

Original Research Article

Effect of Subcritical Water Pre-Treatment on Empty Fruit Bunch for Volatile Fatty Acid Production: A Response Surface Methodology Study

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Abstract: Empty fruit bunch (EFB), a lignocellulosic byproduct from palm oil in the extraction process, offers a promising feedstock to produce volatile fatty acids (VFA), which serve as precursors for biogas production. This study examines the effects of subcritical water pre-treatment of EFB to improve VFA production prior to anaerobic co-digestion. A central composite design (CCD) in response surface methodology (RSM) was used to optimise the pre-treatment parameters. The parameters included temperature ranging from 120 to 180°C, reaction time between 10 and 30 min, and a liquid-to-solid (L/S) ratio of 10:1 to 20:1. Under optimal conditions of 179.94°C for 29.98 min and an L/S ratio of 20:1, the findings indicated that subcritical water pre-treatment significantly improved the digestibility of EFB, yielding a peak VFA production of 1.43 g/L, which corresponds to 16.9 times higher VFA production than untreated. The enhancement of VFA production subsequently improves the co-digestion process, resulting in a 2.87-fold increase in biogas production. The RSM model demonstrated strong predictive capability, achieving a coefficient of determination ($R^2 = 0.8952$). The results indicated that subcritical water pre-treatment is an effective method for converting EFB into VFAs, thereby supporting sustainable waste management and the production of bioenergy.

Keywords: Anaerobic co-digestion; Biogas production; Subcritical water; VFA

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1. Introduction

The palm oil industry generates significant quantities of lignocellulosic waste, particularly in the form of empty fruit bunch (EFB) (Chanthong *et al.*, 2024). This waste poses significant environmental challenges as it is generated in substantial amounts and decomposes at a slow rate through natural processes (Park, 2021). Given its substantial cellulose content, hemicellulose content, and lignin content, EFB presents a promising substrate to produce bio-based products such as volatile fatty acids (VFAs) (Liew *et al.*, 2021; Saelor *et al.*, 2024). Due to their significance in anaerobic digestion, VFAs play a crucial role in the generation of biogas. Acetic, propionic, and butyric acids serve as examples of VFA, which act as versatile intermediates in the production of biogas (Tian *et al.*, 2020). During anaerobic digestion, glucose is converted to acetic acid during acidogenesis. However, deacetylation of the acetyl group in hemicellulose produced acetic acid during pre-treatment (Alonso-Riaño *et al.*, 2023).

Palm oil mill effluent (POME) has been effectively employed in anaerobic co-digestion with EFB to improve biogas production, owing to its elevated nutrient content and biodegradability (Chan *et al.*, 2021; Suksaroj *et al.*, 2023). Research indicates that the co-digestion of EFB with POME enhances microbial activity and biogas yields in comparison to EFB alone, by utilising the synergistic properties of both solid and liquid waste streams (Liew *et al.*, 2021). Given the resilient composition of EFB, it is essential to implement pre-treatment to enhance its biodegradability, thereby facilitating the efficient production of VFAs to increase the accessibility of the substrate for microbial breakdown during co-digestion processes (Aili Hamzah *et al.*, 2024; Chanthong *et al.*, 2024). However, despite the effectiveness of co-digestion, the complex structure of EFB remains an obstacle to microbial hydrolysis efficiency. This indicates the necessity for an effective pre-treatment method that is environmentally sustainable and economically feasible. Acid, alkali, and enzymatic hydrolysis are common methods for breaking down lignocellulosic structures, but each has significant drawbacks. Acid and alkali treatments effectively break down hemicellulose and lignin; however, they often involve the use of harmful chemicals, produce by-products that inhibit the biogas process, and necessitate chemical neutralisation, which ultimately reduces the sustainability of the overall process (Rahmani *et al.*, 2023; Suhartini *et al.*, 2023). On the other hand, subcritical water pre-treatment is a green and chemical-free method that works just as well or better than other approaches to modify lignocellulosic structure without using chemicals.

Subcritical water pre-treatment represents a sustainable method that effectively hydrolyses lignocellulosic biomass while avoiding the need for chemical catalysts (Castro *et*

al., 2024). The pre-treatment occurs at temperatures between 100 and 374°C, with pressures maintained below the critical point of water (Sganzerla *et al.*, 2022). Subcritical water disrupts the connections between lignin and hemicellulose, thereby enhancing the accessibility of cellulose for microbial degradation (Chen *et al.*, 2023). In addition, the characteristics of subcritical water at high temperatures facilitate lignocellulosic hydrolysis, simplifying the conversion of complex polysaccharides into simpler sugars without the need for chemicals (Aili Hamzah *et al.*, 2025). This offers environmental and operational benefits over conventional methods.

