A Lab Scale Optimization of Brewers Surplus Yeast Co-Digested with Brewers Wastewater for the Production of Biogas at Heineken Breweries, Addis Ababa, Ethiopia.

FILAGOT REGASSA FEYE

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Specialization in Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

February, 2024

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled **"A Lab Scale Optimization of Brewers Surplus Yeast Co-Digested with Brewers Wastewater for the Production of Biogas at Heineken Breweries, Addis Ababa, Ethiopia."** is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Recommendation

I, the advisor of this thesis, hereby certify that we have read the revised version of the thesis entitled "**A Lab Scale Optimization of Brewers Surplus Yeast Co-Digested with Brewers Wastewater for the Production of Biogas at Heineken Breweries, Addis Ababa, Ethiopia.**" prepared under my guidance by Filagot Regassa submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Biotechnology. Therefore, I recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	**Signature**	**Date**
_____	_____	_____
Co-advisor	**Signature**	**Date**

APPROVAL SHEET

Thesis I/we, the advisors of the thesis entitled "A Lab Scale Optimization of Brewers Surplus Yeast Co-Digested with Brewers Wastewater for the Production of Biogas at Heineken Breweries, Addis Ababa, Ethiopia." and developed by Filagot Regassa, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor Signature Date

Co-advisor Signature Date

We, the undersigned, members of the Board of Examiners of the thesis by Filagot Regassa have read and evaluated the thesis entitled "A Lab Scale Optimization of Brewers Surplus Yeast Co-Digested with Brewers Wastewater for the Production of Biogas at Heineken Breweries, Addis Ababa, Ethiopia." and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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MAY GOD BLESS YOU ALL!

Table of contents

ABBREVIATIONS AND ACRONYMS

AcoD	Anaerobic co digestion
AD	Anaerobic digestion
ASBR	Anaerobic Sequencing Batch Reactor
BOD	Biological Oxygen Demand
BSY	Brewers Spent Yeast
BWW	Brewery Waste Water
CDM	Clean Development Mechanism
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DO	Dissolved Oxygen
GHG	Green House Gas
HRT	Hydraulic Retention Time
OLR	Organic Loading Rate
TDS	Total Dissolved Solid
TS	Total Solid
TSS	Total Suspended Solid
VFA	Volatile Fatty Acid
VS	Volatile Solid
UASB	Upflow Anaerobic Sludge Bioreactor
WWTP	Waste Water Treatment Plant

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Anaerobic digestion is a promising technology for recovering energy from brewery waste, sewages, and agricultural wastes. The brewing industry typically utilizes batch-type operations to process raw materials into the final beer product, with surplus yeast being recovered through natural sedimentation. The study aimed to investigate the optimum conditions for producing biogas through the anaerobic co-digestion of brewery wastewater and brewers surplus yeast in a lab-scale anaerobic batch reactors. A study was conducted to optimize the mixing ratio and hydraulic retention time for biogas production from brewers' surplus yeast and brewers' wastewater under mesophilic conditions (35℃). The physico-chemical characteristics of raw materials before and after digestion were analyzed using a laboratory scale anaerobic digester system. The study found that a mixing ratio of 75%:25% brewer's wastewater to brewer's surplus yeast resulted in the highest biogas production (2438 ml/800 ml sample) with a 20 days' retention time. In contrast, the lowest production was observed with 100% brewer's surplus yeast (938 ml/800 ml sample) within the same retention time. The highest methane and chemical oxygen demand removal efficiency was achieved at a 3:1 mixing ratio of brewer's wastewater to brewer's surplus yeast (71.6% and 85.08%, respectively) with a 20 days' retention time. The results indicate that co-digestion of brewer's wastewater with brewer's surplus yeast can significantly improve biogas production, particularly at a 3:1 mixing ratio. This approach not only enhances energy recovery from brewery wastes but also helps mitigate disposal difficulties associated with surplus yeast.

Keywords: Anaerobic digestion, biogas, co-digestion, fermentation, sedimentation, surplus yeast, wastewater

1. INTRODUCTION

1.1 Background

In response to increasing environmental concerns, there is a strong push from both political and social as well as economic spheres to minimize pollution caused by industrial activities. This trend is observed across developed and developing countries, as they strive to adjust their operations to enable the recycling of their waste products. Consequently, many major industries now view their waste as valuable resources that can be utilized in other production processes, rather than simply discarding them as unusable materials (Zupančič et al., 2017).

The brewing industry in Ethiopia is currently one of the largest beverage sectors, producing both alcoholic and non-alcoholic drinks. The brewing process utilizes various raw materials such as malted barley/cereals, un malted grains, adjuncts, hops, sugar/corn syrups, water, and yeast to create beer. Malted barley is commonly used as the primary raw material in most breweries Rachwał et al. (2020). This process involves steps like malting, mashing, fermentation, and packaging. Throughout beer production, three main byproducts are generated: brewers spent grain (BSG), spent hops/hot trub, and residual brewer's yeast. Yeast is added during fermentation to convert sugars into alcohol and carbon dioxide, resulting in surplus yeast as a byproduct (Hejna, 2021).

Brewer's spent yeast (BSY) is a common byproduct of the brewing industry. Despite releasing large amounts of polluting effluents, the brewing industry remains an economically significant sector in any country (Hejna, 2021). The beer brewing process consists of two main stages: brewing and packaging (Jacob et al., 2019). Byproducts like spent grains from mashing and surplus yeast from fermentation and maturation contribute to pollution when mixed with effluents. Additionally, cleaning processes in breweries produce high volumes of polluted water (Olajire, 2020).

Many countries are now prioritizing the adoption of environmental policies that promote the production and utilization of alternative, sustainable, and renewable energy sources from locally available resources (Kaifa and Parawira, 2019). Agricultural feedstock's such as manure and food industry waste products are being recognized as valuable sources for green energy production through anaerobic digestion, aimed at producing biogas. Ethiopia is grappling with environmental challenges stemming from pollution caused by organic waste generated by agriculture, households,

and industries, particularly due to the absence of waste treatment plants in most operating industries. Consequently, these industries discharge untreated effluents into nearby rivers or drainage facilities, contravening established standards (Ghebretekle, 2015).

Biogas technology presents a solution for utilizing organic waste for energy generation, with the digested substrate (digestate) serving as a valuable fertilizer for managing organic waste. The emerging technology of anaerobic co-digestion enables enhanced biogas production in digesters through the simultaneous use of two or more substrates, thereby facilitating the management of by-products (Solé-Bundó et al., 2019).

Co-digestion has the potential to significantly increase biogas production compared to mono-digestion, with improvements ranging from 25% to 400% (Hagos et al., 2017). This process not only boosts the load of biodegradable organic matter but also enhances nutrient balance and process stabilization. Moreover, biogas production systems are adaptable to various scales, making them suitable for both urban and rural settings (Kaifa and Parawira, 2019). This research focusing on the Anaerobic Co-Digestion of residual flows, specifically Brewers surplus yeast and brewer's wastewater, the primary objective was to optimize the best feeding ratio of brewer's surplus yeast to brewer's wastewater.

1.2 Statement of the Problem

The brewing industry in Ethiopia, particularly at Heineken Breweries, generates significant quantities of surplus yeast and wastewater as by-products of the beer production process. The disposal of these wastes poses environmental challenges, including potential pollution of water bodies and landfills (Olajire, 2020). Despite the potential for energy recovery through anaerobic digestion, there is a lack of optimized strategies for co-digesting brewers' surplus yeast with brewers' wastewater at a lab scale to produce biogas efficiently.

Co-digestion of brewers' surplus yeast with brewers' wastewater can enhance the overall biogas production efficiency by providing a diverse range of organic substrates for microbial activity. Optimizing the co-digestion process at a lab scale will allow for the identification of key parameters such as feedstock ratios, retention times, and operating conditions that can maximize biogas yield. Implementing an efficient co-digestion strategy not only reduces waste disposal costs

for the brewery but also contributes to sustainable energy generation and environmental protection (Rachwał et al., 2020).

The problem addressed in this study is the need to optimize the co-digestion process of brewers' surplus yeast with brewers' wastewater to maximize biogas production at Heineken Breweries in Addis Ababa, Ethiopia. The surplus yeast generated during the brewing process is currently disposed of as waste, leading to potential environmental issues and missed opportunities for resource recovery (Aranguiz et al., 2019). The wastewater generated from the brewing process contains organic compounds that can be utilized for biogas production through anaerobic digestion.

Addressing the challenge of surplus yeast and wastewater management through biogas production aligns with the brewery's commitment to sustainability and resource efficiency. Developing a tailored co-digestion approach specific to Heineken Breweries will serve as a model for other breweries facing similar waste management issues in the industries.

1.3 Objectives

1.3.1 General Objective

➢ To optimize the biogas production from co-digestion of brewers' surplus yeast and brewers' wastewater on a lab scale anaerobic digester at Heineken breweries, Kilinto Addis Ababa Ethiopia.

1.3.2 Specific Objectives

- To characterize the physicochemical characteristics of the substrates brewer's wastewater and brewer's surplus yeast.
- To characterize the effect of Hydraulic Retention Time and substrate mix ratio on biogas production.
- To analyze the treatment process parameters like BOD, Total Solids, Volatile solids, Total Phosphorous, Total nitrogen and the Chemical Oxygen Demand removal efficiency of the substrates.

1.4 Significance of the Study

The importance of this study lies in its ability to aid the brewery industry in protecting water resources from pollution and improving the effectiveness of wastewater treatment plants. The

findings can be applied by breweries across the country and can be utilized by environmental scientists, the Ethiopian Environmental Protection Authority (EEPA), and other stakeholders working in environmental pollution control. The anaerobic co-digestion treatment (ASBR) of surplus yeast has emerged as a crucial option for producing biogas in brewing industries, while also addressing environmental pollution. Proper treatment of surplus yeast not only facilitates energy recovery but also serves as an alternative resource for agricultural purposes such as fertilizer and offer an alternative energy source that reduces energy consumption and helps mitigate climate change by decreasing greenhouse gas emissions.

1.5 Scope of the Study

This study was conducted from June 1 - August 30, 2023 by focusing on the production of biogas from the Heineken beer share company. The study was a laboratory-scale experiment with batch scale of anaerobic digesters (Erlenmeyer flask) in which characterization of the two substrates and the produced biogas will be addressed. This study is limited to optimization and enhance biogas production from brewery waste and surplus yeast aiming to produce methane for energy recovery in brewing industry. Additionally, all experiments are done on a laboratory scale. This research focused on the potential of brewer's surplus yeast for the production of biogas and characterizes the brewer's yeast and brewers waste water and sludge. Moreover, the study investigated the major factors affecting biogas production, such as hydraulic retention time (HRT) and substrate mixing volume ratio.

2. LITERATURE REVIEW

2.1 Industrial Wastes

In recent decades, the world has experienced a significant rise in energy consumption as a result of population growth and industrial progress, despite its rising, the primary source of energy continues to be non-renewable fossil fuels. Nevertheless, the utilization of fossil fuels poses several challenges as they are not sustainable, heavily contribute to pollution, and are expected to decline in production over the coming decades (Achinas et al., 2017).

The brewing process uses standard basic processes and raw materials such as, malted barley/cereals, un malted grains, adjuncts, hops, sugar/corn syrups, water and yeast to produce beer. Most breweries use malted barley as their principal raw material. The brewing industry produces a wide range of by-products and wastes (Sosa et al., 2016). Main byproducts of the brewing industry are spent grains, spent yeast, spent hops and insoluble proteins (trub) resulting from the brewing process (Jatunarachchi et al., 2006).

Due to the rising cost of energy, the brewing industry, which consumes a lot of energy for beer production from raw materials to finishes, has to convert most of its waste into alternative energy sources. There are various ways of recycling, but anaerobic digestion has proven to be effective and efficient. This is because of its ability to generate energy by converting waste into a valuable resource such as biogas (Rasmeni et al., 2022).

2.2 The Brewing Industry

As a process aimed at beer production, brewing is known for thousands of years (Behre, 1999). Depending on the times and mainly the development of humanity and science, the specifics of this process were changing (Unger, 2001). Today, Ethiopia is home to several large breweries, as well as a thriving craft beer scene (Shahidur et al., 2015). One of the largest breweries in Ethiopia is the Ethiopian brewery share company (EBSC), which is a subsidiary of the global brewing giant, Heineken. Heineken produces popular brands such as Bedele, Harar, and Walia. There is also an increasing demand for craft beer among Ethiopian consumers. Craft breweries such as Habesha and Dashen brewery have gained popularity for their unique and flavorful beers.

Therefore, the increased energy consumption based on fossil fuels affects our environment through the greater amounts of greenhouse gas emissions, the environmental pollution of water, air and

soil, and the climate changes, which dramatically influence the quality of life and the health of people (Ghebretekle, 2015). The brewing industry in Ethiopia has been growing steadily in recent years. The country has a rich history of beer production, with traditional brewing methods dating back centuries.

Overall, the brewing industry in Ethiopia is thriving and has a promising future. With the governments support and the increasing demand for beer among Ethiopian consumers, the industry is expected to continue growing in the coming years (Shahidur et al., 2015).Generally, the brewing process consists of the following stages, starting from the malt: crushing of malt, mashing, lautering, filtration, boiling, and fermentation (Fig.1).

The first step of brewing is milling which takes place when the malt grains are transported from the storage (silo) and milled in a wet dry process to ensure that one can obtain a high yield of extracted substances by naturally occurring enzymes in the malt (UNEP, 1996). Malt is crushed to break apart the kernel and facilitate the extraction of sugars during mashing. The mixture of milled malt, gelatinized adjuncts, and hot water (76°C) is called mash. The purpose of mashing is to obtain high yield of extract (sweet wort) from the malt grist and to ensure product uniformity. Following the completion of mash conversion (saccharification) the wort is separated from the mash by a lautertun or mash filter.

The grains are also sparged (sprayed and mixed) with hot water to recover and residual extract adhering to the grain bed. Such processes are implemented to reduce mash viscosity, free up more starch and extract additional sugar. The extracted grain is termed as spent grain; most often used as animal feed. In mash filter, the mash is charged from the mash mixer. The filter is fitted with fine pore polypropylene sheets that forms a tight filter bed and allows for very high extraction efficiency which is up to 100% laboratory extract (Galitsky and Martin, 2003).

The next step is boiling of wort which involves the boiling and evaporation of the wort (about 412 % evaporation rate) over a 1 to 1.5 hr. period. The wort is moved to the kettle, where it is boiled with hops (and sometimes other ingredients). This step is the most fuel intensive step of the beer production process. The boiling process aims to terminate the enzymatic processes, precipitate proteins, concentrate and sterilize the wort, as well as extract compounds from hops to beer and isomerize hop resins to add bitterness to beer (Keukeleire, 2000). Moreover, boiling may induce caramelization and Maillard reactions in wort (Hellwig et al., 2016).

After boiling in order to remove the hot break the boiled wort is transferred to whirlpool to clarify the wort through sedimentation, filtration and centrifugation. The clarified and cleared hopped wort is cooled and separated from the trub, which includes the hop residues and colloidal proteins coagulated during boiling (Keukeleire, 2000). The cooling system may use air or liquids as a cooling medium. Atmospheric cooling uses stripping columns while liquid cooling uses plate heat exchangers. A secondary cold clarification step is used in some brewers to settle out trub, an insoluble protein precipitate present in the wort obtained during cooling (Hellwig et al., 2016).

Once the wort is cooled, it is oxygenated and pitched with yeast on its way to the fermenter. The wort is then put in a fermentation vessel. During fermentation, the yeast metabolizes the fermentable sugars in the wort to produce alcohol and carbon dioxide (CO_2) as shown in the equation below (Pasqualino et al., 2011) :

$$C_6H_{12}O_6 + 2PO_4^{3-} + 2ADP \longrightarrow 2C_2H_5OH + 2CO_2 + 2ATP \quad \text{..... (eq. 2.1)}$$

where ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Behind this simplified chemical reaction is a series of complex biochemical reactions. These reactions, known as the “Glycolytic pathway” or “Embden-Myerhof-Parnas pathway”, involve a number of enzymes and the reactions take place anaerobically inside the cells of brewing yeast. There are five sugars which may be present in wort which are readily utilized by standard brewer’s yeast in fermentation, and these include glucose, fructose, sucrose, maltose and malttriose (Olajire, 2020).

The fermentation process also generates significant heat that must be dissipated in order to avoid damaging the yeast. Fermenters are cooled by coils or cooling jackets in a closed fermenter, CO_2 can be recovered and later reused. Fermentation time will vary from a few days for ales to closer to 10 days for lagers (Goldammer, 2008). The rate is dependent on the yeast strain, fermentation parameters (like the reduction of unwanted di acetyl levels) and taste profile that the brewer is targeting (Podpora et al., 2016). At the conclusion of the first fermentation process, yeast is removed by means of a suction, a conical collector, settling or centrifugation. Some of the yeast is reused while other yeast is discarded.

