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Posted Date: October 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8495281/v1

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Additional Declarations: No competing interests reported.

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Abstract

Water hyacinth is an invasive aquatic plant that poses significant environmental and water management challenges. Converting this abundant biomass into bioenergy through anaerobic digestion offers a sustainable solution; however, limited studies have evaluated its performance in liquid form under short hydraulic retention times (HRTs), particularly with different inoculum sources. This study examined energy production and nutrient recovery from water hyacinth juice through anaerobic digestion, comparing activated sludge and digested cow dung as inocula. Experiments were carried out in a lab-scale upflow anaerobic sludge blanket (UASB) reactor at HRTs of 6, 4, and 2 days under mesophilic conditions (37 °C) and near-neutral pH. Performance was evaluated by measuring biogas and methane production, solids content, nutrient levels, and chemical oxygen demand (COD). Activated sludge produced a higher biogas yield (0.278 L/g

VS) compared to digested cow dung (0.109 L/g VS), with COD reductions from approximately 3377 mg/L to 1195 mg/L and 1369 mg/L, respectively. Methane content was similar, at 73.4% and 72.0%. Nitrate levels declined, while phosphate levels increased in both systems. These results show that water hyacinth juice can be effectively treated in a UASB reactor at short HRTs, producing measurable biogas and nutrient changes, with activated sludge offering better performance.

Keywords: Anaerobic digestion, Activated sludge inoculum, COD removal efficiency, Cow dung inoculum, Hydraulic retention time, Waste-to-energy

1. Introduction

The worldwide energy need has augmented radically due to industrial growth and The population has risen worldwide over the last century. Fossil fuels (coal, oil, and natural gas) provide 85% of the world's energy needs (Daniel et al. 2023; Manigandan et al. 2023). However, the continued reliance on fossil fuels as the primary energy source has led to environmental degradation, global climate change, and health issues. Global energy consumption exceeds 542 quadrillion British thermal units and is projected to increase by approximately 50% by 2050. In response to these challenges, renewable energy sources have gained increasing attention (Arora and Shi 2016; Gbiete et al. 2024; Unpaprom et al. 2021). Renewable energy is defined as energy generated from naturally replenishing sources that are not depleted by continuous use. These sources include solar, wind, hydropower, geothermal energy, and biomass (Ellabban et al. 2014).

Biomass is one of the earliest energy sources utilized by humankind and has long served as a primary source of energy for more than half of the world's population (Kalak 2023). Biomass can be combusted directly to produce heat or used as a feedstock for producing various liquid and gaseous biofuels. It can be converted into useful energy through thermochemical and biochemical pathways, with common technologies including combustion, pyrolysis, gasification, and anaerobic digestion (Kumar et al. 2020; Shahzad et al. 2024; Yu et al. 2021).

Anaerobic digestion (AD) is a biological process that degrades organic matter by a consortium of microorganisms under oxygen-free conditions to produce biogas composed methane predominantly (CH_4) and carbon dioxide CO_2). AD is used as a management strategy to convert biomass into other usable materials (e.g., energy, fertilizer) (Hernández-Shek et al. 2016). In addition, AD has the advantage of reduced natural methane emissions from the

self-decomposition of biomass in landfills or other open environments, because methane's global warming potential is estimated to be 20 times that of carbon dioxide (Zheng et al. 2014).

Water hyacinth (Eichhornia crassipes) is a fast-growing, freshwater, lignocellulosic floating weed native to the Amazon basin that has since become one of the world's most invasive aquatic species (Koley et al. 2025; Nahar et al. 2024). Its rapid proliferation, capable of doubling biomass in just 6 to 18 days, leads to dense mats that disrupt aquatic ecosystems, increase water loss through enhanced evapotranspiration, and hinder navigation, irrigation, fishing, and recreational activities (Abie and Alemu 2025; Ingabire et al. 2023). In regions such as tropical and subtropical countries, including Ethiopia, water hyacinth is abundantly available and poses serious environmental and economic challenges, particularly in water bodies such as Lake Tana.

On the other hand, water hyacinth represents an attractive biomass resource due to its high moisture content, rapid growth rate, and substantial organic matter content, making it a promising candidate for bioenergy production (Barua and Kalamdhad 2018; Koley et al. 2024). While efforts have been made to eliminate water hyacinth in a variety of ways, including by using it to make biofuels, biofertilizers, animal feed, crafts, and paper, very few of these strategies are both practical, financially, and environmentally (Roopnarain et al. 2019).

Water hyacinth has the potential to be used as a feedstock for anaerobic digestion (AD); however, its direct utilization is limited by its recalcitrant lignocellulosic structure, which restricts hydrolysis and results in long digestion periods and low biogas yields (Bhatia et al. 2025; Getachew et al. 2025; Liu et al. 2021). In contrast, liquid anaerobic digestion (LAD) offers several advantages over solid-state AD, including shorter hydraulic retention time (HRT), lower mixing energy requirements, smaller reactor volume, and suitability for treating liquid wastes such as wastewater sludge and food-processing effluents (Liu et al. 2021). To overcome the

limitations associated with the solid structure of water hyacinth, this study employed crushing followed by compression to extract water hyacinth juice, which was used as the sole feedstock for the AD process.

