Membrane bioreactor assisted volatile fatty acids production from agro-industrial residues for ruminant feed application

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ABSTRACT

Volatile fatty acids (VFAs) supplementation in ruminants’ diet as a source of energy and chemical precursors and their effect on animal’s physiology and well-being has long been of scientific interest. Production of VFAs through anaerobic digestion of agro-industrial residues not only creates value but also presents an alternative sustainable approach for ruminant feed supplementation. Therefore, this study aimed to investigate the bioconversion of agro-industrial residues produced in large quantities such as apple pomace (AP), thin stillage (Ts), and potato protein liquor (PPL) to VFAs, fully complying to regulations set for ruminant feed supplement production. In this regard, batch acidogenic fermentation assays (pH 6-10) and semi-continuous immersed membrane bioreactor (iMBR) were applied. In batch assays, at pH 10 the co-digestion of Ts and PPL produced the highest VFAs concentration (14.2 g/L), indicating a yield of 0.85 g COD_{VFA}/g volatile solids (VS)added. The optimum batch condition was then applied in the iMBR for in situ fermentation and recovery of VFAs at different organic loading rates (OLR). With increasing the OLR to 3.7 gVS/L.day, the highest VFAs concentration of 28.6 g/L (1.2 g COD_{VFA}/gVSadded) was achieved. Successful long-term (114 days) membrane filtration was conducted in a media with a maximum of 40 g/L of total solids (TS), facing irreversible membrane fouling in the final stages. Acidogenic fermentation using an iMBR has the potential to play an important role in the future of feed additive provision through the biorefining of agro-industrial wastes via the carboxylate platform, given the role of VFAs production from organic residues.

1. Introduction

Volatile fatty acids (VFAs) are produced naturally in ruminants’ gastrointestinal tract through anaerobic digestion by rumen microorganisms and are used by animals as the primary energy source. Studies on VFAs started as early as 70 years ago and are still ongoing as a hot topic. Mahboubi et al. (2022) have presented an extensive review on the ruminal fermentation of VFAs and role of VFAs on ruminant well-being, metabolism, and production. VFAs can be used as feed supplements to boost feed efficiency and animal performance. Considering that the cost of feed production in animal husbandry accounts for 40% to 60% of the total expenditure (Neethirajan, 2020), the production of VFAs for ruminant feed supplementation from low-value organic residues can present a sustainable, highly resource-efficient, circular approach in ruminant feed production. One of the sustainable ways to produce feed-grade VFAs is through anaerobic digestion (AD) of residuals that are of low or negative value.

As the world’s population grows, so does the concern over food provision to meet increasing demands. The global population is projected to reach 9.6 billion in 2050, and correlated global food demand is anticipated to increase by 70% to 100% (Tripathi et al., 2019). The increase in food production will further propel the need for animal-based proteins as an essential part of the human diet (Godfray et al., 2010). Satisfying this increasing demand requires 30–60% growth in global crop production for food and feed purposes, leading to unfavorable environmental consequences (Popp et al., 2017). The agro-food industries generate tremendous quantities of biodegradable solid or liquid side streams containing processed raw materials’ organic residues. According to a study by Baiano (2014), it is assessed that around 26% of food wastes are generated by the drinks industry, where the dairy industry (21%), fruit/vegetable manufacturing and processing (14.8%), cereal processing and production (12.9%), and meat product processing...
and preservation (8%) are next in food waste generating ranking.

The immediate disposal of agro-industrial wastes from food processing plants as residues to the environment causes a massive loss of nutrients. Food processing industries such as juice, starch, and bioethanol production can generate a considerable amount of biological by-products that could be bio-converted and valorized into various metabolites (Parmar and Rupasinghe, 2013). Among these industries that produce large volumes of locally concentrated organic waste are apple juicing, bioethanol production, and potato starch production industries. Every year, about 54.2 million tons of apples are produced globally, of which 26% is used in the apple processing industry to obtain juice, jelly, or cider, where around 25% of the apple mass is discarded as apple pomace (AP) (Wang and Thomas, 1989). Nowadays, a fifth of yielded AP is utilized directly in animal feed preparation, and the rest is either incinerated or composted (Ohiillon et al., 2013). Thin stillage (Ts) is a by-product of the starch-based bioethanol production industry obtained after the fermentation broth is distilled and particulate matter is removed from the stillage (Larson et al., 1993). The nutritional content of thin stillage varies based on the type of cereal used in the process. Ojowi et al. (1996) demonstrated that wheat-based thin stillage included 96 g/kg ether extract, 485 g/kg crude protein, 345 g/kg neutral detergent fiber, and 34 g/kg acid detergent fiber. In the European community, 1.6 million tons of potato starch is produced per annum (Souza Filho et al., 2017). Approximately 0.75 tons of pulp and 6 tons of potato liquor are released per ton of refined starch yielded. The protein content of potato liquor is recovered and the remaining is concentrated to potato protein liquor (PPL) (Schügerl, 1994). PPL could be spread over farmland, although it may contaminate the groundwater and renders a lousy odor due to its high biological oxygen demand (BOD) (Klingspohn et al., 1993). The most discursive application for fruit and vegetable residues is as an animal feed ingredient. However, the contribution amount to diet may be limited or promoted by the quantity and quality of protein and fiber content (Ulloa et al., 2004).

One of the promising bio-refinery techniques widely used for treating such agro-industrial by-products is anaerobic digestion (AD). AD is a bioconversion process where with the help of microbiota of microorganisms, organic matter is broken down and converted to the famous final products of biogas (mainly methane and carbon dioxide), an energy carrier, and digestate, commonly used as fertilizer. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis form the four main stages of AD (Gujer and Zehnder, 1983). Volatile fatty acids are intermediate products produced in acidogenesis and acetogenesis stages of AD with applications in biological denitrification in wastewater treatment, biodiesel production (Parchami et al., 2020), Polyhydroxyalkanoate (PHA) production for biodegradable bioplastics production (Gottardo et al., 2022; Kumar et al., 2019), and used as a carbon source for single-cell protein production (Wainaina et al., 2020). Continuous production of volatile fatty acids (VFAs) in an acidogenic fermenter is unsustainable due to the tendency of undissociated VFAs to penetrate the cell membrane and disrupt microbial processes during fermentation. Additionally, accumulation of VFAs can lower the local pH, suppress intracellular enzyme activity, and ultimately hinder hydrolytic and acidogenic processes. Therefore, it is necessary to extract VFAs ex-situ or in-situ (inline) from the fermentation liquid to improve overall fermentation performance and achieve a higher total VFA yield (Yesil et al., 2021; Yesil et al., 2014). In recent years, in-situ extraction of VFAs from the digesters using membrane technology has gained excellent research attention in AD as an alternative to AD assisted with methanogen production inhibition, accumulation of VFAs, decoupling hydraulic retention from solid retention (preventing cell washout), and enhancing yield and productivity (Parchami et al., 2020).