Beer aging is the final step in beer production. The beer is cooled and stored in order to settle yeast and other precipitates and to allow the beer to mature and stabilize. For beers with a high yeast cell

count, a centrifuge may be necessary for pre clarification and removal of protein and tannin material (UNEP, 1996). Different brewers age their beer at different temperatures, partially dependent on the desired taste profile. According to Olajire, (2020), ideally, the beer at this stage is cooled to approximately -1℃. Beer is held at conditioning temperature for several days to over a month and then chill proofed and filtered. The beer's CO_2 content can also be trimmed with CO_2 that was collected during fermentation. The beer is then sent to a bright beer tank (BBT) before packaging. Finally, the beer must be cleaned of all remaining harmful bacteria before bottling by pasteurization (heated to 140 °F (60 ℃) to destroy all biological contaminants to have the beer a long shelf life (Goldammer, 2008).

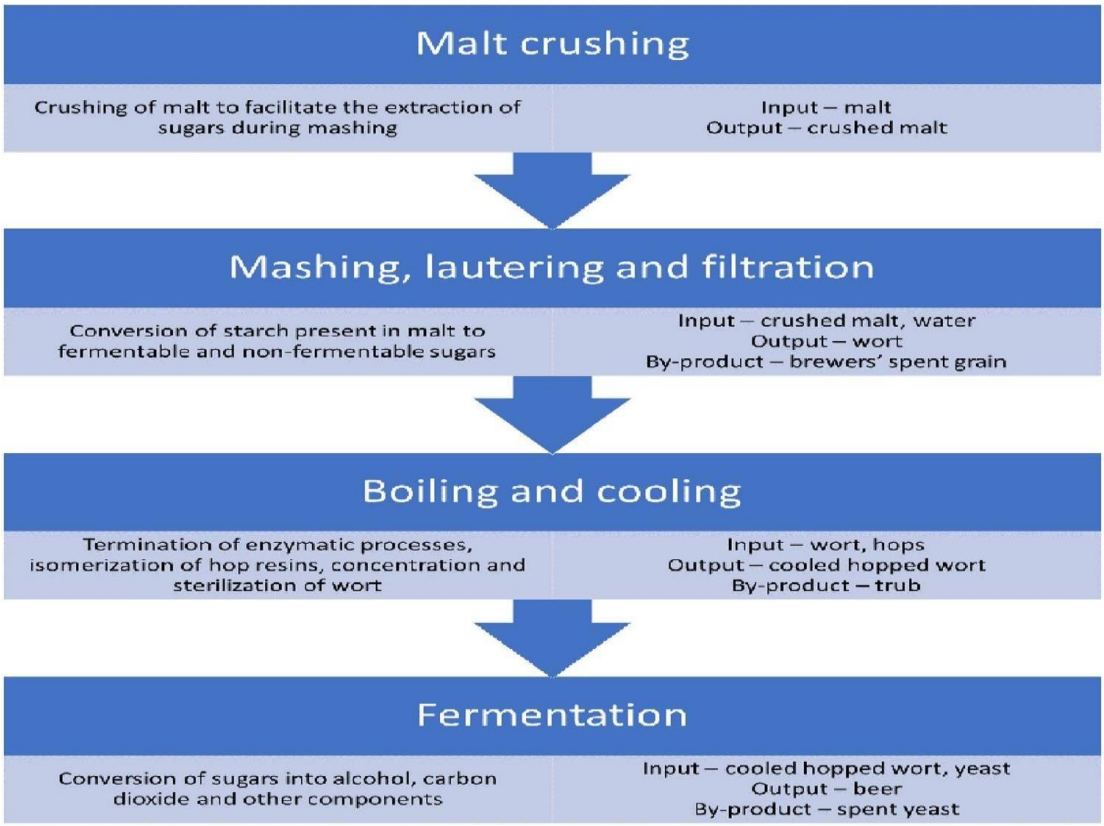

Fig 1:The general scheme of beer production with an indication of generated byproducts (Hejna, 2021).

According to the brewing scheme presented in Fig. 1, the main by-products of beer manufacturing are the brewers' spent grain, trub removed after wort boiling, and spent yeast. The generation of brewing by-products is very similar globally, with possible differences in their composition, depending on the location. These materials are currently often unutilized and present hardly any

market value. Generally, considering the current pro-environmental trends in scientific and industrial activities partially stimulated by the changing law regulations established worldwide, waste and by-products management are essential (Olajire, 2020).

2.3 Yeast

Yeast is a single-celled organism used in beer manufacture for the transformation of sugars from grain to alcohol. Brewer's spent yeast (also known as residual yeast or surplus yeast) is a prevalent by-product of the brewing industry, created when the yeast used in fermentations is no longer useful and must be disposed of. It is estimated that 15 to 18 tons of surplus yeast are produced per 10,000 hl of finished beer (Kunze et al., 2014). Brewers surplus yeast is a rich source of B vitamins, proteins (45–60%) and other compounds that could be applicable to both food and nonfood products (Podpora et al., 2016).

2.3.1 Surplus Yeast

During the initial stage of fermentation, the intensive proliferation of yeast is occurring, so after fermentation and maturation, the surplus of yeast is present and should be removed from beer. Partially, they are recovered by natural sedimentation, but for higher efficiency, additional filtration or centrifugation may be applied (Rachwał et al., 2020). The actual amount of this by-product depends on the type of yeast used for fermentation, but it can account even for 15% of total by-products generated during beer production Clare (Kerby and Vriesekoop, 2017). Depending on the properties of yeast, in particular their flocculation, this by-product is characterized by the high moisture content in the range of 75–90%. Therefore, portion of beer is removed along with yeast, which may generate the 1.5–3.0% loss of beer (Olajire, 2020).

During the production of beer, yeast ferments the simple sugars present in the cooled wort to alcohol. Yeast is separated from the bulk at the end of fermentation by a process known as flocculation which occurs towards the end of the fermentation process. During this process, thousands of yeast cells form 'flocs' which then either rise to the surface (top-fermenting) or sediment on the bottom of the vessel (bottom fermenting) (Verstrepen et al., 2003).

Good flocculation properties are essential in an industrial strain as yeast can be reused multiple times during the brewing process (Ferreira et al., 2010). A small amount of yeast from the previous fermentation is used to start the next fermentation in a process known as re-pitching. Due to only

a small percentage being re-used in the manufacturing process, there remains a large excess of spent yeast. Due to Brewers surplus yeast having a high chemical oxygen demand (COD), it cannot be disposed of into wastewater streams without prior treatment as it would have a severe negative effect on the environment (Kunze et al., 2014).

2.3.2 Composition of Surplus Yeast

Brewer's spent yeast is a high-protein by-product. It contains 45–60% protein and is generally regarded as safe (GRAS). In a study by Vieira et al. (2016), Brewers surplus yeast was found to contain 64.1% protein with a high percentage of essential amino acids. It was determined that 40% of the total amino acid count was comprised of essential amino acids (Rachwał et al., 2020). High level (34%) of flavor-enhancing amino acids was also determined, namely, glutamic acid, aspartic acid, glycine and alanine.

A high level of these particular amino acids presents the potential of Brewers surplus yeast extracts as flavor-enhancing ingredients (Hejna, 2021). Considering the composition, spent yeast contains a noticeable amount of carbohydrates and proteins. Among the first group, the most abundant are non-cellulose compounds (25–35%), mainly β-glucans, mannoproteins, and glycogen, followed by cellulose (17–25%) (Liepins et al., 2015). Proteins may account for more than 50% of the spent yeast dry mass (Jacob et al., 2019).

Brewery spent yeast are also a great source of micro- and macro elements, as well as vitamins. Nevertheless, the mineral composition of yeast can differ depending on the length of fermentation and fermentation cycles (Karlović et al., 2020) . Among the most abundant minerals are sodium, potassium, and magnesium, but noticeable amounts of phosphorous may be present, which could be associated with yeast nutrients in breweries (Mussatto, 2014). Considering the vitamins, the B type is the most popular, especially niacin, thiamin, pantothenate, and riboflavin (Vieira et al., 2016).

However, they show great potential due to the high contents of nucleic acids (6–15%), in particular ribonucleic acid, their use in an unmodified state is somewhat limited (Felix et al., 2018). Other, less popular uses of spent yeast from beer production include methane production or application as bio sorbent. Considering the energy production, spent yeast can be considered an excellent

substrate for co-digestion purposes with other materials, including swine manure Burns et al. (2010), spent grains Costa, (2018), or crude glycerol Gonçalves et al. (2017).

2.4 Brewery Waste Water Treatment Technologies

Wastewater is one of the most significant waste products of brewery operations. Even though substantial technological improvements have been made in the past, it has been estimated that approximately 3-10 L of waste effluent is generated per liter of beer produced in breweries. Solid waste consists of organic material residuals from the process including spent grains and hops, trub, sludge, surplus yeast, diatomaceous earth slurry from filtration (Kieselguhr sludge), and packing materials (Fig. 2).

The principal objective of any wastewater treatment is generally to allow industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment. From environmental, economic, political and social perspectives, wastewater treatment represents one of the most pressing issues facing industries today. Wastewater treatment technologies range from simple clarification in a settling pond to a complex system in advanced treatment requiring ample equipment and highly skilled operators (Simate et al., 2011).

Basically, wastewater treatment methods can be divided into three categories which are physical, chemical and biological wastewater treatment processes. For almost all combinations of requirements in terms of effluent quality, land availability, construction and running costs, mechanization level and operational simplicity there will be one or more suitable treatment processes (Metacalf and Eddy, 2003).

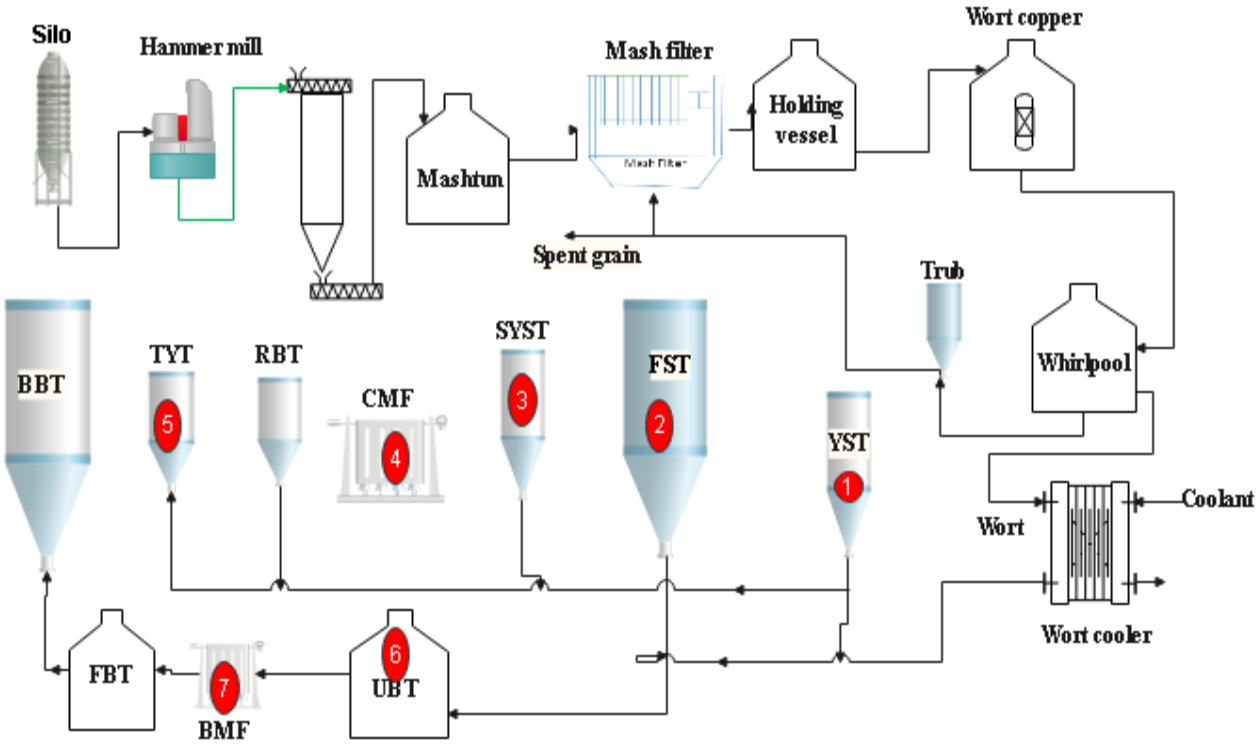

Fig 2: Brewers surplus yeast source of area mapping of Heineken breweries; Kilinto.

2.4.1 Physical Wastewater Treatment Process

Physical treatment is for removing coarse solids and other large materials, rather than dissolved pollutants. It may be a passive process, such as sedimentation to allow suspended pollutants to settle out or float to the top naturally. The physical units most commonly used in wastewater treatment include screening, grit removal, mixing and flocculation, sedimentation, clarification, aeration, and volatilization and stripping of volatile organic compounds. These processes are mostly used at Pre-treatment stage (Arcadio and Gregoria,2002) .

2.4.2. Chemical Wastewater Treatment Process

Chemical treatment processes are the processes used for the treatment of wastewater in which change is brought about by means chemical reactions. The principal chemical processes used for wastewater treatment include chemical coagulation, precipitation, disinfection and oxidation, ion exchange and others. This type of treatment mainly relies on addition of chemicals and is applied when the wastewater cannot be treated biologically (Angassa et al., 2011). A significant disadvantage of this treatment process is its additive. As a result, there is a net increase in the dissolved constituent and secondary pollutants in the wastewater (Omondi, 2017). Besides that,

another disadvantage of chemical treatment process is that the cost of most chemicals is related to the cost of energy (Gupta et al., 2012).

2.4.3. Biological Wastewater Treatment Process

Due to its simplicity and flexibility, biological wastewater treatment is the most utilized and the best economical solution for treatment of biodegradable wastewater. Organic components in brewery effluent are generally easily biodegradable as these mainly consist of sugars, soluble starch, ethanol, volatile fatty acids (Driessen and Vereijken, 2003). The effluent has a low content of non-biodegradable components. Brewery wastewater normally has a COD: BOD ratio of 1.5 to 1.7 indicating that the wastewater is easily degradable Brewers of Europe (2002). The principal process used for the biological treatment of wastewater can be classified with respect to their metabolic function as aerobic and anaerobic (Sperling, 2007).

2.5 Anaerobic Process

The anaerobic digestion consists of complex biological processes that occur mainly in the absence of oxygen, under controlled conditions, and in digesters in which the interactions of various microorganisms allow the degradation of organic matter present in the substrate (Mehariya et al., 2018). Anaerobic digesters are available in various designs and configurations, being classified based on the solids content of the raw material and the feeding mode, the organic load rate, the number of stages/phases and the interaction mode of the biomass with the substrate (Nizami et al., 2013).

2.5.1 Anaerobic Digestion

The biological break down of organic materials can be classified into two major groups: anaerobic (without oxygen) and aerobic (with oxygen). Any process, which comprises the biological conversion of organic waste into another form in the absence of oxygen called Anaerobic digestion. Anaerobic digestion, also known as biomethanation, is a biochemical degradation process that converts complex organic materials into simpler constituents in a series of metabolic interactions that involve a wide range of microorganisms that catalyze the process in the absence of oxygen.

The organic fraction of almost any form of biomass, including sewage, sludge, food wastes, animal wastes and industrial effluents can be broken down through anaerobic digestion (Iacovidou et al., 2012). The organic dry matter can be divided into proteins, fats and carbohydrates all of which have different degradation characteristics. Typically, between 40% and 60% of the organic matter present in the feedstock is converted to biogas and microbial biomass (Kulichkova et al., 2020).

The anaerobic digestion process is carried out by the involvement of different types of microorganisms which possess a very close syntrophic relationship. The production of CH_4 is a slow and sensitive process. Favorable environmental conditions such as temperature and pH are very essential factors for the growth of micro-organisms. The steady conversion and utilization of organic acids are essential as the accumulation of these acids or decrease of pH can lead to the inhibition of the methanogenesis process (Camacho and Ruggeri, 2018).

Anaerobic digestion is currently being one of the most used technologies worldwide in the management of biomass and waste, as in addition to producing biogas, it also results in a nutrient-rich effluent, the digestate (Samun et al., 2017). The digestate is a mixture of undegraded substances with the bacterial community itself. Its direct application in agriculture is dependent on the fulfillment of the established quality requirements. The solid fraction is valued through incorporating into a stabilization process such as composting, giving rise to a compost. This product has enormous agronomic and environmental value and can be used as an organic soil corrector, as it improves its texture and workability, containing nutrients such as N and potassium (K) (Jagadevan et al., 2018).

2.5.2 Biogas Production

Biogas is a colourless combustible gas that is produced by the biological breakdown of organic matter; occurring in the absence of oxygen. The biogas comes from “biogenic materials” and it is generated from AD of biodegradable materials such as biomass, cow dung green waste and agricultural residue such as cassava, sugar cane etc. (Sawyerr et al., 2019a). Biogas comprises a mixture of different gases, Typical biogas contains 40–75% (v/v) of CH_4, 25–55% (v/v) of CO_2 and traces of other gases, such as H_2, oxygen (O_2), nitrogen (N_2), sulfide hydrogen (H_2S), water and ammonia (Praveenkumar et al., 2018).

In addition, it also contains dust particles, siloxanes, aromatic and halogenated compounds, although in very small quantities (Rasi et al., 2020). Biogas is a multilateral renewable energy source that can replace conventional fuels to produce heat and power; it can also be used as gaseous fuel in automotive applications. Bio methane (upgraded biogas) can also substitute for natural gas in chemicals production. Recent findings indicate that biogas produced via anaerobic digestion provides significant advantages over other forms of bioenergy because anaerobic digestion (AD) is an energy-efficient and environmentally friendly technology (Atelge et al., 2020).

