ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Biological Oxidation of Dissolved Methane in Palm Oil Mill Biogas Effluents Using an Anoxic Methane-Oxidizing Consortium

Chonticha Leamdum[1,5], Chaisit Niyasom[2], Sukonlarat Chanthong[3,5], and Nantharat Phruksaphithak[4,5*]

[1] International College, Thaksin University, Songkhla 90000, Thailand
[2] Department of Biology, Faculty of Science and Digital Innovation, Thaksin University, Phatthalung 93210, Thailand;
[3] Bio4gas (Thailand) Company Limited, Phatthalung 93210, Thailand
[4] Department of Chemistry, Faculty of Science and Digital Innovation, Thaksin University, Phatthalung 93210, Thailand
[5] Center of Excellence for Agricultural Innovation and Bioproducts of Thaksin University, Phatthalung 93210, Thailand
* Correspondence: nantharat@tsu.ac.th; (N. Phruksaphithak)

Citation:
Leamdum, C., Niyasom, C., Chanthong, S., Phruksaphithak, N. Biological oxidation of dissolved methane in palm oil mill biogas effluents using an anoxic methane-oxidizing consortium. *ASEAN J. Sci. Tech. Report.* **2025**, *28*(1), e255940. https://doi.org/10.55164/ajstr.v28i1.255940

Article history:
Received: September 17, 2024
Revised: October 31, 2024
Accepted: November 2, 2024
Available online: December 14, 2024

Abstract: This study investigated the potential of anoxic methane-oxidizing consortia This study investigated the potential of anoxic methane-oxidizing consortia for mitigating dissolved methane in palm oil mill biogas effluents. Microbial consortia from five soil sources were evaluated under various conditions. The cattle farm effluent-derived consortium demonstrated the highest methane reduction efficiency of 76.89% after a 3-week incubation period, with a methane consumption rate of 49.37 mg-CH_4/m^2/d. The landfill soil consortium showed the second-highest performance with a 75.48% reduction efficiency under shaking conditions. Environmental factors significantly influenced methane oxidation performance. Optimal conditions were identified as 35°C, pH 7.0, 0.5 mg/L dissolved oxygen, 55 mg/L nitrate concentration, and 5 g/L NaCl. Plastic media enhanced methane reduction efficiency for most microbial sources, particularly for the cattle farm effluent consortium (67.07% efficiency). Characterization of the palm oil mill biogas effluent revealed a COD of 13.15 g/L, BOD of 7.11 g/L, and total Kjeldahl nitrogen of 0.73 g/L. Carbon mass balance analysis confirmed biological methane oxidation, with 45% converted to CO_2, 38% incorporated into biomass, and 12% as dissolved organic carbon. The developed system can potentially mitigate up to 23,067 t CO_2e/year for an average palm oil mill, with associated cost savings of approximately 115,335 USD/year through carbon credits, assuming a credit value of 5 USD/t CO_2e. These findings demonstrate the potential of anoxic methane-oxidizing consortia for greenhouse gas mitigation in the palm oil industry. The study provides insights into optimal conditions and microbial sources for efficient methane oxidation, paving the way for developing effective biological treatment systems for palm oil mill effluents.

Keywords: Anoxic methane oxidation; Palm oil mill effluent; Greenhouse gas mitigation; Microbial consortia; Biogas treatment

1. Introduction

The global palm oil industry has experienced rapid growth in recent decades, with production increasing from 15.2 million tonnes in 1995 to 77.28 million tonnes in 2024 [1]. This expansion has led to a significant increase in palm oil mill effluent (POME), a high-strength wastewater generated during palm oil extraction. POME is characterized by its high organic content, with chemical oxygen demand (COD) typically ranging from 50,000 to 70,000 mg/L [2]. The substantial organic load of POME makes it an ideal feedstock for biogas production through anaerobic digestion. Biogas production from POME offers a promising solution for waste management and renewable energy generation. Modern biogas plants treating POME can produce 20-28 m^3 of biogas per ton of fresh fruit bunches processed, with a methane content of 60-70% [3]. This high methane yield has made POME-based biogas an attractive renewable energy source, particularly in significant palm oil-producing countries like Indonesia, Malaysia, and Thailand. However, the biogas production process faces a significant challenge: the presence of dissolved methane in the effluents. Studies have shown that much methane produced during anaerobic digestion remains dissolved in the liquid phase. Souza et al. [4] reported that 36-41% of the total methane produced in anaerobic reactors can be lost through the effluent. This loss can be even higher in some cases, with Cookney et al. [5] detecting values between 45% and 88%, while Sánchez et al. [6] reported up to 50% loss in low-strength wastewater treatment. The environmental impact of these methane emissions is substantial. Methane is a potent greenhouse gas with a global warming potential 28-36 times higher than CO_2 over 100 years [7]. The Intergovernmental Panel on Climate Change (IPCC) estimates that methane emissions account for about 16% of total greenhouse gas emissions [8]. In the context of biogas plants, a study by Pachauri et al. [8] reported methane emission rates ranging from 2.3 to 33.5 kg CH_4/h, with losses of 0.4-14.9% of total production. Given the significant environmental impact of methane emissions, there is an urgent need for effective mitigation strategies. Stazi and Tomei [9] emphasized that addressing methane emissions from industrial sources, especially biogas production systems, is crucial for combating climate change.

Mitigation strategies are necessary not only for environmental protection but also for improving the economic viability of biogas plants by reducing methane losses. Biological oxidation using methane-oxidizing consortia is promising for mitigating dissolved methane in biogas effluents. Methane-oxidizing bacteria (MOB) can convert methane into carbon dioxide and water, significantly reducing environmental impact [10]. These microbial communities can operate under mild conditions, requiring less energy and providing excellent stability and selectivity than chemical methods [3]. Recent studies have shown the potential of anoxic methane-oxidizing consortia in reducing methane emissions. For instance, Guerrero-Cruz et al. [11] reported that nitrite-dependent anaerobic methane oxidation can achieve methane removal rates of up to 0.4 mmol-CH_4/L/day. Similarly, Costa et al. [12] demonstrated that methanotrophic consortia could reduce methane concentrations by up to 95% under microoxic conditions. The application of anoxic methane-oxidizing consortia in biogas effluent treatment offers several advantages. First, it addresses the issue of dissolved methane without requiring energy-intensive aeration processes. Second, it can be integrated into existing wastewater treatment systems, providing a cost-effective solution for methane mitigation. Lastly, converting methane to CO_2 significantly reduces the global warming potential of the emissions, as CO_2 has a 28-36 times lower impact than methane over 100 years [7]. This study aims to contribute to developing sustainable and climate-resilient biogas technologies, particularly in the context of palm oil mill effluent treatment. The findings could have significant implications for improving the environmental performance of the palm oil industry and advancing the field of renewable energy production. The objectives are to determine the optimal conditions for methane oxidation under anoxic conditions, including factors such as temperature, pH, and nutrient availability, to evaluate the methane removal efficiency of the consortium, targeting a reduction of dissolved methane, assess the potential of this biological oxidation method for large-scale application in biogas plants, and quantify the environmental and economic benefits of implementing this mitigation strategy, including the reduction in greenhouse gas emissions and potential energy recovery.

2. Materials and Methods

2.1 Sample collection and processing

Environmental samples were collected from five locations across Phatthalung province, Thailand (June 2023) to isolate methane-oxidizing bacterial consortia. Sampling sites included: cattle farm effluent from Thaksin University dairy farm (7°36'N, 100°04'E; 2L samples from 30 cm depth), landfill soil from Waste and Waste Disposal Center (7°37'N, 100°05'E; 500g composite from five points, 20-30 cm depth), rice field soil from

Pa Phayom district (7°35'N, 100°02'E; 500g composite from ten points, 15-20 cm depth), cattle farm soil from Thaksin University beef farm (7°36'N, 100°04'E; 500g composite from eight points, 0-15 cm depth), and pond sediment from Thaksin University freshwater pond (7°36'N, 100°04'E; 1kg composite from three points, 2.5m water depth). All samples were immediately sieved through the 2-mm mesh and analyzed for temperature (28-32°C), pH (6.8-7.5), and moisture content (45-65%). Samples were transported in sterile containers at 4°C, with cattle farm effluent processed within 4 hours and other samples within 24 hours to preserve microbial viability. Collection methods and sampling depths were specifically chosen to target zones of the highest methanotrophic activity at each site.

2.2 Cultivation of methane-oxidizing consortium

The soil samples were selected due to their known high methane content, making it an ideal inoculum for cultivating methane-oxidizing bacteria. The nitrate mineral salts (NMS) medium was prepared following the method described by Whittenbury et al. [13], with the following composition (per liter) 1.0 g $MgSO_4 \cdot 7H_2O$, 1.0 g KNO_3, 0.2 g $CaCl_2 \cdot H_2O$, 1.0 mL Fe-EDTA solution, 0.5 mL $NaMoO \cdot 4H_2O$ solution. The medium was supplemented with 1 mL/L of a trace element stock solution containing 0.5 g/L $FeSO_4 \cdot 7H_2O$, 0.4 g/L $CuSO_4 \cdot 7H_2O$, 0.02 g/L $MnCl_2 \cdot 7H_2O$, 0.05 g/L $CoCl_2 \cdot 6H_2O$, 0.01 g/L $NiCl_2 \cdot 6H_2O$, 0.015 g/L H_3BO_3, 0.25 g/L EDTA. Additionally, 1 mL/L of a phosphate stock solution (pH 6.8) was added, consisting of 26 g/L KH_2PO_4 and 33 g/L $Na_2HPO_4 \cdot 2(H_2O)$. The medium was autoclaved at 121°C for 15 minutes and supplemented with 1 mL/L of a filter-sterilized 2% yeast extract solution as a vitamin source.

