

SUSTAINABLE BIOETHANOL PRODUCTION FROM OIL PALM EMPTY FRUIT BUNCH FIBRE USING MICROWAVE-ASSISTED PHOSPHINIC ACID PRETREATMENT

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ABSTRACT

*This study examines the conversion of oil palm empty fruit bunch (OPEFB) fibre into fermentable sugars for bioethanol production using microwave-assisted phosphinic acid (H_3PO_2) pretreatment. Optimal conditions: 8% H_3PO_2 at 180°C for 60 min yielded 32.62 g L⁻¹ of reducing sugars, with response surface modelling showing strong accuracy ($R^2 > 0.90$). Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analyses confirmed structural changes and increased crystallinity (62.22%–74.06%) after pretreatment. Fermentation with *Saccharomyces cerevisiae* produced up to 62.17 g L⁻¹ of ethanol in 48 hr. The results highlight microwave-assisted phosphinic acid pretreatment as an effective method for enhancing sugar recovery and bioethanol yield from OPEFB fibre.*

Keywords: Box-Behnken design, phosphinic acid hydrolysis, OPEFB fibre, reducing sugar yield, *Saccharomyces cerevisiae* fermentation.

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INTRODUCTION

Bioethanol, a leading alternative to petrol, is valued for its eco-friendly and renewable nature. Traditionally produced from crops such as corn, sugarcane and sugar beet, the growing demand for bioethanol has driven the exploration of alternative raw materials. Lignocellulosic substrates, abundant and eco-friendly, are now a focal point for bioethanol production due to their rich content of convertible components for energy, biofuels and chemicals (Beluhan et al., 2023).

Thailand, the world's third-largest palm oil producer, has limited influence on global prices, which are dominated by Indonesia and Malaysia.

Palm oil, with a higher yield than crops such as rapeseed and sunflower, is the most cost-effective and widely produced vegetable oil in Thailand (Statista, 2024). The oil palm industry generates significant biomass waste; particularly empty fruit bunches (EFB). Although often left to decompose, causing environmental issues, EFB has considerable potential as a raw material for energy fuel production. Thailand has cultivated over 1.00 million hectares of oil palm, producing 15.66 million tonnes of fresh fruit bunches (FFB), equivalent to 2.82 million tonnes of crude palm oil (CPO) (Office of Agricultural Economics, 2021). Pradeepkumar et al. (2008) reported that EFB constitute approximately 23% of FFB in palm oil mills. Based on this ratio, 15.66 million tonnes of FFB in Thailand would generate approximately 3.6 million tonnes of EFB. Researchers are exploring its utilisation in biofuel production, including biodiesel, bioethanol, biohydrogen and biomethane (Ahmad et al., 2016).

Oil palm empty fruit bunch (OPEFB) fibres, obtained after fruit removal during palm oil production, represent a major lignocellulosic biomass resource in mills (Momoh & Osofero, 2020).

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These fibres consist of 23.70% cellulose, 35.89% hemicellulose and 29.20% lignin (Xiang et al., 2015), making them a promising feedstock for bioethanol production due to their abundance and favourable properties. A key step in bioethanol production from EFB is sugar fractionation, whereby cellulose and hemicellulose are hydrolysed into glucose and xylose for subsequent fermentation into ethanol (Sweeney & Xu, 2012).

The primary challenge for second-generation biorefineries is the costly and energy-intensive pretreatment of lignocellulosic substrates. There is a need for eco-friendly approaches that reduce energy and chemical consumption, water usage, inhibitor formation, and improve delignification and carbohydrate recovery (Beluhan et al., 2023). Researchers have used various reducing agents such as oxaloacetic acid (OAA), hydrogen peroxide (H_2O_2) and formic acid to enhance sugar yields (Ramaiah et al., 2020; Tareen et al., 2020; Xiong et al., 2023). Phosphinic acid, also known as hypophosphorous acid (H_3PO_2), is a colourless, low-melting compound (m.p. 26.5°C), which is a commercially available inorganic acid and is usually supplied as a 50% aqueous solution (Petrucci et al., 2007). Phosphinic acid is a strong reducing agent and its aqueous solution can be used under moderate conditions compared with other strong reducing agents.

Microwave-assisted pretreatment combines physical and chemical methods, utilising thermal and non-thermal effects to accelerate biomass pretreatment and improve process efficiency (Agu et al., 2018). It effectively removes hemicelluloses and lignin, thereby increasing cellulose availability for enzymatic saccharification (Bundhoo, 2018). Solihat et al. (2020) demonstrated the effectiveness of microwave-assisted oxalic acid pretreatment combined with enzymatic hydrolysis. Fatriasari et al. (2021) optimised microwave-assisted pretreatment of OPEFB fibre using Response Surface Methodology (RSM), reducing hemicellulose content and crystallinity for enhanced cellulose hydrolysis. Microwave-assisted maleic acid pretreatment was also found to significantly boost the reducing sugar yield (RSY) of OPEFB fibre (Fatriasari et al., 2018). For greater efficiency and economic viability, microwave pretreatment is often combined with chemical techniques (Jędrzejczyk et al., 2019).

RSM is a valuable tool for optimising experimental designs, as it effectively analyses the impact of multiple variables on one or more response variables (Aydar, 2018). Fatriasari et al. (2021) used RSM to optimise microwave-assisted pretreatment of OPEFB fibre, focusing on acid concentration, temperature and duration. Pasma et al. (2013) used the Box-Behnken design (BBD) to optimise conditions for utilising OPEFB, achieving

a peak glucose concentration of 168.71 g L^{-1} . This study explored enhancing bioethanol yield from OPEFB fibre using microwave-assisted phosphinic acid pretreatment. It investigated pretreatment-induced structural changes in OPEFB fibre as well as bioethanol fermentation. This is the first study to report using phosphinic acid for OPEFB fibre pretreatment and its application in bioethanol production.

MATERIALS AND METHODS

Oil Palm Empty Fruit Bunch (OPEFB) Substrate

OPEFB was sourced from the palm oil mill industry in Trang province, Thailand. The collected OPEFB fibre was thoroughly rinsed with tap water to remove debris, dust and other contaminants and then dried in an oven at 70°C – 80°C for 24 hr to achieve a consistent dry weight. The dried OPEFB fibre was crushed using a hammer mill to attain a particle size of 1–2 mm. This crushed fibre was then processed through a sieve shaker with 18 mm mesh and stored in sealed plastic bags at room temperature until used in the experiment.

Microwave-Assisted Phosphinic Acid Pretreatment

Pretreatment of OPEFB fibre was conducted using a 20 L microwave oven (model MS20A3010AL/ST, Samsung, Thailand) equipped with a power output of 700 W. Temperature and duration settings were established based on the design model using the oven's automatic control panel.

Phosphinic acid concentration was varied according to the design of the experimental matrix across 17 experimental runs. The required concentrations were prepared by diluting phosphinic acid with distilled water. For example, an 8% (v/v) solution was prepared by mixing 8 mL phosphinic acid with 92 mL distilled water. For each run, 10 g of OPEFB biomass was mixed with 100 mL of the prepared phosphinic acid solution in a 250 mL beaker, maintaining a 1:10 (w/v) solid-to-liquid ratio. The mixture was homogenised and then subjected to microwave pretreatment as per the experimental design.

After treatment, both solid and liquid fractions were obtained. The mixture was adjusted to neutral pH (≈ 7.0) by adding 50 mM sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl) as needed, based on the initial pH, to neutralise. The solid fraction was subsequently separated using Whatman Grade 1 filter paper. The solid residue was washed with distilled water and dried overnight at 50°C . The dried solid material was used for subsequent investigations.

Response Surface Methodology

The study utilised the BBD experimental approach, incorporating three variables: Phosphinic acid concentration ranging from 2% to 8%, pretreatment duration between 40–80 min and temperature fluctuating from 60°C to 180°C. This design involved three duplicate trials at the central point. The goal of this study was to optimise the conditions using phosphinic acid to maximise the total reducing sugar (TRS) yield from OPEFB fibre. The experiment encompassed 17 runs, including three centre points. Data on TRS yield from OPEFB fibre were analysed using a second-order polynomial function model to accurately represent and predict the results obtained from the varying parameters tested in the experiment (Equation [1]).

