Application of biogas recirculation in anaerobic granular sludge system for multifunctional sewage sludge management with high efficacy energy recovery

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Abstract

This study investigated the possibility of biogas recirculation-driven anaerobic granular sludge system for sewage sludge treatment, aiming to develop an energy sufficient and multifunctional anaerobic digestion (AD) system for sewage sludge with biogas upgrading, sludge stabilization and self-aggregation. Results show that biogas recirculation could enhance the CH_4 production rate by 31–44% and shorten the lag-phase duration to 0.08–0.2 day with simultaneous increment of CH_4 content (> 83% in this study). The reason is mainly associated with the stronger interspecies electron transfer under the biogas recirculation condition. In addition, 37–40% better dewaterability of the digested sludge was achieved, implying the occurrence of self-aggregation of microbial cells induced by biogas recirculation. Energy balance analysis reflects that this sewage sludge treatment system could enhance the net energy recovery by 78–85%. Moreover, almost no obvious influence was noticed on the seed granules' composition and properties. These findings suggest that the biogas recirculation-driven anaerobic granular sludge system could be a promising alternative for sewage sludge treatment, which can improve biogas quality and sludge dewaterability simultaneously towards sludge self-aggregation with no addition of other chemicals.

Keywords: Sewage sludge; Up-flow anaerobic granular sludge system; Biogas recirculation; Energy recovery; Sludge dewaterability

1. Introduction

The production of sewage sludge, i.e., the major by-product from wastewater treatment, is increasing globally along with the stringent requirements for wastewater treatment plants (WWTPs). Nowadays, sewage sludge is treated and/or disposed mainly through landfilling, recycling as building materials, and biogas production via anaerobic digestion (AD). Sludge-to-energy systems have been widely recognized as a favorable technology for sewage sludge management and energy recovery [1]. Taking Japan as an example, about 24% of sewage sludge has been re-utilized as biomass energy (16% biogas + 8% sludge fuel) in 2019 [2]. Energy from renewable wastes is of great concern and popularity to overcome the global energy crisis. AD of sewage sludge can simultaneously achieve energy recovery (mainly as CH4), solid content reduction, sludge stabilization, pathogens removal, and odor emissions reduction that can ease its final and safe disposal [3]. Up to now, although AD has been widely applied in WWTPs for decades, such problems as the large reactor volume due to low reaction rate, high investment cost, process vulnerability, and low resilience to inhibitors accumulation are still pending for more research works on this process [3]. Additionally, to facilitate its final disposal, anaerobically digested sludge is generally dewatered after coagulation/flocculation, targeting easily dewatering and energy-saving. The decrease of water content in dewatered sludge can largely reduce its volume thus lowering energy consumption and treatment costs during the downstream processes including transportation. Moreover, in WWTPs almost 50% of the total operational cost is contributed by sludge management, especially the sludge dewatering unit which usually consumes a large amount of expensive chemicals [4]. The high cost of sludge management is becoming a big issue for the sustainable management of WWTPs, especially in developing regions and countries.

Biogranulation is a phenomenon that microbial cells are self-aggregated into dense and rapid settling granules, beneficial for the separation of treated liquid from sludge particles [5]. Up-flow anaerobic sludge blanket (UASB) is currently the most popular anaerobic granular sludge (AnGS) system, which has many advantages such as low energy consumption, moderate capital and operating costs, low biomass yield, and high tolerance to toxic substances [5], [6]. The UASB reactor, developed by Lettinga and his colleagues in the late 1970s, was firstly and successfully utilized in a beet sugar refinery in the Netherlands [7], [8]. Now the UASB systems have been popularly applied in the treatment of high strength industrial wastewaters containing phenolic compounds [6] and those from food processing [9], potato-starch [10], etc. If there's no acclimatized (granular) sludge, the start-up of UASB is usually performed by seeding anaerobically digested sludge [7], [11], [12] or septic tank sludge [13] together with the feeding of organic wastewaters. In general, the seed sludge used for granulation should possess appropriate stability and density in addition to high methanogenic activity, which is usually required to be acclimated and cultivated at first under anaerobic condition. Nevertheless, it is always difficult to find enough anaerobically digested sludge to quickly start up a full-scale AnGS system. As known, the digested sludge is the effluent sludge from the anaerobic digester treating sewage sludge like primary sludge, waste activated sludge, and/or their mixture. Sewage sludge containing a large amount of organic matters is considered as the potential alternative to organic wastewater for UASB in this study. After being anaerobically treated, the digested sludge might be further functioned as the seed of UASB for further granule formation. An early study by Wu et al. [14] shows that activated sludge can be used as seed to start up the granulation in UASB treating prepared glucose molasses solution and citrate wastewater. In this study, a new concept was put forward regarding whether sludge stabilization and granulation could be achieved simultaneously during the treatment of sewage sludge by UASB. The first quick and important step to take with this new concept was probably to examine the possibility of using an AnGS system for sewage sludge treatment. Up to the present, however, little information is available on the use of granular UASB to treat sewage sludge.

As pointed out by Bhunia and Ghangrekar [13], the upward shear force is crucial for sludge granulation in UASB, which is mainly determined by the liquid and biogas up-flow velocity, and the concentration of inoculum in the reactor. A successful start-up and stable operation of UASB can be achieved at hydraulic retention time (HRT) < 6 h. In the stable AD system of sewage sludge, the feedstock, i.e., sewage sludge (instead of wastewater) input velocity can't provide a sufficient upward shear force due to its long HRT of > 10 days [15]. Therefore, how to provide a suitable biogas up-flow condition to create enough upward shear force for sludge granulation under a long HRT condition is the key to the successful UASB operation for sewage sludge treatment.

On the other hand, biogas recirculation can not only provide mixing power but also achieve biogas upgrading for AD systems of organic solid wastes, which has been evidenced previously [16], [17], [18], [19]. Latha et al. [16] claimed that an optimum intermittent biogas recirculation was more promising as an alternative mixing method for the large-scale AD with low power consumption, as it could enhance biogas yield and improve energy efficiency when compared to the conventional mixing mode of impeller. Moreover, Yuan et al. [18], [20] and Zhao et al. [21] found that biogas upgrading and sludge conditioning could be simultaneously achieved during the AD of sewage sludge treatment under biogas recirculation coupled with Fe^{3+} [18], Mg^{2+} [20], or Ca^{2+} [21] addition, reflecting its great potentials and profits for cost-saving sewage sludge management. In fact, biogas recirculation has been successfully applied in UASB systems treating various wastewaters, achieving accelerated reactor start-up with the generation of well settleable granular sludge [11], enhanced biomass retention efficiency [22], and promoted formation of microbial granules [23] as well. In this study, it’s hypothesized that a suitable biogas recirculation may provide enough upward shear force for the granular UASB reactor to treat sewage sludge, which can maintain the whole reactor performance and produce digested sludge with better properties, especially in terms of sludge settleability and dewaterability. As such, it’s necessary to pay more attention to the changes of AnGS and digested sludge in the granular UASB.

Therefore, in this study, a sewage sludge treatment system, i.e., granular UASB coupling with biogas recirculation was proposed, which is expected to maintain biological metabolisms through the dense anaerobic aggregates even under harsh conditions and improve the dewaterability of digested sludge simultaneously for sustainable management of sewage sludge. This work examined the possibility of using the biogas recirculation-driven granular UASB system to treat sewage sludge. More specifically, the effect of biogas recirculation on the granular sludge system performance was mainly concerned in addition to the changes in properties of AnGS and digested sludge so as to elucidate their roles in this system.