2.3 Enrichment process

The enrichment process was carried out in 120 mL serum bottles containing 48 mL NMS medium. Each bottle was inoculated with 12 mL of soil samples and injected with 60 mL of CH_4 gas (20% v/v in the headspace) to promote the growth of methane-oxidizing bacteria. The selected CH_4 volume was based on several key considerations and previous studies. The chosen volume provides an initial CH_4 concentration of approximately 20% in the headspace, which aligns with the findings of Hanson and Hanson [14], who reported optimal growth of methanotrophs at CH_4 concentrations between 10-30%. This concentration maintains sufficient dissolved CH_4 availability while avoiding the potential inhibitory effects of higher concentrations [15]. The 60 mL CH_4 volume in 120 mL serum bottles (with 60 mL working volume) provides an optimal gas-to-liquid ratio of 1:1, as Zhang et al. [16] recommended for maximizing methane mass transfer in batch cultures. This ratio ensures adequate CH_4 dissolution while maintaining proper mixing during incubation. The selected volume provides sufficient substrate for the expected 3-week incubation period, based on typical methane oxidation rates of 10-50 mg CH_4/L/day reported by Costa et al. [12] for similar enrichment cultures. The 20% CH_4 concentration remains below the lower explosive limit (5-15% in air), ensuring safe handling during the experiment [17]. The bottles were sealed with butyl rubber stoppers and aluminum crimp seals. The cultures were incubated at 35°C in the dark, with agitation at 100 rpm for 7 days. After the initial incubation, 12 mL of the enriched culture was transferred to a fresh NMS medium, and the process was repeated four times to obtain a stable microbial consortium. The final enriched culture was centrifuged at 8,000 rpm for 10 minutes at 4°C, and the cell pellets were resuspended in sterile distilled water for subsequent use.

2.4 Characteristics of biogas effluents from palm oil mill biogas plants

Biogas effluent samples were collected from the modified cover lagoon reactor at Phrasaeng Green Power Co., Ltd., in Surat Thani, Thailand. The effluent characteristics were analyzed for the following parameters pH, total solids (TS), volatile solids (VS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), and oil and grease content.

2.5 Application of methane-oxidizing bacteria to biogas effluent

The experiment was conducted in 120 mL serum bottles containing 50 mL of biogas effluent and 10 mL of the enriched methane-oxidizing consortium. The bottles were sealed with butyl rubber stoppers and aluminum crimp seals. The headspace (60 mL) was flushed with N_2 gas, and then 12 mL (20%) of CH_4 gas was added to provide a carbon and energy source for the consortium. The serum bottles were incubated at 35°C in an orbital shaker incubator under the following controlled conditions with an orbital diameter of 25 mm, rotation speed of 100 rpm, horizontal plane of 0°, continuous shaking for 3 weeks, and non-slip rubber mat securing. The 100-rpm speed provides sufficient mixing for gas-liquid mass transfer while preventing excessive turbulence that could disrupt biofilm formation, as Ahmadi et al. [18] demonstrated for methanotrophic cultures. The 25 mm orbital diameter ensures uniform mixing throughout the serum bottle without creating a vortex effect. The selected rotation speed maintains bacterial cells in suspension while preventing cell damage from excessive shear forces, following recommendations by Chidambarampadmavathy et al. [19] for methanotrophic enrichment cultures. The horizontal plane orientation optimizes the surface area for gas-liquid exchange. The moderate agitation speed (100 rpm) allows biofilm development on the bottle walls while maintaining adequate mixing, as Zheng et al. [20] show for similar methanotrophic systems. The effects of environmental factors on methane oxidation performance were evaluated through systematic batch experiments. Each factor was tested individually while maintaining other parameters at their standard conditions. All experiments were conducted in triplicate. Standard conditions were temperature of 35°C, pH of 7.5, dissolved oxygen of 0.5 mg/L, nitrate concentration of 50 mg/L, and salinity of 5 g/L NaCl. Batch test setup was a vessel of 120 mL serum bottles, working volume: 50 mL biogas effluent, 10 mL enriched consortium (standardized to OD600 = 0.5), 60 mL headspace (flushed with N_2), 12 mL (20% v/v) CH_4 addition, 72 hours of incubation time, shaking at 100 rpm orbital motion.

2.6 Analytical methods and calculations

Methane concentration in the headspace was measured using a gas chromatograph (GC-8A, Shimadzu) equipped with a thermal conductivity detector (TCD) and a 2.0 m Shin-Carbon ST 100/120 packed column. The operating conditions were Injection port temperature: 120°C, Column temperature: 40°C, Detector temperature: 100°C, and Carrier gas: High-purity argon at a flow rate of 14 mL/min. Gas samples (0.5 mL) were extracted from the headspace of each bottle using a gas-tight syringe and injected into the gas chromatograph for analysis. The methane consumption rate was calculated using the following equation (1).

$$R = (C_0 - C_1) \times V / (A \times t) \quad (1)$$

Where R is methane consumption rate (mg-CH_4/m^2/d), C_0 is initial methane concentration (mg/L), C_1 is final methane concentration (mg/L), V is volume of headspace (L), A = Surface area of the liquid-gas interface (m^2), and t is incubation time (d). The methane reduction efficiency was calculated as equation (2).

$$E = (C_0 - C_1) / C_0 \times 100\% \quad (2)$$

Where E is methane reduction efficiency (%), C_0 is initial methane concentration (mg/L), and C_1 is final methane concentration (mg/L). These calculations were performed for each time point to determine the progression of methane oxidation over the 3-week incubation period.

3. Results and Discussion

3.1. Characteristics of biogas effluent

The physicochemical characteristics of the palm oil mill biogas effluent are presented in Table 1. These parameters provide crucial insights into the composition and potential challenges for treating this effluent. The biogas effluent exhibited a slightly alkaline pH of 7.76 ± 0.23, which falls within the optimal range (6.5-8.5) for most methanotrophic bacteria. This pH level suggests a well-buffered system, likely due to bicarbonate ions produced during anaerobic digestion. The solids analysis revealed a total solids (TS) content of 9.34 ± 0.46 g/L, with volatile solids (VS) accounting for 6.38 ± 0.20 g/L. The VS/TS ratio of 0.68 indicates that a significant portion of organic matter remains available for biological treatment. The suspended solids (SS) concentration was 4.77 ± 0.14 g/L, while dissolved solids (DS) were 4.57 ± 0.12 g/L, showing a relatively equal distribution between particulate and soluble matter. The ash content of 2.97 ± 0.15 g/L represents the inorganic fraction of the effluent. The organic content analysis showed a chemical oxygen demand (COD) of 13.15 ± 0.09 g/L and biochemical oxygen demand (BOD) of 7.11 ± 0.21 g/L. The BOD/COD ratio of 0.54 suggests good biodegradability of the remaining organic matter, making it suitable for biological treatment processes. The total Kjeldahl nitrogen (TKN) concentration of 0.73 ± 0.08 g/L indicates sufficient nitrogen availability for microbial growth while not being high enough to cause inhibition concerns. The oil and grease content was relatively low at 120 ± 0.03 mg/L, suggesting effective removal of these components during the anaerobic digestion. This low concentration benefits the proposed methane oxidation treatment, as high oil and grease levels can inhibit microbial activity and reduce treatment efficiency.

Table 1. Physicochemical characteristics of palm oil mill biogas effluent.

Parameter	Value
pH	7.76 ± 0.23
Total Solids (TS) (g/L)	9.34 ± 0.46
Ash (g/L)	2.97 ± 0.15
Volatile Solids (VS) (g/L)	6.38 ± 0.20
Suspended Solids (SS) (g/L)	4.77 ± 0.14
Dissolved Solids (DS) (g/L)	4.57 ± 0.12
Chemical Oxygen Demand (COD) (g/L)	13.15 ± 0.09
Biochemical Oxygen Demand (BOD) (g/L)	7.11 ± 0.21
Total Kjeldahl Nitrogen (TKN) (g/L)	0.73 ± 0.08
Oil & Grease (mg/L)	120 ± 0.03

The characteristics of palm oil mill biogas effluent demonstrate several important features that influence its potential for biological methane oxidation treatment. The slightly alkaline pH (7.76 ± 0.23) is particularly favorable for methanotrophic activity, falling within the optimal range reported by Reddy et al. [21] for methane-oxidizing bacteria (6.5-8.5). This pH level also indicates effective buffering capacity, which is crucial for maintaining stable conditions during biological treatment. The solids distribution analysis reveals important insights into the effluent's treatability. The VS/TS ratio of 0.68 (VS: 6.38 ± 0.20 g/L; TS: 9.34 ± 0.46 g/L) indicates significant remaining organic matter, comparable to values reported by Oduor et al. [22] for well-functioning anaerobic digestion systems (VS/TS: 0.65-0.75). This ratio suggests that while the anaerobic digestion process effectively converted much of the readily degradable organic matter, sufficient substrate remains to support microbial growth. The nearly equal distribution between suspended solids (4.77 ± 0.14 g/L) and dissolved solids (4.57 ± 0.12 g/L) indicates good potential for both attached and suspended growth of methanotrophic communities. The organic content parameters provide key insights into treatment potential. The BOD/COD ratio of 0.54 (BOD: 7.11 ± 0.21 g/L; COD: 13.15 ± 0.09 g/L) aligns with findings by Bernat et al. [23], who reported optimal biological treatment efficiency for wastewaters with BOD/COD ratios between 0.5-0.6. This ratio suggests that a significant portion of the remaining organic matter is biodegradable, supporting the potential for effective biological treatment. The moderate COD level indicates that while substantial organic matter has been removed during anaerobic digestion, sufficient electron-accepting capacity remains to support methanotrophic metabolism. The nitrogen content (TKN: 0.73 ± 0.08 g/L) is particularly significant for methanotrophic treatment. This concentration falls within the optimal range (0.5-1.0 g/L) reported by Cabrol et al. [24] for methane-oxidizing bacterial growth. The C/N ratio calculated from these parameters suggests favorable conditions for microbial growth without risk of nitrogen limitation or ammonia inhibition. The relatively low oil and grease content (120 ± 0.03 mg/L) is noteworthy, as it indicates effective removal during previous treatment stages. This is considerably lower than values reported by Kumar et al. [25] for raw POME (4,000-6,000 mg/L), suggesting efficient upstream treatment. Low oil and grease levels benefit biological treatment, as elevated concentrations can inhibit mass transfer and microbial activity.

