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Performance and Energy Utilization Efficiency of an Expanded Granular Sludge Bed Reactor in the Treatment of Cassava Alcohol Wastewater

Guoqin Xu [1,2,†], Junlin Ji [1,†], Zhanyao Zheng [3], Hongchuan Song [1], Hong Yang [1], Jing Liu [1], Fang Yin [1,*], Wudi Zhang [1] and Shumei Hao [1,*]

1 School of Energy and Environment Science, Yunnan Normal University, Kunming 650500, China; xuguoqin06@126.com (G.X.); 13529250572@163.com (J.J.); shc@ynnu.edu.cn (H.S.); yanghong25red@126.com (H.Y.); lj@ynnu.edu.cn (J.L.); wootichang@163.com (W.Z.)
2 College of Biological and Agricultural Science, Honghe University, Mengzi 661100, China
3 Ningjin County Agriculture and Rural Bureau, Dezhou 253400, China; 18487086621@163.com
* Correspondence: yf6709@ynnu.edu.cn (F.Y.); haosmbio@126.com (S.H.)
† These authors contributed equally to this work.

Abstract: In recent years, expanded granular sludge blanket (EGSB) reactor has been widely used in the treatment of high-concentration organic wastewater, but its research mainly focused on treatment efficiency and microbial community composition. There were few studies on the relationship of operation conditions and energy utilization efficiency. Therefore, the methanogenic characteristics and energy utilization efficiency of EGSB reactor were studied by using cassava alcohol wastewater (CAW) as a raw material at (36 ± 1) °C. The results show that the degradation of volatile fatty acids (VFAs) is an important step affecting methane generation compared to the hydrolysis stage. When organic load rate (OLR) was 12.73 gCOD/L·d, the chemical oxygen demand (COD) removal rate was above 95%, the methane production efficiency of raw material was 202.73 $mLCH_4$/ gCOD·d, the four-stage conversion efficiency was the highest, and the energy utilization efficiency was 62.26%, which was the optimal stage for EGSB reactor to treat CAW. These findings support high-efficiency bioenergy recovery from CAW in practice and highlight the potential wide application of high-performance anaerobic reactors for CAW.

Keywords: EGSB; energy utilization efficiency; methane production; volatile fatty acids

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1. Introduction

The EGSB reactor, developed by Lettinga et al. at Wageningen Agricultural University in the 1980s as a third-generation high-efficiency anaerobic reactor based on the UASB reactor, enhances liquid flow rate by employing a large height-to-diameter ratio and incorporating effluent reflux [1,2]. The simple reactor design, user-friendly equipment, reduced sludge production, high treatment efficiency, strong impact resistance, small footprint, and ability to generate biogas energy garnered significant attention from researchers [3]. Therefore, researchers conducted extensive studies on reactor structure, factors affecting performance, fluid dynamics, and microbiology.

In terms of the EGSB reactor structure, it comprised four distinct sections: the influent zone, reaction zone, separation zone, and recirculation zone. During operation of the EGSB reactor, the influent and reflux water were thoroughly mixed and introduced into the distribution area via a well-designed system, ensuring uniform dispersion at the bottom of the reactor and generating an enhanced up-flow velocity. The liquid up-flow velocity could reach 6 m/h or even as high as 30 m/h, while, for gas, it could attain a maximum of 7 m/h. The anaerobic granular sludge (AGS) and wastewater had increased contact, facilitated by the structure of the EGSB reactor that allows for wastewater recycling. These favorable conditions enabled it to effectively treat fresh leachate at an exceptionally high OLR of up to 37.94 g COD/L·d and achieve a remarkable COD degradation rate exceeding 80% [4]. Additionally, high-concentration wastewater (such as cheese whey wastewater [5], high-salt fatty acid organic production wastewater [6], corn starch processing wastewater [7], and soft drink industry wastewater [8]) could function normally even under OLRs exceeding 7 gCOD/L·d, and the COD removal rate reached more than 80% (details provided in Table 1). Therefore, the EGSB reactor can be an efficient and sustainable alternative for wastewater treatment compared to other conventional methods.

Table 1. Different types of wastewater for treatment of the EGSB reactor.

Type of Wastewater	Raw Material COD (mg/L)	Temperature (°C)	OLR (gCOD/L·d)	Removal COD (%)	Methane Production Rate (mLCH4/gCOD·d)	Reference
Cheese Whey Wastewater	43,000–49,700	25–27	7.3–8.3	90	328	[5]
High-Salt Fatty Acid Organic Production Wastewater	15,000–23,400	35 ± 5	8–10	80–90	286	[6]
Corn starch processing wastewater	3014–12,462	38–40	1.3–18.7	90.7	-	[7]
Soft drink industry wastewater	4637	35–37	11	93	-	[8]

Extensive research has been conducted by scholars on various factors influencing reactor performance, including wastewater characteristics, acclimatization of AGS, bioreactor configuration, and operational parameters such as hydraulic retention time (HRT) and OLR. Furthermore, environmental factors like temperature and pH have also been thoroughly investigated. According to the literature, EGSB bioreactors typically operate within a mesophilic temperature range of 35–37 °C, irrespective of any potential variations in wastewater temperature [3]. Xu et al. investigated the formation mechanism of AGS in an EGSB reactor and identified aromatic protein-like substances as crucial contributors to this process. Moreover, a transition from hydrogenotrophic methanogens (*Methanobacterium*) to acetoclastic methanogens (*Methanosaeta*) was observed during the sludge granulation process [9]. The study conducted by Cruz-Salomón also highlights the critical importance of maintaining optimal substrate pH levels in EGSB reactors, such as through the addition of sodium bicarbonate to cheese whey wastewater [5]. The impact of long-term operation (>100 days) on the stability of bioreactors and their resistance to OLR shocks has been evaluated by scholars [10]. The research findings indicated that reactors operating in a stable manner exhibit greater resilience towards high-intensity OLR stimulation [10]. The performance of the EGSB reactors in methane production could be enhanced through the integration of co-digestion or pretreatment techniques. For example, the system of high-pressure homogenization-EGSB reactor was employed by Nabi et al. for the treatment of residual sludge, resulting in a 24% reduction in the rate of sludge degradation compared to the original treatment process, and it also stimulated the proliferation of acetoclastic methanogens and hydrogenotrophic methanogens. The hydraulic retention time (HRT) was shortened to 5 days, leading to an enhanced biogas production rate (240 mL/(gTCOD)·d), increased methane content in biogas (57%), improved total chemical oxygen demand (TCOD) removal efficiency (58%), and elevated volatile solid (VS) removal efficiency (43%) [11]. Meanwhile, Liu et al. successfully achieved the objective of enhancing biogas production through co-digestion of food waste and mature leachate [12].

Computational fluid dynamics (CFD) have gained recognition as an emerging modeling tool for wastewater treatment processes in terms of their dynamic behavior [13]. The commonly used model in the field of anaerobic digestion is ADM1 [14]. In recent years, numerous novel models have emerged, and Pérez-Pérez's research suggested that the modified Stover–Kincannon model was better suited for the operational characteristics of EGSB reactors [15]. The study conducted by Aaneh et al. also employed CFD to assess the distribution of flow velocity, identification of dead zones, and characterization of residence time distribution (RTD) within the reactor. Additionally, they proposed a compartment model that accurately represents RTD by considering distinct regions of plug flow, continuous mixing zones, dead volumes, and recirculation flow patterns [16].