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

In our current research, the response variable (Y) is represented by a model with different coefficients. In this context, β_0 represents a constant, β_i stands for linear coefficients, β_{ii} denotes squared coefficients, and β_{ij} represents interaction coefficients.

The BBD provided actual and coded value levels for the factors in the experiment (Table 1). These values were used to investigate their impact on TRS production from OPEFB fibre during microwave-assisted phosphinic acid hydrolysis (Table 2). The results were analysed with Design-Expert software (Stat-Ease, Inc., 2023), and analysis of variance (ANOVA) was performed to evaluate the model's statistical significance and the impact of the model's coefficients on TRS variability.

Model Validation

The validity of the second-order polynomial model created using RSM was evaluated through a validation study that utilised randomly taken factor values within the optimal levels. Experimental actual data were compared to expected values of the BBD's second-order model to assess its goodness of fit and accuracy.

Yeast Strain and Cultivation

The yeast strain employed for ethanol generation was *Saccharomyces cerevisiae* BCC 15270, obtained from the Thailand Network on Culture Collection, Bangkok. To maintain the yeast cultures, we used yeast extract peptone dextrose (YPD) medium with 2% dextrose, 0.5% yeast extract and 1% peptone, and kept them for 48 hr at 25°C. Before sterilisation at 121°C for 20 min, the pH was adjusted to 6.0.

Yeast Inoculum Preparation and Fermentation Process

The inoculum yeast was grown in YPD broth medium in Erlenmeyer flasks with 5 g L⁻¹ peptone, 10 g L⁻¹ glucose, 3 g L⁻¹ yeast extract and 3 g L⁻¹ malt extract. The flasks were then shaken at 140 rpm (2 × g) for 14 hr at 25°C. Subsequently, yeast cells were added to the fermentation medium at a concentration of approximately 1.0 × 10⁷ cells mL⁻¹.

The fermentation procedure took place in 500 mL Erlenmeyer flasks with 100 mL of fermentation medium. The initial pH was 6.0, and the medium contained 1% (w/v) substrate treated with microwave-assisted phosphinic acid, 0.5% (w/v) peptone and 0.25% (w/v) yeast extract. The yeast culture grown previously was then added to maintain the initial yeast cell density of 1.0 × 10⁷ cells mL⁻¹. The fermentation was conducted under continuous orbital shaking at 120 rpm for 84 hr at pH 4.5 and 30°C. Shaking was employed to ensure uniform mixing, although the fermentation conditions were maintained as anaerobic. Every 12 hr, aseptic samples were withdrawn for ethanol analysis. All fermentation parameters, including pH, temperature, shaking speed and medium composition, were maintained at fixed and previously optimised values based on literature reports (Poludasu, 2024; Poludasu et al., 2024b). The present study did not evaluate the effects of varying these parameters; instead, standardised conditions were employed to assess the impact of microwave-assisted phosphinic acid pretreatment on ethanol production from OPEFB fibre.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR (Perkin Elmer Spectrometer, USA) was used to identify the surface functional groups of both untreated and treated OPEFB fibre. Prior to analysis, the test sample was mixed with potassium bromide (KBr), and the mixture was then compressed into a pellet. Using a resolution value of 4 cm⁻¹, the infrared spectrum was collected throughout a 500–4,000 cm⁻¹ range.

Scanning Electron Microscopy (SEM)

Morphological and structural differences between untreated and pretreated OPEFB fibre were identified using SEM (Thermo Fisher Scientific, USA) imaging. Initially, the solid samples were adhered to the aluminium sample stubs using double-sided carbon tape. After that, a 10 kV acceleration voltage was used to take a photograph.

X-ray Diffraction (XRD) Studies

The complicated crystalline structure of OPEFB fibre was seen both before and after pretreatment using an X-ray diffractometer (XRD, PHILIPS, X'Pert MPD, Netherlands) with an applied voltage of 40 kV and a current of 30 mA with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) as the X-ray source. The samples were used to compute the crystallinity index (CrI), using a step size of 0.03° in the range of $2^\circ = 5^\circ\text{--}50^\circ$ and the Equation (2) given below (Segal et al., 1959) as a reference.

$$\text{CrI (\%)} = \frac{(I_{002} - I_{\text{am}})}{I_{002}} \times 100 \quad (2)$$

where, $I_{18.0^\circ}$ indicates the maximum diffraction intensity at 18.0° (2θ), I_{002} indicates the maximum diffraction intensity of the (002) lattice plane, and I_{am} represents the intensity of the amorphous region.

Reducing Sugars Analysis

The dinitrosalicylic acid (DNS) method quantifies reducing sugar levels during saccharification. Glucose solutions ($50\text{--}2,500 \text{ mg L}^{-1}$) were prepared to generate standard test solutions. Each solution received 1 mL of DNS reagent, was heated in a water bath at 100°C for 10 min, then cooled and diluted with 3 mL of demineralised water. Absorbance of the reddish-brown colour at 550 nm was measured using a UV spectrophotometer (Limayem & Rieke, 2012). Reducing sugar concentration was determined by comparing the absorbance to a glucose calibration curve, with colour intensity directly correlating to sugar concentration (Miller, 1959).

Ethanol Quantification

The Agilent 6890 gas chromatography system (Agilent Technologies, USA), equipped with a flame ionisation detector (FID), was employed for this process. For ethanol detection, a glass-packed phase-five GC column (Agilent configuration "A") was used, packed with Carbowax 20M (80/120 Carbowax B AW support), with dimensions of $1.8 \text{ m length} \times 6.35 \text{ mm outer diameter} \times 2.0 \text{ mm inner}$

diameter. Nitrogen was used as the carrier gas at a constant flow rate of 20 mL min^{-1} . Hydrogen was used as the fuel gas at a steady flow rate of approximately 40 mL min^{-1} . Secondary butyl acetate was used as the internal standard (Cutaia, 1984).

RESULTS AND DISCUSSION

Chemical Composition of Oil Palm Empty Fruit Bunches (OPEFB) Fibre

The chemical composition of untreated OPEFB fibre has been reported in our previous study as $32.6 \pm 0.4\%$ cellulose, $28.4 \pm 0.2\%$ hemicellulose, and $25.0 \pm 0.4\%$ lignin (Poludasu et al., 2024a). In the present study, chemical composition analysis was additionally performed on phosphinic acid (H_3PO_2)-pretreated OPEFB fibre obtained from Run No. 8 (8% phosphinic acid, 60 min and 180°C), which yielded the highest reducing sugar concentration.

Full Factorial Design

Table 1 lists the input factors and their levels. A factorial experimental design, detailed in Table 2, was used to test critical factors at low, medium and high levels, focusing on phosphinic acid concentration, pretreatment time and temperature. A total of 17 tests, each repeated twice, were conducted to ensure data accuracy. The lowest reducing sugar levels were observed with 2% phosphinic acid for 60 min at 60°C . The highest levels were achieved with 8% phosphinic acid for 60 min at 180°C . Higher temperatures and 8% acid concentration significantly increased reducing sugar yields by accelerating cellulose and hemicellulose hydrolysis. Longer reaction times also improved yields by allowing more thorough breakdown of the biomass.

Response Surface Modelling (RSM)

The study aimed to optimise reducing sugar production by analysing phosphinic acid concentration, pretreatment time and temperature using BBD. The factors tested were 2%, 5% and 8%

TABLE 1. FACTORS AND LEVELS FOR BOX-BEHNKEN DESIGN (BBD)

Factors/variables	Unit	Notation	Lowest	Centre	Highest
Phosphinic acid	% w/v	A	2	5	8
Time	Min	B	40	60	80
Temperature	$^\circ\text{C}$	C	60	120	180

Note: w/v - weight per volume.

