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Enhancing Biogas Production and Toxicity Reduction of Sugarcane Vinasse from an Alcohol Plant

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ABSTRACT

Objective: This experimental-intervention study was conducted at a pilot scale in 2022 to optimize the biogas production process from sugarcane vinasse, specifically focusing on reducing the inhibitory effects of toxic compounds and optimizing operational parameters.

Methods: The experiments were carried out based on the Biochemical Methane Potential (BMP) method at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The effect of six variables, including temperature (37 and 55 °C), residence time (14 and 21 and 28 days), C/N ratio (1:20, 1:25, and 1:30), pH (6, 7, and 8), hydrogen peroxide concentration (2, 3 and 5 mg/L) and initial vinasse loading volume (5000 and 10,000 mL) was investigated. Response surface methodology (RSM) and central composite design (CCD) were used to design the experiments. The amount of biogas produced in the process was measured using gas chromatography (GC).

Results: The results showed that the optimal conditions for biogas production were as follows: temperature 37 °C (mesophilic), time: 21 days, pH equal to 7, C/N ratio equal to 1:25, H2O2 amount equal to 3 g/L and loading volume 10,000 mL. Under these conditions, the maximum biogas production efficiency reached 43.2%. Using a hydrogen peroxide concentration of 3 mg/L under these conditions helped to reduce the inhibitory effects of toxic compounds present in the vinasse and facilitated the biogas production process.

Conclusions: These results indicate that the addition of hydrogen peroxide can be considered an effective strategy to reduce toxicity and increase the efficiency of the biogas production process.

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Introduction

Due to the increasing need for sustainable energy sources and the shortage of fossil fuels, on the one hand, and the abundance of perishable materials and liquid and solid waste in sectors such as agriculture and industry, on the other hand, the establishment and use of biogas plants in many countries of the world as a bioenergy production technology have received serious attention (Deng et al., 2020; Ahmad and Jabeen, 2023). Improving the fermentation process and biogas production is an important concern in this process. In this regard, in addition to the raw material used in biogas production, other factors affecting the process should also be studied (Banerjee et al., 2022). Biogas has various advantages, including sustainable energy production (Pavičić et al. 2022), reduced energy production costs (Mengistu et al., 2016), pollution control from urban, industrial, and agricultural wastewater, and production of suitable and nutrient-rich fertilizer (Katuwal and Bohara, 2009).

Various materials are used in biogas production, including municipal waste (Curry and Pillay, 2012), livestock waste (Chowdhury et al., 2020), slaughterhouse waste (Afazeli et al., 2014), food industry waste (Ferdeș et al., 2022), wheat straw and chaff (Satpathy and Pradhan 2023), sewage effluent (Demirbas et al., 2016), and algae (Montingelli et al., 2015; Noweuzi et al., 2025).

The energy production efficiency in the process varies. For example, the efficiency of biogas production from food waste is on average 60 to 80 percent of the dry weight of the waste and 100 to 400 cubic meters per ton of waste (Ruile et al., 2015). This amount is estimated for sugarcane bagasse between 50 and 70 percent and 200 to 300 cubic meters per ton (Joppert et al., 2017), wheat straw 40 to 60 percent and 150 to 250 cubic meters per ton (Kouzi et al., 2020), gas waste 30 to 50 percent of dry weight, poultry waste 50 to 70 percent of dry weight and about 100 to 150 cubic meters per ton (Camargo et al., 2023), and for urban wastewater, 40 to 60 percent and 20 to 30 cubic meters per cubic meter of urban wastewater (Sieling et al., 2013). Among the materials used in the biogas production process, sugarcane waste includes bagasse and vinasse. Bagasse is the dry fiber and residue obtained after the extraction of sugar from sugarcane. It mainly consists of cellulose, hemicellulose, and lignin (Agarwal et al., 2022; Toghraee Samiri et al., 2026). Vinasse is a thick, brown liquid obtained after the sugar refining process from sugarcane. This liquid contains minerals, non-fermentable sugars, and other organic compounds. Vinasse is created in the final stage of the sugar production process when crystalline sugar is separated from the sugarcane juice. The remaining liquid, which contains non-fermentable sugars and nutrients, is called vinasse (Parsaee et al., 2019). Vinasse is one of the most polluted types of industrial wastewater due to its high pollutant load, especially the biological oxygen demand (BOD) (the average BOD of vinasse from molasses distillation is about 35,000 ppm). Disposal of untreated vinasse to natural waters is not possible due to the high amount of acidic waste (Sica et al., 2024). Therefore, its use for energy conversion and biogas production is considered an effective solution (Kiani et al., 2022). Sugarcane vinasse is considered a suitable material for biogas production due to its rich nutrient composition such as nitrogen and phosphorus, organic matter, suitable pH, high moisture content, and

digestibility (Junior et al., 2022). In previous studies, such as those by Pereira et al. (2020) and Longati et al. (2020), the efficiency of biogas production from vinasse has been investigated. However, vinasse has a low carbon-to-nitrogen ratio and therefore, the efficiency of bioga

s production by this material is lower than that of other materials such as sugarcane bagasse (Moreira et al., 2022). Therefore, optimizing the reaction conditions for biogas production can play an effective role in increasing its efficiency. The results of various studies have shown that various components such as temperature, pH, humidity, organic matter content, carbon to nitrogen ratio, time, mixing rate, additives, and even pressure are effective on the efficiency of biogas production (Fenske et al., 2023; Kong et al., 2023).

Currently, 22.4 gigaliters of vinasse are produced worldwide, which has the potential to produce 407.68 gigaliters of biogas. This potential can be considered a significant source of renewable energy (Mazaheri et al., 2024; Karami Moghadam et al., 2026).

