

Research Article

Biogas Production through Anaerobic Codigestion of Distillery Wastewater Sludge and Disposable Spent Yeast

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The ongoing industrial transformation in developing countries, including Ethiopia, has resulted in a significant increase in harmful pollutants in the environment. Various industrial activities release toxic wastewater sludge and spent yeast into the surrounding ecosystem, posing risks to public health and the environment. However, these waste materials have the potential for energy extraction and recycling. This study aimed to investigate and harness the biogas potential through anaerobic codigestion of distillery wastewater sludge and waste yeast. The researchers employed a response surface approach utilizing Box-Behnken experimental designs (BBD) to assess the three key experimental parameters influencing biogas yield: pH levels (6, 7, and 8), volume ratio (85, 92, and 99%), and temperature (33, 36.5, and 40°C). Before and after the digestion process, the researchers measured the total solids (TS), biological oxygen demand (BOD₅), chemical oxygen demand (COD), and pH of all substrates. Additionally, measurements of temperature, total nitrate, and total phosphate were taken before digestion. The methane yield was modeled using a second-order polynomial through the BBD method in Design Expert software, with a p value threshold of \leq 5%. The results showed that the maximum methane yield of 61.18% was achieved at a pH of 7, a temperature of 36.5°C, and a volume ratio of 92%. Conversely, the lowest methane yield of 40.13% was obtained at a pH of 6, a temperature of 33°C, and a volume ratio of 92%. The linear and quadratic values of the model (A, B, C, A^2 , B^2 , and C^2) were determined to be significant terms, with p values ≤5%. Overall, the biogas yields obtained from the anaerobic codigestion of distillery wastewater and waste yeast were promising. This process has the potential to effectively remove BOD₅, COD, and TS from distillery spent wash and sludge. The findings suggest that anaerobic codigestion could be a viable approach for both energy production and waste management in the setting of distillery waste.

1. Introduction

1.1. Background. Global greenhouse gas emissions from fossil fuels are a significant threat to the environment. The combustion of fossil fuels releases carbon dioxide (CO_2) and other greenhouse gases into the atmosphere, which trap heat and contribute to global warming and climate change [1–3]. Fossil fuels, including coal, oil, and natural gas, are unsustainable energy sources because they are finite and take several years to form. Their extraction and use have numerous negative environmental impacts. In addition to greenhouse gas emissions, burning fossil fuels releases air pollutants that contribute to air pollution and

have detrimental effects on human health [4, 5]. The reliance on fossil fuels as the primary source of energy globally is a major factor driving environmental pollution and climate change. It is worth noting that the energy sector is the largest source of greenhouse gas emissions worldwide [6]. Fossil fuels are the main source of energy worldwide, with approximately 81.1% supplied by petrochemical resources [3]. Renewable energy, such as solar, wind, hydro, and geothermal power, offers cleaner alternatives that produce little to no greenhouse gas emissions during operation [7]. These sources of energy are also considered sustainable because they naturally replenish over time. The use of renewable energy technology can have several positive effects on the environment and energy sector such as reducing reliance on fossil fuels and decreasing greenhouse gas emissions [8]. This transition to a more sustainable energy system is essential for addressing climate change, protecting the environment, and ensuring a more sustainable future [9]. It is worth mentioning that government policies, technological advancements, and public awareness and participation play vital roles in accelerating the adoption of renewable energy and transitioning away from fossil fuels.

Renewable energy production indeed has the potential to address several pressing issues and contribute to sustainable development [2, 8]. Renewable energy sources such as solar, wind, hydropower, and geothermal can provide reliable and affordable energy for various purposes such as electricity generation, heating, cooling, and transportation [2, 8]. By ensuring access to clean and affordable energy, renewable sources can meet the basic energy needs of communities, especially in remote or underdeveloped areas [10]. The use of renewable energy sources can significantly reduce environmental problems associated with energy consumption [11]. Fossil fuel combustion releases pollutants into the air, contributing to air pollution, smog formation, and adverse health effects [10]. Renewable energy technologies produce minimal or no emissions during operation, mitigating air pollution and its associated risks [12]. Furthermore, renewable energy plays a crucial role in reducing greenhouse gas emissions, thereby helping to combat climate change and its wide-ranging environmental impacts [13]. Bioenergy, derived from organic waste materials, can be a valuable form of renewable energy. By utilizing environmentally hazardous waste from industries and other sources, bioenergy production can help address waste management challenges while providing a sustainable energy source [13, 14]. Properly managed bioenergy systems can help reduce the environmental impact of waste disposal, minimize greenhouse gas emissions from decomposition, and contribute to a circular economy approach [15].

Waste management is indeed a significant environmental issue in modern society, and waste utilization as a renewable energy source can offer an effective alternative [15]. Waste-to-energy technologies, such as anaerobic digestion, have gained prominence as a resourceful and effective way to utilize waste for renewable energy production [16, 17]. These technologies convert organic waste materials into biogas through processes like anaerobic digestion or biological conversion [18, 19]. By harnessing the energy content of waste, these technologies can generate heat, electricity, or biofuels, reducing the reliance on fossil fuels and mitigating environmental issues associated with waste disposal.

Anaerobic digestion is a renewable energy technology that converts organic waste, such as food waste, agricultural residues, or wastewater sludge, into biogas [18, 20]. Compared to other renewable energy sources such as hydropower, solar, or wind power, anaerobic digestion offers certain advantages. It typically requires lower capital investment and occupies less installation area, making it a favorable option in areas with limited space or financial resources. Anaerobic digestion has attracted significant attention in the global community due to its relatively low capital costs. The technology is relatively mature and widely implemented, offering a cost-effective solution for waste management and renewable energy production [21]. The utilization of waste as a resource through anaerobic digestion can provide economic benefits by reducing waste management costs, creating revenue streams from energy generation, and potentially offsetting fossil fuel expenditures [17].

Ongoing technological advancements in anaerobic digestion have further improved its efficiency and economic viability. Innovations such as codigestion (combining different waste streams), pretreatment methods, and process optimization have contributed to enhancing the performance and cost-effectiveness of anaerobic digestion systems [22, 23]. These advancements help reduce operational costs and improve the overall feasibility of waste-to-energy methods [24].

