. The batch test with the highest initial apparent viscosity required about 50 days to reach the final methane production value, while the other tests achieved the final

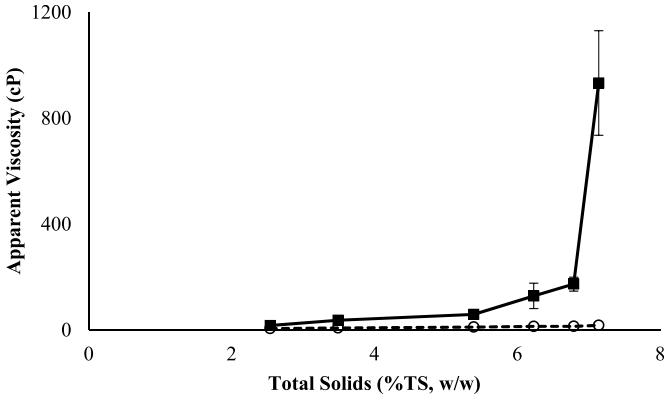

Fig. 1. Apparent viscosity of the substrate at various TS. Black squares, continuous line: viscosity at the start of the BMP test; empty circles, dashed line: viscosity at the end of BMP test.

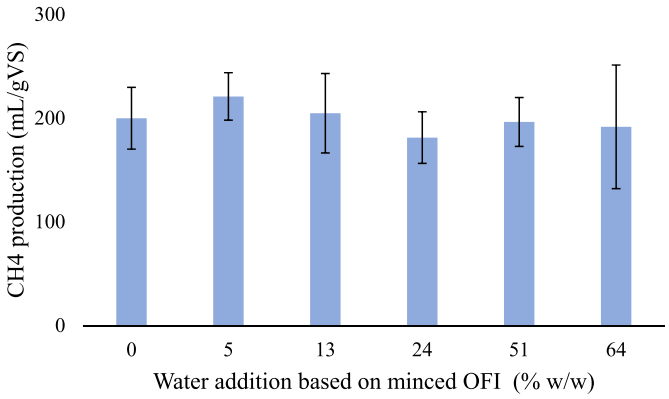

Fig. 2. Biomethane production at various water content.

methane amount in around 25–30 days. This demonstrates that the hydrolysis of the compounds is inhibited with high apparent viscosity. Probably it is due to a lower mass transfer in the reactor.

3.2. Semi-continuous tests

Considering the trends of the batch tests, the 5 % w/w water dilution of the minced OFI had apparent viscosity lower than 200 cP, which was able to assure an adequate reactor mixing with the conventional impellers used in the anaerobic digesters, as explained above. Moreover, the 5 % w/w water dilution led to the highest biomethane production among the batch tests (Fig. 2). For these reasons, this dilution was used for the semi-continuous tests at different HRT.

3.2.1. Discussion on the methane production trend

Table 4 shows the average production of methane, and VFAs and caproic acid concentrations of the semi-continuous tests, when steady state conditions were reached.

The biomethane production is null or negligible at lower HRTs, below 5 days, while it was obtained at higher HRTs (20–40 d). The specific methane productions for the tests at HRT 20, 30 and 40 days, reported in Table 4, were referred to the reaching of the steady state condition, which occurred about 30–40 days after the beginning of the tests. It is fundamental to emphasize that in the first 30–40 days the biomethane production was 15–25 % lower than the values reported in Table 4. This trend can probably be explained considering the heterogeneous chemical composition of the OFI, rich in carbohydrates easily degradable by methanogenic bacteria. At the same time, hemicellulose, and cellulose, the other two major components in OFI [35], are known to be recalcitrant, and their conversion required from 3 to 5 weeks [37]. Consequently, their contribution in the biomethane production started to be observed after about 30 days. With specific reference to biogas production, the best biomethane yield of 210 L/kgVS was achieved at HRT of 20 d. Higher HRT led to a slight decrease of the biomethane production, probably due to the lack of micro- and macro-nutrients which began after 45–50 days of the process, when the C:N ratio increased at around 76. This value was far too high compared to the optimal value of 15–30 [38]. The unbalanced C:N ratio was due to the

Table 4
Biomethane, VFAs and caproic acid production from the semi-continuous tests.