Iran is one of the producers of sugarcane and its by-products in the Middle East. Sugarcane cultivation in Iran is being mechanized and industrialized, with its cultivated area growing by 100 percent between 2010 and 2020 (Zaki et al., 2021). More than 99% of the sugarcane farms in Iran are located in Khuzestan Province in southwestern Iran. According to statistics from the Food and Agriculture Organization of the United Nations (FAO) in 2019, Iran ranked 24th among sugarcane-producing countries with a cultivated area of 60,378 hectares. The yield per unit area of Iranian sugarcane farms was 50.27 tons per hectare, and the sugarcane production that year was about 3 million tons (Baladi et al., 2022). In Iran, due to soil salinity, unlike countries such as Brazil, it is not possible to directly apply vinasse as fertilizer at the field level. Moreover, disposing of vinasse on the soil leads to soil contamination and infiltration into groundwater aquifers, and its contamination ultimately harms aquatic organisms (Hashemi et al., 2022). However, there are challenges in the process of producing biogas from sugarcane vinasse. Organic acids, toxic phenolic compounds, and methanol lead to a decrease in the activity of microorganisms. Moreover, imbalances in the C/N ratio and temperature, as well as inappropriate pH levels, are among the existing challenges. The present study aimed to optimize the process of producing biogas from sugarcane vinasse by reducing the inhibitory effects of toxic compounds and optimizing operational parameters.

Method

This research was designed as an experimental and applied study aimed at optimizing the biogas production process from sugarcane vinasse, with a focus on reducing the inhibitory effects of toxic compounds and optimizing operational parameters, and was carried out in 2022. The experiments were conducted based on the Biochemical Methane Potential (BMP) method in the laboratory of the Faculty of Agriculture, Ferdowsi University of Mashhad (Iran). Vinasse alcohol samples were obtained from Razi Valkol Yeast Company. In this research, five independent variables were investigated, including temperature, retention time, C/N ratio, pH, and hydrogen peroxide concentration. The levels of the variables are presented in Table 1.

Table 1 Intervening variables in the process of biogas production from sugarcane alcohol vinasse in the present research

level			unit	Factor
55		37		**temperature**
28	21	14	day	**retention time**
30:1	25:1	20:1	-	**C/N ratio**
8	7	6	-	**pH**
4	3	2	gr/L	**Hydrogen peroxide**
10000	5000			**Loading volume**

The reasons for choosing the levels of variables are as follows:

Temperature: Temperature is a key factor in the anaerobic digestion process. Microbial activity varies at different temperatures. In the biogas production process, temperature is one of the key factors that can affect the efficiency and yield of biogas production (Noraini et al., 2017). The two main types of microorganisms that play a role in this process are mesophiles and thermophiles. Mesophiles perform best at moderate temperatures (usually between 20 and 45 °C). At 37 °C, these microorganisms function well and decompose organic matter (Murillo-Roos et al., 2022).

On the other hand, thermophilic systems require less heating equipment, are generally more stable and less risky than mesophilic systems, and can perform better under variable conditions. Thermophilic microorganisms thrive at higher temperatures, usually between 45 and 70 °C. At 55 °C, these microorganisms exhibit high activity levels, facilitating faster decomposition of organic matter (Bolzonella et al., 2020). A 700-watt electric rod heater was utilized to supply heat. To establish appropriate convective flow, the heaters were positioned near the bottom of the pilot system. A sufficiently precise thermostat (with a 0.5-degree Celsius error margin in the digestion tank) was employed to control the temperature of the pilot system, calibrated based on temperature index values. To ensure a quicker response from the thermostat, its sensor was placed as close to the heater as possible. The thermostat was calibrated according to the internal temperatures of the tanks. Tests conducted revealed that due to the time delay in heat transfer, the practical error of the thermostat had minimal impact on temperature fluctuations within the tanks.

Retention Time: Adequate retention time is essential for the complete breakdown of organic materials and maximum biogas production. Given the inverse relationship between retention time and temperature, it is crucial to design digesters that can maintain stability or achieve desired changes in influential factors throughout the retention period. The effect of retention time on design and construction is fundamentally related to the continuity of this activity. Short retention times (less than 15 days) can lead to incomplete decomposition and reduced gas production (Atelge et al., 2020). Conversely, extended retention times (over 30 days) can increase operational costs. In this study, retention times of 14, 21, and 28 days were employed.

C/N Ratio (Carbon to Nitrogen Ratio): An appropriate carbon-to-nitrogen (C/N) ratio is vital for microbial growth. Low C/N ratios can result in carbon deficiency and decreased biogas production, while high ratios may lead to nitrogen deficiency and reduced microbial activity.

The ideal range for biogas production in various studies has been reported between 20:1 and 30:1 (Cerón-Vivas et al., 2019; Jos et al., 2018). In this research, the C/N ratios were set at levels of 20:1, 25:1, and 30:1. Since the initial C/N ratio exceeded the upper limit of 30:1, it was adjusted by adding urea (NH_2CONH_2).

pH :The pH level outside the optimal range (between 6 and 8) negatively affects microbial activity. Variations in pH can lead to the production of volatile fatty acids and a reduction in methane production. Maintaining pH within an appropriate range is essential for maximizing biogas production and preventing acidic or alkaline conditions that may harm microorganisms (Ali et al., 2019). In this study, pH levels of 6, 7, and 8 were utilized. Initially, the pH of all samples was below 4, which was subsequently increased to the desired levels by adding NaOH. To validate the experimental data, the pH of samples taken from the units was measured and recorded every six days.

Hydrogen peroxide: Hydrogen peroxide was investigated as an oxidizing agent for inhibiting toxic compounds in this study. Hydrogen peroxide helps to reduce the toxic effects of compounds present in vinasse, which leads to increased microbial activity and biogas production (Rojaya et al., 2024). Hydrogen peroxide levels were 2, 3, and 4 mg/L.

Raw materials and digestive feed

The main raw material of this research was sugarcane alcohol vinasse. Another part of the raw materials is additives that are used to change the chemical properties of vinasse, such as adjusting the pH and supplying digestive bacteria. The mixture of all these materials with water was used as a digestion feed in the digestion process. To feed the digestion system, the input materials were adjusted to suitable chemical and physical properties, such as temperature (mesophilic - 37 °C), pH, concentration, and carbon to nitrogen (C / N) ratio. Micronutrients including urea and phosphoric acid fertilizers and 3 to 5 mg of chicken manure were added to the samples (due to the presence of nitrogen).

