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Novel techniques to improve bio-methanation of rotten grape fruit waste

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***Abstract*—The anaerobic digestion of rotten grape fruit waste presents a viable strategy for mitigating greenhouse gas emissions and generating renewable energy. However, the process is frequently constrained by inhibitory compounds, pH fluctuations, and nutrient deficiencies, which can compromise biomethane yields and process stability. This study undertakes a comprehensive investigation of the synergistic effects of fish waste, prawn peel, bagasse charcoal, and wood ash supplementation on bio-methanation kinetics, methane content, and process robustness. The results reveal that the supplementation of rotten grape fruit waste with fish waste and prawn peel elicited a significant enhancement in biomethane production, concomitant with improved volatile fatty acid (VFA) metabolism and ammonia tolerance. The addition of bagasse charcoal and wood ash further augmented process stability and buffering capacity, thereby mitigating pH fluctuations and promoting a favorable environment for methanogenic archaea. Optimization of the supplementation strategy through response surface methodology (RSM) revealed a synergistic interaction between the supplements, yielding enhanced biomethane production and process stability. The findings of this study provide novel insights into the development of optimized supplementation strategies for enhancing bio-methanation of rotten grape fruit waste, thereby contributing to the advancement of sustainable waste management and renewable energy production.**

***Index Terms*—Anaerobic digestion, Biogas, Bio-methanation, Carbon additives, Metal impregnation, Rotten grape**

I. INTRODUCTION

The escalating global energy crisis, precipitated by the dwindling reserves of fossil fuels and the concomitant escalation of greenhouse gas emissions, has necessitated the exploration of sustainable and eco-friendly energy paradigms. The alarming rate of depletion of fossil fuels, coupled with the devastating impacts of climate change, has underscored the need for a paradigmatic shift towards renewable energy sources. Anaerobic digestion, a microbial process that converts organic waste into biomethane, has emerged as a promising strategy for mitigating the environmental impacts associated with fossil fuel combustion (Ozili et al., 2023).

Grape fruit waste, a significant by-product of the grape processing industry, poses substantial environmental challenges due to its high organic content, propensity for pollution, and potential for contributing to greenhouse gas emissions (Achkar et al., 2023). The grape processing industry generates vast quantities of waste, including grape pomace, grape juice, and other organic residues. If left unutilized, these wastes can lead to environmental pollution, contamination of water resources, and loss of valuable nutrients.

The anaerobic digestion process involves the breakdown of organic matter by microorganisms in the absence of oxygen, resulting in the production of biogas, a mixture of methane and carbon dioxide. Methane, the primary component of biogas, is a potent greenhouse gas, but it can also be utilized as a renewable energy source (Kothari et al., 2014). The anaerobic digestion process offers several advantages, including the production of renewable energy, reduction of waste, and generation of nutrient-rich digestate.

However, the anaerobic digestion process is often hindered by several factors, including the presence of inhibitory compounds, pH fluctuations, and nutrient deficiencies. To overcome these challenges, various supplements and additives can be employed to enhance the anaerobic digestion process. These supplements and additives can include nutrients, buffering agents, and microorganisms that can enhance the growth and activity of methanogenic microorganisms (Yu et al., 2023).

In this context, the present study undertakes a comprehensive investigation of novel techniques to enhance the bio-methanation of rotten grape fruit waste. A multifaceted approach was employed, involving the evaluation of various supplements and additives to augment biomethane production. The study aimed to explore the potential of rotten grape fruit waste as a feedstock for biomethane production, while also investigating the effects of various supplements and additives on the anaerobic digestion process.

Initially, the bio-methanation potential of rotten grape juice was assessed, followed by an investigation of the methane content in the produced biogas. Subsequent experiments involved supplementing the rotten grape juice with fish waste and prawn peel, and evaluating the effect of varying concentrations of these supplements on biomethane production. The use of fish waste and prawn peel as supplements was motivated by their high protein and nutrient content, which can enhance the growth and activity of methanogenic microorganisms.

Furthermore, the study explored the use of bagasse charcoal as an additive to enhance bio-methanation. The impact of varying concentrations of bagasse charcoal on biomethane production was investigated, and the results were compared with those obtained using $FeCl_3$-impregnated bagasse charcoal. The latter involved the impregnation of bagasse charcoal with ferric chloride to enhance its catalytic properties and facilitate the transfer of electrons during the anaerobic digestion process (Yu et al., 2023).

The findings of this study contribute to the development of novel techniques for improving the bio-methanation of rotten grape fruit waste. The results provide valuable insights into the potential of various supplements and additives to enhance biomethane production, and offer a promising solution for reducing waste, generating renewable energy, and promoting a sustainable bioeconomy.

II. MATERIALS AND METHODS

Rotten Grape Juice: Rotten grapes were collected from local fruit shops in Calicut. The grapes were manually sorted, washed, and then crushed to extract the juice. This juice was used as a fermentation substrate in the biogas production process.

Fresh Cow Dung: Fresh cow dung was obtained from a household located near the Calicut University campus. It was collected in clean containers and used as a microbial inoculum due to its natural microbial load suitable for anaerobic digestion.

