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A Statistical Approach for Culture Condition Improvement of Invertase and Inulinase from *Candida guilliermondii* TISTR 5844

Pilanee Vaithanomsat[1,2]*, Molnapat Songpim[2,3] and Sarote Sirisansaneeyakul[2,3]

ABSTRACT

A newly isolated yeast *Candida guilliermondii* TISTR 5844 was shown to be a good producer of invertase and inulinase in an inexpensive, 48-hour batch fermentation. The enzyme production was performed using an orthogonal array experimental design and software for design and analysis of the experiment, based on the Taguchi method. The optimized medium for producing invertase consisted of 5% inulin, 4.8% NH_4Cl and 0.6% $MgSO_4 \cdot 7H_2O$ at pH 6. Maximum activity and productivity were achieved at 552.12 $U.L^{-1}$ and 10.28 $U.L^{-1}.hr^{-1}$, respectively. Inulinase production was maximized using a medium of 1% inulin, 2.4% NH_4Cl and 1.2% $MgSO_4 \cdot 7H_2O$ at pH 5. Under optimum conditions, inulinase activity and productivity were achieved at 56.41 $U.L^{-1}$ and 1.18 $U.L^{-1}.hr^{-1}$, respectively. The optimal temperature was 30 °C for both enzymes.

Keywords: invertase, inulinase, *Candida guilliermondii*, optimization, Taguchi method

INTRODUCTION

The study involved the production of invertase and inulinase using a newly isolated yeast *Candida guilliermondii* TISTR 5844 (Sirisansaneeyakul *et al.,* 2007). Invertase catalyzes the hydrolysis of terminal, non-reducing β-fructofuranoside residues in β-fructofuranosides. In commercial processes, invertase is used to convert sucrose to fructose and glucose. Fructose is sweeter than sucrose and does not crystallize as easily; therefore it is preferred to sucrose in the confectionery industry (Rubio and Navarro, 2006). The second enzyme of interest was inulinase or β-fructan fructanohydrolase, a commercial catalyst for the hydrolysis of inulin. Inulin is a β-(2,1)-linked polysaccharide of fructose with a terminal glucose residue in the polysaccharide chain. Inulinase hydrolyzes inulin to fructose and fructo-oligosaccharides, both of which are commonly used in the food and pharmaceutical industries (Vandamme and Derycke, 1983).

Invertase and inulinase are generally produced using yeasts and filamentous fungi. The yeast *Candida guilliermondii* TISTR 5844 (recently isolated from Jerusalem artichoke tubers) is a novel producer of invertase and inulinase (Sirisansaneeyakul *et al.,* 2007). This strain is capable of producing copious quantities of invertase and lesser levels of inulinase when grown on inulin and has potential for commercial use in producing these enzymes.

Bulk enzymes such as invertase and inulinase are relatively low-value products that

1 Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok 10900, Thailand.

2 Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Kasetsart University, Bangkok 10900, Thailand. (CASTNAR, NRU-KU, Thailand)

3 Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: aappln@ku.ac.th

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must be produced inexpensively. This typically necessitates batch fermentation, ideally without the control of pH. Production of many enzymes by fermentation is highly susceptible to the composition of the culture medium and factors such as feedback inhibition by the carbon source. Therefore, identifying the medium composition to maximize the enzyme titer in a minimally controlled batch fermentation is important.

Statistical methods are increasingly preferred for fermentation optimization because they reduce the total number of experiments needed and provide a better understanding of the interactions among factors on the outcome of the fermentation (Revankar and Lele, 2006). Statistical techniques such as the Taguchi method have gained broad acceptance in fermentation optimization.

Taguchi's method specifies orthogonal arrays for combining the various variables and levels in a minimum acceptable number of experimental trials. This method determines the optimal levels of the important controllable factors based on the concept of robustness and signal-to-noise (*S/N*) ratio (Roy, 1990). The desired design is sought by selecting the best performance under conditions that produce a consistent performance (Roy, 2001). The conclusions drawn from the experiments are valid over the entire experimental space spanned by the levels of the controlled factors (Phadke and Dehnad, 1988). Whereas the traditional experiment design focuses on the average process performance characteristics, Taguchi's method concentrates on the effect of variation on the process characteristics. In addition, Taguchi's approach facilitates the identification of the influence of individual factors and interactive effects of factors on performance with a few well-defined experimental sets (Prasad and Mohan, 2005). In the current study, the focus was on optimizing the production of the enzymes invertase and inulinase by *C. guilliermondii* TISTR 5844 using a low-cost minimally controlled batch fermentation process. The medium compositions were optimized using the Taguchi method.

