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Anaerobic bio-processing of agricultural waste for the biotechnological production of lactic acid and volatile fatty acid by landfill soil inoculums

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Abstract: With the increase in the world population and the ensuing surge in organic waste, effective management strategies are crucial to prevent environmental pollution. This study aims to address this challenge by utilising organic waste (OW) as the substrate for the production of lactic acid (LA) and volatile fatty acids (VFAs) through anaerobic bioprocessing. The substrates used, included grass, starch, and fruit wastes inoculated with non-sterile inoculum landfill soil (LS). The anaerobic bioconversion was performed by varying the substrate to the inoculum. The results unveil that a digester loaded with 150 $g \cdot L^{-1}$ of fruit waste, exhibits the highest concentration of LA, reaching a significance of 25 $mmol \cdot L^{-1}$. A digester fed with 100 $g \cdot L^{-1}$ starch, also manifests significant LA production (18.50 $mmol \cdot L^{-1}$). A digester, supplied with 150 $g \cdot L^{-1}$ starch waste, showcases the highest VFA (92.5 $mmol \cdot L^{-1}$). Intriguingly, the anaerobic bioprocessing of the grass substrate did not produce LA at all, yet al. the substrates showcased VFA production, albeit with fluctuating and lower concentrations. This study highlights the potential of incorporating simple sugar for enhanced LA production and starch-based substrates for increased VFA production when utilising LS as the inoculum. The anaerobic bioprocessing shows promising outcomes for the future development in sustainable waste utilisation.

Keywords: bioproducts; waste conversion; biochemicals; organic acids

The rapid growth of the global population has led to an increase in the amount of organic waste (OW) generated. This OW is derived from various sectors, including agriculture, industry, and urban areas, making it one of the important global environmental issues today. It is estimated that as much as 6 million tonnes of OW will be generated worldwide every day by 2025 (WEC 2016). While OW is rich in organic matter and nutrients, inadequate management practices may generate some problems, such as pol-

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lution, greenhouse gas emissions, and adverse environmental impacts. Hence, the efficient and sustainable utilisation of OW is crucial to minimising the environmental effects and boost its economic value (Darwin et al. 2021a). Utilising OW as a raw material for producing value-added products, including bioplastics, biofuels, biopolymers, and chemicals, through suitable and sustainable methods is an effective approach to OW management (Sharma et al. 2019; Singh et al. 2021; Vinci et al. 2021).

Organic waste management can be carried out through various methods such as direct disposal in landfills, composting, and anaerobic digestion (AD). Currently, AD is widely accepted as the most efficient, flexible, and environmentally friendly method (Uddin and Wright 2023). This process undergoes decomposition without the presence of oxygen and, synergistically, in four distinct stages including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. AD presents opportunities to produce various types of bioproducts and alternative energy sources. Each stage in AD produces different and diverse products (Darwin et al. 2019a,b; Uddin and Wright 2023). The outcome of final anaerobic digestion (methanogenesis) is methane biogas; however, the intermediate by-products of the AD process, such as volatile fatty acids (VFAs) and lactic acid (LA), possess a higher value compared to methane alone (Kleerebezem et al. 2015).

Lactic acid is an organic acid extensively utilised in various sectors, including the chemical industry (Satyanarayana et al. 2012) and packaging industry (Li et al. 2021). LA can be produced through chemical synthesis or anaerobic fermentation with microorganisms (Wee et al. 2006). The production of chemicals has environmental drawbacks, such as the use of petroleum-derived chemicals, low productivity, and high costs. In contrast, microbial fermentation enables cost-effective, high-yield production using eco-friendly substrates (Alves de Oliveira et al. 2018; Tarraran and Mazzoli 2018). Raw materials, derived from carbohydrate-rich agricultural waste like lignocellulose, starch, and simple sugars (e.g., glucose and fructose), serve as carbon sources (Krull et al. 2020; Martins et al. 2023). Conversely, VFAs, short-chain carboxylic acids, are formed during acidogenesis in the AD of OW. Traditionally produced from petroleum derivatives, this method is deemed unsustainable and non-renewable (Pham et al. 2021; Feng et al. 2022). VFAs serve as precursors for the production of various products such as biodegradable plastics (Perez-Zabaleta et al. 2021), and biochemicals (Ramos-Suarez et al. 2021).

