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A Study On The Influence Of Inoculum In The Anaerobic Digestion Of *Xanthium strumarium* Leaves

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ABSTRACT
This study investigates the influence of inoculum on the anaerobic digestion of *Xanthium strumarium* leaves. Sample of fresh leaves of *Xanthium strumarium* were collected within Usmanu Danfodiyo University, Sokoto. Anaerobic digestion was carried out using inoculated and uninoculated substrate of *Xanthium strumarium* leaves by water displacement method, while temperature and pH were determined using pH meter and thermometer. The percentage degradation of each substrate was determined by comparing the initial and final weights after digestion. Data were subjected to descriptive analyses. The inoculated digester had a higher cumulative biogas yield (428.00 cm^3) compared to the uninoculated digester (154.00 cm^3) (Table 1). The inoculated digester had a retention time of (21 days) and a higher average daily gas production of (20.38 mL) than the uninoculated system (25 days; 6.61 mL) (Table 2). The inoculated digester had (66.00%) of Percentage degradation while the uninoculated had (60.00%) (Table 3). The pH of inoculated substrate ranged from 6.69 before digestion to 8.11 after digestion, while the uninoculated substrate ranged from 5.78 to 6.57 (Table 4). The study concludes that inoculum addition shortens the retention period and improves biodegradation. Future work should focus on co-digestion with plant residues to increase biogas yield.
Keywords: *Xanthium strumarium,* inoculum, digester, retention time

1.0 INTRODUCTION

Anaerobic digestion (AD) is an efficient biochemical process that decomposes organic matter in the absence of oxygen, generating biogas, primarily composed of methane (CH_4) and carbon dioxide (CO_2). This sustainable technology plays a vital role in waste management, renewable energy production, and nutrient recycling (Li et al., 2022; Zhang *et al.*, 2021). Various organic feedstocks, such as agricultural

residues, food waste, and invasive plant species, have been explored for AD (Wang *et al.,* 2023). Among these, *Xanthium strumarium* (cocklebur), an invasive plant species with high biomass potential, remains underutilized in bioenergy applications. The efficiency of its digestion depends significantly on the inoculum used, which affects microbial activity, substrate breakdown, and biogas production.

AD proceeds through four key stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Angelidaki *et al.,* 2019). Hydrolysis involves the breakdown of complex organic polymers, including carbohydrates, proteins, and lipids, into simple monomers such as sugars, amino acids, and fatty acids. This step is crucial for lignocellulosic materials like *Xanthium strumarium* due to their rigid structure (García *et al.,* 2022). Acidogenesis follows, where hydrolysis products are further converted into volatile fatty acids (VFAs), alcohols, hydrogen, and CO_2 by fermentative bacteria. Acetogenesis transforms VFAs and alcohols into acetic acid, hydrogen, and CO_2, which serve as precursors for methanogenesis. Finally, methanogenic archaea utilize acetic acid, hydrogen, and CO_2 to produce methane, the primary component of biogas (Appels *et al.,* 2022). The efficiency of these stages is influenced by substrate composition, pH, temperature, and microbial communities. The selection of a suitable inoculum enhances process stability and maximizes methane yield. AD offers numerous environmental and economic benefits, including renewable energy production, waste reduction, nutrient recovery, and carbon footprint reduction. Biogas can be converted into electricity, heat, or upgraded to biomethane for fuel (Zhang *et al.,* 2023). Diverting agricultural waste and invasive plants from landfills reduces methane emissions, while digestate, rich in nitrogen, phosphorus, and potassium, serves as a valuable biofertilizer (Girotto *et al.,* 2023). Moreover, AD offsets fossil fuel use, mitigating greenhouse gas emissions (Li *et al.,* 2023).

