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Effects of short hydraulic retention time on UASB reactor performance inoculated with pre-aggregated anaerobic digestion granular sludge

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Abstract Water hyacinth, a high-strength lignocellulosic macrophyte, poses a serious ecological threat by disrupting aquatic ecosystems and hindering water transport. The juice extracted from water hyacinth, commonly known as water hyacinth juice, is particularly rich in soluble organic compounds and has been demonstrated to enhance hydrolysis and acidogenesis rates when used as a substrate in high-rate anaerobic digestion using up-flow anaerobic sludge blanket reactors. The performance of up-flow anaerobic sludge blanket reactors relies heavily on the formation and maintenance of granules. However, a major challenge in the practical implementation of up-flow anaerobic sludge blanket reactors lies in optimizing the operational conditions like hydraulic retention time (HRT), that balance process kinetics, microbial retention, and system stability. Shortening the HRT can improve volumetric biogas production rates and reduce reactor footprint by utilizing more substrate volume, but it can also disrupt microbial stratification leading to biomass washout and process instability. The use of pre-aggregated anaerobic digestion granules, rich in mature methanogens, has been suggested as a viable strategy to mitigate washout under high loading conditions. This study investigated the effectiveness of up-flow anaerobic sludge blanket reactors for high-rate anaerobic digestion of water hyacinth juice at shortened HRTs, using pre-aggregated anaerobic digestion granular sludge as inoculum. The experiments were conducted in a 430 mL up-flow anaerobic sludge blanket reactors operating for 107 days, at four different HRTs, (2, 1.5, 1.0, and 0.5 days). Despite the high suspended solids concentration of water hyacinth juice (3.3 ± 0.7 g L^{-1}) and increasing organic loading rates at shorter HRTs, the reactor maintained consistent stability and performance. The total organic carbon removal efficiencies ranged from 35.3% at the shortest HRT of 0.5 days to 82.1% at HRT = 1.5 days, indicating effective substrate degradation across varying loading conditions. The suspended solids and volatile suspended solids removal efficiencies were 56.9 ± 14.1% and 74.9 ± 7.0%, respectively, recorded at HRT = 1.0 day. These results highlight the reactor's ability to retain and degrade particulate matter despite short retention times. The Biogas production rates increased significantly with decreasing HRTs, from 201.1 ± 37.4 mL d^{-1} at HRT = 2 days to a peak of 903.8 ± 307.2 mL d^{-1} at HRT = 0.5 days, with methane content consistently above 79%. The maximum SS removal of 56.9 ± 14.1% was achieved at HRT = 1 while the highest biogas production rate (903.8 ± 307.2 mL d^{-1}) was achieved at HRT = 0.5 days. This study demonstrates that the up-flow anaerobic sludge blanket reactors inoculated with pre-aggregated anaerobic digestion granular sludge can effectively treat high-strength lignocellulosic substrates such as water hyacinth juice under high loading conditions. Even at extremely short HRTs, the system maintained stable removal efficiency and robust biogas production, showcasing its potential for efficient valorization of invasive aquatic biomass. These findings support the deployment of the up-flow anaerobic sludge blanket reactor as a viable technology for the dual purpose of environmental management and renewable energy generation, particularly in regions plagued by water hyacinth overgrowth.

Keywords: UASB reactor, hydraulic retention time, anaerobic digestion, granular sludge, biogas production rate, organic loading rate

1. Introduction

In the pursuit of sustainable energy solutions, anaerobic digestion (AD) has emerged as a robust and environmentally friendly biotechnology for the stabilization of organic waste while also producing biogas, primarily methane (Appels et al. 2008, Weiland 2010). Among the variety of biomass resources investigated for anaerobic digestion (AD), aquatic macrophytes particularly water hyacinth (WH), (*Eichhornia crassipes*) have garnered significant attention due to their rapid growth, high photosynthetic efficiency, and ability to absorb excess nutrients and heavy metals from water bodies (Gunnarsson & Petersen 2007, Malik 2007, Kitaka et al. 2022). WH has infested over 100 countries, making it one of the world's most destructive and aggressively reproductive aquatic weeds. Notably, it is estimated that just 10 plants can produce over 600,000 offspring in a single growing season (Lahon et al. 2023), highlighting their extraordinary proliferation potential.

