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Valorization of Palm Oil Sludge And Snail Shell For Enhanced Biogas Production Through Thermophilic Anaerobic Digestion

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Abstract

This study investigated the thermophilic anaerobic digestion of palm oil sludge with snail shell as an additive to enhance biogas yield. 30g of substrate and 3.5g additive were mixed with distilled water in a 500 ml round-bottom flask and heated using a heating mantle. The flask was sealed with a rubber stopper connected to a gas outlet tube, and biogas was collected by water displacement. Proximate analysis confirmed the substrates as effective biogas feed stocks, while XRD, FTIR and XRF characterization of the snail shell revealed carbonate content, supporting its role as a beneficial additive. Optimal conditions were determined as 3.5 g additive dosage, a palm oil sludge-to-water ratio of 0.16 g/ml, digestion time of 60 minutes, and a temperature of 70 °C, yielding 217 ml of biogas. Process optimization using the Central Composite Design (CCD) of Response Surface Methodology (RSM) produced a statistically significant model ($p < 0.0001$) with a high coefficient of determination ($R^2 = 0.9668$). ANOVA confirmed the model's predictive accuracy. Gas chromatography showed the biogas composition as 66.8% methane and 23.5% CO_2. These results highlight palm oil sludge and snail shell as promising feed stocks for efficient and sustainable biogas production at scale.

Keywords: Thermophilic; Biogas; Palm oil sludge; Snail shell; Nanoparticles

Introduction

The increasing generation of organic wastes and the growing demand for renewable energy have intensified global interest in anaerobic digestion as a sustainable technology for simultaneous waste treatment and bioenergy production. In Nigeria, more than 32 million tonnes of solid waste are generated annually, with a significant proportion consisting of biodegradable agricultural residues and agro-industrial wastes. At the same time, increasing energy demand and dependence on fossil fuels continue to contribute to greenhouse gas emissions and environmental degradation (Bakare, 2020; Okonkwo et al., 2021). Converting organic wastes into biogas through anaerobic digestion represents an attractive strategy for advancing waste valorization, energy security, and circular economy objectives. Thermophilic anaerobic digestion offers several advantages over conventional mesophilic digestion, including faster substrate degradation, improved pathogen destruction, enhanced hydrolysis, and higher methane productivity. Nevertheless, thermophilic systems are often constrained by process instability, volatile fatty acid accumulation, ammonia toxicity, and pH fluctuations. These severe issues can inhibit methanogenic microorganisms and reduce overall process efficiency (Ryue et al., 2020). Considerable research has therefore focused on developing additives capable of improving microbial activity, buffering system stability, and enhancing methane production.

Recent studies have demonstrated that conductive nanoparticles (CNPs), including iron, cobalt, and copper based nanomaterials, significantly enhance anaerobic digestion. They do so by facilitating direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogens (Jadhav et al., 2021). This mechanism accelerates electron transport, shortens microbial metabolic pathways, improves substrate conversion efficiency, and increases methane yield. Likewise, trimetallic nanoparticles have been successfully applied during anaerobic digestion of palm oil mill effluent, producing higher biogas yields and improved process stability than conventional digesters. Despite these advantages, nanoparticle-based additives remain expensive and require sophisticated synthesis and characterization. They may also pose environmental and toxicity concerns, making them currently unsuitable for widespread application in many developing countries (Jadhav et al., 2024). Consequently, increasing attention is being directed toward naturally occurring mineral rich wastes that can provide similar process enhancing functions at substantially lower cost. Snail shell waste is an abundant agricultural by-product composed predominantly of calcium carbonate with minor quantities of magnesium and other trace minerals. During anaerobic digestion, calcium based materials buffer pH, reduce volatile fatty acid accumulation, stabilize microbial communities, and provide essential minerals required for methanogenic activity. However, unlike engineered nanoparticles, snail shell waste remains inexpensive, environmentally benign, readily available, and compatible with circular economy principles.

Previous studies have reported the use of calcium rich additives and agricultural residues to improve anaerobic digestion performance. However, limited information exists on the thermophilic anaerobic digestion of palm oil sludge using naturally occurring snail shell as a sustainable calcium rich additive. In particular, there is a lack of comprehensive studies combining physicochemical characterization of the additive (XRD, XRF, and FTIR) with statistical optimization using Central Composite Design of Response Surface Methodology (CCD-RSM). Detailed gas chromatographic evaluation of the resulting biogas composition is also scarce. Furthermore, most recent investigations on palm oil mill wastes have concentrated on engineered conductive nanoparticles, while direct comparisons with naturally occurring mineral additives remain scarce.

Therefore, the novelty of the present study lies in integrating a low-cost, bio waste derived calcium-rich additive (snail shell) with the thermophilic anaerobic digestion of palm oil sludge. This approach provides an environmentally sustainable alternative to conductive nanoparticles. The study combines detailed mineralogical characterization of the additive with CCD-RSM optimization of key operating variables and gas chromatographic assessment of methane quality. This integrated approach provides a practical and economically feasible strategy for improving anaerobic digestion performance. It simultaneously valorizes two abundant agro wastes, thereby contributing to sustainable waste management and renewable energy production in resource constrained regions.