MATERIALS AND METHODS

Microorganism and culture conditions

The yeast *Candida guilliermondii* TISTR 5844 (Sirisansaneeyakul *et al.,* 2007) was used to simultaneously produce invertase and inulinase in a batch fermentation process. The yeast was a stock of the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR) Bangkok, Thailand. The medium composition was comprised of: inulin 10 $g.L^{-1}$; yeast extract 12 $g.L^{-1}$; and $MgSO_4 \cdot 7H_2O$ 2 $g.L^{-1}$ in 0.1 M McIlvaine buffer at pH 5. A loopful of stock culture was inoculated in a 250 mL Erlenmeyer flask containing 50 mL liquid medium. After 24 hr incubation at 30 ^oC, 200 rpm, a 10% v/v inoculum was transferred to a 500 mL Erlenmeyer flask containing 150 mL liquid medium and further incubated at 30 ^oC, 200 rpm, for 24 hr. This was then used as a stock culture for all experimental treatments. All treatments were performed in 500 mL Erlenmeyer flasks containing 200 mL liquid medium. The reaction was incubated as above, for 48 hr (Sirisansaneeyakul *et al.,* 2007).

Analytical method

The activities of invertase and inulinase were measured as previously specified (Sirisansaneeyakul *et al*., 2007). One unit of invertase activity was defined as the quantity of the enzyme that liberated 1 μmol of fructose in 1 min in a 0.5% w/v (g/100 mL) solution of sucrose in 0.5 M McIlvaine buffer at pH 5.0 and 40 °C. One unit of inulinase activity was defined as the quantity of the enzyme that liberated 1 μmol of fructose in 1 min in a 0.5% w/v solution of inulin in 0.5 M McIlvaine buffer at pH 5.0 and 40 °C.

Experimental design and data analysis

Optimization by Taguchi method

The Taguchi method uses various types of signal-to-noise (*S/N*) ratios to measure the variability around the target performance (Engin *et al.,* 2008). A high value of *S/N* implies that the signal is much higher than the random effects of the noise factors. The noise is usually due to the uncontrollable factors that often cannot be completely eliminated. From the point of view of the performance of a process, three possible performance attributes are: 1) the-smaller-the-better; 2) the nominally smaller-the-better; and 3) the larger-the-better (Yang *et al.,* 2007). In this study, the performance attribute of 'the larger-the-better' was used to define the optimum conditions. The *S/N* for 'the larger-the-better' performance attribute was estimated using Equation 1:

$$S/N = \log_{10}\left(\frac{\Sigma\left(\frac{1}{y_i^2}\right)}{n}\right) \quad (1)$$

where y_i is the combination/comparison variable in experiment i for a certain combination of controlled factor levels and n is the number of experiments performed for that combination.

Some times, no optimal conditions can be identified for a process within the entire experimental space selected for the study. In such cases, the balanced characteristics of the orthogonal experimental array can help in predicting the performance value corresponding to the optimum operation conditions, using the additive model shown in Equation 2:

$$Y_i = \beta + X_i + e_i \quad (2)$$

where β is the overall mean of the performance value, X_i is the fixed effect of the quantity level combination used in the i^{th} experiment, and e_i is the random error in the i^{th} experiment.

Because Equation 2 is a point estimation that is used to determine whether the results of the confirmation experiments are meaningful, the confidence interval (*CI*) of Y_i must be calculated using Equation 3 at the selected level of confidence:

$$CI = \left[F_\alpha(1, fe)v_e\left\{(1/n_{eff}) + (1/S)\right\}\right]^{1/2} \quad (3)$$

where $F_\alpha(1, fe)$ is the F-ratio at a confidence level of $(1-\alpha)$ for a given degree of freedom (DOF), $(1, fe)$ is the error DOF, n_{eff} is (N/{1+ total DOF associated with the estimate of the mean}), N is total number of results, S is the larger-the-better sample size for the confirmation test, v_e is error variance, and *CI* is confidence interval (Engin *et al.,* 2008).