Xanthium strumarium is an aggressive, fast-growing invasive species that thrives in nutrient-poor soils. Its high biomass yield makes it a promising AD feedstock (Chen *et al.,* 2023). However, its fruits contain significant amounts of lignin, hemicellulose, and secondary metabolites, which can inhibit microbial activity (Wang *et al.,* 2023). The complex cell wall structure of lignocellulosic biomass limits hydrolysis efficiency, reducing methane production (Zhou *et al.,* 2022; Luo *et al.,* 2023). The inoculum plays a crucial role in establishing and maintaining microbial populations required for efficient digestion. Different inocula, including cattle manure, sewage sludge, and anaerobic digestate, contain distinct microbial consortia that influence digestion kinetics and methane yield (Luo *et al.,* 2023). A suitable inoculum enhances enzymatic activity, promotes lignocellulose degradation, and prevents process inhibition caused by toxic compounds (Zhou *et al.,* 2022). Studies indicate that microbial diversity in the inoculum directly correlates with AD performance, with hydrolytic enzyme-producing microbes significantly improving methane production from lignocellulosic biomass (Wang *et al.,* 2023). Moreover, the inoculum-to-substrate ratio (ISR) is a critical factor, as an optimal ISR ensures balanced microbial activity, prevents excessive acidification, and stabilizes biogas output (Chen *et al.,* 2023). Investigating the influence of inoculum on the AD of *Xanthium strumarium* fruits is essential for optimizing biogas production and promoting sustainable waste management. This study aims to evaluate the effects of different inocula on microbial activity, substrate degradation, and methane yield. The specific objectives are as follows: (i) to determine the cumulative biogas production from inoculated and uninoculated digesters (ii) to compare the influence of inoculum on the anaerobic digestion in terms of hydraulic retention time and average biogas yield (iii) to determine the percentage degradation of substrate (*Xanthium strumarium* leaves) in inoculated and uninoculated digesters and (iv) to determine the pH of the inoculated and uninoculated substrates before and after anaerobic digestion

2.0 MATERIAL S AND METHODS

2.1 Study Area

The Energy Research Center, Usmanu Danfodiyo University, Sokoto is (situated between Longitude 5º 11” 30’E and 5º 14” 30’E and Latitude 13º 8” 30’N and 13º 7”0’N) of Usmanu Danfodiyo University, Sokoto, permanent site in Wamakko LGA of Sokoto State, Nigeria (SERC, 2014). Sokoto lies between Longitude 4^0 8’E and 6^0 54’E and latitude 12^0N and 13º 58’N with an altitude of 351m above the sea level within Sudan Savanna Region of Nigeria. The climate of the area is hot, semi-arid tropical type. It is

characterized by a long and severe dry season lasting from October through May, and short but intensive wet season from May/June until September. The rainfall pattern is characteristically distributed with a peak in August. Sokoto falls under the Sudan savanna agro-ecological zone (North-Western Nigeria) (SERC, 2014).

2.2 Sample Collection

Fresh samples of *Xanthium strumarium* leaves were collected from Usmanu Danfodiyo University, Sokoto. The samples were collected in a clean polythene bags and transported within 24 hours of collection to Sokoto Energy Research Centre, Usmanu Danfodiyo University Sokoto for laboratory analyses.

2.3 Sample preparation

The fresh samples of the *Xanthium strumarium* leaves were air dried under the sun after which were dried in an oven at 105 ± 2^{o}C. The samples were further dried at room temperature for a period of two weeks before they were grounded into powdered form using a pestle and mortar (Rabah *et al.,* 2010).

2.4 Experimental Design

A biogas plant was setup comprising of six (6) empty bama bottles of 470g capacity as biogas digesters for each sample. A hole was made at the center of the lid of each of the three (3) empty bama bottles and a hose pipe (1 inch) was connected to the hole of each digester and covered with epoxy steel gum to avoid leaking of the gas. The pipe conveyed the gas from the digester to a measuring cylinder (1000cm^3 capacity) filled with water and placed in an inverted position in a basin filled with water (water displacement method). The cylinder was held firm in upside down position by a retort stand. The gas produced from the digesters was conveyed through the hose pipe to the measuring cylinder which displaced the water downward. The volume of gas produced was measured by the amount of water being displaced from the measuring cylinder. Daily production temperature was recorded at 12:00 noon throughout the retention period of seven weeks (Baki, 2004).