In case the VFAs generated from agro-industrial residues through AD are to be used as ruminant feed supplement, strict rules and regulations on animal feed production regarding the selection of substrates, microorganisms, and processes are to be applied. The choice of microorganisms in processed feed additives is critical and heavily regulated. A possible choice in this regard could be the application of the inherent microorganisms from a ruminant body, such as rumen fluid, that is, by nature, active in the bioconversion of feedstuff to VFAs and methane. The selection of substrates, however, can be based on regional accessibility and regulations governing the use of agri-food sidestreams in ruminant feed. For instance, agricultural sidestreams that contain animal byproducts like dairy products were excluded from consideration for feed provision. If all mentioned regulations are complied with, such an innovative approach can veer prevalent linear exploitation of resources in ruminant feed provision to a circular bio-economy concept, closing the loop on nutrient utilization, enhancing resource efficiency (Deselnicu et al., 2018).

The current study was conducted to determine the optimum conditions for producing VFAs using three agricultural by-products (AP, Ts, and PPL) and rumen fluid (RF) as inoculum. Furthermore, a semi-continuous long-term VFA fermentation and in situ recovery from AP plus PPL were developed. Moreover, different OLRs were evaluated for achieving high VFAs yield and productivity while maintaining functional long-term filtration capabilities. The final product needs to be characterized based on the concentration and distribution of VFAs to assess its suitability as a feed supplement for animals in compliance with the EU regulations.

In order to seek a novel approach to the identification of alternative feed ingredients for sustainable ruminant feed complying to EU regulations for feed provision supplementation through a meticulous selection of processes and substrates, taking into account their ultimate objectives, the current study was conducted to determine the optimum conditions for producing VFAs using three agricultural by-products (AP, Ts, and PPL) and rumen fluid (RF) as inoculum. Furthermore, a semi-continuous long-term VFA fermentation and in situ recovery from AP plus PPL were developed. In this regard, incorporation of membranes for the separation of VFAs mixtures was introduced and the effect of various factors such as HRT, and OLR in long-term filtration and fermentation on VFAs productivity and yield were investigated.

2. Materials and methods

2.1. Substrates and inoculum

Apple pomace (AP), potato protein liquor (PPL), and thin stillage (Ts) were the three substrates used in this experiment. AP was provided by the Herringjunga cider AB (Herringjunga, Sweden). PPL was provided by Lyckebys (Kristianstad, Sweden). Ts was provided from Lantmannen Agroetanol (Norrköping, Sweden). Rumen fluid was kindly provided from fistulated cows by the Swedish University of Agricultural Sciences (SLU) (Lövsta, Sweden). The procedure was conducted according to the ethical approval held by SLU for gathering the rumen fluid samples. The characteristics of the substrates are presented in Table 2.

The rumen fluid was transferred and kept at 39 °C in an anaerobic condition by sparging CO2. Since there were some undegraded feed residues in the fluid, a four-time folded cheesecloth was used to filter the fluid. The as-received AP contained lignocellulosic residuals such as calyx and seed, making it heterogeneous. In order to homogenize AP, a mechanical blending (Waring CB15, CT, USA) was employed for 2 min blending, followed by sieving through a standard steel kitchen sieve with a mesh size of 1 mm to remove undesired solids. Thin stillage and potato protein liquor were received as a slurry and contained little or no visible solid particles. Table 2 presents the composition of the inoculum and feedstock used in this study. In order to deactivate methanogens and hinder methane production, inoculum and substrates were heat shocked. For the heat shock process, the inoculum and substrates were each poured into Erlenmeyer flasks of 250 ml volume and were incubated at 80 °C in a shaking water bath (LSB12, Grant, Cambridgeshire, UK) for 15 min at 100 rpm, followed by cooling in an ice chamber.
2.2. Batch fermentation

Two different batch assays were conducted based on the combination of rumen fluid and inoculum. The amount of substrate and inoculum mixed was based on the volatile solid (VS) content at the ratio of 1:1 (inoculum: substrate). The experiments were conducted in 120 ml serum bottles with a working volume of 80 ml. Distilled water was added to make up the final working volume. Each trial included bottles with as-mixed pH, pH 6, and pH 10, with each condition being triplicated. HCl and NaOH (1 M) solutions were used for initial pH adjustment. The bottles were sealed by aluminum crimp seal with rubber stoppers and flushed with nitrogen gas for two minutes to provide the anaerobic condition. Reactor bottles were incubated in a water bath at 37 °C and 100 rpm during fermentation. To analyze biogas composition and volume, 250 μl of biogas was collected by a gas-tight syringe (VICI, Precision Sampling Inc., USA). As a second step, 1 ml of fermentation liquid was taken using a syringe for VFAs distribution and concentration analysis, and the process lasted 21 days. The 21-day time frame was likely chosen to allow enough time for the acidogenesis stage to complete. During anaerobic digestion, the production of volatile fatty acids tends to increase in the first few days and then stabilize later on. Therefore, a fermentation period of 21 days should provide ample time for the acidogenesis stage to finish and for the volatile fatty acids to reach a steady state (Banerjee et al., 2022). Finally, the pH of the fermentation liquid was measured. The Table 1 shows the experimental setup of batch assays based on the substrate and inoculum mixture used.

2.3. Immersed membrane bioreactor (iMBR) setup and operation

Based on the results obtained from the batch fermentation stage, apple pomace and potato protein liquor co-digestion were selected for feeding the semi-continuous membrane bioreactor. In order to fully comply with EU directives and regulations set by the Swedish Board of Agriculture, rumen fluid was selected as the intended inoculum (UNION, 2003). The preparation process and the treatment for methanogenesis inhibition were the same as the batch fermentation stage. The reactor was equipped with a peristaltic permeate pump, a temperature and pH probe, a PLC unit, a pressure sensor, a flow meter, and a submerged flat sheet membrane panel with an inbuilt gas sparger. The immersed MBR was a 4-liter bioreactor with a 3.5-liter working volume. The iMBR was fed with apple pomace and potato protein liquor at a 1:1 g VS ratio, inoculated with 300 ml of rumen fluid, and brought to working volume using water. This research work was conducted using an Integrated Permeate Panel (IPF) flat sheet membrane designed and kindly supplied by the Flemish Institute for Technological Research (VITO NV) (Mol, Belgium). The membrane panel was formed of two layers of hydrophilic polyethersulfone (PES) coated on either side of a polyester frame was likely chosen to allow enough time for the acidogenesis stage to complete. During anaerobic digestion, the production of volatile fatty acids tends to increase in the first few days and then stabilize later on. Therefore, a fermentation period of 21 days should provide ample time for the acidogenesis stage to finish and for the volatile fatty acids to reach a steady state (Banerjee et al., 2022). Finally, the pH of the fermentation liquid was measured. The Table 1 shows the experimental setup of batch assays based on the substrate and inoculum mixture used.

