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SUSTAINABLE BIOPRODUCT PRODUCTION VIA ANAEROBIC BIOCONVERSION BY LANDFILL SOIL INOCULUM IN VARIOUS CARBOHYDRATE WASTES

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This study evaluates anaerobic bioconversion using landfill-derived microbes to produce volatile fatty acids (VFAs) as bioproducts. The research was conducted using different substrates, including elephant grass, starch waste, and fruit waste. Landfill-derived microbes collected from the landfill site were used as inoculum. The results show that the carbohydrate composition in the substrate affects VFA production. Simpler substrates, such as starch waste and fruit waste, produced more VFAs (approximately 50 mmol·L^{-1}) than that of more complex substrate of grass (approximately 25 mmol·L^{-1}). The results showed that using simpler substrates (i.e. starch and fruit waste) produce VFAs two times higher than that of the complex substrate or lignocellulosic biomass-based feedstock (i.e. grass). These findings provide valuable insights into the potential use of landfill-derived microbes in the anaerobic bioconversion process to produce VFAs. By understanding the influence of carbohydrate composition, we can enhance the efficiency of VFA production from organic waste, which can be used in various industrial applications. This is a crucial step towards more sustainable waste management and more efficient resource utilisation.

Keywords: waste conversion; organic waste; landfill-derived microbes; waste valorisation

The rapid growth of global population has also led to an increase in the amount of organic waste generated. An improper management of waste may result in problems like pollution, greenhouse gas emissions, and other negative environmental impacts. Therefore, it is essential to utilise organic waste efficiently and sustainably to reduce environmental impact and enhance the economic value of these wastes (Darwin et al., 2021). One approach to achieve this is by using them as raw materials for the production of various value-added products such as bioplastics, biofuels, biopolymers, and chemicals through appropriate and sustainable methods (Sharma et al., 2019; Singh et al., 2021; Vinci et al., 2021).

There are various methods to treat organic waste such as composting, incineration, landfilling, and anaerobic digestion. Currently, anaerobic digestion is widely accepted as the most efficient, flexible, and environmentally friendly method (Darwin et al., 2019a,b). This process involves microorganisms breaking down organic matter in an oxygen-free environment (Darwin et al., 2018a). This method not only handles waste properly but also offers opportunities to produce various types of bioproducts and alternative energy recovery (Gontupil et al., 2012; Darwin et al., 2019c; Janesch et al., 2021; Uddin and Wright, 2022).

The final product of the anaerobic digestion (AD) process is methane biogas, but the intermediate products of the AD process actually have higher value than methane itself. These intermediate products include succinic acid, lactic acid, and volatile fatty acids (VFAs) (Kleerebezem et al., 2015; Darwin et al., 2018b). VFAs are short-chain fatty acids with 2 to 6 carbon atoms. VFAs have the potential to serve as substrates for the production of various useful products such as raw materials for bioplastic or biodegradable plastic manufacturing, biofuels, and biochemicals. VFAs are also used in other sectors, including odour control agents, corrosion inhibitors, and pH regulators. However, the production of VFAs from organic waste depends on the complex carbohydrate composition of organic material. These complex carbohydrates have different structures and chemical properties, which affect the rate and yield of products during anaerobic digestion (Atasoy et al., 2018; Feng et al., 2022; Harirchi et al., 2022; Patel et al., 2021; Pham et al., 2021; Ramos-Suarez et al., 2021; Wainaina et al., 2019).

Anaerobic digestion of organic waste using landfill-derived microbes is one strategy for effectively processing organic waste and producing VFAs. Landfill-derived microbes are microorganisms that inhabit landfills and have the ability to degrade organic matter under anaerobic conditions. These microbes can be used as inoculants to accelerate the anaerobic digestion process of other organic wastes, including agricultural, industrial, and urban waste (Meyer-Dombard et al., 2020; Munir et al., 2022; Rasi et al., 2022; Uddin et al., 2021). However, the production of VFAs from organic waste using landfill-derived microbes depends on the types of complex carbohydrates present in waste, such as cellulose, hemicellulose, and starch. These complex carbohydrates have diverse structures and chemical characteristics, which affect how quickly and to what extent anaerobic conversion can be carried out by the decomposing microorganisms (Luo et al., 2019; Sun et al., 2021).

