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Enhanced anaerobic co-digestion of food waste and solid poultry slaughterhouse waste using fixed bed digester: Performance and energy recovery

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ABSTRACT

This study investigated the effects of organic loading rate (OLR) for anaerobic co-digestion (AcoD) of food waste (FW) and solid poultry slaughterhouse waste (SPSW) was performed in a fixed bed digester (FBD) at controlled pH to improve the methane production not fully discovered. Anaerobic co-digestion FW and SPSW were started up for the first time by gradually increasing OLR. At steady state, the FBD-AcoD reactor at OLR of 23.5 g COD/L/d the methane production was 7.8 L/L/d. Which achieved the highest OLR of 23.5 g COD/L, on the other hand when at OLR of 25.5 to 27.5 g COD/L the digester appeared inhibited and showed low performance in methane yields due to the accumulation of volatile fatty acids (VFAs) and long chain fatty acids (LCFAs). *Methanosaeta* and *Methanosarcina* were dominant over the acidogenic in the digester boosting the FBD-AcoD system to counter the acid effect. The removals of TS and VS around 79% and 76% on a continuous basis with a waste mixing of SPSW 18.5% and OLR of up to 23.5 g COD/L could biogas production 81 g COD/L/d. The FBD-AcoD system produces bioenergy of 875.3 Kj/g COD and the total investment energy utilized in the system was 8.51 Kj/g COD respectively.

1. Introduction

The current report is based on the United Nations Environment Program (UNEP), 931 million tons of food is wasted annually at the retail and consumer level, globally (UNEP, 2021). The food and agriculture organization (FAO) data further reveals that about 13.8% of global food is lost even before reaching consumers owing to inappropriate processing, handling, and storage practices (FAO, 2019). Food wastes (FW) and solid poultry slaughterhouse waste (SPSW) contain high amounts of organic biodegradable substrates and also recalcitrant, toxic materials such as cellulose, humus, and antioxidants (Liu et al., 2022; Abdallah et al., 2022). This waste with high organic content may require effective treatment, nevertheless,

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general treatment disadvantages high energy consumption, generation of toxic gas, bad odour, and slow degradation (Han et al., 2020). Current advances, in technology, to effectively prevent pollution and regenerate energy *simultaneously* together with anaerobic co-digestion attract attention (Negri et al., 2020; Nguyen et al., 2021; Ampese et al., 2022). Anaerobic co-digestion with the waste has a high concentration of biodegradable lipids, proteins, and carbohydrates with COD up to 120 g COD/L due to which it is a significant feedstock for the AD process. Combine FW mono-digestion with SPSW co-digestion offers beneficial such as substrate viability, reduce toxic dilution inhibition, C/N ratio balances, accumulation of volatile fatty acids (VFAs) and ammonia (Negri et al., 2020; Ampese et al., 2022). However, anaerobic co-digestion of FW might be a limitation because of a high amount of VFAs which could promote the growth of acid-forming bacteria it is difficult to form COD conversion and methane production at high OLR.

It is well documented that, anaerobic co-digestion (AcoD) is impacted by pH, C/N ratio, mixing ratio, additives, and microorganisms (Cai et al., 2022). Food waste co-digestion is influenced by the addition of solid poultry slaughterhouse waste (SPSW) to achieve stable conditions and maintain the pH of the system which further increases microbial growth and the high rate of methane production (Mao et al., 2017). SPSW with high protein and lipid content and ammonium nitrogen ($NH4^{+}$-N) is added in FW to improve the co-digestion mass balances, buffer capacity and reduce the risk of acidogenic microbial populations and increase the methanogenic activities (Abouelenien et al., 2014; Mao et al., 2017; Bong et al., 2018; Liao et al., 2021). Co-digestion of FW and SPSW requires specific operating conditions pH, hydraulic/sludge residence time (HRT) organic loading rate (OLR), volatile fatty acids (VFAs), and (long-chain fatty acids) LCFAs all significantly impact treatment success (Luo et al., 2015; Mao et al., 2017; Deng et al., 2021). FW and SPSW are having sufficient nutrients for the growth of microorganisms and impacts on hydrolysis–acidogenesis and acetogenesis–methanogenesis enhance the optimization of process parameters (Luo et al., 2015; Mao et al., 2017; Deng et al., 2021). Researcher claims that a single feedstock or one substrate used in a reactor face challenges that could be an imbalance in the digestion process. AcoD process with food waste, waste-activated sludge, and chicken waste manure can decreases the dilution of toxic chemicals, enhance the balance of nutrients, and synergistic growth of microorganisms (Mao et al., 2017; Liao et al., 2021).

Furthermore, FW and SPSW have a high amount of protein and lipids, and their degradation on produces ammonia which is an inhibitory effect on anaerobic microorganism growth. Lipids also create scum and accumulated VFA and LCFA accumulated could decrease pH in the reactor (Zhen et al., 2022). It required an additional supply of energy is required to run the reactor to maintain the AcoD system resulting in a waste of energy, and resources resulting to save the environment (Kurade et al., 2020).

Based on a systematic literature review, OLRs are helpful to monitor the process stability, microbial community structure, and yield of methane. However, at high OLRs would adversely affect the co-digestion process due to acidification and ammonia (Zhen et al., 2022). The mixing of switch grass and substrate ratio of 1:1 in batch mode operation showed methane yield was 204 mLCH_4/gVS (Ünyay et al., 2022). The system was operated at the mixing ratio of 1:1 switch grass and substrate ratio at OLR 1.0 gVS/L.d, where the methane yield was 35% and energy recovery of 38% was recovered (Ünyay et al., 2022). In continuous stirred-tank reactor (CSTR) operation with poultry slaughterhouse waste at OLR of 1, 5, 10, and 15 g VS L/d, the yield of methane production decreased from 0.48 to 0.10 LCH_4/g VS removed (López-Escobar et al., 2014; Deng et al., 2021). In order to fixed bed reactor (FBD) a high rate biological reactor could be worked in at high OLRs, biomass immobilization, and continuous horizontal flow have shown great efficiency for biogas production and organic matter removal. Furthermore, a drastic decrease in methane yields and microbial community structure was observed at the highest OLR of 6.0 kg/m^3/d VS (Yu et al., 2021; Liu et al., 2022). When the reactor was operated at lower than 4.0 kg/m^3/d VS of OLR, showed good growth of certain bacteria *Methanosaeta* was dominant with respect to its archaeal cell major colony and was the most important acetoclastic methanogen in the reactor. But, when the OLR was 6 kg/ (m^3 day) VS reactor had good growth of hydrogenotrophic methanogens such as *Methanoregula* and *Methanospirillum* were the dominant archaea observed (Feng et al., 2021; Yu et al., 2021). Anaerobic co-digestion *is* the *most* successful technology that could be enhanced the microbial community structure, and their composition and their activities were affected by reactor operational conditions (Feng et al., 2021). *Methanosaeta* and *Methanospirillum* dominated at defined OLRs loading and also dominant microbial archaea acetoclastic and hydrogenotrophic methanogens which could accelerate biogas *production*. *Methanosaeta* almost disappeared at high OLRs, whereas *Methanoculleus* was favored to dominate. *Methanosarcina* is the only methanogen that can switch between acetoclastic and hydrogenotrophic methanogenesis routes, while *Methanosaeta* was known to work on the acetoclastic pathway only. Syntrophic prokaryotes were in high relative abundance (>9%) and their metabolic potential was syntrophic methane production (Zhen et al., 2022; Li et al., 2022). Particularly, there is a significant research gap in determining the effect of various loading of OLRs on methane production, the role of dominant archaea, and theoretical energy recovery with the AcoD system of FW and SPSW.

The novelty of this study identified that the FBD-AcoD system is feasible for long-term operation and significant treatment with solid contents of FW and SPSW. FBD-AcoD was operated for 300 days to evaluate the operation stability with gradually increasing OLR, high performance. Analyses of the FBD-AcoD system performance combined with the microbial community archaea structure, change of microbial community, variations in microbial community composition as well as working methanogens consortia for methane production were investigated. Studied the energy consumption and total energy production from the FBD-AcoD system were calculated. In order to the practical application of the FBD-AcoD system performance and the microbial community was employed for FW and SPSW co-digestion.