2. Materials and methods

2.1. Sludge samples

AnGS used as inoculum in this study was sampled from Asahi Beer Brewery in Ibaraki Prefecture, Japan. Concentrated primary sludge was obtained from the Shimodate WWTP in Ibaraki Prefecture, Japan. The total solids (TS) and volatile solids (VS) (based on wet weight) of AnGS and primary sludge were 5.79 ± 0.28% and 3.77 ± 0.35% (VS/TS = 0.65), and 2.16 ± 0.02% and 1.88 ± 0.02% (VS/TS = 0.87), respectively. The water content of each sludge was equal to 100% minus TS (%). In this study, in order to facilitate samples analysis and calculation especially for the subsequent separation of digested sludge from AnGS, the primary sludge was sieved by a 0.5 mm sieve to remove some large particles, and the AnGS was sieved by the same 0.5 mm sieve to remove the small particulate substances that may not belong to AnGS according to the definition by Alphenaar [24]. Similarly, the size of AnGS was defined physically larger than 0.5 mm in this study.

2.2. UASB reactors

Two identical UASB reactors (biogas-R1 and biogas-R2, in parallel) were fabricated as similar as in the previous study [21], each with an effective volume of 402 mL (D × H = 40 mm × 320 mm). To realize biogas recirculation, each UASB reactor was equipped with a gas flow meter and a gas pump. A gas collector was used to store a certain amount of biogas, and the biogas volume was quantified by displacing the saturated $NaHCO_3$ solution in order to avoid the dissolution of CO_2 in water. During biogas recirculation, the produced biogas was recirculated into the bottom of the UASB reactor through a microporous ceramic gas sparger, which was controlled by a preset timer and conducted intermittently (1 h-on/1h-off) at a constant flow rate of 25 mL/min (with a resultant up-flow velocity of 2 cm/min) according to the preliminary experiments. The third reactor (control), with the same dimension and a gas collector but no other installations, was also set up and used for the AD of sewage sludge without biogas recirculation. The three reactors were operated at 37 ± 2 °C in batch-mode AD experiments simultaneously.

Before being used as the feedstock, the primary sludge pH was adjusted to ~ 9.0 using a combination alkali addition strategy (NaOH and $Ca(OH)_2$) at a resultant added Ca^{2+} concentration of 300 mg Ca/L-sludge, an appropriate Ca^{2+} concentration that could stimulate the formation of granules during the start-up of UASB [25]. The inoculum (AnGS) and feedstock (primary sludge) were mixed at a ratio of 0.29 (VS basis), and the concentrations of AnGS and primary sludge in the reactors were 5.8 and 19.8 kg VS/m^3-reactor, respectively, according to the properly inoculated sludge concentration recommended by Lettinga [26] and Lin and Yang [27]. The initial water content of the sludge mixture loaded into the reactor was about 96.8%. After being fed with 400 mL of the sludge mixture, each reactor was purged with N2 gas for 5 min to create an anaerobic condition. Generally, biogas produced from the AD process mainly consists of 50–60% CH_4 and 40–50% CO_2, in addition to small amounts of other gases like NH_3, H_2S, H_2, CO, and N_2 [17]. In order to rapidly initial the whole AD system, it was assumed that the AD system had been stably operated for a long time with enough biogas containing normal contents of CH_4 and CO_2 in the gas collector. Thus, in this study, synthetic biogas consisting of 60% CH4 and 40% CO_2 was firstly injected into the gas collector with an initial biogas volume of 300 mL to start the biogas recirculation at the beginning of the AD process. During the whole operation, the biogas volume in the gas collector was maintained at the range of 300–600 mL by discharging the produced biogas manually from the gas outlet with a gas-tight injector due to the continuous biogas production. This operation could also ensure the same pressure inside the gas collector during the whole operation period, which approximated to the atmospheric pressure as determined. All the experiments were conducted in triplicate with the similar phenomenon being observed.

During the operation, the sludge was sampled periodically from the AD reactor after well mixed by biogas recirculation. The digested sludge and AnGS were separated carefully as soon as possible through the 0.5 mm sieve. Then a certain amount of digested sludge was taken for further analysis of the related parameters and the remaining digested sludge and AnGS were returned to the reactor right after the separation. At the end of the experiments, after the separation of AnGS from digested sludge, the AnGS on the 0.5 mm sieve was washed with distilled water for three times; then the separated digested sludge and AnGS were used for related analysis.

2.3. Analytical methods

The biogas composition, i.e., CH4 and CO2 and their contents were determined by GC-8A gas chromatography (Shimadzu, Japan) equipped with a stainless-steel column packed with Porapak-Q and a thermal conductivity detector connected to a chromatopac data analyzer (Shimadzu C-R4A, Japan), and N2 was used as the carrier gas. Volatile fatty acids (VFAs) including acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid and n-valeric acid were determined by GC-14B gas chromatography (Shimadzu, Japan) equipped with Unisole F-200 30/60 column and ame ionization detector (FID). A pH meter (FE20, Mettler Toledo, Switzerland) and a conductivity meter (AS710, As One Co., Japan) were respectively used to measure the digested sludge sample pH and conductivity immediately right after being sampled. The measurements of TS and VS were carried out by drying the sample at 105 °C to a constant weight first and then burning at 600 °C for 3 h. Sludge surface charge was determined with the colloid titration technique, and polybrene and polyvinyl sulphate (PVSK) were respectively used as the standard cationic and anionic colloids as described previously [28]. In brief, the sludge sample collected from the AD reactor was firstly diluted with distilled water and then mixed with excess 0.001 N polybrene solution with a small amount of toluidine blue (0.1 mL used in this study) added as the color indicator. Then the above mixture was titrated against 0.001 N PVSK till a subtle color change from blue to pink/purple, signaling its electrical neutrality.

Sludge extracellular polymeric substances (EPS) were extracted and quantified according to Yuan et al. [20] with slight modifications by using distilled water instead of 0.05% NaCl as the re-suspension solution to avoid the influence of cations determination. Soluble EPS (S-EPS) were directly extracted from the sludge sample by centrifugation at 5000 rpm for 10 min; after being washed for three times, the sediment was then re-suspended using distilled water for the followed-up extraction. The loosely bound EPS (LB-EPS) were extracted after the sample being heated at 70 °C for 1 min and centrifuged at 5000 rpm for 10 min. Again, being washed with distilled water for three times, the re-suspended mixture was heated at 60 °C for 30 min and centrifuged at 5000 rpm for 15 min. The resultant supernatant was collected to determine the concentration of tightly bound EPS (TB-EPS). The concentrations of proteins and polysaccharides in the extracted EPS were measured using the Lowry method and phenol-sulfuric acid method, respectively, with bovine serum albumin and glucose as the respective standards. Cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) in the extracted EPS were quantified by ion chromatography (Shimadzu, Japan).

