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Optimization of bioethanol production from sugarcane molasses by the response surface methodology using *Meyerozyma caribbica* isolate MJTm3

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Abstract

Purpose: Yeast strains tolerant to a wide range of stress conditions are needed for the production of bioethanol from substrates rich in sugar. In our earlier research findings, *Meyerozyma caribbica* isolate MJTm3 (OM329077) demonstrated remarkable stress tolerance and fermentative activity. The present study aimed to optimize six fermentation parameters to generate conducive fermentation conditions for ethanol production by *M. caribbica* isolate MJTm3.

Method: The response surface method (RSM) based on central composite design (CCD) was employed to optimize process conditions for higher bioethanol yield. The optimization process was carried out based on six independent parameters, namely temperature (25–35 °C), pH (5.5–6.5), inoculum size (10–20% (v/v)), molasses concentration (25–35 (w/v)), mixing rate (110–150 rpm), and incubation period (48–72-h). Analysis of ethanol concentration was done by HPLC equipped with a UV detector.

Result: The optimal conditions of the parameters resulting in a maximum predicted ethanol yield were as follows: pH 5.5, an inoculum size of 20%, a molasses concentration of 25 °Bx, a temperature of 30 °C, an incubation period of 72-h, and a mixing rate of 160 revolutions per minute (rpm). Using the above optimum conditions, the model predicted a bioethanol yield of 79%, 92% of the theoretical yield, a bioethanol concentration of 49 g L^{-1}, and a productivity of 0.68 g L^{-1} h^{-1}. A batch fermentation experiment was carried out to validate the predicted values and resulted in a bioethanol yield of 86%, 95% of theoretical yield, a bioethanol concentration of 56 g L^{-1}, and productivity of 0.78 g L^{-1} h^{-1}. On the other hand, the surface plot analysis revealed that the synergistic effect of the molasses concentration and the mixing rate were vital to achieving the highest bioethanol yield. These values suggested that the RSM with CCD was an effective method in producing the highest possible output of bioethanol from molasses in actual operation.

Conclusion: The study confirmed the potential of using *M. caribbica* isolate MJTm3 for bioethanol production from sugarcane molasses under the abovementioned optimal fermentation conditions.

Keywords: Yeast, Optimization, Response surface method, Fermentation, Molasses, Bioethanol

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Background

The world is under serious pressure due to the limitation of energy sources, the fluctuation of oil prices, the nonrenewable nature of fossil fuels, and the decreasing amount of reserve oil available. On the other hand, the increment in greenhouse gas emissions from fossil fuels is another growing concern due to its direct negative impact on the environment (Sarris et al. 2014). In addition, nations are looking into alternative energy due to their dependence on petroleum-producing countries (Turhan et al. 2015). In this context, biomass-based energy sources such as bioethanol have emerged as a renewable alternative energy source, because of its eco-friendly nature (Rattanapan et al. 2011), and can be easily blended with gasoline (Hansen et al. 2005). Liquid biofuels, like bioethanol, are widely used in the transportation sector as a mixture of gasoline. Bioethanol has several advantages such as a higher-octane number, higher flame speeds, broader flammability limits, and increased heat of vaporization compared to gasoline (Table 1). Moreover, it is less toxic, readily biodegradable, and produces fewer air pollutants than petroleum (John et al. 2011).

Bioethanol is blended with gasoline at volume percentages of 5, 10, and 85% denoted by the fuel names E5-E85. The use of mixtures of E-5 and E-10 bioethanol does not require any engine modifications, whereas E-85 bioethanol can only be used in flexible fuel vehicles (Bušić et al. 2018). However, using bioethanol as a fuel has several drawbacks, including corrosive effects on electric fuel pumps and fuel injectors, issues starting engines in cold weather, and tribological effects on lubricant characteristics and engine performance. Although there are numerous ways to enhance an engine's performance and lengthen its lifespan by reducing friction and wear, some examples are laser texturing, coatings, mass reduction of engine parts, lubricant composition, and use of synthetic oil (Khuong et al. 2017).

The following are the main sugar-rich biomasses used in bioethanol production: (i) sugar-containing raw materials, such as sugar beet, sugarcane molasses, whey, and sweet sorghum; (ii) starch-containing feedstocks, such as grains like corn, wheat, and root crops like cassava; and (iii) lignocellulosic biomass: straw, agricultural waste, crop, and wood residues (Mussatto et al. 2010). The global bioethanol production indicates that 60% of bioethanol is produced from biomass that contains starch, and the remaining 40% is produced from sugarcane and sugar beet (Miguel et al. 2022). Sugarcane molasses is considered an excellent substrate for bioethanol production (Bouallagui et al. 2013; Shafaghat et al. 2010) due to the presence of high amounts of fermentable sugars (i.e., glucose, fructose, and sucrose). Molasses is also a cheap substrate, noncompetitive with a food stock (Campbell and Block 2010), and abundantly available. It needs less pretreatment during preparation compared to starchy, cellulosic, and hemicellulose materials (Razmovski and Vučurović 2011). Generally, molasses is a byproduct of the sugarcane industry and contains most of the microbial growth factors, including minerals and organic nutrients, and it is largely used as a cost-effective feedstock by the ethanol-producing industry (Ghosh and Ghose 2003). In Ethiopia, a huge amount of sugarcane molasses (542,316 tons/year) is produced annually, and both public and private bioethanol factories use this material to produce bioethanol (Gebreegziabher et al. 2017). According to Hawaz et al. (2022), the Ethiopian bioethanol sector faces serious problems with improper resource usage and waste disposal concerns as a result of low substrate conversion efficiency into the desired ethanol product. Therefore, this problem demands the development of an urgent bioconversion approach for effective resource usage. The current study was conducted in collaboration with Ethiopian distilleries to develop the best wild-type yeast strain with optimal fermentation conditions and industrially robust ethanologenic properties.

Yeasts have been employed for many years to ferment sugar-rich biomass into bioethanol. *Saccharomyces cerevisiae* is the most commonly used yeast species for bioethanol production since it tolerates a wide range of stress conditions (Lin et al. 2012). However, the fermentative capability of the *S. cerevisiae* strain is impaired when exposed to a high ethanol concentration, high temperatures, and high osmotic pressure during the fermentation process (Basso et al. 2008). Therefore, the ethanol fermentation parameters that affect the fermentative characteristics of the yeast need to be optimized. On the other hand, nonconventional yeasts, such

Table 1 The physicochemical specification of gasoline and ethanol

Specification	Gasoline	Ethanol
Chemical formula	C_nH_{2n+2} ($n = 4–12$)	C_2H_5OH
M/(g/mol)	100–105	46.07
Octane number	88–100	108
ρ/(kg/dm^3)	0.69–0.79	0.79
Boiling point/°C	27–225	78
Freezing point/°C	−22.2	−96.1
Flash point/°C	−43	13
Autoignition temperature/°C	275	440
Lower heating value·10^3/(kJ/dm^3)	30–33	21.1
Latent vaporization heat/(kJ/kg)	289	854
Solubility in water	Insoluble	Soluble

Source: (Yüksel and Yüksel 2004)

as *Wickerhamomyces anomalus*, *Pichia stipites* strain NRRL-Y-7124, and *Kluyveromyces marxianus* isolate Kf1 (cited as *Kluyveromyces fragilis*), were reported as being stress-tolerant and good ethanol producers (Atitallah et al. 2020; Mussatto et al. 2012). We previously investigated a variety of stress-tolerant and fermentative wild-type yeast strains that were isolated from biowaste and other byproducts of the Metehara and Fincha sugar factories in Ethiopia (Hawaz et al. 2022). Among the multi-stress-resistant yeast strains, the nonconventional yeast isolate *Meyerozyma caribbica* MJTm3 (accession number OM329077, National Center for Biotechnology Information (nih.gov)) was found to be ethanol tolerant (20%), osmotolerant (50% (w/v)), and temperature tolerant (45 °C) and able to produce bioethanol (42 g L^{-1}) from highly concentrated sugarcane molasses (Hawaz et al. 2022).

The process of bioethanol fermentation is influenced by several factors that affect a series of biochemical reactions involved. Determining the optimum conditions (pH, temperature, substrate concentration, inoculum volume, mixing rate, duration of the fermentation, etc.) is a crucial step during bioethanol production. The use of Design-Expert is widely applied to identify and optimize the key process variables in order to improve systematically the concentration of the aimed product (Jargalsaikhan and Saraçoğlu 2008; Uncu and Cekmecelioglu 2011). Consequently, the response surface methodology (RSM) is chosen as a statistical method that provides a rapid assessment of the key operating factors in which a response parameter is influenced by several independent factors (El-Gendy et al. 2013). Thus, the present study aimed to optimize the fermentation parameters to enhance bioethanol production from sugarcane molasses by using the locally isolated *Meyerozyma caribbica* MJTm3 in a shaker flask using an RSM-based central composite design (CCD).

Results and discussion

Propagation characteristics of *M. caribbica* isolate MJTm3

To achieve a maximal yeast concentration for effective conversion of the substrate to ethanol, the yeast cells have been propagated through four phases that each contain varying concentrations of molasses (viz., 8, 10, 12, and 14 °Bx). Overall, the findings showed that the yeast cell number increased exponentially up to 12 °Bx but started to decline sharply after 14 °Bx. The results revealed that the yeast cell population increased by 41, 51, and 8% at 8 °Bx, 10 °Bx, and 12 °Bx, respectively, after 24-h propagation time.