Table 1
Characteristics of food waste and solid poultry slaughterhouse waste.

Parameters	Food waste	Solid poultry slaughterhouse waste
pH	5.7 ± 0.03	8.3 ± 0.56
COD (g/L)	123 ± 11.76	5.8 ± 0.43
SCOD (g/L)	78 ± 7.89	4.1 ± 0.08
TCOD (g/L)	145 ± 17.98	6.7 ± 0.74
BOD (g/L)	105 ± 9.39	173 ± 16.62
NH_4^+-N (mg/L)	17.3 ± 2.45	3661 ± 23.95
TSS (g/L)	115 ± 10.91	215 ± 11.54
VSS (g/L)	110 ± 13.78	93 ± 3.67
MLSS (mg/L)	1760 ± 117.56	945 ± 19.67
C/N ratio (g/L)	23.70 ± 4.78	14.25 ± 0.89
VS/TS	75.55%	36.23%
Protein	34.7%	14.9%

Table 2
Operational condition and feedstock properties applying the different ratios of Solid poultry slaughterhouse waste (SPSW) of the reactor.

SPSW (%)	OLR (g COD/L/d)	HRT (h)	pH	Influent total COD (g/L)	Feed TS (%)	TS removal (%)	Feed VS (g/L)	VS removal (%)	MLSS (mg/L)	Ammonia NH_4^+-N (g/L)	COD removal (%)	Biogas (L g COD/d)
1.5	0.5	124	6.5	2.95	1.5	23	2.6	21	7810	0.34	13	12
2.5	1.5	124	6.6	4.75	3.5	29	2.6	27	24560	0.55	19	14
3.5	2.5	124	6.5	12.85	3.5	33	2.6	31	34760	0.75	21	19
4.5	4.5	124	6.7	21.33	3.5	39	1.9	36	46786	0.91	35	21
6.5	6.5	124	6.7	29.55	4.5	45	1.8	42	55600	1.32	45	33
8.5	9.5	96	6.6	37.95	4.5	53	1.9	49	65560	1.45	51	41
10.5	12.5	96	6.5	43.15	4.5	59	3.8	53	79340	1.78	63	49
12.5	15.5	96	6.7	43.15	6.1	64	3.8	61	83400	1.89	69	53
14.5	18.5	78	7.1	43.15	6.1	69	4.8	67	91450	1.91	74	60
16.5	21.5	72	6.9	43.15	6.1	72	5.8	71	10500	2.05	78	67
18.5	23.5	60	7.0	43.15	6.5	79	6.9	76	11500	2.11	89	74
20.5	25.5	42	7.3	43.15	2.5	69	4.1	54	98450	2.24	59	23
22.5	27.5	42	7.6	43.15	2.5	52	1.9	33	53500	2.15	35	19

2. Materials and methods

2.1. Substrates

The food waste (FW) was collected from the cafeteria dustbin of the University of Nizwa, Oman. The FW is composed of noodles, rice, cabbage, potatoes, carrots, chicken, lamb, and eggs, which were configured according to the wet mass ratio weight of 11%, 51%, 10%, 11%, 5%, 9%, and 3%. The solid poultry slaughterhouse waste (SPSW) was collected from Alsafwa chicken farmhouse, Nizwa. The SPSW is composed of soft tissue 35%, feather 17%, and ash 11%. The seed sludge was collected from an anaerobic digester sludge of a wastewater treatment plant, Nizwa. The characteristics of FW and solid poultry slaughterhouse waste (SPSW) have been shown in Table 1.

2.2. Experimental setup

A schematic diagram of the FBD reactor is depicted in Fig. 1. The reactor was constructed of plexiglass with an internal diameter of 65 mm and a height of 90 cm. The Experimental setup FBD reactor with 5 L effective volume could effectively mark parts solid, liquid, and gas were placed in the one-fourth part of the reactor (Fig. 1). The fixed bed filled with full active volume and food waste and solid poultry slaughterhouse waste substrate was fed from the bottom using a peristaltic pump. The fixed bed support material was used as solid poultry slaughterhouse waste for the attachment of microbial biomass in the bed zone of the reactor. The bed was immobilized with solid poultry slaughterhouse waste expanded packed into the reactor. The reactor was inoculated to maintain the working volume of 2.5 L of seed sludge AD with a substrate mixture. The reactor was filled with inoculum sludge (VS = 27.25 g/L, TS = 59.35 g/L), and the reactor was run at a 35 °C temperature of condition. The reactor was flushed with nitrogen gas to make sure that anaerobic conditions. The centrifuged FW and ground by grinder partially centrifuged SPSW to remove coarse particles were pumped up-flow from the bottom by a peristaltic pump and run to monitor the biogas and the development of microbial dynamics for different feed ratios. The collected biogas and calculating the volume of biogas and determining the methane contents in biogas. On daily basis biogas, pH, VFAs were monitored, while COD, SCOD, and TS, VS were analyzed at the start and end of sample feeding. The FBD reactor was placed in a water bath layer operating at a temperature was 35 °C. In Table 2, as shown in this experiment, during the OLR 0.5, 1.5, 2.4, 4.5, 6.5, 9.5, 12.5, 15.5, 18.5, 21.5, 23.5, 25.5, and 27.5 g COD/L/d loading, respectively. The OLR of FW in each stage was mixed the SPSW ratio were 1.5, 2.5, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5, 14.5, 16.5, 18.5, 20.5, 22.5% V/W and HRT was changed at constant OLR feeding. To achieve faster growth of microbial biomass in the AcoD system with methanogenic effluent recirculation of 15% of working volume to FBD reactor for methane production and also reducing the energy consumption.

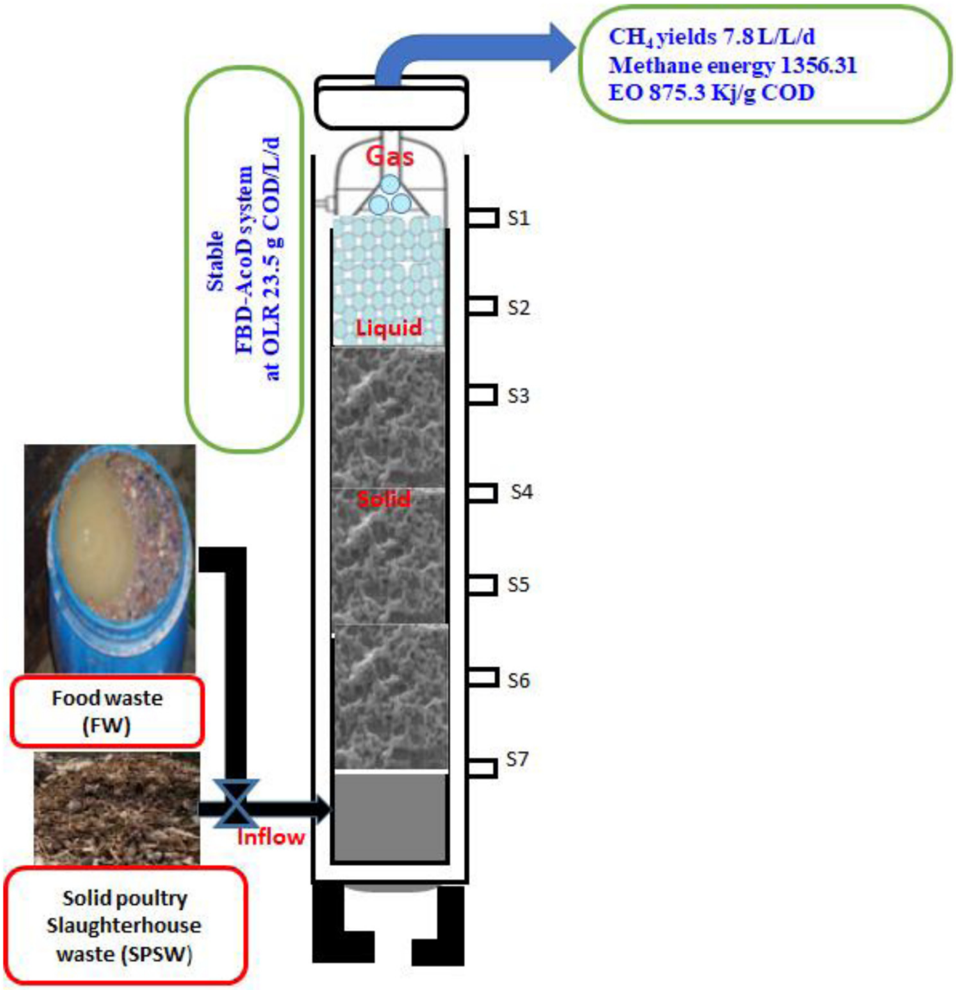

Fig. 1. Experimental setup of FBD-AcoD: FW-food waste; SPSW-Solid poultry slaughter waste; peristaltic pump; Fixed bed sludge: S1–S7: sampling port; methane yields system.