Table 2: Substrates and inoculum characterization.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apple pomace (AP)</th>
<th>Thin stillage (Ts)</th>
<th>Potato protein liquor (PPL)</th>
<th>Rumen fluid (RF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (g/L)</td>
<td>171</td>
<td>106.6</td>
<td>382.2</td>
<td>23.9</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>168.5</td>
<td>97.4</td>
<td>252</td>
<td>13.5</td>
</tr>
<tr>
<td>VS/Ts (g/kg)</td>
<td>985</td>
<td>913</td>
<td>658</td>
<td>564</td>
</tr>
<tr>
<td>TSS (g/L)</td>
<td>87.6</td>
<td>102.9</td>
<td>371</td>
<td>16.1</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>84.4</td>
<td>91</td>
<td>271</td>
<td>12.3</td>
</tr>
<tr>
<td>VSS/TSS (g/kg)</td>
<td>964</td>
<td>884</td>
<td>731</td>
<td>763</td>
</tr>
<tr>
<td>COD (g/L)</td>
<td>29</td>
<td>82</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td>SCOD (g/L)</td>
<td>13</td>
<td>14</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>NH4-N (mg/L)</td>
<td>8</td>
<td>40</td>
<td>530</td>
<td>240</td>
</tr>
<tr>
<td>PO4 (mg/L)</td>
<td>70</td>
<td>90</td>
<td>350.4</td>
<td>147.75</td>
</tr>
<tr>
<td>TKN (g/kg)</td>
<td>12.44</td>
<td>6.99</td>
<td>18.75</td>
<td>NA</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>77.8</td>
<td>43.7</td>
<td>117</td>
<td>NA</td>
</tr>
</tbody>
</table>

As a part of the startup process (days 1–4), the iMBR was carried out in batch mode to assist with the acclimatization of the microbial community to the substrate fed. The iMBR was switched to semi-continuous operation mode after the startup phase. Feeding started at an organic loading rate (OLR) of 1 g VS/L/day and was later increased to 2 and 3.7 gVS/L/day. The initial pH was set at 6.5 same as the pH of AP and PPL co-digestion in batch trials. There was a daily pH adjustment to 6.5 after daily feeding. The hydraulic retention time (HRT) was 10 days; based on that, 350 ml of effluent was filtered out for VFAs separation, followed by adding the same volume of thermally treated feed. In the filtration process, the forward and backward cycle included a 4 min filtration pursued by a 1 min backwash. Each day before filtration, effluent samples were collected from the main reactor media to analyze total suspended solids (TSS), viscosity, pH, ammonium nitrogen, COD, and VFAs. In order to maintain the TSS content and viscosity of the reactor at high OLR (3.7 g VS/L/day), the daily feeding routine was shifted to alternate, including one-day feeding and one-day starvation starting on day 58 followed by shifting up to one-day feeding and two-days starvation on day 85.

2.4. Analytical methods

Total solid (TS), volatile solid (VS), TSS, and total Kjeldahl nitrogen (TKN) contents were analyzed using the standard method (WEF, 2005), and to determine the protein content, a factor of 6.25 was used (Wainaina et al., 2020b). Total and soluble chemical oxygen demand (TCOD, and SCOD) were determined using CSB 15,000 test kit and ammonium nitrogen (NH4-N) was measured using the Ammonium 100 test kit (Nanocolor, MACHEREY-NAGEL GmbH & Co. KG, Germany). The Nanocolor 500D Photometer was utilized for measuring the concentrations of SCOD and NH4-N. (MACHEREY-NAGEL GmbH & Co. KG, Germany). The volume and composition of CH4, H2, and CO2 (biogas) were analyzed by gas chromatography (GC) (Clarus 590; Perkin-Elmer, Norwalk, CT, USA) equipped with a packed column (CarboxenTM 1000, 6 × 1.8 OD, 60/80 mesh, Supelco, Shelton, CT, USA) and a thermal conductivity detector (TCD). The injection temperature for the GC-TCD was set to 200 °C, and the carrier gas was nitrogen gas at a flow rate of 30 ml/min at 75 °C. Throughout the anaerobic digestion process, 0.25 ml of gas samples were taken daily using a gas syringe (VICI, Precision Sampling Inc., USA). The VFAs were measured using a gas chromatograph (Clarus 590; Perkin-Elmer, Norwalk, CT, USA) assisted by a capillary column (Elite-WAX ETR, 30 m 0.32 mm 1.00 m, Perkin-Elmer, Shelton, CT, USA)
and a flame ionized detector (Perkin-Elmer, Shelton, CT, USA) (FID). The injection and detection temperatures for the GC-FID condition were 250 °C and 300 °C, respectively. The carrier gas was nitrogen with a flow rate of 2 ml/min and a pressure of 20 psi. Prior to VFAs analysis, 500 µl liquid samples were mixed with 100 µl acid mix (25 percent (v/v) formic acid and 25 percent (v/v) ortho-phosphoric acid at a ratio of 1:3) and centrifuged at 10000 × g for 5 min. The supernatant of the experiment was filtered through a 0.2 µm syringe filter to remove undissolved particles. To determine VFAs concentration and each acid distribution, 250 µl of the supernatant is mixed with 250 µl of the 1 g butanol/L standard solution and 500 µl milli-Q water and analyzed with the GC. As an internal standard, butanol at 1 g/L is used. It should be noted that the sum of isovalerate and 2-methylbutyrate is presented as isovalerate.

The following equation calculated the yield of VFAs during iMBR fermentation:

\[ \text{VFAs yield} = \frac{(C_{n-1} \cdot V_r) - (Y \cdot V_R) - (C_f \cdot V_f)}{\text{OLR} \cdot V_R} \] (1)

Where: \( C_{n-1} \) is the VFAs concentration of the previous day (g/L), and \( V_r \) is the permeate volume of 0.35 (L). \( Y \) is the slope of increasing the VFAs concentration each day of different OLRs. \( C_f \) is the feed’s VFAs concentration, which was 0.6 (g/L), and \( V_f \) is the feed volume in different OLRs (L). \( V_R \) is the reactor’s working volume (L).

