Enhancement of biogas production from rotten grape juice with metal and carbon additives

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Abstract—This study investigates the potential of rotten grapes as a sustainable substrate for enhanced bio-gas production through anaerobic digestion, leveraging metal impregnated carbon additives. Optimizations include carbon sources, metal ions of ferric chloride, nickel chloride and pH correction using wood ash. Results show a 6% increase in bio-gas yield with optimal carbon additive concentrations (5% w/v). Rotten grapes demonstrate excellent bio-gas production potential, with improved bio-gas yield and pH stability achieved through metal impregnation and wood ash. This research contributes to the development of cost-effective, sustainable bio-gas production technologies, addressing environmental, economic, and energy security concerns. The findings provide valuable insights for scaling up biogas production from rotten grapes, promoting a circular economy and mitigating climate change.

Index Terms—Anaerobic digestion, Biogas, Carbon additives, Metal impregnation, Rotten grape

I. INTRODUCTION

The biological fixation of solar energy provides the energetic basis for almost all organisms and ecosystems. Energy is critical to the functioning of physical processes throughout the universe, and of ecological processes in the biosphere of Earth. There are different forms of energy resources on the earth. All energy sources have some impact on our environment. An ever-growing population means an ever-growing requirement for energy. Energy sources can be broadly classified as renewable and nonrenewable. Knowing the dreadful fact that nonrenewable sources will eventually deplete, the importance of renewable sources cannot be underestimated. Key factors like such as cleanliness, cost, stability, efficiency and environmental effects must be taken into account while choosing the source

of energy for various applications. Global warming is one of the major hazards caused by burning of coal, oil and natural gas which has the potential to negatively affect the planet and the living beings on it. Nonrenewable energy, such as coal, natural gas and oil are available in finite quantities, expensive and require costly explorations and potentially dangerous mining and drilling operations before being available for use. Renewable energy emits lower levels of carbon and therefore is a better alternative considering the potential for climatic changes likely be caused by burning fossil fuel. As per FAO reports of 2020, China is among the leading producers of grapes among others and had contributed to production of 14,769,088 metric tons that year. Raw grapes are 81% water, 18% carbohydrates, 1% protein, and have negligible fat (0.16g/100g). 100g of raw grapes supplies 288 kilojoules (69 kilocalories) of food energy and a moderate amount of vitamin K (14% of the Daily Value), with no other micronutrients in significant amounts. Grapes with an annual production of 2.48 million tonnes, is cultivated in an area of approximately 34,000 hectares in India, of which approximately 30-35% is wasted due to improper cold storage facilities, transportation, lack of appropriate post-harvest technologies and damage caused due to pests and diseases. In the grape processing industry, the major product is grape juice. Huge quantities of grape pomace (grape pulp) are accumulated which cause environmental pollution. Since grapes are rich in nutrients, these can be channelized through bio methanogenic pathway for the production of valueadded products like biomethane. The objective of this work was to explore the possibility of developing a cost-effective bio-methanation process using rotten grapes as raw material and metal impregnated carbon materials as additives. Activated carbon (AC) in

granulated (GAC) and powdered (PAC) forms is a highly porous material with larger surface area, generated by heating biomass (wood, coal, and coconut shell) under anaerobic condition. From the various studies conducted, it can be presumed that due to high electrical conductivity and large surface area, effectively activated carbon can enhance methanogenesis, metabolism of alcohols and VFAs, reduce souring of reactor, participate in DIET by making better contacts with syntrophic bacteria, thus allowing stronger electron transfer. It also bypasses the requirement of natural biological connections such as pili or cytochromes. Studies have also reported a reduction in lag phase and enhanced metabolism of alcohol in various microbial cultures. It was also reported that powdered and granulated activated carbon could promote syntrophic metabolism of alcohol and VFAs. Due to entangled structure of carbon fibers, they render high surface area for adsorption and attachment of microbes. Hexagonal arrangement of rings lying parallel to the longitudinal axis of fiber could help provide support for microbial growth, such as biofilms, as well as enhance their physico-chemical properties. Recent investigations encourage the possible use of carbon fibers for enhancing DIET process of anaerobic digestion. Similarly, iron oxides are prevalent in nature and performs a significant part in several biological and geological processes. Magnetite has been widely used for facilitating DIET by establishing electrical conduits syntrophically between various microbial population.

