ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Efficient Bioconversion of Mango Waste into Ethanol Employing Plackett–Burman and Central Composite Models

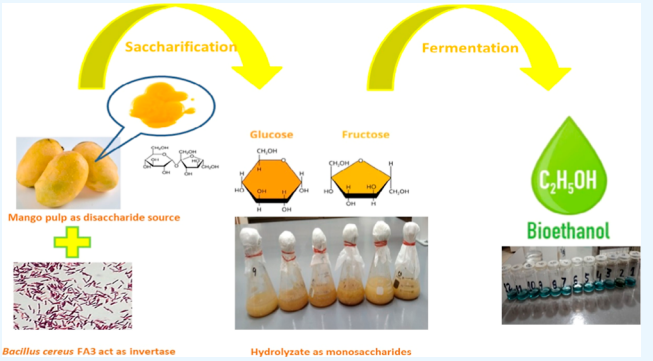
Asma Chaudhary,* Zawar Hussain, Hafsa Ajmal, Rahat Abdul Rehman, Ghulam Abbas, Ayesha Aihetasham, and Syeda Anjum Tahira

Cite This: *ACS Omega* 2024, 9, 39652–39662

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: The current study focuses on the idea of "Energy from Waste" that intends to address energy crises and manage waste. Fruit waste is one of the most common forms of organic waste due to its inedible portion and perishable nature. In Pakistani regions, an extensive amount of mango pulp (MP)/juice waste is produced due to excessive consumption during summers, which poses huge environmental challenges. The study aims at effective valorization of perishable waste and elimination of deteriorating waste that causes a polluting environment. Experimental work has been conducted to evaluate the sucrolytic potential of *Bacillus cereus* FA3 for the bioconversion of sucrose from mango waste into reducing sugars for ethanologenesis. The Plackett–Burman model was designed to analyze enzymatic hydrolytic parameters for sugar conversion. The model was significant for reducing sugars with F and p values of 43.99 and 0.0013 correspondingly. 11.43 ± 0.068 g/L maximum reducing sugars were analyzed in MP after hydrolysis with 12.58 IU of crude enzyme dosage of *B. cereus* FA3 at 30 °C within 5 days with a 22% enzyme conversion rate. Additionally, the ethanologenic potentials of experimental *Metschnikowia cibodasensis* Y34 and standard *Saccharomyces cerevisiae* K7 yeasts were investigated from mango hydrolyzate when subjected to central composite design as a statistical optimization tool. These findings exhibited significantly higher response outcomes and good development for waste management.



1. INTRODUCTION

In many countries around the world, particularly in Asian regions, seasonal fruit is the most significant aspect of agribusiness. In Pakistan, it is quite significant from an economic and social perspective due to its export and preparation of juices, beverages, and other products at the industrial level. Waste production is observed at every step of the food supply chain, from agricultural production to final consumption and disposal. This includes postharvest transport, storage, fruit processing, fruit packaging, distribution, and consumption.[1−4] The peels and pulp waste that remain after the extraction of juices become new and alternative substrates for the synthesis of biofuel. Pakistan is a leading exporter and producer of mangoes in the world with approximately 2.3 million tons of annual production contributing 2.48% of agricultural GDP.[5−7] The global mango market is projected to increase from $63.65 billion in 2023 to $67.95 billion in 2024 at a compound annual growth rate of 6.7%. Due to their highly perishable nature, high production, poor organoleptic standards, pest infestation, low opening in the local and international markets, and improper postharvest management, massive quantities of mangoes are wasted during the peak season. Mangoes that are undersized, with marked and spotty peels, grading-rejected, and mechanically damaged are deemed waste and must be disposed of properly to avoid serious environmental or economic problems.[8]

Farmers can diversify their harvests and increase their profitability by using discarded mangoes as a low-cost, concentrated biomass feedstock and as a raw material for the production of value-added byproducts.[9] Mango postharvest and processing wastes have the potential to be employed as feedstocks for the synthesis of bioethanol, which makes them a desirable substitute for polluting disposed residues. Vegetable and fruit wastes can be utilized directly as substrates for microbiological growth or treated with enzymes to produce bioenergy.[10] It is economically and environmentally feasible to produce bioethanol from waste materials in order to replace

Received: May 8, 2024
Revised: August 31, 2024
Accepted: September 5, 2024
Published: September 13, 2024

petroleum-based products.[11,12] The fruit is soft and tender textured, which makes it more susceptible to microbial degradation. Being a sugar-rich perishable substrate, it is crucial to harvest promptly, extract the juice instantly, store and transport the juice, and waste it after processing properly to carry out the sugar preservation and fermentation processes.[13,14] Cold food chain technology plays a crucial role in maintaining the quality of perishable food items during storage and transportation. In the supply chain, refrigerated storage of perishable food/waste is necessary to prolong its shelf life and safety from microbial contamination.[15,16]

Mango juice fermentation is an economically feasible way to produce an appropriate quantity of ethanol due to its high reducing sugar content. Generally, high sugar contents (15−20% w/v) are preferred for industrial ethanol production as yeast cells have the capability to metabolize these sugars directly.[9,17−19] The major sugars in mature or ripened mangoes are sucrose, fructose, and glucose (in descending order of their concentration), as well as small quantities of cellulose, hemicellulose, pectin, protein (0.8%), and other dietary fibers (1.6%).[20] The relative contributions of nonreducing (sucrose) and reducing (glucose and fructose) sugars to the overall sugar content of mangoes vary as reducing sugars are observed at the beginning of their ripening process while sucrose is observed at the end.[21] The harvesting and transportation costs were minimized by collecting the mango waste from the packaging units.

The hydrolysis of cellulose, hemicellulose, and other polysaccharides into their respective monomers, called saccharification, is an important step in bioethanol production and involves the use of microorganisms or their enzymes, namely, cellulases, hemicellulases, xylanases, amylases, and invertases.[22] As compared to conventional chemical processes, enzyme-based procedures produce less hazardous byproducts, side effects, and polluting toxic wastes. Invertases can be used to carry out the hydrolytic process enzymatically on sucrose-enriched substrates for the hydrolysis of disaccharides.[23] Hydrolysis of sucrose in mango pulp (MP) with invertase is a step in producing fructose and glucose. The hydrolases known as invertases (β-fructofuranosidase, EC 3.2.1.26), also referred to as β-D-fructofuranosidefructohydrolase or saccharases or sucrases, catalyze the conversion of sucrose (β-D-fructofuranosyl α-D-glucopyranoside) into glucose and fructose.[24,25] The sucrose monosaccharides function as signaling molecules and regulate stress conditions, in addition to serving as the main substrates for the biosynthesis of starch and cellulose.[26] The invertase not only performs hydrolysis but also exhibits transferase activity, particularly when sucrose concentrations are high. This characteristic places invertase in the fructosyltransferase (EC 2.4.1.9) class of enzymes. Additionally, invertase hydrolyzes other oligosaccharides like raffinose, stachyose, and kestose.[27] Many different types of organisms, including bacteria, fungi, yeast, plants, and animals, produce invertase enzymes. For many years, yeast species among microbes have been used extensively for generation and industrial applications of invertases. Furthermore, there is not much literature available on invertases from strains of bacteria. Both extracellular (exo) and intracellular (endo) invertases can be produced by bacteria. Due to their great diversity, bacterial invertases most likely differ from other microbes in terms of degree of glycosylation, glycoprotein subunit polymerization, and location of phosphorylation.[28−30] A number of microorganisms have been studied for their ability to produce invertases and ethanol from sugar juices, including *Zymomonas mobilis*, *Klebsiella oxytoca* strain P2, *Escherichia coli* KO11, dried yeast or *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Pichia kudriavzevii*, and *Kluyveromyces marxianus*.[31−35] In addition, *Bacillus* species are considered as promising candidates for industrial applications and enzyme production, specifically invertases. The Food and Drug Administration (FDA) has granted *Bacillus* the GRAS (generally regarded as safe) status, attributing this to its ability to secrete extracellular enzymes and an enhanced growth rate. Employing living organisms (microbes) through biotechnology techniques is highly efficient as it not only yields a diverse range of enzymes at a low cost but also hydrolyzes sucrose in a single step, thereby preventing the production of intermediate products.[36]

In general, bioethanol is produced by fermenting any biomass rich in sucrose, sugars, or carbon-derived compounds. Although lignocellulosic biomass has been widely studied recently, the production of bioethanol from it is still limited to pilot plants or laboratories. Compared to starch or lignocellulosic biomass, free sugar-containing pulp and juices are simpler and more economical ethanol feedstocks because they do not require expensive steps like hydrolysis, pretreatment, or inhibitor detoxification.[37,38] Hence, MP serves as an excellent source of fermentable sugars, in addition to sucrose. Furthermore, invertases hold significant value in the market for their role in hydrolysis of sucrose and are extensively utilized in the beverage and food sectors. The current study aims to achieve positive outcomes by utilizing bacterial invertases to increase the concentration of fermentable sugars in the pulp, which will subsequently lead to the production of bioethanol. This work is an exploratory study of the conversion of MP to bioethanol, which involves the use of statistical models for the enzymatic hydrolysis of sucrose into its monomers and the optimization of the fermentation conditions.