Regarding microorganisms, Li et al. suggested that *Methanobacterium* and *Methanomassiliicoccus* are the predominant archaea in the treatment of high-concentration cephalosporin wastewater, while hydrogenotrophs and methylotrophs served as the primary pathways for methane production [17]. Identical findings were also reported by Nabi et al. [11] and Chen et al. [18].

However, there was a lack of comprehensive reports on the conversion efficiency of EGSB reactors in terms of energy utilization and methane production across all four stages. Therefore, this study aimed to investigate the operational characteristics and energy utilization efficiency of methane production from CAW using a laboratory-made EGSB reactor with an effective volume of 3.3 L. The objective of this study was to investigate the impact of an EGSB reactor on the overall efficiency of CAW treatment, analyze the influence of different organic loads on the energy utilization efficiency of the EGSB reactor, and compare the treatment effects of various methods on this wastewater, thereby providing valuable insights for anaerobic digestion treatment.

2. Materials and Methods

2.1. Experimental Equipment

The EGSB reactor was formed of transparent Plexiglas. The height-to-diameter ratios and effective volumes were 10:1 and 3.3 L, respectively. The reactor involved a sludge discharge port at the bottom and sampling ports in the lower, middle, and upper sections. In the periphery of the reactor, a tightly wound PVC hose was used to circulate water at a constant temperature for insulation. A controller (36 ± 1 °C) was used to monitor the temperature of the circulating water. The experimental setup for each reactor is shown in Figure 1.

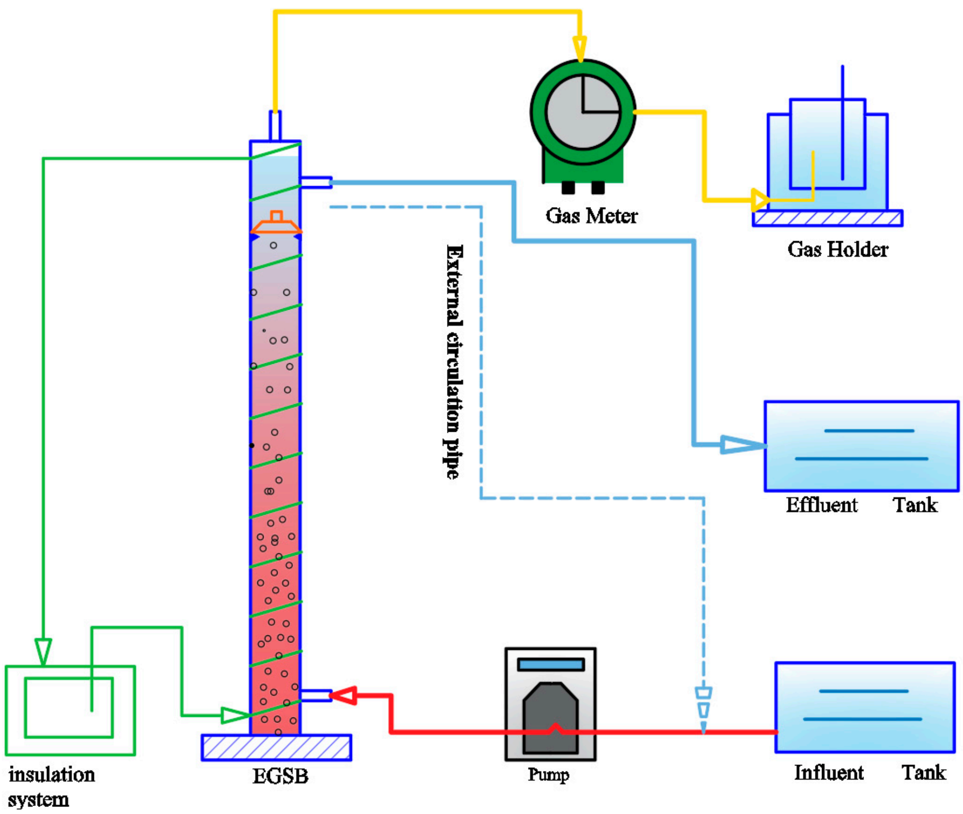

Figure 1. Diagram of experimental device.

2.2. Experimental Materials

2.2.1. Raw Materials

The raw materials employed in this study were CAW, which was obtained by following the subsequent procedures. Firstly, cassava from Guangxi province was utilized as the fermentation substrate, and alcoholic fermentation was conducted at a temperature of 30 °C for a duration of 5–7 days, employing a dual-enzyme approach (liquefying enzyme and saccharifying enzyme). Subsequently, alcohol was obtained through distillation of the fermented broth. Finally, the remaining waste mash was ultimately subjected to solid–liquid separation, resulting in the production of cassava alcohol wastewater as the liquid fraction, and CAW was subsequently stored at a temperature of 4 °C within a refrigerator. CAW properties were as follows: TCOD, 26,386–56,685 mg/L; volatile suspended solids (VSS), 3.51–17.02%; suspended solids (SS), 5.67–20.72; and pH, 3.7–6.5. The CAW was stored in a refrigerator at 4 °C for further analysis.

2.2.2. Inoculum

The inoculum was acquired through extensive laboratory domestication. First, utilizing the residual sludge from Luolong River Wastewater Treatment Plant in Kunming, Yunnan Province, as a substrate, anaerobic digestion was conducted by incorporating 30% of the total sludge quantity with pig manure. Once the sludge reached an optimal anaerobic digestion state, regular supplementation of CAW was introduced into the fermentation system. After 3 months of domestication, the inoculum was obtained. The moisture, mixed liquor volatile suspended solids (MLVSS), and mixed liquor suspended solids (MLSS) of the inoculum obtained after the acclimation protocol were 91.89%, 45.55 g/L, and 82.14%, respectively.

2.3. Experimental Methods

Prior to experiments, acclimated inoculation sludge was filtered using a 2 mm screen to remove impurities and then poured into the reactors. The inoculum volume represented one third of the effective volume of each reactor; the remaining volume was filled with CAW.

A continuous feeding method was adopted, which involved start-up and load lifting phases. In the start-up phase, the influential COD concentration was increased from 5000 mg/L to 23,000 mg/L while maintaining a fixed HRT of 3.9 days. During the load lifting phase, the HRT was reduced from 3.9 days to 1.4 days by fixing the influent-water COD concentration at a range of 20,000–23,000 mg /L. Details on the operation parameters are shown in Table 2.

Table 2. Operation parameters of the EGSB reactor.

Temperature (°C)	Operate Phase	Name	Time (d)	COD_{inf} Concentration (mg/L)	HRT (d)	OLR (g COD/L·d)
36 ± 1	Star-up phase	S-I	1–4	6062 ± 380	3.9	1.55 ± 0.10
		S-II	5–7	7513 ± 306	3.9	1.93 ± 0.08
		S-III	8–13	11,208 ± 900	3.9	2.87 ± 0.23
		S-IV	14–24	14,516 ± 829	3.9	3.72 ± 0.21
		S-V	25–29	17,502 ± 417	3.9	4.49 ± 0.11
		S-VI	30–38	21,346 ± 1630	3.9	5.47 ± 0.42
	Load lifting phase	L-I	39–58	21,785 ± 1083	3.9	5.58 ± 0.28
		L-II	59–69	21,188 ± 939	2.5	8.48 ± 0.38
		L-III	70–83	21,506 ± 1134	1.7	12.65 ± 0.67
		L-IV	84–107	18,873 ± 2839	1.4	16.14 ± 0.87

2.4. Analysis Methods

The COD, gas production, pH, and temperature of the influent and effluent of the reactor were continuously measured on a daily basis. Methane concentration in biogas and VFAs of effluent of the reactor were measured every 3 days.