TABLE 2. ACTUAL LEVELS OF PROCESS FACTORS IN STANDARD RUN ORDER

Std.	Phosphinic acid (% w/v)	Time (min)	Temperature (°C)	Reducing sugars (g L ⁻¹)
1	2	40	120	15.31 (16.09)
2	8	40	120	26.42 (25.95)
3	2	80	120	17.22 (17.69)
4	8	80	120	28.64 (27.86)
5	2	60	60	10.28 (10.10)
6	8	60	60	12.05 (13.12)
7	2	60	180	16.85 (15.78)
8	8	60	180	32.62 (32.79)
9	5	40	60	11.13 (10.53)
10	5	80	60	12.52 (12.22)
11	5	40	180	22.84 (23.14)
12	5	80	180	24.36 (24.96)
13	5	60	120	19.82 (19.76)
14	5	60	120	19.62 (19.76)
15	5	60	120	19.88 (19.76)
16	5	60	120	19.62 (19.76)
17	5	60	120	19.86 (19.76)

Note: Std. - standard run order; values in parentheses represent model-predicted values; w/v - weight per volume.

phosphinic acid concentrations; 40, 60 and 80 min pretreatment times; and 60°C, 120°C and 180°C temperatures. RSM and surface plots (*Figures 1*) revealed that phosphinic acid concentration and temperature significantly affected saccharification rates and reducing sugar yields from OPEFB fibre, with process time having a lesser impact.

The study used analysis of variance to optimise process conditions for OPEFB fibre with microwave-assisted phosphinic acid. Adjusting phosphinic acid concentration (2%–8%), pretreatment duration (40–80 min) and temperature (60°C–180°C) improved reducing sugar yields. The optimisation was represented by a second-order polynomial equation (Equation [3]), and experimental values confirmed the accuracy of the quadratic model.

$$\text{Reducing sugar (\%)} = 19.76 + 5.01A + 0.880B + 6.34C + 0.0775AB + 4.50AC + 0.0325BC + 1.19A^2 + 0.0950B^2 - 3.00C^2 \quad (3)$$

The coded factors equation predicts responses at specific factor levels, assigning +1 for high and –1 for low levels. It uses factor coefficients to determine the relative impact of each factor (Stat-Ease, Inc., 2023). The quadratic polynomial for reducing sugar yield was statistically significant at a 95% confidence level. The R^2 value exceeded 0.90 for all outcomes, indicating high model accuracy (*Table 3*). The model achieved 87.46% accuracy in predicting reducing sugar yields.

Analysis of Variance (ANOVA)

ANOVA estimates parameters and measures variation to assess model efficiency. Significance tests using the F-value and p -value evaluate the importance and impact of regression model components. The analysis examines the model's fit to the data and its ability to account for variability. Three degrees of freedom are found for the combined linear components as given in *Table 3*. The linear factors, phosphinic acid concentration (A), pretreatment time (B) and pretreatment temperature (C), were deemed statistically significant at a 5% significance level, with p -values below 0.05. This suggests that changes in these linearly independent variables result in significant alterations in the dependent variable. The two-way interaction includes interaction terms among the independent variables in the model, such as AB, AC and BC, which collectively contribute three degrees of freedom. The significant p -value for the interaction term AC highlights its considerable impact on the dependent variable. Additionally, the squared terms A^2 , B^2 and C^2 also contribute three degrees of freedom collectively. The F-value for these squared terms is 21.27, with a corresponding p -value of 0.007. This suggests that the dependent variable is not greatly affected by the squared term B^2 and as a result, its variability is not significantly increased. However, both A^2 and C^2 are statistically significant, with p -values of 0.0233 and 0.002, respectively.

The model's F-value of 97.28 indicates its significance, with just a 0.01% chance that this large F-value could occur by chance. The p -values

below 0.0500 confirm significance of model terms, including A, B, C, AC, A² and C² in this instance. Conversely, values above 0.1000 indicate insignificance for model terms. If many terms are found insignificant, simplifying the model could improve its performance. The Lack-of-fit F-value of 97.41 indicates a 0.03% chance that such a large F-value could occur randomly (Stat-Ease, Inc., 2023). The Predicted R² of 0.8746 closely matches the Adjusted R² of 0.9819, differing by less than 0.2. The Adeq Precision ratio of 35.086, well above the desirable threshold of 4, indicates a robust model suitable for exploring the design space.

Optimisation of Reducing Sugar Production

Table 3 displays the effects of factors A, B and C on reducing sugar yield using BBD, with concentrations ranging from 10.28 to 32.62 g L⁻¹ (Table 2). The ANOVA results for reducing sugar production during microwave-assisted phosphinic acid hydrolysis of OPEFB fibre are also shown (Table 3). The factors with a *p*-value below 0.05 are statistically significant, indicating a greater impact on the process (Di Leo & Sardanelli, 2020).

Response surface graphs. The yield of reducing sugars from biomass pretreatment depends on phosphinic acid concentration, temperature and treatment time (Figure 1). As shown in Figure 1a,

higher acid concentrations (up to 8%) initially boost sugar yields by enhancing biomass breakdown, with an optimal hydrolysis time of about 60 min for maximum sugar production.

Reducing sugar yield from biomass depends on temperature and phosphinic acid concentration during pretreatment (Figure 1b). Higher acid concentration (8%) and higher temperature (e.g., 180°C) help break down complex sugars into simpler ones by breaking glycosidic bonds. This increases the yield of sugars like glucose and fructose, with higher temperatures speeding up the process and improving efficiency.

The production of reducing sugars from biomass is strongly influenced by the right hydrolysis time and higher temperatures. Figure 1c shows that a 60-min hydrolysis time strikes a balance, avoiding too much sugar breakdown. Shorter times may not break down the biomass fully, while longer times can lead to sugar loss. Higher temperatures (180°C) speed up reactions, improving efficiency and increasing sugar yields.

The effectiveness of pretreatment is influenced by microwave power, treatment time, biomass type and acid concentration. Response surface plots show that too short a hydrolysis time can lead to incomplete breakdown, while too long a time causes sugar degradation. Optimal hydrolysis time is crucial for maximising sugar yield and minimising degradation. Higher temperatures and high

TABLE 3. ANALYSIS OF VARIANCE (ANOVA) OF QUADRATIC MODEL

Source	Sum of squares	df	Mean square	F-value	<i>p</i> -value
Model	622.47	9	69.16	97.28	< 0.0001
A	200.70	1	200.70	282.29	< 0.0001
B	6.20	1	6.20	8.71	0.0213
C	321.18	1	321.18	451.76	< 0.0001
AB	0.0240	1	0.0240	0.0338	0.8594
AC	49.00	1	49.00	68.92	< 0.0001
BC	0.0042	1	0.0042	0.0059	0.9407
A ²	5.94	1	5.94	8.35	0.0233
B ²	3.80	1	3.80	5.34	0.0540
C ²	37.83	1	37.83	53.21	0.0002
Residual	4.98	7	0.7110		
Lack-of-fit	4.91	3	1.64	97.41	0.0003
Pure error	0.0672	4	0.0168		
Cor Total	627.45	16			
Fit statistics					
SD	0.8432		R ²		0.9921
Mean	19.36		Adjusted R ²		0.9819
CV %	4.36		Predicted R ²		0.8746
PRESS	78.66		Adeq Precision		35.0860

Note: A - phosphinic acid; B - time; C - temperature; df - degree of freedom; SD - standard deviation; CV - coefficient of variance.