The novelty of this study lies in the combined application of physical pretreatment and a comparative evaluation of different inoculum sources to enhance the anaerobic digestion of water hyacinth. In this research, mechanical crushing followed by compression was applied to water hyacinth to produce a liquid feedstock, thereby enhancing substrate solubilization and increasing the bioavailability of organic matter for microbial degradation. In addition, the effects of two different inoculum sources (anaerobic sludge and digested cow dung) on digestion performance were systematically evaluated in a UASB reactor. This approach enhanced biogas production at short hydraulic retention times while enabling effective nutrient recovery from the liquid effluent, demonstrating a sustainable pathway for energy and resource recovery from invasive aquatic biomass.

2. Materials and Methods

2.1. Substrate and inoculum preparation

Freshwater hyacinth samples were manually collected from Lake Tana, located in the northwestern part of Ethiopia (latitude 12.0266° N, longitude 37.3035° E), and thoroughly washed with clean water to remove sand, mud, and other adhering debris. The inoculum consisted of digested cow dung obtained from Bahir Dar (latitude 11.5936° N, longitude 37.3908° E) and anaerobic sludge sourced from Addis Ababa (latitude 9.02497° N, longitude 38.74689° E). The cleaned biomass was then size-reduced using a Jeffco grinder (CG03 model, India) to facilitate efficient juice extraction. The juice was manually extracted and

collected in a plastic jar for temporary storage. The juice was manually extracted and collected in a plastic jar for temporary storage. The average juice yield was 0.7 L/kg of fresh water hyacinth**.** To remove remaining solids and ensure a cleaner substrate, the raw water hyacinth juice was further filtered through a cloth. The extracted juice was stored in a chest freezer (BD-410) under cooled conditions (approximately 4 °C) to minimize microbial deterioration. The water hyacinth (WH) was characterized in terms of key physicochemical properties relevant to the experimental procedures, including pH (4.285 ± 0.352), moisture content (91.708 ± 2.650%), total solids (10.408 ± 2.650 g/L), total suspended solids (2.547 ± 0.791 g/L), soluble chemical oxygen demand (3377.857 mg/L), and volatile solids (4.176 ± 0.822 g/L). Figure 1 illustrates the raw water hyacinth, the crushing and compression steps, and the final extracted juice. The inoculums used for the anaerobic digestion experiments were digested cow dung collected from the Bahir Dar poultry farm and anaerobic sludge obtained from the Kaliti wastewater treatment plant in Addis Ababa, Ethiopia. Prior to use, the pH of both inocula was adjusted to 7.0 ± 0.5 using a 1 N potassium hydroxide (KOH, 85%) solution.



Figure 1: Water hyacinth and compressed juice

2.2 Experimental set up

The experiment was conducted using a continuous liquid-phase upflow anaerobic sludge blanket (UASB) reactor. The reactor was cylindrical, made of glass, and equipped with a gas–liquid–

solid separator, feed inlet, sludge sampling port, effluent outlet, and gas outlet. It had a total volume of 7 L and an effective working volume of 6.6 L, with an inner diameter of 32 cm and a height of 1.09 m. A mesh sieve installed at the bottom inlet prevented the entry of large particles, while a peristaltic pump (BT100-3J) was used to deliver the substrate at controlled flow rates. Prior to operation, the reactor was filled with inoculum to establish anaerobic conditions. After the start-up phase, water hyacinth juice (WHJ), previously stored under refrigerated conditions, was continuously fed into the reactor. The reactor temperature was maintained at 37 °C under mesophilic conditions using an electric heater (TH0023) and monitored with a thermocouple with an accuracy of ±1.5 °C. The system was operated at different hydraulic retention times (HRTs) to evaluate performance under varying loading conditions. For the sludge inoculum, HRTs of 6, 4, and 2 days were applied, while for the digested cow dung inoculum, HRTs of 6 and 3 days were used. Each HRT condition was maintained for a specific duration, with 44 days at 6 days HRT, 16 days at 4 days HRT, 21 days at 3 days HRT, and 6 days at 2 days HRT. The corresponding feed flow rates were determined based on the influent chemical oxygen demand (COD) and the desired organic loading rates (OLR). The corresponding organic loading rate (OLR) values were calculated from the influent COD and the hydraulic retention time. With an average influent COD of 3.378 kg/m³, the applied OLRs were 0.56, 0.84, 1.13, and 1.69 kg COD/m³·d for HRTs of 6, 4, 3, and 2 days, respectively. Performance data were collected after the system exhibited stable trends in key parameters. Biogas produced during the process was collected in gas-tight plastic bags, and the daily volume was recorded.