Biogas can be used to produce thermal or electrical energy, or subjected to a purification process to obtain bio methane, that can be used as a substitute for natural gas (Kulichkova et al., 2020). Biogas has an elevated methane content, which makes it an attractive source of energy. The energy that is released from biogas makes it a suitable fuel in any country for heating and cooking purpose. Biogas can also be used in an anaerobic digester where the energy in the gas is converted into electricity and heat using gas engine (Sorathia et al., 2012). In as much as the biogas constitutes mainly methane and carbon dioxide, which are greenhouse gases that are harmful to the environment. It is therefore important that it undergoes a burning process before releasing it to the atmosphere.

2.5.3 Biogas Composition

Biogas contains approximately 50–70% methane, 30–40% carbon dioxide, traces of hydrogen sulfide and fractions of water vapour (Table1). Biogas production processes by anaerobic fermentation can be divided into two steps: the first step is an initial hydrolytic step to promote complete degradation of the material; and secondly, a methanogenic step in which acidogenic microorganisms convert the macromolecules released from the previous hydrolytic stage to volatile fatty acids, acetate, butyrate and propionate, and subsequently methanogenic bacteria convert these volatile acids to methane (Hagos et al., 2017).

Table 1: Composition of biogas production (Bhattacharjee et al., 2013).

Gas	Formula	Percentage
Methane	CH_4	50-70
Carbon dioxide	CO_2	30-40
Hydrogen sulfide	H_2S	traces
Hydrogen	H_2	5-10
Water vapour	H_2O	0.3
Nitrogen	N_2	1-2
Oxygen	O_2	0-2

2.6 Stages of Biogas Production Using Anaerobic Digestion

Anaerobic digestion has four different operational aspects include pretreatment, digestion, gas upgrading, and digestive treatment. There are four basic stages involved in anaerobic digestion, each of those are accompanied by different bacteria groups (Fig.3). These four basic stages make up the process of biogas production from various organic materials as it occurs in an anaerobic digester. The anaerobic digestion process is characterized by the decomposition of organic matter into methane, carbon dioxide, inorganic nutrients and compost in an anaerobic environment (Saputra et al., 2019).

The process of anaerobic digestion involves hydrolysis, acidogenesis, acetogenesis and methanogenesis reaction (Ravichandran et al.., 2017). These four main stages of this degradation can be distinguished:

2.6.1 Hydrolysis

The hydrolysis process in anaerobic digestion is the first step and involves the solubilization and degradation of complex organic compounds into smaller, water-soluble compounds (Fig. 3). This process is achieved through the action of enzymes produced by fermentative bacteria (Parawira and Parawira, 2012). Optimization of the hydrolysis process is important to prevent inefficient degradation of macromolecules, which could negatively impact the rate of digestion and biogas yield. Physicochemical treatments can also promote solubilization of organic matter. It is crucial

to maintain an anaerobic environment in the system for the microorganisms to perform their duties effectively (Xiao et al., 2020).

Hydrolysis is a slow process that depends on the nature and size of the organic matter (Morgenroth et al., 2018). It converts complex organic compounds into simpler ones, such as sugars, amino acids, and fatty acids. Proteins are broken down into amino acids, peptides, ammonia, and CO_2, while polysaccharides are converted into sugars. Hydrolytic bacteria produce enzymes like protease, lipase, and cellulase to facilitate these breakdown processes. Proper hydrolysis allows the substrate to become available for further degradation by fermentative bacteria in the acidogenesis step (Christy et al., 2014). Equation 2.2 illustrates the biochemical reaction involved in the hydrolysis stage (Bajpai, 2017)**.**

$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2$ …….....(eq.2.2)

2.6.2 Acidogenesis

Acidogenesis is a critical stage in anaerobic digestion as it links the fermentation phase with methane production. It involves the transformation of organic acids into acetic acid and other byproducts through the activity of acidogenic microorganisms (Li et al., 2019). Acidogenesis involves the activity of acid-producing bacteria known as acidogenic microorganisms (Sawyerr et al., 2019a). Maintaining a symbiotic relationship between acidogens and methanogens is essential for the successful operation of anaerobic digesters (White et al., 2008b). The complex interaction of various bacteria groups drives the degradation of organic matter and the production of biogas.

In a balanced bacterial process, approximately 50% of monomers (glucose, xylose, amino acids) and long-chain fatty acids (LCFA) are broken down into acetic acid (CH_3COOH) (Fig. 3). About 20% is converted into carbon dioxide (CO_2) and hydrogen (H_2), while the remaining 30% is broken down into short-chain volatile fatty acids (VFA) (Wainaina et al., 2019). This breakdown is facilitated by a variety of obligate and facultative fermentative microorganisms (Gerardi, 2003).

During acidogenesis, the products of the hydrolysis stage undergo further breakdown by fermentative microorganisms. This results in the production of weak acids such as acetic acid, propionic acid, butyric acid (VFAs), lactic acid, alcohols, hydrogen, and carbon dioxide (Dobre and Nicolae, 2014). These products are essential for the subsequent stages of anaerobic digestion. Equation 2.3, 2.4 and 2.5 illustrates the biochemical process involved in this stage (Bajpai, 2017).

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ …….....(eq.2.3)

$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O$ …….....(eq.2.4)

$C_6H_{12}O_6 \rightarrow 3CH_3COOH$ …….....(eq.2.5)

2.6.3 Acetogenesis

During acetogenesis, alcohols and volatile fatty acids with more than two carbon atoms are converted by acetate-forming bacteria into acetate (Fig. 3). The main byproducts of this process are hydrogen and carbon dioxide. Acetogens play a vital role in reducing hydrogen accumulation, ensuring a favorable environment for acetogenic bacteria (Karekar and Stefanini, 2022).To maintain low hydrogen partial pressure during acetogenesis, a mutually symbiotic relationship between acetogens and hydrogenotrophic methanogens is necessary. Acetogens produce acetate, which serves as a substrate for methanogens. This symbiosis ensures the smooth transition from fermentation to methanogenesis (Kurade et al., 2018).

Acidogenesis and Acetogenesis in anaerobic digestion is a sequential process for organic acid transformation. The acidogenesis and acetogenesis stages play crucial roles in anaerobic digestion, converting organic acids produced during the initial stages into acetic acid and other byproducts (Lohani et al., 2018). This sequential process is essential for the efficient breakdown of organic matter and the production of biogas. Acetogenesis serves as the final phase of fermentation before methanogenesis in anaerobic digestion. Acetogenic organisms bridge the gap between acidogenesis and methanogenesis by providing the necessary substrates, such as acetate and hydrogen, for the last step of organic material conversion (Li et al., 2019).

The biochemical reaction that occurs in this stage is illustrated in Equation 2.6, 2.7, and 2.8 (Anukam et al., 2019).

$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^-$ ……...(eq.2.6)

$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2$ …….....(eq.2.7)

$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 3H_2 + H^+$ …….....(eq.2.8)

2.6.4 Methanogenesis

Methanogenesis is the final step in anaerobic digestion, where methanogenic bacteria convert acetate and hydrogen/carbon dioxide into methane and carbon dioxide (Fig. 3). Methanogenic bacteria belong to the Archaea kingdom and utilize acetate, hydrogen, carbon dioxide, methanol, methylamines, and formate to produce methane and carbon dioxide (Roberto, 2022). These end products are the primary components of biogas, which typically consists of 50-75% methane, 25-50% carbon dioxide, and trace amounts of nitrogen, hydrogen, and hydrogen sulfide. In typical anaerobic digesters, around 70% of methane formation comes from acetate, while the remaining 30% originates from hydrogen/carbon dioxide (Reith, 2003).

Equation 2.9, 2.10, and 2.11 illustrate the biochemical reaction involved in the Methanogenesis stage Bajpai, (2017), Anukam et al. (2019).

$CH_3COOH \rightarrow CH_4 + CO_2$ …….....(eq.2.9)

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ …….....(eq.2.10)

$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH$ …….....(eq.2.11)

The interaction between hydrogen and acetate, facilitated by homoacetogenic bacteria, also plays a significant role in methane formation. Hydrogenotrophic methanogenesis is more efficient at high hydrogen partial pressure, while aceticlastic methanogenesis is independent of hydrogen partial pressure. Methanogenic bacteria are more sensitive to changes in temperature compared to other organisms in the digester. Acetogens, for example, have a faster growth rate and can perform substantial catabolism even at low temperatures (Kotsyurbenko, 2005). At higher temperatures, the oxidation pathway of acetate becomes more favorable. The production of methane indicates the biological activity and performance of an anaerobic system. A higher methane production indicates a more stable and well-performing system.

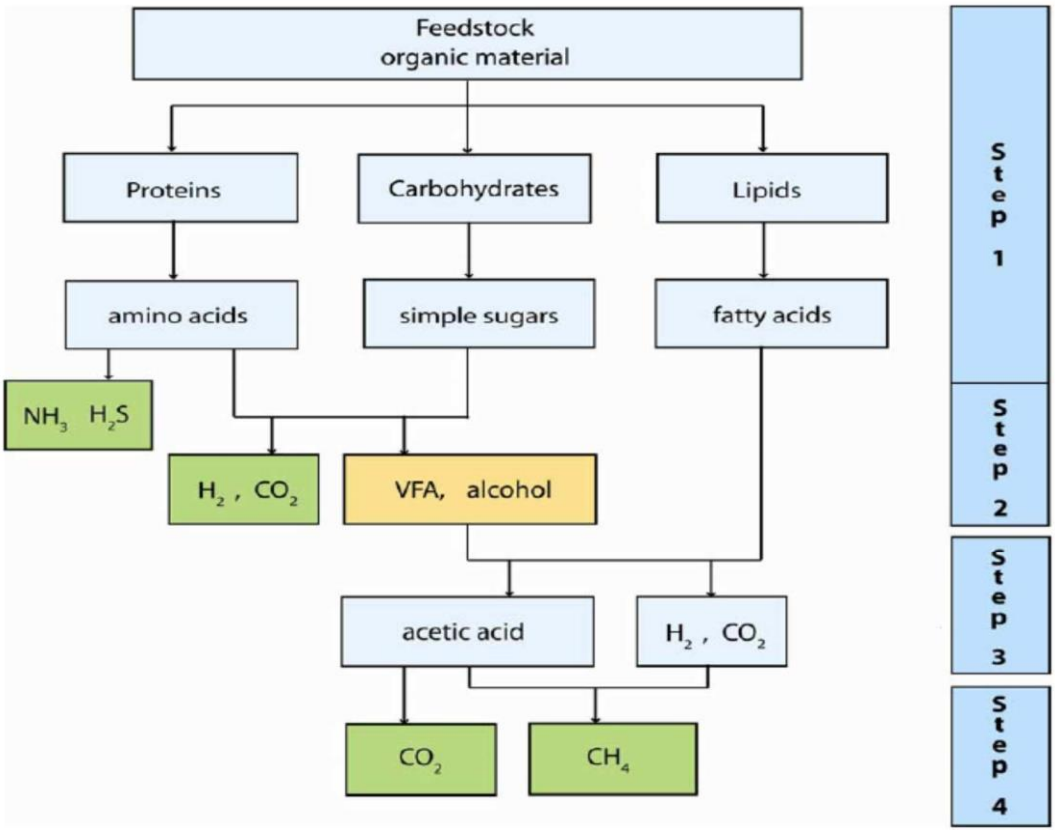

Fig 3: Biogas production process and steps.

2.7 Factors Affecting Biogas Production

The factors affecting the biogas production are mainly caused by the characteristics of the feedstock and operating condition of the process. In order for anaerobic reactors to perform at their best, they should be operated under steady state conditions. An understanding of the parameters that can adversely affect the digestion process is critical because even small changes in conditions can disturb or permanently damage microbial interactions and performance (Bharathiraja et al., 2018).The production of biogas is influenced by many factors. These factors may slow or stall the process of biogas production if the values of the factors are not within a certain range (Sawyerr et al., 2019b). Some of the factors are: -

2.7.1 pH

In anaerobic digestion, the pH plays a crucial role in maintaining the optimal conditions for the methanogenic bacteria responsible for the production of biogas. These bacteria require a slightly alkaline environment to thrive (Christy et al., 2014). To adjust the pH in anaerobic digestion,

chemicals such as sodium bicarbonate, potassium bicarbonate, calcium carbonate (lime), and calcium hydroxide (quick lime) can be used. These chemicals slowly release bicarbonate alkalinity, which is essential for the growth of methanogenic bacteria.

Among these chemicals, sodium bicarbonate and potassium bicarbonate are more preferred due to their solubility, ease of handling, and minimal adverse impacts on the anaerobic digestion process (Gouwanda et al., 2019). Overall, adjusting the pH in anaerobic digestion is crucial for maintaining the activity and efficiency of methanogenic bacteria, and chemicals like sodium bicarbonate and potassium bicarbonate are commonly used for this purpose (Dewil et al., 2008).

2.7.2 Nutrients

Nutrients are essential for the successful production of biogas in anaerobic digestion. These nutrients can be divided into two categories: macronutrients and micronutrients. Macronutrients, such as nitrogen and phosphorus, are required in larger quantities by all bacteria involved in the process (Möller, 2012). On the other hand, micronutrients like potassium, magnesium, calcium, iron, sodium, chlorine, zinc, manganese, and molybdenum are needed in smaller quantities by most bacteria (Khalid, 2014**).** Inorganic nutrients play a critical role in the conversion of acetate to methane. The lack of specific elements required for microbial growth can limit biogas production.

The bacteria involved in anaerobic digestion require trace amounts of various elements, including nitrogen, phosphorus, sulfur, calcium, magnesium, iron, nickel, cobalt, zinc, manganese, and copper (Płuciennik-koropczuk, 2018). Although these elements are needed in very small concentrations, their absence can have negative effects on microbial growth and performance. Macro-nutrients should ideally be present in concentrations around 10-4 M in the bacterial cells, while micronutrients like nickel, cobalt, and copper are required in even smaller amounts (Ying, 2012).

Insufficient nutrient availability in energy crops can lead to issues such as low methane yields, acidification, and process instability. This often results in the need for lower organic loading rates and longer hydraulic retention times (Mao et al., 2015). Adequate amounts of both macro- and micronutrients are crucial for the continuous and efficient performance of the biogas production process.

2.7.3 Hydraulic Retention Time

Hydraulic retention time (HRT) indicates the mean residence time for solids and liquids wastes remaining in a digester (reactor) to contact with the microbial biomass allowing for the conversion of organic matter into biogas. In flow-through systems without recycle, such as the CSTRs adopted in Phase II, the HRT and retention time of the microbial biomass or sludge (SRT) are the same. In situations where the influent streams contain high solids concentrations, longer retention times are required to maximize bioenergy production (Sawyerr et al., 2019b)**.**

The HRT can be understood as the treatment time for a waste that undergoes AD, the higher the HRT the higher the removal efficiency because the biomass has enough time to be in close contact with the waste, therefore removing high amounts of contaminants from the waste being treated. Additionally, a longer HRT allows for better nutrient utilization by the bacteria involved in anaerobic digestion. This is because the longer contact time allows for more efficient uptake and utilization of nutrients, resulting in increased biogas production (Mao et al., 2015).

On the other hand, a shorter HRT can lead to incomplete digestion and reduced biogas production. Insufficient contact time between the waste and microbial biomass can result in inadequate nutrient utilization and lower overall efficiency of the process. Regular monitoring and optimization of HRT can help maximize biogas production and overall process performance (Rocamora et al., 2020).

2.7.4 Temperature

Regular monitoring of temperature is crucial, especially during weather changes, as it indicates the rate of biological reactions taking place in the biogas production process. Ensuring a suitable temperature for the microorganisms used in waste treatment is essential for optimizing biogas production (Sorathia et al., 2012). In the anaerobic digestion process temperature directly affects the activity of methane-forming bacteria and the overall rate of biogas production. The first stages of digestion, hydrolysis, and acidogenesis, are not significantly influenced by temperature fluctuations. However, the subsequent stages, performed by specialized microorganisms such as acetogenic and methanogenic bacteria, are highly sensitive to temperature changes (Sergio and Ponsa ,2008).

The rate of biochemical processes, including biogas production, generally increases with temperature. A rule of thumb is that the rate doubles for every 10 degrees rise in temperature within certain limits. Different types or strains of bacteria are involved in the biogas process, adapted to different temperature ranges: psychrophilic (0-20°C), mesophilic (15-45°C), and thermophilic (40-65°C) (Beshir, 2016). Thermophilic digestion offers advantages over mesophilic digestion, such as improved reaction rates and pathogen reduction. However, microorganisms in mesophilic digestion have lower nutrient demands and can function similarly to thermophilic digestion (Ryue et al., 2020). The choice between mesophilic and thermophilic temperatures depends on the desired outcome and the suitability of microorganisms for waste treatment.