2.3. Reactor performance

The performance of the FBD-AcoD reactor continuously operated at 35 °C and various hydraulic retention time (HRT). A gas–solid–liquid (GSL) separator was installed at top of the reactor for biogas capturing. Sampling ports were installed at suitable heights of the reactor. The experimental setup of the FBD-AcoD reactor is shown in Fig. 1. Continuous feeding was started with an initial organic loading rate (OLR) of g COD/L/d loading and various HRT. The influent COD concentration was 0.5 g COD/L/d loading for the first 5.5 days, and then it was increased stepwise based on OLR. The reactor was monitored daily for COD removal, VFAs, LCFAs effluent VSS, MLVSS, and methane, and biogas production, and sludge was sampled periodically from the sampling points arranged along the height of the reactors to determine according to standard methods (APHA, 1995). Anaerobic co-digestion of the substrate in specific methane production and the ratio of the co-digestion efficiency hydrolysis %, acidogensis%, acetognesis%, and methanogensis% were calculated for a mixed substrate COD mass balance.

$$Hydrolysis\% = \frac{COD_{methane} + SCOD_{effluent} - SCOD_{influent}}{COD_{total\ influent} + SCOD_{influent}} \quad (1)$$

$$Acidogensis\% = \frac{COD_{methane} + COD_{VFA\ effluent} - SCOD_{VFA\ influent}}{COD_{Influent\ total} + COD_{VFA\ influent}} \quad (2)$$

$$Actognesis\% = \frac{COD_{methane} + COD_{acetic\ acid\ effluent} - SCOD_{acetic\ acid\ influent}}{COD_{total\ influent} + COD_{acetic\ acid\ influent}} \quad (3)$$

$$methanogensis\% = \frac{COD_{methane}}{COD_{total\ influent}} \quad (4)$$

2.4. Methods of microbial community analysis

Bacterial genes and archaeal sequences were analyzed by high throughput sequences. The sequencing was performed and assess the bacteria diversity, relative abundance, and archaea in the samples. Amplification and sequencing of genes were used in the 16S rRNA genes (V3–V4 region) of bacteria and the V4–V5 region of archaea barcoded (He et al., 2021). The bacterial primers used for amplification were 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), and the archaeal primers were Arch524F (TGYCAGCCGCCGCGGTAA) and Arch958R (CCYGGCGTTGAVTCCAATT) sequencing data processing and analysis.

2.5. COD mass balance and energy estimations

In this work, we calculated the COD mass balance

CODinput = CODmethane + CODeffluent + CODaccumulation + CODeffluent Where CODDeffluent is the COD of the sludge discharged from the reactor (mg/L); CODaccumulation is the COD accumulated during the stable operation of the reactor (mg/L); CODeffluent is the COD of the reactor effluent (mg/L).

For two-stage efficiencies, energy consumption, and energy recovery on the investigation with OLR of 1.5–23.5 g COD/L/d were analyzed. The highest energy recovery and acidogenesis to methanogenesis at OLR of 23.5 g COD/L/d of the FBD-AcoD system. So that the FBD-AcoD efficiency was analyzed based on COD mass balance, energy production, and energy consumption at OLR of 23.5 g COD/L/d. The FBD-AcoD efficiencies of hydrolysis, acidogenesis, acetogenesis, and methanogenesis were also calculated from the COD mass balance according to Ruggeri et al. (2010). The conversion rate of COD in COD methane (0.67 m^3 CH_4/Kg COD); COD influent and COD effluent are the COD of the reactor influent and effluent respectively (mg/L); COD influent VFAs (acetic acid-LCFAs) and effluent VFAs (acetic acid-LCFAs) of the reactor influent and effluent respectively (mg/L). The reactor system has three components (pump, feed pump, and water bath) and consumption energy was calculated according to Ruggeri et al. (2010). The energy recovered from methane yield (L/gCOD) with the calorific value of methane (35.8 Kj/l) and energy consumed by the system (feeding, heating, refluxing) in h/d.

2.6. Analytical instrumental analysis

Physicochemical characterization of the substrates showed that the pH of the anaerobic digestion sludge was neutral, which is a notable quality of wastewater. pH was measured by a pH meter (Hatch, 1472). The total solids (TS) and volatile solids (VS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), soluble chemical oxygen demand (SCOD), and $NH4^+$-N concentration, total nitrogen (TN), total Kjeldahl nitrogen (TKN) and BOD were analyzed by the standard method (APHA, 1995). COD was measured by the dichromate method. Effluent sludge samples for volatile fatty acid (VFA), and long-chain fatty acid (LCFA) analysis were initially centrifuged at 1000 g for 10 min. The volume of biogas generation was measured and recorded on a basis using a gas analyzer (APHA, 1995). The methane and carbon dioxide determined in the gas were used Shimadzu GC 2010 plus gas chromatography.

3. Result and discussion

3.1. The digestate of the FBD-AcoD system

Studied the anaerobic co-digestion of the food waste (FW) by mixing solid poultry slaughterhouse waste (SPSW), particularly the effect of organic loading rate (OLR) hydraulic retention time (HRT) on the process (Table 2). The prerequisite of the feed properties of the reactor and its removal is an important parameter of COD removal and biogas production. When the OLR was gradually increased from 0.5 to 23.5 g COD/L/d by reducing the HRT from 120 to 60 h, the performance of the further improved. The COD removal rates were 89%, biogas production 74 mL, TS 79%, VS 76%, MLSS 11500 mg/L, and ammonia 2.11 g/L at OLR of 23.5 g COD/L/d used as feedstock FW and SPSW (Table 2). (Kim et al., 2017), reported that when the OLR was more than 17.35 g COD/L/d, the COD removal rate was 76%, and our studies achieved more than 80% by using AD of FW and reactor become stable. In this study, treating the FW mixing with SPSW co-digested by using the FBD reactor was efficient removal of COD and biogas production under high OLR. With the OLR of 23.5 g COD/L/d, the FBD-AcoD system performance was evaluated in SCOD removal at 77.3% and MLSS 11500 mg/L. This indicates that the co-digestion of organics at the mixture of SPSW to achieved higher acetogensis to methanogensis at 240–260 d (Fig. 2). Using FBD reactor for the disposal of solid and FW-SPSW needs consideration to the settling of solid by employing a parameter as MLSS and MLVSS. The reactor showed on day 50, the concentration of MLVSS was 102 mg/L and further concentration increased up to 4335 mg/L at 260–280 d respectively. The parameter involved finding the maximum separation of liquid and solids mean mature acetogensis from anaerobic co-digestion (Fig. 2 and Table 2). The FBD-AcoD system still maintains a high range MLVSS throughout the operation without being significantly affected by