The drawbacks associated with single-substrate digestion systems in anaerobic digestion are well recognized. These drawbacks include limitations in terms of substrate properties and the optimization of the overall system [21, 25, 26]. By implementing anaerobic codigestion, these limitations can be mitigated, particularly in developing countries like Ethiopia where waste management practices are poor [27]. In such contexts, anaerobic codigestion holds significant importance as it offers opportunities to improve energy production and waste management practices. Ethiopia has witnessed socioeconomic growth in recent decades, leading to increased waste generation rates across various sectors, including agriculture, industry, households, and commercial activities [12]. This surge in waste generation necessitates effective waste management strategies. In this particular study, the focus is on using distillery wastewater sludge and waste yeast from the National Alcohol and Liquor Factory (NALF) as substrates for codigestion in biogas production.

Both distillery spent yeast and wastewater sludge are characterized by high levels of pollutants, including biological oxygen demand (BOD₅), total phosphate (TP), chemical oxygen demand (COD), total nitrate (TN), and total solids (TS) content [12, 26]. These substances are considered environmental pollutants and can lead to issues such as eutrophication, foul odors, and adverse health effects on animals and other organisms [16, 27]. The objective of the study is to convert these potential environmental pollutants into valuable bioenergy in the form of methane or biogas production. By employing anaerobic codigestion, the organic materials present in the distillery wastewater sludge and waste yeast can be effectively and sustainably utilized to generate biogas. This process not only helps in waste management by reducing the pollutant load but also harnesses the potential energy contained in these substrates, thereby contributing to renewable energy production. Therefore, the study aims to address the challenges posed by the disposal of distillery wastewater sludge and waste yeast by utilizing anaerobic codigestion to convert these waste materials into valuable bioenergy. This approach aligns with the principles of sustainable waste management and renewable energy production, which are particularly important in developing countries like Ethiopia.

The novelty of this study was described as focusing on enhancing biogas production and methane yield through the application of codigestion and anaerobic digestion techniques. The researchers employed a codigestion process involving two different substrates: distillery wastewater sludge and spent yeast. These substrates were subjected to anaerobic digestion at a temperature range of 33 to 40°C. To obtain biogas through the anaerobic digestion process, the researchers characterized both substrates using advanced instruments and standard methods. The characterization involved measuring various parameters such as pH, total solids (TS), total nitrate (TN), biological oxygen demand (BOD₅), total phosphate (TP), temperature, and chemical oxygen demand (COD). The study utilized the response surface methodology Box-Behnken design (RSM-BBD), which is a statistical technique that allows for the systematic examination of operating parameters and the identification of optimal conditions. By applying this methodology, the researchers aimed to optimize the anaerobic codigestion process. One significant aspect of the study is the analysis of the chemical oxygen demand (COD) removal efficiency of the anaerobic codigestion process. This analysis provides information on the effectiveness of the process in removing organic pollutants. The study also highlights the potential for multiple applications of the anaerobic codigestion process. Overall, the study explores the possibility of utilizing distillery waste, which is both environmentally and health hazardous, for bioenergy production. By converting this waste into biogas, the researchers aim to reduce the ecological impacts associated with the National Alcohol Liquor Factory (NALF). The study demonstrates promising results in terms of biogas production through the simultaneous digestion of both substrates in the anaerobic digestion process.

2. Material and Methods

The study input raw material samples were collected from the National Alcohol and Liquor Factory (NALF) premises located on the south bank of the little Mekanisa River in the capital city of the country. The geographic coordinates of the sampling site are 8° 58′ 32″ N and 38° 44′ 00″ E at an altitude of 2229 m above sea level. This is described by the relative humidity of 57.97% and the annual mean temperature of 16.12°C with 1092 mm of rainfall, which is indicated in Figure 1.

2.1. Collection of Distillery Wastewater Sludge and Waste Yeast. This study focuses on using distillery wastewater sludge and spent yeast as feedstock for anaerobic biogas production. The distillery wastewater sludge was obtained directly from the sludge storage facility of the National Alcohol and Liquor Factory (NALF). The spent yeast was obtained directly from the yeast growth tanks at NALF. The distillery wastewater sludge and spent yeast samples were collected in precleaned and acid-washed plastic bottles. This ensures that the samples are free from contaminants that could affect the analysis. The collected samples were stored in a refrigerator at 4 degrees Celsius until they were used for analysis. A time-composite sampling method was used for analyzing physicochemical parameters such as total solids (TS), chemical oxygen demand (COD), biological oxygen demand (BOD₅), total nitrate (TN), and total phosphate (TP). This method involves collecting multiple samples over a specific time and combining them to obtain a representative composite sample.

The collected composite samples were analyzed in the laboratory to measure the physicochemical parameters mentioned above. These parameters provide valuable information about the composition and characteristics of the distillery wastewater sludge and spent yeast. In addition to the composite sampling approach, a grab-sampling approach was used to measure physicochemical parameters such as pH and temperature directly at the sites where the samples were collected. This approach involves taking instantaneous samples at specific locations and times to obtain immediate measurements. By analyzing the physicochemical parameters of the distillery wastewater sludge and spent yeast, the study aims to evaluate their potential for anaerobic biogas production. Anaerobic digestion processes can convert organic materials, such as sludge and yeast, into biogas, which primarily consists of methane and carbon dioxide.

2.2. Analytical Methods. Throughout the experiment, equipment and devices such as a rubber hose, incubator, drying oven, furnace, spectrophotometer, test tube, and measuring cylinder were used to measure total solids (TS), chemical oxygen demand (COD), biological oxygen demand (BOD₅), total nitrate (TN), and total phosphate (TP). The physicochemical parameters of both raw materials used in this analysis and their test methods are summarized in Table 1.

2.2.1. Analysis of Physicochemical Parameters of the Substrates. Using standard procedures as outlined in the WHO (2011) guidelines, measurements of temperature (T) and hydrogen potential (pH) were made on-site during sample collection. The T and pH of the samples were measured using the portable digital multiparameter probe, HACH instrument (model HQ440D, USA) in duplicate having the respective electrodes.