2.4.1. Gas Production

A wet gas holder was used to collect biogas produced in the reactors; a wet gas flowmeter requiring manual release of gas to set again to the zero point measured the daily production.

2.4.2. Methane Content

A GC9790II gas chromatograph (Fuli Co., Wenling, Zhejiang Province for China) equipped with a TDX-01-type stainless steel packed column was used to determine the concentrations of methane in biogas samples. The temperatures of the column, detector, and injector were 105 °C, 140 °C, and 110 °C, respectively; the flow rate of the carrier gas (nitrogen) was 30 mL/min.

2.4.3. COD Content

A maxII (HACH Co., Loveland, CO, USA) on-line COD monitor was used to determine COD concentrations in reactor inlet and outlet waters based on the potassium dichromate digestion-spectrophotometric method.

2.4.4. VFAs

A GC9790II gas chromatograph (Fuli Co., Wenling, Zhejiang Province for China) equipped with a KB-FFAP capillary column was used to measure concentrations of VFAs in reactor water. The temperatures of the column, detector, and injector were 130 °C, 250 °C, and 200 °C, respectively; the flow rates of nitrogen (carrier gas), air, and hydrogen were 20, 300, and 40 mL/min, respectively. In addition, the preparation sampling procedure prior to measuring VFA involved initially withdrawing approximately 1 mL of effluent from the discharge outlet using a 5 mL needle, followed by centrifugation at 9000 r/min for 9 min at 4 °C. Subsequently, precisely 500 μL of the supernatant in the centrifuge tube was collected and acidified with the addition of 100 μL of formic acid to prevent organic acid dissociation. Finally, 500 μL of extractant (dichloroethane) was added, mixed thoroughly, and allowed to stand for 5 min. The extraction solution (2 μL) was then absorbed by an injection needle and injected into the gas chromatograph injector for detection and analysis using the external standard method.

2.4.5. pH

A pHS-3C meter (Lei Ci Co., Shanghai, China) was used to measure the pH of water from the inlet and outlet of the reactors during the experiments.

2.5. Caculation

2.5.1. Calculation of Conversion Efficiency of Hydrolysis, Acidogenesis, Acetogenesis, and Methanogenesis

Anaerobic digestion included hydrolysis, acidogenesis, acetogenesis, and methanogenesis in four stages [19]. In this experiment, the four-stage conversion efficiency in the methanogenic phase was calculated as follows [12]:

$$\text{Hydrolysis efficiency } (\%) = \frac{\text{COD}_\text{M} + \text{SCOD}_\text{Eff} - \text{SCOD}_\text{Inf}}{\text{TCOD}_\text{Inf} - \text{SCOD}_\text{Inf}} \times 100 \quad (1)$$

$$\text{Acidogensis efficiency } (\%) = \frac{\text{COD}_\text{M} + \text{COD}_\text{Eff VFAs} - \text{COD}_\text{Inf VFAs}}{\text{TCOD}_\text{Inf} - \text{COD}_\text{Inf VFAs}} \times 100 \quad (2)$$

$$\text{Acetogensis efficiency } (\%) = \frac{\text{COD}_\text{M} + \text{COD}_\text{Eff acetic acid} - \text{COD}_\text{Inf acetic acid}}{\text{TCOD}_\text{Inf} - \text{COD}_\text{Inf acetic acid}} \times 100 \quad (3)$$

$$\text{Methanogensis efficiency } (\%) = \frac{\text{COD}_\text{M}}{\text{TCOD}_\text{Inf}} \times 100 \quad (4)$$

where COD_M is the chemical oxygen demand of the produced methane, which can be calculated by methane production (m^3) and a conversion rate (0.35 m^3CH_4/kg COD); SCOD is the soluble chemical oxygen demand in the influent and effluent, respectively; $TCOD_{Inf}$ is the total chemical oxygen demand in the influent; COD_{VFAs} and $COD_{acetic\ acid}$ represent the chemical oxygen demand of VFAs and acetic acid, respectively, in both the influent and effluent. All units are mg/L.

2.5.2. Energy Utilization Characteristics

In practice, the potential to achieve positive net energy is vital for the acceptance of technology. In this experiment, total HHVs of methane and VFAs were deemed as output energy; total input energy included electric energy and thermal energy. It should be mentioned that input electric energy contains power consumption associated with pumping incoming and outgoing materials. The thermal energy input is used to raise the temperature of inlet water to the digestion temperature (36 °C) and to compensate for heat losses through walls, floors, and lids, excluding losses through the pipes.

The output energy values of the reactors were calculated based on methane yields using the equation:

$$E_0 = \frac{P_{CH_4} \times \xi \times \eta_m + P_{ac} \times a + P_{pr} \times b + P_{but} \times c + P_{val} \times d}{Q \times COD_{in}} \quad (5)$$

where E_0 is the output energy (kJ/g fed COD), P_{CH4} is the daily methane production (m^3 CH_4/m^3.d), ξ is the HHV of methane (39,700 kJ/m^3 CH_4), P_{ac}, P_{pr}, P_{but}, and P_{val} are the production of acetic acid, propionic acid, butyric acid, and valeric acid, respectively; a, b, c, and d are the HHV of acetic acid, propionic acid, butyric acid, and valeric acid and are 874, 1527, 2184, and 2837 kJ/mol, respectively. η_m is the energy conversion factor of methane (0.9), Q is the influent flow rate (m^3/d), and COD_{in} is the concentration of the substrate (g Fed COD/m^3).

Input electricity was estimated using Equation (6); input heat was determined using Equations (7) and (8):

$$E_{i,electricity} = \frac{Q \times \theta + V \times \omega}{Q \times COD_{in}}, \quad (6)$$

$$E_{i,\ heat} = E_{h,r} + E_{h,c}, \quad (7)$$

$$E_{h,r} = \frac{\rho \times Q \times \gamma \times (T_d - T_i)}{Q \times COD_{in}}, \quad (8)$$

$$E_{h,c} = \frac{k \times A \times (T_d - T_i) \times 86.4}{Q \times COD_{in}}, \quad (9)$$

where $E_{i,electricity}$ is input electricity (kJ/g COD), θ is electricity consumed by pumping (1800 kJ/m^3), ω is electricity consumed by mixing (300 kJ/m^3·d), E_h is input heat (kJ/gCOD), $E_{h,r}$ is input heat to raise the influent temperature to the digestion temperature, $E_{h,c}$ is input heat to compensate for heat losses, ρ is density of the influent (1000 kg/m^3), γ is specific heat of the influent (4.18 kJ/kg·°C), T_d is temperature in the anaerobic digester (36 °C), T_i is temperature of the influent (22 °C), k is the heat transfer coefficient (W/m^2·°C), and A is surface area of the reactor.

The energy balance (ΔE) and energy ratio (R_e) were calculated using Equations (10) and (11):

$$\Delta E = E_0 - E_{i,electricity} - E_{i,heat} \quad (10)$$

$$R_e = \frac{E_0}{E_{i,electricity} + E_{i,heat}} \quad (11)$$

where ΔE is the energy balance (kJ/gCOD) and R_e is the energy ratio.

