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Contributed Paper

Ethanol Production from Sweet Sorghum Juice Under Very High Gravity Fermentation by *Saccharomyces cerevisiae* : Aeration Strategy and Its Effect on Yeast Intracellular Composition

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ABSTRACT

A new technique of aeration during ethanol fermentation from sweet sorghum juice under very high gravity (VHG) condition was investigated to improve ethanol production efficiency of *Saccharomyces cerevisiae* NP 01. Response surface methodology (RSM) analysis showed that the optimal aeration conditions for ethanol production were an aeration rate, aeration time and agitation rate at 2.50 vvm, 4.44 h and 197 rpm, respectively. When the verification experiment under the optimal conditions was performed in a 5-L bioreactor, ethanol concentration (P), productivity (Q_p) and ethanol yield ($Y_{p/s}$) were 131.75 ± 0.76 g l^{-1}, 2.53 ± 0.01 g l^{-1} h^{-1} and 0.00 ± 0.76, respectively. Under an unaerated condition (control treatment), the P, Q_p and $Y_{p/s}$ values were 120.72 ± 1.37 g l^{-1}, 2.24 ± 0.03 g $l^{-1}h^{-1}$ and 0.50 ± 0.00, respectively. Additionally, total fatty acids (TFAs) and ergosterol concentrations in the yeast cells at the fermentation time under the optimal aeration conditions were 2637.14 ± 64.39 and 282.37 ± 18.46 μg g_{DCW}^{-1}, respectively. These values under the control conditions were 1587.05 ± 25.33 and 201.09 ± 24.33 μg g_{DCW}^{-1}, respectively. The results clearly indicated that optimal aeration can improve ethanol production efficiency of yeast cells, which might be related to an increase in their intracellular components, i.e., TFAs and ergosterol.

Keywords: aeration, agitation, ergosterol, *Saccharomyces cerevisiae*, fatty acid content, VHG fermentation

1. INTRODUCTION

A high ethanol concentration obtained with a short fermentation time is economically desirable for industrial ethanol production [1]. In addition to the typical factors, i.e. yeast strain used, fermentation processes, cell density, temperature, sugar concentration and enrichment of the medium used [2], one approach that can improve the productivity and cost effectiveness of ethanol production is the use of a very high gravity (VHG) fermentation. This technique is defined as the use of media containing 25 to 40% (w/v) of total sugars and it aims to achieve greater than a 15% (v/v) ethanol concentration [3-4]. The potential benefits of VHG fermentation technology over traditional fermentations include a considerable savings of water, a higher alcohol yield, reduced labor and energy needs, less capital costs and minimized risk of bacterial contamination [3]. However, the high sugar content can cause an increase in osmotic pressure, leading to a damaging effect on cell growth and fermentative activities [5-6]. Additionally, the increase in osmotic pressure can cause an increased intracellular ethanol accumulation in the yeast cells [7]. Ethanol tolerance is strictly related to lipid and ergosterol contents of yeast cell membranes, and the loss of membrane integrity decreases the level of ethanol tolerance, eventually causing cell death [8-9].

Yeast growth normally requires proper aeration at certain fermenting stages for lipid (sterol and unsaturated fatty acids) synthesis, which is essential for cell integrity [10-11]. Additionally, ethanol production efficiencies in terms of ethanol concentration and its productivity also depend on initial cell concentration of the yeast in an ethanol production medium [12-13]. In large scale fermenters, obtaining very high initial cell concentrations ($\geq 1\times10^{8}$ cells ml^{-1}) is rarely impossible [14] except by using yeast in a dried form. To reduce the initial yeast cell concentration for ethanol fermentation, an appropriate degree of aeration at the beginning of the fermentation is required. It was reported that an aeration rate of 0.2 vvm enhanced the cell concentration in an ethanol fermentation by about 23%, [15] resulting in an increase in ethanol production and its yield. Additionally, higher ethanol yield can be achieved at higher aeration rates (0.67 to 1 vvm) and impeller speeds (150 to 250 rpm) [16]. However, a high degree of aeration and agitation rate required in fermentation may lead to foam formation which is undesirable. Therefore, a high level of ethanol production under a VHG fermentation can be achieved only with appropriate aeration strategies (aeration and agitation rates) due to high concentrations of healthy yeast cells.