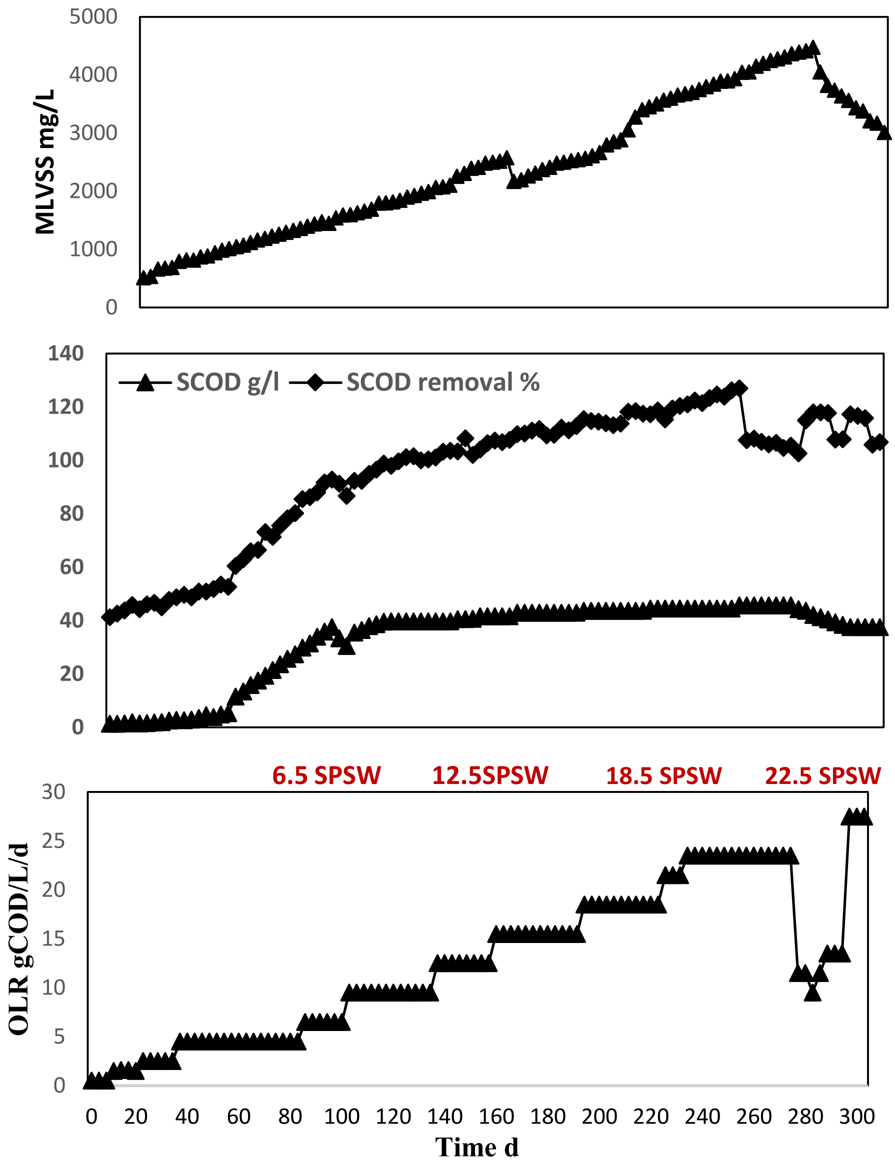

Fig. 2. Loading, methane yield, soluble COD, pH and ammonia in digesters Solid poultry slaughterhouse waste (SPSW).

the high volume of mixed liquor and low solid content. The OLR was elevated to a higher range of 17.3–22.5 g COD/L/d, and the system acidified showed the lowest performance in COD removal and methane production (Bong et al., 2018; Amha et al., 2019; Basak et al., 2021). When we used the FW with SPSW addition of 6.5, 8.5, 12.5, and 18.5% to feedstock volume, the performance of the FBD-AcoD system was stable and further acidified mean inhibition with the lowest COD removal was 89%, biogas production 74 L g COD/d at 18.5% SPSW and in system NH_4^+-N (2.11 g/L) could effectively reduce the acidification and maintain the pH (Table 2).

3.2. OLR on VFAs degradation for methane yields

The digester characteristics based on the present experiment, pH remained between 7.2 and total volatile fatty acids (VFAs) varied under different OLR conditions was around 8.54 g/L COD. Acetic acid is the most abundant in the reactor. The methane production was monitored daily and the methane content of the FBD-AcoD reflected the process performance under different OLRs. The acetic acid and methane yield was higher at 23.5 g COD/L compared to a lower OLR and more than 23.5 g COD/L OLR. The *unlikely to decrease the methane* yield, when the OLR was raised to 25.5 g COD/L, the methane yields decreased quickly to 2.5 L/L/d due to accumulation of VFA, propionic, butyric, Caproaic, Palmitic, and LCFA (Table 3). Our study confirmed that the highest methane yield of 19 L/L/d was co-digested with 18.5% of SPSW. Several studies

Table 3
The effect of elevated organic loading rate with changes pH on digested materials characteristics.

Organic loading rate (g COD/L/d)	pH	Methane yield (L/L/d)	Acetic acid (g/l)	Total VFA as COD (g/l)	Propionic (g/l)	Butyric (g/l)	Iso-butyric (g/l)	Iso-valeric (g/l)	Valeric (g/l)	Caproic (g/l)	Myristic (g/l)	Palmitic (g/l)	Oleaic (g/l)	Steareic (g/l)	Total LCFA (g/l)
0.5	6.3	1.4	0.99	0.31	0.23	0.07	0.04	0.03	0.002	0.11	0.004	1.01	0.1	0.12	1.4
1.5	6.4	2.1	1.31	0.85	0.35	0.09	0.06	0.05	0.005	0.13	0.005	1.05	0.1	0.19	1.6
2.5	6.2	3.5	1.97	1.12	0.67	0.12	0.07	0.06	0.009	0.15	0.006	1.09	0.2	0.21	1.9
4.5	6.6	4.9	2.87	1.85	0.79	0.16	0.08	0.07	0.02	0.14	0.008	1.14	0.2	0.31	2.4
6.5	6.5	5.3	3.17	2.10	0.92	0.19	0.09	0.07	0.04	0.17	0.009	1.51	0.2	0.45	2.9
9.5	6.7	5.7	3.95	2.96	1.25	0.23	0.11	0.08	0.05	0.18	0.011	2.75	0.3	0.56	4.6
12.5	6.5	5.9	4.19	3.76	1.74	0.28	0.21	0.09	0.08	0.19	0.014	2.91	0.3	0.75	5.9
15.5	6.7	6.3	5.96	4.75	1.97	0.33	0.32	0.18	0.09	0.13	0.015	2.99	0.3	0.82	7.3
18.5	6.8	6.7	6.83	5.89	2..14	0.37	0.49	0.34	0.15	0.16	0.018	3.12	0.5	0.91	8.4
21.5	6.9	6.9	8.57	7.65	2.67	0.43	0.56	0.56	0.35	0.19	0.019	3.98	0.5	0.97	9.5
23.5	7.2	7.8	9.32	8.54	2.01	0.49	0.45	0.23	0.13	0.22	0.002	2.11	0.2	0.21	9.9
25.5	7.0	4.4	5.21	9.85	1.13	0.13	0.23	0.14	0.12	0.9	0.007	1.09	0.21	0.11	10.3
27.5	6.3	1.2	3.87	10.76	0.24	0.08	0.11	0.11	0.11	0.1	0.003	0.071	0.03	0.029	12.8

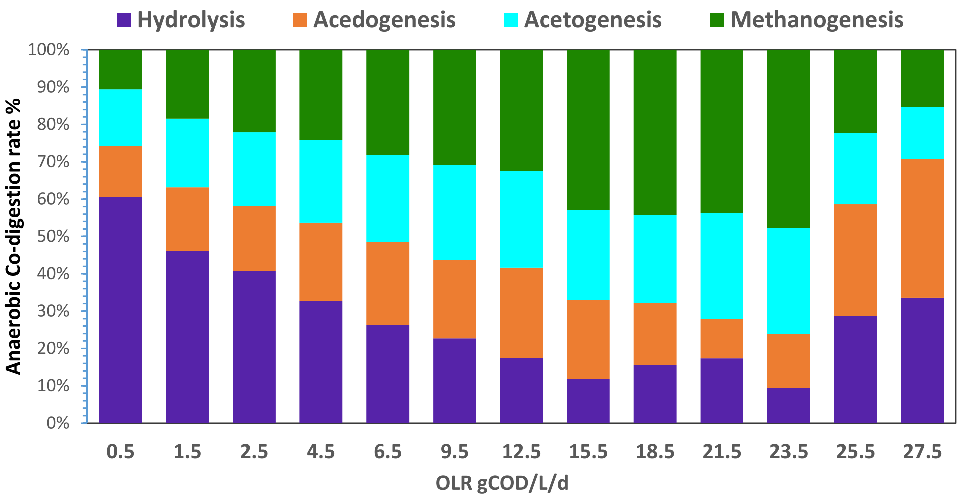

Fig. 3. Effect of OLR for the performance of hydrolysis, acidogenesis, acetogenesis, and methanogenesis of the FBD-AcoD system.

