A Comparative Study on the Effects of Co-Digesting Molasses with Palm Oil Mill Effluent (POME) or Chicken Manure on Biomethane Yield

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Abstract. This study evaluates the performance of molasses (MS) co-digested with palm oil mill effluent (POME) and chicken manure (CM) to enhance methane production. The co-digestion of MS and CM at an optimum ratio of 7:1 yielded the highest biogas (520.2 mL/g VS) and methane (450.0 mL CH_4/g VS), with a methane content of 86.5%, significantly outperforming the co-digestion of MS and POME, which achieved a maximum methane yield of 208.4 mL CH_4/g VS and a methane content of 61.1%. Kinetic modelling using the modified Gompertz equation demonstrated a strong correlation between predicted and observed results, with R^2 values ranging from 0.986 to 0.996, confirming the model's reliability for predicting biogas yields. The MS and CM combination also exhibited faster biogas production initiation, shorter lag phases, and more excellent process stability, attributed to the balanced carbon-to-nitrogen ratio and CM's buffering capacity. These findings highlight the superior performance of the MS and CM combination in anaerobic co-digestion, offering a more efficient approach to biogas production compared to MS and POME. This research contributes to optimising co-digestion systems and provides a foundation for the scaling up of bioreactor designs, enhancing renewable energy production from organic waste.

1 Introduction

The growing demand for renewable energy highlights the importance of anaerobic digestion (AD) for biomethane production. Co-digestion enhances AD by optimizing the carbon-to-

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nitrogen (C/N) ratio, pH, and trace element availability while diluting toxic compounds. Molasses (MS), a sugar-rich by-product, is promising for AD due to its high biodegradability. However, its high chemical oxygen demand (COD) and ion concentration may limit its performance unless co-digested with substrates such as POME and CM. CM offers high nitrogen content, buffering capacity, and microbial diversity, enhancing biogas yields and stability. Meanwhile, POME, a by-product of palm oil processing, contains organic acids and residual oil, which can contribute to biogas production but may lead to process inhibition due to long-chain fatty acid accumulation. The selected ratio for molasses and chicken manure co-digestion testing is referred to a study done by [1] with inoculum-to-substrate ratio (ISR) of 1:3, increasing the ratio of molasses (MS: CM 1-7) has higher daily biogas production with 55.4% of CH_4, whereas increasing the ratio to 9:1, shows a reduction in AD performance. Despite studies on co-digestion of molasses with animal manure and industrial waste, limited information exists on molasses-POME co-digestion in batch processes. This study aims to address this knowledge gap by evaluating the performance of MS with POME or CM as co-substrates.

2 Materials and Methods

The molasses was obtained from an ethanol producer company based in Ipoh, Perak, Malaysia, and chicken manure was collected from Teong Choon Poultry Farm Sdn Bhd, Selangor, Malaysia. The raw POME and inoculum (POME sludge) were supplied by Green Lagoon Technology Sdn Bhd, Selangor, Malaysia. The POME inoculum was pre-incubated at 38°C without feeding for 7 days for inoculum degassing.

For the Biomethane Potential (BMP) setup, 125 mL serum bottles were used as batch anaerobic digesters for 100 mL of working volume. The co-digestions of MS: POME and MS: CM were tested at ratios of 7:1 and 9:1. After adding 25 mL of pre-incubated POME inoculum, the sample pH was adjusted to neutral with 3M NaOH and 2M HCl. A blank was also prepared using inoculum only. The prepared samples were then transferred into the serum bottles, and the headspace was purged with 99.9% nitrogen gas (N_2) for 1 minute (3 L/min). The serum bottles were sealed with a rubber stopper and incubated in a water bath at a controlled temperature of 38 ± 1 °C for 50 days. The biogas produced in the digester's headspace was collected daily using a gas-tight syringe, and the biogas composition was determined using gas chromatography equipped with a thermal conductivity detector (GC-TCD) after scrubbed with 3M NaOH (Fig. 1). All experimental sets were tested in duplicate.