Sweet sorghum has been noted for an alternative feedstock for ethanol production because it contains high levels of fermentable sugars (sucrose, fructose and glucose), which can be directly converted to ethanol by yeast cells [17-18]. Additionally, it can be cultivated at nearly all temperatures and tropical areas with a growing period of 120-150 days [19].

The objectives of this research were to determine an optimal aeration strategy for a VHG ethanol fermentation from sweet sorghum juice by *Saccharomyces cerevisiae* NP 01. A correlation between agitation rate, aeration rate, aeration time and the ethanol concentration (*P*) under a VHG fermentation was developed by the application of statistical methods, i.e., response surface methodology (RSM) using the Box-Behnken design. The experimental prediction of ethanol concentration under optimal condition giving the maximum *P* value

was then verified. Intracellular composition (fatty acids and ergosterol) of the yeast cells under the optimal and control (no aeration) conditions for ethanol fermentation were also investigated.

2. MATERIALS AND METHODS

2.1 Microorganism and Inoculum Preparation

Active *S. cerevisiae* NP 01 cells were transferred into the sweet sorghum juice containing 150 g l^{-1} of total sugar. Then, they were incubated at 30 °C, 150 rpm for 15 h [20] before use as an inoculum for ethanol fermentation.

2.2 Ethanol Production (EP) Medium

The juice (18°Bx of total soluble solids) squeezed from sweet sorghum stalks (cv. KKU 40) was concentrated to 75°Bx and stored at 4 °C until use. The concentrated juice was diluted to 290 g l^{-1} of total sugar and supplemented with 9 g l^{-1} of yeast extract before use as an EP medium.

2.3 Experimental Design

The three independent variables, i.e., agitation rate (X_1), aeration rate (X_2) and aeration time (X_3), were optimized using RSM. Box-Behnken design has three levels of each variable (low, middle and high values, coded as -1, 0 and 1). The three variables at three levels (X_1: 100, 200 and 300 rpm; X_2: 0.5, 1.5 and 2.5 vvm; X_3: 2, 4 and 6 h) were used in a total of 14 runs in the current study (Table 1). Design-Expert 7.0 software (trial version, Stat Ease, Inc., Minneapolis, MN) was used for experimental design, data analysis, and quadratic model building. The quadratic equation used for this model is:

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

where Y is the estimated response and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for the intercept, linear, square and interaction, respectively while X_i and X_j are the coded independent variables.

Table 1. Experimental and predicted values for ethanol concentration (*P*) with coded factor values according to Box-Behnken design.

Run	Coded factor values*			*P* (g l^{-1})	
	X_1	X_2	X_3	Experimental values	Predicted values
1	0	1	1	128.98	128.25
2	-1	-1	0	118.96	119.46
3	-1	0	1	119.24	119.51
4	1	0	-1	122.55	122.28
5	0	1	-1	124.98	125.75
6	0	0	0	125.41	125.70
7	1	-1	0	129.22	128.76
8	1	0	1	121.36	122.59
9	0	0	0	125.98	125.70
10	1	1	0	125.99	125.49
11	0	-1	1	126.64	125.87
12	-1	0	-1	117.66	116.43
13	0	-1	-1	124.24	124.97
14	-1	1	0	125.41	125.87

2.4 Fermentation Conditions

A batch fermentation was performed in a 2-L fermenter (Biostat®B, B. Braun Biotech, Germany) at 30 °C and the initial yeast cell concentration in the sterile EP medium was ~ 2×10^7 cells ml^{-1}. Samples in the fermenter were taken at regular time intervals for analysis. Additional experiments with the corresponding parameters at their optimal conditions were done in a 5-L fermenter (Biostat®B, B. Braun Biotech, Germany) to verify the reliability of the results from the response surface analysis (RSA),

2.5 Analytical Methods

Total soluble solids, residual sugars and ethanol concentration in the fermentation broth were determined using a hand-held refractometer, phenol sulfuric acid method and gas chromatography, respectively [18]. The viable yeast cell counts were determined using an optical microscope and a haemacytometer following a methylene blue staining method [21]. Biomass concentration was determined using a standard calibration curve between cell concentrations (cells ml^{-1}) and dry cell weight (g ml^{-1}).