3. Results and Discussion

3.1. Operation Characteristics of CAW

3.1.1. COD Removal Efficiency Analysis

As shown in Figure 2, the OLR of CAW treated by EGSB increased from 1.39 to 16.99 gCOD/L·d by fixing the retention time in water or increasing the influent COD concentration. In the start-up phase (1–38 d), the fixed HRT was 3.9 d, and the influent COD concentration increased from 5420 to 20,000 mg/L; the average COD removal rate was (97.62 ± 1.52)%. In the load lifting phase (39–108 d), the COD concentration of fixed inlet water was 21,688 ± 1207 mg/L, the HRT was gradually shortened from 3.9 d to 1.4 d, and OLRs increased from (5.58 ± 0.28)g COD/L·d to (16.14 ± 0.87)g COD/L·d. The average COD removal rate was (96.07 ± 0.96) when the OLR was (5.58 ± 0.0.28–12.65 ± 0.67) gCOD/L·d. It is worth noting that, when the load was relatively high (12.65 ± 0.67 gCOD/L·d), the COD removal rate reached (95.75 ± 1.16)%, which was much higher than the research result of Jiang et al. (70.13 ± 0.16%) [20]. At the same time, it was also higher than the effect of Wang et al. (90.4% ± 0.8%) using UASB to treat CAW under the same load [21]. However, when the HRT was reduced to 1.4 days, the OLR increased to (16.14 ± 0.87) gCOD/L·d, leading to the pH value dropping below 5.5 within the system and a decline in COD removal efficiency to 60%. The system was subsequently subjected to attempts aimed at restoring it by reducing the inflow COD load and adjusting the pH value of influent. However, these endeavors proved unsuccessful. The above statement indicates that HRT had a significant impact on the efficiency of wastewater treatment, as it directly affects the growth and metabolic activity of microorganisms [22]. When the influent COD concentration was kept constant, the organic loading rate gradually increased as the hydraulic retention time (HRT) decreased. Once the organic loading rate surpassed (12.65 ± 0.67) gCOD/L·d, the treatment efficiency of the EGSB reactor declined from (95.75 ± 1.16)% to 60%.

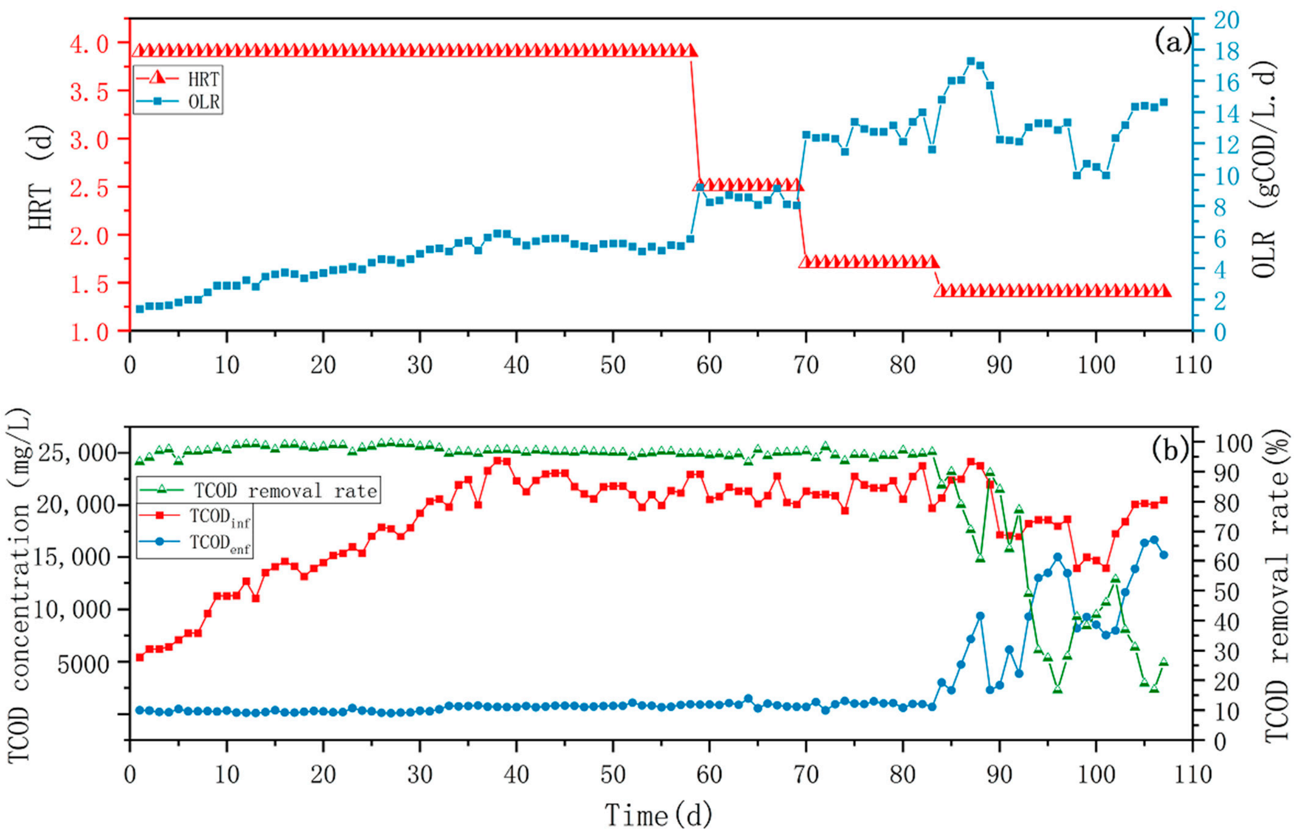

Figure 2. Organics removal of the EGSB system: OLR and HRT (**a**), and TCOD concentration and TCOD removal (**b**).

3.1.2. Analysis of Methane Production

During the initial start-up phase (days 1–13), there was significant variation in methane yield, production, and content within the EGSB reactor. This could be attributed to a necessary adaptation period for microorganisms within the system to acclimate to their new environment. As the fermentation progresses, during the late start-up stage (25–38 days), the methane content stabilized at approximately 50%, while maintaining a consistent methane production rate of around 180 $mLCH_4/gCOD{\cdot}d$. The successful commissioning of the EGSB reactor was observed to take place after 38 days of operation.

In the ascending phase of organic load rate, apart from days 84 to 107, there was a positive correlation between organic load rate and methane production. When OLRs were (5.58 ± 0.28), (8.48 ± 0.38), and (12.65 ± 0.67) gCOD/L·d, the corresponding methane productions were (3605 ± 357), (5477 ± 658), and (7170 ± 738) mL, respectively, with average methane contents of 52%, 52%, and 54%. Additionally, the average methane yields were (203 ± 22), (204 ± 24), and (180 ± 21) $mLCH_4/gCOD{\cdot}d$. When the EGSB reactor was operated for 56 days with an OLR of 5.48 gCOD/L·d, the methane yield reached its peak at 247 $mLCH_4/g$. When the OLR was increased to 8.03 gCOD/L·d, the methane yield remained high at 235 $mLCH_4/gCOD{\cdot}d$. The value obtained in this study was slightly higher than the reported value of 225 mL CH4/gCOD·d for raw material with an organic loading rate of 6.8 gCOD/L·d using a CSTR reactor at 55 °C by Li et al. [23] but lower than that achieved using a three-step anaerobic sequencing batch reactor (3S-ASBR) at 37 °C (methane production rates of 343 mL $CH_4/gCOD{\cdot}d$ (at an OLR of 10 gCOD/L·d)) [24] and Zheng et al.'s [25] study (437 mL $CH_4/gCOD{\cdot}d$) using EGSB for corn alcohol wastewater treatment was also higher. Starting from day 70, the methane yield began to decline due to an increase in OLR. The EGSB reactor was operated for 83 days (OLR = 12.73 gCOD/L·d); the methane production efficiency of raw material was 202.73 $mLCH_4$/ gCOD·d. These findings suggest that the methanogenic capacity of the EGSB reactor decreases when the organic load exceeds (8.48 ± 0.38) gCOD/L·d. When considering Figures 2b and 3 together, it could be observed that, at an OLR of (12.65 ± 0.67) gCOD/L·d, the COD removal efficiency remained above 95%, but there was a decrease in gas production efficiency, indicating potential changes in microorganisms within the EGSB reactor as a result of a sharp increase in organic load. It is possible that some portion of the organic matter present in CAW may serve as a source for microbial growth or become retained within the reactor.