Tap Water: Tap water was sourced from the university laboratory facilities and used for dilution and cleaning purposes.

Measuring Cylinder, Petri Plates, Glass Beaker, and 1000 mL Conical Flask: These standard laboratory glassware items were used for measuring, mixing, culturing, and conducting the fermentation experiments. They were obtained from the Calicut University Department of Biotechnology.

Spatula, Rubber Stopper (with One and Two Holes), Rubber Tubes, Wooden Stand for Gas Collection: These were used to construct the biogas setup and facilitate gas transfer and collection. The rubber stoppers ensured an airtight seal for the fermentation flask.

Grinder: Used to grind dried prawn peels into a fine powder. The grinder was cleaned thoroughly before and after use.

Weighing Balance: Used to accurately weigh all solid materials, including fish waste, bagasse charcoal, and additives.

pH Strip and pH Meter: Used to monitor the pH changes throughout the fermentation process to ensure optimal conditions for biogas production.

Biogas Slurry: Previously fermented slurry was used as a seeding material to enhance the microbial activity in the setup.

Centrifuge Tubes and Centrifuge: Used to separate solid and liquid fractions from the slurry for further analysis. Test Tubes and Distilled Water: Used for preparing test samples and dilutions during the experimental process.

Bagasse Charcoal: Purchased online from a certified supplier. It was used as an additive to enhance microbial activity and gas yield.

Wood Ash: Collected from the canteen at the Calicut University Ladies Hostel. It was sieved and stored in airtight containers to prevent moisture absorption.

Ferric Chloride ($FeCl_3$): Used as a chemical additive.

NaOH: Used for methane gas content determination.

Hot Air Oven: Used for drying samples such as prawn peel and fish waste before grinding and storage.

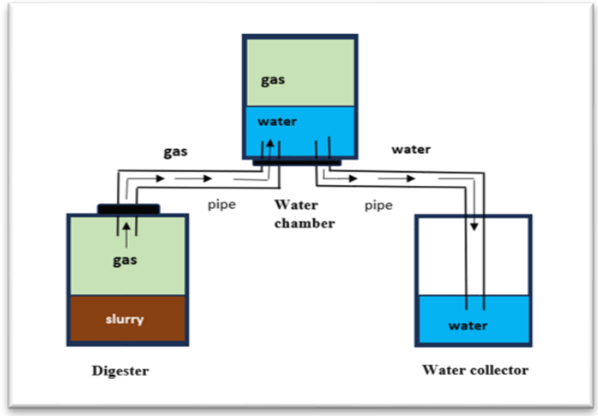
Fish Waste: Collected from the fish market in Calicut. The waste included uneaten parts like fish heads and entrails, which were washed and stored in airtight containers at 4°C before use.

Prawn Peel Powder: Prawn peels were collected from the same fish market, thoroughly cleaned, sun-dried, and then ground into a fine powder using a grinder. The powder was used as a nitrogen-rich additive.

Water Displacement Method

Fermentation mixture was poured in 1000mL Conical flask enclosed with one hole cork with rubber tube connecting an inverted 1000mL Conical flask filled with water by two holes rubber cork and this bottle is connected to glass vessel filled with 100mL water.

Biogas was collected in inverted bottle by downward displacement method, that is equal amount of water was displaced as biogas produced. The daily displaced water was measured accurately with measuring cylinder which is equal to daily biogas production from particular experiment set up (Mamun et al., 2015).



A. Bio-methanation of rotten grape juice

Biogas production from fermented grape juice was assessed via the water displacement method. A 1000 mL conical flask was utilized, containing a reaction mixture comprised of 300 mL of grape juice (*Vitis vinifera*) and 60 g of fresh cow dung (*Bos taurus*). The mixture was diluted to 600 mL with tap water, and the initial pH was determined using pH indicator strips (Kassongo et al., 2020).

B. Determination of methane content in biogas with rotten grape juice as substrate (Deheri et al., 2021)

A 600 mL reaction mixture was prepared by combining 300 mL grape juice and 60 g fresh cow dung, diluted with tap water. Initial pH was measured using a pH strip. The mixture was transferred to a 1000 mL conical flask, and methane production was quantified by water displacement using an inverted glass bottle filled with 5% NaOH solution.

C. Supplementation of fish waste (carbon-nitrogen optimization) (Nges et al., 2012)

Negative Control: A 600 mL mixture of 60 g fresh cow dung and tap water was prepared. Biogas production was measured via water displacement in a 1000 mL conical flask.

Control Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was diluted to 600 mL with tap water. Initial pH was measured, and biogas production was quantified via water displacement.

Test Treatment: A mixture of 300 mL grape juice, 60 g fresh cow dung, and 1% fish waste was diluted to

600 mL with tap water. Initial pH was measured, and biogas production was quantified via water displacement.

D. Bio-methanation of rotten grape juice with varying concentrations of fish waste

A negative control was prepared by mixing 60 g fresh cow dung with tap water to a final volume of 600 mL, and biogas production was measured via water displacement in a 1000 mL conical flask.

