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Research Article

Cultivation of Microalgae *Spirulina platensis* Biomass Using Palm Oil Mill Effluent for Phycocyanin Productivity and Future Biomass Refinery Attributes

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Palm oil mill effluent is a type of wastewater which contains a high concentration of organic nitrogen, phosphorus, and different supplement substances. These substances support and enhance the growth of microalgae. Cyanobacteria *Spirulina platensis* is a blue-green microalga with two phycobiliproteins as its primary constituents. Phycocyanin is a natural blue colourant used in biomedicines, cosmetics, diagnostics, treatments, and environmental protection. Chlorophyll pigment plays a significant role in photosynthesis where the photosynthetic process uses atmospheric carbon to produce starch and lipid which can eventually be converted into desirable products such as biodiesel. Therefore, in this study, *Spirulina platensis* was cultivated in different concentrations of diluted POME (10-50%, *v/v*) at $26 \pm 1°C$ room temperature, $90\,\mu$mol photon m^{-2} s^{-1}, and aerated for 24 h continuously. The growth of *Spirulina platensis* was monitored through optical density at 680 nm for 15 days. The highest biomass yield obtained in the control medium and 30% POME medium were 1.27 ± 0.02 and 1.16 ± 0.01 g/L, respectively. The highest phycocyanin yield obtained from the biomass harvested from 30% nutrient media was 175.12 ± 22.32 mg and followed by 163.55 ± 20.15 mg from control media with purified phycocyanin of 87.11 ± 5.20 mg and 85.31 ± 4.33 mg, respectively. The peak properties of phycocyanin such as the amide group at 1655.17 cm^{-1} (C=O stretching), FT-IR analysis revealed well-formed *Spirulina platensis* with all characteristic peaks and distinct fingerprints of phycocyanin. The ultrasound method produces the highest lipid yield ($28.6 \pm 0.7\%$) which consists of stearic (38.45%), palmitoleic (25.72 ± 0.02), and palmitic (17.71 ± 0.04) fatty acid methyl esters. The FAME produced from the extracted lipid has the potential to be used in biodiesel applications. Since POME contains the essential nutrients which can support the growth of *Spirulina platensis* in the optimum environment for biomass and lipid productivity, it revealed the potential for biodiesel production.

1. Introduction

The cost of nutrient media is one of the most significant economic restrictions on algae cultivation for biomass generation [1, 2]. As a result, efforts are being made to replace more expensive nutritional media with less costly supplemental sources. The use of various types of wastewater for biomass production is one of the current solutions. Utiliza-tion of wastewater, especially palm oil mill effluent (POME), for algae cultivation and due to the presence of nutrients like nitrogen (N) and phosphorus (P), it can absorb by microalgae as nutrients and transform solar energy into biomass through photosynthesis [3–5]. Microalgae biomass has a variety of useful compounds like proteins, amino acids, fatty acids, polysaccharides, vitamins, and pigments like phycocyanin that are employed as a source of nourishment

as well as an active ingredient in pharmaceuticals and cosmetics [6]. Among microalgae, *Spirulina platensis* has gain high attention in the commercial market due to its high protein and phycocyanin, a natural pigment used in food, beverages, and cosmetics. Even without extensive genetic engineering, some cyanobacterial strains, particularly those with abnormalities in starch production, can produce large amounts of lipids. Different acyl groups can be found in the storage lipids of microalgae. These starches and lipids can be converted to biofuels through fermentation and transesterification. Algal biodiesel has the potential to be a greener alternative fuel for CI engines because it is renewable, biodegradable, and nontoxic. As compared to petroleum fuel, the combustion and emission profile of biofuels are satisfactory. Microalgae have a number of advantages over other biofuel sources, including the ability to thrive in wastewater and a significantly lower cultivation area.

POME is one of the major biomasses produced in a palm oil mill in the form of wastewater generated during the sterilising, extraction, and clarification processes [7]. Wastewater is naturally high in nutrients, but it also contains pollutants that can lead to culture collapse [8, 9]. It needs proper treatment before being released into the environment because of its atrophying characteristics like acidity, high acidity, dark brownish appearance, high discharging temperature, high biological oxygen demand, high chemical oxygen demand, oil crease content, total suspended solid, and bad odour [10–12]. Khalid et al. [13] reported that successfully removed 21.5% of COD, 80.0% of total Nitrogen and 89.9% of total Phosphorus within five days by cultivating *Characium* sp. in POME. Cheah et al. [14], obtained 2.04 gL^{-1} of *Chlorella sorokiniana* biomass using POME as a nutrient medium for cultivation. Therefore, POME contains a high amount of nutrients which can support the growth of microalgae [15–17], but raw POME presents with high organics and turbidity which reduce light penetration and resist the growth of microalgae [18]. Therefore, pretreatment of POME is required before allowing the mixture with microalgae inoculums. It might be grown in wastewater, and bioactive compounds accumulated by digesting nutrients present in the effluent.