2.7.5 Organic Loading Rate

The amount of substrate (biomass) fed into the unit reactor system is called the OLR and is commonly expressed in terms of chemical oxygen demand kg/m^3 /day, volatile solids (VS) of total solids (TS)/ L/day or VS/m^3 /day (Sawyerr et al., 2019b). It has been reported that the AD of solid wastes in a single stage may encounter problems if the OLR is increased above the system capabilities and that the hydrogen and the VFAs formed by the acidogenic bacteria are not consumed at the same rate by the methanogens. This is because acidogenic activity and the VFA intermediates produced in the acid forming stages triggers an increase in the acidogenic bacteria at higher OLRs, thereby reducing the growth of the methanogenic population (Magdalena et al., 2019).

The increase in OLR and acidogenic activity (production of VFA, CO_2 and H_2) can result in an accumulation of organic acids and a decrease in pH and gas production. This in turn affects the biological activity of methane-producing methanogens as their growth is inhibited below a pH of 6.6, therefore reducing the production of methane, which is the main product of biogas. Therefore, determining the correct OLR for a particular substrate is critical for the optimization of reactor performance and maximizing methane production. The methane yield is generally measured by the amount of gas that can be produced per unit volume of VS contained in the feedstock after exposing it to AD for a sufficient amount of time under a given temperature and specific conditions (Nosrati et al., 2004).

2.7.6 Retention Time

Retention time in anaerobic digestion reactors refers to the amount of time that feedstock remains in the digester. A longer retention time allows for better sludge stabilization and increased contact between the biomass and liquid flow during the treatment process. The appropriate retention time depends on factors such as the type of feedstock, environmental conditions, and intended use of the digested material (Liao et al., 2006).The retention time in AD systems is influenced by process temperature and total solid content.

Mesophilic digesters typically have longer retention times (10-40 days) compared to thermophilic digesters. Similarly, high solid content systems ("dry" processes) have longer retention times than low solid content systems ("wet" processes). Mixing the digester is a common method used to shorten the residence time in AD reactors (Vanwonterghem et al., 2015). There are two types of retention time; hydraulic retention time and solids retention time (SRT). HRT refers to the theoretical time that the influent liquid phase stays in the digester, while SRT is the ratio of solids maintained in the digester to solids wasted in the effluent. The required retention time for complete AD reactions varies depending on technology, process temperature, and waste composition (Zamri et al., 2021).

The conversion of organic matter to gas is more closely related to SRT than HRT. In mesophilic digesters, the retention time for wastes ranges from 10 to 40 days. If the retention time is too short, bacteria in the digester are washed out faster than they can reproduce, resulting in fermentation coming to a standstill. Keeping a substrate under proper reaction conditions for a longer period of time leads to more complete degradation, but the reaction rate decreases with increasing residence time (Clara et al., 2005). However, a longer retention time requires a larger reactor size for a given amount of substrate to be treated.

2.7.7 Volatile Fatty Acid

During start-up or when the digester is overloaded with organic material, high concentrations of volatile fatty acids are typically observed. These VFAs can have toxic and inhibitory effects on the anaerobic microbes in the digester. While it is commonly believed that VFA inhibition is due to their accumulation and subsequent reduction in pH, some VFAs themselves are toxic to the microbes (Nosrati et al., 2004).

The stability of the anaerobic digestion process can be assessed by monitoring the concentration of intermediate products like VFAs. VFAs are intermediate compounds with a carbon chain of up to six atoms (such as acetate, propionate, butyrate, and lactate) that are produced during the acidogenesis phase of digestion. In cases where the AD process becomes unstable, there is often an accumulation of VFAs inside the digester, which can lead to a drop in pH (Orozco et al., 2013).The presence of a high lipid content in the digester leads to an increased concentration of VFAs, which can severely inhibit the anaerobic digestion process. To ensure proper digestion, the concentration of VFAs should not exceed 250 mg/l. However, methane production can still be achieved at VFA concentrations up to 6000 mg/l, although this is the maximum tolerable value (Xiao et al., 2020).

2.7.8 Free Ammonia

Free ammonia concentrations above 100 mg/l can cause inhibition, although the ionic form, NH_4^+ , will only cause inhibition at much higher concentrations (above 3000 mg/l) (Nishio and Nakashimada, 2007). Free ammonia, which is the un-ionized form of ammonia (NH_3), can also have a significant impact on biogas production in anaerobic digestion systems. Free ammonia is known to be toxic to the anaerobic microbes responsible for biogas production. When organic material is broken down during anaerobic digestion, nitrogen-containing compounds such as proteins and amino acids are converted into ammonia. Ammonia can exist in two forms: ammonium (NH_4^+) and free ammonia (NH_3). The proportion of ammonia present as free ammonia is dependent on the pH and temperature of the system (Chen et al., 2014).

To mitigate the negative effects of free ammonia, it is crucial to maintain appropriate pH levels in the digester. The optimal pH range for anaerobic digestion is typically between 6.5 and 7.5, as this helps to minimize the formation of free ammonia (Chen and Neibling ,2014). High concentrations of free ammonia can occur when the pH of the digester is too high or when there is an excess of nitrogen-rich feedstock. Free ammonia is more toxic to the anaerobic microbes than ammonium, and its toxicity increases with higher pH and temperature. Additionally, controlling the nitrogen content in the feedstock and ensuring a balanced carbon-to-nitrogen ratio can help prevent excessive ammonia production (Rajagopal et al., 2013).

2.7.9 Carbon: Nitrogen (C<N ratio)

The carbon-to-nitrogen (C/N) ratio plays a crucial role in biogas production during the anaerobic digestion process. Anaerobic digestion utilize carbon at a much faster rate than nitrogen, with a ratio of approximately 25 to 30 times. Therefore, it is important to ensure that the carbon content in the feedstock is 25 to 30 times higher The Optimal C/N ratio for anaerobic digesters is typically between 20 to 30 (Sensai et al., 2014).

Microorganisms involved in the digestion process than the nitrogen content. Insufficient supply of nutrients, particularly carbon and nitrogen, can hinder the growth of microorganisms and ultimately reduce biogas production. To achieve the desired C/N ratio, feedstock materials are selected accordingly. The concentration of organic dry matter to total solids should be between 70 to 95 percent. Even when the C/N ratio is close to the ideal range, efficient anaerobic fermentation can only occur if the waste materials are biodegradable at the same time. A high C/N ratio leads to rapid nitrogen consumption by methanogens, resulting in lower gas production. Conversely, a low C/N ratio can cause ammonia accumulation and pH values exceeding 8.5, which are toxic to methanogenic bacteria (Nurliyana et al., 2015).

Therefore, it is important to achieve an optimal C/N ratio by mixing materials with high and low C/N ratios. This can be achieved by combining organic solid waste with sewage or animal manure. so, maintaining an appropriate C/N ratio is essential for optimizing biogas production in anaerobic digesters. By carefully selecting and mixing feedstock materials, the desired C/N ratio can be achieved, leading to efficient and effective biogas production (Sreekrishnan et al., 2004).

2.7.10 Toxicity

Mineral ions, heavy metals and detergents are among the toxic materials that inhibit the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g., sodium, potassium, calcium, magnesium, ammonium and Sulphur) also stimulates the growth of bacteria, while very heavy concentration of these ions leads to toxic effects. For example, presence of NH_4^+ ions in the concentration range of 50 to 200 mg/l stimulates the growth of anaerobic microbes, whereas its concentration above 1500 mg/l produces toxicity (Kigozi et al., 2014).

Similarly, heavy metals such as copper, nickel, chromium, zinc, lead etc., in small quantities are essential for the growth of bacteria but are toxic at higher concentration. Detergents including

soap, antibiotics, organic solvents etc. also inhibit the activity of methane producing bacteria and hence addition of these substances in the digester should be avoided (Mahanta et al., 2005).

2.8 Environmental and Economic Benefits of Biogas

2.8.1 Energy and Climate Concerns

Biogas is a renewable source of energy. The carbon dioxide that is released when biogas is combusted and mixed with the oxygen in the air does not contribute to the greenhouse effect. The carbon in the methane molecule produced by the biogas process originates from carbon dioxide in the air that growing plants have previously taken up by photosynthesis. The use of biogas is thus an important step in climate change mitigation. The development of biogas represents a strategically important step away from oil dependency that will contribute to a sustainable energy supply in the long term. Renewable means that there will be no “peak biogas”; rather biogas will be continuously available and thus offers improved energy security. Biogas is also produced locally meaning that it is not dependent on trade relationships. This also contributes to improved energy security (Olajire, 2020).

2.8.2 Increase in Agricultural Productivity

Anaerobic digestion increases nitrogen fixation and reduces the release of greenhouse gases such as methane, making it an environmentally sustainable way to manage organic waste. The digestate is used as fertilizer, providing valuable nutrients to soil and promoting plant growth. Anaerobic digestion kills certain bacteria, parasites and weed seeds that otherwise might have had negative effects on crop production. Organic bio-gas production helps to ensure food security (Piao et al., 2010).

In Ethiopia, using cow dung and crop residues as fuel leads to a reduction in agricultural productivity. Due to the use of dung as a source of domestic energy it is estimated that 10 % of the annual grain production is lost for the Tigray region. Through the bio-gas Program the utilization of slurry is promoted, thus contributing to increased crop production (Mishra et al., 2015).

2.8.3 Sustainable Development

Production of bio-gas offers many benefits to society and is an important contribution to a sustainable development. One of the most important tasks we face today is to reduce our

exploitation of the earth's finite resources and to develop systems for re-cycling of nutrients and energy that are sustainable in the long term. In the bio-gas process, waste is converted into energy and nutrients and hence, the exploitation of finite resources is reduced. The biogas process has many advantages from the point of view of the environment, especially since it results in two environmentally friendly final products: biogas and bio fertilizer (Holm-Nielsen et al., 2009).

2.8.4 Carbon Revenues

A biogas installation results in greenhouse gas (GHG) abatement. This abatement is denoted as "carbon offsets" and has a value under the clean development mechanism (CDM) or the voluntary carbon market. These offsets can be sold as carbon credits and utilized for policies to stimulate bio digester adoption by for instance, providing subsidies or soft loans. Consequently, these carbon revenues can cover a part of the required capital investments to tackle the impact of the low ambient temperature on biogas production (Kindler et al., 2011). All the CDM certified and biogas projects under validation are studied to determine the claimed carbon reduction per digester to estimate carbon income.

2.8.5 Biogas and Recycling of Nutrients

When biogas is produced from organic waste, manure or food waste, the residue, digestate, contains all the nutrients in the original substrate. These nutrients are retained in soluble and plant available forms in the residue, and cannot be lost by leaching, since the digestion takes place in closed containers. Using the digestion residue as a bio fertilizer reduces the need for mineral fertilizers. The return of the bio fertilizer to arable land constitutes an excellent case of recycling of a natural resource (Mussatto, 2014).

2.8.6 Biogas as a Bio-fuel

Biogas is a high quality bio-fuel. As any fuel it can be used to produce electricity and heat, or both in combusted heat and power (CHP) equipment. As it consists of methane it is easily adaptable to existing processes where natural gas, also methane, is used. Methane is a fuel in demand by industry, partly because it is a gas, which gives a high-quality combustion that can be precisely controlled (Su et al., 2005). Methane burns with a clean and pure flame, which means that boilers and other equipment are not clogged by soot and cinders. This leads to a cleaner workplace environment and less wear and tear on the plant. Biogas is the most environmentally friendly

vehicle fuel on the market today (White et al., 2008a)**.** Biogas gives the smallest emissions of carbon dioxide and particulate matter of all vehicle fuels on the market. Emissions of carbon monoxide, hydrocarbons, Sulphur compounds and nitrogen oxides are less than when petrol or diesel is used as fuel.

2.8.7 Decrease Eutrophication

If manure is just unloaded in the environment, it will leak and be carried by water to the nearest water course. Leaking manure is a main cause of Eutrophication of surface waters in the region. Besides from this, anaerobic digestion also to a great extent reduces the pathogenic contents of the manure. Also, the process greatly reduces the smell of the manure (Nishio and Nakashimada, 2007).

2.8.8 Used as Waste Management Option

Biogas is also produced with organic waste as substrate. This is a great advantage in waste management; the waste does not need to be land filled or just incinerated for recovery of its heat content. When fermenting organic waste, the two important resources are recovered, the biogas and the nutrients in the residue (Achinas et al., 2017).

2.8.9 Reduce Deforestation

The energy saving aspect and thus saving on cost for firewood is from the point of view of the farmer household an important aspect. Moreover, it is one of the major considerations of a government to promote this technology because it reduces the burden on the environment. It saves trees and helps thereby to combat erosion and to store carbon (Sorathia et al., 2012a).

2.8.10 Reduce Greenhouse Gas

The conversion of animal wastes and manure to methane or biogas can yield significant health and environmental benefits. Methane is a GHG that has 21 times more global warming potential than carbon dioxide in trapping heat in the atmosphere. By trapping and utilizing the methane, GHG impacts are avoided (Achinas et al., 2017).

3. MATERIALS AND METHODS

3.1 Study Area

The present study was conducted in Heineken Brewery which is located in southern periphery of Addis Ababa, the capital city of Ethiopia. Addis Ababa is located at the geographical coordinate of 9º N and 38º 45'E, covering a total area of over 527 km^2 (527,000 hectares). As can be seen from the location map below, Heineken Brewery is located in Kilinto, woreda 9, Akaki-Kaliti Sub City in the southeastern part of Addis Ababa, Ethiopia (Fig 4). The brewery produces 3 million Hectoliters of beer annually and uses electric power and fuel burner for energy. The raw materials used are malt, hops, yeast and water. Its main products and by-products are alcoholic and non-alcoholic beer, spent grains, yeast, wastewater, and carbon dioxide. Heineken Brewery uses treated ground water for the production of beer, which meets the international brew standard. The factory produces substantial volume of wastewater per day. The wastewater treatment system employed at Heineken Brewery is UASB reactor coupled with post aeration tank. The treated effluent from the system is released in to nearby river.

Fig 4: Location map of Heineken Breweries; Kilinto site map data @2023 AfriGIS (Pty) Ltd.

3.2 Materials and Chemicals

Spectrophotometer HACH (DR2800) was used to measure Chemical oxygen demand (COD), Total phosphorous (TP) and Total Nitrogen (TN). Muffle Furnace (0-1000°C) was used to measure volatile solid (VS). Oven (0-200°C) was used to measure the total solid (TS). BOD Digester was

used for measurement of BOD5 at 20 °C. Electronic balance was used to measure mass. Refrigerator was used to keep the brewery waste, spent yeast and inoculum at 4°C until used in the experimentation. Desiccator was used to cool crucibles after being heated in the oven or the muffle furnace, crucible tongs to take crucibles out of oven or muffle furnace. Pipette Evaporating dishes 50ml, Crucibles 100 ml and weighing dishes 100 ml for total solid measurement. Portable pH meter was used to measure pH of the samples. The characterization took place at Heineken Breweries, Kilinto laboratory. The reagents used during the experiment are NaOH, HCl, and Buffer solution.

3.2.1 Experimental Design

The study was limited to two substrates; brewers' wastewater (BWW) as the main substrate and brewers surplus yeast (BSY) as the co-substrate. The experiment was designed using design expert software based on response surface method (RSM) with a factorial design of two factors (volume ratio and HRT) having four levels (100% BWW, 75% BWW + 25% BSY, 50% BWW + 50% BSY, and 25% BWW + 75% BSY) Chukwuma et al. (2013), brewer's wastewater to brewers surplus yeast mixing ratio. Each are analyzed for the different combinations of their test levels.

Table 2**: Optimal (custom) design**

	Name	**Units**	**Type**	**Levels**	**L[1]**	**L[2]**	**L[3]**	**L[4]**
A [Numeric]	A	days	Discrete	4	5	10	15	20
B [Numeric]	B	%	Discrete	4	25	50	75	100

Table 3: Custom Design layout

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Run	A:A	B:B	Biogas	Methane	COD Removal
	days	%	ml	%	%
2	5	50			
3	5	50			
10	5	75			
12	5	100			
1	10	25			
4	10	100			
5	10	50			
6	10	100			
9	10	75			
11	10	25			
7	15	75			
8	15	50			
16	15	75			
17	15	75			
18	15	25			
13	20	50			
14	20	25			
15	20	75			
19	20	100			
20	20	100			

3.3 Sample collection and preparation

The Raw materials was collected from Heineken breweries and transferred to Addis Ababa Science and Technology University College of Engineering, Department of Chemical Engineering

laboratory for sample preparation and water bath utilization. The physicochemical and analytical characteristics of the substrate was identified and measured at Heineken breweries wastewater laboratory; after that the desired proportion of the collected wastes can be prepared for anaerobic digestion. The anaerobic digestion was hold in one litter (1000 ml) Erlenmeyer flask which were putted inside a temperature-controlled water bath. The prepared sample arranged and labelled and mixed according to the given ratio with brewers' wastewater and brewers surplus yeast. The 20 digesters of 1000 ml each with their lids were used and marked with a working volume of 800 ml, leaving a headspace of 200 ml for gas production Casallas-ojeda et al. (2020) and sealed with rubber stoppers. The reactors were then fed with inoculum, wastewater sludge and brewer's surplus yeast with the co-fermentation ratios mentioned above. Conditions were set at mesophilic temperatures of 35 ℃ and a working pH of 6.8–7.2 (Brown and Li, 2013). The thermostatic water bath was filled with water to cover the fermenters. After incubation for 5 days, the amount of biogas volume produced was measured by water displacement method. The substrates were mixed once a day for 5 minutes to maintain intimate contact with microorganism and the substrate. The gas produced was stored in a gas bag and analyzed using a digital portable gas analyzer (Fig.5). The measurement was continued until the last day of HRT 20th day. The sample of measurement for biogas volume, methane composition and COD was at 5th, 10th, 15th and 20th day of the digestion process (Rasmeni et al., 2022).