Subcritical water effectiveness has been shown in earlier research to pre-treat several kinds of biomass for use in bioenergy applications (Rosly *et al.*, 2024; Vakalis *et al.*, 2022). For example, Chanthong *et al.* (2024) found that subcritical water pre-treatment of EFB at 190°C increased VFA and sugar yields and subsequently improved biogas production by 15% in comparison to untreated EFB, which was linked to the solubilisation of hemicellulose. In a similar vein, Rahmani *et al.* (2023) demonstrated that pre-treating wheat straw with subcritical water pre-treatment at 175°C enhanced the efficiency of enzymatic hydrolysis, resulting in an increase in available VFA. Despite these successes, there is still a lack of sufficient studies on how to enhance subcritical water conditions for EFB, particularly in an EFB-POME co-digestion system. Current research predominantly emphasises either biomass conversion or digestion performance, failing to comprehensively connect subcritical water - associated physicochemical alterations to VFA production, biogas yields, and overall process efficiency. Additionally, there is an inadequate number of studies that integrate optimisation studies in this area, resulting in gaps regarding the practical viability of implementing subcritical water in the valorisation of palm oil waste.

To address these gaps, the present research employs RSM to optimise the subcritical water pre-treatment parameters, such as temperature, reaction time, and liquid-to-solid (L/S) ratio, to maximise VFA production from EFB. The response surface methodology (RSM) is a statistical tool that makes it easier to model and optimise a wide range of variables while needing just a small number of experimental runs (Ahmad *et al.*, 2024). This work offers novel insights into the role of subcritical water as a sustainable method for sustainable palm oil waste management by correlating structural and compositional modifications in EFB with improved methane yield, net energy recovery, and process economics. Specifically, the objectives are as follows: (1) to establish the influence of temperature, reaction time, and L/S ratio on the generation of VFA; (2) to develop a predictive RSM model; and (3) to identify

the optimal pre-treatment parameters for VFA production to improve the biogas production of EFB co-digested with POME.

2. Materials and Methods

2.1. Preparation of Raw Material

The EFB and POME used as substrates in this project originated from the palm oil mill in Selangor, Malaysia. The inoculum was treated with POME from a biodigester. Prior to usage, the inoculum had been adapted at a temperature of $37 \pm 1^\circ\text{C}$ in a water bath (WTB35, Memmert, Germany). The EFB's moisture was removed by drying at a temperature of 60°C and subsequently reduced to a particle size of 0.5 mm using a milling machine (Pulverisette 19, Fritsch, Germany). The substrates and samples used in this study were kept at 4°C in the cold room prior to usage.

2.2. Subcritical Water Pre-treatment

In this study, EFBs were pre-treated in a system comprised of an oil bath (OB-30, Protech, Malaysia) equipped with a rocking bed using silicone oil as the heating medium. The subcritical water reactor has a diameter of 0.75 in. and a length of 0.2 m and is made of SS316L steel tube. For each pre-treatment setting, EFB and water were poured into the reactor to make up a specified L/S ratio. Subsequently, the reactor caps were fitted together and securely fastened following the purging of nitrogen gas for a duration of one minute at a flow rate of 0.002 L/min. The EFB pre-treatments were subjected to temperatures and times specified in Table 1 within a pressure range of 0.1 to 1.5 MPa. Prior to the reaction, the temperature inside the oil bath was raised to the desired level in the system. The reactors were fastened to the platform, and after the pre-treatment time elapsed, they were instantly removed and submerged in a cooling tub where each run was tripled. The liquid for each run was collected for VFA analysis.

2.3. Response Surface Methodology

The subcritical water factors affecting the response variable (VFA production) in this study were refined through the application of RSM utilising Central Composite Design (CCD). The factors were assigned to one of three levels: low, mean, or high, as displayed in Table 1. Factorial points, axial points, and centre points were incorporated into the CCD design matrix. Axial points evaluate quadratic effects; centre points help measure experimental error; factorial points represent all combinations of high and low levels. The

experiments were conducted in accordance with this technique, generating 20 trial runs, and the response variable was recorded for each run as displayed in Table 2.

Table 1. Low and high coded values for the selected factors.

Factors	Name	Units	Coded Low	Coded High	Mean
A	Temperature	°C	-1 ↔ 120.00	+1 ↔ 180.00	150.00
B	Time	min	-1 ↔ 10.00	+1 ↔ 30.00	20.00
C	L/S ratio	-	-1 ↔ 10.00	+1 ↔ 20.00	15.00

Each factor's importance was evaluated using analysis of variance (ANOVA) following the experimental data evaluation through the development of a second-order polynomial regression model. The ideal combinations of factors were determined through the development of response surface 3-dimensional (3D) and contour plots derived from the fitted model.