The production of lactic acid and volatile fatty acids from OW depends on the sugar composition, where the applied substrate concentration significantly influences the production rates and yields. (Atasoy et al. 2018). The AD of OW using landfill soil (LS) has emerged as an effective treatment strategy. LS acts as an undefined mixed inoculum serving as the source of microorganisms in the AD process, which provides a multitude of living microorganisms capable of anaerobically degrading organic matter into valuable bioproducts (Meyer-Dombard et al. 2020; Rasi et al. 2022). The situation is similar to rumen fluid, containing diverse microorganisms (archaea, bacteria, protozoa, fungi, and viruses) interacting within the ruminant digestive system (Lobo and Faciola 2021). In healthy ruminants, this process can generate LA and VFAs (valeric acid, propionate, butyrate) (Bergman 1990; Jaramillo-López et al. 2017).

Previous studies utilising rumen fluid as inoculum in the AD process have described that, after a 48-hour incubation period, the fermented substrate may generate lactic acid and acetic acid as end products (Darwin et al. 2018b). Other studies have revealed that lactic acid may be generated as a primary product in rumen fermentation, but it also could be in relatively low amounts depending on the operational conditions and the types of substrates that are fed into the process (Darwin et al. 2018b, 2019b). Previous studies have overlooked anaerobic bioprocessing for LA and VFA production utilising LS as a microbial source. Additionally, there is a gap in understanding how the types and compositions of carbohydrates in OW impact the efficiency of the LA and VFA production when LS serves as an undefined mixed inoculum. This study aims to address these gaps by investigating the anaerobic bioprocessing of OW using LS for the production of LA and VFA.

MATERIAL AND METHODS

The experiments were conducted at the Post-Harvest Engineering and Bioprocess Laboratory, Department of Agricultural Engineering, Syiah Kuala University. Various materials were employed, including H_3BO_3, NaOH, HCl, H_2SO_4, phenol (Merck), H_2O, lactic acid, indicators (PP, Methyl Red, Methylene Blue), D-(+)-glucose anhydrous (VWR BDH Prolabo Chemicals).

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Substrate preparation. The study utilised feedstock comprised grass (*Pennisetum purpureum*), starch waste, fruit waste, and landfill soil as the inoculum. Grass was collected from the Lieue Plantation, Aceh Besar Regency, Indonesia. It was finely ground before use. In this study, cassava waste was collected and represented the starch waste. The fruit waste used in this study included pears, melons, watermelons, bananas, and papayas, which were collected from the vegetable and fruit local market in Rukoh Village, Banda Aceh City, Indonesia. These wastes were meticulously separated from impurities, finely crushed, and blended to a reduced size (± 0.1 cm) before experimentation.

Inoculum preparation. The anaerobic bioprocessing employed an undefined mixed culture using landfill soil as the inoculum. The LS was obtained from the Banda Aceh Landfill Site, Aceh, Indonesia. The collection involved gathering LS from areas with waste accumulation. Any undesired materials, such as plastics, stones, and wood, were removed from the LS. The collected LS was filtered to remove any particles and/or contaminants and then placed in sterile tubes. The LS was then spun at 3 000 rpm for 10 min. The LS was then stored in the containers and kept in a fridge at 3.5 ± 0.5 °C for the subsequent analysis and application. Before the start of the experiments, the LS was acclimated under the anaerobic condition to activate the anaerobic microbial community for the subsequent anaerobic bioprocesses.

Anaerobic bioprocessing. The anaerobic bioprocess was conducted without any pH control or adding acids or bases, allowing natural bioconversion. The experiments were performed in sealed digesters with a 250 mL working volume and placed in a thermostatic water bath at 35 ± 0.5 °C for 48 h. Before starting the anaerobic bioconversion process, each digester was purged with nitrogen gas (N_2) to remove the oxygen contamination. Eighteen digesters were prepared, each receiving different substrate and inoculum combinations (50, 100, and 150 g·L^{-1}), as detailed in Table 1. Each treatment was applied in duplicate, and the sampling occurred every 12 h. All the samples were centrifuged for further analysis (Darwin et al. 2018a, b, 2019b, c).

Table 1. Experimental design

Substrate	Substrate concentration (g·L^{-1})	Code
G	50	P1
	100	P2
	150	P3
S	50	P4
	100	P5
	150	P6
F	50	P7
	100	P8
	150	P9

G–grass; S– starch waste; F–fruit waste

Analysis methods. The bioprocess samples underwent centrifugation for 10 min at 2 000 rpm, the supernatant was carefully transferred into tubes and stored at 2 °C in a refrigerator for the subsequent analyses. The analytical procedures encompassed a pH analysis using a laboratory benchtop pH meter equipped with an MW 101 PRO multifunction complete probe, Probe Milwaukee (Darwin et al. 2023). Additionally, assessments were made for the total solids (TSs), and VFAs. For the analysis of the electrical conductivity (EC), examined using the laboratory benchtop pH meter with a multifunction complete probe, oxidation-reduction potential (ORP) (using an ORP sensor) and total ammonia (NH_4^+), a 1 mL sample of the effluent was diluted tenfold with deionised water (DI H_2O) following standard methods (APHA 2012). The lactic acid concentration was measured employing an Accutrend Plus lactate biosensor meter (Darwin 2019). The microbial growth analysis involved determining the optical density (OD) at 600 nm using a Shimadzu 1200 UV spectrophotometer (Shimadzu Corporation, Japan). Furthermore, the carbohydrate content in the samples was quantified using the standard phenol-sulfuric method (Herbert et al. 1971).