In this study, four factors at three levels of variations (Table 1) were used in the experiments. The factors optimized included the concentrations of inulin, NH_4Cl, $MgSO_4{\cdot}7H_2O$ and H^+ (that is the pH). The upper and lower limits of each variable were chosen to encompass the range in the literature and to reflect what was done in practice after a preliminary investigation of the limits. The various combinations of factors and levels were in accordance with Taguchi's L9 orthogonal array. The factor level combinations for all the experiments are shown in Table 2. Submerged

Table 1 Experimental factors and their levels for optimizing invertase and inulinase production.

Factor	Level		
	1	2	3
A: Initial concentration of inulin ($g.L^{-1}$)	10	30	50
B: Initial concentration of NH_4Cl ($g.L^{-1}$)	12	24	48
C: Initial concentration of $MgSO_4{\cdot}7H_2O$ ($g.L^{-1}$)	2	6	12
D: Initial pH	4	5	6

Table 2 Factor levels in the experimental design for the optimization.

Experiment no.	Factor							Enzyme activity	
	Inulin	NH_4Cl	$MgSO_4 \cdot 7H_2O$	pH	Inulin ($g.L^{-1}$)	NH_4Cl ($g.L^{-1}$)	$MgSO_4 \cdot 7H_2O$ ($g.L^{-1}$)	pH	Invertase ($U.L^{-1}$)	Inulinase ($U.L^{-1}$)
1	1	1	1	1	10	12	2	4	173.15	1.28
2	1	2	2	2	10	24	6	5	327.66	39.08
3	1	3	3	3	10	48	12	6	440.47	135.42
4	2	1	2	3	30	12	6	6	209.01	ND
5	2	2	3	1	30	24	12	4	125.45	27.00
6	2	3	1	2	30	48	2	5	224.70	ND
7	3	1	3	2	50	12	12	5	427.84	55.05
8	3	2	1	3	50	24	2	6	430.67	ND
9	3	3	2	1	50	48	6	4	838.20	ND

ND = not determined.

batch fermentations were conducted using various media formulations (1, 3 and 5% inulin; 1.2, 2.4 and 4.8% NH_4Cl; 0.2, 0.6 and 1.2% $MgSO_4 \cdot 7H_2O$) in 0.1 M McIlvaine buffer at pH values of 4, 5 and 6. Enzyme activities were assayed after 48 hr of incubation. The broth was harvested, cooled to 4 ^oC, and centrifuged (8,000×*g*, 20 min) to remove the biomass. The supernatant was held at –20 ^oC. Cell dry weight was estimated as follows: a 5 mL sample was collected in a pre-weighed tube and centrifuged at 8,000 rpm for 10 min. Supernatant was discarded and the pellet was washed twice with sterile distilled water, followed by drying the pellets at 105 ^oC till constant weight and expressed in dry cell weight (DCW, $g.L^{-1}$). The experimental data (enzyme activity) was processed using the Qualitek-4 software (Nutek, Inc., Bloomfield Hills, MI, USA).

Experimental validation

The optimization conditions were studied in 500 mL Erlenmeyer flasks containing 150 mL optimum medium and 10% inocula. Estimations of cell dry weight, enzyme production and concentrations of inulin (total carbohydrate by the phenol sulfuric method (Doboris *et al*., 1956) was carried out every 12 hr.

RESULTS AND DISCUSSION

Optimization by Taguchi methodology

The experimental data was processed using the Qualitek-4 software with the larger-the-better attribute selected for establishing the optimum composition of the fermentation medium and identifying the individual factors that influenced enzyme production. The percentage contributions of the factors in the production of invertase and inulinase are shown in Table 3 and Table 4, respectively. The carbon source (that is inulin, factor A) and the nitrogen source (that is NH_4Cl, factor B) were significant factors for the production of invertase (Table 3), whereas the

strong influences for the production of inulinase (Table 4) appeared to be the carbon source and the concentration of magnesium sulfate (factor C). This result, therefore, indicated that inulin, as a carbon source, was important for enzyme activity, and the enzyme was inducible. Inulin has been reported to be the best carbon source for inulinase production using *Pichia guilliermondii* (Gong *et al.,* 2007) and *Kluyveromyces* sp. Y-85 (Wei *et al.,* 1998). It has also been established that inulin induces enzyme production. Furthermore, it has been reported by Vandamme and Derycke (1983) that microbial inulinases are usually inducible. Selvakumar and Pandey (1999) have reported that the presence of inulin as a carbon source was essential for the synthesis of inulinase.

