Research article

Effects of food wastes based on different components on digestibility and energy recovery in hydrogen and methane co-production

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A B S T R A C T

This study was conducted for four organic fractions (carbohydrates, proteins, cellulose, lipids) at an inoculum concentration of 30 % and a total solid (TS) of 8 % to investigate the effect of the main components of food waste on the performance of the two-stage anaerobic digestion. The results showed that the gas phase products were closely related to the composition of the substrate, with the carbohydrate and lipid groups showing the best hydrogen (154.91 $\pm$ 2.39mL/gVS) and methane (381.83 $\pm$ 12.691mL/gVS) production performance, respectively. However, the increased protein content predisposes the system to inhibition of gas production, which is mutually supported by changes in the activity of dehydrogenase and coenzyme F420. Butyric acid (53.19 %) dominated the liquid phase products in both stages, indicating that all four organic fractions were butyric acid-based fermentation and that the final soluble chemical oxygen demand degradation reached 72.97 %–82.86 %. The carbohydrate and cellulose groups achieved the best energy recovery performance, with conversion rates exceeding 65 %. The above results can provide a useful reference for the resource utilization of food waste.

1. Introduction

The overexploitation of fossil fuels has brought about environmental problems, such as the greenhouse effect and acid rain. Therefore, environmentally friendly new energy sources are inevitable for sustainable development [1]. Anaerobic digestion is one of the effective means of producing clean energy from various agricultural wastes [2], and its resource gas products are usually hydrogen, methane and carbon dioxide [3]. Hydrogen is a clean fuel with a high energy density (142 kJ/g) [4]. However, the traditional treatment method places the digestion system in the same reactor. This method ignores the collection of product hydrogen and tends to have problems such as mutual disturbance of population organisms [5] and imbalance of product conversion. These problems lead to the ineffective conversion of both biomasses into valuable waste resources [6,7].

The two-stage anaerobic digestion eparated the phases of the system [8]. It relies on the first stage of dark fermentation, where hydrogen and volatile organic acids (VFAs) are produced from organic substances by acidogenesis and homoacetogenesis through hydrolysis. It not only avoids the limitations of light conditions [9] but also provides abundant digestive material for the second stage (methanogenic stage), which better solves the inhibition effect of the reaction system and facilitates the recycling of nutrients and energy [10].

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Food waste (FW) is a multi-sourced organic waste produced globally at over 100 million tonnes per year [11], and its main components are carbohydrates, protein, cellulose and lipids [12]. Studies have shown that the content of each component of food waste varies over a wide range [13]. There is some difference in the components and properties of food waste due to geographical conditions, customs and collection sources [14–17]. Previously, scholars had studied the effect of food waste organic composition on anaerobic digestion performance [18–20]. The results showed that carbohydrate and cellulose substrates have a more significant hydrogen production potential than proteins and lipids due to their structural simplicity [21,22]. However, this also causes it to be efficiently inhibited by system acidification and reduced gas production efficiency during the methane production process [23,24]. Although the latter is not as effective in hydrogen production, it is a suitable substrate for methane production [20,25]. Previous studies have typically evaluated only one phase, such as hydrogen or methane production, ignoring the acidification that occurs during digestion and the impact of the combination of the two on energy recovery. In addition, existing studies usually choose a single material to represent a component of food waste, hoping to show the role of this component in the anaerobic digestion process. However, this does not correspond to the characteristics of food waste in real life.

Therefore, in this study, two kinds of substances were selected to represent each composition and were mixed in specific proportions to form food wastes with different enriched compositions (carbohydrates, proteins, cellulose, and lipids). Meanwhile, the dark fermentation-anaerobic digestion process is utilized.

The gas yield, composition, dissolved organic matter, enzyme activity, and energy conversion efficiency were observed. Through these observations, the effect of substrate ratio on digestive performance and the effect of substrate structure on the energy conversion efficiency was signified. The optimal digestive substrate for dark fermentation-anaerobic digestion was also clarified through this process. This study provides a theoretical basis for the resource utilization of food waste.

2. Materials and methods

2.1. Inoculum and materials

The inoculum used for the tests was enriched from the anaerobic digester of pig manure from a research base greenhouse at Shenyang Agricultural University, China. After that, the inoculum was screened through a 100 mm sieve to reduce particulate substances. Sludge used for dark fermentation (DF) tests were submitted to a heat treatment at 105 °C for 30 min in an autoclave to inhibit hydrogenotrophic methanogens [26,27]. On the other hand, the untreated sludge was used for the anaerobic digestion (AD) tests.

The test materials were fresh materials purchased from a supermarket in Shenyang, Liaoning Province, China, which were separated into four types of food waste samples with different compositions according to their properties: (1)Carbohydrates: rice, noodles; (2)Proteins: chicken, lean pork; (3)Cellulose: cabbage, celery; (4)Lipids: lard, cooking oil. All materials were prepared in a refrigerator at 4 °C before the test. The physicochemical characteristics of substrates and inoculum are shown in Table 1.