Ethanol production efficiencies were expressed in terms of ethanol produced (P, g l^{-1}), ethanol yield ($Y_{p/s}$, g g^{-1}) and ethanol productivity (Q_p, g $l^{-1}h^{-1}$) [18].

Fatty acid content in yeast cells was determined by Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand by gas chromatography using a Hewlett-Packard 5890 GC (Hewlett-Packard Company, Waldbronn, Germany) equipped with a flame ionization detector (FID). Their methyl esters were prepared according to AOAC [22]. A capillary column (SP2560; Sigma Aldrich Co.LLC, USA), 100 m long × 1.25 mm with a 0.2 m film was used to separate methyl esters. Chromatography was performed with an initial oven temperature of 100 °C, maintained for 4 min, to a final temperature of 240 °C at temperature a gradient of 3 °C min^{-1}. The injector and detector temperatures were 225 and 285 °C, respectively, and the carrier gas (helium) flow rate was 0.75 ml min^{-1}. Total ergosterol of yeast cells was extracted and its quantity determined using an HPLC equipped with a UV-SPD-10A detector at λ of 205 nm (Shimadzu, Japan) [23]. The separation was performed in a Water spherisorbs ODS2 C18 column (256 mm × 4.6 mm; particle size 5 μm) with a methanol:water ratio of 94:1, v/v as the mobile phase at the flow rate of 1 ml min^{-1}. The results were expressed as μg g^{-1} of dry cell weight.

2.6 Statistical Analysis

All experiments were performed in triplicate and the results were expressed as the mean values ± SD. Statistical analysis was done using SPSS 17.0 for Windows.

3. RESULTS AND DISCUSSION

3.1 Analysis of the Response and Model Fitting

RSM is a useful model for studying the influence of several variables affecting the responses of a treatment by simultaneously varying them and investigating them in a limited number of experiments. In this study, 14 experimental runs of a batch ethanol fermentation were carried out (Table 2). For the fermentation of Run 1 (agitation rate, 200 rpm; aeration rate, 2.5 vvm and aeration time, 6 h), the yeast cell numbers significantly increased from 1.95×10^7 to 2.20×10^8 cells ml^{-1} in 6 h, and the values had slightly increased to 3.02×10^8 cells ml^{-1} at 36 h (Figure 1). The initial pH of the medium was 4.56 and it slightly decreased over 12 h. The initial sugar concentration was 292.70 g l^{-1} and the sugar remaining in the fermented broth was 44.43, corresponding

to ~85% sugar consumption. The ethanol concentration slightly increased in the first 6 h and continuously increased until 54 h (including 6 h of the aeration) to value of 128.98 g l^{-1}. The calculated Q_p and $Y_{p/s}$ were found to be 2.39 g $l^{-1}h^{-1}$ and 0.50, respectively. These results showed that an aeration rate of 2.5 vvm for the first 6 h caused dissolved oxygen to be present in the broth, and the prior aerobic growth of yeast cells was important to increase yeast cell numbers and to dictate the fermentation. Changes of the viable yeast cell count, pH, sugar and ethanol during the ethanol fermentation of the remaining 13 runs were similar to those of Run 1 (data not shown).

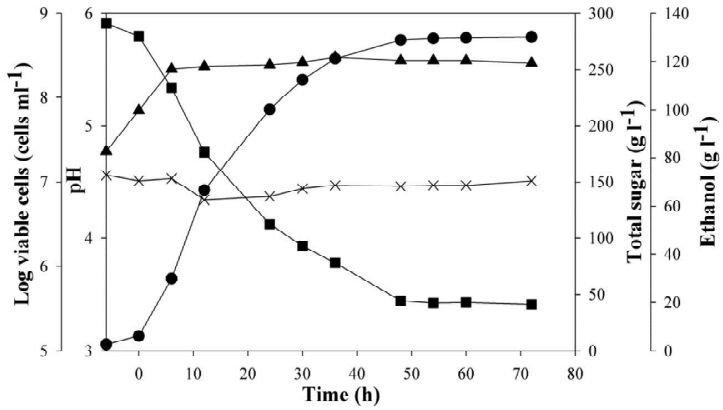

Figure 1. Ethanol fermentation from sweet sorghum juice under an agitation rate of 200 rpm, aeration rate of 2.5 vvm and aeration time of 6 h (Run 1) (▲ = log viable cells, × = pH, ■ = total sugar and ● = ethanol).