Materials and Methods

2.1. Collection and Pretreatment of Feedstock and Additive (snail shell)

Fresh palm oil sludge weighing 8kg was obtained from a palm oil mill in Amansea, Anambra State, Nigeria, using high-density polyethylene bags. It was subjected to physical pre-treatment to improve its biodegradability and enhance the release of readily available organic matter for anaerobic digestion. The collected POS was initially spread in a clean tray and sun dried for 36 h to reduce its moisture content. The partially dried material was then manually ground using mortar and pestle. It was screened through a sieve of 3.5mm to obtain a relatively uniform particle size. Foreign materials and other visible impurities were removed during the preparation process. The pre-treated palm oil sludge was stored in clean, airtight container and kept at room temperature until required for physicochemical characterization and anaerobic digestion experiment.

Similarly, 5 kg of snail shells were sourced from Eke Market, Awka. The shells were initially crushed with a mortar and pestle, sieved to a size of about 5 mm, and soaked in 10 L of water for three days before being thoroughly washed. Afterward, they were sun-dried for 24 hours and further ground into a fine powder using a 2 hp electric mill. The resulting material was passed through a 500 µm mesh sieve to achieve a uniform particle size, producing 550 g of powdered snail shell for subsequent laboratory applications.

2.2. Physico-chemical characterization of substrates

The physicochemical characterization investigations conducted on Palm oil sludge are outlined in Table 1.0, along with the standard methods applied.

Table 1.0: Standard methods for physico-chemical analyses

Tests	Standard method	References
Total solids	ASTM E1756-08(2020)	(Staber et al., 2008)
Ash content	ASTM D4906-95 (2017)	(Ismail, 2024)
Moisture content	AOAC (1990)	(Slight, 1989)
Volatile matter content	ASTM D3175-20 (2020)	(Brown, 2002)
Fixed carbon content	ASTM D3172-13(2021)	(Staber et al., 2008)
Energy value	ASTM D5865-12	(Tyrrell & Reid, 1965)

2.3. Procedure for instrumental characterization

The prepared snail shell powder was characterized using X-ray Diffraction (XRD) in Theta–Theta configuration with a reflection–transmission spinner stage. The analysis was performed over a 4°–75° 2θ scanning range at a step size of 0.026261 and a dwell time of 8.67 seconds per step, operating at 40 mA current and 45 VA tension. Diffraction data, expressed as intensity versus angle, were used to identify mineral phases based on peak positions and X-ray counts (Owoyemi & Owoyemi, 2020). Elemental composition was determined using X-ray Fluorescence (XRF) spectroscopy on pelletized samples operated at 30 kV, following ASTM C114 standards to quantify major oxides and chemical constituents (Kolawole et al., 2017). Functional group analysis was carried out using Fourier Transform Infrared (FTIR) spectroscopy with the KBr pellet technique. Approximately 10 mg of powdered sample was blended with 500 mg of potassium bromide, compressed at 22–30 MPa, and examined with a Nicolet 6700 M spectrometer. The spectra were collected across 400–4000 cm^{-1} at a resolution of 4 cm^{-1}, and the absorption peaks were interpreted to identify functional groups (Ndibe et al., 2025).

2.4. Biogas production procedure and laboratory digester setup

The thermophilic anaerobic digestion method was employed for biogas production. Palm oil sludge was mixed with distilled water in a 500 mL round-bottom flask to form a slurry. 3.5g of snail shell powder was added as an additive, and the mixture was stirred thoroughly with a glass rod to ensure homogeneity. The flask was placed on a heating mantle to maintain a steady thermophilic temperature, promoting efficient microbial digestion. It was sealed with a rubber stopper, fitted with a gas outlet tube, and tightly covered to prevent leakage. The produced biogas was directed through the outlet tube into an inverted, water-filled graduated cylinder placed in a trough. As gas accumulated, it displaced the water, enabling direct collection and measurement of biogas volume. The water displacement method ensured accurate monitoring, and the displaced water was recorded at regular intervals to quantify gas production.

2.5. Determination of Biogas Quality and Composition

Gas composition analysis was carried out using a BUCK M910 Gas Chromatograph equipped with a Flame Ionization Detector (FID) and a RESTEK MXT-1 column (15 m × 250 µm × 0.15 µm). The injector temperature was maintained at 280 °C and operated in splitless mode with injection volumes ranging from 0.5 to 2 µL. Helium, at 5.0 Pa·s, was used as the carrier gas at a flow rate of 40 mL/min, corresponding to a linear velocity of 30 cm/s. The oven temperature was programmed to start at 200 °C, then ramped up to 330 °C at a rate of 3 °C/min and held for five minutes. The detector temperature was set at 320 °C. Gas constituents were analyzed over a total run time of 45 minutes, and their concentrations were quantified by comparing peak areas to those of an internal standard, with results expressed in µg/mL (Ndibe et al., 2025).