2.3 Characterization of influents and effluents

All physicochemical parameters were determined in accordance with standard procedures described by the American Public Health Association (Standard Methods for the Examination of

Water and Wastewater, 23rd ed. 2017). The moisture content (MC) of the biomass was determined by oven-drying at 105 °C until a constant weight was reached, followed by cooling in a desiccator, in accordance with APHA Method 2540 B. Total solids (TS) of the water hyacinth juice were measured similarly by drying a known volume in a pre-weighed dish at 105 °C, following APHA Method 2540 B. Volatile solids (VS) were determined by igniting the dried residue in a muffle furnace at 550 °C, and calculating the mass loss attributed to organic matter volatilization, according to APHA Method 2540 E. Total suspended solids (TSS) in both the substrate and effluent were measured by filtering samples through a 0.45 μm glass-fiber filter, followed by oven drying at 105 °C to a constant weight, as described in APHA Method 2540 D. Chemical oxygen demand (COD) was analyzed using the closed-reflux colorimetric method in accordance with APHA Method 5220 D. Prior to analysis, samples were filtered (0.45 μm), and the filtrate was digested at 150 °C using a DRB200 reactor and measured with a DR 3900 spectrophotometer. COD removal efficiency was used to evaluate digester performance. Soluble parameters (e.g., COD, nitrate, and phosphate) were analyzed using filtered samples, while total parameters (e.g., TS, VS, and TSS) were determined using unfiltered samples, as appropriate.

Table 1: Sample specifications

Sample	**Description**
C1	Substrate with digested cow dung inoculum
C2	Effluent with digested cow dung inoculum
C3	Substrate with sludge inoculum
C4	Effluent with sludge inoculum

2.4 Biogas yield

The biogas produced from the UASB reactor was quantified using a gas meter (model W–NK 0.5B, Japan), which recorded the gas flow rate twice daily. Biogas volumes were measured at ambient operating conditions and were not corrected to standard temperature and pressure (STP). The specific biogas yield was calculated based on volatile solids (VS) removed and expressed as the volume of biogas per unit VS removed. To determine the methane content, the raw biogas collected in gas bags was purified using an alkaline absorption method. Carbon dioxide and hydrogen sulfide were selectively removed by passing the gas through an absorption solution consisting of 85% NaOH (1 N) with thymolphthalein indicator. The biogas was drawn into the absorption solution using a vacuum pump, and the change in gas volume before and after absorption was measured. The observed volume difference corresponded to the methane fraction, as CO_2 and H_2S were completely absorbed by the sodium hydroxide solution, leaving methane and other trace gases unabsorbed.

3. Results and Discussion

3.1 Physico-chemical characteristics of substrate and effluent

The proximate analysis of substrate and effluent with (digested cow dung and wastewater treatment sludge) inoculum was characterized as shown in Table 1. Samples were taken at three-day intervals to analyze pH, total solids (TS), suspended solids (SS), volatile solids (VS), volatile suspended solids (VSS), soluble chemical oxygen demand (COD), nitrate, and phosphate.

Table 1: Result of substrate and effluent parameters for both inocula

Parameter	**Mean values of substrate**	**Effluent for the digested cow dung inoculum**	**Removal efficiency %**	**Effluent for sludge inoculum**	**Removal efficiency %**
MC	91.708±2.650	-	-	-	-
TS (g/L)	10.408±2.650	4.652±0.567	55.322	4.368±2.323	58.03

TSS (g/L)	2.547±0.791	1.435±0.522	43.660	1.185±0.574	53.34
VS (g/L)	4.176±0.822	1.278±0.552	69.391	0.907±0.463	78.31
COD (mg/L)	3377.857	1369.230±78.451	59.461	1195.429	64.44
Nitrate (mg/L)	61.361±0.251	52.340±2.780	14.700 loss	51.972±1.96	15.301 loss
Phosphate (mg/L)	2.49±0.256	5.49±0.941	120.482 gain	7.46±0.439	199.598 gain
pH	4.285±0.352	7.200+0.1926	-	6.826±0.226	-

Table 1 shows that the substrate moisture content was 91.708±2.65%. This suggests that water hyacinth biomass is a suitable feedstock for wet anaerobic digestion to produce biogas. So, in this research, liquid phase anaerobic digestion was done with 91.708±2.65% of moisture, which is within the range of liquid phase anaerobic digestion (Naji et al. 2024).

3.1.1 Total solid content (TS)

The cumulative mean value of the total solid content obtained for the substrate and effluent of anaerobic digestion was 10.408±2.650 g/L and 4.65±0.567 g/L for digested cow dung inoculum and 10.408±2.650 g/L and 4.368±2.323 g/L for municipal wastewater sludge inoculum, respectively. The corresponding total solid removal efficiencies of the anaerobic digestion for the two inocula were 55.322% and 58.031%, respectively. According to (Hudakorn and Sritrakul 2020) The total solids of water hyacinth juice is 10.76%, which is close to our result. On the other hand, by the work of (Soeprijanto Soeprijanto et al. 2020) the total solids of water hyacinth compressed juice is 5.65%, which is lower than our result, and the possible reason may be due to variation of sample type, crushing method, seasonal variation, the (water and soil) quality, and weather conditions of the environment. According to (Ji et al. 2014), the optimal total solids

content for a liquid-phase anaerobic bioreactor ranges from 5–15%, and the total solids values obtained in this study fall within this recommended range.