While there is not existing research on anaerobic bioconversion and volatile fatty acids (VFAs) production

using landfill-derived microbes and there is limited understanding of how the specific composition of complex carbohydrates in organic waste materials influences the efficiency and selectivity of VFA production, this research could provide valuable insights of anaerobic bioconversion process by landfill-derived microbes to produce VFAs.

Material and methods

The research was conducted at the Post-Harvest Engineering and Bioprocess Laboratory, Department of Agricultural Engineering, Faculty of Agriculture, Syiah Kuala University, Banda Aceh. The materials used in this research included HCl, H_2SO_4, H_3BO_3, NaOH, phenol, peptone (Merck), nutrient agar (Oxoid), lactic acid, indicator (PP, methyl red, methylene blue), D-(+)-glucose anhydrous (VWR BDH Prolabo Chemicals), and H_2O.

Substrate and inoculum preparation

The inoculum used for the study was landfill soil obtained from the TPA Kampung Jawa. Feedstock used consisted of elephant grass (G), cassava waste (S), and fruits waste (F). Elephant grass (*Pennisetum purpureum*) was obtained from the Village Lieue Plantation, Aceh Besar Regency, Aceh Province, Indonesia. Before use, the grass was finely ground (±0.2 cm). The starch waste used in this experiment is cassava waste. The fruit waste used included pears, melons, watermelons, bananas, and papayas. All those wastes were collected from the Vegetable and Fruit Market in Rukoh Village, Banda Aceh City, Aceh Province, Indonesia. The wastes collected were separated from impurities (i.e. sand, plastic, and stones) and then finely crushed and blended (±0.1 cm) to reduce their size.

Inoculum preparation

The anaerobic bioconversion process was carried out using an undefined mixed microbial culture. The undefined mixed culture used landfill derived microbes. The landfill-derived microbes were obtained from the Kampung Jawa Landfill Site in Banda Aceh, Aceh, Indonesia. Landfill soil was collected from areas where waste had accumulated and separated from contaminants such as plastic, stones, and wood. The collected soil was then placed in sealed containers and stored in a refrigerator for subsequent analysis.

Anaerobic bioconversion process

The anaerobic bioconversion process was conducted without pH control, no acids or bases were added. This was done to allow the anaerobic bioconversion process to occur naturally. The tests were carried out in a closed digester with a working volume of 250 mL, placed on a thermostatic water bath for 48 hours at a temperature of 35 ±0.5 °C. Before bioconversion began, nitrogen gas (N_2) was introduced into the digester to remove oxygen contamination (Darwin et al., 2018b; Darwin et al., 2019d; Darwin et al., 2018a; Darwin et al., 2019d). Each digester was filled with a substrate-inoculum mixture at a concentration of 100 $g \cdot L^{-1}$. The first digester (C1) was fed with 100% of grass. The second digester (C2) was loaded with 50% of grass, 25% of starch waste, and 25% of fruit waste (C2), respectively. The third (C3) was loaded with 100% of starch waste. The fourth reactor (C4) was added with 25% of grass, 50% of starch waste, and 25% of fruit waste. The fifth digester was supplied with 100% of fruit waste, and the sixth digester (C6) was fed with 25% of grass, 25% of starch waste, and 50% of fruit waste. The details of experimental design and substrate composition is presented in Table 1.

Table 1 Experimental design

Substrate composition G : S : F	Code
100 : 0 : 0	C1
50 : 25 : 25	C2
0 : 100 : 0	C3
25 : 50 : 25	C4
0 : 0 : 100	C5
25 : 25 : 50	C6

