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Enhanced biogas production through co-digestion of tapioca starch wastewater and duckweed in a continuous stirred tank reactor

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Abstract

This study investigates the biogas production performance of co-digesting duckweed with tapioca starch wastewater (TSW) in a laboratory-scale continuous stirred tank reactor (CSTR). Duckweed, with its rich nutrient composition, represents an underutilized biomass resource for renewable energy production in Thailand, where the tapioca starch industry constitutes a significant economic sector. The experimental setup utilized a 3-liter CSTR operated at mesophilic conditions (35°C) with a hydraulic retention time (HRT) of 28 days. Initial mono-digestion of TSW at an organic loading rate (OLR) of 0.31 gCOD/L-d resulted in a specific methane production of 0.28 NL-CH_4/g COD removed (NL = liter of gas at 273 K and 1 atm). Subsequent co-digestion with duckweed (1.0 g dry weight per liter of TSW) under identical operational conditions, enhanced methane production to 0.35 NL CH_4/g COD removed—corresponding to a 1.3-fold increase in specific methane production yield. These findings demonstrate that co-digestion of duckweed with TSW significantly enhances methane yield compared to mono-digestion of TSW, offering a promising approach for simultaneous wastewater treatment and renewable energy generation in Thailand's tapioca processing industry.

Keywords: Duckweed, Tapioca starch wastewater, Co-digestion, Methane production, CSTR

1. Introduction

Improving alternative energy is a global priority due to increasing energy demands and environmental concerns linked to fossil fuel consumption. Duckweed, a small floating aquatic plant commonly found in freshwater bodies worldwide—including those in Thailand—offers a promising option for sustainable energy production. Duckweeds are classified into five genera: *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis*, *Lemna tenera*, and *Wolffia globosa* [1]. Its efficient nutrient absorption enables it to use these nutrients in photosynthesis, generating carbohydrates and proteins that accumulate in its tissues. Comprising 34% carbohydrates, 24% protein, 6% fat, and other compounds, duckweed provides essential nutrients for microbial growth [2]. For instance, anaerobic microorganisms break down these organic components, supporting their growth and enhancing methane production. This process helps increase biogas production efficiency.

Duckweed has shown potential for biogas production through anaerobic digestion, either mono-digestion or co-digestion with other organic materials, such as animal manure, sludge, wastewater, other [3]. Studies have shown that the type of duckweed species, cultivation conditions, and mixing ratios have a significant impact on both the amount and quality of biogas produced. The carbon-to-nitrogen ratio (C/N ratio) is a crucial factor in co-digestion, with an optimal range of 15-35 [4], playing a key role in anaerobic digestion. If the C/N ratio is not optimal, it can cause problems like volatile fatty acid buildup or ammonia inhibition. Among aquatic plants studied for biogas production, water hyacinth demonstrated the highest potential, followed by water lettuce, duckweed, and water spinach [3]. Biogas production from aquatic plants must account for lignin content, which prolongs degradation and may require pretreatment. Duckweed is preferred for anaerobic digestion due to its quicker breakdown compared to other aquatic plants. [3]

Tapioca, also known as cassava, is a starch-rich root crop widely cultivated in tropical regions. Thailand, as the world's third-largest producer of cassava products in 2021 [5], relies heavily on the tapioca starch industry, which extracts starch from cassava roots. This industry plays a crucial role in Thailand’s economy and generates significant wastewater, which is commonly treated through anaerobic digestion to produce biogas. In 2018, Thailand’s cassava starch factories produced approximately 1,618 million cubic meters of biogas [6]. However, challenges such as insufficient biogas production to meet factory electricity demands and nutrient accumulation in treated wastewater persist.