Statistical analysis. The experiment was conducted in duplicate (replication), and the data were presented as the mean ± standard deviation (SD). The data analysis was performed using an analysis of variance (ANOVA) with a 95% confidence interval (α: 0.05) using the statistical software Statistical Product and Service Solutions (IBM© SPSS 25).

RESULTS AND DISCUSSION

To examine the characteristics of the substrates and inoculum utilised in the bioprocessing process, a series of tests were conducted and are presented in Table 2. Each substrate exhibited a different total solid content. The substrate starch (S), in particular, had the highest total solid content at 67.88%,

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Table 2. Substrate and inoculum characteristics

Parameters	LS	G	S	F
Total solid (%)	64.65 ± 0.65	16.81 ± 0.1	67.88 ± 0.1	21.695 ± 0.02
Moisture content (%)	35.35 ± 0.65	83.19 ± 0.1	32.12 ± 0.1	78.305 ± 0.02
Ammonia ($mg \cdot L^{-1}$)	6 ± 0	–	–	–
EC ($\mu s \cdot L^{-1}$)	13 ± 0	–	–	–
pH	8.6 ± 0.1	–	7 ± 0.1	7 ± 0.1

LS – landfill soil; G – grass; S – starch waste; F – fruit waste; EC – electrical conductivity; mean ± standard deviation

surpassing the other two substrates including fruit waste (F, 21.695%) and grass (G, 16.81%). The pH values of the substrates S and F used were neutral (7 ± 0.1), while the employed LS inoculum had a basic pH of 8.6 ± 0.1. This agrees with the study reporting that pH values of the studied leachates, from active as well as in active landfills, were in the range of 7.4–8.7, which is representative of leachates from older and/or mature landfills (Jorstad et al. 2004). A pH altering from acid to alkaline would be associated with landfills that age over time (Słomczyńska and Słomczyński 2004; Wdowczyk and Szymańska-Pulikowska 2021). The high pH level in the landfill soil inoculum used in this study may be attributed to the significant accumulation of ammonia present in the landfill soil (Darwin et al. 2019b, c). Additionally, the LS exhibited a high electrical conductivity (EC) value of 13 µs/m due to the abundance of mineral ions/salts within the LS (Haarstad and Mæhlum 2007).

The pH profile is crucial for understanding the digester's environmental dynamics during anaerobic bioprocessing. The pH significantly influences the microorganism activity and can dictate the end-product outcomes. Figure 1 shows that during the 0–48 h bioprocessing period, digesters P4–P9 experienced a shift from alkaline to acidic conditions. This change resulted from the microorganisms in the LS converting the substrate into metabolites, such as VFA and LA. VFAs typically accumulate under acidic to neutral conditions (Garcia-Aguirre et al. 2017), while LA tends to accumulate under acidic pH conditions (Pau et al. 2024). However, a different trend was observed in digesters P1–P3, where the pH gradually decreased, reaching only 7.5. The microorganisms in LS require a longer time to convert the substrate grass as a complex carbohydrate rather than the other substrates. Despite the relatively minor pH decrease, VFA produc-

Table 3. Volatile fatty acid (VFA) production of the different substrates and concentrations during the anaerobic processes

Digester	VFA production of various substrates and concentrations ($mmol \cdot L^{-1}$)				
	0 h	12 h	24 h	36 h	48 h
Substrate G					
P1	0 ± 0[a]	35 ± 0.07[a]	40 ± 0[a]	45 ± 0.07[a]	25 ± 0.07[a]
P2	0 ± 0[a]	25 ± 7.07[a]	35 ± 0.07[a]	40 ± 0[a]	15 ± 0.07[a]
P3	0 ± 0[a]	30 ± 0[a]	25 ± 0.07[a]	45 ± 0.07[a]	25 ± 0.07[a]
Substrate S					
P4	0 ± 0[a]	30 ± 0[a]	60 ± 0[a]	30 ± 0[a]	27.5 ± 3.54[a]
P5	0 ± 0[a]	25 ± 7.07[a]	57.5 ± 3.54[a]	65 ± 3.54[a]	35 ± 7.07[a]
P6	0 ± 0[a]	15 ± 7.07[a]	65 ± 7.07[a]	80 ± 0[a]	92.5 ± 3.54[a]
Substrate F					
P7	0 ± 0[a]	20 ± 0[a]	27.5 ± 0[a]	45 ± 7,07[a]	30 ± 0[a]
P8	0 ± 0[a]	55 ± 7.07[a]	32.5 ± 0[a]	42.5 ± 3.54[a]	35 ± 7.07[a]
P9	0 ± 0[a]	30 ± 0[a]	30 ± 0[a]	47.5 ± 3.54[a]	25 ± 7.07[a]