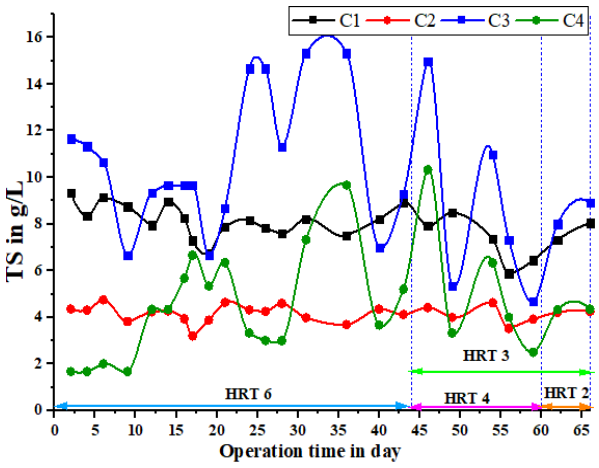


Figure 2: Total solids of substrate and effluent for both inocula

Figure 2 shows the daily variation of total solids (TS) in the substrate and effluent during the anaerobic digestion process in the UASB reactor. Changes in TS directly affect biogas production and overall reactor performance. The high TS conversion observed indicates that the reactor operated under stable conditions and that microbial activity was effective. The observed variations in TS may be due to the settling of suspended solids over time, the absence of a mixing mechanism, the characteristics of the upflow process, and the influence of microbial activity under the given operating conditions.

3.1.2 Total suspended solids (TSS)

Total suspended solids (TSS) are an important parameter used to assess particulate matter removal efficiency in the reactor and serve as an indirect indicator of substrate degradation during anaerobic digestion. The cumulative suspended solid content of water hyacinth juice substrate using digested cow dung inoculum and municipal waste water sludge inoculum was 2.547±0.245 g/l, and the respective effluent was 1.435±0.522 g/L, 1.185±0.574g/L respectively. The average reduction of total suspended solids for the respective inoculum was 43.660 % and

53.342 %, respectively. According to (Zahller et al. 2007), the reduction of total suspended solids was 55.2%, which is approximately similar to our result. On the other hand, the reduction of suspended solids of water hyacinth juice with the addition of biochar during biogas production was 85.7%, which is higher than our result. The conversion of total suspended solids in our experiment is lower than the above result, but this value may not reflect the actual rate of biogas yield because the converted solids may instead become volatile fatty acids, which may not further convert into methane gas due to methanogenic bacterial activity. The possible reasons for this problem may be the process condition, time of extraction, compression machine efficiency, and raw material type (Xu 2010). As shown in Figure 3, the total suspended solids of substrate and effluent have a slight variation with consecutive measurements; the source of the variation in the substrate may be the properties of the solids. In the case of effluent, the variation may be due to microorganism activity and digester performance.

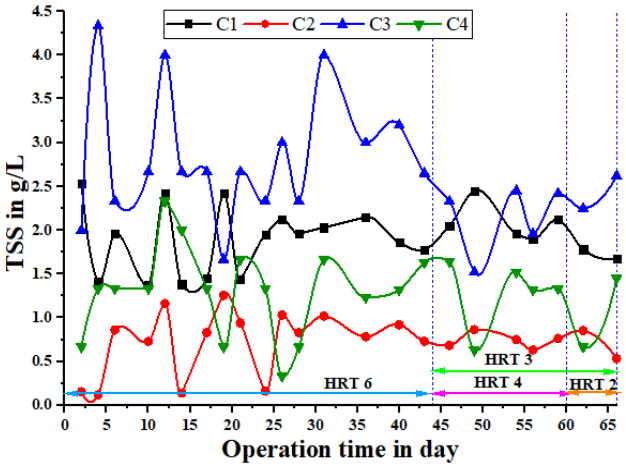


Figure 3: Total suspended solids of substrate and effluent for both inoculum

3.1.3 Total volatile solids (TVS)

In this study, the cumulative mean volatile solids for the substrate and effluent with digested cow dung inoculum were 4.176±0.822 g/L and 1.278±0.552 g/L, respectively, and the corresponding cumulative mean conversion was 69.391%. Whereas the volatile solids of the substrate were

reduced from 4.176±0.822 g/L to 0.9066±0.463 g/L using municipal wastewater sludge inoculum, with a cumulative mean conversion of 78.3%. This shows that the activity of microorganisms from municipal wastewater treatment sludge was relatively high compared to the digested cow dung inoculum. According to (Suthar et al. 2022), the reduction of total volatile solids of anaerobic digestion of water hyacinth was 77.4%. Similarly, (Mathew et al. 2015) reported a volatile solids conversion of 74.6 ± 1.4% during the anaerobic digestion of water hyacinth using digested cow dung as inoculum. So, our results are approximately similar to the above results. Despite the above findings, these results differ from those obtained in our experiments. This variation may be attributed to differences in feedstock type, raw material volatile solids concentration, weather conditions, crushing efficiency, and overall raw material quality. As shown in Figure 4, the consecutive values of total volatile solids (TVS) exhibit only slight variation, yet they have a significant impact on biogas production. Higher conversion of volatile solids is associated with increased biogas yield, highlighting the importance of substrate degradability for reactor performance.