The main objectives of microalgae cultivation are to achieve high biomass and produce commercial-value products. Bioactive chemicals, particularly those derived from natural sources (as opposed to synthetic ones), are gaining popularity in the market, business, and research domains. Microalgae are sources of protein, fatty acids, essential amino acids, vitamins, and minerals which are used in the food industry [19]. Among that, phycocyanin is a water-soluble natural pigment-protein complex that belongs to the phycobiliprotein family of light-harvesting proteins [20, 21], and it is used as a food colouring; however, due to its fluorescent qualities, small amounts are used as biochemical tracers in immunoassays. Silveira et al. [22] reported that among microalgae, *Spirulina platensis* is a great source of phycocyanin. Chlorophycocyanins present a major portion of the phycobiliproteins as compared to allophycocyanin and phycoerythrins in *Spirulina platensis* [23, 24]. Phycobiliproteins are photosynthesis-related auxiliary pigments that accumulate in cells as phycobilisomes linked to the chloroplast thylakoid membrane [25].

Therefore, in this study, suitable POME concentration was investigated for culturing *Spirulina platensis*. The growth and biomass production were monitored under laboratory environmental conditions. The presence of phycocyanin compounds was extracted from the collected biomass and followingly perform quantification and qualification purified phycocyanin compounds. The lipid content of *Spirulina platensis* was studied by applying various extraction processes for gas chromatography analysis.

2. Material and Methods

2.1. Collection of POME Sample. The POME was collected from the LCSB Lepar Palm Oil Mill of the facultative pond which is located at coordinates (3°35′34.0″ N, 103°04′58.8″ E) in Kuantan, Pahang. The effluent was filtered using a 0.45 μm filter cloth and collected in a 5 L bottle. In the laboratory, the samples were centrifuged at 7168 g for 10 min and separated from the supernatant from the pellet. The characteristics of the sample were analysed and provided in Table 1. The dark brownish POME was diluted in a 1 : 1 ratio with distilled water, and the pH of the samples was adjusted for 7.0 ± 0.2 by using 1 M NaOH. Then it was sterilised at 121°C for 20 min.

2.2. Cultivation of Spirulina platensis. The mother culture of *Spirulina platensis* was obtained from the Algae Culture Collection Center and Laboratory of Universiti Malaysia Pahang. The culture was scaled up in a 2 L Erlenmeyer flask using a sterilised BG-11 medium with a 3 : 1 ratio, and it was maintained at $26 \pm 1°C$ room temperature, 90 μmol photon m^{-2}s^{-1}, and aerated for 24 h continuously. The purity of the culture was examined under a fluorescence microscope and scanning electron microscope (FEI Quanta 450), as shown in Figure 1. The BG-11 medium was prepared with compositions such as NaNO$_3$, 1.5; K$_2$HPO$_4$, 0.04; MgSO$_4$.7H$_2$O, 0.075; CaCl$_2$.2H$_2$O, 0.036; citric acid, 0.006; Na$_2$MoO$_4$.2H$_2$O, 0.00039; CuSO$_4$.5H$_2$O, 0.000079; and Co(NO$_3$)$_2$.6H$_2$O, 0.000049 [26]. The purity of the culture was maintained regularly by handling the culture under aseptic conditions and ensured by observing it under a light microscope.

2.3. Effect of POME Concentrations on Spirulina platensis. The prepared, sterilised POME medium was inoculated with fresh *Spirulina platensis* culture in different concentrations of 10%, 20%, 30%, 40%, and 50% v/v using a 1 L conical flask. The culture was aerated by pumping filtered air through an inlet glass tube and also provided an outlet with a syringe filter at the top mouth of the flasks, covered by cork and sealed by parafilm to prevent air from entering the environment which helps to avoid contamination. The culture flasks were placed at $26 \pm 1°C$ room temperature and provided 90 μmol photon m^{-2}s^{-1} light intensities continuously for 2 weeks of time. The experiments were repeated in sets $n = 3$. The growth rate of cultures was monitored by taking optical density at 680 nm using a spectrophotometer.

