



Evaluating the Influence of Transition Metal Oxides on Anaerobic Digestion Performance

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Citation:

Wardani, A.N.; Amalia, D.; Ramadhan, R.M.; Jaya, D.; Widayati, W.T.; Nursubiyantoro, E.; Putra, A.R.; Khaliq, A.M.; Najla, S.Q.; Syailendra, R.N. Evaluating the influence of transition metal oxides on anaerobic digestion performance. *ASEAN J. Sci. Tech. Report.* **2025**, 28(4), e257685. <https://doi.org/10.55164/ajstr.v28i4.257685>.

Article history:

Received: January 28, 2025

Revised: May 25, 2025

Accepted: June 11, 2025

Available online: June 30, 2025

Publisher's Note:

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Abstract: Palm oil, the world's most widely consumed edible oil, produces palm oil mill effluent (POME) as a byproduct, which poses significant environmental risks if untreated due to its high organic content and pollutants. Anaerobic digestion (AD) is a process that converts organic waste into biogas, a promising renewable and sustainable energy source, especially for areas with abundant feedstock. Accelerators play a vital role in enhancing the performance of AD systems through various mechanisms. The high conductivity of TMOs facilitates efficient electron transfer, providing the fastest pathway for electron exchange between microorganisms. MnO_2 and Fe_2O_3 are abundantly available in Indonesia. This study compared MnO_2 and Fe_2O_3 to identify the most effective TMO for improving mesophilic batch AD performance in POME treatment. Results indicated that Fe_2O_3 was superior, increasing methane production volume by 21% and methane yield by 32% compared to AD without TMOs.

Keywords: Accelerator; Electron transport; Biogas; Renewable energy; Waste treatment

1. Introduction

Palm oil is the most widely demanded edible oil globally [1]. Indonesia ranks as the world's leading exporter of palm oil. The land area dedicated to oil palm plantations and the production of crude palm oil (CPO) experienced significant growth in 2018 compared to previous years. By 2022, the total area of oil palm plantations was estimated to have reached 15.34 million hectares, as illustrated in Figure 1 [2]. Each hectare of oil palm yields 10–35 tons of fresh fruit bunches (FFB) annually, indicating that in 2022, Indonesia produced approximately 153.4–536.9 million tons of FFB [3]. The production of palm oil generates a byproduct known as palm oil mill effluent (POME) during its extraction process. For every ton of FFB processed in palm oil mills, the resulting waste comprises 23% empty fruit bunch fibers, 12% mesocarp fibers, 5% shells, and 60% POME [4]. These proportions indicate that POME constitutes the most significant fraction of waste in the palm oil industry, amounting to

approximately 92.04–322.14 million tons in Indonesia in 2022. The wet extraction process of palm oil requires a substantial volume of water, with about 1.5 m³ of water used per ton of FFB processed [5]. Of the estimated 5.0–7.5 tons of water needed to produce one ton of crude palm oil, more than 2.5–3.75 tons are converted into POME [6], [7]. According to A. A. Z. Lorestani [8], the processes contributing the most to POME generation are FFB sterilization (36%), crude palm oil extraction and clarification (60%), and the separation of kernels and shells in hydrocyclones (4%).