Using a dichromatic reflux method, the substrate sample's chemical oxygen demand (COD) was determined under the American Public Health Association (APHA) by the addition of 10 mL of 0.25 N potassium dichromate (K₂Cr₂O₇) and 30 mL H₂SO₄ + Ag₂SO₄ reagent in 20 mL diluted sample. The mixtures were refluxed for 2 hours and cooled at room temperature. Then, the solutions were diluted to 150 mL by using distilled water, and excess K₂Cr₂O₇ remained were titrated with ferrous ammonium sulfate (FAS) using a ferroin indicator.



FIGURE 1: The location of the distillery wastewater sludge and spent yeast sampling site of NALF.

TABLE 1: Standard testing methods for physicochemical parameters of distillery spent sludge and yeast.

S. no	Parameters	Standard testing methods used
1	TN	TNT persulphate digestion method, HACH's UV-VIS spectrophotometer (model DR6000, USA)
2	TP	APHA 5220 B, molybdovanadate method, HACH's UV-VIS spectrophotometer (model DR6000, USA)
3	TS	APHA 5220 B, total solid dried at 103-105°C
4	COD	APHA 5220 B, open reflux method
5	BOD ₅	APHA 5210 B, 5-day BOD test
6	pH	HACH instrument (model HQ440D, USA) field case
7	Temperature	HACH instrument (model HQ440D, USA) field case

The chemical oxygen demand (COD) of the substrates sample was measured using the dichromatic reflux and titration methods in the presence of potassium dichromate ($K_2Cr_2O_7$) and $H_2SO_4 + Ag_2SO_4$ reagent diluted sample or buffer solution and ferrous ammonium sulfate (FAS) using as an indicator by the addition of 10 mL of 0.25 N potassium dichromate ($K_2Cr_2O_7$) and 30 mL $H_2SO_4 + Ag_2SO_4$ reagent in a 20 mL diluted sample. The mixtures were refluxed for 2 hours and cooled at room temperature. Then, the solutions were diluted to 150 mL by using distilled water, and excess $K_2Cr_2O_7$ remained were titrated with ferrous ammonium sulfate (FAS). The COD values were determined using the following equation:

$$COD = \frac{((A - B) \times N \times 8 \times 1000 \text{ mL/L})}{V}, \qquad (1)$$

where A is the mL of FAS used for blank, B is the mL of FAS used for the sample, V is the volume of the sample, N is the normality of FAS, and 8 is the mill equivalent weight of oxygen APHA (2005).

Biological oxygen demand (BOD₅) was estimated by preparing the required volume of dilution water with the addition of nutrients namely phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride. The diluted samples were transferred to BOD bottles. After determining the initial dissolved oxygen, the final dissolved oxygen was estimated in the bottles kept in the incubation at a constant temperature of 20 degrees Celsius (68 degrees Fahrenheit) for five days. This method follows the standard procedure for estimating BOD_5 , as outlined in the WHO (World Health Organization) guidelines.

Using HACH's UV-VIS spectrophotometer (model DR6000, USA) at the wastewater quality control laboratory of National Alcohol and Liquor Factory (NALF), Addis Ababa, Ethiopia, according to the procedure described by American Public Health Association (APHA), measurements of total nitrate (TN) and total phosphate (TP) were determined by using TNT persulphate digestion and molybdovanadate methods, respectively. Before measurement, the samples were digested in 1:1 HCl on a sand bath. After digestion, distilled water was used to mark the complex, reagents were added, and color change was observed, and then, the samples were read by using a UV-visible spectrophotometer.

2.3. Experimental Design

2.3.1. Response Surface Methodology (RSM). Response surface methodology (RSM) is indeed a statistical technique used for modeling and optimizing multiple independent variables that affect an outcome variable [28, 29]. It is widely

employed to improve the optimization of process parameters and assess product effectiveness. In this study, RSM can be utilized to develop a mathematical model that predicts the optimal values of process variables, thus maximizing biogas yield. By analyzing contour plots or response surface plots generated from experimental design data, RSM enables the identification of the most effective conditions for achieving optimal yield [28]. These visual representations illustrate the influence of different factors on the dependent variable, assisting in determining the ideal conditions for biogas production [29]. The implementation of RSM offers the advantage of reducing the number of experiments required to optimize the biogas production process [26]. This reduction in experimentation can lead to significant savings in time and resources. Additionally, RSM allows for the identification of variable influences on the outcome variable, such as biogas yield.

Response surface methodology, along with techniques like BBD, offers a systematic and efficient approach to optimizing biogas production processes. It allows for the modeling of influential variables, prediction of optimal process conditions, and reduction of experimental efforts, ultimately leading to improved yield and potential cost savings. The Box-Behnken design (BBD) is a useful experimental design method for researchers to systematically manipulate independent variables and study their effects on a response variable. It is particularly effective when the relationship between the independent variables and the response variable is nonlinear [26]. The independent variables being manipulated were the volume ratio (A), pH (B), and temperature (C), and the response variable being measured is the percentage of biogas yield. Selvankumar et al. used BBD in conjunction with RSM to maximize CH₄ yield output from cow dung with alkali-treated coffee pulp. A three-dimensional (3-D) response surface is presented in the study to explain the influence of processing factors on biogas generation. According to Malik et al. and Elazhar et al., the BBD method was used because the extreme points are unknown, and there is a need to consider the effect of curvature. BB is a spherical design with all the points sitting on the radius of the sphere and does not include any points on the extreme corner of a cube [29]. The BBD generates a response surface that helps assess the impact of interaction terms between the independent variables on the response variable. To conduct the BBD and analyze the outcomes, the study utilized Design Expert version 13 software. This software is specifically designed for experimental design and analysis, providing tools to generate the BBD and evaluate the results.

The BBD has been chosen in this study to strike a balance between exploring the experimental limits, avoiding extreme treatment combinations, and optimizing resource utilization [30, 31]. The BBD typically utilizes mid-levels of other factors, which allows the study to focus on the expected optimum regions [31]. This concentration of experimental runs around the anticipated optimal conditions improves the efficiency of obtaining methane yield or biogas and increases the likelihood of obtaining meaningful outcomes (CH₄). One possible reason for using the BBD could be the desire to explore a spherical experimental region [32, 33]. The BBD is known for providing a relatively balanced and efficient allocation of experimental points within a design space, allowing for a more reliable estimation of response surfaces [28, 33]. This balanced allocation of points can help ensure a full examination of the experimental region, including the exploration of various combinations of factors within a feasible and safe range [29]. These extreme combinations of temperature, pH, and volume could potentially lead to hazardous or extreme values of the response. By utilizing the BBD, which avoids extreme factor combinations, the researchers could ensure that the experiments were conducted within a safe and feasible range.