II. MATERIALS

Rotten grape juice, fresh cow dung, tap water, measuring cylinder, petri plates, glass beaker, 1000 mL conical flask, spatula, rubber stopper with one and two holes, rubber tubes, wooden stand for gas collection, grinder, weighing balance, pH strip and pH meter, biogas slurry, centrifuge tubes, centrifuge, test tubes, distilled water, wood charcoal, bagasse charcoal , wood ash, nickel chloride, ferric chloride, activated charcoal, hot air oven, 5% NaOH, fish waste and prawn peel powder.

III.METHODS

Water Displacement Method

Fermentation mixture was poured in 1000mL conical flask. The initial pH was checked with a pH strip and pH meter. The 1000-mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Biogas was collected in the conical flask by downward displacement method. The daily displaced water was measured accurately with measuring cylinder which is equal to daily biogas production from particular experiment set up.



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.

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

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 method using an inverted conical flask filled with 5% NaOH solution.

C. Bio-methanation of pH adjusted rotten grape juice Biogas production from fermented, pH adjusted 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 (pH adjusted using 1% wood ash) and 60 g of fresh cow dung. The mixture was diluted to 600 mL with tap water, and the initial pH was determined using pH indicator strips.

D. Effect of wood charcoal on biogas production

The experiment consisted of two treatments: control and test. In the control treatment, 300 mL of grape juice was mixed with 60 g of fresh cow dung in a beaker. The volume of the reaction mixture was made up to 600 mL with tap water. The initial pH was measured using pH strips. The reaction mixture was then transferred to a 1000 mL conical flask, and biogas production was measured using the water displacement method.

A negative control was also prepared by mixing 60 g of cow dung with tap water to make a final volume of 600 mL. The mixture was transferred to a 1000 mL conical flask, and biogas production was measured.

In the test treatment, 300 mL of grape juice was mixed with 60 g of fresh cow dung and 1% wood charcoal in a beaker. The volume of the reaction mixture was made up to 600 mL with tap water. The initial pH was measured using pH strips. The reaction mixture was then transferred to a 1000 mL conical flask, and biogas production was measured using the water displacement method.

E. Determination of methane content in biogas upon the addition of wood charcoal

A 600 mL reaction mixture was prepared by combining 300 mL grape juice, 60 g fresh cow dung, and 5% wood charcoal, 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 conical flask filled with 5% NaOH solution.

F. Bio-methanation of rotten grape juice with varying concentrations of wood charcoal

The experiment consisted of a control, negative control, and four test treatments.

Control: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared, made up to 600 mL with tap water. Initial pH was measured using pH strips. The mixture was transferred to a 1000 mL conical flask, and biogas production was measured by water displacement.

Negative Control: A mixture of 60 g cow dung and tap water (600 mL) was prepared. Initial pH was measured, and biogas production was assessed.

Test Treatments: Four individual reaction mixtures were prepared by mixing 300 mL grape juice, 60 g fresh cow dung, and varying concentrations of wood charcoal (1%, 3%, 5%, and 7% w/w). The mixtures were made up to 600 mL with tap water, and initial pH was measured. The slurries were transferred to 1000 mL conical flasks, and biogas production was measured by water displacement.

G. Effect of ferric chloride on bio-methanation of rotten grape juice

Effect of metal ions on biogas production from rotten grapes was assessed via the water displacement method. Control was prepared by utilizing a 1000 mL conical flask, containing a reaction mixture comprised of 300 mL of grape juice and 60 g of fresh cow dung. The mixture was diluted to 600 mL with tap water, and the initial pH was determined using pH indicator strips. Test mixture was prepared by adding 10ppm ferric chloride to 300mL grape juice and 60g fresh cow dung was added. The mixture was diluted to 600mL with tap water and initial pH was determined.