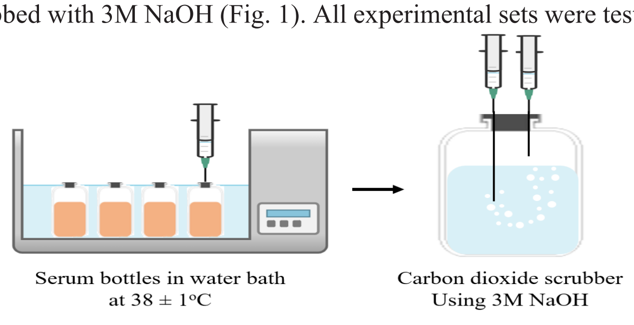

Fig. 1. Biogas collection and CO_2 scrubbing with NaOH.

For the characterization of samples before and after digestion, the analysis of total solids (TS), volatile solids (VS), COD, ammoniacal-N was conducted according to the standard methods outlined in the [2]. The carbon-to-nitrogen (C/N) ratio was obtained from the CHN628 Series Elemental Determina. The pH was determined using pH meter. Following gas collection, the biogas was transferred into Hungate tubes via the water displacement method, then tightly sealed. The gas composition, including methane (CH_4), hydrogen (H_2), carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2), was analysed using an Agilent 6890N Network Gas Chromatograph (GC) equipped with a thermal conductivity detector (TCD) and argon as the carrier gas. The modified Gompertz model as in Equation (1) was employed in this study to describe the behaviour and dynamics of the anaerobic co-digestion process involving molasses. This model effectively captures the microbial growth and biogas production trends, making it a suitable choice for analysing the co-digestion performance and predicting biogas yields under the specific conditions used in this research.

$$B_t = B_m\, exp\left\{-exp\left[Rm\,.\, \frac{e}{B_m\,(\lambda - \mathrm{t})} + 1\right]\right\} \tag{1}$$

Where B_t is the cumulative biogas production yield (mL/gVS), B_m is the maximum biogas yield (mL/gVS), Rm is the maximum biogas production rate (mL/gVS.day), e is Euler's number with a value of 2.71828, λ is the lag phase time (day), and t is the incubation time (day). All statistical analyses were performed using Excel software (Microsoft Inc., Redmond, WA) with a specific Microsoft Excel solver tool used to calculate the kinetics constant of B_t, B_m, and λ.

3 Results and Discussion

3.1 Characterization of Inoculum and Substrates

The characterisation for molasses, raw POME, chicken manure, and POME inoculum are presented in Table 1.

Table 1. Characteristics of substrates and inoculum.

	Molasses	Raw POME	Chicken Manure	POME Inoculum
Total Solid (g/L)	42.8	49.3	278.7	22.4
Volatile Solid (g/L)	27.4	34.4	157.7	9.25
Chemical Oxygen Demand (mg/L)	29700	23200	57600	4650
Ammoniacal Nitrogen (mg/L)	2.0	3.0	6.0	3.5
pH	3.93	3.38	7.95	7.05

3.2 Performance of the Anaerobic Co-Digestion on Biogas and Methane Production

As shown in Fig. 2, the trend of biogas yield corresponding to the different combinations of co-substrates with different ratios is similar to an S-curve or sigmoidal curve [3,4]. A significant difference in biogas yield between MS's co-digestion with POME and CM can be observed. The cumulative biogas yield for the co-digestion of MS and CM ranged from 380.9 to 520.2 mL biogas/g VS, whereas the co-digestion of MS with POME resulted in a lower yield, ranging from 220.6 to 314.0 mL biogas/g VS.