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

Total cell counts of inoculum

A total of 28 g of nutrient agar, containing 1.0 g of Lab Lemco powder, 2 g of yeast extract, 5 g of peptone, 5 of sodium chloride, and 15 g of agar, was dissolved in a 1000 mL volumetric flask using distilled water and homogenised. The media was transferred to glass bottles (Duran®). In other hand, 0.5 g of peptone was dissolved in a 500 mL volumetric flask using distilled water and homogenised. The solution was then transferred to a 500 mL glass bottle (Duran®). The medium and peptone solution were sterilised using an autoclave at a temperature of 121 °C for 15 minutes and then cooled to 50 °C. After reaching the desired temperature, 20 mL of the medium was poured into sterile petri dishes. The medium in the petri dishes was allowed to solidify and cool at room temperature. Once the medium had solidified, it was placed in a refrigerator. The inoculum was then serially diluted (10^{-1}–10^{-10}) using the prepared peptone solution. A 1 mL sample of the diluted sample was inoculated to the medium and then incubated at 30 °C for 48 hours. The treatment was performed in duplicate. After 48 hours, the total of colonies was counted as $CFU \cdot mL^{-1}$.

Analysis methods

The bioconversion samples were collected and then centrifuged for 10 minutes at 2000 rpm. The supernatant was then transferred to tubes and stored in the refrigerator at 2 °C for further analysis. The analyses conducted included the pH analysis (using a Laboratory Benchtop pH Meter with a Multifunction Complete Probe), total solids (TS), volatile fatty acids (VFAs), and oxidation-reduction potential (ORP) (using an ORP sensor). A 1 mL sample of the effluent was diluted with deionised water (DH_2O) tenfold and then analysed for electrical conductivity (EC), total dissolved solids (TDS) (using a Laboratory Benchtop pH Meter with a Multifunction Complete Probe), and total ammonia (NH_4^+) based on standard methods (Baird et al., 2017). The microbial growth analysis was carried out

Table 2 Characteristics of substrates and inoculum

Parameters	Unit	Landfill soil	Starch waste	Fruit waste	Grass
Total solids	%	64.19	87.81	21.68	16.88
Moisture content	%	35.81	58.14	87.71	86.30
Ammonia	$mg \cdot L^{-1}$	6	–	–	–
Total cell count	$CFU \cdot mL^{-1}$	$305 \cdot 10^9$	–	–	–
pH	–	8.6 ±0.2	7 ±0.2	7 ±0.2	–

by determining the optical density (OD). The OD analysis was performed by using a Spectrophotometer at a wavelength of 600 nm (UV Shimadzu 1200). To determine the carbohydrate content of the samples, the analysis was performed according to the standard phenol-sulfuric method (Herbert et al., 1971). The lactic acid yield was determined using a Lactate Biosensor Accutrend Plus meter (Darwin, 2019d).

Results and discussion

To evaluate the characterisation of various substrate and inoculum characteristics used in the bioconversion process, a series of tests were conducted. The results of the analysis can be seen in Table 2. The pH of the landfill soil used in this experiment was alkaline condition (pH 8.6). This occurred due to the accumulation of ammonia in the landfill soil (Darwin et al., 2019c). The experiment also analysed the amounts of bacterial cells accumulated, which was $305 \cdot 10^9$ $CFU \cdot mL^{-1}$. These results are supported by previous research where landfill soil contains various types of bacteria (Zhang et al., 2011). This microbial community will play a significant role in converting organic waste into VFAs.

During anaerobic bioconversion, the pH profile plays a crucial role in understanding the digester environment dynamics. pH is a key parameter that can influence the activity of microorganisms and determine the end-products generated. The results of the current study showed that as the bioconversion time progresses, the pH in the digester decreases from 8 to 5 (Fig. 1). This occurs because during bioconversion, the microorganisms present in landfill-derived microbes convert the substrate into VFAs. Eventually, VFAs accumulate in the digester (Atasoy and Cetecioglu, 2022). VFAs typically accumulate under acidic to neutral conditions (Garcia-Aguirre et al., 2017). The results of this study indicate that VFAs accumulate at higher concentrations under acidic conditions. However, even when the digester is in a basic condition, VFAs still accumulate (at lower concentrations) due to the complex metabolic pathways of the microbial community present in landfill-derived microbes (Pham et al., 2021).