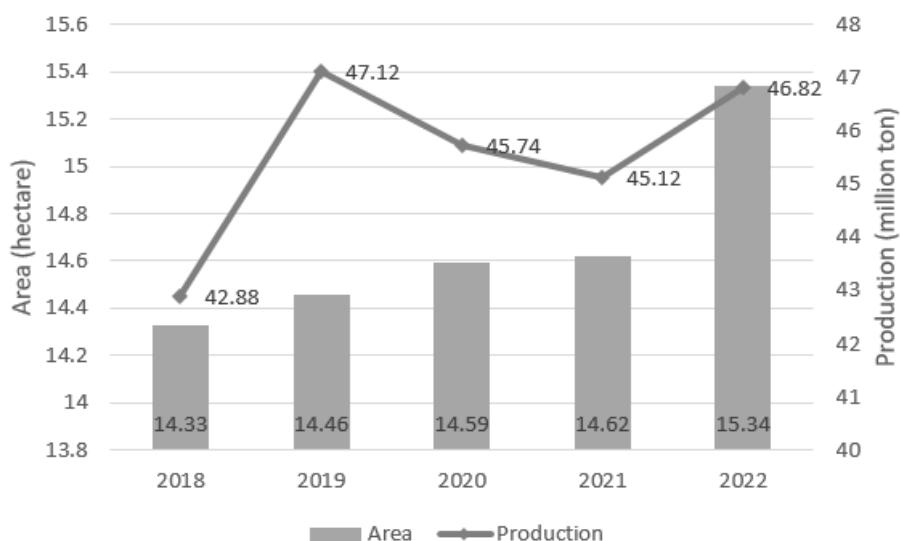


Figure 1. Palm oil plantation area in Indonesia [2]

If left untreated, industrial waste from POME poses a significant environmental risk due to its high concentration of organic matter and other pollutants, which can harm both fauna and flora, as well as compromise water quality. Exposure to POME has been linked to reduced plankton diversity and physiological and reproductive issues in fish [9]. Additionally, it can severely impact aquatic ecosystems by creating highly acidic conditions or triggering eutrophication, characterized by excessive algal growth on water surfaces. Traditional POME treatment methods typically employ anaerobic-aerobic lagoon systems, comprising at least two sequentially connected ponds, to reduce the organic content before discharge into surface waters. However, these systems face limitations, including solid accumulation, methane emissions, sludge and foam formation (which decrease treatment efficiency), and the requirement for large land areas. When POME is stored in open-air holding ponds for remediation, it releases methane, carbon dioxide, and hydrogen sulfide, contributing to global climate change [3].

The anaerobic digestion (AD) process converts organic waste into biogas, a promising renewable and sustainable energy source, particularly in regions with abundant feedstock. AD can be utilized for various organic materials, including agricultural waste, the organic fraction of municipal solid waste, sewage sludge, and industrial waste. POME has also been investigated as a potential substrate for AD systems due to its high organic content. Figure 2 shows the AD process scheme. The AD process involves several sequential microbial stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During methanogenesis, the collaborative interaction among diverse microorganisms is essential for efficient digestion, relying on effective interspecies electron transfer [10]. The process begins with fermentation, establishing a complex network of interspecies electron transfer to sustain cooperative microbial activity. Within this network, electron exchange between syntrophic bacteria (secondary fermenting bacteria) and methanogens is a critical step, addressing the intermediate bottleneck and ensuring the successful completion of final methanogenesis.

Accelerators play a vital role in enhancing the performance of AD systems through various mechanisms. These accelerators can be categorized into several types, including biological accelerators (such as enzymes, microbial consortia, and fungi), chemical reagents, macronutrients, minerals, trace elements, transition metal oxides (TMOs), and carbon-based materials [11], [12]. Under natural conditions, Direct Interspecies Electron Transport (DIET) occurs only through direct physical contact between bacteria and

methanogens. However, the addition of TMOs to AD systems eliminates the requirement for direct contact due to their conductive properties [13]. The high conductivity of TMOs facilitates efficient electron transfer, providing the fastest pathway for electron exchange between microorganisms [12], [14]. MnO_2 and Fe_2O_3 are abundantly available in Indonesia. This study aims to compare Fe_2O_3 and MnO_2 to determine the most effective TMO for enhancing the AD process in POME treatment.