2.5 Slurry preparation

Fifty grams (50 g) of *Xanthium strumarium* leaves was weighed using a digital weighing scale and poured into empty bama bottles of 473 grams capacity serving as digesters, followed by the addition of 500ml of water to give a ratio of 1:10 of substrates to water. The mixtures were all continuously stirred with a rod for five (5) minutes, until it's fully dissolved to obtain homogeneity (Baki, 2004).

2.6 Addition of Inocula

About 15 grams of Cow dung was used as an inocula in the digester. The first three digesters (D1, D2 and D3) received 15 ml of inoculum each respectively. The fourth,fifth and Sixth digester (D4, D5 and D6) respectively did not receive any inoculum and was set up as a control. The contents of each digester were thoroughly mixed to distribute the seeding agent. The mouths of all the digesters were tightly sealed.

2.7 Determination of pH

The pH values of the substrates was determined before and after anaerobic digestion using a digital pH meter. Sample of substrate were mixed with water in a clean beaker to form a slurry (Agrawa *et al.,* 2023). The pH meter was calibrated using standard buffer solutions, and the pH of the slurry was measured before anaerobic digestion by immersing the pH electrode into the sample and pH of the slurry after each week of anaerobic digestion was measured in triplicates and recording the reading (Onwuliri *et al.,* 2014).

2.8 Determination of temperature

To monitor the temperature, a wall-mounted thermometer, was installed near the biogas digester. The wall-mounted thermometer was first calibrated to ensure accurate temperature readings. At every 12:00 PM, the ambient temperature for each substrate were recorded for the period of retention time (Masinde *et al.,* 2020).

2.9 Statistical Analysis

The data obtained on biogas production and average daily gas yields from the inoculated digesters were first subjected to descriptive statistical analysis in order to summarize the mean values, retention times, and total gas volumes.

3.0 RESULTS

3.1 Weekly Biogas Yield from Inoculated and Uninoculated Substrates of *Xanthium strumarium* leaves

The table 1 showed that, the inoculated substrate at the first week (7 days), had the highest biogas yield with (240.00 cm^3), followed by second week (14 days), with (84.00) and the least was (44.00 cm^3) at fourth week (28 days). The uninoculated substrate had (80.00 cm^3) at the first week (7 days), followed bu second week (14 days) with (28.00 cm^3) and the least was (22.00 cm^3) at fourth week (28 days).

Table 1: Weekly Biogas Yield from Inoculated and Uninoculated Substrates of *Xanthium strumarium* leaves

Days (Retention Period)	Biogas Yield (cm^3)		Mean Weekly Temperature (^0C)
	Inoculated	Uninoculated	
7	240.00	80.00	30.10
14	84.00	28.00	29.50
21	60.00	24.00	28.20
28	44.00	22.00	27.10
Total	**428.00**	**154.00**	