H. Effect of nickel chloride on bio-methanation of rotten grape juice

Effect of metal ions on biogas production from rotten grapes was assessed via the water displacement method. Control was prepared by utilizing a 1000 mL conical flask, containing a reaction mixture comprised of 300 mL of grape juice and 60 g of fresh cow dung. The mixture was diluted to 600 mL with tap water, and the initial pH was determined using pH indicator strips. Test mixture was prepared by adding 10ppm nickel chloride to 300mL grape juice and 60g fresh cow dung was added. The mixture was diluted to 600mL with tap water and initial pH was determined. *I. Effect of varying concentrations of ferric chloride on bio-methanation*

A positive 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, and 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.

Five test treatments were prepared by mixing 300 mL grape juice, 60 g fresh cow dung, and varying concentrations of ferric chloride (10, 25, 50, 70, and

79

90 ppm). Each mixture was diluted to 600 mL, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

J. Effect of varying concentrations of nickel chloride on bio-methanation

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, and biogas production was quantified via water displacement in a 1000 mL conical flask.

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

Five test treatments were prepared by mixing 300 mL grape juice, 60 g fresh cow dung, and nickel chloride (10, 25, 50, 70, and 90 ppm). Each mixture was diluted to 600 mL, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

K. Synergistic effect of ferric chloride & nickel chloride on bio-methanation of rotten grape juice

Negative Control: A mixture of 60 g fresh cow dung and 600 mL tap water was prepared. The mixture was transferred to a 1000 mL conical flask, and biogas production was measured via water displacement.

Positive 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 the mixture was transferred to a 1000 mL conical flask. Biogas production was measured via water displacement.

Test Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride and nickel chloride. The mixture was blended, diluted to 600 mL with tap water, and initial pH was measured. The mixture was transferred to a 1000 mL conical flask, and biogas production was measured via water displacement.

L. Effect of varying concentrations of ferric chloride impregnated wood charcoal on bio-methanation

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. Test Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride-impregnated activated 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.

M. Effect of varying concentrations of nickel chloride impregnated wood charcoal on bio-methanation

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

Test Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with nickel chloride-impregnated activated charcoal (10-90 ppm). The mixture was diluted to 600 mL, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

N. Synergistic effect of ferric chloride & nickel chloride impregnated wood charcoal on biomethanation of rotten grape juice

Control Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride and nickel chloride to investigate synergistic effects. The mixture was blended, diluted to 600 mL with tap water, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

Test Treatment: A mixture of 300 mL grape juice and 60 g fresh cow dung was prepared with varying concentrations of ferric chloride and nickel chlorideimpregnated activated charcoal to investigate synergistic effects. The mixture was blended, diluted to 600 mL with tap water, and initial pH was measured. Biogas production was quantified via water displacement in 1000 mL conical flasks.

IV. RESULTS AND DISCUSSION

A. Bio-methanation of rotten grape juice



Figure 4. 1 Bio-methanation of rotten grape juice

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

Table 4.1 Determination of biogas production withRotten Grape Juice as substrate

Fig. 4.1 shows the bio-methanation data of rotten grapes. It is evident that the test which contains rotten grapes as substrate produced more gas compared to control with a percentage increase of 4500. India ranks as one of the largest producers of fruits and vegetables in the world. Grapes with an annual production of 2.48 million tonnes, is cultivated in an area of approximately 34,000 hectares in India, of which approximately 30-35% is wasted due to improper cold storage facilities, transportation, lack of appropriate post-harvest technologies and damage caused due to pests and diseases. In the grape processing industry, the major product is grape juice. Huge quantities of grape pomace (grape pulp) are accumulated which cause environmental pollution. Since grapes are rich

in nutrients, and also due to the availability throughout the year, these can be channelized through biomethanogenic pathway for the production of valueadded products like biomethane. The grape pulp contains more of carbon and less of nitrogen.

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

 Table 4.2 Determination of methane content inbiogas

 with rotten grape juice as substrate

The amount of methane content in the biogas was determined using CO_2 scrubbing method using 5% NaOH. In this experiment, the methane content in the biogas was found to be 3520ml which is 58.80% (fig 4.2).