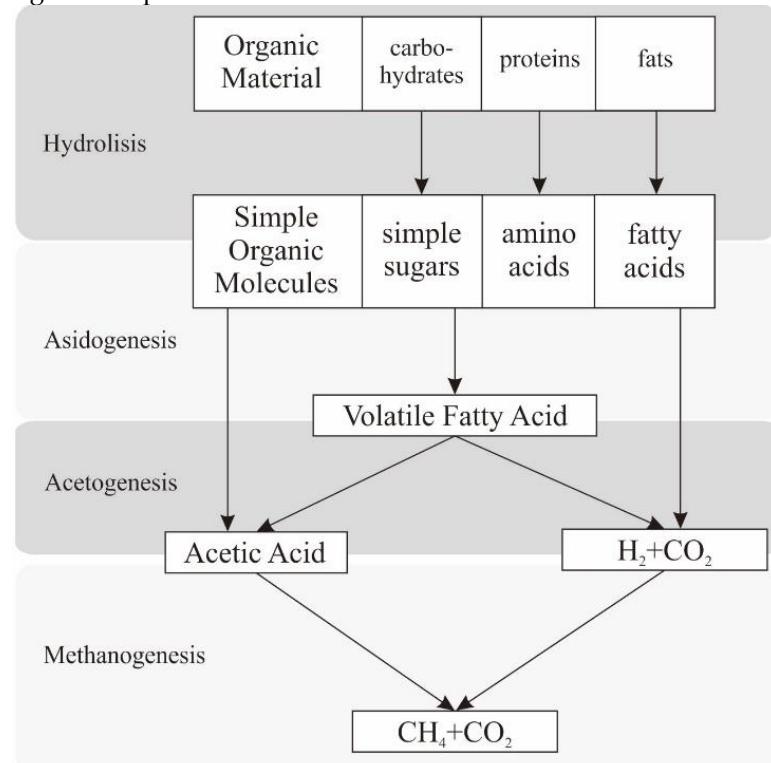


Figure 2. Anaerobic Digestion Scheme

2. Materials and Methods

2.1 Inoculum

The inoculum used in this research was digested cow manure (DCM) obtained from a biodigester at a cattle farm in Hargobinangun, Kaliurang, Yogyakarta, Indonesia. The inoculum was filtered to remove impurities and prevent clogging. The COD concentration of the inoculum was analyzed (with a measured value of 62,000 mg COD/L), and the inoculum was used on the same day the reactor was started.

2.2 Substrate

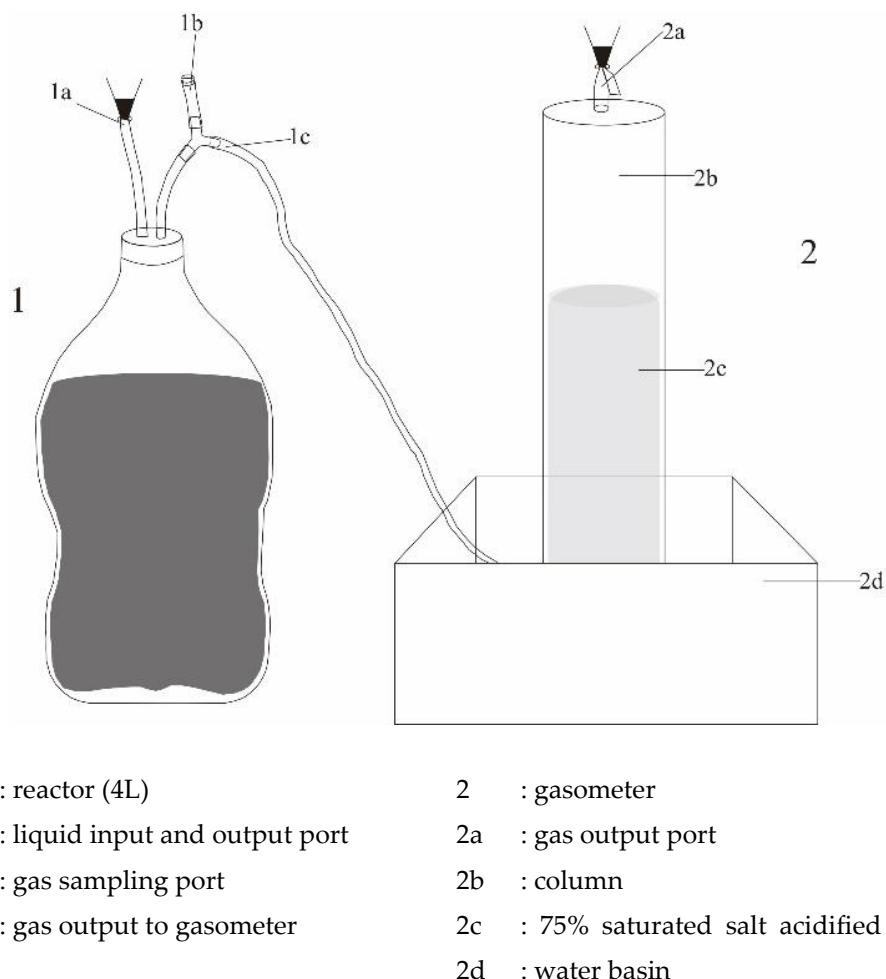
POME was used as the substrate for anaerobic digestion in this study. It was collected from a palm oil mill in Riau, Indonesia. To remove impurities, the POME was filtered using a 2 mm pore filter. The COD concentration of the POME was analyzed to determine the precise mixing ratio between the inoculum and the substrate. Table 1 presents the physical and chemical properties of the POME. The pH of the POME in this research was 4.5, indicating an acidic nature, which is typical for raw POME and aligns with the values reported by Saelor [15] (4.68 ± 0.27) and Suksong [16] (5.6). The COD concentration observed in this study was significantly higher (81,000 mg/L) compared to the values reported by Saelor [15] and Suksong [16]. The carbohydrate content in this research was 0.91%, equivalent to 73.71 g/L, which was also significantly higher than the values reported by Saelor [15] and Suksong [16]. The variation of both COD and carbohydrate may be attributed to differences in the palm oil milling process or feedstock composition [17].