The control treatment consisted of a 600 mL mixture of 300 mL grape juice and 60 g fresh cow dung, diluted with tap water. Initial pH was recorded, and biogas production was quantified via water displacement.

Four test treatments were prepared by combining 300 mL grape juice, 60 g fresh cow dung, and varying concentrations of fish waste (1%, 3%, 5%, and 7% w/w). Each mixture was diluted to 600 mL with tap water, and initial pH was measured. Biogas production was then quantified via water displacement in 1000 mL conical flasks (Nges et al., 2012).

E. Supplementation of prawn peel powder (carbon-nitrogen optimization) (Mathew et al., 2021)

A control treatment was prepared by mixing 300 mL grape juice and 60 g fresh cow dung, diluted to 600 mL with tap water. Initial pH was measured, and biogas production was quantified via water displacement in a 1000 mL conical flask.

A negative control was also prepared, consisting of 60 g cow dung diluted to 600 mL with tap water. Biogas production was measured using the same method.

The test treatment involved mixing 300 mL grape juice, 60 g fresh cow dung, and 1% prawn peel powder, diluted to 600 mL with tap water. Initial pH was measured, and biogas production was quantified via water displacement in a 1000 mL conical flask.

F. Bio-methanation of rotten grape juice with varying concentrations of prawn peel powder (Mathew et al., 2021)

Control Treatment: A 600 mL mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with tap water. Initial pH was measured, and biogas production was quantified via water displacement in a 1000 mL conical flask.

Negative Control: A 600 mL mixture of 60 g cow dung and tap water was prepared. Biogas production was measured using the same method.

Test Treatment: Four mixtures of 300 mL grape juice, 60 g fresh cow dung, and prawn peel powder (1%, 3%, 5%, and 7% w/w) were prepared. Each mixture was blended, diluted to 600 mL with tap water, and initial pH was measured. Biogas production was quantified via water displacement method in 1000 mL conical flasks (Mathew et al., 2021).

G. Effect of bagasse charcoal on biogas production (Raziya et al., 2020)

Control Treatment- A 300 mL sample of grape juice was mixed with 60 g of fresh cow dung in a beaker. The volume was adjusted to 600 mL with tap water. Initial pH was measured using pH strips. The mixture was then transferred to a 1000 mL conical flask, and biogas production was measured by water displacement.

Negative Control- A 600 mL mixture of 60 g cow dung and tap water was prepared. Initial pH was measured, and biogas production was assessed using the same method as the control treatment.

Test Treatment- A 300 mL sample of grape juice was mixed with 60 g of fresh cow dung and 1% bagasse charcoal in a beaker. The volume was adjusted to 600 mL with tap water. Initial pH was measured using pH strips. The mixture was then transferred to a 1000 mL conical flask, and biogas production was measured by water displacement.

H. Bio-methanation of rotten grape juice with varying concentrations of bagasse charcoal (Raziya et al., 2020)

A control treatment was prepared by mixing 300 mL of grape juice with 60 g of fresh cow dung in a beaker. The volume was adjusted to 600 mL with tap water. Initial pH was measured using pH strips. The mixture was then transferred to a 1000 mL conical flask, and biogas production was quantified by water displacement.

A negative control was also prepared by mixing 60 g of cow dung with 600 mL of tap water.

Four test treatments were prepared by mixing 300 mL of grape juice, 60 g of fresh cow dung, and varying concentrations of bagasse charcoal (1%, 3%, 5%, and 7% w/w). Each mixture was adjusted to 600 mL with tap water, and initial pH was measured. Biogas production was quantified by water displacement in 1000 mL conical flasks.

I. Effect of varying concentrations of ferric chloride impregnated bagasse charcoal on bio-methanation (Park and Novak, 2013)

Control Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride (10, 25, 50, 70, and 90 ppm). The mixture was diluted to 600 mL with tap water, and initial pH was measured using pH strips. Biogas production was quantified via water displacement in 1000 mL conical flasks.

(Metal ion-impregnated charcoal was prepared by mixing 5 g of corresponding metal chloride (ferric chloride) with 5 g of charcoal in 100 mL of water. The mixture was kept for 1 hour, then centrifuged, and the residue was dried in a hot air oven. The dried metal ion-impregnated charcoal was stored in a dry glass bottle.)

Test Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride-impregnated bagasse charcoal (10, 25, 50, 70, and 90 ppm). The mixture was diluted to 600 mL with tap water, and initial pH was measured using pH strips. Biogas production was quantified via water displacement in 1000 mL conical flasks (Park and Novak, 2013).s

J. Effect of wood ash on biogas production (Cimon et al., 2020)

A control treatment was prepared by combining 300 mL of grape juice with 60 g of fresh cow dung in a beaker. The reaction mixture was diluted to 600 mL with tap water. Initial pH was determined using pH indicator strips. The mixture was then transferred to a 1000 mL conical flask, and biogas production was quantified via water displacement.

A negative control was prepared by mixing 60 g of cow dung with 600 mL of tap water, followed by transfer to a 1000 mL conical flask for biogas measurement.