The pH value showed a decreasing trend when the propagation period was extended to 24-h. Specifically, the pH demonstrated decreasing dynamics from 5.5 to 3.5, 3.6, and 3.5, at 8 °Bx, 10 °Bx, and 12 °Bx, respectively after a 24-h propagation time (Fig. 1a, b, c, and d, respectively). This is due to the rapid consumption by yeast of buffering capacity (i.e., amino acids) and the related production of an acidic material such as organic acids. Furthermore, the pH value of the propagated broth decreased to 3.2 as propagation time exceeded to 24-h, making it more acidic. As a result, the number of viable yeast cells started to decrease. Although the molasses propagated media (MPM) has an acidic pH, the yeast's cellular morphology is maintained (Fig. 2a and b). This is consistent with our earlier findings, which stated that *M. caribbica* MJTm3 was found to be tolerant to acidic pH in YPD broth (Hawaz et al. 2022).

Regarding the reduction of molasses concentration in function to cell viability and incubation period, the Brix was decreased to 4.25 °Bx, 4.5 °Bx, 5.5 °Bx, and 6.25 °Bx from 8 °Bx, 10 °Bx, 12 °Bx, and 14 °Bx, respectively, during a 24-h propagation period. Consequently, viable yeast cell values of a density of 4.26×10^8 cells/mL, 8.75×10^8 cells/mL, 9.52×10^8 cells/mL, and 2.1×10^7 cells/mL were produced (Fig. 1a, b, c, and d, respectively). In this experiment, the molasses concentration was reduced by half after 24 h of propagation, yet lesser viable yeast cells were counted at 14 Bx, and this is due to the cause of longer propagation duration. The propagated yeast density under different molasses concentration can be seen in Fig. 3. Even though changes in pH and cell density were observed until the end of the propagation time, the molasses concentration (Brix content) stabilized after 20-h propagation time.

Model validation and optimization of fermentation parameters

The aim of this study was to maximize the yield of bioethanol produced from sugarcane molasses by optimizing the conditions of the fermentation parameters essential for efficiently converting all of the available fermentable sugar to ethanol. The complete design matrix of the independent variables in actual values corresponding with predicted responses of the bioethanol yield is presented in the supplementary data. A second-order quadratic model equation was generated based on the experimental data and CCD, and this indicated linear, interaction, and quadratic effect of variables on bioethanol yield as (+ve) or (−ve) under Eq. 1. The quadratic equations had high regression coefficients, and the lack of fit values was insignificant ($p > 0.05$), demonstrating that the quadratic models fit the data well.

$$\begin{aligned} Y = {} & + 24.30 + 0.4401A + 0.6653B + 0.4703C - 3.41D + 4.00E + 2.47F \\ & - 3.54A^2 + 0.3438B^2 + 0.8547C^2 + 1.04D^2 + 0.9509E^2 + 1.21F^2 \\ & + 1.90AB - 3.68AC + 0.7165AD + 0.8818AE - 1.13AF \\ & + 1.67BC + 0.3925BD - 0.9626BE + 1.12BF \\ & + 0.1981CD + 0.4089CE + 2.27CF \\ & - 1.53DE - 0.6768DF \\ & - 0.4527EF \end{aligned} \quad (1)$$

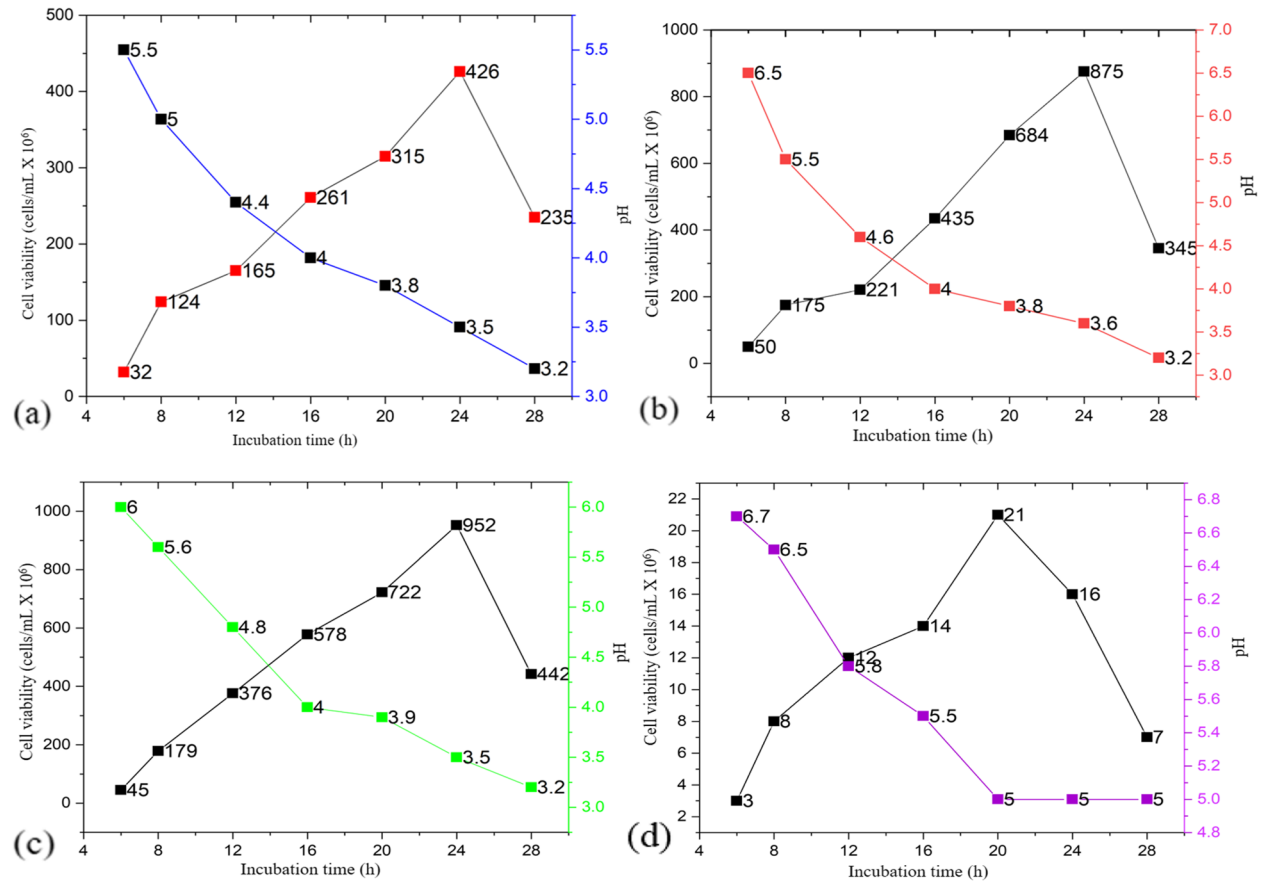

Fig. 1 The dynamics of yeast cell propagation as a function of pH and incubation period at 8 °Bx (**a**), 10 °Bx (**b**), 12 °Bx (**c**), and 14 °Bx (**d**). A maximum yeast cell density of 9.52 × 10^8 cells/mL was achieved at 12 °Bx molasses concentration after 24h

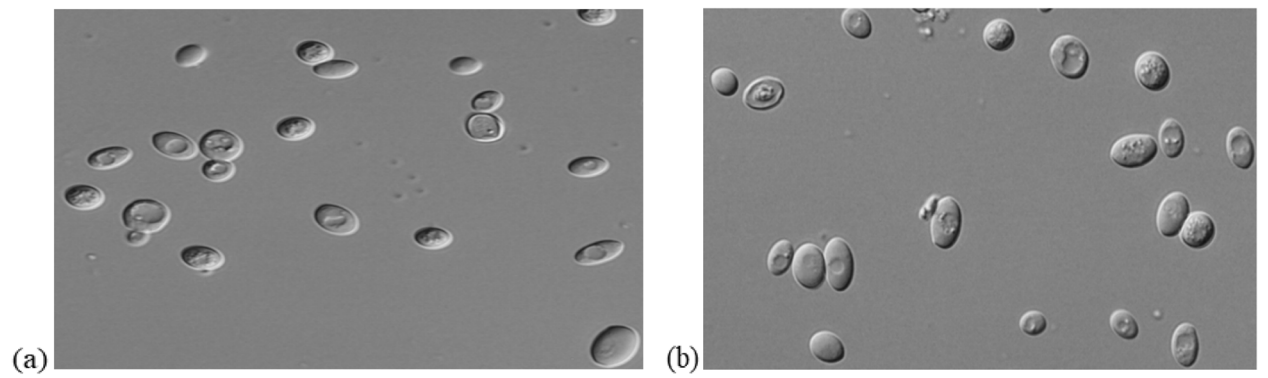

Fig. 2 The cellular morphology of *M. caribbica* isolate MJTm3 photographed at 100× magnification with a bright field microscopy while being exposed to pH 5.5 (**a**) and 3.2 (**b**) after 24-h

where Y is the bioethanol yield (%) and a positive sign indicates a synergetic effect, whereas a negative sign indicates an antagonistic effect.