2. RESULTS

2.1. Proximate Composition of MP Waste. The proximate contents of MP (without any treatment) are presented in Table 1. The percent ash contents were 0.31 ± 0.15, while moisture was found to be 75.4 ± 0.241. Different content values (g/L), viz., 7.03 ± 0.11, 20.0 ± 1.89, and 0.48 ± 0.23, were recorded for reducing, total sugars, and protein, correspondingly.

Table 1. Estimated Biochemical Contents of Untreated MP[a]

constituents	contents
moisture (%)	75.4 ± 0.241
ash (%)	0.31 ± 0.15
reducing sugars (g/L)	7.03 ± 0.11
total sugars (g/L)	20.0 ± 1.89
total protein (g/L)	0.48 ± 0.23

[a]Values denoted mean of three replicates with standard error means.

2.2. Plackett−Burman Design (PBD) to Screen Enzymatic Hydrolytic Parameters. The reducing sugars in the saccharified samples were determined spectrophotometrically. High levels of reducing sugar contents were identified in enzymatic hydrolysis, reaching 11.43 ± 0.068 g/L, and total sugar contents were 25.01 ± 0.013 g/L after 5 days with 12.58 IU of crude enzyme dosage at 30 °C and pH 5 as presented in Table 2. The conversion of total sugars into reducing sugars was calculated as 22%, with the released sugars

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

Table 2. Plackett–Burman Matrix Depicting Hydrolysis Parameters and Responses in MP[a]

runs	F1: buffer (mL)	F2: MP (mL)	F3: enzyme dosage (IU)	F4: temp (°C)	F5: time (days)	F6: pH	red sugars (g/L)	total sugars (g/L)
1	40	25	6.29	30	1	4.0	6.64 ± 0.062	15.41 ± 0.033
2	55	25	12.58	30	1	4.0	7.71 ± 0.007	17.76 ± 0.113
3	55	50	6.29	37	1	4.0	9.23 ± 0.019	21.02 ± 0.102
4	40	50	12.58	30	5	4.0	10.32 ± 0.005	22.00 ± 0.031
5	55	25	12.58	37	1	5.0	7.48 ± 0.011	16.01 ± 0.011
6	55	25	6.29	30	5	5.0	6.75 ± 0.036	15.58 ± 0.003
7	55	50	12.58	30	5	5.0	11.43 ± 0.068	25.01 ± 0.006
8	40	25	6.29	37	5	5.0	5.74 ± 0.093	15.53 ± 0.005
9	40	25	12.58	37	5	4.0	6.88 ± 0.012	16.00 ± 0.002
10	55	50	6.29	37	5	4.0	8.21 ± 0.005	19.56 ± 0.011
11	40	50	12.58	37	1	5.0	107.26 ± 0.003	19.78 ± 0.005
12	40	50	6.29	30	1	5.0	9.19 ± 0.049	18.99 ± 0.001

[a]Values denoted mean of three replicates with standard error means.

Table 3. Analysis of Variance for the Responses after Hydrolysis Using PB Design

treatments	responses	source	sum of squares	DF	mean square	*F* value	*p* value
enzymatic hydrolysis of MP	reducing sugars	model	42.68	7	6.10	43.99	0.0013 significant
		residual	0.55	4	0.14		
		cor total	43.24	11			
	total sugars	model	49.27	7	7.04	200.8	<0.0001 significant
		residual	0.14	4	0.035		
		cor total	49.41	11			

Table 4. Regression Model for Various Responses after Hydrolysis Using PB Design

treatments	responses	CV	press	*R*-square	Adj-*R* square	Pred-*R* square	Adeq precision
enzymatic hydrolysis of MP	reducing sugars	6.68	4.99	0.9872	0.9647	0.8846	14.923
	total sugars	2.42	1.26	0.9972	0.9922	0.9745	30.369

Table 5. Validation of Predicted Parameters for Enzymatic Hydrolysis Using PB Design[a]

treatments	responses	predicted value (g/L)	experimental value (g/L)	residual	error (%)
enzymatic hydrolysis	reducing sugars	9.98	11.96 ± 0.13	1.98	10.96
	total sugars	22.32	25.41 ± 0.001	3.09	0.14

[a]Residual = experimental value − predicted value. Error = residual/predicted value × 100.

at 4.40 g/L. From the design runs, reducing sugar improvement was seen. It is hypothesized that invertases of *Bacillus cereus* FA3 may work more efficiently at a low temperature (30 °C), at higher enzyme doses and pH. A higher enzyme dose (12.58 IU) may be required to convert more substrate (50 mL MP) efficiently, whereas the same enzyme dose and a lower substrate concentration (25 mL MP) produced the opposite effect. From the experiment, it was found that the *B. cereus* FA3 enzyme performed efficiently at 30 °C and pH 5.

The data for ANOVA to interpret the appropriateness of the model for enzymatic hydrolysis are presented in Table 3. The PB model for reducing sugars after enzymatic hydrolysis was significant due to *F* values of 43.99, with a 13% chance that it occurred due to noise. The cor total (corrected total sum of squares) of 43.24 was close to the sum of squares of the model (42.68), which explained the variation in the response sum of the squares with individual observations (mean of the observations). The cor total indicates the part of variations in the model that can be explained, whereas the residual shows the unexplained variations. However, the cor total is as important as *R*-square in statistics as it helps quantify the total variability of different factors. The model for total sugars was significant due to the 200.8 *F* value, with a 0.01% chance that it occurred due to noise. The cor total for the total sugar response was 49.41, which was close to the sum of the squares of the model (49.27).

Statistical data for regression coefficients are recorded in Table 4. As far as the enzymatic hydrolysis of MP is concerned, the *R*-square for reducing sugars is 0.9872, with an Adj *R*-square of 0.9647, which might indicate the significance of the model for this response. The value of 14.923 elucidated an adequate signal for design space navigation, whereas the greater ratio of 30.369 explained the adequate signal for the total sugars. This model navigated the design space.

Table 5 shows the predicted and experimental values, along with the percent errors. In the enzymatic hydrolysate of MP, the predicted values for total and reducing sugars were 22.32 and 9.98 g/L, respectively, with 50% MP and buffer with 12.58 IU of enzyme load at 30 °C and pH 4 for 5 days. The experimental values improved when MP was subjected to hydrolysis with predicted parameters.

2.3. CCD-Based Optimization for Fermentation Conditions. The values presenting ethanol titer and yield according to different conditions planned by CCD are illustrated in Table 6. Both yeasts generate maximum ethanol contents at 25 °C, with 25 mL of hydrolyzate when incubated for 15 days. The ethanol yields recorded were 0.39 ± 0.01 and 0.38 ± 0.05 g/g of consumed sugars by *Metschnikowia*

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

Table 6. CCD Matrix Representing Fermentation Parameters and Responses for the MP Hydrolyzate