Considerable variation in the ethanol concentration or *P* value from the *S. cerevisiae* NP 01 cultivated under the different conditions is shown in Table 2. A low value of Coefficient of the Variation (CV, 1.03%) indicated a high degree of precision and a good deal of reliability of the experimental values. Applying multiple regression analysis, the results were fitted to a second-order polynomial equation. Thus, the resulting mathematical regression model for *P* was as follows:

$$Y = 125.70 + 2.23X_1 + 0.79X_2 + 0.85X_3 - 2.42X_1X_2 - 0.69X_1X_3 + 0.40X_2X_3 - 3.40X_1^2 + 2.60X_2^2 - 2.09X_3^2 \quad (2)$$

Analysis of variance (ANOVA) was done to analyze the experimental data (Table 2). The model *F* value of 11.10 implied that the model was highly significant, and there was only a 1.68% chance that "the model *F* value" this large could occur because of noise. The *p*-values < 0.05 indicated that the model terms were significant. The linear model terms of agitation rate (X_1), the second order effect of agitation rate (X_1^2), aeration rate (X_2^2) and aeration time (X_3^2) and interactive model terms of aeration rate and aeration time (X_2X_3) were statistically significant ($p < 0.05$), while the linear model terms (X_2 and X_3), and the interactive model terms (X_1X_2 and X_1X_3) were not statistically significant ($p > 0.05$) (Table 2). The experimental vs. predicted values (calculated from Equation (2)) in Table 1 showed a close linear correlation (Figure 2). The R^2 (correlation coefficient) for the *p*-value in our study was 0.9615, indicating that the

regression model modeled the fermentation precisely [24]. The *F*-value of the lack of fit was 13.03 (Table 2), implying that it was not significant relative to the pure error and/or difference between the central points. According to the regression analysis (Equation 2), the optimal conditions for the maximum predicted *P* value of 129.22 g l^{-1} were an agitation rate of 197 rpm, aeration rate of 2.50 vvm and aeration time of 4.44 h.

Table 2. Analysis of variance (ANOVA) of the model.

Source	*Df* *	SS	MS	*F*-value	*p*-value
Model	9	162.68	18.08	11.10	0.0168
X_1	1	39.83	39.83	24.46	0.0078
X_2	1	4.96	4.96	3.05	0.1558
X_3	1	5.76	5.76	3.54	0.1331
X_1^2	1	23.43	23.43	14.39	0.0192
X_1X_2	1	1.92	1.92	1.18	0.3388
X_1X_3	1	0.64	0.64	0.39	0.5647
X_2^2	1	37.07	37.07	22.77	0.0088
X_2X_3	1	21.69	21.69	13.32	0.0218
X_3^2	1	13.96	13.96	8.57	0.0429
Lack of fit	3	6.35	2.12	13.03	0.2002
Pure of error	1	0.16	0.16		
R^2		0.9615			

* *df*: degrees of freedom; SS: sum of squares; MS: mean sum of squares.

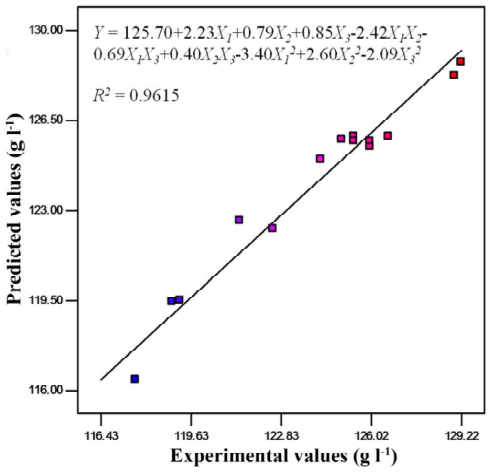

Figure 2. Correlation between predicted and experimental values of ethanol concentration (*P*).

