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Addis Ababa Institute of Technology

School of Chemical and Bio-Engineering

Waste Management in Sugar and Ethanol Production: Evaluation of Anaerobic Co-Digestion of Sugarcane Vinasse and Bagasse for Biogas Production Under Different Parametric Conditions

By

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A Thesis Submitted as a Partial Fulfillment to the Requirements for the Award of the Degree of Master of Science in Environmental Engineering

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Approval Page

This is to certify that the thesis prepared by Ms. Hermela Birhanu Gebretsadik entitled "**Waste Management in Sugar and Ethanol Production: Evaluation of Anaerobic Co-Digestion of Sugarcane Vinasse and Bagasse for Biogas Production Under Different Parametric Conditions**" and submitted as partial fulfillment of the requirements for the award of the degree of master of science in Environmental Engineering complies with the regulations of the university and meets the accepted standards concerning content, quality, and originality.

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Declaration

I hereby declare that this research entitled "**Waste Management in Sugar and Ethanol Production: Evaluation of Anaerobic Co-Digestion of Sugarcane Vinasse and Bagasse for Biogas Production Under Different Parametric Conditions**" is based on my original work, to the best of my knowledge, it contains no material previously published by another person nor material which has been previously submitted entirely or in part for obtaining any other degree of the University and all sources of the materials used for this thesis has been properly acknowledged.

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Abstract

In this thesis work the anaerobic co-digestion of sugarcane vinasse with bagasse was investigated under varying conditions to evaluate biogas (methane) production as well as sludge characteristics for selected batch digestors. Anaerobic digestion offers a sustainable approach for managing sugarcane residues such as vinasse & bagasse by utilizing them to generate biogas and organic fertilizer from the sludge while addressing environmental concerns related to their disposal. Sugarcane vinasse is a highly pollutant liquid due to its high organic matter content, low pH, high salt content, dark brown color and high temperature when discharged from the distillation unit. It is produced in large amount with a 1: 10-20 ratio of alcohol to vinasse from alcohol/ethanol factories. The study employs an experimental design to analyze the effects of mixing ratios of vinasse (V) to bagasse (B); (75%V:25%B, 50%V:50%B & 25%V:75%B), pH levels (6.8, 7.4 & 8.0) and temperature levels (25°C, 35°C & 45°C) on biogas production and methane yield resulting in 27 anaerobic batch digestors. Through batch experiments conducted over a period of more than 35 days (until the end of gas production), quantitative and qualitative biogas yields were measured. The results showed that the highest methane yield (203.6774ml/gVS_{added}) was at mixing ratio of 25%V:75%B, pH=7.4 and T=45^{o}C. The sludge characteristics for the batch sample were determined in terms of its NPK content, TOC, TOM and C: N to compare with FAO's recommendation for an organic fertilizer. Its TOC & TOM values are well above the minimum amount recommended by FAO (12% and 30% respectively) however the nutrient content is below the recommended value (N+P+K ≥5%). The research findings contribute for optimizing anaerobic co-digestion of sugarcane vinasse with bagasse to maximize methane production and improve sludge management practices to address energy demands, waste management challenges and limitations of inorganic fertilizers by nutrient recycling.

Keywords: Anaerobic Co-digestion, Bagasse, Biogas, Sugarcane Vinasse, Waste management

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List of Abbreviation and Acronyms

APHA	American Public Health Association
AD	Anaerobic Digestion
B	Bagasse
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
ECAE	Ethiopian Conformity Assessment Enterprise
FAO	Food and Agriculture Organization
FS	Fixed Solid content
K	Potassium
Mg/L	Milligrams per Liter
M.C	Moisture Content
N	Nitrogen
pH	Power of Hydrogen
TOC	Total Organic Carbon
TOM	Total Organic Matter
TP	Total Phosphorus
TS	Total Solid content
V	Sugarcane Vinasse
VFAs	Volatile Fatty Acids
VS	Volatile Solid content
WHO	World Health Organization
%V	Percentage composition of Vinasse
% (w/w)	Weight percent or percent weight of solute by weight of solution
% (v/v)	Volume percent or percent volume of solute by volume of solution

Chapter One

1. Introduction

1.1. Background Information

Sugar and Ethanol production is a vital component of Ethiopia’s agricultural and industrial sectors, contributing significantly to the country’s economic growth and job creation. However, these industries face substantial environmental challenges due to the large volume of waste they generate, primarily in vinasse and sugarcane bagasse. As Ethiopia strives to balance industrial growth with environmental sustainability, there is an increasing need to develop effective waste management practices for the sugar and ethanol industries. Ethiopia's sugar industry has been expanding rapidly in recent years, with major producers of ethanol Metehara and Fincha Sugar Factory playing crucial roles in the country's sugar and ethanol production[1, 2]. However, this dual production system generates significant waste, posing environmental challenges that need urgent attention.

The most problematic waste these factories generate is vinasse, a byproduct of ethanol production from molasses. For every liter of ethanol produced, up to 20 liters of vinasse is generated as wastewater[3]. This dark, malodorous liquid is characterized by its high organic matter content, low pH, elevated levels of salts, and recalcitrant compounds like phenols and melanoidins[4, 5]. The environmental impact of untreated vinasse discharge is profound and multifaceted. When released into water bodies, vinasse can lead to eutrophication, causing algal blooms that deplete oxygen levels and devastate aquatic ecosystems[6, 7]. Its high chemical oxygen demand (COD), often ranging from 50,000 to 150,000 mg/L, and biological oxygen demand (BOD) significantly strain the self-purification capacity of receiving water bodies. This indicates its potential for severe environmental impact if discharged untreated[4, 6, 8, 9].

Another byproduct of the sugar industry is bagasse, the fibrous residue left after sugarcane stalks are crushed to extract their juice, which is produced in enormous quantities. For every 10 tons of sugarcane crushed, a sugar factory produces nearly 3 tons of wet bagasse[10]. While often used as a fuel source for the sugar mills, this practice is not without its environmental costs[11]. The combustion of bagasse, while reducing waste volume, contributes to air pollution by releasing

particulate matter and greenhouse gases. Furthermore, the ash from bagasse combustion requires careful disposal to prevent soil and water contamination[8, 9, 12].

Current waste treatment methods for vinasse often fail to address the full spectrum of environmental concerns. The disposal of vinasse on land, a practice known as fertirrigation, is common in many sugar-producing regions due to its high nutrient content[13]. However, this approach is not without risks. Continuous vinasse application can lead to soil salinization, altering soil structure and reducing fertility over time[14]. Moreover, the potential for groundwater contamination through leaching of nitrates and other pollutants poses a significant threat to human health and ecological balance[13].

Coagulation, flocculation, and chemical precipitation commonly remove suspended solids and some dissolved contaminants from vinasse. However, they generate chemical sludge requiring further treatment and fail to address the high organic load[15]. Chemical oxidation and ozonation can reduce organic content and color, but they are energy-intensive, expensive, and may produce harmful byproducts[16]. While reducing vinasse volume, evaporation is energy-intensive and merely concentrates the pollutants rather than treating them[9, 15]. Aerobic and anaerobic treatments have been employed to produce biogas and reduce the organic load of vinasse before discharge which still may not fully mitigate all environmental risks. While burning bagasse in sugar mills offers some energy benefits, it doesn’t maximize its overall potential. Composting can be a sustainable option, but the lengthy process and potential costs make it challenging for large-scale implementation.

In light of these challenges, there is a growing interest in more sustainable and holistic approaches to manage sugar/ethanol production wastes. Anaerobic digestion is a promising solution that offers multiple benefits[9, 17-19]. This biological process that decomposes organic matter in the absence of oxygen, effectively manages the waste while producing valuable resources[9, 17]. The main product is biogas, a renewable energy source composed of methane and carbon dioxide, which can generate electricity or heat which can help to meet the sugar mill's needs or supply local communities.

The anaerobic digestion process for biogas production involves four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis[9, 20]. In hydrolysis, complex organic

compounds are broken down into simpler molecules. Acidogenesis then converts these molecules into volatile fatty acids, alcohols, and other intermediates. During acetogenesis, these intermediates are transformed into acetic acid, carbon dioxide, and hydrogen. Finally, methanogenic bacteria convert these products into methane and carbon dioxide, resulting in the production of biogas[20].

Various factors, including temperature, pH, substrate composition, and microbial community dynamics, influence each stage of this process. Optimizing these parameters is crucial for maximizing biogas production and ensuring process stability[21]. In addition to biogas, anaerobic digestion produces a nutrient-rich effluent known as digestate which can be used as an organic fertilizer, thereby closing the loop in resource utilization[22]. The digestate retains most of the nutrients in the original feedstock, particularly nitrogen, phosphorus, and potassium, making it a valuable soil amendment. However, using digestate as a fertilizer requires careful consideration of its composition and potential environmental impacts, particularly regarding soil health and potential nutrient runoff.

While several studies have explored the anaerobic digestion of vinasse or bagasse individually[9, 21, 23], research on their co-digestion is limited. Co-digestion, the simultaneous anaerobic digestion of two or more substrates, offers several potential advantages as it can improve the nutrient balance of the feedstock, enhance the stability of the anaerobic digestion process, and potentially increase biogas yield[24, 25]. However, the optimal conditions for co-digesting vinasse and bagasse remain largely unexplored. Previous research has indicated that co-digestion can enhance biogas yield[23, 26], but several knowledge gaps persist. The optimal mixing ratios of vinasse and bagasse for maximum biogas production have not been established. The effects of varying operational parameters such as temperature and pH on methane yield in co-digestion systems are not fully understood. Moreover, the nutritional and environmental quality of the resulting digestate as a fertilizer needs comprehensive evaluation to ensure its safe and effective use in agriculture.

This thesis addresses knowledge gaps by studying the anaerobic co-digestion of sugarcane vinasse and bagasse under various conditions, specifically examining the impacts of temperature, pH, and mixing ratios on biogas production. It also evaluates the potential of the resulting digestate as

organic fertilizer. The research targets the waste management needs of Ethiopian sugar and ethanol producers, aiming to create locally relevant solutions. By focusing on this co-digestion process, the study promotes circular economy principles within the sugar industry, transforming waste into valuable resources and fostering sustainable economic and environmental practices.

1.2. Statement of the Problem

Potable alcohol/ ethanol production is an activity which results in a large amount of vinasse in a very short period of time. This is because after the byproduct from sugar production i.e., molasses or other sources is used as a raw material for ethanol production about 10-20 liters of vinasse is produced per each liter of ethanol produced[4, 20]. In Ethiopia, around 20 million liters of ethanol is expected to be produced from molasses every year by two dominant sugar factories (Metehara and Fincha). There are also other potable alcohol producing factories such as National alcohol and liquor factory having the same disposal problem for their waste product; vinasse. Assuming the average range of vinasse production i.e., 14 liters per each liter of ethanol, 280 million liters of vinasse is produced annually by the two sugar factories alone. This causes a problem of land scarcity for large sugar-ethanol industries in relation to disposal and/or handling.

Vinasse is a pollutant due to its high chemical oxygen demand (COD), biological oxygen demand (BOD), high salt content, unpleasant odor, high acidity, and dark color which results in the reduction of sunlight penetration when disposed into rivers and lakes, thus decreasing photosynthetic activity and dissolved oxygen concentrations and causing harmful conditions for the aquatic life. These wastewaters also contain phytotoxic, antibacterial and recalcitrant compounds, such as phenols, polyphenols and heavy metals, which have negative effects on micro-organisms and plants at disposal sites[4].

Studies have pointed out that vinasse permeation in soil and groundwater resources caused soil hardening, soil acidification, salinization and growing concentration of inorganic salts and organic carbon[4]. For this reason, it needs pretreatment before releasing to the environment. However, vinasse treatment for disposal is additional expense for the factories. Therefore, a more feasible way of handling this waste will be anaerobic co-digestion with other byproducts from sugar factories such as bagasse and filter cake to increase the low C: N ratio of vinasse as it is evidenced by previous works to create favorable condition for a better biogas yield[20]. Its conversion into

energy source will be an advantage for the factories to at least substitute their energy consumption while the digestate resulting after the anaerobic digestion will be used as an organic fertilizer for their sugarcane farm in addition to solving the environmental problems.

1.3. Objectives

1.3.1. General Objective

The general objective of this research is to evaluate the effectiveness of anaerobic co-digestion of sugarcane vinasse and bagasse as a waste management strategy for sugar and ethanol/alcohol production by investigating the process under different parametric conditions to identify the parameters for best biogas production and evaluate the potential of the resulting digestate as an organic fertilizer.

1.3.2. Specific Objectives

The specific objectives of this thesis work are;

- ✓ Physico-chemical characterization of raw materials i.e., vinasse and sugarcane bagasse;
- ✓ Investigating the effects of operating parameters; temperature, pH and mixing ratio on biogas quantity and quality;
- ✓ Evaluation of the digestate for potential use as an organic fertilizer

1.4. The Scope of the Study

The focus area of the research ranged from the characterization of the two raw materials i.e., vinasse and sugarcane bagasse; for the anaerobic co-digestion regarding their potential as substrates for biogas production and the experimental design for investigation of the effects of pH, temperature, and substrate mixing ratio among others on the quality and quantity of biogas production to the characterization and evaluation of the digestate for use as an organic fertilizer.

1.5. Significance of the Study

This study will play a significant role in sustainable waste management as the sugar and ethanol industries generate substantial amounts of waste, primarily in the form of vinasse and bagasse. Effective management of these by-products is crucial for reducing environmental impact and promoting sustainability within the industry. This research aims to explore value added waste

management technique that can mitigate pollution and enhance resource recovery by investigating anaerobic co-digestion to produce biogas, a renewable energy source. This not only contributes to energy sustainability but also aligns with global efforts to transition to cleaner energy alternatives. Understanding the parametric conditions that influence the co-digestion process can lead to best operational parameters for anaerobic digesters. This understanding can enhance the efficiency of biogas production, reduce operational costs, and improve the economic viability of sugar and ethanol/alcohol production factories.

Effective utilization of vinasse and bagasse through co-digestion can significantly decrease greenhouse gas emissions associated with traditional waste disposal methods in addition to its impacts on the receiving land & water bodies. The research provides valuable insights into the biochemical processes involved in anaerobic co-digestion of sugarcane by-products. This contributes to the broader body of knowledge in environmental engineering, waste management, and renewable energy, and can serve as a reference for future studies in related fields. Evaluation of the bio-digestate for use as an organic fertilizer in terms of its NPK, Carbon and organic matter is performed which is essential for the factories to effectively use the byproduct from the co-digestion process ensuring value-added waste management.

Chapter Two

2. Literature Review

2.1. Renewable Energy Sources

For a comfortable daily life and the fulfillment of basic needs human beings need energy. However a vast majority of countries specially developing countries have energy crisis with over dependence on fossil fuels caused by the growing fossil fuel consumption[27]. Environmental pollution and energy resources reduction are two major crises as a result of growing global fossil fuels consumption (80% from the required energy) in recent years[28]. Hence, looking for clean and renewable energy sources such as wind energy, solar energy, hyropower energy, tidal energy and biomass energy is the highest priority globally. One of the major renewable energy sources in the world is Biomass having a potential to substitute fossil fuel[20]. Electricity generation and heat production are the major uses of the energy resulting from biomass. Through combustion, pyrolysis, and gasification biomass can be used to extract thermal energy. Energy rich fuels can also be produced by chemical and biochemical treatment such as biogas and bioethanol.