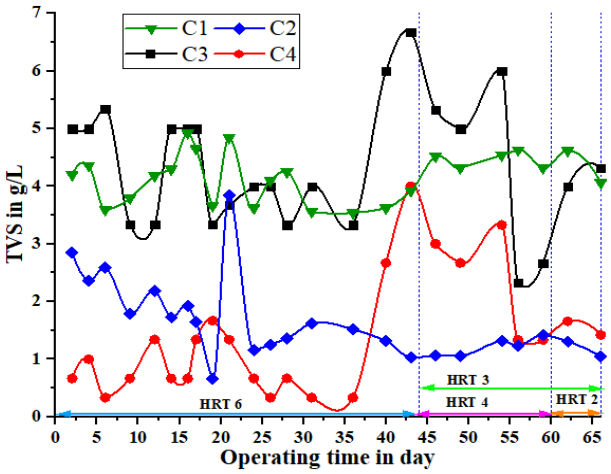


Figure 4: total volatile solids of substrate and effluent for both inocula

3.1.4 pH variation during anaerobic digestion

The pH values of the substrate and the corresponding effluent varied with the reactor's feeding rate and operational conditions. In this study, the substrate pH ranged from 3.5 to 5.6, while the effluent pH remained within 6.3 to 7.5. The near-neutral effluent pH is favorable for anaerobic digestion and biogas production, indicating generally stable reactor performance. The difference between the substrate and effluent pH reflects the system's buffering capacity and the progression of microbial degradation processes within the reactor. Effluent quality for use as an organic fertilizer depends on both pH and nutrient content, as pH influences soil fertility and plant growth. According to (Alexander et al. 2019), liquid fertilizers suitable for crop production typically have a pH between 6.3 and 7.6. The effluent pH observed in this study falls within this range, suggesting its suitability for agricultural application. Although some temporal fluctuations in pH were observed during operation, these variations are likely attributable to changes in feeding conditions and digestion dynamics rather than to process instability. Overall, the consistent near-neutral effluent pH suggests that the digester maintained stable operating conditions throughout the experiment.

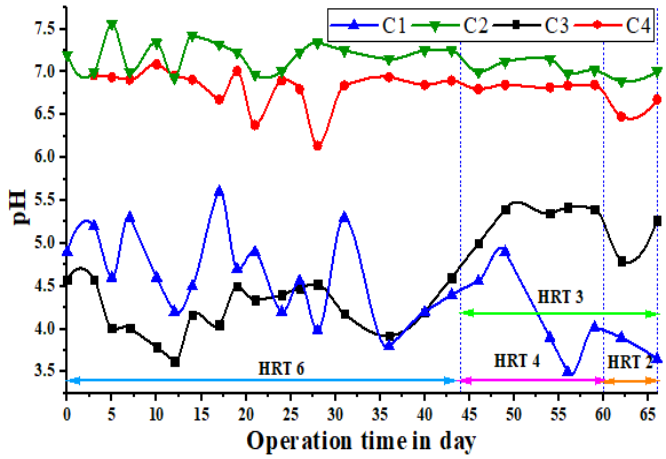


Figure 5: pH value of substrate and effluent for both inoculum

3.1.5 Chemical oxygen demand (COD)

Table 2: summary of COD removal efficiency with respect to inoculum and HRT

Digested cow dung inoculum			
HRT in the day	Mean values of COD for Substrate(mg/L)	Mean values of COD Effluent (mg/L)	Mean removal efficiency in %
6	3328 ±65.45	1265 ±42.25	61.99
3	3319.2 ±35.75	1473.25±36.21	55.62
Sludge inoculum			
6	3389.85±46.58	1165.42±45.32	65.56
4	3378.85±56.25	1186.56±35.24	64.89
2	3388.75±45.23	1285.45±54.35	62.07

In this study, the mean COD of floating water hyacinth juice inoculated with digested cow dung decreased from 3377.54 ± 115.03 mg/L to 1369.23 ± 240.95 mg/L, corresponding to a mean removal efficiency of 59.46%. When municipal wastewater sludge was used as inoculum, the mean COD of the substrate and effluent were 3377 ± 196.87 mg/L and 1195.88 ± 575.29 mg/L, respectively, yielding a higher mean removal efficiency of 64.44%. The maximum COD conversion achieved was 70.70% for digested cow dung inoculum and 87.74% for sludge inoculum, demonstrating a direct relationship between COD conversion and methane yield. COD was measured three times per week, and as shown in Figure 6, removal efficiency decreased with decreasing HRT from 6 to 4 to 2 days for sludge inoculum and from 6 to 3 days for digested cow dung inoculum, despite an increase in the absolute amount of COD removed at lower HRTs due to higher flow rates and organic loading. Substrate COD showed minimal variation, whereas effluent COD varied more noticeably, indicating changes in conversion rates; at an HRT of 6 days, COD conversion was initially high and gradually declined for both inocula. Compared with previous studies, (Trisakti et al. 2017) reported a higher COD removal efficiency (79.82 ± 6.03%) during anaerobic digestion of water hyacinth, while (Liu et al. 2020) observed a significant reduction in COD removal (25.17%) at a very short HRT of 0.6 days. These findings

are consistent with the present results, confirming that decreasing HRT reduces COD removal efficiency, even though biogas production increases due to higher organic loading.

