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Research Article

Effects of the PSB-to-OW Ratio and Light Intensity on Biogas Composition during Phototrophic-Assisted Anaerobic Fermentation of Organic Waste

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Abstract

The integration of phototrophic processes into anaerobic fermentation offers a promising strategy for improving biogas quality; however, the combined effects of the photosynthetic bacteria-to-organic waste (PSB-to-OW) ratio and light intensity remain unclear. In this study, the photosynthetic bacteria (PSB)-assisted anaerobic fermentation of municipal organic waste under varying PSB-to-OW ratios and light intensities was evaluated. An optimal PSB-to-OW ratio of 1:2 resulted in significantly higher CH_4 concentrations and lower CO_2 and H_2S concentrations than the other tested ratios did ($p < 0.05$). At this ratio, moderate illumination (2000 lx) resulted in the highest CH4 concentration (68.2 ± 0.3%), compared with 1000 lx (61.5 ± 0.4%) and 4000 lx (63.1 ± 0.5%). Under illumination, the CO_2 and H_2S concentrations were lower than those under dark conditions, indicating a more favorable biogas composition. Kinetic analysis indicated that CO_2 and H_2S exhibited apparent first-order behavior under illumination, with the highest rates occurring at 2000 lx ($kH_2S = 0.093$ day^{-1}; $kCO_2 = 0.059$ day^{-1}), suggesting light-dependent transformation processes. Metagenomic analysis revealed a microbial community dominated by sulfide-oxidizing (*Chlorobium* sp.), photoheterotrophic (*Rhodopseudomonas* sp.), and syntrophic bacteria, supporting coupled carbon conversion and sulfur transformation. Overall, optimization of the PSB-to-OW ratio and light intensity improved the biogas composition by increasing the CH_4 concentration while reducing the CO_2 and H_2S concentrations. These findings demonstrate a coupled mechanism of light-driven sulfide oxidation and carbon conversion, providing a basis for integrating phototrophic processes into anaerobic systems for improved energy recovery and biogas purification.

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Introduction

Municipal organic waste constitutes a major biodegradable fraction of solid waste streams, particularly in developing countries, where it accounts for approximately 45–70% of total municipal waste generation (Pollution Control Department, 2022). Anaerobic digestion (AD) is widely used for the stabilization of such substrates and for the production of methane-rich biogas (Angelidaki et al., 2011; Appels et al., 2008). Since the methane (CH_4) content determines the calorific value of biogas, enhancing the CH_4 fraction and overall gas quality remains a key objective in AD process optimization.

However, anaerobic fermentation of food-derived substrates is often constrained by process instability, characterized by the accumulation of intermediate

products and reduced sulfur compounds (Chen et al., 2008; Wang et al., 2014). Sulfide (H_2S) is a well-known inhibitor of methanogenic activity, resulting in a reduced methane concentration (Vu et al., 2022). In addition, CO_2 dilutes the methane content and reduces the calorific value of biogas, highlighting the need for strategies that simultaneously increase the methane concentration and reduce undesirable gas components.

Anoxygenic phototrophic bacteria have attracted increasing attention because of their ability to utilize light energy under anaerobic conditions (Gunaseelan et al., 2024). Sulfur-oxidizing phototrophs, such as *Chlorobium* spp., utilize reduced sulfur compounds as electron donors and convert them primarily into elemental sulfur or other oxidized sulfur species, thereby decreasing H_2S accumulation (Frigaard and Dahl, 2008; Lens et al., 1998). Photoheterotrophic bacteria, including *Rhodopseudomonas* spp., can assimilate organic intermediates and contribute to carbon transformation under illuminated anaerobic conditions (Gunaseelan et al., 2024; Madigan and Jung, 2009). Previous studies have applied phototrophic microorganisms to wastewater treatment, biological desulfurization, and in situ biogas upgrading through the removal or transformation of CO_2 and H_2S (Alarcon et al., 2024; Egger et al., 2020; Lens et al., 1998). These studies have demonstrated the potential of phototrophic processes for improving biogas quality; however, they have primarily investigated individual functions, such as sulfide oxidation, carbon assimilation, or gas upgrading, under predefined operating conditions. Consequently, the interactions between key operational parameters governing phototrophic-assisted anaerobic fermentation remain insufficiently understood.

In particular, the combined effects of the PSB-to-OW ratio and light intensity on CH_4, CO_2, and H_2S proportions during phototrophic-assisted fermentation of food waste have not been systematically investigated. It remains unclear whether the PSB-to-OW ratio that provides the most favorable biogas composition also responds consistently to different light intensities. Furthermore, limited information is available regarding the relationship between temporal gas transformation behavior and microbial community composition under different operational conditions. Addressing these knowledge gaps is essential for distinguishing the effects of substrate availability from those of light-dependent microbial activity and for improving the understanding of phototrophic-assisted anaerobic fermentation for enhancing biogas quality.