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Results and Discussion

3.1. Physico-chemical characterization of substrates

Table 2.0 presents the physicochemical characteristics of palm oil sludge, emphasizing its suitability for biogas generation. The moisture content, recorded at below 10%, falls within the recommended limit for long-term storage (Onuora et al., 2023). Maintaining low moisture levels prevents mold development, reduces biochemical degradation, and enhances material stability. The ash content of 7.98% reflects moderate mineral concentrations while indicating substantial organic matter availability (Onuora et al., 2023). When combined with low moisture, this composition supports improved biogas yield by providing more biodegradable material for microbial conversion. The volatile matter content, measured at 68.37%, demonstrates the high degradability of the substrate, which is essential for sustaining microbial activity during anaerobic digestion (Sambo et al., 2015). Similarly, the total solids content of 90.14% points to a significant organic load, favouring increased methane production (Godfrey, 2024). The energy value further confirms the substrate's potential for bioenergy applications (Lee et al., 2019). Meanwhile, the fixed carbon fraction, associated with lignocellulosic components, represents the remaining carbon after volatile matter

release and influences the overall efficiency of the digestion process (Wan et al., 2022).

Table 2.0: Proximate Characteristics of the samples

Parameters	Palm Oil Sludge
Volatile matter content (%)	68.37
Ash content (%)	7.98
Moisture content (%)	9.86
Fixed carbon (%)	13.79
Energy value (KJ/100g)	2395.47
Total solid (%)	90.14

3.2. Instrumental characterization of snail shell (SS)

3.2.1. Phase constituent analysis using X-ray diffractometer (XRD)

The X-ray diffraction (XRD) analysis (Figures 1 and 2) revealed a dominant diffraction peak at 36.36° with an interplanar spacing of 2.471 Å and a relative intensity of 100%, confirming aragonite as the primary crystalline phase with a score of 27. Aragonite is largely responsible for the hardness and structural strength of snail shells (Owoyemi & Owoyemi, 2020). The mineral composition indicated that calcium carbonate accounted for 75.56% of the sample, primarily as aragonite, along with minor phases including lime (CaO), quartz, periclase, and orthoclase. Calcium carbonate and lime play a critical role in stabilizing anaerobic digestion by neutralizing acidic environments and reducing volatile fatty acid (VFA) accumulation (Liu et al., 2021). Furthermore, calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions act as cofactors for microbial enzymes, thereby supporting metabolic processes that enhance biogas production. Calcium based compounds also help precipitate ammonium ions (NH_4^+) into stable salts, mitigating ammonia toxicity and improving microbial stability. Additionally, the alkaline nature of snail shells prevents excessive acidification, maintaining optimal pH conditions for microbial activity and ultimately increasing methane yields (Ning et al., 2019).