It was observed that biogas production initiated more rapidly (less than 10 days) in the co-digestion of MS and CM, yielding a significant amount of biogas early in the process. The co-digestion of MS and CM resulted in a shorter lag phase and higher methane yield due to enhanced microbial synergy. CM provides a diverse microbial community, accelerating hydrolysis and methanogenesis compared to POME. The nitrogen-rich environment of CM supports microbial proliferation, leading to faster biogas initiation.

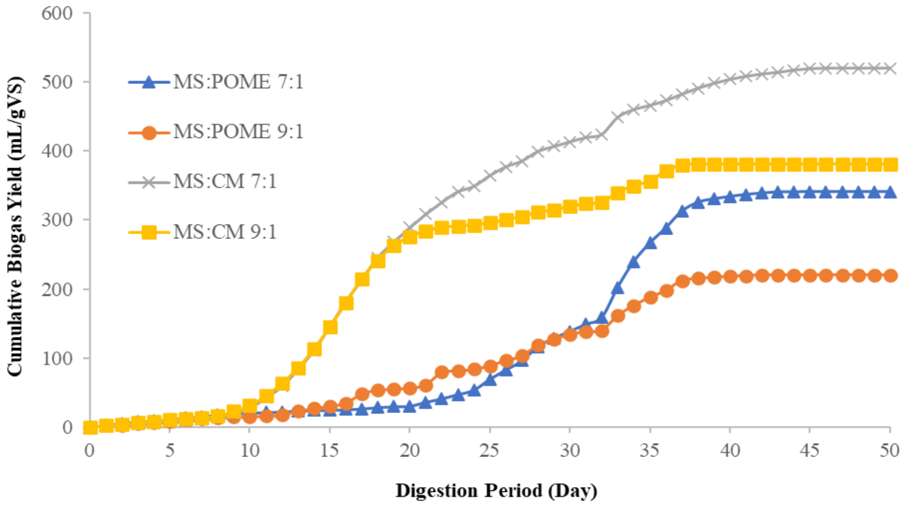

Fig. 2. Graph of cumulative biogas yield of all samples with different mixing ratios.

In contrast, the co-digestion of MS and POME exhibited a more prolonged lag phase, with slower microbial acclimatisation to the mixed substrates, leading to delayed biogas production [5] This finding suggests that POME's high lipid content may have inhibited methanogenesis, prolonging the lag phase and reducing methane yields [6]. The optimum molasses (MS) ratio to chicken manure (CM) in this experiment was 7:1. These results are consistent with the findings of [1], who also observed improved anaerobic digestion (AD) performance when the MS ratio increased from 1:1 to 7:1, with further increases (e.g., 9:1), resulting in a decline in performance. Notably, the methane content obtained in this study (Table 2) exceeds the range reported by [1], where methane yields were between 30% and 61.7%. Despite having a lower biogas yield, the methane content for the MS (7:1) ratio was the highest at 88.8%, in contrast to the MS (7:1) ratio, which produced the lowest methane content at 61.1%. This suggests that while the overall biogas volume was lower for MS, the proportion of methane in the gas was significantly higher, indicating a more efficient conversion of organic matter into methane in this specific combination.

A balanced C/N ratio is crucial for optimal methanogenesis. CM's high nitrogen content complements MS's carbon-rich composition, enhancing microbial metabolism. The 7:1 MS:CM ratio provided the best balance, yielding the highest methane content (86.5%). POME, with a lower nitrogen content, resulted in suboptimal microbial growth, limiting methane production. These findings align with previous studies on manure-based co-digestion, which reported improved methane yields with optimized nutrient balances.

Table 2 and Fig. 3 shows CM performs superior to POME as co-substrates for MS with higher methane yield (311.6 and 450 mL CH_4/gVS). This is due to its nutrient-rich composition, particularly its high nitrogen content, which supports microbial growth and enhances biogas production. The higher nitrogen levels in chicken manure help balance the carbon-to-nitrogen (C/N) ratio when co-digested with carbon-rich substrates like MS, optimising microbial metabolism and methane yield [7,8,9].