The statistical significance of the quadratic model was determined using analysis of variance (ANOVA). The statistical significance was controlled by *F*-test and *p*-values,

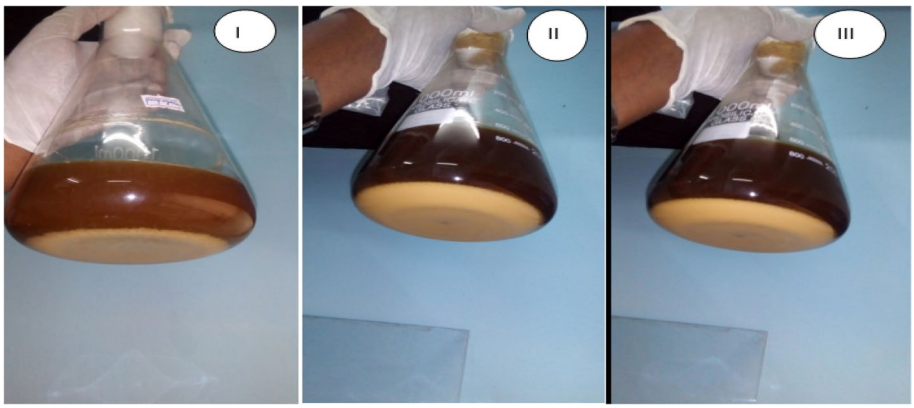

Fig. 3 *M. caribbica* isolate MJTm3 propagation at different concentrations of molasses propagation medium (MPM) containing 8 °Bx (phase 1), 10 °Bx (phase 2), and 12 °Bx (phase 3) at 24-h propagation time

and the model was found to be highly significant at a 95% confidence level (Eq. 5), with an *F*-value of 65.48 and a very low probability ($p < 0.0001$) (Table 2). This indicated that there is less than 0.01% chance that this error is caused by noise. The significance of the current model agrees with results of Hamouda et al. (2015) who developed a model for optimization ethanol production parameters with statistically significance at a 95% confidence level, an *F*-value of 29.1, and a very low probability of $p < 0.0001$ with < 0.01% chance that of which error caused by noise.

The model fitting reliability was evaluated using R^2 and adjusted R^2 and found to be 0.9682 and 0.9534, respectively (Table 2). These values indicated that approximately 96.82% of the variability in the response obtained is explained by the model ensuring perfect fit of the model to the observed data. This is in line with results from Hamouda et al. (2015), the R^2 value 0.953, which demonstrated the high significance of the model with 95.3% of confidence for bioethanol production from sugarcane molasses. Cavalaglio et al. (2016) have developed a model fitted with R^2 equal to 0.970 with 97% significance for optimization of ethanol production from a cellulosic substrate. Flayeh (2017) has designed a model that fitted with a slightly lower confidence of 90.25% compared to the present reported values.

The regression model's suitability between the experimental and predicted data of the response parameter suggests a reasonable correlation over the tested experimental ranges (Table 2). According to the current analysis, a ratio of 38.42 adequate signals (Eq. 4) was achieved for the CCD consisting of 86 trials, for which the overall experimental bioethanol yield ranged between 8.41 and 46.59% and corresponding predicted value of 8.28 to 39.98%, respectively. An adequate signal-to-noise ratio of greater than four ($R > 4$) and an adequate accuracy value of 38.42 were found using ANOVA measurement. The current model can therefore be reliable and employed to navigate the design space. This is in agreement with Hamouda et al. (2015), who obtained an experimental ethanol yield of 8.20–41.4% with the corresponding predicted values ranging from 9.26–39.1%, respectively, with an adequate signal of 17.1. On the other hand, the current developed model indicated that the lack of fit ($F = 0.0525$) was found significant relative to the pure error.

The actual and predicted values, as well as the normal plot of experimental design residuals, which are shown in Fig. 4a and b, further supported the aforementioned ANOVA analysis. A plot of the predicted and experimental values of the bioethanol yield is shown in Fig. 4a. The plot showed a strong correlation ($R^2 = 0.9682$) between the experimental and predicted data, demonstrating that the model accurately predicted the bioethanol production within the experimental range under consideration. This demonstrates that the experimental outcomes were largely consistent. The residuals for the bioethanol production are normally distributed on a normal plot, as shown in Fig. 4b, with results extremely closely spaced to a straight line with no substantial departure.

In the current study, the temperature (A), pH (B), inoculum size (C), molasses concentration (D), mixing rate (E), and incubation period (F) were selected as key factors to maximize the bioethanol yield (%) using CCD. Results revealed that all linear and interaction factors, except for temperature (A), inoculum size (C), pH and molasses concentration (BD), inoculum size and molasses concentration (CD), inoculum size and mixing rate (CE), and mixing rate and incubation period (EF), were significant at the 95% confidence level. The independent factors, such as linear (B, D, E, F), interactive (AB, AC,

Table 2 Analysis of variance for response surface of quadratic model for the CCD experiments

Source	SS	Df	MS	*F*-value	*P*-value	
Model	4731.99	27	175.26	65.48	< 0.0001	Significant
A	13.39	1	13.39	5.00	0.0292	
B	30.96	1	30.96	11.57	0.0012	
C	15.29	1	15.29	5.71	0.0201	
D	802.05	1	802.05	299.65	< 0.0001	
E	1101.06	1	1101.06	411.36	< 0.0001	
F	422.51	1	422.51	157.85	< 0.0001	
AB	230.10	1	230.10	85.97	< 0.0001	
AC	866.95	1	866.95	323.90	< 0.0001	
AD	32.85	1	32.85	12.27	0.0009	
AE	49.76	1	49.76	18.59	< 0.0001	
AF	81.03	1	81.03	30.27	< 0.0001	
BC	178.25	1	178.25	66.60	< 0.0001	
BD	9.86	1	9.86	3.68	0.0599	
BE	59.31	1	59.31	22.16	< 0.0001	
BF	79.90	1	79.90	29.85	< 0.0001	
CD	2.51	1	2.51	0.9383	0.3367	
CE	10.70	1	10.70	4.00	0.0503	
CF	328.57	1	328.57	122.76	< 0.0001	
DE	150.18	1	150.18	56.11	< 0.0001	
DF	29.31	1	29.31	10.95	0.0016	
EF	13.12	1	13.12	4.90	0.0308	
A^2	190.47	1	190.47	71.16	< 0.0001	
B^2	2.79	1	2.79	1.04	0.3117	
C^2	11.11	1	11.11	4.15	0.0462	
D^2	16.45	1	16.45	6.15	0.0161	
E^2	12.50	1	12.50	4.67	0.0348	
F^2	21.44	1	21.44	8.01	0.0064	
Residual	155.24	58	2.68			
Lack of fit	145.56	49	2.97	2.76	0.0525	Not significant
Pure error	9.68	9	1.08			
Corrected total	4887.23	85				

R-squared (R^2), 0.9682; adjusted R^2, 0.9534; adeq. precision, 38.42

SS Sum of squares, *DF* Degree of freedom, *MS* Mean square

AD, AE, AF, BC, BE, BF, CF, DE, and DF), and quadratic (A^2) were found significant model terms ($p < 0.05$), whereas all quadratic variables were showed insignificant ($p > 0.05$), except for temperature (A^2) ($p < 0.0001$) (Table 2). This is in agreement with Kamal et al. (2021) who reported that linear pH, interactive inoculum size, and pH were significant model terms for efficient bioethanol production from sugarcane molasses.

The Pareto chart was plotted to highlight the most significant independent variables and their main and interaction effects on bioethanol production (Fig. 5). The effect of each independent parameter on bioethanol production was confirmed by the coefficient of the quadratic equation (Eq. 1). As a result, linearly, mixing rate (rpm) and incubation period (h) were considered significant variables ($p < 0.0001$). The mixing rate revealed the highest positive impact on the bioethanol yield (%), followed by the incubation period. This suggests that increasing the mixing rate and incubation time beyond their preset values will increase the bioethanol yield. Other fermentation factors, i.e., inoculum size, initial pH, and temperature, showed a slightly positive impact on the bioethanol production ($p = 0.0201$, 0.0012, and 0.0292, respectively). In the present study, molasses concentration exerted an adverse negative impact ($p < 0.0001$) on the ethanol production process, while its quadratic effect demonstrated the highest positive impact on ethanol fermentation ($p = 0.0161$). The quadratic effects of the incubation period,

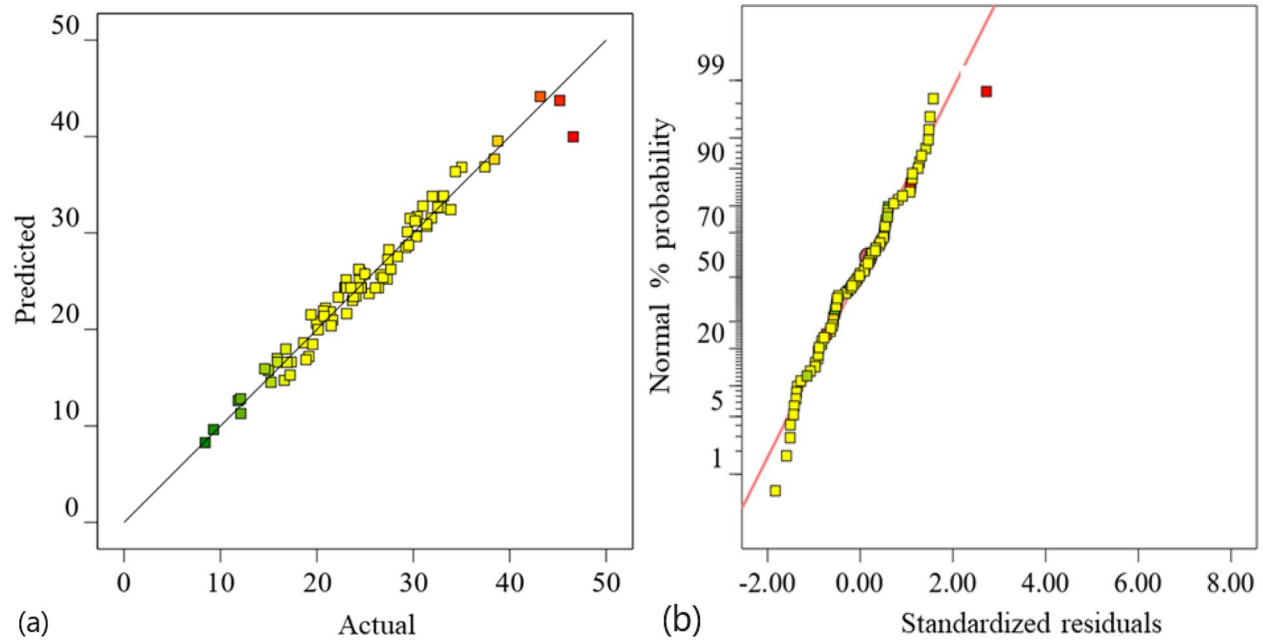

Fig. 4 Predicted versus actual values (**a**). Normal probability plot versus standardized residuals (**b**)

