

Research Article

Extraction of Municipal Sewage Sludge Lipids Using Supercritical CO₂ for Biodiesel Production: Mathematical and Kinetics Modeling

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Demand for determining renewable lipids feedstock for the production of biodiesel is increasing with the rapid depletion of petroleum diesel. The present study was conducted to assess the feasibility of utilizing municipal sewage sludge (MSS) as a potential lipids feedstock for biodiesel production. The lipids' extraction and separation from MSS were conducted using supercritical CO₂ (scCO₂) with varying treatment time (15–120 min), temperature (30–80°C), pressure (10–50 MPa), and addition of cosolvents (1–10 wt.%). The modified Gompertz equation and Arrhenius equation were employed to evaluate lipids' extraction and kinetics behavior from municipal sewage sludge using scCO₂. About 27% of lipids were extracted from MSS with scCO₂ at a temperature of 60°C, pressure of 30 MPa, treatment time of 60 min, and 5 wt.% of ethanol (EtOH) as cosolvent. The modified Gompertz equation was adequately fitted with experimental data of the lipids' extraction from MSS using scCO₂. The kinetics properties analyses revealed that the scCO₂ extraction technology was highly dependent on pressure than the temperature for the extraction of the lipids from MSS. The physicochemical characteristics and fatty acid contents of the scCO₂ extracted lipids from MSS and sewage sludge biodiesel were determined using a variety of analytical techniques. The physicochemical properties of the sewage sludge biodiesel were compared with the international standard specifications of biodiesel, such as the American Society for Testing and Materials specifications for diesel fuel (ASTM D6751) and European Standard (EN 14214) specifications.

1. Introduction

There is an increasing concern on the rapid diminution of fossil fuel energy with increasing energy consumption due to rapid population growth, urbanization, and expanding economies. It is being reported that the estimated daily consumption of diesel fuel is about 5.7 million barrels per

day, which may increase to 109.4 million barrels per day by 2040 [1]. Therefore, it is urgent to determine alternate sources of diesel fuel to reduce rising demands on petrodiesel, since energy demand and consumption patterns are expanding in an unsustainable manner [2, 3]. Renewable energy is considered a significant energy resource in many countries worldwide. This approach is dependent on natural

resources, which can produce the required energy and protect surrounding environments. The major challenge is to expand the amount of renewable energy in our existing supply system. Biofuel is an alternative energy resource that has the advantage of a flexible supply system as fossil fuel [4]. Biomass feedstock can be used to produce a range of gaseous biofuels, like hydrogen and methane, and liquid biofuels, like ethanol, methanol, and biodiesel. Biofuel is considered eco-friendly fuel as its emissions are biodegradable and create a little impact on the environment. As such, using biofuel as an energy source provides a substitute and a sustainable supply of clean energy [1, 3].

Biodiesel has become a viable substitute for petrodiesel in recent years. In addition, it is considered an eco-friendly energy resource that can meet high worldwide energy demands, achieve sustainability goals, and decrease environmental pollution [2, 5]. Generally, biodiesel can be synthesized from multiple lipids feedstock, including those generated from plants and animal fats [5, 6]. Transesterification is the main biochemical process for synthesizing biodiesel, which occurs when the alkoxy group of an ester is dislodged by the alcohol group [3]. In the process of catalytic transesterification, alcohol combines with triglycerides in the existence of an acid or basic catalyst producing biodiesel and glycerol as by-products [1, 6]. Studies reported that biodiesel isolated from edible crops, such as oil palm and grape seeds, had a similar engine performance to diesel fuel [7, 8]. Therefore, biodiesel could be utilized as a viable replacement for petroleum diesel due to its environmentally favorable features and is a secure and sustainable energy resource for the foreseeable future [9]. Municipal sewage sludge (MSS) is a by-product of a wastewater treatment plan. The concern on the safe disposal and sustainable utilization of MSS are increasing with increasing MSS generation due to the increase in urbanization and industrialization. Existing practices of treating MSS and its disposal include an application as organic fertilizer in crop production, ocean, landfill disposals, and incineration [10, 11]. These inappropriate disposal practices of MSS pose potential hazards to the environment and human health. However, the MSS contains 5–30 wt.% of lipids, which is close to the lipid content in some vegetative feedstock of lipids [10, 12]. Kwon et al. [13] reported that the biodiesel conversion of lipids extracted from MSS would be a new paradigm for mining renewable energy from municipal hazardous waste. Melero et al. [14] implemented simultaneous extraction and conversion of lipids from MSS and obtained about 15.5 wt.% biodiesel from primary MSS and about 10 wt.% from secondary MSS. Khalil et al. [15] utilized supercritical carbon dioxide (scCO₂) as a waterless extraction technology for the extraction of lipids from MSS for biodiesel production. Thus, the MSS could be utilized as a potential lipids feedstock for biodiesel production.