2.4. Biogas Production

The batch anaerobic co-digestion tests were used to study the biogas production from EFB and POME under mesophilic conditions. Tests were carried out in 125 mL digestion vials fitted with a rubber stopper and aluminium cap. The vials were filled with a mixture of EFB and POME at a substrate loading of 11 gVS/L and a pH of 7, and co-digested under a controlled temperature bath at 37±1°C (WTB35, Memmert, Germany) for 60 days (Liew *et al.*, 2021). The mixing ratio for EFB and POME was 0.6:1 with a substrate-to-inoculum ratio of 0.5 on a VS basis (Aili Hamzah *et al.*, 2024). The biogas content was measured via gas chromatography (GC) (6890 N, Agilent, US). The gas composition was detected using a thermal conductivity detector, and peak identification was performed using a fused-silica capillary wall-coated open tubular GC column using argon acting as the transport gas. The composition of methane was determined using GC data.

2.5. Analytical Analysis

The batch anaerobic solid and liquid fractions of EFB were analysed to determine their characteristics. Pre-treated liquid was separated by centrifugation using a benchtop centrifuge (Kubota, Japan) for 5 min. The VFA concentrations were determined using Rezex ROA (Phenomenex, USA) at 0.005 N sulphuric acid at 0.6 mL/min. Calibration curves from the reference were used to determine the concentrations of VFA. The carbon (C) and nitrogen (N) compositions were measured using the CHNS analyser (LECO, United States). Then, the lignocellulosic composition of EFB was analysed using the standard methods as described in

Aili Hamzah *et al.* (2024). The standardised method described in the American Public Health Association's standard procedure for water and wastewater testing was used to conduct the total solids (TS) and volatile solids (VS) calculations (APHA, 1998).

3. Results and Discussions

3.1. VFA Production from Subcritical Water Pre-treated EFB using RSM

The characteristics of the EFB used in this study are shown in Supplementary Table S1. According to Table S1, the EFB contains organic material with a VS concentration of 25.52 g/L. The untreated form of EFB exhibits a carbon content of 43.24%, a mildly acidic pH of 5.35, and a VFA concentration of 0.08 g/L. Due to its lignocellulosic composition and the resistance of its components, EFB is difficult to break down (Saelor *et al.*, 2024). It needs to be pre-treated to make EFB more degradable from VFA production (Chanthong *et al.*, 2024). Subcritical water pre-treatment effectively degrades lignin, hemicellulose, and cellulose, enhancing their decomposition and hydrolysis of carbohydrates to VFA (Chen *et al.*, 2023; Náthia-Neves & Alonso, 2024). Water in the subcritical water region acts as both a solvent and an acid; water autoionises to hydronium and hydroxide ions, which hydrolyse hemicellulose and partially degrade cellulose by loosening lignin bonds (Alonso-Riaño *et al.*, 2023). The lower viscosity and polarity enhance the penetration and solubilisation, causing structural modification (Čolnik *et al.*, 2025). These changes make cellulose easier to access and to break down and utilise for bioenergy. To evaluate the influence of the subcritical water pre-treatment process on EFB regarding VFA production, a total of twenty subcritical water sets has been developed utilising the RSM-CCD, as detailed in Table 2.

As observed in Table 2, the highest VFA production was at pre-treatment for 30 min at 180°C with a 20:1 L/S ratio (Run 17) with 1.43 g/L VFA production compared to all runs tested. In contrast, run 9 produced the lowest VFA, with a pre-treatment condition of 150°C for 3 min and a 15:1 L/S ratio, resulting in only 0.08 g/L VFA production. No changes were observed in the VFA production in Run 9 when compared to the VFA levels in the untreated EFB. According to the observation in Table 2, this study revealed that higher temperatures (150-180°C), longer pre-treatment durations (15-30 min), and an ideal L/S ratio (15:1 to 20:1) have been shown to increase VFA yields from EFB. Chen *et al.* (2023) reported comparable findings, indicating that total VFA levels increased from 132.95 mg/L at 119°C to 2849 mg/L at 159°C, highlighting the substantial impact of higher temperatures on VFA production. Similarly, Sganzerla *et al.* (2022) carried out subcritical water pre-treatment on brewer's spent grains at 160°C, demonstrating that this technique enhances the solubilisation of

hemicellulose, resulting in the release of greater amounts of monomeric sugars and VFA, which then increases biogas production.