Biogas is a byproduct of microbial metabolism which can be produced by anaerobic digestion of different biomass such as agricultural crop wastes, poultry droppings, and wastewaters having high amount of organic matter. It can be applied for electricity generation, fuel production, and can be injected in natural gas pipelines after biomethane production through further processing and concentration. The process and the type of feedstock used in the production of biogas influences its methane composition which determines its energy content and calorific value. Constituents such as sulfur may be found in biogas which prohibits its industrial, chemical and thermal application as wel as its use as a fuel for internal combustion engines[27].

Bioethanol is a clear liquid produced by fermentation of sugar containing biomass used for many applications in place of the non- renewable fuels. It can be produced from a variety of resources, comprising sugar crops (sugarcane, sugar beet, molasses, sweet sorghum, and grapes), starch crops (corn, wheat, rice, cassava, and barley), tuber crops (potato and cassava), dairy products and cellulose materials (rice straw, bagasse, wood, and municipal solid waste) as well as agave plant[4, 9, 20] in mesophilic fermentation using a yeast called Saccharomyces cerevisiae[29].

Alcoholic fermentation is a biological process in which sugars such as glucose, fructose and sucrose are converted into cellular energy to produce ethanol and carbon dioxide as a byproduct. The general chemical reaction is;

$$C_{12}H_{22}O_{11} + H_2O + \text{enzyme} \longrightarrow 2C_6H_{12}O_6 \longrightarrow 4C_2H_5OH + 4CO_2$$

Sucrose fructose and glucose ethanol and carbon dioxide

Ethanol can be classified as first and second-generation ethanol based on the raw materials used for its production. Ethanol produced from sugarcane juice which can be used for human consumption or molasses (or their mixture) depending on the processing plant is called first-generation ethanol. In autonomous distilleries sugar- cane juice is used for ethanol production, while in annexed plants a portion of the sugarcane juice is diverted for sugar production, and the rest combined with the molasses is used [30].

Second generation ethanol is produced from lignocellulosic material of sugarcane such as bagasse and straw which is less important as it cannot be directly used for human consumption and it has been facilitated to increase ethanol production per sugarcane planted area though it is not well advanced as a result of the production cost from this feedstock being overly high [30]. Figure 2.1 and 2.2 show production of first-generation ethanol from sugarcane juice and molasses.

Molasses is obtained from the production of white sugar by repeated crystallization of sucrose in the mother liquor. The molasses that is obtained from the last massecuite is commonly used for the production of ethanol[31]. It varies by the amount of sugar, method of extraction, and age of plant. Sugarcane molasses is primarily used for sweetening and flavoring foods. The development and use of high rate bioreactors is critical for ethanol production through successful anaerobic fermentation of molasses[32].

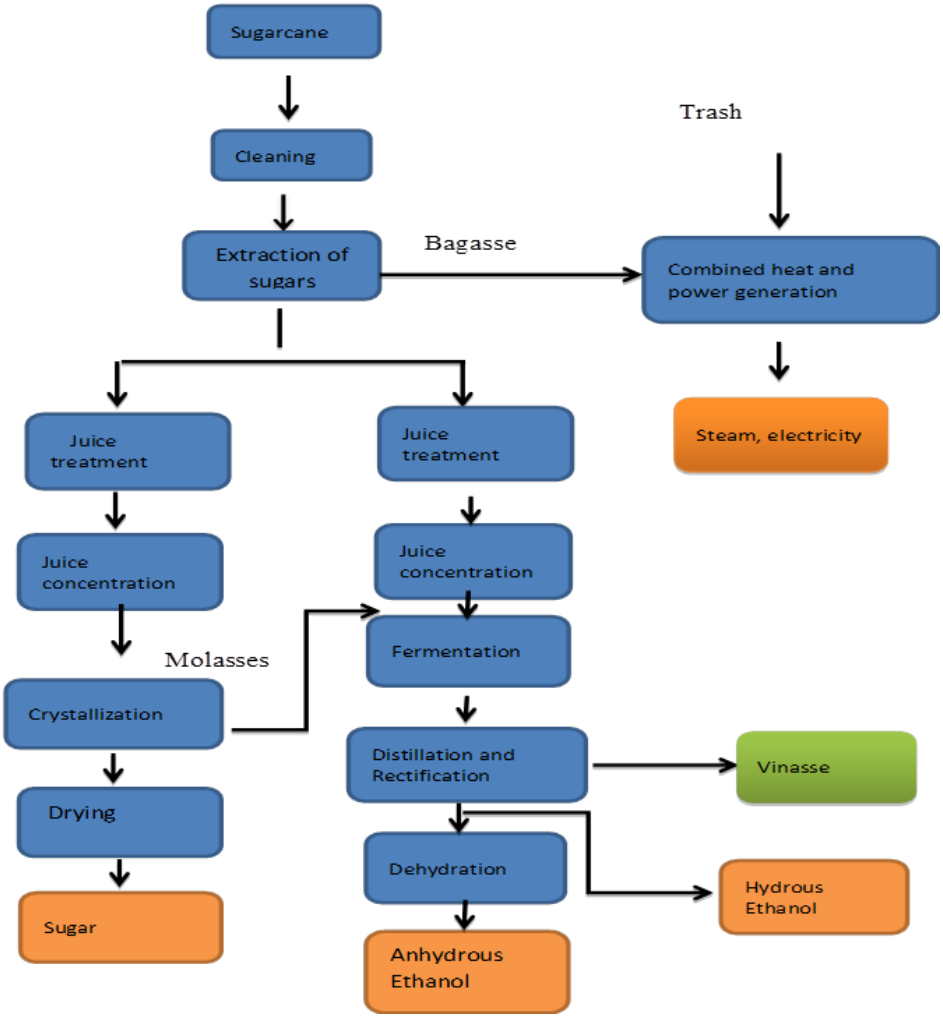

Figure 2.1 Block flow diagram of sugar production, first-generation ethanol, and electricity from sugarcane[30]

The agricultural residues from burned or crashed sugarcane during harvesting process and the industrial byproducts namely ash, bagasse, filter cake, molasses, and vinasse are the two major wastes resulting from sugar and bioethanol production from sugarcane[20]. While producing bioethanol the largest source of contamination is Vinasse which is produced in large amount and has the potential of polluting water bodies due to its high organic matter content, nutrients, heavy metals and other pollutants that can also be toxic for the soil as a result of uncontrolled land disposal for long period of time. As it contains some essential plant nutrients and organic matter it can be used as fertilizer in a controlled manner by reducing its harmful effects.

Generally, the ethanol plant using molasses as feedstock consists of four major processing steps; molasses treatment, fermentation, distillation and molecular sieve dehydration[2, 31]. Furthermore evaporation plant is very vital for concentration of vinasses and it needs a special consideration[33].

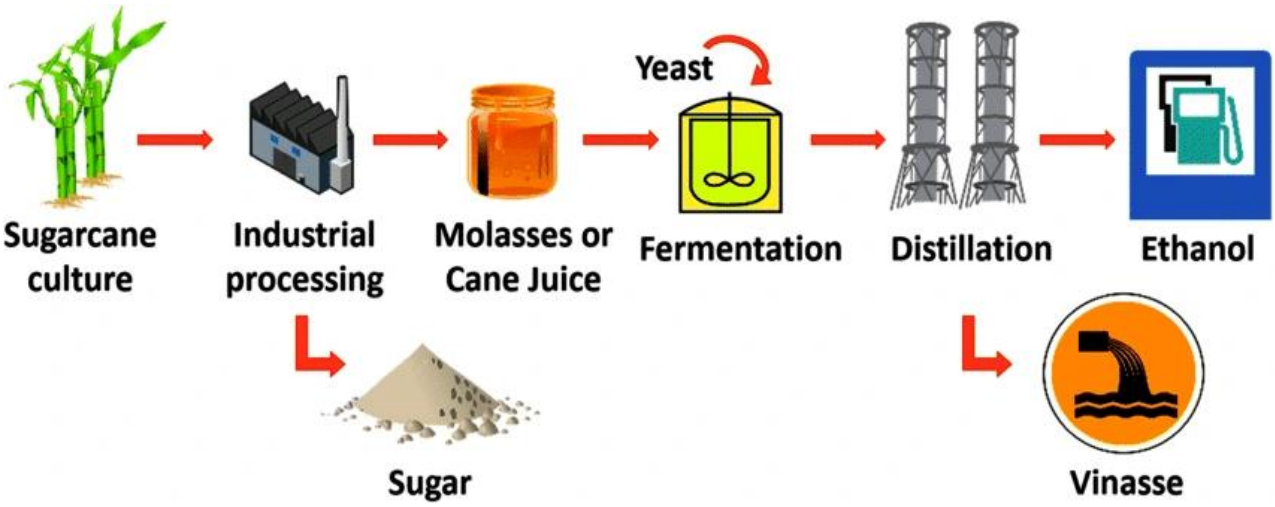

Figure 2.2 The process of producing ethanol from molasses
adopted from (https://pwonlyias.com/wpcontent/uploads/2023/12/Untitled-2023-12-09T153945.704.webp)

2.1.1. World Ethanol Production

Ethanol production in the world is increasing very fast as a result of the search for clean with neutral CO_2 emission, sustainable and competitive alternative energy source due to the unsustainability, high cost and environmental concerns related to the use of fossil fuels. Currently in Brazil, United States, majority of the European Union Countries and South Africa policies are promoting ethanol production since it is used as automobile fuel as it has high octane count[31].

As in the data taken from Renewable Fuels Association in 2020 the United States has produced 13.9 billion gallons of ethanol which made it the world's largest ethanol producer followed by Brazil with 7.9 billion gallons. The two countries together cover 83% of the world's ethanol production. In the U.S. the major raw material used for ethanol production is corn, where as in Brazil sugarcane is used as the main feed stock. From the total world sugarcane based bioethanol production Brazil takes 50 percent share[34].

2.1.2. Ethanol Production Potential in Ethiopia

In Ethiopia ethanol production is directly incorporated in sugar factories that use sugarcane as raw material and the molasses byproduct which contains about 50% of the total sugar is the only raw material used for ethanol production in the country[2]. Though the sugar in the molasses can be recovered by further crystallization through boiling of the final process it is not economically feasible and therefore, ethanol production is used as a means to recover this loss by fermenting the sugars into ethanol alcohol being the best solution to the current shortage and high cost of energy[31].

In Ethiopia about 700,000 hectares of total land is identified for sugarcane cultivation, having an expected one billion liters of ethanol production potential and yet the current annual production capacity is around 32 million liters with only two of the sugar factories namely Fincha and Metehara[2, 35]. On the other hand, based on the data from Ethiopian Sugar Corporation 10 sugar manufacturing projects will have their own integrated ethanol plant with an expected 307.4 million liters of ethanol production per year.

2.1.3. Environmental Impacts of Ethanol Production

There are two main phases in the process of Ethanol production: agricultural cultivation and industrial processing. The main environmental impacts recognized during cultivation consist of: soil degradation as a result of monoculture practices; biodiversity loss due to unsustainable expansion; water contamination due to the use of pesticides and fertilizers; and greater atmospheric emissions and injury to both wildlife and humans during the practice of pre-harvest burning.

During industrial processing, the adverse impacts of ethanol production include: intensive water consumption and subsequent water contamination during cane cleaning of soot; huge waste generation as only certain constituents of the cane are required for ethanol; vinasse which is a huge amount of corrosive waste generated from the distillation and rectification units and can contaminate soil and water and affect biodiversity; and also carbon dioxide emission is increased as a result of burning bagasse for energy generation. The major environmental problem caused by ethanol production is vinasse which is produced in large amount as high as 20L per 1L of ethanol produced. Therefore, it is important to study its physico-chemical properties to identify the

pollutant component for treatment and looking for methods that may allow its transformation into a value-added product.

2.2. Vinasse Production

Vinasse which is also called stillage, molto, thin stillage, distillery wastewater, distillery spent wash, and distillery slope[20, 36]is a liquid produced in the unit of rectification and distillation in bioethanol production[20]. It accounts for 80 mass percent of the raw materials used in producing alcohol[37]. Vinasse is produced as much as 10-20 liters [38, 39] per each liter of ethanol produced. Since the annual production of bioethanol is about 0.23, 0.82, 0.18, and 0.37 giga liters in Africa, Asia, Central America, and South America, respectively[20] which is a total of 1.6 giga liters is produced annually throughout the world and that will produce about 24 giga liters of vinasse.

The large volume production of vinasse is a difficulty for almost all ethanol plants for disposal and thus, concentration of this liquor can add values which can be used for animal feeds and also certain cation such as potassium and sodium can be recovered by drying and crystallization followed by precipitation. In some ethanol plants concentrated vinasse is mixed with filter cake from sugar factory and digested with bacteria and applied to cane field as fertilizer after full digestion[31]. At Metehara sugar factory vinasse produced from primary column is sent to evaporators for water reduction which will be partially mixed with filter cake and the remaining surplus amount of vinasse is used for fertirrigation and discharged to water bodies without further treatment[31].

2.2.1. Properties of Vinasse

Vinasse is a severe and recalcitrant wastewater[4, 9] which when derived from the ethanolic distillation has a temperature of 65–107°C and a pH of 3-5[29]. Vinasse is classified as a class II residue, not inert but not dangerous[40]. Higher COD is observed at sugarcane vinasse when compared to vinasse found from other feedstocks such as sugar beet, sweet sorghum, grape, and agave[20]. The pH is highly dependent on the cane cultivation situations and its processing as well as contamination of ethanolic fermentation by acid forming bacteria that lower the pH. Vinasse has density, viscosity, conductivity, boiling point, specific heat capacity, and thermal value of 1.031 kg/L, 0.00138 Pa s, 16.4 dS/m, 100.25 °C, 0.934 Cal/g. C, and 3.39 Cal/g, respectively[20].

Vinasse contains phytotoxic, antibacterial, and recalcitrant compounds such as phenols, polyphenols, and heavy metals[4]. Vinasse has 93–97% water[30, 41], 5% organic matter (sugars and carbohydrates), and 2% inorganic insoluble solids[42]. It is strongly soluble in water. The major organic components of sugar cane vinasse are glycerol, lactic acid, ethanol and acetic acid[4]. It contains nutrients such as nitrogen (up to 4.2 g/L), phosphorus (up to 3.0 g/L) or potassium (up to 17.5 g/L) and important organic matters (organic acids, amino acids, sugars, polysaccharides, proteins) that can improve soil properties when used as fertilizer [30, 43, 44].

Vinasse from molasses shows higher potassium concentrations in addition to calcium, magnesium, and phosphorous, as a result of adding such nutrients in the course of sugar processing which includes; the addition of magnesia, calcium and phosphoric acid for clarification, carbonation and liming, and phosphating respectively[30]. Applying sulfuric acid to yeast cell suspensions for bacterial control in the process of alcoholic fermentation is responsible for the presence of sulfate in vinasse from both sugarcane juice and molasses. Mineral salts are added that are used as nutrients for the yeast to increase its activity during fermentation and it's the unused salts that are responsible for the occurrence of those minerals in the vinasse[40].

High organic matter content and potassium concentrations are observed followed by sulfate, nitrogen, phosphorus, calcium, and magnesium irrespective of the sugarcane vinasses origin [30]. Table 2.1 lists the main characteristics of sugarcane vinasse reviewed by Parsaee, 2019[20] and those taken from Ayalew, 2017 [31]. Generally different authors and researchers have pointed out that the chemical composition of sugarcane vinasse is variable and relies on the raw materials whose characteristics are also dependent mainly on the preparation, alcoholic fermentation system, types of yeast, distillation and separation[40].