The results showed that both digester C3 and C4 have lower pH (5) or more acidic in comparison to that of other digesters (Fig. 1). This is because both digesters contain more starch waste as substrate, not only accumulated VFAs but also lactic acid in the digester (Darwin et al., 2019a). The accumulation of lactic acid can lower the pH of the digester due to the substrate oxidation of substrate to lactic acid, finally increasing proton concentration in the digester. This significant pH decrease is also attributed to the fact that lactic acid has a low pKa value, approximately 3.86 (Robergs et al., 2018).

As shown in Fig. 2, when bioconversion time increases, carbohydrate concentration rapidly decreases. This is because microbial communities utilise the substrate as a carbon source and convert it into bioproducts such as lactic acid and VFAs (Tang et al., 2017). A different phenomenon occurred in digester C3, where after 12 hours of bioconversion, the total carbohydrate increased from 16.11 $mmol \cdot L^{-1}$ to 20.79 $mmol \cdot L^{-1}$, possibly due to a low rate of acidogenesis. The low rate of acidogenesis is caused by the low population of acidogenic bacteria in the digester. In other words, the pH in the digester dropped, and the microorganisms present in the landfill soil were not tolerant of an acidic pH, resulting in carbohydrates not being converted into the desired products (Tang et al., 2016). Some microorganisms may exhibit higher activity levels or become more dominant within

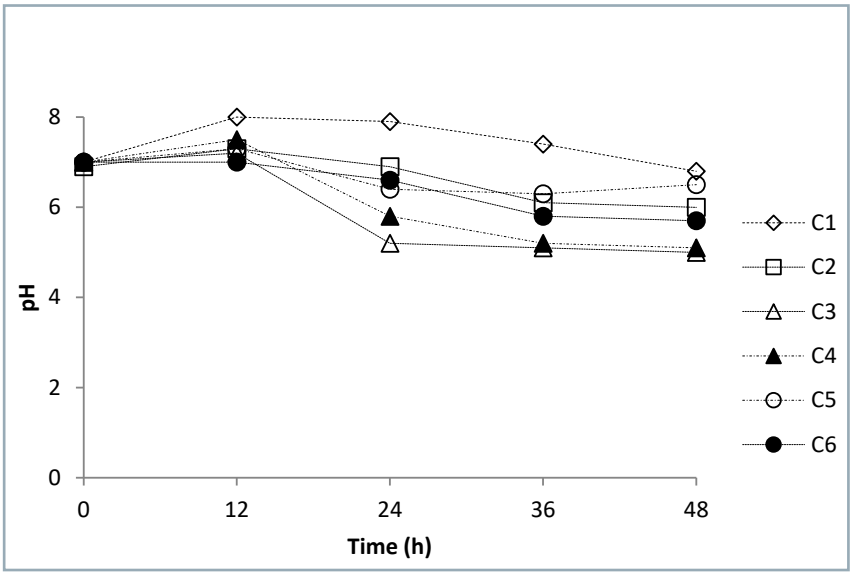


Fig. 1 pH profile during the anaerobic bioconversion processes

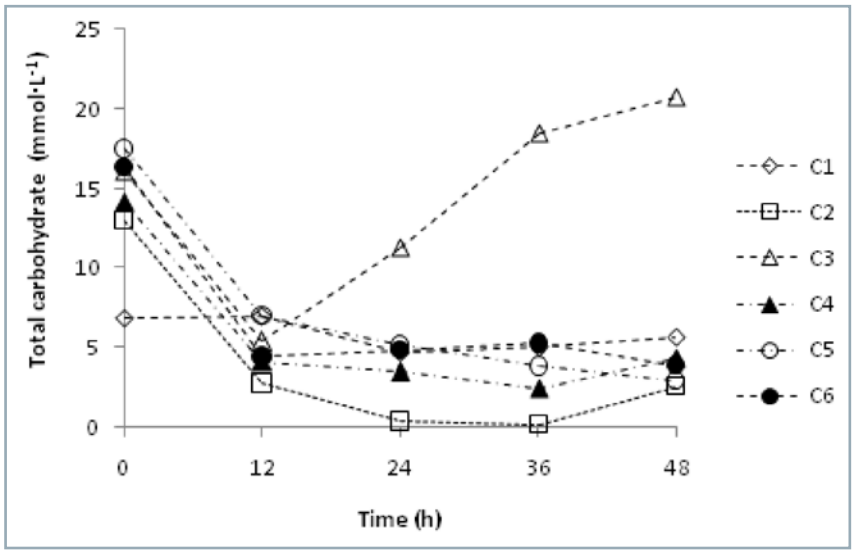


Fig. 2 Total carbohydrate concentration during the anaerobic bioconversion process