The four components mentioned above were combined to make digestive substrates with different rich components and named carbohydrate-rich (CR), protein-rich (PR), cellulose-rich (ER) and lipid-rich (LR), where the main components of each group accounted for 40 % of the total raw material. The rest accounted for 20 % to ensure the content variation was within the effective range.

2.2. Fermentation methods

2.2.1. First-stage dark fermentation (DF)

Other enriched food wastes were used as substrates (i.e., CR, PR, ER, LR) and heat-treated sludge as inoculum. DF tests were carried out in brown glass vessels with a sufficient volume of 600 mL, each reactor having a dry matter concentration of 8 % and an inoculum of 30 % of the digestion broth. All reactors were adjusted to pH 6.5 ± 0.1 using 6 M HCL and placed in a 37 °C water bath for incubation. Three replicate trials were set up for each case, lasting 52 h. The produced gas and biomass samples were collected every 4 h, and the gas was used to determine the gaseous composition. The biomass samples determine pH, soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), polysaccharides and protein content.

2.2.2. Second-stage anaerobic digestion (AD)

At the end of the first stage, the hydrogenogenic effluent of each group was introduced into new glass containers with the untreated sludge. The pH of each bottle was adjusted to 7.5 ± 0.1 with 6 M NaOH, and the rest of the parameters were the same as DF. After that, all test groups were placed in a constant temperature water bath at 37 °C for 40 days of second-stage AD. Gas production was measured every 24 h and sampled every 4 days. Fermentation broth gas composition, broth pH, SCOD, VFAs, dehydrogenase activity and

Table 1
Physicochemical properties of raw materials.

Parameter	FW-Carbohydrates	FW-Protein	FW-Cellulose	FW-Lipids	Inoculum
TS/%	30.88 ± 0.11	32.57 ± 0.13	7.69 ± 0.07	100	12.2 ± 0.09
VS/%TS	89.29 ± 0.24	96.97 ± 0.21	92.86 ± 0.36	1	50 ± 0.06
C/N	27.07	3.84	4.94	–	11.59
pH	–	–	–	–	7.2 ± 0.12

Note: VS is volatile solids.

coenzyme F420 concentration were measured.

2.3. Analysis method

The TS and VS data were determined using standard methods (APHA, 2005). A portable pH meter (PHS-3G, Leici, China) measured the pH during digestion. Gas chromatography (Aligent 6890 N, USA) was used to determine the composition of the gases, with argon as a carrier gas and column and detector temperatures set at 170 °C and 220 °C, respectively, with a 2-min equilibration time.

An analysis of SCOD was carried out following (ISO 6060:1989) [28]; phenol-sulphuric acid was used to measure polysaccharide concentrations [29]; Folin-phenol reagent was used to measure protein content [30], and UV spectrophotometry and TTC (2,3, 5-triphenyl tetrazolium chloride) were used to measure coenzyme F420, dehydrogenase activity. By using a gas chromatography system (Aligent 6890 N, USA), volatile fatty acid (VFA) concentrations were determined. 240 and 250 °C were set as the injector and detector temperatures, respectively, with an initial column chamber temperature of 80 °C for 5 min and then ramped up to 220 °C at a rate of 10 °C min^{-1}.

The following equation calculates hydrogen and methane's energy conversion efficiency (ECE%) [24,25].

$$\text{ECE}(\%) = \frac{a \times b}{1000 \times 22.4 \times c} \times 100\% \tag{1}$$

where "a" is cumulative H_2/CH_4 production per unit mass of material (mL/gVS), "b" is the H_2/CH_4 calorific value (H_2:242 kJ/mol; CH_4: 801 kJ/mol) and "c" is the calorific value of the raw material for the substrate.

3. Results and discussion

3.1. Hydrogen production during the DF process

Fig. 1 shows the hydrogen production from four different organic components of food waste as digestion substrates. As can be seen in Fig. 1A, the hydrogen production of each test group showed a trend of increasing and then decreasing with time, and there was a significant decrease in gas production after 12 h. The whole hydrogen production cycle lasted until 48 h. The total hydrogen production was in the order of CE > ER > PR > LR.