Vinasse is an acidic substance with a high carbon content. Due to its acidity, the carbon present in vinasse exists in an organic rather than an inorganic form. However, vinasse is deficient in bacteria required for digestion. Therefore, poultry manure was utilized to supply methanogenic bacteria.

COD adjustment

In the initial samples of alcohol vinasse, the Chemical Oxygen Demand (COD) exceeded 120,000 milligrams per liter. High organic loading can lead to decreased biogas production, as microorganisms may not effectively produce methane under such conditions. Consequently, 100 milliliters of vinasse was diluted with 800 milliliters of sugarcane bagasse wastewater, resulting in a COD reduction to below 10,000 milligrams per liter.

The flow diagram of the experimental procedure is presented in Figure 1, which summarizes all major steps from sample collection and feedstock preparation to reactor setup and biogas measurement. Figure 2 shows a view of the experimental pilot.

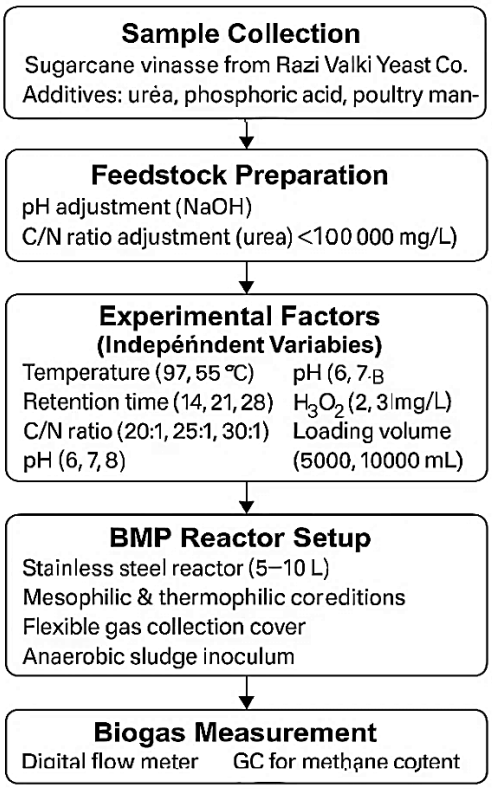


Figure 1- The flow diagram of the experimental procedure

Experimental BMP reactor

Biogas devices were designed to perform BMP experiments at the pilot scale.

Table 2- BMP specifications

Factor	Value
Reactor Type	Continuous Tank
Material	Stainless Steel (304)
Internal Dimensions	25 cm
Internal Pressure	1 bar
Type of Cover	Flexible cover for gas collection
Gas Discharge System	PVC pipe with one-way valve
Working Volume	5000 and 10,000 mL
Temperature	35 and 55 °C
Retention Time	14, 21, 28
Inoculum Source	Anaerobic sludge

Figure 2- A view of the experimental bench scale

Measurement of total biogas and methane gas produced

The total gas volume was measured using a digital flow meter.

The percentage of total biogas production was calculated as follows:

Percentage of biogas production = ((Volume of biogas produced / Volume of vinasse input)) × 100

The methane content was also determined using gas chromatography (GC).

Experiment design

Response surface methodology (RSM) based on a Central Composite Design (CCD)

was employed to systematically optimize the biogas production process from sugarcane vinasse. RSM is a robust mathematical and statistical technique for the evaluation of the main effects, interaction effects, and quadratic effects of multiple independent variables on a response variable.

In this study, the experimental design was formulated to investigate the effects of six independent variables: pH (A), temperature (B), retention time (C), H2O2 concentration (D), C/N ratio (E), and loading volume (F). As detailed in Table 1, the variables were evaluated at different levels to effectively explore the parameter space. The primary response variable (Y) was the percentage of biogas production efficiency.

To predict the optimal conditions, a second-order polynomial equation was fitted to the experimental data to describe the relationship between the independent variables and the response. The generalized quadratic model is expressed as Eq. 1:

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}X_I^2+\sum_{i<j}\beta_{ij}X_iX_{ij}+\varepsilon \quad (1)$$

Where:

Y represents the predicted response (Biogas production percentage),

X_i and X_j are the coded independent variables,

β_0 is the intercept term,

β_i represents the linear coefficients,

β_{ii} represents the quadratic coefficients,

β_{ij} represents the interaction coefficients, and

ε is the statistical error.

The design of the experiments, model fitting, and statistical analyses were performed using Design-Expert software (Version 13.05, Stat-Ease Inc.). Analysis of variance (ANOVA) was used to evaluate the statistical significance and adequacy of the developed model. The significance of the model and each coefficient was determined using F-values and p-values, with a 95% confidence interval.

The quality of the model fit was expressed by the coefficients of determination (R^2) and adjusted R^2. Three-dimensional (3D) response surface plots were generated from the polynomial model to visualize the interaction and individual effects of the variables on biogas production and to identify the optimal operational conditions.

Results

In this study, the properties of sugarcane vinasse as a raw material for biogas production have been investigated. Sugarcane vinasse, as an industrial wastewater with a high concentration of organic matter, has great potential for biogas production. The results of the experiments are shown in Table 3, which presents the properties of sugarcane vinasse as a raw material for biogas production.

Table 3- Specifications of the primary Vinas used

Parameter	unit	Value
TS (Total Solids)	g/L	128±5
COD	mg/L	4800±500
BOD	mg/L	2900±200
Carbon (C)	%	14.2±1.5
Hydrogen (H)	%	6.3±0.2
Total N	mg/L	102±9
Total P	mg/L	119±10
Regenerating sugars	mg/L	2250±70
Phenol	mg/L	14.2±1.7
Oxygen (O)	mg/L	<1
Sulfur (S)	mg/L	128±6
pH	–	6.9±0.3
Relative humidity	%	82±3.1

The total solids (TS) content was measured at 128 grams per liter with a standard deviation of 5 grams, indicating a high concentration of solids in the vinasse. This value indicates that sugarcane vinasse can be used as a rich source of organic matter for biological processes. The COD value of 4800±500 mg/L and BOD of 2900±200 mg/L indicate a high concentration of biodegradable organic matter in this sample. This high COD value indicates a relatively favorable potential of sugarcane vinasse for biogas production. A higher COD value also increases the methane production potential. The BOD to COD ratio in this sample is about 0.60. Considering the ratio between BOD and COD, a significant portion of the organic matter in vinasse is biodegradable and can be effective in biogas production. In the study of Utami et al. (2016), the initial COD and BOD levels in sugarcane vinasse were 9360 and 4013 mg/L, respectively. Sugarcane type, cultivation conditions, and sugar production process have a great influence on the composition of vinasse (Sawatdeenarunat et al., 2019).