Therefore, in this study, (i) the effect of the PSB-to-OW ratio on the biogas composition under illuminated and dark conditions and (ii) the effect of light intensity at the selected PSB-to-OW ratio were sequentially evaluated. Temporal changes in CH_4, CO_2, and H_2S proportions were further evaluated in conjunction with apparent gas-transformation kinetics and microbial community composition. This integrated approach extends the methods of previous studies by systematically evaluating the combined effects of the PSB-to-OW ratio and light intensity and by relating these operational factors to temporal gas-transformation behavior and the dominant phototrophic and anaerobic microorganisms within a single experimental framework.

Materials and methods

1) Substrate preparation and characterization

Organic waste (OW), primarily composed of fruit and vegetable residues, was collected from a local fresh market. Nonbiodegradable materials were manually removed, and the collected waste was immediately homogenized using a laboratory blender to ensure uniformity (Zhang et al., 2014). The homogenized samples were stored at −20 °C to prevent biological degradation prior to analysis and experimentation (APHA, 2017). Before use, the samples were thawed at room temperature.

The physicochemical characteristics of both the solid and liquid fractions were analyzed. The solid fraction was analyzed for total volatile solids (TVS), while the leachate was analyzed for pH, volatile fatty acids (VFA), ammonia, sulfide, and soluble chemical oxygen demand (sCOD) according to standard methods (APHA, 2017). These parameters reflect substrate characteristics, including organic strength and sulfur content, which influence biogas composition and sulfide transformation during anaerobic fermentation. The measured values are summarized in Table 1.

2) Enrichment of photosynthetic bacterial inoculum

Photosynthetic bacteria (PSB) inoculum was enriched from microbial sludge collected from a facultative pond at a piggery farm in Nakhon Pathom Province, Thailand. The sludge was introduced into 110-mL serum bottles, and the headspace was purged with high-purity nitrogen gas (99.99% N_2) to establish anaerobic conditions. The bottles were then sealed with rubber stoppers and aluminum caps.

Illumination was performed using a cool white fluorescent lamp (Philips TL-D 14 W/840 1SL/20, CRI = 82) at 100–200 lx for 16 days. The selected light intensity was based on our previous study, which demonstrated enhanced photosynthetic bacterial growth under low-light conditions, as evidenced by increased biomass and pigment production (Jirasansawat et al., 2024).

Table 1 Physicochemical characteristics of organic waste (n = 3)

Fraction	Parameter	This study	Previous studies	References
Solid (Organic waste)	TVS (g kg^{-1}, wet weight basis)	98.67 ±0.31	194.00 – 252.00	Bolzonella et al. (2005)
Liquid (Leachate)	pH	5.97 ± 1.23	6.60 ± 0.10	Bolzonella et al. (2005)
	Soluble COD (mg L^{-1})	64,000 ± 120.65	8,200 ± 800	Aromolaran et al. (2023)
	VFA (mg L^{-1})	450 ± 54.26	366.00 ± 52	Law et al. (2023)
	Ammonia (mg L^{-1})	1,960 ± 79	1,500 – 3,000	Yang et al. (2025)
	Sulfide (mg L^{-1})	747 ± 132	< 500	Vu et al. (2022)

Biomass development was monitored by measuring the optical density at 660 nm (OD_{660}). A biomass-specific calibration curve was constructed by correlating the OD_{660} with the VSS concentration according to standard methods. The resulting relationship was OD_{660} = 0.0041VSS + 0.0238 (R^2 = 0.9946), where VSS is expressed in mg L^{-1}. Biomass concentrations were estimated from the measured OD_{660} values using this calibration curve. After 16 days of enrichment under anaerobic conditions and continuous illumination (100–200 lx), the inoculum reached an OD_{660} of 2.5, corresponding to an estimated biomass concentration of approximately 604 mg VSS L^{-1}. A constant inoculum volume of 25 mL was subsequently used in all **the** batch anaerobic digestion experiments, providing an initial microbial loading of approximately 189 mg VSS L^{-1} in each reactor.

3) Microbial community analysis using 16S rRNA amplicon sequencing

The microbial community composition of the enriched PSB inoculum was analyzed using 16S rRNA amplicon sequencing. DNA extraction, library preparation, sequencing, and bioinformatics analysis were performed by a commercial sequencing service provider (AFTER LAB Co., Ltd., Thailand). In accordance with the service protocol, DNA quality was assessed prior to library preparation. The V3–V4 regions of the 16S rRNA gene were amplified using the universal primers 341F and 805R with 2× KAPA HotStart ReadyMix. PCR products were purified using AMPure XP beads (Illumina) and indexed with Nextera XT index primers (Illumina). Sequencing was performed on an Illumina MiSeq platform (2 × 250 bp paired-end reads), and the raw sequence data were processed using the QIIME2 pipeline (version 2019.10), including quality filtering, denoising, and taxonomic assignment (Bolyen et al., 2019). The resulting taxonomic profiles were used to determine the relative abundance of the dominant microbial groups in the enriched consortia.