The CR has better hydrogen production than the other three, reaching a peak of (54.05 ± 1.614)mL/gVS at 12h. The cumulative hydrogen production of CR during the DF stage was 2.34 and 2.66 times higher than that of PR and LR. It is due to the high content of carbohydrates being more easily utilized by microorganisms and thus degraded to starch or simple sugars, which become the preferred substrate for the DF process [18]. It agrees with Mustafa Aslan et al. [31] whose report showed that carbohydrate-rich biomass was the best substrate for hydrogen production and that the efficiency of biohydrogen production increased with the total amount of carbohydrates. In contrast, proteins and lipids showed poor hydrogen production as DF substrates, which peaked at the 8th and 12th hour. The maximum hydrogen production was only 47.9 % and 38.7 % of that of the CR substrate. It is because the amino acids produced during the hydrolysis of proteins consume some of the H_2 [32,33]. At the same time, lipids are only converted to H_2 by long-chain fatty acids at shallow partial pressures of hydrogen [34]. The ER had a moderate hydrogen production ability from the overall perspective, although it did not have a higher peak (21.72 ± 0.86mL/gVS) during the DF. The cumulative hydrogen production (Fig. 1B) was (83.26 ± 1.32)mL/gVS, 62.44 % higher than the LR. It may be due to the high water content of the vegetables and the high levels of cellulose and hemicellulose in the solid composition. It has a more straightforward structure than proteins and lipids, facilitating a more suitable working environment for hydrogen-producing bacteria [35].
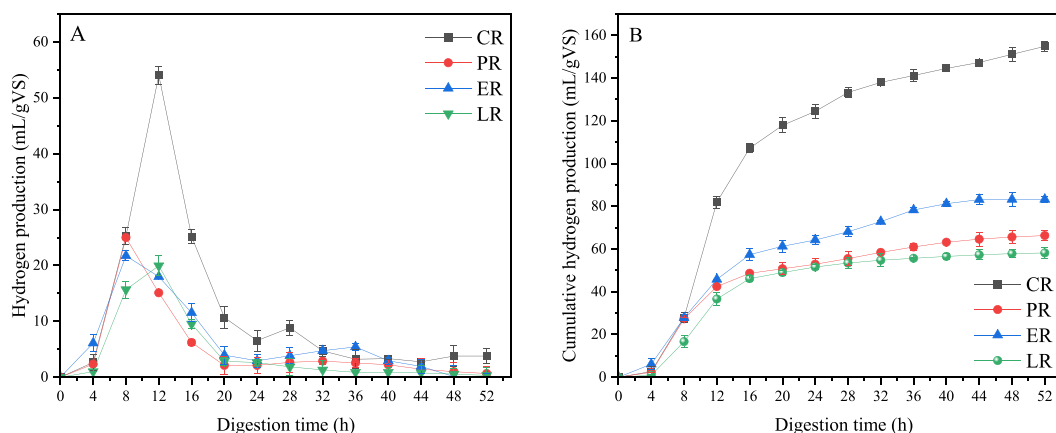

Fig. 1. Hydrogen production (A), cumulative hydrogen production (B) for different components of the DF phase.
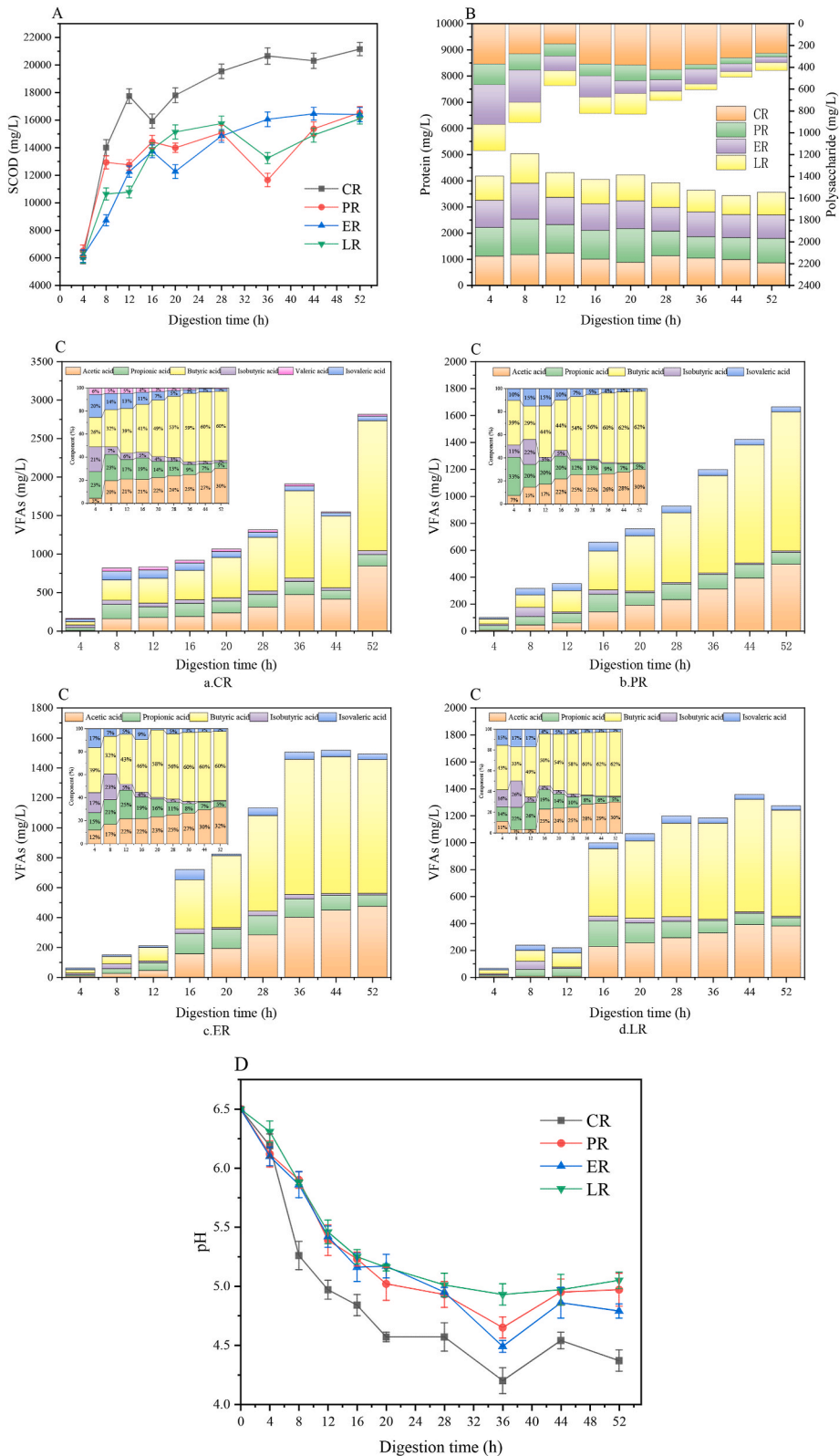
Fig. 2. SCOD concentrations (A), polysaccharide and soluble protein concentrations (B), VFAs (C), pH (D) for different components of DF stages.