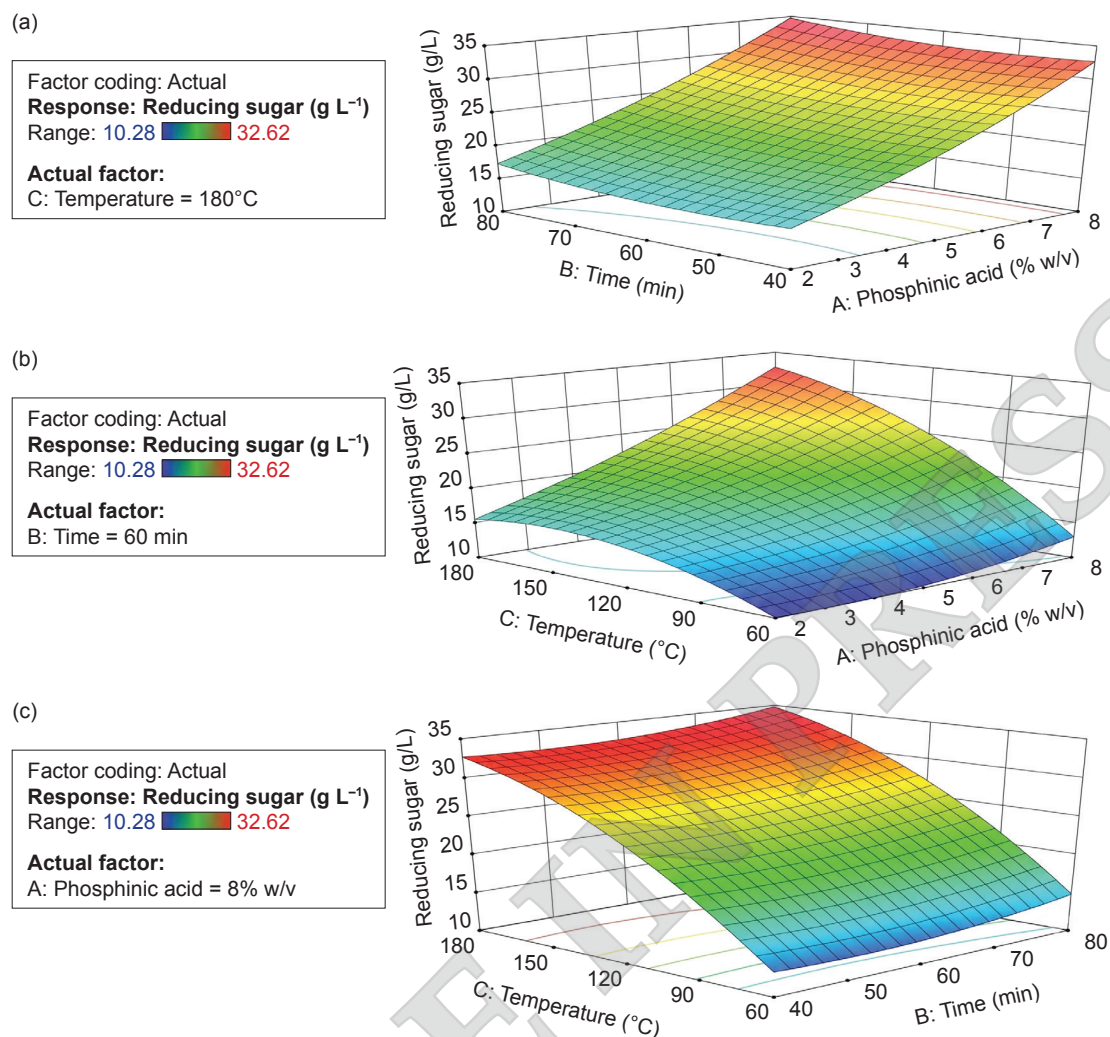


Figure 1. Response surface plots showing the interaction of process factors; (a) phosphinic acid and time, (b) phosphinic acid and temperature and (c) time and temperature on reducing sugar production.

phosphinic acid concentrations improve biomass breakdown and enhance hydrolysis efficiency, resulting in higher initial yields of reducing sugars. Fan et al. (2013) found that strong hydrogen bonding prevents $-\text{CH}_2\text{OH}$ groups on cellulose from interacting with microwaves below 180°C. Above 180°C, these groups can rotate, promoting cellulose decomposition and increasing sugar yield. However, temperatures above 200°C significantly reduce sugar yield due to sugar degradation. In our investigation, increasing the temperature to 180°C resulted in a higher concentration of reducing sugars. At this temperature, microwave radiation causes polar groups to rotate, leading to effective biomass degradation. Our study shows that the best applicable condition for microwave-assisted pretreatment was at 180°C, resulting in impressive sugar yields (Fan et al., 2013). However, the duration may vary depending on the type of substrate and the pretreatment technique used. Chen et al. (2012) treated sugarcane bagasse with dilute sulphuric acid in a microwave for 30 min at 180°C. Microwave

use accelerated reaction times and enhanced hemicellulose hydrolysis, with minimal impact on cellulose crystallinity and lignin structure. The pretreatment selectively yielded glucose and xylose. Fatriasari et al. (2021) applied RSM to optimise maleic acid pretreatment of OPEFB fibre, identifying optimal conditions as 176.57°C, 70 min and 1.5% acid concentration, yielding 22.32% reducing sugar. Harahap et al. (2020) found that NaOH two-stage pretreatment was more effective than single-stage or acetic acid two-stage pretreatments for enhancing reducing sugar production through enzymatic hydrolysis of OPEFB fibre. The optimal conditions for the NaOH two-stage pretreatment were 125°C for 60 min, yielding a maximum reducing sugar output of 0.73 g g⁻¹ OPEFB fibre. The variation in temperature in this study demonstrated a rise in reducing sugar yield with increasing temperature. The present results indicate that higher temperature, increased phosphinic acid concentration and optimal duration improve outcomes with minimal negative effects.

Validation experiments. We validated our predictive model by using RSM to identify optimal parameters and then forecasting and verifying the model's performance. Validation experiments were repeated twice and results averaged, as shown in Table 4. The average error for TRS between predicted and actual values was -0.49% and -0.71% , showing negligible discrepancies. These results confirm that the response surface design effectively identifies optimal processing parameters, validating the BBD model as a reliable tool for predicting TRS.

TABLE 4. VALIDATION STUDIES OF THE PRESENT MODEL

Sample	Experimental value	Predicted value	Error %
1	32.62	32.78	-0.49
2	10.28	10.10	1.75
3	19.62	19.76	-0.71

Oil Palm Empty Fruit Bunch (OPEFB) Fibre Characterisation Studies

Scanning electron microscopic (SEM) image analysis. SEM analysis was used to conduct a qualitative investigation into the morphological modification of pretreated OPEFB fibre; the results are shown in Figure 2. The whole surface strand of untreated OPEFB fibre was covered with a thick layer of waxy substance, giving the surface morphology a rough appearance. On the surface of OPEFB fibre that had not been treated, many pointed silica entities were shown to be randomly embedded and distributed. Because numerous strong structures, including silica bodies, waxes and lignin, were present, the entire structure of untreated OPEFB fibre was tough and strong.

Pretreatment of OPEFB fibre with phosphinic acid severely disrupted the biomass fibre structure (Figure 2b). Silica bodies were removed, leaving exposed pores. Greater heating temperatures and longer irradiation times resulted in more noticeable silica removal and surface disruption (Solihat et al., 2020). Removing silica bodies can enhance enzymatic hydrolysis and pulping chemical penetration by widening channels and exposing more amorphous fibre. Chemical treatment roughens the fibre surface and removes fatty deposits, increasing surface area and improving interlocking with the matrix (Mohammed et al., 2022; Namondo et al., 2018). An appropriate pretreatment may improve the efficiency of converting cellulose to sugars. Despite increased porosity, silica bodies remained, suggesting that the 120 min pretreatment was insufficient for complete lignocellulosic breakdown.

