JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(9), 170–185
https://doi.org/10.12911/22998993/204559
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.04.25
Accepted: 2025.06.13
Published: 2025.06.23

Valorization of fruit and starch wastes via anaerobic acidification for the sustainable production of bio-products

Andriy Anta Kacaribu[1], Yuliani Aisyah[2], Febriani[3], Ramayanty Bulan[4], Darwin[4*]

[1] Doctoral Program of Agricultural Science, Postgraduate School Program, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[2] Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[3] Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[4] Department of Agricultural Engineering, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
* Corresponding author's email: darwin_ae@usk.ac.id

ABSTRACT

Global agricultural activities generate a substantial amount of organic waste, particularly from fruit and starch-based residues. This study investigates the anaerobic acidification (AC) of these wastes using microbiota derived from rumen fluid, aiming to produce value-added bioproducts such as lactic acid (LA) and volatile fatty acids (VFAs) for sustainable waste management. The AC process was conducted over 48 hours at 35 ± 0.5 °C without pH control, utilizing six digesters with varying substrate-to-inoculum ratios. Results showed that starch-based substrates led to higher lactic acid accumulation compared to fruit waste, while both substrate types produced VFAs in comparable concentrations. LA and VFA accumulation ranged from 2–6 and 20–60 mmol/L, respectively. These findings underscore the feasibility of converting organic waste into economically viable, value-added products through bioconversion, with implications for environmental sustainability and resource efficiency. Further research is recommended to optimize the process and assess its scalability for industrial applications.

Keywords: Anaerobic acidifications, bio-based products, organic wastes, waste conversion, waste management.

INTRODUCTION

The global agricultural sector produces vast amounts of organic waste annually, including substantial fractions from fruit and starch-based food processing. These residues not only represent a severe threat to the environment through the release of greenhouse gases, leachate pollution, and resource inefficiency but also pose significant economic and logistical burdens to waste management systems (Cheung et al., 2021; FAO, 2013). The widespread consumption of fruit and starch-based products globally has led to the continuous accumulation of waste materials such as peels, pulp, seeds, and processed starch residues, demanding urgent attention and sustainable mitigation strategies.

Fruit and starch residues are particularly rich in carbohydrates, especially glucose, which is readily liberated during hydrolysis. Starch, a polysaccharide comprising glucose units, is one of the dominant constituents in agro-industrial waste streams (Chisenga et al., 2019; Martins et al., 2023). Likewise, fruit waste contains considerable amounts of fermentable sugars originating from the breakdown of complex carbohydrates during ripening and processing (Zia et al., 2022). Given their biochemical profile, these waste types possess immense valorization potential, provided that efficient bioconversion strategies are employed. Addressing the escalating burden of organic waste requires solutions aligned with the principles of the circular economy, which emphasize not only waste minimization but also resource

regeneration and economic viability (Kacaribu et al., 2025; Kacaribu and Darwin, 2024b).

Several organic waste management approaches have been explored in recent decades, including landfilling (Kharola et al., 2022), composting (Darwin et al., 2022), incineration, and biological treatments such as anaerobic digestion (AD) and co-digestion (Darwin et al., 2021a; 2021b). Among these, AD stands out due to its dual functionality: it reduces the organic load while simultaneously generating value-added products. During AD, hydrolyzed organic materials are converted into intermediate bio-products such as lactic acid (LA), volatile fatty acids (VFA), and alcohols—compounds with significant industrial and commercial applications (Darwin et al., 2019b; 2019c; Uddin and Wright, 2023). The subsequent stages of the AD process yield methane, further enhancing its attractiveness for sustainable waste-to-energy schemes (Ali Shah et al., 2014; Alkaya and Demirer, 2011).

The acidogenic phase of anaerobic digestion is particularly critical in determining the profile and yield of intermediate products. In this phase, complex organics are converted into simpler compounds, primarily LA and VFA, through the enzymatic action of specific microbial consortia (Kacaribu et al., 2025). Prior studies have demonstrated that various microbial inocula, such as activated sludge and anaerobic digestate, can efficiently drive acidogenic fermentation to produce these bio-products from diverse organic substrates (Darwin et al., 2019a; Zhang et al., 2020). Moreover, recent advancements suggest that rumen fluid—a microbiota-rich byproduct of ruminant digestion—can serve as a potent inoculum, offering superior hydrolytic and acidogenic activity compared to conventional sludge. For instance, Pourbayramian et al. (2021) reported high VFA yields from potato waste using rumen fluid, along with the production of nutrient-rich digestate suitable for animal feed (Pourbayramian et al., 2021).

Despite these promising developments, a significant research gap remains concerning the use of fruit and starch-based residues as substrates in combination with rumen fluid inoculum, particularly when sourced from slaughterhouse waste. This finding is a critical oversight, considering the high microbial diversity and enzymatic potential of rumen consortia, as well as the abundance and accessibility of slaughterhouse waste in many regions.

The novelty of this study lies in its integrated use of fruit and starch-based residues with rumen fluid derived from slaughterhouse waste for anaerobic acidification—an approach that has not been systematically explored in previous research. This synergistic valorization pathway combines underutilized organic waste streams with a highly active microbial inoculum to lactic acid and VFA production. By elucidating the metabolic behavior of rumen microbiota in mixed-substrate environments, the study offers new insights for optimizing process efficiency and resource recovery in decentralized biorefineries. Ultimately, this research addresses urgent issues of organic waste accumulation while contributing to sustainable waste management strategies and bio-based circular economy development.

MATERIALS AND METHODS

The present study was conducted at the Post-Harvest Engineering and Bioprocess Laboratory, Department of Agricultural Engineering, Universitas Syiah Kuala. The study was conducted Indonesia at July – October, 2024. The various materials were employed, including Lactic Acid, D-(+)-Glucose Anhydrous (VWR BDH Prolabo Chemicals), nutrient Agar (Oxoid) Phenol, H_2SO_4, NaOH, H_3BO_3, HCl, Indicator (PP, Methyl Red, Methylene Blue), was procured from Merck, Co, Ltd (Selangor, Malaysia), H_2O, Fruit Waste, Cassava Waste, and Rumen fluid.

Substrate and inoculum preparations

The study utilized feedstock comprised fruit and cassava waste. Cassava waste served as the starch waste. Fruit waste included randomly fruit waste such as pears, melons, watermelons, bananas, and papayas, both collected from the Vegetable and Fruit Local Market in Rukoh Village, Banda Aceh City, Indonesia. Wastes were meticulously separated from impurities, finely crushed, and blended to reduce size (± 0.1 cm) before experimentation.

Anaerobic acidification employed an undefined mixed inoculum, which is rumen fluid waste obtained from the Banda Aceh City slaughterhouse, Aceh, Indonesia. The rumen fluid used in this study was obtained from 2.5-year-old local Acehnese female cattle that were not subjected to feed control. The collected fluid was then

filtered, transferred into dark glass bottles, and transported to the laboratory. The rumen fluid was stored in a refrigerator at a temperature of 1–2 °C for subsequent analysis. Characteristics of substrate and inoculum employed in this study presented in Table 1.

The characteristics of the substrate and inoculum utilized before initiating the anaerobic acidification process were meticulously examined, as shown in Table 2. The rumen fluid, serving as the key inoculum, displayed a pH of 7 ± 0.10, with MC of 90.4 ± 0.10%, a TS content of 9.60 ± 0.10%, and total viable cells of 4.85×10^8 CFU/mL. Furthermore, its ORP value was recorded at 40.00, while ammonia content was absent. Conversely, both cassava waste and fruit waste exhibited similar pH values of 7 ± 0.10. Cassava waste presented a lower MC of 12.12 ± 0.10% and higher TS content of 87.88 ± 0.10%, and negligible ammonia content at 0 ppm. Similarly, fruit waste demonstrated MC of 78.31 ± 0.02% and a TS content of 21.69 ± 0.02%, with no measurable ORP and negligible ammonia content at 0 ppm. These comprehensive characterizations provide crucial insights into the composition of the substrates and inoculum, laying the foundation for the subsequent anaerobic acidification process to valorize fruit and starch waste for bio-based product development. The initial characterization of the substrates and inoculum provided a foundation for understanding their composition and suitability for anaerobic acidification. The absence of ammonia and suitable pH levels indicated a conducive environment for microbial activity.

Anaerobic acidification process

The anaerobic acidification process was conducted without pH control (no acid or base was added), allowing the process to occur naturally. The process was carried out using covered digesters with a working volume of 250 mL placed on a thermostat-controlled water bath for 48 h, maintaining a constant temperature of 35 ± 0.5°C as depicted in Figure 1. The substrate was added to the digesters, followed by rumen fluid inoculum. A total of 6 digesters were prepared, each containing substrate and inoculum with different concentrations (50, 100, and 150 g) of substrate per liter of inoculum as listed in Table 2. Before sealing the digesters, a heating process was conducted on the water bath to remove oxygen. Once oxygen was removed from the digesters, they were tightly sealed, and the anaerobic acidification process commenced (Kacaribu and Darwin, 2024a).