Although the VFA concentration at 180°C for 30 min in this study was 1.43 g/L, it is significantly lower than the 2.85 g/L observed at 159°C for the same duration, as reported by Chen *et al.* (2023). This discrepancy may be attributed to differences in the material composition of the waste analysed, particularly the high lignin content in EFB, which complicates the delignification process. Moreover, the results of this study were comparable to VFA production from EFB at a slightly higher temperature of 190°C (Chanthong *et al.*, 2024), where the concentration of VFAs, including acetic, lactic, and propionic acids, reached 2.36 g/L. This finding demonstrates the impact of subcritical water pre-treatment on the production of VFA. Additional investigation into the influence of subcritical water parameters and their interactions is essential to comprehensively grasp the ideal conditions for enhancing VFA production and optimising the overall process efficiency.

Table 2. Experimental design of the CCD matrix with the corresponding factors and response

Run Order	Factors			Response	
	Temperature (°C)	Time (min)	L/S Ratio (-)	Actual Value (g/L)	Predicted Value (g/L)
1	150	20	15	0.20	0.31
2	120	30	20	0.60	0.59
3	150	20	15	0.47	0.31
4	180	10	20	0.62	0.76
5	150	20	15	0.27	0.31
6	150	20	15	0.30	0.31
7	150	20	23	0.86	0.87
8	150	20	15	0.34	0.31
9	150	3	15	0.08	0.13
10	180	10	10	0.29	0.26
11	120	10	10	0.40	0.35
12	120	10	20	0.41	0.22
13	150	37	15	0.73	0.74
14	150	20	7	0.25	0.30
15	150	20	15	0.27	0.31
16	200	20	15	1.16	0.99
17	180	30	20	1.43	1.43
18	100	20	15	0.13	0.35
19	180	30	10	0.48	0.62
20	120	30	10	0.57	0.40

3.2. Statistical Analysis

The ANOVA analysis was implemented to assess the statistical significance of the subcritical water factors, thereby confirming the appropriateness of the chosen quadratic model (Table 3). The ANOVA results revealed an F-value of 9.49, accompanied by a p-value of less than 0.05 ($p < 0.0001$), indicating the model's significance. The ANOVA study revealed that the model consisted of significant factors such as temperature, time, L/S ratio, the interaction between temperature and L/S ratio, as well as the squared terms of both temperature and L/S ratio (A, B, C, AC, A², C²). Adequate precision (Adeq Precision) quantifies the signal-to-noise ratio. Precision ratios exceeding 4 are preferred (Venkatachalam *et al.*, 2021). The higher Adeq precision (12.105) for the current model suggests a sufficient signal from the selected model.

Good correlation between the experimental and predicted values was indicated by high coefficient determination (R^2) values, which were closer to 1, and the model was well-fitted by the data (Ahmad *et al.*, 2024). The findings suggest that the model successfully accounted for 90% of the variation in VFA production that results from subcritical water pre-treatment, with approximately 10% remaining that exceeded its parameters. For the present study, the R^2 exceeded the values reported for the optimisation of VFA using sludge (0.83) (Nabaterega *et al.*, 2022) and subcritical water pre-treated pineapple waste (0.84) (Aili Hamzah *et al.*, 2022), suggesting a stronger correlation in this analysis. Though a large gap was observed between adjusted and predicted, this difference still indicates the model fits well; however, it is unable to provide good predictions for upcoming outcomes. This could be because of variability in the experiments, missing interactions between factors in the quadratic model, or an uneven distribution of data points, especially at the extreme conditions (Chen *et al.*, 2022; Okwu *et al.*, 2021).

Table 3. ANOVA results for VFA production from subcritical water pre-treated EFB

Source	Sum of Squares	Mean Square	F-value	p-value
Model	1.9800	0.2202	9.4900	0.0008
A-Temperature	0.4841	0.4841	20.8700	0.0010
B-Time	0.4453	0.4453	19.1900	0.0014
C-L/S Ratio	0.4037	0.4037	17.4000	0.0019
AB	0.0498	0.0498	2.1500	0.1736
AC	0.1947	0.1947	8.3900	0.0159
BC	0.0514	0.0514	2.2200	0.1674
A²	0.2368	0.2368	10.2100	0.0096

B²	0.0293	0.0293	1.2600	0.2871
C²	0.1399	0.1399	6.0300	0.0339
Residual	0.2320	0.0232		
Lack of Fit	0.1901	0.0380	4.5400	0.0612
Pure Error	0.0419	0.0084		
Cor Total	2.2100			
R²	0.8952	Predicted R ²	0.2888	
Adjusted R²	0.8009	Adeq Precision	12.1053	

To evaluate the normality of the regression coefficients, a normal probability plot (Figure 1a) was used. A straight line with points distributed symmetrically on either side suggests that the data follows a normal distribution (Ahmad *et al.*, 2024). Meanwhile, the adequacy of the model can be assessed using Figure 1b, where the points on the straight line represent the experimental data, demonstrating that the results of the experiment were consistent with the anticipated values. Therefore, the adequacy of the improved model was verified by these plots. In an optimisation study of biologically treated palm oil wastes by Tan *et al.* (2021), their report indicated that the experimental data were consistent with the projected data, as evidenced by the linear trend of the predicted versus actual plots. Given that all the data points are positioned on or near the line of best fit in Figure 1b. This additionally strengthens the high R² achieved for this model.