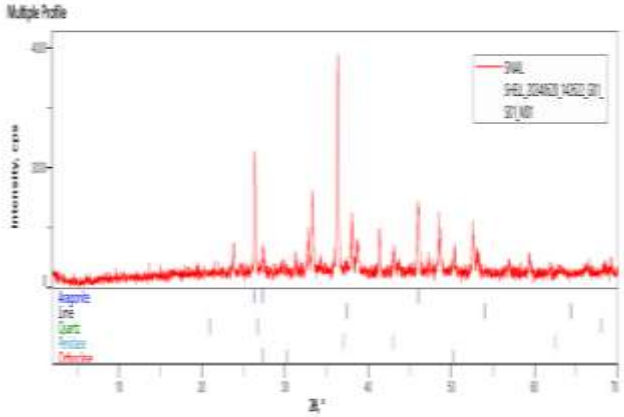

Fig 1: XRD pattern of snail shell

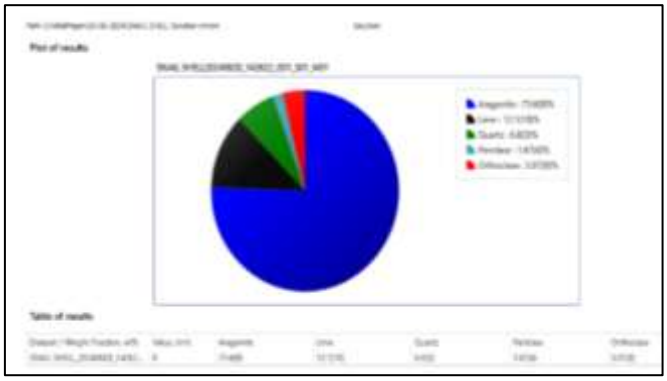

Fig 2: XRD pie chart of snail shell

3.2.2. Fourier transform infrared (FTIR) analysis of snail shell

The infrared (IR) spectrum of the snail shell sample (Fig. 3) exhibited distinct absorption peaks characteristic of CO_3^{2-} ions present in $CaCO_3$. Prominent bands appeared at 1826, 1635, 1374, 1221, 1056, and 811 cm^{-1}, confirming the presence of carbonate ions, in agreement with findings by Udomkan and Limsuwan (2008). The absorption band within 1390–1416 cm^{-1} corresponded to the $v_3(E)$ vibrational mode of CO_3^{2-}, while the $v_1(A_1)$ mode was absent as noted by Zuliantoni et al. (2022). Broad absorption features between 3234–3817 cm^{-1} and 3400–3600 cm^{-1} indicated the presence of water molecules. The 400–1800 cm^{-1} region reflected CO_3^{2-} vibrations, with a dominant C=O stretching band observed at approximately 1404–1423 cm^{-1}. Peaks at 811 and 1374 cm^{-1} were linked to calcite phases, with the latter also associated with the $v_1(A_1)$ vibrational mode in aragonite. Hence, the FTIR analysis confirmed that the snail shell material was predominantly composed of calcium carbonate, with minor contributions from water and organic residues. These properties make it an effective additive for anaerobic digestion by buffering pH

fluctuations and promoting stable microbial activity (Liu et al., 2021).

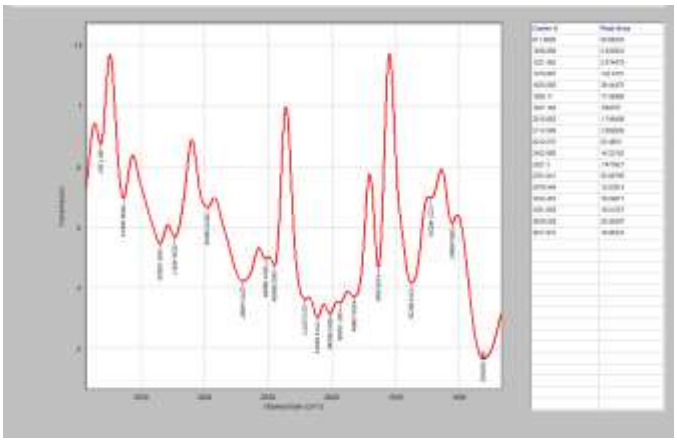

Figure 3: FTIR spectrum of raw snail shell

3.2.3. XRF compositional analysis

Table 3.0 presents the oxide composition of snail shells as determined by Energy Dispersive X-ray Fluorescence (XRF) spectroscopy. The analysis revealed the presence of several oxides, including CaO, K_2O, Fe_2O_3, P_2O_5, Al_2O_3, ZnO, SrO, Cr_2O_3, TiO_2, NiO, Ag_2O, Pb_2O, and MnO, outlining the mineralogical characteristics of the shells and their suitability for biogas production (Owoyemi & Owoyemi, 2020). Among these, CaO recorded the highest concentration (89.884%), while Pb_2O showed the lowest (0.002%), consistent with earlier findings. The predominance of calcium aligns with the known composition of snail shells, which primarily consist of calcium carbonate ($CaCO_3$) in aragonite or calcite forms. Magnesium oxide (MgO), although present in smaller quantities, is important as a cofactor for microbial enzyme activities during anaerobic digestion. Trace elements such as potassium, iron, silicon, and aluminum were also detected. While silicon and aluminum are naturally occurring with limited direct roles in digestion, potassium is a critical nutrient for hydrolytic and acidogenic bacteria, and iron facilitates electron transfer processes in methanogens, thereby enhancing methane production (Zhou et al., 2020).

Table 3.0: XRF analysis report

S/NO	Chemical Formula	Concentration (%)
1	SiO_2	0.315
2	V_2O_5	0.009
3	Cr_2O_3	0.007
4	MnO	0.064
5	Fe_2O_3	0.154
6	CoO	0.044
7	NiO	0.023
8	CuO	0.075
9	Nb_2O_5	0.011
10	WO_3	0.043
11	CaO	89.884
12	SO_3	0.024
13	MgO	4.802
14	K_2O	0.029
15	Al_2O_3	3.122
16	Ta_2O_5	0.066
17	Cl	0.392
18	SnO2	0.617
19	SrO	0.307
20	Pb_2O	0.002
21	PbO	0.005

3.3. Effect of process variables on the biogas yield

Figure 4 illustrates the effect of temperature on biogas production, showing a steady increase in yield from 50 °C to 70 °C, where the maximum output of 197 ml was recorded. Beyond 70 °C, production declined slightly, likely due to thermal inhibition of methanogenic microbes. Similar trends were reported by Zhang et al. (2020), who observed optimal gas generation between 55 °C and 70 °C, and by Sambo et al. (1995), who recorded peak yields near 70 °C before inhibition set in. Excess heat (>70 °C) can denature microbial enzymes (Ryue et al., 2020) and destabilize microbial communities (Cheng et al., 2018). Figure 5 highlights the effect of retention time. Biogas production increased from 100 ml at 40 minutes to 197 ml at 60 minutes before leveling off. The initial rise reflects active microbial metabolism, while the plateau beyond 60 minutes suggests substrate depletion and reduced microbial activity (Zhang et al., 2020; Ezekoye et al., 2011).