While water hyacinth (WH) poses a serious ecological threat by disrupting aquatic ecosystems and hindering water transport (Bhatia et al. 2025), its removal and subsequent valorization through AD presents a promising strategy for biomass management and renewable energy recovery (Nigam 2002, Kashyap et al. 2003). Although WH is a high-strength lignocellulosic macrophyte, it also contains a significant proportion of biodegradable components such as cellulose, hemicellulose, and soluble sugars, making it a suitable substrate for AD (Kushwaha et al. 2020). The juice extracted from WH, commonly known as WH juice (WHJ) is particularly rich in soluble organic compounds and has been demonstrated to enhance hydrolysis and acidogenesis rates when used as a substrate in high-rate anaerobic digestion (Singh et al. 2023, Bhatia et al. 2025). However, its effective bioconversion depends largely on reactor configuration and operational parameters, among which hydraulic retention time (HRT) plays a pivotal role (Bhatia et al. 2025).

The up-flow Anaerobic Sludge Blanket (UASB) reactor, originally developed by Lettinga et al. (1970), represents a landmark innovation in anaerobic treatment technology. The UASB system is characterized by the development and accumulation of dense microbial granules that settle well and support high biomass retention, thereby allowing for elevated organic loading rates and shorter HRTs (van Lier et al. 2001, Hulshoff Pol et al. 2004). The performance of UASB reactors relies heavily on the formation and maintenance of these anaerobic granules, which are complex consortia of microbial communities embedded in a matrix of extracellular polymeric substances (EPS) (Barros et al. 2007, Fang et al. 2011). These granules facilitate efficient syntrophic interactions between acidogens, acetogens, and methanogens, promoting process stability and biogas yield (Fang et al. 2011).

A major challenge in the practical implementation of UASB reactors lies in optimizing the operational conditions that balance process kinetics, microbial retention, and system stability. HRT, defined as the average time the substrate remains in the reactor, directly influences the washout rate of slow-growing methanogenic archaea and the contact time between substrate and biomass (Seghezzo et al. 1998). Shortening HRT can improve volumetric biogas production rates and reduce reactor footprint by utilizing more substrate volume, but it can also disrupt microbial stratification and granule integrity, leading to biomass washout and process instability (Lettinga 1995, Liu & Tay 2004).

Previous studies have shown that operating UASB reactors at shorter HRTs can yield promising results when conditions are carefully managed. For instance, Jiraprasertwong et al. (2020) demonstrated that a three-stage UASB reactor treating ethanol wastewater achieved high methanogenic activity and stable performance at low HRTs due to the presence of mature granular sludge. Similarly, Sarti et al. (2017) highlighted that granule characteristics such as size, density, and EPS composition significantly determine the system's ability to withstand hydraulic and organic shocks. The use of pre-aggregated AD granules, rich in mature methanogens, has been suggested as a viable strategy to mitigate washout under high loading conditions (Hulshoff et al. 2004).

Despite these advances, the literature still lacks systematic investigations into the performance of UASB reactors inoculated with pre-aggregated granular sludge and operated at reduced HRTs using lignocellulosic-rich feedstocks such as WHJ. While several researchers have studied the effect of pretreatment techniques on WH to improve biodegradability (Hendriks & Zeeman 2009, Kushwaha et al. 2020), there are limited studies on how such substrates interact with granular sludge under short retention times.