A carbon content (%C) of about 15 and a hydrogen content (%H) of 6.3% indicate a suitable carbon-to-hydrogen ratio for the methane production process. The presence of high carbon and hydrogen contents indicates the high potential of sugarcane vinasse in methane production (Battista et al., 2016).

Total nitrogen (Total N) and total phosphorus (Total P) are 102 mg/L and 119 mg/L, respectively, which are known as essential nutrients for the growth of microorganisms in the anaerobic digestion process.

Regenerating sugars (Rs) are 2250 mg/L, indicating the presence of fermentable sugars in sugarcane vinasse. These sugars act as an energy source for microorganisms in the biogas production process (Patil et al., 2022).

The presence of phenol at 14.2±1.7 mg/L indicates the presence of toxic compounds in vinasse that may negatively affect microbial activity. Phenol is one of the organic compounds that, due to its cyclic structure and hydroxyl group, has significant negative effects on microbial activity in biogas processes (Levén et al., 2012). Phenol, by penetrating the cell membrane of microorganisms, disrupts their normal function. This disruption leads to changes in membrane permeability and disruption in the transport of nutrients and metabolic products (Caroca et al., 2021). It also inhibits enzymatic processes that play a role in carbohydrate metabolism and methane production. Phenol also causes oxidative stress in microbial cells, leading to damage to cellular and protein structures. This damage can lead to cell death and a decrease in the microbial population (Wei et al., 2018).

An oxygen content of <1 mg/L indicates anaerobic conditions in sugarcane vinasse, which is essential for the biogas production process. In addition, a sulfur (S) content of 128 mg/L can be considered another nutrient element, although high sulfur concentrations may lead to the production of toxic compounds in some conditions (Tabak et al., 2020).

The pH of sugarcane vinasse is 6.9 with a standard deviation of 0.3, which is within the appropriate range for microbial activity. This appropriate pH can help facilitate the anaerobic digestion process. Finally, a relative humidity of 82% with a standard deviation of 3.1% indicates a high water content in sugarcane vinasse, which is considered a positive factor for the fermentation process.

Results of experimental design

The results of the experimental design based on the RSM-CCD method are presented in Table 4. The results obtained from the RSM-CCD experimental design indicated that the independent variables had a significant effect on biogas production. The overall model had an F value of 110.38 and a p-value of less than 0.00189, indicating that the model was highly significant. These results indicate that the independent variables effectively affect biogas production and can be considered key factors in this process. Among the independent variables, pH and temperature were particularly influential. The F value for pH was 849.19 and for temperature was 792.10, both with a p-value of less than 0.0001. These results indicate the importance of these two parameters in optimizing biogas production. Also, residence time and hydrogen peroxide concentration had a significant effect on biogas production, with F-values of 25.61 and 42.8, respectively, and p-values less than 0.0001. These findings indicate that fine-tuning these parameters can lead to increased biogas production.

C/N ratio and sample volume were also identified as effective variables. The C/N ratio, with an F-value of 84.13 and a p-value of less than 0.0001, and sample volume, with an F-value of 34.62 and a p-value of 0.0134, indicate the importance of these parameters in the biogas production process. These results emphasize that the appropriate balance between carbon and nitrogen as well as the appropriate sample volume can help increase the efficiency of the process.

In addition, the interaction effects between some variables were also investigated. The interaction between pH and hydrogen peroxide concentration (AB) had an F of 39.35 and a p-value of less than 0.0001, indicating a positive effect of this interaction on biogas production. Also, other interactions such as AD and BE had a significant effect on biogas production, which can help develop optimization strategies.

Table 4- Results of experiment design based on RSM-CCD method

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1772.21	55	71.217	110.38	0.00189
A-pH	564.54	1	499.92	849.19	< 0.0001
B- Temperature	501.83	1	485.15	792.10	< 0.0001
C-time	18.31	1	16.31	25.61	< 0.0001
D- H_2O_2	25.31	1	23.19	42.8	< 0.0001
E-C/N	45.64	1	32.4	84.13	< 0.0001
F-Volume	26.31	1	17.67	34.62	0.0134
AB	21.45	1	22.74	39.35	< 0.0001
AC	0.9845	1	0.6918	1.08	0.3451
AD	9.03	1	9.12	14.35	0.0002
AE	7.34	1	6.57	9.25	< 0.0001
AF	2.16	1	2.12	2.89	< 0.0001
BC	1.43	1	1.79	2.83	0.1347
BD	3.87	1	3.96	6.12	0.0231
BE	1.87	1	1.52	4.27	0.0349
BF	0.84	1	0.82	1.20	< 0.0001
CD	0.1296	1	0.138	0.14	0.5761
CE	1.24	1	1.21	2.38	< 0.0001
CF	5.31	1	4.78	7.04	0.0031
DE	1.32	1	1.31	2.14	< 0.0001
DF	1.27	1	1.27	2.56	< 0.0001
EF	1.57	1	1.5	1.89	0.0043
A^2	228.17	1	241.98	348.91	0.002
B^2	3.17	1	5.63	6.46	0.012
C^2	0.928	1	1.95	1.28	0.2539
D^2	1.47	1	1.96	1.99	0.137
E^2	1.10	1	1.03	1.42	< 0.0001
F^2	1.72	1	1.63	1.89	0.00037
Residual	29.02	22	2.21	35.42	< 0.0001
Lack of Fit	11.48	6	4.89	14.11	< 0.0001
Pure Error	0.00	15	0.00	0.00	0.0001
Cor Total	3291.022	125	1466.6768		

Table 5- Compatibility statistics of calculated and observed values

Std. Dev.	**4.91**	**R^2**	**0.8080**
Mean	13.79	Adjusted R^2	0.8191
C.V. %	34.74		

The goodness-of-fit statistics for the calculated and observed values are presented in Table 5. Statistical values such as R^2 of 0.8080 and adjusted R^2 of 0.8191 indicate a good alignment of the model with the experimental data. Additionally, the standard deviation of 4.91 and the coefficient of variation (C.V.) of 34.74% reflect significant variability in the data, which may

arise from the influence of environmental factors or fluctuations in experimental conditions. Overall, the results of this study demonstrate the high potential of sugarcane vinasse for biogas production and underscore the importance of optimizing operational parameters in this process. The predicted-versus-actual curve shown in Figure 3 further supports the alignment between observed data and model predictions.