Table 1. Physical and Chemical Properties of POME

Properties	This Research	[15]	[16]
pH	4.5	4.68 ± 0.27	5.6
COD (mg/L)	81000	42550 ± 6140	44000
C/N ratio	n/a	27.59	22.85
Carbohydrate	0.91%	9.00 ± 0.01 g/L	14.11 g/L

2.3 Reactor

Batch reactors with a working volume of 4 liters were used in this study. The reactors were operated at room temperature (27 °C). Each reactor was connected to a gasometer, based on the principle of water displacement. The gasometer was filled with a 75% salt-saturated solution at pH 2 to prevent gas absorption into the water [18]. Figure 3 illustrates the reactor setup used in this study.

**Figure 3.** Reactor Scheme in This Research

2.4 Experimental Part

2.4.1 Anaerobic Reactor Start Up

All reactors underwent a leakage test before use. The inoculum and substrate were mixed at an inoculum-to-substrate ratio of 4:1 (%COD). After mixing, the mixture was divided into 4-liter batches. The first two batches were loaded into two identical reactors without the addition of any TMO, serving as the control (RC). The second two batches were mixed with Fe_2O_3 (60 mg/L) until homogeneous and then loaded into two identical reactors (RFe). Similarly, the last two batches were mixed with MnO_2 (60 mg/L) and loaded

into two identical reactors (RMn). Each reactor was flushed with nitrogen to eliminate oxygen from the sludge and headspace. Following nitrogen flushing, each reactor was connected to a gasometer.

2.4.2 Anaerobic Reactor Operation

The batch reactors were operated under mesophilic conditions at 27 °C. Gas production volume was measured daily using the gasometer tube scale and basin scale [18]. The pH was maintained within the range of 7.0–7.5. If the pH dropped to 7.0 or below, 1 M NaOH was added to the reactor to adjust the pH.

2.4.3 Gas Analysis

Gas samples were collected weekly to analyze their CH₄ and CO₂ content. The analysis was performed using a Shimadzu GC-8A gas chromatograph equipped with a thermal conductivity detector (GC-TCD), manufactured in Japan.

2.4.4 Liquid Analysis

Liquid samples were collected twice a week. COD and sCOD analyses were conducted using the titrimetric method [19], while VFA analysis was performed using the distillation method [20].

3. Results and Discussion

3.1. Proximate Analysis

Proximate analysis was performed to characterize the substrate and inoculum. Table 2 presents the proximate analysis results for POME and DCM.