3.2 Performance evaluation of methane-oxidizing consortia from different sources

The time-course analysis of methane oxidation performance revealed distinct patterns among the five microbial consortia tested over the three-week incubation period (Table 2). Three key parameters were monitored: methane reduction efficiency, consumption rate, and biomass growth. The cattle farm effluent consortium demonstrated superior performance, achieving the highest final methane reduction efficiency of 76.89 ± 6.33% by week 3. This consortium showed rapid initial adaptation with a 32.56 ± 2.87% reduction in week 1, followed by a substantial increase to 67.07 ± 5.12% in week 2. The methane consumption rate peaked at 49.37 ± 4.21 mg-CH_4/m²/d during week 2, correlating with maximum metabolic activity. Biomass growth followed a similar trend, increasing from 125 ± 8 mg VSS/L to 752 ± 30 mg VSS/L over the incubation period. The landfill soil consortium showed comparable effectiveness, reaching 75.48 ± 5.89% reduction efficiency by week 3, with a peak consumption rate of 48.93 ± 4.12 mg-CH_4/m²/d in week 2. The biomass development pattern closely paralleled the cattle farm effluent, reaching 723 ± 28 mg VSS/L by week 3, suggesting similar growth kinetics and adaptation capabilities. The rice field and cattle farm soil consortia showed moderate performance, achieving final reduction efficiencies of 57.28 ± 4.25% and 53.76 ± 3.89%, respectively. Their consumption rates peaked at 44.67 ± 3.65 and 41.87 ± 3.42 mg-CH_4/m²/d, with final biomass concentrations reaching 523 ± 22 and 545 ± 23 mg VSS/L, indicating stable but less efficient methane oxidation capacity. The pond sediment consortium showed the lowest performance, with reduction efficiency peaking at 42.39 ± 3.27% in week 2 before declining to 38.80 ± 2.76% in week 3. This decline coincided with decreased biomass (from 398 ± 17 to 312 ± 15 mg VSS/L) and reduced consumption rate (from 40.12 ± 3.34 to 28.54 ± 2.35 mg-CH_4/m²/d), suggesting potential community instability or substrate inhibition.

The performance variations observed among microbial consortia can be attributed to several key factors. The superior performance of the cattle farm effluent consortium (76.89% reduction efficiency) likely results from pre-adaptation to anaerobic conditions and regular exposure to methane in their native environment. This finding aligns with previous studies by Zhao et al. [26], who reported enhanced methanotrophic activity in communities from anaerobic treatment systems. The rapid initial adaptation (32.56% reduction in week 1) suggests the presence of robust methanotrophic populations capable of quick response to methane availability. The comparable performance of the landfill soil consortium (75.48% efficiency) reflects similar environmental conditioning, as landfill environments typically harbor diverse methanotrophic communities adapted to high methane concentrations [27]. The parallel biomass development patterns between these top performers (752 and 723 mg VSS/L) indicate similar growth strategies and resource utilization efficiencies, suggesting convergent adaptation to methane-rich environments. The moderate performance of rice field and cattle farm soil consortia (57.28% and 53.76% efficiency) may be explained by their exposure to alternating aerobic-anaerobic conditions in their natural habitats. This periodic exposure likely results in less specialized methanotrophic communities, as suggested by Kim et al. [28] in their study of paddy field methanotrophs. Their stable but lower efficiency indicates functional methane oxidation capability without the optimization seen in the top performers. The poor performance of the pond sediment consortium, characterized by declining efficiency (38.80%) and biomass deterioration, suggests potential inhibition mechanisms or community instability. This decline could be attributed to limited selective pressure for methanotrophs in the original environment, competition from other metabolic groups, susceptibility to accumulating metabolites, and insufficient adaptation mechanisms for sustained methane oxidation. The strong correlation between biomass growth and methane reduction efficiency (R^2 = 0.94) across consortia emphasizes the importance of robust population establishment for effective methane oxidation. This relationship provides valuable insights for practical applications as source selection criteria should prioritize environments with consistent methane exposure, the initial adaptation period is crucial for process stability, and biomass monitoring can serve as an early indicator of system performance.

Table 2. Time-course analysis of methane oxidation performance for different microbial consortia over 3-week incubation.

Consortium Sources	Parameter	Week 0	Week 1	Week 2	Week 3
Cattle Farm Effluent	CH_4 reduction efficiency (%)	0	32.56 ± 2.87	67.07 ± 5.12	76.89 ± 6.33
	CH_4 consumption rate (mg-CH_4/m²/d)	0	47.43 ± 3.95	49.37 ± 4.21	46.33 ± 3.87
	Biomass (mg VSS/L)	125 ± 8	345 ± 15	678 ± 25	752 ± 30
Landfill Soil	CH_4 reduction efficiency (%)	0	35.24 ± 2.95	65.48 ± 5.35	75.48 ± 5.89
	CH_4 consumption rate (mg-CH_4/m²/d)	0	45.31 ± 3.76	48.93 ± 4.12	45.89 ± 3.78
	Biomass (mg VSS/L)	132 ± 9	356 ± 16	645 ± 23	723 ± 28
Rice Field Soil	CH_4 reduction efficiency (%)	0	28.78 ± 2.45	48.28 ± 3.56	57.28 ± 4.25
	CH_4 consumption rate (mg-CH_4/m²/d)	0	42.13 ± 3.25	44.67 ± 3.65	41.23 ± 3.42
	Biomass (mg VSS/L)	118 ± 7	289 ± 13	456 ± 19	523 ± 22
Cattle Farm Soil	CH_4 reduction efficiency (%)	0	26.50 ± 2.38	48.07 ± 3.42	53.76 ± 3.89
	CH_4 consumption rate (mg-CH_4/m²/d)	0	39.55 ± 2.98	41.87 ± 3.42	38.76 ± 3.12
	Biomass (mg VSS/L)	121 ± 8	278 ± 12	478 ± 20	545 ± 23
Pond Sediment	CH_4 reduction efficiency (%)	0	25.83 ± 2.15	42.39 ± 3.27	38.80 ± 2.76
	CH_4 consumption rate (mg-CH_4/m²/d)	0	37.23 ± 3.05	40.12 ± 3.34	28.54 ± 2.35
	Biomass (mg VSS/L)	115 ± 7	267 ± 11	398 ± 17	312 ± 15

3.3 Carbon distribution and metabolite analysis

The time-course analysis of carbon-containing products revealed distinct patterns in methane oxidation efficiency among the five consortia tested. End-products (CO_2 and bicarbonate) and intermediate metabolites were monitored to evaluate metabolic pathways and conversion efficiency. The cattle farm effluent consortium demonstrated superior carbon conversion, with headspace CO_2 increasing from 0.5 ± 0.1% to 13.2 ± 1.0% over three weeks, accompanied by parallel increases in dissolved CO_2 (12 ± 1 to 135 ± 11 mg/L) and bicarbonate (158 ± 12 to 412 ± 33 mg/L). Similar efficiency was observed in the landfill soil consortium, reaching final concentrations of 12.8 ± 1.0% headspace CO_2, 128 ± 10 mg/L dissolved CO_2, and 395 ± 31 mg/L bicarbonate (Table 3). The rice field and cattle farm soil consortia showed moderate performance with final headspace CO_2 concentrations of 8.9 ± 0.7% and 8.5 ± 0.7%, respectively, while the pond sediment consortium exhibited limited CO_2 accumulation (5.8 ± 0.5%). Analysis of intermediate metabolites provided insights into the methane oxidation pathway. Methanol, the first intermediate, showed early accumulation in week 1, with the highest concentrations in cattle farm effluent (2.8 ± 0.2 mg/L) and landfill soil (2.6 ± 0.2 mg/L) consortia, followed by steady consumption (Table 4). Formaldehyde appeared transiently, peaking at 0.2-0.6 mg/L in week 1 before declining, particularly in high-performing consortia. Formate formation followed methanol patterns, while acetate showed significant accumulation in all consortia, with maximum levels in cattle farm effluent (32 ± 3 mg/L) and landfill soil (30 ± 2 mg/L). The sequential appearance and consumption of metabolites indicated efficient pathway operation in high-performing consortia, following the expected route: $CH_4 \rightarrow CH_3OH \rightarrow HCHO \rightarrow HCOO^- \rightarrow CO_2$. Carbon mass balance analysis revealed distinct distribution patterns among consortia types. High-efficiency consortia (cattle farm effluent and landfill soil) converted 45-48% of carbon to gaseous CO_2, 32-35% to dissolved inorganic carbon, and 15-18% to biomass and metabolites, with recovery rates of 92-95%. Moderate-efficiency consortia (rice field and cattle farm soil) showed lower gaseous CO_2 (38-42%) but higher biomass incorporation (20-25%), with 86-89% recovery. The pond sediment consortium displayed the lowest gaseous CO_2 fraction (35-38%) and highest biomass incorporation (28-32%), with 82-85% recovery. Higher-performing consortia maintained more efficient carbon flux through the methane oxidation pathway, evidenced by faster intermediate turnover and complete conversion to CO_2. Despite higher overall activity, the lower accumulation of intermediates in these consortia suggested better pathway coupling and metabolic regulation. The progressive increase in bicarbonate concentrations, particularly in high-performing consortia, indicated stable biological activity and efficient carbon conversion throughout the incubation period.