Duckweed, especially *Spirodela polyrhiza*, is a promising resource for alternative energy production, particularly as a co-substrate in biogas production [7, 8]. However, previous studies have not investigated the application of duckweed for co-digestion with tapioca

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starch wastewater. Our research group proposes cultivating duckweed in tapioca starch treated wastewater [9] and utilizing its biomass as a co-substrate to enhance biogas production while reducing nutrient accumulation in wastewater. This strategy aligns with the Bio-Circular-Green (BCG) economic model [10], promoting the sustainable use of biomass and waste materials for energy production. This approach fosters industrial self-sufficiency, reduces costs, and enhances both economic and environmental sustainability.

This study investigates the co-digestion of duckweed and wastewater from tapioca starch factories in Khon Kaen Province, Thailand. A lab-scale continuous stirred tank reactor (CSTR), which offers advantages for treating semi-solid biomass [11], was employed to evaluate biogas production efficiency, wastewater treatment performance, chemical oxygen demand (COD) balance and specific methane production under operational conditions. The findings will contribute to optimizing biogas production processes and enhancing sustainability in Thailand's tapioca starch industry.

2. Materials and methods

2.1 Feedstock and inoculum

Tapioca starch wastewater (TSW) was obtained from a tapioca starch factory in Khon Kaen, Thailand, and stored at 4°C. Fresh duckweed (*Spirodela polyrhiza*) were collected from cultivation ponds in Khon Kaen University. It was cultivated in 220-liter outdoor plastic ponds containing soil and tap water under ambient Khon Kaen weather conditions without temperature or light control. The experiment was conducted in two phases: during the mono-digestion phase (Phase I, day 0–85), undiluted TSW was used. After day 85, the experiment transitioned to co-digestion phase (Phase II, day 86–275). For co-digestion, harvested fresh duckweed was homogenized using a blender and added to TSW at a concentration of 10 g fresh weight (equivalent to 1.0 g dry weight) per liter of TSW. Sodium bicarbonate (1,250 mg-$CaCO_3$/L) was added to optimize alkalinity from day 15 to 275. The addition of duckweed increased TCOD, TN, and TP concentrations by 10.3%, 18.0%, and 48.4%, respectively, compared to sole TSW. This modification adjusted the COD:N:P ratio from 300:4.4:0.8 in Phase I to 16.6 and 300:4.7:1.0 in Phase II. Table 1 presents the complete physicochemical characteristics of the feedstock during both experimental phases.

Table 1 Characteristics of TSW and TSW mixed with duckweed (TSW + duckweed)

Parameter	TSW	TSW + duckweed
pH	3.76	3.91
SS (mg/L)	1,660	2,620
VSS (mg/L)	1,650	2,480
TCOD (mg/l)	11,700	12,900
SCOD (mg/L)	8,150	10,000
TN (mg/L as N)	172	203
TP (mg/L as P)	31	46
COD: N: P ratio	300 : 4.4 : 0.8	300 : 4.7 : 1.0

The seed sludge was collected from a covered lagoon at the tapioca starch factory that supplied TSW. The lagoon operated at an organic loading rate (OLR) of 2 kg COD/m^3-d under ambient conditions in Khon Kaen, Thailand, where temperatures averaged 27°C (ranging from 17°C to 36°C). Prior to use, the seed sludge was preserved at 4°C for 6 months, then acclimated at 35°C for 3 days before reactor start-up. The CSTR was inoculated with 3 L of the seed sludge, which MLSS 2,250 mg/L, MLVSS 1,700 mg/L, and SVI 89 mL/gSS.

2.2 The CSTR set up and operation

The experimental setup was shown in Figure 1. Feedstock was stored at 4±2ºC in the substrate tank and pumped into the CSTR from the top of the reactor. The effluent was pumping out at the bottom of the reactor. The CSTR has a liquid volume of 3 L and continuously stirred at a speed of 45±3 rpm. The influent and effluent flow rates were each 110 mL/day, resulting in a hydraulic retention time (HRT) of 28 days. Since the sludge concentration in the reactor is equivalent to the solids concentration in the effluent, the sludge retention time (SRT) is equal to the hydraulic retention time (HRT). The temperature of CSTR was controlled at 35±2ºC by water jacket. Produced biogas was stored in a gas bag.