3.2 The Verification Experiments

Under the optimal conditions above, the fermentation parameters measured in the 5-L fermenter were almost constant after 52 h (Figure 3). The residual viable cell count was high, 2.73×10^8 cells ml^{-1}, with ~90% sugar consumption. The experimental *P* value was 131.75 ± 0.76 g l^1, which was in good agreement with the predicted value (129.22 g l^{-1}). The Q_p and $Y_{p/s}$ values were 2.53 ± 0.01 g $l^{-1}h^{-1}$ and 0.50 ± 0.00, respectively (Table 3). When the control (without aeration) treatment was done under the same optimal condition, the fermentation time (54 h) was slightly longer than under the optimal conditions (Figure 3). The viable yeast cell count under the optimal conditions reached its maximum value earlier than that under the control condition (2.86×10^8 cells ml^{-1} at 12 h vs. 2.58×10^8 cells ml^{-1} at 24 h). The viable cell count at the end of the fermentation of the optimal conditions was slightly higher than that under the control conditions, while the total sugar remaining of the former was about 23 g l^{-1} lower than

that of the latter (Figure 3). Under both conditions, yeast viability gradually declined at the end of the fermentation. This could be due to high levels of ethanol accumulated in the fermented broth. The P and Q_p values under the optimal aeration strategy increased ~9 to 13% compared to those of the control conditions, while the $Y_{p/s}$ values of both conditions were the same (Table 3).

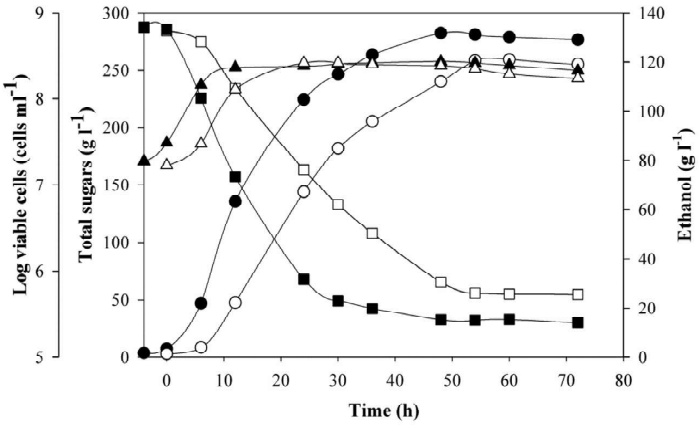

Figure 3. Ethanol fermentation in a 5-L fermenter from sweet sorghum juice under optimal aeration (closed symbols) and control (open symbols) conditions: (▲, △) = log viable cells; (■, □) = total sugar and (●, ○) = ethanol.

Table 3. Kinetic parameters of ethanol production from sweet sorghum juice under optimal aeration and control conditions in a 5-L fermenter.

Conditions	P^* (g l^{-1})	Q_p (g l^{-1} h^{-1})	$Y_{p/s}$ (g g^{-1})	t (h)
Optimal (aeration rate, 2.50 vvm; aeration time, 4.44 h and agitation rate, 197 rpm)	131.75 ± 0.76[a]	2.53 ± 0.01[a]	0.50 ± 0.00[a]	52
Control (agitation rate, 200 rpm)	120.72 ± 1.37[b]	2.24 ± 0.03[b]	0.50 ± 0.00[b]	54

* P, ethanol concentration; Q_p, ethanol productivity; $Y_{p/s}$, ethanol yield and t, fermentation time.

The experiments were performed in triplicate and the results were expressed as the mean value ± SD.

[a, b] Means superscripted with the same letter within the same column are not significantly different using Duncan's multiple range test at the 0.05 level.

Our results clearly showed that an appropriate aeration rate was necessary because it enhanced the viability of yeast cells and increased the *P* value. Yan et al. [25] found that aeration slightly improved the final *P* values from 75.8 g l^{-1} (unaerated and static condition) to 85.2 g l^{-1} (aerated and static condition), and aeration with agitation had stronger positive effects on fermentation process. Lin et al. [26] reported that aerating a yeast population between the early log and mid log growth phase had a more positive effect on ethanol production efficiency in terms of Q_p than aerating at other phases. Ethanol fermentations with proper aeration using various sugar-utilizing yeasts have been studied by other investigators and their findings are summarized in Table 4. It can be concluded that an optimal aeration strategy has a significant potential to enable a high level of ethanol production in batch systems, especially under VHG fermentations.

Table 4. Comparison of literature values of parameters in batch ethanol fermentation using different optimal aeration conditions.