This study builds on previous own findings by evaluating the effect of short HRTs (ranging from 0.5 to 2 days) on the performance of a lab-scale UASB reactor fed with WHJ and inoculated with pre-aggregated AD granular sludge. The objective is to elucidate the relationship between shortened HRT, organic matter removal, and biogas productivity under high OLRs. In doing so, this research aims to provide deeper insight into the operational thresholds of UASB systems for high-rate treatment of plant-based substrates.

2. Materials and Methods

2.1. Substrate and inoculum preparation

WH plants were harvested from a pond in Suijo Park, Saitama, Japan (same sampling site reported in our previous study (Bhatia et al. 2025)). The whole plant (including leaves, stem and roots) was then shredded and compressed using a milling machine (RSC-3500, R-ing Co. Ltd., Japan) and a screw dewatering machine (Dash-1, R-ing Co. Ltd., Japan), respectively to obtain the WHJ. WHJ was then filtered through a 106 μm mesh; thereafter, the juice was stored at 4 °C and used as a substrate for AD. Bhatia et al. (2025) reported the chemical parameters and the ions concentration of WHJ as shown in Table 1. In this study, the pre-aggregated anaerobic digestion granular sludge obtained from our previous study on UASB treating WHJ (Bhatia et al. 2025) was used as an inoculum. The sludge was then stored in a temperature-controlled room at 37 ℃ for a few days to remove residual organic compounds from the sludge.

Table 1. The chemical composition of WHJ reported in our previous study (Bhatia et al. 2025). The harvested site was the same with the current study, but the harvesting season differed.

Parameters	Measurements
pH	5.0 ± 0.7
Suspended solids (SS)	6.1 ± 2.0
Volatile suspended solids (VSS)	4.3 ± 1.5
Chemical oxygen demand (COD)	10616.9 ± 2068.8
Total organic carbon (TOC)	4210.5 ± 1467.7
Total nitrogen (TN)	512.8 ± 156.1
Dissolved organic carbon (DOC)	1411.4 ± 220.3
Dissolved nitrogen (DN)	103.6 ± 44.4

All parameters were measured in mg L^{-1} except pH.

2.2. Reactor set up and operation

A 430-mL lab-scale UASB reactor was used in this study. Fig. 1 shows the schematic representation of a UASB reactor used for WHJ treatment. Primarily 430 mL of inoculum was introduced into the reactor. The substrate was then fed continuously into the reactor's bottom inlet throughout the HRT of 2.0 to 0.5 days (Table 2). The substrate was then diffused into the reactor using inert alumina beads (diameter =10 mm) for homogeneous supply. The resulting effluent passed through a U-shaped pipe attached to the reactor's upper part before proceeding to an effluent (digestate) storage tank (Fig. 1). On day 53, the sampling point was changed from 1 to 2 (Fig. 2a) to avoid significant

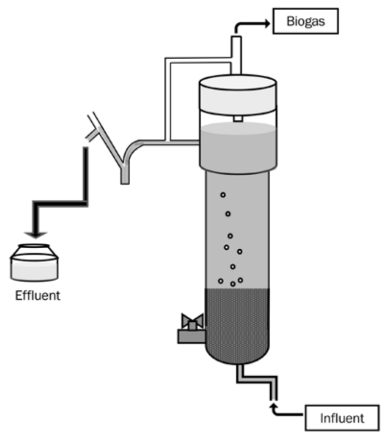

Fig 1. Schematic representation of a UASB reactor used for WHJ treatment. Influent flows upward through a sludge blanket, producing biogas and treated effluent through microbial degradation.

SS accumulation in sampling point 1, as at point 2, the SS concentration was comparable to the SS in the reactor (Fig. 2b). The volume of biogas produced was determined using a wet gas meter (W-NK-0.5B, Shinagawa, Japan) and kept in a 5 L aluminum gas bag (GL Science, Japan). The reactor operated at 37 ± 1° C in a temperature-controlled laboratory for 107 days.

Table 2. Loading parameters during each phase of anaerobic digestion.