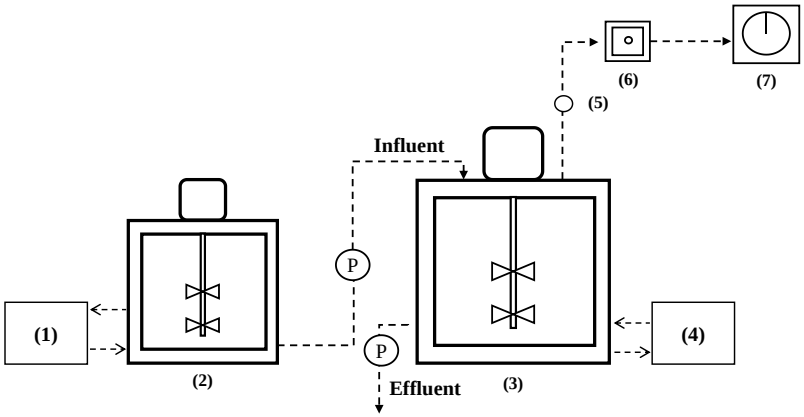

Figure 1 Experimental set-up diagram; (1) cooling water circulator controlled at 4±2°C, (2) substrate tank, (3) CSTR, (4) water bath controlled at 35±2°C, (5) gas sampling port, (6) gas bag, (7) gas meter.

2.3 Analysis methods

Biogas volume was measured daily using a wet test gas meter (Shinagawa W-NK-0.5BE). Gas composition was analyzed every three days via gas chromatography (Shimadzu GC-2014) equipped with a thermal conductivity detector (TCD). Influent and effluent samples were also analyzed at three-day intervals for multiple parameters, including pH, total COD (TCOD), soluble COD (SCOD), suspended solids (SS), volatile suspended solids (VSS), and volatile fatty acids (VFAs), all according to standard methods [12]. The suspended solids COD (SS-COD) was calculated as the difference between TCOD and SCOD.

2.4 COD balance

Based on the COD balance presented in Equation (1), the TCOD in the influent during the stable period was considered as 100% ($Influent_{COD}$). Methane (CH_4) production was converted to its COD equivalent using a stoichiometric factor, where 1 NL of CH_4 at standard temperature and pressure (STP; 273 K and 1 atm) is equivalent to 2.857 g COD. The unaccounted COD fraction, referred to as $Unknown_{COD}$, was determined by subtracting the sum of the daily effluent TCOD ($Effluent_{COD}$) and the COD equivalent of methane produced ($CH_{4\,COD}$) from the $Influent_{COD}$.

$$Influent_{COD} = Effluent_{COD} + CH_{4\,COD} + Unknown_{COD} \quad (1)$$

Where, $Influent_{COD}$ = influent TCOD (mg/L) × flow rate (L/d)
$Effluent_{COD}$ = effluent TCOD (mg/L) × flow rate (L/d)
$CH_{4\,COD}$ = CH_4 in biogas (%) × biogas volume (NL/d) × 2.857 (gCOD/L-CH_4)
$Unknown_{COD} = Influent_{COD} - Effluent_{COD} - CH_{4\,COD}$