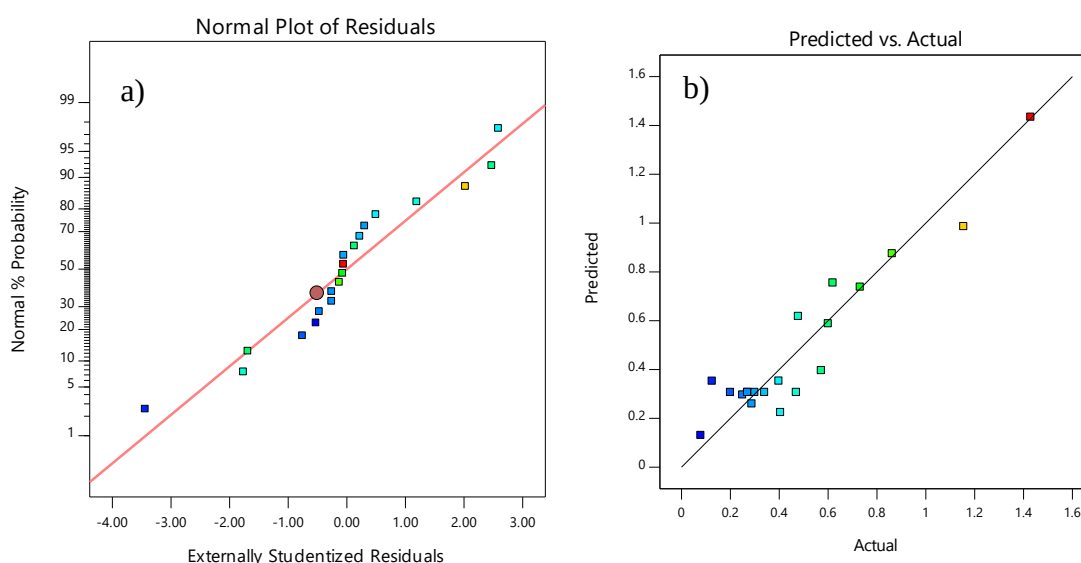


Figure 1. a) Normal plot and b) Predicted versus actual plot

A quadratic model that represented the pre-treatment's coded influencing factors was used to assess the experimental data. The results were correlated with the mathematical

equations associated with VFA production using the final second-order polynomial equation, which was expressed in terms of coded factors in Equation 1. The software was used to regress the equation. One may use the coded factor equation for predicting how each factor will respond at a certain level (Iweka *et al.*, 2021). Both the coded and actual versions of the equation have their respective uses; the former allows one to compare parameter coefficients to find out how each parameter affects the system, while the latter predicts how the system will respond to different values of the parameters (Hamzah *et al.*, 2024).

$$\begin{aligned} \text{VFA} = & 0.3067 + 0.1883A + 0.1806B + 0.1719C + 0.0789AB + 0.1560AC + 0.0802BC \\ & + 0.128A^2 + 0.0451B^2 + 0.0985C^2 \end{aligned} \quad (1)$$

3.3. Interaction Between Subcritical Water Pre-Treatment Factors

To better understand the impact of subcritical water pre-treatment parameters on VFA production, the present study assessed contour plots and 3D visualisations. Regression analysis of the simulated data was employed to generate 3D response surface plots, which were accompanied by perturbation plots (Yan *et al.*, 2025). At each given point in the design space, these visualisations made it possible to compare the impacts of any single factor. A perturbation plot's response is generated by changing one variable over its range while holding all other variables constant (Tan *et al.*, 2021). As noted by Hong *et al.* (2021), a pronounced slope or curvature in the plot signifies the sensitivity of the dependent response variable. To further visualise the effects of the factors on the response, perturbation plots for the VFA production from subcritical water pre-treatment are presented in Supplementary Figure S1. Temperature (A) and L/S ratio (C) exhibit greater curvatures in comparison to time (B), which presents a relatively flat line. The production of VFA is more sensitive to temperature and the L/S ratio. Nonetheless, even though the L/S ratio shows a less steep slope, it still affects VFA production, suggesting its influence is not as substantial as that of temperature. The significance of reaction time appears to be limited within the examined range. The sensitivity ranking for VFA production is as follows: $A > C > B$.

The 3D plot (Figure 2a) and contour plot (Figure 2b) of the interaction between temperature and time of the pre-treatment for VFA production show that the curved contour lines move from the blue region to the green region. As time increases, the VFA production increases, signifying the influence of time and temperature. However, the interaction is not significant, as the p-value of this interaction is not statistically significant (Table 3).