Phase	Period (day)	Assuming HRT (day)	Actual HRT (day)	Average OLR (g-TOC $L^{-1}d^{-1}$)
Phase 1	0 - 16	2.0	2.1 ± 0.1	1.1 ± 0.1
Phase 2	17 - 70	1.5	1.6 ± 0.3	1.3 ± 0.2
Phase 3	71 - 86	1.0	1.0 ± 0.1	2.1 ± 0.1
Phase 4	87 - 107	0.5	0.5 ± 0.4	3.2 ± 0.1

HRT: Hydraulic retention time; TOC: Total organic carbon; OLR: Organic loading rate.

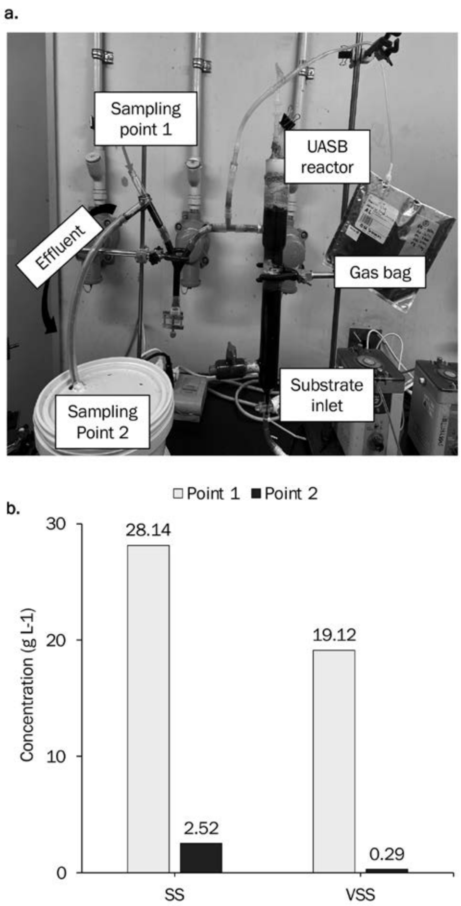

Fig 2. Actual representation of a UASB system used for WHJ treatment in this study (a), and SS and VSS concentration measured at different sampling points (b). The sampling point was shifted from point 1 to 2 on day 51.

2.3. Analytical methods

The pH, suspended solids (SS), volatile suspended solids (VSS), total organic carbon (TOC) and total nitrogen (TN) of the substrate and effluent were measured during the experimental period. The pH was measured with a pH meter (SevenCompact pH/Ion meter, S220, Mettler Toledo, Japan), and TSS, VSS, and TS, were determined by the standard methods of the American Public Health Association (Bhatia et al. 2025). TOC and TN were measured using a TOC analyzer equipped with nitrogen measurement (TOC-L CPH/CPN, TNM-L, Shimadzu, Japan). The biogas composition in the aluminum gas bag was measured using a gas chromatograph (GC-14B, Shimadzu, Japan) equipped with a packed column (Micropacked-ST, Shimadzu, Japan) and a thermal conductivity detector. The injector and detector temperatures were maintained at 100° C and 200° C, respectively. Argon was used as the carrier gas at a flow rate of 10 mL min^{-1}.

3. Results and Discussion

3.1. Reactor performance

The WHJ used in this study had the same sampling site as WHJ reported in our previous study (Bhatia et al. 2025) although the harvesting season differed (Table 1). The pH of WHJ used in previous studies varied depending on the plant's habitat or growing conditions, with ground-type WHJ having a pH of 4.02 and floating-type WHJ ranging from 7.0 to 7.5 (Liu et al. 2021, Maruyama et al. 2023). In this study, floating-type WH was used, and its juice exhibited an average pH of 5.9 ± 0.8 throughout the experimental period. Notably, despite fluctuations in influent pH within the range of 5.1 to 7.5, no significant impact was observed on the effluent pH, which remained within the neutral to mild alkaline range (7.3 to 8.8) as shown in Fig. 3. The average effluent pH was 7.7 ± 0.2 and the pH at HRTs 2, 1.5, 1 and 0.5 were 7.9 ± 0.3, 8.0 ± 0.4, 7.7 ± 0.3, and 8.0 ± 0.4, respectively. Recent studies have identified that the pH regime for methanogenic bacteria falls within the range of 7.2 to 8.8 (Antwi et al. 2017). Despite the fluctuations in HRT and OLR, the effluent pH remained within the optimal range.