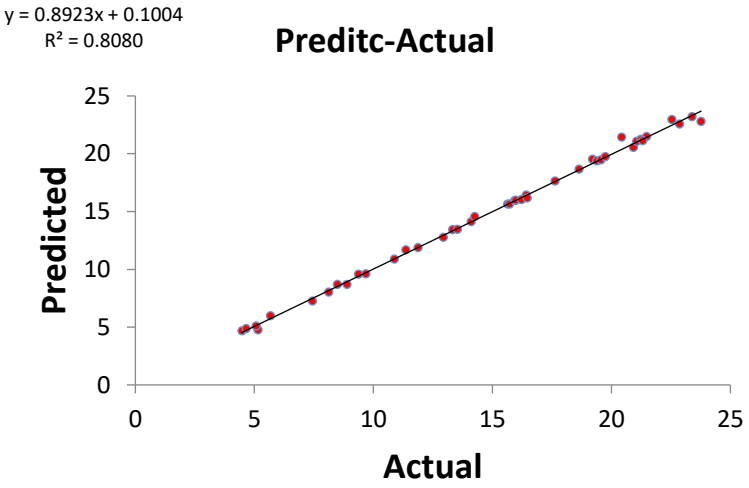
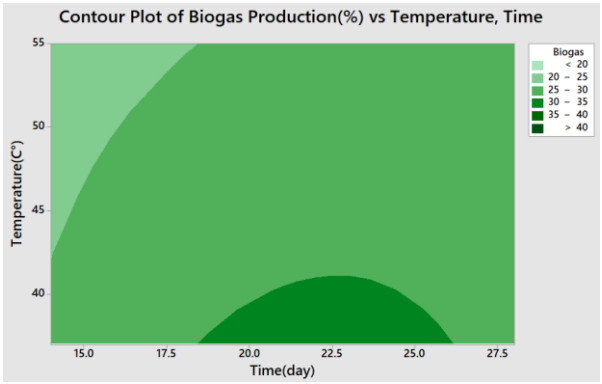
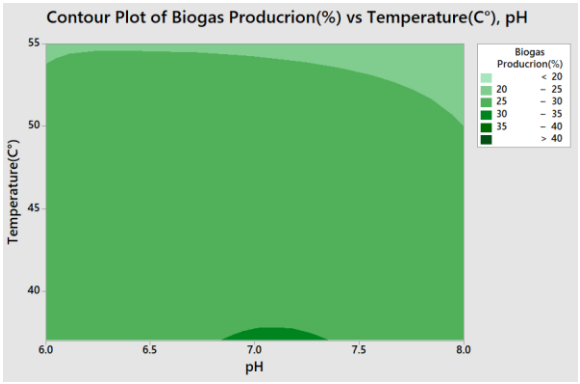


Figure 3- Predicted Actual curve

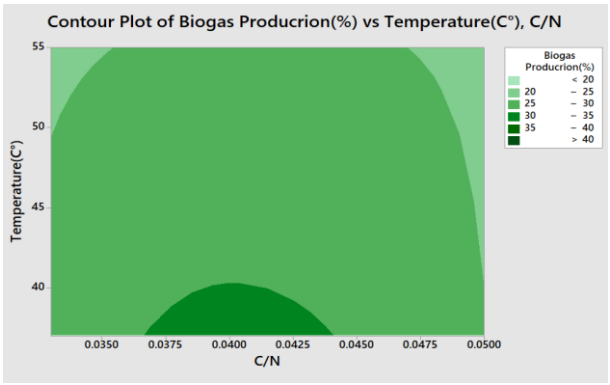
The three-level diagrams of the percentage of biogas production from sugarcane vinasse under the influence of variables based on reaction temperature are presented in Figure 4.



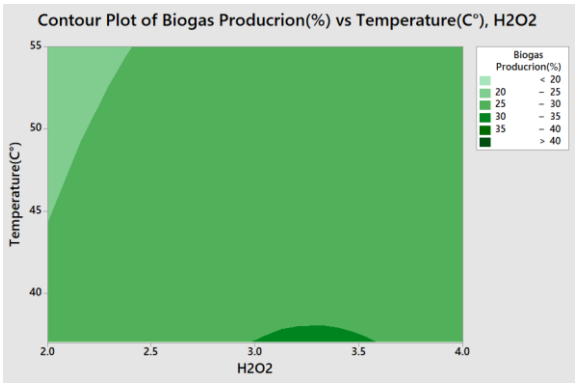
A: Temperature, Time, Biogas Production



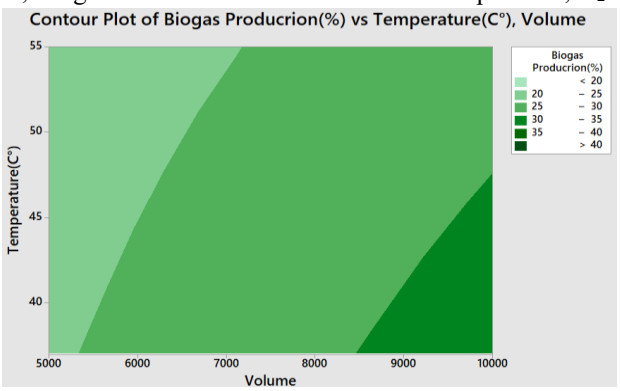
B: Temperature, pH, Biogas Production



C: Temperature, C/N, Biogas Production



D: Temperature, H_2O_2, Biogas Production



A: Temperature, Volume, Biogas Production

Fig4- Three-level graphs of response percentage of biogas production from sugarcane vinasse under the influence of variables based on reaction temperature