Analytical methods

The anaerobic acidification samples were subjected to centrifugation at 2000 revolutions per minute (rpm) for 10 min to separate the supernatant, which was then meticulously transferred into analytical tubes and stored at 2 °C for further analysis. The pH levels were determined employing a high-precision Laboratory Benchtop pH Meter equipped with a Multifunction Complete Probe Milwaukee MW 101 PRO (Darwin et al., 2023), ensuring accurate measurement of the acidic or basic nature of the samples. Additionally, assessments were also made for total solids (TS). For the analyses of electrical conductivity (EC), total dissolved solids (TDS), oxidation-reduction potential (ORP), and ammonia concentration (NH_4^+), a preparatory step involved diluting a 1 mL aliquot of the effluent tenfold with deionized water (DH_2O). All analysis parameters were determined following established standard methods (APHA, 2012). Analysis was performed in duplicate to produce reproducible results.

The total carbohydrate content of the samples was determined using the Phenol-Sulfuric method (Herbert et al., 1971), which is crucial for assessing the availability of sugar in anaerobic acidification process. The quantification of VFAs followed established procedures to assess

Table 1. Characterization of inoculum and substrate

Inoculum/ Substrate	pH	MC (% wb)	TS (%)	ORP (mV)	TVC (CFU/mL)	Ammonia (mg/L)
Rumen fluid	7 ± 0.10	90.4 ± 0.10	9.60 ± 0.10	40.00	4.85×10^8	0
Cassava waste	7 ± 0.10	12,12 ± 0.10	87.88 ± 0.10	-	-	0
Fruit waste	7 ± 0.10	78.31 ± 0.02	21.69 ± 0.02	-	-	0

Note: Data presented as mean value ± standard deviation; MC – moisture content; TS – total solid; ORP – oxidation reduction potential; TCC – total cell counts.

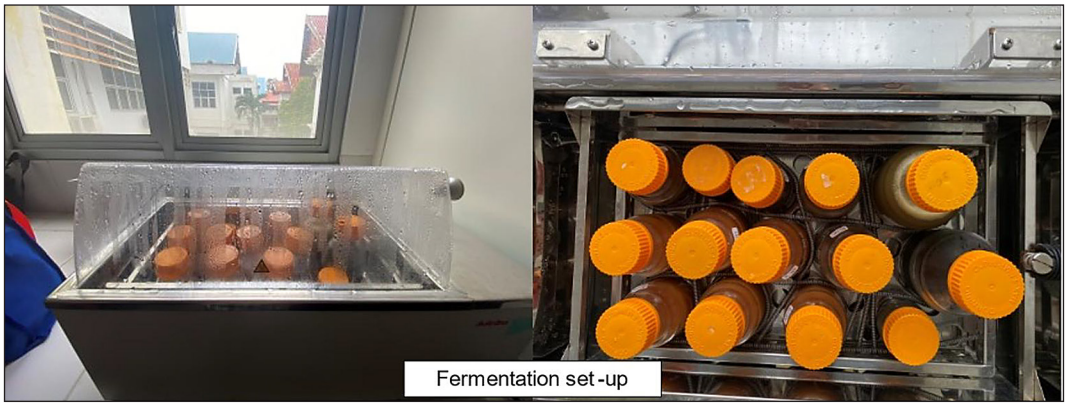

Figure 1. Laboratory installation set-up for anaerobic acidification processes

the volatile acid fraction present in the samples, and was determined using titrimetric methods as described previous study (Lützhøft et al., 2014). The concentration of lactic acid, measured using a Lactate Biosensor Accutrend Plus meter (Darwin, 2019). The microbial growth was quantified by measuring the optical density (OD) at 600 nm using a Spectrophotometer UV Shimadzu 1200, offering direct insights into the microbial population dynamics. All analysis was performed in duplicate to produce reproducible results.

Statistical analysis

All experimental data were obtained from duplicate runs. Mean values and standard deviations were calculated and used to evaluate the reproducibility of the measurements. Given the preliminary nature of this investigation and the focus on process trends rather than statistical inference, no further statistical analyses were performed.

RESULTS AND DISCUSSIONS

pH profile during anaerobic acidification

The pH profile is a critical factor in the anaerobic acidification process of organic waste using Ruminal fluid inoculum. The pH analysis results from this study are presented in Figure 2, which illustrates distinct dynamic changes between the two types of substrates employed. Throughout the process, the pH of the digester fluctuated, initially decreasing from neutral (pH 7) to acidic levels (approximately pH 5) and subsequently, in some cases, returning to around neutral. This trend is consistent with previous studies, which

Table 2. Experimental design

Substrates	Substrate concentration/ inoculum (g/L)	Code
S	50	P1
	100	P2
	150	P3
F	50	P4
	100	P5
	150	P6

Note: S – starch waste; F – fruit waste.

have reported similar patterns of pH reduction due to microbial adaptation and the hydrolysis of substrates into acidic metabolites, including lactic acid and volatile fatty acids (VFAs) (Franke-Whittle et al., 2014; Grzelak et al., 2018; Tang et al., 2017; Villanueva-Galindo et al., 2024). The observed pH decline can be attributed to lactic acid accumulation, which results in proton release due to substrate oxidation during the incubation period. Moreover, the low pKa value of lactic acid (3.86) contributes significantly to the acidification of the digester environment (Robergs et al., 2018).

The results presented in Figure 2 demonstrate that digesters supplied with starch waste substrates (P1–P3) experienced a continuous pH decline throughout the 48-hour incubation period, dropping from pH 7 to pH 5. This significant decrease in pH is corroborated by the accumulation of lactic acid metabolites, as illustrated in Figure 4. These findings are consistent with previous studies, which reported that a pronounced pH decline during the incubation of rice and corn starch substrates with rumen fluid inoculum was attributed to the accumulation of lactic acid (Darwin et al., 2018a). Similarly, a separate study reported that

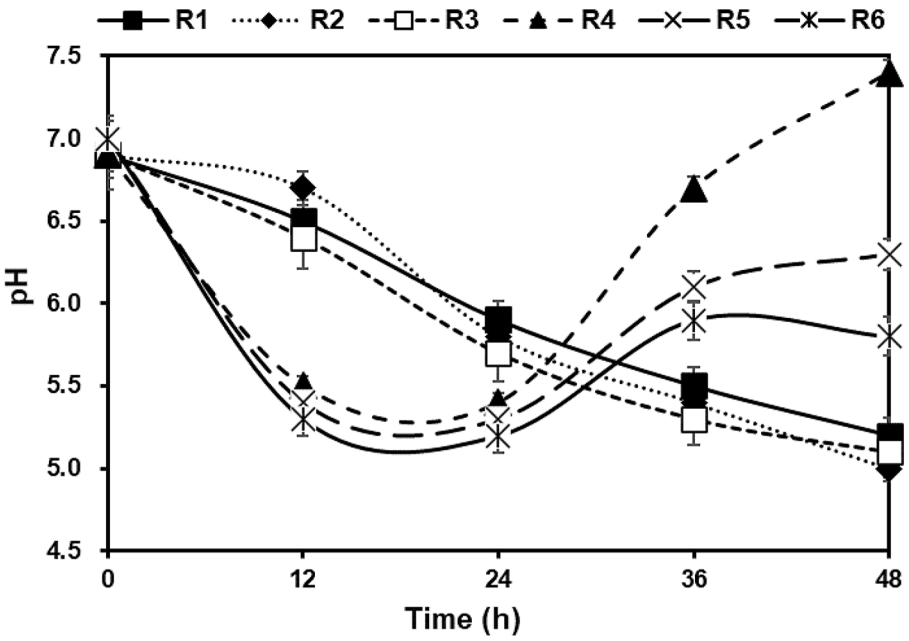

Figure 2. pH profile during anaerobic acidification

the anaerobic acidogenesis of food waste using aerobic primary sludge from municipal wastewater exhibited a comparable trend, where the drop in pH within the digester was also associated with lactic acid accumulation (Pau et al., 2024).

A contrasting phenomenon was observed in the other set of digesters (P4–P6), which were supplied with fruit waste substrates. In these reactors, the pH declined only during the initial 24 hours of incubation, from pH 7 to 5.3. During this period, lactic acid accumulation was evident; however, beyond 24 hours, no further lactic acid production was detected due to pH recovery. Previous research has indicated that the pH increase (pH recovery) within anaerobic digesters occurs once the acidogenic phase has passed and the system transitions into the subsequent stage (Adekunle and Okolie, 2015). At this stage, the microbial community within the digester begins to utilize lactic acid as a secondary substrate for the biosynthesis of other metabolic products (Ayudthaya et al., 2018).

TDS profile

During the anaerobic acidification of organic waste using rumen fluid inoculum, a significant decrease in TDS was observed within the digesters. This reduction is primarily attributed to microbial activity breaking down substrates such as starches and sugars, which gradually reduces the concentration of dissolved solids. The decline in TDS throughout the bioconversion process is further supported by the concurrent production of lactic acid and volatile fatty acids (VFAs), as depicted in Figures 5 and 6.