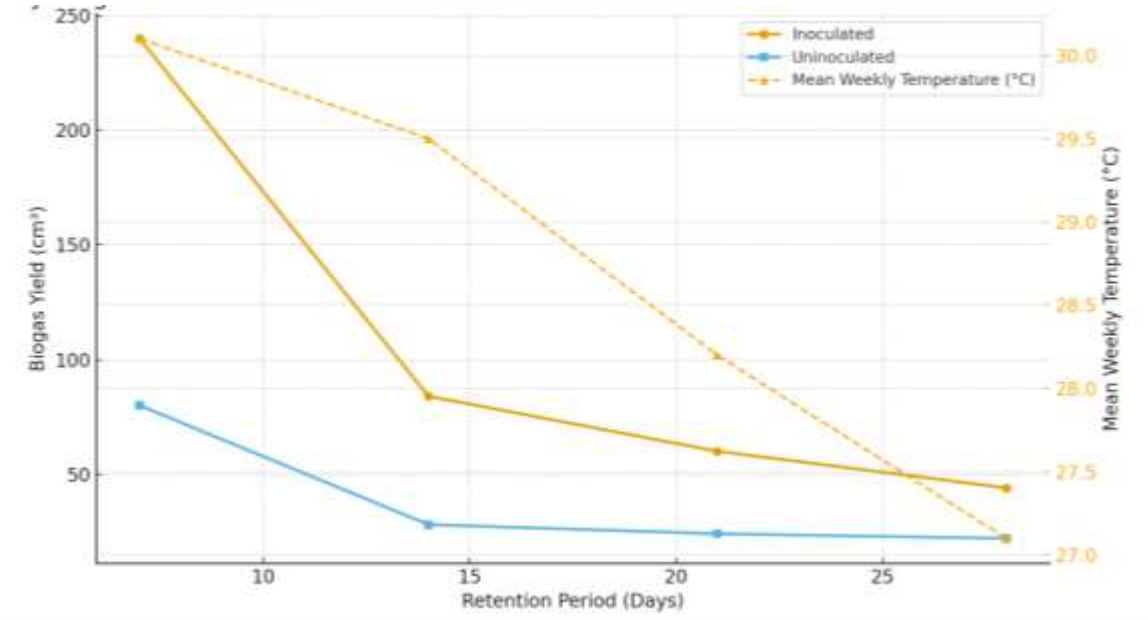


Figure 1: Line graph showing the trends of Biogas Yield and Temperature Variation

3.2 Comparison of the Influence of Inoculum on Anaerobic Digestion Parameters in Inoculated and Uninoculated Substrates

The table (Table 2) showed that, the inoculated substrate had the highest daily gas production at a retention time of 21 days with (20.38%) while the uninoculated substrate had the least daily gas production at a retention time of 25 days with (6.61%).

Table 2: Comparison of the Influence of Inoculum on Anaerobic Digestion Parameters in Inoculated and Uninoculated Substrates

Digesters	Hydraulic Retention Time (Days)	Cumulative Gas Production (cm^3)	Average Daily Gas Production (ml)
Inoculated	21	428.00	20.38
Uninoculated	25	154.00	6.61

3.3 Percentage Degradation of Inoculated and Uninoculated Substrates

The table 3 showed that, the inoculated substrate had the highest percentage of degradation with (66.00%) while the uninoculated substrate had the least percentage of degradation with (60.00%).

4.3 Percentage degradation of Inoculated and Uninoculated Substrates

Digesters	Weight of Substrates Before Digestion	Weight of Substrates After Digestion	Percentage Degradation (%) (So - Se/ So ×100)
Inoculated	50.00	17.00	66.00
Uninoculated	50.00	20.00	60.00

3.4 pH of Inoculated and Uninoculated Substrates Before and After Anaerobic Digestion

The table 4 showed that, the pH of inoculated substrate before digestion had the highest value (6.69) while the uninoculated substrate had the least value with (5.78). The pH after digestion showed that, the inoculated substrate had the highest value with (8.11), while the least value is (6.57) for the uninoculated.

Table 4: pH of Inoculated and Uninoculated Substrates Before and After Anaerobic Digestion

Digesters	Before Digestion	After Digestion
Inoculated	6.69	8.11
Uninoculated	5.78	6.57

4.1 DISCUSSION

The weekly biogas yield from inoculated and uninoculated substrates of *Xanthium strumarium* leaves showed that the total biogas yield for the inoculated substrate was considerably higher than that of the uninoculated substrate. This represents an increase of about 178%, indicating that the addition of inoculum enhanced microbial activity and gas production. The highest yield was recorded in the first week, after which the production rate gradually decreased with time. This decline may be attributed to substrate depletion and a reduction in microbial population density as digestion progressed. Similar production patterns have been observed in several studies on plant-based substrates, where biogas generation peaked during the first week and decreased in subsequent weeks due to reduced availability of easily degradable organic matter (Li *et al.*, 2024).

Temperature during the digestion period ranged between 27 °C and 30 °C, which is within the mesophilic range suitable for optimal anaerobic microbial activity. According to Wang *et al*. (2022), mesophilic temperatures between 30–37 °C promote stable biogas generation and prevent inhibition of methanogenic bacteria. The slight temperature decrease across the four weeks may have contributed to the gradual decline in gas output, since microbial metabolism slows at lower temperatures.