Supercritical carbon dioxide (scCO₂) is reported to be a promising and eco-friendly extraction technology for the extraction and separation of lipids from various matrices [16, 17]. There are some distinct advantages of the scCO₂ extraction technology over the conventional extraction technologies, including the absence of organic solvents and

eco-friendliness, and it does not produce any waste residue after extraction [3, 17]. The fluid CO₂ is considered as an ideal supercritical fluid due to its nontoxic, inflammable, lipophilic nature, low critical temperature (31.1°C), and moderate critical pressure (7.38 MPa) [2, 18, 19]. ScCO₂ has a faster reaction rate, easier lipids separation, and higher quality lipids than solvent extraction, as indicated by many studies [3, 19, 20]. Studies have been conducted for the extraction of lipids from MSS using conventional solvent extraction methods [21, 22]. For example, Zhu et al. [21] used soxhlet extraction, acid hydrolysis, and water-bath shaking to extract lipids from sewage sludge. The percentage of lipids' extraction obtained was 1.30%, 6.35%, and 4.10% for acid hydrolysis, soxhlet extraction, and water-bath shaking, respectively. There are rare studies in the literature on the extraction and separation of lipids from MSS using scCO₂ extraction technology. Therefore, the present study was conducted to determine the influence of scCO₂ on lipids' extraction from MSS. The modified Gompertz mathematical model and Arrhenius equations were employed to elucidate the extraction behavior and kinetics for the lipids' extraction from MSS. Subsequently, biodiesel was produced from the lipids extracted with scCO₂ in the alkaline catalytic transesterification process. The physicochemical properties and fatty acid compositions in scCO₂ extracted lipids from MSS and sewage sludge biodiesel were determined with various analytical means. The physicochemical properties of the sewage sludge biodiesel were then compared with the biodiesel standards specifications of EN 14214 and ASTM D6751. The present study's findings will be a harmony in implementing the sustainable deployment of MSS for biodiesel production.

2. Materials and Methods

2.1. Sludge Sampling and Preparation. Primary MSS was collected from Indah Water Konsortium, Lebu Permai, Pulau Pinang, Malaysia. The sludge collected from the wastewater treatment plant was concentrated by settling. Subsequently, the concentrated sludge was dewatered by centrifuging at 1968 g for 10 min. The dewatered sludge was then oven-dried overnight at 70°C and mechanically pounded and sieved to fine particle size (0.5–1.0 mm). The fine sludge was stored at 4°C prior to further studies.

2.2. Extraction of Lipids from Municipal Sewage Sludge by scCO₂. The lipids from MSS were extracted using scCO₂ with varying temperature (32–80°C), pressure (10–50 MPa), treatment time (15–120 min), and cosolvents (H₂O, C₂H₅OH, and H₂O₂). Commercial liquefied CO₂ with a purity of 99% was used as a solvent to extract lipids from MSS. The scCO₂ reactor used in the present study comprised an extraction vessel (300 mL), a chiller, a reservoir to recycle CO₂, a CO₂ pressurize pump, and a separation vessel. A certain amount of MSS was placed into the extraction vessel, and the temperature of the extraction vessel and separation vessel was set to the desired experimental temperature. Once the temperature of extraction and separation vessels reached

set temperatures, the liquefied CO₂ was pumped into the extraction vessel to obtain the desired pressure. After the extraction time elapsed, the extracted lipids from MSS were collected from the separation vessel. The experiments were conducted three times, and the outcomes were presented as means ± standard error. The percentage of lipids extracted from MSS using scCO₂ was determined using the following equation.