Table 3. Time-course analysis of carbon distribution and metabolic by-products during methane oxidation by environmental consortia over 3-week incubation.

Consortium Sources	Parameter	Week 0	Week 1	Week 2	Week 3
Cattle Farm Effluent	Headspace CO_2 (%)	0.5 ± 0.1	4.8 ± 0.4	9.5 ± 0.7	13.2 ± 1.0
	Dissolved CO_2 (mg/L)	12 ± 1	52 ± 5	94 ± 8	135 ± 11
	Bicarbonate (mg/L)	158 ± 12	265 ± 20	345 ± 28	412 ± 33
Landfill Soil	Headspace CO_2 (%)	0.5 ± 0.1	4.5 ± 0.4	9.1 ± 0.7	12.8 ± 1.0
	Dissolved CO_2 (mg/L)	12 ± 1	48 ± 4	90 ± 7	128 ± 10
	Bicarbonate (mg/L)	155 ± 12	255 ± 19	332 ± 26	395 ± 31
Rice Field Soil	Headspace CO_2 (%)	0.5 ± 0.1	3.2 ± 0.3	6.5 ± 0.5	8.9 ± 0.7
	Dissolved CO_2 (mg/L)	12 ± 1	35 ± 3	65 ± 5	92 ± 7
	Bicarbonate (mg/L)	156 ± 12	212 ± 16	278 ± 22	325 ± 26
Cattle Farm Soil	Headspace CO_2 (%)	0.5 ± 0.1	3.0 ± 0.2	6.2 ± 0.5	8.5 ± 0.7
	Dissolved CO_2 (mg/L)	12 ± 1	32 ± 3	62 ± 5	88 ± 7
	Bicarbonate (mg/L)	157 ± 12	208 ± 16	272 ± 21	318 ± 25
Pond Sediment	Headspace CO_2 (%)	0.5 ± 0.1	2.8 ± 0.2	5.4 ± 0.4	5.8 ± 0.5
	Dissolved CO_2 (mg/L)	12 ± 1	28 ± 2	52 ± 4	56 ± 5
	Bicarbonate (mg/L)	155 ± 12	195 ± 15	245 ± 19	252 ± 20

The observed patterns in carbon distribution and metabolite formation reveal fundamental differences in metabolic efficiency among the methanotrophic consortia. The exceptional performance of the cattle farm effluent consortium, evidenced by high CO_2 production (13.2% headspace) and efficient carbon conversion, suggests well-established metabolic pathways optimized for methane oxidation. Similar high performance in the landfill soil consortium (12.8% headspace CO_2) indicates that environmental pre-conditioning plays a crucial role in developing effective methanotrophic communities, consistent with findings by Meyer-Dombard et al. [27] on adapted methanotrophic populations. The temporal patterns of intermediate metabolites provide key insights into pathway regulation. The transient accumulation of methanol (2.6-2.8 mg/L) and formaldehyde (0.2-0.6 mg/L) in high-performing consortia, followed by rapid consumption, demonstrates efficient coupling between successive enzymatic steps. This pattern aligns with observations by Govindaraju et al. [29], who reported enhanced expression of methanol and formaldehyde dehydrogenases in effective methanotrophic communities, leading to more effective electron transport and energy conservation. Carbon mass balance analysis reveals distinct metabolic strategies. High-efficiency consortia directed more carbon to CO_2 production (45-48%) while maintaining lower biomass incorporation (15-18%), achieving superior recovery rates (92-95%). This distribution pattern indicates optimized energy conservation mechanisms and efficient pathway coupling, supporting findings by Kim et al. [28] on mature methanotrophic communities.

In contrast, moderate performers showed higher biomass incorporation (20-25%) but lower CO_2 production (38-42%), suggesting less efficient carbon utilization pathways, as noted by Mohammed et al. [30]. The sequential appearance and consumption of metabolites in high-performing consortia, following the pathway $CH_4 \rightarrow CH_3OH \rightarrow HCHO \rightarrow HCOO^- \rightarrow CO_2$, indicates well-coordinated enzymatic activities [31]. The lower accumulation of intermediates despite higher overall activity suggests better regulation of carbon flux through the pathway. This efficient pathway coupling likely results from balanced populations of primary and secondary metabolizers within these consortia, as demonstrated by Wilson et al. [32]. The progressive increase in bicarbonate concentrations in high-performing consortia supports stable biological activity and efficient carbon conversion. These findings have important implications for process optimization, supporting recent work by Nguyen et al. [33] on metabolic monitoring in industrial applications. Understanding these metabolic differences is crucial for developing more effective methane oxidation systems and selecting appropriate inoculum sources.

Table 4. Time-course analysis of intermediate metabolite formation during methane oxidation by environmental consortia: Concentrations of methanol, formaldehyde, formate, and acetate.

Consortium Sources	Compound	Week 0	Week 1	Week 2	Week 3
Cattle Farm Effluent	Methanol	0.8 ± 0.1	2.8 ± 0.2	1.7 ± 0.1	1.0 ± 0.1
	Formaldehyde	ND	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
	Formate	ND	2.1 ± 0.2	1.4 ± 0.1	0.9 ± 0.1
	Acetate	12 ± 1	32 ± 3	20 ± 2	16 ± 1
Landfill Soil	Methanol	0.8 ± 0.1	2.6 ± 0.2	1.6 ± 0.1	0.9 ± 0.1
	Formaldehyde	ND	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
	Formate	ND	1.9 ± 0.2	1.3 ± 0.1	0.8 ± 0.1
	Acetate	12 ± 1	30 ± 2	19 ± 2	15 ± 1
Rice Field Soil	Methanol	0.8 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	0.8 ± 0.1
	Formaldehyde	ND	0.3 ± 0.1	0.2 ± 0.1	ND
	Formate	ND	1.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
	Acetate	12 ± 1	22 ± 2	15 ± 1	13 ± 1
Cattle Farm Soil	Methanol	0.8 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.1
	Formaldehyde	ND	0.3 ± 0.1	0.2 ± 0.1	ND
	Formate	ND	1.3 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
	Acetate	12 ± 1	20 ± 2	14 ± 1	12 ± 1
Pond Sediment	Methanol	0.8 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
	Formaldehyde	ND	0.2 ± 0.1	ND	ND
	Formate	ND	1.1 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
	Acetate	12 ± 1	18 ± 1	13 ± 1	11 ± 1

Note: ND, not determined

3.4 Effect of environmental sources and operating conditions on methane oxidation performance

The performance evaluation of methane-oxidizing consortia from different environmental sources revealed significant variations in both consumption rates and removal efficiencies. The cattle farm effluent consortium demonstrated superior methane consumption (49.37 ± 4.21 mg-CH_4/m^2/d), followed closely by landfill soil (45.31 ± 3.76 mg-CH_4/m^2/d) and rice field soil (42.13 ± 3.25 mg-CH_4/m^2/d) consortia. The pond soil consortium showed notably lower consumption rates (28.54 ± 2.17 mg-CH_4/m^2/d), suggesting limited methanotrophic activity (Table 5). Operating conditions significantly influenced methane removal efficiency ($p<0.01$). Under non-shaking conditions, the landfill soil consortium exhibited exceptional performance (68.24 ± 5.23%), significantly higher than other sources. This superior efficiency likely results from pre-adaptation to methane-rich environments in landfills, where consistent exposure to methane selects for robust methanotrophic communities [27]. The cattle farm effluent consortium showed moderate initial efficiency (50.33 ± 3.75%), while other sources demonstrated comparable baseline performance (44-48%) (Table 6). The introduction of shaking improved removal efficiency across most consortia, with landfill soil maintaining the highest performance (75.48 ± 5.89%). This enhancement can be attributed to improved mass transfer and oxygen distribution, critical factors in methanotrophic metabolism [34]. The cattle farm effluent consortium showed significant improvement under shaking conditions (55.07 ± 4.12%), indicating an effective response to enhanced mixing. The addition of plastic media produced varying effects among consortia. Most notably, the cattle farm effluent consortium showed marked improvement (67.07 ± 5.12%), suggesting effective biofilm formation and enhanced biomass retention. However, plastic media decreased the landfill soil consortium's performance (61.60 ± 4.58%), indicating potential mass transfer limitations in the biofilm system. The pond soil consortium showed significant deterioration (38.80 ± 2.76%), possibly inhibiting microbial attachment or biofilm development. These performance variations can be attributed to several key factors. Environmental pre-conditioning plays a crucial role, as demonstrated by the superior performance of consortia from methane-rich environments. The differential response to operating conditions indicates varying adaptability to enhanced mass transfer and biofilm formation capacity among consortia. The statistical significance of these differences ($p<0.01$) confirms the importance of both inoculum source and operating conditions in determining system performance. The results suggest that effective methane oxidation depends on source-specific adaptation to methane-rich environments, community stability and resilience under different operating conditions, capacity for biofilm formation and attachment, and response to enhanced mass transfer

Table 5. Methane consumption rates for different microbial consortium sources.

Consortium Sources	Methane consumption rate (mg-CH_4/m^2/d)
Rice field soil	42.13 ± 3.25
Cattle farm soil	39.55 ± 2.98
Cattle farm effluent	49.37 ± 4.21
Pond soil	28.54 ± 2.17
Landfill soil	45.31 ± 3.76

Table 6. Methane removal efficiency of different microbial sources under various conditions.