Figure 6 examines additive dosage, showing yield improvements as snail shell dosage increased from 2.5 g to 3.5 g, after which no significant gains was observed. Snail shells likely stabilized pH and supplied bacteria supporting methanogens (Liu et al., 2021; Onuora et al., 2023; Çalhan & Ulutaş, 2023). Figure 7 shows that increasing the palm oil sludge/water ratio to 0.20 g/ml enhanced yields up to 195 ml; however, higher loadings reduced output due to volatile fatty acid accumulation

and water limitation (Sambo et al., 1995; KeChrist et al., 2017).

The experimental conditions for the process variables were as follows: additive dosage ranged from 2.5 g to 4.5 g, palm oil sludge/water ratio ranged from 0.08 to 0.24 g/mL, digestion time ranged from 40 to 80 minutes, and temperature ranged from 50 to 90 °C. To study the effect of temperature, the palm oil sludge/water ratio was fixed at 0.16 g/mL, additive dosage at 3.5 g, and digestion time at 60 minutes. Optimal conditions were 70 °C, 60 minutes, 3.5 g additive, and 0.20 g/ml palm oil sludge/water ratio, beyond which inhibition effects became evident.

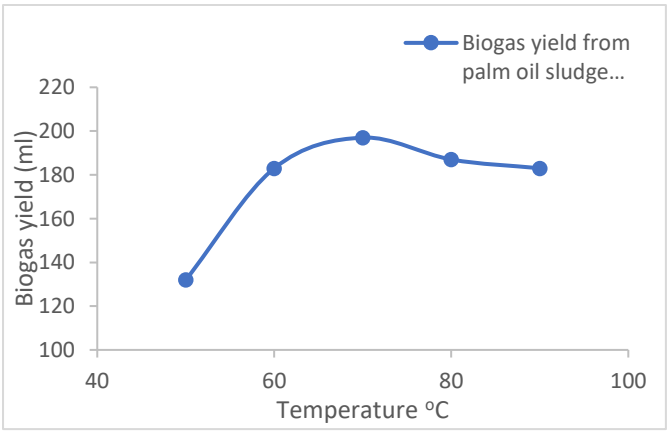

Fig. 4: Effect of temperature on the biogas yield

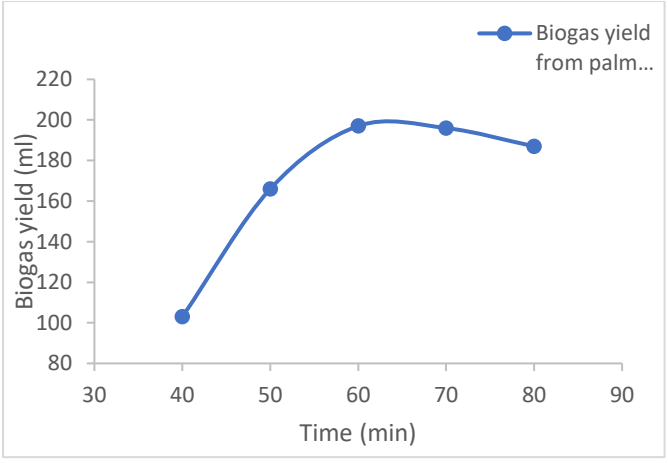

Fig. 5: Effect of time on the biogas yield

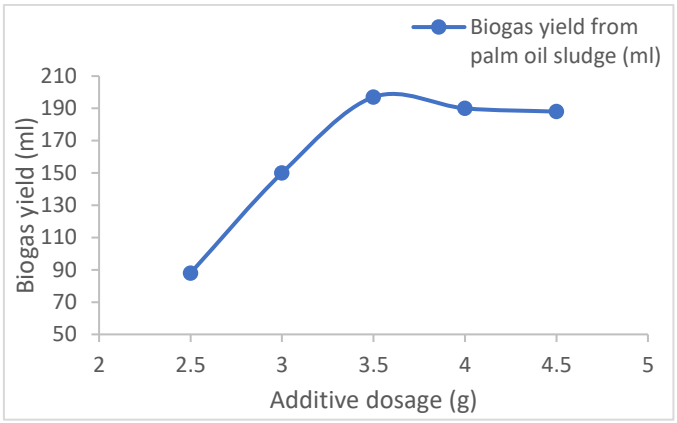

Fig. 6: Effect of Additive dosage on the yield

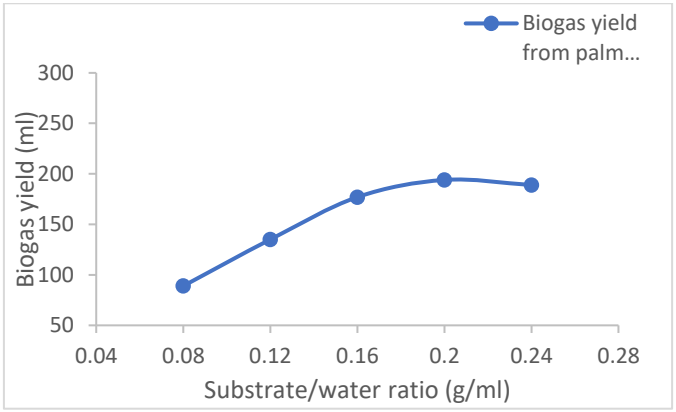

Fig. 7: Effect of palm oil sludge/water ratio on the biogas yield

3.4. Determination of biogas quality and composition using gas chromatography

Biogas produced from palm oil sludge supplemented with snail shell additive was primarily composed of methane (66.81%), indicating efficient anaerobic digestion and suitability for energy recovery. Although this value is slightly below the >70% methane content typically reported for highly optimized digesters, it still falls within the standard range of 50–70% for biogas quality. Carbon dioxide accounted for 23.53%, consistent with expected microbial metabolism, while the calcium carbonate in snail shells likely contributed to pH stabilization, enhancing methane conversion efficiency. The detection of acetic acid (1.78%) suggests active acetogenic pathways, with minimal residual intermediates as evidenced by trace concentrations of lactic acid (0.003%) and ethanol (0.001%). Other minor components included phenol (1.23%) and acetonitrile (0.26%), both at levels too low to inhibit microbial activity. Ethylene (3.84%), may result from incomplete degradation of complex organics.

Hydrogen sulfide (2.52%) remained moderate, implying partial suppression due to the buffering capacity of snail shells. Hence, the composition demonstrates that the snail shell additive improves methane yield, gas quality, and overall digestion stability.