HRT (d)	CH_4 (L/kgVS)	VFAs (gCOD/L)	Caproic Acid (gCOD/L)
1.05	0.00 ± 0.00	3.82 ± 1.41	0.20 ± 0.06
2.50	0.00 ± 0.00	16.32 ± 2.35	0.50 ± 0.11
5.00	0.00 ± 0.00	26.09 ± 2.46	7.85 ± 1.02
10.00	1.86 ± 0.52	23.16 ± 1.07	1.55 ± 0.21
20.00	210.20 ± 20.73	12.68 ± 1.73	0.37 ± 0.04
30.00	201.59 ± 12.83	8.30 ± 0.66	0.00 ± 0.00
40.00	176.66 ± 17.52	4.47 ± 0.99	0.00 ± 0.00

feeding stream, constituted by a single substrate, the OFI cladodes which are rich in carbon content and low in nitrogen compounds (proteins). Nitrogen was essentially supplied just at the beginning of the test with the inoculum of the reactors [4]. These results emphasized that the mono-digestion of OFI is not sustainable. Therefore, in practical terms, co-digestion is an obligatory choice. A co-digestion of OFI with rich nitrogen substrates, like livestock effluents, will be evaluated in the future steps of this research. A recent work confirmed that co-digestion can improve biomethane production. Espinosa-Solares et al. [1] demonstrated that OFI and cow manure co-digestion can lead to a biomethane production 80 % higher than cow manure mono-digestion, obtaining the best result of 152.7 L/kg VS biomethane with 50:50 cow manure to OFI ratio. Cow manure mono-digestion only produced 79.5 L/kg VS of biomethane. As an alternative to co-digestion, pretreatments can help the degradation of the most recalcitrant OFI compounds in methane. Iris Saldovan Rojas et al. [39] tested different chemical, physical and thermal pretreatments on OFI. Demonstrating that a combination of mechanical, chemical, and thermal operations increased the methane production by 160 %, obtaining around 200 L/kg VS. This is an interesting result similar this study, which were achieved by a simple water dilution, without expensive and high environmental impacting pretreatments. Finally, very interesting is the work by Tarrisse et al. [40], which focused the attention to a new OFI feature. Their work showed a methane production in the range of 200–350 L/kgVS. This large range can be justified considering the tested OFI came from different Countries, which can have differences in the soil calcium content and, consequently in the cladodes. The authors emphasized the calcium action as it could be able to facilitate the precipitation of CO_2 bubbles as calcium carbonate, increasing the methane content in the biogas.

The biomethane productivity was also evaluated. Clearly it had a consistent trend with the specific biomethane production: its value was maximized at the HRT of 20 days, with about 10 $mLCH_4/gVS \times d$ (Fig. 2A), decreasing to 4.5–7 $mLCH_4/gVS \times d$ at HRT of 40 and 30 d, respectively. Having achieved the best biomethane yield, the mass balance was performed for this test. Regarding the calculation, it was considered the COD daily flow entering the system and the biomethane theoretical conversion of 350 $mLCH_4/gCOD$. This yield is referred to a biogas composed for 55–60 % v/v in methane and 40–45 % v/v in carbon dioxide. So, the same theoretical yield referred to biogas corresponds to about 580–600 mL/gCOD [41]. Consequently, a biomethane specific yield of 232.59 ± 41.53 $mLCH_4/gCOD_{in}$ was found. Fig. 3 reported the daily COD inlet in the system, the COD converted into biomethane and the COD which left the reactor in the effluent streams. The COD amount sum leaving the reactor fitted perfectly with the entering COD amount, validating the consistence of the experimental data and of the mass balance.