Table 3 Analysis of variance (ANOVA) of factors affecting the production of invertase.

Factor	DOF	*SS*	*MS*	*F*	*S*'	Contribution (%)
Inulin	2	137.30	68.65	0	137.30	65.94
NH_4Cl	2	44.81	22.403	0	44.81	21.52
$MgSO_4 \cdot 7H_2O$	2	16.09	8.045	0	16.09	7.73
pH	2	10.01	5.005	0	10.01	4.81
Error	0	0	0			
Total	8	208.21				
Pooled	2	137.30	68.65	10.52	124.25	59.68
Inulin						
NH_4Cl	2	44.81	22.403	3.43	31.76	15.25
$MgSO_4 \cdot 7H_2O$	(2)	(16.09)	POOLED (*CL* = 61.88%)			
pH	(2)	(10.01)	POOLED[a]			
Error	4	44.17	11.04			25.07
Total	8	230.85				100.00

DOF = Degrees of freedom; CL = Confidence limit; *S*' = Pure sum of squares; [a] = CL was not calculated.

Table 4 Analysis of variance (ANOVA) of factors affecting the production of inulinase.

Factor	DOF	*SS*	*MS*	*F*	*S*'	Contribution (%)
Inulin	2	443.11	216.55	0	443.11	16.47
NH_4Cl	2	98.89	49.44	0	98.89	3.76
$MgSO_4 \cdot 7H_2O$	2	1892.67	946.34	0	1892.67	71.95
pH	2	205.76	102.88	0	205.76	7.82
Error	0	0	0			
Total	8	2630.42				
Pooled	2	443.11	216.55	2.84	280.78	10.67
Inulin						
NH_4Cl	(2)	(98.89)	POOLED[a]			
$MgSO_4 \cdot 7H_2O$	2	1892.67	946.34	12.43	1740.35	66.16
pH	(2)	(205.76)	POOLED[a]			
Error	4	304.65	76.16			23.16
Total	8	2630.43				100.00

DOF = Degrees of freedom; *S*' = The pure sum of squares; [a] = Confidence limit was not calculated.

In addition, the concentration of magnesium sulfate was included in the optimization because the Mg^{2+} ion has been reported to affect the production of microbial invertase and inulinase. The addition of magnesium ions is assumed to affect the permeability of cell walls (Burnett and Trinci, 1979; Jung *et al*., 1987; Vanadakova *et al*., 2004).

As the number of degrees of freedom of the error was zero (Table 3, Table 4), information regarding the sum of the squares could not be determined for the error and the *F*-ratios for the factors could not be calculated. To complete the analysis, smaller factorial effects were added together, or pooled, to obtain a nonzero estimate of the error term (Table 3, Table 4). Pooling was done until the degrees of freedom (DOF) of the error term became close to half of the total DOF. Thus $MgSO_4 \cdot 7H_2O$ (factor C) and pH (factor D) were pooled. Based on the pooled data, the initial concentration of inulin and NH_4Cl had a clear substantial influence on the results. However, when interactions of different factors were calculated (Table 5), factors that had low influence individually (such as factor C, $MgSO_4 \cdot 7H_2O$ and factor D, pH) had interactively the strongest influence on invertase production. For example, the interactive effect of the factors C and D had the highest severity index (Table 5). Similarly, the severity index for the interaction of pH (factor D, the least important factor individually) with inulin (factor A, the most influential factor individually) was only 0.2%. For inulinase, the interactive effect of the concentration of ammonium chloride (factor B) and the concentration of magnesium sulfate (factor C) had the strongest influence on activity (Table 6). These results suggest that the influence of one factor on enzyme production depended on the value of the other factors in the production process.

The above-mentioned pooling was used to identify the optimum fermentation conditions shown in Table 7. The relevant main effect plots are shown in Figure 1. A main effect plot reveals how the changes in a factor level affect the response of the fermentation process. For four factors (A–D), each at three levels (1–3), only one of the levels maximized the value of the mean *S/N* ratio (Figure 1). Figure

Table 5 Interaction of factors in terms of severity index for invertase.