4) Batch experimental study

4.1) Batch I: Optimization of the PSB-to-OW ratio

Batch experiments were conducted to evaluate the effect of the PSB-to-OW ratio on the composition of the biogas (CH_4, CO_2, and H_2S). The PSB-to-OW ratios were set at 1:1, 1:2, 1:4, and 1:8 (w/w, based on solids). In all the experiments, the PSB inoculum was kept constant, while the OW loading was varied to evaluate the effect of substrate availability. All assays were performed in 110-mL serum bottles, with a working volume of 80 mL and a headspace of 30 mL. The headspace was purged with high-purity nitrogen gas (99.99% N_2) to establish anaerobic conditions, and the bottles were sealed with butyl rubber septa and aluminum crimp caps. The experiments were conducted under both illuminated and dark conditions. Under illuminated conditions, the bottles were exposed to a cool white fluorescent lamp (Philips TL-D 14 W/840, CRI = 82) at a light intensity of 170 ± 29 lx. In the dark, the bottles were completely wrapped with aluminum foil to prevent light exposure and served as dark controls. All the bottles were incubated at 31 ± 2 °C for 15 days. Biogas composition was monitored periodically. Gas samples (300 µL) were withdrawn from the headspace using gas-tight syringes, and CH_4, CO_2, and H_2S concentrations were determined by gas chromatography as described in the following section.

4.2) Batch II: Effect of light intensity

Batch experiments were conducted to evaluate the effects of light intensity on biogas composition and gas transformation behavior under phototrophic-assisted anaerobic conditions. The experiments were performed at the optimal PSB-to-OW ratio (1:2) identified in Batch I, while all other operational conditions were kept constant. All assays were carried out in 110-mL serum bottles with the same configuration as described in Batch I. The headspace was purged with high-purity nitrogen gas (99.99% N_2), and the bottles were sealed with butyl rubber septa and aluminum crimp caps. The incubation temperature was continuously monitored throughout the experimental period for all treatments, and the average temperature was maintained at 31 ± 2 °C, regardless of the applied light intensity. Two experimental conditions were evaluated: dark and illuminated conditions. The dark condition served as the control, with the serum bottles completely wrapped in aluminum foil to exclude light. Under illuminated conditions, the bottles were exposed to a cool white fluorescent lamp

(Philips TL-D 14 W/840, CRI = 82). Light intensities of 1000, 2000, and 4000 lx were established by adjusting the distance between the light source and the serum bottles and were verified using a calibrated lux meter.

The experiments were conducted over 20 days, and gas samples were collected on days 5, 10, 15, and 20 to evaluate temporal changes in biogas composition. Gas samples (300 μL) were withdrawn from the headspace and analyzed for CH_4, CO_2, and H_2S concentrations as described in the following section. The temporal profiles of CO_2 and H_2S were analyzed using kinetic modeling to evaluate gas transformation behavior under different light intensities. The kinetic parameters were estimated by fitting the experimental data to the selected kinetic models.

5) Biogas composition analysis

The biogas volume was measured using a gas-tight syringe. Biogas composition (CH_4, CO_2, and H_2S) was analyzed by gas chromatography (GC; Agilent 6890, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and a HayeSep Q 80/100 column, using helium as the carrier gas. For each GC analysis, a 300-μL headspace gas sample was withdrawn from each bottle using a gas-tight syringe and directly injected into the gas chromatograph (Yamada et al., 2006).

6) Statistical analysis

All experiments were conducted in triplicate, and the results are presented as the mean ± standard deviation (SD). Statistical analyses were performed to evaluate differences in biogas concentrations (CH_4, CO_2, and H_2S) under different experimental conditions. For Batch I, independent two-tailed t tests ($\alpha = 0.05$) were used to compare the biogas concentration between illuminated and dark conditions at each PSB-to-OW ratio (1:1, 1:2, 1:4, and 1:8). For Batch II, pairwise independent t tests were performed to compare each illuminated condition (1000, 2000, and 4000 lx) with the dark control. In addition, pairwise comparisons among light intensities were conducted to identify the optimal illumination level. Differences were considered to be statistically significant at $p < 0.05$. For kinetic analysis, rate constants (k) obtained from model fitting were compared across different light intensities to evaluate the effect of illumination on gas transformation rates. For substrate characterization, the measured values were qualitatively compared with those reported in the literature to assess their relative magnitude compared with that of typical organic waste substrates. All the statistical analyses were performed using Microsoft Excel 365.

Results and discussion

1) Characteristics of organic waste

The physicochemical characteristics of the organic waste are summarized in Table 1. The analyzed parameters included the TVS in the solid fraction and the pH, sCOD, VFA, ammonia, and sulfide in the liquid fraction (leachate). Literature ranges are provided for comparison with those of reported values for organic waste substrates in anaerobic digestion systems.

As summarized in Table 1, the solid fraction exhibited a lower TVS content than that previously reported for food waste, suggesting a relatively lower biodegradable organic fraction in the substrate (Bolzonella et al., 2005). TVS represents the biodegradable organic fraction available for microbial degradation during anaerobic digestion; therefore, higher TVS contents generally indicate a greater availability of biodegradable organic matter, which may support higher CH_4 concentrations in biogas under suitable operating conditions (Appels et al., 2008).