3. Results and discussion

3.1 The CSTR performance

3.1.1 pH and VFAs

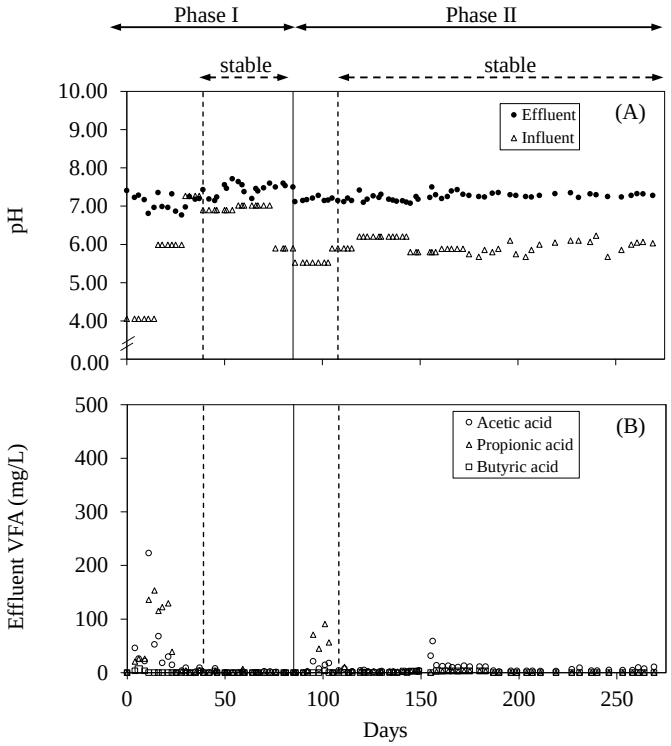

Figure 2 Time course of (A) influent and effluent pH, and (B) effluent VFA concentration

Figure 2 illustrates the changes in influent pH and effluent pH and effluent VFA over the experimental period**.** During the start-up in phase I, TSW was fed without the addition of alkalinity, the influent pH was at 4.0 during day 0 to day 14. As a result, the effluent pH decreased from 7.28 to 6.81 after day 11, leading to the accumulation of acetic acid and propionic acid, which reached peak concentrations of 50 mg/L and 150 mg/L, respectively. Since no alkalinity was added and the influent had a pH of 4, the effluent pH dropped below the recommended range for anaerobic digestion (6.8–7.8) [11]. On day 15, sodium bicarbonate was introduced at a concentration of 1,250 mg $CaCO_3$/L, raising the influent pH from 4.06 to an average of 6.66 between days 15 and 85. It took 24 days for the effluent pH to return to the recommended range. From days 39 to 85, the effluent pH stabilized at an average of 7.45 ± 0.16, while the concentrations of acetic acid, propionic acid, and butyric acid remained below 20 mg/L.

In Phase II (days 86–275), after nine days of feeding with co-digestion substrates, the concentrations of acetic acid, propionic acid, and butyric acid increased, reaching maximum levels of 15 mg/L, 91 mg/L, and 3.24 mg/L on day 101, respectively**.** However, VFA

concentrations declined within 14 days. After day 108, each VFA remained below 20 mg/L, and the average effluent pH stabilized at 7.26 ± 0.09, without notable fluctuations throughout the study.

Based on the effluent pH and VFA results from both mono-digestion and co-digestion, the CSTR exhibited strong performance in methanogenic fermentation, following acidogenic fermentation in the anaerobic digestion process. A previous study on acidogenic fermentation at 34 ± 1ºC, using different concentrations of feed TSW (influent TCOD 4,910 mg/L, with pH adjusted to 5.4), reported that acidogenic fermentation—occurring before methanogenesis—produced a maximum total VFA concentration of 2,650.19 mgHAc/L (0.45 gCOD-VFA/gCOD) at a substrate-to-microorganism (S/M) ratio of 4 gCOD-VFA/gVS. Acetic (40.9%), butyric (29.8%), and propionic (29.3%) acids were identified as the primary metabolites [13].

In contrast, for this study, the S/M ratio in Phase I was only 0.25 gCOD-VFA/gVSS, with a maximum total VFA concentration of 0.01 gCOD-VFA/gCOD. The composition of VFAs differed, with acetic (80.7%), propionic (9.2%), and butyric (8.1%) acids as dominant products.

The stable period was determined based on effluent pH and VFA stability, with a standard deviation lower than 10%. Consequently, the stable period for Phase I was observed from days 39 to 85, while for Phase II, stability was achieved after day 108 and continued until the end of the experiment on day 275.