3.3.1 Inoculum (Sludge)

The seed sludge was taken from an operational upflow granular anaerobic sludge bed (UASB) reactor at the Heineken brewery Kilinto in which the brewery wastewater is treated at (35±2) °C, depending on the seasonal weather. The sludge is typically granular and a total of 400ml of the sludge was used as inoculum, which constitutes 50 % of operational reactor volume.

3.3.2 Sampling Techniques and Laboratory Analysis

The brewery feedstock's for the present study was collected from Heineken brewery share company, Kilinto; Addis Ababa. The sample was conducted from July 2023 to August 30, 2023. Overall, a total of 20 experiments were done. The brewery's wastewater sample was taken from the Heineken brewery's UASB waste water treatment plant influent pit and the brewers Surplus yeast samples were taken from the brewery's cross flow filtration (CFF) plant sample point with

a 5L polypropylene jerikan. The fresh collected sample was stored in a refrigerator at 4℃ until the substrate characteristic was identified. The grab sampling technique was used for physicochemical parameters measured at the sites whereas a time-composite sampling technique was applied for biogas production measurement in the laboratory.

3.4 Laboratory Analysis

Before any analysis of samples, instruments are calibrated using standard solutions. Determinations of the parameters are done according to the analytical methods described in Standard Methods for the Examination of Wastewater (APHA, 2005), using laboratory grade reagents. For all the methods that require the use of the spectrophotometer, both reagent blanks and sample blanks was used. Characteristics of the brewery waste was determined BOD5 at 20 °C (mg/l), COD (mg/l), Total Solids (mg/l), Total nitrogen (N) mg/l, and Total phosphorus (P) mg/l. Total solid (TS), Volatile Solids (VS), and pH of the brewery waste, BSY and inoculum was also separately characterized.

3.4.1 Total Suspended Solids (TSS)

The filter paper was placed in the oven and the temperature was set to 105℃. After a period of 2 hours, the filter paper was removed from the oven and weighed, resulting in a weight of A (g). Subsequently, the filter paper was placed in a funnel and used to filter a 100 ml sample, with the resulting filtrate being collected in a beaker. Once the filtration process was complete, the filter paper was placed back in the oven for a period of 4 hours to dry. After drying, the filter paper was removed from the oven and weighed, resulting in a weight of B (g) (Guangzhou advance environment technology, 2014).

Calculation: $TSS = \frac{B-A}{v} X 10^6$ ..... (eq.3.1)

Here: v is the sample volume, 100ml

3.4.2 Total Solids (TS):

Total solids was determined by gravimetric method and then suspended solids were calculated by using the equation below: (American Public Health Association (APHA AWWA, 2005).

$TS = TSS + TDS$ ..... (eq. 3.2)

3.4.3 Chemical Oxygen Demand (COD)

The DRB200 Reactor was started and preheated to 150°C. Then, 100mL of the sample was homogenized for 30 seconds before being poured into a 250 mL beaker and gently stirred. A sample of 2.0 ml pipette was added of into a vial, capped it, cleaned the outside of the vial, and inverted it gently several times to mix. The vial was then placed in the preheated DRB200 reactor and heated for two hours then place the vial in rack to cool to room temperature. Then installed the light shield in Cell Compartment #2 and inserted a blank into the 16-mm cell holder. After inserted the sample vial into the 16-mm cell holder and pressed zero after cleaning the outside of the vial then select the high range test. After inserting the sample in the Light Shield in Cell Compartment #2 and pressed read. The results were read in mg/l COD (Guangzhou advance environment technology, 2014).

3.4.4 Biological Oxygen Demand (BOD)

The pH of the sample was adjusted to 7 and a 27 ml sample of the brewery effluent was taken. The sample was added with nitrification inhibitor and 1 ml of KOH solution to preserve the dissolved oxygen levels during the incubation period. After that, the sample was sealed in a bottle with a pressure sensor and electronic system for measurement and kept in a thermostat at 20°C for 5 days. Place the bottles on the BOD Track apparatus, connect tubes, and tighten caps. Place the instrument in the incubator and start it. Ensure stir bars are rotating correctly before beginning the test. Set the test duration and BOD range on the instrument display, then start the test by selecting the bottle channel number and pushing ON. A graph will display to monitor the BOD analysis process. The data were automatically recorded every 24 hours by the BOD digester. Finally, BOD5 value was obtained on the fifth day (Guangzhou advance environment technology, 2014).

3.4.5 Volatile Suspended Solids (VSS):

The crucible was placed in the muffle furnace at 605 ℃ for 15 minutes and then dried and cooled. After cooling the crucible was weighed and its weight was recorded as A(g). Then 50 mL of the sample was added to the crucible and dried in an oven at 105 ℃ for 24 hours. The crucible with the sample was then dried and weighed, and the weight was recorded as B(g). The crucible was placed back in the muffle furnace at 605 ℃ for 15 minutes, then dried and cooled. The crucible

was weighed again and its weight was recorded as C(g). The change in weight was used to determine VSS.

Calculation: TS (mg/l) = $\frac{B-A}{v} X^{10^6}$ ……. (eq.3.3)

VSS (mg/l) = $\frac{B-c}{v} X^{10^6}$ ……...(eq.3.4)

Here: V is the sample volume 50 ml

TS means total solids, including TSS and TDS

The residue produced by the method of total solid determination was ignited in a muffle furnace according to ASTM D 2017 (1998). Then, it was transferred to desiccator used to store moisture-sensitive substances in a dry environment for final cooling in a dry atmosphere. The volatile solid sample was calculated by subtracting the before muffle furnace and the after and dividing by the before muffle furnace (Guangzhou advance environment technology, 2014).

3.4.6 pH

The brewery waste and spent yeast were directly measured by using the portable pH meter immediately after sampling. The sample was subjected to pH measurement by inserting the sensor of the pH meter, opening the pH meter, and waiting for the reading to stabilize before reading the result. The pH was adjusted to 6.8-7.2 Brown and Li (2013), using 2N NaOH and 0.5N HCl solution.

3.4.7 Total Nitrogen (TN)

TN was an analyzed using the DRB200 Reactor and HACH Spectrophotometer. The DRB200 reactor stir the sample mixture and control the temperature to allow precise regulation of the reaction temperature to ensure efficient mixing of the reaction components. First, the DRB200 Reactor was turned on and heat to 105 °C. Then total Nitrogen Persulfate Reagent Powder Pillow was added to Total Nitrogen Hydroxide Digestion Reagent vials contains a pre-measured amount of hydroxide digestion reagents, which were used to break down the sample matrix and release the nitrogen compounds. Sample or blank was then added to the vials, which were capped and shaken

vigorously. The vials were heated in a reactor for 30 minutes and cooled to room temperature. Next, Total Nitrogen Reagent a Powder Pillow was added to the vials, capped, and shaken for 15 seconds. The timer was pressed for 3 minutes, then Total Nitrogen Reagent B Powder Pillow was added to the vials, capped, and shaken for 15 seconds. After 2 minutes, the digested, treated sample was added to TN Reagent C vial and capped. The reaction was allowed to proceed for 5 minutes before inserting a reagent blank and pressing zero on the cell holder. Finally, a reagent vial was inserted and read was pressed to display results in mg/LN (Guangzhou advance environment technology, 2014).

3.4.8 Total Phosphorous(TP)

5.0 mL brewery sample was added to a reactive phosphorus test N Tube Dilution Vial. The test tube was capped and mixed. The outside of the vial was wiped with a damp towel, followed by a dry one, to remove fingerprints or other mark. The vials were inserted in round cell holder and pressed zero. The display showed 0.00 mg/l PO_4^3. Using a funnel, the contents of one PhosVer 3 Phosphate Powder Pillow was added to the vial and immediately capped tightly and shake. The reaction proceeded for two-minute. The samples were read between two and eight minutes after adding the PhosVer 3 reagent. The vials were inserted into the holder and the read button was pressed. The result showed in mg/l $PO4^{3-}$ (Guangzhou advance environment technology, 2014).

3.4.9 VFA (Volatile Fatty Acids)

VFA analysis was performed using acid-base titration, before testing the following materials and equipment should be ready; electromagnetic stirrer, pH meter, titrator, timer, 0.08N HCl, 0.023N NaOH. The detailed testing process is;

Samples were filtered coarsely for measurement, and about 80 ml was taken, with 25 ml filtrate added into a 100 ml graduated cylinder, then pure water was added to make it 80 ml. The pH meter sensor was then inserted into the sample, and the sample was titrated with 0.08N HCl to pH = 4.0, with the HCl dosage (ml) recorded. It was advised to carefully and slowly add HCl when the pH reached 5.5. The sample was then titrated with 0.08 mol/L HCl to pH = 3.5, but this dosage was not needed to be recorded. After removing the pH meter sensor, the sample was placed on a magnetic stirrer with several boiling beads added and covered with glass. The temperature setting was adjusted when boiling, and the timer was set for heating to stop after 15 minutes. After cooling

for 10 minutes, the beaker was put into cold water for cooling. Once completely cooled, the pH meter sensor was inserted into the sample, and the sample was titrated with 0.023 mol/L NaOH to pH = 4.0. It was important to note that this was the start point of NaOH dosage (ml) from pH = 4.0 to pH = 7.0.

Calculation: VFA(mg/l) = NaOH dosage (ml) X NaOH (concentration, 0.023N) X F (60,000)/ sample (volume, 25ml) ……...(eq.3.5)

Notes: when VFA analysis, acid dosing operation should be accomplished in half an hour to avoid VFA evaporation and affect the measurement result; and the pH meter used should be calibrated every day (Guangzhou advance environment technology, 2014).

3.4.10 Ammonia

The light shield was installed in cell compartment #2 after selected the test. The sample was prepared by adding 0.1ml of sample to one AmVer™ diluent reagent Test N tube for high range ammonia nitrogen. Then, the content of one ammonia salicylate reagent pillow for 5ml sample was added to each vial, followed by the content of one ammonia cyanurate reagent powder pillow to each vial. The vials were then capped tightly and shaken thoroughly to dissolve the powder. After that, a 20-minute reaction period began. After waiting, the blank was wiped and inserted into the 16-mm round holder. The display was pressed zero, and it showed: 0.0 mg/l NH_3-N. Then, the sample vial was wiped and inserted into the 16-mm round cell holder, followed by pressing read to obtain the results in mg/l NH_3-N (Guangzhou advance environment technology, 2014).

3.4.11 COD Removal Efficiency

The removal efficiency of the selected wastewater quality parameters is calculated using the following formula.

Removal Efficiency (%) = $\frac{C_i - C_e}{C_i} x^{100}$.....(eq.3.6)

Where, Ci = is the concentration of COD before digestion, Ce = is the concentration of COD after digestion (Guangzhou advance environment technology, 2014).

3.4.12 Water Displacement Measurement Method

A measuring cylinder was filled with water up to the brim and inverted into a container filled with water, making sure that no air bubbles were trapped inside. After that, the gas-tight syringe was attached to the outlet of the anaerobic digester and the plunger was slowly pulled to collect the biogas sample. Then, the tip of the syringe was carefully inserted into the inverted measuring cylinder, taking care not to let any gas escape. Next, the plunger of the syringe was slowly released to allow the biogas to displace the water in the measuring cylinder. The volume of displaced water in the measuring cylinder was recorded, and the initial volume of water in the measuring cylinder was subtracted from the final volume of displaced water to obtain the volume of biogas collected.

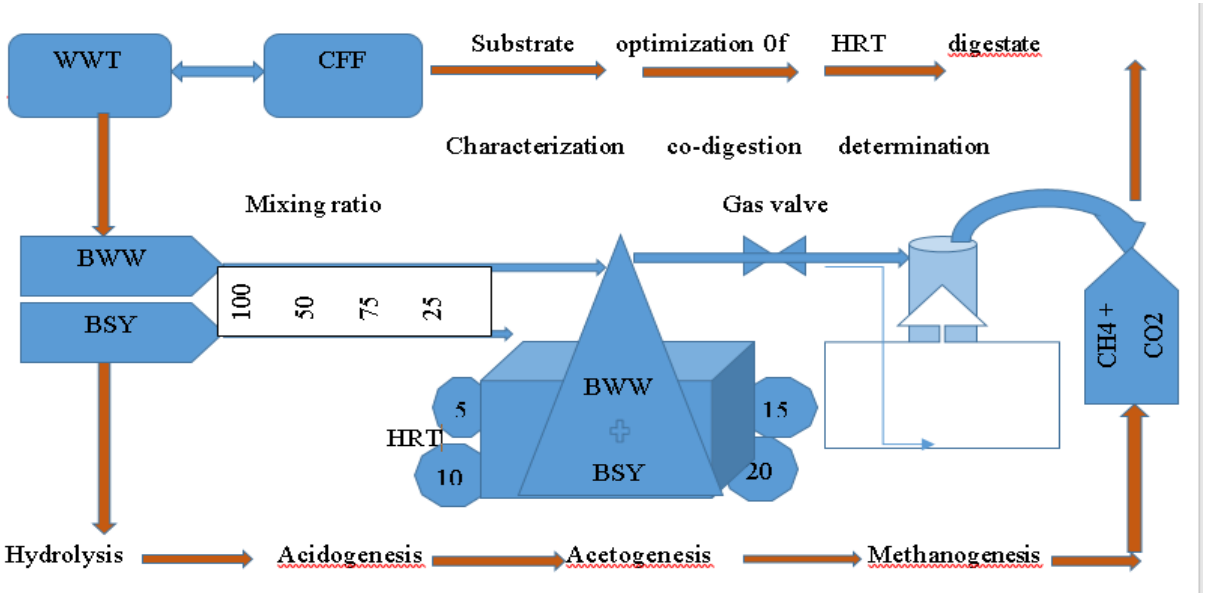

Fig 5: Schematic representation of Laboratory scale ASBR experiment.

Note: The temperature and pressure of the biogas should be measured and corrected to standard conditions (e.g. 0°C and 1 atm) for accurate volume calculations.

3.5 Data Analysis

The data obtained from the study with different parameters were summarized and the results were expressed in terms of mean and standard deviation. The analysis of variance and comparison of means, the models and graphs were performed using design expert software, Model 13. The comparison between the mean was performed at a 95% level of confidence.

4. RESULTS AND DISCUSSION

4.1 Characterization of the Substrates

This chapter presents the physiochemical characteristics of brewery wastewater and surplus yeast in which the variations in the concentration and mixing ratio of these parameters and their effect on biogas production have been discussed. In the section below, the experiment executed and their results were presented.

4.1.1 Characterization of Brewery Wastewater

Brewery wastewater from the Heineken breweries treatment plant was analyzed for COD, BOD5, TSS, TN, TP, VFA, and pH. Table 2 shows results obtained from the raw brewer's wastewater sample before digestion and results showed that BOD5 and COD were the main contents, with values of 1089 ±120 mg/l and 5940±510 mg/l, respectively. These values were within the reported range for brewery wastewater by (Driessen and Vereijken, 2003) which resulted 2000 mg/l to 6000 mg/L for COD and the BOD result was also found in range related with a result reported 500 mg/l to 1,500 mg/l by Ariola et al., (2018).

The high organic loads in the wastewater were attributed to raw materials, trub discharge, weak wort, surplus yeast, tank emptying and rinsing and filtration, dissolved carbohydrates and alcohol from beer wastes (Noukeu et al., 2016). Brewery wastewater was analyzed for TN and TP, with results showing an average of 53.3 mg/l and 62.8 mg/l, respectively. The high level of P was attributed to the use of phosphorus-containing chemicals in CIP units, while the sources of nitrogen were from raw materials and CIP chemicals (Driessen and Vereijken, 2003). Brewery wastewater had a pH range of 9.7 ±1.2, which could be due to batch processing and chemicals used in cleaning which were within the wide range (2-12) pH values (Driessen and Vereijken, 2003).

4.1.2 Characterization of Brewers Surplus Yeast

The BSY was collected from the brewery cross flow filtration (CFF) plant and subjected to COD, BOD, TSS, TS, VFA, VS, TN, and TP before experiment. Table 2 showed that results obtained during analysis of brewers spent yeast for various parameters before conducting the experiment.

The total COD of spent yeast ranges from 197,120 to 199,920 mg/l, with an average of 198,520 ± 1400 mg/l. The brewer's spent yeast is a concentrated suspension of yeast cells in beer (Hejna,

2021). The cell or solids concentration of the suspension differs according to the beer recovery process after fermentation.

Carbohydrates and phospholipids make up the majority of COD, contributing about 80% of the cell mass as such, the degradation of protein is important in achieving high degradation efficiencies (Hejna, 2021).The disparities in concentrations are due to varying solids concentrations in the spent yeast suspensions, influenced by the brewery's beer recovery strategy. Brewery Laško in Slovenia reported an average total solids concentration of 188 g TS/L, of which 95% were volatile solids, and an average total COD concentration of 200,000 + 642 mg/l (Zupančič et al., 2017).