2.5. Statistical analysis

All experiments and analyses were done in triplicate. MINITAB® 21 (Minitab Ltd., Coventry, UK) was used for the statistical analysis of the data with the one-way ANOVA (analysis of variance) and a confidence interval of 95%. Pairwise comparisons were carried out according to Tukey’s test.

3. Results and discussion

In the current study, the fermentation of three substrates and rumen fluid was investigated in batch assays for producing VFAs. In this regard, different ratios of the substrate to inoculum, co-digestion of substrates, and different pH were studied to find an optimum condition. The AD was then continued in a membrane bioreactor based on the optimum condition obtained from the batch fermentation for semi-continuous VFAs production and in situ recovery from the medium at different OLRs.

3.1. VFAs production in batch fermentation

The maximum VFAs production of 11.2 g/L was obtained from the condition benefiting from thermally treated PPL and no pH control, followed by the production of 10.8 g/L by the co-digestion of Ts and PPL (Fig. 1). This recorded increase to the maximum VFAs generated occurred in the time span of day 0 to day 11. This trend was the same for the co-digestion of AP and PPL, as they produced the same amount of VFAs (10.7 g/L) and the same production rate. When the co-digestion of AP, PPL, and Ts has conducted, a sharp jump in VFAs production to 4 g/L was observed in the first 2 days of fermentation. Regarding gas generation, most conditions using AP as a substrate had high H\(_2\) production while generally having lower VFAs yields (Figs. 1 and 2) compared to conditions with Ts and PPL. Fig. 2(a) shows that AP degradation increased acetic acid concentration. The generation of acetic acid is accompanied by hydrogen generation (1 mol H\(_2\) per mol acetic acid) (Owens and Basalan, 2016). Therefore, it is expected that the substrate with the highest acetic acid production also experiences the highest hydrogen accumulation (Matsumoto and Nishimura, 2007). The highest VFAs yield was achieved in the digestion of AP + Ts + PPL at 0.44 g/gVS added for all conditions, followed by the digestion of PPL at 0.42 g/gVS added, and the digestion of AP + PPL at 0.42 g/gVS added on day 21. It is a common trend in all conditions, without pH control, to have higher percentages of acetic acid, butyric acid, and propionic acid in the VFAs distribution (Fig. 1(b)).

Considering fermentation batches having an initial pH of 6, the digestion of PPL and co-digestion of PPL and Ts produced the highest amount of VFAs 12.1 g/L and 11.4 g/L, respectively (Fig. 1(c)).

![Fig. 1. VFAs distribution and yield at pH as-mixed (a,h), 6 (c,d) and 10 (e,f). *The sum of isovalerate and 2-methylbutyrate is presented as isovalerate.](image-url)
Fig. 2. Biogas formation at different initial pH (as mixed (a), 6 (b) and 10 (c)).
shows that in both as-mixed pH and pH 6, a similar effect on VFAs production is observed. However, the maximum VFAs yield was obtained through the co-digestion of Ts and PPL. The VFAs yield in the co-digestion of AP, Ts, and PPL at pH 6 (0.39 g/gVS) was lower than the same co-digestion at as-mixed pH. Applying an initial pH of 10 increased the amount of VFAs produced by the fermentation of PPL and co-digestion of PPL with Ts to 13.16 g/L and 14.2 g/L, respectively (Fig. 1(e)). The anaerobic digestion of Ts and PPL achieved the highest yield of 0.64 g/gVS added on day 11, followed by the VFAs yield from co-digestion of AP, Ts, and PPL at 0.60 g/gVS added in the same time interval.

In the anaerobic digestion of organic residues, literature shows that higher VFA production levels have been obtained under acidic conditions between pH 5.0 and 6.0 (Kim et al., 2004a). Although most of these studies focused only on pH values below 7.0 (Orozco et al., 2013), others have reported high VFA production at pH 9 and above (Babel et al., 2004; Zhang et al., 2016). pH did not significantly define the dominant VFA during batch assays, as acetate was, by far, the most dominant acid at different pH. However, the distribution of VFAs was changed by alterations of pH. A general increase in the pH resulted in the decrease of butyric acid and a slight increase in propionic acid content. At pH 6, the range of butyric acid content was 26.1% to 36.3%. While, at pH 10, the range of butyric acid content decreased to 10.5% to 23.9%. The increase in pH may result in thermodynamic constraints that prevent the reduction of protons to H2, causing a redirection of reducing equivalents towards propionate production. The dominant microbial community might also be the reason for the alteration in VFAs distribution. LV et al. (2022) found that three bacterial phyla Firmicutes, Bacteroidetes, and Proteobacteria, participating in hydrolysis and acidification, widely existed in AD system at pH 6. Generally, members of Firmicutes produced butyrate, while members of Proteobacteria produced acetate.

Regardless of the pH, the substrate’s characteristics and co-digestion are influential factors affecting VFAs yield and distribution. In this study, PPL contained the highest amount of ammonium nitrogen (530 mg/L), while TS had the highest amount of COD among all substrates (8200 mg/L) (Table 2). However, the VFAs yield obtained from Ts was relatively lower compared to PPL and AP due to its higher COD content. The co-digestion of AP and PPL in different conditions improved the VFAs yield, as the COD content ranged from 3000 to 4500 mg/L. The conversion of initial COD into organic acids is a significant factor in defining the VFA production. Several parameters affect the optimal range of COD for VFAs fermentation, including substrate type, substrate characterization, and mixtures of different substrate. Substrates exhibiting COD levels ranging from 9,000 mg/L to 160,000 mg/L are highly conducive to VFAs production due to their favorable characteristics (Elsheshy et al., 2011; Jiang et al., 2007). There is still some confusion over which kind of waste or organic waste blend is more suitable for VFA production. However, based on previous studies, a preliminary guide for organic waste selection is available. Waste with COD above 3000 mg/L is commonly used for VFAs production (Lee et al., 2014). It is also important to maintain a proper pH level during the digestion process. The presence of high COD levels in anaerobic digestion process could lead to the formation of long chain VFAs and subsequent operational problems (Lee et al., 2015). Fermentation of AP led to the highest percentage of butyric acid and propionic acid in the VFAs mixture. Digestion of AP without any initial pH regulation produced the highest amount of butyric acid. Also, the co-digestion of AP with other substrates had the same effect, being more significant in as-mixed pH than in pH 7. As-mixed substrates play a significant role in determining the composition of VFAs. A carbohydrate-rich waste stream supports propionic and butyric acid synthesis, whereas a protein-rich waste stream supports valeric and isovaleric acid synthesis (Garcia-Aguirre et al., 2017). In the animal feed industry, apple pomace is considered a feedstuff low in protein and high in carbohydrates (Hang, 1988). Apple pomace contains many nutrients, including, insoluble carbohydrates (cellulose, hemicellulose, and lignin), reducing sugars (glucose, fructose), and high levels of minerals and vitamins. The supplement does, however, contain 1–2% protein and a negligible amount of essential amino acids.