The three-level response analysis of the effects of temperature, retention time, and biogas production (Figure 3A) reveals that both temperature and retention time significantly affect biogas production. At a temperature of 37°C (mesophilic conditions), biogas production from vinasse was significantly higher than that under thermophilic conditions (55°C). At a retention time of 21 days, biogas production peaked at 43.2%, whereas at 55°C, the maximum recorded biogas production was only 31.8%. Data analysis indicates that increasing retention time, particularly at lower temperatures, has a positive and significant impact on biogas production. Under mesophilic conditions, increasing retention time from 14 to 28 days increased average biogas production. In contrast, under thermophilic conditions, the effect of increased retention

time on biogas production was less pronounced, possibly due to the negative effects of high temperatures on microorganisms.

The three-level response analysis of the effects of temperature, pH, and biogas production (Figure 3B) showed that, at a pH of 7, maximum biogas production reached 43.2%. This indicates that optimal temperature and pH conditions can significantly influence the fermentation process and biogas production from sugarcane vinasse. Analysis of variance (ANOVA) demonstrated a significant effect of pH on biogas production. Furthermore, Pearson correlation calculations revealed a negative correlation between temperature and biogas production, along with a positive correlation between pH and biogas production.

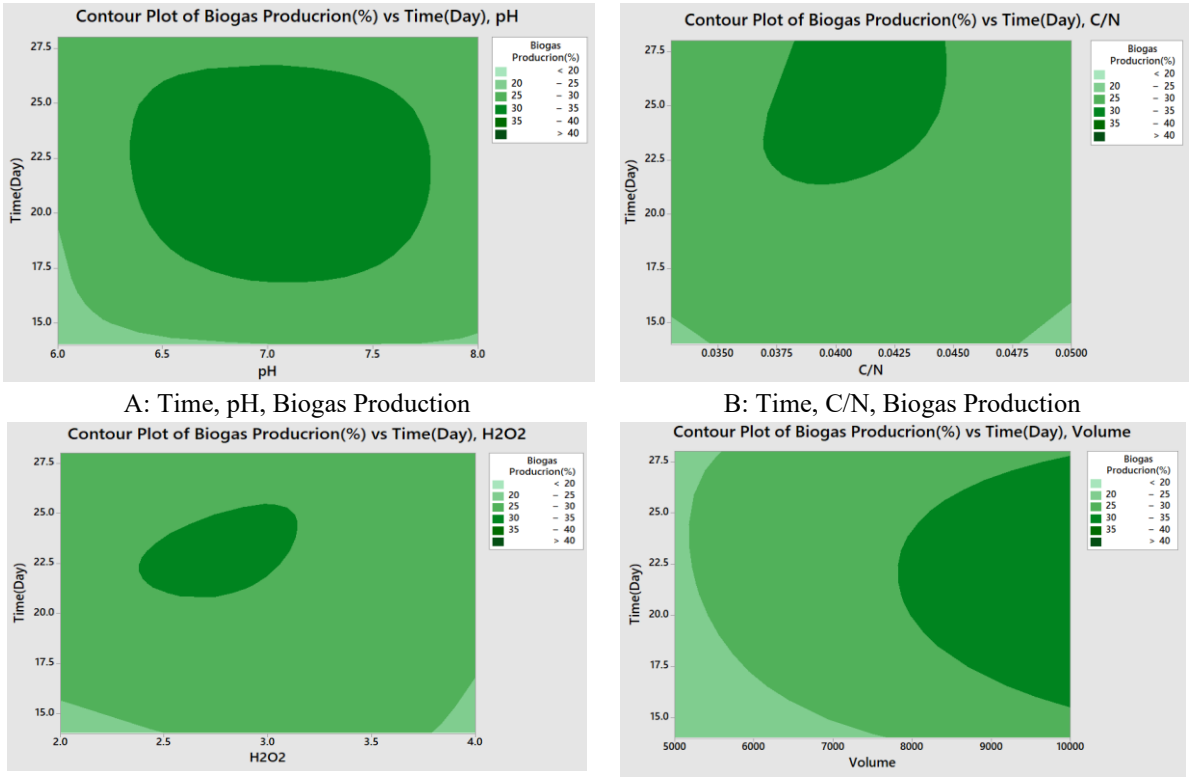
Results of the Three-Level Response Analysis of the Effects of Temperature, C/N Ratio, and Biogas Production (Figure 3C): The composite analysis results based on experimental design indicated that increasing the C/N ratio from 1:20 to 1:25 at a temperature of 37°C significantly enhanced biogas production. The maximum biogas production observed under these conditions was 43.2%, demonstrating the positive impact of increasing the C/N ratio to 1:25 on the fermentation process and biogas yield. In thermophilic conditions, variations in the C/N ratio had a lesser effect on biogas production, which may be attributed to the inhibitory effects of toxic compounds present in sugarcane vinasse. The study's findings revealed that under mesophilic conditions with a C/N ratio of 1:25, biogas production reached its maximum at various retention times. One-way ANOVA results indicated a significant effect of the C/N variable on biogas production, with a significance value (Sig) of 0.034 and an F-value of 0.576.

Results of the Three-Level Response Analysis of the Effects of Temperature, H_2O_2 Concentration, and Biogas Production (Figure 3D): The results obtained from experiments on biogas production from sugarcane vinasse demonstrated a significant effect of hydrogen peroxide (H_2O_2) concentration on biogas yield. At a concentration of 3 mg/L H2O2, the maximum recorded biogas production was 43.2%. ANOVA results indicated that H2O2 concentration had a significant effect on biogas production ($p < 0.05$). Under mesophilic conditions, an H_2O_2 concentration of 4 mg/L resulted in a biogas yield of 26.5%, showing a substantial increase compared to the concentration of 2 mg/L (18.5%). These findings suggest that an appropriate concentration of H2O2 can facilitate in the fermentation process, helping to mitigate the inhibitory effects of toxic compounds. Based on the optimal-level results, increasing H_2O_2 concentration up to 3 mg/L led to enhanced biogas production; however, beyond this level, negative effects on the fermentation process became evident.