Brewers surplus yeast has an average VFA concentration of 2860 mg/L, which is within the range suitable for anaerobic digestion reported by Xiao et al., (2020); Methane production can be achieved at VFA concentrations up to 6000 mg/L. Spent yeast liquor is essentially unrecovered beer, containing easily digestible volatile compounds and low amounts of other compounds.

4.1.3 Sludge Characterization

The sludge or inoculum was obtained from the UASB reactor sample point of Heineken breweries wastewater treatment plant, which operates at a mesophilic temperature range of 30-35℃. The sample comprised 50% of the digester volume. The seed sludge was tested for TSS, VS, BOD5, COD, TP, and TN, and the results are shown in Table 2. The BOD5 and COD of the sludge were measured on an average concentration of 1130 mg/L and 2940 mg/L, respectively. The reduction of these parameters was due to the conversion of anaerobic digestion into the mineral form of the nutrient (Herbert et al., 2016).

The pH of the brewery wastewater sludge was close to neutral (pH = 7.3) and it was on range reported in previous study by Vijayakumar and Ramya, (2015), the pH value of brewery sludge ranged from 6.68 to 7.62. The sludge had higher levels of nutrients, with TP and TN values of 56 and 72 mg/L respectively. According to Vijayakumar and Ramya, (2015), the TN content of the sludge was higher than the TP content, which could be the result of a computability impact with phosphate anions and an increase in anions' absorption upon inhabitation. Brewers excess yeast and effluent from the brewery had substantially higher concentrations of TSS than the brewery sludge, which had 850 mg/L.

A decrease in BOD5 was directly correlated with a decrease in TSS in the sludge, indicating a clear proportional link between the two parameters. The reason behind this is because when BOD5 falls, microbial activity rises and total suspended particles in the sludge digester break down (Chow et al., 2020).

Table 4: Characteristics of the substrates collected from Heineken brewery factory, Addis Ababa Kilinto from wastewater treatment plant and the crossflow filtration line July 2023.

Analysis	**BWW (mg/l)**	**BSY (mg/l)**	**Sludge (mg/l)**
COD	5940 ± 510	198,520 ± 1400	2940 ± 660
BOD	1089 ± 120	122,887 ± 1482	1130 ± 78
TP	62.8 ± 16.2	358 ± 15.8	56 ± 14.5
TN	53.3 ± 8.2	605 ± 37.5	72 ± 11
TSS	2312 ± 455	65,450 ± 1820	850 ± 305
TS	1428 ± 208	37,160 ± 1284	1400 ±245
VFA	442 ± 44	2860 ± 262	
VS	1170±185	26755 ± 339	930 ±185
pH	9.7 ±1.2	4.3 ± 0.3	7.3 ± 0.5

Key: COD (chemical oxygen demand), BOD (biological oxygen demand), TP (total phosphorous), TN (total nitrogen), TSS (total suspended solids), VFA (volatile fatty acids), VS (volatile solids), TS (total solids).

4.1.4 Characteristics of Mixtures Before Digestion

The results of the characterization of monocultures and co-digestion of brewer's yeast and sewage sludge are shown in Table 3 presents the results of characterizing brewer's wastewater and co-digestion of brewer's yeast and sewage sludge. Characteristics of Brewery waste and surplus yeast

sample used in the study were determined with different mixing ratios and the observed results were executed.

Four experiments were conducted with different mixing ratios of brewery wastewater and surplus yeast. The addition of surplus yeast resulted in a significant increase in COD and BOD values, as well as TS and TSS values. This is due to the high organic content of yeast (Rachwał et al., 2020) Yeast cells are composed of a significant amount of organic matter such as protein lipids and carbohydrates when surplus yeast is added to wastewater which contributes to the overall TSS and TS value in the system (Rasmeni et al., 2022). The presence of surplus yeast can improve the efficiency of biogas production and wastewater treatment process.

Table 5: Characteristics of the substrates brewers' wastewater (BWW)and Brewers surplus yeast (BSY) at 100, 75, 50 and 25% mixing ratio before digestion.

Analysis	50:50 BWW/BSY (mg/l)	75:25 BWW/BSY (mg/l)	25:75 BWW/BSY (mg/l)	0:100 BWW/BSY (mg/l)
COD	105000± 2300	79800 ± 1565	138400± 2250	198000± 4400
BOD	51467 ± 343	33870 ±448	48270 ± 358	67830 ± 622
TS	11060 ±225	7000 ±430	11760 ±512	15120 ±810
TSS	35156 ±928	22672 ±1646	53884 ±2150	67460 ±1920
VS	8627 ± 577	6454 ±410	8762 ±402	9358 ±390
VFA	2860 ±715	2448±556	7662 ±184	7612 ±836
$NH4^+$	104 ±26	102 ±35	108 ±28	32.6 ± 8
TN	409 ±32	218 ±22	473 ±15	705 ±42
TP	287.67 ±46	146 ±27	346.33 ±21	426.67 ±65

The measurement of VS, VSS showed that a mean value of 2228 mg/l, 8627 mg/l, 6454 mg/l, and 8762 mg/l for 100%:0%, 50%:50%, 75%:25% and 25%:75% brewers wastewater to brewers' surplus yeast respectively. it also increased when the yeast percentage increased this was because of the high organic and nutrient content nature of the yeast. There was also increased in TP and TN during the addition of yeast accordingly so, the TN, COD, BOD, and TP increases in all the mixtures as concentration or contribution of yeast increases. The result was expected and this is

due to the rich nutrient content nature of spent yeast; surplus yeast have high nutrients and mineral content such as N,P,C (Hejna, 2021).

4.1.5 Characteristics of Mixtures After Digestion

The characteristics of the substrates after anaerobic digestion were showed on table 5 and resulted in significant reduction of BOD, COD, and TS. BOD decreased by 84.58%, 86.43%, 74.85%, and 66.79% at 0%, 25%, 50%, and 75% brewers surplus yeast mixing ratio respectively. COD were decreased by 87.81%, 84.26%, 72.43%, and 44.82% at the same mixing ratios. Total solids also decreased by 28%, 32.8%, 21%, and 14.3% at the same ratios. The results are comparable to other studies, with lower removal rates than (Bula, 2014) and higher removal rates than (Gizachew, 2011).

Table 6: Characteristics of the substrates Brewers wastewater (BWW) and Brewers surplus yeast (BSY) with 100, 75, 50 and 25 % mixing ratio with 20 days of HRT at 35℃ after digestion.

Analysis	**100:0 BWW/BSY (mg/l)**	**50:50 BWW/BSY (mg/l)**	**75:25 BWW/BSY (mg/l)**	**25:75 BWW/BSY (mg/l)**	**0:100 BWW/BSY (mg/l)**
COD	780 ±160.5	28950 ±1200	11560 ±2,132	76365 ±2869	143000 ± 2230
BOD	434 ±26	12944 ±43	4597 ±28	16,031 ±102	24402 ± 1150
TSS	1690 ±225	18985 ±308	8162 ±154	23180±578	34950 ±1710
TS	1771±115	8738±226	4704±108	9378±296	12768±715
VS	1487±346	5978±162	3980±138	6349±285	7286±351
VFA	365± 65	982±152	458±110	4150±275	5874±321
$NH4^+$	680±92	1238±198	1645±332	2130±342	4196±509
TN	42.8±12	122.5±18	80±6.5	176±33	216±29
TP	46.4±8.6	89.2±21.8	68.8±8.2	155±39	237±12

The results obtained from table 4 shows a reduction in TN and TP due to methanogenic bacteria decomposing organic matter and biogas production from substrates. The study and referenced studies indicate good organic matter reduction and high biogas production. There was an increase in COD, VS, TS, nutrient content, and alkalinity with increasing Brewers surplus yeast mixing

volume ratio. However, brewers surplus yeast concentration increased for some parameters such as COD and decreased for pH due to the type of yeast used and beer pH value.

4.2 Statistical Modeling

Biogas production, percentage of methane, and COD removal efficiency were evaluated using an experimental run involving the two independent factors (substrate mix ratio and HRT). The experiment's yield was using the raw material combination of brewers' wastewater and brewers surplus yeast as shown in Table 7.

The biogas yield exhibited significant variation, ranging from 389 to 2438 ml per 800 ml of sample. The lowest yield was recorded when using a mixture of 25% brewers' wastewater and 75% brewers' surplus yeast at a 5-day Hydraulic Retention Time (HRT) and 35°C, while the highest yield was achieved with a 75% brewers' wastewater and 25% brewers' surplus yeast mixture at run number 12. In contrast, the maximum methane percentage yield of 71.6% was observed under the conditions of a 75% brewers' wastewater and 25% brewers' surplus yeast mixture at a 20-day HRT.

The removal efficiency of COD was ranged from 12.85 to 85.08 %, which were observed at 25 % of brewers' wastewater with 75 % of brewers' surplus yeast at the 5 days of HRT and the highest was observed at a mixing ratio of 75 % brewers' wastewater and 25 % of brewers' surplus yeast with 20 days of HRT respectively.

Table 7: Results of the Experimental Factors with Corresponding Levels showing biogas production, methane composition and COD removal for Brewers wastewater and Brewers surplus yeast from Experimental design.

	Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**
Run	A: A HRT	B: B Mixing ratio	Biogas	Methane	COD Removal
	days	%	ml	%	%
1	20	50	1832	67.3	76.25
2	20	25	1062	51.2	44.33
3	15	75	1827	69.8	56.53
4	15	100	1225	68.2	65.38
5	5	100	694	44.6	28.38
6	5	25	389	36.7	12.85
7	15	50	1508	65.6	61.52
8	15	25	895	46.8	39.75
9	10	50	1222	55.2	42.02
10	10	75	1558	56.9	40.81
11	5	75	937	42.2	26.6
12	20	75	2438	71.6	85.08
13	15	25	895	46.8	39.75
14	10	50	1227	55.5	42.02
15	5	50	605	40.5	25.4
16	15	50	1508	65.6	61.52
17	10	100	1102	49.3	43.58
18	20	100	1443	71.8	84.5
19	10	75	1557	56.9	40.81
20	15	75	1824	69.5	56.3

Where; A=hydraulic retention time, B=mixing ratio, COD=chemical oxygen demand

4.2.1 Biogas Production

Based on Table 8, An ANOVA was used to determine the significant impact of each factor on the production of biogas based on the p-value. Table displays the cubic model that RSM recommended (Appendix B), with p-value and F-value of less than 0.0001 and 191.57, respectively. Consequently, the model was deemed reliable with a confidence interval of greater than 99.9% based on the ANOVA suggested model analysis p-value of less than 0.0001. P-values less than 0.0500 indicate model terms are significant (Appendices F (A)).

In this case A, B, A^2, B^2, AB^2, A^3, B^3 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The Lack of Fit F-value of 1521.27 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise.

Table 8: Coefficients in Terms of Coded Factors for biogas production.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1613.54	1	19.93	1569.14	1657.94	
A-A	433.32	1	55.44	309.79	556.84	10.74
B-B	587.72	1	52.60	470.51	704.92	9.69
AB	38.06	1	23.64	-14.62	90.74	1.04
A^2	-107.28	1	27.34	-168.20	-46.37	1.06
B^2	-596.27	1	27.69	-657.97	-534.57	1.09
A^2B	57.12	1	39.64	-31.20	145.43	2.66
AB^2	-361.65	1	39.17	-448.92	-274.38	2.58
A^3	286.35	1	59.07	154.73	417.97	10.50
B^3	-461.12	1	55.20	-584.12	-338.12	9.17
Std. Dev.	51.61			**R^2**		0.9942
Mean	1287.40			**Adjusted R^2**		0.9890
C.V. %	4.01			**Predicted R^2**		0.9395
				Adeq Precision		52.7098

The R^2 and adjusted R^2 values indicate the degree of variance around the mean that the model examines. The biogas production model has R^2 and R^2 (adj) values of 0.9942 and 0.9890, respectively, indicating a high level of data analysis. Adeq Precision is used to analyze the signal to noise ratio, with a ratio higher than 4 preferred. The model equation in terms of coded factors can be used to predict responses for given factor levels. The equation exhibits cubic forms and coded factors are useful for identifying their relative impact by comparing coefficients. The model exhibits cubic forms of the equation as shown below.

Final Equation in Terms of Coded Factors

$$\text{Biogas} = +1613.54+433.32\,A+587.72\,B+38.06\,AB-107.28\,A^2-596.27\,B^2+57.12\,A^2B-361.65\,AB^2+286.35\,A^3-461.12\,B^3 \quad \text{(eq.4.1)}$$

4.2.2 Methane Composition

ANOVA's p-value was used to determine each factor's significance with a (0.0001) p-value and a (42.33) F-value, implies the model is significant and the quadratic model was suggested by RSM. individually as shown in the following (Appendices B).

Table 9 : Coefficients in Terms of Coded Factors for methane composition.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	62.65	1	1.26	59.95	65.35	
A-A	12.48	1	1.08	10.17	14.78	1.01
B-B	6.52	1	1.08	4.21	8.82	1.02
AB	3.35	1	1.48	0.1717	6.52	1.02
A^2	-4.66	1	1.70	-8.31	-1.00	1.04
B^2	-9.32	1	1.71	-12.99	-5.66	1.04
Std. Dev.	3.26		**R^2**			0.9380
Mean	56.43		**Adjusted R^2**			0.9158
C.V. %	5.78		**Predicted R^2**			0.7749
			Adeq Precision			22.2276

The proposed model's ANOVA, which looked at the p-value to be less than 0.0001, as needed, demonstrated the model's dependability and greater than a 99.9% confidence interval. The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable (table 9).

The Predicted R^2 and Adjusted R^2 are reasonably close at 0.7749 and 0.9158, respectively, i.e. the difference is less than 0.2. with an Adeq Precision ratio of 22.2276 indicating adequate signal. A ratio greater than 4 is desirable. The ratio of 22.228 indicates an adequate signal. This model can be used to navigate the design space. The quadratic equations in Eqn. (4.2) express the percentage of methane, and the developed model equation predicts methane percentage in produced biogas using a coding unit. The developed model equation is used for predicting the methane percentage in produced biogas with a coding unit as follows:

Final Equation in Terms of Coded Factors

$$\text{Methane} = +62.44 + 12.85A + 6.86B + 4.02AB - 4.27A^2 - 8.88B^2 \quad \text{……. (eq. 4.2)}$$

For specific levels of each factor, predictions regarding the response can be made using the equation expressed in terms of coded factors. The factors are coded as +1 for high levels and -1 for low levels by default. By comparing the factor coefficients, the coded equation helps determine the factors' relative impacts.

4.2.3 COD Removal Efficiency

The models' accuracy or significance for COD removal efficiency was evaluated using the p-value and F-a value, which is extremely small in comparison to the software's suggested significance level (p-value greater than 0.1 is not significant and p-value less than 0.05 best fit). Model terms are significant if the P-value is less than 0.0500. Significant model terms in this instance are A, AB, B2, A^2B, AB^2, and B^3. The model terms are deemed not significant if their values exceed 0.1000. Model reduction could help the model if it has a lot of unnecessary terms (aside from those

needed to support hierarchy). The significance of the Lack of Fit is indicated by the F-value of 2783.73 (Appendices F (C)). A large F-value for Lack of Fit could only happen in 0.01% of cases because of noise.

Table 10 : Coefficients in Terms of Coded Factors for COD removal efficiency.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	51.76	1	1.05	49.42	54.09	
A-A	28.59	1	2.92	22.09	35.08	10.74
B-B	-4.34	1	2.77	-10.50	1.83	9.69
AB	5.85	1	1.24	3.08	8.62	1.04
A^2	1.19	1	1.44	-2.01	4.39	1.06
B^2	-9.05	1	1.46	-12.29	-5.80	1.09
A^2B	5.38	1	2.08	0.7400	10.03	2.66
AB^2	-5.33	1	2.06	-9.92	-0.7459	2.58
A^3	-1.01	1	3.11	-7.93	5.91	10.50
B^3	13.43	1	2.90	6.96	19.90	9.17
Std. Dev.	2.71		**R^2**			0.9898
Mean	48.67		**Adjusted R^2**			0.9807
C.V. %	5.58		**Predicted R^2**			0.9115
			Adeq Precision			38.2725

Where A= HRT, B= Mixing ratio, CI= confidence interval and VIF= variance inflation factor

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable table 10.

Predicted R^2 (0.9115) agrees reasonably with Adjusted R^2 (0.9807), with a difference of less than 0.2. Adeq Precision ratio of 38.273 indicates a desirable signal-to-noise ratio, allowing this model

to navigate the design space. The adjusted R^2 0.9807 for COD removal and predicted R^2 (0.9827) are in reasonable agreement with the predicted R^2 (0.9918).

The equation expressed in terms of coded factors can predict the response for specific levels of each factor, with relative impact determined by comparing factor coefficients. By comparing the factor coefficients, the coded equation can be used to determine the relative impact of the factors. Final Equation in Terms of Coded Factors as shown below:

$$CODRemoval=+51.65+28.41A-5.01B+5.63AB+0.7679A^2-8.76B^2+4.94A^2B-4.57AB^2-1.63A^3+14.42B^3 \quad \text{(eq.4.3)}$$

The model is significant with a 0.01% chance that F-values resulted from noise. Coded factors are used to create the equation, which predicts responses for specific factor levels. High (+) and low levels (–) of factors are automatically coded. Factor coefficients in the coded equation determine relative importance of factors.