According to Ahmed, 2013[40] different microbial groups such as bacteria, yeast, molds and thermophilic bacteria weren't identified in vinasse samples which could be because of the high temperature (105-110˚C) of vinasse during its production that doesn't support microbial growth and the presence of significant amount of alcohol which can be a strong inhibitor for most of the microorganisms that even the thermophilic bacteria couldn't grow on it.

Apart from antibiotics that are added during the fermentation process to inhibit bacterial actions that compete with yeasts responsible for the fermentation, vinasse might comprise other significant

organic pollutants, including agrochemicals applied on the cane fields, by-products or additives used during the ethanol production process which can be environmental threats[45].

Table 2.1 Physico-chemical characteristics of vinasse

Parameters	Abbreviation	Units	Values , ref [20]	Values, ref [31]
Power of Hydrogen	pH	–	3.25–4.97	3.8-4.4
Chemical oxygen demand	COD	kg/m^3	27.5–299.250	70 – 98
Biochemical oxygen demand	BOD	kg/m^3	5.046–47.636	45 – 60
Total organic carbon	TOC	kg/m^3	20.16	
Carbon/nitrogen	C/N	–	11-15/1	
Mineral Solid	MS	kg/m^3	31	
Volatile Solid	VS	kg/m^3	46.39	
Total solids	TS	kg/m^3	27–81.5	
Mineral Suspended Solid	MSS	kg/m^3	5.3	
Volatile Suspended Solid	VSS	kg/m^3	1.62–15.86	
Volatile Solid	VA	Mgacetic	1.5	
Phenols	Phenols	kg/m^3	0.45–0.469	
Volatile Matter	VM	kg/m^3	60	
Fixed matter		kg/m^3	21.5	
Volatile fatty acids	VFA	Kg	1.31	
Total fixed solids	TFS	Kg	8-10	
Volatile Suspended solids	VSS	Kg	0.890–1.62	
Total volatile solids	TVS	Kg	19–22	45-65
Suspended solids	SS	Kg/m^3	1.25	
Electrical conductivity	EC	μS/cm	8294–15110	
Bicarbonate alkalinity	Ba	Mg	295–276	
Total lipids	TL	mg/L	250–6894	
Total proteins	TP	mg/L	2750–6894	
Dissolved sugars	DS	mg/L	80	
Betaine		mg/L	22530	
Glycerol		mg/L	3333	
Carbohydrates		mg/L	9117	

2.2.2. Environmental Impacts of Vinasse

As discussed above the physicochemical properties and components of vinasse such as its high COD, BOD, low pH, heavy metals, nutrients and high temperature during production are good indicators for its polluting potential when disposed to the environment such as water bodies and the soil. Overall wastewater quality in terms of its components such as salts, pathogens, organic matter, nutrients (nitrogen, phosphorus, potassium), metals, suspended solids, pH and toxic organic compounds can somewhat describe its environmental impacts [32]. Vinasse disposal into the soil or running waters caused environmental damage which was an incentive to studies directing towards finding out alternative economic uses for this wastewater[39]. The studies show that vinasse when used properly, contributes to soil quality improvements[46, 47] and agricultural productivity[48, 49].

Many negative effects may be seen on the environment mainly the soil by land disposal of sugarcane vinasse but the magnitude of the impacts is directly dependent on the soil type and the climatic conditions [50]. Some of its negative impacts are in the seed germination and changes in the genetic material of exposed organisms[9]. Continuous land disposal of vinasse mainly causes soil salinization and organic overloading, due to its high salt and biodegradable organic matter content, the high concentrations of specific elements, such as Al and Fe may also have phytotoxic effects[50].

Some metals, such as Cd, Pb and Zn, will be found in vinasse due to the leaching of the metals when using low quality materials and/or old machineries and the low pH of vinasse will strengthen the impacts of the metals by increasing their mobility in the soil as their solubility increases in acidic conditions[50]. Impacts associated with land disposal or soil fertirrigation with sugarcane vinasse are listed in the table 2.2.

Reduced sunlight penetration and hence inhibition in plant production due to the dark color, clogging of fish gills due to the increase of turbidity, decrease in dissolved oxygen and eutrophication will be caused by vinasse disposal into rivers and lakes while corrosion in water system and pipe line and crop yield reduction will occur when used for irrigation [4, 31].

Table 2.2 Breakdown of the potential impacts associated with the soil fertirrigation with sugarcane vinasse[50].

Adverse effect	Implications	Reference parameters	Characteristics of the vinasse	Risk
Soil salinization	• Reduction in the osmotic potential of the soil • Toxicity of specific ions (SO_4^{2-}, Na^+, K^+) • Reduction in the uptake of water and nutrients by plants • Disruption of the soil structure • Leaching of salts to groundwater	EC > 3.0 dS/m TDS > 500mg/L	EC > 6.7 dS/m TDS > 4,000 mg/L	High
Soil sodification	• Disruption of the soil structure • Severe reduction in the water infiltration rate • Burning and necrosis of the leaf tissue in plants	Na: Ca >3:1 SAR > 15	Na: Ca < 0.09:1 SAR < 0.51	Low-to-null
Organic overloading (soil and water bodies)	• Depletion of oxygen levels • Establishment of local anaerobic conditions • Reduction in the microbial activity • Increase in the structural instability of the soil	BOD > 400 mg/L TDS > 500 mg/L	BOD > 14,400 mg/L TDS > 4,000 mg/L	High
Soil over fertilization (excess of N and P)	• Increase in the succulence of the plants • Alterations in both nitrifying and denitrifying activity of the soil • Depletion of oxygen levels by nitrifying bacteria	N: effects may be observed when TKN >30 mg /L	TKN > 629 mg /L	High

	• Potential release of nitrogen oxides (N_2O) • Toxicity of ammonia (NH_3) (aquatic organisms) • Leaching of nitrates (NO_3^-) • Eutrophication of water bodies (excess of P)	P: the literature does not indicate reference values		
Permanent acidification (soil and water)	• Alteration in the buffer capacity of the soil • Solubilization of phytotoxic metals (Al and Fe) • Reduction in the productivity of the crop • Reduction in the microbial activity	pH < 6.5–8.0	pH < 4.6	High

Reference parameters are values above which adverse impacts are triggered or amplified.

SAR- sodium adsorption ratio, relates the concentrations of sodium, calcium and magnesium.

Vinasse comprises high amount of BOD that leads to its anaerobic decomposition consuming the amount of dissolved oxygen available in the water bodies. This lowers the level of dissolved oxygen which threatens the aquatic life and causes the release of highly objectionable gases such as methane, ammonia, and hydrogen sulfide[31]. Large quantity application of Vinasse on the soil can create ponds of vinasse, which provides best conditions for the development of insects such as stable fly that feeds on bovine blood causing stress to the cows and reducing milk production and weight loss[38].

Table 2.3 discusses the impacts of disposing wastewaters as an irrigation water and the effects of the different compounds found in the wastewaters on ground and surface waters.

Table 2.3 Impacts of different compounds on ground and surface waters due to irrigation with wastewater [51]

Compound	Impact	Impacts on	
		Ground water	surface water
Nitrogen	May contaminate underground and surface water through infiltration and irrigation runoff. The amount depends on crop demand, hydraulic load due to rain and irrigation, soil permeability and N content in the soil.	High	Medium
Phosphorus	Causes eutrophication which leads to the deterioration of irrigation and wastewater treatment infrastructures.	Not significant	Medium
Biodegradable organic matter	Too much organic matter can cause depletion of dissolved oxygen.	Not significant	Medium
Salinity	Saline soil leachates contaminate both surface and groundwater; up to a certain level it can limit water use. TDS > 500mg/l do not cause health problems in water supplies only flavor change. Very high concentration causes laxative effects on consumers and corrosion on pipes.	Medium	Low
Boron	It's essential but when it exceeds the limit it can easily became toxic.	Medium	Low
Heavy metals	By leaching from acidic soils, they can reach aquifers and surface water.	Low	Low
Toxic organic compounds	Mostly removed by soils.	Not significant	Not significant

A study conducted by Da silva,2021[45] to identify organic contaminants in vinasse, soil and ground water where vinasse is used for fertirrigation in order to relate the presence of contaminants in the wastewater showed that there was no overlap of suspected contaminants indicating the

contents in the soil and ground water were not due to vinasse fertilization and therefore vinasse reuse as fertilizer in sugarcane crops cause minor environmental risks in terms of organic contaminant dissemination.

2.3. Overview of Management Practices and Treatment Methods for Vinasse

2.3.1. Vinasse Management Practices

The US is the largest ethanol producer using corn as the main feedstock followed by Brazil which uses sugarcane with the resulting high production of vinasse. In Ethiopia vinasse is derived from ethanol and potable alcohol production from sugarcane molasses as the main feedstock.

During the 1970s, among the main management options practiced for vinasse was fertirrigation; land disposal as it was found to contain nitrogen, phosphorus and organic content that will enhance yield of certain crops and sugarcane productivity. In Brazil the effluent was capable of replacing application of inorganic fertilizers under controlled conditions. However, for the high strength molasses based spent wash, the odor, putrefaction and unpleasant landscape due to unsystematic disposal become concerns of land application[36].

From other practices in Brazil the main alternatives that can be implemented at large scale are anaerobiosis and recycling in fermentation and fertirrigation or irrigation of agricultural fields through the filtration of vinasse to the soil with the subsequent nutrient transfer from vinasse to the plants. Yeast production, vinasse combustion for power generation and potassium rich ash, livestock feed production, anaerobic digestion for biogas production and reduced organic matter vinasse digestate, and incineration are also some of the methods that are under different stages of research and development [9]. Figure 2.3 summarizes different methods of vinasse application followed by their advantages and disadvantages in the next table that are adopted from a review made by Christofoletti, 2013[9].

Vinasse application as fertilizer or fertirrigation is one of the easiest, economical and more likely way of avoiding pollution related to its disposal as it partially substitutes the use of chemical fertilizers. However, some studies have revealed that its direct application for long periods of time without control causes soil salinity, heavy metals leaching to the underground water and unbalance of nutrients on the soil. The other problem related to fertirrigation is the large volume of vinasse

which is more than the holding capacity of the land that will cause vinasse pond formation and unpleasant land scape. Since its transportation to faraway places is not economical concentration through evaporation of the water content is important. The concentrated vinasse can also be used for livestock feed production and can be burned in special boilers for energy generation in addition to its use as improved quality fertilizer in which the condensed water can be treated and reused by the factory[9].

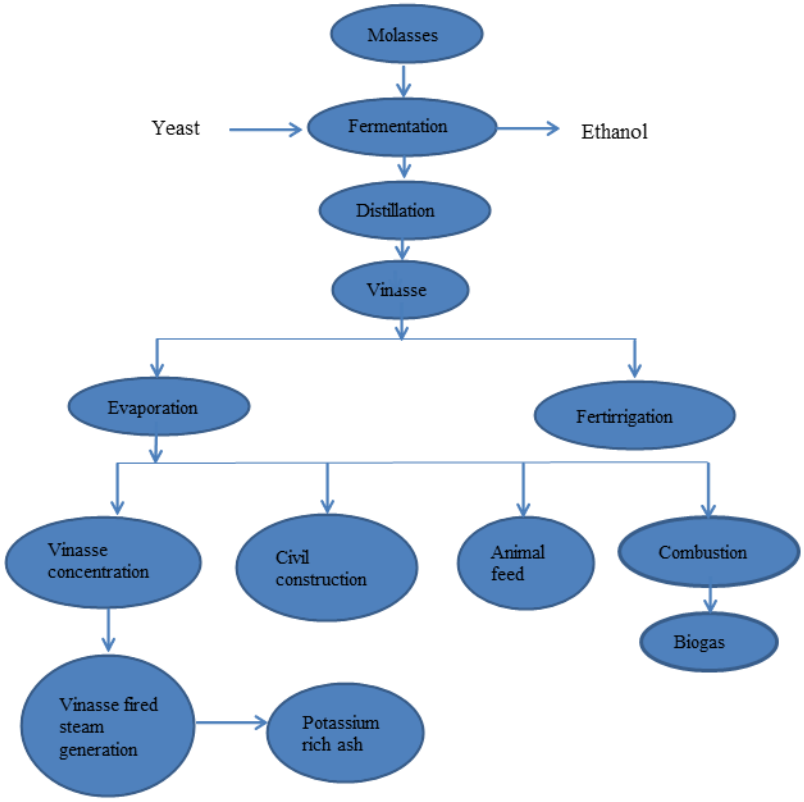

Figure 2.3 Main alternatives for the use/discharge of sugar cane vinasse

Source: adapted from Christofoletti, 2013[9]

As reviewed from studies energy generation through biogas production from vinasse is a good option to contribute for sustainable energy security for countries like Ethiopia. This practice not only reduces cost for treating vinasse but also contributes to the energy saving of the factory by substituting part of their energy use with biogas. The digestate after anaerobic digestion can also be used as an organic fertilizer to improve the soil's physical and chemical properties by adding organic matter and nutrients such as N, P and K.

2.3.2. Treatment Methods for Vinasse

Many proposals have been forwarded for physicochemical and biological treatments of vinasse that can reduce its pollutant characteristic such as COD, color and turbidity[9] by degrading organic toxic substances into biodegradable wastes. In general many reactants are used by the physicochemical treatments like coagulation and flocculation, chemical precipitation, chemical oxidation and ozonation process to oxidize organic contaminants [48] whereas many types of highly contaminated industrial wastewaters can be effectively treated by biological treatment methods that are classified as aerobic and anaerobic systems[4]. Even though aerobic systems, such as aerated lagoons or activated sludge units, generate large quantities of sludge which needs repeated elimination, they are frequently used. Anaerobic systems are relatively cheaper alternatives that are able to produce biogases such as hydrogen or methane replacing the energy consumption of the plant itself[52].

The biological processes turn out to be particularly favorable as they are able to produce interesting metabolites from living cells. Among them, production of biogas through anaerobic digestion is by far the most developed and economically viable process for treatment of vinasse[43]. The high organic content of vinasse makes anaerobic treatment the most attractive in comparison with aerobic treatment[52, 53]. Nitrogen loss in the form of ammonia in anaerobic conditions can be prohibited by maintaining proper initial C: N ratio (range 38±40), pH, moisture content and oxygen concentration[53, 54].

During the past 20 years using a combination of the chemical, physical and biological methods has been advanced as a law to get the most effective and ecological technological systems. Anaerobic digestion, anaerobic filters, lagoons, activated sludge and trickling filters have all been successfully applied to treat distillery wastewater. Membrane and membrane separation techniques with immobilized microorganism or enzyme have also very significant role in distillery wastewater treatment[36]. Other new proposals such as electro dialysis for potassium recovery from vinasse [55], integrated ozonation and digestion for methane production[29], coagulation/flocculation using vegetable tannin followed by photo catalysis[56] and hydrothermal carbonization[38] are also tested to be effective for vinasse treatment.

Anaerobic digestion is a sustainable alternative to process sugarcane vinasse as it allows energy recovery as hydrogen and methane production via a two-stage acidogenic and methanogenic system without interfering in its quality as organic fertilizer[57].

