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SCREENING PHYSICAL FACTORS TO ENHANCE BIOETHANOL PRODUCTION IN OIL PALM TRUNK SAP FERMENTATION

(Saringan Faktor Fizikal Untuk Penghasilan Bioetanol Melalui Fermentasi Air Perahan Batang Kelapa Sawit)

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Abstract

Fermentation is a simple process that can produce a high–demand byproduct such as bioethanol. To produce a high yield concentration of bioethanol by *Saccharomyces cerevisiae* (*S. cerevisiae*) in the fermentation of oil palm trunk (OPT) sap as a sole carbon source, an experimental design by using a two–level full factorial design (2^{k-1}) was conducted at a laboratory scale to screen important factor in fermentation. The experiment was conducted to study the effect of pH, temperature, agitation rate, incubation time, and inoculum size as important physical factors in fermentation. The factors were exploited, respectively, at low (–1) and high (+1) level parameter ranges of 3.5 to 7.5 for pH, 20°C to 40°C for fermentation temperature, 0 to 50 rpm for agitation rate, 20 to 48 hours for the time of incubation, and 5% v/v to 15% v/v of inoculum size in the fermentation media. Thirty–two combinations of experimental design with a 2^{5-1} full factorial design reflected in 32 flasks of OPT sap with *S. cerevisiae* were conducted for the fermentation process. The bioethanol yield concentration was investigated in these experiments using gas chromatography with flame ionization detection (GCFID). In this study, the maximum bioethanol yield concentration was 37.8383mg/mL with pH media at 3.5, 5% v/v inoculum size, temperature at 40°C, agitation rate at 50rpm, and incubation length of 48 hours. Temperature, pH, agitation rate, incubation time, and inoculum size in the fermentation media were significant contributing factors in the fermentation of *S. cerevisiae* in OPT sap to produce a high yield concentration of bioethanol. These factors can be further optimized to increase bioethanol yield concentration in the fermentation by *S. cerevisiae* in OPT sap.

Keywords: fermentation, bioethanol, full factorial design, oil palm trunk sap, physical factor

Abstrak

Fermentasi adalah proses yang mudah dan boleh menghasilkan produk sampingan yang sangat diperlukan seperti bioetanol. Untuk menghasilkan hasil bioetanol oleh *Saccharomyces cerevisiae* (*S. cerevisiae*) dalam proses fermentasi cecair perahan batang kelapa sawit (OPT) sap sebagai sumber karbon utama, satu reka bentuk eksperimen dengan menggunakan reka bentuk faktorial penuh dua peringkat (2^{k-1}) telah dijalankan di skala makmal untuk menyaring faktor penting dalam proses fermentasi. Eksperimen ini dijalankan untuk mengkaji kesan pH, suhu, kadar pengadukan, masa, dan kandungan *S. cerevisiae* sebagai faktor fizikal penting dalam penapaian. Faktor–faktor tersebut dieksploitasi masing–masing pada julat parameter paras rendah (–1), dan tinggi (+1) 3.5 hingga 7.5 untuk pH, 20°C hingga 40°C untuk suhu penapaian, 0 hingga 50 rpm untuk kadar pengadukan, 20 hingga 48 jam untuk masa pengeraman, 5% v/v hingga 15% v/v kandungan *S. cerevisiae* dalam media fermentasi. Tiga puluh dua kombinasi reka bentuk eksperimen dengan 2^{5-1} reka bentuk faktorial penuh yang dijalankan di dalam 32 kelalang yang mengandungi larutan perahan OPT bersama *S. cerevisiae* telah dijalankan untuk proses fermentasi, dan tindak balas hasil bioetanol telah disiasat dalam eksperimen ini. Dalam kajian ini, kepekatan hasil bioetanol maksimum ialah 37.8383 mg/mL dengan media pH pada 3.5, 5% v/v kandungan *S. cerevisiae*, suhu pada 40°C, kadar pengadukan pada 50rpm, dan panjang pengeraman selama 48 jam. Suhu, pH, kadar pengadunan, masa penyejukan, dan saiz inokulum merupakan faktor penyumbang penting dalam fermentasi oleh *S. cerevisiae* dalam sap OPT untuk menghasilkan hasil bioetanol yang tinggi. Kesemua faktor ini boleh dioptimumkan lagi untuk meningkatkan pengeluaran bioetanol dalam proses fermentasi oleh *S. cerevisiae* dalam sap OPT.

Kata kunci: fermentasi, bioetanol, reka bentuk faktorial penuh, air perahan batang kelapa sawit, faktor fizikal

Introduction

The development of biofuels as a sustainable replacement for fossil fuels has become increasingly important in recent years. Various microorganisms can be used in fermentation to make bioethanol used in biofuel [1–2]. Various biomass sources produce bioethanol, such as plant sugars, lignocellulosic materials, and agricultural waste [3]. Employing microorganisms like *Saccharomyces cerevisiae* (*S. cerevisiae*) to ferment carbohydrates is a typical way to make bioethanol in fermentation [4–5]. Carbohydrates (including total sugar content) undergo anaerobic metabolism to produce bioethanol and carbon dioxide [6]. Robust fermentation capabilities make the *S. cerevisiae* strain widely used in bioethanol synthesis and an excellent choice for large–scale bioethanol production [7–8]. *S. cerevisiae* has several beneficial industrial properties, including rapid growth, efficient anaerobic glucose metabolism, and high resistance to various environmental stressors such as high yield concentration of bioethanol, low pH, and low oxygen [9–12]. Thus, it is crucial to understand the influence of physical factors throughout the fermentation process to achieve a high yield concentration of bioethanol.

Physical factors such as temperature, pH, agitation, inoculum size, and inoculum time play crucial roles in *S. cerevisiae* enzyme activity in fermentation. In the optimum temperature range, the bacteria's growth and metabolism speed up as the temperature rises [13]. Therefore, the rate of the fermentation reaction rises. However, the enzymes are inactivated when the temperature exceeds the optimum range and cause mortality of the microbe, the fermentation cycle is shortened, and the yield concentration of bioethanol is lowered [13, 14]. The pH can influence *S. cerevisiae* fermentation by altering enzyme activity and the charge state of cell membranes. This may affect the metabolic and physiological changes produced at high or extreme values, preventing yeast growth [14]. The correlation of these factors may influence the growth and metabolic activity of *S. cerevisiae*. In this work, the Design of experiments (DOE) was employed to screen and determine the significant physical factors that affect *S. cerevisiae*'s ability to produce bioethanol. Understanding their impact on bioethanol production is essential for enhancing process efficiency and yield.