As shown in Figure 3, all digesters exhibited a downward trend in TDS during the bioconversion process. This trend indicates the hydrolysis of dissolved solids into metabolites—lactic acid and VFAs—facilitated by microbial activity during anaerobic digestion (metabolism) and metabolite synthesis (Litti et al., 2024). Digesters P5 and P6 recorded the highest TDS concentrations, reaching 110 mg/L, due to the higher substrate concentrations of fruit waste (100 and 150 g/L, respectively). Fruit waste is predominantly composed of soluble sugars such as glucose and fructose, which account for approximately 75% of its total organic content, as confirmed by previous research (Zia et al., 2022). The high carbohydrate content in these substrates is also consistent with the data presented in Figure 3, which shows a substantial level of total carbohydrates in these digesters. Earlier studies have also reported elevated TDS levels associated with high organic matter input in such systems (Butler and Ford, 2018). Conversely, digester P4, which also received fruit waste as a substrate, exhibited a lower initial TDS of 70 mg/L due to a lower substrate concentration of 50 g/L. This variation further

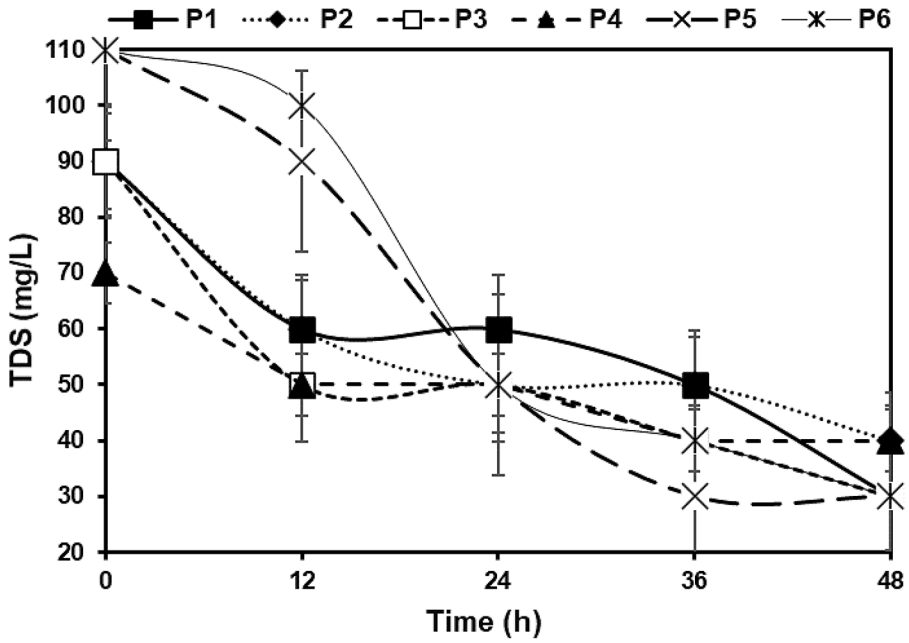

Figure 3. TDS profile during anaerobic acidification

highlights the influence of substrate concentration on TDS levels and hydrolysis rates.

On the other hand, digesters P1–P3, which were supplied with starch waste substrates, exhibited an initial TDS value of approximately 90 mg/L. This relatively lower TDS may be because starch is an insoluble carbohydrate. This finding contrasts with previous reports, which noted that starch in aqueous media exhibited a TDS of only 0.365 mg/L (Airlangga et al., 2021). However, the relatively high initial TDS observed in digesters P1–P3 could also be attributed to the contribution of rumen fluid used as the inoculum. Rumen fluid is known to have high TDS levels, primarily due to the presence of VFAs, minerals, and other soluble compounds generated from microbial activity during ruminant digestion (Beede, 2012).

Total carbohydrate content

Total carbohydrate analysis was conducted using the standard phenol–sulfuric acid method to determine the glucose concentration in carbohydrate-containing samples (Herbert et al., 1971). The initial glucose content varied depending on the substrate used, with substrate F (fruit waste) exhibiting the highest glucose concentration, followed by substrate S (starch-based waste), as shown in Figure 3. This study investigated the capacity of microbial communities in rumen fluid to convert these substrates into lactic acid metabolites by analyzing the glucose consumption profile over the bioconversion period. According to Figure 4, a consistent decrease in glucose concentration was observed across all digesters, indicating microbial utilization of organic matter as a primary carbon source (de Almeida et al., 2018; Sanjorjo et al., 2023).

Digesters P4, P5, and P6, which were fed with substrate F (fruit waste) at substrate/inoculum ratios of 50, 100, and 150 g/L, exhibited initial glucose concentrations of 21.8, 27.0, and 30.0 mmol/L, respectively. These can be attributed to the high content of simple sugars—primarily fructose and glucose—in fruit waste, which comprises approximately 75% of its total organic content (Zia et al., 2022). These sugars are readily assimilated by microorganisms as carbon and energy sources (Ren et al., 2018). In contrast, digesters P1, P2, and P3, which were supplied with substrate S (cassava-based starch waste) at equivalent concentrations, had lower initial glucose levels of 17.12, 20.89, and 24.75 mmol/L, respectively. This finding is consistent with the composition of cassava waste, which contains approximately 70.66% starch, primarily composed of the glucose polymers amylose and amylopectin (Chisenga et al., 2019; Martins et al., 2023).

Interestingly, increasing the substrate concentration did not correspond to increased lactic acid production, as shown in Figure 5. This phenomenon is likely due to substrate inhibition at higher

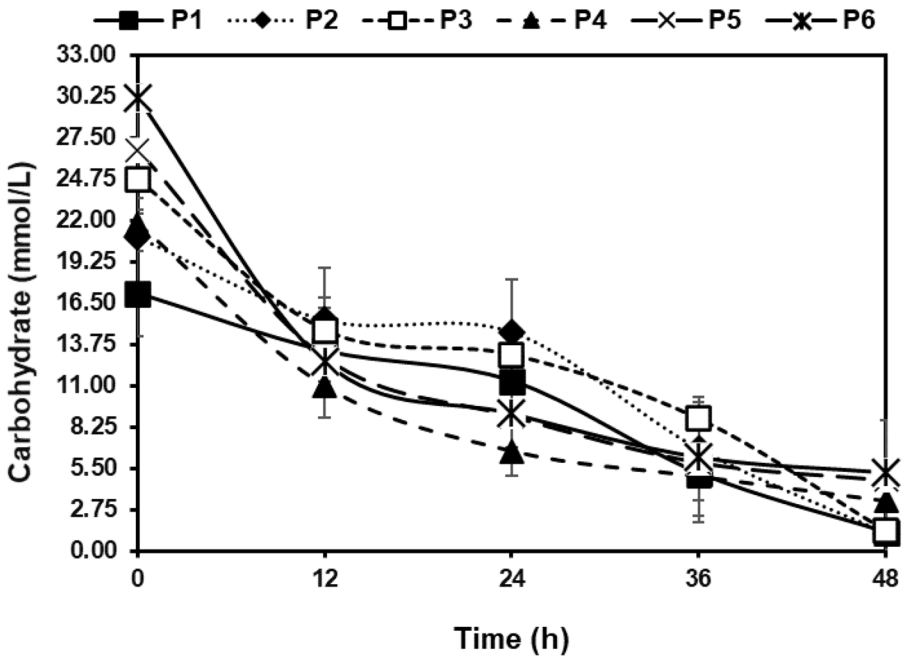

Figure 4. Total carbohydrate content profiles in the digesters during bioconversion

concentrations, which can impair microbial enzymatic activity and reduce the overall bioconversion rate through osmotic stress mechanisms (Amha et al., 2018; Zabed et al., 2017). Previous studies have proposed potential mitigation strategies, including the development of microbial strains tolerant to toxic or high-substrate conditions (Li et al., 2024) or the application of fed-batch bioconversion techniques to regulate substrate loading (Son and Kwon, 2013). Notably, starch-based substrates tended to yield higher lactic acid production at lower substrate concentrations compared to fruit-based substrates. These results emphasize the importance of optimizing substrate concentrations to maximize lactic acid yields while minimizing the risk of substrate inhibition (Kacaribu and Darwin, 2024a).

Lactic acid accumulation

Utilizing rumen fluid microbiota as an inoculum for anaerobic acidification of starch and fruit waste-based substrates holds great promise for producing lactic acid, a valuable commodity widely utilized in industries (Ojo and de Smidt, 2023). The rumen fluid microbiota is known to contain various types of lactic acid bacteria, such as *Streptococcus* spp. (Ayudthaya et al., 2018), and *Prevotella* (Darwin et al., 2018a), which are capable of converting organic waste into lactic acid via anaerobic fermentation pathways.

The findings of the present study (Figure 5) indicate that all digesters produced lactic acid during the anaerobic acidification process, although production was limited to specific bioconversion periods. This finding suggests the existence of substrate-specific biotransformation mechanisms. Digesters P1, P2, and P3, which utilized starch-based substrates, began lactic acid accumulation at 36 hours of incubation, with concentrations of 2.8, 3.3, and 3.9 mmol/L, respectively. As bioconversion time and substrate concentration increased, lactic acid production also rose. However, at 48 hours, digester P3—containing the highest substrate concentration (150 g/L) – showed a slight decrease in lactic acid concentration from 3.9 to 3.8 mmol/L. This decline is likely due to substrate inhibition, a condition in which high substrate concentrations hinder microbial or enzymatic activity due to osmotic stress (Dumbrepatil et al., 2008).