The test treatment involved combining 300 mL of grape juice, 60 g of fresh cow dung, and 1% (w/w) wood ash in a beaker. The reaction mixture was diluted to 600 mL with tap water, and initial pH was determined. The mixture was then transferred to a 1000 mL conical flask, and biogas production was quantified via water displacement**.**

K. Bio-methanation of rotten grape juice with varying concentrations of wood ash (Cimon et al., 2020)

A control treatment consisted of 300 mL grape juice and 60 g fresh cow dung, diluted to 600 mL with tap water. Initial pH was measured using pH strips. Biogas production was quantified via water displacement in a 1000 mL conical flask.

A negative control was prepared with 60 g cow dung in 600 mL tap water.

Four test treatments were prepared by mixing 300 mL grape juice, 60 g fresh cow dung, and varying concentrations of wood ash (1%, 3%, 5%, and 7% w/w). Each mixture was diluted to 600 mL, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

Each experiment was conducted three times for accuracy.

III. RESULTS AND DISCUSSION

A. Bio-methanation of rotten grape juice

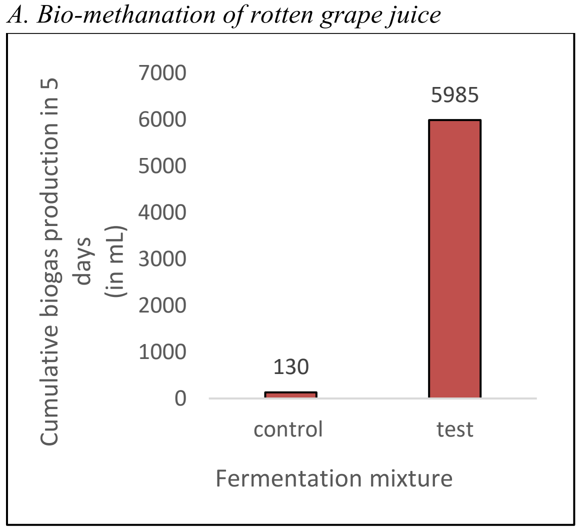


Figure 4. 1 Bio-methanation of rotten grape juice

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase in biogas production
Control	60g fresh cow dung + 600mL tap water	130	
Test	300mL grape juice + 60g cow dung + 300mL tap water	5985	4500%

Table 4.1 Determination of biogas production with Rotten Grape Juice as substrate

India is among the world's largest fruit and vegetable producers, with grape cultivation spanning approximately 34,000 hectares and yielding 2.48 million tonnes annually. However, around 30-35% of this production is lost due to inadequate cold storage, transportation issues, lack of suitable post-harvest technologies, and damage from pests and diseases. The grape processing industry primarily produces grape juice, generating substantial quantities of grape pomace (grape pulp) that contribute to environmental pollution (Sah et al., 2022). Fortunately, grapes' nutrient-rich composition and year-round availability make them ideal for conversion into valuable products like biomethane through bio-methanogenic pathways. Notably, the grape pulp's high carbon content and low nitrogen content make it a suitable substrate for bio-methanation, as evident from Figure 4.1, which shows a significant increase in gas production when using rotten grapes as a substrate, with a remarkable 4500% increase compared to the control.

B. Determination of methane content in biogas with rotten grape juice as substrate

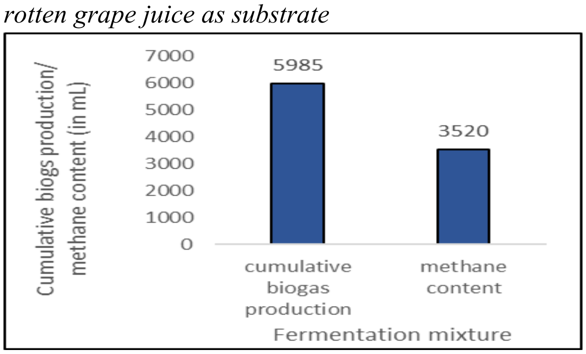


Figure 4. 2 Determination of methane content in biogas with rotten grape juice as substrate

Fermentation mixture	Cumulative biogas production	Methane content	Percentage of methane
300mL grape juice+ 300mL water+ 60g cow dung	5985mL	3520mL	58.80%

Table 4.2 Determination of methane content in biogas with rotten grape juice as substrate

Biogas, a renewable fuel, is generated through the anaerobic digestion of diverse organic feedstocks, encompassing municipal waste, farm waste, food waste, and energy crops. The composition of raw biogas typically comprises methane (ranging from 50-75%), carbon dioxide (25-50%), and smaller proportions of nitrogen (2-8%), accompanied by trace amounts of hydrogen sulfide, ammonia, hydrogen, and various volatile organic compounds, which vary depending on the feedstock utilized. To determine the methane content in the biogas, a CO2 scrubbing method employing 5% NaOH was employed, revealing a methane content of 3520ml, which corresponds to a percentage of 58.80%, as illustrated in Figure 4.2.