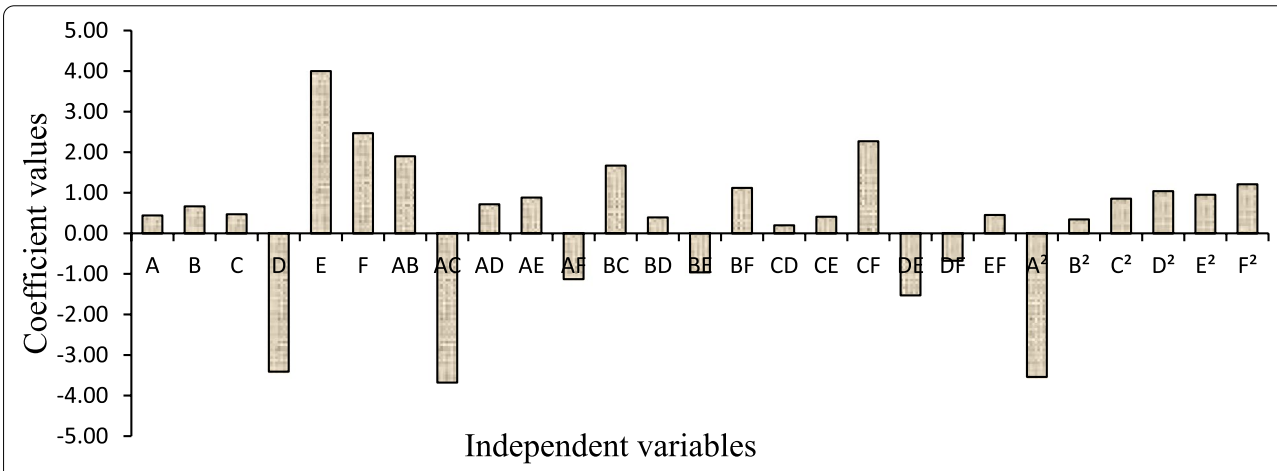

Fig. 5 Pareto chart showing the linear, interactive, and quadratic effects of different factors on the response bioethanol yield ($Y_{E/S}$)

initial molasses concentration, mixing rate, and inoculum size showed a positive impact on the fermentation process in declining order ($p = 0.0064$, 0.0161, 0.0348, and 0.0462, respectively). On the other hand, the incubation temperature showed the highest negative quadratic effect ($p < 0.0001$) on the bioethanol yield.

The positive interactive effect of all the parameters within the studied range of the experiment ranked in declining order: inoculum size and mixing rate ($p < 0.0001$) > temperature and initial pH ($p < 0.0001$) > initial pH and inoculum size ($p < 0.0001$) > initial pH and incubation period ($p < 0.0001$) > temperature and mixing rate ($p < 0.0001$) > temperature and initial molasses concentration ($p = 0.0009$) > inoculum size and mixing rate ($p = 0.0503$) > initial pH and initial molasses concentration ($p = 0.599$) > inoculum size and initial molasses concentration ($p = 0.3367$). The negative interactive effect of the investigated parameters ranked in the following decreasing order: temperature and inoculum size ($p < 0.0001$) > initial molasses concentration and mixing rate ($p < 0.0001$) > temperature and incubation period ($p < 0.0001$) > initial pH and mixing

rate ($p < 0.0001$) > initial molasses concentration and incubation period ($p = 0.0016$) > mixing rate and incubation period ($p = 0.0308$).

Surface plot analysis

An extensive experimental trial was conducted over the considered range of 86 experimental runs to determine the optimum operating value for the factors that will maximize the bioethanol yield from sugarcane molasses by *M. caribbica* isolate MJTm3. To elucidate the optimum condition of each factor for a maximum bioethanol yield (%) production, thee-dimensional response surfaces (3D) were plotted based on the predicted second-degree polynomial equation (Fig. 6).

According to the response surface plots for bioethanol yield as a function of temperature and molasses concentration in Fig. 6a, lowering the temperature from 35 to 30.25 °C and the molasses concentration from 35 to

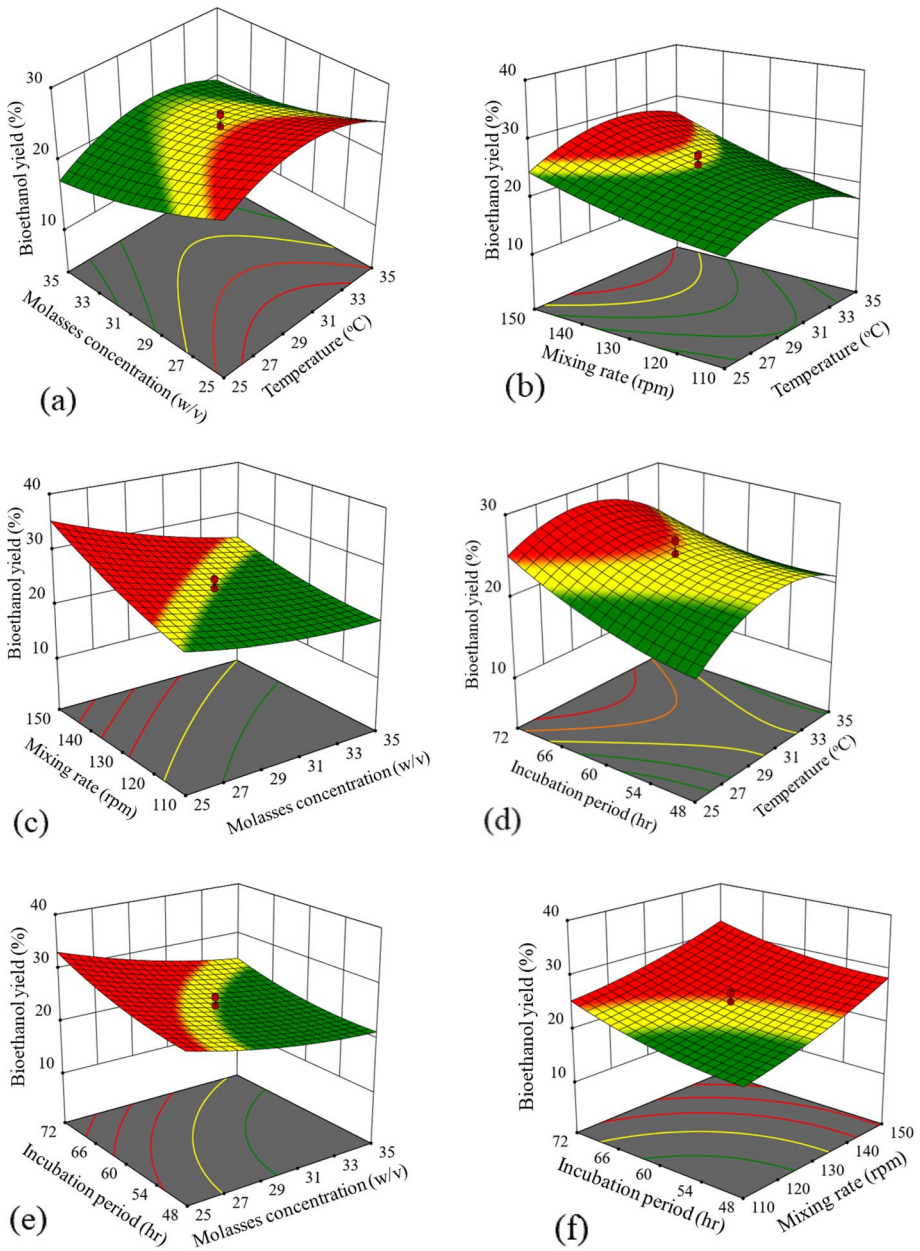

Fig. 6 The 3D response surface plots of ethanol yield of molasses concentration and temperature (**a**), mixing rate and temperature (**b**), mixing rate and molasses concentration (**c**), incubation period and temperature (**d**), incubation period and molasses concentration (**e**), and incubation period and mixing rate (**f**). Green, yellow, and red colors represent lower, medium, and higher levels of ethanol yield, respectively

25.13 °Bx resulted in an increase of the bioethanol yield. At 34.90 °Bx and 25.05 °C, a lower bioethanol production of 17.40% was observed, but a higher bioethanol yield of 28.70% was shown at 25.06 °Bx of the molasses and 29.78 °C. There is a negative correlation between molasses concentration and bioethanol yield, meaning that a decrease in molasses concentration will result in higher bioethanol yield. The yield began to decrease at 25.09 °Bx and 29.96 °C, which is because high temperatures and high substrate concentration have a negative effect on the fermentation capacity of the yeast cells (Cazetta et al. 2007). Low bioethanol yield signified that the yeast was sensitive to the inhibitory compound present in the fermentation medium. Moreover, the enzymes that regulate the fermentation process are sensitive to high temperatures which can result in the denaturation of their tertiary structure (Phisalaphong et al. 2006). According to Liu and Shen (2008), the optimal operating temperature for free fermenting yeast cells was near 30 °C. It has been shown that increasing the initial molasses concentration to 25 °Bx resulted in a maximum bioethanol yield of 25% (Hamouda et al. 2015). In agreement with our finding, Morimura et al. (1997) reported the highest ethanol yield and ethanol concentration at an optimum of 25 °Bx molasses concentration by a yeast strain K211 of *Saccharomyces* species.