Table 2. Proximate Analysis Result of POME and DCM

Compound	POME	DCM
Protein	0.66% ± 0.01%	0.58% ± 0.03%
Lipid	0.85% ± 0.01%	0.08% ± 0.00%
Carbohydrate	0.91% ± 0.06%	0.84% ± 0.01%
Water	97.09% ± 0.06%	97.97% ± 0.01%
Ash	0.45% ± 0.42%	0.54% ± 0.03%

3.2 Biogas Analysis

Three reactors were used in this study: RC (control reactor), RFe (AD reactor with Fe₂O₃ addition), and RMn (AD reactor with MnO₂ addition). Figure 4 illustrates the biogas production of all three reactors. Two distinct peaks were observed, occurring around day 1 and day 9, which are associated with the degradation of carbohydrates and subsequently of complex macromolecules such as crude proteins, lignocelluloses, and aromatics. As reported by Yun [12], these complex compounds generally decompose more slowly compared to carbohydrates. Additionally, POME contains 32,505–36,894 ppm of long-chain fatty acids (LCFA), and the hydrolysis of lipids has been identified as the rate-limiting step in the anaerobic digestion of POME, which may explain why the second peak was more pronounced than the first [21]. Biogas production decreased significantly after Day 21. In line with this, cumulative methane production began to level off as shown in Figure 5. The lag phase lasted less than one day, and approximately 90% of the methane was produced by Day 12, indicating that the substrate was readily biodegradable [22]. RFe achieved the highest methane production, reaching 793.4 mL CH₄, which was 21% higher than that of the control reactor (RC). In contrast, RMn produced 7.6% less methane than the RC. Methane production volume was measured under controlled conditions at 25 °C and 1 atm.