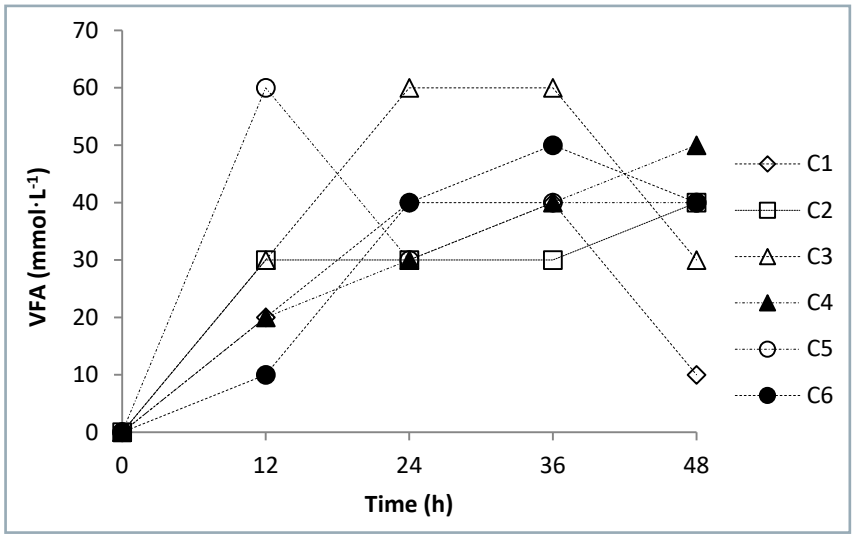


Fig. 3 VFAs production during the anaerobic bioconversion process

specific pH ranges. Changes in pH can lead to changes in the composition of the microbial community, which can impact the carbohydrate conversion process in the bioconversion system (Cho et al., 2016).

Another factor to consider in this phenomenon is that in the early stages of bioconversion, the substrate may have been converted by microorganisms in the landfill soil into specific products. However, in the subsequent stages, microorganisms may start converting other substrates, such as organic compounds produced during previous bioconversions, into additional carbohydrates. By the 36-hour bioconversion time, almost all digesters experienced an increase in total carbohydrates. A similar situation may have occurred in digester C3 during the 12-hour bioconversion, where microorganisms in the digester were not tolerant of acidic pH. This is consistent with the data in Fig. 1, where after 36 hours of bioconversion, the pH in the digester continues to decrease to the acidic condition.

A significant variation was shown in the VFAs production profiles during bioconversion. The results revealed fluctuations in the amount of VFAs produced at different time in the bioconversion process. The VFAs production in all digesters tended to increase from 12 to 36 hours of bioconversion. After 36 hours, the amount of VFAs produced slowly decreased. However, in contrast in digester C4, the VFAs produced continued to increase until the 48-hour bioconversion time. This may be due to the complex nature of bioconversion pathways involving mixed microorganisms (Pham et al., 2021). The research results showed that VFAs could be produced within a concentration range of 10–60 mmol·L^{-1} during the 12–36-hour bioconversion period. The VFAs produced during bioconversion were within the pH range of 5–7. Similar findings have been reported previously, where VFAs can accumulate during bioconversion in the pH range of 5.5–6.5 (Cavinato et al., 2017; Yin et al., 2021).