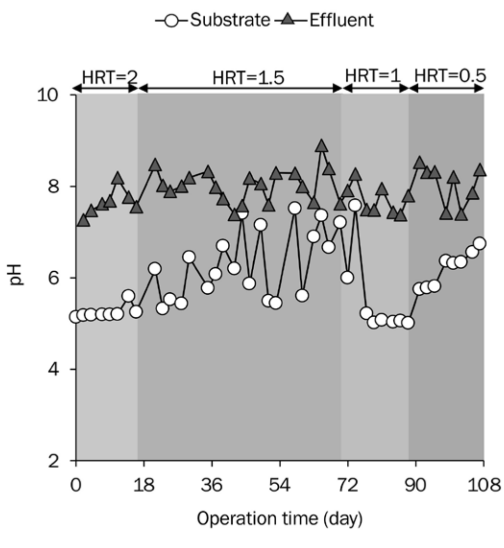

Fig 3. pH variation within the timeline of anaerobic digestion treatment process

The observed pH stability suggests that the AD process in this study demonstrated strong operational resilience, maintaining consistent performance even under high OLR (HRT 0.5). However, variations in substrate pH may still influence other operational parameters of the UASB system (Bhatia et al. 2025).

The effective removal of SS and VSS is essential for the optimal operation of a UASB reactor, as it minimizes the risk of clogging, floating and washout of active biomass (Mohan et al. 2022). As mentioned in the materials and methods section, the sampling point for effluent SS and VSS was shifted from point 1 to 2 (Fig. 2a) on day 51 due to high accumulation of SS observed in point 1 (Fig. 2b). The accumulation of SS and VSS in point 1 could be attributed to the small diameter of the U-tube. Therefore, the effluent SS and VSS measurements were considered for comparison after day 51. Fig. 4 depicts the timeline of SS and VSS throughout the operational period. The average SS concentration of the influent and effluent was 3.3 ± 0.7 g L^{-1} and 1.8 ± 0.8 g L^{-1}, respectively (Fig. 4a). The average VSS concentration of influent and effluent was 2.1 ± 0.5 g L^{-1} and 0.8 ± 0.6 g L^{-1}, respectively (Fig. 4b). Previous studies have shown that UASB reactors are typically designed for the treatment of liquid substrates, such as industrial and municipal wastewaters, which generally fall within an SS concentration range of 0.17 to 1.80 g L^{-1} (Turkdogan-Aydinol et al. 2011). In contrast, WHJ used in this study exhibited significantly higher SS concentrations, with influent values ranging from 1.4 to 5.2 g L^{-1}. Despite this high SS load, even under low HRT of 0.5, the reactor achieved an average SS and VSS removal efficiency of 51.6 ± 18.6% and 67.8 ± 21.8%, respectively, over the operational period. This level of performance reflects the reactor's strong resilience and capacity to operate effectively under high solids loading conditions. The react SS and VSS removal rate at HRT

1.5, 1 and 0.5 was 55.3 ± 19.2 and 74.0 ± 18.2, 56.9 ± 14.1and 74.9 ± 7.0, and 38.3 ± 17.7 and 51.4 ± 25.1, respectively (Table 3). The lower SS and VSS removal rates observed at HRT 0.5 might be attributed not only to excessive organic and solids loading, but also to reduced biomass-substrate contact time, hydraulic overloading that hindered solid retention, increased turbulence leading to resuspension of settled solids (Liu et al. 2008, Rajagopal et al. 2013).