TABLE 1: Physicochemical parameter of palm oil mill effluent.

Parameter	Value
Chemical oxygen demand (COD, mgL^{-1})	1870 ± 15.0
Total suspended solids (TSS, mg L^{-1})	83 ± 5.0
Nitrate (mg L^{-1})	3.9 ± 0.3
Nitrite (mg L^{-1})	1.1 ± 0.1
Ammonium (mg L^{-1})	10.3 ± 0.5
Phosphate (mg L^{-1})	6.4 ± 0.2
pH	7.3

2.4. Measurement of Biomass Growth. The growth of *Spirulina platensis* cells at different POME concentrations was measured by taking absorbance reading at 680 nm using a Thermo Scientific Genesys 10S UV-VIS Spectrophotometer at room temperature for 15 days (every 2 days once). The wet biomass of the culture was centrifuged at 3200 g for 5 min using a Hybrid high-speed refrigerated centrifuge Kubota B6200. The collected biomass was rinsed with distilled water 2-3 times and centrifuged again. Then, the biomass was placed at -20°C, freezing and thawing the cell 5 times to rupture the cell walls.

2.5. Extraction of Phycocyanin. The maceration method was used to extract phycocyanin from *Spirulina platensis* biomass. Phycocyanin extraction was performed with phosphate buffer solution as a solvent in a 1 : 20 (*w/v*) ratio. The biomass of *Spirulina platensis* was weighed up to 5 g and placed in Erlenmeyer flasks. A 100 mL phosphate buffer solution was then added and agitated for 20 min at 4°C using a magnetic stirrer. After that, the solution was chilled for 20 min at 4°C. At a speed of 2500 g, the solution was centrifuged for 20 min. The crude extract of phycocyanin was centrifuged and kept in a dark bottle at 4°C.

2.6. Purification of Phycocyanin. Based on the previous work by Munowarah et al. [27], phycocyanin purification was carried out utilising the salting out method using ammonium sulphate $(NH_4)_2SO_4$. The crude extract was purified in a single step with 65% $(NH_4)_2SO_4$ and stored at 4°C overnight. The article was recovered by centrifugation at 5000 g for 15 min at 4°C, diluted in 10 ml of the same extraction buffer, and dubbed ammonium sulphate extract. A dialysis membrane from Hi-Media was used to dialyze 10 ml of ASE against the extraction buffer. Dialyses were done twice against 1000 ml of extraction buffer, the first at room temperature and the second at 4°C overnight. The resulting extract was filtered through a 0.45 lm filter and recovered from the dialysate membrane. In anion exchange chromatography, DEAE-cellulose was used to purify the phycocyanin. A $30 \times 9 \times 2$ cm column was constructed and equilibrated with 150 ml of acetate buffer (pH - 5.10). On the column, a dialyzed, filtered sample (10 ml) was put. The column was constructed using a linear gradient of acetate buffer with a pH range of 3.76 to 5.10, and elutes were collected in 5 ml portions. The flow rate was kept

constant at 20 ml h^{-1}. Used a Thermo Scientific Genesys 10S UV-VIS spectrophotometer for scanning the sample at the range of the absorption spectrum at 300–750 nm. The concentration of phycocyanin was measured using the following formula.

$$PC(mg \cdot mL - 1) = \frac{A620 - nm\, 0.474\,(A652)nm}{5.34} \quad (1)$$

2.7. Fourier Transform Infrared Spectroscopy. The crude extract and purified phycocyanin were crushed homogenously with potassium bromide (KBr) to obtain powders. These samples were compressed into pellets using a hydraulic pellet press with a 10-ton pressure load to produce translucent pellet samples that enable IR radiation to flow through. The phycocyanin substances were determined using a Spectrum 100 series FTIR spectrometer. The spectra were acquired in the region of 400 cm^{-1} to 4500 cm^{-1} using an infrared spectrophotometer.