Finally, a graphical representation was generated to investigate the correlation between organic load and actual gas production in EGSB reactors. The figure represents the linear relationship between daily methane production and its corresponding COD removal load for different OLRs. Data analysis revealed a strong linear correlation between daily methane production and corresponding COD removal load (except for the HRT = 1.4 d stage). The derived relationship equation was "y = 606.74x + 34.491 (R^2 = 0.9849)", suggesting that high methane production from EGSB treatment of CAW can be attributed to OLR. However, exceeding the system's tolerance load would lead to system collapse.

3.1.3. COD Balance

In the process of anaerobic digestion, a part of the organic matter was converted into methane, a part was drained with water, a part was fed to microorganisms for growth, and another part was deposited in the sludge [26,27]. Therefore, in order to reveal the transformation of organic matter in anaerobic digestion, the COD balance during the operation of the system was calculated (Figure 4). First, in HRT = 3.9 d, 2.5 d, 1.7 d, 1 the COD–methane was between 40% and 70%, and the overall trend was to increase first and then decrease. When the HRT was 2.5 d, the COD–methane was higher (65%). Second, because the COD removal rate was above 93% (except the acidification stage), the COD–effluent ratio was low, only between 0.45% and 6.94%. In the acidification stage, due to the production of a large number of VFAs, the COD content in the discharge water increased, accounting for more than 60%. COD–others include sludge loss and growth consumption of inoculants (there was no waste sludge discharge in this paper, so COD–others were COD–sludge accumulation). As can be seen from COD–others, it basically accounts for 30–60%. However, the results of Liu et al. showed that there were few COD–others [12]. The EGSB treatment of CAW system was 50% converted to methane, which was conducive to efficient energy recovery. Methane was the main form of carbon in anaerobic digestion. Monitoring methane content in biogas is an important indicator to judge COD balance, that is, carbon conversion.

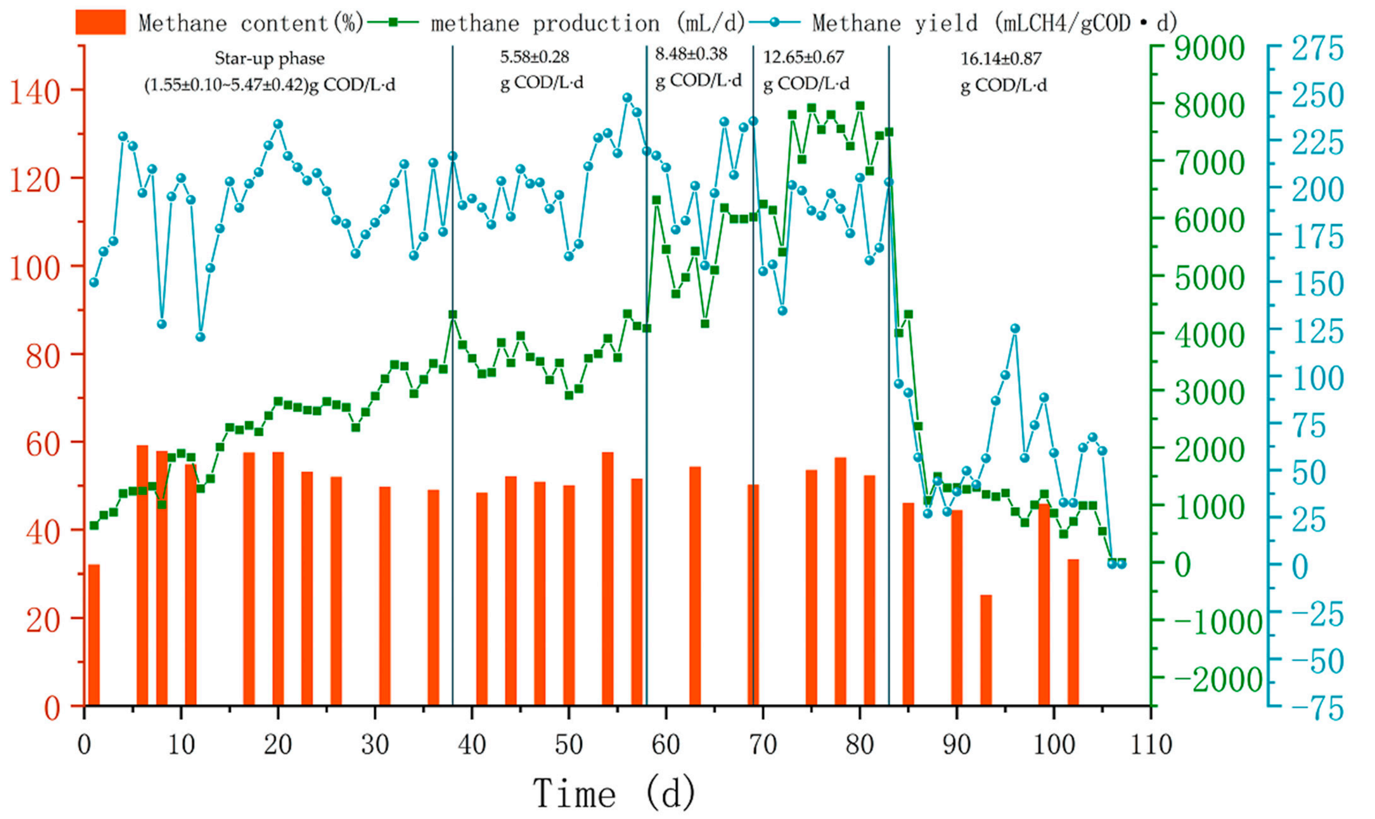

Figure 3. Methanogenic performance of EGSB reactor for treatment of CAW.