In this study, capillary suction time (CST) of digested sludge measured by a CST meter (CST-1, Yamato Scientific Co., Ltd., Japan) was used to evaluate the sludge dewaterability. A smaller CST value indicates better sludge dewaterability. The settling velocity of granules was determined by monitoring the falling of granules in tap water [12]. Granular strength was measured by determining the TS distribution in the supernatant and the remaining part after being shaken at 200 rpm for 5 min [12]. The proportion of TS in the supernatant could indicate the strength of the granules: the smaller value, the greater the granular strength. Morphology of granules was observed with Leica M205 C Microscope (Leica Micro-systems, Switzerland). The granular size distribution was estimated by image analysis according to Tassew et al. [29] assisted by Leica M205 C Microscope but with a simplified procedure, in which the diameter of granules was estimated by vernier caliper with the granules being assumed to be spherical.

In addition, the effects of biogas recirculation on electron transport system (ETS) activity during the AD of sewage sludge was assessed using the reduction method of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to formazan (INTF) according to Zhang et al. [30].

2.4. Statistical analysis

The statistical difference of the experimental data was analyzed by an independent sample t-test using SPSS 17.0 software, and $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Performance of the up-flow granular sludge systems

3.1.1. CH_4 production

Fig. 1A illustrates the cumulative CH_4 production and CH_4 content from the reactors during the 24 days' AD operation. The CH_4 contents in the reactors with biogas recirculation (biogas-R1 and -R2, in parallel) significantly increased during the AD process compared to the control, achieving biogas upgrading as similar as previous studies [19], [20], [21]. The average CH_4 contents in the control, biogas-R1, and -R2 during the whole operation were $76.2 \pm 2.2\%$, $83.6 \pm 4.2\%$, and $84.5 \pm 2.5\%$, respectively. Although the same cumulative CH_4 production was obtained from the three UASB reactors at the end of the tests, the two biogas recirculation-driven UASB systems progressed much faster. In this study, kinetic models including the first-order, modified Gompertz, and Cone kinetic models were adopted to fit and evaluate the UASB of sewage sludge, which have been widely applied for the AD process [31]. The experimental CH_4 yields were fitted to the above three kinetic models, with the data fitting and the meanings of the corresponding parameters being shown in Table 1. As seen, the modified Gompertz and Cone models exhibited a higher correlation coefficient (R^2), lower residual sum of squares, and lower Diff. value in comparison to the first-order model, indicating their better fit to the CH_4 production process of sewage sludge under the test conditions. Compared to the control, a much higher specific rate constant (k, increased by 80–100%) from the Cone model and maximum CH_4 production rate (μ, increased by 31–44%) from the modified Gompertz model were obtained in the two biogas recirculation systems. This observation suggests that biogas recirculation could shorten the duration time of the AD process thus favored organic matter degradation and CH_4 production. As seen from Table 1, the lag-phase duration (λ) of the control reactor, about 2.4 days, was shortened to 0.08–0.2 day in the biogas recirculation systems according to the modified Gompertz model, signaling the desirable condition for the AD of sewage sludge under the test biogas recirculation. This observation also implies that biogas recirculation could facilitate the quick start-up of the granular UASB system. The increased CH_4 production rate and shortened lag-phase duration via biogas recirculation could be attributable to various aspects, such as the promoted contact and interaction between substrates and microorganisms [16], and the increased buffering capacity in the AD system, especially during the hydrolysis stage due to enhanced dissolution of CO_2. Besides the functions of mixing and enhanced contact between the microorganisms and substrates induced by biogas recirculation, CO_2-containing biogas recirculation may be responsible for the enrichment of hydrogenotrophic methanogens and their enhanced activities [18], [32], resulting in better AD performance of biogas-R1 and -R2.

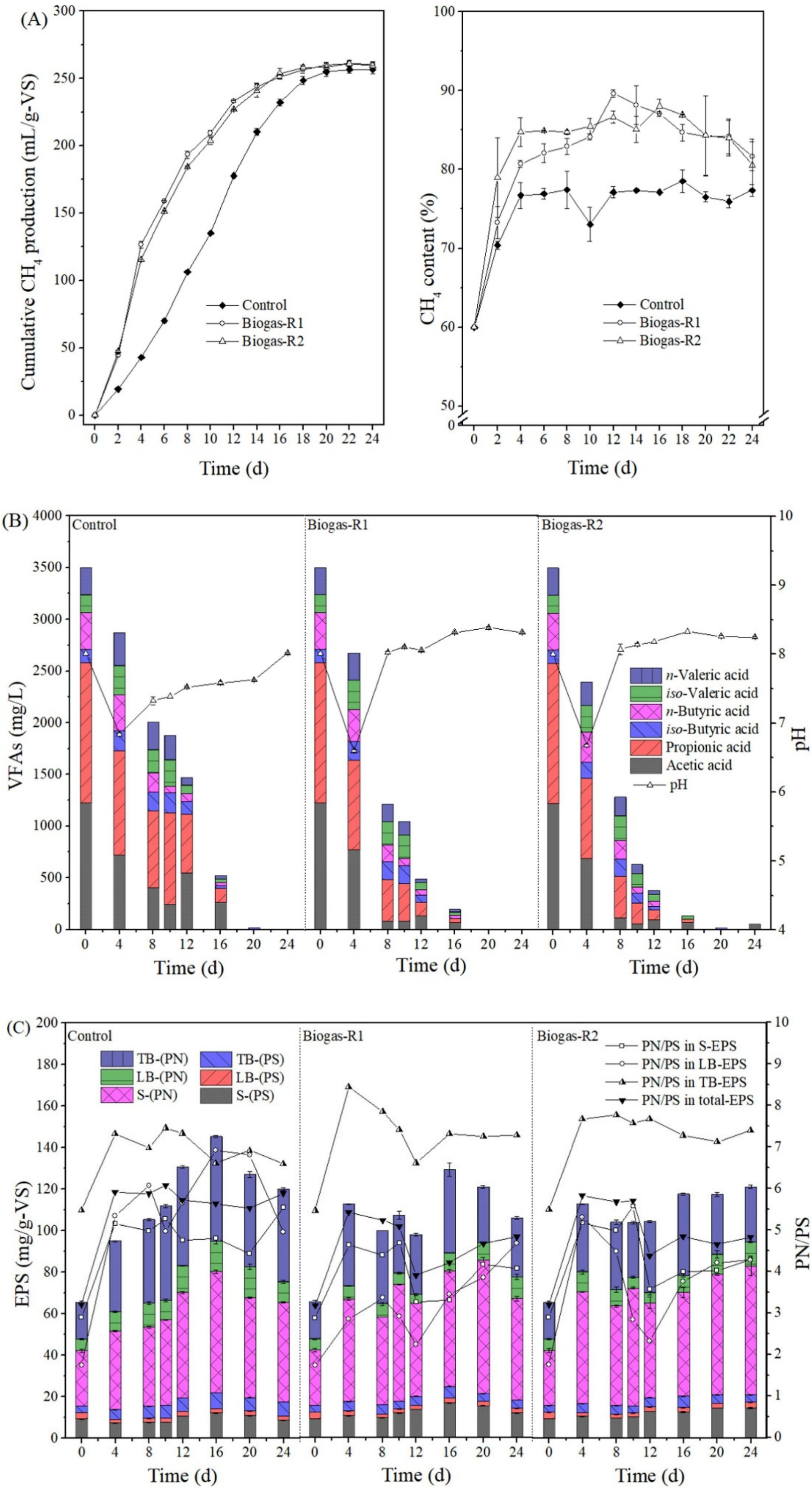

Fig. 1. Changes in (A) cumulative CH_4 production and its content, (B) VFAs and pH, and (C) EPS during the operation of UASB reactors. Control, with no biogas recirculation; Biogas-R1 and -R2, the two identical UASB under biogas recirculation (in parallel operation).