Fourier transform infrared spectroscopy (FTIR). Based on the FTIR spectra of untreated and phosphinic acid pretreated OPEFB fibres, the structural alterations of the fibres were examined, as shown in Figure 3. A strong and broad absorption band was observed around $3,320\text{--}3,400\text{ cm}^{-1}$, corresponding to hydrogen-bonded O–H stretching. The O–H stretching region ($3,600\text{--}3,800\text{ cm}^{-1}$) was more similar to cellulose I than cellulose II (Oh et al., 2005), and its reduced intensity after pretreatment indicates decreased hydroxyl groups and hydrogen bonding. High-intensity bands were also observed at $2,854$, $2,922$ and $2,985\text{ cm}^{-1}$, which are attributed to aliphatic C–H stretching of $-\text{CH}_2$ and $-\text{CH}_3$ groups from cellulose, hemicellulose and lignin. Specifically, the band at $2,985\text{ cm}^{-1}$ is assigned to asymmetric CH_2 stretching of the $\text{CH}_2\text{--OH}$ group (Isroi et al., 2012), while the band at

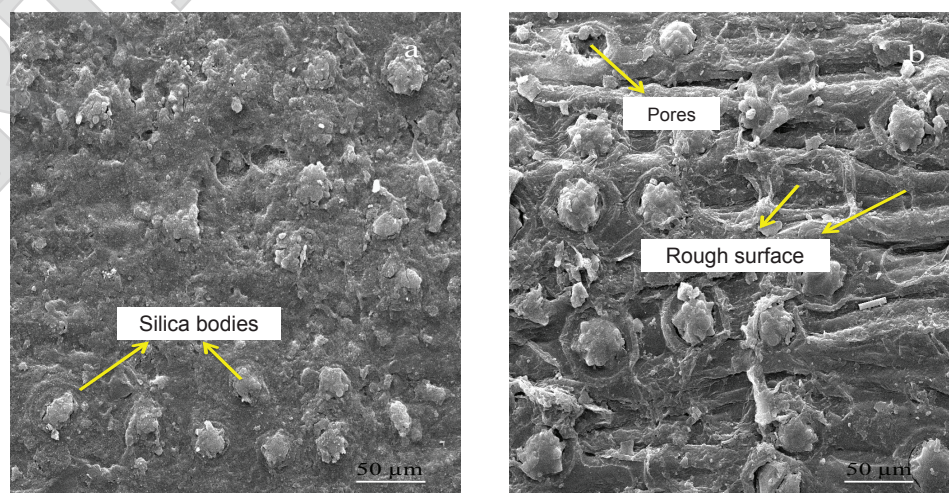


Figure 2. Scanning electron microscopic (SEM) images of oil palm empty fruit bunch (OPEFB); (a) untreated and (b) pretreated with phosphinic acid, showing silica bodies, pores and surface roughness. Images were recorded at $1,000\times$ magnification (scale bar = $50\text{ }\mu\text{m}$).

2,920–2,922 cm^{-1} corresponds to symmetric CH_2 vibration (Ciumac et al., 2017). The slight reduction in these peaks suggests partial removal of aliphatic components, while indicating that the polysaccharide structure remains intact after pretreatment.

The peak at 1,730–1,735 cm^{-1} is attributed to $\text{C}=\text{O}$ stretching of acetyl and uronic ester groups in hemicellulose and p-coumaric acids in lignin (Li et al., 2009). The decrease in intensity and the presence of shoulder peaks at 1,730 and 1,733 cm^{-1} after pretreatment indicate significant hemicellulose dissolution. The band around 1,510 cm^{-1} corresponds to aromatic $\text{C}=\text{C}$ vibrations in lignin and shows a decrease, confirming partial lignin removal. Absorption bands at 1,425–1,428 cm^{-1} are associated with CH_2 bending in cellulose and indicate its crystalline structure (Poletto et al., 2014; Xu et al., 2013); the increased prominence of this peak after pretreatment suggests greater cellulose exposure. Other cellulose-related bands at 1,367, 1,334, 1,027 and 896 cm^{-1} are attributed to stretching and bending vibrations of $-\text{CH}_2$, $-\text{CH}$, $-\text{OH}$, and $\text{C}-\text{O}$ bonds (Xu et al., 2013), where the band at 897 cm^{-1} corresponds to the amorphous region of cellulose (Poletto et al., 2014).

Variations in intensity were also observed near 1,260 and 1,230–1,247 cm^{-1} . The band at $\sim 1,260 \text{ cm}^{-1}$ is assigned to guaiacyl lignin, while the band at $\sim 1,230$ – $1,247 \text{ cm}^{-1}$ corresponds to cellulose and syringyl lignin deformation. The greater decrease at $\sim 1,230 \text{ cm}^{-1}$ after pretreatment indicates preferential solubilisation of syringyl lignin compared to guaiacyl lignin, suggesting that cellulose becomes more exposed for sugar release. The $\text{C}-\text{O}$ stretching band around 1,033 cm^{-1} , associated with cellulose and hemicellulose, shifts to $\sim 1,025 \text{ cm}^{-1}$ and decreases in intensity after pretreatment, indicating reduced hemicellulose content. The peak at 895 cm^{-1} corresponds to $\text{C}-\text{H}-\text{O}$ stretching of β -(1 $\rightarrow$ 4)-glycosidic linkages, confirming the preservation of

the cellulose backbone. Peaks at 750 and 716 cm^{-1} are attributed to CH_2 vibrations in cellulose $\text{I}\alpha$ and $\text{I}\beta$, respectively, while a distinct peak at 769 cm^{-1} is present in all spectra (Hu & Li, 2014). Additionally, the band at 650 cm^{-1} is assigned to aromatic $\text{C}-\text{H}$ bending of lignin.

Only the phosphinic acid-pretreated samples exhibited clearer bands at 1,730, 1,425 and 1,247 cm^{-1} , where the 1,730 cm^{-1} peak indicates cleavage of lignin-hemicellulose linkages, the 1,425 cm^{-1} band reflects increased crystalline cellulose content and the 1,247 cm^{-1} peak corresponds to $\text{C}-\text{O}$ stretching of aryl-alkyl ether linkages, suggesting lignin modification. Overall, these spectral changes confirm that phosphinic acid pretreatment removes hemicellulose, partially degrades lignin, disrupts lignin-carbohydrate linkages and enhances cellulose accessibility while preserving the main cellulose structure.

X-ray diffraction (XRD) spectroscopy. To assess changes in the crystalline structure of raw and pretreated OPEFB fibre, analysis and CrI determination were conducted. The XRD results showed amorphous peaks at $2\theta = 18^\circ$ – 19° and crystalline peaks at $2\theta = 22^\circ$ – 23° . The CrI of pretreated OPEFB fibre was higher (74.06%) compared to raw OPEFB fibre (62.22%) (Figure 4). The diffractogram shows that pretreated OPEFB fibre has decreased diffraction intensity in amorphous regions and increased intensity in crystalline regions, reflecting higher crystallinity. This rise is due to the removal of amorphous components like lignin and hemicellulose. Higher pretreatment temperatures further increased cellulose crystallinity. The increase in crystallinity results from the removal of amorphous components, leaving behind more crystalline cellulose (Hermiati et al., 2020). This degradation of amorphous fractions is reflected in the CrI (Kłosowski & Mikulski, 2023). Previous studies

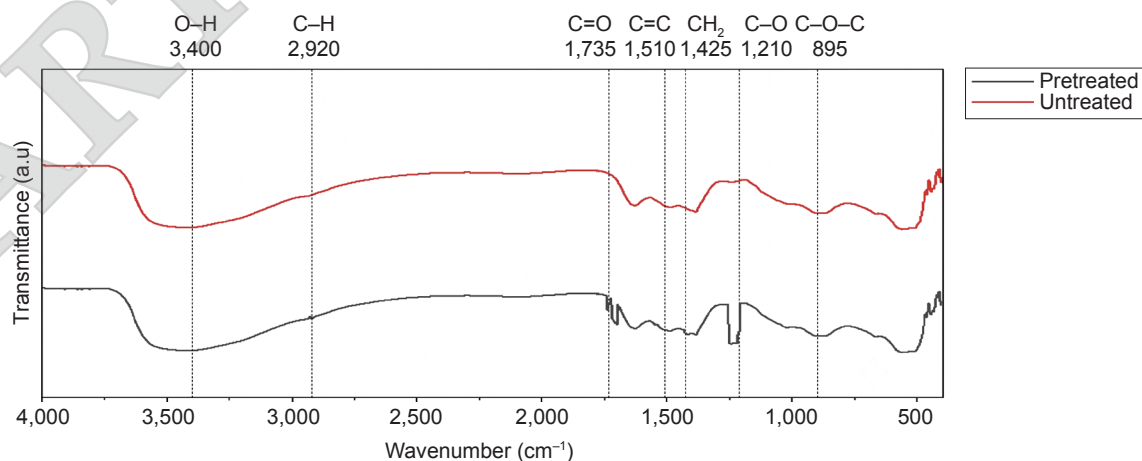


Figure 3. Fourier transform infrared spectroscopy (FTIR) spectrum of untreated and phosphinic acid pretreated oil palm empty fruit bunch (OPEFB) fibre.

by Solihat et al. (2020) and Hermiati et al. (2020) also reported an increase in the CrI of OPEFB fibre after pretreatment.