G–grass; S– starch waste; F–fruit waste; mean ± standard deviation; [a]no significant difference in each measurement within the respective row

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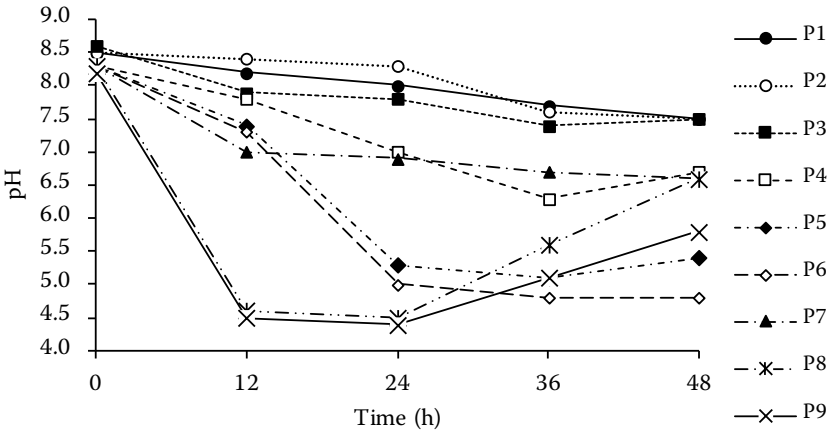

Figure 1. pH profile of the various substrates and applied concentrations
For P1–P9 explanation see Table 1

tion still occurred. This study demonstrates that higher concentrations of VFAs accumulate under acidic conditions (pH 5–6), particularly in digesters P4, P5, and P6 during the 24–36 h bioprocessing period (Table 3). Even in alkaline conditions (pH 7.5–8.5), VFAs still accumulate, albeit at lower concentrations, influenced by the complex metabolic pathways of the microorganism community in the LS (Pham et al. 2021).

Based on Figure 1, digesters P8 and P9 exhibit an extreme pH decrease (8.5–4.5) during the 0–24 h bioprocessing period, representing more acidic conditions among all the digesters. This is due to the accumulation of LA, as depicted in Figure 3. The build-up of LA can lower the digester pH as the substrate undergoes oxidation, increasing the proton concentration. This observation is supported by the ORP data in Figure 6. The significant pH decrease is further influenced by the low pKa value of the LA, approximately 3.86 (Robergs et al. 2018). The substrate F (fruit wastes), comprising simple sugars (fructose and glucose), is more readily utilised by microorganisms as a carbon source. After 24 h of bioprocessing, the pH in this digester rebounds to a range of 5.6–6.6. This phenomenon, known as pH recovery, commonly occurs in the AD process, indicating the completion of acidogenesis and the progression to subsequent stages (Moosbrugger et al. 1993; Adekunle and Okolie 2015; Cioabla et al. 2012).

As shown in Figure 2, it becomes apparent that, with a prolonged bioprocessing time, the concentration of total carbohydrates diminishes. This phenomenon arises from the microbial community utilising the substrate as a carbon source, thereby transforming it into bioproducts, such as lactic acid and volatile fatty acids. Figure 2 shows that digesters supplied with substrate F (fruit waste), namely P7, P8, and P9, with substrate/inoculum compositions of 50, 100, and 150 g·L^{-1}, respectively, exhibit the highest initial total carbohydrate concentrations compared to others: 17.49, 22.75, and 32.64 mmol·L^{-1}, respectively This is due to the simple sugar composition of the fruit waste, containing around 75% simple sugars including fructose and glucose (Zia et al. 2022). Notably, in these digesters, the LA is more dominantly produced than VFA.