Table 1. Kinetic models and related parameters estimated from the experimental data of the three UASB systems.

Model	Parameter	Control	Biogas-R1	Biogas-R2
Experimental value	G_{max0} (mL CH_4/g-VS_{fed})	256.37 ± 3.08	260.3 ± 2.12	259.46 ± 2.3
The first order $G(t) = G_{max}[1 - exp(-kt)]$	G_{max} (mL CH_4/g-VS_{fed})	298.65 ± 73.36	275.83 ± 6.12	280.24 ± 5.59
	k (d^{-1})	0.06 ± 0.03	0.14 ± 0.01	0.13 ± 0.01
	R^2	0.908	0.990	0.994
	Reduced Chi-Square	306.82	71.80	47.78
	Residual Sum of Squares	9630.71	789.84	525.58
	Diff. value	16.5	6.0	8.0
Modified Gompertz $G(t) = G_{max} \cdot exp\left\{-exp\left[\frac{\mu}{G_{max}}(\lambda - t)e + 1\right]\right\}$	G_{max} (mL CH_4/g-VS_{fed})	277.04 ± 6.34	257.89 ± 4.54	260.21 ± 4.42
	μ (mLCH_4/g-VS/d)	19.47 ± 0.94	28.08 ± 2.25	25.55 ± 1.78
	λ (d)	2.42 ± 0.32	0.20 ± 0.38	0.08 ± 0.36
	R^2	0.996	0.988	0.990
	Reduced Chi-Square	40.99	90.60	72.38
	Residual Sum of Squares	409.99	906.05	723.84
	Diff. value	8.1	0.9	0.3
Cone $G(t) = \frac{G_{max}}{1 + (kt)^{-n}}$	G_{max} (mL CH_4/g-VS_{fed})	310.35 ± 19.85	283.41 ± 6.49	293.51 ± 7.82
	k (d^{-1})	0.10 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
	n	2.21 ± 0.24	1.68 ± 0.11	1.57 ± 0.10
	R^2	0.992	0.996	0.997
	Reduced Chi-Square	79.06	27.31	24.43
	Residual Sum of Squares	790.62	273.13	244.31
	Diff. value	21.1	8.9	13.1

Note: G (mL CH_4/g-VS), cumulative specific methane production; G_{max} (mL CH_4/g-VS), ultimate methane production; k (d^{-1}), specific rate constant; t (d), digestion duration; μ (mL CH_4/g-VS/d), maximum methane production rate; λ (d), lag-phase duration; e = 2.7182; *n*, dimensionless shape factor.
Diff. value is the absolute value of the difference between experimental value and modified value of maximum CH_4 yield. Diff. value = $100 \times |(G_{max0} - G_{max})|/G_{max0}$.

3.1.2. Changes in VFAs and pH

The AD process mainly consists of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Among the four steps, hydrolysis is regarded as the rate-limiting one [4] that can decompose complex organic substances into soluble monomers. In the acidogenesis step, soluble monomers can be further converted into intermediate products like VFAs [33]. Thus, the changes in pH and VFAs concentration in the reactors were monitored during the AD process as shown in Fig. 1B. The initial feedstock (*i.e.*, primary sludge) was initially adjusted to ~ pH 9.0, but this pH value dropped to ~ 8.0 within two hours during the loading into the AD reactor with high VFAs being detected on day 0 simultaneously because of alkaline hydrolysis. During the AD process, the pH value dropped sharply during the first 4 days from the same initial pH (~ 8.0), followed by a gradual increase and finally to a relatively stable value. In general, VFAs accumulation would result in a sharp pH drop and this phenomenon lasted to day 4, and on day 8 the pH value was detected to gradually increase with continuous decline of VFAs concentration. Obviously, the lowest pH did not correspond to the highest VFAs on day 4, which might occur during day 0 to day 4. Being different from the control (no biogas recirculation, pH = 6.83 ± 0.02) on day 4, the pH values in the biogas recirculation UASB systems were a little bit lower, averagely 6.74 ± 0.05, which is attributable to the 40 times higher water solubility of CO_2 than CH_4 [16] and the increase in contact time between CO_2 and digestate under biogas recirculation. The dissolution of CO_2 in the digestate might remarkably decrease the bulk liquor pH [34]. However, CO_2-containing biogas recirculation may also form a carbonate buffer system [35] which brings about the accumulation of alkalinity in the AD system as previously reported [19]. More specifically, during the whole operation, a lower VFAs accumulation in the biogas recirculation reactors was always detected when compared to the control. The enhanced carbonate buffer capacity in the biogas recirculation-driven UASB reactors may play a key role in their stable operation [19], and they were also detected to have higher pHs and lower VFAs accumulation during the remaining operation period (excepting the first 4 days). Therefore, the performance of granular UASB could be enhanced due to the improvement of hydrolysis via enhanced CO_2 dissolution together with the carbonate buffering system.

3.1.3. Changes in EPS extracted from digested sludge

EPS consisting of variable proportions of proteins (PN) and polysaccharides (PS) are an important part of the sludge biomass, which can influence the matrix structure and stability of granular sludge to a great extent [36]. EPS may protect the cell-matrix and resist the adverse influence of the environment with their composition being dependent on the nature of aggregates [37]. PN and PS are also the intermediates in the AD process, which are produced during the hydrolysis and acidogenesis steps, and then consumed in the acidogenesis and acetogenesis steps [38]. During the AD of sewage sludge, EPS were extracted from the digested sludge and quantified as shown in Fig. 1C. As seen, no significant difference ($p > 0.05$) in the amount of total-, LB-(PS)-, and S-(PN)-EPS, while a significant difference ($p < 0.05$) in the amount of S-(PS)-, TB-(PS)-, LB-(PN)-, and TB-(PN)-EPS was noticed in the digested sludge between the control and biogas recirculation systems. A higher S-(PS)-EPS, and lower TB-(PS)-, LB-(PN)-, and TB-(PN)-EPS contents were detected in biogas-R1 and -R2 when compared to the control. It is known that PN are mainly localized in the core region of the granules, while PS are easy to loosen from the EPS in the presence of upward shear force, which may negatively affect the sludge granulation [9], [39]. Being different from the traditional UASB reactors treating high strength organic wastewater under the combined shear force from hydraulic and biogas uplifting, the UASB reactors in this study were applied for sewage sludge stabilization only under the shear force from biogas recirculation. In this process, the sludge particles may possess the potentials to form granular sludge, during which organic matters including PN and PS from sludge are degraded simultaneously. Therefore, the amount and components of EPS in the sludge may be different from the formation process of granular sludge, which are influenced by the degree of sludge degradation and other factors. Moreover, the PN/PS ratio in EPS may indicate the formation and stabilization of granular sludge [9]. As claimed by Hulshoff Pol et al. [40], a high proportion of PN is favorable for sludge granulation because of its high negatively charged amino acids. Basuvaraj et al. [39] stated that the PN/PS ratio should be about 1.4 for granular sludge with a good settleability. In this study, the PN/PS ratios in TB-EPS were obviously higher in the digested sludge from biogas-R1 and -R2 compared to the control, while their S- and LB-EPS were lower than the control, implying that the relative degradation degrees of PN and PS were different in different layers of sludge particles, or there might be some movement of PN towards the central layer. In addition, the relative higher PN in the central layer of sludge particles from the biogas recirculation UASB systems may favor the formation of new granules. Still, more in-depth works should be conducted, including the changes in PN and PS types and their properties that are crucial for the sludge granulation process and particle stability.