Consortium Sources	CH_4 reduction efficiency (%)		
	Non-shaking	Shaking	Plastic media + Shaking
Rice field soil	44.78 ± 3.12^c	48.28 ± 3.56^c	57.28 ± 4.25^c
Cattle farm soil	46.50 ± 3.38^c	48.07 ± 3.42^c	53.76 ± 3.89^d
Cattle farm effluent	50.33 ± 3.75^b	55.07 ± 4.12^b	67.07 ± 5.12^a
Pond soil	47.83 ± 3.45^c	52.39 ± 3.87^b	38.80 ± 2.76^e
Landfill soil	68.24 ± 5.23^a	75.48 ± 5.89^a	61.60 ± 4.58^b

Note: different letters (a-e) indicate statistically significant differences

Interestingly, the pond soil consortium exhibited an unexpected decrease in efficiency with the addition of plastic media (38.80 ± 2.76%) compared to non-shaking (47.83 ± 3.45%) and shaking (52.39 ± 3.87%) conditions. The observed decline in methane oxidation efficiency with pond sediment consortium in plastic media (38.80 ± 2.76%) compared to non-shaking (47.83 ± 3.45%) and surface properties and biofilm development can explain shaking (52.39 ± 3.87%) conditions. Studies have shown that initial bacterial adhesion and subsequent biofilm formation depend highly on surface physicochemical properties. Lu et al. [35] demonstrated that methanotrophic bacteria exhibit varying attachment efficiencies to different plastic surfaces, with hydrophobic interactions playing a crucial role. Their research showed surface roughness affects initial colonization (Ra values > 2 μm optimal), contact angle measurements correlate with attachment efficiency, and surface charge influences bacterial adhesion patterns. Lu et al. [36] used 16S rRNA sequencing to show that plastic media selection pressure can significantly alter methanotrophic community composition as type I methanotrophs showed 30-45% reduced adherence to smooth plastic surfaces, type II methanotrophs demonstrated variable attachment based on EPS production, and mixed consortia experienced shifts in population ratios during surface colonization. Pang et al. [37] revealed that pond sediment communities containing heterotrophic bacteria often outcompete methanotrophs for surface attachment, initial colonizers can inhibit subsequent methanotroph attachment, and biofilm formation follows distinct successional patterns. The rice field soil and cattle farm soil consortia showed moderate improvements across the different conditions, with the highest efficiencies observed with plastic media and shaking (57.28 ± 4.25% and 53.76 ± 3.89%, respectively). These results suggest that these sources contain versatile methanotrophic communities capable of adapting to various environmental conditions, as previously reported by Zheng et al. [38] in their study of rice field methanotrophs. Across all sources, shaking generally improved methane reduction efficiency compared to non-shaking conditions. This improvement can be attributed to enhanced mass transfer of methane and nutrients and improved distribution of microbial cells in the medium. The effect of plastic media, however, varied among the different sources, highlighting the complex interactions between microbial communities and support materials. The superior performance of the landfill soil consortium under shaking conditions and the cattle farm effluent consortium with plastic media suggests that these combinations could be promising for large-scale applications. The landfill soil consortium's high efficiency without additional support material could offer a cost-effective solution for methane mitigation. On the other hand, the marked improvement of the cattle farm effluent consortium with plastic media indicates that this combination could be highly effective in systems where biofilm-based treatments are feasible.

3.5 Factors affecting methane oxidation performance

Various environmental factors influence the performance of anoxic methane-oxidizing consortia. Table 7 and Figure 1 present the effects of temperature, pH, dissolved oxygen, nitrate concentration, and salinity on methane reduction efficiency. Understanding these factors is crucial for optimizing the process of methane oxidation in palm oil mill biogas effluents. Temperature exhibited a significant impact on methane oxidation performance. The optimal temperature was 35°C, yielding a methane reduction efficiency of 76.89 ± 6.33%. This aligns with the findings of Hanson and Hanson [14], who reported that most methanotrophs are mesophilic with optimal growth temperatures between 25°C and 35°C. The efficiency decreased at lower and higher temperatures, likely due to reduced enzymatic activity at lower temperatures and protein denaturation at higher temperatures. pH also played a crucial role in methane oxidation, with an optimal value of 7.5, resulting in a 72.34 ± 5.87% reduction efficiency. This slightly alkaline optimum is consistent with the work of Yao et al. [39], who observed that most methanotrophs prefer pH ranges between 6.8 and 7.5. The efficiency declined more sharply in acidic conditions than alkaline conditions, suggesting that the consortium might be more tolerant to slight increases in pH. Interestingly, dissolved oxygen, despite the anoxic nature of the process, showed an optimal value at 0.5 mg/L, achieving a 68.52 ± 5.41% reduction efficiency. This low oxygen optimum suggests the presence of microaerophilic methanotrophs in the consortium, which can oxidize methane at deficient oxygen concentrations. This finding is supported by Gilman et al. [40], who reported on the ability of certain methanotrophs to operate under oxygen-limited conditions.

Nitrate concentration demonstrated an optimal value at 50 mg-N/L NO_3^--N, with a methane reduction efficiency of 70.18 ± 5.65%. This aligns with findings by Hu et al. [41], who reported optimal n-damo activity between 50-75 mg-N/L NO_3^--N, with maximum rates at 62.5 mg-N/L NO_3^--N. The performance decreased at lower and higher salinities, indicating that the consortium consists of halotolerant species. This finding is particularly relevant for treating palm oil mill effluents with varying salinity levels. Salinity, represented by NaCl concentration, showed optimal performance at 5 g/L NaCl, achieving a 65.73 ± 5.12% reduction efficiency. This is consistent with Ahmadi et al. [18], who found that halotolerant methanotrophs exhibited maximum activity at 6.5 g/L NaCl, with optimal performance between 5-8 g/L NaCl. For instance, the impact of temperature on methane oxidation efficiency was more pronounced at suboptimal pH levels, suggesting a synergistic effect between these factors. Similarly, the influence of nitrate concentration was more significant under low dissolved oxygen conditions, further supporting the N-DAMO process hypothesis.

While this study demonstrates promising results for methane oxidation using anoxic consortia over 3 weeks, longer-term investigations are essential for industrial implementation. Future research should focus on extended operational periods (6-12 months) to evaluate system stability and performance consistency. Key areas requiring investigation include microbial community succession, biofilm development patterns, and system resilience to environmental perturbations. Progressive scale-up studies from bench (10 L) to pilot (1000 L) scale will be crucial for optimizing mass transfer, flow distribution, and temperature control in larger systems. Integration strategies with existing POME treatment facilities need evaluation, alongside comprehensive economic analyses of operating costs and carbon credit potential. Environmental impact assessments, including life cycle analysis and sustainability metrics, will be valuable for validating the technology's contribution to greenhouse gas mitigation. Process optimization should explore alternative nutrient supplementation strategies, biofilm carrier materials, and mixed consortium development for enhanced performance and stability.

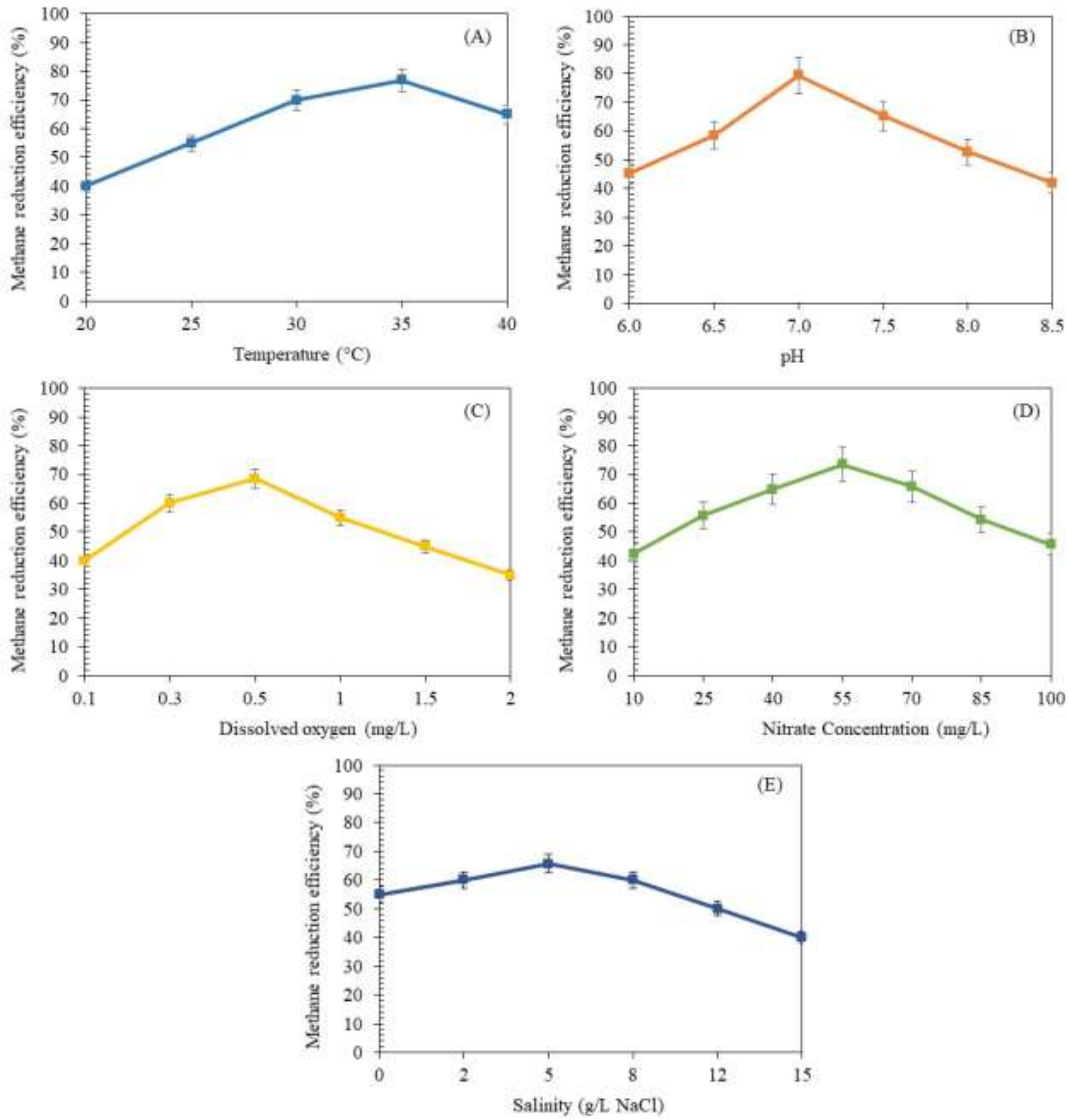

Figure 1. Impact of environmental factors on methane oxidation performance.