3.1.2 COD and Solids

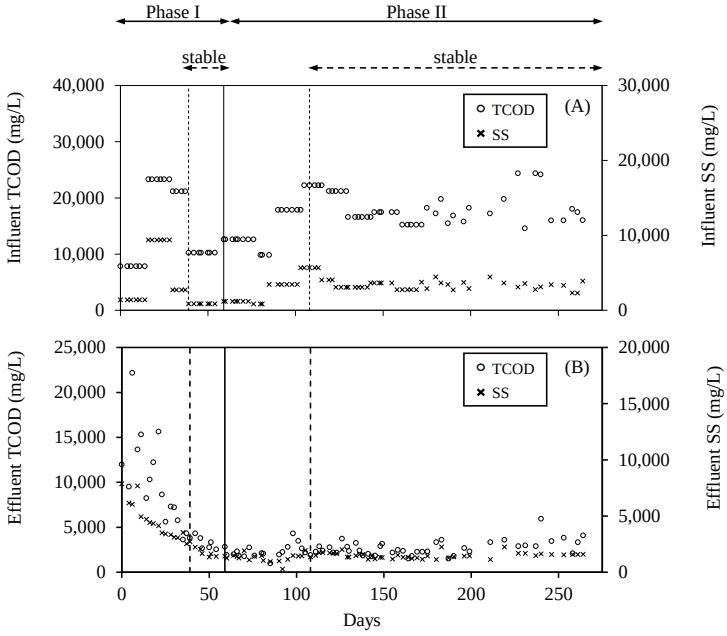

Figure 3 Time course of (A) influent TCOD and SS and (B) effluent TCOD and SS

Figure 3 illustrates the TCOD and SS concentrations in both the influent and effluent throughout the experiment. During the initial start-up period (days 0–14), the average SS concentration in the effluent was 7,800 mg/L, while in the influent, it averaged 1,800 mg/L. As a result, the effluent TCOD exceeded the influent TCOD. This occurrence was attributed to the sludge selection process, which led to the release of microbial-inactive sludge into the effluent. This step plays a crucial role in maintaining the quality and efficiency of the treatment system during start-up [14].

During this initial phase, these conditions hindered an accurate evaluation of TCOD removal efficiency, resulting in a calculated removal rate of -84% (data not shown). However, after alkalinity addition began on day 15, TCOD removal gradually improved and stabilized after day 39.

Table 2 COD SS VSS removal performance in stable periods of both 2 phases (N=19)

Parameter	Mono-digestion (Phase I)			Co-digestion (Phase II)		
	Influent	**Effluent**	**Removal (%)**	**Influent**	**Effluent**	**Removal (%)**
TCOD (mg/L)	8,500 ± 970	2,000 ± 700	76 ± 10	13,150 ± 1,810	2,100 ± 580	84 ± 5
SCOD (mg/L)	6,200 ± 310	140 ± 60	98 ± 1	7,100 ± 1,330	160 ± 120	98 ± 1
SS-COD (mg/L)	2,300 ± 860	1,850 ± 690	-1 ± 71	6,050 ± 1,930	1,910 ± 550	65 ± 16
SCOD/TCOD	0.73	0.07	-	0.54	0.08	-
SS-COD/TCOD	0.27	0.93	-	0.46	0.91	-
SS (mg/L)	1350 ± 230	1920 ± 680	-47 ± 56	4710 ± 1160	1860 ± 300	59 ± 9
VSS (mg/L)	1320 ± 240	1520 ± 510	-19 ± 44	4460 ± 1140	1610 ± 240	62 ± 9
VSS/SS	0.98 ± 0.01	0.79 ± 0.02	-	0.94 ± 0.05	0.87 ± 0.07	-

Table 2 summarizes the average and standard deviation of COD and solids during the stable period of phase I and phase II. From the influent TCOD and flow rate, the OLR calculated and averaged to 0.31±0.04 gCOD/L-d for phase I and 0.50±0.08 gCOD/L-d for phase II (the data was not summarise in Table 2). The average OLR increased by 1.6 times. However, comparison of the organic

removal in various COD fraction during stable period in phase I with II, SCOD removal were almost similar, the significant differences were with TCOD and SS-COD removal. This study indicates that the addition of duckweed for co-digestion was not evidentially effect to the removal efficiency of organic matter like SCOD, but improve TCOD removal in mesophilic CSTR.