3.1.4. Changes in sludge surface charge, conductivity, and ETS activity

Fig. 2A and B show the changes in sludge surface charge and conductivity during the operation of UASB systems. A significant difference ($p < 0.05$) was found in both sludge surface charge and conductivity between the biogas recirculation systems (biogas-R1 and -R2) and the control. Compared to the control (with no biogas recirculation), the sludge from the biogas recirculation systems showed relatively lower negative surface charge and conductivity.

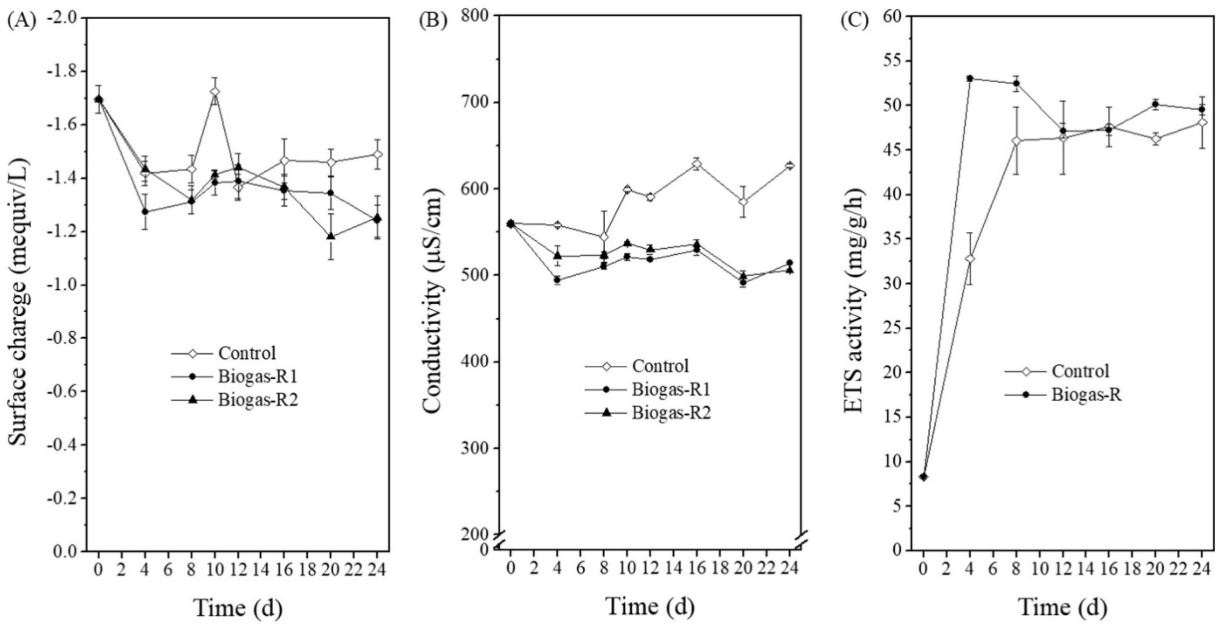

Fig. 2. Changes in (A) surface charge, (B) conductivity, and (C) electron transport system (ETS) activity during the operation of UASB reactors. Control, with no biogas recirculation; Biogas-R1 and -R2, the two identical UASB under biogas recirculation (in parallel operation); Biogas-R, one

reactor for repeated experiments of biogas recirculation AD system operated under the same conditions as for biogas-R1 and -R2

Sludge surface charge has long been believed to be closely associated with the stability of microbial aggregates [36]. In general, the surface of sludge particles is negatively charged, which may gradually decrease along with the formation of granules [41]. Granular sludge was also found to be less negatively charged than activated sludge [28]. During the AD process, the presence of some cations such as Ca^{2+} could enhance microbial adhesion through compressing the electrical double layers on the cell surface and linking exocellular polymers [25]. Most recently, Liang et al. [6] found that Ca^{2+} may reduce electrostatic repulsion between negatively charged bacteria, which can also bind to EPS accelerating bacterial adhesion. The formed microbial aggregates may have the potential to further form granules due to the biogas upward shear force. Therefore, in this biogas recirculation-driven granular UASB system (using AnGS as inoculum) for sewage sludge treatment, a positive tendency to form granular sludge was observed during the AD of sewage sludge.

The conductivity of microbial aggregates can reflect the ability of microorganisms to transport electrons, which is beneficial for AD [42], especially for the direct interspecies electron transfer within flocs or granular microbial aggregates [43]. At the end of AD operation, the conductivity of digested sludge in the control was higher than that in the biogas recirculation systems (biogas-R1 and -R2, Fig. 2B), indicating a worsening ability of sludge to transport electrons under biogas recirculation. On the contrary, a better AD performance was obtained in the two biogas recirculation systems (Section 3.1.1), probably due to more interspecies electron transfer occurred within the granules or sludge flocs (not in the bulk solution used for conductivity determination). Another possible reason for this phenomenon might be associated with some soluble cations that also greatly influence the determination of conductivity. Clearly seen, much lower soluble cations were detected in the biogas recirculation systems (biogas-R1 and -R2, Fig. 3A), and a significant correlation at the 0.01 level was noted between the conductivity and soluble total cations, probably leading to the lower conductivity of sludge. The effect of soluble cations on conductivity might be greater than the interspecies electron transfer within the granules or sludge flocs, resulting in the contradictory observations between the AD performance and bulk conductivity. Therefore, the ETS activity of digested sludge was determined in this study to further explain this phenomenon.

The ETS activity can more intuitively reflect the activity of the electron transfer in microorganisms, namely, the electron transport efficiency [30]. In this study, as shown in Fig. 2C, initially the ETS activity increased rapidly in all the reactors, which was consistent with the occurrence of methanogenic processes. A higher ETS activity was always detected in the biogas recirculation UASB system, especially during the first 8 days, which was approximately 1.62 times on day 4 and 1.14 times on day 8 that of the control reactor, further confirming that the ETS activity is closely associated with biogas production rate. Consequently, the better AD performance, lower negative surface charge and conductivity, and higher ETS activity in the biogas recirculation UASB systems indicated that more interspecies electron transfer occurred within sludge flocs or granules under the test biogas recirculation conditions.