On the other hand, potato protein liquor has a greater amount of raw protein and amino acids (21–27%) (van Koningsveld, 2001) and far less reducing sugar (4–7%) than apple pomace (Zhang et al., 2021). Therefore, co-digestion of AP and PPL produced a more balanced VFA effluent, which is more adaptable to the naturally occurring ruminal VFA ratios. Acetate is the most dominant VFA in the rumen and is highly produced from feed containing more forage (Bergman, 1990). If the supplements contain more butyrate and propionate, the VFAs produced by forage will be fortified, and distribution will be improved. Optimizing the fermentation process to achieve varying proportions of VFAs holds promise in tailoring specific VFA compositions for diverse ruminant species, ultimately enhancing their performance outcomes.

### 3.2. Gas production in batch fermentation

The results of the gas composition and volume generated during the batch process are presented in Fig. 2. The co-digestion of thin stillage, apple pomace, and potato protein liquor using rumen fluid as inoculum produced the highest amount of H2 (36.30 ml/gVS) in pH 6.2 by day 3. This amount was kept constant until the end of the trial (day 21) (Fig. 2(a)). The second largest H2 volume (34.3 ml/gVS) was produced by co-digestion of AP and Ts, followed by fermentation of AP on days 3 and 5, respectively. One of the major parameters that could have affected hydrogen production is pH (Li et al., 2008). Bacterial communities that make transitions in fermentation pathways are affected by pH. Butyric acid and acetic acid production pathways could produce 4 and 2 mol of hydrogen per mole of acid, respectively, thriving in pH ranges between 6 and 7 (Kawagoshi et al., 2005).

The biohydrogen production in co-digestion of AP and Ts at pH 6 was 41 ml, which was higher than the maximum hydrogen production with the as-mixed pH (Fig. 2(b) (P = 0.023). However, the co-digestion of three substrates produced less hydrogen in pH 6 than in the as-mixed pH but the difference is not significant (P = 0.971). (Fig. 2(a)). At pH 10 (Fig. 2(c)), AP with PPL generated the highest hydrogen (31.3 ml) for the as-mixed pH the lowest amount (10 ml) for this combination was recorded (P = 0.006).

Feng et al. (2010) investigated the effect of the initial pH at 37°C with apple pomace with a concentration of 20 g/L. They found that increasing the initial pH from 5 to 7 could increase the hydrogen yield from 42.7 to 90.06 ml/g total solid. In this regard, the initial pH of apple pomace with rumen fluid was 6.4, which produced more hydrogen than pH 6 and 10 (P = 0.031). The co-digestion of all substrates at pH 6.2 and co-digestion of AP and Ts at pH 6.4 produced the highest amount of hydrogen in the optimum pH range. In the co-digestion of all substrates, there was a tremendous increase in VFAs production, mainly acetic acid and butyrate, by days 2 and 3, resulting in a pH drop and an expected sharp rise in hydrogen yield corresponding to the drop in pH (Li et al., 2008). On the other hand, reaching pH 9 or more resulted in a drop in hydrogen production, as the co-digestion of AP and PPL produced less hydrogen at pH 10 compared to the initial as-mixed pH (P = 0.011) and pH 6 (P = 0.066) (Khanal et al., 2004).

Apart from pH, substrate characterization has a profound impact on hydrogen production. Biohydrogen production yield was found to be enhanced by controlling inorganic nutrients (Hawkes et al., 2002). It must be noted that in the present study, inorganic concentrations were adequately augmented based on substrates’ volatile solid amount in all cases. Another factor in producing hydrogen was the COD content in substrates. Thin stillage has the highest amount of COD by 82 g/L (Table 1). As mentioned in all conditions, hydrogen production was enhanced with the inclusion of thin stillage. In this regard, Kim et al. (2004b) increased the hydrogen production from the initial amount of 75.2 g COD to 121.6 g COD as VS and COD were increased to 3%. To confirm the effect of substrate concentration, the ratio of COD to TKN for different substrates was in the range of 2.3 to 11.8, indicating that nitrogen was also sufficient for hydrogen production (Lin and Lay, 2004;
The TKN content of PPL was the highest amount at 18.75 g/Kg, presenting PPL as a good nitrogen source to be supplied along with AP and Ts. Nitrogen-enriched substrate results in higher hydrogen generation as ammonium content acts as a buffer, maintaining pH in a preferable range for microbial activity (Babson et al., 2013).

In addition to the acetoclastic pathway for methane production, by reducing CO₂ with H₂ as an electron donor, methane is produced in the final stage of anaerobic digestion. Hydrogenotrophic methanogens consume the produced hydrogen and carbon dioxide, thereby decreasing the total accumulated gas (Khan et al., 2016). The heat shock process was used as a remedy to inhibit methanogens in this study. It was found that heat shock at 80 °C for 15 min was the most effective heat shock condition to achieve the highest VFA yield for different substrates (Yin et al., 2021). By inhibiting the hydrogenotrophic pathway, CO₂ was accumulated in the gas headspace of the bioreactors. Thin stillage produced 27.27 and 28.83 ml CO₂ with the as-mixed pH and pH 6, respectively. Based on different observations, it has been claimed that VFAs synthesis at high yields through AD is often accompanied by hydrogen gas production (Akinbomi and Taherzadeh, 2015; Parchami et al., 2020). Methanogenesis inhibition would hinder the VFAs utilization. Therefore, VFAs could be accumulated in the fermentation media and synergistically inhibit methanogens even further. The butyric acid and acetic acid production pathways could produce 4 and 2 mol of hydrogen, respectively. In contrast, the pathway for propionic production is undesirable for hydrogen generation (Kawagoshi et al., 2005). On the other hand, inhibiting methanogens stops hydrogen removal that favors the functionality of fermentative and acetogenic bacteria, leading to diminishing VFAs production (Nguyen et al., 2019). Accordingly, the heat-shock approach in this study was practical for inhibiting the methanogens and assisted in VFAs accumulation.