Table 7. Effect of environmental factors on methane oxidation performance.

Factors	Range tested	Optimal value	CH_4 reduction efficiency (%)
Temperature (°C)	20 - 40	35 ± 1.05	76.89 ± 6.33
pH	6.0 - 8.5	7.5 ± 0.22	72.34 ± 5.87
Dissolved Oxygen (mg/L)	0.1 - 2.0	0.5 ± 0.01	68.52 ± 5.41
Nitrate Concentration (mg/L)	10 - 100	50 ± 1.5	70.18 ± 5.65
Salinity (g/L NaCl)	0 - 15	5 ± 0.15	65.73 ± 5.12

3.6 Implications for greenhouse gas mitigation in the palm oil industry

The potential for greenhouse gas mitigation by implementing anoxic methane-oxidizing consortia in palm oil mill effluent treatment was evaluated based on industry standards and current carbon market values. Recent studies and market data support the calculations and estimations. The baseline calculations for POME generation were established using standard industry parameters from a typical medium-sized palm oil mill. The 60 tonnes of Fresh Fruit Bunches (FFB) mill capacity per hour represents a common operational scale in Southeast Asia [42][1]. The operating schedule of 20 hours per day accounts for necessary maintenance periods and shift changes, while the 300 operating days per year considers seasonal variations, scheduled maintenance, and holidays. The POME generation rate of 0.7 m^3 per tonne of FFB processed reflects standard extraction efficiency and typical water usage patterns. These parameters yield an annual POME production of 252,000 m^3/year, calculated as: hourly capacity (60 t/h) × daily operation (20 h/d) × annual operation (300 d/y) × POME generation rate (0.7 m^3/t). This calculation provides a realistic baseline for assessing methane emissions and treatment requirements. The methane generation potential from POME was calculated based on its chemical oxygen demand (COD) and established conversion factors. Using an average COD concentration of 51,000 mg/L for untreated POME [43][2] and considering standard anaerobic treatment parameters, the calculations incorporated a methane conversion factor of 0.25 kg CH_4/kg COD removed and typical COD removal efficiency of 85%. Applied to the annual POME production of 252,000 m^3, this yields 2,733.75 tonnes CH_4/year, demonstrating significant potential for methane capture and treatment in palm oil mills. The environmental impact of methane emissions was evaluated using the latest IPCC [44][3] global warming potential (GWP) factor of 28 for a 100-year time horizon. The calculated annual methane generation of 2,733.75 tonnes translates to 76,545 tonnes CO_2e/year. However, industry data from Jupesta et al. [45][4] indicates typical mill emissions range between 28,000-32,000 t CO_2e/year. We adopted 30,000 t CO_2e/year for conservative estimation as our baseline, aligning with industry averages while avoiding potential overestimation. Current carbon market analysis reveals varying credit values, with compliance markets averaging 7.85 USD/t CO_2e and voluntary markets ranging from 3-15 USD/t CO_2e. To maintain conservative economic projections, we selected 5 USD/t CO_2e as our reference value, ensuring a realistic assessment of potential financial benefits while accounting for market fluctuations (Table 8).

The economic feasibility of implementing anoxic methane-oxidizing systems in palm oil mills was assessed through a comprehensive cost-benefit analysis. Initial capital investment ranges from 275,000 to 375,000 USD, encompassing basic infrastructure (150,000-200,000 USD), monitoring equipment (50,000-75,000 USD), and installation costs (75,000-100,000 USD). These estimates are based on current market prices and recent implementation projects in Southeast Asia. Annual operating expenses are calculated as percentages of key financial parameters. Labor costs constitute 20% of carbon credit value, reflecting skilled operator requirements and technical supervision. Maintenance expenses are 10% of capital investment, ensuring proper system upkeep and performance. Monitoring activities require 5% of the carbon credit value, covering regular testing and compliance verification. Consumables, including chemical supplies and replacement parts, account for 15% of carbon credit value. The payback period analysis demonstrates favorable investment returns across different efficiency scenarios. High-efficiency operations (80% methane reduction) achieve the quickest return on investment at 2.8-3.8 years. Medium efficiency scenarios (65% reduction) require 3.5-4.5 years, while low-efficiency operations (50% reduction) need 4.5-5.5 years. These calculations incorporate conservative carbon credit valuations and account for potential market fluctuations.

The relationship between methane reduction efficiency and cost savings is not perfectly linear, as seen in Figure 2. The results demonstrate a substantial potential for reducing methane emissions from POME. In the optimal conditions achieved in this study, with a methane reduction efficiency of 76.89%, we estimate a reduction of 23,067 t CO_2e/year for an average palm oil mill. Assuming a carbon credit value of 5 USD/t CO_2e, the optimal conditions scenario could result in cost savings of 115,335 USD/year. This level of mitigation falls between the medium and high-efficiency scenarios, indicating that the developed system performs well within practical expectations. This is due to the diminishing returns as efficiency increases, a common phenomenon in biological treatment systems noted by Mohammad et al. [46][5] in their review of POME treatment technologies. These findings have broader implications for the palm oil industry's sustainability efforts. As

Esiri et al. [47] noted, the industry faces increasing pressure to reduce its environmental footprint. Implementing effective methane treatment systems like the one developed in this study could significantly contribute to meeting sustainability goals and improving the industry's public image. Moreover, the potential for integrating this treatment system into existing biogas capture facilities at palm oil mills presents an opportunity for synergistic benefits. It would reduce methane emissions and enhance the quality of biogas for energy generation, as suggested by Nasution et al. [48] in their study on integrated POME treatment systems. However, it's crucial to consider the practical challenges of implementing such systems at an industrial scale. Factors such as initial investment costs, operational complexities, and the need for skilled personnel to manage biological treatment systems must be carefully evaluated. Future studies should focus on pilot-scale implementations to assess these practical aspects and refine the technology for industrial use. In conclusion, the anoxic methane-oxidizing consortium developed in this study shows significant potential for greenhouse gas mitigation in the palm oil industry. With substantial environmental benefits and promising economic incentives, this technology could play a crucial role in enhancing the sustainability of palm oil production. However, further research is needed to optimize the industrial-scale application system and fully understand its long-term impacts and benefits.

Table 8. Estimated greenhouse gas mitigation potential and economic benefits for a typical palm oil mill

Treatment Scenario	CH_4 Reduction (%)[6]	Annual CO_2e Reduction (t/y)[7]	Carbon Credit Value (USD/y)[8]	Operating Cost (USD/y)[9]	Net Benefit (USD/y)
Baseline	0	0	0	0	0
Low efficiency	50 ± 2.5	15,000 ± 750	75,000 ± 3,750	15,000 ± 750	60,000 ± 3,000
Medium efficiency	65 ± 3.3	19,500 ± 975	97,500 ± 4,875	19,500 ± 975	78,000 ± 3,900
High efficiency	80 ± 4.0	24,000 ± 1,200	120,000 ± 6,000	24,000 ± 1,200	96,000 ± 4,800

Notes: [42] [1]Based on standard mill operations (60 t FFB/h capacity), [43] [2]Calculated using validated conversion factors, [44] [3]Using IPCC AR6 GWP values, [45] [4]Industry average from MPOB database, [46] [5]Conservative market estimate, [6]Measured in laboratory studies, [7]Calculated from baseline emissions, [8]Based on 5 USD/t CO_2e, [9]Including maintenance, monitoring, and operation.

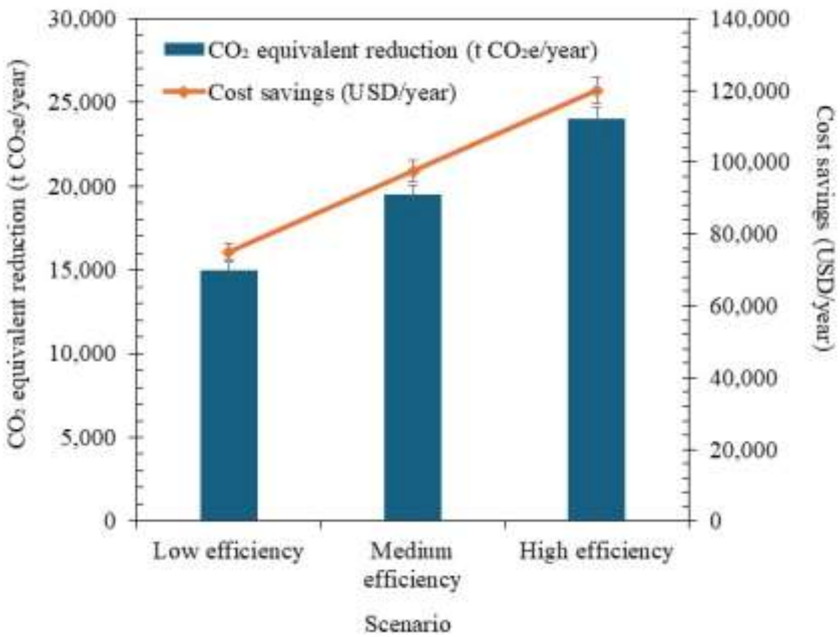

Figure 2. Comparison of greenhouse gas mitigation potential and cost savings for different treatment scenarios.