Results of the Three-Level Response Analysis of the Effects of Temperature, Loading Volume, and Biogas Production (Figure 3E): The results from experiments on biogas production from sugarcane vinasse under varying temperature and loading volume conditions indicated significant effects of loading volume on biogas yield. Under mesophilic conditions, increasing the loading volume from 5,000 mL to 10,000 mL resulted in a substantial increase in biogas production, reaching a maximum of 43.2% at a loading volume of 10,000 mL. Conversely, in thermophilic conditions, biogas production decreased, with the maximum yield

at this optimal loading volume (10,000 mL) being 31.16%. Analysis of variance revealed a negative correlation between temperature and loading volume.

The three-level response graphs depicting the percentage of biogas production from sugarcane vinasse under the influence of various variables based on reaction time are presented in Figure 5.



A: Time, pH, Biogas Production

B: Time, C/N, Biogas Production

C: Time, H_2O_2, Biogas Production

A: Time, Volume, Biogas Production

Fig5- Three-level graphs of response percentage of biogas production from sugarcane vinasse under the influence of variables based on reaction time

Results of the Three-Level Response Analysis of the Effects of Time, pH, and Biogas Production (Figure 4A): The results of the three-level response analysis indicated a decrease in the maximum biogas yield when both time and pH were considered as variables. The optimal biogas production achieved was 34.9%. Additionally, the findings demonstrated a significant impact of retention time and pH on biogas production from sugarcane vinasse ($p < 0.05$). The optimal conditions were observed at a retention time of 21 days and a pH of 8 (notably, the optimal pH level based on reaction temperature analysis was found to be 7). These results suggest that extended retention times in alkaline conditions have a more favorable effect on biogas production. Previous studies have shown that certain microorganisms, such as hydrolytic bacteria and methanogenic archaea, exhibit enhanced fermentation and biogas production capabilities under alkaline conditions. This environment positively influences enzyme activity and facilitates metabolic processes (Azman et al., 2015; Čater et al., 2015).

Results of the three-level response of the effects of time, C/N, and biogas production (Figure 4B): The results of the three-level analysis showed a significant effect of time and carbon to

nitrogen ratio on the percentage of biogas production ($p < 0.05$). A C/N ratio of 1:25 led to a significant increase in biogas production, indicating a suitable balance between carbon and nitrogen in the fermentation medium. The optimal percentage of biogas production in response to the levels of these three variables was 38.7%. In general, the positive effect of the C/N ratio on biogas production is due to the high activity of methanogenic bacteria (Ghaleb et al., 2021). The results of numerous studies have shown that the optimal C/N ratio for biogas production is 1:25 (Janke et al., 2015).

Results of the three-level response of the effects of time, H_2O_2, and biogas production (Figure 4C): The results of the three-level analysis indicate a significant effect of residence time and hydrogen peroxide (H2O2) concentration on biogas production from sugarcane vinasse ($p < 0.05$). The optimal biogas production under these conditions was 43.2%, indicating that hydrogen peroxide played the most effective role in improving the biogas production process from sugarcane vinasse. The addition of H_2O_2 converts these toxic compounds into less toxic forms by oxidizing them. On the other hand, H_2O_2 acts as a stimulating agent in biological processes. This compound can help accelerate the anaerobic digestion process by increasing the activity of certain enzymes that play a role in the decomposition of organic matter. Therefore, the improvement of the biogas production process by microorganisms under the influence of hydrogen peroxide is considered an effective outcome in the present study. Previously, studies such as McCollam (2009) and Hakika et al. (2022) have suggested the use of H_2O_2 to remove some toxic compounds produced in the biogas process, such as H_2S. At the same time, the presence of large amounts of H_2O_2 in the process damages the biogas production process due to the production of free radicals and the creation of severe oxidative conditions (Achouri et al., 2021). Therefore, determining its optimal values plays a key role in optimizing biogas production.

Results of the three-level response of the effect of time, loading volume, and biogas production variables (Figure 4D):

The results of the three-level effect analysis indicate a significant effect of residence time and loading volume on biogas production from sugarcane vinasse ($p < 0.05$). The maximum biogas production of 33.8% was recorded under conditions with a residence time of 21 days and a loading volume of 10,000 ml. A loading volume of 5,000 mL resulted in a decrease in biogas production. This increase in biogas production at a higher loading volume may be due to the increased contact between microorganisms and vinasse, thus facilitating the fermentation process (Marafon et al., 2020). These findings are in agreement with Atelge et al. (2020), who reported that retention times between 20 and 30 days are generally optimal for the complete anaerobic digestion of complex organic wastes, whereas shorter times often lead to incomplete degradation and reduced gas yields. Parsaee et al. (2019) emphasized that providing an adequate residence time during sugarcane vinasse digestion is a crucial operational parameter to prevent the accumulation of volatile fatty acids (VFAs) and maintain system stability.

Optimization of Operational Conditions for Biogas Production from Sugarcane Vinasse

Figure 6 illustrates the comparison of biogas production from sugarcane vinasse across various levels of operational parameters (A: Temperature, B: Time, C: pH, D: C/N Ratio, E: H_2O_2, F: Volume).