2.4. Overview of Anaerobic Digestion

It is indicated form historical evidences recorded in Assyria and Persia that biogas was used for heating bathing water as early as the 10^{th} century. In the Mid Ages, Jean Baptiste van Helmont detected combustible gas production as the organic matter in lakes decomposes. Later on, a series of experimental analysis was conducted by Alessandro Volta on combustible gas which was collected from marsh sediments. The experiment reveals that there is a direct relation between organic matter decomposition and gas production. In1808, Humphry Davy discovered that when cattle manure is digested anaerobically i.e. without the presence of oxygen, it produced methane, which became an initiation to further investigate the possibility of combustible gas production from manure[58].

Anaerobic digestion (AD) is a biotechnological process that allows waste stabilization while methane-rich biogas is produced by the conversion of the organic matter in the absence of free or molecular oxygen[31]. During AD more than 90% of the available energy for direct oxidation is converted into methane while only 10% is used for bacterial growth. The benefits of the process include; odor and greenhouse gases emission reduction, low production of by-products, not utilizing chemical compounds, low operational costs and positive energy balance [59].

Bacteria are responsible for the various chemical reactions during the anaerobic digestion as a result of the activity of enzymes or “ferments” elaborated by the bacterial cells. Different bacteria are tested to indicate that they are composed of about 80% water and 20% dry material, of which 90% is organic and 10% inorganic. The organic fraction that will be converted into biogas during the digestion has an approximate formula of $C_5H_7O_2N$[31].

An organism such as bacteria must have source of energy, carbon for the synthesis of new cellular material, and inorganic elements (nutrients) such as nitrogen, phosphorus, sulfur, potassium, calcium and magnesium to continue reproducing and function properly. Organic nutrients (growth factors) may also be required for cell synthesis as precursors or constituents of organic cell material that cannot by synthesized from other carbon sources. Among the major growth factors are amino

acids, purines and pyrimidines, and vitamins. Two of the most common sources of cell carbon for microorganisms are organic matter and carbon dioxide. The energy needed for cell synthesis may be supplied by light or by a chemical oxidation reaction[31].

If best operating conditions are applied a biogas comprising 60-70% methane, 30 to 40% carbon dioxide and <1% nitrogen can be generated typically from organic waste while some quantity of hydrogen sulfide and ammonia is also produced for non-ideal operating conditions[60].

2.4.1. Process Steps of Anaerobic Digestion (AD)

The process of biogas formation is a result of linked process steps, in which the initial material is broken down into smaller units continuously. There are specific groups of micro-organisms that are responsible for each step of the process in which they consecutively decompose the products of the preceding steps. **Hydrolysis**, **acidogenesis**, **acetogenesis**, and **methanogenesis** are the four main biological process stages of anaerobic digestion [31]. The simplified pathways for the process are shown in figure 2.4.

Hydrolysis is theoretically the first stage in the conversion of organic material to biogas, where certain bacteria (hydrolytic bacteria) breakdown the complex organic matter (polymers) such as carbohydrates, lipids, and proteins into smaller units (mono- and oligomers) namely sugars, long chain fatty acids (LCFAs), and amino acids respectively so that the products can be further processed by the next group of bacteria. The hydrolytic step can be rate-limiting in wastes with high organic matter while it has also been revealed in past study that methanogenesis might be the one determining the rate of the process based on the ratio of hydrolytic to methanogenic microorganisms[58].

Acidogenesis is the second stage in which the products of hydrolysis are absorbed by the cell mebranes of acidogenic microorganisms to be converted into intermediate volatile fatty acids (VFA) and smaller amounts of ethanol and lactate. VFAs include a class of organic acids such as acetates, and larger organic acids such as propionate and butyrate, usually in a ratio ranging from 75:15:10 to 40:40:20. This stage of AD is believed to be the fastest of the other stages as the acidogenic bacteria have a regeneration time of less than 36 h[31, 58, 59].

For wastes that contain higher protein such as sewage wastewaters, it is important to inspect the degradation of amino acids into VFA which generally occurs in pairs through the Stick land reaction, while single amino acid degradation is also possible in the presence of hydrogenotrophic bacteria which is slower than the Stick land reaction. During the breakdown of amino acid ammonia is also produced from deamination which is identified as inhibitor of AD when it is produced in sufficiently high concentration[58].

Acetogenesis is the third stage of AD which follows the conversion of hydrolytic products into acetate which is methanogenic substrate, and higher volatile fatty acids and other intermediates which cannot be directly converted to methane. In this step the products that can't be directly converted to methane are converted into methanogenic substrate mainly acetate with the production of Hydrogen as well[58].

Methanogenesis is the last stage of AD in which the production of methane and carbon dioxide is carried out by methanogenic bacteria from intermediate products i.e., acetate produced both at the acidogenesis and acetogenesis stages, Hydrogen and Carbon dioxide. Methane production from acetate, by splitting two acetic acid molecules to produce methane and CO_2, accounts for 70% of the formed methane, while the remaining 30% is originated from the reduction of CO_2 with hydrogen (H). When biogas production stops it can be recognized that methanogenesis has ended in batch reactors which can last for about 40 days. By taking the sludge's volatile solids content and its ability to dewater, the extent of its digestion can also be evaluated [31, 58].

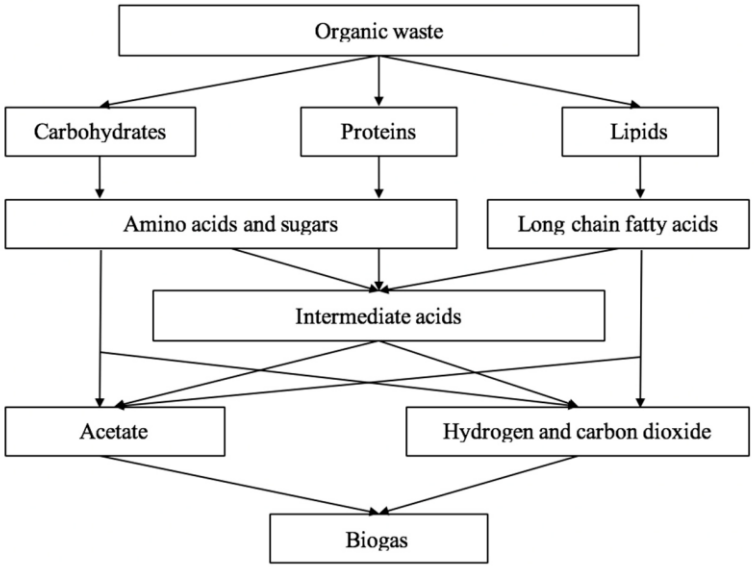

Figure 2.4 The simplified scheme of pathways in anaerobic digestion[58].

2.4.2. Main Factors Affecting Anaerobic Digestion

There are a lot of factors that govern anaerobic digestion which is directly affected by the microbial activity, these are; temperature, pH, buffering capacity, Carbon to nitrogen ratio (C: N), volatile fatty acid concentration, total solid content (TS), Retention time, Organic loading rate (OLR), Particle size and moisture content of substrate.

2.4.2.1. Temperature

The three temperature ranges under which anaerobic digestion can occur are psychrophilic, Mesophilic and Thermophilic Temperature with working temperature ranges of < 25^{o}C, 25^{o}C – 45^{o}C, 45^{o}C – 70^{o}C respectively each supporting a specific type of methanogenic bacteria that are sensitive to temperature variations. Temperature directly affects the physico-chemical properties of all components in the digester and the thermodynamic and kinetic of the biological processes as well. Increasing the solubility of organic compounds making them available for the microbes, increasing rates of chemical and biological reactions to accelerate conversion processes which allow the use of smaller reactor and short retention time are some of the advantages of increasing

temperature. However since the solubility of some gases (NH_3, H_2, CH_4, H_2S and VFA) increases with temperature, it can affect the system negatively if the gas has an inhibitory effect[31, 60].

2.4.2.2. Power of Hydrogen (pH)

The optimum pH for the growth of fermentative bacteria, that are responsible for the first stage of AD process, is generally low (5 to 6.5) while they can perform at pH ranging from 8.5 down to 4. Methanogens which are responsible for producing methane are more pH sensitive than the fermentative bacteria performing in the range of 5.5-8.5 with an optimum neutral pH of 6.5-8.0 for their growth[31]. Since the change in pH affects the toxicity of intermediates such as ammonia and short chain fatty acids it is an important parameter that must be taken into account. As a general rule, a pH range between 6.8 and 8 is suggested to be optimum as pH above 8 can be toxic to most anaerobic microorganisms while pH below 5 is lethal to methanogens. Methane formation is identified to be optimized between pH values ranging from 6.7 to 7.4[31, 60].

2.4.2.3. Buffering Capacity

Major and rapid pH changes of a system are resisted by an important parameter called the buffering capacity which can be expressed in terms of alkalinity. The difference in the rate of acid production and acid consumption is usually related to the buildup of short chain fatty acids (SCFA) and therefore the pH decreases in a system where the buffering capacity is low. As process imbalance causes pH reduction, alkalinity or pH can be used as an indicator of process imbalance to monitor the system. However if a system is highly buffered, pH can no longer be used as an indication of process imbalance as the change in pH will be very small even when the system is highly stressed[31].

2.4.2.4. Carbon to Nitrogen ratio

As carbon is an important substrate for anaerobic digestion at certain concentration, Nitrogen is also important at certain concentration in case the protein formation for microorganisms is compromised. Bearing in mind the composition of carbohydrates, lipids, and proteins, degradation of proteins is the greatest available source of nitrogen in anaerobic digester[58]. Fast consumption of nitrogen by methanogens causes high C: N ratio and low production of gas while the ammonia

accumulation causes low C: N with the optimum C: N ratio for a good anaerobic digestion ranging from 20 to 30.

2.4.2.5. Hydraulic Retention Time

The hydraulic retention time (HRT) is a term commonly used to represent the average length of time that liquids remain in a digester, which often appears as *q* in literature and can be calculated by dividing digester volume, V, with digester flow rate, Q[58]. The HRT, which is affected by substrate characteristics, reactor design, digester temperature and environmental conditions, should be lengthy enough to allow metabolism by microorganisms to degrade the organic material and produce biogas. For substrates that degrade slowly, the HRT is typically longer to efficiently let the organic material solublized and in this situation, hydrolysis is considered as a rate limiting step. Continuous stirred tank reactor (CSTR) is run at longer HRT (10-60 days)[31].

2.4.2.6. Anaerobic Co-digestion and Its Advantages

Anaerobic co-digestion (AcoD) is the simultaneous anaerobic digestion of two or more substrates that have complimentary characteristics for combined treatment. AcoD can be a solution to avoid inhibitions thereby increasing biogas production as a result of enhanced carbon accessibility, nutrient balance and buffering capacity. It is helpful to correct the limitations of mono digestion as it involves operations taking lower retention time bringing about economic feasibility for industrial applications due to higher methane production[59]. Due to the variation of waste composition and increased demand for a waste, constant supply of homogeneous feedstock is not always achievable in some regions, which is an important requirement for the economic applicability of anaerobic digestion. Therefore, in order to overcome feedstock composition fluctuation, co-digestion of different substrates is needed as it allows composition balance and availability[61].

For the purpose of this thesis work the methane production potential of sugarcane vinasse anaerobically co-digested with bagasse will be tested by changing the operational parameters pH, temperature and composition or mixing ratio of the two substrates which will be tested in three levels. The pH will be tested in the optimum range suggested by literatures reviewed and the

average, the temperature will be tested in the mesophilic range and the average and the mixing ratio will be tested at 75% Vinasse to 25% Bagasse, 50% V: 50% B & 25%V: 75%B.

Chapter Three

3. Materials and Methods

In this section all the materials, chemicals and equipment used as well as the methods and experimental procedures employed during substrate characterization and experimental approaches for anaerobic co-digestion of sugarcane vinasse with bagasse are explained briefly.

3.1. Materials and Reagents

3.1.1. Substrates for Biogas Production

3.1.1.1. Sugarcane Vinasse

Sugarcane vinasse, a byproduct of alcohol/ethanol production, was obtained from National Alcohol and Liquor Factory's distillation unit which uses molasses as a raw material for alcohol production. The vinasse was stored in refrigerator at 4^{o}C prior to use. It was then characterized to test its initial Total Solid content (TS), volatile solid content (VS), fixed solid content (FS), power of Hydrogen (pH), chemical oxygen demand (COD), biological oxygen demand (BOD), total Nitrogen (TN), Carbon to Nitrogen ratio (C: N), Potassium (K) as well as heavy metals contents.

3.1.1.2. Bagasse

Bagasse, the fibrous residue left after sugarcane crushing, was collected from Wonji sugar factory's sugar mill. It was dried, and crushed to obtain finer particles for better digestion. It was then characterized in terms of its; moisture content (MC), volatile matter contents, ash content, fixed carbon content and bulk density.

3.1.1.3. Inoculum

Anaerobic sludge obtained from a biogas plant at Addis Ababa University, 4 kilo campus was used as the inoculum. The viscous liquid/sludge was collected and stored anaerobically at room temperature prior to use. It was then characterized to get its Total solid, Volatile solid, Ash content and moisture content.

3.1.1.4. Chemicals, Reagents and Equipment

The following chemicals, reagents and equipment were used for the sample preparation and characterization of all the above three substrates.

Chemicals such as 1M sodium hydroxide (NaOH), 0.5 M Hydrochloric Acid (HCl), H_2SO_4, $HgSO_4$ hydrated ferrous sulphate ($FeSO_4.7H_2O$), KOH, K_2Cr_2O7, H_2SO_4, $HgSO_4$, Ag_2SO_4, Standard ferrous ammonium sulfate titrant (FAS), Potassium hydrogen phthalate standard, diluted nitric acid, Sulfamic acid, Phosphate buffer solution, Magnesium sulfate solution, Calcium chloride solution, Ferric chloride solution, Sodium sulfite solution, Nitrification inhibitor, Ammonium chloride solution were used.

Laboratory equipment such as Reagent bottles of 500ml capacity were used for the digestion, pharmaceutical urine bag was used to collect generated gas, Buffer tablets, pH 4 and 8 for pH meter calibration, Grinder, analytical balance, pH meter, spatula, filter paper, BOD incubator, incubation bottles, evaporating dishes, oven, muffle furnace, crucibles, tongs, desiccator, beakers, pipettes, flasks, graduated cylinder, beakers and other glassware, magnetic stirrer, water bath, and gas analyzer were used during substrate characterization and anaerobic digestion processes.

3.2. Methodology

The experimental method includes substrates preparation and characterization, anaerobic digestor setup, experimental conditions as well as product [biogas and sludge] analysis which is summarized in figure 3.1.