Compared with previous reports, the leachate exhibited a slightly acidic pH, a higher VFA concentration, and a substantially higher sCOD concentration, suggesting that acidogenic reactions had progressed prior to the fermentation experiment and led to the accumulation of dissolved oxidizable organic matter that was available for subsequent anaerobic conversion (Aromolaran et al., 2023; Law et al., 2023).

The ammonia concentration was within the typical range reported for anaerobic digestion systems, suggesting sufficient nitrogen availability to support microbial growth without apparent inhibitory effects (Yang et al., 2025). In contrast, the total dissolved sulfide concentration exceeded that reported in previous studies (Lens et al., 1998; Vu et al., 2022). However, sulfide toxicity cannot be inferred from the total dissolved sulfide concentration alone because it is influenced by pH, free H_2S concentration, temperature, and methanogen acclimation (Vu et al., 2022). Considering the slightly acidic pH of the leachate, the measured sulfide concentration should therefore be interpreted with caution when evaluating its inhibitory potential.

Overall, the organic waste substrate was characterized by high organic strength (TVS and sCOD) and elevated sulfide content, suggesting the potential for favorable biogas composition under appropriate operating conditions and a significant risk of sulfide-related inhibition. These characteristics highlight the importance of process conditions that simultaneously increase carbon conversion and regulate sulfur compounds. Therefore, the interaction between substrate availability and microbial activity, particularly the PSB-to-OW ratio, may influence system performance, as further evaluated in the section on the effect of the PSB-to-OW ratio on the biogas composition.

2) Metagenomic analysis

The microbial community structure of the enriched inoculum is presented in Figure 1, which shows a community dominated by phototrophic and filamentous anaerobic bacteria. The dominant taxa included *Chlorobium* sp. (57.25%) and *Rhodopseudomonas* sp. (25.07%), followed by filamentous anaerobic bacteria such as *Pelolinea* sp. (11.45%) and *Leptolinea* sp. (5.19%). Minor populations included *Ectothiorhodospira* sp. (0.57%), indicating that the enriched inoculum consisted of a mixed phototrophic microbial consortium.

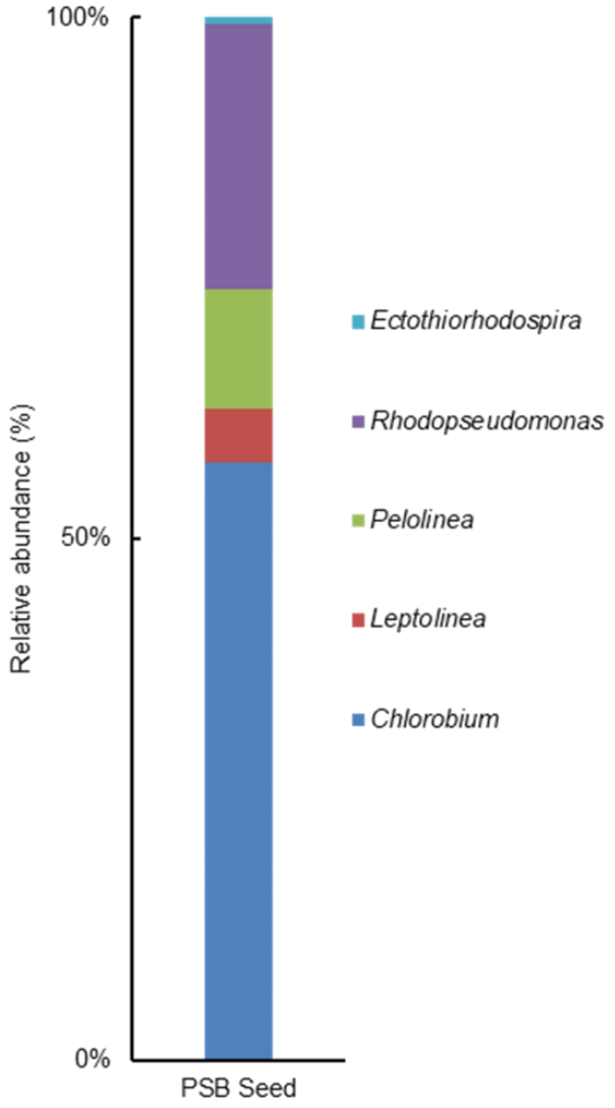


Figure 1 Microbial community composition of the PSB inoculum based on relative abundance.

The dominance of *Chlorobium* sp., a green sulfur bacterium (GSB), suggests the potential for sulfide oxidation under illuminated anaerobic conditions. These bacteria utilize H_2S as an electron donor during anoxygenic photosynthesis, converting it primarily into elemental sulfur and, under certain conditions, into more oxidized sulfur species, thereby contributing to sulfide transformation in the system (Frigaard and Dahl, 2008; Lens et al., 1998). This metabolic capability is particularly relevant in the present study, as the substrate contained elevated sulfide levels (747 ± 132 mg L^{-1}; Table 1), which have been reported to inhibit methanogenic activity if not effectively controlled.