Similarly, the interaction of time and L/S ratio is illustrated in Figures 2e and 2f. VFA production increases as both factors approach the optimal point (red dot); however, beyond

this point, the enhancement in VFA production becomes less pronounced. The curved shape of the 3D surface indicates that the relationship between time and L/S ratio is not linear, and the interaction effect is insufficient to draw a conclusion about a significant impact on VFA production (p-value of 0.1674).

Meanwhile, the interaction between temperature and L/S ratio on VFA production in Figures 2c and 2d demonstrates that the curvature of the contour lines increases as the responses (VFA production) approach the optimal region. This indicates that the combined effects on VFA are significant, as the closer contour lines suggest higher sensitivity and a statistically significant interaction (p value of 0.0159).

The subcritical water pre-treatment temperature plays a significant role in the extent of the subcritical water pre-treatment effect on the waste structural disruption. Depending on the pre-treatment temperature, the pre-treatment breaks apart the packs of cellulose microfibrils, which increases the specific surface area, surface porosity, fractures, and cracks on the deformed surface (Chanthong *et al.*, 2024; Saelor *et al.*, 2024). According to de Oliveira *et al.* (2022), the efficacy of subcritical water pre-treatment is highly dependent on temperature since temperature can enhance the mass transfer between the biomass and water to promote better hydrolysis. A study on VFA production from food waste using subcritical water pre-treatment found that total VFA levels increased from 132.95 mg/L to 2849 mg/L as the temperature of pre-treatment increased (Chen *et al.*, 2023).

Though the interaction between the factors with time is not significant, the individual effects are significant. Reaction time plays a critical role in subcritical water pre-treatment, as it directly impacts the extent of lignocellulosic structural breakdown. Insufficient reaction time during pre-treatment can lead to incomplete degradation or hydrolysis, preventing optimal conversion of biomass (Saritpongteeraka *et al.*, 2022). Ma *et al.* (2024) highlighted the importance of reaction time associated with subcritical water pre-treatment. They observed that extended reaction times led to degradation of cellulose, resulting in the formation of soluble compounds. In contrast, shorter pre-treatment times were insufficient for the effective depolymerisation of lignocellulosic components. These statements are further supported by Vakalis *et al.* (2022).