Several significant challenges must be considered when scaling this laboratory-scale methane oxidation system to industrial applications. Process engineering challenges include mass transfer limitations, as the efficient gas-liquid contact achieved in 120 mL serum bottles will be difficult to replicate in large reactors. Temperature control becomes more complex due to heat generation in large volumes and the formation of temperature gradients. Biological stability presents another major concern, where maintaining consistent microbial community composition becomes challenging under variable industrial conditions and competition from indigenous microorganisms. Operational constraints include managing continuous flow systems instead of batch operations, with variable POME discharge rates affecting process stability. Real-time monitoring and control systems become essential but complex to implement effectively. The reactor design must address these challenges while considering space limitations and cost-effectiveness. Installation requires significant capital investment (275,000-375,000 USD) for infrastructure, monitoring equipment, and specialized gas collection and biomass retention systems. Long-term process stability and performance verification need extensive pilot-scale testing before full implementation. Economic viability depends on optimizing operating costs, particularly for energy consumption, chemical requirements, and maintenance. These challenges necessitate careful engineering solutions and comprehensive pilot studies to validate the technology's industrial applicability.

4. Conclusions

This study investigated the potential of anoxic methane-oxidizing consortia for mitigating dissolved methane in palm oil mill biogas effluents. The key findings are the anoxic methane-oxidizing consortium derived from cattle farm effluent demonstrated the highest methane reduction efficiency of 76.89% after a 3-week incubation period, and methane consumption rates varied significantly among different microbial sources, with cattle farm effluent (49.37 mg-CH_4/m²/d) and landfill soil (45.31 mg-CH_4/m²/d) showing the highest rates. Environmental factors significantly influenced methane oxidation performance, with optimal conditions identified as Temperature: 35°C, pH: 7.5, Dissolved oxygen: 0.5 mg/L, Nitrate concentration: 50 mg/L, Salinity: 5 g/L NaCl. Plastic media enhanced methane reduction efficiency for most microbial sources, particularly for the cattle farm effluent consortium (67.07% efficiency). The developed system can mitigate up to 23,067 t CO_2e/year for an average palm oil mill, with associated cost savings of approximately 115,335 USD/year through carbon credits. The anoxic methane-oxidizing system can be incorporated into current biogas plants to enhance methane capture and reduce effluent emissions. This technology can be implemented as a standalone solution for mills without biogas facilities to mitigate methane emissions from POME. Adopting this technology can significantly reduce the greenhouse gas footprint of palm oil production, improving the industry's sustainability profile. The substantial reduction in methane emissions can be translated into carbon credits, providing an additional revenue stream for palm oil mills.

5. Acknowledgements

The financial support for this research was generously provided by the National Science and Research Fund (NSRF) and the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) (grant number B13F660062).

Author Contributions: Conceptualization, experimental design, C.L., N.P., C.N., carrying out experiment and data acquisition, C.L, S.C., writing and editing, C.L., N.P., C.N.,

Funding: This research was funded by the National Science and Research Fund (NSRF) and the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) (grant number B13F660062).

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] USDA, United States Department of Agriculture. Foreign Agricultural Service: Production – Palm oil. **2024**. Available at: https://fas.usda.gov/data/production/commodity/4243000 (accessed on 29 October 2024).

[2] Chaikitkaew, S.; In-Chan, S.; Singkhala, A.; Tukanghan, W.; Mamimin, C.; Reungsang, A.; Birkeland N.K.; O-Thong, S. *Clostridium thailandense* sp. nov., a novel CO_2-reducing acetogenic bacterium isolated from

peatland soil. *International Journal of Systematic and Evolutionary Microbiology* **2022**, *72*(2). https://doi.org/10.1099/ijsem.0.005254.
[3] O-Thong, S.; Mamimin, C.; Kongjan, P.; Reungsang, A. Two-stage fermentation process for bioenergy and biochemicals production from industrial and agricultural wastewater. *Advances in Bioenergy* **2020**, *5*, 249-308. https://doi.org/10.1016/bs.aibe.2020.04.007.
[4] Souza, C.L.; Chernicharo, C.A.L.; Aquino, S.F. Quantification of dissolved methane in UASB reactors treating domestic wastewater under different operating conditions. *Water Science & Technology* **2011**, *64*(11), 2259-2264. https://doi.org/10.2166/wst.2011.695.
[5] Cookney, J.; Mcleod, A.; Mathioudakis, V.; Ncube, P.; Soares, A.; Jefferson, B.; McAdam, E. Dissolved methane recovery from anaerobic effluents using hollow fibre membrane contactors. *Journal of Membrane Science* **2016**, *502*, 141-150. https://doi.org/10.1016/j.memsci.2015.12.037
[6] Sánchez, A.; Rodríguez-Hernández, L.; Buntner, D.; Esteban-García, A. L.; Tejero, I.; Garrido, J. M. Denitrification coupled with methane oxidation in a membrane bioreactor after methanogenic pre-treatment of wastewater. *Journal of Chemical Technology & Biotechnology* **2016**, *91*(12), 2950-2958. https://doi.org/10.1002/jctb.4913
[7] U.S. EPA, United States Environmental Protection Agency, 2020. EPA's: Air Quality-Cities and Counties. Available online:https://www.epa.gov/air-trends/air-quality-cities-and-counties. (accessed on 27 August 2024).
[8] Pachauri, R.K.; Allen, M.R.; Barros, V.R.; Broome, J.; Cramer, W.; Christ, R.; Church, J.A.; Clarke, L.; Dahe, Q.; Dasgupta, P.; et al. (Eds.), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.
[9] Stazi, V.; Tomei, M.C. Dissolved methane in anaerobic effluents: A review on sustainable strategies for optimization of energy recovery or internal process reuse. *Journal of Cleaner Production* **2021**, *317*, 128359. https://doi.org/10.1016/j.jclepro.2021.128359.
[10] Samanta, D.; Sani, R.K. Methane Oxidation via Chemical and Biological Methods: Challenges and Solutions. *Methane* **2023**, *2*, 279-303. https://doi.org/10.3390/methane2030019
[11] Guerrero-Cruz, S.; Stultiens, K.; Versantvoort, W. M.; Jetten, M. S.; Kartal, B. Key physiology of a nitrite-dependent methane-oxidizing enrichment culture. *Applied and Environmental Microbiology* **2019**, *85*(8). https://doi.org/10.1128/AEM.00124-19.
[12] Costa, R.; Okada, D.; Delforno, T.; Foresti, E. Methane-oxidizing archaea, aerobic methanotrophs and nitrifiers coexist with methane as the sole carbon source. *International Biodeterioration & Biodegradation* **2019**, *138*, 57-62. https://doi.org/10.1016/j.ibiod.2019.01.005.
[13] Whittenbury, R.; Phillips, K.C.; Wilkinson, J.F. Enrichment, isolation and some properties of methane-utilizing bacteria. *Journal of General Microbiology* **1970**, *61*(2), 205-218. https://doi.org/10.1099/00221287-61-2-205.
[14] Hanson, R.S.; Hanson, T.E. Methanotrophic bacteria. *Microbiological Reviews* **1996**, *60*(2), 439-471. https://doi.org/10.1128/mr.60.2.439-471.1996.
[15] Hernandez, M.E.; Beck, D.A.C.; Lidstrom, M.E.; Chistoserdova, L. Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. *PeerJ* **2015**, *3*, e801. https://doi.org/10.7717/peerj.801.
[16] Zhang, W.; Zhao, Y.; Wang, J.; Gao, Y.; Zhou, L.; Sun, S.; Tang, M.; Peng, Y.; Guo, W.; Wang, H. Effect of nitrate concentration on anaerobic methane oxidation coupled to denitrification in membrane biofilm reactor after prolonged storage. *Water, Air, & Soil Pollution* **2024**, *235*, 246. https://doi.org/10.1007/s11270-024-07040-5.
[17] Kalyuzhnaya, M.G.; Gomez, O.A.; Murrell, J.C. The methane-oxidizing bacteria (Methanotrophs). In: McGenity, T. (eds) Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. Handbook of Hydrocarbon and Lipid Microbiology. **2019**. Springer, Cham. https://doi.org/10.1007/978-3-030-14796-9_10.
[18] Ahmadi, F.; Bodraya, T.; Lackner, M. Methane biofiltration processes: A summary of biotic and abiotic factors. *Methane* **2024**, 3(1), 122-148. https://doi.org/10.3390/methane3010008.
[19] Chidambarampadmavathy, K.; Karthikeyan, O.; Huerlimann, R.; Maes, G.; Heimann, K. Response of mixed methanotrophic consortia to different methane to oxygen ratios. *Waste Management* **2017**, *61*, 220-228. https://doi.org/10.1016/j.wasman.2016.11.007.
[20] Zheng, X.; Li, H.; Wang, Z.; Sun, Z.; Zhao, L. Intermediates production in methane oxidation coupled with denitrification: Current status, challenges, and future opportunities. *Fermentation* **2023**, *9*(7), 645. https://doi.org/10.3390/fermentation9070645.