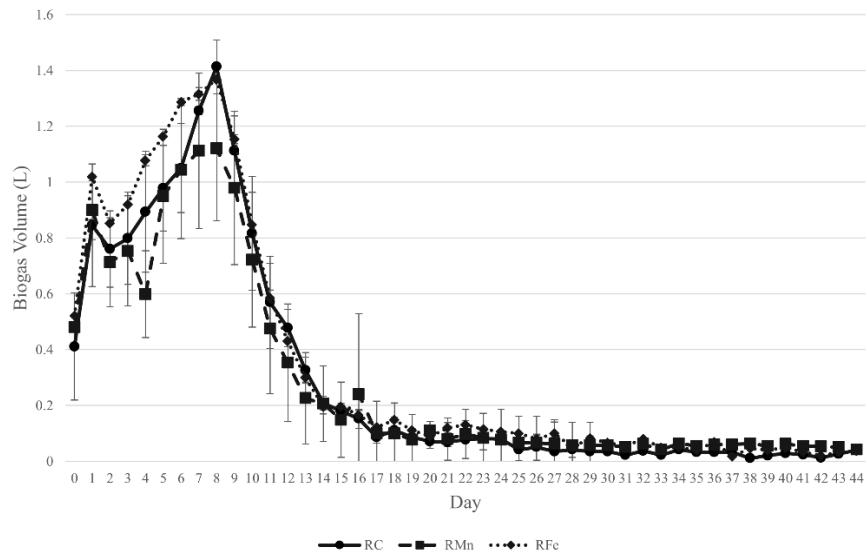


Figure 4. Biogas Production

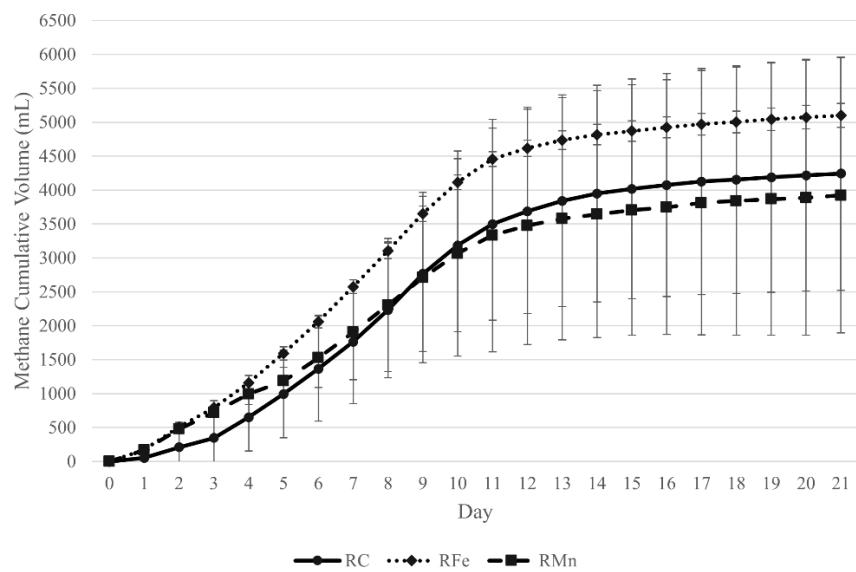


Figure 5. Cumulative Methane Production

3.3 Liquid Analysis

Figure 6 shows the volatile fatty acid (VFA) concentrations of all reactors, which remained stable and predominantly below 1,000 mg/L. VFAs are intermediate compounds in the AD process generated from acidogenesis and acetogenesis. However, VFAs accumulation can lower pH, becoming toxic to methanogens [23]. The optimal concentration of organic acids is less than 1,000 mg/L, with propionic acid levels below 200 mg/L [24]. All reactors experienced a sharp decline in VFA concentrations on Day 10, corresponding to the peak methane production on Day 9. This indicates high methanogen activity, as large amounts of VFAs were consumed and converted into methane.

Among the reactors, RMn had the lowest VFA concentration, yet its methane production was the lowest. Conversely, RFe exhibited the highest VFA concentration on Day 10 and also achieved the highest methane production, suggesting efficient acidogenic and acetogenic activity. After the peak methane production, VFA levels returned to their regular concentrations. On Day 21, when methane production was significantly reduced, RFe had the lowest VFA concentration.

At the beginning of the process, the VFA concentration in all reactors was 1,079 mg/L. By Day 21, VFA levels remained consistent across all reactors, indicating stable systems. The pH profile, shown in Figure 7, reflects this stability. Due to steady VFA levels, the pH remained stable in all reactors throughout the process. Figure 8 presents the profiles of COD and sCOD. Both COD and sCOD concentrations declined over time, with no accumulation of sCOD, indicating that the rates of acidogenesis and acetogenesis were higher than hydrolysis. On Day 10, when methane production peaked and VFA concentrations dropped, sCOD levels remained stable. This stability suggests that the reduction in VFA was due to enhanced methanogenic activity, with no inhibition of acidogenesis or acetogenesis. Similar trends of declining VFA during peak methane production have been observed in other studies [25], [26]. The type of substrate influences the rate-limiting step in anaerobic digestion. The observed trends in VFA and sCOD concentrations indicate that acidogenesis, acetogenesis, and methanogenesis proceeded rapidly. However, due to the high content of long-chain fatty acids (LCFA) in POME, lipid hydrolysis was identified as the rate-limiting step, as supported by these patterns [11], [21]. Figure 9 illustrates the percentage of COD removal for all reactors. The reactor without TMO (control reactor) exhibited the highest COD removal efficiency at 55.4%, compared to 50.6% for RFe and 43.7% for RMn.