3.2. Characteristics of manure digestive liquid during the DF process

3.2.1. SCOD

SCOD is an essential parameter for changes in the concentration of dissolved organic matter during reaction digestion [36], which consists mainly of soluble sugars, dissolved proteins and VFAs. As shown in Fig. 2A, the increasing concentration of SCOD after DF start-up indicates that hydrolysis is significant in the system at this time [37]. The significant decrease in polysaccharide concentrations in each test group in the first 12 h (Fig. 2B) indicates that the pioneering hydrolysis of substances such as starch causes the elevated phenomenon.

Compared to the CR group, the other groups showed a later and lower peak, after which the SCOD concentrations all showed an increasing trend, which may be attributed to this phenomenon, including (1) The polysaccharide concentration of each experimental group showed a specific rise after 12h, indicating that the dissolution of polysaccharides in the system at this time was more significant than the consumption. The accumulation of polysaccharide concentration would cause an increase in SCOD concentration. (2) After 12h, the hydrogen production of each experimental group showed a substantial decrease, which may be due to the accumulation of VFA in the early stage inhibited the activity of hydrogen-producing and acetic acid-producing bacteria, which in turn led to the reduction of the conversion efficiency of microorganisms to soluble organic matter, causing the increase of SCOD concentration. However, it is notable that the SCOD concentrations in the PR and LR groups showed a significant trough at 36 h, which may be attributed to the low amount of glycans contained in the PR and LR substrates and the extremely slow hydrolysis of proteins and lipids. After the pre-hydrolytic acidification, the remaining soluble organic matter was insufficient to support their consumption, hence the decreasing trend. This is also evident from the changes in polysaccharide concentration, where the production of soluble polysaccharides decreased by 54.23 %, 38.11 % in the PR and LR groups at 36h. In contrast, the dissolved protein concentration (Fig. 2B) showed a smoother trend throughout the digestion process with only a small decrease in each test group before and after digestion. It may be due to the difficulty of biodegradation of proteins as organic matter in a short period, which is generally consistent with the results of Yanan Yin et al. [38].

3.2.2. VFAs

VFAs are the main products of hydrolysis and acidification processes and are essential intermediates in methane production from AD [39]. The content of VFAs and their fractions for each time of dark fermentation are shown in Fig. 2C, with total acid production in order of magnitude CR > ER > PR > LR. This sequence is consistent with the hydrogen production results, indicating that hydrogen is inextricably linked to the production of VFAs. Butyric acid (55.22 %) was the primary volatile fatty acid produced at this stage, followed by acetic acid, propionic acid, isobutyric acid and isovaleric acid, thus showing that all four substrates were butyric acid type fermentations during the DF stage. All groups showed a gradually increasing trend in VFAs concentration. Among them, CR had the best acid production with VFAs reaching (2846.13 ± 132.14)mg/L at the end of the hydrogen production phase, 1.71, 1.91 and 2.24 times higher than the other three groups, respectively. In contrast, although PR produces more VFAs, the system still has some buffering capacity, owing to the nitrogenous organic matter generated by its degradation [40].

It is interesting to notice that the ER group has a similar volatile acid concentration as the PR group, but produces more H_2. Analysis of the reasons for this phenomenonProteins are often hydrolyzed to peptides and amino acids by microorganisms under anaerobic conditions, and the degradation process is mainly dominated by the Strickland pathway [33]. Liu et al. [41]used a stoichiometric coefficient method to derive the theoretical equation (Eq.(1)) for acid-producing digestion of proteins under ideal conditions.

$$CN_{0.29}H_{2.05}O_{0.74}+H_2O \rightarrow 0.235Ac+0.009Pr+0.038Bu+0.012Va \quad (1)$$
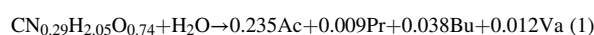
As can be seen from the equation, no additional hydrogen is produced. However, the ER group is rich in celluloses and hemicelluloses, partially converted to monosaccharides (C5–C6 sugars) during hydrolysis. It has been shown that the conversion of glucose to VFAs involves three pathways (Eqs. (2)–(4)) and the ratio of pathway contributions is 1:0.9:1.1 [42].

$$C_6H_{12}O_6+2H_2O \rightarrow 2CH_3COOH+2H_2+2CO_2+4H^+ \quad (2)$$

$$1.5C_6H_{12}O_6 \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2+H_2O \quad (3)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2COOH+2H_2+2CO_2 \quad (4)$$

Considering that only part of the cellulose and hemicellulose in the ER group can be converted to glucose, the production of VFAs is lower than that of the CR. However, it maintains a similar concentration to that of the PR. However, the glucose conversion process is accompanied by H_2 production, so there is some significance in obtaining higher H_2 production in the ER group than in the PR group. In addition, the substrate used in this study is a composite material, which may have some synergistic relationship between different components during digestion.