runs	parameters			*Saccharomyces cerevisiae* (K7)		*Metschnikowia cibodasensis* (Y34)	
	hydrolyzate (mL)	incubation (days)	temp (°C)	ethanol yield (g/g)	ethanol prod. (g/L)	ethanol yield (g/g)	ethanol prod. (g/L)
1	50	8	45.11	0.33 ± 0.01	8.05 ± 0.01	0.32 ± 0.00	8.03 ± 0.01
2	25	1	40	0.29 ± 0.02	7.6 ± 0.001	0.28 ± 0.01	7.6 ± 0.04
3	75	1	40	0.25 ± 0.01	7.45 ± 0.001	0.26 ± 0.01	7.24 ± 0.01
4	25	15	40	0.33 ± 0.01	8.73 ± 0.004	0.3 ± 0.01	9.81 ± 0.003
5	75	15	25	0.28 ± 0.00	8.28 ± 0.05	0.29 ± 0.00	8.41 ± 0.01
6	7.96	8	32.5	0.31 ± 0.01	6.79 ± 0.28	0.25 ± 0.01	6.12 ± 0.001
7	75	1	25	0.33 ± 0.01	7.13 ± 0.05	0.28 ± 0.02	6.81 ± 0.08
8	50	8	32.5	0.35 ± 0.00	8.29 ± 0.008	0.36 ± 0.00	8.35 ± 0.00
9	25	15	25	0.38 ± 0.00	9.16 ± 0.01	0.39 ± 0.01	10.02 ± 0.009
10	50	8	19.8	0.37 ± 0.01	8.09 ± 0.01	0.31 ± 0.00	7.63 ± 0.03
11	50	19.7	32.5	0.3 ± 0.00	7.75 ± 0.02	0.3 ± 0.02	6.72 ± 0.03
12	50	8	32.5	0.36 ± 0.02	8.31 ± 0.002	0.36 ± 0.01	8.34 ± 0.001
13	50	−3.77	32.5	0.08 ± 0.02	5.33 ± 0.01	0.08 ± 0.02	5.34 ± 0.00
14	50	8	32.5	0.36 ± 0.01	8.32 ± 0.01	0.36 ± 0.00	8.32 ± 0.01
15	50	8	32.5	0.36 ± 0.01	8.31 ± 0.003	0.37 ± 0.01	8.4 ± 0.08
16	92.0	8	32.5	0.14 ± 0.02	6.01 ± 0.11	0.13 ± 0.01	5.95 ± 0.01
17	50	8	32.5	0.36 ± 0.00	8.31 ± 0.01	0.37 ± 0.00	8.45 ± 0.06
18	25	1	25	0.28 ± 0.02	7.25 ± 0.05	0.31 ± 0.00	7.61 ± 0.01
19	50	8	32.5	0.36 ± 0.01	8.32 ± 0.01	0.37 ± 0.02	8.42 ± 0.08
20	75	15	40	0.31 ± 0.01	8.31 ± 0.08	0.32 ± 0.01	8.4 ± 0.01

Table 7. Fitted Quadratic Regression Model for Various Responses in Fermentation of WRW

responses	yeast isolates	source	sum of squares	DF	mean of square	*F* value	*p* value
ethanol yield	standard *S. cerevisiae* K7	model	0.087	9	9.555	3.79	0.025 significant
		residual	0.026	10	2.524		
		lack of fit	0.026	5	5.032	301.96	<0.0001 significant
		pure error	0.015	5	2.90		
		cor total	0.14	19			
	experimental *M. cibodasensis* Y34	model	0.085	9	9.57	3.11	0.046 significant
		residual	0.030	10	3.081		
		lack of fit	0.030	5	6.14	204.50	<0.0001 significant
		pure error	1.499	5	2.99		
		cor total	0.12	19			
ethanol titer	standard *S. cerevisiae* K7	model	13.88	9	1.53	3.18	0.0439 significant
		residual	4.90	10	0.50		
		lack of fit	4.90	5	0.97	8138.25	<0.0001 significant
		pure error	5.99	5	1.199		
		cor total	18.73	19			
	experimental *M. cibodasensis* Y34	model	16.68	9	1.84	2.17	0.124 not significant
		residual	8.60	10	0.87		
		lack of fit	8.60	5	1.73	659.98	<0.0001 significant
		pure error	0.014	5	2.59		
		cor total	25.23	19			

cibodasensis Y34 and *S. cerevisiae* K7, correspondingly. During fermentation, the concentration of sugars in the medium can have a significant effect on the final end product, i.e., ethanol contents. The presence of a suitable concentration of sugars in the medium will lead to an improvement in the fermentation efficiency. In a 20-run experiment, optimum ethanol contents were recorded at 25 mL of MP hydrolyzate, while lower contents were found at 92 and 7.96 mL. High and low concentrations may have a negative impact on yeast growth. Higher ethanol yield in the experiment may indicate that both yeast isolates performed in the MP hydrolyzate medium with greater fermentation efficiency because ethanol yield and fermentation efficiency are directly proportional to each other. This may reveal that the sugars in the fermentation medium were used optimally and fermentation conditions took place efficiently. The optimum ethanol yield was obtained after 15 days at 25 °C. It may be hypothesized that yeast cells adapted to the nutrients slowly and tolerated the ethanol contents present in the medium as an end product of the experiment.

The appropriateness of the CCD model for optimized conditions was evident by the 3.79, 0.025 *F*, and *p* values. This 2.50% chance could happen due to noise. The 301.96 *F* value inferred the significance of the "Lack of Fit". Similarly, experimental yeast indicated the significance of the ethanol yield model and lack of fit by showing 3.11 and 204.50 *F*-values, respectively. This 4.62% chance for the ethanol yield model could happen due to noise. The 3.18 *F* values implied the significance of the standard yeast model for the second

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

Table 8. Analysis of Variance for Responses in Fermented Hydrolyzate by Yeast Isolates

responses	yeast isolates	CV	press	R^2	Adj R^2	Pred R^2	Adeq precision
ethanol yield	*S. cerevisiae* K7	16.39	0.20	0.7731	0.5688	−0.7604	6.980
	M. cibodasensis Y34	18.48	0.23	0.7363	0.4989	−1.0071	5.170
ethanol titer	*S. cerevisiae* K7	24.48	36.88	0.7396	0.5052	−0.9671	6.352
	M. cibodasensis Y34	32.44	64.84	0.6597	0.3535	−1.5679	4.768

response, i.e., ethanol titer, whereas the experimental yeast implied the nonsignificance of the 2.17 *F* value (Table 7).

The data for regression analysis for coefficients, CV, and adequate precision is recorded in Table 8. The standard yeast yield model's reliability was demonstrated by 0.7731 (R^2) and 0.5688 (Adj *R*-square) values. The variables of the model corresponded with 6.980 Adeq Pre. and 16.39 CV. In a similar way, *R*-square (0.7363), Adj *R*-square (0.4989), adequate precision (5.170), and CV (18.48) values exhibited the yield model appropriateness for experimental yeast. For the ethanol titer produced by standard yeast K7, the values of *R*-square, Adj *R*-square, and adequate precision were 0.7396, 0.5052, and 6.352. On the other hand, the *R*-square, Adj *R*-square, and adequate precision values for the experimental yeast were 0.6597, 0.3535, and 4.768.

Predicted values for ethanol yield (g/g) and contents (g/L) produced by both yeast strains were 0.37 and 9.37 (K7) and 0.38 and 10.32 (Y34) under optimized conditions, viz., 50% hydrolyzate and synthetic medium, 25 °C, and 15 days. As compared with the predicted parameters, the experimental values for ethanol yield and titer were improved (Table 9).

Table 9. Validation of Optimum Fermentation Responses[a]

contents	experimental value	predicted value	residual	% error
ethanol yield K7	0.39 ± 0.01	0.38	0.01	2.6
ethanol yield Y34	0.40 ± 0.01	0.39	0.01	2.5
ethanol titer K7	9.99 ± 0.092	9.37	0.62	6.62
ethanol titer Y34	11.34 ± 0.012	10.32	1.02	9.88

[a]Residual = experimental value − predicted value. Error = residual/predicted value × 100.

2.4. Interrelationship of Different Variables for the Ethanol Yield Model. The variable interrelationship for both yeasts is presented by eq 1

$$\begin{aligned} \mathrm{Yi(K7)} = &+ 0.37 - 0.029A + 0.038B - 0.011C \\ &- 0.037A^2 - 0.047B^2 + 0.011C^2 - 0.014\mathrm{AB} \\ &- 2.49\mathrm{AC} + 7.49\mathrm{BC} + 0.015 \end{aligned} \quad (1)$$

Likewise, the ethanol yield of Y34 (eq 2) came out as

$$\begin{aligned} \mathrm{Yi(Y34)} = &+ 0.35 - 0.023A + 0.041B - 6.821C \\ &- 0.046A^2 - 0.046B^2 - 7.60C^2 - 3.749\mathrm{AB} \\ &+ 0.018\mathrm{AC} - 1.252\mathrm{BC} + 1.499 \end{aligned} \quad (2)$$

The antagonistic and synergistic relationships within variables in equation form were denoted by negative and positive signs, correspondingly.