The oil palm trees aged above 25 decrease their oil production; thus, replantation of the oil palm trees is essential to maintaining oil production. Thus, maintaining stable and high levels of palm oil production within the current palm oil extent is a potential strategy to relieve deforestation pressure [15]. The oil palm trunk (OPT) is a byproduct of the oil palm industry and is rich in ready–to–ferment sugars, which can serve as a potential low–cost fermentation medium for bioethanol yield concentration. Selecting a suitable fermentation medium is essential for practical and long–lasting bioethanol production. Shahirah et al. [16] found that glucose (75.51g/L) was the dominant sugar in OPT sap, followed by sucrose (62.68g/L), fructose (29.41g/L), and a small amount of galactose (4.49g/L). OPT sap contains a small amount of micronutrients such as P (0.001%) and Mg (0.014%) and other micronutrients in sufficient amounts such as Mo, Na, Ca, Zn, and vitamins that promote the growth of yeast cells [16–17]. Liu et al. [18] suggested that the bioethanol yield produced from fermentation improves by adding nutrients such as magnesium sulfate, ammonium sulfate, and disodium hydrogen phosphate. At the same time, these micronutrients were contained in OPT sap liquid as a buffer [19]. Utilizing this resource not only adds value to the oil palm industry but also promotes the use of sustainable feedstocks, reducing the reliance on food crops for bioethanol yield concentration. The felled OPT sap produced by the oil palm industry could become a versatile, affordable, and renewable waste material. However, its effectiveness as a fermentation medium for *S. cerevisiae's* bioethanol yield concentration needs more research.

The optimization of physical factors during fermentation using OTP sap as a medium for *S. cerevisiae* to produce bioethanol was attempted to address in this work. Examining one variable at a time may be acceptable in some circumstances, but it occasionally ignores the combined impact of several factors, mainly when doing experiments involving physiological systems [20–27]. Therefore, DOE was used as a statistical approach for efficient screening and optimization of multiple factors simultaneously. Operational variables interact during fermentation and influence their respective effects on response [28]. The experimental method must account for these interactions so that a set of optimal research conditions can be determined. Full factorial design involves systematically varying all possible combinations of factors, providing comprehensive visions into the interactions between factors and their impact on the response variable. This approach enables the identification of critical process parameters and their optimal levels. Empirical models and statistical analysis are significant, thus providing better control and understanding of the optimization of physical factors to produce bioethanol from OTP sap [21, 26–29]. Hence, this study aimed to optimize the physical factors of bioethanol production by *S. cerevisiae* during fermentation using the DOE full factorial design approach. The fermentation process can be optimized to maximize bioethanol yield concentration by identifying the critical factors and their optimal levels.

Materials and Methods

Preparation of OPT sap

The 25 years old *Elaeis guineensis* oil palm tree from Ladang Sawit Kampung Sungai Ranggam Muar was felled, and OPT was squeezed using a sugar cane press machine within 12 hours to produce sap. Homogenized OPT sap liquid was filtered using a 9.0 filter and autoclaved at 121°C for 20 minutes. Sterilized OPT sap was kept at 4 °C before usage.

Microorganisms and preadaptation

S. cerevisiae, a commercial baker’s yeast from AB Mauri Malaysia, was used as a fermenter. *S. cerevisiae* was cultured in 10% w/v yeast extract, 20% w/v peptone, 20% w/v glucose as sole carbon source, and 15% w/v agar and incubated at 30 °C for 24 hours. One colony was recultured onto a new yeast extract peptone glucose agar (YPGA) and incubated at 30 °C for 24 hours. Five colonies from the pure culture plate were introduced into sterile OPT sap and incubated at 30 °C for 18 hours before usage.

Two–level factorial design

Fermentation was performed in sterilized OPT sap media in a 250 mL shake flask with 50 mL fermentation media as working volume. Experimental design and statistical analysis were analyzed using Design–Expert® v12 software. Five physical factors in the fermentation by *S. cerevisiae* were exploited, respectively, at low (–1) and high (+1) level parameter ranges 3.5 to 7.5 for pH (factor A), 20°C to 40°C for fermentation temperature (factor B), 0 to 50rpm for agitation rate (factor C), 20 to 48 hours for time of incubation (factor D) and 5% v/v to 15% v/v of inoculum size (factor E) in the fermentation media. In contrast, the other remaining factors were kept constant. A full factorial design, 25, comprising 32 experimental runs, was performed to evaluate the effect of a physical factor of fermentation by *S. cerevisiae* in producing a high yield concentration of bioethanol by using OPT sap as the sole carbon source medium. Fermentation parameters for each experiment set were performed with five physical factors, as in Table 1.

Samples were collected in all experiments, and bioethanol yield concentration was analyzed.

Determination of bioethanol yield concentration

The bioethanol content was determined using gas chromatography (GC) with flame ionization detection (FID). The Agilent 7890B GC System and ZB–WAX Plus column (60m × 0.25mm × 0.25µm) from Phenomenex, USA, were used. 100 µL of supernatant from the fermentation product was mixed with 900 µL of n–propanol and filtered through a 0.22 µL nylon filter before being injected into GCFID for analysis. The minimum and maximum temperatures were 40 and 200 °C, respectively. The temperature rate in the oven was 15 per minute up to 140 °C and 50 per minute up to 200 °C. Nitrogen was the carrier gas with a flow rate of 1.4 mL/min and a temperature of 250 °C for the injection opening. The result of the data was recorded, and the graphic was created.

Results and Discussion

Five physical factors and parameters expected to influence bioethanol yield concentration were employed in the fermentation of sugar in OPT sap by *S. cerevisiae* to produce a high yield concentration of bioethanol. Table 1 shows the design matrix covering five variables to evaluate their effect on bioethanol yield concentration as the response for bioethanol yield concentration in mg/mL; the runs were randomized for statistical reasons. The physical factor variables included in the screening experiment, their setting, and the results of all 32 experimental two–level factorials are shown in Table 1. Each independent variable was investigated at a high (+1) and a low (–1) level. The variables affecting bioethanol yield concentration were identified based on confidence levels above 94% (p <0.05). The experiments were performed to optimize the physical factor of fermentation by *S. cerevisiae* on producing a high bioethanol yield concentration by using OPT sap as the sole carbon source medium. The variables having the most significant effect on bioethanol yield concentration in the fermentation of OPT sap were identified using a 2–level factorial design.