Typically, specific pH levels result in the accumulation of certain types of VFAs, which is why the VFA concentrations obtained in this study varied. Acetic acid is normally dominantly accumulated in the digester at pH 5, followed by butyric acid and propionic acid. At pH 6–7, accumulation is dominated by butyric acid, followed by acetic acid, propionic acid, and valeric acid (Jiang et al., 2013; Ye et al., 2018). Other research mentions that at pH 6.5, propionic acid is dominantly accumulated in the digester (Gameiro et al., 2016). Digester C3 (100% S) and C5 (100% F) respectively had the highest VFA production (±50 mmol·L^{-1}) compared to the other digesters. This was followed by the highest VFAs produced by digester C4 (25% G : 50% S : 25% F) and C6 (25% G : 25% S : 50% F) (approximately 35 mmol·L^{-1}). These four digesters were dominated by starch waste and fruit waste substrates. According to (Darwin et al., 2019a), digesters fed with starch and simple sugar substrates can produce more VFAs. In contrast, digester C1 and C2, each fed with 100% G and 50% G : 25% S : 25% F, respectively, resulted in lower VFA production (25 mmol·L^{-1}).

The results showed that not all digesters produced lactic acid during bioconversion. Lactic acid accumulated merely in 2 digesters (C3 and C4), both were primarily fed with starch waste substrates. The results are in agreement with some studies revealing that digesters fed with substrates containing mainly starch tended to produce more lactic acid (Darwin et al., 2019a; Darwin et