showed a lower methane yield of 2.42 L/L/d using co-digestion of wastes at a higher OLR of 10 KgCOD (Gao et al., 2020; Kong et al., 2019). However, a high OLR leads to maximum VFA production, showing significant inhibition of acidogenesis and methanogenesis due to high VFA can hinder microbial growth and metabolism (Otero et al., 2021; Kazimierowicz et al., 2021). AcoD system digested components of TVFA and their degradation was mediated for VFA accumulation (Li et al., 2017; Zhang et al., 2020). Results presented in Table 3, butyric acid with combined formation was found (propionic, butyric, and Iso-butyric) became a main constituent of VFA.

3.3. OLR on hydrolysis to methanogenesis

Explore the efficiency of the FBD-AcoD system is divided into four stages hydrolysis, acidogenesis, acetogenesis, and methanogenesis might be performing, well, and the stability of each stage at various OLRs. The high efficiency of the reactor was an effective way to improve the organic loading; average stabilization stages were estimated as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Poszytek et al., 2019; Zhen et al., 2022). The co-digestion of FW with SPSW conversion in four stages namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, and improved hydrolysis ratio 83%, acidogenesis 79%, acetogenesis 84%, and methanogenesis 89% at OLR 23.5 g COD/L. Co-digestion to form a more efficient synergistic metabolic process to improve the methanogenesis ratio by microbial activity determined the stability of the AD process as shown in Fig. 3. Furthermore, with an increase in the OLRs 1.5–21.5 g COD/L/d, the efficiency of the hydrolysis gradually improved is a rate-limiting step of microbial activity. However, the elevation of *OLR* 25.5–27.5 g COD/L/d *significantly* the inhibitions of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The hydrolysis ratio might be higher than acidogenesis and less than acetogenesis and methanogenesis due to microbial accumulation of VFA, and LCFA, important to *maintain a balance between* the four stages. The hydrolysis decreased when the OLR exceeded the threshold, due to an imbalance of microbial growth might decrease the efficiency of acidogenic, acetogenic, and methanogenic (Gao et al., 2020; Liu et al., 2022). In this study, the FBD-AcoD retaining optimum hydrolysis efficiency was vital to excellent mass transfer and microbial retention of the reactor. O*ur* investigations on the potential of the addition of SPSW was fruitful in the accumulation of VFA reducing acidification and supporting microbial growth.

Table 4
Mechanism of mass balance COD and net energy recovery from FBD-AcoD system of per g COD.

Organic loading rate (g COD/L/d)	COD converted methane (%)	COD in sludge (%)	COD final discharge (%)	COD effluent (%)	Methane Production rates (%)	Energy from methane (KJ/d)	EnergyFrom system (KJ/d)	Total Energy recovery (KJ/gCOD) EO	Total Energy Consumed System (KJ/gCOD) EI
0.5	37.4	12	9.1	28.3	19	73.12	17.80	−29.4	130.26
1.5	49.3	10	8.6	23.5	23	109.46	73.73	−13.6	120.92
2.5	51.5	9	7.9	21.7	31	178.45	184.33	91.7	110.31
4.5	63.7	8.7	7.2	19.7	34	262.78	256.12	156.9	97.54
6.5	69.5	7.2	6.3	17.6	36	375.90	375.75	276.8	87.4
9.5	73.5	6.4	5.5	15.5	39	523.05	489.45	376.3	73.87
12.5	78.9	5.3	4.7	13.8	42	631.56	698.3	496.7	63.53
15.5	83.2	4.5	4.1	12.9	56	790.34	765.33	571.5	51.82
18.5	85.8	3.4	3.5	10.4	69	945.65	962.59	690.5	30.46
21.5	87.9	2.7	2.7	8.5	77	1185.35	1139.90	745.8	19.62
23.5	93.9	13.3	1.4	7.5	89	1356.31	1456.92	875.3	8.51
25.5	76.4	3.7	7.9	14.5	59	934.87	1032.34	563.9	56.35
27.5	69.4	7.5	11.5	23.3	47	556.21	636.77	325.8	79.91

The performance of the FBD-AcoD system

The performance of the reactor FBD-AcoD showed high degradation of organics in COD removal and methane production under different OLRs. According to Abdallah et al. (2022) and Zhao et al. (2019), the FBD reactor compared with CSTR, UASB has excellent mass balance and good solids-liquid separation effect on the retention of solid and microorganisms. Furthermore, co-digestion of FW and SPSW provides benefits in NH_4^+-N increase to maintain pH. Thus, high concentrations of $NH4^+$-N may *lead to* a bottleneck in *acidogenesis* due *to* decreased specific *acidogenic* enzyme activities, which might be the decomposition of proteins could produce NH_4^+-N (Fig. 4). However, the acidogenesis also improve digestate stability with fixed bed recycled refluxed effluent into the inlet to uniform distribution of NH_4^+-N and reduced the chances of acidification in the reactor.

To assess the co-digestion, mixed substrate FW with SPSW was beneficial to reduce of toxic chemicals maintaining the buffer capacity due to ammonium of the AcoD system (Fig. 4). The co-digestion, VFAs were the intermediate product and accumulation by microbes, decreasing pH, causing unstable operational performance, inhibiting the methanogens activity, might be a decrease in methane production (Jabeen et al., 2015; Li et al., 2017; Zhang et al., 2022). As illustrated in Table 3 and Fig. 5, under mesophilic conditions, studying the VFA and LCFA concentrations and the methane yields under the continuous mode in the long-term operation of the FBD reactor. The butyric acid (n-Butyric and iso-Butyric) was accumulated and accounted for more than 90% VFA in stable conditions under the hydrolysis stage. Which might be decreased to 60%–70% in a methanogenic stage. Acetic acid was the most abundant VFA, indicating that acetoclastic methanogenesis was the rate-limiting step that showed a rich amount of feedstock. However, the system demonstrated in VFA profiles under varying process conditions, indicating different degradation steps being rate-limiting could result in variations in the methanogenic communities (Jabeen et al., 2015; Cheng et al., 2020). Acetic acid and propionate acids showed almost no accumulation in the reactor but accounted for 10%–15% of total VFA in the reactor (Fig. 5). Based on our findings the *effects* of *pH* on the production VFAs concentrations and the substrate condition also effect on the stability of metabolic pathway process. The VFAs accumulation has an impact feel stressed, leads to a decrease in pH due to rises in H_2 concentrations, creates toxicity (inhibiting the methanogenesis) might be decreasing methane production (Jabeen et al., 2015; Li et al., 2017; Dyksma et al., 2020). It was found that the highest methane production rate and VFAs steeply precursor rises when the OLR of 4.5 g COD/L/d (20 days), further increases to an OLR of 23.5 g COD/L/d (280 days). Due to long-term acclimation and adaptation of microorganisms for effective degradation of VFA at OLR of 23.5 g COD/L/d (280 days) were observed methane production was 8.23 L/L/d, biogas 81 L/d (Fig. 4). In other cases, digester with waste mixture resume that LCFA inhibited the propionate acid degradation and the methanogenesis steps of AD (Zhen et al., 2022). Furthermore, with the high VFA and LCFA concentrations, methanogenesis was partially affected (Xu et al., 2014; Wu et al., 2021). Long-term operation of the reactor with higher OLR rates and at the same time better methane yield and low effluent VFA and LCFA are the aims of this study.