The experiment showed that bioethanol yield concentration varied from 5.79 mg/mL to 37.84 mg/mL from various combinations of five parameters. The results were analyzed using the analysis of variance (ANOVA) as appropriate to the experimental design used. The regression equation obtained after the variance analysis showed the bioethanol production level as a function of different variables. The interaction of variables pH (A), temperature (B), agitation rate (C), incubation time (D), and inoculum size (E) on bioethanol production in the fermentation of OPT sap were summarized in regression Equation (1) as below:

$$\text{Bioethanol yield concentration (mg/mL)} = 19.9919 - 0.544538A + 3.87677B + 0.512207C + 7.60135D + 0.970862E - 0.756333AB - 0.119174AC - 0.569173AD + 0.686418AE + 0.359868BC + 0.219447BD - 0.254717BE - 0.569908CD - 0.280364CE - 0.610352DE \quad (1)$$

The half–normal plot can be utilized to determine the significant factors affecting bioethanol yield concentration in the fermentation of OPT sap by *S. cerevisiae*. The half–normal plot showed the symbol of factors far away from the linear line as the most significant factors toward bioethanol yield concentration. The half–normal plot of effects revealed two large main effect factors, incubation time (D) and temperature (B), with a p–value of 0.007, as in Figure 1. Incubation time (D) fell far from the line and represented the strongest factor affecting bioethanol yield concentration, followed by temperature (B). Zhang et al. [30] and Silva et al. [31] also reported that incubation time and temperature were some of the most important factors in the fermentation by *S. cerevisiae* to produce bioethanol.

Table 1. Full factorial design of fermentation physical factors

Run Order	Factor					Response Bioethanol (mg/mL)
	A	B	C	D	E	
1	− 1	+ 1	− 1	− 1	− 1	14.42
2	+ 1	+ 1	+ 1	− 1	+ 1	17.92
3	+ 1	+ 1	− 1	− 1	− 1	10.53
4	+ 1	+ 1	+ 1	− 1	− 1	15.68
5	+ 1	+ 1	+ 1	+ 1	+ 1	31.22
6	+ 1	− 1	+ 1	− 1	− 1	6.70
7	− 1	− 1	− 1	− 1	+ 1	7.92
8	− 1	− 1	+ 1	+ 1	+ 1	24.96
9	− 1	+ 1	+ 1	− 1	+ 1	19.54
10	+ 1	+ 1	− 1	− 1	+ 1	17.48
11	+ 1	− 1	+ 1	+ 1	+ 1	22.76
12	+ 1	− 1	− 1	+ 1	− 1	20.64
13	− 1	+ 1	+ 1	+ 1	− 1	37.84
14	+ 1	− 1	− 1	− 1	− 1	8.40
15	− 1	+ 1	− 1	− 1	+ 1	16.43
16	+ 1	+ 1	+ 1	+ 1	− 1	29.19
17	− 1	+ 1	− 1	+ 1	− 1	32.12
18	− 1	− 1	+ 1	+ 1	− 1	22.03
19	+ 1	− 1	+ 1	+ 1	− 1	22.12
20	− 1	− 1	− 1	+ 1	+ 1	23.22
21	+ 1	+ 1	− 1	+ 1	− 1	29.06
22	+ 1	− 1	− 1	+ 1	+ 1	27.38
23	+ 1	+ 1	− 1	+ 1	+ 1	29.46
24	− 1	− 1	+ 1	− 1	+ 1	9.87
25	− 1	− 1	− 1	− 1	− 1	5.79
26	+ 1	− 1	− 1	− 1	+ 1	9.48
27	+ 1	− 1	+ 1	− 1	+ 1	13.13
28	− 1	+ 1	+ 1	+ 1	+ 1	30.16
29	− 1	+ 1	− 1	+ 1	+ 1	34.47
30	− 1	+ 1	+ 1	− 1	− 1	16.38
31	− 1	− 1	− 1	+ 1	− 1	24.86
32	− 1	− 1	+ 1	− 1	− 1	8.57

The conversion process took time, and sufficient incubation was required to ensure that all available sugars were metabolized and converted into bioethanol. The length of the incubation time influenced the growth and activity of *S. cerevisiae*, affecting the bioethanol yield concentration. However, longer incubation times could also lead to the formation of undesirable byproducts and secondary metabolites, such as higher alcohols and esters, which could inhibit *S. cerevisiae* cells and negatively affect bioethanol quality [11]. Incubation temperature played a crucial role in the fermentation process of *S. cerevisiae* for bioethanol yield concentration. At optimal temperatures, *S. cerevisiae* cells could effectively use sugar and convert it into bioethanol. Higher temperatures could speed up yeast metabolism, resulting in faster fermentation rates, but could also increase the production of unwanted byproducts [32]. On the other hand, lower temperatures could slow yeast metabolism and fermentation, reducing bioethanol yield concentration. Additionally, temperature influences the solubility and diffusion of gases like oxygen and carbon dioxide, which are essential for yeast growth and fermentation. Higher temperatures generally reduced the solubility of gases in the fermentation medium, thus reducing oxygen availability for yeast respiration [33]. Higher temperatures could also increase the rate of carbon dioxide production, impacting pH and fermentation performance. Furthermore, higher temperatures could improve yeast's ability to tolerate higher bioethanol concentrations, allowing for increased bioethanol yield concentration. However, excessive temperatures could cause thermal stress, affecting yeast viability and fermentation efficiency [34].

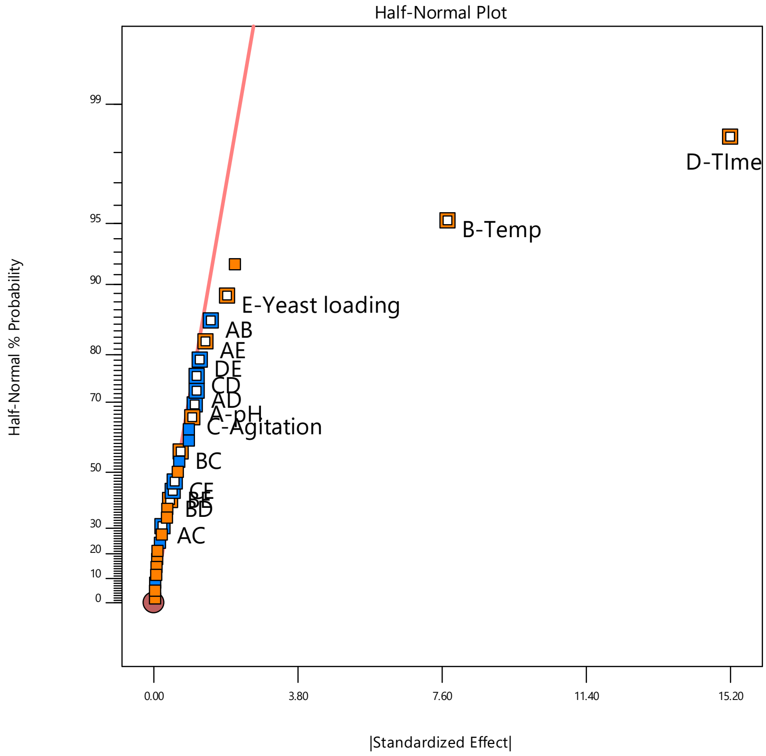

Figure 1. Half–normal plot effect of factor A (pH), B (incubation temperature), C (agitation rate), D (incubation time) and E (inoculum size) in fermentation. Orange dots indicate a positive effect, while blue dots indicate a negative effect