The interaction of model variables with the ethanol yield response was illustrated by plotting 3D graphs. The relationship of three factors in ethanol yield by standard yeast K7 is presented in Figure 1 and by experimental yeast Y34 in Figure 2. Figure 1a shows that response is directly proportional to

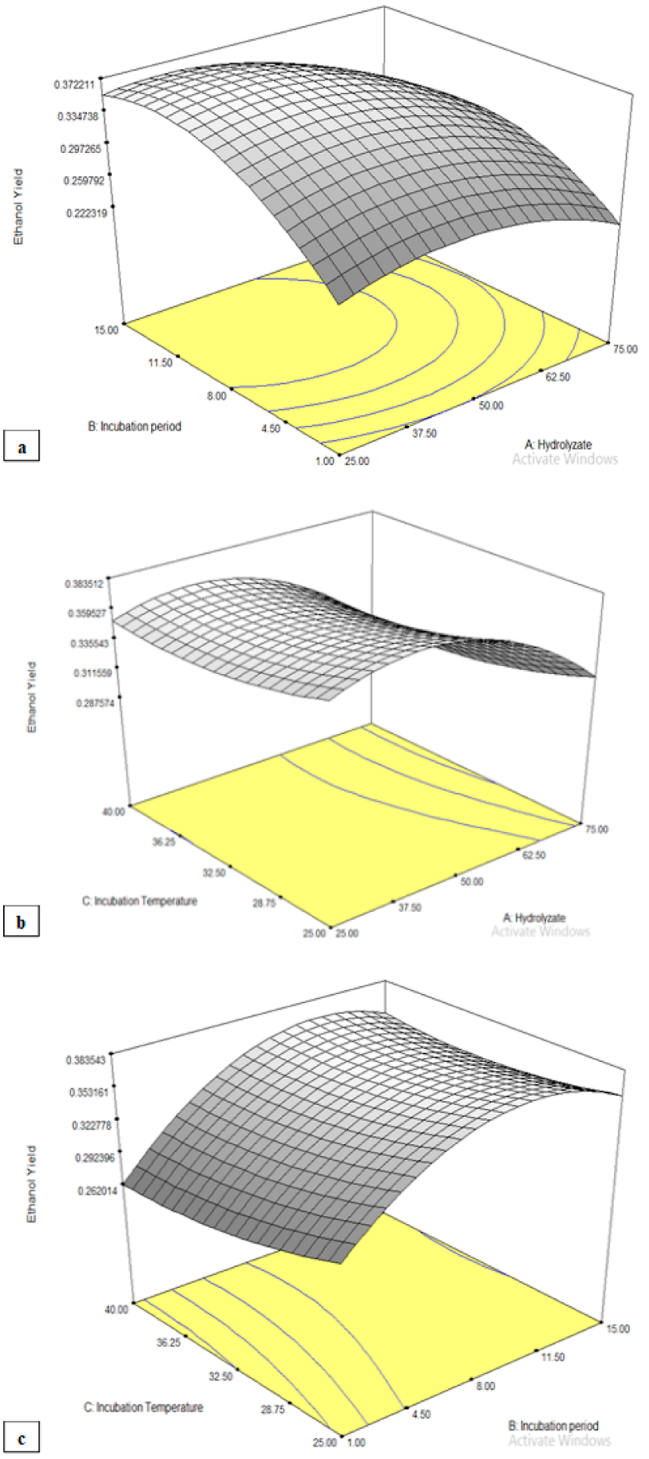


Figure 1. 3D graph of the interrelationship of standard yeast K7 ethanol yield on varying the incubation period, temperature, and hydrolyzate volume (a−c).

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

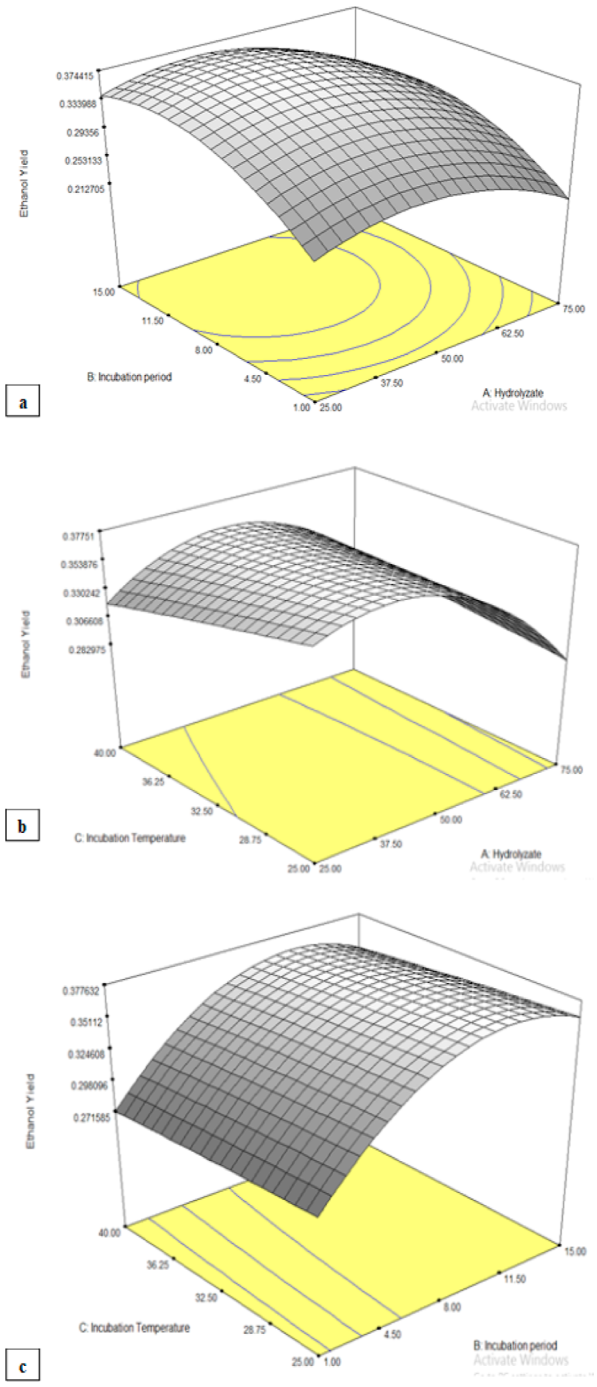


Figure 2. 3D graph of the interrelationship of experimental yeast Y34 ethanol yield on varying the incubation period, temperature, and hydrolyzate volume (a–c).

incubation time, while the hydrolyzate tends to increase the yield up to the midpoint (50 mL), followed by a decrease. Figure 1b shows the positive correlation between temperature and response. On the other hand, a rise in ethanol yield was noticed by increasing the hydrolyzate up to the central point, which led to a further decrease. Figure 1c shows a sharp rise in yield as incubation days passed up to 11 days and remained constant until the time of termination. When the temperature was increased, the yield fluctuated by an increase as well as decreased slowly.

Figure 2a exhibits an increase in yield up to the central point (50 mL) and then a decrease as the hydrolyzate volume is increased further. Similarly, yields increased sharply as the days of the experiment passed, followed by a decrease on the 11th day. Figure 2b illustrates that temperature influenced the ethanol yield by a slight increase. The yield fluctuated (from increasing to decreasing) with varied concentrations of the hydrolyzate. As evident from Figure 2c, the temperature caused a slight increase in the yield of ethanol, whereas a sharp increase in yield was observed up to day 11 of incubation, followed by a decrease.

3. DISCUSSION

Fruit waste is the most readily available and least expensive source for producing bioethanol. The presence of significant fermentable soluble sugars, viz., fructose, glucose, and sucrose, as well as structural cellulose and hemicellulose, makes fruit waste a desirable source for the generation of bioethanol.[39,40] Higher ethanol yields could be achieved, provided that the conversion of all sugars was achieved. This would improve the economic viability of the bioconversion process, which would increase the bioconversion process's economic sustainability.[41] MP is readily available in Pakistan and appears as an ideal and inexpensive substrate for fermentation with high carbohydrate contents. The sugar contents are present in a degradable form, and yeast cells can directly metabolize sugars.[18] Fruit waste has been used by other authors. For example, Hossain and Fazliny[42] reported that 68.64 g/L ethanol could be produced from the waste of rotting pineapples. Agulejika et al.[43] stated that *Z. mobilis* produced ethanol contents of 64.01 and 21.14 g/L, respectively, when they used fresh fruit and waste fruit. Fresh fruit has higher fructose and glucose concentrations than corn husk, millet husk, and wasted fruit, which accounts for the higher ethanol yield.

In addition to fermentable sugars, MP contains 16–20% disaccharides or sucrose. A significant biocatalyst that has received a lot of attention in the field of enzyme kinetics is the sucrolytic enzyme, invertase. It is responsible for hydrolyzing sucrose into equimolar quantities of D-glucose and D-fructose, which results in the formation of an inverted sugar. Bacterial invertases have been shown to exhibit activity in both acidic and alkaline pH environments, as previously reported by Yoon et al.[44] and Warchol et al.[45] correspondingly.

The sucrolytic agent *B. cereus* FA3 was used to hydrolyze sucrose in MP into simple sugars. *B. cereus* FA3 had an invertase potential of 6.29 ± 0.07 IU. Numerous investigations have determined that *Bacillus* species are the ideal microbes for the synthesis of invertase.[46,47] The study found the total and reducing sugars (g/L) of the untreated MP to be 20.0 ± 1.89 and 7.03 ± 0.11, respectively. According to Santiago-Urbina et al.,[19] the average total and reduced sugar contents in the MP were 18 and 4.8%, respectively, which is in accordance with the current findings.