In the coefficient table of a statistical model, the p-values associated with each coefficient provide information about the significance of that particular predictor variable HRT and mixing ratio in the model. The p-value indicates the probability of observing the coefficient estimate (or a more extreme value) if the null hypothesis is true, i.e., if there is no relationship between the predictor variable and the response variable.

Table 11: Coefficients table of biogas production, methane composition and COD removal of the predictor variables of HRT and Mixing ratio.

p-value shading: $p < 0.05$ $0.05 \leq p < 0.1$ $p \geq 0.1$

	Intercept	A	B	AB	A^2	B^2	A^2B	AB^2	A^3	B^3
Biogas	1613.54	**433.316**	**587.716**	38.06	**-107.283**	**-596.268**	57.1155	**-361.65**	**286.351**	**-461.12**
p-values		**< 0.0001**	**< 0.0001**	0.1385	**0.0028**	**< 0.0001**	0.1802	**< 0.0001**	**0.0007**	**< 0.0001**
Methane	62.6503	**12.4791**	**6.51672**	**3.34501**	**-4.65797**	**-9.32374**				
p-values		**< 0.0001**	**< 0.0001**	**0.0402**	**0.0162**	**< 0.0001**				
COD Removal	51.7583	**28.5867**	-4.33572	**5.85164**	1.19076	**-9.04549**	**5.38394**	**-5.3349**	-1.00661	**13.4303**
p-values		**< 0.0001**	0.1480	**0.0008**	0.4268	**< 0.0001**	**0.0273**	**0.0269**	0.7526	**0.0009**

Where A= HRT, B = mixing ratio

Based on the table 11, there is strong evidence to reject the null hypothesis and conclude that the predictor variable has a significant effect on the response variable ($P < 0.05$). If the p-value associated with a coefficient is less than 0.05, it is considered statistically significant at the 95% confidence level. The p-value for HRT and mixing ratio was <0.0001, in this case, there is a statistical significance on HRT and Mixing ratio and have evidence to suggest that the predictor variable has an effect on the response variable.

A 95% PI High value represents the upper bound of the prediction interval at a 95% confidence level. It indicates the maximum predicted value that the response variable is expected to reach with 95% confidence. 95% PI Low also represents the lower bound of the prediction interval at a 95% confidence level. It indicates the minimum predicted value that the response variable is expected to reach with 95% confidence. These prediction interval values are useful for assessing the uncertainty associated with the predicted response values and for determining the range within which future observations are likely to fall. The wider the prediction interval, the greater the uncertainty in the predictions.

Table 12: A summary table for key factors of response variables based on the experimental design and analysis.

Analysis	Predicted Mean	Predicted Median	Std Dev	n	SE Pred	95% PI low	95% PI high
Biogas	1613.54	1613.54	51.6138	1	55.3271	1490.27	1736.82
Methane	62.6503	62.6503	3.26131	1	3.4952	55.1539	70.1468
COD Removal	51.7583	51.7583	2.71397	1	2.90922	45.2761	58.2404

Where PI= prediction interval, SE pred= standard error predicted

Table 13 showed that a two-sided confidence interval at a confidence level of 95% indicates that 95% of confident that the true parameter falls within the interval. If we are looking for a point prediction with a two-sided confidence interval at 95% confidence level for a population with 99% coverage, it means to estimate a single value (point estimate) for a parameter of interest with a certain level of confidence, while considering that the population coverage is 99 %. It is important

to note that the confidence level of 95% refers to the likelihood that the interval contains the true parameter value, while the population coverage of 99% suggests that the prediction is made for a large proportion of the population.

Table 13: Point Prediction of biogas production, methane composition and COD removal

Two-sided Confidence = 95% Population = 99%

Analysis	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Biogas	1613.54	1613.54	51.6138	19.9276	1569.14	1657.94	1365.63	1861.46
Methane	62.6503	62.6503	3.26131	1.25709	59.9541	65.3465	48.174	77.1267
COD Removal	51.7583	51.7583	2.71397	1.04784	49.4235	54.093	38.7224	64.7941

Where CI= confidence interval, TI= tolerance interval, SE mean= standard error mean

4.2.4 Model fitting and Analysis of Variance Using ANOVA

To obtain the regression curve, the experimental data was fitted into various models (linear, interactive, quadratic and cubic) and the sequential model sum of squares and model summary statistics tests were used to determine the adequacy of models (Appendices B). The quadratic model for the responses was evaluated by analysis of variance (ANOVA). In the analysis of the study, the F-value of the model for the responses (biogas production, methane composition and COD removal efficiency) is 191.57, 42.33, and 108.09 respectively showing statistical significance with p-value < 0.0001 (Appendices F). The highest f-value and the lowest p-value indicates that the proposed models important and the responses are significantly affected by the factors. The p- value of the lack of fit tests for response was found < 0.0001, which were significantly indispensable that implies the quadratic model were well fitted with the experimental data. The cubic models are well fitted with the experimental data.

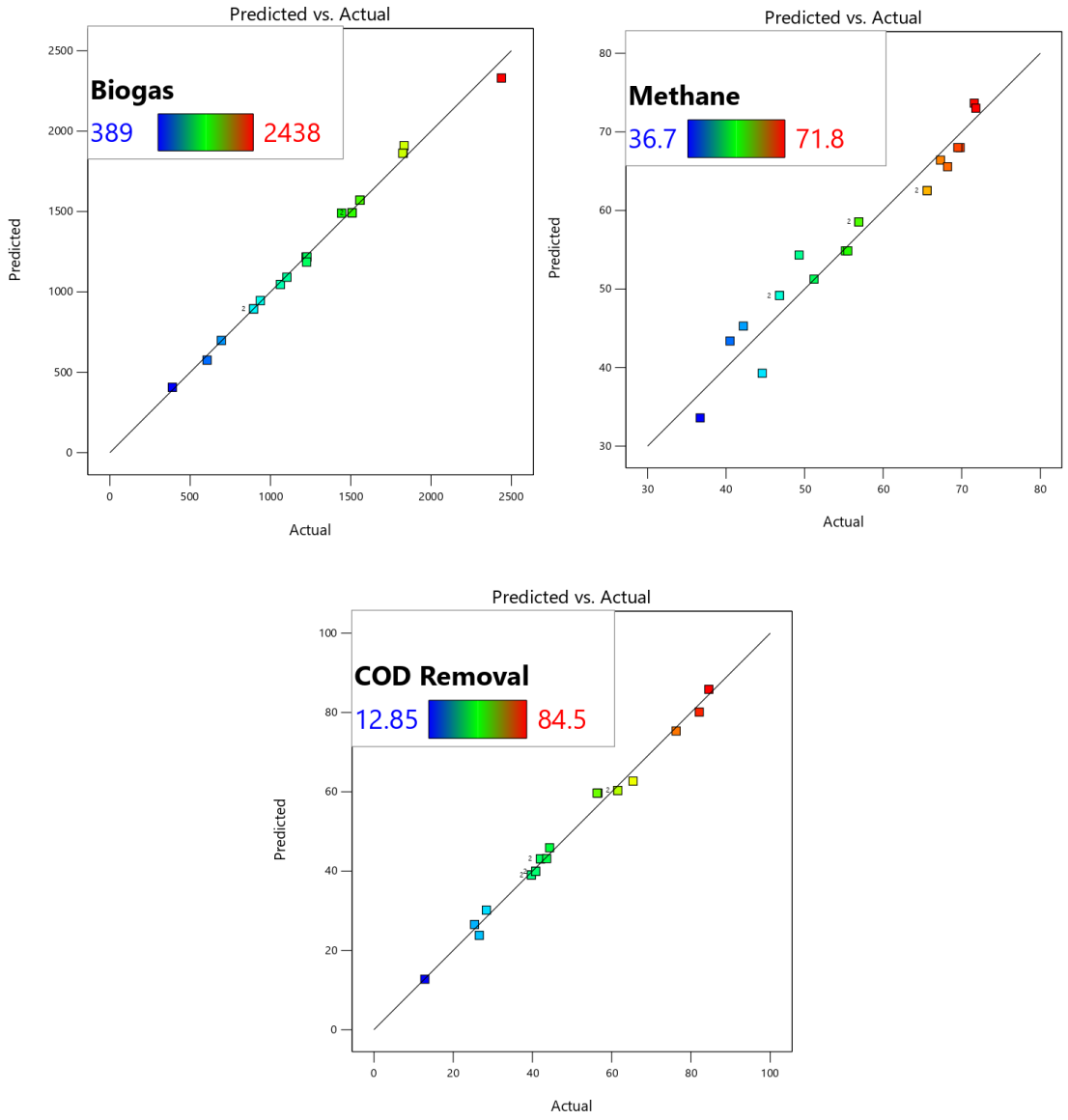

Fig 6: The Predicted vs Actual model Graph of the mean biogas, methane production and COD removal efficiency.

The study used a normal probability plot to check the impact of residuals on surface response and diagnose mathematical models. Good correlation between experimental and predicted values requires a straight line distribution (Fig. 6). Graphical analysis verified model suitability, with normally distributed errors without outliers, independent and randomly dispersed residuals, and all residual values falling within control ranges. The experimental value fit the model well. A three-dimensional wireframe graph represented the functional relationship between the response and experimental factors, while 3D plots displayed the relationship between a response variable

and two factors based on a model equation (Fig. 7). 3-D plots were also used to visualize changes in response surface related to different experimental factors.

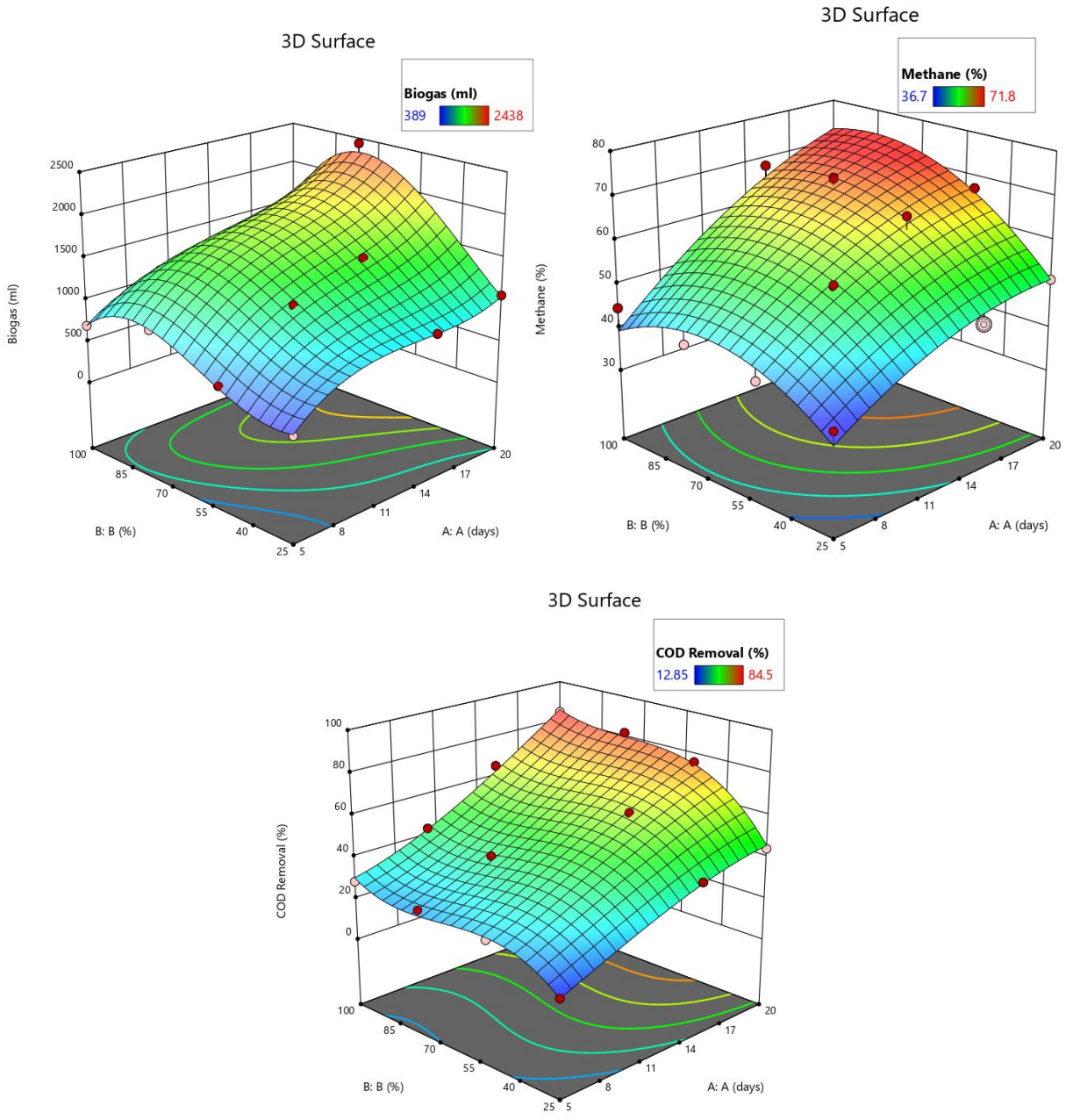

Fig 7: Interaction Effects of hydraulic retention time (A), and mixing ratio (B) on biogas production, methane composition and COD removal efficiency in a response surface methods of 3D Plots .

4.3 Discussion

4.3.1 Effect of HRT and Mixing Ratio on Biogas Production

The study evaluated different mixing ratios of brewery wastewater and brewery surplus yeast for biogas production. The availability and quality of organic matter is the fundamental factor in generating biogas. The amount of biogas produced per kilogram (kg) differs depending on the substrate's composition and nature (Weiland, 2010). The results, as seen in Table7, indicated that the addition of surplus yeast in to wastewater samples shows a significant increase in COD and BOD values, as well as TS and TSS values. This is due to the high organic content of the yeast (Rachwał et al., 2020). Yeast cells are composed of a significant amount of organic matter such as proteins, lipids and carbohydrates when surplus yeast is added to wastewater which contributes to the overall TSS and TS value in the system (Rasmeni et al., 2022). The presence of surplus yeast can improve the efficiency of biogas production and wastewater treatment process.

This findings demonstrated that mixing the substrates resulted in better biogas production compared to mono-digestion similar to previous reports (Alvarez et al., 2014). The peak biogas production was observed in the 3:1 mix of BWW: BSY which was 2438 ml. However, relatively low amount of biogas was produced after 20 days for digestions of 1:3 BWW: BSY (895 ml/800ml of sample and 100% BSY (655 ml/ 800ml sample). The results showed that the mixing of substrates plays a significant role on the biogas production. The least biogas production was observed at 100% BSY due to monomers and small molecular compounds are depleted and larger once take a long time to degrade as hydrolysis Zhu, (2010) and 1:3 BWW :BSY mixing this was due to the flocculation nature of the BSY affected the degradation process and reduced biogas production because insufficient contact time between the waste and microbial biomass could result in inadequate nutrient utilization and lower overall efficiency of the process (Mao et al., 2015). This study was slightly higher than reported by Zupančič et al., (2017) which allows mixing up to 17 % BWW and BSY and lower than Rasmeni et al. (2022) with a mixing ratio of 1:2 of WWS: BSY with the highest bio methane production of 338.2 NmL CH_4/g VS.

The measurement of VS, VSS showed that an increase in yeast percentage resulted in an increase in VS and VSS, which can be attributed to the high organic and nutrient content of the yeast. There was also increased in TP and TN during the addition of yeast accordingly so, the TN, COD, BOD,

and TP increases in all the mixtures as concentration or contribution of yeast increases. The result was expected and this is due to the rich nutrient content nature of spent yeast; which contains high nutrients and mineral content such as N,P,C (Hejna, 2021).

The major point of the result showed in Table 6 was that anaerobic digestion significantly reduces BOD, COD, and TS, with higher reductions at higher mixing ratios of spent yeast. This suggests that anaerobic digestion can effectively treat wastewater containing spent yeast. This study found that anaerobic digestion effectively reduces BOD, COD, and TS in wastewater containing spent yeast, with higher reductions observed at higher mixing ratios of spent yeast. These results are consistent with other research reports and suggest that anaerobic digestion can be an effective treatment method for wastewater containing spent yeast. However, the percentage of removal reported in this study is lower than that reported by Bula, (2020) for different parameters after digestion from an anaerobic wastewater plant and higher than the study which reported 82% of COD, and 66.1% of the total solid removal efficiency after digestion (Gizachew, 2011).

There was also a significant reduction in TN and TP due to methanogenic bacteria decomposing organic matter and biogas production from substrates. The study and related research suggest that effective organic matter reduction leads to increased biogas production. This study indicates that higher BSY mixing volume ratios resulted in increased COD, VS, TS, nutrient content, and alkalinity but the flocculation nature of the yeast cells may affect the decomposition of organic matter through AD (Bauer et al., 2010). However, the concentration of BSY varied for certain parameters such as COD and pH, the pH decreased due to the type of yeast used and beer pH value.