A positive interacting effect between temperature and mixing rate was seen for the bioethanol yield Fig. 6b. The bioethanol yield increased with an increase of the mixing rate from 111 to 149.77 rpm and temperatures ranging from 25 to 30 °C. Results showed that at 110 rpm and 25 °C, a bioethanol production of 18.20% was obtained. At a mixing rate of 149.77 rpm and a temperature of 30 °C, a maximum yield of 29.26% of bioethanol was observed. When the mixing speed and temperature reached 30.2 °C and 149.83 rpm, respectively, the bioethanol production started to decline. It is obvious that as the agitation rate increases, the diffusion of the necessary nutrient from the fermentation broth to the yeast cells is increased. However, at the same time, this also increased the release of ethanol from the cells to the fermentation broth. The ideal agitation rate for ethanol fermentation by yeast cells was reported as 150–200 rpm (Zabed et al. 2014). In the current situation, the fermentation broth was efficiently mixed and distributed when the agitation rate increased ≈150 rpm.

Figure 6c demonstrates the negative interactive effect of molasses concentration and mixing rate for bioethanol production from molasses. The bioethanol yield increased with an increase in mixing rate from 110.19 to 149.67 rpm and a decrease of the molasses concentration from 35 to 25.18 °Bx. This finding revealed that the maximum bioethanol yield of 34.84% was demonstrated at 25.19 °Bx and 149.67 rpm, which is because the yeast cells are in contact with vital nutrients, like sugars, which resulted in an effective bioethanol yield (Kopsahelis et al. 2007). Moreover, at 25.25 °Bx and 147.32 rpm, the bioethanol output started to decrease.

Figure 6d shows the negative interactive effect of temperature and incubation period on bioethanol production. Results showed that the yield increased with a decrease of the incubation temperature from 35 to 29.86 °C, and a slight decrease in the incubation period from 72 to 71.78 h. At 29.86 °C and a 71.78 h incubation period, a maximum yield of 27.85% ethanol was produced. However, at 25.05 °C and 48.19 h, the lowest bioethanol yield of 18.04% was measured. An earlier study using *Saccharomyces* species indicated that fermentation at 31 °C for 72-h produced 26.4% of bioethanol yield (Flayeh 2017). According to Zabed et al. (2014), a longer fermentation period is required to recover a high ethanol yield with the highest productivity using a batch fermentation system. This suggests that the use of a short fermentation time and a short incubation temperature cause inefficient ethanol fermentation due to inadequate growth of microorganisms (Zabed et al. 2014).

Figure 6e illustrates the interaction effect of molasses concentration and incubation time on the bioethanol yield. The plot showed that ethanol production increased as the incubation period was extended from 48.16 to 71.99-h, while the concentration of molasses decreased from 35 to 25.10 °Bx. At 48.16-h of incubation time and 25.11 °Bx, a lower ethanol yield of 26.71% was obtained. A maximum bioethanol yield of 32.98% was recorded at 25.10 °Bx and 71.99-h of molasses concentration and incubation period, respectively. When the fermentation flask was overloaded with the substrate, a continuous fermentation rate caused the yeast cells to experience osmotic shock that has an inhibitory effect on the yeast (Azhar et al. 2017; Cavalaglio et al. 2016). The results revealed that the production of bioethanol started to decline immediately after 72-h and 26 °Bx of incubation time and molasses concentration, respectively.

A positive interactive effect of mixing rate and incubation time on the production of bioethanol from sugarcane molasses is demonstrated in Fig. 6f. The production of bioethanol improved by increasing the mixing rate from 110.43 to 149.94 rpm and the incubation time from 48.15 to 71.96-h. A lower bioethanol yield of 19.60% was shown at 48.15-h and 110.43 rpm. However, a maximum bioethanol production of 32.45% was produced at 149.94 rpm and 71.96-h of mixing rate and incubation time, respectively. The plot also demonstrated that the bioethanol yield started to decrease at 147.92 rpm and 70.89-h of mixing rate and incubation time, respectively. Furthermore, when the incubation period reached 72-h, the

production of bioethanol started to decline. This might be due to ethanol oxidation, organic acid formation, and substrate depletion in the fermentation broth that could potentially accelerate the deactivation of enzymes and thereby lowering both ethanol yield and yeast cell viability (Kopsahelis et al. 2007). Low ethanol yield may also occur due to the formation of secondary byproducts that limit ethanol productivity (Ergun and Mutlu 2000; Hamouda et al. 2015).

Fermentation under optimum conditions

Ethanol fermentation parameters, such as pH, inoculum size, molasses concentration, temperature, mixing rate, and incubation period, were optimized in the above described experiments and applied to evaluate the reliability of the model equation using batch fermentation. In the maximum predicted bioethanol concentration of 49 g^{-1} L, bioethanol yield of 78.6% was obtained under the predicted optimal conditions of pH 5.5, 20% inoculum size, 25 °Bx initial molasses concentration, 30 °C temperature, 72-h incubation period, and 159 rpm with the desirability of 1.0.

Fermentation was conducted using a bioreactor with a working volume of 5 L to validate the predicted optimal conditions in the actual experiment using the parameters specified above. Results showed that, during the fermentation process, the pH value slightly declined from 5.5 to 5.27 at 24-h. However, after 48-h of the incubation period, the pH returned back to the optimal condition (i.e., 5.5) without adjustment and was kept constant until completion of the ethanol fermentation process, which might be due to the production of enzymes and other chemicals required for adaptation to the new environment to facilitate the overall ethanol fermentation process. On the other hand, molasses might exhibit a buffering effect attributed to its acid composition (weak acids and amino acids) and phosphates that would regulate the pH values to 3-5 and 6-7, respectively (Cazetta et al. 2007).

In the present study, the key process variables were experimentally supported to produce a maximum bioethanol concentration of 56 $g.L^{-1}$ with a bioethanol yield of 86% and a percent theoretical yield of 95% from the 25 °Bx molasses concentration within 72-h in Fig. 7d. Figure 7b and c shows the chromatogram of the internal ethanol standard at 30% dilution and the highest ethanol concentration throughout all experimental runs, respectively. The overview of the fermentation steps under optimum fermentation conditions is demonstrated in Fig. 8. Hamouda et al. (2015) obtained a bioethanol concentration and bioethanol yield of 32.32 $g.L^{-1}$ and 44%,

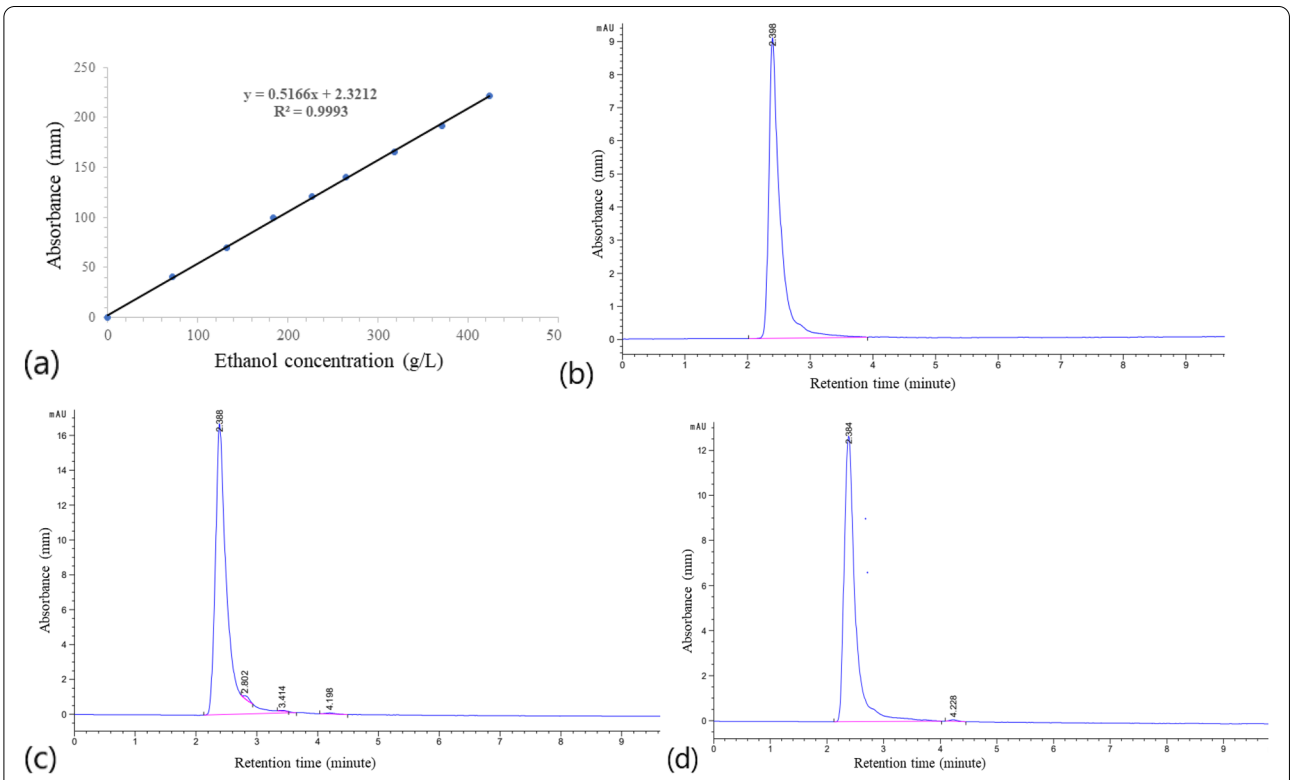

Fig. 7 Standard ethanol calibration curve (**a**), standard ethanol concentration chromatogram at 30% dilution (**b**), maximum ethanol concentration chromatogram among experimental runs (**c**), and validation experiment under optimum operating conditions (**d**)