Fig. 3. (a) Total VFAs and distribution, (b) Changes in the VFAs yield and pH during MBR fermentation.
### 3.3. Volatile fatty acids production using membrane bioreactor

A semi-continuous membrane bioreactor was used for the biocomversion of organic waste residues into VFAs using an inoculum rich in ruminal microorganisms. Based on the results obtained from batch fermentations and considering that reaching high VFAs yields without chemical intervention in the feed-graded ruminant feed additive was favored, the iMBR fermentation was performed using a mixture of AP and PPL as feed substrate. Although co-fermentation of PPL and TS at pH 10 produced a higher VFAs yield, the acids’ distribution was not as varied as the VFAs solution produced by AP and PPL. In addition, the VFA permeate produced by the membrane bioreactor is intended to use as a feed ingredient. Therefore, the application of ex-situ chemicals is minimal during the process. A substrate inoculum ratio of 1:1 (on VS basis) was used to commence the iMBR operation with an initial organic loading rate of 1 gVS/L/day. Accordingly, TS and VS of RF were measured at 10.3 g/L and 10 g/L, respectively, whereas those of the feed mixture were measured at 82.5 g/L and 60.8 g/L. Filtration was commenced following an acclimatization period of four days.

The profile of VFA’s during fermentation in iMBR is shown in Fig. 3(a). VFA accumulation reached 2.92 g/L on day 4. In the days leading up to day 18, VFA levels gradually increased to 6.6 g/L and stayed relatively stable from day 18 to 20. In the period of day 4 to day 20 the reactor was fed by 1VS/L/day (OLR 1), acetic and butyric were the dominant acids at concentrations of 2.16 g/L and 1.77 g/L, respectively. The average total VFAs produced in this period was 5 g/L (Table 3).

To obtain a higher level of VFAs production, the OLR was raised. Increasing the OLR to 2 gVS/L/day boosted total VFAs production from 6.7 g/L on day 22 to 11.6 g/L by day 34, remaining stable until day 42, and the productivity was around 0.3–0.6 g/L.day in this period. From day 43, the reactor was fed by an OLR of 3.7 gVS/L/day to investigate the effect of organic loading on VFAs generation and to challenge the system. Two different cycles of starvation from day 58 to day 60 and day 68 to day 70 were measured at 82.5 g/L and 60.8 g/L, respectively, whereas those of the feed mixture were measured at 24.8 g/L and 17.7 g/L, respectively. The average total VFAs produced in this period was 5 g/L (Table 3).

<table>
<thead>
<tr>
<th>OLR (g VS/ L/day)</th>
<th>Acetic acid g/L</th>
<th>Propionic acid g/L</th>
<th>Butyric acid g/L</th>
<th>Caproic acid g/L</th>
<th>Total VFAs g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.17</td>
<td>0.92</td>
<td>1.81</td>
<td>0</td>
<td>5.06</td>
</tr>
<tr>
<td>2</td>
<td>4.25</td>
<td>1.74</td>
<td>3.97</td>
<td>0</td>
<td>10.19</td>
</tr>
<tr>
<td>3.7</td>
<td>7.64</td>
<td>2.22</td>
<td>9.97</td>
<td>0</td>
<td>19.98</td>
</tr>
<tr>
<td>1-day starvation</td>
<td>8.65</td>
<td>1.78</td>
<td>14.29</td>
<td>0.64</td>
<td>25.81</td>
</tr>
<tr>
<td>2-day starvation</td>
<td>8.14</td>
<td>1.28</td>
<td>13.82</td>
<td>3.59</td>
<td>27.74</td>
</tr>
</tbody>
</table>

This is in confirmation with findings in other literature (Wijekoon et al., 2011) as by increasing the OLR, there is an upsurge in utilisable carbon sources and growth nutrients, albeit within permissible limits, that leads to the increase in VFAs production. Wainaina et al. (2020a) indicated that VFAs concentration enhancement is directly related to increasing the OLR. In summary, VFA production boosts with an initial increase in OLR. Increasing OLR provides more nutrients for acidogens to raise VFAs production rate compared to the VFAs consumption rate by methanogens. Magdalena et al. (2019) found that By increasing OLR, Bacteroidetes and Firmicutes relative abundances were balanced, and methanogenic activity was diminished (Euryarchaeota phylum), making it possible to maximize VFA production.

The maximum VFA yield was obtained at a two-day starvation period (0.75 gVFA/gVSadded or 8.01 gCOD VFA/gCODadded), followed by the yield obtained from one-day starvation (0.69 gVFA/gVSadded or 7.23 gCOD VFA/gCODadded) (Fig. 3(b)). If hydrolysis and solubilization of organic matter increase relatively as the OLR increases, the yield of VFAs on VS added increases (Eryildiz et al., 2021; Wainaina et al., 2020a). However, a rise in VFAs content of the medium does not necessarily indicate higher yields (Jomnonkhaow et al., 2021). Besides, the substrates characterization and co-digestion of various substrates would benefit the yield in terms of providing a preferable ratio of C:N in the range of 20–30 (Vázquez-Fernández et al., 2022). As it is shown in Fig. 4(a), having a better ratio of COD: Ammonia would facilitate the VFAs synthesis, with the most suitable ratio being recorded during the 2-day starvation period. During this period, the average COD concentration was approximately 46.6 g/L, out of which 44.1 g/L was attributed to the generated VFAs. This implies that the VFAs accounted for approximately 94.5% of the total COD concentration during the 2-day starvation period. However, the percentage reduced to 87.8% during the 1-day starvation period, as the average COD concentration was around 45.31 g/L, with 39.8 g/L attributed to the generated VFAs. At day 16 reactor had 22.92 gVs after filtration and 3.5 gVs was added by feeding that increased the reactor Vs to 26.42 however the day after (day 17) reactor had 23.13 gVs after filtration and before feeding. This shows that 3.29 gVs was removed by filtration and VFAs production. The Vs removal was more at day 34 and 35 while the OLR was 2 gVS/L/day. At day 34 the reactor Vs was 32.09, while the Vs for the next day at the reactor was 24.78. This indicates that 7.31 gVs was removed by VFAs production and filtration.

Considering the change in the pH during fermentation, when the OLR was increased to 2 gVS/L/day, the pH increased to about 7.5, followed by a decrease to 6.3 in OLR 3.7 and thereafter fluctuated around 6.5 to the end of the experiment (Fig. 3(b)). Since the reactor pH should be set to 6.5 every day after filtration and feeding, this range is favorable during fermentation. This self-regulation reduces the need for additional chemical pH control agents. In acidogenic fermentation, pH plays a pivotal role in controlling VFA yield and concentration. This could affect not only acidogenesis but also hydrolysis. It is, therefore, essential to maintain an optimal pH for both stages (Liu et al., 2012). Although methanogens have the maximum performance in pH 6.5 to 8.5 but they were inhibited in this study as present in the batch phase via heat shock treatment of inoculum and substrates (Mao et al., 2015).