Notably, there was no sludge floating observed in this study even at high OLR load (HRT 0.5) as reported in previous studies (Liu et al. 2020, Jeganathan et al. 2006). This might be due to the compact and porous structure of granules (from granular sludge) which allows them to trap suspended particles as the influent passes through the sludge bed (Lettinga et al. 1980).

TOC and TN are critical indicators of the organic and nitrogenous load of the substrate, and their removal efficiency reflects the reactor's capacity for simultaneous carbon and nitrogen stabilization (Tchobanoglous et al. 2003). The TOC and TN concentrations in the influent and effluent exhibited similar trends as shown in Fig. 5a and 5b, respectively. The average TOC and TN concentrations of influent were 2060.2 ± 160.2 mg L^{-1} and 15.0 ± 0.7 mg L^{-1}, respectively. Conversely, the TOC and TN concentration in the effluent was higher than the influent from day 2 to day 51, with an average concentration of 2187.4 ± 2651.5 mg L^{-1} and

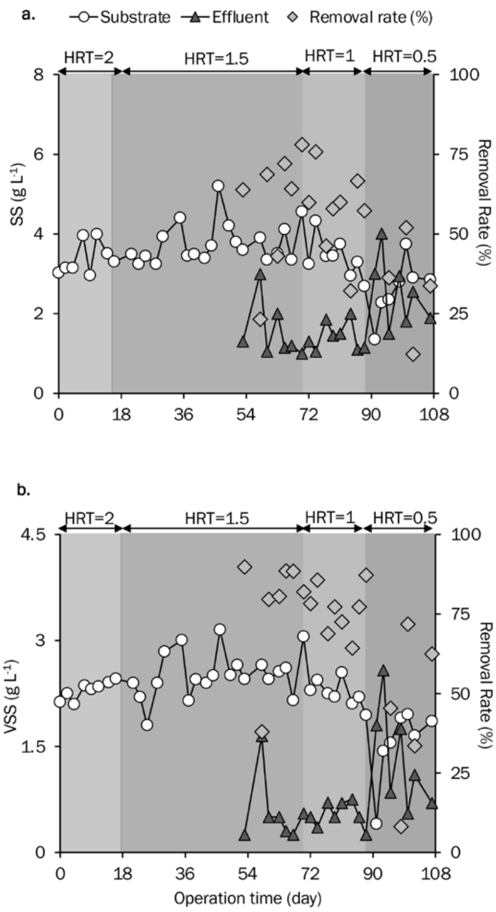

Fig 4. Timeline of anaerobic digestion process performance. (a) SS and its removal variation and, (b) VSS and its removal variations.

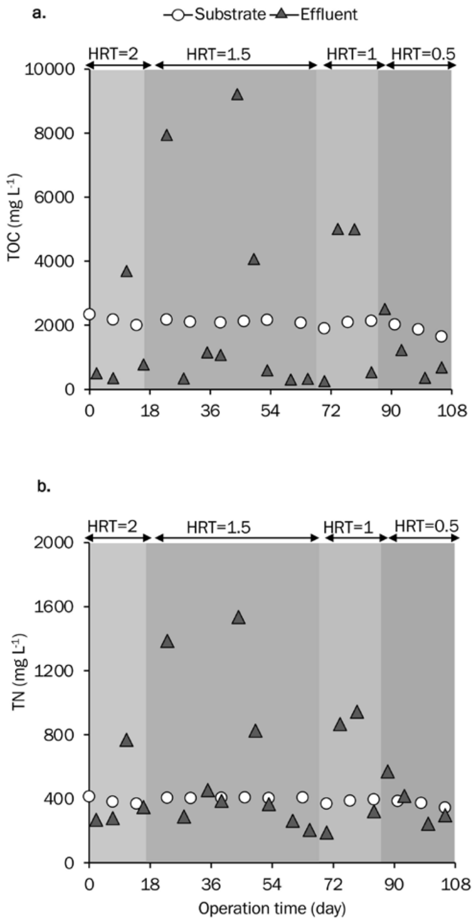

Fig 5. Timeline of anaerobic digestion process performance. (a) TOC concentration (b) TN concentration.