3.1.4. VFAs and pH Analysis

The pH variations in VFAs in the effluent and influent/effluent water of the EGSB reactor are depicted in Figure 5. VFAs, encompassing acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid, represented crucial intermediates generated through hydrolysis and acidification during anaerobic digestion [28]. Their metabolism and turnover played a pivotal role in the process of anaerobic digestion. If the rate of methane production was lower than the efficiency of acid production, accumulation of VFAs would occur, thereby inhibiting the process of methanogenesis. Consequently, the degradation of VFAs was considered to be another crucial limiting factor in anaerobic digestion [29]. In the initial stage of start-up (1–38 days), the concentration of VFAs was below 300 mg/L, with acetic acid being the predominant component, indicating a balanced rate between acid consumption by methanogenic bacteria and acid production by other bacteria. Despite an influent water pH ranging from 4 to 5, the effluent water maintained a pH above 6.5, demonstrating successful initiation of EGSB reactor. During the load lifting phase, the levels of VFAs were significantly elevated compared to those observed during the initial phase. From day 39 onwards, the concentration of VFAs remained below 500 mg/L. Acetic acid continued to dominate; however, there was a noticeable increase in propionic acid and isobutyric acid. Particularly after day 70, propionic acid and butyric acid (including isobutyric acid) accounted for more than half of the total acidic content. The inhibition of acid has been demonstrated to be directly caused by propionate and butyric acid [30]. As the OLR increased, the risk of acidification in EGSB reactors also increased. However, within a certain range of OLRs, methane production could still occur normally as long as the concentration of VFAs in the effluent remains below 1000 mg/L. Nevertheless, an increase in propionic and butyric acids (including isobutyric acid) proportions among VFAs led to a decrease in methane production efficiency. On the 84th day of operation, the organic load increased to (16.14 ± 0.87) gCOD/L·d, while VFAs surged from 400 mg/L to over 4000 mg/L and effluent pH dropped below 5.0 from its initial level of 7.5. The interaction metabolism of microorganisms in the EGSB reactor was disrupted, leading to inhibition of methanogen activity, and the acidic environment favored the production of VFAs. Gradually, VFAs accumulated in the system, causing a cessation in methane production and a decrease in COD removal rate within the EGSB reactor. Even attempts to restore normal functioning by adjusting influent pH to 7.0 and reducing organic loading rate proved unsuccessful.

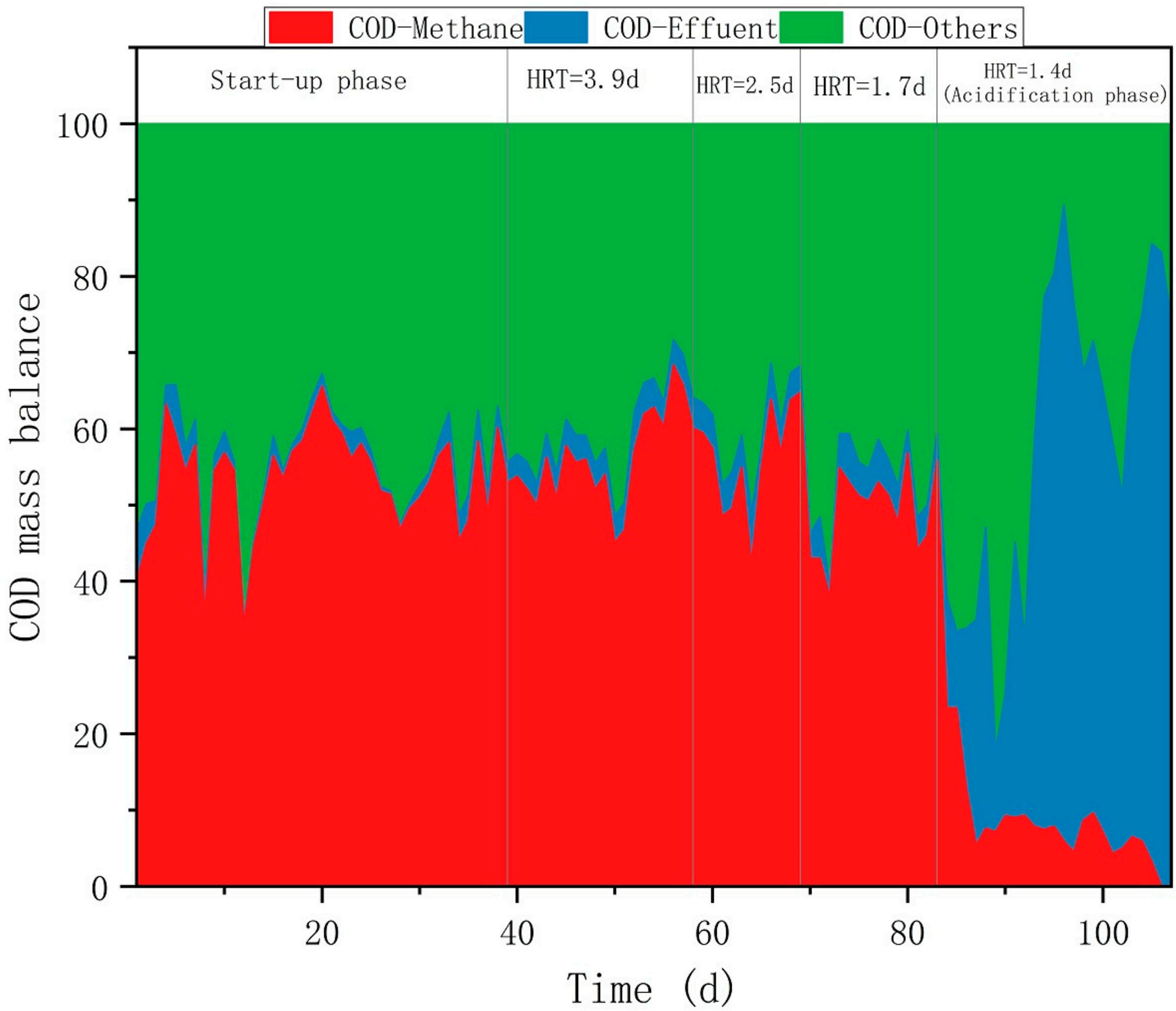

Figure 4. COD balance of EGSB reactor for treatment of CAW. COD–effluent: COD in the effluent, COD–others include COD–discharge (COD discharged as waste sludge) and COD–sludge accumulation (COD accumulated in the reactor), and COD–methane: COD converted to methane.