Characterisation studies using SEM, FTIR and XRD reveal that the removal of silica bodies from OPEFB fibre enhances the lignocellulosic structure's surface area, increasing cellulase enzyme accessibility for breaking down polysaccharides into fermentable sugars. Furthermore, the pretreatment process selectively removes amorphous lignin and hemicellulose, increasing crystallinity, reducing substrate recalcitrance and exposing cellulose chains for enzymatic hydrolysis. These improvements boost substrate digestibility, enhance sugar release during saccharification and significantly increase ethanol yields during fermentation, optimising bioethanol production from OPEFB fibre.

Comparison of Sugar Production from Previous Study

Table 5 compares the optimal biomass pretreatment parameters and reducing sugar yields from this study with those reported in other studies. Within the present study, a longer pretreatment time (60 min) and a higher temperature (180°C) resulted in a higher yield of reducing sugars. The study highlights the role of pretreatment conditions, raw material composition, temperature and time in achieving high sugar yields. Using low-cost and readily available raw materials improves the practicality of the process. Although this study optimised key pretreatment parameters within selected ranges using RSM, it was limited to laboratory conditions. Future study should expand optimisation to wider operating ranges, include more process variables and test scale-up conditions to further improve sugar yield and economic feasibility.

Ethanol Fermentation Studies

Ethanol fermentation was carried out under standardised and optimised conditions of pH (4.5), temperature (30°C), shaking speed (120 rpm) and medium composition to evaluate the effect of phosphinic acid pretreatment severity on ethanol production by *S. cerevisiae* BCC 15270. Figure 5 shows that ethanol production from *S. cerevisiae* BCC 15270 was higher with more extensively phosphinic acid-pretreated (8%) OPEFB fibre, producing 62.17 g L⁻¹ ethanol compared to 44.38 g L⁻¹ from less phosphinic acid-treated (2%) fibre. This increase is attributed to higher levels of fermentable sugars from cellulose. Ethanol production rose steadily for both substrates, peaking at 48 hr before levelling off.

Substrates with more extensive pretreatment produced higher ethanol concentrations due to increased reducing sugars essential for fermentation. Extensive pretreatment enhances sugar availability, optimising ethanol yields (Kumar & Sharma, 2017). In batch fermentation with alkali-pretreated OPEFB fibre hydrolysate, 21 g L⁻¹ of ethanol was produced in 28 hr, yielding 0.102 g ethanol g⁻¹ of dry OPEFB fibre or 0.458 g ethanol g⁻¹ of glucose (Kim, 2018). Ningthoujam et al. (2023) found that using *S. cerevisiae* with pretreated substrates achieved a maximum ethanol concentration of 21.45% with 20% w/v biomass, an enzyme ratio of 2:1:1 and fermentation for one week at 30°C and pH 4.5. This was significantly higher than the 3.67% yield from untreated rice straw, demonstrating the effectiveness of microwave-assisted phosphinic acid pretreatment in enhancing bioethanol productivity from OPEFB fibre. Han et al. (2011) reported that the fermentation of pretreated OPEFB fibre using *S. cerevisiae* achieved an ethanol concentration of 48.54 g L⁻¹. This was accomplished with a 20% (w/v) pretreated biomass loading through

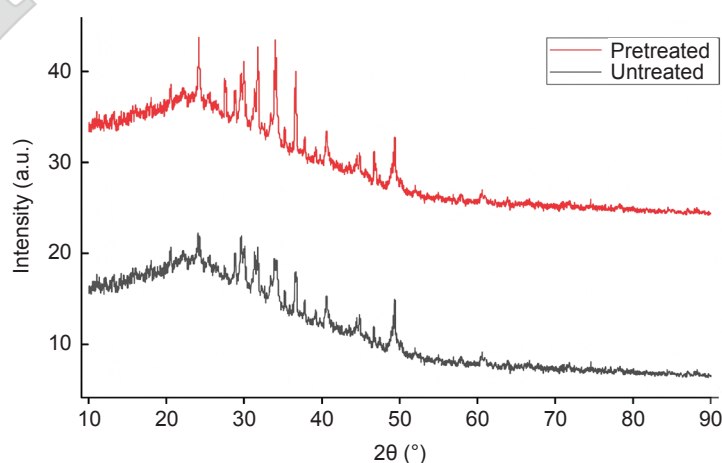


Figure 4. X-ray diffraction (XRD) spectrum of untreated and phosphinic acid pretreated oil palm empty fruit bunch (OPEFB) fibre.

TABLE 5. COMPARISON STUDY OF VARIOUS STUDIES ON REDUCING SUGAR PRODUCTION

Substrate	Catalyst-concentration (%)	Time (min)	Temperature (°C)	Reducing sugar concentration	Reference
<i>Typha latifolia</i>	Oxalic acid - 1.20	20	120	22.32 mg mL ⁻¹	Ramaiah et al. (2020)
OPEFB fibre	Oxalic acid - 1.13	3.01	190	59.92 g L ⁻¹	Anita et al. (2023)
<i>Bambusa balcooa</i> culm	Oxalic acid - 3.00	15	121	48.33 g L ⁻¹	Sivamani et al. (2022)
OPEFB fibre	Phosphinic acid - 8.00	60	180	32.62 g L ⁻¹	Present study

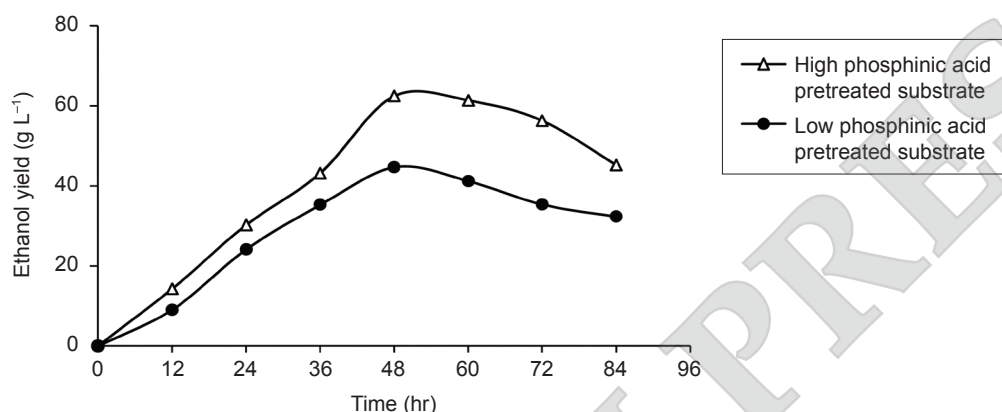


Figure 5. Ethanol yield from both high- and low-phosphinic acid-pretreated oil palm empty fruit bunch (OPEFB) fibre.

simultaneous saccharification and fermentation (SSF) over a 48-hr fermentation period. In the present study, the ability of phosphinic acid pretreatment to efficiently enhance cellulose accessibility while reducing recalcitrance demonstrates its superiority in optimising ethanol production. This method not only increases yield but also shortens the time to peak ethanol production, making it a more effective and practical solution for bioethanol production from OPEFB fibre compared to conventional methods. Zhang et al. (2013) optimised key fermentation parameters (temperature, yeast concentration, pH, enzyme loading) for ethanol production from wheat straw using BBD, clearly demonstrating statistical standardisation and optimisation of fermentation conditions.