This result could have been further interpreted in the Pareto Chart, as in Figure 2, a graphical tool used to display the magnitudes of the effects from the results obtained from the largest to the smallest effects. Referring to the Pareto charts, orange bars represent a positive effect, and blue bars represent a negative effect. A positive effect meant the response variable increased as the factor level increased. This suggested that higher levels of the factor were beneficial for maximizing the response. On the other hand, an adverse effect meant that the response variable decreased as the factor level increased. In this case, lower factor levels were preferred to maximize the response. The main factors or two–factor interactions were displayed on the top of the bar. The residual's degree of freedom (df) was 16, so the critical t–value was 2.1199. The Bonferroni limit was a more conservative t–value that accounted for the estimated effects by dividing the risk value alpha by the desired probability, producing a value of 3.7880. The Pareto chart showed that incubation time was the most critical factor affecting bioethanol yield concentration, followed by the incubation temperature. Both positively affected bioethanol yield concentration in the fermentation process by *S. cerevisiae*. These two factors exceeded even the more conservative Bonferroni limit, thus providing a high confidence level greater than 95%. Inoculum size also marked a significant factor that exceeded the t–value line, as shown in Figure 2.

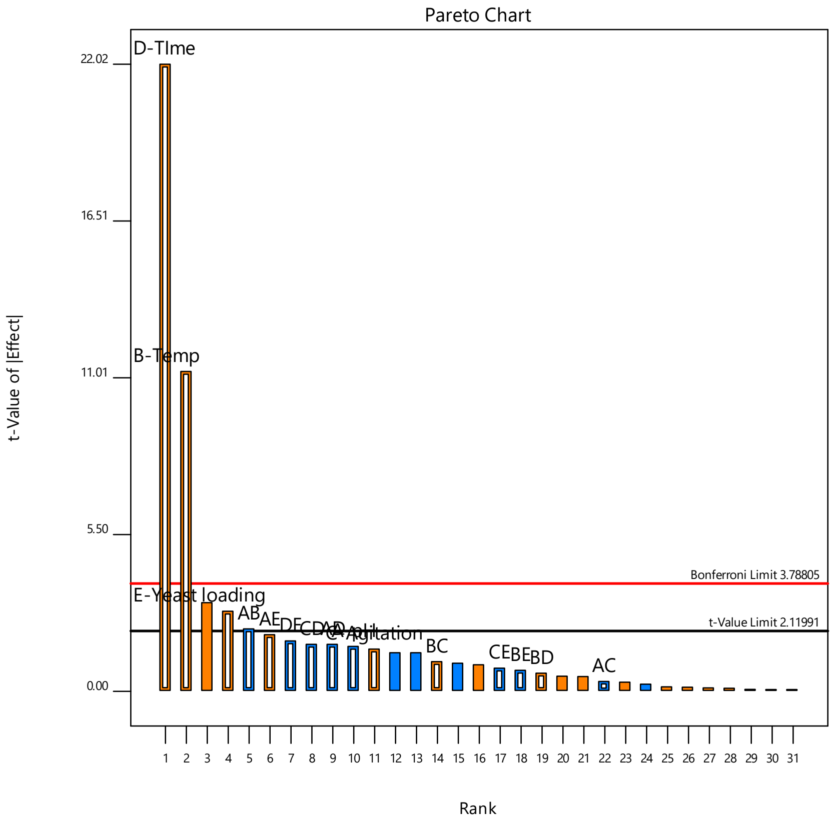

Figure 2. Pareto chart of factors A (pH), B (incubation temperature), C (agitation rate), D (incubation time), and E (inoculum size) that affect fermentation. Orange indicates a positive effect, while blue indicates a negative effect

The ANOVA was used to evaluate the adequacy of the fitted model. It can be used to assess the findings of a full factorial design, which manipulates possible combinations of the factors being studied. Table 2 shows the ANOVA of bioethanol yield concentration for the desired response. The Model F–value of 42.91 implied that the model was significant. There was only a 0.01% chance that an F–value this large could occur due to noise. P–values less than 0.0500 indicated that model terms were significant. Three main factors were found to have a significant effect on the fermentation of OPT sap to produce a high yield concentration of bioethanol by *S. cerevisiae*, which was incubation temperature p–value <0.0001, incubation time p–value <0.0001 and inoculum size with p–value = 0.0125 whereas interaction of two factors, pH and temperature (AB) also shown a significant effect on bioethanol yield concentration in the fermentation of OPT sap by *S. cerevisiae.* The significance of each coefficient was determined using a p–value (p <0.05), and the smallest p–value indicated a high significance of the corresponding coefficient. Variables with the most significant effect were incubation time (D) and incubation temperature (B), followed by inoculum size (E). Chol et al. [35] and Dasgupta et al. [36] both reported that incubation time was one of the significant factors in fermentation to produce a high yield concentration of bioethanol, while El–Gendy et al. [37]

reported that both incubation time and temperature resulted in a significantly high yield concentration of bioethanol product in the fermentation by *S. cerevisiae*. The media's pH did not significantly affect bioethanol yield in the fermentation of OPT sap as media. This is because OPT sap contains a rich composition of amino acids, organic acids, vitamins, and minerals essential for the growth and metabolism of yeast cells. Therefore, this composition may contribute to maintaining pH levels to regulate physiological processes **[38, 39].**

Table 2. ANOVA for bioethanol production as the desired response for unreduced models.

Source	**Model**	**A**	**B**	**C**	**D**	**E**	**AB**
Sum of Squares	2454.760	9.490	480.940	8.400	1848.970	30.160	18.310
df	15	1	1	1	1	1	1
Mean Square	163.650	9.490	480.940	8.400	1848.970	30.160	18.310
F–value	42.910	2.490	126.090	2.200	484.770	7.910	4.800
p–value	< 0.0001	0.1343	< 0.0001	0.1573	< 0.0001	0.0125	0.0436

Table 3 shows the percentage of factors contributing to the fermentation of OPT sap by *S. cerevisiae*. Higher percentages of contribution indicated that the element has a more significant impact on high bioethanol yield concentration. Small changes in this element have a significant impact on bioethanol yield concentration. The factor contributing the most to bioethanol generation during the fermentation of OPT sap by *S. cerevisiae* was incubation time, at 73.5%, followed by incubation temperature, at 19.1%. Inoculum size (1.2%), pH (0.4%), and agitation rate (0.3%) were the factors that had the most negligible impact on the outcome.