2.8. Lipid Content and Transesterification. The lipid content of the dried biomass of *Spirulina platensis* was extracted using three different methods: Bligh and Dyer, Soxhlet, and ultrasound. For the Bligh and Dyer method, 500 mg of weighed biomass was mixed and homogenised with 10 ml of organic solvent (hexane) in 50 ml of a falcon tube and vortexed for 5 min. Then, the mixture was covered with aluminium foil and placed overnight at room temperature. The suspension was centrifuged after the whole night directly. New organic solvents were added again with pellet biomass and vortexed for 5 min continuously. Repeat this step until the supernatant turns colourless. The collected supernatant was centrifuged again and filtered through a syringe filter (0.45 μm) to eliminate the remaining biomass. The extraction was performed at room temperature (25°C). For the ultrasound method, the biomass was dissolved in 10 ml of hexane solvent and placed under a water bath at 60°C with ultrasound at 20 kHz of frequency wave sound for 120 min. A rotary evaporator was applied at temperatures 70°C, and 65 rpm to separate the lipid from the solvent for both above methods. For the Soxhlet method, the samples were transferred to the thimble placed in the Soxhlet holder and its flask containing 250 ml of hexane solvent. The extraction was performed for 3 h. The solvents keep contacting the sample and breaking down the cell to release the lipid. The collected 0.5 g of lipid was transesterified using an alkali catalyst of methanol KOH at 75°C by placing it in a water bath. The separated top layer was collected using a pipette. The fatty acid samples were further analysed in gas chromatography using the method mentioned in the previous study [16].

3. Results and Discussions

3.1. Characterization of POME. The characteristics of the collected POME were evaluated to determine the composition which can help as a medium for the growth of *Spirulina platensis*. The physicochemical characteristics are provided in Table 1. A high concentration of COD reduces the dissolved oxygen content in the balance of aquatic ecosystems
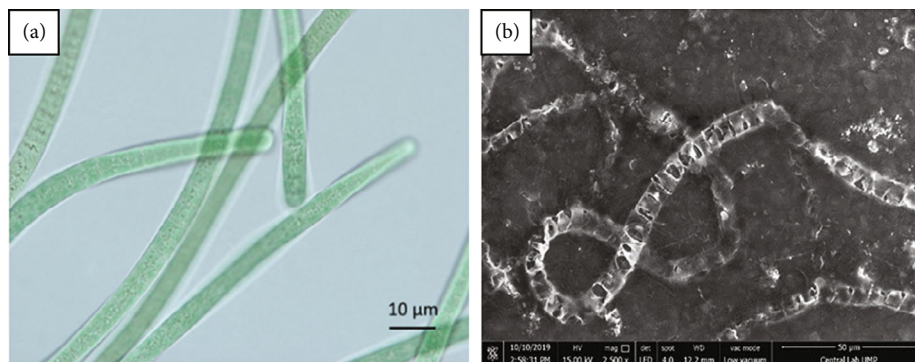
Figure 1: Photographs of (a) *Spirulina platensis* under a fluorescence microscope (1000×) and (b) scanning electron microscope (2,500x).

if it releases without proper treatment. Phosphorus and nitrogen are present in POME more than enough than in standard algal media; due to that, it required dilution modification where the content can be equal to the standard growth medium. Fernando et al. [28] reported that the presence of minute concentrations of toxic heavy metals such as cadmium, arsenic, lead, and mercury in POME helps synthesize microalgae biomass. Therefore, it is showing that POME has great potential to be a nutrient medium for the cultivation of *Spirulina platensis.*

3.2. Growth of Spirulina platensis in POME. Different microalgae species have different tolerances for organic chemicals, especially in highly concentrated media such as anaerobic digested POME [29]. Microalgae, *Spirulina platensis* (Figure 1), were inoculated with different concentrations of POME as per the condition described in Section 2.1. The growth of *Spirulina platensis* was monitored at regular intervals in terms of $OD_{680nm.}$ All the experiments were repeated ($n = 3$) and recorded the result and compared with the expected outcomes gathered from the literature review. The result obtained by cultivating *Spirulina platensis* in various concentrations is shown in Figure 1.

It was found that 30% POME medium highly supports the growth of *Spirulina platensis* as compared to other concentrations of POME medium; however, culture in standard medium found the highest growth than POME medium. Minimal growth was found at 10% and 20% *v/v* due to lacking nutrient content in the limited diluted POME. Based on Figure 2(a), in the initial days, the culture was in the lag phase where the growth rate was slightly slower during the first 3 days. This is due to the direct contact between cell culture and POME exerting physical and chemical forces on microalgal cells. As a result, the culture requires a longer time to adapt to the POME medium [30, 31]. Photosynthesis is an important element in the formation of microalgal biomass. The pigment of the algae's chlorophyll increases in the first few days. The exponential growth initiated on day 3, which is a rapid growth of biomass, continued until day 10 (initial stationary phase) in all the flasks except the 50% POME media flask. The utilization of nutrients increased by *Spirulina platensis* and produced more biomass concentration was monitored. The rate of cell division and photo-

synthesis of cells is based on nutrient availability and the surrounding environment in the presence of light. From days 10 to 15, the cultures were stationary and moving towards the log phase due to the limitation of nutrients in it and the accumulation of waste in the culture. The culture started to turn diluted form and the density of dark green began to turn pale on day 13 which referring to be the log phase of culture.