Interacting factor pairs	Severity index (*SI*) for invertase (%)		
	Activity	Yield	Productivity
$MgSO_4 \cdot 7H_2O \times pH$	31.62	59.01	49.42
Inulin × NH_4Cl	30.04	24.56	32.36
$cNH_4Cl \times MgSO_4 \cdot 7H_2O$	12.05	23.75	12.96
$MgSO_4 \cdot 7H_2O \times pH$	1.35	43.53	9.27
Inulin × pH	0.20	1.62	17.14

Table 6 Interaction of factors in terms of severity index for inulinase.

Interacting factor pairs	Severity index (*SI*) for inulinase (%)		
	Activity	Yield	Productivity
$NH_4Cl \times MgSO_4 \cdot 7H_2O$	80.80	76.39	75.26
$MgSO_4 \cdot 7H_2O \times pH$	79.35	74.71	73.40
Inulin × pH	53.93	49.87	55.09
Inulin × NH_4Cl	35.76	31.09	31.65
Inulin × $MgSO_4 \cdot 7H_2O$	10.75	11.34	13.87
$NH_4Cl \times pH$	9.92	5.36	12.80

1 suggests that the conditions for attaining high activity, yield and productivity of any of the two enzymes are different.

The equations in Table 7 were used to estimate the expected activity ($Y_{expected}$) of the enzymes under various conditions. The highest predicted inulinase activity of (174 $U.L^{-1}$) was for the conditions CON-1 (that is, 10 $g.L^{-1}$ inulin, 24 $g.L^{-1}$ NH_4Cl, 12 $g.L^{-1}$ $MgSO_4 \cdot 7H_2O$, pH 5, 30 °C). This set of conditions was therefore selected for further experiments. In contrast, two different sets of conditions were revealed in relation to invertase. One set of conditions (CON-2) maximized the predicted final activity of invertase. These conditions were (CON-2): 50 $g.L^{-1}$ inulin, 48 $g.L^{-1}$ NH_4Cl, 6 $g.L^{-1}$ $MgSO_4 \cdot 7H_2O$, pH 6 and 30 °C. A different set of conditions (CON-3) maximized both the predicted invertase yield on inulin and invertase productivity. This set of conditions (CON-3) was: 10 $g.L^{-1}$ inulin, 24 $g.L^{-1}$ NH_4Cl, 6 $g.L^{-1}$ $MgSO_4 \cdot 7H_2O$, pH 6 and 30 °C.

Under the optimal conditions identified by the Taguchi method for maximizing the invertase activity, (CON-2), the predicted value of the response parameter, (the invertase activity $Y_{expected}$) could be calculated using the Equations 4 and 5:

$$Y_{expected} = A3 + B3 + C2 + D3 - 3\overline{T} \quad (4)$$

where $\overline{T}$ was calculated using the following equation:

$$\overline{T} = (\Sigma(S/N)_{invertase})/N = 444.95/9 = 49.438 \quad (5)$$

For the pooled ANOVA (factor C, $MgSO_4 \cdot 7H_2O$, and D, pH, pooled) for invertase activity, Equation 4 was revised to Equation 6:

$$Y_{expected} = A3 + B3 - \overline{T} \quad (6)$$

From Equations 5 and 6, $Y_{expected}$ = (54.361 + 52.583) – 49.438 = 57.506. This $Y_{expected}$ value is dimensionless and needs to be converted to units of enzyme activity using the value of *MSD* (mean squared deviation, $U.L^{-1}$) and Equation 7:

$$Y_{expected}\left(U\ L^{-1}\right) = \left(\frac{1}{MSD}\right)^{1/2} \quad (7)$$

where $MSD = 10^{-Y_{expected,\ dimensionaless}/10} = 10^{-57.506/10} = 1.776 \times 10^{-6}$.

Therefore $Y_{expected}$ is 750.4 $U.L^{-1}$. The predicted and the expected values of $Y_{expected}$ for the two enzymes are shown in Table 8.

Table 7 Optimized factor levels for the production of invertase and inulinase.