3.2.3. pH

pH is one of the most important parameters in determining hydrogen production efficiency from DF [43]. Generally, the optimum pH for hydrogen fermentation is between 5.0 and 6.5 [44]. Fig. 2D illustrates the changes in pH during DF in each test group. At the beginning of the reaction, the pH of all groups showed a decreasing trend. The CR group showed the fastest and lowest pH drop, dropping to (4.20 ± 0.09) at 36 h. The other three groups showed more or less the same trend, with pH ranging from 4.6 to 5.0 at 36 h. It occurs because hydrogen-producing bacteria produce VFAs during the degradation of the substrate, which leads to a decrease in pH

within the system [45]. Due to the most degradable nature of carbohydrates, they accumulate more volatile organic acids than other components and, therefore, have a more significant impact on pH in the system. It is important to note that there is a significant recovery of pH in the system after the 36th hour. It is the same trend as the pH change in the hydrogen production phase of food waste reported by Xianpu Zhu et al. [46]. This occurrence may be due to the decomposition of macromolecules, such as proteins in the substrate, leading to an increase in ammonia nitrogen concentration [47].

3.3. Methane production during the AD process

Fig. 3 shows each group's gas production from the AD of the hydrogen-producing residues. The entire gas production cycle lasted 36 days, and the order of magnitude of methane production was LR > ER > CR > PR.

As shown in Fig. 3A, CR and ER's daily biogas production patterns are similar, with both having lower gas production rates in the early stages. It may be due to the reaction rate limitation caused by the higher concentration of VFAs accumulated by both substrates in the first stage [48]. The PR test group reached peak gas production earlier (11th day), but its peak was significantly lower than the other fractions, at (78.53 ± 2.3) mL/gVS. It is also seen in Fig. 3B that despite the high gas production rate in the early stages of the PR group, the final total biogas production is still lower than the other three groups. It may result from the low water content of such substrates and the difficulty of completely degrading large molecules such as proteins [49]. Lipids are considered an ideal substrate for enhancing methane production, and lipid-rich food waste yields higher methane than other enriched components [50].In this study, LR showed a better methanogenic capacity. As shown in Fig. 3A, there were two significant peaks throughout the digestion phase on days 13 and 18. It may be due to the sequential degradation of the carbohydrates and fats present in the substrate [51]. Eventually, the total biogas production for the LR cycle reached (1405.64 ± 18.51) mL/gVS, which is 1.09, 1.11, and 1.20 times higher than ER, CR, and PR, respectively. However, it has been shown that high lipid concentrations can also inhibit microorganisms, mainly due to the hydrolysis of long-chain fatty acids such as LFCA, the accumulation of which can be toxic to further reactions and reduce methane yield [12]. In this study, the LR group did not show adverse effects of LFCA throughout the fermentation cycle, showing that it does not affect microbial activity and even promotes methanogenesis when the proportion of lipids in the substrate is maintained at about 40 %.

3.4. Characteristics of manure digestive liquid during the AD process

3.4.1. pH

As seen in Fig. 4A, the pH trends for the organic fractions were essentially the same, all dropping rapidly on day one after the start of the AD phase and dropping to a minimum on day 3. It may be attributed to the high concentration of organic acids in the first phase. Gradually, as the methanogenic bacteria in the substrate adapted to the system environment and began to increase their consumption of VFAs and convert them to methane and carbon dioxide [52], the pH in the system began to rise back up, eventually stabilising at around 8.0 gradually. The PR substrate had a faster pH recovery rate and a higher pH after stabilisation than the other three groups. However, it did not show the desired yield effect. This situation may occur because the ammonia nitrogen produced by protein hydrolysis does act as a pH regulator. However, as the concentration of ammonia nitrogen increases, the activity of methanogenic microorganisms is correspondingly inhibited, leading to a decrease in methane production [6].