However, a high concentration of POME did not support microalgae growth due to the properties of POME, which is in the form of a black beverage and prevents light penetration through the culture. Researchers reported that the shade effect reduced the light penetration; as a result, elevated POME concentrations slow growth and require more time in the lag period for adaptation. A longer lag phase might lead to a longer exponential and stationary phase, reducing overall cell growth [14, 26, 32]. The pigment content reduction could be attributed to a significant amount of energy supplied by the oxidation of organic contents and other compounds in the medium. As a result, the light must provide less energy. As a result, the microalgal cells require less pigment in order to produce biomass. A longer period of cultivation will reduce the nutritional content of the culture media, and as a result, the biochemical compositions of microalgal biomass may change due to photoinhibition and nutrient limitation [29].

Based on Figure 2(b), the highest biomass yield was obtained in the control medium, and 30% POME yielded 1.27 ± 0.02 and $1.16 \pm 0.01\,g\,L^{-1}$, respectively, followed by 20% media with $0.91 \pm 0.02\,g\,l^{-1}$ average. Lower biomass harvested from 10% and 40% of POME medium was 0.71 and $0.59\,g\,L^{-1}$, respectively. There is less yield found in 50% of POME media culture since it has limited photoaccumulation for growth in it. The biomass was harvested at an early stage of the stationary phase in order to obtain the maximum biomass from the culture. The culture was ensured to be freshness and healthy before harvesting. From the repeated cultivation of *Spirulina platensis* in various POME, we obtained the biomass and calculated its average.

3.3. Phycocyanin Content. The effect of different concentrations of POME influences the biomass production and crude extraction of phycocyanin. The phycocyanin was extracted
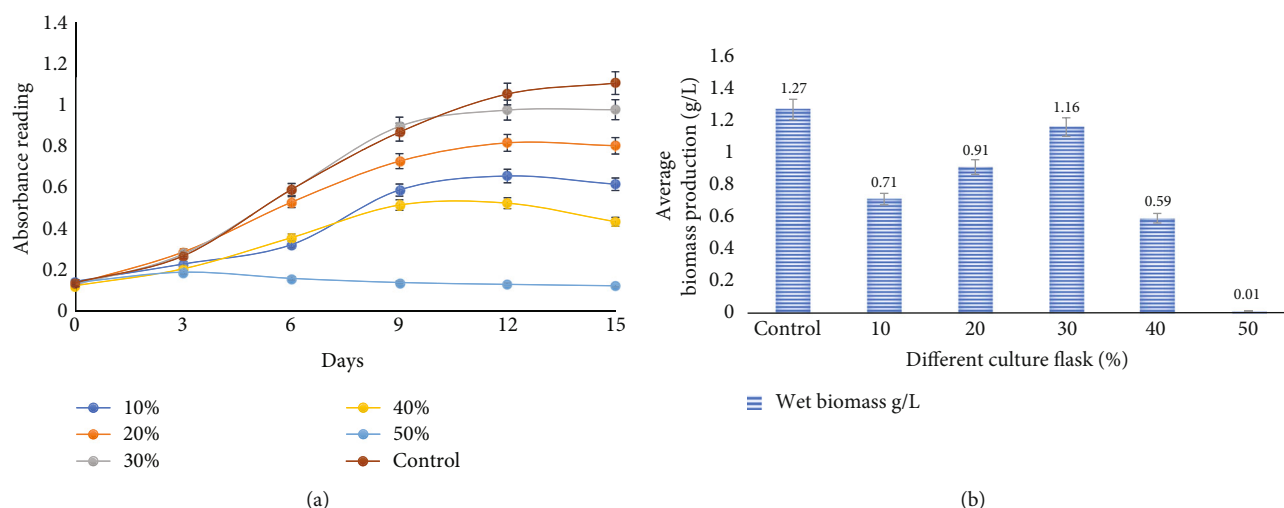
(a)

(b)