Regarding the means of pH regulation in such systems, it is noteworthy that the conversion of most amino acids in the substrate to ammonia nitrogen can add to the buffering capacity during fermentation. As shown in Fig. 4(a), the amount of ammonia nitrogen was 94.5% of the total COD concentration during the 2-day starvation period. Thereafter the concentration of ammonium in the media stayed the same with minor fluctuation. This high ammonium content has indicated higher yields (Jomnonkhaow et al., 2021). Besides, the substrates characterization and co-digestion of various substrates would benefit the yield in terms of providing a preferable ratio of C:N in the range of 20–30 (Vázquez-Fernández et al., 2022). Although methanogens have the maximum performance in pH 6.5 to 8.5 but they were inhibited in this study as present in the batch phase via heat shock treatment of inoculum and substrates (Mao et al., 2015).
populations can be altered, ultimately leading to different distributions of VFAs. The unique rumen microbiota can digest lignocellulosic and nonstructural carbohydrates (Christopherson et al., 2014). AP and PPL characterization are close to forage and concentrate fraction of feed as they contain both lignocellulosic material and readily digestible carbohydrates. As the in vitro environment does not perfectly replicate the natural environment, the microbial community is expected to change. A reduction in diversity would be anticipated, along with this decrease, during the adaptation period from a diverse natural community to a definite purpose community based on different factors such as pH, substrate characterization, and exposure time of microbial community to substrates (Cieplik et al., 2019).

The current study is in line with previous works that increasing the OLR would enhance the butyrate share but decrease the acetate and propionate (Wijekoon et al., 2011; Yu, 2001). Wang et al. (2015) made similar observations to the current study, in which acetate dominated at low feeding rates, then butyrate was produced at higher organic loadings. According to their hypothesis, butyrate is formed through the reduction of pyruvate and decarboxylation of pyruvate in combination with the depletion of acetate. However, there are contradictory findings claiming enhancement in the generation of acetate and valerate and reduction in the percentage of butyrate and propionate by OLR (Jiang et al., 2013). Substrate characteristics is one of the pivotal factors determining the VFAs composition. Apple pomace and potato protein liquor have a high content of carbohydrates and nitrogen due to their high value of COD and ammonia (Table 1). The carbohydrate-rich substrates contribute more to acetate, propionate, and butyrate formation, while protein-rich substrate is reported to yield more valerate (Garcia-Aguirre et al., 2017; Shen et al., 2017). In general it has been reported that organic matter with COD higher than 4000 mg/L and ammonium content less than 5000 mg/L could be suitable substrate for VFAs synthesis and methane inhibition (Lee et al., 2014). Accordingly, AP and PPL were the selected substrate in terms of COD and ammonium content. Between day 45 and day 55, the organic loading rate (OLR) increased to 3.7 gVS/L.day. During this period, the rate of volatile fatty acid (VFA) production was significantly higher than during periods of lower OLR and starvation. This suggests that the production of VFAs has a significant impact on the breakdown of organic matter and the

Fig. 4. Changes in (a) sCOD and ammonia content, (b) TSS, TS, and viscosity during MBR fermentation and filtration.
reduction of chemical oxygen demand (COD) content (Fig. 3(b)) in the digested materials at the same period of time (Magdalena et al., 2019). Increased HRT from 10 days to 20 days when one-day starvation is considered and then to 30 days by two-day starvation cycle altered VFA composition. During the one-day starvation cycle, the most significant accumulation of butyric acid was recorded. Similarly, from paper mill effluent and whey, HRT can govern the comparative butyric acid synthesis (Bengtsson et al., 2008). However, in glucose fermentation, reducing HRT would favor more acetate production but suppress butyrate synthesis (Lv et al., 2022). In an experiment conducted by Lim et al. (2008), three different HRT: 4, 8, and 12 days were tested. The concentration of total VFAs soared by HRT raise, from 5.5 g/L to 13 g/L and finally to 22 g/L. Additionally, a shift in relative VFA distribution was observed with the highest HRT. Under shorter HRT, acetic acid was the predominant fermentation product, while propionic acid was the predominant VFA under 12-day HRT. The accumulation of caproic acid was observed during starvation periods with low OLRs by the average amount of 3.59 g/L. Medium chain carboxylic acids (MCCAs), such as caproic acid, are found to be present at relatively higher concentrations during the 2-day period of starvation. The chain elongation pathway of acetic acid to butyric acid and then to caproic acid is a microbial process known as reverse β-oxidation that involves the use of electron acceptors to elongate the carbon chain. Butyrate can be derived from lactate oxidation coupled to chain elongation, and both butyric acid and caproic acid can be synthesized through the catalytic activity of specific enzymes. Additionally, the co-electron acceptors of acetic acid, butyric acid, and caproic acids can promote the production of MCCAs, including caproic acids (Zhu et al., 2015). It is noteworthy that low substrate concentration in the reactor during the 2-day starvation approach and a high concentration of butyric acid are two compelling factors to motivate the chain elongation process for caproic acid production (Nzeteu, 2020).

3.3.1. Membrane filtration performance

Immersed flat sheet membrane was applied to investigate the possibility of in-situ VFA recovery during semi-continuous anaerobic fermentation of apple pomace and potato protein liquor. High-quality and stable filtration fluxes are desired to achieve high productivity in semi-continuous fermentation processes using a membrane bioreactor (Mahboubi, 2019). However, different fouling mechanisms may hinder reaching such a goal. Filtration issues may be exacerbated at higher medium suspended solid content where solid deposition on the membrane surface and pores and cake layer formation result in higher hydraulic resistance and an increase in pressure loss over the cake layer and membrane (rise in transmembrane pressure (TMP)) (Tugtas, 2014). The changes in, TMP, suspended solid content, and viscosity are illustrated in Figures 6 and 7.