Table 3. Percentage contribution based on the full factorial design for factors in fermentation

Factor	**Standardized Effect**	**Sum of Squares**	**Percentage Contribution**
pH	–1.089	9.489	0.377
Temperature, °C	7.756	480.94	19.117
Agitation rate, rpm	1.024	8.3954	0.334
Time, h	15.203	1848.97	73.495
Inoculum size, % v/v	1.942	30.1623	1.199

Incubation time and incubation temperature were indeed essential factors affecting bioethanol yield concentration by *S. cerevisiae* during fermentation. Research showed that bioethanol yield concentration increased with increasing fermentation time, reaching the maximum bioethanol yield concentration after a certain period, such as 72 hours of incubation [40]. Bioethanol yield concentration increased gradually during fermentation at certain temperatures, with an optimal temperature range and a subsequent decline at higher temperatures [41]. These results were consistent with the broader understanding that bioethanol yield concentration during fermentation depended on several factors, including temperature, incubation time, and other variables [32]. With a longer incubation time, bioethanol yield concentration decreased when the substrate had been depleted, causing the accumulation of waste byproducts. Moreover, the optimal temperature for bioethanol yield concentration by *S. cerevisiae* depended on the strain and fermentation conditions. Most studies reported that the optimal temperature for bioethanol yield concentration by *S. cerevisiae* was between 30 and 40 °C, with some strains reaching the highest bioethanol yield concentration at 30 °C. In contrast, some strains produced more bioethanol yield concentration optimally at higher temperatures, such as 34°C [9, 40, 42, 43]. Temperature significantly influenced yeast cells' enzymatic activity and membrane turgidity. Thus, higher temperatures could cause denaturation of enzymes and ribosomes and problems with membrane fluidity, resulting in reduced bioethanol yield concentration [9].

Figure 3 shows the tendency of the primary effects diagrams when the variable level is different. The two points of the experiment setting were determined at low and high values from the test runs. For overall individual influence, these graphs showed that factors B– and D– produced the least sufficient amounts of bioethanol in fermentation, in contrast to A–, A+, B+, C–, C+, D+, E–, and E+. This resulted in a positive influence on bioethanol yield concentration during fermentation. Variables with steeper slopes calculated the main effects and significantly influenced the data experimentally. Subsequently, the impact of variable D, the incubation time of OPT sap fermentation by *S. cerevisiae*, was determined to be the primary contributing variable compared to other effects.

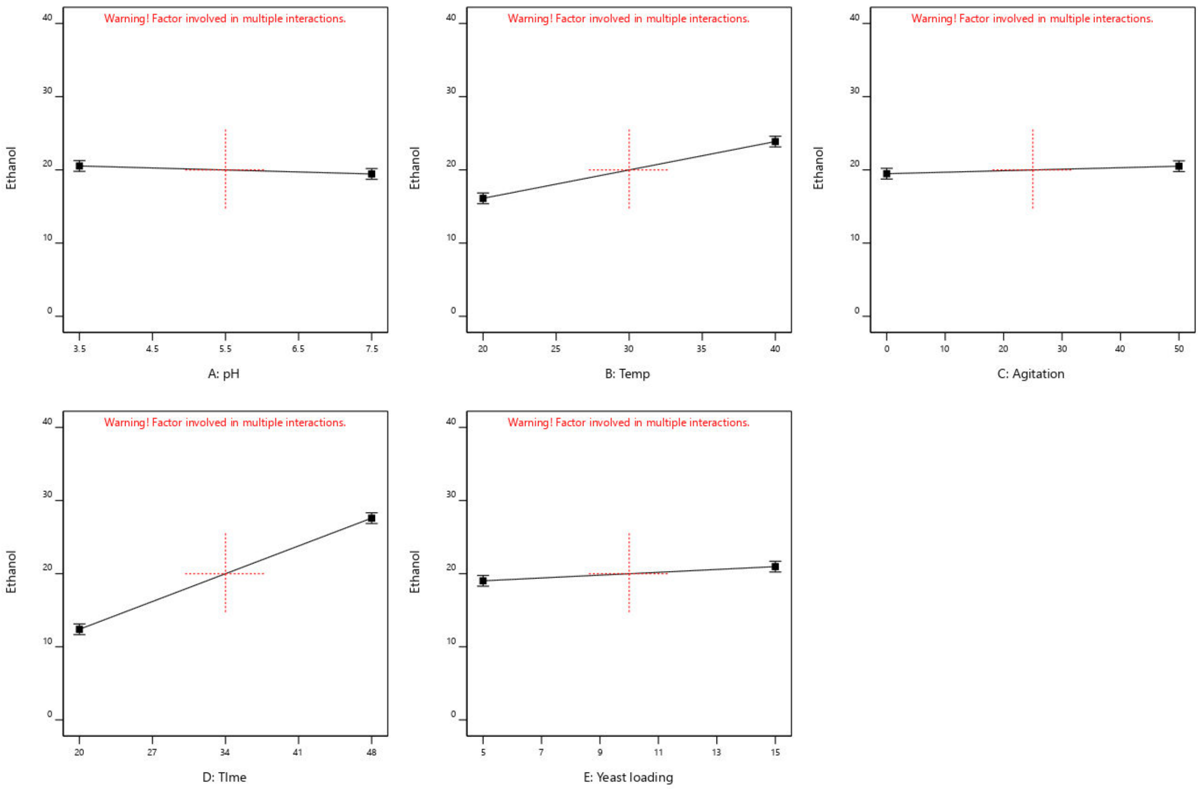

Figure 3. The main impact plots of physical factors of fermentation that produce a high bioethanol yield concentration during the fermentation of OPT sap by *S. cerevisiae*.

Figure 4 shows response prediction for bioethanol yield concentration in mg/mL as a function of fermentation temperature (B) and initial pH (A) of OPT sap and agitated rate (C) for the actual factor of 10% volume over volume (v/v) inoculum size and 34 hours of fermentation. The figure shows that without agitation rate, the temperature from 20 °C to 40 °C increased bioethanol yield concentration during fermentation at initial pH 3.5 from 15.63mg/mL to 24.18mg/mL. In contrast, the bioethanol yield concentration was slightly lower in the same condition, with a difference in initial pH at 7.5. A slight agitation at 50rpm of the fermentation broth provides a better condition for fermentation, which at an initial pH of 3.5, with increased temperature from 20 °C to 40 °C, showed increases of bioethanol yield concentration from 16.18 mg/mL to 26.16 mg/mL. Agitation rate may cause homogenization of fermentation media that can cause the collision of cells and sugar that can drive better conditions for the cell to ferment sugar compared to batch fermentation, where dense cells are sediment at the bottom of the flask and minimize the collation of cells and sugar to happen.