In contrast, digesters P4, P5, and P6, which used fruit waste as a substrate, showed lactic acid accumulation only during the 24-hour bioconversion period, with respective concentrations of 2.3, 2.8, and 3.4 mmol/L. Beyond this period, no further lactic acid accumulation occurred, likely due to microbial conversion of the previously accumulated lactic acid into VFAs. This phenomenon is supported by pH analysis (Figure 2), which showed a drop in pH to 5.2–5.4 during the first 24

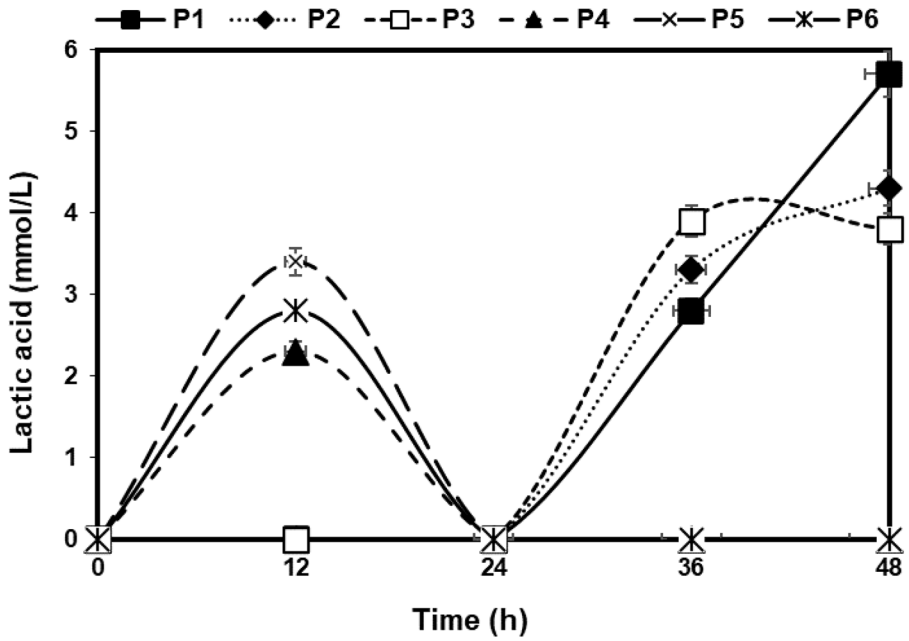

Figure 5. Lactic acid accumulation during anaerobic acidification

hours, a range considered optimal for lactic acid production by undefined mixed inocula (Tang et al., 2017). After 24 hours, the pH increased to between 6 and 6.7 (Figure 2), indicating a pH recovery phase during which lactic acid was converted into VFAs such as acetic, butyric, and valeric acids (Parchami et al., 2023; Wainaina et al., 2019).

The starch-to-lactic acid pathway likely involves enzymatic hydrolysis of starch into glucose by amylolytic enzymes (e.g., alpha-amylase), followed by glucose fermentation by facultative anaerobes. Previous studies have shown that alpha-amylase activity is enhanced in starch- or maltose-based environments (Higuchi et al., 2005). This explains the sustained lactic acid production at 50–100 g/L substrate concentrations. However, at 150 g/L (P3), osmotic inhibition may have reduced enzyme activity and microbial efficiency. For fruit-based substrates, lactic acid production depends on the presence of simple sugars like glucose and fructose, which are rapidly consumed during early fermentation. However, rapid sugar depletion, coupled with rising pH and microbial community shifts, may suppress continued lactic acid accumulation. As such, substrate composition, sugar availability, and microbial resilience to environmental stress are key determinants of lactic acid productivity.

Recent literature provides additional insights into these dynamics. For example, Jodhani et al., (2024) demonstrated that inoculum type and pre-treatment significantly affect fermentation outcomes; untreated anaerobic digester sludge yielded higher hydrolysis efficiency than waste-activated sludge. This aligns with the present study's use of rumen fluid, which is naturally rich in hydrolytic and fermentative microbes, supporting both starch hydrolysis and lactic acid fermentation (Jodhani et al., 2024). Moreover, Blasco et al. (2020) emphasized the importance of microbial community composition in acidogenic fermentation. Orders like *Lactobacillales* and *Clostridiales* play critical roles in VFA production, and managing their activity – e.g., by inhibiting methanogens – can shift metabolic pathways to favor acid accumulation (Blasco et al., 2020). This concept is relevant in explaining the transformation of lactic acid to other VFAs in fruit-based digesters after 24 hours.

Innovative strategies such as the Community and Single Microbe Optimisation System (COSMOS) (Raajaraam and Raman, 2024), a microbial community optimization system, has shown that mixed microbial cultures often outperform monocultures, particularly under stress conditions such as high organic loads or pH fluctuations – conditions mirrored in the starch and fruit waste digesters of this study. Furthermore, substrate-inhibition effects, such as those observed in digester P3, are also discussed by Radadiya (2022), who highlighted the need for granular activated carbon and microbial enrichment to

counteract long fermentation times and low product yields under high organic loading rates (Radadiya, 2022). In light of these findings, future process improvements may focus on microbial inoculum engineering, pH control, and substrate optimization to sustain lactic acid production and reduce conversion to secondary metabolites. For instance, the application of free nitrous acid as a pre-treatment (Akaniro et al., 2024) or co-digestion strategies (Choudhury et al., 2024) may further improve lactic acid yields and align with circular bioeconomy goals.

Volatile fatty acid production

This study compared the accumulation of VFAs during anaerobic acidification using starch-based and fruit-based organic waste substrates. The results (Figure 6) show that VFA accumulation increased during the first 24 hours of incubation. Thereafter, some digesters continued to exhibit increasing concentrations, while others experienced a decline. During the bioconversion process, the accumulated VFA concentrations ranged from 20 to 60 mmol/L. Specifically, in digesters P2 and P3, VFA levels continued to rise until 36 hours of incubation. The VFA production rate varied across treatments, with some digesters demonstrating more rapid accumulation than others. These findings indicate that substrate type significantly influences the rate and extent of VFA production.

The results depicted in Figure 6 illustrate the dynamics of VFA production during anaerobic acidification with starch-based substrates (P1–P3) and fruit-based substrates (P4–P6). In general, all digesters showed a sharp increase in VFA concentration within the first 24 hours. Following this, distinct patterns emerged depending on the substrate used. Digesters P1–P3, which received starch-based waste, exhibited a continuous increase in VFA concentration, peaking at 36 hours with the highest concentrations (60 mmol/L) recorded in P2 and P3. In contrast, digesters P4–P6, fed with fruit-based waste, reached peak VFA accumulation earlier, at 24 hours, with the same maximum concentration of 60 mmol/L. After reaching their peaks, VFA concentrations in most digesters began to decline, indicating further conversion or changes in digester environmental conditions.

As shown in Figure 2, pH levels during the initial bioconversion phase dropped to an acidic range of 5.2–5.4. This condition is conducive to the accumulation of VFAs, particularly acetic acid. This finding aligns with the anaerobic acidification process, where microorganisms break down organic matter, producing VFAs and consequently decreasing the pH (Darwin et al., 2018b). Another study has reported that mixed microbial cultures typically produce acetic, propionic, butyric, and valeric acids under such pH conditions (Atasoy and Cetecioglu, 2022). Another study

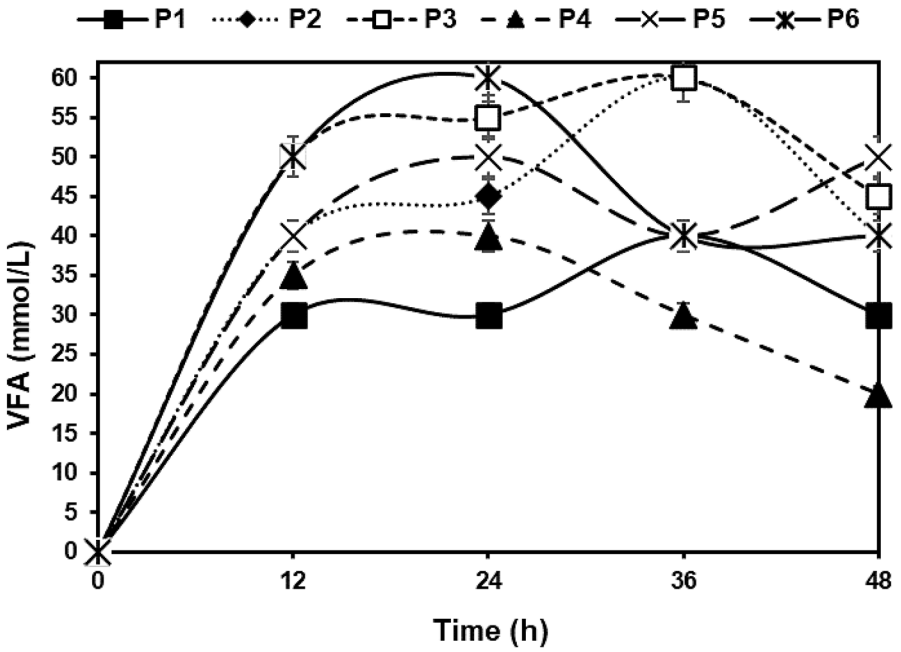

Figure 6. VFA accumulation during the incubation period (0–48 hours)

also noted that anaerobic fermentation of food waste at pH 4–5 mainly results in acetic and butyric acid accumulation (Feng et al., 2018). However, after 24–36 hours, the pH began to rise toward near-neutral levels, from 6.0 to 6.7, indicating a transition to the acetogenesis stage.