Table 5 shows that addition of additive improved the quality of the biogas by raising methane concentration and lowering unwanted constituents. The methane fraction increased from 64.224% to 66.805%, suggesting improved activity of methanogenic microorganisms and more effective transformation of intermediate compounds into usable gas. Conversely, carbon dioxide content declined from 25.009% to 23.533%, indicating a corresponding improvement in calorific value and fuel performance. A slight decrease in acetic acid implies more efficient substrate conversion during methanogenesis, while the low lactic acid concentration shows minimal influence on the digestion pathway. Trace compounds, including phenol, acetonitrile, ethanol, and ethylene, were also slightly reduced, pointing to fewer inhibitory effects and a more stable digestion environment. Although the total biogas volume increased only slightly, the additive's main impact was on improving gas composition. Hence, the additive supported a more efficient anaerobic digestion process and produced higher-quality biogas suitable for energy use.

Table 4: Biogas from palm oil sludge using snail shells as an additive

Components	Concentration(ug/ml)	% Concentration
Lactic acid	0.0022	0.003
Methane	52.0532	66.805
Acetic Acid	1.3899	1.784
CO_2	18.3523	23.533
Phenol	0.9606	1.233
Ethanol	0.0004	0.001
Acetonitrile	0.2032	0.261
H_2S	2.9941	3.843
Ethylene	1.9627	2.519
Total	77.9187	100

Table 5: Comparative analysis of biogas quality and composition with and without additive

	WITH ADDITIVE		WITHOUT ADDITIVE	
Components	**Conc. (ug/ml)**	**% Conc.**	**Conc. (ug/ml)**	**% conc.**
Lactic acid	0.0022	0.003	0.00229	0.003
Methane	52.0532	66.805	49.4505	64.224
Acetic acid	1.3899	1.784	1.4038	1.822
CO_2	18.3523	23.533	19.2699	25.009
Phenol	0.9606	1.233	0.9746	1.265
Ethanol	0.0004	0.001	0.00044	0.001
Acetonitrile	0.2032	0.261	0.2053	0.267
H_2S	2.9941	3.843	3.0243	3.927
Ethylene	1.9627	2.519	1.9818	2.573
Total	77.9187	100	76.3129	100

3.5 Optimization with Response Surface Methodology (RSM)

The CCD of RSM was employed to design the experiment (DOE) and model biogas production under anaerobic conditions. Table 6 shows biogas yields from palm oil sludge, considering variations in sludge-to-water ratio, additive dosage, temperature, and digestion time. The highest cumulative biogas yield of 217 mL was obtained at a substrate-to-water ratio of 0.16 g/mL, an additive dosage of 3.5 g, and a temperature of 70°C for 60 minutes. The Model F-value of 17420.33 indicates the model is highly significant, with a p-value < 0.0001, showing it explains most of the variation in biogas yield. There is only a 0.01% chance that such an F-value occurs due to noise. Terms A, B, C, D, AB, A^2, B^2, C^2, and D^2 are significant. The Predicted R^2 of 0.8889 reasonably agrees with the Adjusted R^2 of 0.9359, and Adeq Precision of 18.123 indicates an adequate signal for navigating the design space.

Statistical parameters in Table 7 confirm model fitness. The R^2 of 0.9668 shows that 96.68% of variability is explained by the model, affirming reliability and consistency. The sum of squares for the model source was 17420.33 with a total DF of 14, giving a mean square of 1244.31. The lack-of-fit p-value of 0.900 (greater than 0.05) indicates that the model fits the data well. Therefore, the CCD-RSM model accurately predicts biogas yield, identifies significant factors, and provides a reliable framework for optimizing anaerobic digestion conditions.