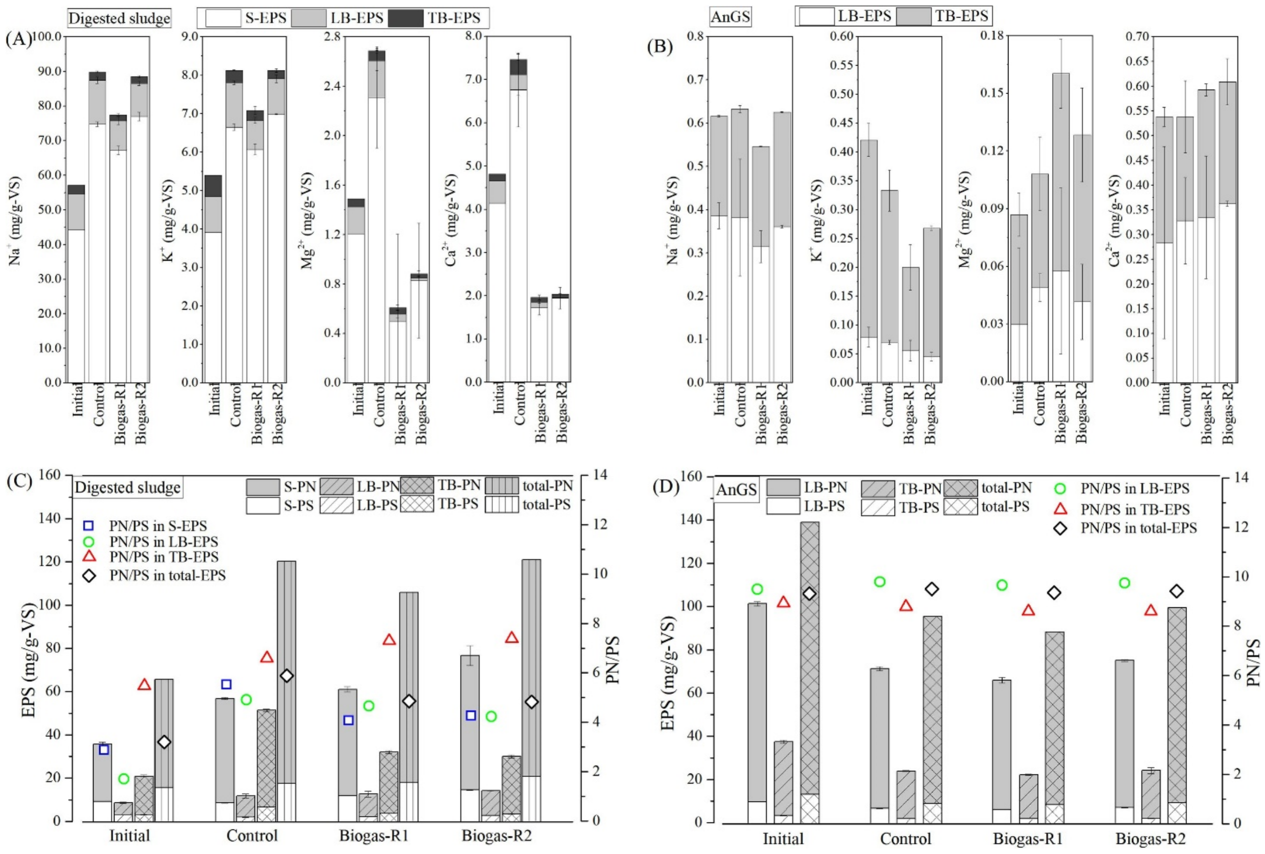

Fig. 3. Changes in concentrations of cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) (A and B), proteins (PN) and polysaccharides (PS) (C and D) in EPS extracted from digested sludge and anaerobic granular sludge (AnGS), respectively.

In this work, the biogas recirculation-driven granular UASB for sewage sludge treatment was operated in batch mode for a short time (24 days) with one-off feeding of sewage sludge. As a consequence, this operation might have limited detectable effects on the granulation of digested sludge. But its existing influence could not be ignored, particularly on the betterment of sludge dewaterability that will be discussed in Section 3.3. It is expected to achieve granular digested sludge (at least the digested sludge with strong self-aggregation ability) after a long-term operation of the biogas recirculation-driven granular UASB during the treatment of sewage sludge, which demands further exploration and confirmation in the followed-up experiments.

3.2. Comparison of AnGS and digested sludge from UASB reactors

It has been reported that the formation of granular sludge plays a crucial role in the maintenance of stable process and performance of UASB during wastewater treatment [9]. In this study, the UASB reactor was applied to treat sewage sludge, and biogas recirculation instead of hydrodynamic shear force from influent wastewater provided the upward shear force. To better evaluate the changes in sludge properties during the AD operation under biogas recirculation, the digested sludge (with AnGS being removed) and the AnGS were separated and analyzed comparatively at the end of the AD experiments.

3.2.1. Cations in EPS

Generally, sludge flocs or sludge particles are composed of multivalent cations, EPS, microorganisms, biogenic and inorganic substances, etc., in which cations play a vital role in sludge stability and dewaterability. Besides, cations may help accelerate the granulation process through bridging between negatively charged groups on cell surfaces and linking the exocellular polymers [25]. Therefore, the changes of cations in EPS might have some implication for this sludge treatment system. As shown in Fig. 3A, after the AD process, the concentrations of cations including Na^+, K^+, Mg^{2+}, and Ca^{2+} in the total EPS increased in the digested sludge from the control reactor, while Na^+ and K^+ increased but Mg^{2+} and Ca^{2+} decreased in the digested sludge from the biogas recirculation systems (biogas-R1 and -R2) when compared to their initial values. The decreases in Mg^{2+} and Ca^{2+} concentrations may be associated with their changes in S-EPS, reflecting more Mg^{2+} and Ca^{2+} uptake by microbes or their combination with some inorganics or organics (via precipitation or conjugation), which is conductive to microbial cells self-aggregation or sludge re-flocculation. In contrast, as shown in Fig. 3B, the amount of cations in the extracted EPS from AnGS was much less and relatively stable than that from digested sludge, indicating the better stability of AnGS than digested sludge and some difficulty of cations release from granules. Less cations were detected to release during the extraction of EPS with relatively long error bars for the data from > 3 times batch extraction experiments, however, the same trend was observed for the same batch extraction. For instance, no obvious difference was noticed in the concentration of Na^+ in AnGS between the control and biogas recirculation systems; a lower K^+ concentration was detected in the EPS of AnGS from the biogas recirculation systems (biogas-R1 and -R2) which contained higher Mg^{2+} and Ca^{2+} concentrations in comparison to the control. It is clearly evidenced that the presence of divalent ions, such as Ca^{2+} and Mg^{2+}, is a key factor for sludge granulation [12], [44]. Results from this study show that Mg^{2+} and Ca^{2+} can be further accumulated in the EPS of AnGS, especially Ca^{2+} that has been found to effectively speed up the granulation process and be extensively accumulated in the microbial aggregates [25], [45], which would also improve granular strength. In this study, the strength of all the AnGS was below 0.8% with no significant difference among the test reactors (Fig. 5A), suggesting the very strong and stable granules in this study. As reported, the uptake of Mg^{2+} and Ca^{2+} by the sludge might be beneficial for sludge granulation during wastewater treatment, while the higher uptake of Na^+ and K^+ may adversely influence sludge concentration, granular strength, sludge settling velocity, and treatment efficiency. This is inferred from the fact that more uptake of Na^+ and K^+ might have saturated carrier molecules, thus restricting the uptake of Mg^{2+} and Ca^{2+} [12]. Up to the present, still, limited information could be found regarding the effects of various cations contained in the sewage sludge on its anaerobic granulation during the AD process, which needs more in-depth studies.