3.4.2. SCOD

The change in SCOD during the AD phase is shown in Fig. 4B. As can be seen from the graphs, the SCOD concentrations in each test group showed an increase followed by a decrease during the AD process, and all reached the maximum SCOD concentration on day 8. On the one hand, this may be due to an increase in the concentration of organic matter dissolved in the water due to the soluble material remaining at the end of the DF stage. On the other hand, it may be due to some micro-soluble macromolecular organic matter
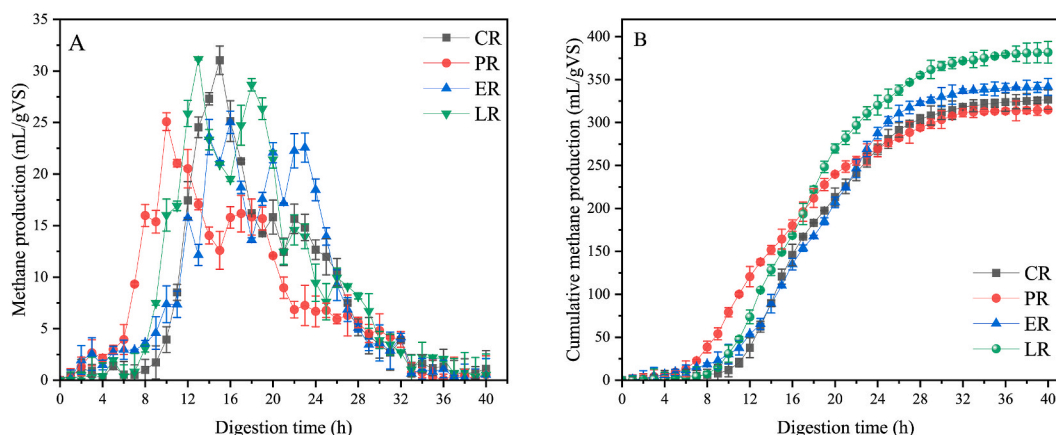

Fig. 3. Daily methane production (A), cumulative methane production (B) for different components of AD stages.
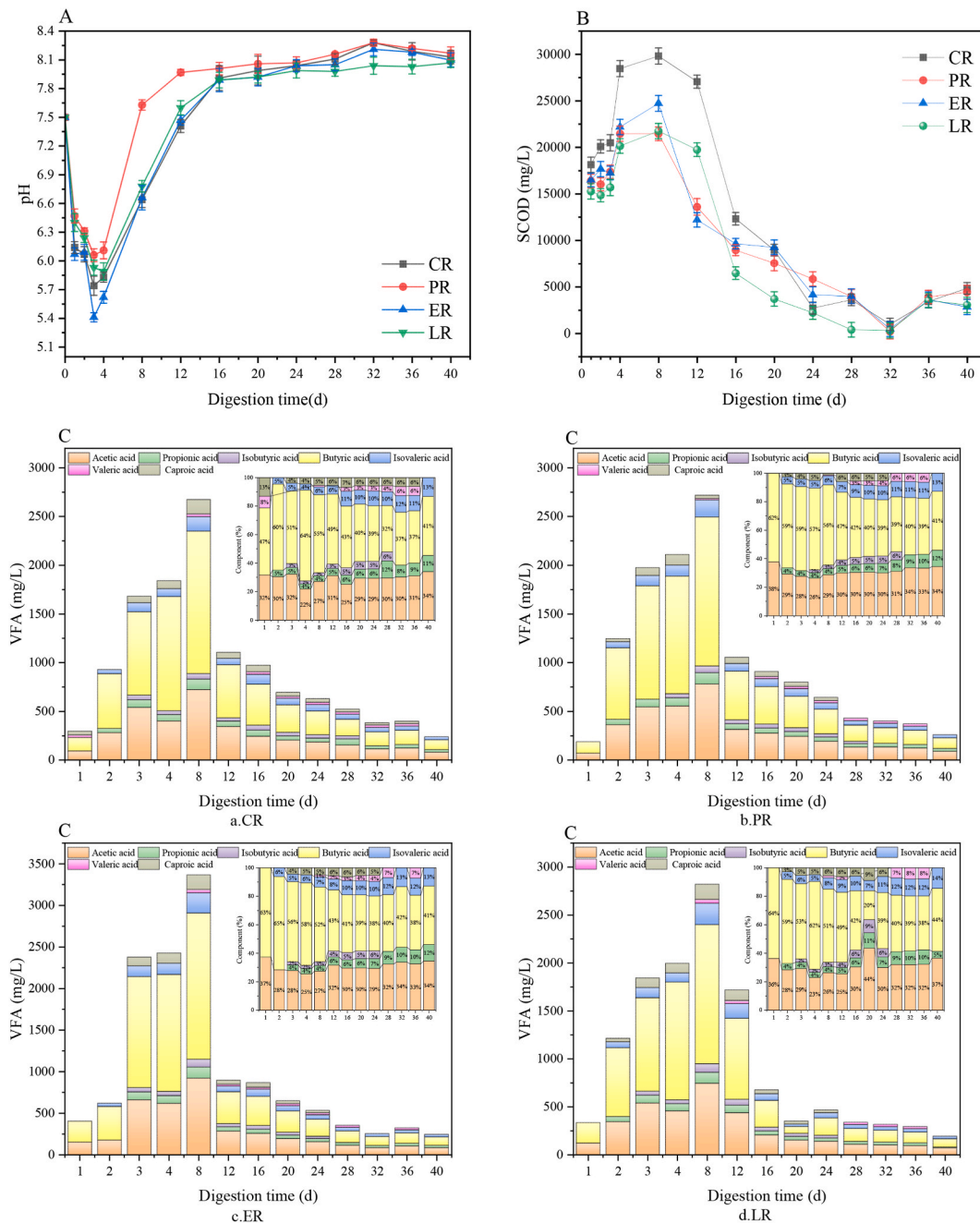
Fig. 4. pH (A), SCOD concentration (B), VFAs (C) for different components of the AD stage.