Table 6: Biogas yield for palm oil sludge under varying conditions

Run	A:POS/water ratio (g/ml)	B:Additive dosage. (g)	C: Time (mins)	D:Temp. (°C)	Biogas yield (ml)
1	0.12	3	70	80	159
2	0.16	3.5	60	70	197
3	0.20	3	70	60	146
4	0.16	3.5	60	70	197
5	0.12	4	50	60	133
6	0.16	3.5	60	90	190
7	0.12	3	70	60	131
8	0.16	3.5	60	70	197
9	0.08	3.5	60	70	131
10	0.16	3.5	60	70	197
11	0.16	4.5	60	70	183
12	0.20	4	50	60	166
13	0.12	3	50	60	130
14	0.16	3.5	60	50	150
15	0.12	4	70	60	151
16	0.12	4	70	80	165
17	0.12	3	50	80	161
18	0.20	4	70	80	207
19	0.20	4	70	60	188
20	0.20	3	50	60	142
21	0.20	3	70	80	178
22	0.16	3.5	60	70	217
23	0.16	3.5	80	70	181
24	0.16	3.5	60	70	197
25	0.20	4	50	80	198
26	0.12	4	50	80	159
27	0.16	2.5	60	70	154
28	0.16	3.5	40	70	170
29	0.24	3.5	60	70	182
30	0.20	3	50	80	180

Table 7: ANOVA for quadratic model of the biogas yield

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	17420.33	14	1244.31	31.23	< 0.0001	Significant
A-POS-water ratio	4225.46	1	4225.46	106.05	< 0.0001	
B-Additive. dos.	1653.41	1	1653.41	41.49	< 0.0001	
C-time	249.12	1	249.12	6.25	0.0245	
D-temp.	3780	1	37.80	94.86	< 0.0001	
AB	439.02	1	439	11.02	0.0047	
AC	4.82	1	4.82	0.12	0.7328	
AD	25.57	1	25.57	0.64	0.4356	
BC	170.39	1	170.39	4.28	0.0564	
BD	89.13	1	89.13	2.24	0.1555	
CD	66.46	1	66.46	1.67	0.2161	
A^2	3855.21	1	3855.21	96.76	< 0.0001	
B^2	2151	1	2151	53.98	< 0.0001	
C^2	1380	1	1380	34.63	< 0.0001	
D^2	1990.15	1	1990.15	49.95	< 0.0001	
Residual	598	15	39.85			
Lack of Fit	264.35	10	26.43	0.40	0.9000	not significant
Pure Error	333.33	5	66.67			
Cor Total	18018.18	29				

Std. Dev.	6.31	R-Squared	0.9668
Mean	171.68	Adj R-Squared	0.9359
C.V. %	3.68	Pred R-Squared	0.8889
PRESS	2002.64	Adeq Precision	18.123

The regression equation in terms of coded factors is developed to establish a mathematical relationship between the response of anaerobic digestion (biogas amount) and different independent process variables as represented in Eq. (1). The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. The actual factor equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$\text{Biogas yield} = +200.73 + 13.27*A + 8.30*B + 3.22*C + 12.55*D + 5.24*AB + 3.26*BC - 11.86*A^2 - 8.86*B^2 - 7.09*C^2 - 8.52*D^2 \quad (1)$$

The purpose of a normal probability plot of residuals is to assess whether the residuals from a statistical model (like a regression) are approximately normally distributed. From Fig 8 it is observed that the straight diagonal line represents a perfectly normal distribution. The data points generally follow the diagonal line, suggesting that the residuals are reasonably close to being normally distributed. However, some deviations are present at the end of the plot.

Fig 9 is a plot of predicted vs. actual values, which is used to evaluate the performance of a regression model. The data points are generally clustered closely around the diagonal line. This indicates that the model has a good fit and is predicting the actual values relatively well. The colors likely represent different value ranges, but the trend appears to be well-aligned with few potential outliers.