3.2.2. Organic matters in EPS

As discussed in Section 3.1.3 and shown in Fig. 3C, the changes in EPS from digested sludge at the end of the AD experiments suggest its potential for granule formation in the biogas recirculation systems. Compared to the digested sludge, no significant change in the EPS from AnGS was noticed for the control and biogas recirculation systems after the AD process (Fig. 3D). This observation implies that biogas recirculation exerted little influence on the composition of EPS from AnGS. It is known that PS are hydrophilic polymers, while PN, especially the amino acids in PN, contribute to the hydrophobic character of flocs [39]. Since the large number of negatively charged amino acids contained in PN can form electrostatic bonds with multivalent cations, the aggregate structure may become stable [46]. EPS can help promote the adhesion of microorganisms through chemical bonds or physical entanglement, and the granules with a higher TB-EPS content usually possess better mechanical strength and physical stability [9], [39]. In this study, the high PN/PS ratio in the TB-EPS from digested sludge together with little change in the EPS from AnGS, as well as the decrease of Ca^{2+} content in digested sludge and the accumulation of Ca^{2+} in AnGS suggest the great potential of sewage sludge to form aggregates and even granules in the granular UASB system under biogas recirculation condition.

3.2.3. Changes in dewaterability of digested sludge

As shown in Fig. 4, a better dewaterability of digested sludge indicated by a lower CST was detected in both biogas recirculation systems (biogas-R1 and -R2). To exclude the effect of TS on CST, the CST value based on TS content was also calculated, which shows the similar trend. Basuvaraj et al. [39] claimed that LB- and TB-EPS control the floc behavior, and PN-rich TB-EPS extracted from granular sludge contribute to the improvement of sludge dewaterability. In this study, a high PN/PS in TB-EPS was noticed in the biogas recirculation systems, which is probably the main reason for better sludge dewaterability. Besides, the multivalent cations may condense the diffusion within the electrical double layers, promoting the aggregation of microbial pellets due to Van der Waals forces [25]. Ca^{2+} may also play a major role in the resultant better sludge dewaterability, indicative of less soluble Ca^{2+} in the biogas recirculation systems because of the aggregation or bridging between Ca^{2+} and organics like PN.

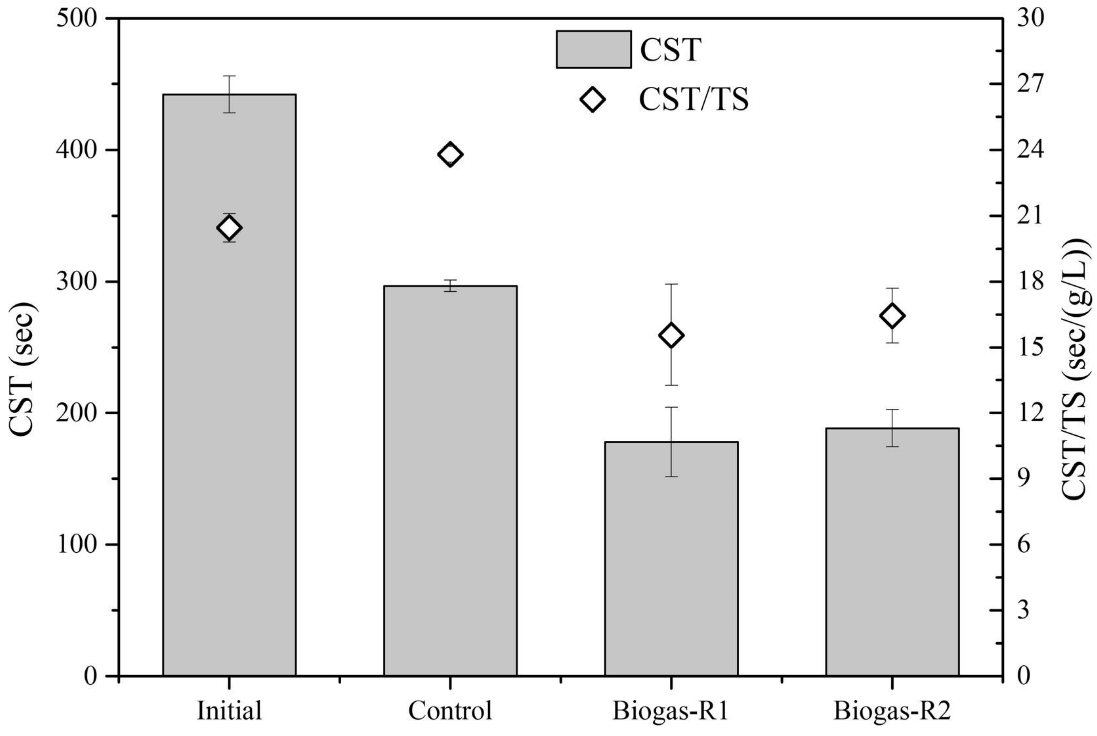

Fig. 4. Sludge dewaterability indicated by capillary suction time (CST) of digested sludge at the initial and the end of the AD process. Initial, sampled primary sludge; Control, with no biogas recirculation; Biogas-R1 and -R2, the two identical granular UASB with biogas recirculation (in parallel operation).

3.3. Changes in the characteristics of AnGS

AnGS was collected from the UASB reactors before and after batch AD tests to check their characteristics in terms of granular strength, VS/TS, particle size distribution, settling velocity, and morphology.

3.3.1. Granular strength and biomass content

Granular strength is a key parameter to characterize the sludge quality. Granules with higher strength could resist the upward shear force, while the granules with lower strength might break up and cause biomass loss thus worsening the reactor performance [47]. In this study as shown in Fig. 5A and B, the applied AnGS was very strong with little changes in granular strength (< 0.8%) and VS/TS ratio being noticed under the test conditions, indicating that biogas recirculation had no obvious effect on granular strength and biomass content in AnGS.