These findings are consistent with previous research suggesting that starch-based substrates tend to produce higher VFA concentrations under low pH conditions due to the activity of amylolytic enzymes that break down starch into simple sugars, which are then fermented by facultative anaerobic bacteria. For fruit waste substrates, the higher content of readily available simple sugars may explain the faster, but shorter, VFA production window (Garcia-Aguirre et al., 2017; Higuchi et al., 2005). This study highlights that the specific characteristics of the substrate influence the timing and extent of peak VFA production. Starch-based waste supports longer-duration VFA production compared to fruit-based waste. This difference may be attributed to the nutritional composition of the substrates; fruit waste, rich in simple sugars, decomposes more rapidly into VFAs but accumulates over a shorter period.

Microbial growth profile

The microbial growth patterns observed in each digester varied depending on the type of substrate used, which also influenced the production of metabolites. A prolonged growth phase was observed in digesters fed with starch-based substrates, supporting the production of lactic acid and VFAs. In contrast, fruit waste substrates exhibited different growth dynamics, as shown in Figure 7.

Microbial growth followed distinct phase patterns across the digesters, as presented in Figure 7. Digesters P1–P3 demonstrated an extended growth phase lasting up to 48 hours. This prolonged activity was accompanied by lactic acid production during the 36–48-hour incubation period (Figure 4) and continued accumulation of VFAs until 36 hours (Figure 5). Over the incubation period, amylolytic enzymes present in the rumen fluid converted the starch-based substrates into glucose, which could be readily utilized by microorganisms for sustained growth (Chen and Hsu, 1998). These findings indicate that the metabolite-producing microorganisms in these digesters maintained their activity for a longer period, delaying entry into the death phase. The availability of carbon sources from starch-based substrates likely provided more accessible fermentable sugars, extending microbial activity.

In contrast, digester P4 exhibited a sharp decline in metabolite production after 24 hours, suggesting the early onset of the death phase due to substrate depletion or the presence of

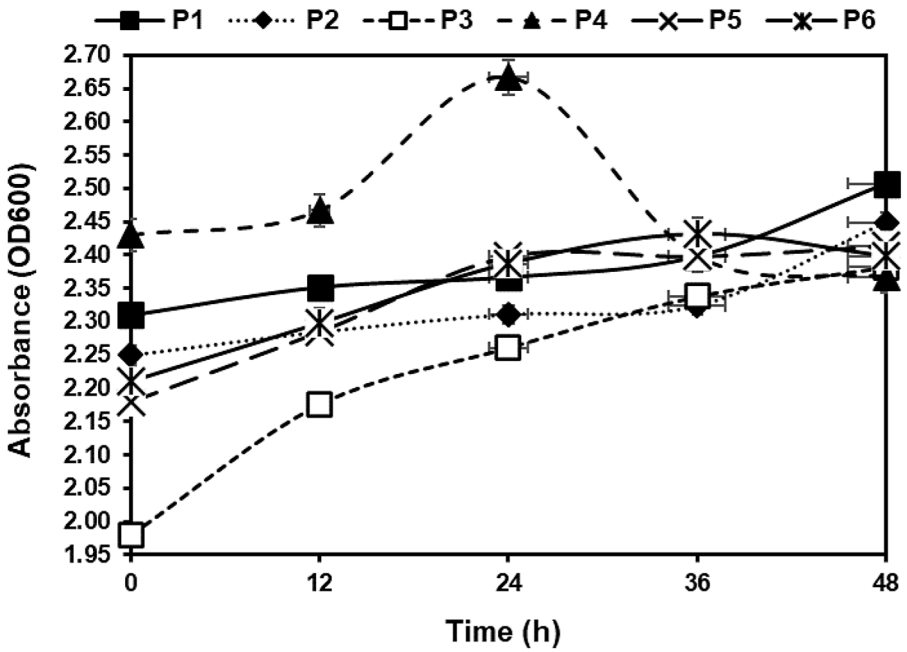

Figure 7. Microbial growth during the incubation period (0–48 hours), measured by optical density at 600 nm (OD600)

inhibitory conditions within the system (Cheng et al., 1991; Kamke et al., 2016). Previous studies have reported that rapid substrate exhaustion or the accumulation of inhibitory by-products, such as low pH, can limit microbial activity (Söllinger et al., 2018). Digesters P5 and P6 maintained microbial growth until around 36 hours; however, a decline in VFA production was observed beyond that point. These suggest that although microbial populations continued to grow, the active metabolite-producing populations may have decreased or been replaced by others with lower metabolic accumulation (Gado, 2024). The variations in microbial growth trends across digesters were influenced by both the substrate type and environmental conditions. Biotransformation using rumen fluid with starch-based substrates (P1–P3) appeared to better support sustained metabolite production compared to fruit waste substrates (P4–P6), likely because the microbial diversity in rumen fluid is more suited for starch degradation and consistent metabolite biosynthesis (Ayudthaya et al., 2018; Darwin et al., 2025; Darwin et al., 2018a).

ORP profile

Fluctuations in ORP values during anaerobic acidification are influenced by microbial metabolism and the oxidation of organic matter into metabolites. Variations in substrate type and concentration significantly affect microbial activity and the redox conditions within the digesters. The ORP profiles obtained in this study are shown in Figure 8.

Based on Figure 8, initially, ORP values in all digesters were low, indicating a reductive environment. These values transitioned toward more oxidative conditions as microbial activity increased and lactic acid and VFA accumulation occurred (Shin et al., 2022; Viet et al., 2008). In general, ORP values increased within the first 24 hours due to ongoing oxidation processes. However, digester P3 exhibited a more rapid decline in ORP after 24 hours, possibly due to substrate interactions or unique microbial responses within that system (Shin et al., 2022). The type and composition of substrates significantly influenced microbial metabolic pathways, thereby altering ORP dynamics (Silva et al., 2013). Previous studies have reported that anaerobic microorganisms, such as *Clostridium* strains, adapt their metabolic pathways according to substrate availability, optimizing the conversion of organic matter into metabolites and affecting ORP fluctuations (Martínez-Ruano et al., 2024). While ORP serves as a useful indicator for monitoring microbial activity, it does not always accurately predict VFA production, as other factors, such as temperature and substrate characteristics, also play important roles in the outcome of anaerobic digestion (Lee, 2008).

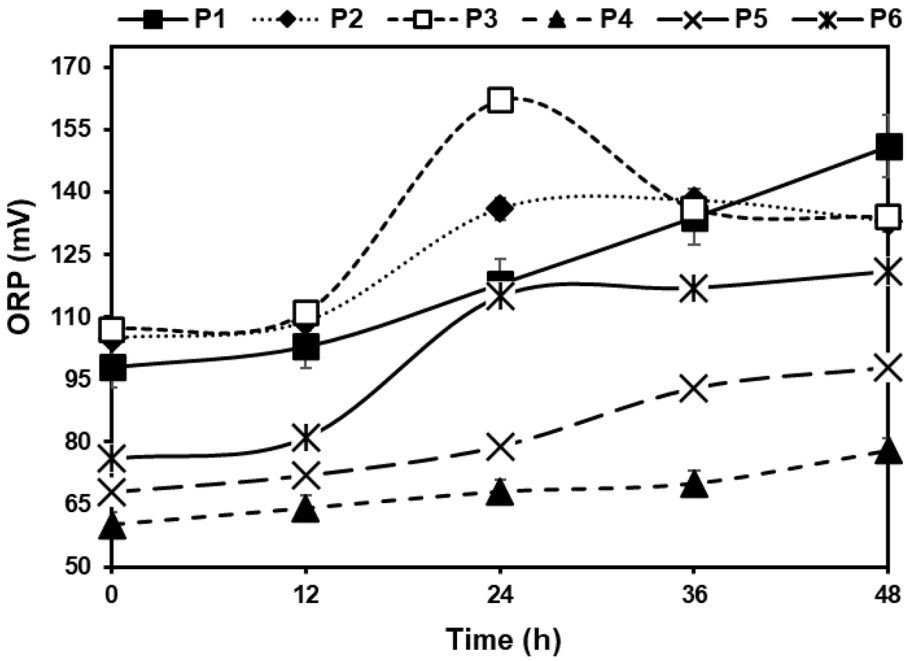

Figure 8. Redox potential during the incubation process

EC profile

The electrical conductivity (EC) profile during anaerobic acidification provides crucial insights into ion uptake by microorganisms and the effect of substrates on bioconversion efficiency. The observed decline in EC during the first 24 hours reflects active ion consumption by microorganisms to support metabolic activity. Variations in EC reduction across different substrates highlight the significance of substrate composition in influencing ion dynamics during this process. The EC analysis results from this study are presented in Figure 9.