Besides the COD, is interesting to observe that the VS/TS ratio evolution before and after the AD at HRT 20 d. The minced OFI had a VS/TS ratio of about 70 % before entering the reactor. This value is low compared to other organic substrates, due to the high presence of inert and minerals in the OFI, especially calcium and magnesium [42]. At the end of the AD, the VS/TS ratio was about 45–50 %, demonstrating the degradation of only the most edible organic compounds. The most recalcitrant compounds were not or partially degraded. Besides the cellulose and the hemicellulose, OFI has also a good number of mucilaginous polysaccharides, high molecular weight heteropolysaccharides composed of up to 30,000 different sugars, which are not easily degradable [43].

3.2.2. Discussion on VFAs and caproic acid production trends

VFAs and caproic acid production showed a reverse trend than the biomethane yield, achieving the best concentrations at low HRTs, typically below 10 days. VFAs and caproic acid concentrations peaked at HRT 5 with 26.09 and 7.85 gCOD/L respectively. Regarding the VFAs productivity (Fig. 4B), the best value was at HRT 2.5 with around 6 gCOD/(Ld), closely followed by HRT 5 test, which had the best caproic acid productivity. In particular, the best VFAs yield of 79 % on influent COD was achieved by the HRT 5, followed by HRT 10 with 70 %. Focusing on the caproic acid production, the best yield was 30 %, obtained at HRT 5 too.

The overall results of both biomethane and VFAs yields are consistent with the acidogenic and methanogenic steps of the AD process. In fact, HRTs of 1–10 days leads to the wash out of methanogenic bacteria, which have higher reproduction time than the considered HRTs [44]. Instead, acidogenic bacteria have smaller reproduction time, ranging from few hours to 5 days and, for this reason, they were not washed out by low HRT. In particular, low HRT in combination with a high OLR represent the optimum condition for VFAs production [45].

The washout of the methanogenic bacteria at low HRT also allowed for the instauration of the reverse β-oxidation processes for the chain elongation of short VFAs. It was observed that the acetic acid appeared immediately, at the very beginning of the fermentation, while the butyric acid accumulation in the bioreactor started after 4–5 days. In the first 5 days since the beginning of the test, the total concentration of the VFAs was 13.7 g_{COD}/L; acetic acid represented the 84 % w/w of them, followed by propionic and butyric acids which accounted for the 11 % and 5 % w/w, respectively. Then, the appearance of valeric and caproic acid production after 7–10 days since the beginning of the tests was observed, demonstrating the occurring of the implementation of the reverse β-oxidation reactions. The process consists in the elongation of the VFAs chain as effect of the acetyl-CoA molecule, supplied by an electron donor compound, typically ethanol or lactic acid. These electron-donor compounds allow for the increasing of the VFAs by two carbons (C2) at a time. Consequently, acetic acid and propionic acids are converted into butyric and valeric acids in a first cycle elongation, respectively. Then butyric acid is further elongated into caproic acid along the second cycle [46,47]. Consequently, when the steady state of

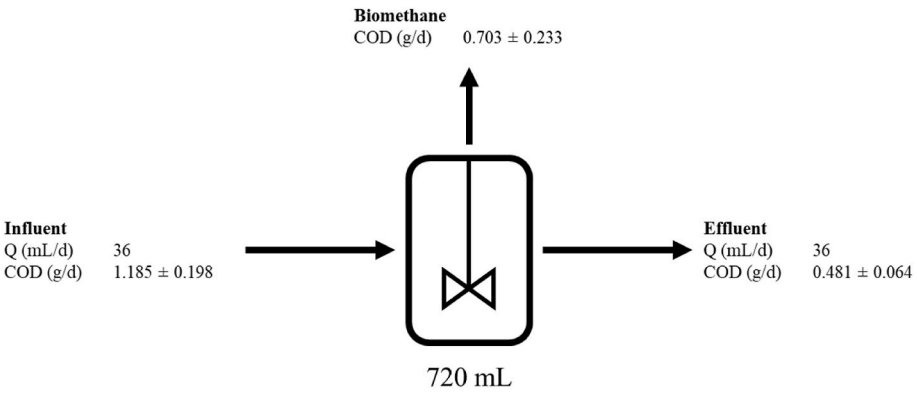

Fig. 3. Mass balance for HRT 20.