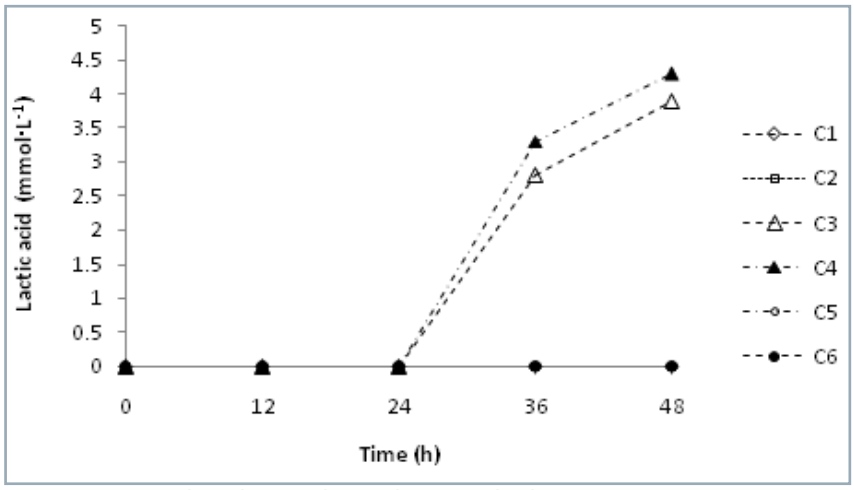


Fig. 4 Lactic acid production during the anaerobic bioconversion process

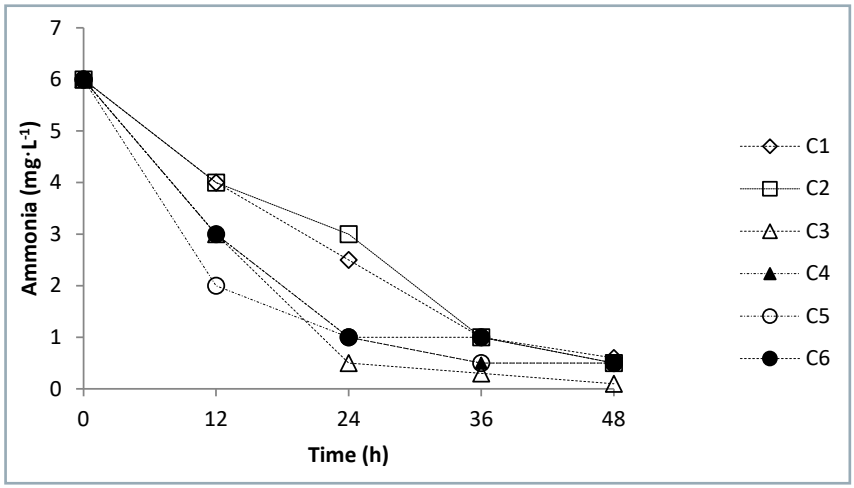


Fig. 5 Ammonia concentration profile during the anaerobic bioconversion process

al., 2018b; Palakawong Na Ayudthaya et al., 2018). In this study, during the 36-hour bioconversion, digester C3 produced 2.8 mmol·L^{-1} of lactic acid, while digester C4 produced 3.3 mmol·L^{-1}. Slightly higher results were observed at the 48-hour bioconversion, where digester C3 produced 3.9 mmol·L^{-1} of lactic acid, and digester C4 reached 4.3 mmol·L^{-1}. These results are also supported by pH conditions in the digesters. Based on Fig. 1, during bioconversion, both digesters had the lowest pH values. When lactic acid accumulates in the digester, the pH in the digester drops and becomes more acidic (Darwin et al., 2022). Other digesters especially containing mainly grass (lignocellulosic biomass) did not produce lactic acid during the anaerobic bioconversion. This is because grass or lignocellulosic biomass typically contain complex carbohydrates, including cellulose and hemicelluloses, in which pretreatment processes were required to become fermentable sugar (Abdel-Rahman et al., 2011; Velvizhi et al., 2022). On the other hand, simple sugar (fructose) also did not accumulate lactic acid. This is suspected to be because the microorganisms present in landfill-derived microbes cannot convert fructose in fruit waste into lactic acid but produce VFAs and ethanol as the main products (Darwin et al., 2018b).

During bioconversion, the total ammonia (NH_4^+) content in the digesters was evaluated (Fig. 5). The results revealed the total ammonium concentration inside the digesters rapidly decreased during bioconversion from 6 mg·L^{-1} to 0 mg·L^{-1}. This is suspected to be because there were no nitrogen-containing organic materials such as amino acids, proteins, and urea in the substrate and inoculum used. The decrease in ammonia concentration is also supported by the pH data during bioconversion, where the initial pH of the digester (0 hours of bioconversion) was 8 (alkaline), and as bioconversion time increased, the pH of the digester became 5 (acidic) (Fig. 1). This is unlike the typical scenario in anaerobic digestion (AD), where ammonium usually accumulates in the digester from the degradation of nitrogen-containing raw materials, typically from proteins, and can inhibit methane production in AD processes (Bujoczek et al., 2000; Gallert and Winter, 1997).

The profile of electrical conductivity (EC) during anaerobic bioconversion to produce VFAs (volatile fatty acids) was also observed. This analysis can provide insights into the process. Electrical conductivity is typically used as a parameter to monitor the concentration of ions in a solution during bioconversion. During the bioconversion of organic matter into VFAs, EC can undergo changes that reflect changes in the chemical composition and ion concentration in the digester. The data on the EC profile during bioconversion can be used to understand the chemical dynamics in the digester, monitor the progress of the process, and enable better control over VFA production. The results of the EC analysis during the process are shown in Fig. 6. It can be seen that as bioconversion time increases, the EC of each digester experiences a significant decrease. The results show that the EC value decreases from approximately 10 mS·m^{-1} to 4 mS·m^{-1}. This is because when the accumulation of VFAs occurs, EC can decrease. This happens because the soluble salts present in the digester are utilised by the microorganisms present in landfill-derived microbes as a source of minerals (Chergui et al., 2021). Besides that, the decrease in EC values is often caused by the accumulation of alcohol in the digester (Li et al., 2019).

The determination of microbial growth in a digester using optical density (OD600) was also assessed in this study. The results of the OD600 analysis can be seen in Fig. 7. Based on Fig. 7, it is evident that each digester exhibited different growth phases. Digester C1 (100% G) had a short