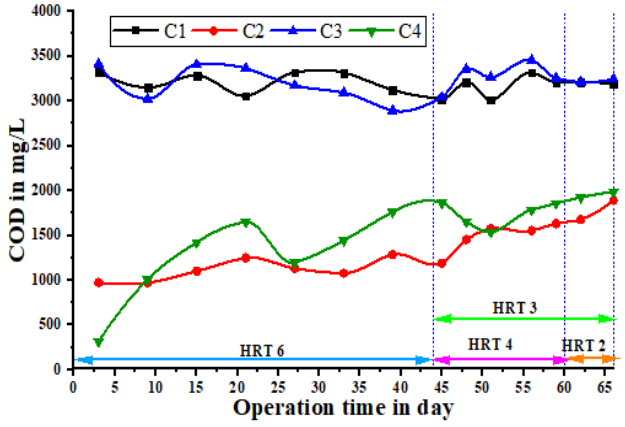


Figure 6: COD of substrate and effluent for both inocula

3.2 Biogas yield

The cumulative biogas production using digested cow dung inoculum was 43.44 L at an HRT of 6 days over 44 days of operation and 3.8 L at an HRT of 3 days over 21 days. The corresponding average biogas yield was 0.1094 L/g VS removed. In contrast, biogas production using municipal wastewater sludge inoculum was higher, reaching 62.6 L, 10.54 L, and 8.52 L at HRTs of 6, 4, and 2 days, with operating periods of 44, 16, and 6 days, respectively. The average biogas yield for sludge inoculum was 0.278 L/g VS removed. Overall, biogas production from water hyacinth juice (WHJ) using sludge inoculum was higher than that obtained with digested cow dung inoculum, and the digester reached stable operation more quickly with sludge inoculum. Previous studies reported biogas yields of 0.185 L/g VS removed with 71% methane (Simbayi et al. 2023) and 0.3145 L/g VS removed from water hyacinth (S. Soeprijanto et al. 2021). These values are comparable to the present results obtained using sludge inoculum, whereas yields with digested cow dung inoculum were lower, indicating that inoculum quality strongly influences microbial activity and biogas generation. As shown in Figure 7A, biogas production with sludge inoculum at an HRT of 6 days increased rapidly during the initial phase,

peaked around day 10, declined until day 15, and then stabilized up to day 44. With digested cow dung inoculum, biogas production increased rapidly until day 5 and then gradually declined throughout the digestion period. Changes in HRT further influenced production trends, with higher biogas output observed at shorter HRTs due to increased organic loading. The average daily biogas production was 0.697 L/day for digested cow dung inoculum and 1.119 L/day for sludge inoculum. Stable daily production indicates that the continuous anaerobic digestion process reached steady-state conditions at each operating flow rate. Consistent with (Vijin Prabhu et al. 2020), biogas yield increased with increasing organic loading rate, achieved by reducing HRT. Thus, decreasing HRT enhanced biogas production for both inocula, with the sludge inoculum consistently outperforming the digested cow dung inoculum.

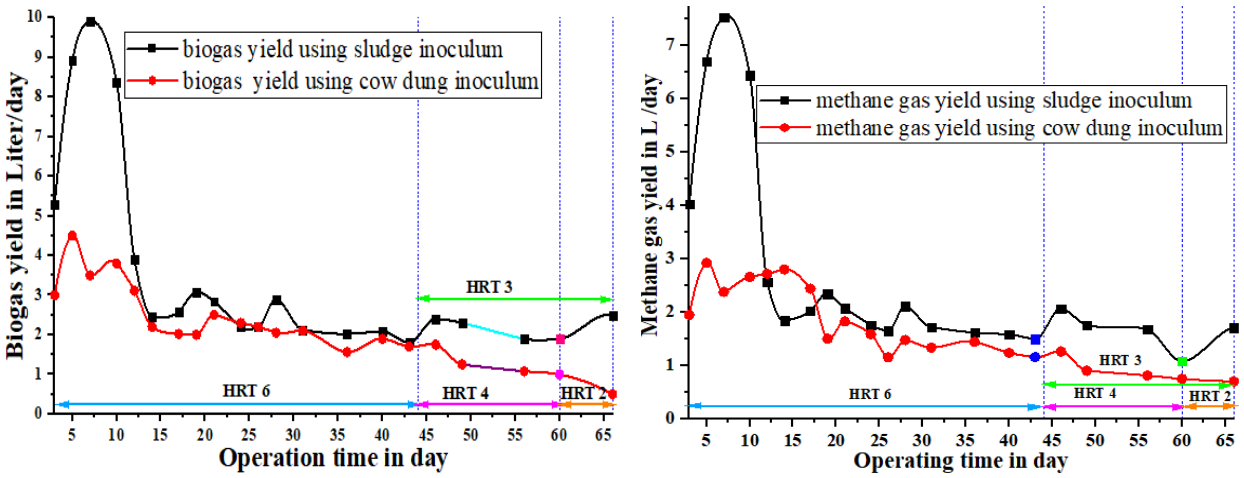


Figure 7: A) Production of biogas from floating WHJ B) Methane yield from floating water hyacinth juice