Despite its high biodegradability, POME may have introduced inhibitory compounds such as long-chain fatty acids (LCFAs) and phenolics, which can disrupt methanogenic pathways. CM, with its buffering capacity, mitigated process acidification and ammonia inhibition, resulting in higher methane yields. Previous studies have shown that excessive ammonia levels from manure can inhibit methanogenesis, but in this study, CM's NH_3-N levels remained within tolerable limits, ensuring stable biogas production.

Table 2. Biogas and methane yield for each experimental set.

Substrates	Biogas Yield (mL/gVS)	Methane Content (%)	Methane Yield (mL CH_4/gVS)
MS and POME (7:1)	341.0	61.1	208.4
MS and POME (9:1)	220.6	88.8	195.9
MS and CM (7:1)	520.2	86.5	450.0
MS and CM (9:1)	380.9	81.8	311.6

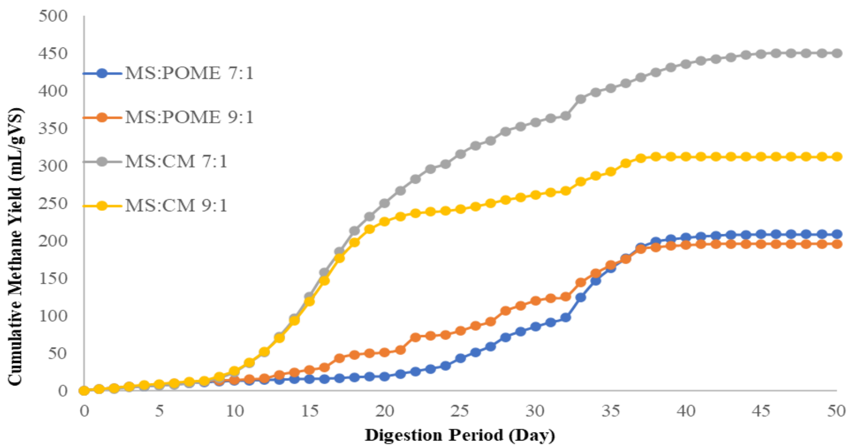

Fig. 3. Graph of cumulative methane yield of all samples with different mixing ratios.

Table 3 shows the difference between the predicted and measured biogas yields, which was between 0.88 and 7.34%. To evaluate the soundness of the model results in the modified Gompertz model, the expected values of biogas yield were plotted against the measured values in Fig. 3. All experimental results were fitted to the modified Gompertz kinetic model. The R-squared (R^2) values of the fitted graphs, presented in Table 3, ranged from 0.986 to 0.996, indicating that the modified Gompertz model provides an excellent fit for all experimental sets. This high correlation coefficient confirms the model's suitability for this study, reflecting a strong relationship between the predicted and observed data[10,11]. The data fitting into the sigmoid curve is implied by [12] that the biogas production did not increase linearly with time due to the lag phase.

Similar results were observed in previous studies on co-digestion with animal manure, such as the co-digestion of apple waste with swine manure, palm pressed fibre (PPF) with cattle manure [12], and empty fruit bunch (EFB) with POME [13], all of which demonstrated high R^2 values (>0.95). These findings confirm the strong correlation between experimental data and the kinetic models used, highlighting the suitability of co-digestion in enhancing biogas production performance. Overall, the cumulative and actual biogas yields from the co-digestion of MS with CM outperformed those from the co-digestion with POME. Notably, the 7:1 mixing ratio of MS showed that the cumulative biogas yield predicted by the model closely matched the actual yield, indicating efficient anaerobic digestion for this substrate combination. The high utilisation rate of raw materials and the shorter lag phase for most experimental sets were all less than 13 days except for MS (7:1), which had a lag phase of approximately 22 days.

Table 3. Kinetic parameters of modified Gompertz modelling on the anaerobic co-digestions.