Conditions		Initial total sugar ($g\,l^{-1}$)	Yeast strain	P^* ($g\,l^{-1}$)	Q_p ($g\,l^{-1}\,h^{-1}$)	$Y_{p/s}$ ($g\,g^{-1}$)	t (h)	References
Aeration	Agitation Aeration time							
2.88 vvm	100 rpm -[a]	200	*P. stipitis*	9.66	0.20	0.41	48	[34]
0.20 vvm	300 rpm 12 h	220	*K. marxianus* DMKU 3-1042	50.73	1.30	0.31	40	[35]
1.50 vvm	250 rpm -[a]	50	*S. cerevisiae*	9.91	0.16	0.23	62	[16]
0.13 vvm	200 rpm -[a]	340	*S. cerevisiae* SC1024	160	4.44	0.47	-	[36]
4-9 mg l^{-1}	200 rpm -[a]	305	*S. cerevisiae* CCTCC M206111	143.8	2.66	0.47	54	[25]
0.15 vvm	200 rpm 12 h	350	*S. cerevisiae*	126.24	2.63	0.36	48	[37]
2.5 vvm	200 rpm -[a]	50	*P. stipitis* NRRL Y-7124	16	0.39	0.37	40	[38]
0.82 vvm	-[a] 10 h	302	*S. cerevisiae*	83.88	2.33	0.43	36	[26]
0.25 vvm	250 rpm -[a]	90	*P. stipitis* NRRL Y-7124	26.7	0.32	0.32	83	[39]
2.50 vvm	197 rpm 4.44 h	287	*S. cerevisiae* NP 01	131.75	2.53	0.50	52	This study

* *P*, ethanol concentration; Q_p, ethanol productivity; $Y_{p/s}$, ethanol yield and *t*, fermentation time.

[a] not reported.

3.3 Effects of Aeration on Fatty Acids and Ergosterol Contents in Yeast Cells

Microscopic examination of *S. cerevisiae* NP 01 morphology under the optimal aeration and control conditions showed no differences (data not shown). Fatty acid and ergosterol contents were analyzed at different phases of the ethanol fermentation to evaluate the differences and changes in composition of the yeast cells subjected to the optimal aeration and control conditions, (Figure 4). In the initial stage (Phase I), total fatty acids (TFAs) and ergosterol contents under both conditions were the same. Aeration at 2.5 vvm for 4.44 h (Phase II) showed a 16.36% increase in the TFA content (Figure 4A). Subsequently, the TFA content fluctuated slightly throughout the fermentation with the values ranging from 2386.31 to 2637.14 μg g_{DCW}^{-1}. In contrast, the TFA content under the control condition significantly decreased to a value of 1410.48 ± 27.33 μg g_{DCW}^{-1} at the end of the fermentation (72 h, Phase V). Ergosterol is the primary sterol found in yeast cells and moderates cellular membrane fluidity [27]. Under normal growth conditions, it constitutes approximately 80% of total sterols [28]. In the current study, ergosterol content under the optimal aeration condition in all phases was relatively constant with values of 273.21 to 294 .09 μg g_{DCW}^{-1} (Figure 4B), while those under the control condition decreased by 22% from 273.21 to 212.00 μg g_{DCW}^{-1} in the midlog phase (Phase III) and then was relatively constant throughout the remainder of the experiment. Under the optimal conditions, TFA and ergosterol contents during ethanol fermentation were higher than those under the control condition. This might be attributed to a higher ethanol concentration under the optimal conditions [29-30].