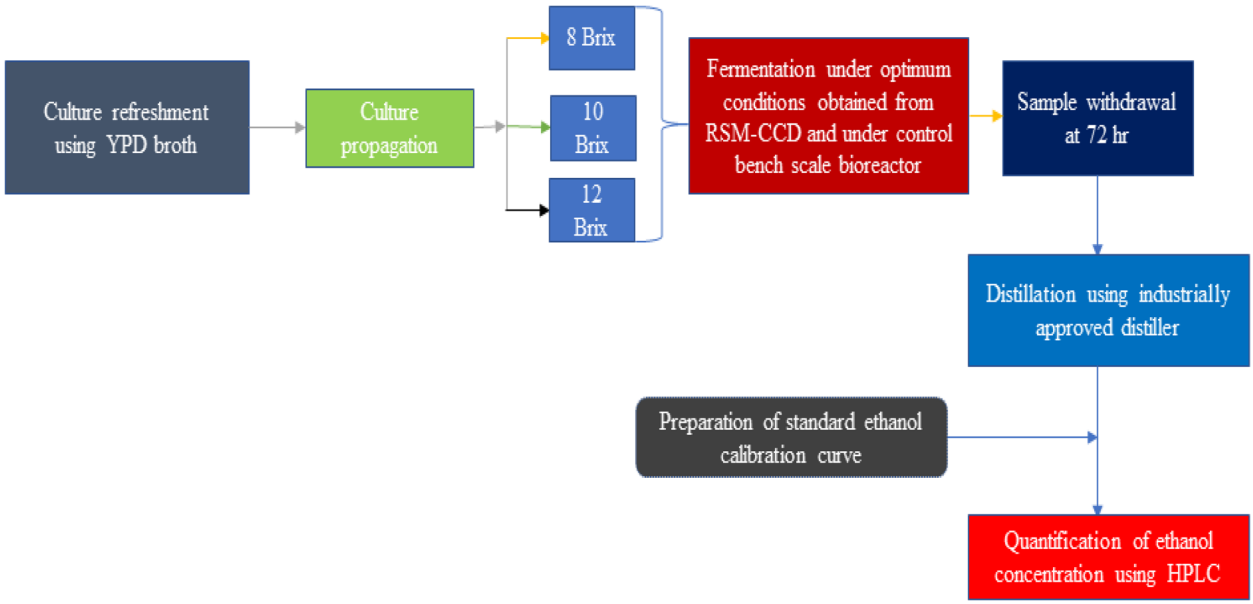

Fig. 8 Overveiw of complete set experiments under optimum fermentation conditions obtained from response surface methodology (RSM) based on the central composite design (CCD). Under these optimum condition, a bioethanol concentration 56 g.L^{-1} and a bioethanol yield of 86% were produced

respectively, from sugarcane molasses using *Pichia veronae* strain HC-22 during 60-h fermentation time. The bioethanol yield in our study was found higher due to the differences in fermentation period, inoculum size, fermenting microorganisms, and pretreatment methods. Over all, the current study suggests that the process optimization using RSM was applicable to optimize the bioethanol fermentation reliably from molasses by *M. caribbica* isolate MJTm3.

Materials and methods

Feedstock collection

For testing the bioethanol fermentation of the yeasts, 20 L of raw and fresh concentrated sugarcane molasses (63 °Bx) was obtained from the molasses storage tanker at the Fincha Sugar Factory using sterile plastic containers on March 2020. Prior to collection, the automated agitator was used to homogenize the biomass. Fincha Sugar Factory is located in Wollega province in West-Central Ethiopia (8° 31′ N 39° 12′ E) that has a humid subtropical climate with average annual temperatures of 31 °C. The biomass was immediately transported in an icebox to the Fermentation Laboratory, Department of Biotechnology, Addis Ababa Science and Technology University. After arrival at the laboratory, the biomass was kept at room temperature for 24-h in tightly closed containers for further propagation and fermentation use.

Pretreatment of molasses

Raw molasses was pretreated with 99.8% sulfuric acid (H_2SO_4) and heated at 90 °C for 2-h (Zohri et al. 2022) to remove unwanted particles, dirt, and retarding microbial contaminants (De Vasconcelos et al. 2004; Malik 2016; Rahman et al. 2013). To achieve the desired Brix level, various amounts of raw molasses were diluted with distilled water. In agreement with Hawaz et al. (2022), for yeast cell propagation, the concentration of molasses was adjusted to 8, 10, and 12 °Bx, while it was diluted in accordance with the design matrix for ethanol fermentation. Diammonium phosphate (DAP) was added to the medium and homogenized with a magnetic stirrer at the optimum concentration of 4 g L^{-1}. Finally, the pH was adjusted using 99.8% H_2SO_4 and autoclaved at 121 °C for 15 min in line with the design matrix. The treated medium was standing overnight under laminar air flow for decantation (Arshad et al. 2008). The clear upper suspension of the broth was then carefully poured into a newly sterile fermentation flask under the safety cabinet.

Medium preparation

For inoculum preparation, yeast extract peptone dextrose (YPD) broth supplemented with 100 mg L^{-1} chloramphenicol was utilized with a composition of (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} dextrose, and 20 g L^{-1} agar) (Ramos et al. 2013). The pH of the YPD broth

was adjusted to 5.5 using 1N HCl and/or 1N NaOH and sterilized at 121 °C for 15 min. The strain was maintained at 4 °C on YPD agar slants.

To prepare the propagation and fermentation medium, concentrated molasses were diluted with various amounts of distilled water to reach the desired Brix level (degree of dissolved soluble solids in water). The pH of the propagation medium was then adjusted to 5.5 using 99.8% of concentrated sulfuric acid (H_2SO_4) (De Vasconcelos et al. 2004), while the pH of the fermentation medium was adjusted in accordance with the set experimental conditions (supplementary data), and sterilization at 121 °C for 15 min was then performed.

Culture source, inoculum preparation, and propagation

In this study, *Meyerozyma caribbica* isolate MJTm3, a wild indigenous yeast with the accession number OM329077, was used from the culture collection at Department of Biotechnology, Addis Ababa Science and Technology University, Ethiopia. This yeast was previously isolated from the Metehara sugar factory's sugarcane mill juice tanker three denoted as MJTm3 and was used in this experimental study because it demonstrated a remarkable tolerance to ethanol (20% of ethanol), sugar (50% (w/v) of glucose), and temperature (45 °C) (Hawaz et al. 2022).

M. caribbica isolate MJTm3 was refreshed from the stock culture in 10 mL of yeast extract peptone dextrose (YPD) broth at pH 5.5 and incubated at 30 °C for 24 h in a rotary shaker incubator operating at 150 rpm (Flayeh 2017). The yeast cells were harvested by centrifuging at 5000 g and resuspended in a sterile 10 mL of 1% peptone water followed by a second centrifugation. The harvested cells were resuspended in 10 mL of 1% peptone water and used as an active culture for inoculation (Boboye and Dayo-Owoyemi 2009).

Inoculum propagation was carried out in 250 mL of sterilized and cotton plugged Erlenmeyer flasks. An amount of 1 mL of active viable yeast cells (2.51×10^8 cells/mL) was inoculated into the sterilized molasses propagation medium (MPM) adjusted at four concentration (8, 10, 12, and 14 °Bx). Finally, 4 g L^{-1} of diammonium phosphate was added to the fermentation medium (Hawaz et al. 2022). Propagation of *M. caribbica* isolate MJTm3 was carried out under vigorous shaking at 150 rpm and 30 °C for 24-h.

Fermented samples were collected every 6-h for 24-h to measure the Brix, alcohol content (v/v), residual sugar (RS%), pH, and cell viability of the propagated medium. These measurements were made using a refractometer (ATAGO densimeter model 2312; ATAGO Co., Ltd., Tokyo, Japan), a pH meter, ebulliometer, and a hemocytometer, respectively. For molasses fermentation, propagated cultures containing 3.0×10^8 cells/mL, RS% 3.0%, and an alcohol concentration between 3 and 4% (v/v) were selected (Mukhtar et al. 2010).

Batch fermentation

A batch fermentation was carried out in 1000 mL Erlenmeyer flasks containing different concentrations (degree Brix) of molasses and pH that were adjusted in accordance with the experimental design (supplementary data). The molasses fermentation medium (MFM) was inoculated with propagated yeast cells as an inoculum as per the experimental design specification. Bioethanol fermentation was performed under a shaker incubator regulated at different shaking speeds and temperatures according to the experimental conditions established in the complete design matrix. Samples for analysis were taken according to the prescribed incubation period (h), and the ethanol concentration (g L^{-1}) of each experimental run was quantified using high-performance liquid chromatography (HPLC) equipped with a UV detector (Duarte et al. 2009).

Quantitative estimations

The total reducing sugar (TRS%) and residual sugar content (RS%) of treated molasses and the fermented broth samples were determined using the 3,5-dinitrosalicylic acid method (DNS) (Miller 1959) at Wonji Research and Development Center, Ethiopian Sugar Corporation, Ethiopia. The ethanol concentration analysis was carried out at the Department of Food Engineering, Addis Ababa Science and Technology University, Ethiopia. In order to accomplish a standard calibration curve, different volumes of the internal standard were diluted with the standard solution (acetone) in a 2 mL vial. Six different concentrations (5, 10, 15, 20, 25, and 30%) were prepared (Fig. 7a). The same percentage of the internal standard solution for sample preparation was added (Mohammed et al. 2018).