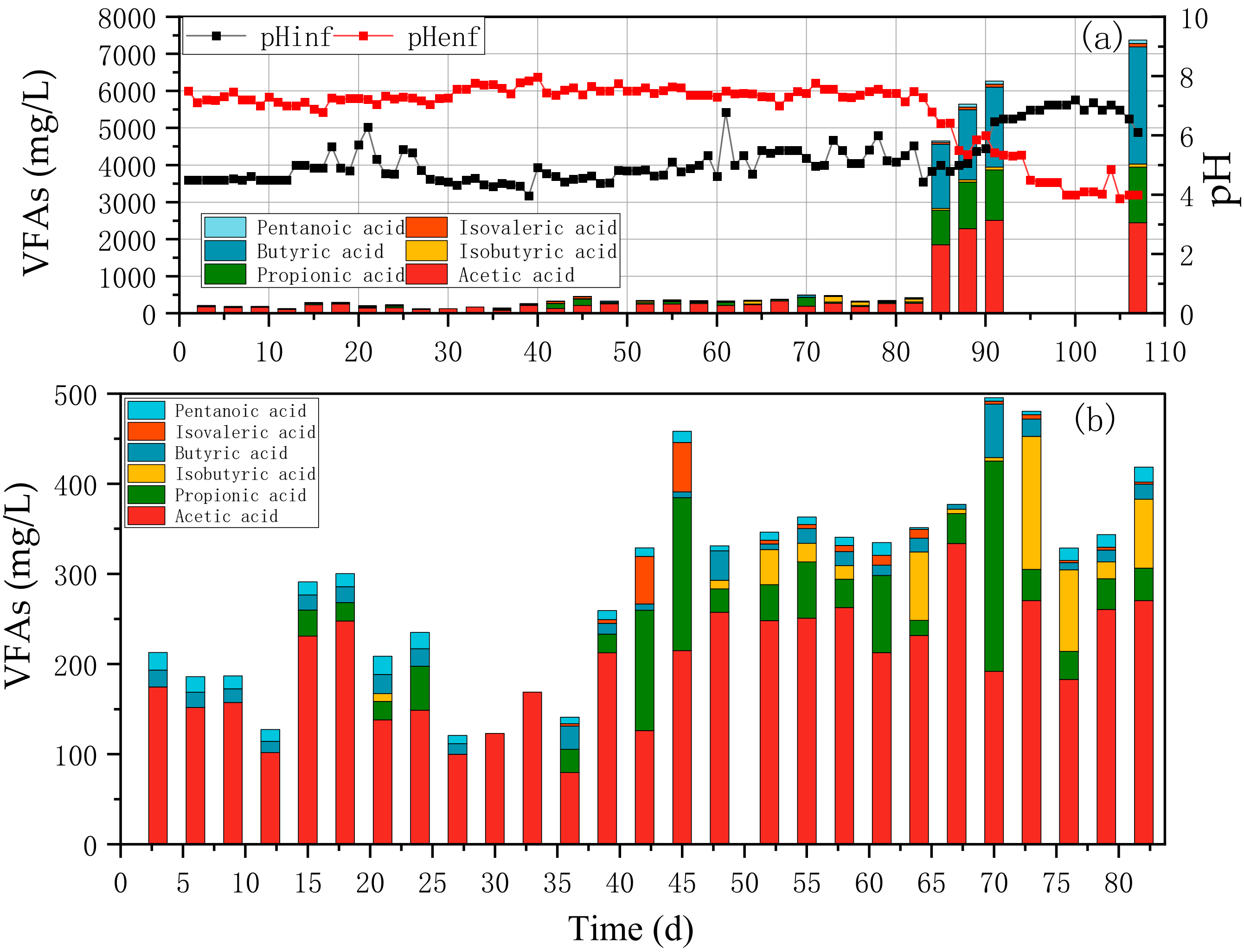

Figure 5. Variation in VFA content and pH in EGSB reactor effluent, where (**b**) is a magnified image from day 1 to day 83 in (**a**).

3.2. Efficiency of Four-Stage Anaerobic Digestion

In order to better understand the treatment of CAW by EGSB, we calculated the treatment efficiency of four stages. It can be seen from Figure 6, except for the stage of HRT = 1.4 d, the fourth-stage efficiency of the system was gradually improved. The findings suggest that VFAs generated during the acidification stage in the EGSB reactor can be promptly utilized and converted into methane by methanogens. When the organic load was 12.65 ± 0.67 gCOD/L·d, the fourth-stage treatment efficiency of the system was the highest, the COD removal rate reached 95%, and the methane production rate reached 202.73 mLCH_4/gCOD·d. However, when the OLR increased to 16.14 ± 0.87 gCOD/L·d, the hydrolysis efficiency, acidification efficiency, acetate production efficiency, and methane production efficiency were recorded as 26.81%, 64.09%, 24.03%, and 16.70%, respectively. The acidification stage exhibited an efficiency that was 3.84 times higher than that of the methane production stage and also surpassed the acetate production efficiency by a factor of 2.67, indicating significant acidification occurred within the system. Zhang et al. [29] had also reported the same finding. The aforementioned analysis indicated that VFA accumulation in the system impeded methane production. In anaerobic digestion processes, hydrolysis not only served as a rate-limiting stage but, also, the degradation of VFAs represented another crucial limiting step.

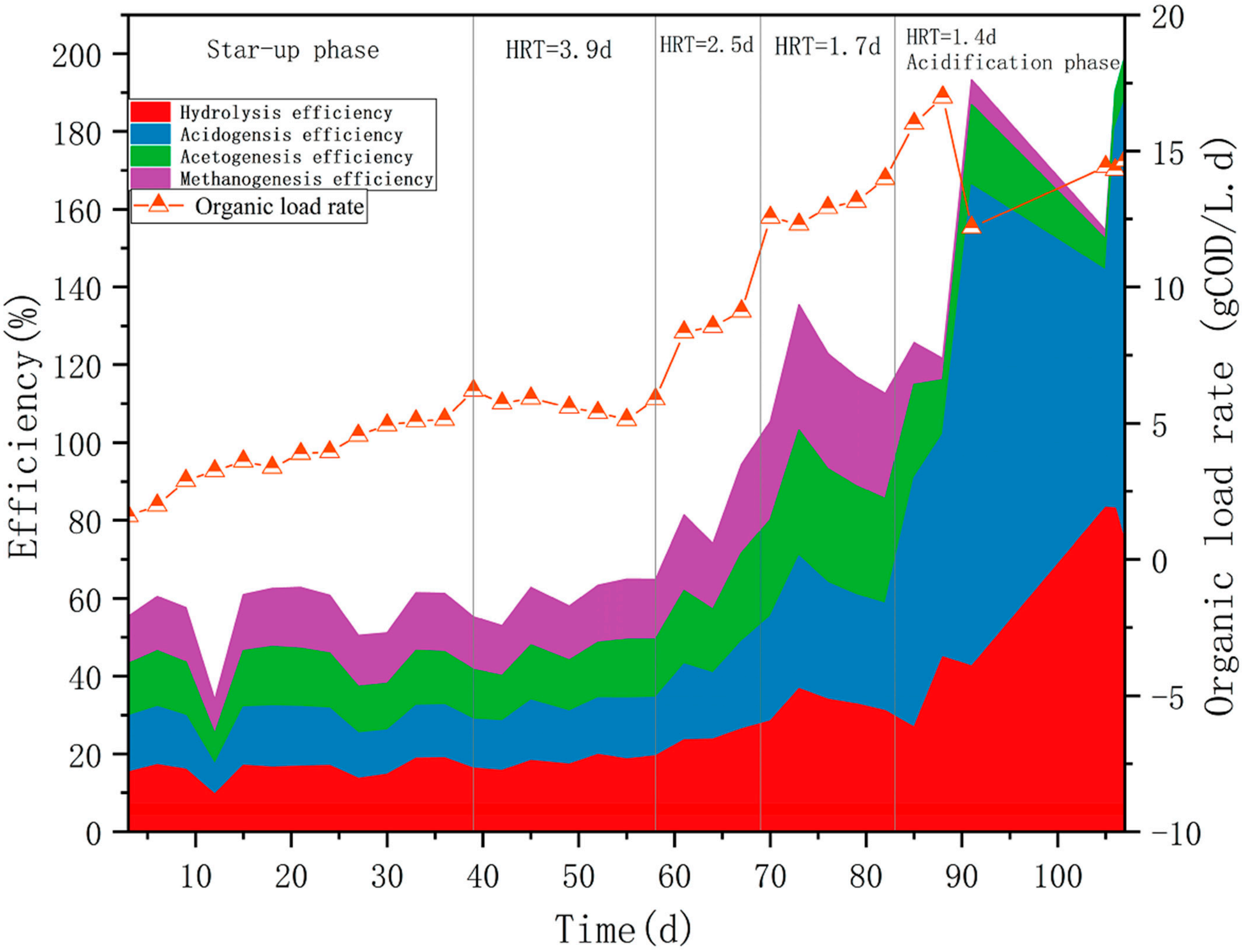

Figure 6. Four-stage efficiencies of EGSB system: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

3.3. Analysis of Energy Utilization Characteristics

Under different OLRs, the energy yield is shown in Figure 7. As can be seen from the figure, except the stage that the OLR was (16.14 ± 0.87) gCOD/L·d, methane was the main energy carrier, which accounts for more than 50%, followed by acetic acid, which accounts for between 3% and 12%. When OLR was (12.65 ± 0.67) gCOD/L·d, the total energy yield was the highest, reaching 618.64 kJ, and the energy conversion efficiency was 57.80%. In the acidification stage (OLR was (16.14 ± 0.87) gCOD/L·d), VFAs (total 568 kJ) were the main energy carrier, of which butyric acid was the main component (456.49 kJ), followed by acetic acid (295.36 kJ) and propionic acid (223.15 kJ), and methane output was almost 0.