FIGURE 2: Growth of *Spirulina platensis* in different concentrations of POME was measured by (a) absorbance reading and (b) average biomass production.

from all the flasks with different concentrations of POME except 50% due to a lack of biomass production. The extracted and purified yield of phycocyanin is provided in Figure 3. The highest phycocyanin yield obtained from the biomass harvested from 30% nutrient media was 175.12 ± 22.32 mg, followed by 163.55 ± 20.15 mg from control media with purified phycocyanin of 87.11 ± 5.20 mg and 85.31 ± 4.33 mg, respectively. The cells were disrupted by freezing and thawing cycles to minimize the loss of phycocyanin. When the intracellular fluid freezes, ice crystals develop, increasing the cell volume, which is followed by cell contraction after thawing. Furthermore, due to variations in electrolyte centration in certain places, freezing causes changes in cell membrane pressure conditions and osmotic chocks, adding to cell membrane damage [33, 34]. According to the literature, the freeze-thawing approach is commonly used to extract phycocyanin from fresh biomass, which improves extraction efficiency. However, literature reported that the loss of phycocyanin in *Spirulina platensis* was estimated to be 50% due to various drying parameters such as temperature and drying duration. The considerable loss of phycocyanin in dried samples could be attributed to its peripheral position in phycobilisomes on the thylakoid membrane as well as its temperature sensitivity [20, 35]. Therefore, the crude phycocyanin content was investigated from the biomass harvested from the different nutrient media cultures.

There are four steps such as crude extract preparation, ammonium sulphate precipitation, dialyses, and anion exchange chromatography are performed and obtained purified phycocyanin from the extract. The crude extract and purified phycocyanin were scanned using a UV-VIS spectrophotometer at a wavelength between 300 and 800 nm. Based on the analysis, the presence of protein, chlorophyll, and carotenoids was found, as shown in Figure 4. There is a big peak was found at a wavelength between 590 and 620 nm which reflects the presence of phycocyanin in a large amount. Because both pigments are found in the thylakoid
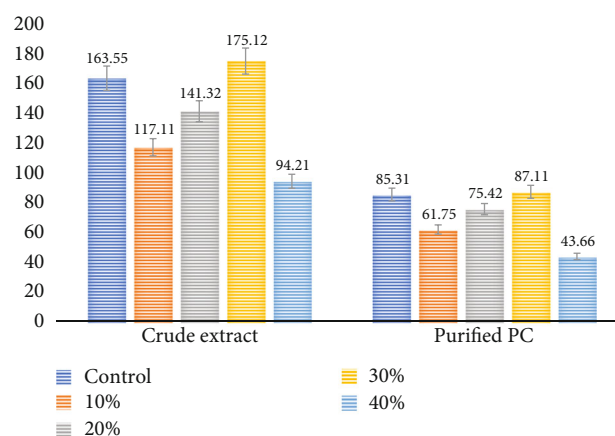

FIGURE 3: The extracted crude and purified phycocyanin (mg) from *Spirulina platensis*.

membrane, the presence of carotene and chlorophyll in a crude extract is relatively similar. The limited solubility of the chemicals resulted in a weak and expanding absorbance pattern. Munawaroh et al. [27] reported that phycocyanin is present at peak absorbance at 619 nm which is similar. The type, amount, and content of bilin that is covalently linked to the protein also influence the absorbance pattern of phycocyanin [36]. Phycocyanin has three chromophores (bilin) groups in each of its monomers, with a maximum absorbance of 600 nm [37]. Each monomer is made up of subunits. There is one bilin in the subunit, while there are two bilins in the subunit as reported [27, 38].