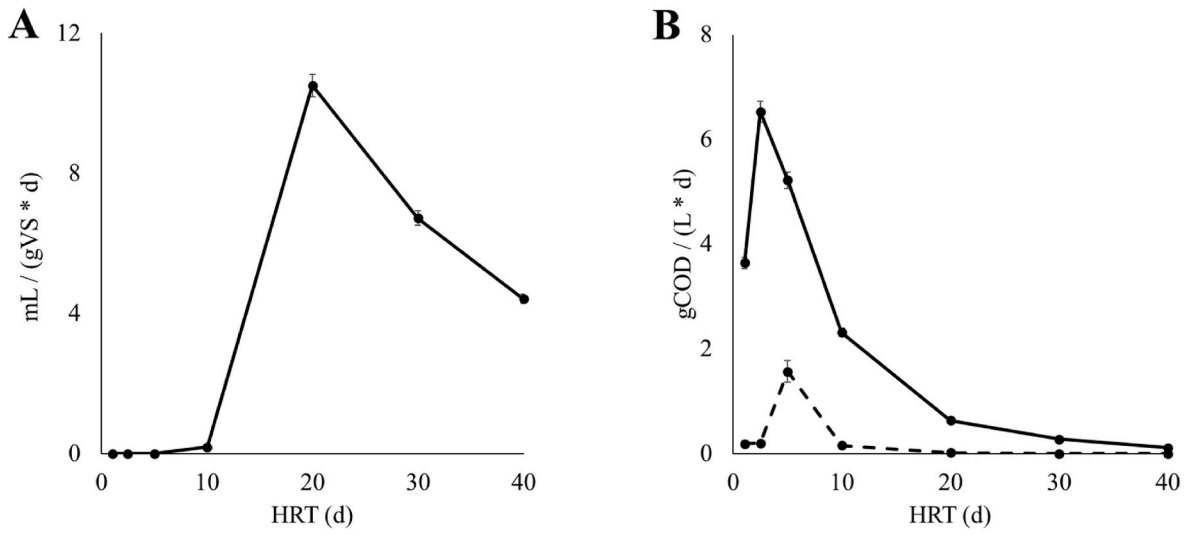

Fig. 4. A: Biomethane productivity. B: VFAs (continuous line) and Caproic Acid (dashed line) productivity.

the HRT 5 test was reached, the total concentration of the VFAs was of acids was 26.09 g_{COD}/L, as previously reported. Regarding the VFAs composition, acetic acid represented 41.5 % w/w, caproic acid 30.2 % w/w, butyric acid 15.6 % w/w, valeric acid 6.9 % w/w and propionic acid 5.8 % w/w of the total VFAs.

If the HRT 5 showed the best condition for caproic acid accumulation, the HRT 2.5 led to an accumulation of acetic and butyric acids (data not shown), meaning that longer HRT are necessary for the complete instauration of the chain elongation process with OFI [48].

Besides the VFAs presence, the reverse β-oxidation reactions require an electron donor compound. Olokede et al. (2022) [49] reported a decrease in ethanol and lactic acid concentrations, which can be justified by the instauration of the chain elongation process. Similarly, the HRT 5 test of the present research work showed the presence of one electron-donor compound, the lactic acid which accounted at about 67.5 g/L. Lactic acid presence can be easily explainable by the hydrolysis of the carbohydrates and cellulose contained in OFI into glucose, which was further converted into lactate through both different metabolic routes: the glycolysis, the bifidus and the 5 *P*-gluconate pathways [50]. Considering the acetic acid concentration of about 12 g/L, the molar ration of the acetate and lactate was 1: 3.8. The molar concentration between acetic acid and the electron donor compound (ethanol or lactate) is one of the fundamental parameters for the instauration of the reverse β-oxidation [47]. Different authors found the optimal molar ratio between the VFAs, and the electron donor compound is in the range 1:3–1:6 [51,52], which fits well with the one found along the HRT 5 test.