The experiments in this study are carried out utilizing the Box–BB experimental design method. Factors such as volumetric ratio (%), pH, and temperature (°C) are denoted as L_1 , L_2 , and L_3 , respectively. The experimental range of the true level of the factor level of the three independent variables is shown in Table 2.

The above design elements are selected based on rationale. The volume ratio of distillery wash sludge to distillery waste yeast, 85%, 92%, and 99%, was chosen based on the biodegradability index (BI) of the waste materials. A biodegradability index (BI) of 0.6 or higher suggests that the waste is more easily degraded and digested by bacteria [34, 35]. The pH value and temperature were selected based on the behavior of the anaerobic bacteria involved in the digestion process. A pH range of 6-8 and a temperature range of 33-40°C were chosen as they have been found to increase the activity and reproduction of these anaerobic bacteria according to various literature sources [36-40]. To experiment, Design Expert 13 software was used to calculate the experimental design, taking into account three treatment parameters: volume ratio, pH value, and temperature. Additionally, five central replication points were considered to enhance the reliability and validity of the experimental results.

2.4. Experimental Operation. The researchers conducted the study using a batch anaerobic reactor and performed a total of 17 tests. During the tests, different combinations of temperature, distillery wastewater sludge to waste yeast ratios, and pH values were examined. The temperature, pH, and volume ratios are important factors that can influence the efficiency of the anaerobic digestion process and biogas production. To maintain consistency, the researchers used a constant hydraulic retention time of 24 days throughout all the tests. Hydraulic retention time refers to the average amount of time that the feedstock remains in the reactor [38]. At the end of the 24-day operation period for each test, the researchers measured the amount of biogas produced. Biogas typically consists of methane (CH₄) and carbon dioxide (CO₂), among other trace gases, and is generated as a result of the anaerobic decomposition of organic matter [39]. The purpose of the study was to identify the optimal combination of operating conditions that maximized biogas production during the codigestion of distillery wastewater sludge and waste yeast. By systematically varying the

TABLE 2: Factors and their levels that can affect the methane yield.

Variables	Symbol	Actual value		
variables		-1	0	1
Volumetric ratio (%)	L_1	85	92	99
pН	L_2	6	7	8
Temperature (°C)	L_3	33	36.5	40

temperature, pH, and volume ratios, the researchers aimed to determine the most favorable conditions for efficient biogas generation in the batch anaerobic reactor. The specific results and conclusions of the study, including the effects of temperature, pH, and volume ratios on biogas production, were described in this study.

The characterization process likely involved analyzing various parameters such as chemical composition, organic content, nutrient content, and other relevant factors. By characterizing the raw materials, the researchers obtained a detailed understanding of the composition and quality of each feedstock component. Additionally, the researchers also characterized the mixtures of the two components, which refer to the distillery wastewater sludge and spent yeast combined. This step was crucial to determine the composition and properties of the feedstock mixture before it was used for anaerobic digestion. After characterizing the raw materials and mixtures, the researchers determined the required proportion of the collected samples. This helps to decide on the specific ratio or mixture ratio of distillery wastewater sludge and spent yeast that was used for anaerobic digestion. The purpose of characterizing the raw materials and mixtures and determining the required proportion of the samples was to ensure the consistency and accuracy of the experimental setup [40].

Anaerobic digestion was conducted in 1-liter polyethylene plastic bottles. These bottles were placed in a temperature-controlled water tank in the NALF wastewater testing laboratory. The 1-liter polyethylene plastic bottle was divided into two parts: a headspace of 0.3 liters and a working volume of 0.7 liters. The headspace allows for the accumulation of biogas produced during the anaerobic digestion process. Before the anaerobic digestion process, the pH of the distillery wastewater sludge and spent yeast was adjusted once to a specific value. This adjustment is likely to ensure optimal conditions for the anaerobic bacteria involved in the digestion process. To measure the amount of biogas generated, the water displacement method was employed. This method involves collecting the biogas in a graduated cylinder or beaker filled with water, causing the water level to rise due to the displacement of the gas. The percentage methane yield, a crucial parameter in anaerobic digestion, was determined using a BIOGAS 5000 gas analyzer. This analyzer is specifically designed to measure the composition of biogas and determine the methane content. To optimize and predict the optimal point of the response influenced by the independent variables, a quadratic polynomial equation (2) was used. This type of mathematical model can help analyze the relationship between the independent variables such as volume ratio, pH value, and temperature and the response variable.

$$\Upsilon A = a_0 + a_1 L_1 + a_2 L_2 + a_3 L_3 + a_{12} L_1 L_2 + a_{13} L_1 L_3 + a_{23} L_2 L_3 + a_{11} L_1^2 + a_{22} L_2^2 + a_{33} L_3^2,$$
(2)

where ΥA is the predicted responses, a_0 is constant, a_1 , a_2 , and a_3 are linear coefficients; a_{12} , a_{13} and a_{23} are crossproduct coefficients, a_{11} , a_{22} , and a_{33} are the quadratic coefficients, and L_1 , L_2 , and L_3 are independent variables [30, 33].

3. Results and Discussion

3.1. Distillery Spent Wash (Wastewater) Sludge and Spent (Waste) Yeast Characterization. The physical and chemical characteristics of the NALF effluents indicate that organic matter was the primary pollutant in both types of refinery waste. The mean concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅) exceeded the maximum permissible discharge standards set by the Ethiopian regulatory authority, which are 250 mg/L for COD and 80 mg/L for BOD₅. The mean temperature for one type of effluent was $35.00 \pm 1.72^{\circ}$ C, while for the other type, it was $44.39 \pm 1.11^{\circ}$ C. The prescribed temperature limit for effluent

in Ethiopia is below 40°C, and the temperature of the distillery wastewater sludge falls within this range. However, the temperature of the second effluent (44.39 \pm 1.11°C) exceeds the prescribed limit of 40°C for waste yeast. These results indicated that the NALF effluents contain high concentrations of organic pollutants, which exceed the permissible discharge standards in Ethiopia. The temperature of the effluents also varies, with one type falling within the prescribed limit and the other type exceeding it.