Factor	Factors level optimized for enzyme production					
	Activity	Invertase yield	Productivity	Activity	Inulinase yield	Productivity
Inulin	50	10	10	10	10	50
NH_4Cl	48	24	24	24	48	48
$MgSO_4 \cdot 7H_2O$	6	6	6	12	12	12
pH	6	6	6	5	6	6
$Y_{expected}$[a]	Predicted activity and productivity based on optimal pooled conditions					
	Invertase			Inulinase		
Activity ($U.L^{-1}$)	$Y_{expected} = A3 + B3 - \overline{T}$			$Y_{expected} = A1 + C3 - \overline{T}$		
Productivity ($U.L^{-1}.hr^{-1}$)	$Y_{expected} = A1 + B2 - \overline{T}$			$Y_{expected} = A3 + C3 - \overline{T}$		

[a] = See Tables 3 and 4 for details of the pooled results.

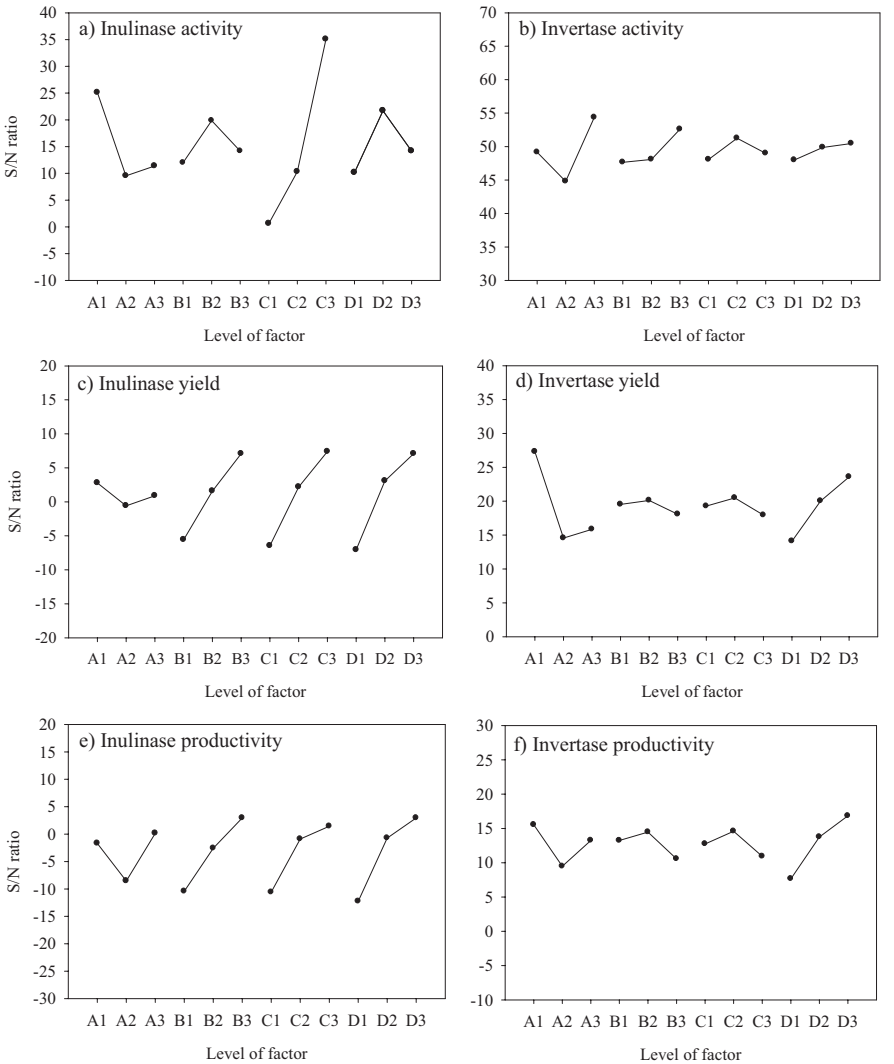

Figure 1 Signal-to-noise (*S/N*) ratios for the various factors (A–D, Table 1) and levels (1–3, Table 1): (a) inulinase activity; (b) invertase activity; (c) inulinase yield; (d) invertase yield; (e) inulinase productivity; and (f) invertase productivity. Optimal conditions are indicated by the peak values of the *S/N* ratio.

Table 8 Comparison between predicted and experimental $Y_{expected}$ values at the optimal conditions for production of invertase (CON-2) and inulinase (CON-1).