3.2.1 Biogas composition

Biogas produced from floating water hyacinth juice (WHJ) with digested cow dung inoculum contained 72% methane, with the remainder primarily carbon dioxide, yielding 0.079 L CH_4/ g VS removed. In contrast, biogas generated using sludge inoculum showed a higher cumulative methane content of 73.4%, with trace amounts of other gases, and a methane yield of

0.204 L CH_4/g VS removed. These results are consistent with previous studies. For example, (Soeprijanto Soeprijanto et al. 2020) reported a biogas composition of 70.57% CH_4 during anaerobic digestion of water hyacinth biomass. Additionally, (S. Soeprijanto et al. 2021) reported methane, carbon dioxide, and hydrogen sulfide contents of 71.23%, 25.79%, and 1.71%, respectively, for biogas produced from water hyacinth, banana peel, and water spinach wastes. The observed increase in methane concentration despite variations in total biogas yield, along with the relatively stable carbon dioxide fraction, aligns well with these reported findings. As shown in Figure 7B, methane production using sludge inoculum at an HRT of 6 days began early and increased rapidly until day 10, then declined sharply through day 15, followed by a gradual decrease through day 32, and subsequent stabilization through day 44. In contrast, methane production with digested cow dung inoculum also started early but increased rapidly only until day 5, after which it gradually declined throughout the digestion period. At shorter HRTs (4 and 2 days), methane production increased during the initial phase and then remained relatively stable. Overall, methane content tended to increase over the digestion period with only minor fluctuations. The observed nonlinear trends in methane yield can be attributed to variations in COD loading and removal efficiency during anaerobic digestion.

3.3 Effect of inoculum on anaerobic digestion

In this study, municipal wastewater sludge and digested cow dung were used as inocula in a UASB reactor. Overall, the sludge inoculum consistently outperformed digested cow dung in biogas yield and process stability. This superior performance is likely due to the higher microbial activity and better adaptation of the sludge inoculum to the water hyacinth juice, enabling more efficient degradation of organic matter. Although both inocula achieved comparable overall COD reductions, the higher maximum COD conversion observed with the sludge inoculum

suggests more complete substrate utilization and less accumulation of intermediate products, such as volatile fatty acids, compared to digested cow dung. These results underscore the critical role of inoculum quality in establishing an active microbial community and achieving stable anaerobic digestion. Previous studies also highlight the influence of inoculum type on biomethanation performance. For example, (Gaur and Suthar 2017) demonstrated that inoculum combinations, including digested cow dung and activated sludge, significantly affect methane production, while (Rajput and Sheikh 2019) reported superior digestion performance using digested manure compared to acclimatized and septic tank sludge. The present findings, however, show that municipal sludge can achieve higher biogas yields than digested cow dung, likely reflecting differences in reactor configuration, substrate characteristics, inoculum adaptation, and operating conditions.

3.4 Nutrient recovery

In our study, the mean nitrate (NO_3^-Concentrations in the substrate were 61.36 ± 2.52 mg/L and 51.97 ± 1.96 mg/L for digested cow dung and sludge inocula, respectively, while the corresponding effluent values were 52.38 ± 2.79 mg/L and 51.97 ± 1.96 mg/L. Similarly, the mean phosphate (PO_4^-) concentrations in the substrate were 2.49 ± 0.26 mg/L for both inoculums, increasing to 5.49 ± 0.71 mg/L and 7.46 ± 0.44 mg/L in the effluent for digested cow dung and sludge inoculums, respectively. The pressed water hyacinth juice studied here contained significant amounts of nitrate and phosphate, highlighting its potential as a nutrient-rich organic fertilizer. According to (Ji et al. 2014), the available nutrients obtained from anaerobic digestate during biogas production used for micro algae growth specially (Chlorella species) are 59.6 mg/L NO_3^- and 6.21mg/L PO_4. According to (Alexander et al. 2019), the application of liquid organic fertilizers provides an effective and sustainable approach for reusing agricultural wastes, while supporting plant growth and maintaining soil health (Allouzi et al.

2022; Satyanarayana et al. 2025). The results of the present study are consistent with these findings, indicating that the effluent has strong potential for use as an organic agricultural biofertilizer.