The presence of *Rhodopseudomonas* sp., a purple nonsulfur bacterium (PNSB), suggests active photo-heterotrophic metabolism, through which organic intermediates such as VFAs are assimilated and converted into intermediates that can be further utilized by methanogens. Under illuminated conditions, these bacteria contribute to carbon conversion and organic carbon assimilation, thereby indirectly supporting methane formation and potentially reducing CO_2 accumulation (Gunaseelan et al., 2024; Madigan and Jung, 2009). Filamentous bacteria such as *Pelolinea* sp. and *Leptolinea* sp. are not phototrophic but play important roles in the degradation of complex organic matter and syntrophic interactions. These organisms are involved in the breakdown of macromolecular substrates into smaller intermediates, which are subsequently utilized by fermentative bacteria and methanogens. This process supports the utilization of the organic fraction of the substrate, as reflected by its high TVS and sCOD values, as described in the section on the characteristics of organic waste.

Overall, the microbial community indicates a synergistic system integrating sulfide oxidation (*Chlorobium* sp.), photoheterotrophic carbon conversion (*Rhodopseudomonas* sp.), and syntrophic degradation (*Pelolinea* sp. and *Leptolinea* sp.). These interactions may contribute to the simultaneous regulation of sulfur compounds and carbon conversion under illuminated conditions, providing a plausible explanation for the observed increase in CH_4 concentration and the reduction in CO_2 and H_2S concentrations described in the following sections.

3) Effect of the PSB-to-OW ratio on the biogas composition

The effects of the PSB-to-OW ratio on the composition of biogas (CH_4, CO_2, and H_2S) under illuminated and dark conditions are presented in Figure 2. At all the tested ratios (1:1, 1:2, 1:4, and 1:8), illuminated conditions consistently resulted in higher CH_4 concentrations and lower CO_2 and H_2S concentrations than dark conditions did. Independent two-tailed t tests confirmed that the differences in CH_4, CO_2, and H_2S concentrations were statistically significant at each ratio ($p < 0.05$).

Among the tested conditions, the PSB-to-OW ratio of 1:2 resulted in the highest CH_4 concentration under illumination, suggesting the most favorable balance between substrate availability and microbial activity. At this ratio, the CH_4 concentration was significantly greater than those at 1:1 and 1:4–1:8 ($p < 0.05$), which may reflect substrate limitation at lower ratios and substrate

overloading at higher ratios. In addition, CO_2 and H_2S concentrations were simultaneously reduced.

The observed improvement at the PSB-to-OW ratio of 1:2 may be associated with the interaction between substrate characteristics and microbial community structure as described in the previous section. The high organic content of the substrate (TVS 98.67 ± 0.31 g kg^{-1}; sCOD 64,000 ± 120.65 mg L^{-1}) provides abundant biodegradable carbon, which may support efficient microbial carbon conversion and reduce the accumulation of intermediate compounds under appropriate operating conditions.

At a lower substrate loading (1:1), the limited availability of organic matter may limit methane enrichment despite adequate microbial activity. In contrast, at higher substrate loadings (1:4 and 1:8), the excessive organic load may have promoted the accumulation of intermediate products (e.g., VFAs), which may have inhibited methanogenesis and resulted in increased CO_2 and H_2S concentrations. Similar trends have been reported in anaerobic digestion systems, where imbalanced PSB-to-OW ratios are associated with process instability and reduced CH_4 content (Appels et al., 2008; Zhang et al., 2014).