This study dealt with the mathematical models used to select parameters for the hydrolysis of waste MP into monomers employing *B. cereus* FA3's invertase potential. The substrate, MP, does not need any kind of pretreatment or detoxification. The optimized conditions were interpreted by using the PBD. Up to day five of incubation, the highest reducing sugars of 11.43 + 0.068 g/L were recorded at 30 °C, 5 pH, and 12.58 IU of enzyme load. Invertases were stable at 30–50 °C, whereas most production and performance of enzymes were gained at 30 °C. High temperatures have a negative impact on enzyme

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652–39662

activity. Similarly, most invertase enzyme production and activity occurred at pH 5 in *Aspergillus niger*. These findings corroborated the results of enzyme activity in the present study.[48] Awad et al.[27] determined the nutritional requirements for invertase production using *Lactobacillus brevis* Mm-6 and employed fractional factorial design and Plackett−Burman to optimize the parameters. According to Yan et al.,[49] the maximum reducing sugars were 164.8 g/L with the following parameters: a pH of 4.82 for saccharification, a glucoamylase load of 142.2 IU, an enzyme reaction temperature of 55 °C, and an enzyme reaction time of 2.48 h. These results were contrary to the current study's findings. The PBD is an orthogonal array that enables testing the greatest number of factors with the fewest number of observations.[50,51] The current PB model showed an improvement in the sugar contents. Arrizon and Gschaedler[52] found enhanced fermentation efficiency at high sugar concentrations with low nitrogen levels. The production of ethanol was positively impacted by the total sugar concentration.[53]

In this study, a *B. cereus* FA3-treated MP hydrolyzate was fermented using *S. cerevisiae* K7 and *M. cibodasensis* Y34 isolates under different conditions, including concentration of the hydrolyzate, temperature, and incubation time. A number of studies and reviews have been published regarding the fermentation of microorganisms to produce ethanol.[49,54,55] According to Lin and Tanaka,[18] a variety of bacteria, yeasts, and fungi have been employed to produce ethanol. *S. cerevisiae* is the most widely utilized organism for ethanol production. Both yeasts generated maximum ethanol contents at 25 °C with 25 mL of hydrolyzate when incubated for 15 days. The high and low hydrolyzate extremes caused a decrease in ethanol yield. Yeast cannot perform optimally in high concentrations of sugars by lowering the fermentation efficiency. A suitable substrate concentration will lead to better fermentation efficiency.[56] During fermentation, the reducing sugars in the medium were converted into CO_2 and ethanol.[57] By increasing the incubation time, the ethanol content tends to increase. The number of yeast cells started to increase gradually due to the availability of nutrients.[58] After 19 days, the ethanol content decreased. The yeast cells might start to die due to the exhaustion of nutrients and the accumulation of ethanol contents in the fermentation medium. However, the yeasts could reassimilate ethanol as a carbon source once the sugars were depleted.[59] The temperature of the incubation period, the pH of the fermentation medium, the concentration of cells and sugars, the availability of nitrogen sources, and the aeration are the factors that affect the growth of yeast and ethanol production.[60,61]

As compared to *S. cerevisiae* K7 (9.16 ± 0.01 g/L and 0.38 ± 0.01 g/g ethanol contents and yield), *M. cibodasensis* Y34 displayed an ethanol yield and contents of 0.39 ± 0.01 g/g and 10.02 ± 0.009 g/L, respectively. *S. cerevisiae* generates bioethanol from reducing sugars employing the Embden−Meyerhof−Parnas pathway of glycolysis.[57] The reported percent contents and yield of ethanol were 9.56 and 71.52, respectively, with a percent fermentation efficiency of 139.95% in 30% sugar cane molasses by *S. cerevisiae*.[62] Khalil et al.[63] recorded the optimum bioethanol contents, 39.2 g/L, from the juice of sweet sorghum SS-301 through coculture of *Z. mobilis* and *S. cerevisiae*. Tan et al.[64] studied the bioethanol contents and yields of 45.75 g/L and 0.33 g/g via a bioreactor system in the juice of banana fronds, comprising 14% total sugars, 18.9 g/L glucose, and 13.29 g/L sucrose contents. The results differed from the current study findings because bioreactor systems and cocultures might be factors in bioethanol improvement.

Chaudhary et al.[55] investigated the ethanol yield g/g and titer g/L, which were found to be 0.30 ± 0.003 and 11.1 ± 0.12; 0.32 ± 0.005 and 11.78 ± 0.1; 0.29 ± 0.003 and 10.80 ± 0.13 by *S. cerevisiae* K7, *Metschnikowia* sp. Y31, and *M. cibodasensis* Y34, correspondingly, in pomegranate peel waste. These results varied slightly from those of our study. The findings of Maina et al.[65] on pomegranate ethanol contents of 5.58 g per liter with *Saccharomyces cerevisiae* were consistent with our research. Mazmanci[66] assessed the production of ethanol of 25 g/L with a yield of 71.42 ± 1.4 g/kg, and reducing sugars (105 g/L) using *Washingtonia robusta* fruits, 2−12 pH, 1−24 h contact times, 20−50 °C temperature, and 121 °C, 10 min, and 1.2 atm autoclave pretreatment.

Without adding any nutrients, Reddy and Reddy[9] recorded 8.5−10% (w/v) of ethanol by fermenting the mango juice. The maximum ethanol yield of T-S and T-KM3 was found to be 0.49 and 0.38 g/g sugar, respectively.[67] Using strains of *K. marxianus* and *S. cerevisiae*, Limtong et al.[60] described a fermentation efficiency of 77.5−86% and 80%. Experiments were conducted to verify the model's predicted conditions in the current investigations. The design's validity was confirmed when the experimental ethanol yield (0.40 ± 0.01 for Y34 and 0.39 ± 0.01 for K7) and titer values were found to be in good agreement with the expected values and conditions. Santiago-Urbina et al.[19] obtained an 8% increase in ethanol production by following the model's predicted parameters.

4. CONCLUSIONS

This study concluded that the highest reducing sugar contents, 11.43 ± 0.068 g/L, were achieved by a Plackett-Burman-designed enzymatic hydrolysis model in MP using *B. cereus* FA3. *M. cibodasensis* Y34 is a fermentative agent yielding 0.40 ± 0.01 g of ethanol per gram of reducing sugar after fermenting the hydrolyzate for 8 days. It is implied that *B. cereus* FA3 and *M. cibodasensis* Y34 have promising capacities to tolerate ethanol and convert waste into bioethanol. The study should be extended to simultaneous saccharification and fermentation in order to design fermenters for production of bioethanol on a commercial scale, in addition to batch and continuous fermentation.

5. MATERIALS AND METHODS

5.1. Raw Material Collection. *Mangifera indica*, also known as Chaunsa, is a widely used fruit during the summer and was collected as the raw material for the study. The peel and fibrous material of the pulp after juice extraction from whole fruit is termed pulper waste. For the current study, peels were removed from pulper waste that will be proceeded for ethanol production in another study. The collection of damaged, deshaped, discolored, and rotten mangoes was made from a local fruit market in Township, Lahore, Pakistan. Fruit pulp was used as the substrate. The mango was peeled, seeded off, sliced into tiny cubes to make the puree, and sterilized by autoclaving. After that, it was stored in the freezer to be used for future experiments.

5.2. Content Estimation of MP. Various contents of MP were analyzed by using biochemical methods. Total sugars (carbohydrates) and total protein were calculated using the phenol−sulfuric and Lowry methods.[68,69] The DNS (3,5-

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

dinitrosalicylic acid) protocol was applied to estimate reducing sugars.[70] The recommended protocol by the AOAC[71] was followed to calculate the moisture contents of MP.