Biogas is a renewable fuel produced from the anaerobic digestion of organic feedstocks including municipal waste, farm waste, food waste, and energy crops. Raw biogas typically consists of methane (50–75%), carbon dioxide (25–50%), and smaller amounts of nitrogen (2–8%). Trace levels of hydrogen sulfide, ammonia, hydrogen, and various volatile organic compounds are also present in biogas depending on the feedstock.

C. Bio-methanation of pH adjusted rotten grape juice



Figure 4. 3 Bio-methanation of pH adjusted rotten grape juice

Sample	Fermentation	Cumulative	Percentage
	Mixture	production	increase
		of Biogas	
		over a	
		period of 5	
		days (in	
		mL)	
Control	300mL grape	5985	
	juice + 60g		
	cow dung +		
	300mL water		
	(initial pH 5.5)		
Test	300mL grape	7300	21.97%
	juice + 60g		
	cow dung +		
	300mL water		
	(pH adjusted		
	to 7.0 with		
	0.1N NaOH)		

Table 4.3 Bio-methanation of pH adjusted rotten grape juice

In the experiment 3.3, pH of the fermentation mixture was adjusted using wood ash. pH was made to 7.0 from 5.5 which was the initial one and a 21.97% increase in cumulative biogas production was observed (fig. 4.3) when compared to control.For anaerobic digestion to take place profoundly, pH value of the composition of food waste (taken into consideration) is of huge importance. It is a pivotal factor in the digestion. The modern world, post its urbanization has resulted in an excessive release of food waste which contains a large amount of organic

matter which can be decomposed, hence leading to the harnessing of biogas from it. The biogas generation is highly affected by the parameters like its pH value range, optimum operating temperature, retention time, loading capacity and the composition of the food waste used. It has been experimentally proved that the biogas production yield and the degradation efficiency is said to be higher for the substrates having an optimum range value of pH 7.0 comparing with other pH range values. The pH value plays an important part as the micro-organism i.e. the methanogens are highly sensitive to acidic environmental conditions. As an acidic environment inhibits their growth and methane production. On the other hand, increasing the pH value more than 7.5 and towards 8 can lead to proliferation of methanogens which inhibits acetogenesis process. In order to keep the pH value in an equilibrium condition, a certain amount of buffer solution is added to the system such as CaCO₃ or lime. Although the optimum pH value should be maintained between 7.5 to 8.0, in order to obtain higher yield of biogas. D. Effect of wood charcoal on biogas production



Figure 4. 4 Effect of wood charcoal on biogas production

Sample	Fermentatio	Cumulativ	Percentag
	n Mixture	e	e increase
		production	
		of Biogas	
		over a	
		period of 5	
		days (in	
		mL)	
Negativ	60g fresh	130	
e	cow dung +		
Control	600mL tap		
	water		
Control	300mL	5985	
	grape juice		
	+ 60g cow		
	dung +		
	300mL tap		
	water		
Test	300mL	9680	61.73%
	grape juice+		
	300mL		
	water+ 60g		
	cow dung+		
	бg wood		
	charcoal		
	(1% (w/v))		

Table 4.4 Effect of wood charcoal on biogas production

It is evident from fig.4.4 that the addition of wood charcoal has significant effect on the bio-methanation using rotten grapes. There is 61.73% increase in the production of biogas when 1% (w/v) wood charcoal was added compared to control.

The anaerobic methanogenesis process is mediated by microorganisms of the three major bacterial groups, which form a symbiotic relationship between microorganisms, thus overcoming the thermodynamic barriers of the metabolic process. In symbiotic relationships, interspecies electron transfer (IET) is a new type of mutualistic symbiosis that has been discovered in recent years. Electron donor microorganisms transfer electrons to electron acceptor microorganisms by direct means of cell contact or indirect pathways mediated by intermediates, thus enabling metabolic processes that are difficult for a single microorganism to accomplish. IET can be divided into Direct Interspecies Electron Transfer (DIET) and Indirect Interspecies Electron Transfer (MIET) according to the different modes of electron transfer. Studies conducted so far show that DIET can be accelerated by conductive materials like carbon nanotubes, biochar, carbon cloth, granular activated carbon (GAC), and magnetite. The conductive materials mediated DIET has shown to be highly efficient in enhancement of methane yield than indirect interspecies electron transfer (IIET) in case of conventional anaerobic digestion (AD) process.