Comparing the solids in both SS and VSS forms in influent, during phase II they were higher than in phase I around 3.4 - 3.5 times, however the effluent was average at almost same concentration. In phase I, the SS and VSS removal were averaged negative with high standard deviation show the fluctuations in the solids removal, coherent with the SS-COD removal results as discussed in aboved paragraph. In contrast in phase II, the influent had 2.5 times higher solids content than the effluent, but the higher in stability in solids removal and SS-COD removal compared to Phase I, refer to the standard deviations at ±9 for SS and VSS removal, while at ±16 for SS-COD removal.

The ratio of VSS/SS in the influent during phase I and phase II did not differ significantly. However, the VSS/SS ratio of the effluent increase 9% compared to the Phase I. The higher effluent VSS/SS ratio in phase II indicates increased organic content in solids, suggesting greater microbial biomass in the system, possibly resulting from changes in substrate composition or the extended system operation time [11].

3.1.3 Methane production

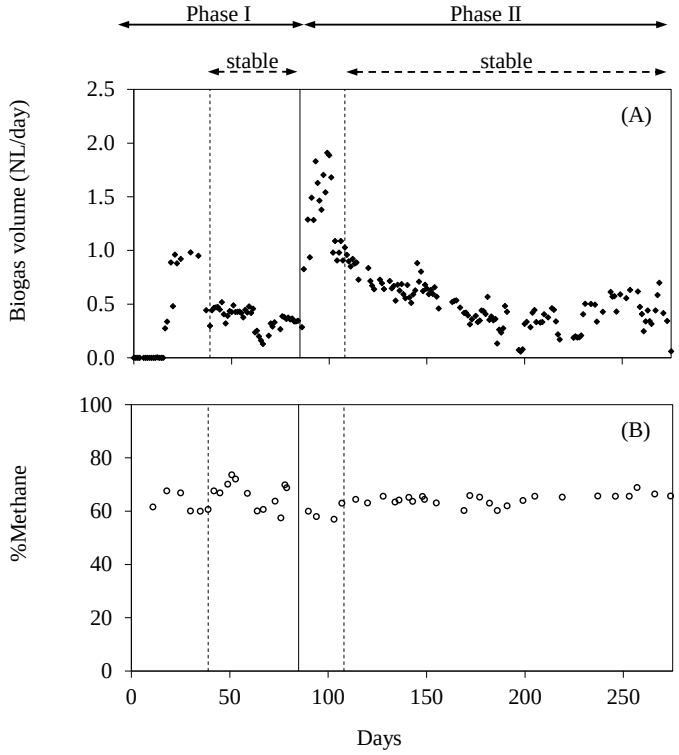

Figure 4 Time course of (A) biogas volume and (B) methane composition in biogas

During the Phase I, no biogas was detected after start-up. A small amount of biogas production began on day 19 of operation and increased linearly up to 1 NL/d as shown in Figure 4. Due to the instability of effluent pH, gas production was initially inconsistent. After day 39, the effluent pH and gas production stabilized, reaching a stable from day 39 to 85. The average biogas production during the stable period was 0.370±0.14 NL/d, with methane content comprising 65±6% of the biogas.

After introducing co-digestions into the system, biogas production sharply increased, reaching a peak of 1.911 NL/d on Day 99 of operation (14 days after shift to co-digestion). By Day 108, the system reached a stable, with biogas production at 0.474±0.22 NL/d and methane content of 64±2%.

A comparative study between Phase I and II revealed a significant difference in biogas production. The addition of duckweed as a co-substrate resulted in a 1.3-fold increase in biogas volume. The methane content in biogas is related to the substrate composition, including protein, carbohydrate, and lipids content [15]. Different substrate compositions yield varying biogas characteristics: high protein substrates produced biogas with 60% methane and 40% carbon dioxide, high carbohydrate content generated 50% methane and 50% carbon dioxide, while high lipids content resulted in 72% methane and 28% carbon dioxide. Algae feedstock demonstrated a biogas composition of 60% methane and 40% carbon dioxide [16].