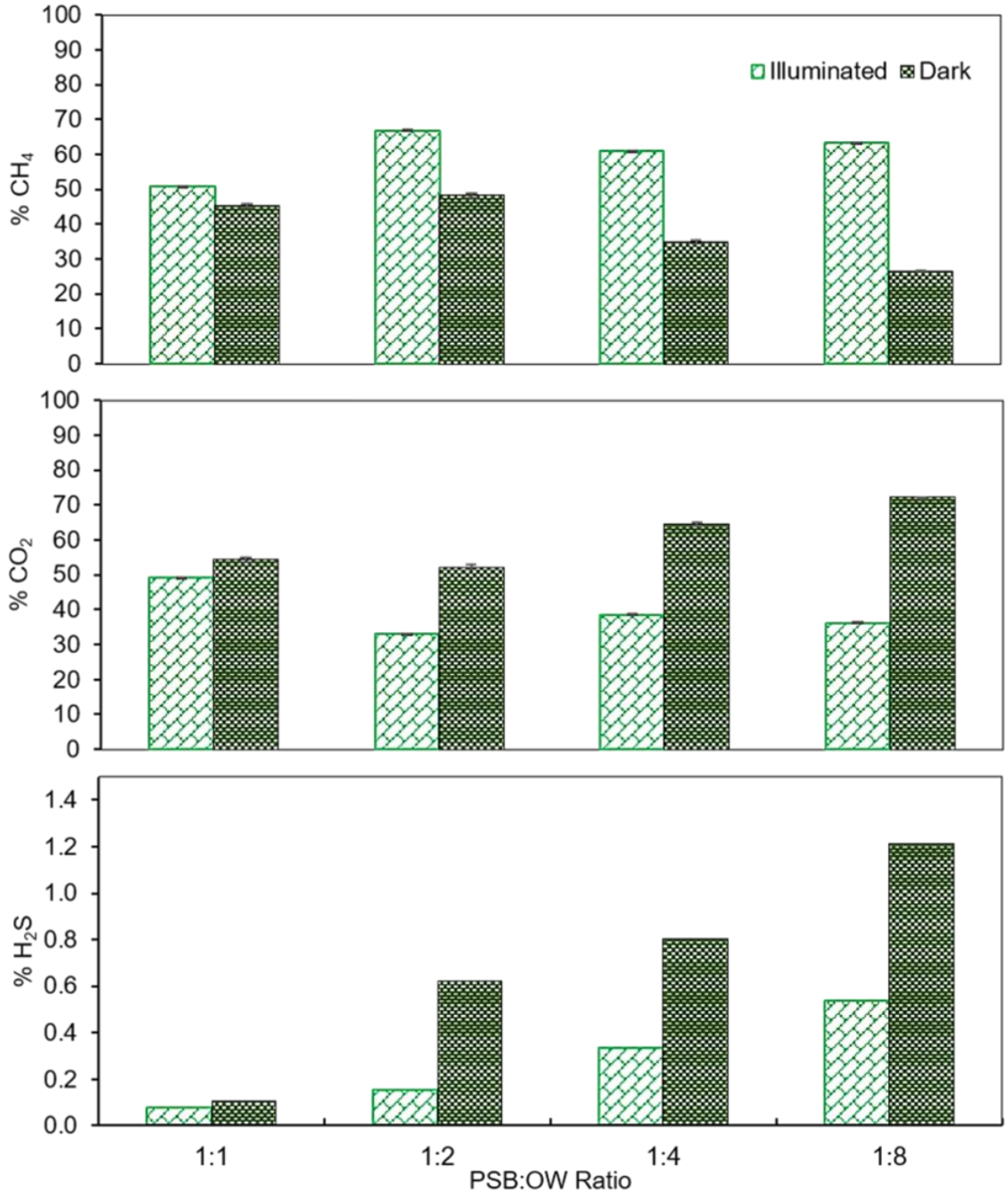


Figure 2 Effects of the PSB-to-OW ratio on the composition of biogas (CH_4, CO_2, and H_2S) under illuminated and dark conditions.

Under illuminated conditions, the increase in CH_4 concentration at the optimal ratio (1:2) was accompanied by a significant decrease in CO_2 and H_2S, suggesting enhanced carbon conversion and sulfur transformation. This behavior is consistent with the metabolic activity of the dominant phototrophic bacteria identified in the previous section. Sulfide-oxidizing bacteria such as *Chlorobium* sp. utilize H_2S as an electron donor during anoxygenic photosynthesis, contributing to sulfide transformation and potentially reducing sulfide accumulation (Frigaard and Dahl, 2008; Lens et al., 1998). Moreover, photoheterotrophic bacteria such as *Rhodopseudomonas* sp. contribute to the assimilation of organic intermediates (e.g., VFAs), promoting their conversion into metabolic intermediates available for methanogens while potentially reducing CO_2 accumulation (Madigan and Jung, 2009; Yamada et al., 2006). Filamentous anaerobic bacteria such as *Pelolinea* sp. and *Leptolinea* sp. further support system performance by degrading complex organic matter and enabling syntrophic interactions, facilitating the utilization of the organic fraction of the substrate, as reflected by its high TVS and sCOD values (Yamada et al., 2006). These synergistic interactions may have contributed to the observed improvement in the methane concentration and biogas quality at the optimal PSB-to-OW ratio (1:2) under illumination.

In contrast, under dark conditions, the CH_4 concentration remained lower across all ratios, while CO_2 and H_2S accumulated, suggesting less favorable carbon conversion and the absence of light-driven phototrophic activity. The elevated H_2S levels are consistent with the elevated sulfide concentration of the substrate (Table 1), suggesting that sulfide transformation by phototrophic microorganisms was limited in the absence of illumination. Similar accumulation behavior has also been reported under imbalanced or nonoptimized conditions in anaerobic digestion systems Appels et al., 2008; Zhang et al., 2014).

Overall, the results indicate that the biogas concentration is governed by the interplay between substrate loading and microbial activity. The optimal PSB-to-OW ratio (1:2) provided a favorable balance between substrate availability and microbial conversion capacity, thereby promoting efficient carbon conversion. Under illuminated conditions, phototrophic processes were associated with higher CH_4 concentrations and lower CO_2 and H_2S concentrations, which is consistent with enhanced carbon conversion and sulfur transformation. These findings provide a basis for evaluating the effects of light intensity at the optimal PSB-to-OW ratio, as discussed in the following section.