Ira *et al.* (2024) reported that inoculum prepared from acclimatized cow dung and heat-treated organic wastes produced higher methane content compared to uninoculated systems. Similarly, Zhang *et al*. (2023) observed that co-digestion of plant residues with inoculated manure significantly increased gas yield due to improved microbial synergism and nutrient balance.

The relatively lower yield in the uninoculated digester could be due to the slow establishment of anaerobic microflora. The absence of a ready methanogenic community leads to an extended lag phase before biogas production begins. This agrees with the findings of (Li *et al.,* 2024), who demonstrated that inoculated digesters had shorter start-up times and higher gas production rates compared to uninoculated

controls. Additionally, Chukwuma *et al.* (2021) reported that inoculation using cow dung slurry enhanced gas output from leafy substrates by over 60% compared to uninoculated systems.

The comparison on the influence of inoculum on anaerobic digestion parameters between inoculated and uninoculated substrates of *Xanthium strumarium* leaves showed that the inoculated digester had a shorter hydraulic retention time (21 days), a higher cumulative gas production, and a greater average daily gas yield than the uninoculated digester. This demonstrates that the addition of inoculum significantly enhanced the rate and efficiency of anaerobic digestion. The shorter retention time in the inoculated system suggests that microbial activity commenced rapidly due to the presence of active methanogenic consortia. According to Ira *et al.* (2024), inoculated digesters experience faster microbial colonization and shorter adaptation phases, leading to earlier methane generation and higher biogas yields. The findings of this study are consistent with those of Li *et al.* (2024), who reported that co-digestion of cow manure with rice straw under low inoculum-to-substrate ratios increased total methane yield while reducing digestion time..

The percentage degradation of inoculated and uninoculated substrates of *Xanthium strumarium* leaves. The inoculated digester recorded a higher percentage degradation compared to the uninoculated system. The higher degradation rate in the inoculated digester clearly indicates that inoculum addition enhanced microbial hydrolysis and biodegradation efficiency during the anaerobic digestion process (Zhang *et al.,* 2023).

According to Ira *et al*. (2024), the presence of an inoculum increases the abundance of hydrolytic and methanogenic microorganisms that accelerate the decomposition of complex organic compounds into simpler intermediates.

The initial slightly acidic pH observed before digestion may be attributed to the presence of organic acids in the substrate and inoculum. As digestion progressed, these acids were metabolized into biogas components such as methane and carbon dioxide, leading to a gradual increase in pH. According to Ira *et al.* (2024), the conversion of volatile fatty acids (VFAs) into methane during the methanogenesis stage results in an alkaline shift in digester pH.

In contrast, the uninoculated digester exhibited a lower pH, which is close to the lower limit for efficient methane production. This suggests that acid accumulation occurred due to the delayed establishment of methanogenic bacteria. Li *et al.* (2024) observed that uninoculated digesters often experience transient acidification during the early phase of fermentation, which inhibits methanogenesis until microbial adaptation occurs. The inoculum's buffering capacity likely helped maintain the pH balance, preventing process inhibition and sustaining microbial stability throughout digestion.

4.2 CONCLUSION

The findings of this study clearly demonstrate that inoculation plays a vital role in enhancing the anaerobic digestion of *Xanthium strumarium* leaves. The inoculated digester produced a markedly higher cumulative biogas yield compared to the uninoculated digester, with a shorter hydraulic retention time and a higher average daily gas production. The higher percentage degradation and improved pH stability further confirm that inoculum addition accelerates microbial activity, enhances substrate breakdown, and maintains favorable conditions for methanogenesis.

4.3 RECOMMENDATIONS

1. Subsequent designs should adopt shorter hydraulic retention times to improve process efficiency and reduce operational costs.
2. Continuous pH and temperature monitoring should be maintained within mesophilic ranges to ensure stable methanogenic activity and prevent process inhibition.
3. Further studies should explore co-digestion of *Xanthium strumarium* with other organic wastes such as animal manure or agricultural residues to balance nutrient ratios and enhance gas yield.
4. Detailed microbial analyses of inocula should be conducted to identify dominant methanogens and optimize conditions for their growth and activity.

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