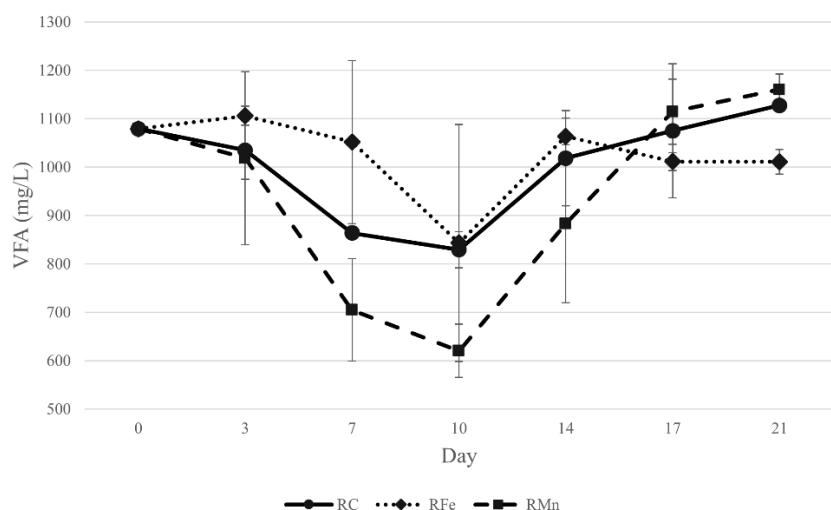


Figure 6. VFA Concentration Profile

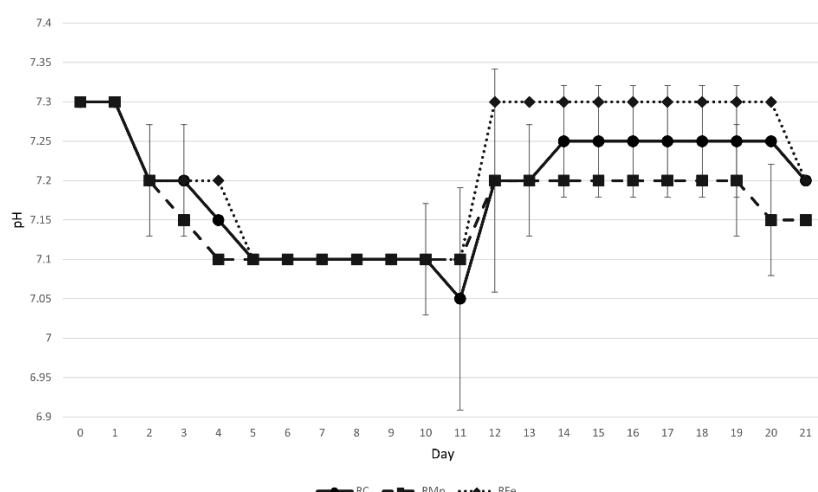


Figure 7. pH Profile

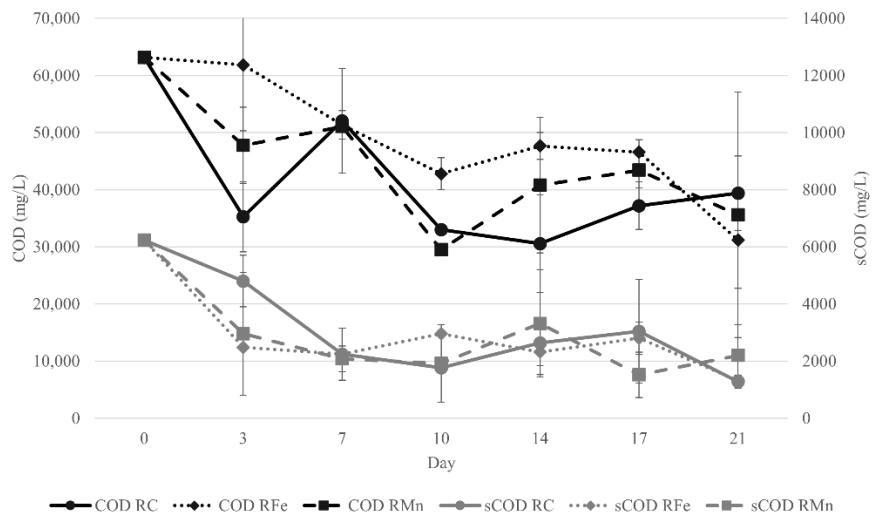


Figure 8. COD and sCOD Profile

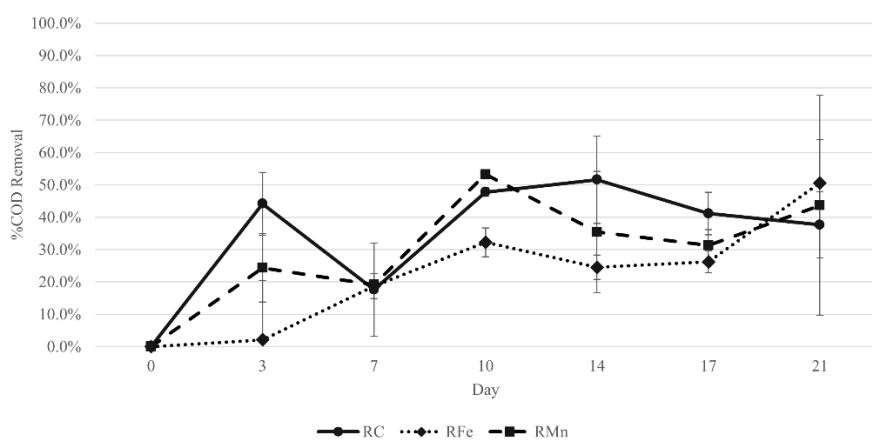


Figure 9. COD Removal Profile

3.4 TMO Effect Analysis

All reactors exhibited similar trends, but the effectiveness of the TMOs can be evaluated based on several parameters, as summarized in Table 3. While RC showed the highest COD removal, its methane production was relatively unsatisfactory. The methane yield of RFe, at $0.0391 \text{ mL CH}_4/\text{mg COD}$, was 32% higher than that of RC. Although RMn had lower methane production and %COD removal, its methane yield was 17.2% higher than RC. Based on these parameters, Fe_2O_3 was identified as the most effective TMO for anaerobic digestion. A study conducted by Tian (2019) [27] reported a 21.7% increase in methane production volume using MnO_2 as a TMO in AD. In another study, Kokdemir Ünsar and Perendeci (2018) [28], observed a 28.9% increase in methane production volume in AD with Fe_2O_3 addition. These findings align with the results of this study, confirming that Fe_2O_3 is more effective than MnO_2 .

Table 3. Anaerobic Digestion Performance of All Reactors

No	Reactor	Methane Production (mL)	%COD removal	Methane Yield (mL/g COD)
1	RC	4153.5	55.4%	0.0297
2	RFe	5003.2	50.6%	0.0391
3	RMn	3839.2	43.7%	0.0348

As shown in Table 3, RC exhibited higher methane production than RMn, although the methane yield was lower. A similar phenomenon was reported by Chaiprapat [29], where, at a cycle time of 24 hours, the biogas production volume, methane concentration, and methane yield were 3.87 L gas/L wastewater, 44.9%, and 0.02 L CH₄/g TCOD removed, respectively. In contrast, at a 12-hour cycle time, the respective values were 2.55 L gas/L wastewater, 35.8%, and 0.12 L CH₄/g TCOD removed. Chaiprapat found that although methane production was higher at a 24-hour cycle time, the percentage of total chemical