5.3. Microbe Selection. Two yeast isolates, *S. cerevisiae* K7 (standard) and *M. cibodasensis* Y34 (experimental), and a sucrose-degrading bacterium, *B. cereus* FA3 (with accession no. OQ450350) were collected from the Microbiology Lab of the Department of Zoology at the University of Education, Lahore, Pakistan. The standard yeast strain, *S. cerevisiae* K7, was a gift from the Brewing Society of Japan in Tokyo. According to Chaudhary and Karita,[72] *M. cibodasensis* Y34 was isolated from *Abelia* flowers and screened for efficient ethanol production (1.80 ± 0.05%) through ethanologenic processes. *B. cereus* FA3 is a sucrolytic bacterium with a sucrase/invertase potential of 0.629 IU[73] MP contains sucrose, which was hydrolyzed by *B. cereus* FA3, whereas yeast isolates were used for fermentation tests. The molar quantity of the enzyme (eq 3) was calculated as

$$\text{Enzyme activity (IU)} = \frac{\text{O. D. of sample} \times \text{standard curve value} \times 1000 \times \text{reaction volume (mL)}}{\text{molecular weight of sucrose} \times \text{incubation time (min)} \times \text{total crude enzyme (mL)}} \quad (3)$$

5.4. Experimental Design for Saccharification/Enzymatic Hydrolysis. Response surface methodology (RSM) employs statistical and mathematical tools to optimize experimental parameters efficiently, considering multiple factors for maximum system impact analysis with a minimum no. of trials.[74,75] Design Expert software (ver. 8.0. Stat-Ease Inc.) was used here for the model designs. The present investigation focused on the enzymatic hydrolysis of MP with *B. cereus* FA3. Hence, the PBD was used with two levels encoded (+1, −1) for screening and pinpointing the influential parameters, i.e., MP concentration, enzyme dose, pH, buffer/salt concentration, temperature, and incubation period, in a straightforward and simple manner. According to the PBD and the CCD, 12 and 86 runs are required for the six-factor analysis.[51] The PB-based hydrolysis experiment was performed in 12 runs. It was efficiently used by the researchers to screen experiments, detect the highly significant effects on various variables, consider the negligible impact of interaction effects, reduce the number of experimental runs, use limited chemicals, and most importantly save time as well as human resources. However, due to its significant confounding of main effects, PBD is not regarded as a suitable tool for factor optimization.[76,77] For this reason, the CCD with levels encoded (−1.0, 0, +1.0) is selected here because of its more accurate elucidation of optimization studies involving ethanologenesis that is the prime focus of the investigation. Parameters for enzymatic treatment were 30−37 °C, 1−5 days hydrolysis time, 6.29−12.58 IU of enzyme load, 40−55% acetate buffer, 4−5 pH buffer, and 25−50% MP. For enzymatic hydrolysis, the crude enzyme was prepared in a basal medium having percent composition such as 0.10 g of yeast extract, 0.20 g of potassium dihydrogen phosphate, 0.05 g of $Na_3C_6H_5O_7$, and 0.02 g of $MgSO_4$ followed by incubation at 37 °C for 72 h.[78] Acetate buffer (0.2 M) was used to serve as the substrate buffer.[79] ANOVA and regression analyses were used to evaluate the coefficients and assess the significance of the independent variables by computing *F* and *p* values.

The yield/conversion of total sugars into monomers (reducing sugars) is computed and presented in percentage by eqs 4 and 5[80]

$$\text{Percent yield or conversion} = \frac{\text{reducing sugars released after treatment}}{\text{total sugars before treatment}} \times 100 \quad (4)$$

$$\text{Reducing sugar released} = \text{R. S. after treatment} - \text{R. S. before treatment} \quad (5)$$

5.5. Central Composite Design for Fermentation Parameter Optimization. The primary focus of this research is to improve ethanologenesis, and CCD was selected for this purpose because it provided a more precise explanation and optimization of the fermentation factors. In the bioethanol production process, hydrolysis and fermentation are linked to one another, even though they are different processes. The parameters that the hydrolysis narrows down will help to maximize the fermentation. CCD is primarily used as an optimization tool because of its main effects that are not confounded. Generally, the CCD approach for optimization purposes should only be selected later in the RSM application once the total number of significant variables has been decreased to an acceptable figure. It necessitates a comparatively large number of sample points. The CCD involves maximum information with minimal experimental result data, interpretation of interaction among different variables, and reduction of needed experiment numbers to predict quadratic terms in the model.[81] A 20-run experiment with three parameters was designed by CCD. The parameters for fermentation were 25−75% hydrolyzate with 75−25% minimal medium, 25−40 °C, and 1−15 days incubation period. The experiment was analyzed for three responses, namely, ethanol titer, ethanol yield, and yeast growth. The yeast inoculum was prepared in a MYG medium, while the minimal medium was composed of 0.65 g of yeast extract, 0.042 mg of zinc chloride, 0.25 g of $(NH_4)_2SO_4$, 0.50 g of sodium citrate, 0.025 g of calcium chloride, 0.27 g of KH_2PO_4, 0.15 g of citric acid, and 0.08 g of magnesium sulfate heptahydrate.[72] Ethanol and reducing sugar contents were estimated following the DNS and potassium dichromate methods.[70,82] Ethanol yield (g/g) was computed by dividing the ethanol contents (g/L) by the amount of sugar consumed (g/L). The CCD statistical tools were used to compute the theoretical ethanol yield under optimized conditions. The selected optimum conditions were validated by performing a fermentation experiment, and the actual yield was calculated. Three-dimensional graphs were plotted to interpret the interactions of factors with responses.

AUTHOR INFORMATION

Corresponding Author

Asma Chaudhary − *Department of Zoology, Division of Science and Technology, University of Education, Lahore 54770, Pakistan;* orcid.org/0000-0001-6837-7611; Email: asma.ch@ue.edu.pk

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

Authors

Zawar Hussain − *Department of Zoology, Division of Science and Technology, University of Education, Lahore 54770, Pakistan*

Hafsa Ajmal − *Department of Zoology, Division of Science and Technology, University of Education, Lahore 54770, Pakistan*

Rahat Abdul Rehman − *Department of Forensic Medicine/Medical Jurisprudence, University of Health Sciences, Lahore 54600, Pakistan*

Ghulam Abbas − *Department of Biotechnology, University of Agriculture, Dera Ismail Khan, Khyber Pakhtunkhwa 29111, Pakistan*

Ayesha Aihetasham − *Institute of Zoology, University of the Punjab, Lahore 54590, Pakistan*

Syeda Anjum Tahira − *Department of Botany, University of Okara, Okara 56300, Pakistan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c04374

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research work was a part of the MS thesis of H.A. and was facilitated by the University of Education, Lahore, Pakistan, under the supervision of A.C. Z.H., R.A.R., and G.A. provided assistance for model design and analysis of statistical models. S.A.T. supported substrate collection and processing, while A.A. guided manuscript preparation.

■ REFERENCES

(1) Gustavsson, J.; Cederberg, C.; Sonesson, U.; Otterdijk, R. V.; Meybeck, A. *Global Food Losses and Food Waste—Extent, Causes and Prevention*; FAO: Rome, Italy, 2011; p 37.

(2) FAO (Food and Agriculture Organization). *Toolkit: Reducing the Food Wastage Footprint*; FAO: Rome, Italy, 2013; p 119.

(3) Thi, N. B. D.; Kumar, G.; Lin, C. Y. An overview of food waste management in developing countries: Current status and future perspective. *J. Environ. Manage.* **2015**, *157*, 220−229.

(4) Salihoglu, G.; Salihoglu, N. K.; Ucaroglu, S.; Banar, M. Food loss and waste management in Turkey. *Bioresour. Technol.* **2018**, *248*, 88−99.

(5) Mehdi, M.; Ahmad, B.; Yaseen, A.; Adeel, A.; Sayyed, N. A. comparative study of traditional versus best practices mango value chain. *Pak. J. Agric. Sci.* **2016**, *53*, 733−742.

(6) FAO. *Food and Agricultural, Statistics, Italy*. Available online with updates at www.fao.org/faostat/en/, 2019.

(7) Bashart, R.; Aslam, M.; Khan, A.; Rehman, A.; Bhatti, H. A.; Imran, M. A.; Raza, M.; Munir, A. Exports of mangoes from Pakistan: determinants and competitiveness. *J. Arable Crops Mark.* **2023**, *5* (2), 19−27.

(8) The Business Research Company. *Mango Global Market Report 2023*; ID: 5792898; Research and Markets, The World's Largest Market Research Store, 2023.

(9) Reddy, L. V.; Reddy, O. V. S. Production of ethanol from mango (*Mangifera indica* L.) fruit juice fermentation. *Res. J. Microbiol.* **2007**, *2* (10), 763−769.

(10) Calıskan Eleren, S.; Öziş Altınçekiç, Ş.; Altınçekiç, E. Biofuel potential of fruit juice industry waste. *J. Hazard., Toxic Radioact. Waste* **2018**, *22* (4), 05018002.

(11) Walia, N. K.; Bedi, S. S.; Kundu, K.; Karmakar, R. Production of bioethanol from mango peel. *Int. J. Eng. Res. Technol.* **2013**, *2*, 1−7.

(12) Tesfaye, T. Valorisation of mango fruit by-products: Physicochemical characterization and future prospect. *Chem. Process Eng. Res.* **2017**, *50*, 22−34.

(13) Maan, I.; Kaur, A.; Singh, H. P.; Batish, D. R.; Kohli, R. K. Evaluating the role of phenology in managing urban invasions: a case study of *Broussonetia papyrifera*. *Urban For. Urban Green.* **2020**, *48*, 126583.