Even if there are few works on VFAs and caproic acid productions, the comparison with the scientific literature highlights the very promising results obtained by the present work. Medjekal et al. [53] performed batch acidogenic fermentation of OFI obtaining a VFAs yield of about 0.6 g/gTS. But, analyzing the VFAs profile, emerged that they were essentially due to acetic (71 %) and propionic (21 %) acids. Thus, the process was not optimized for chain elongation which led to longer and higher value fatty acids. Tenci et al. [54] adopted *Euphorbia tirucalli* as substrate for VFAs production in batch test. *E. tirucalli* is a CAM plant and has similar chemical characteristics to OFI. The VFAs productivity was 0.3–0.4 gVFAs/gCOD when *E. tirucalli* was fermented alone, while reached 0.65 gVFAs/gCOD in co-digestion with pig blood, characterized by high proteins content. After 16 days of fermentation, the VFAs profile was characterized by around 50 % w/w of acetic acid, followed 15–20 % w/w of butyric acid. Valeric and caproic acids were detected in lower concentrations (5–10 % w/w). It demonstrated that, even if co-digestion can increase VFAs yield, this work demonstrated that the simple mono-fermentation of OFI performed in semi-continuous mode at optimized HRT can lead to higher production yields of high added value compounds.

3.2.3. Determination of the kinetical parameters of the process

The experimental data were interpolated to find the kinetical parameters of the fermentation process according to the modified Stoker-Kicannon model (Fig. 5), whose equation was reported before (Equation (3)).

As shown in Fig. 5, the trend line, obtained from the experimental data, had a slope value of 0.0528 and an intercept of 0.054. From these values, the kinetic parameters were calculated as $K_b = 67.64$ g_{VS}/L d and $R_{max} = 32.15$ g_{VS}/L d, respectively.

The modified Stover-Kicannon model plot can be used to predict the theoretical effluent concentrations of the reaction medium ($S_{e\ theor}$) for the different HRT considering the following Equation (5), which can be easily obtained from Equation (3):

$$S_{e\ theor} = S_0 - \frac{Rmax\ S0}{kb + \left(\frac{S0}{HRT}\right)} \quad (5)$$

The deviation between the theoretical values and the experimental ones were in the range 5–15 % for all the HRT. These slow deviations demonstrated the suitability of the modified Stover–Kicannon model to predict the effluent concentration and ultimately the process performance.

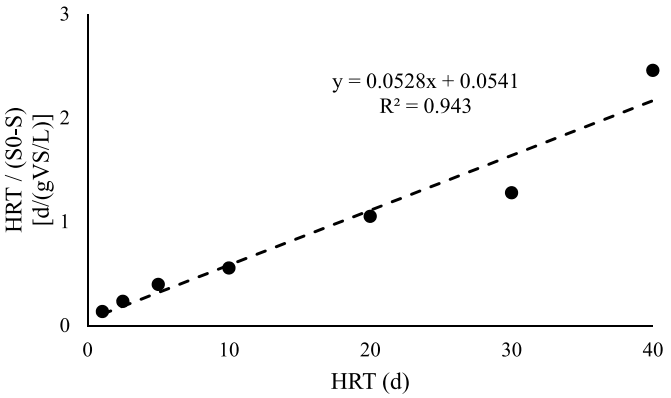

Fig. 5. Modified Stover–Kicannon model plot.