3.4. FTIR Analysis. FT-IR spectra analysis of purified phycocyanin from *Spirulina platensis* is shown in Figure 5. There are fifteen peaks found in the FTIR spectrum with changes in intensity at % T. The peak properties of phycocyanin such as the amide group at $1655.17 \, \text{cm}^{-1}$ (C=O stretching), the position and form of the amide I band are used to analyse
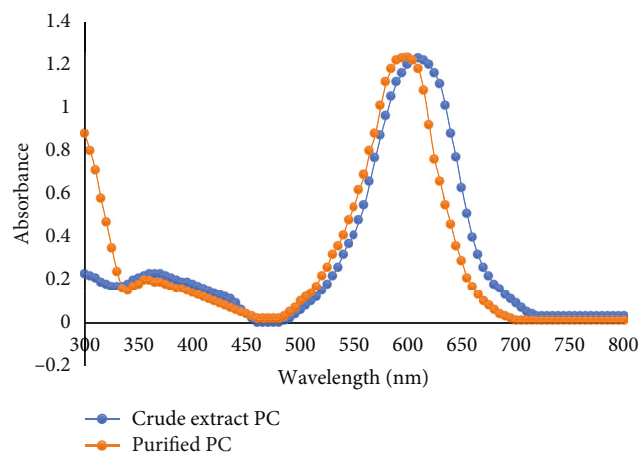
FIGURE 4: Absorption spectrum of extracted and purified phycocyanin. The fluorescence emission was excited at 620 nm. The spectra were measured at room temperature.
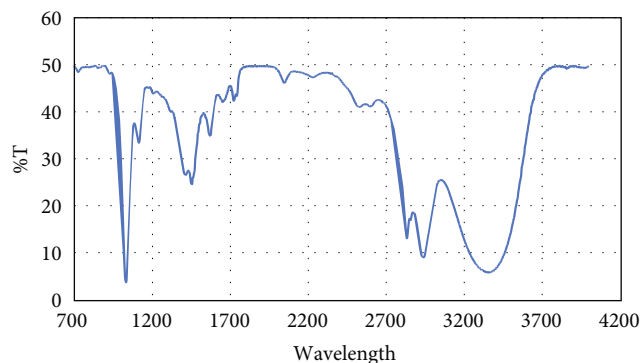

FIGURE 5: FTIR spectra of crude extract and purified phycocyanin of *Spirulina platensis*.

the secondary structure of proteins. The amide band I at $1655.17\,\mathrm{cm^{-1}}$ reflects that the a-helix is the primary secondary structural element. There is a sharp peak at $3310.12\,\mathrm{cm^{-1}}$ derived from alcohols (O-H stretch). Patel et al. [39, 40] reported that IR spectra revealed the absence of inorganic contaminants, as well as the presence of a -helix as the primary secondary structural element and the absence of -sheets.

3.5. Effect of Lipid Extraction Method and Fatty Acid Analysis. Lipid extraction is a crucial step in the production of microalgal biodiesel. To optimise the lipid production from the dried biomass, three methods were applied. Figure 6 shows the total lipid extracted from the ultrasound, Bligh and Dyer, and Soxhlet extraction methods and the fatty acid methyl ester (biodiesel) yield from the extracted lipid. The greatest lipid content was obtained with the ultrasound method ($28.6 \pm 0.7\%$) as compared to Bligh and Dyer ($20.5 \pm 0.5\%$) and Soxhlet ($25.8 \pm 0.8\%$). In this study, the ultrasound method highly contributed to lipid extraction. Lipids are extracted from microalgae mostly through mass
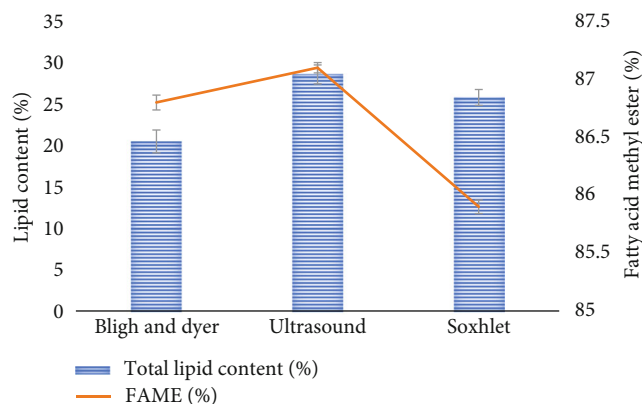

FIGURE 6: Lipid extraction and fatty acid methyl ester yield obtained from the different methods.

transfer, which is dependent on the composition of the solute and solvent, the solvent's selectivity, and the degree of convection in the medium. The experiment has been conducted three times repeatedly and obtained various results. Figure 6 provides the average value calculated. When using the Soxhlet method, a solid mass is washed with a solvent that is highly soluble and selective for the solute. Diffusion plays a major role in the Soxhlet extraction method, and the biomass is not subjected to any shear stress during the process. Therefore, a low yield was obtained from the Soxhlet as compared to Ultrasound. The findings demonstrate that relying solely on the slow and poor oil-yielding mechanism of lipid diffusion across the cell membrane, Bligh and Dyer are high-potential methods for lipid extraction according to many kinds of literature [34, 41, 42]. However, it produces a lower yield since it is not supported by ultrasound-assisted in order to enhance the cell disruption by the cavitation process. Araujo et al. [42] reported that the old method does not involve any shear stress to break down the cells, and it produces a lower yield of lipids.