(14) Klasson, K. T.; Boone, S. A. Bioethanol fermentation of clarified sweet sorghum (*Sorghum bicolor* (L.) Moench) syrups sealed and stored under vegetable oil. *Ind. Crops Prod.* **2021**, *163*, 113330.

(15) Selamoglu, M.; Memon, A. R. Transportation of food by cold chain methods one of the Cause of reoccurrence Covid-19 Infection during its pandemic. *TURJAF* **2021**, *9* (12), 2376−2378.

(16) Uysal, I.; Mohammed, F. S.; Selamoglu, M.; Sevindik, M. Microbial spoilage in food and its agents, cold chain and measures to prolong microbial spoilage. *J. Food Sci. Technol.* **2023**, *20* (140), 16−27.

(17) Reddy, L. V. A.; Reddy, O. V. S. Production and characterization of wine from mango fruit (*Mangifera indica* L.). *World J. Microbiol. Biotechnol.* **2005a**, *21*, 1345−1350.

(18) Lin, Y.; Tanaka, S. Ethanol fermentation from biomass resources: current state and prospects. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 627−642.

(19) Santiago-Urbina, J. A.; Ventura-Canseco, L. M. C.; Ayora-Talavera, T. D. R.; Ovando-Chacón, S. L.; Luc Dendooven, G. M. F. Optimization of ethanol production from mango pulp using yeast strains isolated from "taberna": A Mexican fermented beverage. *Afr. J. Microbiol. Res.* **2011**, *5* (5), 501−508.

(20) Maldonado-Celis, M. E.; Yahia, E. M.; Bedoya, R.; Landázuri, P.; Loango, N.; Aguillón, J.; Restrepo, B.; Guerrero Ospina, J. C. Chemical composition of mango (*Mangifera indica* L.) fruit: Nutritional and phytochemical compounds. *Front. Plant Sci.* **2019**, *10*, 1073.

(21) Carrillo-Nieves, D.; Ruiz, H. A.; Aguilar, C. N.; Ilyina, A.; Parra-Saldivar, R.; Torres, J. A.; Martínez Hernández, J. L. Process alternatives for bioethanol production from mango stem bark residues. *Bioresour. Technol.* **2017**, *239*, 430−436.

(22) Demain, A. L.; Newcomb, M.; Wu, J. H. D. Cellulase, clostridia, and ethanol. *Microbiol. Mol. Biol. Rev.* **2005**, *69* (1), 124−154.

(23) Vitolo, M. Invertase. In *Enzymes as Biotechnological Agents*; Said, S., Pietro, R. C. L. R., Eds.; Legis Summa: Ribeirao Preto, Sao Paulo, Brazil, 2004; pp 207−221.

(24) Heil, M.; Buchler, R.; Boland, W. Quantification of invertase activity in ants under field conditions. *J. Chem. Ecol.* **2005**, *31* (2), 431−437.

(25) Hanumaiah, S.; Shirnalli, G. Optimization of growth conditions for production of saccharolytic enzymes by cellulolytic fungi. *Karnataka J. Agric. Sci.* **2013**, *26* (3), 379−383.

(26) Ruan, Y. L.; Jin, Y.; Yang, Y. J.; Li, G. J.; Boyer, J. S. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Mol. Plant* **2010**, *3*, 942−955.

(27) Awad, G. E.; Amer, H.; El-Gammal, E. W.; Helmy, W. A.; Esawy, M. A.; Elnashar, M. M. Production optimization of invertase by *Lactobacillus brevis* Mm-6 and its immobilization on alginate beads. *Carbohydr. Polym.* **2013**, *93*, 740−746.

(28) Shankar, T.; Thangamathi, P.; Rama, R.; Sivakumar, T. Characterization of invertase from *Saccharomyces cerevisiae* MTCC 170. *Afr. J. Microbiol. Res.* **2014**, *8*, 1385−1393.

(29) Shankar, T.; Thangamathi, P.; Rama, R.; Sivakumar, T. Characterization of invertase from *Saccharomyces cerevisiae* MK obtained from toddy sample. *J. Bioproc. Chem. Eng.* **2014**, *1*, 1−6.

(30) Nadeem, H.; Rashid, M. H.; Siddique, M. H.; Azeem, F.; Muzammil, S.; Javed, M. R.; Ali, M. A.; Rasul, I.; Riaz, M. Microbial invertases: a review on kinetics, thermodynamics, physiochemical properties. *Process Biochem.* **2015**, *50*, 1202−1210.

(31) Cazetta, M. L.; Celligoi, M. A. P. C.; Buzato, J. B.; Scarmino, I. S. Fermentation of molasses by *Zymomonas mobilis*: Effects of temperature and sugar concentration on ethanol production. *Bioresour. Technol.* **2007**, *98* (15), 2824−2828.

(32) Silva, G. P. D.; Araújo, E. F. d.; Silva, D. O.; Guimarães, W. V. Ethanolic fermentation of sucrose, sugarcane juice and molasses by