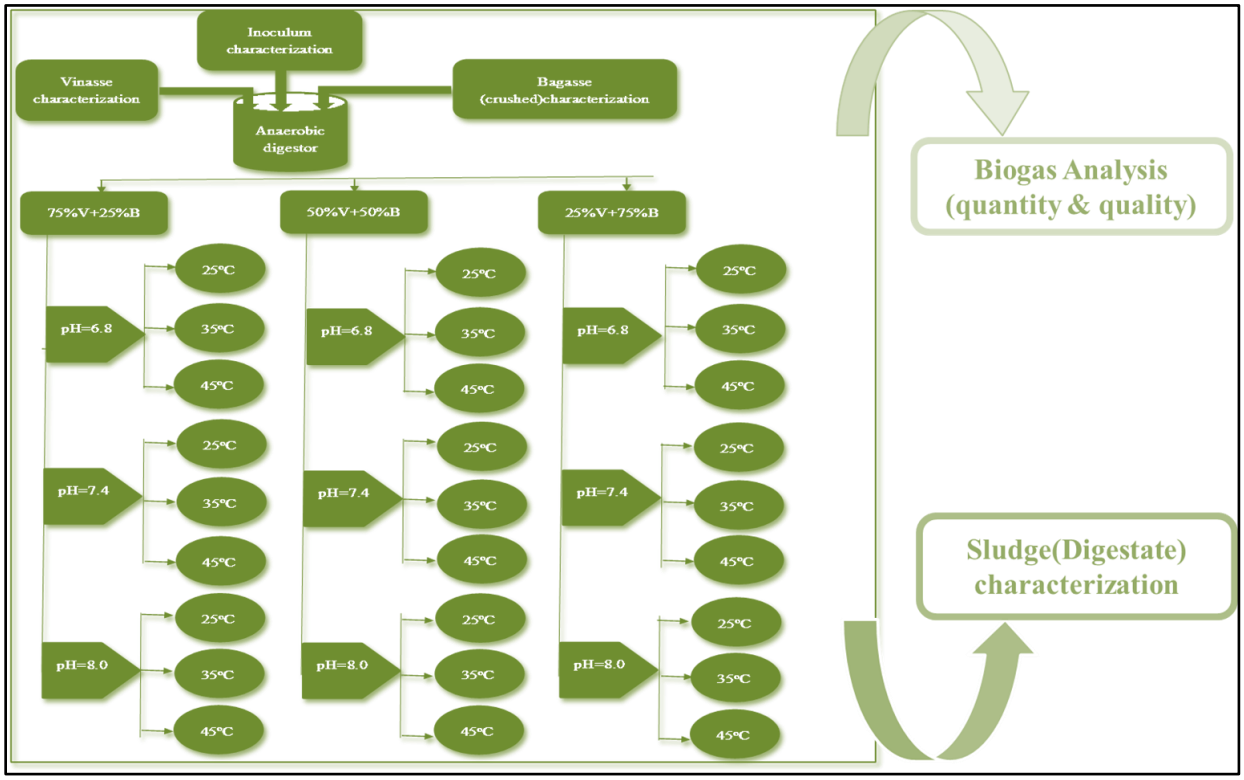

Figure 3.1 Summary of methodology for anaerobic co-digestion of sugarcane vinasse with bagasse

3.2.1. Vinasse Characterization

Vinasse was characterized in terms of its pH (measured using pH meter), Total solid (TS), Volatile solid (VS), fixed solid (FS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Total Nitrogen, Carbon to Nitrogen ratio, and Potassium using standard methods. Total solids, volatile solids, and fixed solids tests were performed at AAIT environmental engineering laboratory while the remaining tests were conducted at Ethiopian Conformity Assessment Enterprise (ECAE). The concentration of heavy metals namely; Arsenic, Chromium, Lead, Copper, Cadmium and Mercury were tested at ECAE's laboratory.

3.2.1.1. Total Solid, Volatile Solid and Fixed Solid Determination

TS determination was performed according to APHA 2540B method. A clean and dried crucible was weighed and recorded. 5 g of vinasse, homogenized was then placed in the crucible and put into an electric oven set at 105ºC. After significant amount of water was removed it was cooled in a desiccator to avoid moisture reabsorption and weighed. It was then dried, cooled in a desiccator and weighed in every three hours interval until its mass remains constant. After that, the dried sample was ignited in a muffle furnace at 550ºC for an hour. After ignition the sample was cooled in a desiccator and weighed repeatedly until constant weight or below 4% weight difference was obtained. The investigations were carried out in triplicate. Lastly, the TS, VS & FS were determined by using the equations below.

$$\%TS = \frac{A-B}{C-B} * 100 \quad 3.1$$

$$\%VS = \frac{A-D}{A-B} * 100 \quad 3.2$$

$$\%FS = \frac{D-B}{A-B} * 100 \quad 3.3$$

Where;

TS: total solid content, %

VS: volatile solid content, %

FS: fixed solid content, %

A: weight of the dried sample and the crucible, g

B: weight of the crucible, g

C: weight of the wet sample and the crucible, g

D: weight of ignition residue and the crucible, g

3.2.2. Proximate Analysis of Bagasse

Bagasse sample was crushed with a grinder for better digestion and analyzed prior to use in terms of its properties listed below.

3.2.2.1. Moisture Content Determination (MC)

It was measured by using ASTM standard D 3173-03,2013. 5g of the bagasse sample was measured and placed in a weighted clean and dry crucible. It was then placed in an oven at 105^{o}C for 24 hr. This was done in triplicates. MC was determined as per equation 3.

$$MC\% = \frac{M_w - M_d}{M_w} \quad 3.4$$

Where;

MC% is percentage of moisture content

M_w is mass of wet sample

M_d is mass of dried sample

3.2.2.2. Volatile Matter Content (VMC)

The VMC was determined according to ASTM D 3175 -89a, 2012 by measuring 2g of the oven dried bagasse and placing it in a pre-weighed and dried crucible. The crucible containing the sample was placed in a muffle furnace at 950^{o}C for 7 minutes. It was then withdrawn and placed in a desiccator to cool down without moisture absorption and weighed again. It was done in triplicates. The VMC was then calculated using equation 3.5.

$$VMC\% = \frac{W_{ods} - W_{mfs}}{W_{ods}} * 100\% \quad 3.5$$

Where;

VMC% is percentage of Volatile matter content

W_{ods} is weight of oven dried sample

W_{mfs} is weight of the sample after burning in the muffle furnace

3.2.2.3. Ash Content Determination (AC)

AC% was determined according to ASTM standard D 3174 – 02,2012 by first washing, drying and weighing a crucible to record its mass. And then 2g of dried bagasse sample was placed in the crucible and put into a muffle furnace to burn for 3hrs at 550^{o}C. It was then withdrawn and placed in a desiccator to cool without absorbing moisture. After cooling to room temperature, the sample and crucible was weighed and recorded to determine the AC% using equation 3.6. This was done in triplicates.

$$\mathrm{AC}(\%) = \frac{\mathrm{W}_{ar}}{\mathrm{W}_{ods}} * 100 \qquad 3.6$$

Where;

AC% is percentage of ash content

W_{ar} is weight of ash residue

W_{ods} is weight of oven dried sample

3.2.2.4. Fixed Carbon Content Determination

The fixed carbon composition was determined by subtracting the percentage of the moisture content, volatile matter and ash content of the sample from the total mass as per ASTM standard D 3172-89 R02,2002.

3.2.2.5. Bulk Density (ρ)

Bulk density was measured according to ASTM 7481 by using glass measuring cylinder of 50ml capacity that was cleaned, dried and weighted. Crushed bagasse was packed (not compressed) by using a piston like material without leaving space for air in the cylinder and weighed to calculate the bulk density using equation 3.7;

$$\rho(\mathrm{kg/m3}) = \frac{M_{pc} - M_{ec}}{V_c} \qquad 3.7$$

Where;

ρ: bulk density

M_{pc}: weighted mass of packed cylinder

M_{ec}: weighted mass of empty cylinder

V_c: Volume of cylinder

3.2.3. Anaerobic Digester Setup

The anaerobic digesters were set up using sterile 500 ml reagent bottles, with 400 ml working volume. After adding the weighted number of substrates based on the experimental design, the bottles were closed with rubber corks (pierced) and sealed with parafilm to avoid air entry and create anaerobic conditions. Tubes of the urine bags were fitted into the cork holes to allow gas collection in the bags from the digestors which will then be analyzed. The digesters were placed in a water bath to control temperature, with the bath temperature set 1°C higher than the experimental target to compensate for cooling caused by exposure of part of the bottles to ambient air. To ensure uniformity and bacterial digestion, the digesters were shaken twice daily.

3.2.4. Experimental Conditions

The experimental conditions for the anaerobic co-digestion experiments were conducted at three different operating parameters; substrate mixing ratio (Vinasse to bagasse), pH and temperature in three levels based on the one variable at a time approach. Three levels for substate mixing ratio of vinasse to bagasse were selected as 75%:25%, 50%:50% and 25%:75%. Temperature levels of the mesophilic range [25-45] °C and the average 35 °C were tested to assess the effect of temperature on biogas and methane production. pH levels were taken from the best range (6.8-8) assessed from previous works and the average (7.4) to test for three pH levels. It was adjusted to the desired level using 0.5M sodium hydroxide (NaOH), and 1M Hydrochloric Acid (HCl). These levels of the three parameters result in 27 anaerobic digestors which was done on 500ml capacity reagent bottles with working capacity of 400ml.

For 400ml of working volume a constant volatile solid of 4.03 gram which is contributed by the two substrates based on the substrate mixing ratio level and total solid and volatile solid content of the substrates as well as 125.6 ml of inoculum was fed. The amount (gram) of substrates for each level used for the anaerobic co- digestion preparation is summarized in table 3.1. The table shows that the quantity of bagasse (in grams) is lower than that of vinasse (also in grams) for the same percentage composition. This difference arises from bagasse having a higher total solid content compared to vinasse. The calculations were based on the total solids and the volatile solids available for conversion to biogas, as the remainder of both samples consists primarily of water.

Consequently, for the same percentage composition, vinasse has a greater amount since most of its composition is water, unlike bagasse. Additional information about the calculation is found in the appendix.

Table 3.1 Substrate preparation for anaerobic co-digestion

No.	Substrate mixing ratio, %		Amount of substrate		Inoculum amount (ml)	Working volume
	Vinasse	Bagasse	Vinasse (gm)	Bagasse (gm)		
1	75	25	30.6	1.22	125.6	400ml
2	50	50	20.4	2.44	125.6	400ml
3	25	75	10.2	3.66	125.6	400ml

The two substrates were weighed and added to each of the 27 digestors according to the mixing ratio (9 similar mixing ratios for each level) and labelled. Equal amount of inoculum, calculated based on the TS and VS values of the substrates, was added to all the 27-batch anaerobic digestors. The mixtures were then filled with tap water to a bit less than 400ml (working volume) to give space for NaOH and HCl addition for pH adjustment.

The pH was adjusted to the three levels of pH; 6.8, 7.4 and 8.0 for all the 27 digestors (9 for each pH level). And then, three temperature levels, 25^{o}C, 35^{o}C and 45^{o}C were maintained using water bath. The operating parameters and their levels of analysis are summarized in table 3.2.

Table 3.2 Operating parameters and levels of analysis

No.	Operating parameter	Unit	Level		
1	Mixing ratio	% Vinasse	75	50	25
2	pH	-	6.8	7.4	8.0
3	Temperature	^{o}C	25	35	45

The gas produced by each digester was collected in urine bags securely fitted to each digester, ensuring no gas leakage. The closing corks were also covered with parafilm, as illustrated in Figure 3.2.

Figure 3.2 Anaerobic digestion setup

3.2.5. Biogas Measurement

Once complete digestion was confirmed, indicated by stable bag volume and the disappearing of the bubbles in the digestors, the process was halted to analyze the gas collected in the urine bags, and the remaining sludge in the digestor was also characterized to evaluate its potential use as an organic fertilizer. The gases collected from each digester were quantitively and qualitatively measured. Quantitative measurement involved using a liquid displacement method: the gas-filled urine bags were placed in graduated container, and the change in volume was recorded after deducting the initial volume of the empty bags.

Figure 3.3 Portable Gas Analyzer

Qualitative analysis was performed using a portable gas analyzer (Biogas 5000, Geotech), figure 3.3 to determine the composition of methane, carbon dioxide, oxygen, hydrogen sulfide and other residual gases. The results were recorded for each of the anaerobic digestor batches.

3.2.6. Sludge Analysis

After the end of the batch anaerobic digestions, two selected sludges resulted from the digestors with the highest methane production from the two temperature levels (36^{o}C and 45^{o}C) were analyzed in terms of their total Nitrogen, Phosphate and Potassium (NPK) contents, Total organic carbon content (TOC), and Carbon to Nitrogen ratio (C: N) in AAU 4kilo campus chemistry laboratory to compare the results with the requirements recommended by FAO for use as an organic fertilizer.

Chapter Four

4. Results and Discussion

In this chapter the results obtained from the physico-chemical characterization of the substrates as well as the results from the experimental operations for anaerobic co-digestion of vinasse with bagasse are discussed briefly.

4.1. Physico-Chemical Characteristics of Vinasse

Using the corresponding standard methods for their determination, the results of the physico-chemical characterization of sugarcane vinasse used for the current experiment are summarized in table 4.1 below. Additional information can be found in the Appendix part.

Table 4.1 Physico-chemical characteristics of sugarcane vinasse

No.	Parameter	Standard test method	Value	Unit
1	pH	APHA 4500-H+B	3.58	-
2	Total solid (TS)	APHA 2540B	9.905	%
3	Volatile solid (VS)	APHA 2540B	99.631	%
4	Fixed solid (FS)	APHA 2540B	0.369	%
5	Chemical oxygen demand (COD)	APHA 5220-B	36,275	mg/l
6	Biological oxygen demand (BOD)	APHA 5210-B	1046.5	mg/l
7	Total Carbon	ASTM D7573-09	22.4975	%
8	Total Nitrogen	SLC 208	1233.3	mg/l
9	Carbon to Nitrogen ratio	-	32.2452	-
10	Potassium Oxide (as K_2O)	MP-AES	0.45	% by m/v
11	Arsenic (As)	EPA 200 B-ICP-MS	0.054	mg/l
12	Chromium (Cr)	MP-AES	<0.4	mg/l
13	Lead (Pb)	EPA 200 B-ICP-MS	0.24	mg/l
14	Copper (Cu)	MP-AES	<0.4	mg/l
15	Cadmium (Cd)	MP-AES	0.02	mg/l
16	Mercury (Hg)	EPA 200 B-ICP-MS	0.006	mg/l

From the results in table 4.1, it can be seen that vinasse has a pH value of 3.58, which is in the range of the values reported in many studies, indicating its acidic behavior having a potential of causing soil and water acidity if disposed uncontrollably to water bodies and the soil as fertirrigation. It's nutrient and organic matter contents are very high compared to the limits of wastewater disposal causing oxygen depletion to receiving water bodies, the dark color and high COD/BOD values are evident for its potential to pollute the environment if not treated or used for other purposes. These results show that vinasse management/treatment before discharging to the environment is needed as stated in the problem statement part of the thesis.

The Total solid (TS), Volatile solid (VS), Fixed solid (FS), Total Carbon, Total Nitrogen, Carbon to Nitrogen ratio, and Potassium Oxide (as K_2O), were determined to be 9.905%, 99.631%, 0.369%, 22.4975%, 1233.3 mg/l, 32.2452 and 0.45 % by m/v respectively. The concentration of the heavy metals Arsenic (As), Chromium (Cr), Lead (Pb), Copper (Cu), Cadmium (Cd) and Mercury (Hg) were 0.054, <0.4, 0.24, <0.4, 0.02, and 0.006 mg/l which has low risk for use as fertirrigation water as mentioned in table 3.2[51].

The higher organic matter content as well as the total solid content makes vinasse suitable for biogas production as evidenced by its high COD/BOD values which signifies that there is enough food available for the bacteria during the anaerobic conversion into biogas. The heavy metals contents have also been tested to check the pollution potential of soil and ground water upon discharge to the environment and use as irrigation water with fertilizer values. The results have shown that low amount of heavy metals are present which suggests the examination of the sludges for use as an organic fertilizer to meet with WHO's recommendation for the use of organic manures as organic fertilizers [51].