In this study, added 20% SPSW with FW in the reactor, the 465 mg/L NH_4^+-N increased in feedstock, resulting in the influenced activity of microorganisms for VFA being neutralized by the action of NH_4^+-N. Fig. 5 and Table 4, the observed for VFA including acetic acid > butyric > propionic > isobutyric > valeric > isovaleric and LCFA palmitic > oleic > stearic > myristic > caproic appear at 280 d. The findings suggested that the acetic acid might be converted to methane more efficiently than propionate, butyric and propionic. As shown in Fig. 5, the operation of the FBD-AcoD system, at the OLR of 1.5 and 4.5, total VFA concentrations were under 0.9 and 2.87 g/L. At the OLR of 23.5, VFA accumulation arises, and the concentration jumps up to 9.32 g/L. The experiment indicated that acetic acid was a leading component that could be converted to methane, which could be methanogens weak due to the loss of sludge at higher OLRs (Basak et al., 2021). This result agrees with Li et al. (2017), Poszytek et al. (2019), who recommend that the microbes grow well in acetic acid conditions and highest specific growth. The lower to higher concentrations of VFA and LCFA suggested that the progressively improved methanogenic metabolic activity in the FBD-AcoD system. When the OLR increased, the progressively produced VFAs were elevated with the action of hydrolysis and acidification, and also had an impressive rate of VFAs consumption by methanogens. At high OLR 23.5 g COD/L/d, the levels of VFA and LCFA concentration hit 9.34 and 9.9 g/L, the biogas 81 L/L/d, methane production was with methane content 78%, at this stage positively support

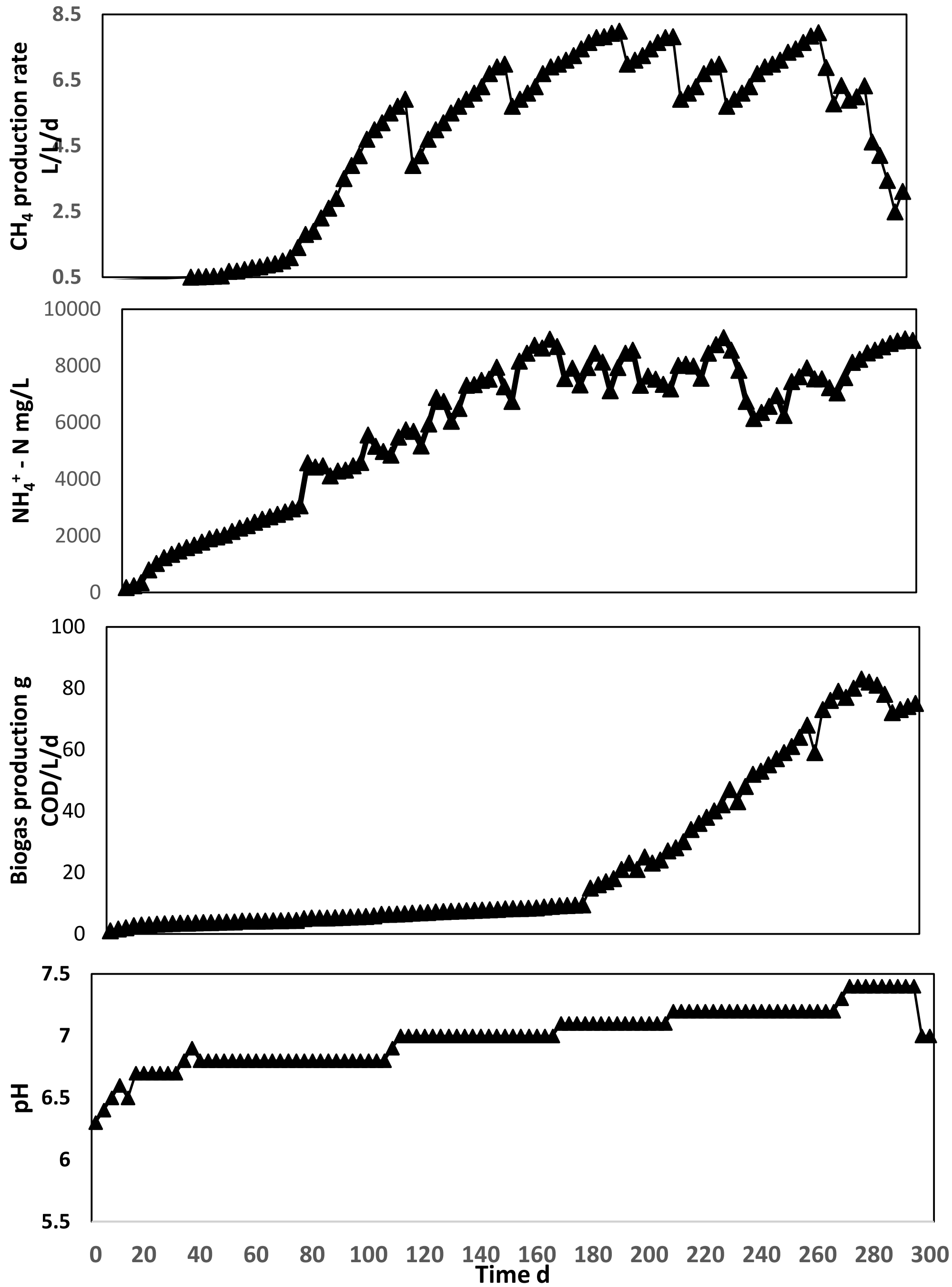

Fig. 4. FBD-AcoD system showed performance CH_4 production rate, NH_4^+-N concentration, Biogas production, changes of pH.

the growth of methanogens (Fig. 5). The levels of free ammonia (FA) could be increased with the increase of NH_4^+-N concentration. Rocamora et al. (2020) suggested that methanogenesis is affected due to FA could diffuse microbial cells and releasing a proton polarity that may enhance the enzymatic response of methanogens. The working environment of methanogens might be affected by FA concentrations in the range of 300–800 mg/L. As shown in Fig. 5, the progressive FA concentration achieved 679 mg/L in our novel study, which could be lower than the cause of inhibitory methanogenesis, it is a good sign that the FBD-AcoD system is stable.