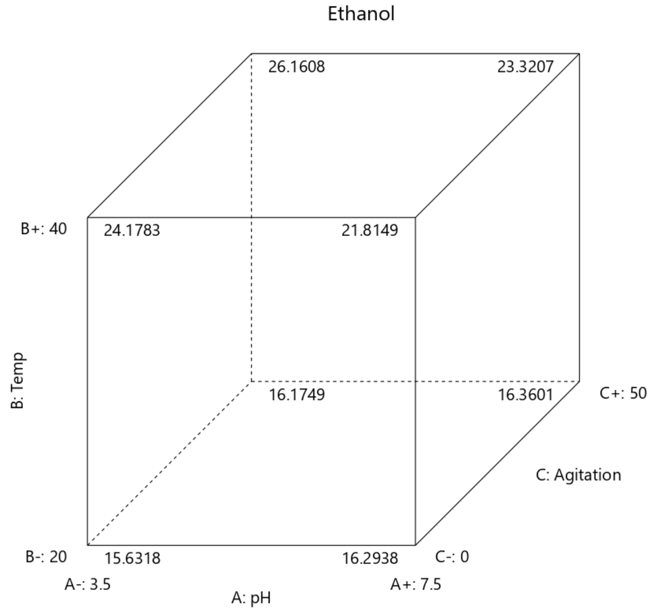

Figure 4. Response prediction for bioethanol yield concentration in mg/L as a function of fermentation temperature (B) and initial pH (A) of OPT sap for fermentation and agitated fermentation (C)

The comparative analysis of bioethanol production across various studies illuminated the critical role of yeast strain selection, incubation parameters, and process conditions in optimizing yield. *S. cerevisiae* emerged as a preferred organism due to its robust fermentative capabilities, yet its bioethanol yield varied significantly across different research efforts (Table 4). This variation underscored the impact of genetic differences within the species and possibly the conditions under which fermentation was conducted. The yields reported by Kumneadklang et al. [44] and Kusmiyati et al. [45] were identical, reflecting a potential for standardization in bioethanol production when conditions were kept consistent. Conversely, Nutongkaew et al. [46] reported a lower yield, emphasizing the influence of yeast strain variability or process conditions on bioethanol production efficiency. The range in yields from 0.350 to 150 g/L, as seen in the study by Edeh [47] further highlighted the potential for optimizing production through strain selection or genetic enhancement.

The incubation duration also played a pivotal role, varying widely across studies. Ezzatzadegan et al. [48] achieved substantial yields within a relatively short time frame, suggesting that bioethanol production efficiency may only sometimes correlate directly with longer incubation times. This insight pointed to the potential for reducing production cycles, thereby enhancing the economic viability of bioethanol as a renewable energy source. Moreover, these studies' agitation speed and temperature conditions suggested a consensus towards higher agitation speeds (around 150rpm) and a temperature range favorable for *S. cerevisiae* activity, generally around 30 ºC to 40 ºC. These conditions reflected the yeast's requirements for optimal oxygen transfer, nutrient uptake, and metabolic activity conducive to bioethanol production. However, the broad temperature range explored in some studies indicated the adaptability of yeast to varying conditions, offering flexibility in industrial bioethanol production settings. The pH levels across the studies remained relatively consistent, with slightly acidic conditions around 4.80, except in the study by Gimbun et al. [49], with a higher pH of 5.79. This consistency underlined the importance of maintaining specific pH levels for optimal yeast performance.

The variation in yields, incubation times, and operation al parameters across these studies illustrated the comple x interplay of factors influencing bioethanol production . These comparative studies highlighted the importance of strain selection and process optimization and pointed to the potential for further research into fermentation str ategies. Optimizing these factors could significantly en hance bioethanol's efficiency, sustainability, and econo mic feasibility as a renewable fuel source.

Table 4. Recent literature findings on bioethanol production from OPT.

Studies	Yeast Strain	Bioethanol Yield	Incubation Time (hours)	Agitation (rpm)	Temperature (ºC)	pH
[44]	*S. cerevisiae*	2.648%	120	100.00	37.00	4.80
[45]	*S. cerevisiae*	2.648%	120	100.00	37.00	4.80
[46]	*S. cerevisiae* TISTR5055	0.350g bioethanol/g	12–54	150.00	30.00±2	4.80
[47]	*S. cerevisiae*	150.000g/L	–	150.00–200.00	20.00–35.00	4.00–5.00
[48]	*S. cerevisiae*	95.000%	24	150.00	27.34	4.54
[49]	*S. cerevisiae* SC90	44.250g/L	96	150.00	40.00–50.00	4.80
[50]	*S. cerevisiae* NCYC 479 & *Pichia stipitis* NCYC 15411	2.180% (v/v)	120	100.00	30.00	5.00
[51]	*S. cerevisiae* SC90	0.469g EtOH/ g cellulose	72	150.00	40.00	4.80
[52]	*S. cerevisiae* SC901	0.320g/g	24	150.00	40.00	4.80
[53]	*S. cerevisiae*	~77.670%	24	164.38	31.05	5.79

Conclusion

Analysis of the physical factor fermentation of OPT sap by *S. cerevisiae* using a full factorial design reveals that the maximum bioethanol production can be achieved at a longer incubation time of 48 hours. As shown above, incubation time has the most significant effect on bioethanol production during fermentation. The highest bioethanol yield at 37.84 mg/mL was achieved during fermentation of OPT sap by *S. cerevisiae* at 48 hours of incubation with 3.5 for initial pH of OPT sap media, 40°C of incubation temperature, agitation of 50rpm, and 5% vv of inoculum size. Therefore, the significant physical factors in the fermentation of OPT sap by *S. cerevisiae* associated with bioethanol production in this study served as the foundation for further optimization studies to achieve a high bioethanol yield during fermentation.