Escherichia coli strain KO11 and *Klebsiella oxytoca* strain P2. *Braz. J. Microbiol.* **2005**, *36*, 395−404.
(33) Maruthai, K.; Thangavelu, V.; Kanagasabai, M. Statistical screening of medium components on ethanol production from cashew apple juice using *Saccharomyces diasticus*. *Int. J. Chem. Biol. Eng.* **2012**, *6* (6), 108−111.
(34) Dhaliwal, S. S.; Oberoi, H. S.; Sandhu, S. K.; Nanda, D.; Kumar, D.; Uppal, S. K. Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of *Pichia kudriavzevii*. *Bioresour. Technol.* **2011**, *102* (10), 5968−5975.
(35) Nonklang, S.; Abdel-Banat, B. M.; Cha-aim, K.; Moonjai, N.; Hoshida, H.; Limtong, S.; Yamada, M.; Akada, R. High-temperature ethanol fermentation and transformation with linear DNA in the thermotolerant yeast *Kluyveromyces marxianus* DMKU3−1042. *Appl. Environ. Microbiol.* **2008**, *74* (24), 7514−7521.
(36) Lincoln, L.; More, S. S. Bacterial invertases: occurrence, production, biochemical characterization, and significance of trans-fructosylation. *J. Basic Microbiol.* **2017**, *57* (10), 803−813.
(37) Nikolov, T.; Bakalova, N.; Petrova, S.; Benadova, R.; Spasov, S.; Kolev, D. An effective method for bioconversion of delignified waste-cellulose fibers from the paper industry with a cellulase complex. *Bioresour. Technol.* **2000**, *71* (1), 1−4.
(38) Robak, K.; Balcerek, M. Review of second generation bioethanol production from residual biomass. *Food Technol. Biotechnol.* **2018**, *56* (2), 174−187.
(39) Janani, K.; Ketzi, M.; Megavathi, S.; Vinothkumar, D.; Ramesh Babu, N. G. Comparative studies of ethanol production from different fruit wastes using *Saccharomyces cerevisiae*. *Int. J. Innovative Res. Sci. Eng. Technol.* **2013**, *2* (12), 7161−7167.
(40) Vishwakarma, H. S.; Kumar, A.; Singh, J.; Dwivedi, S.; Kumar, M. Production of ethanol from fruit wastes by using *Saccharomyces cerevisiae*. *Int. J. Renewable Energy Technol. Res.* **2014**, *3* (10), 1−5.
(41) Van Dyk, J. S.; Gama, R.; Morrison, D.; Swart, S.; Pletschke, B. I. Food processing waste: Problems, current management and prospects for utilisation of the lignocellulose component through enzyme synergistic degradation. *Renew. Sustain. Energy Rev.* **2013**, *26*, 521−531.
(42) Hossain, A. B. M. S.; Fazliny, A. R. Creation of alternative energy by bio- ethanol production from pineapple waste and the usage of its properties for engine. *Afr. J. Microbiol. Res.* **2010**, *4* (9), 813−819.
(43) Agulejika, E. O.; Olabode, F. I.; Babatunde, K. A. Ethanol production from waste fruits. *Int. J. Food Agric. Res.* **2005**, *2* (2), 190−194.
(44) Yoon, M. H.; Choi, W. Y.; Kwon, S. J.; Yi, S. H.; Lee, D. H.; Lee, J. S. Purification and properties of intracellular invertase from alkalophilic and thermophilic *Bacillus cereus* TA-11. *J. Appl. Biol. Chem.* **2007**, *50* (4), 196−201.
(45) Warchol, M.; Perrin, S.; Grill, J. P.; Schneider, F. Characterization of a purified *β*-fructo furanosidase from *Bifidobacterium infantis* ATCC 15697. *Lett. Appl. Microbiol.* **2002**, *35* (6), 462−467.
(46) Xu, Z. W.; Li, Y. Q.; Wang, Y. H.; Yang, B.; Ning, Z. X. Production of B-fructo furanosidase by *Arthrobacter* sp. and its application in the modification of stevioside and rebaudioside A. *Food Technol. Biotechnol.* **2009**, *47*, 137−143.
(47) Schallmey, M.; Singh, A.; Ward, O. P. Developments in the use of *Bacillus* species for industrial production. *Can. J. Microbiol.* **2004**, *50* (1), 1−17.
(48) Manoochehri, H.; Hosseini, N. F.; Saidijam, M.; Taheri, M.; Rezaee, H.; Nouri, F. A review on invertase: Its potentials and applications. *Biocatal. Agric. Biotechnol.* **2020**, *25*, 101599.
(49) Yan, S.; Li, J.; Chen, X.; Wu, J.; Wang, P.; Ye, J.; Yao, J. Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate. *Renew. Energy* **2011**, *36* (4), 1259−1265.
(50) Montgomery, D. C. *Design and Analysis of Experiments*, 5th ed.; John Wiley and Sons, Inc: NY, USA, 1997; p 303.
(51) Vanaja, K.; Shobha, R. H. Design of experiments: concept and applications of Plackett-Burman design. *Clin. Res. Regul. Aff.* **2008**, *24*, 1−23.
(52) Arrizon, J.; Gschaedler, A. Increasing fermentation efficiency at high sugar concentrations by supplementing an additional source of nitrogen during the exponential phase of the tequila fermentation process. *Can. J. Microbiol.* **2002**, *48*, 965−970.
(53) Shuler, M. L. *Bioprocess Engineering*; Prentice-Hall, 1992; pp 412−420.
(54) Oyeleke, S. B.; Jibrin, N. M. Production of bioethanol from guinea cornhusk and millet husk. *Afr. J. Microbiol. Res.* **2009**, *3* (4), 147−152.
(55) Chaudhary, A.; Hussain, Z.; Aihetasham, A.; El-Sharnouby, M.; Abdul Rehman, R.; Azmat Ullah Khan, M.; Zahra, S.; Saleem, A.; Azhar, S.; Alhazmi, A.; El Askary, A.; et al. Pomegranate peels waste hydrolyzate optimization by Response Surface Methodology for bioethanol production. *Saudi J. Biol. Sci.* **2021**, *28* (9), 4867−4875.
(56) Vasconcelos, J. N. D. *Ethanol Fermentation in Sugarcane Agricultural Production, Bioenergy and Ethanol*; Academic Press: USA, 2015; Chapter 15.
(57) Raharja, R.; Murdiyatmo, U.; Sutrisno, A.; Wardani, A. K. Bioethanol production from sugarcane molasses by instant dry yeast. In *International Conference on Green Agro-industry and Bioeconomy*; IOP Conference Series: Earth and Environmental Science; IOP Publishing: United Kingdom, 2019; Vol. *230*, p 012076.
(58) Osei, J. A.; Manohar, S.; Kitur, E. Effects of different incubation methods on Ethanol production from selected food wastes products. *IJEMS* **2020**, *4* (3), 64−69.
(59) Hawaz, E.; Tafesse, M.; Tesfaye, A.; Kiros, S.; Beyene, D.; Kebede, G.; Boekhout, T.; Groenwald, M.; Theelen, B.; Degefe, A.; Degu, S.; et al. Bioethanol production from sugarcane molasses by co-fermentation of *Saccharomyces cerevisiae* isolate TA2 and *Wickerhamomyces anomalus* isolate HCJ2F-19. *Ann. Microbiol.* **2024**, *74* (1), 13.
(60) Limtong, S.; Sringiew, C.; Yongmanitchai, W. Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. *Bioresour. Technol.* **2007**, *98* (17), 3367−3374.
(61) Nuanpeng, S.; Thanonkeo, S.; Yamada, M.; Thanonkeo, P. Ethanol production from sweet sorghum juice at high temperatures using a newly isolated thermotolerant yeast *Saccharomyces cerevisiae* DBKKU Y-53. *Energies* **2016**, *9* (4), 253.
(62) Raby, H. S.; Saadat, M. A.; Sakib, A. N.; Moni Chowdhury, F.; Yousuf, A. Bioethanol production from sugarcane molasses with supplemented nutrients by industrial yeast. *Biofuels* **2024**, *15* (2), 129−135.
(63) Khalil, S. R.; Abdelhafez, A. A.; Amer, E. A. M. Evaluation of bioethanol production from juice and bagasse of some sweet sorghum varieties. *Ann. Agric. Sci.* **2015**, *60* (2), 317−324.
(64) Tan, J. S.; Phapugrangkul, P.; Lee, C. K.; Lai, Z. W.; Abu Bakar, M. H.; Murugan, P. Banana frond juice as novel fermentation substrate for bioethanol production by *Saccharomyces cerevisiae*. *Biocatal. Agric. Biotechnol.* **2019**, *21*, 101293.
(65) Maina, S.; Pateraki, C.; Kopsahelis, N.; Paramithiotis, S.; Drosinos, E. H.; Papanikolaou, S.; Koutinas, A. Microbial oil production from various carbon sources by newly isolated oleaginous yeasts. *Eng. Life Sci.* **2017**, *17* (3), 333−344.
(66) Mazmanci, M. A. Ethanol production from *Washingtonia robusta* fruits by using commercial yeast. *Afr. J. Biotechnol.* **2011**, *10* (1), 48−53.
(67) Buenrostro-Figueroa, J.; Tafolla-Arellano, J. C.; Flores-Gallegos, A. C.; Rodríguez-Herrera, R.; De la Garza-Toledo, H.; Aguilar, C. N. Native yeasts for alternative utilization of over ripe mango pulp for ethanol production. *Rev. Argent. Microbiol.* **2018**, *50* (2), 173−177.
(68) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(69) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28* (3), 350−356.

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662

(70) Miller, G. L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* **1959**, *31* (3), 426−428.
(71) AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis of AOAC International*, 19th ed., WA, 2012.
(72) Chaudhary, A.; Karita, S. Screening of yeast isolates from flowers for effective ethanol production. *Turk. J. Biol.* **2017**, *41* (6), 890−900.
(73) Shaheen, I. Screening of Efficient Sucrose Degrading Bacterial Ethanologen. BS Thesis, University of Education, Lahore, Pakistan, 2020.
(74) Myers, R. H.; Montgomery, D. C.; Anderson-Cook, C. M. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*; John Wiley and Sons, Inc.: New York, 2016.
(75) Dean, A.; Voss, D.; Draguljic, D. Response surface methodology. In *Design and Analysis of Experiments*; Springer: Cham, 2017; pp 565−614.
(76) Chaudhari, S. R.; Shirkhedkar, A. A. Application of Plackett-Burman and central composite designs for screening and optimization of factor influencing the chromatographic conditions of HPTLC method for quantification of efonidipine hydrochloride. *J. Anal. Sci. Technol.* **2020**, *11*, 48.
(77) Ahuja, V.; Macho, M.; Thabet, J.; Banerjee, A.; Ewe, D.; Saha, S.; Saurav, K. Optimization and characterization of biosurfactant from Streptomyces. *Methods Actinobacteriol.* **2022**, 647−659.
(78) Bai, L.; Hu, H.; Xu, J. Influences of configuration and molecular weight of hemicelluloses on their paper-strengthening effects. *Carbohydr. Polym.* **2012**, *88* (4), 1258−1263.
(79) Abu-Gharbia, M. A.; El-Sawy, N. M.; Nasr, A. M.; Zedan, L. A. Isolation, optimization and characterization of cellulases and hemicellulases from *Bacillus cereus* LAZ 518 isolated from cow dung using corn cobs as lignocellulosic waste. *J. Pharm. Appl. Chem.* **2018**, *4* (2), 67−79.
(80) Khandekar, D. C.; Palai, T.; Agarwal, A.; Bhattacharya, P. K. Kinetics of sucrose conversion to fructo-oligosaccharides using enzyme (invertase) under free condition. *Bioproc. Biosyst. Eng.* **2014**, *37*, 2529−2537.
(81) Atta-Allah, A. A.; Ahmed, R. F.; Shahin, A. A.; Hassan, E. A.; El-Bialy, H. A. A.; El-Fouly, M. Z. Optimizing the synthesis of yeast Beta-glucan via response surface methodology for nanotechnology application. *BMC Microbiol.* **2023**, *23* (1), 110.
(82) Bennett, C. Spectrophotometric acid dichromate method for the determination of ethyl alcohol. *Am. J. Med. Technol.* **1971**, *37* (6), 217−220.

https://doi.org/10.1021/acsomega.4c04374
ACS Omega 2024, 9, 39652−39662