in the newly added inoculum, whose hydrolysis increases the solubility of organic matter in the system [53]. Subsequently, the reduced amount of macromolecular organic matter in the system and the weakening of the associated microbial life activities by the generated VFAs caused the SCOD levels to decrease [54]. In the end, the degradation rate of each component SCOD in the system ranged from 72.97 % to 82.86 %. LR and ER degradation were better, both of which exceeded 80 %. It means that the organic material has been fully utilized by the microorganisms and converted to methane [55], which supports the gas production results in the previous study.

3.4.3. VFAs

Fig. 4C illustrates the VFAs concentrations and proportions changes for each test group during AD. The concentration of VFAs showed a trend of increasing and decreasing in all test groups during this phase. The difference in total yield was not significant. Butyric acid and acetic acid were the main components detected at this stage, accounting for 78.67–81.00 % of the total VFAs

concentration. This result further proves that this study was a typical butyric acid fermentation process. In contrast to the DF phase, the ER showed the best acid production energy. It reached a maximum peak of (3366.43 ± 625.902) mg/L on day 8, 1.26 times higher than the CR group. It may be that the more structurally complex organisms have sufficient time to convert during the AD phase. The VFAs concentrations in each test group decreased by 1767.11 mg/L, 1664.81 mg/L, 2468.56 mg/L and 1101.27 mg/L after day 8, respectively. It indicates that methanogenic bacteria recovered their activity and began deleting small molecules of organic matter (e. g. VFAs) in the system to CH_4 and CO_2 [56]. It is equally evident from the daily biogas production (Fig. 3A) and the changes in butyric acid content.

3.5. Changes in enzyme activity during the AD process

3.5.1. Dehydrogenase

Dehydrogenase is a suitable parameter for reacting to changes in microbial activity in AD systems [57]. The changes in dehydrogenase activity during AD in the different groups are shown in Fig. 5A. As can be seen from the graph, there are two distinct peaks in dehydrogenase activity throughout the cycle, which follows the same trend as the results of Xueling Ran et al. [58]. Nanwen Zhu et al. reported that the appropriate pH range for the activated sludge dehydrogenase reaction was 7–9, and the optimum was 7.6. In this study, each component's first peak of dehydrogenase activity was reached on days 8–16, when the pH range was optimal. LR showed the best performance with a dehydrogenase activity of 80.24 ± 2.76 TFμg/(L-h), an increase of 30.2 % compared to PR. It is generally consistent with the trend in daily methane production. As the AD process proceeds, dehydrogenase activity decreases until it drops to a minimum around day 24. It may result from the constant depletion of nutrients in the substrate [59]. However, some undegraded proteins and other more complex organic matter remain in the substrate, which has hydrolytic and methanogenic effects. The dehydrogenase increases its activity to adapt to this environment. Therefore, a second peak is reached at day 32, gradually decreasing again [60].

3.5.2. Coenzyme F_{420}

Coenzyme F_{420} is a functional enzyme specific to methanogenic bacteria. It can react to the number and activity of methanogenic bacteria and plays an important role in the formation of CH_4 [61]. Fig. 5B shows the change in Coenzyme F_{420} concentration over time. During the start-up phase, all test groups showed a decreasing and then increasing trend, possibly due to the VFAs remaining at the end of DF inhibiting the metabolism of methanogenic bacteria at the beginning, leading to a decrease in coenzyme F_{420} concentration As AD proceeded, coenzyme F_{420} activity reached its highest level in all groups on days 8–12. It then gradually decreased, with the peak coenzyme F_{420} concentration in LR being 1.68 times higher than in PR. It is interesting to see that the daily methane production of each test group reached a corresponding peak at this time. It shows a correlation between the concentration of coenzyme F_{420} and the activity of methanogenic bacteria and methane production.