E. Determination of methane content in biogas by the effect of wood charcoal





Sample	Fermentation	Cumulative	Methane	Percentage	Percentage
	mixture	biogas	content	of	increase of
		production		methane	methane
					gas
Control	300mL	5985mL	3520mL	58.80%	
	grape juice+				
	300mL				
	water+ 60g				
	cow dung				
Test	300mL	12720mL	8190mL	64.38%	5.5%
	grape juice+				
	300mL				
	water+ 60g				
	cow dung+				
	30g wood				
	charcoal				

There was 6% increase in methane content in the experiment with 5% (w/v) wood charcoal when compared to the control.

F. Bio-methanation of rotten grape juice with varying concentrations of wood charcoal



Figure 4. 6 Bio-methanation of rotten grape juice with varying concentrations of wood charcoal
There was 61.73% increase in the production of biogas when 1% (w/v) wood charcoal was added compared to control. And also, optimization was carried out using 1%, 3%, 5% and 7% (w/v) wood charcoal. It's clear from fig.4.6 that the addition of 5% (w/v) wood charcoal produced more biogas compared to other concentrations. It was found that the cumulative biogas production was 12270 ml (Table 4.6).

Sample	Fermentation	Cumulative	Percentage
	Mixture	production of	increase
		Biogas over a	
		period of 5	
		days (in mL)	
Control	300mL grape	5985	
	juice + 60g cow		
	dung + 300mL tap		
	water		
Test 1	300mL grape	9680	61.73%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 6g wood		
	charcoal (1%		
	(w/v))		
Test 2	300mL grape	10780	80%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 18g wood		
	charcoal (3%		
	(w/v))		
Test 3	300mL grape	12720	112.5%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 30g wood		
	charcoal (5%		
	(w/v))		
Test 4	300mL grape	6980	16.62%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 42g wood		
	charcoal (7%		
	(w/v))		

Table 4.6 Bio-methanation of rotten grape juice with varying concentrations of wood charcoal

G. Effect of ferric chloride on bio-methanation of rotten grape juice





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+ 0.017g ferric chloride (10ppm ferric ion)	9140	52.71%

 Table 4.7 Effect of ferric chloride on bio-methanation
 of rotten grape juice

There was 52.71 % increase in biogas production upon addition of ferric chloride on comparison with control (table 4.7).

H. Effect of nickel chloride on bio-methanation of rotten grape juice



Figure 4. 8 Effect of nickel chloride on biomethanation of rotten grape juice

Sample	Fermentatio	Cumulativ	Percentag
	n Mixture	e	e increase
		production	
		of Biogas	
		over a	
		period of 5	
		days (in	
		mL)	
Contro	300mL	5985	
1	grape juice +		
	60g cow		
	dung +		
	300mL tap		
	water		
Test 1	300mL	8970	49.87%
	grape juice+		
	300mL		
	water+ 60g		
	cow dung+		
	10ppm		
	nickel ion		
	(0.024g		
	nickel		
	chloride)		

Table 4.8 Effect of nickel chloride on biomethanation of rotten grape juice

There was 49.87 % increment in biogas production on addition of nickel chloride to the fermentation mixture (table 4.8).