The total chemical oxygen demand (COD), biological oxygen demand (BOD₅), and total solid (TS) of waste yeast used in this study ranged between 126576.53 ± 310.68 mg/L, 32849.37 ± 501.80 mg/L, and 140664.83 ± 358.38 mg/L, with an average of 17 samples (Table 3), respectively. Another study on distillery waste sludge reported comparable results for certain characteristics. The BOD₅ in the distillery waste sludge ranged between 50,000 and 60,000 mg/L, while the COD and total solids ranged between 110,000 and 190,000 mg/L, respectively [35, 41]. These results indicate

TABLE 3: Physical and chemical characteristics of distillery spent wash sludge and waste yeast.

S. no	Parameters	Distillery spent wash sludge (mg/L)	Distillery spent yeast (mg/L)
1	COD	1623.72 ± 228.98	126576.53 ± 310.68
2	BOD ₅	1119.91 ± 222.71	32849.37 ± 501.80
3	BI	0.68	0.26
4	Temperature (°C)	35.00 ± 1.72	44.39 ± 1.11
5	pH	7.49 ± 0.42	5.40 ± 0.46
6	TP	23.53 ± 1.87	69.76 ± 2.32
7	TN	15.36 ± 0.76	47.81 ± 1.90
8	TS	1538.83 ± 221.19	140664.83 ± 358.38

that both the waste yeast and the distillery wastewater sludge have high concentrations of organic pollutants, as evidenced by high values of COD and BOD₅. The total solids content is also significant in both cases. These results indicated the pollution potential of these waste materials and underlined the importance of proper management and treatment to minimize their environmental impact.

The average levels of total phosphate (TP) and total nitrate (TN) in NALF distillery wastewater sludge and waste yeast are 23.53 ± 1.87 mg/L, 69.76 ± 2.32 mg/L, and 15.36 ± 0.76 mg/L, 47.81 ± 1.90 mg/L, respectively (Table 3). The results indicated that both NALF distillery wastewater sludge and waste yeast contain measurable levels of TP and TN. These nutrients are important for the distillation process, and their presence in the waste materials suggests that they may contribute to the nutrient load in the effluents.

The nutrient content of the distillery wastewater sludge was 23.53 ± 1.87 mg/L and 15.36 ± 0.76 mg/L for TN and TP, respectively. This high concentration of TP was associated with variations in the use of cleaning agents containing phosphorus-derived compounds from phosphoric acid in CIP (Clean-in-Place) units. The TN content of distillery wastewater sludge was higher than the TP content. This could be due to the computability effect of phosphate anions, which inhibits their absorption.

The nutrient content of the distillery waste yeast was 15.36 ± 0.76 and 47.81 ± 1.90 mg/L for TN and TP, respectively. The high TN concentrations come from the use of DAP in the fermentation process for the propagation of yeast, and the high TP concentrations come from phosphoric acid, which is used to adjust the broth pH in the fermentation process.

The pH of the distillery wastewater sludge ranged from neutral to weakly alkaline, with an average pH of 7.49 ± 0.42 . Another study [35] reported a pH range of 6.68 to 7.62 for distillery spent sludge, which is also in the neutral to weakly alkaline range. This alkaline property of the sludge is beneficial in neutralizing the strongly acidic distillery waste yeast, which had a pH range of 4.96 to 5.86. The acidity of the waste yeast is attributed to the use of phosphoric acid during molasses fermentation to adjust the pH below 5 and create favorable conditions for yeast propagation [35]. Mixing both substrates, the distillery wastewater sludge and waste yeast, for anaerobic digestion serves the purpose of creating a more neutral pH environment before initiating the biological process of biogas production. This neutral pH environment is essential for the activity of methanogenic microorganisms, which are responsible for methane production during anaerobic digestion [36]. Therefore, combining the two substrates helps to establish a neutral pH environment, which is conducive to the growth and activity of methanogenic microorganisms, ultimately facilitating biogas production.

The average values of chemical oxygen demand (COD) and biological oxygen demand (BOD₅) in NALF distillery wastewater sludge are 1623.72 ± 228.98 mg/L and $126,576.53 \pm 310.68 \text{ mg/L}$, respectively. For distillery waste yeast, the average values of BOD5 and COD are $1119.91 \pm 222.71 \text{ mg/L}$ and $32,849.37 \pm 501 \text{ mg/L},$ respectively. According to [35, 36], the minimum COD requirement for biological treatment is 1000 mg/L. Both the NALF distillery wastewater sludge and the distillery waste yeast meet this requirement, as their COD values exceed the minimum threshold. This shows that both substrates contain sufficient organic matter to support biological treatment processes. Therefore, both NALF distillery wastewater sludge and distillery waste yeast satisfy the condition for effective biological treatment of organic matter.

The BOD₅/COD ratio is commonly used as an indicator of the degradability of organic matter by microbial species. The concept of biodegradability is expressed through the biodegradability index (BI), which provides information for selecting appropriate treatment methods for different types of effluents [34]. A BOD₅/COD ratio greater than 0.6 is typically expected for fairly degradable and effective biological treatment. A BI value between 0.3 and 0.6 indicates that seeding of adapted microorganisms is necessary. When the BOD₅/COD ratio is less than 0.3, biological treatment becomes difficult because biodegradation has not progressed [34, 35]. Based on the BI values obtained, it was determined that the distillery wastewater sludge had a BI of 0.68, indicating that it was fairly degradable. However, the distillery waste yeast had a BI of 0.26, suggesting that biological treatment of this waste is challenging. To address the nonbiodegradability situation of the distillery waste yeast, a possible solution proposed in the study is to mix it with the distillery wastewater sludge for codigestion. Codigestion involves combining different substrates to enhance the overall degradation process. Similarly, distillery spent wash sludge and spent yeast contained high total solid (TS) levels (1538.83 ± 221.19 and 140664.83 ± 358.38 mg/L). This is attributed to the presence of filterable and nonfilterable particulate matter in both samples. Table 3 presents the experimental results related to the investigation, while Table 4 provides information regarding the mixing of substrates for codigestion.