533.5 ± 383.0 mg L^{-1}, respectively. The high effluent concentration of TOC is attributed to the excess accumulation of SS at sampling point 1 (Fig. 2a). After the sampling point shifted to point 2, there was a notable reduction in effluent TOC and TN concentrations observed (1528.9 ± 1833.5 mg L^{-1} and 424.9 ± 260.2 mg L^{-1}, respectively). This shift highlights the importance of accurate sampling location in evaluating reactor performance, as emphasized by Latif et al. (2011), who noted that sedimentation dynamics in UASB reactors could substantially influence effluent quality measurements. In general, the TOC concentration in the effluent fluctuated (day 2 to day 51) and increased as the HRT decreased. However, the TOC removal efficiency remained relatively high after the sampling point was shifted to point 2, ranging from 35.3% at HRT = 0.5 days to 82.1% at HRT = 1.5 days, demonstrating the system's capacity to manage higher OLR (lower HRT). Findings from Liu et al. (2020) and Lettinga et al. (1980), highlighted that HRT lower than 2days can hinder complete biodegradation due to limited retention and microbial uptake time, as well as the potential washout of slow-growing microbes such as methanogens. Interestingly, the UASB reactor reported in the present study-maintained system stability even at HRT=1. Show et al. (2012) reported that Granular sludge has a high microbial density and excellent settling properties, which enhance the retention of active biomass even under conditions of high OLR. The system's stability, even at high OLR, highlights the effectiveness of granular sludge in enhancing organic and nitrogenous matter degradation in high-strength substrates.

The evaluation of biogas yield and biogas production rate is widely regarded as a key indicator of methanogenic microbial activity and overall reactor performance during anaerobic digestion (Angelidaki et al. 2011). The biogas yield fluctuated throughout the AD process, with a slight increase observed as HRT increases (Fig. 6). As shown in Fig. 6, the average biogas production rate increased significantly by decreasing HRT, from 201.1 ± 37.4 mL d-1 at HRT = 2 to 903.8 ± 307.2 mL d^{-1} at HRT = 0.5. This trend is consistent with the findings of Liu et al. (2020), who investigated the long-term energy efficiency and microbial community dynamics of various reactors under increased WHJ loadings. They reported that higher OLR, resulting from shorter HRTs, can stimulate microbial activity, particularly among hydrolytic and acidogenic communities, thereby enhancing the volumetric biogas production. Interestingly, while the biogas production rate varied significantly across different HRTs, the biogas yield remained relatively stable, ranging from 0.8 ± 0.2 L L-substrate^{-1} to 1.2 ± 0.2 L L-substrate-1. In addition, the average methane content was above 80%. These results suggest that substrate conversion efficiency was largely maintained across the different HRTs, highlighting the robustness of the methanogenic community and the stability of the granular sludge (Van Lier et al. 2001, Show et al. 2012). Granular sludge has high microbial density and excellent settling properties which enhance the retention of active biomass under dynamic loading conditions (Tay et al. 2001). This

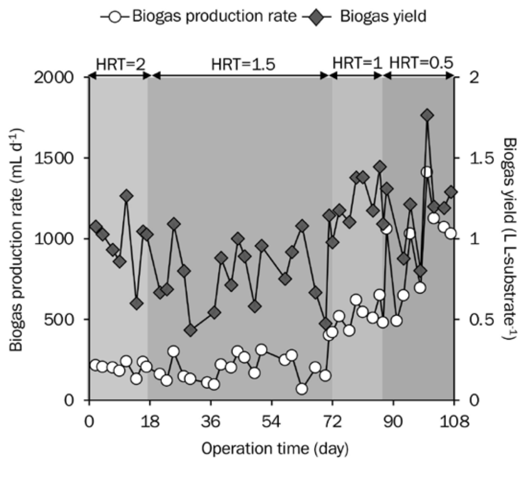

Fig 6. Timeline of anaerobic digestion process performance production rates and Biogas yield