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



Figure 4. 9 Effect of varying concentrations of ferric chloride on bio-methanation

Sample	Fermentation	Cumulative	Percentage
	Mixture	production	increase
		of Biogas	
		over a period	
		of 5 days (in	
		mL)	
Control	300mL grape juice	5985	
	+ 60g cow dung +		
	300mL tap water		
Test 1	300mL grape	9140	52.71%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 10ppm		
	ferric ion		
	(0.017g ferric		
	chloride)		
Test 2	300mL grape	9980	66.75%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 25ppm		
	ferric ion (0.043g		
	ferric chloride)		
Test 3	300mL grape	10630	73%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 50ppm		
	ferric ion (0.087g		
	ferric chloride)		
Test 4	300mL grape	11590	93.6%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 70ppm		
	ferric ion (0.12g		
	ferric chloride)		
Test 5	300mL grape	9350	56.22%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 90ppm		
	ferric ion (0.15g		
	ferric chloride)		

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

Maximum biogas production was observed when ferric chloride was added at the levels of 0.12 g where the increment observed in biogas production was 93.6 %.

J. Effect of varying concentrations of nickel chloride on bio-methanation



Figure 4. 10 Effect of varying concentrations of nickel chloride on bio-methanation

Table 4.10 Effect of varying concentrations of nickel chloride on bio-methanation

Sample	Fermentation Mixture	Cumulative production of	Percentage increase
		Biogas over a	
		period of 5	
		days (in mL)	
Control	300mL grape juice +	5985	
	60g cow dung +		
	300mL tap water		
Test 1	300mL grape juice+	8970	49.87%
	300mL water+ 60g		
	cow dung+ 10ppm		
	nickel ion		
	(0.024g nickel		
	chloride)		
Test 2	300mL grape juice+	9430	57.56%
	300mL water+ 60g		
	cow dung+ 25ppm		
	nickel ion (0.060g		
	nickel chloride)		
Test 3	300mL grape juice+	11520	92.48%
	300mL water+ 60g		
	cow dung+ 50ppm		
	nickel ion (0.12g		
	nickel chloride)		
Test 4	300mL grape juice+	9380	56.72%
	300mL water+ 60g		
	cow dung+ 70ppm		
	nickel ion (0.17g		
	nickel chloride)		
Test 5	300mL grape juice+	8860	48.03%
	300mL water+ 60g		
	cow dung+ 90ppm		
	nickel ion (0.21g		
	nickel chloride)		

Nickel chloride levels at 0.17 g could yield maximum biogas, as can be observed from table 4.10.

The term heavy metals refer to metals and metalloids having densities greater than 5 g cm-3 and is usually associated with pollution and toxicity although some of these elements (essential metals) are required by micro-organisms at low concentrations. Heavy metals toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems of our modern society. Heavy metals affect the biochemical reactions that take place during anaerobic digestion processes of organic matter. Heavy metals like copper, nickel, zinc, cadmium, chromium and lead have been overwhelmingly reported to be inhibitory and under certain conditions toxic in biochemical reactions depending on their concentrations. Heavy metals like iron may exhibit inhibitory effects beyond certain levels, as can be seen from the various results from this study. Fig 4.7 shows the effect of ferric chloride on biomethanogenic pathway and it was found that there has 52.71% increase in biogas production when 10ppm ferric chloride is added. There is 93.6% increase in biogas production when 0.12g ferric chloride (70ppm ferric ion) is used (fig 4.9). Also, it is evident from fig 4.8 that the addition of nickel chloride (10ppm) produces 49.87% more biogas when compared to the control. Also there occurred a percentage increase of 92.48 by the addition of 0.12g nickel chloride (50ppm nickel ion).

K. Synergistic effect of ferric chloride & nickel chloride on bio-methanation of rotten grape juice



Figure 4. 11 Synergistic effect of ferric chloride & nickel chloride on bio-methanation of rotten grape juice

Synergistic effect of ferric chloride and nickel chloride was found out (fig 4.11) and it is clear that the addition

of 50ppm ferric chloride along with 70ppm nickel chloride produces maximum biogas (10740 mL).