3.1.1. Characterization of the Mixing of Substrate before Digestion. Mixtures of both distillery wastewater sludge and waste yeast substrates were characterized. The result of the effect of the substrate mixing ratio on the characteristics of the two wastes is presented in Table 4.

S. no	Parameters	Unit	Substrate mixing ratio DWWS:DWY (%)		
			99:1	92:8	85:15
1	COD	mg/L	1334.50 ± 152.94	3158.67 ± 462.64	4460.50 ± 39.20
2	BOD ₅	mg/L	752.25 ± 19.92	1741.11 ± 32.03	2699.50 ± 15.86
3	BI	_	0.6 ± 0.05	0.6 ± 0.07	0.61 ± 0.00
4	Temperature	(°C)	32.04 ± 0.52	35.99 ± 0.34	39.71 ± 0.54
5	pH	_	7.55 ± 0.21	6.69 ± 0.16	6.00 ± 0.18
6	TS	mg/L	793.92 ± 49.49	1789.57 ± 32.12	4117.00 ± 96.49

TABLE 4: Mixture characterization before digestion.

Note. DWWS: distillery wastewater sludge and DWY: distillery waste yeast.

From Table 4, it can be observed that as the percentage of distillery waste yeast increased from 1% to 15% in the mixture with distillery wastewater sludge, the concentrations of COD, temperature, TS, and BOD₅ increased. However, there was a decrease in the pH value from 7.55 to 6.00 as the percentage of distillery waste yeast increased. The pH value of the anaerobic digestion (AD) process is recommended to be within the range of 6.5 to 7.5 according to the study [42]. In this case, the pH value of the mixture was agreed with this recommended range. The average COD levels ranged from 1334.5 mg/L to 4460.50 mg/L in the mixed substrates. The BI value for the mixed substrates was found to be 0.68, which indicates that the organic matter in the mixture is fairly degradable, as it falls within an acceptable range (Tables 3 and 4). Furthermore, the concentrations of total nitrogen (TN), COD, BOD₅, and total phosphate (TP) increased in all mixtures as the yeast concentrations increased. This result was expected since spent yeast is known to have high TN, COD, BOD₅, and TP content.

3.1.2. Characterization of the Mixture after Digestion. According to Table 5, at the end of the 24-day experiment, the anaerobic codigestion process resulted in significant removal of COD, BOD₅, and TS. At 99, 85, and 92% volume ratios of distillery sludge to distillery waste yeast, the COD was reduced by 83.09, 86.32, and 87.92 (%), respectively. Similarly, at 99, 85, and 92% volume ratios of distillery sludge to distillery waste yeast, organic matter as measured by BOD₅ was reduced by 84.90, 87.3, and 88.84%, respectively. At 99, 85, and 92% volume ratios of distillery sludge to distillery waste yeast, the total solids of the mixture were reduced by 80.01, 84.38, and 87.88%, respectively. These results indicate that the anaerobic codigestion process was effective in removing a significant number of COD, BOD₅, and TS, regardless of the specific volume ratio of distillery sludge to distillery waste yeast.

The findings of the current study align with previous research reports. According to [35, 43], those studies reported similar results, with a COD removal of 86.2% and a BOD₅ reduction of 84.80%. These results are comparable to the findings of the current study in terms of COD and BOD₅ removal. On the other hand, the authors of [44–46] discovered a lower COD removal efficiency of 69% in the codigestion of sewage sludge and primary clarifier

skimming. This result is significantly lower than the results obtained in the current study. In a study by Tafesh and Najami, a UASB reactor was used to investigate the codigestion of olive mill wastewater and swine manure. They achieved a high COD removal efficiency of 85-95% with a mixture containing 33% olive mill wastewater and 67% swine manure. This result is comparable to the highest COD removals obtained in the current study at a volume ratio of 92:8%, which was 87.92%. This indicates that the amount of organic matter reduction achieved in the current study is sufficient for biogas production, as mentioned in [43]. The amounts of total solids (TS) and BOD₅ are directly proportional. As the BOD₅ content decreases, the TS also decreases. This is because microorganisms degrade the total solids in the anaerobic digester, leading to a decrease in BOD₅ as microbial activity increases [47, 48].

3.2. The Yield of the Experiments. To examine the primary and interactional effects of volume ratio (A), pH (B), and temperature (C) on methane yield, a three-factor Box–Behnken design (BBD) was implemented. The design consisted of 17 experimental runs, each with varying numerical values of the aforementioned factors. Table 6 displays the outcomes of the Box–BBD investigation, while the analysis of variance (ANOVA) outcomes are presented in Table 7. The highest methane yield of 207 ml (61.18%) was found at run number 12.

According to Table 6, the highest percentage yield observed in the current study was 61.18%, which was obtained at a volume ratio of 92% distillery spent sludge, a pH of 7, and a temperature of 36.5°C. On the other hand, the lowest percentage yield was 40.13% and was observed at a volume ratio of 92% distillery sludge, a pH of 6, and a temperature of 33°C in run number 5. In a study conducted by Oladejo et al. [49], a lower methane content of 0.0488 L was observed during the anaerobic codigestion of cow dung (CD) with pig manure (PM). This indicates that the methane content achieved in the current study is higher than that reported in the reference study. Similarly, Noutsopoulos et al. [50] reported a methane content of 55% from the digestion of grease sludge and sewage sludge. This result is comparable to the findings of the current study. Therefore, the methane content achieved in the current study is relatively high and comparable to the methane content reported in the reference studies [46].

S. no	Parameters	Unit	Substrate mixing ratio DWWS:DWY		
			99:1	92:8	85:15
1	COD	mg/L	226.10 ± 30.60	382.93 ± 68.04	610.14 ± 22.21
2	BOD_5	mg/L	112.70 ± 5.74	193.00 ± 7.89	342.89 ± 16.94
3	TS	mg/L	158.81 ± 12.75	216.91 ± 7.75	643.27 ± 32.42

TABLE 5: Characterization of the mixture after digestion.