4) Effect of light intensity on biogas composition and reaction kinetics

The effect of light intensity on biogas composition was evaluated at the selected PSB-to-OW ratio of 1:2 as described the previous section. The temporal profiles of CH_4, CO_2, and H_2S under the dark control and illuminated conditions are presented in Figure 3a and b, respectively. Compared with the dark control (Figure 3a), illumination (Figure 3b) significantly increased the CH_4 concentration and decreased the CO_2 and H_2S concentrations ($p < 0.05$). Among the illuminated treatments, 2000 lx resulted in significantly higher CH_4 concentrations and lower CO_2 and H_2S concentrations than 1000 and 4000 lx did ($p < 0.05$). On day 20, the CH_4 concentration reached 68.2 ± 0.3% at 2000 lx, while lower CH_4 concentrations were observed at both 1000 and 4000 lx. These results indicate that 2000 lx was associated with the most favorable biogas composition among the evaluated light intensities.

The increase in CH_4 concentration under illumination was accompanied by a simultaneous decrease in CO_2 and H_2S concentrations, suggesting enhanced carbon conversion and sulfur transformation. This behavior is consistent with the activity of phototrophic bacteria identified in Section 3.2. Sulfide-oxidizing bacteria such as *Chlorobium* sp. utilize H_2S as an electron donor during anoxygenic photosynthesis, contributing to sulfide transformation and potentially reducing sulfide accumulation (Frigaard and Dahl, 2008; Lens et al., 1998), while photoheterotrophic bacteria such as *Rhodopseudomonas* sp. assimilate organic intermediates (e.g., VFAs) and convert them into metabolic intermediates that can be further utilized by methanogens (Appels et al., 2008; Madigan and Jung, 2009). Filamentous bacteria (*Pelolinea* sp. and *Leptolinea* sp.) further support these processes through the degradation of complex organic matter and syntrophic interactions, supporting the utilization of the organic fraction of the substrate, as reflected by its high TVS and sCOD values (Yamada et al., 2006).

In contrast, under dark conditions, the CH_4 concentration remained significantly lower, while CO_2 and H_2S accumulated (H_2S up to 1.21 ± 0.02%), suggesting less favorable carbon conversion and the absence of light-driven phototrophic activity. The accumulation of H_2S is consistent with the elevated sulfide concentration in the substrate (747 ± 132 mg L^{-1}; Table 1), suggesting that sulfide transformation by phototrophic microorganisms was limited in the absence of illumination. However, the inhibitory effect of sulfide cannot be inferred from the total dissolved sulfide concentration alone because it is influenced by pH, free H_2S concentration, temperature, and methanogen acclimation (Vu et al., 2022).

To further elucidate gas transformation behavior, the temporal profiles of CO_2 and H_2S were analyzed using kinetic modeling. Under illuminated conditions, both CO_2 and H_2S exhibited apparent first-order decay behavior following an initial increase, whereas no clear first-order decay behavior was observed under dark conditions. The highest rate constants were obtained at 2000 lx (k_{H_2S} = 0.093 day^{-1}; k_{CO_2} = 0.059 day^{-1}), followed by 1000 lx (k_{H_2S} = 0.069 day^{-1}; k_{CO_2} = 0.033 day^{-1}) and 4000 lx (k_{H_2S} = 0.061 day^{-1}; k_{CO_2} = 0.044 day^{-1}). These values are comparable to the reported ranges for biological sulfide oxidation (k ≈ 0.05–0.12 day^{-1}) and CO_2 conversion in phototrophic systems (k ≈ 0.02–0.07 day^{-1}), supporting the observed first-order transformation behavior under illuminated conditions (Egger et al., 2020; Lens et al., 1998), as summarized in the Supplementary Table S1.