	MS: POME 7:1	**MS: POME 9:1**	**MS: CM 7:1**	**MS: CM 9:1**
B_t **(mL biogas/gVS)**	362.5	238.1	515.6	373.5
B_m **(mL biogas/gVS)**	378.1	267.9	522.8	374.6
Rm **(mL/gVS.day)**	20.8	8.3	24.9	22.8
λ (day)	22.2	12.8	9.2	8.5
$\mathbf{R^2}$	0.9866	0.9870	0.9959	0.9888
Experimental Biogas Yield (mL/gVS)-50 days	341.0	220.6	520.2	380.9
Difference between measured and predicted biogas yield (%)	5.93	7.34	0.88	1.94

Notes: B_t= cumulative predicted biogas production yield for 50 days, B_m= maximum biogas yield, Rm= maximum biogas production rate, λ= lag phase time, R= correlation coefficient.

The long lag phase in anaerobic digestion is typically due to the time required for microbial communities, particularly methanogenic bacteria, to acclimate to the new substrate environment [14]. A prolonged lag phase appears to be a defence mechanism that allows bacteria to tolerate stress, mainly due to substrate complexity, inhibitory conditions such as high organic load or ammonia levels, and the need for microbial populations to adjust to the specific nutrient profile of the feedstock [15,16]. Findings by [17] clarified that when ammonia concentration was high, up to 6.8 g TAN/L resulted in ammonia inhibition, which can prolong the lag phase. In cases like co-digestion of MS and POME, the acidic nature of molasses and POME may slow down microbial adaptation, delaying the onset of active biogas production. When comparing the R^2 values (Fig. 4 and Table 3), the co-digestion of MS and CM at a 7:1 ratio demonstrated the best fit with the modified Gompertz model. The calculated kinetic parameter for total biogas yield (B_t) was 515.6 mL biogas/gVS, closely aligning with the actual biogas yield of 520.2 mL biogas/gVS, with only a 0.88% difference. These results confirm the modified Gompertz model effectively predicted biogas production trends, with R^2 values exceeding 0.98 for all experimental conditions. The best kinetic fit was observed for the MS:CM 7:1 ratio, confirming its efficiency in methane production. The models assume that biogas production is directly related to the specific growth rate of methanogenic bacteria within the digester [13] These results validate the suitability of kinetic modeling for scaling up anaerobic digestion processes.

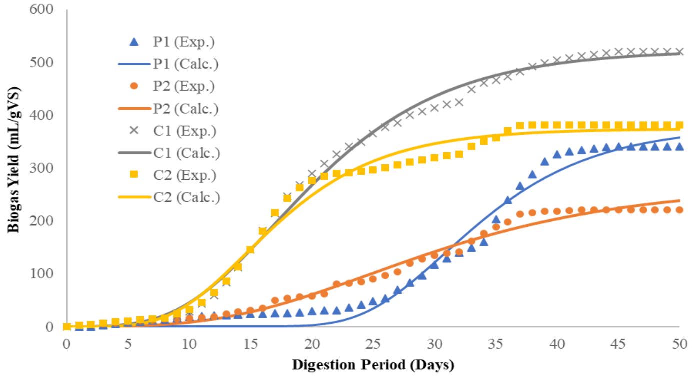

Fig. 4. Fitted curves of biogas yield from the Modified Gompertz kinetic model.

Note: set P= MS: POME, set C= MS: CM, Exp.= experimental data, Calc.= calculated (predicted) data.

4 Conclusion

The study demonstrated that co-digestion of MS with CM significantly enhances biomethane production compared to POME. The MS:CM 7:1 ratio achieved the highest methane yield, shortest lag phase, and superior process stability. These findings support the use of CM as an optimal co-substrate for molasses, offering a scalable solution for efficient biogas generation. Further research should explore microbial community dynamics and long-term reactor performance to optimize large-scale implementation.

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