4.2. Proximate Analysis Results of Bagasse

The results of bagasse analysis are given in table 4.2. The Total solid (TS) which is 100-M.C., Volatile solid (VS), Bulk density, fixed solid content (FS) and the Ash content (AC) were 88.52%, 93.25%, 0.21 g/ml, 4.71% and 2.06 % respectively showing a higher TS and VS value which is believed to complement the low TS in vinasse for biogas production. This is the main reason for co-digestion to overcome feedstock composition fluctuation. This will increase production of biogas as a result of enhanced carbon accessibility, nutrient balance and buffering capacity.

Table 4.2 Results for proximate analysis of bagasse

No.	Parameter	Value	Unit	Standard test method
1	Total solid (TS), (100-M.C)	88.52	%	ASTM D 3173-03,2013
2	Volatile solid (VS)	93.25	%	ASTM D 3175 -89a, 2012
3	Bulk density	0.21	g/ml	ASTM 7481
4	Fixed solid content (FS)	4.71	%	D 3172-89 R02,2002
5	Ash content (AC)	2.06	%	ASTM D 3174 – 02,2012

4.3. Characterization Results of Inoculum

The inoculum was characterized to determine its total solids, volatile solid, ash contents and moisture contents and the results were 8.60662 wt.%, 73.9895 wt.%, 26.0105 wt.% and 91.3934 wt% as in the table 3.1.

Table 4.3 Characteristics of inoculum

Parameter	Total solids (%)	Volatile solids (%)	Ash content (%)	Moisture content (%)
Value	8.60662	73.9895	26.0105	91.3934

4.4. Biogas Production

Biogas production (volume) measurement includes quantitative measurement of all gases produced including methane, carbon dioxide, oxygen and other trace volumes of gases of which methane is the most important for energy production. The quantitative biogas production was measured in terms of the total volume of gases to identify which operating condition results in high amount of gas production. The data collected from all digestors can be found in Appendix A.

4.4.1. Effect of Temperature on Biogas Production

The experimental results demonstrated a significant variation in the volume of gas production with changes in temperature. At a lower temperature of 25°C, there was no observable gas production across all batches, regardless of the pH and substrate mixing ratio levels. This was evident from the lack of gas accumulation in the urine bags as well as the absence of bubbles in the digestors even after a prolonged retention time of over 35 days. However, as the temperature increased to 35°C and 45°C, there was a marked increase in gas production. This increase was visually confirmed by the daily pumping of the bags, indicating enhanced bacterial activity at these higher temperatures. This observation aligns with the findings of S. P. Lohani and J. Havukainen, who reported that bacterial activity is significantly boosted at higher temperatures in the mesophilic range[62].

Figure 4.1 Volume of gas at 25ºC

Temperature plays a crucial role in affecting the physico-chemical properties of all components within the digester. It influences both the thermodynamics and kinetics of the biological processes involved. Higher temperatures increase the solubility of organic compounds, making them more accessible to microbes. This, in turn, accelerates the rates of chemical and biological reactions, facilitating faster conversion processes[62, 63]. Consequently, this allows for the use of smaller reactors and shorter retention times, which are advantageous for efficient gas production. However, it is important to note that the solubility of certain gases, such as Ammonia (NH_3), Hydrogen (H_2), methane (CH_4), hydrogen sulfide (H_2S), and volatile fatty acids (VFA), also increases with temperature. This can have a negative impact on the system if these gases exhibit inhibitory effects on the microbial activity.

Figure 4.2 a) Volume of gas produced at 35ºC b) Volume of gas produced at 45ºC

The experimental data clearly illustrated these changes at the temperature levels of 35°C and 45°C, particularly after more than 35 days of retention time which can also be seen from the pictures in figures 4.1 and 4.2. At this point, gas production ceased for all digesters, as indicated by the disappearance of gas bubbles in the digestors and no further change in the volume of gas collected in the urine bags. The accompanying chart in figure 4.3 highlights the effect of temperature on biogas production, showing the highest yield at 45°C, followed by 35°C.

Batch	A	B	C	D	E	F	G	H	I
pH	6.8	6.8	6.8	7.4	7.4	7.4	8.0	8.0	8.0
Mixing ratio (%V)	75	50	25	75	50	25	75	50	25

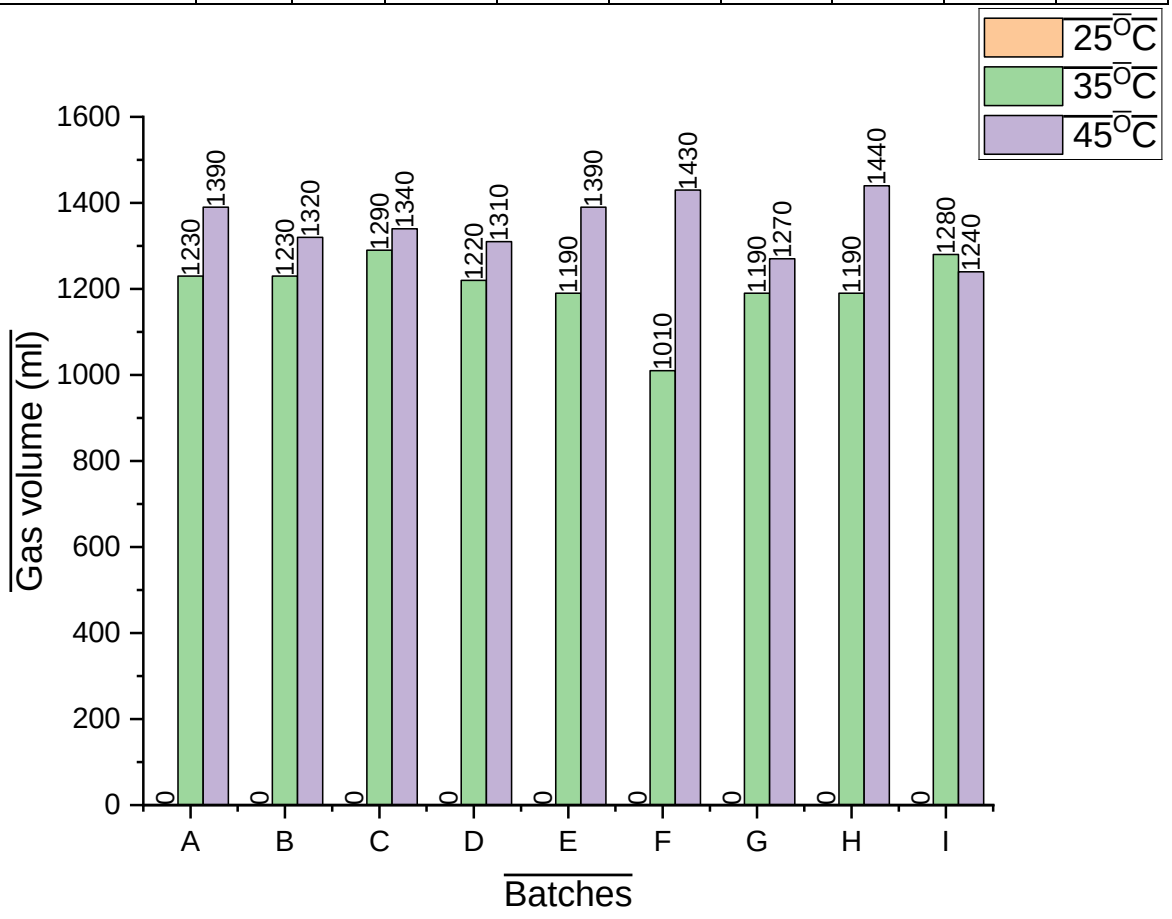

Figure 4.3 The effect of temperature on volume production of gas produced

4.4.2. Effect of Mixing Ratio on Biogas Production

The effect of substrate mixing ratio was analyzed by comparing the volumes of the gases for the same temperature and pH values, designated as batches A-F which is because all the 9 batches at 25^{o}C were zero. From the chart in figure 4.4 it can be seen that there is no obvious trend as we increase the mixing ratio of sugarcane vinasse from 25% - 75%. For batches A, D & E, the volume of biogas produced increases as we increase the mixing ratio, for batch C it decreases and for batches B and D it changes after the 50% vinasse. From this varied response, we can infer that the change in biogas volume was influenced not just by the alteration in the substrate mixing ratio, but

also by its interaction with the other two variables. This implies that optimization techniques should be employed to examine the interactive effects of the parameters.

Batch	A	B	C	D	E	F
Temperature	35	45	35	45	35	45
Ph	6.8	6.8	7.4	7.4	8.0	8.0

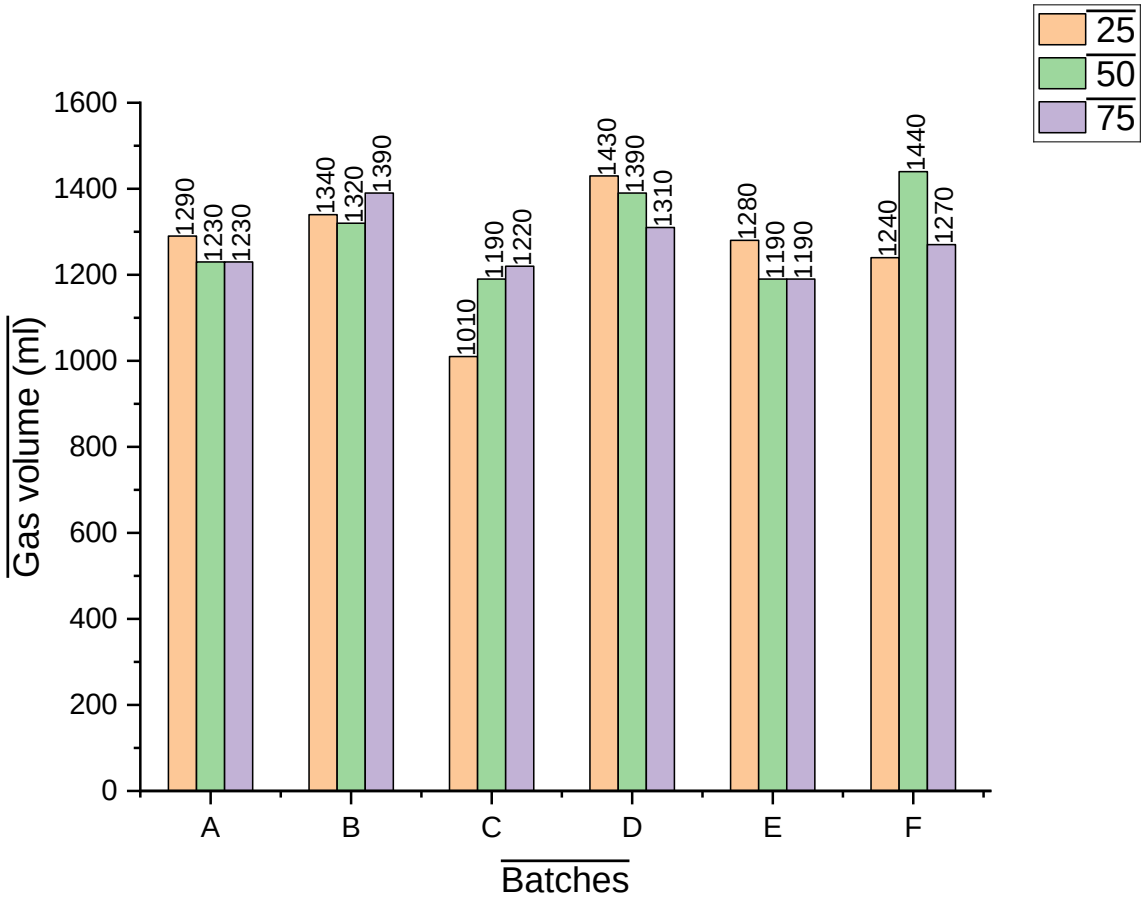

Figure 4.4 The effect of substrate mixing ratio on the volume of gas produced

4.4.3. Effect of pH on Biogas Production

The effect of pH is shown in the chart from figure 4.5, for the three pH levels, while keeping the other two parameters constant (represented as batches A-F because 3 batches at $25^{O}C$ were not considered as there was no gas production). As we increase the pH from 6.8 to 8.0; for batches A-D the highest gas production was observed at pH=6.8 while it was at pH= 8.0 & 7.4 for batches E

& F respectively that the maximum volume was obtained. Both the highest and the lowest values were at pH value of 7.4. The highest average biogas production was 1300ml @pH = 6.8, followed by 1268.33ml @pH = 8.0, and the lowest was 1258.33ml @pH = 7.4.

Batch	A	B	C	D	E	F
Mixing ratio (%V)	75	50	25	75	50	25
Temperature	35	35	35	45	45	451

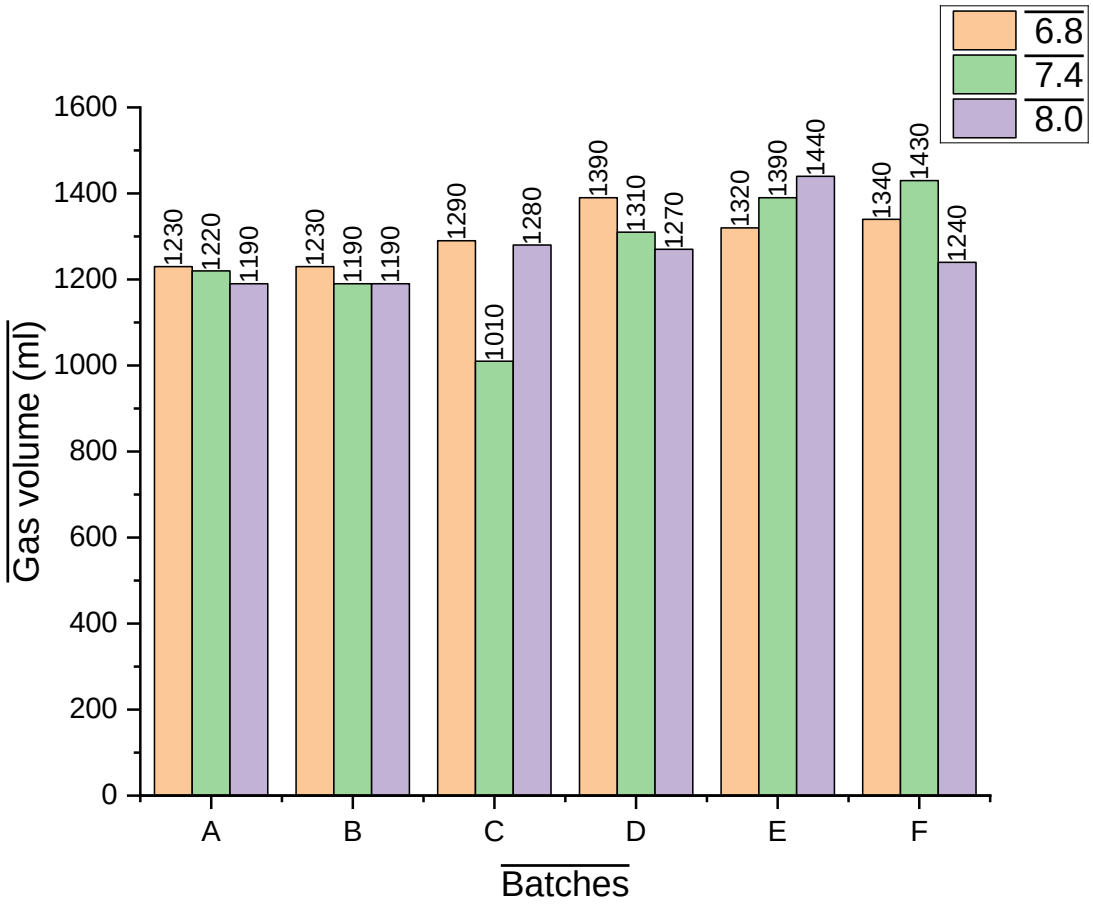

Figure 4.5 The effect of pH on the volume of biogas produced.