To quantitatively evaluate fermentation performance, ethanol yield (Y_{etoh}) was defined as the ratio of experimentally produced ethanol to the amount of fermentable sugar consumed and expressed as grams of ethanol per gram of sugar (g g⁻¹). Fermentation efficiency (%) was calculated by comparing the observed ethanol yield to the theoretical maximum yield of 0.51 g ethanol g⁻¹ hexose. Although the reducing sugar concentrations obtained during BBD optimisation ranged from 10.28 to 32.62 g L⁻¹ (Table 2 and 3), ethanol fermentation was conducted using concentrated hydrolysate generated under the optimised pretreatment conditions. Based on the measured

ethanol concentration of 62.17 g L⁻¹, the calculated fermentation efficiency was approximately 88%–90%, corresponding to an effective fermentable sugar utilisation of about 135–139 g L⁻¹. These results indicate a high conversion efficiency of fermentable sugars to ethanol and highlight the positive impact of phosphinic acid pretreatment on improving ethanol production from OPEFB fibre.

CONCLUSION

This study demonstrates the effectiveness of microwave-assisted phosphinic acid pretreatment for enhancing reducing sugar yield from OPEFB fibre. Using factorial design and RSM, optimal conditions produced 32.62 g L⁻¹ of reducing sugars. Higher acid concentration and temperature significantly improved hemicellulose and cellulose breakdown. Structural analyses (SEM, FTIR) confirmed effective biomass disruption, leading to increased ethanol production. This method is adaptable to other plant wastes like rice or wheat straw, with microwave heating enabling efficient sugar release. Phosphinic acid is effective and environmentally friendly and RSM helps identify optimal process conditions. Future work should focus on cost reduction, scalability and testing different yeast strains to boost ethanol yields. This approach supports sustainable bioethanol production from agricultural waste.

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REFERENCES

- Agu, O. S., Tabil, L. G., Meda, V., Dumonceaux, T., & Mupondwa, E. (2018). Pretreatment of crop residues by application of microwave heating and alkaline solution for biofuel processing: A review. In E. Jacob-Lopes & L. Q. Zepka (Eds.), *Renewable resources and biorefineries* (pp. 47–68). IntechOpen. <https://doi.org/10.5772/intechopen.79103>
- Ahmad, A., Buang, A., & Bhat, A. H. (2016). Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): A review. *Renewable and Sustainable Energy Reviews*, 65, 214–234. <https://doi.org/10.1016/j.rser.2016.06.084>
- Anita, S. H., Oktaviani, M., & Hermiati, E. (2023). Utilization of pretreated oil palm empty fruit bunches and their hydrolysate for ethanol production by Indonesian ethanologenic yeast. *Biodiversitas Journal of Biological Diversity*, 24(10), 5243–5252. <https://doi.org/10.13057/biodiv/d241004>
- Aydar, A. Y. (2018). Utilization of response surface methodology in optimization of extraction of plant materials. In V. Silva (Ed.), *Statistical approaches with emphasis on design of experiments applied to chemical processes* (pp. 157–170). IntechOpen. <https://doi.org/10.5772/intechopen.73690>
- Beluhan, S., Mihajlovski, K., Šantek, B., & Ivancic Santek, M. (2023). The production of bioethanol from lignocellulosic biomass: Pretreatment methods, fermentation and downstream processing. *Energies*, 16(19), 7003. <https://doi.org/10.3390/en16197003>
- Bundhoo, Z. M. A. (2018). Microwave-assisted conversion of biomass and waste materials to biofuels. *Renewable and Sustainable Energy Reviews*, 82, 1149–1177. <https://doi.org/10.1016/j.rser.2017.09.066>
- Chen, W.-H., Ye, S.-C., & Sheen, H.-K. (2012). Hydrolysis characteristics of sugarcane bagasse pretreated by dilute acid solution in a microwave irradiation environment. *Applied Energy*, 93, 237–244. <https://doi.org/10.1016/j.apenergy.2011.12.014>
- Ciumac, D., Campbell, R. A., Clifton, L. A., Xu, H., Fragneto, G., & Lu, J. R. (2017). Influence of acyl chain saturation on the membrane-binding activity of a short antimicrobial peptide. *ACS Omega*, 2(11), 7482–7492. <https://doi.org/10.1021/acsomega.7b01270>
- Cutaia, A. J. (1984). Malt beverages and brewing materials: Gas chromatographic determination of ethanol in beer. *Journal of the Association of Official Analytical Chemists*, 67(1), 192–193. <https://doi.org/10.1093/jaoac/67.1.192>
- Di Leo, G., & Sardanelli, F. (2020). Statistical significance: *P* value, 0.05 threshold, and applications to radiomics – Reasons for a conservative approach. *European Radiology Experimental*, 4, 18. <https://doi.org/10.1186/s41747-020-0145-y>
- Fan, J., De Bruyn, M., Budarin, V. L., Gronnow, M. J., Shuttleworth, P. S., Breeden, S., Macquarrie, D. J., & Clark, J. H. (2013). Direct microwave-assisted hydrothermal depolymerization of cellulose. *Journal of the American Chemical Society*, 135(32), 11728–11731. <https://doi.org/10.1021/ja4056273>
- Fatriasari, W., Raniya, R., Oktaviani, M., & Hermiati, E. (2018). The improvement of sugar and bioethanol production of oil palm empty fruit bunches (*Elaeis guineensis* Jacq.) through microwave-assisted maleic acid pretreatment. *BioResources*, 13(2), 4378–4403. <https://doi.org/10.15376/biores.13.2.4378-4403>
- Fatriasari, W., Ulwan, W., Aminingsih, T., Sari, F. P., Fitria, Suryanegara, L., Iswanto, A. H., Ghozali, M., Kholida, L. N., Hussin, M. H., Fudholi, A., & Hermiati, E. (2021). Optimization of maleic acid pretreatment of oil palm empty fruit bunches (OPEFB) using response surface methodology to produce reducing sugars. *Industrial Crops and Products*, 171, 113971. <https://doi.org/10.1016/j.indcrop.2021.113971>
- Han, M., Kim, Y., Kim, S. W., & Choi, G. W. (2011). High efficiency bioethanol production from OPEFB fibre using pilot pretreatment reactor. *Journal of Chemical Technology and Biotechnology*, 86(12), 1527–1534. <https://doi.org/10.1002/jctb.2668>
- Harahap, B. M., Maulid, M. R., Dewantoro, A. I., Mardawati, E., & Huda, S. (2020). Moderate