Compositional analysis of duckweed (*Spirodela polyrhiza*) revealed a nutrient profile consisting of protein ranging from 25.6-34.5%, carbohydrates between 11.1-29.8%, and fats at 4.5% [17]. TSW analysis indicated protein at 1,625 mg/L, carbohydrates at 1,770 mg/L, and TCOD at 13,500 mg/L [18]. The macro nutrient composition of TSW and duckweed (*Spirodela polyrhiza*) are high similarity with high protein and carbohydrate with low fat content. The experimental results aligned with reference, showing a methane content in Phase I and II with almost similar at 65±6% and 64±2%, in order. These findings suggest that supplementing duckweed as a co-digestion substrate did not significantly alter the biogas composition from mono-digestion of TSW but notably increased the overall biogas volume.

3.2 COD balance and Specific methane production

Refer to Figure 5(A), in Phase I during the stable phase (day 39-85), the $CH_{4\ COD}$ accounted for 60.9% of the total, while the $effluent_{COD}$ decreased to 14.6%. Additionally, 24.5% of unknown $_{COD}$ was observed, which may have resulted from the accumulation of SS-COD. This can be evaluated by examining the ratio of influent SS-COD/TCOD, which was 0.27, indicating that 27% of TCOD consisted of SS-COD. During this stable period, SS-COD removal efficiency was notably low at -1 ± 71%, in stark contrast to the high SCOD removal efficiency of 98 ± 1%.

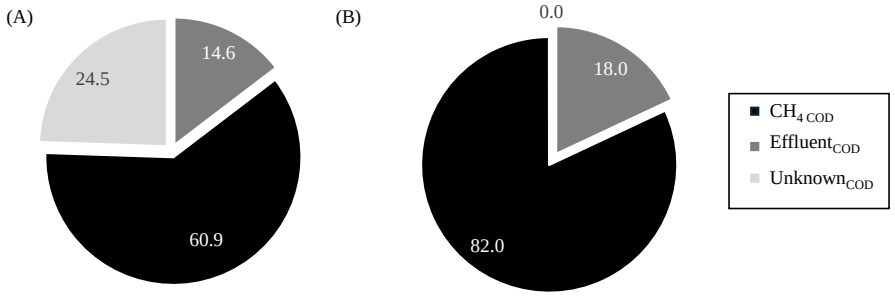

Figure 5 COD balance of (A) phase I and (B) phase II

As shown in Figure 5(B), during the stable period of Phase II (days 108–275), $CH_{4\ COD}$ accounted for 82.0%, representing a 34% increase—or approximately a 1.3-fold improvement—compared to the $CH_{4\ COD}$ percentage observed in Phase I.