The impact of pH changes on one batch differs from its effects on another batch that has a different substrate mixing ratio and temperature. The results did not reveal a clear trend across all batches, making it impossible to determine that variations in biogas volume are solely due to changes in pH. The results indicated that biogas production was achievable within the pH range of 6.8 to 8.0, as all batches produced over 1000 ml, suggesting this range is conducive to bacterial activity which

is consistent with the pH values recommended in previous studies [31, 60]. To gain a better understanding of these effects and identify the optimal pH value, it is necessary to investigate the combined effects with the other parameters.

4.5. Methane Yield

As the quality of the biogas is determined by its methane content, methane production was measured (in percentage) with the help of a portable gas analyzer to evaluate the biogas quality produced from each anaerobic digestor in addition to the quantity which was measured in the previous section. The methane content is also a factor of investment costs for biogas production facilities as high methane content production implies the requirement of smaller digestor volume. The effect of the three parameters on methane production is discussed below.

Methane yield was calculated by multiplying the volume of biogas produced by the respective percentage composition of methane and dividing it by the amount of total volatile solid fed to the system which is 4.03 gram. This value is the recommended value of total volatile solid for batch digestors.

$$Methane\ yield = \frac{Biogas\ Volume * methane\ concentraion}{4.03gVS} * 100$$

4.5.1. Effect of Temperature on Methane Yield

The effect of temperature on methane production was evident from the observations of the urine bags used for gas collection from the digestors operating at three different temperatures. The results indicated that no gas production occurred at 25°C, suggesting that anaerobic co-digestion of sugarcane vinasse with bagasse is not feasible at this temperature, regardless of pH changes or substrate mixing ratios.

At the same pH values and substrate mixing ratios, methane production was generally higher at 45°C compared to 35°C, except for batches B and D, where the yield was lower at 45°C. This indicates that higher temperatures in the mesophilic range generally enhance methane production, but there are exceptions based on specific batch conditions. Similar studies have shown that temperature plays a crucial role in anaerobic digestion processes. For instance, research by

[64]demonstrated that methane production increases with temperature up to an optimal point, beyond which the yield may decrease due to thermal inhibition[64]. Generally, it was observed that higher methane yield was obtained at 45^{o}C as shown in figure 4.6 which shows the gas compositions and there was no production of H_2S for all batches.

Figure 4.7 which shows the effect of temperature on methane yield indicates the increase in methane yield as the temperature was increased from room temperature 25^{o}C to 45^{o}C while the yield was better for 35^{o}C than 45^{o}C for batch B at 50% vinasse concentration and pH of 6.8. this deviation from the increasing trend can be interpreted as by changing the other two parameters it is possible to get better yield at 35^{o}C which will be helpful in reducing energy cost for large scale applications.

Batch	A	B	C	D	E	F	G	H	I
pH	6.8	6.8	6.8	7.4	7.4	7.4	8.0	8.0	8.0
Mixing ratio (%V)	75	50	25	75	50	25	75	50	25

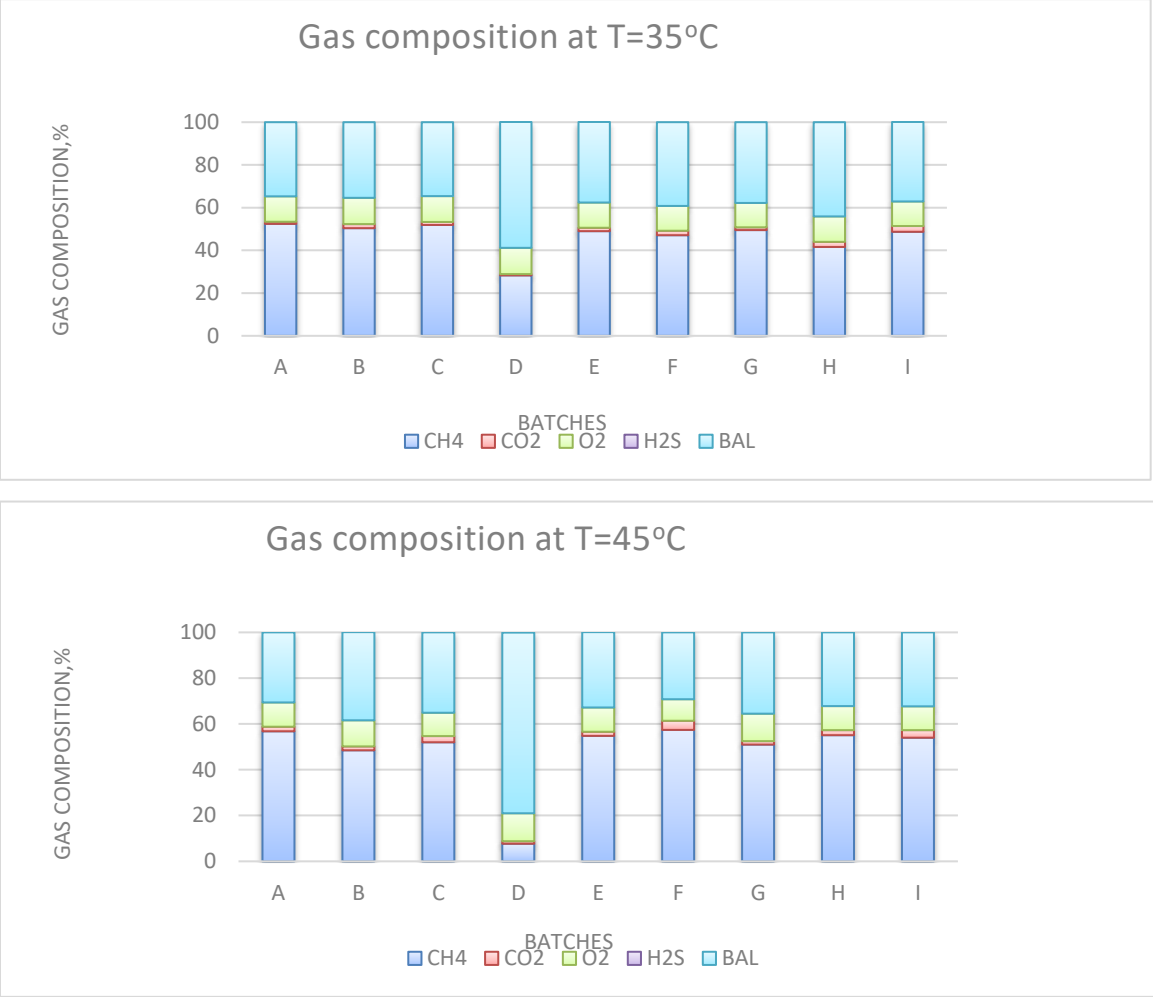

Figure 4.6 The effect of temperature on gas composition.

The findings align with existing literature, emphasizing the importance of maintaining optimal temperatures for maximizing methane production in anaerobic digestion processes. The observed variations in methane yield at different temperatures highlight the need for tailored approaches based on specific conditions and substrates.

Batch	A	B	C	D	E	F	G	H	I
pH	6.8	6.8	6.8	7.4	7.4	7.4	8.0	8.0	8.0
Mixing ratio (%V)	75	50	25	75	50	25	75	50	25

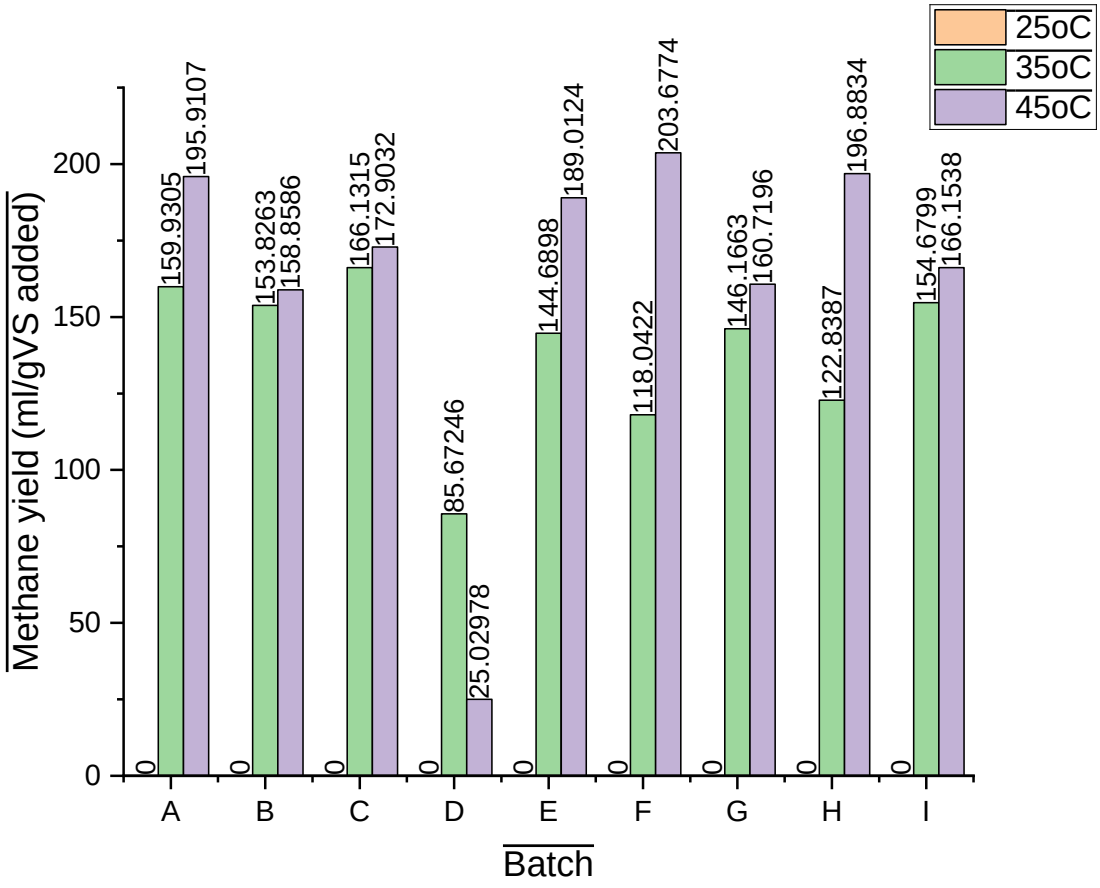

Figure 4.7 The effect of temperature on methane yield

4.5.2. Effect of Substrate Mixing Ratio on Methane Yield

The effect of substrate mixing ratio on methane production was evaluated by comparing the percentage of methane produced from digestors under identical temperature and pH conditions, as illustrated in Figure 4.8. Notably, there was no gas production at 25℃ across all nine batch digestors, thus the comparison focuses on the two remaining temperature levels and three pH levels.

Batch	A	B	C	D	E	F
Temperature	35	45	35	45	35	45
Ph	6.8	6.8	7.4	7.4	8.0	8.0

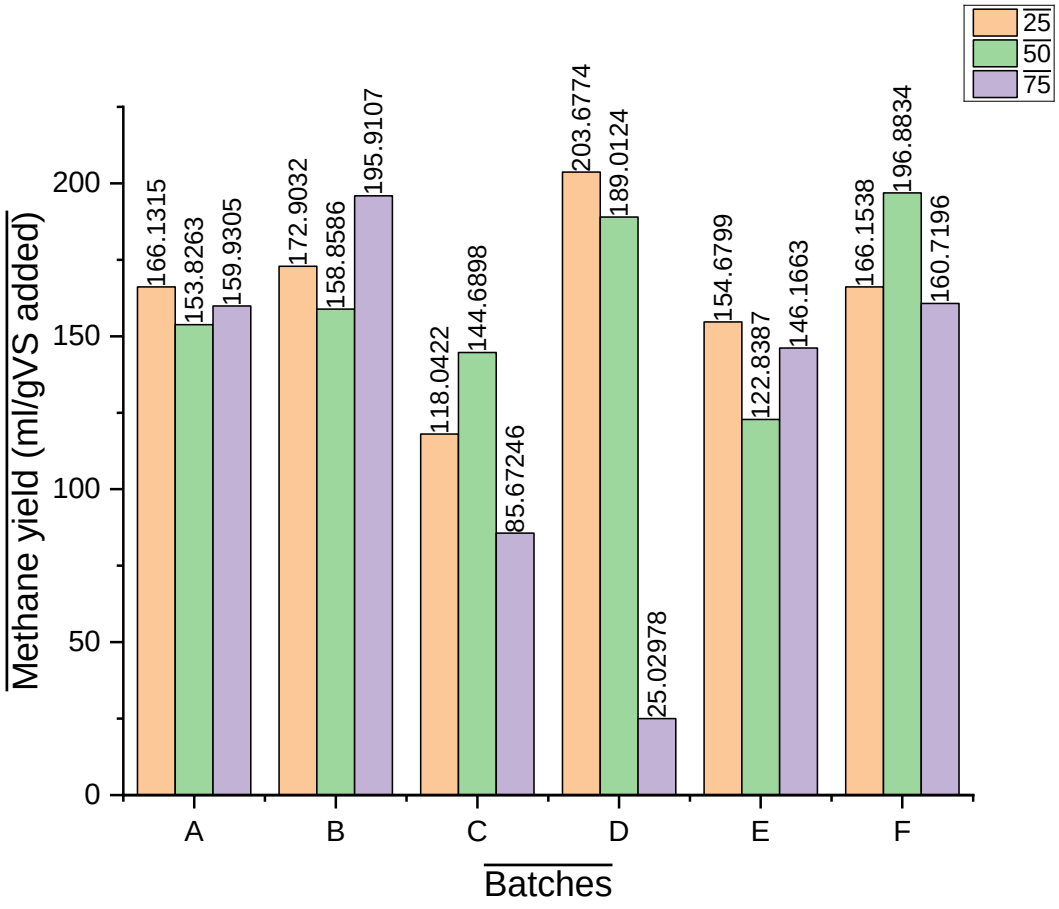

Figure 4.8 The effect of substrate mixing ratio on methane yield

From the graph chart it can be seen that there is no obvious trend of methane yield with the change in the mixing ratio looking at individual values which is difficult to conclude mainly because the effect is not only caused by the mixing ratio but also the interactive effect of temperature and pH. However, the data reveals that the highest methane yield of 203.6774 $mlCH_4/gVS_{added}$ was achieved at a mixing ratio of 25% Vinasse, with an average yield of 163.598 $mlCH_4/gVS_{added}$. This is followed by a 50% Vinasse mixing ratio, which had an average yield of 161.018 $mlCH_4/gVS_{added}$, and the lowest average yield of 128.905 $mlCH_4/gVS_{added}$ was observed at a mixing ratio of 75% Vinasse.