- pretreatment strategies for improvement of reducing sugar production from oil palm empty fruit bunches. *IOP Conference Series: Earth and Environmental Science*, 443, 012081. <https://doi.org/10.1088/1755-1315/443/1/012081>
- Hermiati, E., Laksana, R. P. B., Fatriasari, W., Kholida, L. N., Thontowi, A., Yopi, Arniefanto, D. R., Champreda, V., & Watanabe, T. (2020). Microwave-assisted acid pretreatment for enhancing enzymatic saccharification of sugarcane trash. *Biomass Conversion and Biorefinery*, 12, 3037–3054. <https://doi.org/10.1007/s13399-020-00971-z>
- Hu, S., & Li, Y. (2014). Two-step sequential liquefaction of lignocellulosic biomass by crude glycerol for the production of polyols and polyurethane foams. *Bioresource Technology*, 161, 410–415. <https://doi.org/10.1016/j.biortech.2014.03.072>
- Isroi, Ishola, M. M., Millati, R., Syamsiah, S., Cahyanto, M. N., Niklasson, C., & Taherzadeh, M. J. (2012). Structural changes of oil palm empty fruit bunch (OPEFB) after fungal and phosphoric acid pretreatment. *Molecules*, 17(12), 14995–15012. <https://doi.org/10.3390/molecules171214995>
- Jędrzejczyk, M., Soszka, E., Czapnik, M., Ruppert, A. M., & Grams, J. (2019). Physical and chemical pretreatment of lignocellulosic biomass. In A. Basile & F. Dalena (Eds.), *Second and third generation of feedstocks: The evolution of biofuels* (pp. 143–196). Elsevier. <https://doi.org/10.1016/B978-0-12-815162-4.00006-9>
- Kim, S. (2018). Enhancing bioethanol productivity using alkali-pretreated empty palm fruit bunch fibre hydrolysate. *BioMed Research International*, 2018(1), 5272935. <https://doi.org/10.1155/2018/5272935>
- Kłosowski, G., & Mikulski, D. (2023). Changes in various lignocellulose biomasses structure after microwave-assisted hydrotropic pretreatment. *Renewable Energy*, 219, 119387. <https://doi.org/10.1016/j.renene.2023.119387>
- Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. *Bioresources and Bioprocessing*, 4, 7. <https://doi.org/10.1186/s40643-017-0137-9>
- Li, R., Fei, J., Cai, Y., Li, Y., Feng, J., & Yao, J. (2009). Cellulose whiskers extracted from mulberry: A novel biomass production. *Carbohydrate Polymers*, 76(1), 94–99. <https://doi.org/10.1016/j.carbpol.2008.09.034>
- Limayem, A., & Ricke, S. C. (2012). Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. *Progress in Energy and Combustion Science*, 38(4), 449–467. <https://doi.org/10.1016/j.pecs.2012.03.002>
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428. <https://doi.org/10.1021/ac60147a030>
- Mohammed, M., Rahman, R., Mohammed, A. M., Adam, T., Betar, B. O., Osman, A. F., & Dahham, O. S. (2022). Surface treatment to improve water repellence and compatibility of natural fibre with polymer matrix: Recent advancement. *Polymer Testing*, 115, 107707. <https://doi.org/10.1016/j.polymertesting.2022.107707>
- Momoh, E. O., & Osofero, A. I. (2020). Recent developments in the application of oil palm fibers in cement composites. *Frontiers of Structural and Civil Engineering*, 14, 94–108. <https://doi.org/10.1007/s11709-019-0576-9>
- Namondo, B. V., Foba-Tendo, J., Etape, E. P., & Krause, R. W. M. (2018). Potential of blended biomass feedstock from some species of raffia palm (*Raffia farinifera*, *Raffia hookeri* and *Raffia vinifera*) and oil palm empty fruit bunch (OPEFB) from Cameroon. *African Journal of Pure and Applied Chemistry*, 12(4), 25–33. <https://doi.org/10.5897/AJPAC2018.0753>
- Ningthoujam, R., Jangid, P., Yadav, V. K., Sahoo, D. K., Patel, A., & Dhingra, H. K. (2023). Bioethanol production from alkali-pretreated rice straw: Effects on fermentation yield, structural characterization and ethanol analysis. *Frontiers in Bioengineering and Biotechnology*, 11, 1243856. <https://doi.org/10.3389/fbioe.2023.1243856>
- Office of Agricultural Economics. (2021). *Agricultural statistics of Thailand 2020*.
- Oh, S. Y., Yoo, D. I., Shin, Y., Kim, H. C., Kim, H. Y., Chung, Y. S., Park, W. H., & Youk, J. H. (2005). Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy. *Carbohydrate Research*, 340(15), 2376–2391. <https://doi.org/10.1016/j.carres.2005.08.007>
- Pasma, S. A., Daik, R., Maskat, M. Y., & Hassan, O. (2013). Application of Box-Behnken design in optimisation of glucose production from oil

- palm empty fruit bunch cellulose. *International Journal of Polymer Science*, 2013(1), 104502. <https://doi.org/10.1155/2013/104502>
- Petrucci, R. H., Harwood, W. S., Herring, G. E., & Madura, J. (2007). *General chemistry: Principles and modern applications* (9th ed.). Prentice Hall.
- Poletto, M., Ornaghi, H. L., & Zattera, A. J. (2014). Native cellulose: Structure, characterization and thermal properties. *Materials*, 7(9), 6105–6119. <https://doi.org/10.3390/ma7096105>
- Poludasu, R. M. (2024). Potassium hydroxide pretreatment of Napier grass: Conditions for enhanced reducing sugar and bioethanol production. *Journal of Microbiology, Biotechnology and Food Sciences*, 14(3), e11653. <https://doi.org/10.55251/jmbfs.11653>
- Poludasu, R. M., Cheirsilp, B., & Boonsawang, P. (2024a). Structural alteration of oil palm empty fruit bunch by peracetic acid combined with ultrasonic pretreatment for xylanase production by *Neosartorya* sp. *Journal of Oil Palm Research*, 36(4), 674–686. <https://doi.org/10.21894/jopr.2023.0050>
- Poludasu, R. M., Reddy, O. V. S., Boonsawang, P., & Mathakala, V. (2024b). Optimization of carboxymethyl cellulase production by *Cladosporium* sp. with food waste in bagasse solid-state fermentation using response surface methodology. *Journal of Microbiology, Biotechnology and Food Sciences*, 14(2), e10132. <https://doi.org/10.55251/jmbfs.10132>
- Pradeepkumar, T., Suma, B., Jyothibhaskar, & Satheesan, K. N. (2008). *Management of horticultural crops*. New India Publishing Agency.
- Ramaiah, S. K., Thimappa, G. S., Nataraj, L. K., & Dasgupta, P. (2020). Optimisation of oxalic acid pre-treatment and enzymatic saccharification in *Typha latifolia* for production of reducing sugar. *Journal of Genetic Engineering and Biotechnology*, 18(1), 28. <https://doi.org/10.1186/s43141-020-00042-w>
- Segal, L., Creely, J. J., Martin Jr., A. E., & Conrad, C. M. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal*, 29(10), 786–794. <https://doi.org/10.1177/004051755902901003>
- Sivamani, S., Kaveri, R., & Umaa Nandhini, S. (2022). Production of total reducing sugars from *Bambusa balcooa* through oxalic acid pretreatment. *Biosciences Biotechnology Research Communications*, 15(1), 236–242. <https://doi.org/10.21786/bbrc/15.1.36>
- Solihat, N. N., Hermiati, E., & Adi, D. T. N. (2020). Microwave-assisted dilute organic acid pretreatment of oil palm empty fruit bunch to improve enzyme accessibility. *IOP Conference Series: Materials Science and Engineering*, 935, 012046. <https://doi.org/10.1088/1757-899X/935/1/012046>
- Stat-Ease, Inc. (2023). *Design-Expert (version 23.0.1) [computer software user guide]*. <https://www.statease.com/docs/latest/>
- Statista. (2024). *Palm oil industry in Thailand – statistics and facts*. <https://www.statista.com/topics/12224/palm-oil-industry-in-thailand/#statisticChapter>
- Sweeney, M. D., & Xu, F. (2012). Biomass converting enzymes as industrial biocatalysts for fuels and chemicals: Recent developments. *Catalysts*, 2(2), 244–263. <https://doi.org/10.3390/catal2020244>
- Tareen, A. K., Punsuvon, V., & Parakulsuksatid, P. (2020). Investigation of alkaline hydrogen peroxide pretreatment to enhance enzymatic hydrolysis and phenolic compounds of oil palm trunk. *3 Biotech*, 10, 179. <https://doi.org/10.1007/s13205-020-02169-6>
- Xiang, L. Y., Hanipah, S. H., Mohammed, M. A. P., Baharuddin, A. S., & Lazim, A. M. (2015). Microstructural, mechanical and physicochemical behaviours of alkali pre-treated oil palm stalk fibres. *BioResources*, 10(2), 2783–2796. <https://doi.org/10.15376/biores.10.2.2783-2796>
- Xiong, B., Ma, S., Chen, B., Feng, Y., Peng, Z., Tang, X., Yang, S., Sun, Y., Lin, L., Zeng, X., & Chen, Y. (2023). Formic acid-facilitated hydrothermal pretreatment of raw biomass for co-producing xylo-oligosaccharides, glucose, and lignin. *Industrial Crops and Products*, 193, 116195. <https://doi.org/10.1016/j.indcrop.2022.116195>
- Xu, F., Yu, J., Tesso, T., Dowell, F., & Wang, D. (2013). Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: A mini-review. *Applied Energy*, 104, 801–809. <https://doi.org/10.1016/j.apenergy.2012.12.019>
- Zhang, W., Lin, Y., Zhang, Q., Wang, X., Wu, D., & Kong, H. (2013). Optimisation of simultaneous saccharification and fermentation of wheat straw for ethanol production. *Fuel*, 112, 331–337. <https://doi.org/10.1016/j.fuel.2013.05.064>