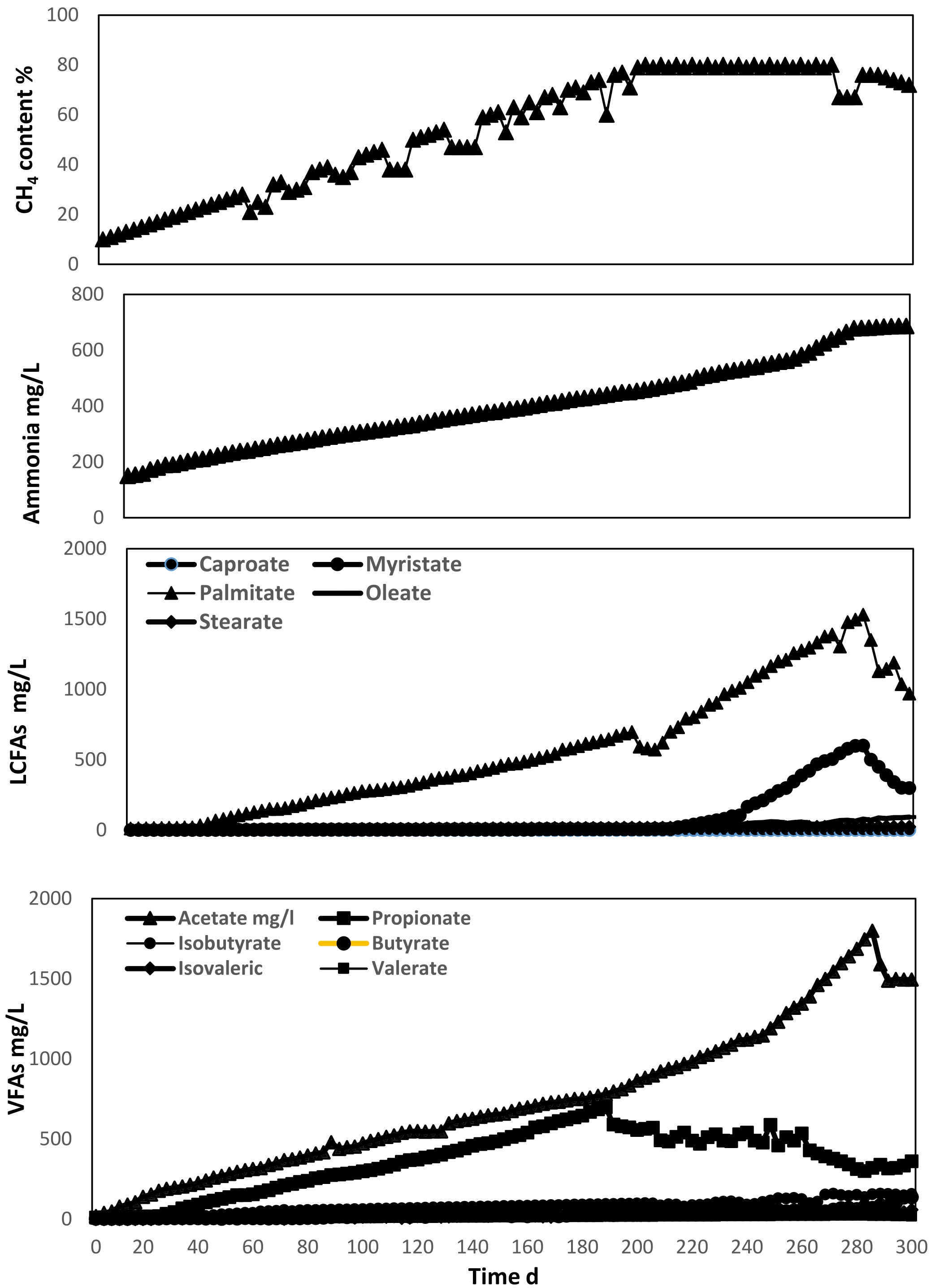

Fig. 5. FBD-AcoD system showed performance CH_4 content, Ammonia content, changes in LCFAs and VFAS.

Bacterial community diversity

To analyze the microbial structure composition and relative abundance (RA) of the bacterial community at the phylum level in response to the increase of OLRs were presented in Fig. 6a. In our study, *Acidobacteria* species RA of 47.31% in digested sludge was observed, and readily enriched in acid accumulation condition at OLR 23.5 g COD/L/d. The degradation of biological macromolecules might be degraded by *Chloroflexi*, to improve the hydrolysis process and reduce the organic acids in the system (Nguyen et al., 2020; Zhen et al., 2022). The relative abundance showed positive growth during the

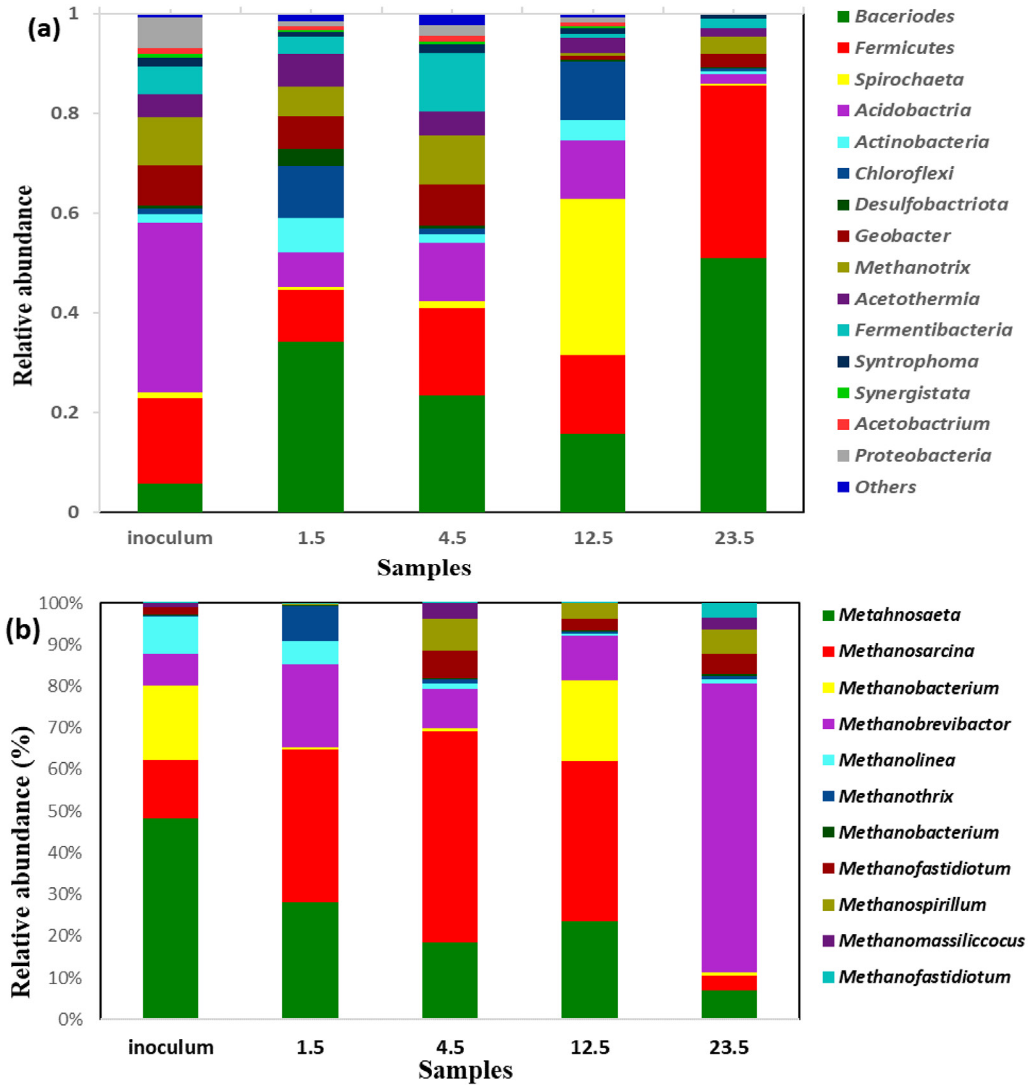

Fig. 6. Bacterial community diversity of the FBD-AcoD system (a) Relative abundance of dominance major methanogenic genus (b) Archaeal Community structure genus genera with relative abundances.

increasing order of OLRs. Researchers observed that the AcoD system showed good methanogenic activities and efficient mass transfer due to the availability of reasonable amounts of buffering effects of NH_4^+-N and FA (Magdalena et al., 2020; Ma et al., 2021). In this study supported by Ma et al. (2021) *Firmicutes and Bacteroidetes* were the main dominant bacteria in the AD process significantly responsible for the generation of propionate and acetate in the AcoD system. *Firmicutes* phylum is renowned for its ability to degrade a wide range of complex proteins, cellulose, and sugars with help of enzymes proteases, cellulases, and lipases (Kurade et al., 2020; Wu et al., 2021). The previous works have shown that *Bacteroidetes and Clostridium* are the main bacteria that degraded the macromolecules into VFA (Acetic acid, butyric, propionic) and LCFA (palmitic) (Ma et al., 2021). The RA of *Firmicutes* has a strong correlation with a positive impact on the methane production rate (Ma et al., 2021). The OLR gradually increased from 1.5 to a further 23.5 g COD/L/d, and the RA of *Firmicutes* progressively increase (11.34, 17.47, 23.91, and 47.31%). The influent OLR further increased to 25.5 g COD/L/d, and the RA decreased due to the inability to adapt to acidified conditions (Fig. 6a). *Firmicutes* remolding structure has a thick cell wall with peptidoglycans muropeptides can survive in an extreme environment. RA gradually increases at higher or low pH and showed a great impact on methane production such as a unit percent increase of *Firmicutes* can produce methane 2.9 mL (Wang et al., 2018; Ma et al., 2021; Zhen et al., 2022). *Spirochaeta* is the major group of bacteria capable of degrading organic macromolecules such as VFA and LCFA into Acetic acid, H_2, and CO_2. *Spirochaeta* showed a high presence at OLR of 12.5 g COD/L/d and RA achieved a maximum of 29.4%, promoting hydrolysis with gradually increasing OLR. *Acidobacter* is susceptible to an acidogenic environment and degrades to LCFA to produce acetic acid, H_2, and CO_2

(Zhou et al., 2021). *Actinobacteria* was present in inoculated sludge in the AcoD system consistently, mostly accumulation of VFA of the acidogenic environment and promoting degradation of cellulose, proteins of FW (Wang et al., 2018; Zhou et al., 2021). *Chloroflexi* was observed in both conditions of hydrolysis and acidogenesis and might be degrade various organic macromolecules (Nguyen et al., 2020; Ma et al., 2021). According to Magdalena et al. (2020), observed that *Chloroflexi* disappearance due to the toxicity of higher concentrations of NH_4^+-N at higher OLRs. The enrichment of RA in the AD system strong correlation with methane yields, and furthermore improvement of the AD process by hydrolysis, acidogenesis, acetogenesis, and methanogenesis to increment CH_4 production.