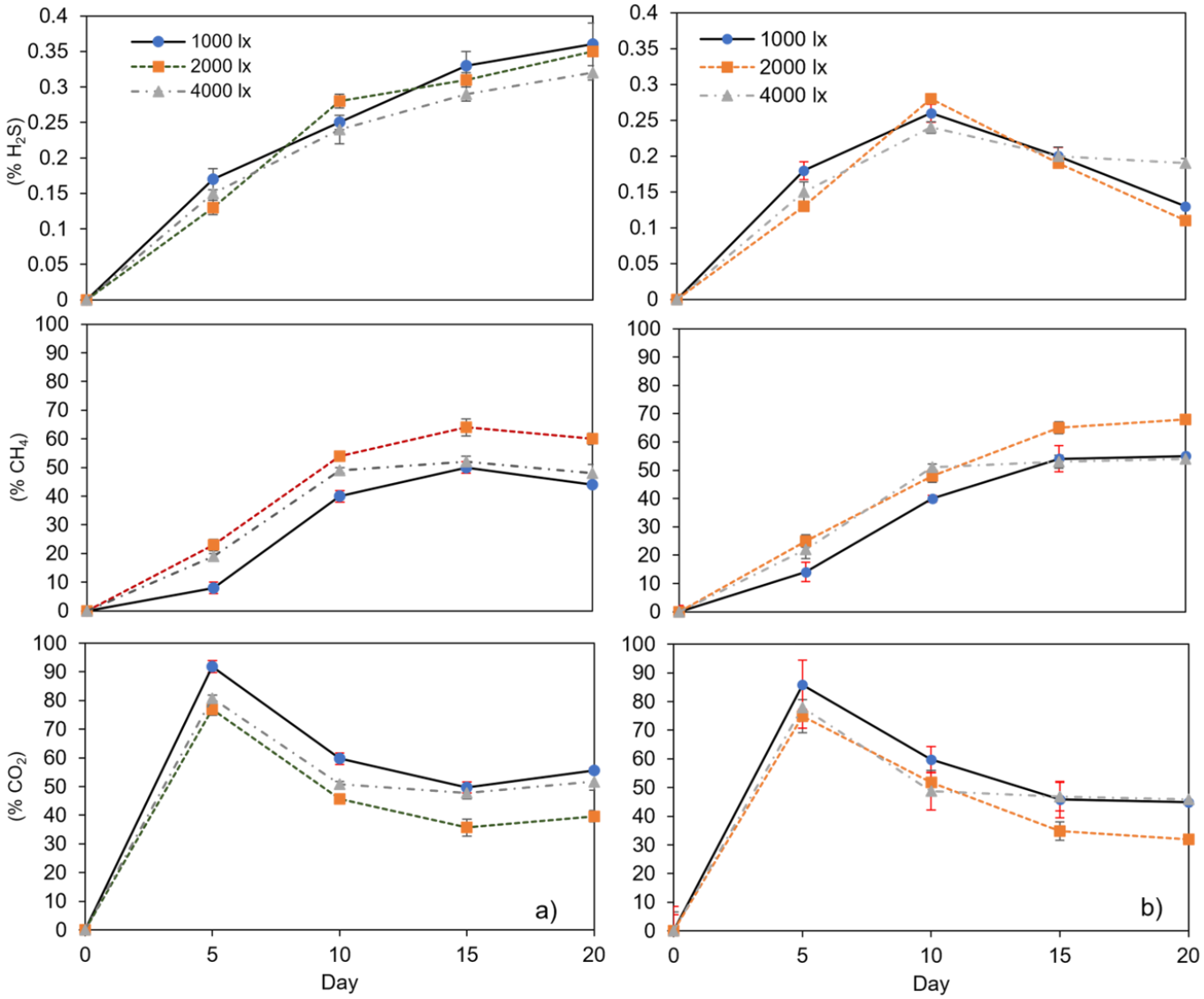


Figure 3 Effects of light intensity on biogas composition (CH_4, CO_2, and H_2S) at the optimal PSB-to-OW ratio (1:2) under a) dark (control) and b) illuminated conditions.

The obtained k values indicate that gas transformation rates were influenced by light intensity, with moderate illumination (2000 lx) corresponding to the most favorable conditions for H_2S and CO_2 transformation. The reduced rate constants observed at 4000 lx suggest that excessive light intensity may have affected microbial activity, although the underlying mechanism was not investigated in the present study. In contrast, the absence of first-order decay behavior under dark conditions supports the role of light-dependent microbial processes in CO_2 transformation and H_2S transformation (Yamada et al., 2006).

Overall, the results indicate that under fixed inoculum conditions, substrate characteristics (high sCOD and sulfide concentrations) influence biogas composition, whereas light intensity plays a key role in regulating the rate of carbon conversion and H_2S transformation. Moderate illumination (2000 lx) corresponds to the most favorable condition, under which microbial processes associated with *Chlorobium* sp., *Rhodopseudomonas* sp., and syntrophic bacteria

may have contributed synergistically to the highest observed CH_4 concentration, together with reductions in CO_2 and H_2S concentrations.

Conclusions

In this study, the effects of the PSB-to-OW ratio and light intensity on biogas composition during PSB-assisted anaerobic fermentation of municipal organic waste were evaluated. Among the tested conditions, a PSB-to-OW ratio of 1:2 resulted in significantly higher CH_4 concentrations and lower CO_2 and H_2S concentrations than the other tested ratios did ($p < 0.05$). Under this ratio, moderate illumination **(**2000 lx) was associated with the highest CH_4 concentration **(**68.2 ± 0.3%**)** and the lowest CO_2 and H_2S concentrations. Kinetic analysis revealed that the apparent transformation rates of CO_2 and H_2S were highest at 2000 lx. Microbial community analysis revealed the presence of sulfide-oxidizing (*Chlorobium* sp.), photoheterotrophic (*Rhodopseudomonas* sp.), and syntrophic filamentous (*Pelolinea* sp. and *Leptolinea* sp.) bacteria under the tested conditions. Overall, these findings indicate that the biogas composition was influenced by both the PSB-to-OW ratio and light intensity during PSB-assisted anaerobic fermentation.

Data availability statement

Information and data used in the study will be disclosed upon request.

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Nitiwist Teangthai: Methodology, Software, Resources, Validation, Visualization, Supervision, Project administration

Conflicts of interest

The authors declare that there are no conflicts of interest in competing financial or personal relationships that could have appeared to influence the work reported in this work.

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