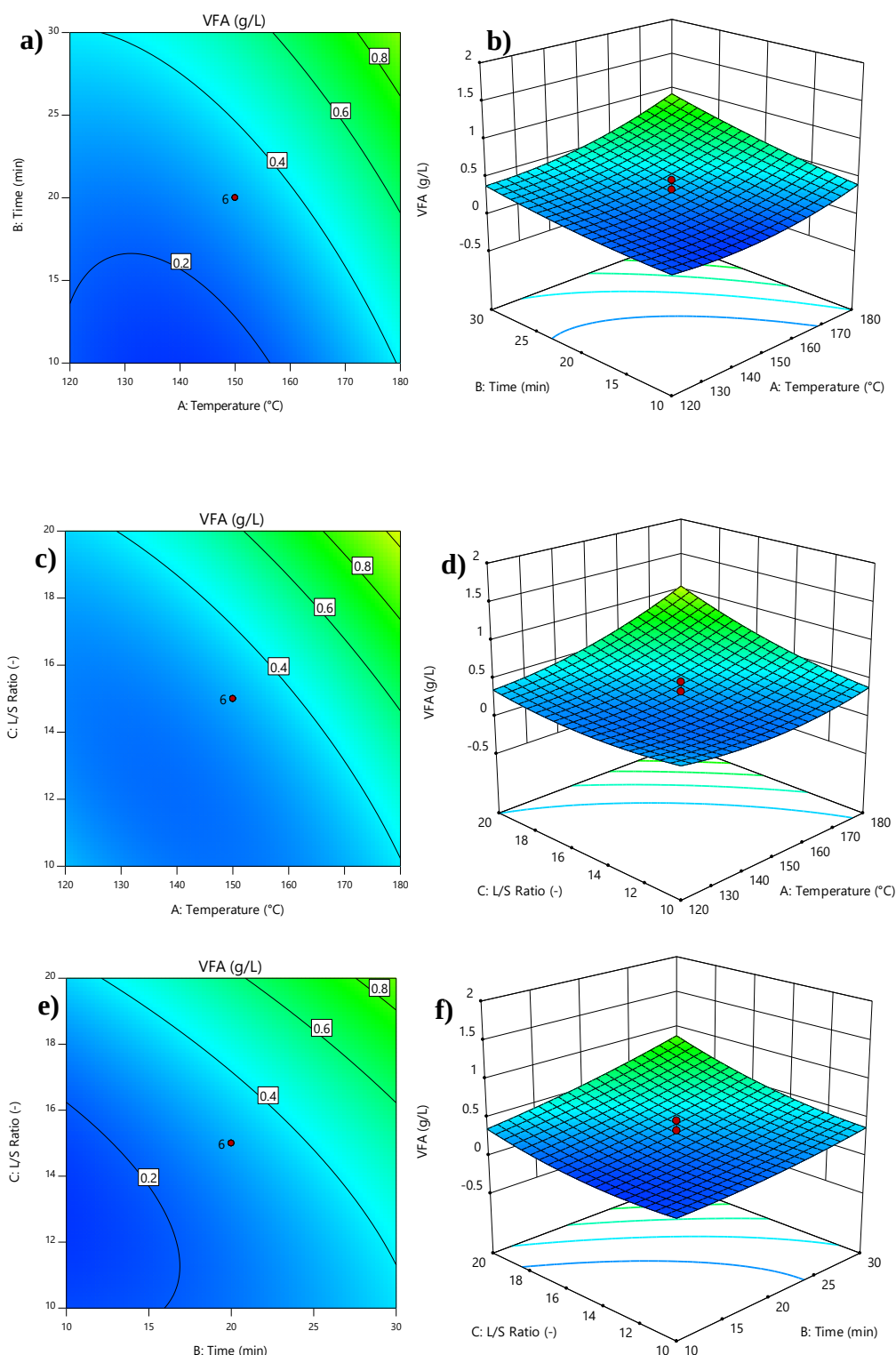


Figure 2. Contour plot and 3D plot interaction a) and b) for interaction between time and temperature, c) and d) for temperature and L/S ratio, e) and f) for L/S ratio and time

The L/S ratio is another important parameter that affects the subcritical water pre-treatment process; because of its low viscosity and high diffusivity, water continues to be an

alternative solvent in the subcritical water state for dissolving substrates (Yue *et al.*, 2022). According to the mass transfer theory, a high concentration gradient increases the diffusion rate due to a high-water content. This, in turn, facilitates the hydrolysis and depolymerisation of biomass. Previously, optimisation of subcritical water hydrothermal pre-treatment for biogas production from EFB reported that the optimal L/S ratio of 20:1 gives optimal conditions with the highest methane production (Saritpongteeraka *et al.*, 2022). The authors highlighted that high water content in the pre-treatment systems provides a better hydrolysis reaction of EFB. In addition, a high L/S ratio indicates that the water diffusion is facilitated and provides a mass transfer gradient between the lignin-cellulose matrix that enhances its solubilisation. These results indicate that while time and L/S ratio are important individually, the most important component in the reaction is the combined influence of temperature and the L/S ratio, and their interaction should be prioritised in optimising the process for maximum VFA yield.

3.4. Process Optimisation and Biogas Production

A numerical optimisation strategy was employed for the subcritical water process parameters, aiming to maximise the VFA production from EFB to be co-digested with POME for biogas production. The numerical constraints for the factors were established as “is in range”, whereas the goal for the response has been specified as “maximise”. The optimisation resulted in 59 solutions. Supplementary Table S2 presents a selection of 10 from a total of 59 solutions, with the selected optimal condition identified as the one exhibiting the highest desirability score of 1. Figure 3a illustrates that the desirability of optimisation grows from 0.2 to 0.8 as the subcritical water pre-treatment temperature and time increase at an L/S ratio of 20. Simultaneously, the production of VFA increased from 0.4 g/L to the predicted levels, as illustrated in Figure 3b. The optimal temperature for subcritical water pre-treatment was found to be 179.94°C for a period of 29.98 min at an L/S ratio of 20, under which settings VFA production was enhanced to 1.431 g/L. This is equivalent to a VFA production corresponding to 16.9 times greater than that of untreated EFB.