Sample	Fermentation	Cumulative	Percentage
	Mixture	production of	increase
		Biogas over a	
		period of 5	
		days (in mL)	
Control	300mL grape juice	5985	
	+ 60g cow dung +		
	300mL tap water		
Test 1	300mL grape	10740	79.44%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 50ppm		
	Ferric chloride +		
	70ppm Nickel		
	chloride		
Test 2	300mL grape	9530	59.23%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 60ppm		
	Ferric chloride +		
	80ppm Nickel		
	chloride		
Test 3	300mL grape	9280	55.05%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 60ppm		
	Ferric chloride +		
	70ppm Nickel		
	chloride		

Table 4.11. Synergistic effect of ferric chloride & nickel chloride on bio-methanation of rotten grape juice

L. Effect of varying concentrations of ferric chloride impregnated wood charcoal on bio-methanation



Figure 4. 12 Effect of varying concentrations of ferric chloride impregnated wood charcoal on biomethanation

Impregnation using wood charcoal was done (fig 4.12) and 50ppm ferric chloride impregnated with wood charcoal was found to be the best for maximum biogas production with a percentage increase of 9.59 with respect to control. For Nickel chloride, it was found that 50ppm was the effective one for maximum biogas production and it was 13890 mL (fig 4.13).

Sampl	Fermentation Mixture	Cumulati	Percentage
e		ve	increase
		productio	
		n of	
		Biogas	
		over a	
		period of	
		5 days	
		(in mL)	
Contro	300mL grape juice+	9140	
11	300mL water+ 60g cow		
	dung+ 10ppm Ferric ion		
	(0.017g ferric chloride)		
Test 1	300mL grape juice+	9320	1.96%
	300mL water+ 60g cow		
	dung+ 10ppm ferric ion		
	impregnated wood charcoal		
	(0.017g ferric chloride)		
Contro	300mL grane juice	9980	
1.2	300mL water $\pm 60 \text{g cow}$	<i>))</i> 00	
12	dung+ 25ppm Ferric ion		
	(0.043g ferric chloride)		
	(0.045g leffic ellipfide)		
T 10	200 1	10150	1 700/
Test 2	300mL grape juice+	10150	1.70%
	300mL water+ 60g cow		
	dung+ 25ppm ferric ion		
	(0.042a farria ablarida)		
	(0.045g terric chioride)		
Contro	300mL grape juice+	10630	
13	300mL water+ 60g cow		
	dung+ 50ppm Ferric ion		
	(0.087g ferric chloride)		
Test 3	300mL grape juice+	11650	9.59%
	300mL water+ 60g cow		
	dung+ 50ppm ferric ion		
	impregnated wood charcoal		
	(0.087g ferric chloride)		
Contro	300mL grape juice+	11590	
14	300mL water+ 60g cow		
	dung+ 70ppm Ferric ion		
	(0.12g ferric chloride)		
Test 4	300mL grape juice+	9170	79.11% of
	300mL water+ 60g cow		control
	dung+ 70ppm ferric ion		
	impregnated wood charcoal		
	(0.12g ferric chloride)		

Table 4.12 Effect of varying concentrations of ferric chloride impregnated wood charcoal on biomethanation

87

M. Effect of varying concentrations of nickel chloride impregnated wood charcoal on bio-methanation



Figure 4. 13 Effect of varying concentrations of nickel chloride impregnated wood charcoal on biomethanation

Table 4.13 Effect of varying concentrations of nickel chloride impregnated wood charcoal on biomethanation

Sample	Fermentation	Cumulative	Percentage
	Mixture	production	increase
		of Biogas	
		over a	
		period of 5	
		days (in mL)	
Control	300mL grape	8970	
1	juice+ 300mL		
	water+ 60g cow		
	dung+ 10ppm		
	Nickel ion (0.024g		
	nickel chloride)		
Test 1	300mL grape	9280	3.45%
	juice+ 300mL		
	water+ 60g cow		
	dung+ 10ppm		
	Nickel chloride		
	impregnated wood		
	charcoal (0.024g		
	nickel chloride)		
Control	300mL grape	9430	
2	juice+ 300mL		
	water+ 60g cow		
	dung+ 25ppm		
	Nickel ion (0.060g		
	nickel chloride)		