TABLE 6: Experimental and predicted values of methane yield value in percentage.

Run		Actual factor	Methane yield value in %		
	VR (%)	pН	Temperature (°C)	Experimental	Predicted
1	85	6	36.5	40.92	42.07
2	99	6	36.5	47.08	47.02
3	85	7	33	45.49	45.83
4	99	7	33	50.06	51.62
5	92	6	33	40.13	38.63
6	99	8	36.5	53.08	51.92
7	92	8	40	46.59	48.09
8	85	7	40	52.78	51.23
9	85	8	36.5	45.89	45.95
10	92	6	40	44.46	44.86
11	92	7	36.5	60.38	60.3
12	92	7	36.5	61.18	60.3
13	92	7	36.5	59.18	60.3
14	92	8	33	44.58	44.18
15	92	7	36.5	60.68	60.3
16	92	7	36.5	60.07	60.3
17	99	7	40	56.7	56.36

TABLE 7: Coefficient in terms of coded factors, fit statistics, and p values of the response of methane yield using RSM.

x7 · 11		Methane yield (%)	
Variable	Coefficient of estimation	<i>p</i> value	Significance
Intercept	60.30	<0.0001 (model)	**
Volume ratio (A)	2.73	0.0011	**
pH (B)	2.19	0.0037	**
Temperature (C)	2.53	0.0017	**
AB	0.2559	0.7352	*
AC	-0.1654	0.8265	*
BC	-0.5812	0.4502	*
A^2	-3.12	0.0031	**
B^2	-10.44	< 0.0001	**
C^2	-5.92	< 0.0001	**
R^2	0.9830		
Adjusted R^2	0.9611		
Predicted R ²	0.7651		
Adequate precision	19.4333		
Lack of fit		0.0405	* *
F value	44.97		

** significant terms and *insignificant terms.

3.2.1. ANOVA Quadratic Model. Methane percentage was calculated using experimental runs that took into account three independent variables. ANOVA was used to determine the significance of each factor on biogas production based on the p value (Table 7).

Based on the statistical analysis conducted in the study, the model developed for predicting methane yield or biogas production demonstrated high accuracy. This was supported by the low probability value of p = 0.05 and the high *F* values of 44.97 obtained for methane yield. When the *P* values are lower than or equal to 0.05, it can be inferred that the terms of the model hold a significant value. The terms included in the model were denoted as A, B, C, AC, BC, A², B², and C², representing the fundamental model parameters in this study. The *R*-squared (R^2) value, which measures the degree of variability in the response explained by the quadratic equation model, was found to be 0.983 for methane yield or biogas, indicating a high level of agreement between the experimental and predicted outcomes as presented in Table 7. Additionally, the p values were used to determine the statistical significance of the main and interaction effects. A p value of less than or equal to 0.05 indicated that the corresponding terms in the model were statistically significant. In this case, terms A, B, C, A², B², and C² were found to be significant based on the p values presented in Table 7. This suggests that these terms had a significant impact on biogas or methane production. The R-squared (R^2) value of 0.983 for the model and the adjusted R-squared value of 0.9611 indicated a high level of concurrence between the experimental and predicted outcomes. The small difference between these values less than 0.2 was considered promising, further supporting the accuracy of the model. The signal-tonoise ratio of 19.433 for methane yield or biogas indicated sufficient precision, signifying that the model is well-suited for exploring design possibilities. To improve the model,

insignificant terms that were not stated in the model were reduced. The regression equation developed using seconddegree polynomials effectively represented the relationship between the response (methane yield or biogas) and the independent variables. The ANOVA results showed that the R^2 value for all adequacy measures was 0.983, indicating that the created model was adequate for the investigation. In summary, the statistical analysis demonstrated the high accuracy and significance of the model terms, indicating that the developed model was suitable for predicting methane yield or biogas production in this study.

An equation in terms of code variables can be utilized to predict the response to various levels of each factor. By default, upper levels of the factors are coded as +1, and lower levels are coded as -1. The *p* values for significant model terms in Table 6 less than or equal to 0.05 were used in the quadratic model equation. In this case, pH², temperature, volume ratio², pH, temperature², and volume ratio are the significant terms. To represent the relationship between the response and independent variables, a coded equation was found using second-degree polynomials as follows:

CH4 Yield =
$$60.29 + 2.73 \times A + 2.19 \times B + 2.53 \times C - 3.12 \times A^2 - 10.44 \times B^2 - 5.92 \times C^2$$
, (3)

where A represents volume ratio, B represents pH, and C represents temperature.

3.3. Effect of Process Parameters on Methane Yield. The study's biogas production experiments were designed using RSM, and the response variance was predicted using a quadratic model. ANOVA was used to examine the effects of the main and interaction effects of each factor on the methane yield. Actual factor terms were used to predict the response for given levels of each factor. Table 6 displays the ANOVA results from the study. BBD generated three factors with a three-level matrix for the response variable, which was the percentage of methane yield. Based on this, response surface quadratic model analyses were carried out, and many significant terms of the main factors were found.

The analysis also revealed that volume ratio was the most influential factor on methane yield, while pH was the least influential. The most influential terms in the second-order quadratic were pH and temperature, while the least influential factor was the volumetric ratio. Generally, only those significant terms have real effects on biogas production.

3.3.1. Volumetric Ratio and pH. The analysis of RSM was used to assess the effect of interactions on the response variable. The ANOVA test revealed that the data were wellfitted. Figure 2 depicts a 3-D plot of the response surface methods used to statistically correlate the interaction impact to the response variable. Figure 2 shows that the pH has a direct proportional relationship with the methane production rate until the pH reaches 7. A maximum methane yield of 61.18% was obtained when a volume ratio of 92% was combined with a pH of 7. As the pH of the digestion process rose from 6 to 7, the methane yield increased from 40.13 to 61.18%. When the volume ratio was held constant at 92% and the pH was kept at 7, it was discovered that increasing the volume ratio from 85 to 92% increased the methane yield from 45.49 to 61.18%, but above this pH 7, the yield began to decrease due to the creation of an unfavorable environment for anaerobic bacteria. Methanogenic bacteria are highly sensitive to changes in pH, especially when it is above 7. The obtained result is comparable to the results of other investigations [40, 47, 51-53], which support the interaction effect of volumetric ratio and pH. The methane percentage yield gradually increased, but as the proportion of spent yeast increased from 8% to 15%, the methane yield decreased. This is because the biodegradation index of waste yeast decreased (Figure 2).