C. Supplementation of fish waste (carbon-nitrogen optimization)

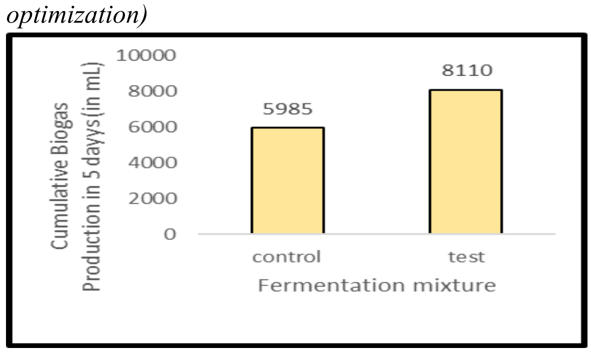


Figure 4.3 Supplementation of fish waste (carbon-nitrogen optimization)

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control	300mL grape juice + 60g cow dung + 300mL water	5985	
Test	300mL grape juice+ 60g cow dung+ 6g fish Waste + 300mL water	8110	35.50%

Table 4.3 Supplementation of fish waste (carbon-nitrogen optimization)

Effective anaerobic digestion and biogas production are significantly influenced by the carbon to nitrogen (C/N) ratio, a critical parameter that requires careful consideration. Optimal anaerobic digestion is typically achieved with a C/N ratio ranging from 20:1 to 30:1, although this can vary depending on the specific substrate and operating conditions. Co-digestion with materials having higher carbon or nitrogen contents than the primary feedstock can help optimize the C/N ratio, leading to enhanced methane production. Conversely, excessively high or low C/N ratios can inhibit the growth of methanogenic microorganisms, resulting in reduced methane generation rates and the accumulation of volatile fatty acids (VFAs) and ammonia in the digestion solution.

D. Bio-methanation of rotten grape juice with varying concentrations of fish waste

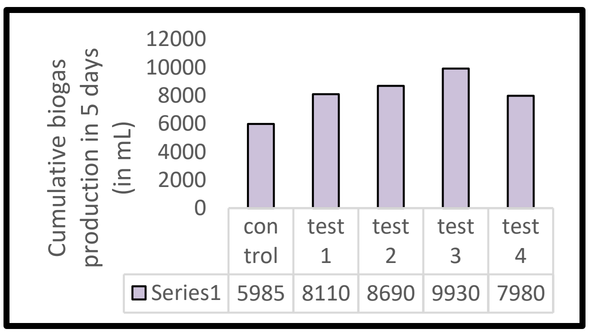


Figure 4.4 Bio-methanation of rotten grape juice with varying concentrations of fish waste

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percent age increase
Control	300mL grape juice + 60g cow dung+ 300mL water	5985	
Test 1	300mL grape juice+ 60g cow dung+ 6g fish waste (1% (w/v)) +300mL water	8110	35.50%
Test 2	300mL grape juice + 60g cow dung+ 18g fish waste (3% (w/v)) + 300mL water	8690	45.19%
Test 3	300mL grape juice + 60g cow dung+ 30g fish waste (5% (w/v)) + 300mL water	9930	65.91%
Test 4	300mL grape juice + 60g cow dung+ 42g fish waste (7% (w/v)) + 300mL water	7980	33.33%

Table 4.4 Bio-methanation of rotten grape juice with varying concentrations of fish waste

Supplementation of fish waste as a nitrogen source was investigated, and the results indicate that its addition can effectively enhance biogas production compared to the control. The optimal concentration for maximum biogas production was found to be 5% (w/w) fish waste (fig 4.4). As shown in table 4.4, the cumulative biogas production using fish waste was 9930ml, demonstrating the potential of fish waste as a nitrogen supplement to improve biogas yields.

E. Supplementation of prawn peel powder (carbon-nitrogen optimization)

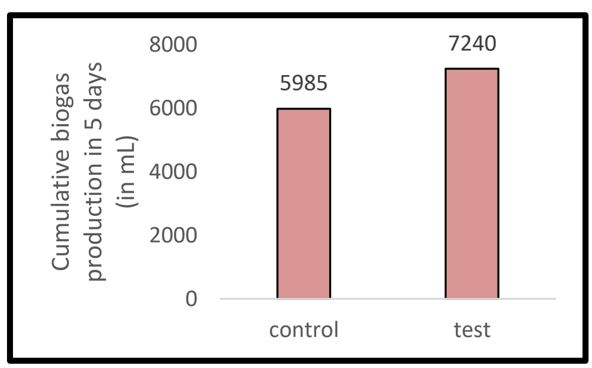


Figure 4.5 *Supplementation of prawn peel powder (carbon-nitrogen optimization)*

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control	300mL grape juice + 60g cow dung + 300mL water	5985	
Test	300mL grape juice+ 60g cow dung+ 6g prawn peel powder + 300mL water	7240	20.96%

Table 4.5 Supplementation of prawn peel powder (carbon-nitrogen optimization)

Prawn peel powder has emerged as a promising nitrogen source in bio-methanation, offering a sustainable and eco-friendly alternative to traditional nitrogen supplements. Rich in protein and nitrogenous compounds, this waste by-product of the seafood processing industry can provide the necessary nutrients for optimal microbial growth and activity, thereby enhancing biomethane production. Utilizing this readily available resource also presents an opportunity for waste valorization, reducing disposal costs and promoting a more circular bioeconomy. Furthermore, its high nitrogen content can help maintain an optimal carbon-to-nitrogen ratio, a critical parameter for efficient bio-methanation.