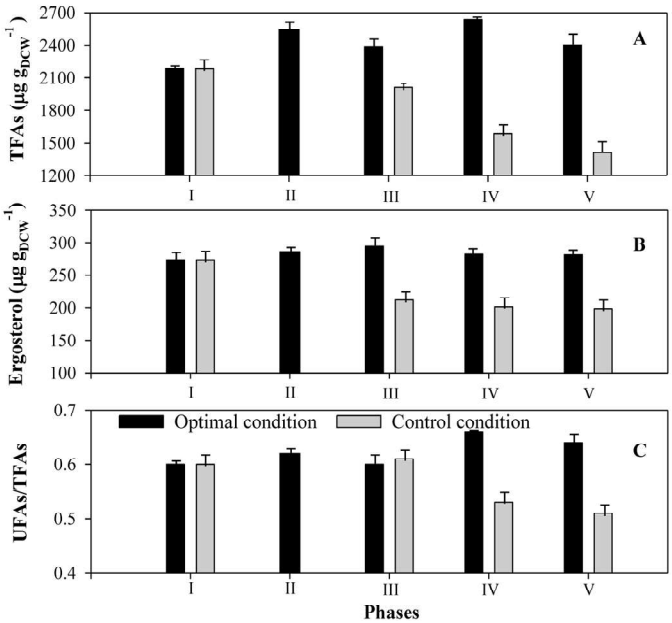

Figure 4. Total fatty acids (TFAs, A), ergosterol (B) and unsaturated index (UFAs/TFAs, C) in *S. cerevisiae* NP 01 cells at different phases during ethanol fermentation under optimal aeration and control conditions: Phase I (the initial stage), Phase II (stop aeration, only in the optimal condition), Phase III (midlog phase), Phase IV (stationary phase) and Phase V (the end of fermentation).

It was reported that a higher unsaturated index (unsaturated fatty acids/total fatty acids or UFAs/TFAs) was associated with an increase in membrane fluidity and yeast activity [31]. In the current study, the unsaturated indices in Phase I to Phase III under both conditions were similar at approximately 0.60-0.62 (Figure 4C). Subsequently, the values under the optimal condition increased to 0.64-0.66, while those values under the control condition decreased to 0.51-0.53. These results implied that higher unsaturated indices under the optimal conditions might increase membrane fluidity of the yeast cells, leading to higher ethanol tolerance and yeast activity.

Analysis of the composition of fatty acids in the cellular membrane of *S. cerevisiae* NP 01 revealed that they consisted of very long-chain (VLCFAs, more than 22 carbon atoms), long-chain (LCFAs, containing 12 - 22 carbon atoms), mid-chain (MCFAs, containing 6 - 12 carbon atoms) and short-chain (SCFAs, less than 6 carbon atoms) fatty acids (Table 5). Under both conditions, the most abundant fatty acid in the yeast cells (Phase I, at 0 h) was palmitic acid (C16:0), which accounted for 28.52% of TFAs, followed by linoleic acid, C18:2n6 (15.92%); oleic acid, C18:1 (15.41%); eicosatrienoic acid, C20:3n3 (12.11%); palmitoleic acid, C16:1 (10.14%); stearic acid, C18:0 (7.77%) and cis11, 14eicosatrienoic acid, C20:2 (5.47%). However, Piper [32] reported that linoleic acid was the main fatty acid in *S. cerevisiae* cells. Among the MCFAs in our study, lauric acid (C12:0) was the largest constituent, followed by capric acid (C10:0), undecanoic acid (C11:0) and caprylic acid (C8:0), in descending order. Minimal amounts of other TFAs were also detected (Table 5). Under the optimal conditions, both SFA and UFA contents (864.21 ± 68.36 and 1537.33 ± 64.39 μg g_{DCW}^{-1}, respectively) at the end of fermentation were significantly higher than those under the control condition (697.38 ± 72.31 and 713.10 ± 24.55 μg g_{DCW}^{-1}, respectively). The level of UFAs under the optimal aeration conditions was twice that of the control conditions. Dihn et al. [33] reported that high contents of UFAs increased membrane fluidity and enhanced ethanol tolerance. This might explain higher ethanol production under the optimal aeration condition in the current study.

Table 5. Fatty acid composition in *S. cerevisiae* NP 01 cells at different phases under the optimal aeration and control conditions.

Fatty acids (μg g_{DCW}^{-1})	Phase I	Phase V (Optimal condition)	Phase V (Control condition)
SFAs	871.96±91.1	864.21±68.36	697.38±72.31
C4:0	ND	5.17±0.04	7.76±0.67
C6:0	ND	ND	ND
C8:0	2.30±0.79	2.30±0.07	ND
C10:0	8.62±0.69	12.07±2.33	17.24±2.34
C11:0	2.59±0.91	ND	ND
C12:0	11.78±2.31	16.09±0.76	12.12±2.19
C13:0	ND	ND	ND
C14:0	9.19±1.18	5.17±0.43	6.90±1.39
C15:0	4.02±0.01	5.46±0.04	2.87±0.07
C16:0	623.15±24.01	515.42±24.31	409.24±16.75

Table 5. Continued.