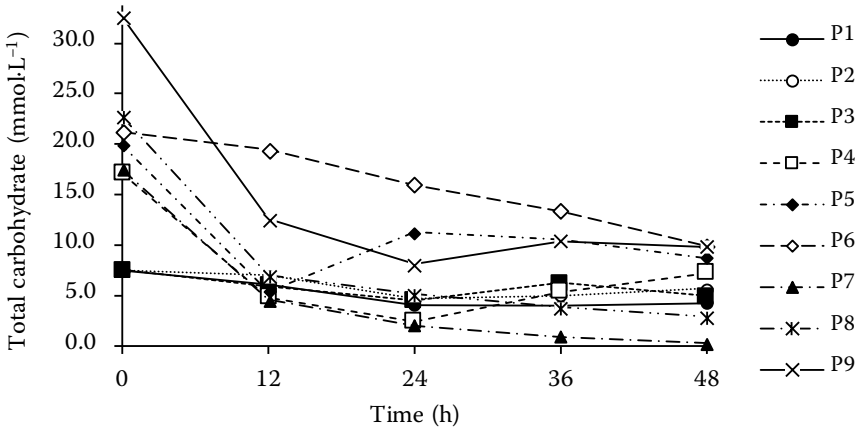

Figure 2. Total carbohydrate content of the various substrates and applied concentration
For P1–P9 explanation see Table 1

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The total carbohydrate analysis in the substrate S (starch wastes) digesters P4, P5, and P6 (with substrate/inoculum compositions of 50, 100, and 150 g·L^{-1}, respectively), shows initial content of 17.11, 19.91, and 21.23 mmol·L^{-1}, respectively. Increasing the substrate concentration leads to a higher total carbohydrate content. The cassava waste contains about 70.66% starch, composed of amylose and amylopectin, resulting in an elevated content in these digesters (Chisenga et al. 2019). As the bioprocessing progresses, the total carbohydrate content decreases. However, a unique occurrence is observed in P5, where, after 12 h, the total carbohydrate content increases. This phenomenon may occur due to the low rate of acidogenesis caused by the limited population of acidogenic bacteria in the digester (Tang et al. 2016).

Digesters supplied with the substrate G (grass), (P1, P2, and P3) show the lowest total carbohydrate concentrations, around 7 mmol·L^{-1}. This is due to substrate G's lignocellulosic nature, comprising cellulose, lignin, and hemicellulose. The robust structure of lignocellulose necessitates pre-treatment for enzymatic hydrolysis, involving a delignification process to break down the structure for the subsequent fermentation into simple sugars (Cubas-Cano et al. 2018). The total carbohydrate analysis, employing the phenol-sulfuric acid method, reveals substrate G has the lowest content, followed by substrate S and substrate F having the highest initial content.

The research findings indicate fluctuating VFA production, with concentrations experiencing both increases and decreases during the bioprocessing period (Table 3). The VFA production shows no significant influence from varying substrate concentrations (50 to 150 g·L^{-1}). After 36 h, the average VFA concentrations decrease, attributed to the onset of the methanogenesis phase, where methanogenic microbes utilise VFAs to produce methane gas (Mir et al. 2016; Zhang et al. 2015). This is supported by some digesters reaching pH values within the methane formation range of 6 to 7.8 (Lay et al. 1997). Contrastingly, digester P6 exhibited the highest VFA production among all the digesters, generating up to 92.5 mmol·L^{-}1 during the 48-hour bioprocessing. Notably, its VFA production concentration did not decrease, suggesting the efficient utilisation of the supplied substrate S by the microorganisms. The sustained low pH value in digester P6 (Figure 1), within the pH 5 range, indicates that the methanogenesis stage did not commence (Qiu et al. 2023). The AD process with starch as a substrate yields more VFA compared to the other substrates, as observed in the digesters with VFA concentrations ranging from 15 to 65 mmol·L^{-1}.

The current study reveals that from 24 to 36 h of incubation period, VFA accumulated in digesters P5 and P6 (Table 3). In this state, the culture was somewhat too acidic with a pH of 5.0 (Figure 1). The VFA build-up in the fermentation culture could be influenced by the complex metabolic pathways of the microorganism community present in the LS (Pham et al. 2021). During anaerobic bioprocess, VFA accumulation frequently occurs when the digester receives a substrate overload, and a drop in pH typically corresponds to the VFA build-up (Basak et al. 2021; Nikita et al. 2022). Some studies mentioned that a high concentration of biodegradable substrates loaded into the anaerobic digester may generate VFA accumulation and lower the pH of the culture (Nguyen et al. 2019; Darwin et al. 2021a). Xu and He (2021) found that the VFA accumulation occurred when a high dosage of glucose as a substrate was loaded to the anaerobic digester. This agrees with the current study that found that high concentration of the soluble as well as insoluble carbohydrates (100–150 g·L^{-1}) represented in the starch and fruit wastes introduced to the anaerobic digester may potentially enhance the production of volatile fatty acids.