F. Bio-methanation of rotten grape juice with varying concentrations of prawn peel powder

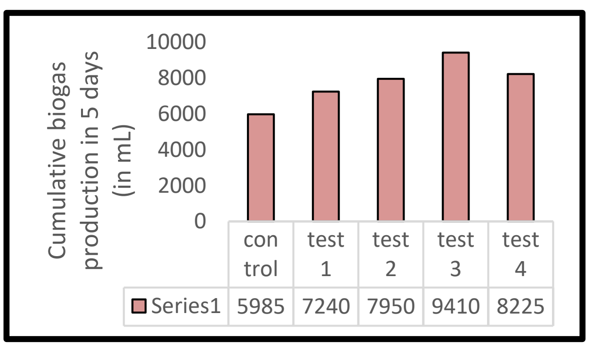


Figure 4.6 *Bio-methanation of rotten grape juice with varying concentrations of prawn peel powder*

Table 4.6 Bio-methanation of rotten grape juice with varying concentrations of prawn peel powder

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control	300mL grape juice + 60g cow dung+ 300mL water	5985	
Test 1	300mL grape juice+ 60g cow dung+ 6g prawn peel powder (1% (w/v)) +300mL water	7240	20.96%
Test 2	300mL grape juice + 60g cow dung+ 18g prawn peel powder (3% (w/v)) + 300mL water	7950	32.83%
Test 3	300mL grape juice + 60g cow dung+ 30g prawn peel powder (5% (w/v)) + 300mL water	9410	57.22%
Test 4	300mL grape juice + 60g cow dung+ 42g prawn peel powder (7% (w/v)) + 300mL water	8225	37.42%

Supplementation of prawn peel powder as a nitrogen source significantly enhanced biogas production, outperforming the control. The optimal concentration for maximum biogas production was found to be 5% (w/w) prawn peel powder (fig 4.6). Notably, the cumulative biogas production using prawn peel powder reached 9410ml (table 4.6), demonstrating its potential as a valuable nitrogen supplement for augmenting biogas yields.

G. Effect of bagasse charcoal on biogas production

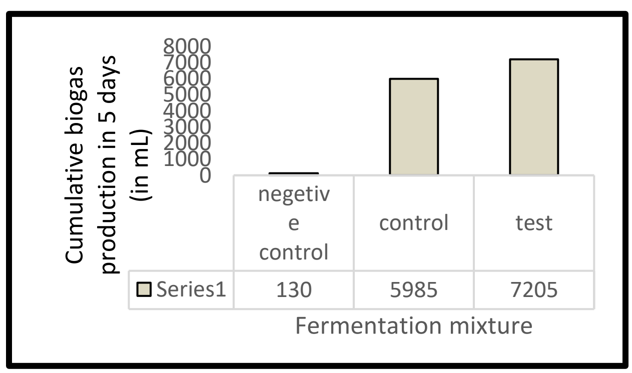


Figure 4.7 Effect of bagasse charcoal on biogas production

Table 4.7 Effect of bagasse charcoal on biogas production

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Negative Control	60g fresh cow dung + 600mL tap water	130	
Control	300mL grape juice + 60g cow dung + 300mL tap water	5985	
Test	300mL grape juice+ 300mL water+ 60g cow dung+ 6g bagasse charcoal (1% (w/v))	7205	20.38%

The incorporation of bagasse charcoal into bio-methanation systems has been shown to have a profoundly positive impact on biogas production. As a porous and inert material, bagasse charcoal provides a favorable environment for microbial growth and activity, leading to enhanced biomethane yields. Additionally, bagasse charcoal's high surface area and adsorption capacity enable it to effectively buffer pH fluctuations, reduce volatile fatty acid (VFA) toxicity, and mitigate ammonia inhibition, thereby maintaining optimal conditions for methanogenic microorganisms. As a result, the addition of bagasse charcoal can significantly improve biogas production rates, increase methane content, and enhance overall process stability.