3.3. VFAs purification by sequential steps of pressure driven membranes

The reaction medium, daily discharged, was centrifuged according to the procedure reported in paragraph 2.3. The supernatant had a PUR degree of 86.11 % w/w (Fig. 6).

The first filtration step at 0.45 μm led to two streams, the RET, which accounted for the 20 % of the inlet supernatant, and the PER, whose represented the remaining 80 % w/w of the inlet supernatant. It is interesting to observe that the 0.45 μm filtration step was very effective in the increasing the purity degree of the supernatant: the PUR was of about 92.73 and 70.73 % w/w for the PER and the RET, respectively (Fig. 6). It means that no-VFAs organic compounds were largely retained by the 0.45 μm filter. A further filtration step (300 kDa) led to an improvement of the PUR which almost reached 96.72 % w/w for the PER. While the PUR was 90.84 % for the RET, slightly lower than the value from the supernatant PER coming from the previous filtration stage. It demonstrated that 300 kDa filter was able to retain the smaller non-VFAs organic matter. On the contrary, no significant differences in PUR parameters were observed in the PER and RET streams from the last filtration step (1 kDa), meaning that the highest possible purity degree were already achieved along the previous 300 kDa stage and the few remaining no-VFAs compounds have dimension similar to the VFAs.

Regarding the distribution of the VFAs between the PER and RET along the purification line, no significant difference was detected. The relative concentrations of the different acids remained almost constant, showing very close values to those reported in the previous paragraph. It demonstrated that micro and ultrafiltration were not able to perform a first separation between VFAs caproic acid. For this reason, a final step of adsorption on PAC, followed by desorption were performed.

3.4. Adsorption/desorption of caproic acids on/from PAC

An adsorption test was performed on the 300 kDa permeate. As mentioned above, the adsorption resin used was PAC, which has a higher affinity with hydrophobic compounds, such as caproic acid whose chemical features differ from shorter fatty acids (acetic, propionic, and butyric ones) [21,55]. The adsorption tests resulted in a complete adsorption of the caproic acid (Table 5), while lower chain and less hydrophobic VFAs had lower adsorption yields, as expected. Going in detail, caproic acid adsorption was higher with 0.5 g PAC (corresponding to 50 g PAC/L) ranging from 97.7 to 99.1 %, and lower for 0.25 and 0.75 gPAC/L, demonstrating that 0.5 was the ideal PAC amount.

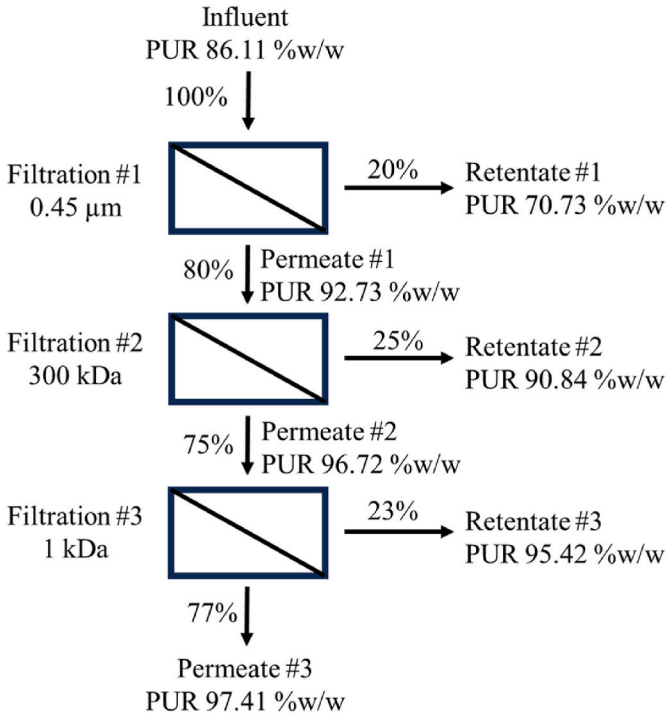

Fig. 6. The distribution and purity degree of the purification line streams.