Fatty acids ($\mu g\ g_{DCW}^{-1}$)	Phase I	Phase V (Optimal condition)	Phase V (Control condition)
C17:0	7.47±1.32	10.06±0.82	6.90±0.99
C18:0	169.79±34.21	237.88±24.88	129.92±13.44
C20:0	7.76±2.03	11.21±0.92	8.33±1.22
C21:0	1.15±0.09	ND	ND
C22:0	5.75±0.00	8.91±0.97	5.46±0.72
C23:0	4.31±0.83	8.33±0.91	21.26±2.64
C24:0	14.08±2.01	26.14±3.91	69.38±3.47
UFAs	1312.96±43.01	1537.33±64.39	713.10±24.55
C14:1	ND	ND	ND
C15:ln10	ND	ND	ND
C16:1	221.51±38.01	212.32±23.84	23.27±2.35
C17:ln10	ND	ND	ND
C18:1	336.72±12.61	253.69±25.69	94.23±12.01
C20:ln11	1.44±0.51	ND	ND
C22:ln9	0.86±0.01	ND	ND
C24:ln9	17.53±2.81	ND	ND
C18:2n6	347.92±62.01	618.42±64.97	418.90±36.05
C18:3n3	ND	ND	ND
C20:2	119.52±24.01	225.81±28.67	115.50±12.14
C20:3n6	1.72±0.01	ND	ND
C20:3n3	264.60±34.01	227.09±26.94	61.20±4.01
C20:4n6	1.15±0.01	ND	ND
C22:2	ND	ND	ND
C20:5n3	ND	ND	ND
C22:6n3	ND	ND	ND
TFAs	2184.92±19.57	2401.54±66.20	1410.48±31.91

Phase I (the initial stage, 0 h) and Phase V (the end of fermentation time)

* The results were expressed as mean ± SD. ND: not detected; SFAs, Saturated fatty acids; UFAs, Unsaturated fatty acids; TFAs, Total fatty acids; C4:0, Butyric acid; C6:0, Caproic acid; C8:0, Capprylic acid; C10:0, Capric acid; C11:0, Undecanoic acid; C12:0, Lauric acid; C13:0, Tridecanoic acid; C14:0, Myristic acid; C15:0, Pentadecanoic acid; C16:0, Palmitic acid; C17:0, Heptadecanoic acid; C18:0, Stearic acid; C20:0, Arachidic acid; C21:0, Heneicosanoic acid; C22:0, Behenic acid; C23:0, Tricosanoic acid; C24:0, Lignoceric acid; C14:1, Myristoleic acid; C15:ln10, cis-10-Pentadecanoic acid; C16:1, Palmitoleic acid; C17:ln10, cis-10-Heptadecanoic acid; C18:1, cis-9-Oleic acid; C20:ln11, cis-11-Eicosenoic acid; C22:ln9, Euric acid; C24:ln9, Nervonic acid; C18:2n6, cis-9,12-Linoleic acid; C18:3n3, a-Linolenic acid; C20:2, cis-11,14-Eicosadienoic acid; C20:3n6, cis-8,11,14-Eicosatrienoic acid; C20:3n3, cis-11,14,17-Eicosatrienoic acid; C20:4n6, Arachidonic acid; C22:2, cis-13,16-Docosadienoic acid; C20:5n3, cis-5,8,11,14,17-Eicosapentaenoic acid; C22:6n3, cis-4,7,10,13,16,19-Docosahexenoic acid.

4. CONCLUSION

Aeration and agitation were the important factors for improvement of ethanol production under the VHG fermentation in the current study. The predicted optimal aeration and agitation conditions for high ethanol production efficiency using RSM analysis were an agitation rate of 197 rpm, an aeration rate of 2.50 vvm and an aeration time, 4.44 h. Under these conditions, the final P (131.75 ± 0.76 g l^{-1}) and Q_p (2.53 ± 0.01 g $l^{-1}h^{-1}$) were ~9 and 13% higher than those of the control condition (unaerated), respectively. A proper aeration in the initial stage of the fermentation not only stimulated growth of *S. cerevisiae* NP 01 but also promoted the yeast to synthesize essential intracellular components (TFAs, UFAs and ergosterol). These factors protected yeast cell membranes from ethanol poisoning, resulting in an increase in ethanol production efficiency.

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