H. Bio-methanation of rotten grape juice with varying concentrations of bagasse charcoal

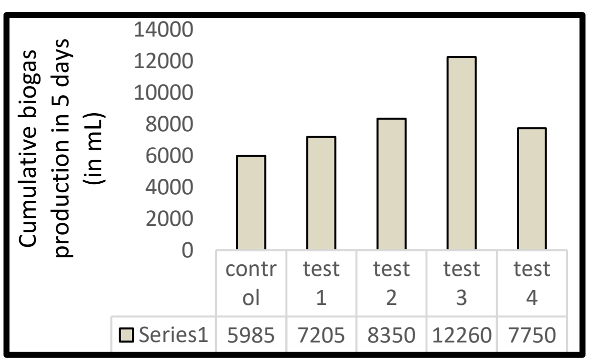


Figure 4.8 Bio-methanation of rotten grape juice with varying concentrations of bagasse charcoal

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control	300mL grape juice + 60g cow dung + 300mL tap water	5985	
Test 1	300mL grape juice+ 300mL water+ 60g cow dung+ 6g bagasse charcoal (1% (w/v))	7205	20.38%
Test 2	300mL grape juice+ 300mL water+ 60g cow dung+ 18g bagasse charcoal (3% (w/v))	8350	39.51%
Test 3	300mL grape juice+ 300mL water+ 60g cow dung+ 30g bagasse charcoal (5% (w/v))	12260	104.84%
Test 4	300mL grape juice+ 300mL water+ 60g cow dung+ 42g bagasse charcoal (7% (w/v))	7750	29.49%

Table 4.8 Bio-methanation of rotten grape juice with varying concentrations of bagasse charcoal

The effect of bagasse charcoal on biogas production using rotten grapes was determined and there is significant increase in the production compared to control (20.38%). Fig. 4.8 shows the data of cumulative biogas production after the addition of varying concentration of bagasse charcoal. It is evident from the figure that 5% (w/v) bagasse charcoal is the optimum concentration for bio-methanation using rotten grape as a substrate (12260ml). There is 104.84% increase in the production of biogas when compared to control.

I. Effect of varying concentrations of ferric chloride impregnated bagasse charcoal on bio-methanation

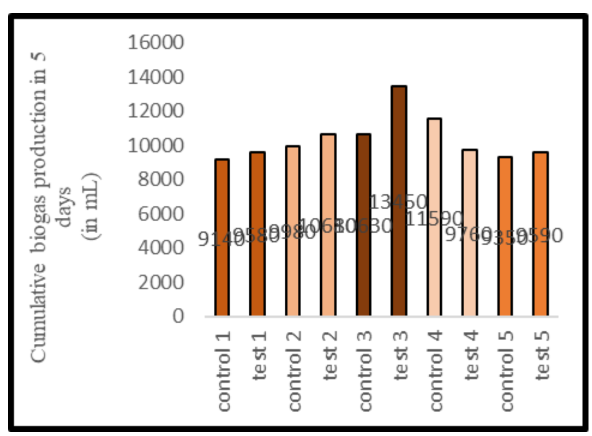


Figure 4.9 Effect of varying concentrations of ferric chloride impregnated bagasse charcoal on bio-methanation

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control 1	300mL grape juice+ 300mL water+ 60g cow dung+ 10ppm Ferric ion (0.017g ferric chloride)	9140	
Test 1	300mL grape juice+ 300mL water+ 60g cow dung+ 10ppm ferric chloride impregnated carbon (0.017g ferric chloride)	9580	4.81%
Control 2	300mL grape juice+ 300mL water+ 60g cow dung+ 25ppm Ferric ion (0.043g ferric chloride)	9980	
Test 2	300mL grape juice+ 300mL water+ 60g cow dung+ 25ppm ferric chloride impregnated carbon (0.043g ferric chloride)	10680	7.01%
Control 3	300mL grape juice+ 300mL water+ 60g cow dung+ 50ppm Ferric ion (0.087g ferric chloride)	10630	
Test 3	300mL grape juice+ 300mL water+ 60g cow dung+ 50ppm ferric chloride impregnated carbon (0.087g ferric chloride)	13450	26.5%
Control 4	300mL grape juice+ 300mL water+ 60g cow dung+ 70ppm Ferric ion (0.12g ferric chloride)	11590	
Test 4	300mL grape juice+ 300mL water+ 60g cow dung+ 70ppm ferric chloride impregnated carbon (0.12g ferric chloride)	9760	85.6% of control
Control 5	300mL grape juice+ 300mL water+ 60g cow dung+ 90ppm Ferric ion (0.15g ferric chloride)	9350	
Test 5	300mL grape juice+ 300mL water+ 60g cow dung+ 90ppm ferric chloride impregnated carbon (0.15g ferric chloride)	9590	2.56%

Table 4.9 Effect of varying concentrations of ferric chloride impregnated bagasse charcoal on bio-methanation

Metal ion impregnated on bagasse charcoal has found to be excellent for maximum biogas production. It is evident that 50ppm ferric chloride impregnated carbon produces more biogas compared to the control (26.5% increase) (Table 4.9).