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References

1. Bukhari, S.K., Loh, A.A.I., Luthfi, A.A.I., Abdul, P.M., Nasrin, A.B., Harun, S., and Jahim, J.M. (2021). Whole slurry saccharification of mild oxalic acid–pretreated oil palm trunk biomass improves succinic acid production. *Industrial Crops and Products*, 171: 113854.
2. Bukhari, S.K., Loh, A.B., Nasrin, A.B., Luthfi, A.A.I., Harun, S., Abdul, P.M., and Jahim, J.M. (2019). Compatibility of utilizing nitrogen–rich oil palm trunk sap for succinic acid fermentation by *Actinobacillus succinogenes* 130Z. *Bioresource Technology*, 293.
3. Alting, S.A., and Zhaoping, Z. (2015). Optimization of bioethanol production by *Saccharomyces cerevisiae* microencapsulated on alginate–delignified cellulose material. *International Journal of Pharma and Bio Sciences*, 6: B1259-B1270.
4. Ansar, Nazaruddin, Azis, A.D., and Fudholi, A. (2021). Enhancement of bioethanol production from palm sap (*Arenga pinnata (*Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum. *Journal of Materials Research and Technology*, 14: 548-554.
5. Mohd Hassan, N.A., Zakaria, K., Salleh, K.M., and Ahmad, S.M. (2023). An empirical analysis of Malaysian palm oil export to world main palm oil importing countries: evidence from a panel cointegration model. *Operations Research and Decisions*, 33: 61-73.
6. Elarbe, B., Elganidi, I., Ridzuan, N., Yusoh, K., Abdullah, N., and Vijaya Kumar, S. (2022). Application of full factorial design to screen the factors influencing the wax deposition of Malaysian crude oil. *Journal of Petroleum Exploration and Production Technology*, 12: 1829-1839.
7. Kamal, S., Rehman, S., Rehman, K., Ghaffar, A., Bibi, I., Ahmed, T., Maqsood, S., Nazish, N., and Iqbal, H.M.N. (2022). Sustainable and optimized bioethanol production using mix microbial consortium of *Saccharomyces cerevisiae* and *Candida cantarelli*. *Fuel*, 314: 122763.
8. Muhamad, A., Fatimah, S., Noor, M., Syahirah, N., Halim, A., Hamid, A.I., Aiman, M., and Zaidi, H.M. (2021). Physical factors optimization of *Saccharomyces cerevisiae* fermentation to enhance the production of bioethanol: A review. *Multidisciplinary Applied Research and Innovation*, 2: 266-277.
9. Tesfaw, A., and Assefa, F. (2014). Current trends in bioethanol production by *Saccharomyces cerevisiae*: Substrate, inhibitor reduction, growth variables, coculture, and immobilization. *International Scholarly Research Notices*, 2014: 1-11.
10. Zani, S.H.M., Asri, F.M., Azmi, N.S., Yussof, H.W., Zahari, M., and Zahari, M. (2019). Optimization of process parameters for bioethanol production from oil palm frond juice by *Saccharomyces cerevisiae* using response surface methodology as a tool. *IOP Conference Series: Materials Science and Engineering*, 702: 012003.
11. Da Silva Fernandes, F., De Souza, É.S., Carneiro, L.M., Silva, J.P.A., and De Souza, J.V.B. (2022). Current ethanol production requirements for the yeast *Saccharomyces cerevisiae*. *International Journal of Microbiology*, 2022: 7878830.
12. Wauters, R., Herrera–Malaver, B., Schreurs, M., Bircham, P., Cautereels, C., Cortebeeck, J., Duffin, P.M., Steensels, J., and Verstrepen, K.J. (2022). Novel *Saccharomyces cerevisiae* variants slow down the accumulation of staling aldehydes and improve beer shelf–life. *Food Chemistry*, 398: 133863.
13. Khongsay, N., Laopaiboon, L., Jaisil, P., and Laopaiboon, P. (2012). Optimization of agitation and aeration for very high gravity ethanol fermentation from sweet sorghum juice by *Saccharomyces cerevisiae* using an orthogonal array design. *Energies*, 5 : 561-576.
14. Peña, A., Sánchez, N.S., Álvarez, H., Calahorra, M., and Ramírez, J. (2015). Effects of high medium pH on growth, metabolism and transport in *Saccharomyces cerevisiae*. *FEMS Yeast Research*,

15(2): 005.

15. Zhao, J., Elmore, A.J., Lee, J.S.H., Numata, I., and Zhang, X. (2023). Replanting and yield increase strategies for alleviating the potential decline in palm oil production in Indonesia. *Agricultural Systems*, 210: 103714.
16. Shahirah, M.N.N., Gimbun, J., Pang, S.F., Zakria, R.M., Cheng, C.K., Chua, G.K., and Asras, M.F.F. (2015). Influence of nutrient addition on the bioethanol yield from oil palm trunk sap fermented by *Saccharomyces cerevisiae*. *Journal of Industrial and Engineering Chemistry*, 23: 213-217.
17. Norhazimah, A.H., Siti, F.M.N., Aida, M., Dilaeleyana, A.B., and Nur Shahirah, M.A. (2020). Direct fermentation of oil palm (*Elaeis guineensis*) trunk sap to bioethanol by *Saccharomyces cerevisiae*. *IOP Conference Series: Materials Science and Engineering*, 943: 012012.
18. Liu, R., Li, J., and Shen, F. (2008). Refining bioethanol from stalk juice of sweet sorghum by immobilized yeast fermentation. *Renewable Energy*, 33: 1130-1135.
19. Mei, X., Liu, R., and Shen, F. (2008). Experimental research on storage of condensed stalk juice and composition analysis of juice of sweet soghum stalk. *Transactions of the Chinese Society of Agricultural Engineering*, 2008: 218-223.
20. Baker, T.B., Smith, S.S., Bolt, D.M., Loh, W. Y., Mermelstein, R., Fiore, M.C., Piper, M.E., and Collins, L.M. (2017). Implementing clinical research using factorial designs: A primer. *Behavior Therapy*, 48(4): 567-580.
21. Muhammad, N., and Siddiqua, S. (2019). Full factorial design for optimization of magnesium alkalinization additive. *Transportation Geotechnics*, 21: 100294.
22. Rezende, C.A., Atta, B.W., Breitkreitz, M.C., Simister, R., Gomez, L.D., and McQueen–Mason, S.J. (2018). Optimization of biomass pretreatments using fractional factorial experimental design. *Biotechnology for Biofuels*, 11: 1-15.
23. Rachmawaty, Syamsiah, Idris, I.S., Pagarra, H., Hartati, Hartati, Salleh, M.M., and Salleh, M.M. (2018). Screening factors influencing chitinase production by *Trichoderma virens* using two level factorial design. *AIP Conference Proceedings*, 2018: 20176.
24. Phanphet, S., and Bangphan, S. (2021). Application of full factorial design for optimization of production process by turning machine. *Journal of Tianjin University Science and Technology*, 54: 35-55.
25. Daud, A.Y., Atikah, W.S., and Omar, W. (2021). Screening of medium compositions for recombinant lipase production via two–level fractional factorial design, *Journal of Academia*, 9: 154-165.
26. Costa, A.C., Atala, D.I.P., Maugeri, F., and Maciel, R. (2001). Factorial design and simulation for the optimization and determination of control structures for an extractive alcoholic fermentation, *Process Biochemistry*, 37(2): 125-137.
27. Illias, R.M., Fen, T.S., Rahman, R.A., Aini, N., Rashid, A., Mohtar, W., Yusoff, W., Hamid, A.A., Hassan, O., and Kamaruddin, K. (2003). Application of factorial design to study the effects of temperature, initial ph and agitation on the production of cyclodextrin glucanotransferase from *Alkalophilic Bacillus sp.* G1. *Science Asia*, 29: 135-140.
28. De Coninck, J., Leclercq, B., Exbrayat, J.M., and Duyme, F. (2004). Factorial designs: An efficient approach to choosing the main factors influencing growth and hydrolase production by *Tetrahymena thermophila*. *Journal of Industrial Microbiology and Biotechnology*, 31: 204-208.
29. Costa, A.C., Atala, D.I.P., Maugeri, F., and Maciel, R. (2001). Factorial design and simulation for the optimization and determination of control structures for an extractive alcoholic fermentation. *Process Biochemistry*, 37: 125-137.
30. Zhang, Q., Wu, D., Lin, Y., Wang, X., Kong, H., and Tanaka, S. (2015). Substrate and product inhibition on yeast performance in ethanol fermentation. *Energy & Fuels*, 29: 1019-1027.
31. Silva, J.P.A., Mussatto, S.I., and Roberto, I.C. (2010). The influence of initial xylose concentration, agitation, and aeration on ethanol production by *Pichia stipitis* from rice straw hemicellulosic hydrolysate. *Applied Biochemistry and Biotechnology*, 162: 1306-1315.
32. Mohd Azhar, S.H., Abdulla, R., Jambo, S.A.,