The results of the current study reveal that not all the digesters performing anaerobic bioconversion produced lactic acid (LA) as the main metabolite (Figure 3). The results showed that digester P1–P3 loaded with the grass substrate did not produce any LA even though their concentration increased from 50 to 150 g·L^{-1}. This is because the grass is lignocellulosic biomass which is not easily hydrolysed during the anaerobic bioconversion (Yankov 2022). This agrees with the study reporting that the conversion of lignocellulosic biomass into sugars is one of the main problems in biochemical and biofuel production, as inherent biomass recalcitrance may prevent efficient conversion. To enhance the conversion efficiency, pre-treatments should be introduced to the lignocellulosic biomass before using it as the substrate in anaerobic bio-processing (Darwin et al. 2016; Qin et al. 2017).

In addition, digester P4 (50 g·L^{-1} of the substrate concentration) and P6 (150 g·L^{-1} of the substrate concentration) fed with starch waste, did not produce LA

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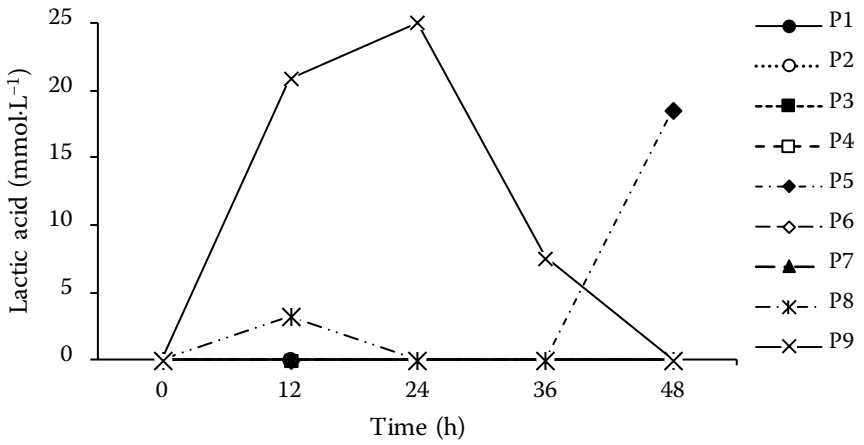

Figure 3. Lactic acid production of the different substrates and concentrations during anaerobic bioprocessing
For P1–P9 explanation see Table 1

also while digester P5 (100 g·L^{-1} of the substrate concentration) produced lactic acid of 18.50 mmol·L^{-1} at 48-h of incubation. This agrees with previous studies finding that digesters supplied with starch-containing substrates can produce lactic acid (Darwin et al. 2019a; Kacaribu and Darwin 2024). In the present study, the statistical analysis (95% confidence interval) revealed no significant influence between the varying concentrations of the substrate S and the LA production. Increasing the substrate concentration of starch waste from 100 to 150 g·L^{-1} generated no lactic acid production, potentially due to the excess substrate or an abundance of the carbon source supply. When the substrate is overloaded, this may hinder lactic acid production, possibly due to substrate inhibition (Dumbrepatil et al. 2008). This is because high substrate concentrations may prolong the lag phase, induce osmotic stress, cause cellular lysis, and reduce the microbial activity (González-Leos et al. 2019).

An interesting result was found in the digesters supplied with fruit waste in which lactic acid was produced where statistical differences were found. The study showed that a significant influence was observed between the varying concentrations of the fruit waste substrate and the lactic acid production. The higher the supplied substrate concentration, the higher the LA concentration within the digester (Figure 3). Within 12 h of incubation, the digester P9 supplied with fruit waste significantly produced lactic acid at around 20.9 mmol·L^{-1}, and the production reached a peak at 24 h of incubation with 25 mmol·L^{-1} of lactic acid. After 24 h of incubation, the production of lactic acid decreased followed by an increase in the pH level from 4.4 to 6.0. At this pH level, the lactic acid production was normally shifted to VFA formation. This agrees with the study reporting that lactic acid production would be optimal at a pH lower than 5.0 while the VFA production tended to be produced at a pH level between 5.5 and 6.0 (Begum et al. 2018; Darwin et al. 2018a, b, 2022).