Despite the absence of a clear trend in individual results, the 25% Vinasse mixing ratio produced the highest yield and the maximum average yield. This could be as a result of reducing the vinasse concentration that resulted to a more balanced C/N ratio, less accumulation of inhibitory byproducts like volatile fatty acids (VFAs) which would be resulted if excessive vinasse was available that can be easily fermented than bagasse, lower salt concentrations as vinasse has high salt contents and better synergy between the two substrates. By optimizing the proportion of easily biodegradable organic material (vinasse) and the more complex lignocellulosic material (bagasse), microbial activity becomes more efficient, leading to increased biogas production and more effective methane generation. Therefore, while the interactive effects of temperature and pH play a significant role, the substrate composition appears to be a crucial factor in optimizing methane production.

In conclusion, while individual results may not show a clear trend, the 25% vinasse mixing ratio stands out for its superior methane yield, likely due to the less accumulation of inhibitory byproducts such as VFAs and salts contained in vinasse. This observation aligns with findings from other studies, underscoring the complex interplay between substrate composition, temperature, and pH in anaerobic digestion processes.

4.5.3. Effect of pH on Methane Yield

The effect of the change in pH is observed by comparing the methane yield for batches with the same temperature and substrate mixing ratio at different pH levels as shown in the bar graph below which shows that for batches A-D methane yield is higher for pH value of 6.8 (average yield of 167.9268 ml) while it is lower than the other two pH levels for batches E&F. The highest methane yield (203.6774 $mlCH_4/gVS_{added}$) is obtained for batch F at pH of 7.4 (average yield of 127.68734 $mlCH_4/gVS_{added}$) however the two lowest methane yields are also at pH of 7.4 for Batch A & D. The average methane yield at pH of 8.0 is 157.90695 $mlCH_4/gVS_{added}$.

As it can be seen in figure 4.9 there is no obvious trend of methane yield as we increase the pH rather there is a fluctuation from one batch to another. The reason for this could be due to the interactive effect of the change in other parameters which suggests the use of optimization techniques. It is also noted that the range of pH from 6.8-8.0 is well aligned with previous studies as good results are found at all the three levels. Therefore, the highest value is obtained at pH 7.4

even though the highest average yield is at pH 6.8 as the interactive effect with other parameters matters.

Batch	A	B	C	D	E	F
Mixing ratio, %V	75	50	25	75	50	25
Temperature	35	35	35	45	45	45

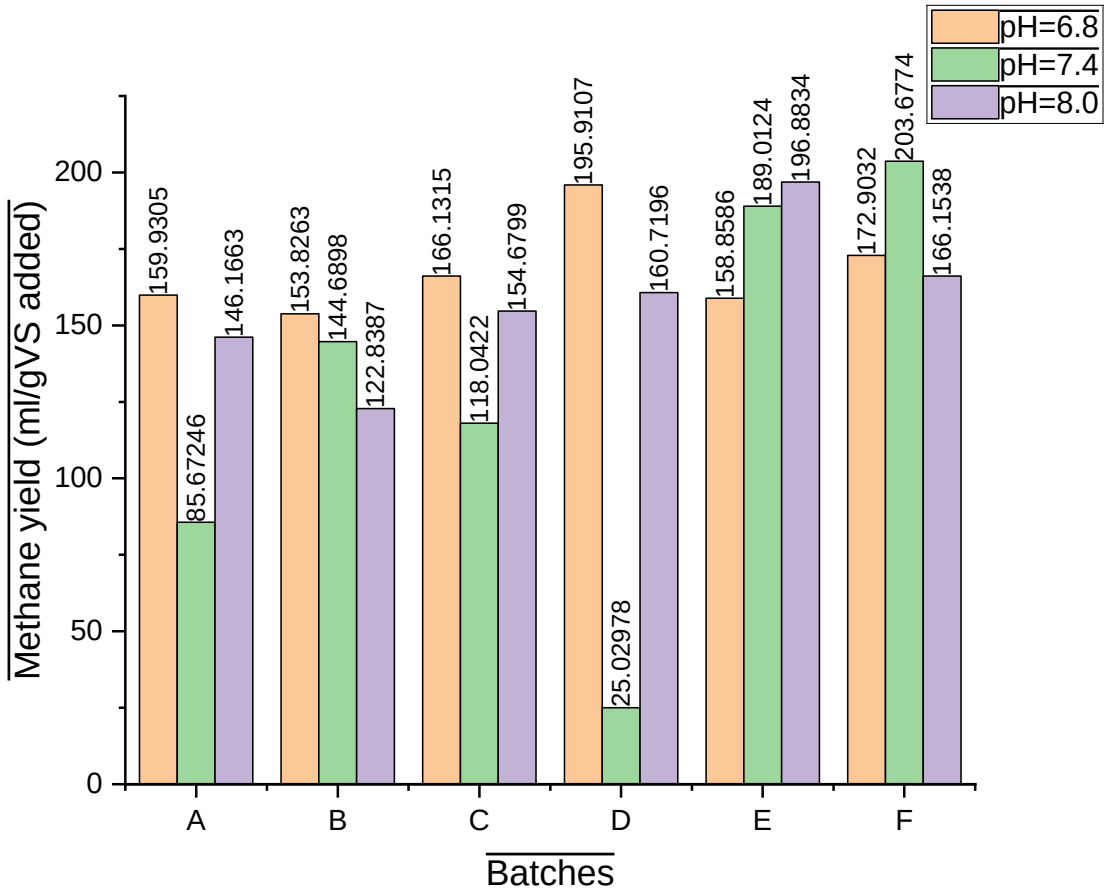

Figure 4.9 The effect of pH on methane yield

4.6. Physico-Chemical Characteristics of Sludge

Chemical analysis was performed on the sludge resulting from the anaerobic digestors with the highest percentage of methane production at two temperature levels (35°C and 45°C). The results are presented in Table 4.3 and compared with the recommendations by FAO for the application of organic manures as organic fertilizers which is shown in Table 4.4. The sludges were characterized to analyze their potential use as organic fertilizers in terms of their Total Organic Carbon, TOC,

Total phosphorus, TP, Total Nitrogen, TN, Carbon to Nitrogen ratio, C: N, Potassium, K, Total Organic matter, and OM to get the values 28.6045%, 0.551 mg/l, 4.151mg/l, 9.01, 46 mg/l, and 49.3145% respectively for the sludge taken with the highest methane yield for the temperature level 35^{o}C and 34.865%, 0.538 mg/l, 4.191 mg/l, 7.105, 40.2 mg/l and 60.1073% respectively for the sludge at 45^{o}C.

Table 4.4 Chemical analysis results of anaerobic digestion sludges

No.	Parameter	Unit	Sludge @ 35^{o}C	Sludge @ 45^{o}C
1	Total Organic matter, OM	%	49.3145	60.1073
2	Total Organic Carbon, TOC	%	28.6045	34.865
3	Total Nitrogen, TN	mg/l, %	4.151, $3.95*10^{-4}$	4.191, $3.99*10^{-4}$
4	Total phosphorus, TP	mg/l, %	0.551, $5.25* 10^{-5}$	0.538, $5.12* 10^{-5}$
5	Potassium, K	mg/l, %	46, $4.38* 10^{-3}$	40.2, $3.82* 10^{-3}$
	N+P+K	%	$4.83* 10^{-3}$	$4.27* 10^{-3}$

According to the FAO, the total nutrient content (sum of nitrogen, phosphorus, and potassium) should be at least 5% by weight. However, the results show that the nutrient content is significantly lower than the minimum value. Therefore, fortifying the sludge with nutrient sources, either inorganic (commercial) or organic wastes with high nutrient value, will help meet the requirements. On the other hand, the organic carbon and organic matter contents are well above the minimum requirement. This suggests that while the organic carbon and organic matter contents are adequate, the nutrient content needs enhancement to be viable as an organic fertilizer. Regarding heavy metals, the sludges were not tested for heavy metal content. It is recommended that future studies include this analysis to facilitate the use of sludges in agriculture as a replacement for inorganic fertilizers, which have their own adverse effects on the soil.

Table 4.5 Quality of organic manures (basic criteria to consider suitability of the material as a nutrient source)

Adapted from www.fao.org

No.	Parameter		Value
1	Organic matter content by weight		minimum 30%
2	Organic carbon content by weight		minimum 12%
3	Total nutrients (N+ P_2O_5+ K_2O) content by weight		minimum 5%
4	Heavy metals content: maximum (mg/kg)	Arsenic (As)	10
		Chromium (Cr)	50
		Lead (Pb)	100
		Copper (Cu)	300
		Cadmium (Cd)	5

Chapter Five

5. Conclusions and Recommendations

5.1. Conclusions

To conclude, in the current work the anaerobic co-digestion of sugarcane vinasse with bagasse was investigated under varying conditions of substrate mixing ratio, pH, and temperature to evaluate the production of biogas by measuring the volume of the gases produced as well as methane yield as percentage using gas analyzer to measure the gases' compositions. The key findings developed from the experimental data are presented as follows.

Generally increasing the temperature from the lower value to the higher in the mesophilic range ($25^{O}C$-$45^{O}C$) resulted in increased biogas production and methane yield though better volume and methane yield are also observed at $35^{O}C$. Maximum average gas volume and methane yield was observed at $45^{O}C$ followed by $35^{O}C$ with no production at $25^{O}C$, indicating the increase in temperature is favorable for the bacterial consortium both for volume and methane yield of the biogas.

Although the substrate mixing ratio impacted both biogas volume and methane yield, the effect was not solely attributable to this variation alone; it was also influenced by interactions with the other two variables. This underscores the importance of optimization techniques to explore these interactive effects. While individual results lacked a clear trend, the 25% vinasse mixing ratio emerged as particularly effective, likely due to reduced accumulation of salts and inhibitory byproducts like VFAs from vinasse, the more easily degradable substrate.

The effect of pH changes on one batch varies from its impact on another batch with a different substrate mixing ratio and temperature. The results did not display a consistent trend across all batches, making it difficult to conclude that changes in biogas volume are solely linked to pH variations. This inconsistency may be attributed to the interactive effects of other parameters, highlighting the need for optimization techniques. Additionally, the pH range of 6.8 to 8.0 aligns well with findings from previous studies, as favorable results were observed at all three levels.

In general, the results emphasize that anaerobic co-digestion of vinasse with bagasse holds significant promise for biogas production as well as waste management for sugar and alcohol producing factories. Important insights have been provided by this study in the experimentation of biogas production from vinasse and bagasse through varied operational parametric conditions to obtain the maximum methane yield at pH of 7.4 by mixing 25% of vinasse with 75% of bagasse at 45^{o}C.

5.2. Recommendations

Based on the findings, several recommendations can be made for future research and industrial applications:

- Optimization of parametric conditions through interactive effect analysis as well as measuring the quality & quantity of the gases in a defined interval of days to avoid the dilution of methane by the production of other gases is highly recommended.
- Substrate Mixing Strategies should be investigated at additional substrate combinations and ratios beyond those explored in this study to identify synergistic effects that further enhance biogas production efficiency.
- A comprehensive economic feasibility study shall be performed to assess the cost-effectiveness of scaling up the anaerobic co-digestion process based on the optimized parameters. Consideration should be given to capital investment, operational costs, and potential revenue from biogas sales as well as environmental benefits gained by utilizing alcohol factory wastes that could otherwise be environmental pollutants.
- The sludge from the digestion shall be studied for use as organic fertilizer to increase its fertilizer values and conform the heavy metal contents with regulations.

Implementing these recommendations will not only advance scientific understanding but also facilitate the practical application of anaerobic co-digestion technology in sustainable waste management and renewable energy production.

By addressing these aspects, future research can contribute to the development of more efficient and economically viable anaerobic digestion processes for biogas production from vinasse and bagasse, thereby promoting sustainable development in the energy sector.

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Appendices

Appendix A

Table A1: Data for COD and BOD result of sugarcane vinasse

No.	Dilution	BOD (mg/l)	COD (mg/l)
1	5ml/50ml	1551	13,890
		1553	13,890
		1548	13,782
2	10ml/50ml	1056	14,843
		1065	14,829.5
		1053	14,981.5
3	2ml/50ml	2100	36,305
		2250	36,300
		2080	36,220

Table A2: Data from the quantitative and qualitative gas analysis

No.	pH	Mixing Ratio/ % Vinasse	Temperature	Gas volume, ml	Composition, %(v/v)					Methane yield (ml/gVS_{added})
					CH_4	CO_2	O_2	H_2S	BAL	
1	6.8	75	25	0	0	0	0	0	0	0
2	6.8	50	25	0	0	0	0	0	0	0
3	6.8	25	25	0	0	0	0	0	0	0
4	7.4	75	25	0	0	0	0	0	0	0
5	7.4	50	25	0	0	0	0	0	0	0
6	7.4	25	25	0	0	0	0	0	0	0
7	8.0	75	25	0	0	0	0	0	0	0
8	8.0	50	25	0	0	0	0	0	0	0
9	8.0	25	25	0	0	0	0	0	0	0
10	6.8	75	35	1230	52.4	1.0	11.9	0	34.7	159.9305

11	6.8	50	35	1230	50.4	1.9	12.3	0	35.4	153.8263
12	6.8	25	35	1290	51.9	1.4	12.1	0	34.6	166.1315
13	7.4	75	35	1220	*28.3*	0.6	12.3	0	58.9	85.67246
14	7.4	50	35	1190	49.0	1.6	11.8	0	37.7	144.6898
15	7.4	25	35	1010	47.1	2.1	11.6	0	39.2	118.0422
16	8.0	75	35	1190	49.5	1.3	11.4	0	37.8	146.1663
17	8.0	50	35	1190	41.6	2.4	11.9	0	44.1	122.8387
18	8.0	25	35	1280	48.7	2.7	11.5	0	37.2	154.6799
			Average	1203.33	46.45					
19	6.8	75	45	1390	**56.8**	2.0	10.6	0	30.6	195.9107
20	6.8	50	45	1320	48.5	1.7	11.4	0	38.5	158.8586
21	6.8	25	45	1340	52.0	2.7	10.2	0	35.1	172.9032
22	7.4	75	45	1310	*7.7*	1.1	12.2	0	78.9	25.02978
23	7.4	50	45	1390	54.8	1.8	10.6	0	32.9	189.0124
24	7.4	25	45	1430	**57.4**	4.0	9.4	0	29.2	**203.6774**
25	8.0	75	45	1270	51.0	1.5	12	0	35.5	160.7196
26	8.0	50	45	1440	**55.1**	2.2	10.5	0	32.2	196.8834
27	8.0	25	45	1240	54.0	3.3	10.4	0	32.3	166.1538
			Average	1347.78	48.59					

Appendix B

Supplementary Pictures

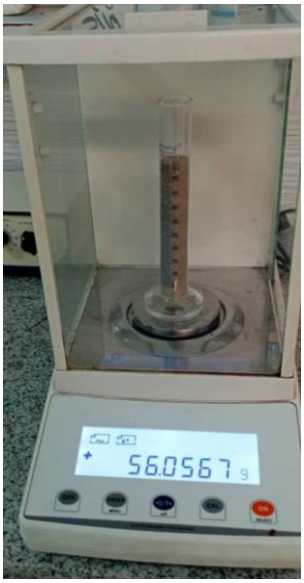
(a) Ethanol/alcohol production plant

(b) Vinasse sample



(c) Vinasse characterization





(d) Bagasse characterization

(e) Sustrate preparation

(f) Anaerobic digestor setup and temperature regulation