The ethanol concentration (g L^{-1}) was measured by high-performance liquid chromatography (HPLC) (1200 Series Agilent HPLC, Germany) equipped with an UV detector at 280 nm (model Agilent 1260 infinity, Germany) and Spherisorb Amino (NH_2) Cartridge column (pore size 80 A, inner diameter 4.6 mm, length 250 mm, and particle size 5 μm, Waters, Germany). The mobile phase was acetonitrile and water (70:30 (v/v)), the flow rate was 0.25 mL/min, and the sample injection volume was 10 μl with a column temperature of 25 °C. Estimation of bioethanol yield ($Y_{E/S}$), volumetric ethanol productivity (P_V), and percent of theoretical ethanol yield was determined using Eqs. 2–4 as described by Hamouda et al. (2015) and Laopaiboon et al. (2009).

$$Y_{E/S}\,(\%) = \frac{bioethanol\ concentration\ (\mathrm{g\,L-1})}{total\ sugar\ utilized\ (\mathrm{g\,L-1})} \times 100 \quad (2)$$

$$\%\ of\ theoretical\ yield = \frac{actual\ ethanol\ content\ (g)}{theoretical\ ethanol\ content\ (g)} \times 100 \quad (3)$$

$$PV\,(\mathrm{g/L/h}) = \frac{maximum\ ethanol\ concentration(\mathrm{g\,L-1})}{fermentation\ time\ (hr)} \quad (4)$$

Experimental design

The RSM based on CCD was used to optimize the bioethanol fermentation parameters from molasses by the stress-tolerant *M. caribbica* isolate MJTm3. Fermentation factors which exerted a significant effect on the ethanol percentage (v/v) were selected based on the data obtained through the single factor experiment, varying one variable at a time optimization. Design-Expert 12.0 (State-Ease, Inc., Minneapolis, USA) was employed to generate experimental runs using CCD with two levels (+1 and −1) for six independent factors (Table 3). In addition, this model was used to develop regression and graphical analysis of the experimental data. Analysis of variance (ANOVA) was applied to evaluate the key contribution and significance of each variable to the bioethanol yield (Cavalaglio et al. 2016).

The model fit statistics described by the equation was confirmed by regression model analysis. RSM was used to identify the optimal operating conditions of each independent variable for ethanol production (Bezerra et al. 2008). According to the CCD, two levels corresponding to low (−1) and high (+1) were used for each experimental variable, namely temperature (A), initial pH (B), inoculum size (C), initial molasses concentration (D), mixing rate (E), and incubation period (F) (Table 3). The central point was replicated 10 times for a total of 86 experimental runs (supplementary data). In the present study, bioethanol yield was selected as a response parameter during optimization of ethanol fermentation parameters.

A response surface experiment was done to produce a prediction model to detect the interaction effects of the independent factors and optimize the fermentation variables with a maximum bioethanol yield. The experimental data were fitted to the respective response variable's second-order polynomial equation as follows:

$$Y = \beta o + \sum_{i=1}^{5} \beta_i x_i + \sum_{i=1}^{5} \sum_{j=i+1}^{6} \beta_{ij} x_i x_j + \sum_{6}^{i=1} \beta_{ii} x_i^2 \quad (5)$$

where Y is the bioethanol yield (%) and βo the value of the center point. βi, βij, and βii are the linear, interactive, and quadratic coefficients, respectively, and xi and xj are the independent factors.

Table 3 Parameters and level of the experiment design

Parameters	Levels		
	−1	0	+1
Temperature, °C	25	30	35
pH	5.5	6.0	6.5
Inoculum size, % (v/v)	10	15	20
Molasses concentration (w/v)	25	30	35
Mixing rate, rpm	110	130	150
Incubation period, h	48	60	72

Fermentation under optimum conditions

A validation experiment was conducted under the optimal fermentation conditions obtained from the response surface plot. After adjusting the pH to 5.5, the molasses fermentation medium (MFM) was sterilized at 121 °C for 15 min and allowed to stand overnight to cool down to room temperature and sedimented unnecessary constituents suspended in the molasses fermentation broth (Arshad et al. 2008). The clear suspension from the upper part was transferred aseptically and inoculated with the previously optimized inoculum size of 20% (v/v) into a bioreactor (ECMA-C20604RS, Taiwan) with a working volume of 5 L containing 25 °Bx of molasses concentration. Initially, the yeast cells were propagated at aeration rate of 3.0 vvm for 24-h (Chang et al. 2018). The pH and dissolved oxygen were measured by a pH meter and oxygen electrode, respectively. The fermentation process was carried out anaerobically at 29 °C and 160 rpm for 72-h. Finally, the ethanol concentration (g L^{-1}) was determined using HPLC according to the previously described method under Quantitative estimations section.

Data analysis

The significance of each fermentation parameters was analyzed using ANOVA analysis under central composite design (CCD). The significant difference in the variables was considered at $p < 0.05$. All of the experiments were done in triplicate.

Conclusion

The RSM were found reliable to identify the key process variables and to optimize bioethanol fermentation parameters using the wild indigenous yeast strain *M. caribbica* isolate MJTm3 isolated from sugarcane mill juice tanker. In addition, the quadratic model and 3-D response surfaces plots were found suitable to predict and investigate the variation of the bioethanol yield as per the experimental design. Depending on the maximum bioethanol yield obtained from the surface plot, the

interaction of five factors, namely molasses concentration and mixing rate, molasses concentration and incubation period, mixing rate and incubation period, temperature and mixing rate, molasses concentration and temperature, and temperature and incubation period, showed a significant effect on the ethanol production. Hence, the obtained bioethanol concentration of 56 g L^{-1} and bioethanol yield of 86% were comparable with the predicted results 49 g L^{-1} and 78.6% of bioethanol concentration and bioethanol yield, respectively, reflecting the accuracy and applicability of RSM to optimize bioethanol production from molasses. Thus, further optimization under large-scale bioreactors with controlled fermentation parameters should be conducted.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-022-01706-3.

Additional file 1: Supplementary data. Experimental design matrix using Response surface central composite design (CCD) and response for each experimental trial.

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Authors' contributions
EH, MT, AT, DB, SK, AA, AD, SD, GK, and DM wrote the proposal and collected the samples. EH conducted the data analysis and prepared the manuscript. DM, AT, SK, MG, and TB edited the manuscript. MG preserved and collected yeast cultures at the Westerdijk Fungal Biodiversity Institute in the Netherlands. The author(s) read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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References

Arshad M, Khan Z, Shah F, Rajoka M (2008) Optimization of process variables for minimization of byproduct formation during fermentation of blackstrap molasses to ethanol at industrial scale. Lett Appl Microbiol 47(5):410–414. https://doi.org/10.1111/j.1472-765X.2008.02446.x

Atitallah IB, Ntaikou I, Antonopoulou G, Alexandropoulou M, Brysch-Herzberg M, Nasri M, Lyberatos G, Mechichi T (2020) Evaluation of the non-conventional yeast strain Wickerhamomyces anomalus (Pichia anomala) X19 for enhanced bioethanol production using date palm sap as renewable feedstock. Renew Energy 154:71–81. https://doi.org/10.1016/j.renene.2020.03.010

Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM, Rodrigues KF (2017) Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep 10:52–61. https://doi.org/10.1016/j.bbrep.2017.03.003

Basso LC, De Amorim HV, De Oliveira AJ, Lopes ML (2008) Yeast selection for fuel ethanol production in Brazil. FEMS Yeast Res 8(7):1155–1163. https://doi.org/10.1111/j.1567-1364.2008.00428.x

Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76(5):965–977. https://doi.org/10.1016/j.talanta.2008.05.019

Boboye B, Dayo-Owoyemi I (2009) Evaluation of dough sensory properties impacted by yeasts isolated from cassava. J Appl Sci 9(4):771–776 ISSN 1812-5654.

Bouallagui H, Touhami Y, Hanafi N, Ghariani A, Hamdi M (2013) Performances comparison between three technologies for continuous ethanol production from molasses. Biomass Bioenergy 48:25–32. https://doi.org/10.1016/j.biombioe.2012.10.018

Bušić A, Marđetko N, Kundas S, Morzak G, Belskaya H, Ivančić Šantek M, Komes D, Novak S, Šantek B (2018) Bioethanol production from renewable raw materials and its separation and purification: a review. Food Technol Biotechnol 56(3):289–311. https://doi.org/10.17113/ftb.56.03.18.5546

Campbell J, Block E (2010) Land-use and alternative bioenergy pathways for waste biomass. Environ Sci Technol 44(22):8665–8669. https://doi.org/10.1021/es100681g

Cavalaglio G, Gelosia M, Ingles D, Pompili E, D'Antonio S, Cotana F (2016) Response surface methodology for the optimization of cellulosic ethanol production from Phragmites australis through pre-saccharification and simultaneous saccharification and fermentation. Ind Crop Prod 83:431–437. https://doi.org/10.1016/j.indcrop.2015.12.089

Cazetta M, Celligoi M, Buzato J, Scarmino I (2007) Fermentation of molasses by Zymomonas mobilis: effects of temperature and sugar concentration on ethanol production. Bioresour Technol 98(15):2824–2828. https://doi.org/10.1016/j.biortech.2006.08.026

Chang Y-H, Chang K-S, Chen C-Y, Hsu C-L, Chang T-C, Jang H-D (2018) Enhancement of the efficiency of bioethanol production by Saccharomyces cerevisiae via gradually batch-wise and fed-batch increasing the glucose concentration. Fermentation 4(2):45. https://doi.org/10.3390/fermentation4020045