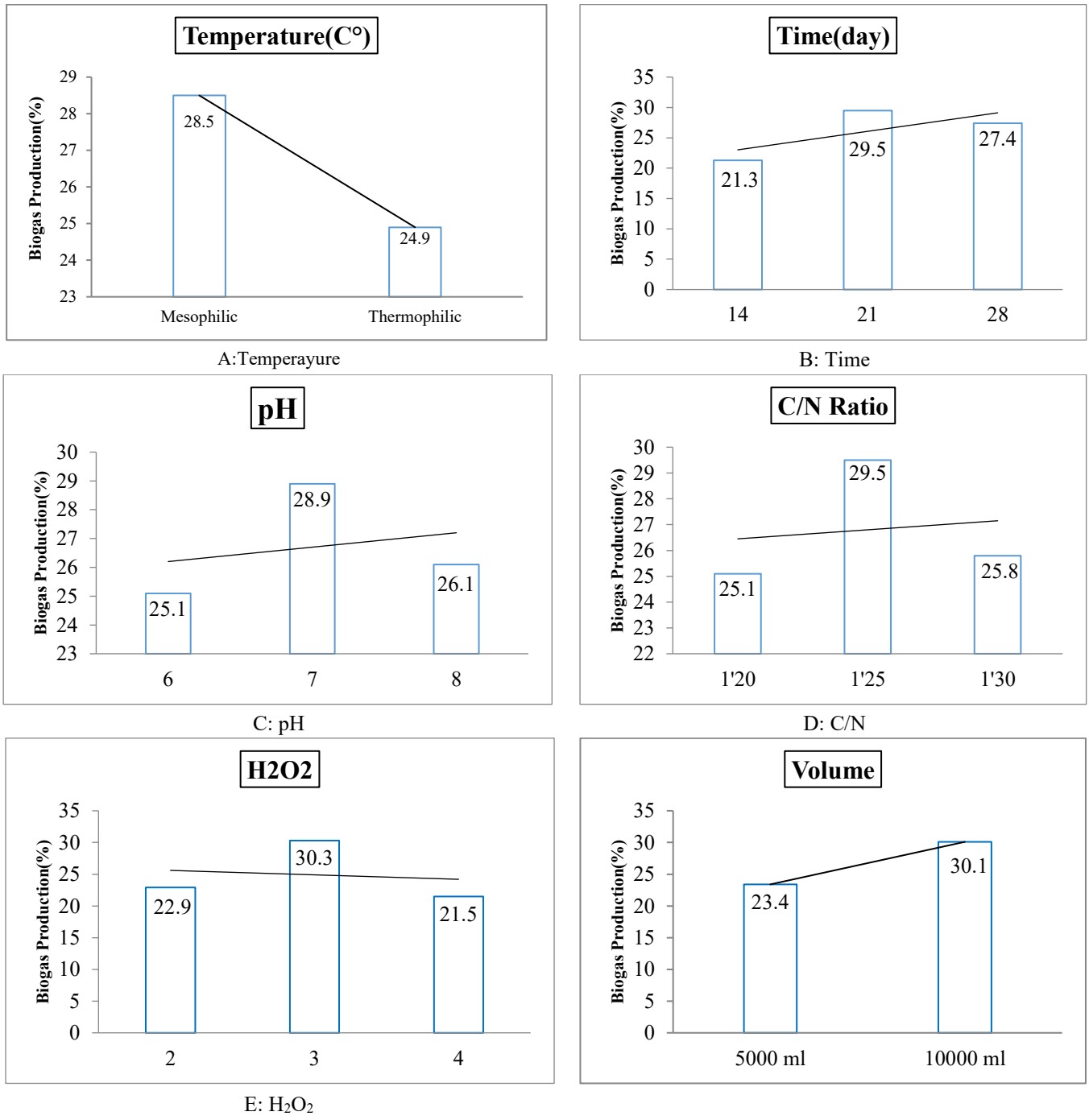


Fig6- Comparison of biogas production from sugarcane vinasse at different levels of operating variables in the process (A: Temperature, B: Time, C: pH, D: C/N, E: H_2O_2, F: Volume)

The results of this study showed that operating parameters had a significant effect on biogas production from sugarcane vinasse. The results showed that the optimal conditions for biogas

production were as follows: temperature 37 °C (mesophilic), time: 21 days, pH 7, C/N ratio 1:25, H2O2 content 3 g/L, and loading volume 10,000 mL. Under these conditions, the maximum biogas production efficiency reached 43.2%. Using a hydrogen peroxide concentration of 3 mg/L under these conditions helped to reduce the inhibitory effects of toxic compounds in vinasse and facilitated the biogas production process. These results indicate that the addition of hydrogen peroxide can be considered an effective strategy to reduce toxicity and increase the efficiency of the biogas production process.

Conclusions

The results of this study show that optimizing operating parameters can significantly affect on biogas production from sugarcane vinasse. By determining the optimal conditions, including temperature, retention time, pH, C/N ratio, hydrogen peroxide concentration, and loading volume, we were able to achieve a maximum biogas production efficiency of 43.2%. These findings highlight the importance of paying attention to details in the design of biogas production processes and show that better results can be achieved by carefully adjusting these conditions. In addition, the use of hydrogen peroxide as a toxicity-reducing agent played a key role in this study. The results show that a concentration of 3 mg/L of this compound can reduce the negative effects of toxic compounds present in vinasse, and thus facilitate the biogas production process. This indicates that the use of appropriate chemical methods can help increase the efficiency of biogas production systems and has considerable potential for improving existing processes.

Future research can investigate the interactions of other chemical compounds and operating conditions to gain a deeper understanding of the biogas production process. Considering the global challenges in energy and waste management, the use of new methods such as biogas production and optimization of its conditions can provide effective solutions for meeting energy needs and protecting the environment.

Author Contributions

Conceptualization, Mohamadali Salehi Rannani and Ashkan Ghafouri. Methodology, Neamatollah Jaafarzadeh Haghighifard. Software, Mohamadali Salehi Rannani. Validation, Afshin Takdastan, Ashkan Ghafouri and Mohamadali Salehi Rannani. Formal analysis, Afshin Takdastan. Investigation, Mohamadali Salehi Rannani. Resources, Mohamadali Salehi Rannani. Data curation, Neamatollah Jaafarzadeh Haghighifard. Writing—original draft preparation, Mohamadali Salehi Rannani. Writing—review and editing, Ashkan Ghafouri and Neamatollah Jaafarzadeh Haghighifard. Visualization, Mohamadali Salehi Rannani. Supervision, Ashkan Ghafouri. Project administration, Afshin Takdastan. Funding acquisition, none.

Data Availability Statement

Not applicable

Ethical Considerations

The authors avoided data fabrication, falsification, and plagiarism, or any other form of misconduct.

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Conflict of Interest

The authors declare no conflict of interest

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