Findings in the present study indicates that there was a high biogas production at the 15^{th} day (667 ml/800ml sample) and this shows that hydraulic retention time had a significant contribution for biogas production in anaerobic digestion (Table7). Biogas production increased with longer retention time up to day 15 due to better sludge stability and increased contact time between biomass and bacteria similar to recent reports (Sawyerr et al., 2019b). The study found that higher HRT led to higher removal efficiency, which is in line with previous research. It is important to note that retention time can vary depending on factors such as feedstock type and environmental conditions, and BSY solubility can limit organic substrate degradation. On the other hand, the study used biogas production as an indicator of optimum production and digestion process

development. The results showed that biogas production decreased after day 15 but continued until day 20. These findings highlight the importance of monitoring biogas production over time to ensure optimal digestion process development (Liu et al., 2018).

4.3.2 Effect of Mixing Ratio and HRT on Methane Composition

The percentage of methane obtained from the biogas also varies based on the type of biomass material used (Weiland, 2010). As a result, combining both wastes may result in improved biogas volume and composition. The production of biogas using AD was determined by methane composition. The methane percentage in the present study showed that a direct relation between biogas production volume (Schnürer and Jarvis, 2010). The volume of biogas increased when an increase on HRT and the biogas production on the 20^{th} day of the experiment results higher methane content for all mixing ratios (Baâti et al., 2018). This study found that the mixing ratio of brewery wastewater and surplus yeast affects biogas and methane production. The highest methane content of 70.6±1.2% was observed at a 75:25% BWW: BSY mixing ratio and a hydraulic retention time (HRT) of 20 days' (Table 7). A better methane composition with an average of 68.4 % and 70.6 % was observed when digestion of BWW alone and 3:1 mix ratio of BWW: BSY respectively so, the obtained result shows mixing ratio and retention time determine the quality of biogas production in the anaerobic digester (Singh et al., 2020).

The methane content in this study was found to be higher than that reported by Mubeshir, (2020), who found a methane (CH_4) content of 69.74% during co-digestion of brewery waste and Khat waste with a 75:25 mixing ratio of anaerobic digestion. However, it was slightly lower than the methane content of 70.8% reported by Maragkaki et al. (2017) during anaerobic co-digestion of sewage sludge (SS) with olive mill wastewater (OMW), cheese whey (CW), and crude glycerol (CG). Furthermore, the study carried out by Dalkilic and Ugurlu, (2015) showed average methane content of produced biogas was 74% during anaerobic digestion of chicken manure at different organic loading rates (OLRs), in a mesophilic-thermophilic two-stage anaerobic system which is higher than the current study.

Therefore, the study found that longer retention times led to increased biogas production and methane content. This relationship was statistically significant, with a notable difference between a retention time of 15 days and other retention times. As the hydraulic retention time (HRT) increased, biogas production and methane content also increased, as demonstrated in Table 7. This

is likely due to the increased contact between the biomass and microbial biomass, which leads to higher biogas production (Kasinath et al., 2021).

4.3.3 COD Removal Efficiency for Brewers Wastewater and Brewers Surplus Yeast

A positive correlation was found between brewers' wastewater (BWW) and brewers surplus yeast (BSY) mixture digestion were combined, significant COD removal efficiencies were typically seen. The highest COD removal efficiency was observed at 3:1 BWW: BSY (85.08%) and the lowest was observed at 1:3 BWW: BSY (44.33%) and 100% BSY (35.39%) mixing ratio respectively. As BSY addition increased, the COD removal efficiency significantly decreased, according to the results. Table 7 shows that the efficiency of COD removal was assessed by considering the BWW: BSY mixing ratio and retention time. The results demonstrate a clear correlation between hydraulic retention time and the percentage of COD removal, with an increase in HRT resulting in improved efficiency. Specifically, the highest COD removals were observed at a 75:25% volume ratio, with a removal efficiency of 85.08% at 20 day HRT.

The present study findings were in line with Tafesh et al. (2011) which revealed that the co-digestion of swine manure and wastewater from olive mills in a UASB reactor and found high COD removal efficiency of 85–95%. On the other hand, the present study results disagree with Hublin et al. (2012), in which a low TCOD removal efficiency of 56.3% during the co-digestion of whey and cow manure, using a mixture containing 33% wastewater from olive mills and 67% swine manure. Similarly, a study conducted by Andualem et al. (2016) on the anaerobic co-digestion of cattle dung and tannery wastewater for the production of biogas showed a slightly lower COD removal efficiency (82%) than the present study.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Anaerobic digestion has the potential to secure energy supplies while satisfying the waste utilization's multifaceted requirements. In this study, bio methane was produced through the anaerobic co-digestion of brewers' wastewater (BWW) and brewers surplus yeast (BSY). Anaerobic co-digestion increases the load of biodegradable organic matter, which in turn increases the production of bio methane, dilution of potentially toxic compounds, and provision of missing nutrients. Low levels of bio methane have been produced by the mono-digestion of BSY and 25% BWW to 75% BSY mixing samples. On the other hand, BWW and BSY produced the most bio methane 2438 ml and the best co-digestion ratio was observed at 75 % BWW and 25 % BSY (3:1 BWW: BSY) with a 20 days of HRT. There was high production of biogas was observed on the 15 days of HRT of digestion. So, inadequate mixing and low HRT leads to the non-uniform distribution of substrates, enzymes and microorganisms, incomplete stabilization of wastes, a decrease of methane production and less efficient pathogen destruction.

Overall, those results suggested that BSY had the potential to serve as a reliable co-substrate for bio methane production through anaerobic co-digestion with BWW. The present finding has shown the importance of proper mixing and management of substrates to ensure uniform distribution of enzymes and microorganisms, complete stabilization of wastes, and efficient pathogen destruction during anaerobic digestion. The findings of the study contributed to the development of sustainable waste management practices and renewable energy production from waste materials.

5.2 Recommendations

- To increase the production of biogas from the co-digestion of brewery wastewater and spent yeast, factors like digester design should be considered.

- The findings of the present study are not all comprehensive. Future research should include a thorough characterization of brewery wastewater and BSY, an assessment of reactor removal performance, and an evaluation of parameters that affect biogas production, such as C/N ratio and sludge characterization.

- Routine monitoring of the bacterial composition and population of the ASBR reactor is required so as to get a balanced operation of the basic processes.

- Characterization of the digestate needs further analysis to use as a bio fertilizer.

❖ Note that while the study's results highlight the system's potential at the laboratory scale for treating brewery wastewater, it is not advised to scale up this system up to full scale without first completing a pilot scale study project.

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7. Appendices

7.1 Appendices A: Experimental pictures which were Undertaken During Laboratory Work

Water bath at AASTU

Conductivity meter

Oven

Sample filtration for TS measurement

COD digester (measuring COD)

BOD digester (BOD measurement)

pH measurement of the samples at Heineken breweries WWT laboratory.

7.2 Appendices B: Model summary statistics of biogas production, methane content and COD removal efficiency

7.2.1 Table Model Summary Statistics of biogas production

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	349.64	0.5501	0.4972	0.3307	3.092E+06	
2FI	358.89	0.5539	0.4703	0.0992	4.161E+06	
Quadratic	176.29	0.9058	0.8722	0.6701	1.524E+06	
Cubic	**51.61**	**0.9942**	**0.9890**	**0.9395**	**2.794E+05**	**Suggested**
Quartic	46.41	0.9967	0.9911	0.4151	2.702E+06	Aliased

Std. Dev.	51.61		**R^2**	0.9942
Mean	1287.40		**Adjusted R^2**	0.9890
C.V. %	4.01		**Predicted R^2**	0.9395
			Adeq Precision	52.7098

7.2.2 Table Model Summary Statistics of methane content

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	6.18	0.7398	0.7092	0.6354	908.73	
2FI	5.87	0.7790	0.7375	0.5967	1005.17	
Quadratic	**3.12**	**0.9455**	**0.9260**	**0.8139**	**463.79**	**Suggested**
Cubic	2.75	0.9697	0.9424	0.4999	1246.29	
Quartic	0.7027	0.9986	0.9962	0.9240	189.52	Aliased

Std. Dev.	3.12		**R^2**	0.9455
Mean	56.60		**Adjusted R^2**	0.9260
C.V. %	5.50		**Predicted R^2**	0.8139
			Adeq Precision	23.4733

7.2.3 Table Model Summary Statistics of COD removal

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	7.01	0.8812	0.8672	0.8251	1229.08	
2FI	6.34	0.9086	0.8915	0.8241	1236.44	
Quadratic	5.03	0.9496	0.9316	0.8802	842.02	
Cubic	**2.40**	**0.9918**	**0.9844**	**0.9331**	**470.14**	**Suggested**
Quartic	1.91	0.9963	0.9901	0.5177	3389.70	Aliased

7.3 Appendices C: - Table of VS, VSS and TSS measurement result and calculation

Sample name	Unit	WA	WB	WC
100WW	gm	42.5470	42.7230	42.6584
100BSY	gm	41.8700	45.2430	42.3751
50/50 BSY:BWW	gm	42.7120	44.4698	42.8845
75/25 BSY:BWW	gm	42.2255	44.9197	42.8845
25/75 BSY:BWW	gm	40.8502	41.9838	40.9983

Where: - WA= weight of crucible

WB=weight of sample before muffle furnace

WC=weight of sample after oven

V=Sample volume= 50ml

Calculation TS (mg/L) = B-A/V * 10^6

VSS (mg/L) = B-C/V * 10^6

VS= C/B * 100

7.4 **Appendices E:** Figures obtained from design expert 13

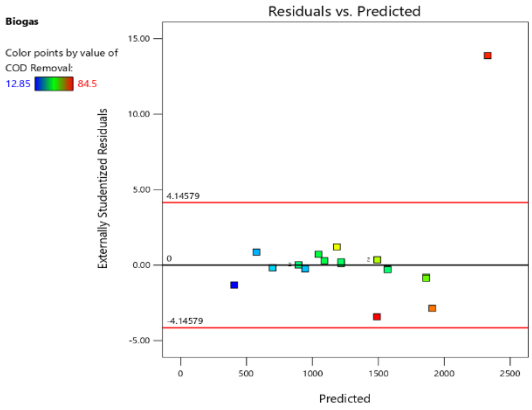

Fig.1A residuals vs predicted graph for biogas production.

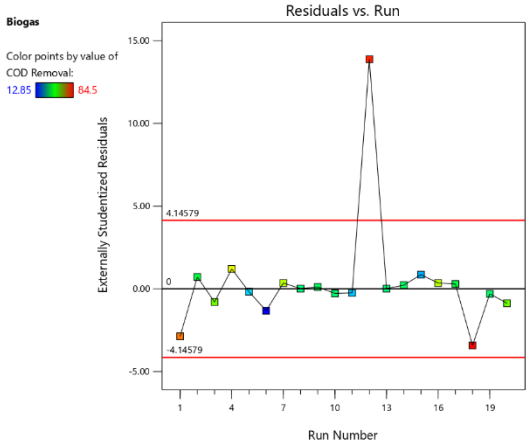

Fig.1B residuals vs run graph for biogas production.

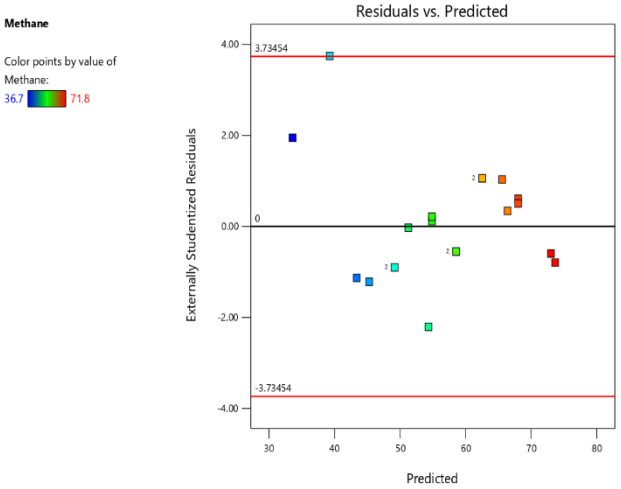

Fig.2A residual vs predicted graph for methane %

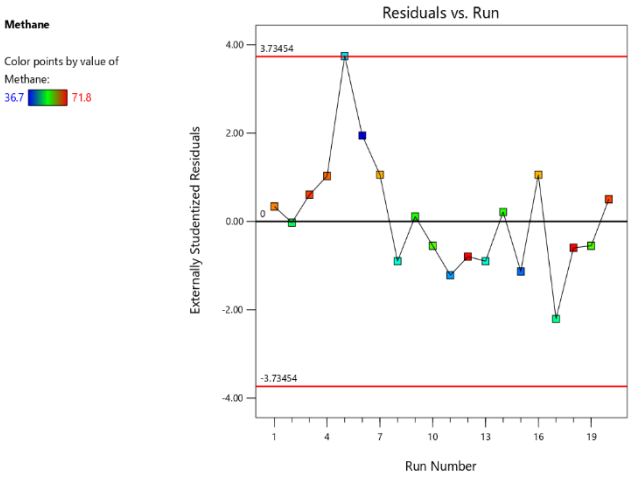

Fig.2B residuals vs run graph for methane%

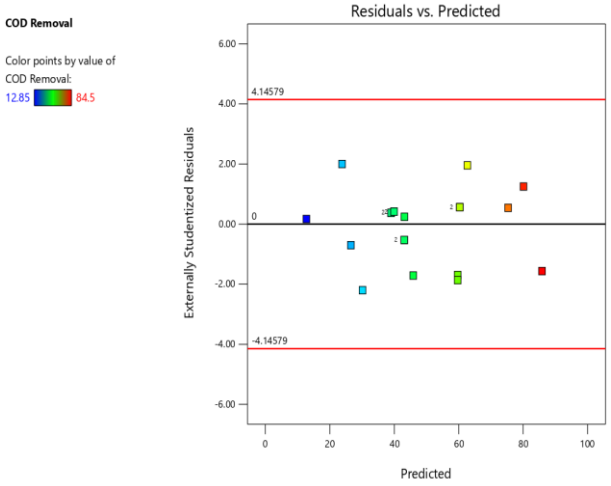

Fig.3A residuals vs predicted graph for COD removal

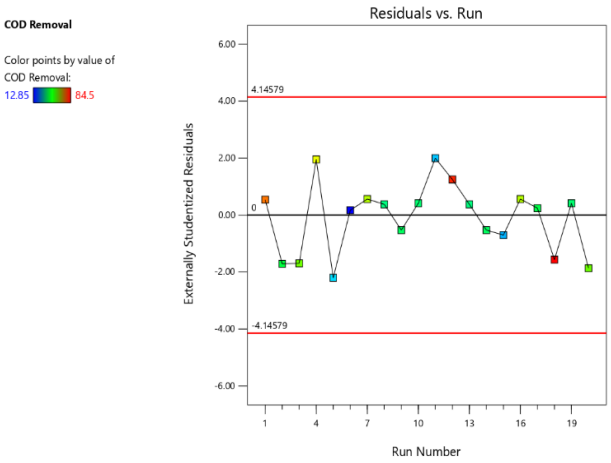

Fig.3B residuals vs run graph for COD Removal

7.5 Appendices F: Model summary

A. Response 1: Biogas

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.593E+06	9	5.103E+05	191.57	< 0.0001	significant
A-A	1.628E+05	1	1.628E+05	61.09	< 0.0001	
B-B	3.326E+05	1	3.326E+05	124.84	< 0.0001	
AB	6903.89	1	6903.89	2.59	0.1385	
A^2	41024.83	1	41024.83	15.40	0.0028	
B^2	1.235E+06	1	1.235E+06	463.69	< 0.0001	
A^2B	5531.31	1	5531.31	2.08	0.1802	
AB^2	2.271E+05	1	2.271E+05	85.25	< 0.0001	
A^3	62599.83	1	62599.83	23.50	0.0007	
B^3	1.859E+05	1	1.859E+05	69.78	< 0.0001	
Residual	26639.81	10	2663.98			
Lack of Fit	26622.31	5	5324.46	1521.27	< 0.0001	significant
Pure Error	17.50	5	3.50			
Cor Total	4.620E+06	19				

B. Response 2: Methane

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2250.92	5	450.18	42.33	< 0.0001	significant
A-A	1433.17	1	1433.17	134.75	< 0.0001	
B-B	390.41	1	390.41	36.71	< 0.0001	
AB	54.37	1	54.37	5.11	0.0402	
A^2	79.40	1	79.40	7.46	0.0162	
B^2	316.25	1	316.25	29.73	< 0.0001	
Residual	148.91	14	10.64			
Lack of Fit	148.82	9	16.54	918.62	< 0.0001	significant

Pure Error	0.0900	5	0.0180			
Cor Total	2399.82	19				

C. Response 3: COD Removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7165.16	9	796.13	108.09	< 0.0001	significant
A-A	708.34	1	708.34	96.17	< 0.0001	
B-B	18.10	1	18.10	2.46	0.1480	
AB	163.20	1	163.20	22.16	0.0008	
A^2	5.05	1	5.05	0.6862	0.4268	
B^2	284.27	1	284.27	38.59	< 0.0001	
A^2B	49.15	1	49.15	6.67	0.0273	
AB^2	49.42	1	49.42	6.71	0.0269	
A^3	0.7736	1	0.7736	0.1050	0.7526	
B^3	157.68	1	157.68	21.41	0.0009	
Residual	73.66	10	7.37			
Lack of Fit	73.63	5	14.73	2783.73	< 0.0001	significant
Pure Error	0.0265	5	0.0053			
Cor Total	7238.81	19				