3.3.2. Temperature and pH. Figure 3 describes the relationship between temperature, pH, and methane yield. Specifically, the focus is on the interaction between temperature and pH on methane production. When the pH was maintained at 7, increasing the temperature from 33 to 36.5° C led to an increase in methane yield from 45.49% to 61.18%. However, beyond 36.5° C, the methane yield started to decline. This decline can be attributed to the creation of an unfavorable environment for anaerobic bacteria, which are responsible for methane production. These bacteria are sensitive to changes in temperature, and beyond a certain threshold, higher temperatures can negatively impact their activity and methane production.



FIGURE 2: The interface impact of volume ratio and pH on methane yield.



FIGURE 3: The interface impact of pH and temperature on methane yield.



FIGURE 4: The interface impact of volume ratio and temperature on methane yield.



FIGURE 5: Comparison of the influence of all factors at the reference point in the design space.

Similarly, while keeping the temperature constant at 36.5°C, raising the pH from 6 to 7 resulted in an increase in methane yield from 40.93% to 61.18%. This suggests that maintaining a slightly higher pH level can create a more favorable environment for the anaerobic bacteria, thereby enhancing methane production. The sensitivity of these bacteria to temperature and pH aligns with the findings of previous studies [54, 55]. Additionally, it notes that temperature influences the digestion rate, which in turn contributes to higher volumetric methane production, as reported in another study [40].

3.3.3. Volume Ratio and Temperature. Figure 4 provides a visual representation of the relationship between volume ratio and temperature concerning methane production. These three-dimensional response surface plots can demonstrate how these factors interrelate and impact the production of methane. According to the results, when the volume ratio was held constant at 92%, it was observed that the methane yield had a directly proportional relationship with temperature up to 36.5°C. Increasing the temperature from 33 to 36.5°C resulted in an increase in methane yield from 45.49% to 61.18%. However, beyond this temperature, the yield started to decrease. This decline in methane yield at higher temperatures can be attributed to the creation of an unfavorable environment for anaerobic bacteria, which are responsible for methane production. Likewise, when the temperature was maintained at 36.5°C, increasing the volume ratio from 85% to 92% led to an increase in methane yield from 40.93% to 61.18%. The findings reported in this passage align with those of previous studies referenced [51, 56–58], suggesting a consistent trend in the relationship between volume ratio, temperature, and methane yield.

3.4. Comparison of the Factor Influence at a Point. Figure 5, the perturbation graph, illustrates the impacts of all independent variables at a specific point in the design space. By varying only one factor while keeping the other variables constant, the response is plotted. The slope and curvature of the line associated with each factor indicate the sensitivity of the response to changes in that particular factor. A steep slope and curvature suggest that the response is highly sensitive to changes in the factor, while a flat line indicates that the factor has minimal effect. In the perturbation plot, it is observed that increasing three factors have a significant effect on methane yield up to the reference point. This means that changes in these factors have a positive influence on methane yield. However, beyond the reference point, the effect of each factor on the yield is reversed. This suggests that increasing the factors beyond a certain point may have a detrimental effect on methane yield. The perturbation plot helps identify the factors that have the greatest influence on the response, which in this case is methane yield. By analyzing the slopes and curvatures of the lines associated with each factor, it becomes apparent which factors are more critical in determining the response.

4. Conclusion

The research findings indicate that the optimal conditions for the anaerobic codigestion of distillery wastewater sludge and waste yeast resulted in a methane yield percentage of 61.18%. These optimal conditions included a volume ratio of 92%, a pH of 7, and a temperature of 36.5°C. The experimental outcomes obtained under these optimal process variables demonstrated great potential and showed a significant level of agreement with the predicted values derived from the actual values. This suggests that the developed model accurately predicted the methane yield under the given conditions. The ANOVA findings indicated that the adequacy measure, represented by the R^2 value, was close to one. This indicates that the developed model had sufficient regression and effectively explained the variability in the methane yield. Furthermore, the linear values (A, B, and C) and quadratic values $(A^2, B^2, and C^2)$ of the model were found to be statistically significant, with p values of less than or equal to 0.05. The degree of impact on biogas production or methane yield followed the order of A > C > B, indicating that parameter A had the highest influence on the process. In conclusion, the study demonstrated encouraging results in terms of methane yield percentage and the reduction of total solids (TS), biochemical oxygen demand (BOD₅), and chemical oxygen demand (COD). Both distillery wastewater sludge and waste yeast were identified as potential substrates for biogas production. The study recommends further exploration of the biogas production potential through isolated and adaptive microbial populations. This approach aims to improve methane yield, which is crucial for scaling up the process at the industrial level. Therefore, the research suggests that anaerobic codigestion treatment, specifically the anaerobic digestion (AD) process, is a suitable method for treating such waste. The study concludes that AD is a promising option, particularly in developing countries, based on the results of biogas production and the benefits it offers in terms of TS, BOD₅, and COD removal [59–61].

Acronyms

- AD: Anaerobic digestion
- BBD: Box-Behnken design
- BI: Biodegradation index
- BOD: Biological oxygen demand
- COD: Chemical oxygen demand
- DAP: Diammonium phosphate
- RSM: Response surface methodology
- TN: Total nitrogen
- TP: Total phosphate
- TS: Total solid.

Data Availability

The data supporting the finding of this study are included within the article. Raw data that support the findings are available from the corresponding author and will be provided upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

A.D. was involved in generating ideas and designing the study; A.D., T.B., and A.G. were involved in methodology; A.D. and A.G. were involved in software; A.D., T.B., and A.G. were involved in formal analysis; A.D. was involved in the investigation; A.D. was involved in writing the manuscript and preparation; T.B., A.D., and A.G. were involved in writing, reviewing, and editing; and A.D. was involved in visualization and monitoring. All authors have read and agreed to the final version of the manuscript.

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