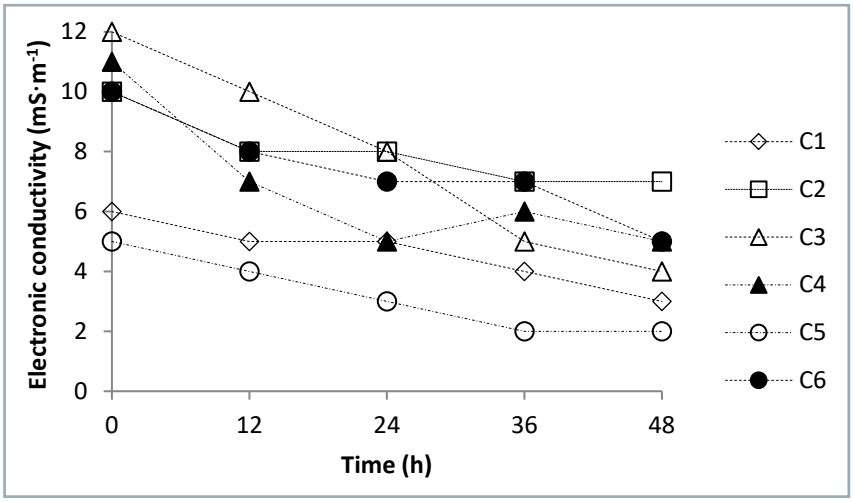


Fig. 6 Electronic conductivity profile during the anaerobic bioconversion process

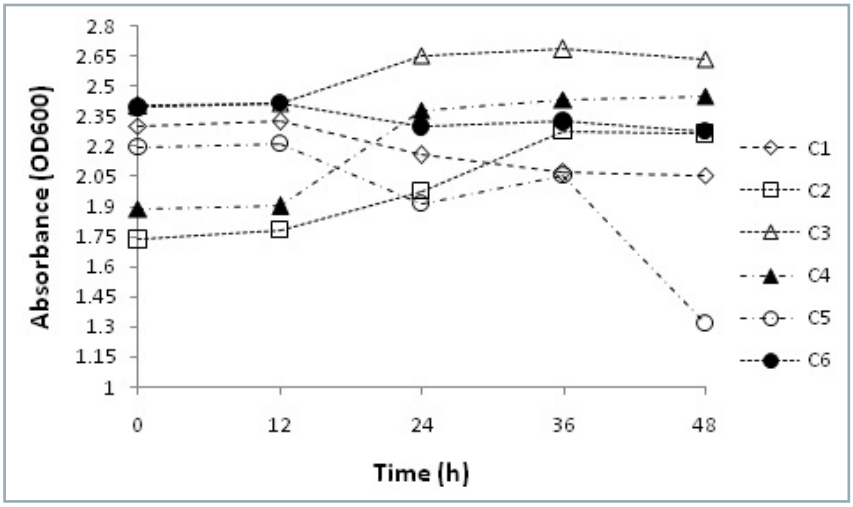


Fig. 7 Microbial growth as measured by absorbance (OD600) during the anaerobic bioconversion process

log phase, where microbial growth from landfill-derived microorganisms decreased after 12 hours of bioconversion. This is evidenced by the lower and continually decreasing VFA production compared to the other digesters over the course of bioconversion. However, a different phenomenon occurred in digester C2. When supplied with a different substrate (50% G : 25% S : 25% F), microorganisms continued to experience significant growth until 36 hours of bioconversion. This explains that the microorganisms in the inoculum used could utilise starch waste and fruit waste as carbon sources, whereas grass (lignocellulosic biomass) could not be directly used by microorganisms in the inoculum as a carbon source due to its complex carbohydrate content (cellulose, hemicellulose, and lignin), which requires pretreatment to convert it into simple and fermentable sugars (Abdel-Rahman et al., 2011; Ajala et al., 2020).

The results of the current study showed that the VFA production derived from digester C2 was also higher and only began to decrease after 36 hours compared to digester C1. This phenomenon was relatively close to other digesters (C3–C6). The results also showed that each digester had a longer growth phase, and VFA production continued to increase until the stationary phase was reached (36-h bioconversion). This stationary phase is a phase where microorganisms undergo death due to a lack of carbon sources or a shortage of mineral media for microorganisms to survive and continue producing specific bioproducts.

Conclusion

The anaerobic bioconversion process allows for the utilisation of organic waste to produce VFAs, offering the potential to reduce the negative environmental impacts of organic waste. The composition of carbohydrates present in the substrate plays a crucial role in VFA production. The chemical composition of complex carbohydrates and their properties can influence the rate and yield of VFAs. The substrate derived from starch waste and fruit waste produced more VFAs compared to that of the grass. During the bioconversion process, utilising substrates with a dominant mixture of starch waste has shown better effectiveness in VFA production (approximately 50 $mmol \cdot L^{-1}$) compared to the other substrates.

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