During bioprocessing, the total NH_4^+ analysis was conducted within the digester, as depicted in Fig-

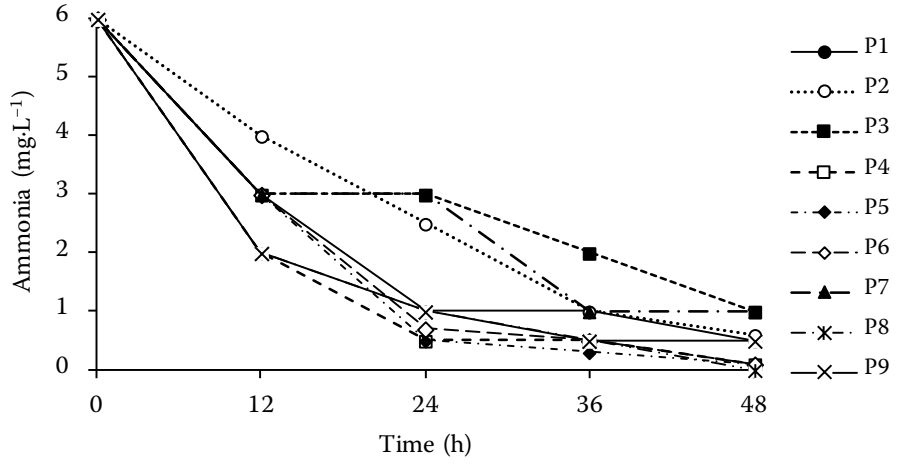

Figure 4. Ammonia profile of the different substrates and concentrations during anaerobic bioprocessing
For P1–P9 explanation see Table 1

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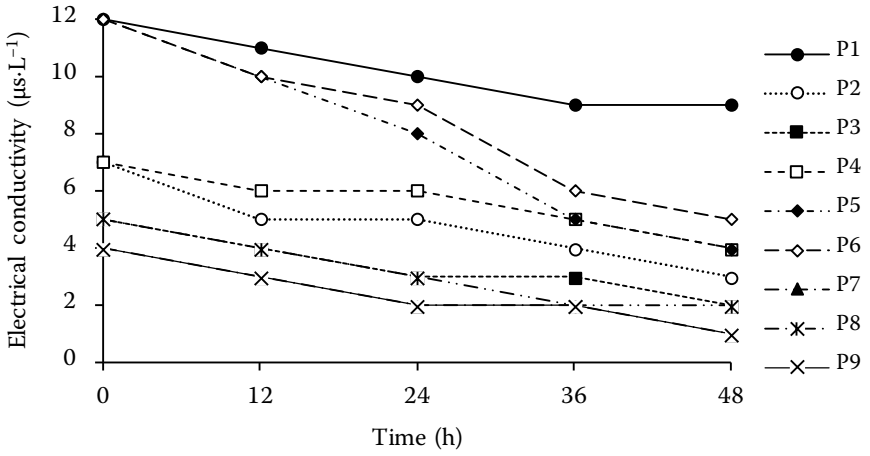

Figure 5. Electrical conductivity profile of the different substrates and concentrations during anaerobic bioprocessing
For P1–P9 explanation see Table 1

ure 4. Figure 4 shows a consistent decrease in the total ammonia throughout the bioprocessing period. This result aligns with the pH conditions in the digester, where pH continued to decrease with the post-substrate bioprocessing. The phenomenon is likely attributed to the LS microorganisms utilising nitrogen-containing organic materials (amino acids, proteins, and urea) as nutrient sources during bioprocessing. No additional nutrients were introduced into the digester before bioprocessing. Lower pH values, typically within the range of 5–6, are associated with reduced ammonia production, as low pH inhibits ammonia production. This finding corresponds with prior research where methane production was notably slower or absent when the digester pH was below 6 during extended digestion times (Gonde et al. 2023).

The EC profile during bioprocessing is shown in Figure 5. The results show that a decline in the EC values across all the digesters. This decrease is attributed to the utilisation of the ions available from the LS by the microorganisms, leading to the reduced electron transport activity among the microorganisms involved in the metabolite formation (Caizán-Juanarena et al. 2020). This finding also aligns with the fact that VFA and LA are produced at low concentrations. This typically leads to an increase in the EC, when these compounds accumulate significantly within the digester, causing an elevation in the proton accumulation due to the oxidation of organic compounds in the digester.

As depicted in Figure 6, digesters P1, P2, and P3 exhibit no significant changes in the ORP values. This is attributed to the absence of substrates oxidising into LA, and the resulting VFAs are also produced in low concentrations (Table 3). Conversely, digesters P5 and P6 follow a different trend. After a 12-h bioprocessing period, the ORP values continue to increase, reaching 150 mV at the 48-h bioprocessing period. This trend corresponds with the LA production in digester P5 and the high VFA production in digester P6. Notably, there is no observed