could explain the system's ability to perform well even at reduced HRTs. The reactor performance at HRT = 0.5 days is particularly noteworthy. Although short HRTs are often associated with washout risks and reduced contact time between microbes and substrates (Lettinga et al. 1980), the system in this study maintained a relatively high biogas production rate (903.8 ± 307.2 mL d^{-1}) and biogas yield (1.0 ± 0.2 L L-substrate^{-1}). In this study, the retention of high biogas production despite an HRT of only 0.5 days suggests that the granular sludge used provided sufficient surface area and structural integrity to immobilize active biomass, enhancing microbial-substrate interactions even under rapid flow conditions (Show et al. 2010). However, a drop in biogas production followed by an increase in pH between days 102 and 107 likely indicates a transient disturbance in reactor performance such as microbial stress under high OLR (HRT=0.5 day). Overall, the reactor demonstrated notable robustness and flexibility, confirming that granular sludge is effective for treating high-rate AD of WHJ even at reduced HRT of 0.5. The ability of the UASB system to maintain process stability and treatment efficiency at HRT=0.5 days attracts further investigation, particularly examining microbial dynamics at different HRTs, granular integrity at long-term operation and a comprehensive carbon mass balance. Such integrated biological approaches would significantly advance our understanding of the fundamental processes governing high-rate anaerobic treatment systems and facilitate their optimization for aquatic biomass utilization.

Table 3. The UASB reactor performance at different HRTs.

Indicators	HRT= 2	HRT=1.5	HRT=1	HRT=0.5	Overall
SS (%)	-	55.3 ± 19.2	56.9 ± 14.1	38.3 ± 17.7	51.6 ± 18.6
VSS (%)	-	74.0 ± 18.2	74.9 ± 07.0	51.4 ± 25.1	67.8 ± 21.8
BPR (mL d^{-1})	201.1 ± 37.4	193.0 ± 74.4	511.4 ± 92.3	903.8 ± 307.2	413.7 ± 331.9
BY (L L^{-1})	1.0 ± 0.2	0.8 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.0 ± 0.3

SS: Suspended solids; VSS: Volatile suspended solids; BPR: Biogas production rate; BY: Biogas yield; L L^{-1}: Liter per liter substrate (L L-substrate^{-1}).

4. Conclusion

This study confirms the feasibility and resilience of UASB reactors inoculated with pre-aggregated AD granular sludge for high-rate AD of WHJ at short HRTs. Despite the high OLR (1.1 ± 0.1- 3.2 ± 0.1 g-TOC $L^{-1}d^{-1}$), findings from this study revealed that even at an HRT as low as 0.5, the UASB system maintained process stability and treatment efficiency. The reactor achieved consistent TOC removal ranging from 35.3% (at HRT = 0.5 days) to 82.1% (at HRT = 1.5 days), while SS and VSS removal reached up to 56.9 ± 14.1% and 74.9 ± 7.0%, respectively. Across all HRTs, the methane content surpassed 79%. In this study, HRT=1 gives a maximum SS and VSS removal of 56.9 ± 14.1% and 74.9 ± 7.0%, respectively while HRT=0.5 yields a maximum biogas production of 903.8 ± 307.2 mL d^{-1}. The use of pre-aggregated AD granular sludge effectively mitigated common issues such as biomass washout and reactor instability under short HRTs. However, it is important to develop new methods to facilitate high removal efficiency of SS at high OLR (HRT=0.5). These findings support the advancement of compact, efficient, and scalable anaerobic digestion technologies for biomass valorization and renewable energy generation.

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