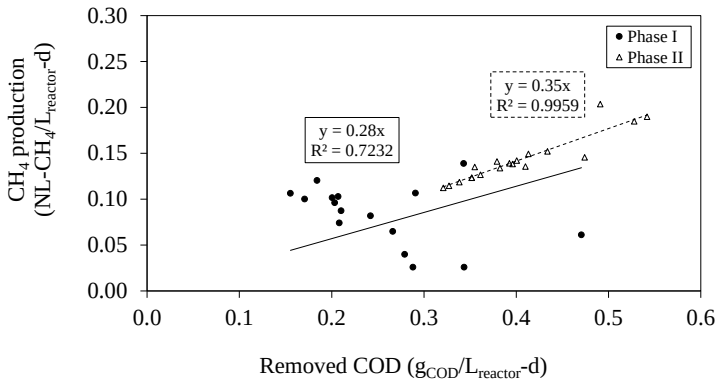

Figure 6 Relationship between removed COD and CH_4 production

To clarify the enhancement of methane production through co-digestion with duckweed, the relationship between the removed COD (influent COD – effluent COD) and methane production rate was analyzed, as shown in Figure 6. In Phase I, the specific methane production was 0.28 NL CH_4/g COD removed, with a coefficient of determination (R^2) of 0.732, indicating notable fluctuation in the data series. In contrast, Phase II showed a higher specific methane production of 0.35 NL CH_4/g COD removed, with an R^2 value of 0.996, reflecting excellent consistency and stability in the data. The specific methane production observed in Phase II was equivalent to the theoretical COD value of methane based on stoichiometric calculations. This high yield may be attributed to favorable experimental conditions for methanogenesis, the use of a readily biodegradable substrate, or the biodegradation of previously accumulated slowly degradable compounds. The 1.3-fold increase in specific methane production in Phase II compared to Phase I clearly demonstrates the positive effect of duckweed co-digestion.

The enhancement of CH_4 production resulting from the addition of duckweed to TSW in this experiment can be attributed to two main factors.

First, the improvement is likely related to the sludge characteristics within the reactor. Due to the limitation of having only one experimental unit, the study was conducted in series, starting with the mono-digestion of TSW in Phase I (days 0–85), followed by co-digestion in Phase II (days 86–275). This setup allowed for more than three hydraulic retention times (HRTs) during Phase I and over six HRTs in Phase II. As the sludge concentration in the continuous stirred tank reactor (CSTR) was equivalent to the effluent solids concentration, the sludge characteristics were evaluated based on the effluent suspended solids (SS) and volatile suspended solids (VSS), as shown in Table 2.

A comparison between the two phases revealed that while the effluent SS concentration in Phase I was 3.2% higher, the VSS concentration was 5.5% lower than in Phase II. The VSS/SS ratio in Phase II increased by 10% compared to Phase I, suggesting a greater proportion of organic (biologically active) material in the retained sludge. This increase in organic content, resulting from a longer operational period, likely improved the viability of the microbial community—especially methanogens—and contributed to higher methanogenic activity. Although methanogenic activity was not directly measured, the enhanced specific methane production and CH_4-COD percentage support this conclusion.

Second, the improvement can be attributed to better nutrient balance achieved through co-digestion. The COD:N:P ratio improved from 300:4.4:0.8 in Phase I to 300:4.7:1.0 in Phase II, approaching the recommended optimal ratio of 300:5:1 for stable long-term anaerobic digestion [11]. In terms of nitrogen, Phase I achieved 88% of the recommended value, while Phase II reached 94%, indicating a 6% increase in nitrogen availability. For phosphorus, Phase I provided only 80% of the recommended level, whereas Phase II achieved

the ideal ratio, representing a 20% improvement. These favorable changes in nutrient composition may have supported increased methanogenic microbial activity, thereby improving CH_4-COD conversion and overall system performance.

4. Conclusions

This study highlights the significant benefits of co-digesting duckweed (*Spirodela polyrhiza*) with tapioca starch wastewater (TSW) in a lab-scale continuously stirred tank reactor (CSTR) under mesophilic anaerobic conditions. Transitioning from mono-digestion to co-digestion led to a 1.3-fold increase in specific methane production, while maintaining a stable methane content of 64–65%. The addition of duckweed improved substrate composition by increasing total COD, total nitrogen, and total phosphorus by 10.3%, 18.0%, and 48.4%, respectively, and optimizing the COD:N:P ratio from 300:4.4:0.8 to 300:4.7:1—closer to the ideal 300:5:1 ratio for stable anaerobic digestion. Additionally, the extended operation during co-digestion enhanced microbial biomass retention, as reflected by an increase in the effluent VSS/SS ratio from 0.79 to 0.87. This co-digestion strategy not only improves biogas production and process stability but also aligns with the principles of Thailand's Bio-Circular-Green (BCG) economy by promoting a sustainable, closed-loop solution that converts agricultural waste into renewable energy while improving environmental performance.

5. Acknowledgements

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