Regarding the desorption, the overall VFAs desorption yield was 69.5 and 57.7 % for ethanol and water respectively. The very interesting fact was that desorption yield with ethanol was around 70–72 %, higher than desorption yield which consider all the VFAs, while it was very low when water was adopted as eluent. This attitude can be explained considering the caproic acid solubility, which is low in water (10.8 g/L), complete in ethanol [56]. Consequently, adsorption and desorption test in ethanol allowed to reach both a very high caproic acid purity degree and to concentrate it until 3.5 times the concentration in the 300 kDa PER, passing from about 7.5 g_{COD}/L to about 26 g_{COD}/L.

4. Conclusions

This work tried to express the full potential of the AD applied to OFI to produce different bioproducts through the simple modulation of the HRT. Moreover, sequential downstream processes were optimized to purify and concentrate the obtained compounds. Considering the high apparent viscosity of the minced OFI, preliminary batch tests were carried out to find the best water-OFI mixture. A 5 % w/w (based on minced OFI amount) of water addition was enough to decrease the apparent viscosity from about 1000 cP to about 200 cP, which was able to assure an adequate mixing of the bioreactor. This water dilution led to the highest biomethane production of 220 mL_{CH4}/gVS. Consequently the 5 % w/w of water addition was used for the implementation of the biorefinery model to produce VFAs, caproic acid and biomethane. The influence of different HRT was evaluated on the typologies of the produced compounds and the process yields.

It emerged that HRT modulation can effectively lead to different bioproducts and biofuels. The best result for biomethane was obtained at HRT 20, with a specific production of 210 mL_{CH4}/gVS and 232.59 $mL_{CH4}/gCOD_{in}$. While the VFAs and caproic acid accumulation reached the best yields at HRT 5, with concentrations of 26.09 and 7.85 g_{COD}/L of VFAs and caproic acid, corresponding to a COD conversion yield of 79 and 30 % w/w, respectively.

The work also investigated the downstream processes to purify and concentrate the VFAs and caproic acid from the semi continuous test at HRT 5. Firstly, pressure-driven membrane filtration was implemented to remove all non-VFAs compound. The process allowed to pass from a purity degree of 86.11 %w/w to 97.41 %w/w of the 300 kDa permeate. Finally, the purified 300 kDa permeate was sent to adsorption process on PAC to separate caproic acid from the other VFAs. It emerged that caproic acid had a very high affinity with PAC, demonstrating that this solid matrix can be used to separate it from the other VFAs. The following desorption test showed a good yield with ethanol (1 NaOH), while water had very poor performance because of the low solubility of caproic acid in it.

Table 5
Adsorption and desorption yields at different eluent and PAC quantity, regarding caproic acid and all the VFAs.

PAC (g)	Adsorption yield (% w/w)		Desorption Eluent	Desorption yield (% w/w)	
	Caproic acid	Global VFAs		Caproic Acid	Global VFAs
0.25	90.8	20.6	H_2O	26.5	66.9
0.50	97.7	25.9	(1 M NaOH)	3.3	60.3
0.75	96.3	33.8		0.76	60.1
0.25	94.7	33.2	Ethanol	72.9	75.8
0.50	99.1	46.0	(1 M NaOH)	70.2	58.9
0.75	96.6	42.4		70.9	76.9

Authors' contribution to the paper

Fabio Rizzioli: management and coordination of the tests, redaction of the manuscript; Claudia Magonara: performing of the test of fermentation of OFI; Gianmarco Mengoli: performing of the test on purification and concentration of the fermentate. David Bolzonella: Supervision of the research work and revision of the manuscript; Federico Battista: conceptualization, supervision of the research work and revision of the manuscript.

Declaration of competing interest

The authors declare that have not conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work.

Data availability

No data was used for the research described in the article.

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