The EC profile during anaerobic acidification was influenced by substrate composition, which governs the availability and dynamics of essential ions for microbial metabolism. The initial EC decline reflects the uptake of ions such as ammonium and volatile fatty acids, which are crucial for microbial energy production (Martin et al., 2018). Substrates P1, P2, and P3 exhibited significant EC reductions followed by stabilization, indicating a balance between ion consumption and ion release due to microbial lysis or the breakdown of complex compounds (Martin et al., 2018). Conversely, substrates P4, P5, and P6 displayed more stable EC trends, likely due to simpler or more easily metabolized compositions (Choi et al., 2024). Additionally, the presence of conductive materials may enhance interspecies electron transfer, thereby influencing ion dynamics and microbial efficiency (Shekhurdina et al., 2023; Wang et al., 2023). While substrate composition is a key factor, variations in microbial community structure and external factors such as pH and electron transfer mechanisms also play significant roles in determining the outcomes of the anaerobic digestion process (Lu et al., 2023; Zhou et al., 2021). These factors may serve as focal points for future research.

CONCLUSIONS

This study demonstrated the effective use of rumen fluid microbiota, sourced from slaughterhouse waste, as a potent inoculum for the anaerobic acidification of fruit and starch-based residues, producing LA and VFAs. Starch-based substrates, particularly cassava waste, enabled more stable and higher LA accumulation at moderate concentrations (50–100 g/L), likely due to the gradual enzymatic hydrolysis of complex carbohydrates. However, increasing the substrate dose to 150 g/L led to substrate inhibition, reducing LA yield. In contrast, rich in simple sugars, fruit waste substrates supported rapid initial LA production, but were less effective in sustaining yields due to fast sugar depletion and pH instability. Notably, VFA production was consistent across all substrate types and concentrations, suggesting that VFA synthesis was less sensitive to substrate dose. These findings emphasize the importance of substrate type and loading in optimizing bioconversion

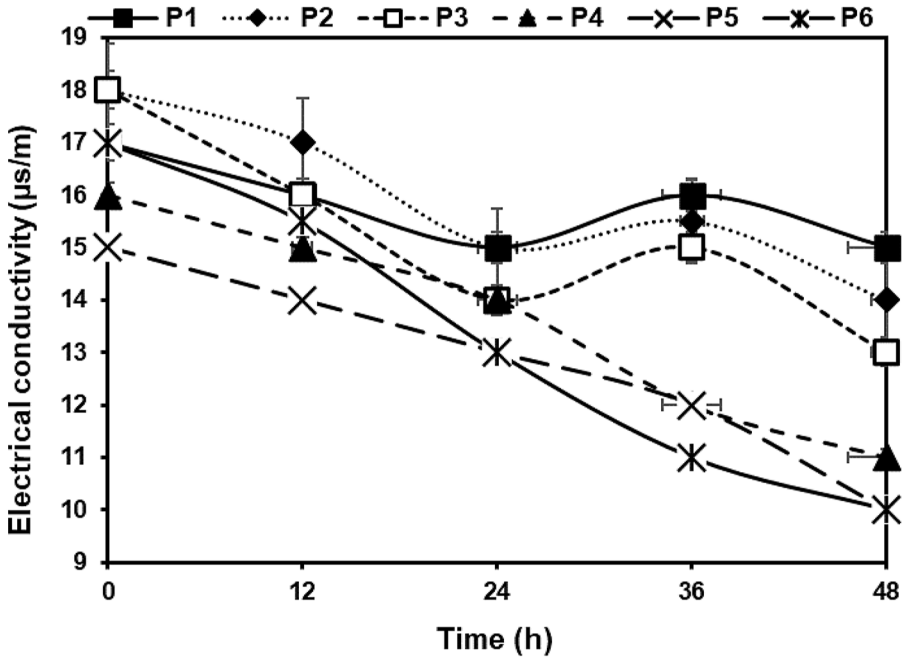

Figure 9. Electrical conductivity profile during anaerobic acidification

efficiency and support the integration of rumen-based inocula into circular bioeconomy strategies for organic waste valorization. Future research should focus on process optimization and scale-up for industrial applications.

Acknowledgements

The authors acknowledge Universitas Syiah Kuala for funding the research project under the PRUUPD scheme (grant number: 444/UN11.2.1/PG.01.03/SPK/PTNBH/2024), established by the Institute for Research and Community Services (LPPM-USK).

REFERENCES

1. Adekunle, K. F., Okolie, J. A. (2015). A review of the biochemical process of anaerobic digestion. *Advances in Bioscience and Biotechnology*, *6*(3), 205–212. https://doi.org/10.4236/abb.2015.63020
2. Airlangga, B., Sugianto, A. M., Parahita, G., Puspasari, F., Mayangsari, N. E., Trisanti, P. N., Sutikno, J. P., Sumarno, S. (2021). Study of cassava starch degradation using sonication process in aqueous sodium chloride. *Journal of the Science of Food and Agriculture*, *101*(6), 2406–2413. https://doi.org/10.1002/jsfa.10864
3. Akaniro, I. R., Zhao, J., Nyoyoko, V. F., Onwosi, C. O. (2024). Free nitrous acid-assisted bioresource recovery from anaerobic digestion of organic materials. *Journal of Environmental Chemical Engineering*, *12*(3), 112624. https://doi.org/10.1016/j.jece.2024.112624
4. Ali Shah, F., Mahmood, Q., Maroof Shah, M., Pervez, A., Ahmad Asad, S. (2014). Microbial ecology of anaerobic digesters: The key players of anaerobiosis. *The Scientific World Journal*, *2014*, 1–21. https://doi.org/10.1155/2014/183752
5. Alkaya, E., Demirer, G. N. (2011). Anaerobic acidification of sugar-beet processing wastes: Effect of operational parameters. *Biomass and Bioenergy*, *35*(1), 32–39. https://doi.org/10.1016/j.biombioe.2010.08.002
6. Amha, Y. M., Anwar, M. Z., Brower, A., Jacobsen, C. S., Stadler, L. B., Webster, T. M., Smith, A. L. (2018). Inhibition of anaerobic digestion processes: Applications of molecular tools. *Bioresource Technology*, *247*, 999–1014. https://doi.org/10.1016/j.biortech.2017.08.210
7. APHA. (2012). *Standard Methods for the Examination of Water and Wastewater*.
8. Atasoy, M., Cetecioglu, Z. (2022). The effects of pH on the production of volatile fatty acids and microbial dynamics in long-term reactor operation. *Journal of Environmental Management*, *319*(July), 115700. https://doi.org/10.1016/j.jenvman.2022.115700
9. Ayudthaya, S. P. N., Van De Weijer, A. H. P., Van Gelder, A. H., Stams, A. J. M., De Vos, W. M., Plugge, C. M. (2018). Organic acid production from potato starch waste fermentation by rumen microbial communities from Dutch and Thai dairy cows. *Biotechnology for Biofuels*, *11*(1), 1–15. https://doi.org/10.1186/s13068-018-1012-4
10. Beede, D. K. (2012). What will our ruminants drink? *Animal Frontiers*, *2*(2), 36–43. https://doi.org/10.2527/af.2012-0040
11. Blasco, L., Kahala, M., Tampio, E., Vainio, M., Ervasti, S., Rasi, S. (2020). Effect of inoculum pretreatment on the composition of microbial communities in anaerobic digesters producing volatile fatty acids. *Microorganisms*, *8*(4), 581. https://doi.org/10.3390/microorganisms8040581
12. Butler, B. A., Ford, R. G. (2018). Evaluating relationships between total dissolved solids (TDS) and total suspended solids (TSS) in a mining-influenced watershed. *Mine Water and the Environment*, *37*(1), 18–30. https://doi.org/10.1007/s10230-017-0484-y
13. Chen, C. Y., Hsu, J. T. (1998). The effects of starch and protein degradation rates, hay sources, and feeding frequency on rumen microbial fermentation in a continuous culture system. *Proceedings of the National Science Council, Republic of China. Part B, Life Sciences*, *22*(4), 159–165.
14. Cheng, K.-J., Forsberg, C. W., Minato, H., Costerton, J. W. (1991). Microbial Ecology and Physiology of Feed Degradation within the Rumen. *Physiological Aspects of Digestion and Metabolism in Ruminants* 595–624. https://doi.org/10.1016/B978-0-12-702290-1.50031-X
15. Cheung, J. T. H., Lok, J., Gietel-Basten, S., Koh, K. (2021). The food environments of fruit and vegetable consumption in East and Southeast Asia: A systematic review. *Nutrients*, *13*(1), 148. https://doi.org/10.3390/nu13010148
16. Chisenga, S. M., Workneh, T. S., Bultosa, G., Alimi, B. A. (2019). Progress in research and applications of cassava flour and starch: a review. *Journal of Food Science and Technology*, *56*(6), 2799–2813. https://doi.org/10.1007/s13197-019-03814-6
17. Choi, Y., Kim, D., Choi, H., Cha, J., Baek, G., Lee, C. (2024). Comparative study of exoelectrogenic utilization preferences and hydrogen conversion among major fermentation products in microbial electrolysis cells. *Bioresource Technology*, *393*, 130032. https://doi.org/10.1016/j.biortech.2023.130032
18. Choudhury, A. R., Singh, N., Lalwani, J., Srinivasan, H., Palani, S. G. (2024). Enhancing biomethanation performance through co-digestion of diverse

organic wastes: a comprehensive study on substrate optimization, inoculum selection, and microbial community analysis. *Environmental Science and Pollution Research*, *31*(23), 34622–34646. https://doi.org/10.1007/s11356-024-33557-7