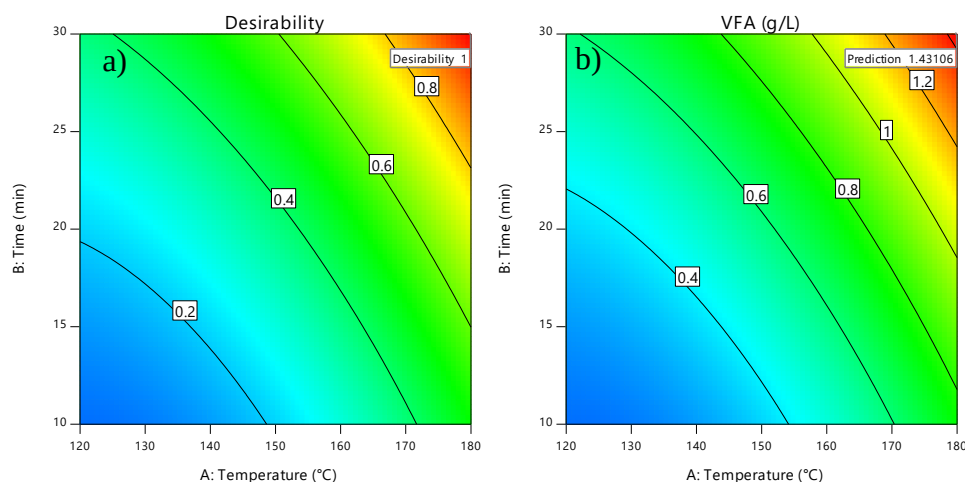


Figure 3. Contour plot of a) desirability and b) VFA production

Biogas was produced by subjecting the pre-treated EFB to anaerobic co-digestion after undergoing pre-treatment with subcritical water. According to Figure 4, the pre-treated EFB exhibited greater biogas production in comparison to EFB that has not been treated. The implementation of subcritical water pre-treatment has enhanced the ability of microorganisms to effectively degrade EFB, thus accelerating the conversion of organic matter into biogas (Aili Hamzah *et al.*, 2024). As a result, co-digestion efficiency was higher than that of untreated EFB. The primary objective of subcritical water pre-treatment is to disrupt the recalcitrant components in the EFB structure, thereby rendering cellulose and hemicellulose more susceptible to microbial degradation (Chen *et al.*, 2023; Sganzerla *et al.*, 2022). Subcritical water pre-treatment enhances the degradation of structures and accessibility of substrates after pre-treatment (Castro *et al.*, 2024).

The subcritical water pre-treated EFB yielded 0.86 L/gVS of biogas as compared to 0.30 L/gVS of biogas yield in the untreated EFB. The improvement in biogas yield from the subcritical water pre-treated EFB compared to the untreated EFB is approximately 2.87 times. Previously, subcritical water pre-treatment has been successfully applied to EFB at 175°C for 20 min and resulted in an increase in biogas production compared to untreated EFB (Rosly *et al.*, 2024). Reduction of the lignocellulosic fraction and crystallinity, and the increase of surface area at 180°C of pre-treated EFB have enhanced the methane production to 56.06%, as reported by Saelor *et al.* (2024). The findings from the study and the literature suggest that subcritical water pre-treatment can enhance VFA production, thereby improving biogas production.

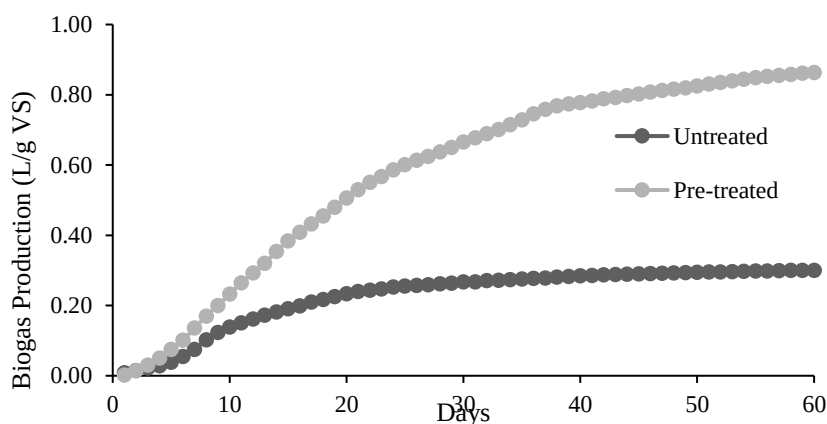


Figure 4. Biogas production from subcritical water pre-treated and untreated EFB

4. Conclusions

This study proved that subcritical water pre-treatment significantly improves the hydrolysis and solubilisation of lignocellulosic components in EFB, resulting in increased VFA production and enhanced co-digestion efficiency with POME. The optimised condition of 179.94°C for 29.98 min with an L/S ratio of 20, resulting in 1.43 g/L of VFA, facilitates a 2.87-fold increase in biogas production. These results show that subcritical water pre-treatment could be a sustainable strategy for improving the anaerobic digestion process. Nonetheless, the energy requirements and scalability of the process in an industrial context necessitate additional evaluation. Future research should investigate energy integration, inhibitor formation, and techno-economic analysis to improve the overall efficiency and viability of subcritical water applications in waste-to-energy systems.

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Supplementary Materials: The following are available online at <https://journals.hhpublisher.com/index.php/AAFRJ/article/view/1136/741>, Figure S1: Perturbation plot, Table S1: Characteristic of EFB used in this study, Table S2: List of optimised parameters generated by numerical optimisation.

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