Marbawi, H., Gansau, J.A., Mohd Faik, A.A., and Rodrigues, K.F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports*, 1: 52-61.
33. Dragone, G., Silva, D.P., and De Almeida E Silva, J.B. (2003). Improvement of the ethanol productivity in a high gravity brewing at pilot plant scale. *Biotechnology Letters*, 25: 1171-1174.
34. Zhao, X.Q., and Bai, F.W. (2009). Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. *Journal of Biotechnology*, 144: 23-30.
35. Garang, J.C., and Ridzuan, P.D. (2015). Optimization of ethanol production process from sugarcane bagasse using genetic algorithm optimization of ethanol production process from sugarcane bagasse using genetic algorithm. *Undergraduate Dessertation,* Universiti Teknologi Petronas.
36. Dasgupta, D., Suman, S.K., Pandey, D., Ghosh, D., Khan, R., Agrawal, D., Jain, R.K., Vadde, V.T., and Adhikari, D.K. (2013). Design and optimization of ethanol production from bagasse pith hydrolysate by a thermotolerant yeast *Kluyveromyces sp*. IIPE453 using response surface methodology. *Springerplus*, 2: 1-10.
37. El–Gendy, N.S., Madian, H.R., and Amr, S.S.A. (2013). Design and optimization of a process for sugarcane molasses fermentation by *Saccharomyces cerevisiae* using response surface methodology. *International Journal of Microbiology*, 2013: 815631.
38. Pagliarani, C., Casolo, V., Beiragi, M.A., Cavalletto, S., Siciliano, I., Schubert, A., Gullino, M.L., and Secchi, F. (2019). Priming xylem for stress recovery depends on coordinated activity of sugar metabolic pathways and changes in xylem sap pH. *Plant, Cell & Environment*, 42: 1775-1787.
39. Verhage, L. (2021). Pump it up! How xylem sap pH controls water transport in leaves. *The Plant Journal*, 106: 299-300.
40. Zaghlou, R.A., Ismail, S.A., Enan, G., El–Meihy, R.M., and Abdel–Rahman, H.M. (2021). Maximization of bio–ethanol production by yeasts using sugar cane and sugar beet molasses. *Advances in Animal and Veterinary Sciences*, 9: 2069-2076.
41. Mohanty, S.K., Behera, S., Swain, M.R., and Ray, R.C. (2009). Bioethanol production from mahula (*Madhuca latifolia L.*) flowers by solid–state fermentation. *Applied Energy*, 86: 640-644.
42. Sivasakthivelan, P., Saranraj, P., and Sivasakthi, S. (2014). Production of ethanol by *Zymomonas mobilis* and *Saccharomyces cerevisiae* using sunflower head wastes–A comparative study. *International Journal of Microbiological Research*, 5(3): 208-216.
43. Tesfaw, A., Oner, E.T., and Assefa, F. (2021). Optimization of ethanol production using newly isolated ethanologenic yeasts. *Biochemistry and Biophysics Reports,* 25: 100886.
44. Kumneadklang, S. (2019). Development of bioethanol production process from oil palm trunk with ethanol membrane separation. Doctoral dissertation, Thaksin University.
45. Kusmiyati, K., Anarki, S. T., Nugroho, S. W., Widiastutik, R., & Hadiyanto, H. (2019). Effects of dilute acid and alkaline pretreatments on enzymatic saccharification of palm tree trunk waste for bioethanol production. *Bulletin of Chemical Reaction Engineering & Catalysis*, 14(3): 705-714.
46. Nutongkaew, T., Prasertsan, P., Leamdum, C., Sattayasamitsathit, S., and Noparat, P. (2020). Bioconversion of oil palm trunk residues for ethanol and acetic acid production. *Waste and Biomass Valorization*, 11: 1333-1347.
47. Edeh, I. (2021). Bioethanol production: An overview. *InTechOpen*.
48. Ezzatzadegan, L., Yusof, R., Morad, N. A., Shabanzadeh, P., Muda, N. S., and Borhani, T. N. (2021). Experimental and artificial intelligence modelling study of oil palm trunk sap fermentation. *Energies*, 14: 2137.
49. Tareen, A. K., Sultan, I. N., Songprom, K., Laemsak, N., Sirisansaneeyakul, S., Vanichsriratana, W., and Parakulsuksatid, P. (2021). Two-step pretreatment of oil palm trunk for ethanol production by thermotolerent Saccharomyces cerevisiae SC90. *Bioresource Technology*, 320: 124298.
50. Wardani, A. K., Sutrisno, A., Faida, T. N., Yustina, R. D., and Murdiyatmo, U. (2021). Ethanol production from oil palm trunk: a combined

strategy using an effective pretreatment and simultaneous saccharification and cofermentation. *International Journal of Microbiology*, 2021(1): 2509443.
51. Laosiriwut, O., Srinophakun, P., Parakulsuksatid, P., and Srinophakun, T. R. (2021). Process simulation of ethanol production from oil palm trunk. *Songklanakarin Journal of Science & Technology*, 43(4): 927-935.
52. Wilaithup, A., Sultan, I. N., Tareen, A. K., Laemsak, N., Sirisansaneeyakul, S., Vanichsriratana, W., and Parakulsuksatid, P. (2022). Bioethanol production from oil palm trunk fibers using activated immobilized Saccharomyces cerevisiae SC90 under simultaneous saccharification and fermentation. *BioEnergy Research*, 15(4): 1972-1981.
53. Gimbun, J., Nasir, N. S. M., Abidin, S. Z., Cheng, C. K., and Mel, M. (2023). Optimization of bioethanol production from oil palm trunk sap. *E3S Web of Conferences*, 422: 01004.