oxygen demand (TCOD) removed was 14.1%. In contrast, a higher percentage of TCOD removal, 16.4%, was achieved at the shorter cycle time of 12 hours, despite lower methane production. A similar trend is observed in Table 3, where RC showed the highest %COD removal, yet lower methane production than RFe. This could be attributed to the formation of other gases such as hydrogen. A study by Abdurahman [30], [31] also reported lower methane yield associated with higher %COD removal, supporting this observation.

4. Conclusions

The addition of TMOs to the AD process can enhance its performance. Fe₂O₃ has the potential to improve both methane production volume and methane yield in the AD process when POME is used as the feedstock. Fe₂O₃ could serve as an effective accelerator for the AD process, supporting the palm oil industry.

5. Acknowledgements

The author would like to thank Lembaga Penelitian dan Pengabdian Kepada Masyarakat, Universitas Pembangunan Nasional Veteran Yogyakarta (contract number 121/UN62.21/DT.07.00/2024) for funding this research. This work was also supported by a grant from the Directorate of Research, Technology and Community Service - Directorate General of Higher Education, Research and Technology – Ministry of Education, Culture, Research and Technology, following Research Contract Number: 080/E5/PG.02.00.PL/2024.

Author Contributions: Conceptualization, N.A.W., M.R.R.; methodology, N.A.W., M.R.R, D.J., T.W.W., and E.N.; validation, N.A.W., and M.R.R.; formal analysis, N.A.W., M.R.R., and D.A., and.; investigation, N.A.W., and D.A.; resources, N.A.W., and D.A.; data curation, N.A.W., M.A.K., R.A.P., Q.S.N., and N.R.S.; writing-original manuscript preparation, N.A.W., M.R.R, D.A.; visualization, N.A.W., M.A.K., R.A.P., Q.S.N., and N.R.S.; supervision, N.A.W., D.A., M.R.R, D.J., T.W.W., and E.N.; funding acquisition, N.A.W., M.R.R. All authors have read and agreed the manuscript.

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

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