19. Darwin, Aldama, S., & Masturi. (2022). Effects of the different loading characters of cattle manure as inoculum towards the composting process of coconut pulp waste. *International Journal of Design & Nature and Ecodynamics*, *17*(3), 475–479. https://doi.org/10.18280/ijdne.170320
20. Darwin, Barnes, A., Cord-Ruwisch, R. (2018). In vitro rumen fermentation of soluble and non-soluble polymeric carbohydrates in relation to ruminal acidosis. *Annals of Microbiology*, *68*(1), 1–8. https://doi.org/10.1007/s13213-017-1307-x
21. Darwin, Bulan, R., Fitria, S. R., Kacaribu, A. A. (2025). Impact of silages made of lignocellulosic biomass on rumen fermentation in-vitro and potential mitigation of acid accumulation by bentonite supplementation. *International Journal of Design & Nature and Ecodynamics*, *20*(2), 265–273. https://doi.org/10.18280/ijdne.200205
22. Darwin, Charles, W., Cord-Ruwisch, R. (2019). Anaerobic acidification of sugar-containing wastewater for biotechnological production of organic acids and ethanol. *Environmental Technology*, *40*(25), 3276–3286. https://doi.org/10.1080/09593330.2018.1468489
23. Darwin, Cord-Ruwisch, R., Charles, W. (2018). Ethanol and lactic acid production from sugar and starch wastes by anaerobic acidification. *Engineering in Life Sciences*, *18*(9), 635–642. https://doi.org/10.1002/elsc.201700178
24. Darwin, D. (2019). Rapid determination of lactic acid in anaerobic biological treatment process using a portable sensitive lactate biosensor. *BioTechnologia*, *100*(2), 115–120. https://doi.org/10.5114/bta.2019.85320
25. Darwin, Diana, N., Mardhotillah, Pratama, A. (2021a). Anaerobic co-digestion of cow manure and palm oil mill effluent (POME): assessment of methane production and biodegradation efficiency. *International Journal of Design & Nature and Ecodynamics*, *16*(6), 671–676. https://doi.org/10.18280/ijdne.160608
26. Darwin, Pratama, A., Mardhotillah. (2021b). Organic waste conversion via continuous anaerobic co-digestion of oil palm empty fruit bunches and cow manure: evaluation of feeding regime on methane production. *Acta Technologica Agriculturae*, *24*(1), 8–13. https://doi.org/10.2478/ata-2021-0002
27. Darwin, Thifal M., Alwi M., Murizal Z., Pratama A., Rizal M. (2023). The synthesis of biodiesel from palm oil and waste cooking oil via electrolysis by various electrodes, Case Studies in Chemical and Environmental Engineering, 8, 100512. https://doi.org/10.1016/j.cscee.2023.100512
28. Darwin, Triovanta, U., Rinaldi, R. (2019). Two-stage anaerobic co-digestion of landfill leachate and starch wastes using anaerobic biofilm reactor for methane production. *Progress in Agricultural Engineering Sciences*, *15*(1), 53–70. https://doi.org/10.1556/446.15.2019.1.4
29. Darwin, Triovanta, U., Rinaldi, R., Pratama, A. (2019). Anaerobic acidification of coconut water waste by *Lactobacillus acidophilus* culture for biotechnological production of lactic acid. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *67*(6), 1433–1440. https://doi.org/10.11118/actaun201967061433
30. de Almeida, R. T. R., do Prado, R. M., Porto, C., dos Santos, G. T., Huws, S. A., Pilau, E. J. (2018). Exploring the rumen fluid metabolome using liquid chromatography-high-resolution mass spectrometry and Molecular Networking. *Scientific Reports*, *8*(1), 17971. https://doi.org/10.1038/s41598-018-36196-4
31. Dumbrepatil, A., Adsul, M., Chaudhari, S., Khire, J., Gokhale, D. (2008). Utilization of molasses sugar for lactic acid production by Lactobacillus delbrueckii subsp. delbrueckii mutant Uc-3 in batch fermentation. *Applied and Environmental Microbiology*, *74*(1), 333–335. https://doi.org/10.1128/AEM.01595-07
32. FAO. (2013). Food Wastage Foot Print Impacts on Natural Resources. In *Fao*.
33. Feng, K., Li, H., Zheng, C. (2018). Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. *Bioresource Technology*, *270*(August), 180–188. https://doi.org/10.1016/j.biortech.2018.09.035
34. Franke-Whittle, I. H., Walter, A., Ebner, C., Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management*, *34*(11), 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020
35. Gado, H. M. (2024). Mathematical models to evaluate the probiotic effect on the ruminant digestive system. *Egyptian Journal of Nutrition and Feeds*, *27*(2), 129–138. https://doi.org/10.21608/ejnf.2024.377394
36. Garcia-Aguirre, J., Aymerich, E., González-Mtnez de Goñi, J., Esteban-Gutiérrez, M. (2017). Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. *Bioresource Technology*, *244*(Pt 1), 1081–1088. https://doi.org/10.1016/j.biortech.2017.07.187
37. Grzelak, J., Ślęzak, R., Krzystek, L., Ledakowicz, S. (2018). Effect of pH on the production of volatile fatty acids in dark fermentation process of organic waste. *Ecological Chemistry and Engineering S*, *25*(2), 295–306. https://doi.org/10.1515/eces-2018-0020
38. Herbert, D., Phipps, P. J., Strange, R. E. (1971). Chapter III chemical analysis of microbial cells. In *Methods in Microbiology* 5, 209–344. https://doi.