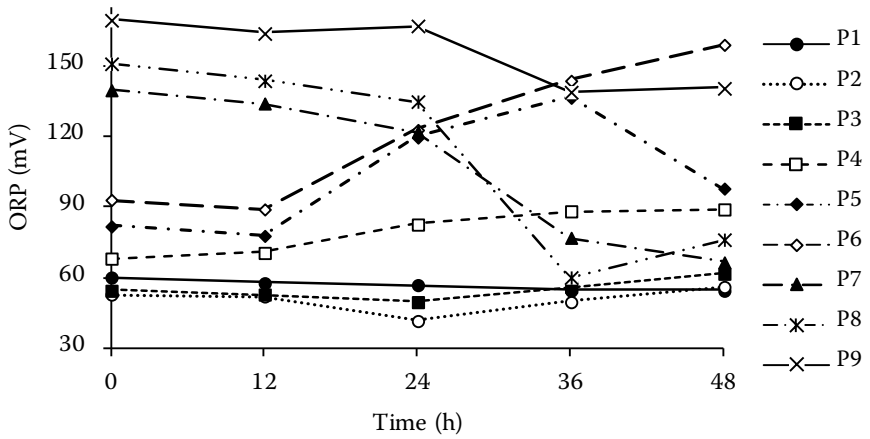

Figure 6. Oxidation-reduction potential (ORP) profile of the different substrates and concentrations during anaerobic bioprocessing
For P1–P9 explanation see Table 1

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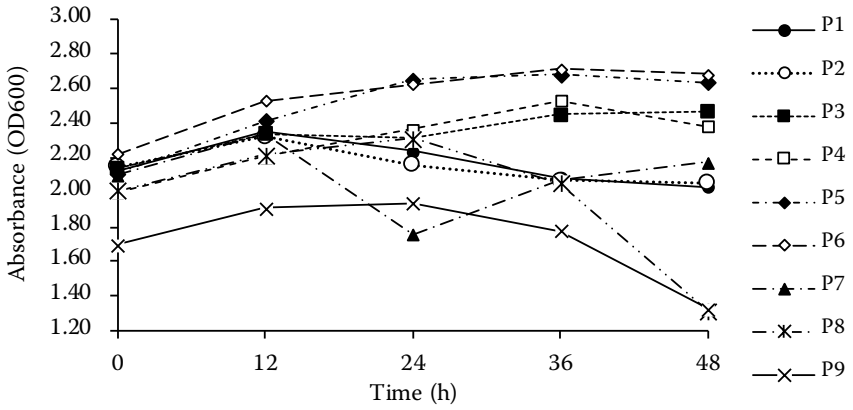

Figure 7. Microbial growth of the different substrates and concentrations during anaerobic bioprocessing
For P1–P9 explanation see Table 1

decrease in the ORP values in both digesters after a 48-hour bioprocessing period. Similar phenomena occur in digesters P8 and P9, where both digesters produce LA at the 12- and 24-h bioprocessing periods, respectively. In the case of digester P8, when the LA is produced (at the 12-h bioprocessing), the ORP value increases, followed by a decrease after 12 h, and thereafter, no more LA is produced from that digester. Meanwhile, digester P9, fed with 150 g·L^{-1} F, shows a high ORP value at the 24-h bioprocessing period, reaching 167 mV, and the produced LA is the highest among all the digesters (25 mmol·L^{-1}). After 24 h of bioprocessing, the ORP value in that digester continues to decrease until the 48-hour bioprocessing period (Liu et al. 2013).

The microbial growth analysis reveals variations in the growth phases among the microorganisms. Some, like P4, P5, and P6, display a continuous growth phase up to the 48-h bioprocessing period. In contrast, P1, P2, and P7 experience a rapid death phase, with microorganisms dying after 12 hours (Figure 7). P8 and P9 undergo a microbial death phase after 24 h, supported by the LA metabolite formation (Figure 3). The differences in the growth and death phases align with the fluctuating concentrations of produced metabolites, especially VFA (Table 3). This is attributed to the lack of nutrient supply in the digesters, as microorganisms require appropriate nutrient supplements for maximum growth, as explained by many researchers based on the phenotypic characteristics and genomic analyses (Blaiotta et al. 2017; Hayek et al. 2019).

CONCLUSION

The anaerobic bioprocessing process presents a viable avenue for converting OW into LA and VFAs, thereby offering potential solutions for mitigating the adverse environmental impacts associated with organic waste disposal. The employment of a non-sterile LS as an inoculum in the OW bioprocessing enhances the production of the LA and VFAs. The intricate interplay between the sugar composition in the substrate and the substrate concentration significantly influences the production rate and yield of the LA and VFAs. The fruit waste substrates demonstrated superior LA production, reaching a concentration of 25 mmol·L^{-1}, while the cassava waste substrates exhibited the highest VFA concentration at 92.5 mmol·L^{-1}. Throughout the bioprocessing, the fruit waste emerged as the most effective substrate for the LA production, whereas cassava waste showcased its promise for VFA production compared to the other substrates.

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