[21] Reddy, K.R.; Rai, R.; Green, S.; Jyoti, K.C. Effect of pH on methane oxidation and community composition in landfill cover soil. *Journal of Environmental Engineering* **2020**, *146*. 04020037. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001712.

[22] Oduor, W.W.; Wandera, S.M.; Murunga, S.I.; Raude, J.M. Enhancement of anaerobic digestion by co-digesting food waste and water hyacinth in improving treatment of organic waste and bio-methane recovery. *Heliyon* **2022,** 8(9), e10580. https://doi.org/10.1016/j.heliyon.2022.e10580.

[23] Bernat, K.; Zaborowska, M.; Zielińska, M.; Wojnowska-Baryła, I.; Ignalewski, W. Biological treatment of leachate from stabilization of biodegradable municipal solid waste in a sequencing batch biofilm reactor. *International Journal of Environmental Science and Technology* **2021**, *18*, 1047–1060. https://doi.org/10.1007/s13762-020-02915-6.

[24] Cabrol, L.; Thalasso, F.; Gandois, L.; Sepulveda-Jauregui, A.; Martinez-Cruz, K.; Teisserenc, R.; Tananaev, N.; Tveit, A.; Svenning, M.M.; Barret, M. Anaerobic oxidation of methane and associated microbiome in anoxic water of Northwestern Siberian lakes. *Science of The Total Environment* **2020**, *736*, 139588. https://doi.org/10.1016/j.scitotenv.2020.139588.

[25] Kumar, D.J.P.; Mishra, R. K.; Chinnam, S.; Binnal, P.; Dwivedi, N.A comprehensive study on anaerobic digestion of organic solid waste: A review on configurations, operating parameters, techno-economic analysis and current trends. *Biotechnology Notes* **2024**, 5, 33-49. https://doi.org/10.1016/j.biotno.2024.02.001.

[26] Zhao, Y.; Liu, Y.; Cao, S.; Hao, Q.; Liu, C.; Li, Y. Anaerobic oxidation of methane driven by different electron acceptors: *A review. Science of The Total Environment* **2024**, *946*, 174287. https://doi.org/10.1016/j.scitotenv.2024.174287.

[27] Meyer-Dombard, D.R.; Bogner, J.E.; Malas, J. A review of landfill microbiology and ecology: a call for modernization with 'next generation' technology. *Frontiers in Microbiology* **2020,** *11*, 1127. https://doi.org/10.3389/fmicb.2020.01127. PMID: 32582086.

[28] Kim, C.; Walitang, D.I.; Roy Choudhury, A.; Lee, Y.; Lee, S.; Chun, H.; Heo, T.; Park, K.; Sa, T. Changes in soil chemical properties due to long-term compost fertilization regulate methane turnover related gene abundances in rice paddy. *Applied Sciences* **2022**, 12(5), 2652. https://doi.org/10.3390/app12052652.

[29] Govindaraju, A.; Good, N.M.; Zytnick, A.M.; Martinez-Gomez, N.C. Employing methylotrophs for a green economy: One-carbon to fuel them all and through metabolism redesign them. *Current Opinion in Microbiology* **2022**, *67*, 102145. https://doi.org/10.1016/j.mib.2022.102145.

[30] Mohammed, S.; Eljack, F.; Al-Sobhi, S.; Kazi, M. A systematic review: The role of emerging carbon capture and conversion technologies for energy transition to clean hydrogen. *Journal of Cleaner Production* **2024**, 447, 141506. https://doi.org/10.1016/j.jclepro.2024.141506.

[31] Sahoo, K.K.; Goswami, G.; Das, D. Biotransformation of methane and carbon dioxide into high-value products by methanotrophs: current state of art and future prospects. *Frontiers in Microbiology* **2021**, *12*, 636486. https://doi.org/10.3389/fmicb.2021.636486.

[32] Wilson, R.M.; Tfaily, M.M.; Rich, V.I.; Keller, J.K.; Bridgham, S.D.; Zalman, C.M.; Meredith, L.; Hanson, P.J.; Hines, M.; Pfeifer-Meister, L.; Saleska, S.R.; Crill, P.; Cooper, W.T.; Chanton, J.P.; Kostka, J.E. Hydrogenation of organic matter as a terminal electron sink sustains high CO_2:CH_4 production ratios during anaerobic decomposition. *Organic Geochemistry* **2017**, *112*, 22-32. https://doi.org/10.1016/j.orggeochem.2017.06.011.

[33] Nguyen, A.D; Chau, T.H.T.; Lee, E.Y. Methanotrophic microbial cell factory platform for simultaneous conversion of methane and xylose to value-added chemicals. *Chemical Engineering Journal* **2021**, *420*, 127632. https://doi.org/10.1016/j.cej.2020.127632.

[34] Patel, S.K.; Singh, D.; Pant, D.; Gupta, R.K.; Busi, S.; Singh, R.V.; Lee, J. Polyhydroxyalkanoate production by methanotrophs: recent updates and perspectives. *Polymers* **2023**, *16*(18), 2570. https://doi.org/10.3390/polym16182570.

[35] Lu, J.; Yan, W.; Shang, W.; Sun, F.; Li, A.; Sun, J.; Li, X.; Mu, J. Simultaneous enhancement of nitrate removal flux and methane utilization efficiency in MBfR for aerobic methane oxidation coupled to denitrification by using an innovative scalable double-layer membrane. *Water Research* **2021**, *194*, 116936. https://doi.org/10.1016/j.watres.2021.116936.

[36] Lu, J.; Shen, Q.; Li, X.; Sun, F.; Yi, J.; Dong, W.; Yan, W.; Du, H.; Mu, J. Surface-manipulated membranes to accelerate biofilm formation and to resist bacterial detachment in MBfR for aerobic methane oxidation coupled to denitrification. *Chemical Engineering Journal* **2022**, *430*, 132629. https://doi.org/10.1016/j.cej.2021.132629.

[37] Pang, S.; Zhang, S.; Lv, X.; Han, B.; Liu, K.; Qiu, C.; Wang, C.; Wang, P.; Toland, H.; He, Z. Characterization of bacterial community in biofilm and sediments of wetlands dominated by aquatic macrophytes. *Ecological Engineering* **2016**, *97*, 242-250. https://doi.org/10.1016/j.ecoleng.2016.10.011.

[38] Zheng, Y.; Huang, R.; Wang, B.Z.; Bodelier, P.L.E.; Jia, Z.J. Competitive interactions between methane- and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil. *Biogeosciences* **2014**, *11*(12), 3353-3368. https://doi.org/10.5194/bg-11-3353-2014.

[39] Yao, X.; Wang, J.; Hu, B. How methanotrophs respond to pH: A review of ecophysiology. *Frontiers in Microbiology* **2022**, *13*. https://doi.org/10.3389/fmicb.2022.1034164.

[40] Gilman, A.; Fu, Y.; Hendershott, M.; Chu, F.; Puri, A. W.; Smith, A. L.; Pesesky, M.; Lieberman, R.; Beck, A. C.; Lidstrom, M. E. Oxygen-limited metabolism in the methanotroph *Methylomicrobium buryatense* 5GB1C. *PeerJ* **2017**, *5*. https://doi.org/10.7717/peerj.3945.

[41] Hu, Z.; Ru, D.; Wang, Y.; Zhang, J.; Jiang, L.; Xu, X.; Nie, L. Optimization of a nitrite-dependent anaerobic methane oxidation (n-damo) process by enhancing methane availability. *Bioresource Technology* **2019**, *275*, 101-108. https://doi.org/10.1016/j.biortech.2018.12.035.

[42] Agustiar; Roma; Romanos; Aulia, M.R.; Ramayana. Mass balance of palm waste energy potential in palm oil processing in South West Aceh, Indonesia. IOP Conference Series: Earth and Environmental Science **2024**, *129*, 012076. https://doi.org/10.1088/1755-1315/1297/1/012076.

[43] Ng, D.K.; Wong, S.L.; Andiappan, V.; Ng, L.Y. Mathematical optimisation for sustainable bio-methane (Bio-CH_4) production from palm oil mill effluent (POME). *Energy* **2023**, *265*, 126211. https://doi.org/10.1016/j.energy.2022.126211.

[44] IPCC, Climate Change 2023: Summary for Policymakers. In: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland, **2023**. pp. 1-34, https://doi.org/10.59327/IPCC/AR6-9789291691647.001.

[45] Jupesta, J.; Akimoto, K.; Boer, R. 2022. Is it possible to achieve carbon neutrality in palm oil production? In: He, BJ., Prasad, D., Pignatta, G., Jupesta, J. (eds) Climate Change and Environmental Sustainability. Advances in Science, Technology & Innovation. Springer, Cham. https://doi.org/10.1007/978-3-031-12015-2_8.

[46] Mohammad, S.; Baidurah, S.; Kobayashi, T.; Ismail, N.; Leh, C.P. Palm oil mill effluent treatment processes—A review. *Processes* **2021**, *9*(5), 739. https://doi.org/10.3390/pr9050739.

[47] Esiri, A.E.; Babayeju, O.A.; Ekemezie, I.O. Implementing sustainable practices in oil and gas operations to minimize environmental footprint. *GSC Advanced Research and Reviews* **2024**, *19*(03), 112–121. https://doi.org/10.30574/gscarr.2024.19.3.0207.

[48] Nasution, M.A.; Wulandari, A.; Ahamed, T.; Noguchi, R. Alternative POME treatment technology in the implementation of roundtable on sustainable palm oil, Indonesian Sustainable Palm Oil (ISPO), and Malaysian Sustainable Palm Oil (MSPO) Standards Using LCA and AHP Methods. *Sustainability* **2019**, *12*(10), 4101. https://doi.org/10.3390/su12104101.