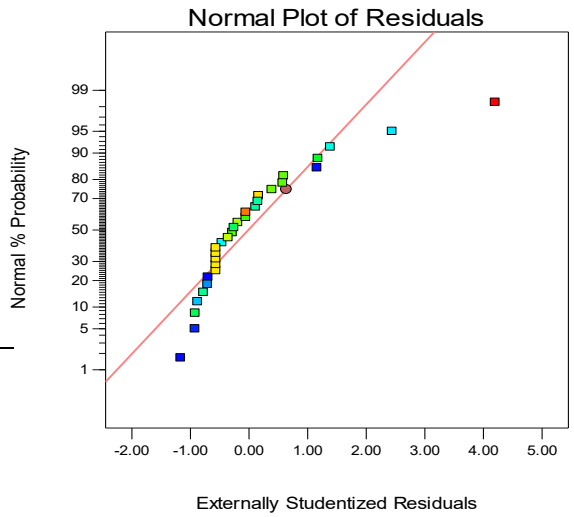

Fig 8: Normal probability plot of residual

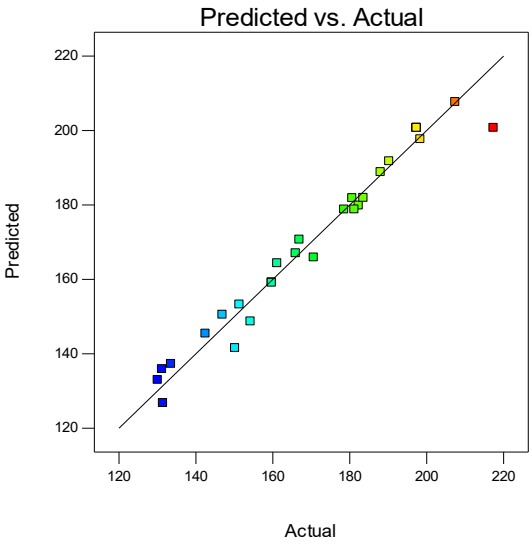

Fig 9: Graph of predicted versus actual Biogas yield

Figure 10 (a,b) shows that contour plot and 3D surface plots can be used for visualizing the relationships between multiple factors and a response variable in experimental design (Anderson-Cook et al., 2016). The progressive shift in color gradient across the contour and 3D surface plots (Fig. 10a,b) corresponds to a systematic increase in biogas yield, with the response surface indicating a positive correlation between increasing additive dosage and biogas production up to the optimal level. When the time is increased, the effect on biogas yield, is noticeable until it peaks and drops gradually because of substrate depletion. At lower additive dosages, increasing time initially increases the yield, but further increases seem to have less impact. At higher additive dosages, the effect is

less pronounced, with the yield remaining high across a wider range of times

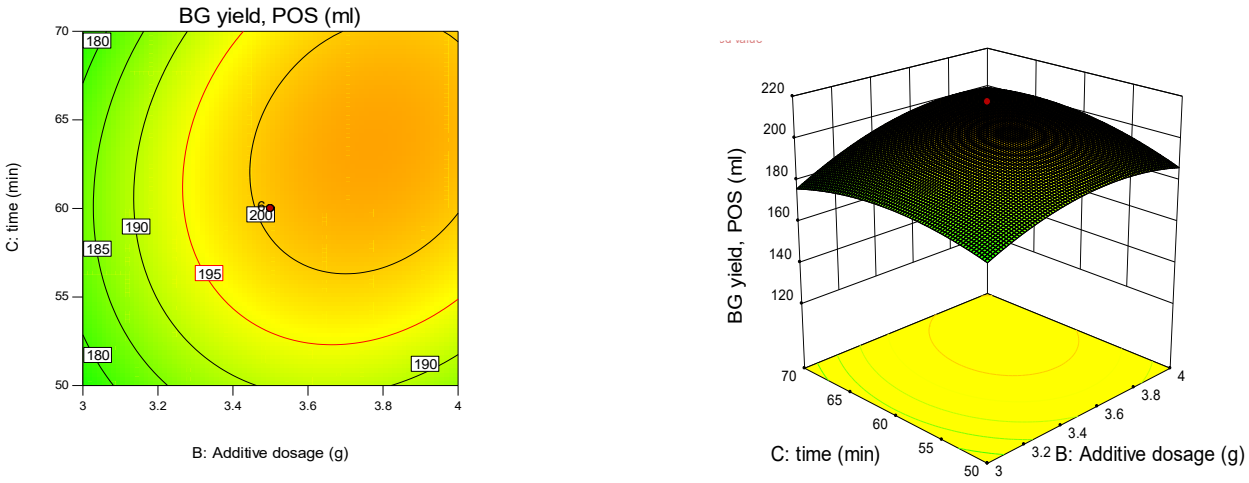

Figure 10 (a) 2D contour and (b) 3D Surface plots for the combined effect of time and additive dosage on the biogas yield.

Conclusions

Thermophilic anaerobic digestion of palm oil sludge with snail shell as an additive has the potential to generate clean, renewable, and environmentally friendly energy. Proximate analysis of the palm oil sludge confirmed its suitability as a biogas feedstock due to its high organic content, considerable energy value, low ash levels, and moderate fixed carbon. However, initial moisture content may require adjustment to optimize microbial performance during digestion. XRD and XRF analyses revealed calcium carbonate content in aragonite and oxide form, in the snail shells, making them a beneficial additive for enhancing methane yield and stabilizing digestion conditions.

Experimental results showed that the highest biogas production (217 ml) occurred under optimal conditions of 3.5 g additive dosage, a palm oil sludge-to-water ratio of 0.16 g/ml, a retention time of 60 minutes, and a temperature of 70 °C. ANOVA confirmed that temperature, substrate ratio, retention time, and additive dosage significantly affected biogas output, with the model achieving strong statistical significance ($p < 0.0001$) and a high coefficient of determination ($R^2 = 0.9668$). Gas chromatography analysis indicated that the biogas contained 66.8% methane and 23.5% CO_2, demonstrating its suitability as a renewable energy source. Furthermore, the digestate generated can be used as a nutrient-rich fertilizer, supporting sustainable agricultural practices.

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Conflicts of Interest

The authors declare no conflict of interest

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