J. Effect of wood ash on biogas production

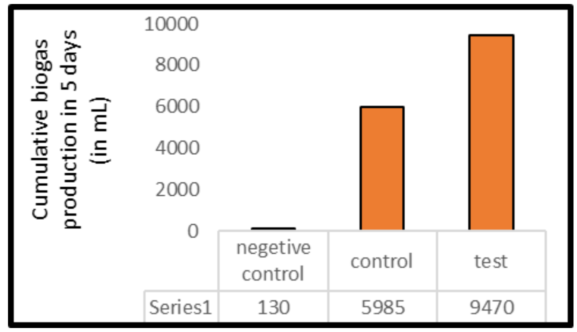


Figure 4.10 Effect of wood ash on biogas production

Table 4.10 Determination of biogas production by the addition of wood ash

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Negative Control	60g fresh cow dung + 600mL tap water	130	
Control	300mL grape juice + 60g cow dung + 300mL tap water	5985	
Test	300mL grape juice+ 300mL water+ 60g cow dung+ 6g wood ash (1% (w/v))	9470	58.22%

The gas yield in anaerobic digestion process depends on number of operating parameters such as solid concentration, temperature, pH, C/N ratio, retention time, etc. Many researchers investigated the effects of operating parameters on biogas production and reported their findings. Kim et al. (2006) investigated the influence of temperature and hydraulic retention time on anaerobic digestion using food waste as feed. They reported that the performance of anaerobic digestion and food waste digestion efficiency increased at 50°C with 12 days hydraulic retention time. Sivakumar et al. (2012) studied the effect of pH on biogas production from spoiled milk. Experiments were conducted with substrate of different pH values (5.0-8.0) and reported that the substrate with 7.0 pH resulted better biogas yield.

Fig.4.10 shows the effect of wood ash on biogas production. Since the wood ash primarily consists of calcium and other alkaline elements, this material can be used to increase the pH of the anaerobic digestate and promote the volatilization of NH_3. It is clear from the figure that there is an increase in the production of biogas after the addition of wood ash (58.22%).

K. Bio-methanation of rotten grape juice with varying concentrations of wood ash

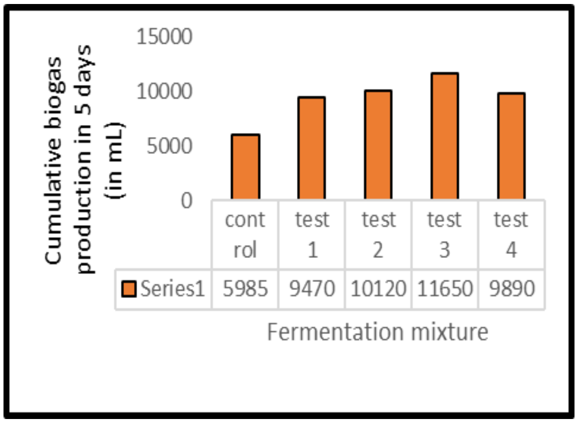


Figure 4.11 *Bio-methanation of rotten grape juice with varying concentrations of wood ash*

Sample	Fermentation Mixture	Cumulative production of Biogas over a period of 5 days (in mL)	Percentage increase
Control	300mL grape juice + 60g cow dung + 300mL tap water	5985	
Test 1	300mL grape juice+ 300mL water+ 60g cow dung+ 6g wood ash (1% (w/v))	9470	58.22%
Test 2	300mL grape juice+ 300mL water+ 60g cow dung+ 18g wood ash (3% (w/v))	10120	69%
Test 3	300mL grape juice+ 300mL water+ 60g cow dung+ 30g wood ash (5% (w/v))	11650	94.6%
Test 4	300mL grape juice+ 300mL water+ 60g cow dung+ 42g wood ash (7% (w/v))	9890	65.24%

Table 4.11 Bio-methanation of rotten grape juice with varying concentrations of wood ash

It is evident from table 4.11 that, the fermentation mixture containing 5% wood ash produced more biogas when compared to other concentrations. Although previous studies, such as that by Podmirseg *et al*., warned against using high concentrations of wood ash in anaerobic digesters due to the risk of volatile fatty acid (VFA) accumulation and disruption of methanogenic activity, the present study showed contrasting results. In this experiment, the fermentation mixture containing 5% wood ash produced a cumulative biogas yield of 11,650 mL, representing a 94.6% increase compared to the control. This suggests that, under the specific conditions of this study, a higher concentration of wood ash not only avoided inhibitory effects but also enhanced biogas production. The observed improvement may be due to better microbial adaptation or a more balanced nutrient environment, indicating that the optimal wood ash concentration could vary depending on substrate composition and operational conditions.

V. CONCLUSION

This study highlights the potential of using rotten grape waste as an effective substrate for biomethane production, contributing to both waste valorization and renewable energy generation. The incorporation of bagasse charcoal as a carbon additive significantly improved biogas yield, while the supplementation of metal ions and metal-impregnated carbons further optimized the efficiency of the anaerobic digestion process. Additionally, the use of wood ash not only stabilized reactor pH but also enhanced overall microbial activity, underscoring its role as a multifunctional additive. Together, these results demonstrate a promising integrated approach to enhancing biogas production using locally available organic wastes and low-cost additives. This work supports the advancement of sustainable and scalable bio-methanation systems, particularly in resource-constrained settings.

APPENDIX

w/v	Weight per volume
%	Percent
g	Gram
DIET	Direct interspecies electron transfer
IIET	Indirect interspecies electron transfer
mL	Milliliter
ppm	Parts per million
AC	Activated carbon
GAC	Granulated activated carbon
PAC	Powdered activated carbon
VFA	Volatile fatty acids

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