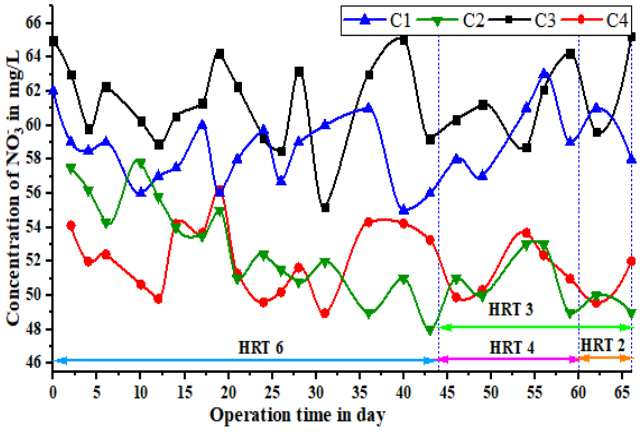


Figure 9: Nitrate concentration of substrate and effluent

As shown in Figure 9, the nitrate concentration in the effluent was lower than that of the influent substrate. This reduction can be attributed to denitrification during anaerobic digestion, in which denitrifying microorganisms utilize nitrate as an alternative electron acceptor under oxygen-limited conditions. As a result, nitrate is biologically reduced to gaseous nitrogen compounds, thereby decreasing nitrate concentration in the effluent. Overall, the mean nitrate concentration declined throughout the digestion process, with the lowest values observed in the effluent. This indicates that nitrate recovery from the anaerobic digestion of water hyacinth juice is limited; however, adjusting operational conditions may enable the effluent to be more effectively utilized for plant nutrient applications.

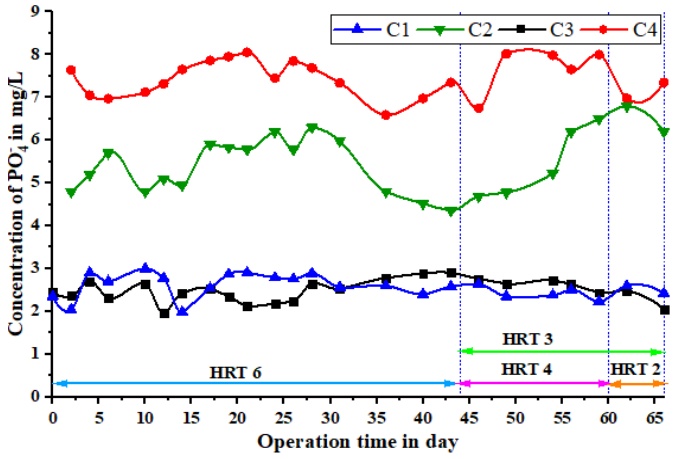


Figure 10: concentration of phosphate for substrate and effluent

As shown in Figure 10, the phosphate concentration increased from the substrate to the effluent during anaerobic digestion. Although the change between consecutive measurements was not large, phosphate recovery was achieved, with values of 5.49 ± 0.708 mg/L for digested cow dung inoculum and 7.46 ± 0.439 mg/L for sludge inoculum. These results indicated that the UASB reactor is effective for phosphate recovery. The increase in phosphate can be attributed to microbial activity and chemical interactions during anaerobic digestion. In this study, the sludge inoculum achieved higher phosphate recovery from water hyacinth juice than the digested cow dung inoculum.

4. Limitations of the study

Strategies to enhance microbial activity, such as the addition of stimulatory materials, including biochar and other substrates, were not investigated. As a result, while biogas and phosphate recovery were achieved, further optimization of reactor design and operational conditions is needed to improve overall efficiency and maximize substrate conversion.

5. Conclusion

Anaerobic digestion of water hyacinth juice in a UASB reactor using municipal wastewater sludge and digested cow dung effectively reduced TS, TSS, TVS, and COD. Sludge inoculum consistently outperformed digested cow dung in biogas yield and process stability, and shorter HRTs further enhanced biogas production due to higher organic loading and improved substrate availability. In addition to energy recovery, phosphate concentrations increased in the digestate, particularly with sludge inoculum, highlighting its potential as a phosphate-rich biofertilizer, while nitrate was largely lost through denitrification. These results demonstrate that water hyacinth juice is a promising substrate for simultaneous biogas and nutrient recovery, emphasizing the importance of inoculum selection and operational optimization. Future work should explore strategies to enhance microbial activity, biogas yield, and phosphate recovery, including inoculum adaptation, process optimization, substrate pre-treatment, and the use of additives such as biochar, which were not addressed in the present study.

CRediT authorship contribution statement

GWA: Writing-original draft, Writing-review & editing, Methodology, Investigation, Validation, Formal analysis. **NGH:** Writing-review & editing, Methodology, Resources, Validation, and Supervision. **HZA:** Writing-review & editing, Methodology, Investigation, Validation. **WMA:** Writing-review & editing, Methodology, Software, Validation

Declaration of interest statement

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

Ethics declaration

Authorization from an ethics board and informed consent were not necessary. Since this research did not involve any experiments on humans or animals requiring ethical approval.

Acknowledgments

We would also like to express our deep gratitude to Bahir Dar Institute of Technology for providing laboratory facilities and essential chemicals.

Data availability statement

Data will be made available on request. For requesting data, please write to the corresponding author.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or execution of this research.

Clinical trial number

Clinical trial number: not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Reference

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