Archaeal Community structure

AD process with methanogens enhanced the methanogenesis for methane production. Furthermore, methanogenic *activity increased* with the methanogens strongly improves the methanogenesis with increasing of OLRs, which might increase the growth of the methanogenic community of methanogens. The strong *correlation* between the relative abundance (RA) with methane production rate and methanogens was quantified at varying organic loading *rates* (OLRS, 0.0, 1.5, 4.5, 12.5, 23.5 g COD/L/d (Fig. 6b). *Methanosaeta, Methanosarcina, Methanobacterium*, and *Methanobrevibacter* are the important methanogens genus at OLRs of 0 (inoculum), 1.5 and 12.5 g COD/L/d, which importantly component acetic acid consumed to produce methane and might increase the RA of *Methanosaeta* uses acetic acid as a substrate for methanogenesis. *Methanosaeta* the dominant RA continuously decreased, but *Methanosarcina* with increasing OLRs increased (Fig. 6b). The addition of SPSW in AD, increased an organic-rich nitrogen protein, might increase ammonium concentrations with increasing OLRs, and continued decreasing the RA of *Methanosaeta*. According to Gatidou et al. (2022), the addition of waste leachate escalates the ammonium concentration and increased the free nitrogen in the system foremost a decline in RA of methanogens. The *Methanobrevibactor* was rich in the AcoD system and showed RA 91.3%, achieving the highest at the OLRs of 23.5 g COD/L/d. However, the RA was consistently increased, moreover, the hydrogenotrophic methanogens (HMs) were resistant *to ammonia inhibition* at higher OLR (Lerm et al., 2012; Zhang et al., 2022). The RA of *Methanolinea, Methanothrix,* and *Methanobacterium* their RA maintained a low level as the OLR gradually increased showing no uses of acetic acid for metabolic substrates. *Methanobacterium, Methanofastidiotum, Methanospirillum,* and *Methanofastidiotum* are RA exhibit *lower* growth and major hydrogenotrophic methanogens have no significant on methane yields, might be using H_2, CO_2, or formate to produce methane. The methanogens inhibited under reduction reaction with sulfides and the presence of hydrogen, therefore lower methane production in the reactor (Ma et al., 2021). Acetic acid mainly consumed by both *Methanosarcina* and *Methanosaeta* is some commonly dominant archaea, *Methanosarcina* has a rapid expansion, a good life span, and faster degradation of acetic acid compared to *Methanosaeta*. Moreover, *Methanosarcina* abundance RA at the highest OLR 23.5 g COD/L/d, optimal feedstock, the optimum biogas, and COD removal were observed. *Methanosarcina* is performed based on an acetic acid substrate, while *Methanosaeta* only depends on acetic acid consumed to produce methane (Ma et al., 2022).

COD mass balance

The analysis of COD mass balance in order to utilize and convert organic matter by microbial metabolic activity to conversion in methane. The mechanisms of COD removal have been considered at various loading of OLR for methane production, and COD removal to attain a stable phase of the FBD-AcoD system (Table 4). The average effluents COD were observed at 21.7–12.9% at the OLR of 2.5–15.5 g COD/L/d, which might not reduce the organic matter, due to the reactor less active sludge, not well microbial enrichment. The COD effluent decreased to 8.5 to 7.5%, while the OLRs increased from 21.5 to 23.5 g COD/L/d, AcoD reaching the highest organic removal could be co-digestion of organic for higher methane yields and distinction microbial structure. Furthermore, the COD effluents rate increased by 14.5 to 23.3% further when the OLRs increased from 25.5 to 27.5 g COD/L/d, due to the inhibition of the growth of methanogens and alteration of microbial structure to observe system failure. The COD methane production rates accounted for 89% was observed at an OLR of 23.5 g COD/L/d, which was more than achieved by 61% by Yang et al. 2015. In conclusion, during the AD process, methane conversions decreased from 69 to 77% at the OLRs of 18.5–21.5 g COD/L/d (Lv et al., 2021; Zhen et al., 2022).

The energy efficiency of the FBD-AcoD system

The recovered energy efficiency and exploration potential of methane reclaim from co-digestion of FW-SPSW using the FBD-AcoD system. To assess the energy balance process, the energy consumption criteria of the system are evaluated as energy input (EI) to energy output (EO) ratio, to estimate the energy efficiency (Hall et al., 2009). Table 4, presents the results indicating that the EI ratio included (feedstock feeding, reactor heating, and refluxing) is the primary main energy consumption of the AcoD system (Chen et al., 2016). However, the energy consumption of different systems assessed in EI 8.51 KJ/gCOD corresponds to EO 875.3 KJ/gCOD. The refluxing and heating of the system are the main energy consumption including refluxing the feed to increase OLRs and heating to maintain the temperature of the reactor system at 35 °C (Braguglia et al., 2018). The energy recovery also impacts by the VFA, LCFA, and characteristics of feedstock. Therefore, FW with SPSW did not require pre-treatment and energy balance recovery on biogas yields of the FBD-AcoD system. The energy from methane results obtained 73.12, 109.46, 178.45, 262.78, 375.90, 523.05, 631.56, 790.34, 945.65, 1185.35, 1356.31, 934.87, 556.21 KJ/d with an increase of OLRs 0.5, 1.5, 2.5, 4.5, 6.5, 9.5, 12.5, 18.5, 21.5, and 25.5 g COD/L/d. Whereas the decreasing energy methane production at higher OLRs for example 25.5 and 27.5 g COD/L/d might be inhibition of microbial metabolism (Table 4). Therefore, the FBD-AcoD system provides a bottleneck to solving the energy issues and the assessment of the methanogenesis process of energy efficiency pathways for methane production could be used in large-scale industrial applications.

4. Conclusion

Food waste and solid poultry slaughterhouse waste co-digestion were studied to *identify* the key parameters for the stable performance of the FBD-AcoD system. The results presume that methane production achieved the highest 8.23 L/L/d at the OLR of 23.5 g COD/L/d, to improve the efficiency of the FBD-AcoD system. During long-term operation, methanogens are increased with NH_4^+-N coupled with generated VFAs, and LCFAs use of FBD-AcoD reactor efficiency was quite appreciable at higher OLRs. The high conversion ratio methane energy recovery is 1356.31 KJ/d, and the net energy recovery of the system EO is 875.3 KJ/g COD at the OLR of 23.5 g COD/L/d. Therefore, the co-digestion of FW with SPSW of these wastes as a resource will empower sustainable development.

CRediT authorship contribution statement

Anwar Ahmad: Analyzed and interpreted the experimental data regarding co-digestion of FW-SPSW. **Roomana Ghufran:** Final edited the manuscript, Evaluated the results microbial structure, relative abundance. **Qazi Nasir:** Reviews the data for methane production, Conclude the discussions. **Fathima Shahitha:** Performed the experiment of the AcoD system dosing strategy, VFA, LCFA analysis,and various parameter. **Mohammed Al-Sibani:** Analysis biogas production at various feed. **Amal S. Al-Rahbi:** Check the quantity of fatty acid and LCFA evaluated the energy potential of FW-SPSW, Writing the manuscript.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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Ethical approval

The authors declare to publish this novel research.

Consent to participate

The authors participate in this research work and publish.

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