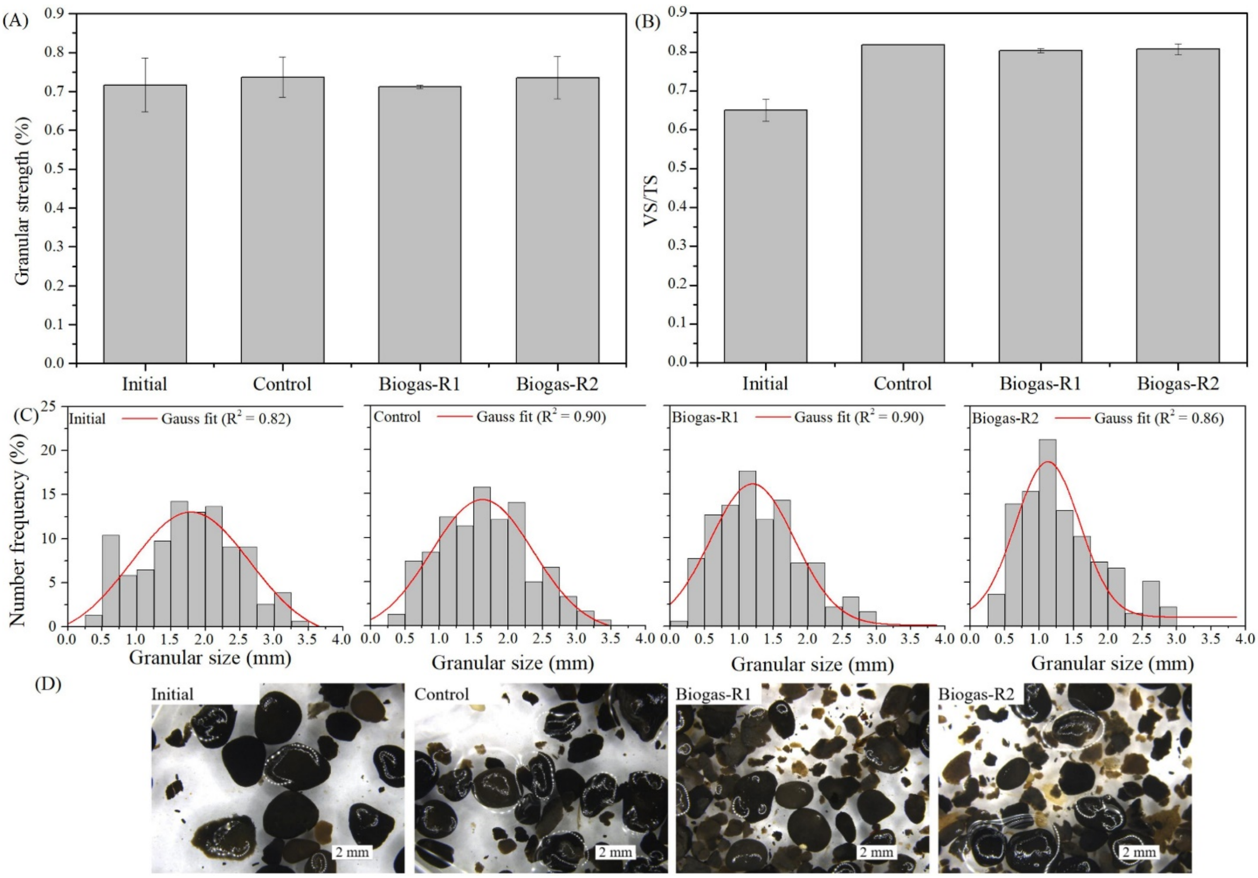

Fig. 5. Changes in granular strength (A), VS/TS ratio (B), granular size distribution (C), and morphology (D) of anaerobic granular sludge (AnGS) at the initial and the end of the AD process. Initial, seed AnGS; Control, with no biogas recirculation; Biogas-R1 and -R2, the two identical granular UASB under biogas recirculation.

3.3.2. Size distribution

The size distribution demonstrates that the granules were normally distributed, which could be well fitted by the Gauss model ($R^2 > 0.82$). As shown in Fig. 5C and D, after 24 days' operation, the main granule size decreased from the initial AnGS with a main size of 1.50–2.25 mm to 1.25–2.00 mm in the control and to 0.75–1.50 mm in biogas-R1 and -R2. As mentioned in Section 2.1, in this study the granular sludge is physically defined as sludge particles with size > 0.5 mm [24]. It can be seen from Fig. 5C that the proportion of > 0.5 mm particles accounted for 98.7%, 98.7%, 91.8%, and 96.4% in the initial, control, biogas-R1, and -R2, respectively. The main granular size became slightly smaller with a slower settling velocity in the biogas recirculation systems that are probably not beneficial for the maintenance of stable operation of larger-scale granular systems. This phenomenon may also indicate the dynamic breakage and growth of granular sludge, which needs further confirmation after a long-term operation of the biogas recirculation-driven granular UASB. Hulshoff Pol et al. [40] claimed that the upward shear force is not responsible for breaking or disintegrating the granules, and only the growth of small particles may change the size distribution of granules. Therefore, the granular size distribution in the UASB reactors is probably brought about by the growth of small aggregates which may be potentially developed into larger granules. In this regard, these small size aggregates might start a new generation of growth nuclei, further promoting anaerobic granulation [6], in which the attachment of cells to these particles is proposed as the initiation step for granulation.

3.4. A proposal for economically sustainable sludge management

The energy recovered from the AD of sewage sludge in WWTPs can be partially utilized for biogas recirculation in the granular UASB system. Namely, the produced biogas can be used to power an electricity generator that is operated to provide power for biogas recirculation, heating digesters, and dewatering of the digested sludge. CH_4 in-situ enrichment can be obtained by biogas recirculation thus improving the heating value of biogas [19]. The amount of energy generation from produced biogas, and energy consumption including heating the digesters and powering the gas pumps in the control, biogas-R1, and -R2 systems can be estimated based on the operation data of a local WWTP (Ibaraki, Japan) as described in detail in Supplementary Materials. As shown in Table 2, the biogas recirculation-driven granular UASB can improve net energy profit by 78–85% from sewage sludge treatment compared to the control (traditional AD system). In addition, much less or no other chemical addition is required during the dewatering of digested sludge due to its better sludge dewaterability and formation of compact and dense granular structure in the UASB system. The current UASB system achieved 37–40% improvement on sludge dewaterability compared to the control, which could be further enhanced after the optimization of operational conditions including biogas recirculation rate, organic solids loading, temperature, etc. As summarized by Wei et al. [48], 37–98% improvement of sludge dewatering could be achieved using chemical or organic flocculants. This biogas recirculation-driven UASB system is expected to realize no additional chemical addition and energy self-sufficiency for sustainable sewage sludge management under its optimal operation conditions. The findings from this work are valuable for sludge management in full-scale WWTPs, which still needs more in-depth research especially on the long-term operation of continuous-flow sewage sludge treatment systems. A key challenge to the successful development of granules is the long start-up time of the bioreactors. This work proved that microbial cells' self-aggregation or sludge re-flocculation of digested sludge could be achieved by this system, which is an important step in the formation of anaerobic granules. Therefore, more detailed research works are still demanding to further explore the possibility of granule formation from digested sludge in this biogas recirculation-driven granular UASB during the long-term treatment of sewage sludge.

Table 2. Energy balance analysis of the three UASB systems.

UASB system	Control	Biogas-R1	Biogas-R2
CH_4 content (%)	76.2	83.6	84.5
LHVbiogas (MJ/m^3)	26.51	28.04	28.26
The peak brake thermal efficiency (η, %)	28.9	32.2	32.7
Energy generated from biogas (MJ/t)	87.19	102.80	105.18
τ (days)	13.96	8.79	9.39
Energy consumption for heating (MJ/t)	71.79	65.95	66.63
Energy consumption by gas pump (MJ/t)	0	9.39	10.03
Net energy profit from UASB system (MJ/t)	15.40	27.46	28.52

Note: LHV_{biogas}, lower heating value of biogas; τ, effective CH_4 production duration, *i.e.*, the time used to produce 80% of the total CH_4 production.

4. Conclusions

A biogas recirculation granular UASB system was applied to treat sewage sludge. Biogas recirculation can help mix the influent sludge and provide biogas upward shear force with simultaneous achievement of biogas upgrading. Compared with the control (no biogas recirculation), the CH_4 production rate in the biogas recirculation system was enhanced by 31–44% with lag-phase duration shortened to 0.08–0.2 day, which was mainly ascribed to the improvement of interspecies electron transfer. Almost no obvious effect of biogas recirculation on the granular characteristics was observed, excepting some decrease in granule size. Besides, the digested sludge with 37–40% better dewaterability may be attributed to the self-aggregation of microbial cells induced by biogas recirculation. Therefore, the results from this work can help establish a sewage sludge treatment system which can couple biogas recirculation with granular sludge system to achieve biogas upgrading and easily dewatered digested sludge with no additional chemical addition. This work can also provide an innovative technique for the enhancement of energy recovery from sewage sludge.

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