Besides that, there are numerous disadvantages to using organic solvents due to their toxic properties. These solvents can be affected negatively on the environment and human health. Therefore, it can be suggested to use green solvent to minimize the harmfulness of the solvent. The extraction techniques had no impact on the lipid composition. The gas chromatography result revealed the ultimate composition of the lipid which consists of fatty acid methyl ester, as shown in Table 2 which compares the fatty acids quantified from all the extracted lipid samples. The composition of *Spirulina platensis* can be influenced by environmental conditions due to physiochemical changes in temperature, light, and nutrient content. POME media which consist of dissolved organic substances such as nitrogen and phosphorus, highly affect the growth and composition of biomass cells. The fatty acids from all samples analysed were almost similar. There are less significant differences in terms of fatty acid quantification. The biodiesel would have a high viscosity due to the high concentration (81%) of FAMEs with a carbon chain of 18 carbons [43].

TABLE 2: Fatty acid composition of *Spirulina platensis*.

Fatty acid	Content (%)					
	10%	20%	30%	40%	50%	Standard media
Palmitic acid (C16:0)	16.54 ± 0.03	19.58 ± 0.03	18.71 ± 0.04	18.77 ± 0.03	18.78 ± 0.02	18.56 ± 0.01
Palmitoleic acid (C16:1)	25.11 ± 0.02	24.67 ± 0.01	25.52 ± 0.02	24.86 ± 0.02	25.43 ± 0.01	25.13 ± 0.01
Stearic acid (C18:0)	37.87 ± 0.01	36.85 ± 0.03	36.35 ± 0.05	38.58 ± 0.03	37.54 ± 0.01	37.10 ± 0.01
Oleic acid (C18:1)	0.34 ± 0.01	1.86 ± 0.02	3.23 ± 0.02	2.41 ± 0.01	1.34 ± 0.02	2.43 ± 0.02
Linoleic acid (C18:2)	5.34 ± 0.02	11.38 ± 0.01	11.24 ± 0.03	10.45 ± 0.02	11.84 ± 0.02	11.18 ± 0.02
Eicosanoic acid (C20:0)	1.12 ± 0.01	2.78 ± 0.02	2.24 ± 0.01	2.65 ± 0.01	2.23 ± 0.01	2.51 ± 0.01
Heneicosanoic acid (C21:1)	2.32 ± 0.01	2.65 ± 0.01	2.71 ± 0.02	2.28 ± 0.01	2.76 ± 0.01	2.32 ± 0.02
Saturated FAME	55.53 ± 0.02	59.21 ± 0.01	57.30 ± 0.02	60.00 ± 0.01	58.55 ± 0.01	58.17 ± 0.01
Unsaturated FAME	27.77 ± 0.01	29.18 ± 0.01	31.46 ± 0.01	29.55 ± 0.01	29.53 ± 0.01	29.88 ± 0.01
Polyunsaturated FAME	5.34 ± 0.02	11.38 ± 0.01	11.24 ± 0.01	10.45 ± 0.02	11.84 ± 0.01	11.18 ± 0.02

4. Conclusions

Palm oil mill effluent is a type of wastewater which contain high concentration of organic nitrogen, phosphorus and different supplement substances. These substances support and enhance the growth of microalgae. Cyanobacteria *Spirulina platensis* is a blue-green microalga with two phycobiliproteins as its primary constituents. Optimising microalgal growth and biomass productivity, extracting lipids at maximum level with efficiency, and converting it into biodiesel and value-added product as phycocyanin are long-term successful commercial processes. This work is focused on determining the concentration of diluted POME (v/v) for higher growth and biomass production of *Spirulina platensis* at optimum conditions. 30% v/v of POME concentration highly supports the growth of cells and accumulation of phycocyanin. The UV-VIS and FT-IR result confirmed the presence of phycocyanin in high quantity due to greater biomass productivity (mg/L). Gas chromatography results revealed that the potential fatty acid methyl ester is suitable for biodiesel application. Furthermore, the findings of this work can be applied to microalgae pigment production using various wastewaters, enhancing both biomass production and effluent treatment functionalities. POME contain the essential nutrients which can support the growth of *Spirulina platensis* an optimum environment.

Data Availability

The research data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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