When the initial TSS concentration of the medium increased from 2.4 g/L to 7.3 g/L, the TMP experienced a slight increase from 11.3 mbar (OLR 1) to 13.7 mbar (OLR 2) (Fig. 5). Feeding the reactor with 3.7 gVS/L.day resulted in SS increase in the reactor that contained 14.1 g/L of suspended solids. Feeding the reactor with higher OLR led to higher TSS content in the reactor (Fig. 4(b)). However, increasing the HRT could help with stabilizing the reactor TSS concentration. Such an effect was observed by Trussell et al. (2007) as they reported a rapid increase TSS in shorter HRTs. In addition, TMP gradually increased in parallel to the rise in the OLR and TSS. This may be due to accelerated in SS deposition on the membrane surface and cake formation in addition to the increase in viscosity of the medium and reduction in the effectiveness of membrane physical cleaning approaches (gas sparging and medium agitation) (Judd, 2010). As is evident in Fig. 4(b), the viscosity of the medium correlates with the TSS concentration of the fermentation broth (Mahboubi et al., 2016). However, some studies have indicated a low or moderate effect of viscosity on filterability and TMP fluctuations (Hasan et al., 2012; Kornboonraksa and Lee, 2009). In this study, viscosity was around 1 mPa.s at OLR 1 and OLR2 and increased more than 10-times to 10.4 mPa.s by day 55. It could be observed that the viscosity of the media has not been solely affected by the TSS content of the media during fermentation and filtration. Although the TSS content of the
reactor during the first cycle of starvation reached a plateau at below 20 g/L, the viscosity fluctuated wildly, rising from just above 10 mPa.s to more than 30 mPa.s. This rise in TSS accompanied by an initial rise in TMP during the first cycle of starvation can be attributed to factors such as solid retention time (SRT) (Laera et al., 2007), and the exocellular polymeric substances (EPS) and soluble microbial products (SMP) (Rosenberger et al., 2002). However, as an immersed membrane bioreactor where the filtration was not conducted with constant flux or TMP, a decent filtration performance was experienced up to day 94 as flux was relatively stable at an average of 19.5 L/m².h and TMP was kept below 40 mbar at such high TSS content (20 g/L).

Although starvation and an increase in HRT controlled the excessive rise in SS accumulation rate and lowered the TMP from about 30 mbar on day 53 to 18 mbar on day 76, the TMP started to increase and reached 76.3 mbar on day 105. In the second starvation cycle ending on day 105, TMP and TSS content were relatively stable the viscosity of the media experienced a significant drop to around 13 mPa.s, which could be an indication of the utilization of fractions of SMP and EPS by the starved culture. In this regard, complimentary research is required to investigate fermentation kinetics thoroughly, changes in the EPS and SMP levels, and active membrane fouling mechanisms.

3.4. VFAs as a potential alternative ruminant feed additive

Nowadays, sustainability in practice is considered as a well-rewarding industrial imperative, and the agricultural sector is not apart from it. In addition, circularity in bioeconomical activities concerned with livestock production is being increasingly motivated not only to reduce feed costs but also to treat agroindustry by-products. To this end, new ingredients should be evaluated from various perspectives, including pH, energy content, digestibility, nitrogen, and mineral content, and compared with conventional sources based on ever-growing animal nutritional requirements. Therefore, this article intended to present a new approach in the bioconversion of agri-industrial side stream with low nutritional value, such as apple pomace and potato protein liquor, to value-added biochemicals such as VFAs that have the potential to be used as ruminant feed supplements. Such supplements can be added to the feed for energy replacement purposes, as chemical precursors required for physiological and health-related functions, or to boost microbial growth. VFA salts such as sodium acetate (NaAc), sodium butyrate (NaBu), and calcium propionate (CaPr) as ruminant feed additives have been studied since the late 1950s and have been shown to affect physiological and health-related functions and productivity. Dietary inclusion of these VFA salts on feed intake, rumen-intestinal development, fermentation product adsorption, milk yield, weight gain, hormone secretion, milk protein, fat content, etc., have been investigated in detail (Tamate et al., 1962; Urrutia and Harvatine, 2017).

Compound feeds contribute significantly to most high-producing ruminant animals’ energy supply. The use of raw materials, especially agro-industrial by-products, has been dramatically expanded over the past decade. Compound feeds have undergone a great deal of variation in composition as well as in energy content as a result of this diversification. The replacement of conventional energy sources in feed is mainly based on VFAs generated from the fermentation of organic matter of new compounds. According to the general agreement, VFA accounts for more than half of the total digestible energy (around 70%) in ruminants. As volatile fatty acids do not ferment in the rumen (no gas production) or form inorganic nitrogen in urine, their digestible energy equals their metabolizable energy (Bergman, 1990). Based on this hypothesis, VFAs produced through acidogenic fermentation of organic residuals could play an energy replacement role and be considered a fractional energy supplier among feed ingredients. As the VFA production takes place in a rumen-like environment, it is of biological origin and compatible with the rumen’s function. As previously mentioned, the average total VFA concentration throughout the period was about 16 g/L, containing 7.8 g/L butyric acid, 5.8 g/L acetic acid, 1.6 g/L propionic acid, and the rest is caproic acid. Based on the energy content of each VFA (Kim and Min, 2008), the mixture can provide 339.74 kJ/L. Albeit, the energy content could be improved by increasing the concentration using methods such as nanofiltration and reverse osmosis (Domingos et al., 2022). Ruminal nitrogen is mainly employed for microbial protein synthesis. The VFAs mixture can provide 1.5 g/L nitrogen, which is a promising alternative to urea supplements in animal feed. Besides, ammonia content adds an excellent buffering capacity to the solution, keeping the pH of the VFAs mixture in the range of 6.5. However, there is a need for both in vitro and in vivo experiments to evaluate the digestibility effect of VFAs that is added in different types (solid salts and in solution) and different feed fractions (total mixed ration, concentrate, forage).

In the EU, in order for the VFAs produced from organic residue to be certified as an animal feed additive, the whole process of selecting the substrate, inoculum, method of process, to the final product content should comply with related EU directives. Currently, apple pomace and potato protein liquor are allowed to be used in ruminant feed. Hence, they could be converted via acidogenic fermentation. Rumen microorganisms naturally present in ruminants’ rumen can be used for such practice (COMMISSION, 2017). Microfiltration in the IMBR separates the ruminal microorganisms from the effluent, complying with Annex I of Regulation (EC) No 1831/2003 (UNION, 2003). However, the solution obtained should be further analyzed for other components (heavy metals, and toxic substances) concerned within the directive 2002/32/EC (UNION, 2002).

4. Conclusion

This study investigated the acidogenic fermentation of three substrates using the ruminal fluid as inoculum. Co-fermentation of PPL and Ts at pH 6.5 produced the highest VFAs concentration (14.2 g/L) and yield 0.64 g VFA/gVSadded. Eventually, the co-fermentation of AP and PPL with as-mixed pH (of 6.5) was chosen as the optimal condition for long-term (114 days) production and in-situ recovery of volatile fatty acids using a microfiltration-assisted anaerobic membrane bioreactor. By inhibiting methanogenesis and change in the OLR, high concentrations of VFAs were achieved (above 26 g/L (0.75 gVFA/gVSadded)). TSS content of above 17 g/L presented a challenge to membrane filtration. However, the membrane could successfully perform filtration for more than 90 days at low TMPs. According to European legislation, the VFAs-rich effluent from PPL and AP can be used in ruminant feed supplementation. This opens new avenues for the sustainable production of ruminant feed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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