org/10.1016/S0580-9517(08)70641-X

39. Higuchi, Y., Ohashi, A., Imachi, H., Harada, H. (2005). Hydrolytic activity of alpha-amylase in anaerobic digested sludge. *Water Science and Technology: A Journal of the International Association on Water Pollution Research*, *52*(1–2), 259–266.
40. Jodhani, S., Sebastian, J., Lee, J., Venkiteshwaran, K., Lee, H.-S., Singh, V., Ormeci, B., Hussain, A. (2024). Acidogenic fermentation of food waste for the production of short-chain fatty acids: The impact of inoculum type and inoculum heat pretreatment. *Fermentation*, *10*(3), 162. https://doi.org/10.3390/fermentation10030162
41. Kacaribu, A. A., Darwin. (2024a). Sustainable bioproduct production via anaerobic bioconversion by landfill soil inoculum in various carbohydrate wastes. *Acta Technologica Agriculturae*, *27*(2), 61–68. https://doi.org/10.2478/ata-2024-0009
42. Kacaribu, A. A., Darwin, D. (2024b). Biotechnological lactic acid production from low-cost renewable sources via anaerobic microbial processes. *BioTechnologia*, *105*(2), 179–194. https://doi.org/10.5114/bta.2024.139757
43. Kacaribu, A. A., Darwin, D., Aisyah, Y., Febriani, F. (2025). Anaerobic bio-processing of agricultural waste for the biotechnological production of lactic acid and volatile fatty acid by landfill soil inoculums. *Research in Agricultural Engineering*, *71*(1), 10–21. https://doi.org/10.17221/52/2024-RAE
44. Kamke, J., Kittelmann, S., Soni, P., Li, Y., Tavendale, M., Ganesh, S., Janssen, P. H., Shi, W., Froula, J., Rubin, E. M., Attwood, G. T. (2016). Rumen metagenome and metatranscriptome analyses of low methane yield sheep reveals a Sharpea-enriched microbiome characterised by lactic acid formation and utilisation. *Microbiome*, *4*(1), 56. https://doi.org/10.1186/s40168-016-0201-2
45. Kharola, S., Ram, M., Goyal, N., Mangla, S. K., Nautiyal, O. P., Rawat, A., Kazancoglu, Y., Pant, D. (2022). Barriers to organic waste management in a circular economy. *Journal of Cleaner Production*, *362*, 132282. https://doi.org/10.1016/j.jclepro.2022.132282
46. Lee, S. J. (2008). Relationship between oxidation reduction potential (ORP) and volatile fatty acid (VFA) production in the acid-phase anaerobic digestion process. In *Thesis* (Issue January).
47. Li, B., Liu, C., Bai, J., Huang, Y., Su, R., Wei, Y., Ma, B. (2024). Strategy to mitigate substrate inhibition in wastewater treatment systems. *Nature Communications*, *15*(1), 7920. https://doi.org/10.1038/s41467-024-52364-9
48. Litti, Y., Zhuravleva, E., Kovalev, A. (2024). Anaerobic fermentation and high-value bioproducts: a brief overview of recent progress and current challenges. *Fermentation*, *10*(11), 537. https://doi.org/10.3390/fermentation10110537
49. Lu, W., Song, Y., Liu, C., Dong, H., Li, H., Huang, Y., Liang, Z., Xu, H., Wu, H., Li, P., Sun, D., Xu, K., Dang, Y. (2023). Microbial electrochemical CO_2 reduction and in-situ biogas upgrading at various pH conditions. *Fermentation*, *9*(5), 444. https://doi.org/10.3390/fermentation9050444
50. Lützhøft, H.-C. H., Boe, K., Fang, C., Angelidaki, I. (2014). Comparison of VFA titration procedures used for monitoring the biogas process. *Water Research*, *54*, 262–272. https://doi.org/10.1016/j.watres.2014.02.001
51. Martin, A. L., Satjaritanun, P., Shimpalee, S., Devivo, B. A., Weidner, J., Greenway, S., Henson, J. M., Turick, C. E. (2018). In-situ electrochemical analysis of microbial activity. *AMB Express*, *8*(1), 162. https://doi.org/10.1186/s13568-018-0692-2
52. Martínez-Ruano, J. A., Suazo, A., Véliz, F., Otalora, F., Conejeros, R., González, E., Aroca, G. (2024). Effect of pH on metabolic pathway shift in fermentation and electro-fermentation of xylose by Clostridium autoethanogenum. *Journal of Environmental Management*, *351*, 119918. https://doi.org/10.1016/j.jenvman.2023.119918
53. Martins, F., Abreu, M. C. C., Diniz, P. A. F., Marques, G. N., Oliveira, M. M., de Jesus Silva Mendonça, C., de Figueredo, G. P., Cavalcante, K. S. B. (2023). Hydrolysis of cassava's (*Manihot esculenta* Crantz) waste polysaccharides using the natural zeolite Stilbite-Ca as catalyst. *Bioresource Technology Reports*, *21*(January). https://doi.org/10.1016/j.biteb.2023.101384
54. Ojo, A. O., & de Smidt, O. (2023). Lactic acid: A comprehensive review of production to purification. *Processes*, *11*(3), 688. https://doi.org/10.3390/pr11030688
55. Parchami, M., Uwineza, C., Ibeabuchi, O. H., Rustas, B.-O., Taherzadeh, M. J., Mahboubi, A. (2023). Membrane bioreactor assisted volatile fatty acids production from agro-industrial residues for ruminant feed application. *Waste Management*, *170*(July), 62–74. https://doi.org/10.1016/j.wasman.2023.07.032
56. Pau, S., Tan, L. C., Arriaga, S., Lens, P. N. L. (2024). Lactic acid fermentation of food waste at acidic conditions in a semicontinuous system: effect of HRT and OLR changes. *Biomass Conversion and Biorefinery*, *14*(10), 10979–10994. https://doi.org/10.1007/s13399-022-03201-w
57. Pourbayramian, R., Abdi-Benemar, H., Seifdavati, J., Greiner, R., Elghandour, M. M. M. Y., Salem, A. Z. M. (2021). Bioconversion of potato waste by rumen fluid from slaughterhouses to produce a potential feed additive rich in volatile fatty acids for farm animals. *Journal of Cleaner Production*, *280*, 124411. https://doi.org/10.1016/j.jclepro.2020.124411
58. Raajaraam, L., Raman, K. (2024). *COSMOS:*

Community and Single Microbe Optimisation System. https://doi.org/10.1101/2024.10.31.621216

59. Radadiya, P. (2022). *Acidogenic Fermentation of Food Waste in a Leachate Bed Reactor at High Organic Loading: Effect of Granular Activated Carbon (GAC) and Inoculum* [Carleton University]. https://doi.org/10.22215/etd/2022-15093
60. Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., Liu, Y. (2018). A comprehensive review on food waste anaerobic digestion: Research updates and tendencies. *Bioresource Technology*, *247*(September 2017), 1069–1076. https://doi.org/10.1016/j.biortech.2017.09.109
61. Robergs, R. A., McNulty, C. R., Minett, G. M., Holland, J., Trajano, G. (2018). Lactate, not Lactic Acid, is Produced by Cellular Cytosolic Energy Catabolism. *Physiology*, *33*(1), 10–12. https://doi.org/10.1152/physiol.00033.2017
62. Sanjorjo, R. A., Tseten, T., Kang, M.-K., Kwon, M., Kim, S.-W. (2023). In pursuit of understanding the rumen microbiome. *Fermentation*, *9*(2), 114. https://doi.org/10.3390/fermentation9020114
63. Shekhurdina, S., Zhuravleva, E., Kovalev, A., Andreev, E., Kryukov, E., Loiko, N., Laikova, A., Popova, N., Kovalev, D., Vivekanand, V., Litti, Y. (2023). Comparative effect of conductive and dielectric materials on methanogenesis from highly concentrated volatile fatty acids. *Bioresource Technology*, *377*, 128966. https://doi.org/10.1016/j.biortech.2023.128966
64. Shin, S. G., Park, S. H., Hwang, S. (2022). Substrate characteristics fluctuations in full-scale anaerobic digesters treating food waste at marginal organic loading rates: a case study. *Energies*, *15*(9), 3471. https://doi.org/10.3390/en15093471
65. Silva, F. C., Serafim, L. S., Nadais, H., Arroja, L., Capela, I. (2013). Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids. *Chemical and Biochemical Engineering Quarterly*, *27*(4), 467–476.
66. Söllinger, A., Tveit, A. T., Poulsen, M., Noel, S. J., Bengtsson, M., Bernhardt, J., Frydendahl Hellwing, A. L., Lund, P., Riedel, K., Schleper, C., Højberg, O., Urich, T. (2018). Holistic Assessment of rumen microbiome dynamics through quantitative metatranscriptomics reveals multifunctional redundancy during key steps of anaerobic feed degradation. *MSystems*, *3*(4). https://doi.org/10.1128/msystems.00038-18
67. Son, M.-S., Kwon, Y.-J. (2013). Direct fermentation of starch to l(+)-Lactic acid by fed-batch culture of Lactobacillus manihotivorans. *Food Science and Biotechnology*, *22*(1), 289–293. https://doi.org/10.1007/s10068-013-0040-x
68. Tang, J., Wang, X. C., Hu, Y., Zhang, Y., Li, Y. (2017). Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. *Bioresource Technology*, *224*, 544–552. https://doi.org/10.1016/j.biortech.2016.11.111
69. Uddin, M. M., Wright, M. M. (2023). Anaerobic digestion fundamentals, challenges, and technological advances. *Physical Sciences Reviews*, *8*(9), 2819–2837. https://doi.org/10.1515/psr-2021-0068
70. Viet, T. N., Behera, S. K., Kim, J.-W., Park, H.-S. (2008). Effects of oxidation reduction potential and organic compounds on anammox reaction in batch cultures. *Environmental Engineering Research*, *13*(4), 210–215. https://doi.org/10.4491/eer.2008.13.4.210
71. Villanueva-Galindo, E., Pérez-Rangel, M., Moreno-Andrade, I. (2024). Lactic acid production from different sources of organic solid waste: evaluation of the inoculum type and operational optimization. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-05563-9
72. Wainaina, S., Lukitawesa, Kumar Awasthi, M., Taherzadeh, M. J. (2019). Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. *Bioengineered*, *10*(1), 437–458. https://doi.org/10.1080/21655979.2019.1673937
73. Wang, M., Ren, T., Yin, M., Lu, K., Xu, H., Huang, X., Zhang, X. (2023). Enhanced anaerobic wastewater treatment by a binary electroactive material: pseudocapacitance/conductance-mediated microbial interspecies electron transfer. *Environmental Science & Technology*, *57*(32), 12072–12082. https://doi.org/10.1021/acs.est.3c01986
74. Zabed, H., Sahu, J. N., Suely, A., Boyce, A. N., Faruq, G. (2017). Bioethanol production from renewable sources: Current perspectives and technological progress. *Renewable and Sustainable Energy Reviews*, *71*(January), 475–501. https://doi.org/10.1016/j.rser.2016.12.076
75. Zhang, L., Loh, K.-C., Dai, Y., Tong, Y. W. (2020). Acidogenic fermentation of food waste for production of volatile fatty acids: Bacterial community analysis and semi-continuous operation. *Waste Management*, *109*, 75–84. https://doi.org/10.1016/j.wasman.2020.04.052
76. Zhou, Q., Yang, N., Zheng, D., Zhang, L., Tian, C., Yang, Q., Li, D. (2021). Electrode-dependent ammonium oxidation with different low C/N ratios in single-chambered microbial electrolysis cells. *Bioelectrochemistry*, *142*, 107889. https://doi.org/10.1016/j.bioelechem.2021.107889
77. Zia, M., Ahmed, S., Kumar, A. (2022). Anaerobic digestion (AD) of fruit and vegetable market waste (FVMW): potential of FVMW, bioreactor performance, co-substrates, and pre-treatment techniques. *Biomass Conversion and Biorefinery*, *12*(8), 3573–3592. https://doi.org/10.1007/s13399-020-00979-5