Parameter	Invertase		Inulinase	
	Predicted	Experiment[a]	Predicted	Experiment[b]
Activity ($U.L^{-1}$)	750.41	552.12	174.06	56.41
$Y_{expected}$[c] + *CI*	$497.96 < Y_{expected} < 1130.83$		$23.10 < Y_{expected} < 1311.60$	
Productivity ($U.L^{-1}.hr^{-1}$)	7.27	10.28	1.43	1.18
$Y_{expected}$[c] + *CI*	$4.31 < Y_{expected} < 12.26$		$0.21 < Y_{expected} < 9.56$	

[a] = See Figure 2b for invertase production (CON-2); [b] = See Figure 2a for inulinase production (CON-1); [c] = The expected ranges for enzyme activity and productivity; CI = Confidence interval.

Confirmation under optimal conditions

Confirmation testing is a necessary requirement of the Taguchi method. A single confirmation test was conducted for enzyme production using the above identified optimum settings of the process parameters. The 90% confidence interval (*CI*) of the confirmation test was calculated using Equation 3 and found to be ± 3.562. Therefore, the predicted optimal range was estimated as $(57.506 - 3.562) < Y_{expected} < (57.506 + 3.562)$, or $53.944 < Y_{expected} < 61.068$.

The confirmation test results for the set of conditions CON 1–3 are shown in Table 9. For both enzymes, the measured activity and productivity were within the expected ranges that were consistent with the predictions. The specific growth rate of the yeast under all three conditions (CON 1–3) was similar at 0.1136, 0.1035 and 0.1199 hr^{-1} (Table 9), but enzyme production varied greatly with the specific conditions used.

Table 9 reveals comparable levels of invertase activity and productivity for the conditions CON-2 and CON-3. In contrast, the confirmation data in Figure 2 showed the highest inulinase activity (261.59 $U.L^{-1}$) and productivity (1.98 $U.L^{-1}.hr^{-1}$) under CON-2 (Figure 2b). CON-1 and CON-3 favored production of invertase. CON-2 maximized the production of both invertase and inulinase.

Under CON-2, maximal production of the two enzymes occurred during the exponential phase of growth within the first 24 hr (Figure 2b). Inulinase production declined continuously with the approach of the stationary phase of growth (Figure 2b). In contrast, under CON-1, the level of inulinase production was relatively low but was not adversely affected by the onset of the stationary phase of growth (Figure 2a). The high level of inulinase attained within the first 24 hr under CON-2 (Figure 2) compared well with the results for the other producer microorganisms where the inulinase activity generally peaked much later in the fermentation. For example, for *Aspergillus niger*-245, Cruz *et al.* (1998) reported peak production after 48–60 hr of fermentation. Similarly, using *K. marxianus* YS-1, Singh and Bhermi (2008) observed the highest inulinase activity after 60 hr of incubation.

CONCLUSION

The yeast, *C. guilliermondii* TISTR 5844, was found to be a good producer of invertase and inulinase. The yields of the enzymes were enhanced substantially by optimization of the medium composition. Conditions were established for preferential production of only invertase, but a different set of fermentation conditions simultaneously afforded high titers of both invertase and inulinase. Concentrations of the carbon source (inulin) and the nitrogen source (NH_4Cl) were found to have the most impact on the production of the enzymes. Under optimal conditions, maximum invertase activity, productivity and yield were achieved at 552.12 $U.L^{-1}$, 10.28 $U.L^{-1}.hr^{-1}$ and 14.86 $U.g^{-1}$, respectively. Maximum inulinase activity was attained at 24 hr (261.59 $U.L^{-1}$) and the highest

Table 9 Summary of invertase and inulinase production under various optimal conditions[a].

Batch	μ (hr^{-1})	Invertase			Inulinase		
		Activity ($U.L^{-1}$)	Yield ($U.g^{-1}$)	Productivity ($U.L^{-1}.hr^{-1}$)	Activity ($U.L^{-1}$)	Yield ($U.g^{-1}$)	Productivity ($U.L^{-1}.hr^{-1}$)
CON1	0.1136	260.33	33.60	5.80	56.41	5.92	1.18
CON2	0.1035	552.12	14.86	10.28	94.91	2.82	1.98
CON3	0.1199	599.30	54.05	10.97	8.00	1.79	1.17

μ = Specific growth rate (hr^{-1}); Yield = Enzyme yield based on substrate (U enzyme per g substrate); Productivity = Volumetric production rate of enzymes ($g\ L^{-1}\ hr^{-1}$); [a] = See Figure 2 for production profiles.