The energy conversion of the EGSB reactor under different OLRs is illustrated in Table 3. The results presented in Table 3 indicated that, when the OLR was below 3 gCOD/L·d, $\Delta E < 0$; when the OLR > 5 gCOD/L·d, $\Delta E > 4$ KJ/gCOD; at an OLR of 16.14 ± 0.87 gCOD/L·d, both ΔE and R_e reach their maximum values of 22.53 ± 4.62 KJ/gCOD and 7.99 ± 1.05, respectively. Notably, under OLR being (16.14 ± 0.87) gCOD/L·d, EGSB solely produces VFAs without generating methane, which indicated that considering VFA production in the EGSB reactor can enhance energy conversion efficiency and maximize CAW's overall energy utilization.

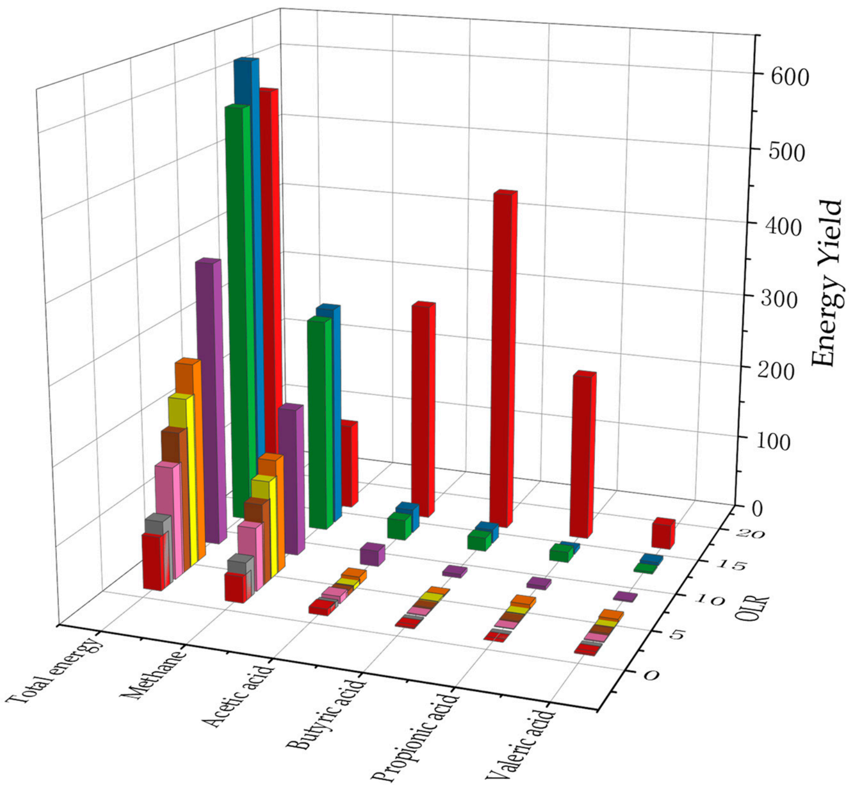

Figure 7. Energy yield under different organic load rates.

Table 3. Energy conversion associated with operation of EGSB reactor at 36 °C.

Operate Phase	OLR (gCOD/L·d)	E_{VFAs} (KJ/gCOD)	E_{CH4} (KJ/gCOD)	E_{out} (KJ/gCOD)	E_{in} (KJ/gCOD)	ΔE (KJ/gCOD)	Re
Star-up phase	1.55 ± 0.10	2.38 ± 0.02	5.96 ± 0.04	8.35 ± 0.15	12.03 ± 0.07	<0	0.69 ± 0.01
	1.93 ± 0.08	1.68 ± 0.02	6.84 ± 0.02	8.52 ± 0.08	9.68 ± 0.05	<0	0.88 ± 0.02
	2.87 ± 0.23	0.71 ± 0.09	4.29 ± 0.01	5.00 ± 0.05	5.89 ± 0.05	<0	0.85 ± 0.01
	3.72 ± 0.21	1.28 ± 0.21	7.36 ± 0.21	8.64 ± 0.18	5.20 ± 0.33	3.43 ± 0.25	1.66 ± 0.09
	4.49 ± 0.11	0.43 ± 0.04	6.43 ± 0.03	6.86 ± 0.07	4.05 ± 0.16	2.80 ± 0.09	1.69 ± 0.09
	5.47 ± 0.42	0.53 ± 0.01	7.32 ± 0.01	7.86 ± 0.03	3.75 ± 0.02	4.10 ± 0.05	2.09 ± 0.02
Load lifting phase	5.58 ± 0.28	1.15 ± 0.22	6.98 ± 0.45	8.13 ± 0.57	3.41 ± 0.21	4.72 ± 0.54	2.39 ± 0.19
	8.48 ± 0.38	1.16 ± 0.08	6.18 ± 0.75	7.34 ± 0.68	3.22 ± 0.12	4.12 ± 0.78	2.29 ± 0.29
	12.65 ± 0.67	1.44 ± 0.32	5.29 ± 1.22	7.53 ± 0.61	3.03 ± 0.13	4.50 ± 0.52	2.49 ± 0.14
	16.14 ± 0.87	25.73 ± 4.88	0.00	25.73 ± 4.88	3.20 ± 0.34	22.53 ± 4.62	7.99 ± 1.05

In addition, according to the analysis in Figure 5, the four-stage conversion efficiency was the highest on the OLR= (12.65 ± 0.67) gCOD/L·d. At the same time, R_e was also the highest when methane was produced in the EGSB reactor (OLR < 12.65 ± 0.67 gCOD/L·d).

4. Conclusions

In this study, an EGSB reactor was used to treat CAW. The EGSB reactor's treatment performance, biogas production, energy utilization characteristics, and efficiency of four-stage anaerobic digestion at different OLRs were studied. It achieved the conversion of CAW into energy, with the energy balance (ΔE) and energy ratio (R_e) reaching (22.53 ± 4.62) KJ/gCOD and 7.99 ± 1.05, respectively. At HRT of 1.7 days, the optimal OLR was found to be 12.65 ± 0.67 gCOD/L·d, resulting in an average COD removal rate and methane production efficiency of (95.75 ± 1.16)% and (180 ± 21) $mLCH_4$/gCOD·d, respectively, and the efficiency of the conversion in four stages was the highest. The daily methane production in the reactor and corresponding COD removal load showed a linear correlation under different OLRs, represented by the equation y = 606.74x + 34.491 (R^2 = 0.9849). The findings will contribute to the acquisition of information pertaining to the treatment of alcohol wastewater through EGSB reactors and offer insights into mitigating environmental pollution resulting from direct discharge of such wastewater. Simultaneously, in light of energy utilization characteristics, it also presents a novel approach for the biological treatment of alcohol wastewater by harnessing its potential for volatile fatty acid.

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