De Vasconcelos J, Lopes C, De Franca F (2004) Continuous ethanol production using yeast immobilized on sugar-cane stalks. Braz J Chem Eng 21:357–365. https://doi.org/10.1590/S0104-66322004000300002

Duarte WF, Dias DR, de Melo Pereira GV, Gervásio IM, Schwan RF (2009) Indigenous and inoculated yeast fermentation of gabiroba (Campomanesia pubescens) pulp for fruit wine production. J Ind Microbiol Biotechnol 36(4):557–569. https://doi.org/10.1007/s10295-009-0526-y

El-Gendy NS, Madian HR, Amr SSA (2013) Design and optimization of a process for sugarcane molasses fermentation by Saccharomyces cerevisiae using response surface methodology. Int J Microbiol 2013. https://doi.org/10.1155/2013/815631

Ergun M, Mutlu SF (2000) Application of a statistical technique to the production of ethanol from sugar beet molasses by Saccharomyces cerevisiae. Bioresour Technol 73(3):251–255. https://doi.org/10.1016/S0960-8524(99)00140-6

Flayeh HM (2017) Optimization of process parameters for bioethanol production from low grade Iraqi dates. Al-Nahrain J Eng Sci 20(3):647–656 https://nahje.com/index.php/main/article/view/257
Gebreegziabher Z, Mekonnen A, Ferede T, Kohlin G (2017) Profitability of bioethanol production: the case of Ethiopia. Ethiop J Econ 26(1):101–122 eISSN: 1993-3681
Ghosh P, Ghose TK (2003) Bioethanol in India: recent past and emerging future. Biotechnol India II:1–27. https://doi.org/10.1007/3-540-36466-8_1
Hamouda HI, Nassar HN, Madian HR, Amr SSA, El-Gendy NS (2015) Response surface optimization of bioethanol production from sugarcane molasses by Pichia veronae strain HSC-22. Biotechnol Res Int 2015. https://doi.org/10.1155/2015/905792
Hansen AC, Zhang Q, Lyne PW (2005) Ethanol–diesel fuel blends—a review. Bioresour Technol 96(3):277–285. https://doi.org/10.1016/j.biortech.2004.04.007
Hawaz E, Tafesse M, Tesfaye A, Beyene D, Kiros S, Kebede G, Boekhout T, Theelen B, Groenewald M, Degefe A, Degu S, Admas A, Muleta D (2022) Isolation and characterization of bioethanol producing wild yeasts from bio-wastes and co-products of sugar factories. Ann Microbiol 72(1):39. https://doi.org/10.1186/s13213-022-01695-3
Jargalsaikhan O, Saraçoğlu N (2008) Application of experimental design method for ethanol production by fermentation of sunflower seed hull hydrolysate using Pichia stipitis NRRL-124. Chem Eng Commun 196(1-2):93–103. https://doi.org/10.1080/00986440802300992
John RP, Anisha G, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102(1):186–193. https://doi.org/10.1016/j.biortech.2010.06.139
Kamal S, Rehman S, Rehman K, Ghaffar A, Bibi I, Ahmed T, Maqsood S, Nazish N, Iqbal HM (2021) Sustainable and optimized bioethanol production using mix microbial consortium of Saccharomyces cerevisiae and Candida cantarelli. Fuel:122763. https://doi.org/10.1016/j.fuel.2021.122763
Khuong L, Masjuki H, Zulkifli N, Mohamad EN, Kalam M, Alabdulkarem A, Arslan A, Mosarof M, Syahir A, Jamshaid M (2017) Effect of gasoline–bioethanol blends on the properties and lubrication characteristics of commercial engine oil. RSC Adv 7(25):15005–15019. https://doi.org/10.1039/C7RA00357A
Kopsahelis N, Agouridis N, Bekatorou A, Kanellaki M (2007) Comparative study of spent grains and delignified spent grains as yeast supports for alcohol production from molasses. Bioresour Technol 98(7):1440–1447. https://doi.org/10.1016/j.biortech.2006.03.030
Laopaiboon L, Nuanpeng S, Srinophakun P, Klanrit P, Laopaiboon P (2009) Ethanol production from sweet sorghum juice using very high gravity technology: effects of carbon and nitrogen supplementations. Bioresour Technol 100(18):4176–4182. https://doi.org/10.1016/j.biortech.2009.03.046
Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H (2012) Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. Biomass Bioenergy 47:395–401. https://doi.org/10.1016/j.biombioe.2012.09.019
Liu R, Shen F (2008) Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized Saccharomyces cerevisiae (CICC 1308). Bioresour Technol 99(4):847–854. https://doi.org/10.1016/j.biortech.2007.01.009
Malik H (2016) Utilization of agro-industrial wastes for the biomass production of baker's yeast. M. Sc. Thesis, Punjab Agricultural University, Punjab
Miguel GA, Carlsen S, Arneborg N, Saerens SM, Laulund S, Knudsen GM (2022) Non-Saccharomyces yeasts for beer production: insights into safety aspects and considerations. Int J Food Microbiol:109951. https://doi.org/10.1016/j.ijfoodmicro.2022.109951
Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426–428. https://doi.org/10.1021/ac60147a030
Mohammed AH, Mohammed AK, Kamar FH, Abbas AA, Nechifor G (2018) Determination of ethanol in fermented broth bu headspace gas chromatography using capillary column. Rev Chim Burcharest-Orig Ed 69(11):2969–2972. https://doi.org/10.37358/RC.18.11.6664
Morimura S, Ling ZY, Kida K (1997) Ethanol production by repeated-batch fermentation at high temperature in a molasses medium containing a high concentration of total sugar by a thermotolerant flocculating yeast with improved salt-tolerance. J Ferment Bioeng 83(3):271–274. https://doi.org/10.1016/S0922-338X(97)80991-9
Mukhtar K, Asgher M, Afghan S, Hussain K, Zia-Ul-Hussnain S (2010) Comparative study on two commercial strains of Saccharomyces cerevisiae for optimum ethanol production on industrial scale. J Biomed Biotechnol 2010. https://doi.org/10.1155/2010/419586
Mussatto SI, Dragone G, Guimarães PM, Silva JPA, Carneiro LM, Roberto IC, Vicente A, Domingues L, Teixeira JA (2010) Technological trends, global market, and challenges of bio-ethanol production. Biotechnol Adv 28(6):817–830. https://doi.org/10.1016/j.biotechadv.2010.07.001
Mussatto SI, Machado EM, Carneiro LM, Teixeira JA (2012) Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Appl Energy 92:763–768. https://doi.org/10.1016/j.apenergy.2011.08.020
Phisalaphong M, Srirattana N, Tanthapanichakoon W (2006) Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation. Biochem Eng J 28(1):36–43. https://doi.org/10.1016/j.bej.2005.08.039
Rahman SS, Hossain MM, Choudhury N (2013) Effect of various parameters on the growth and ethanol production by yeasts isolated from natural sources. Bangladesh J Microbiol 30(1-2):49–54. https://doi.org/10.3329/bjm.v30i1-2.28453
Ramos CL, Duarte WF, Freire AL, Dias DR, Eleutherio ECA, Schwan RF (2013) Evaluation of stress tolerance and fermentative behavior of indigenous Saccharomyces cerevisiae. Braz J Microbiol 44(3):935–944. https://doi.org/10.1590/S1517-83822013005000051
Rattanapan A, Limtong S, Phisalaphong M (2011) Ethanol production by repeated batch and continuous fermentations of blackstrap molasses using immobilized yeast cells on thin-shell silk cocoons. Appl Energy 88(12):4400–4404. https://doi.org/10.1016/j.apenergy.2011.05.020
Razmovski R, Vučurović V (2011) Ethanol production from sugar beet molasses by S. cerevisiae entrapped in an alginate–maize stem ground tissue matrix. Enzym Microb Technol 48(4-5):378–385. https://doi.org/10.1016/j.enzmictec.2010.12.015
Sarris D, Matsakas L, Aggelis G, Koutinas AA, Papanikolaou S (2014) Aerated vs non-aerated conversions of molasses and olive mill wastewaters blends into bioethanol by Saccharomyces cerevisiae under non-aseptic conditions. Ind Crop Prod 56:83–93. https://doi.org/10.1016/j.indcrop.2014.02.040
Shafaghat H, Najafpour GD, Rezaei SP, Sharifzadeh M (2010) Optimal growth of Saccharomyces cerevisiae (PTCC 24860) on pretreated molasses for ethanol production: application of response surface methodology. Chem Ind Chem Eng Q 16(2):199–206. https://doi.org/10.2298/CICEQ100201029S
Turhan O, Isci A, Mert B, Sakiyan O, Donmez S (2015) Optimization of ethanol production from microfluidized wheat straw by response surface methodology. Prep Biochem Biotechnol 45(8):785–795. https://doi.org/10.1080/10826068.2014.958164
Uncu ON, Cekmecelioglu D (2011) Cost-effective approach to ethanol production and optimization by response surface methodology. Waste Manag 31(4):636–643
Yüksel F, Yüksel B (2004) The use of ethanol–gasoline blend as a fuel in an SI engine. Renew Energy 29(7):1181–1191. https://doi.org/10.1016/j.wasman.2010.12.007
Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Nasrulhaq Boyce A (2014) Bioethanol production from fermentable sugar juice. Sci World J 2014. https://doi.org/10.1155/2014/957102
Zohri AE-NA, Soliman MF, Ibrahim OM, Abdelaziz AM (2022) Reducing heavy metals content in sugarcane molasses and its effect on ethanol fermentation efficiency. Egypt Sugar J 18(0). https://doi.org/10.21608/ESUGJ.2022.143054.1011

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