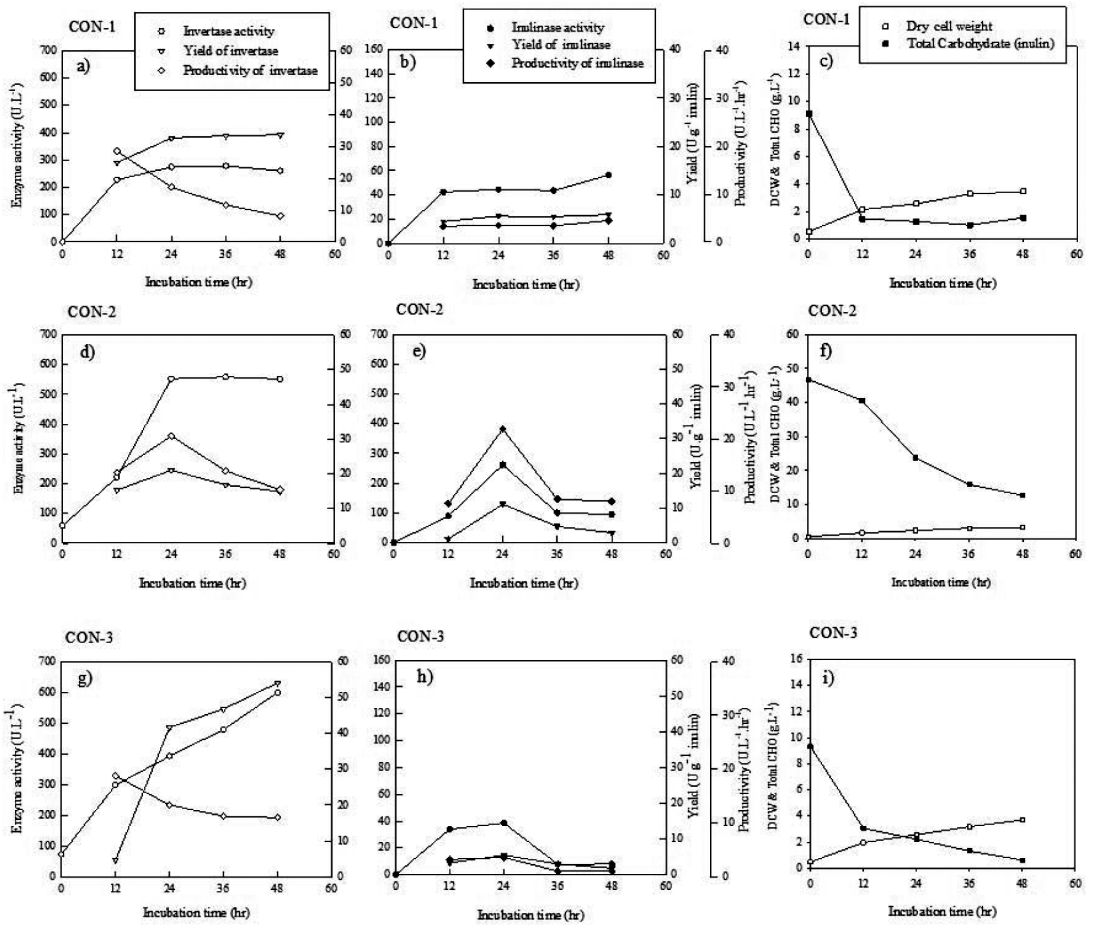

Figure 2 Yield of inulin, activity and productivity of inulinase and invertase under the optimal conditions CON-1 ((a), (b) and (c)), CON-2 ((d), (e) and (f)) and CON-3 ((g), (h) and (i)).

inulinase productivity at 1.98 ($U.L^{-1}.hr^{-1}$) after 48 hr. The titers of both the enzymes peaked much earlier than has been generally attained in fermentations with other microorganisms. In view of its ability to rapidly produce both invertase and inulinase in a minimally controlled batch fermentation, *C. guilliermondii* TISTR 5844 is potentially useful for producing a commercial enzyme cocktail for making sweeteners from inulin.

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