Test 2	300mL grape juice+ 300mL	9990	5.93%
	water+ 60g cow dung+ 25ppm		
	Nickel chloride impregnated		
	wood charcoal (0.060g nickel		
	chloride)		
Control 3	300mL grape juice+ 300mL	1152	
	water+ 60g cow dung+ 50ppm	0	
	Nickel ion (0.12g nickel		
	chloride)		
Test 3	300mL grape juice+ 300mL	1389	20.57
	water+ 60g cow dung+ 50ppm	0	%
	Nickel chloride impregnated		
	wood charcoal (0.12g nickel		
	chloride)		
Control 4	300mL grape juice+ 300mL	9380	
	water+ 60g cow dung+ 70ppm		
	Nickel ion (0.17g nickel		
	chloride)		
Test 4	300mL grape juice+ 300mL	1123	19.72
	water+ 60g cow dung+ 70ppm	0	%
	Nickel chloride impregnated		
	wood charcoal (0.17g nickel		
	chloride)		

N. Synergistic effect of ferric chloride & nickel chloride impregnated wood charcoal on biomethanation of rotten grape juice



Figure 4. 14 Synergistic effect of ferric chloride & nickel chloride impregnated wood charcoal on biomethanation of rotten grape juice

Synergistic effect of metal ion impregnated charcoal was determined (fig 4.14) and it is found that 50ppm Ferric chloride impregnated carbon along with 70ppm carbon impregnated Nickel chloride has maximum production of biogas (14750 mL).

Table 4.14 Synergistic effect of ferric chloride & nickel chloride impregnated wood charcoal on biomethanation of rotten grape juice

Sample	Fermentation Mixture	Cumulative	Percentage
Sumple	i ennentation minitare	production of	increase
		Biogas over a	
		period of 5 days	
		(in mL)	
Control	300mL grape juice+	11740	
1	300mL water+ 60g		
	cow dung+ 50ppm		
	Ferric chloride +		
	70ppm Nickel chloride		
Test 1	300mL grape juice+	14750	25.63%
	300mL water+ 60g		
	cow dung+ 50ppm		
	carbon impregnated		
	Ferric chloride +		
	70ppm Nickel chloride		
	impregnated carbon		
Control	300mL grape juice+	11120	
2	300mL water+ 60g		
	cow dung+ 60ppm		
	Ferric chloride +		
	80ppm Nickel chloride		
Test 2	300mL grape juice+	13250	19.15%
	300mL water+ 60g		-,,
	cow dung+ 60ppm		
	carbon impregnated		
	Ferric chloride +		
	80ppm Nickel chloride		
	impregnated carbon		
Control	300mL grape juice+	10650	
3	300mL water+ 60g		
	cow dung+ 60ppm		
	Ferric chloride +		
	70ppm Nickel chloride		
Test 3	300mL grape juice+	12860	20.75%
10500	300mL water+ 60g	12000	2017070
	cow dung+ 60ppm		
	carbon impregnated		
	Ferric chloride +		
	70ppm Nickel chloride		
	impregnated carbon		
Control	300mL grape juice+	9690	
4	300mL water+ 60g		
	cow dung+ 50ppm		
	Ferric chloride +		
	80ppm Nickel chloride		
Test 4	300mL grape juice+	11550	19.19%
	300mL water+ 60g		
	cow dung+ 50ppm		
	carbon impregnated		
	Ferric chloride +		
	80ppm Nickel chloride		
	impregnated carbon		
	Ferric chloride + 80ppm Nickel chloride impregnated carbon		

V. CONCLUSION

Rotten grapes are excellent substrate which can be can be channelized through bio-methanogenic pathway for the production of value-added products like biomethane. Addition of wood charcoal has found to be efficient carbon additives for increasing the biogas production. The optimum concentration for maximum biogas production was found to be 5% (w/v) for wood charcoal. There is 6% increase in methane content in the experiment with 5% (w/v) wood charcoal when compared with control. Addition of metal ions like ferric chloride and nickel chloride has found to be efficient for maximum biogas production. Addition of metal impregnated carbon additives has found to be much beneficial for higher biogas production. Wood ash was found to be an excellent substance for pH correction.

VI. APPENDIX

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

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