3.6. Energy conversion efficiency

The ECE is the sum of the potential energy of the two-step process of converting biomass to hydrogen and methane from different substrates in the test [62]. Table 2 presents the ECE of other digested substrates at both DF and AD stages. As a result of AD, the whole system receives more energy, whereas DF provides less energy. It is consistent with the findings of Zhiping Zhang et al. [63]. Fig. 6 shows that the CR and ER show better energy recovery performance with an overall ECE of 77.84 % and 66.55 %, related to the higher hydrogen yield obtained at this stage. Notably, The CR and PR groups had very similar methane yields at the anaerobic digestion stage, with a difference of no more than 5 %. However, from the point of view of energy conversion efficiency, the CR group improved by 34.82 % over the PR group. The reason for this may be related to the substrate's molecular structure and chemical bonding properties.
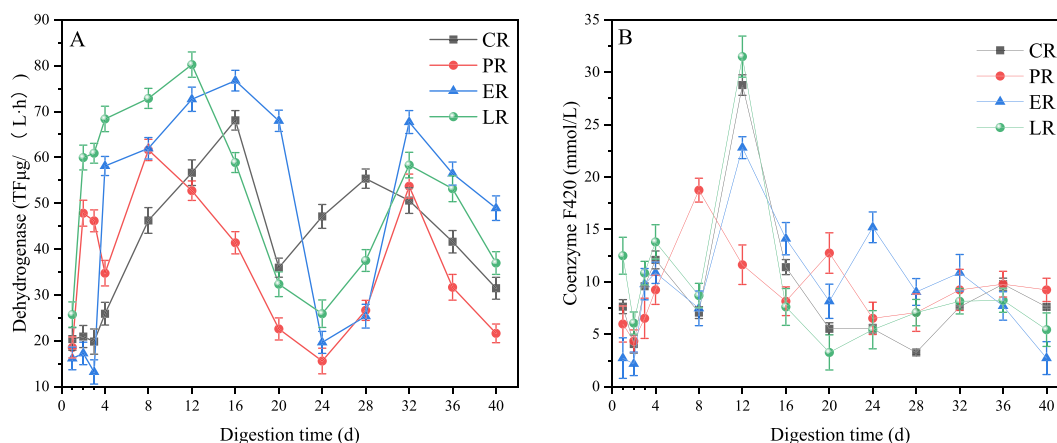

Fig. 5. Changes in the concentration of dehydrogenase, coenzyme F420 in different components.

Table 2
Energy conversion efficiencies of the different digested substrates.

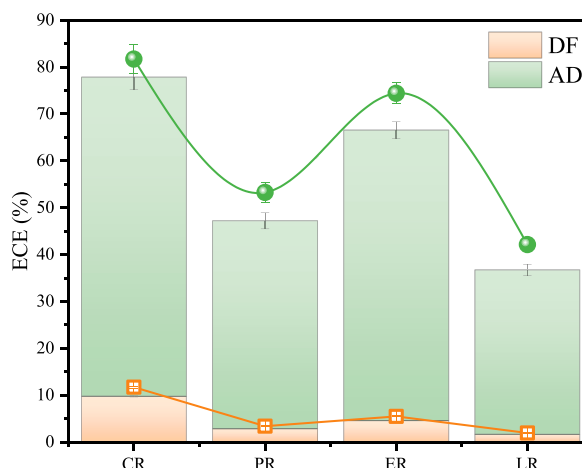| Substrate	Heat value kJ/gVS	DF (H_2)		AD (CH_4)		ECE	
		mL/gVS	kJ/gVS	mL/gVS	kJ/gVS	H_2%	CH_4 %
CR	17.16	154.91 ± 2.39	1.67 ± 0.03	326.77 ± 11.19	11.69 ± 0.44	9.75 ± 0.15	68.09 ± 2.58
PR	25.37	66.39 ± 2.23	0.72 ± 0.01	314.85 ± 11.57	11.26 ± 0.43	2.83 ± 0.05	44.38 ± 1.71
ER	19.70	83.24 ± 1.32	0.90 ± 0.01	341.53 ± 9.89	12.21 ± 0.37	4.56 ± 0.07	61.99 ± 1.86
LR	38.90	58.24 ± 2.57	0.63 ± 0.03	381.83 ± 12.61	13.65 ± 0.45	1.62 ± 0.07	35.10 ± 1.17

Fig. 6. Energy conversion efficiency of different components.

Since protein molecules are more significant and contain more carbon, hydrogen, and oxygen atoms, more heat is released during complete combustion, leading to a sizeable calorific value and low energy efficiency. This explains why the LR group achieved the highest methane yield (381.82 ± 12.61 mL/gVS), but the energy conversion efficiency was 20.91 % lower than that of the PR group with the lowest methane yield.

4. Conclusions

CR and LR substrates showed the best hydrogen (154.91 ± 2.39 mL/gVS) and methane (381.83 ± 12.691 mL/gVS) production performance, respectively. However, a more than 40 % protein ratio tended to lead to ammonia-nitrogen inhibition in the system and reduced gas yield, a result supported by changes in dissolved organic matter content and enzyme activity. Dark fermentation-anaerobic digestion effectively avoided the acid inhibition phenomenon, and the high ratio of butyric and acetic acid production in the liquid phase products proved the butyric acid type of digestion in all four test groups. The CR and ER groups performed better energy recovery with an overall ECE of 77.84 % and 66.55 %. In contrast, PR and LR were limited by the substrate molecular structure and chemical bonding, resulting in lower overall energy efficiency. The results showed that the anaerobic digestion performance was closely related to the physicochemical properties of the food waste and that an increase in carbohydrate concentration benefited hydrogen production and energy recovery. Two-stage anaerobic digestion can effectively prevent acid inhibition and contribute higher value to effectively utilizing food waste.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Shiyan Gu: Writing – review & editing, Supervision, Resources, Funding acquisition. **Huige Xing:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Lei Zhang:** Visualization, Investigation. **Ruji Wang:** Visualization, Investigation. **Ruoyu Kuang:** Investigation. **Yi Li:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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