$$\text{Lipids (\%)} = \frac{\text{Mass of lipids (g)}}{\text{Mass of MSS (g)}} \times 100. \quad (1)$$

2.3. Modified Gompertz Mathematical Model. Gompertz equation is used to describe a sigmoidal curve, which includes the lag phase, growth phase, and asymptotic phase. Generally, the Gompertz equation can be expressed as follows.

$$Y = a \exp[-\exp(b - ct)], \quad (2)$$

where T is the yield of extracted lipids subjected to scCO₂ extraction from MSS. However, equation (2) contains three important parameters, such as a , b , and c . It may be problematic to differentiate these parameters because of the difficulties in calculating the start value of these parameters of the lipids' extraction. In order to elucidate the lipids' extraction from MSS using the Gompertz equation, it is important to reparameterize the equation with the more meaningful parameters of λ (lag phase), k_m (extraction rate), and A (asymptote value). To obtain the inflection point of the curve, equation (2) can be derivatized as

$$\frac{dY}{dt} = ac \exp\{-\exp(b - ct)\} \exp(b - ct), \quad (3)$$

$$\frac{d^2Y}{dt^2} = ac^2 \cdot \exp\{-\exp(b - ct)\} \exp(b - ct) \{-\exp(b - ct)\}. \quad (4)$$

At inflection point, $t = t_i$. Thus, the second derivative can be equal to zero. So, we can write it as

$$\frac{dy}{dt} = 0 \longrightarrow t_t = \frac{b}{c}. \quad (5)$$

An expression of the lipids' extraction rate (k_m) can be derived by calculating the first derivative in equation (3) at an inflection point.

$$k_m = \left(\frac{dY}{dt} \right)_{t_i} \quad (6)$$

$$= \frac{ac}{e}, \quad (7)$$

$$c = \frac{k_m e}{a}$$

where e is the dummy constant. Thus, the parameter c in the Gompertz equation can be substituted by $c = k_m e/a$. The description of the tangent line through the inflection point is

$$Y = k_m t + \frac{a}{e} - k_m t_i. \quad (8)$$

The lag time is defined as the t -axis intercept of the tangent through the inflection point. Thus,

$$0 = k_m t + \frac{a}{e} - k_m t_i. \quad (9)$$

From equations (6)–(9), the lag page can be expressed as below.

$$\lambda = \frac{b - 1}{c}. \quad (10)$$

Thus, the parameter b in the Gompertz equation can be substituted as

$$b = \frac{k_m e - 1}{a} \lambda + 1. \quad (11)$$

Assume that the asymptotic value will be reached as t approaches infinity; thus,

$$t \longrightarrow \infty; y \longrightarrow a \Rightarrow A = a. \quad (12)$$

By substituting the parameter values of “ a ,” “ b ,” and “ c ” in the Gompertz equation, yielding the modified Gompertz equation can be expressed as below [23].

$$Y = A \exp \left[-\exp \left\{ \frac{k_m e}{A} (\lambda - t) + 1 \right\} \right], \quad (13)$$

where Y is the yield of extracted lipid (g/100 g sludge) subjected to scCO₂ extraction. A is the higher asymptote, k_m denotes the maximum extraction rate (g/min), λ refers to the lag phase, and t represents the treatment time. t_i refers to the time length required to obtain the maximum lipids' extraction from MSS. The t_i can be calculated using the following equation:

$$t_t = \lambda + \frac{A}{k_m}. \quad (14)$$

The values for A , λ , and k_m were determined using experimental data. However, the estimation of the starting value for computation of λ , k_m , and A was carried out using graphical software, namely, Kaleida Graph 4.5.4 (Synergy software ver. 2021). The model fitting accuracy was determined using the regression coefficient (R^2) values at the 95% confidence level.

2.4. Biodiesel Production from Municipal Sewage Sludge. Extracted MSS lipids may contain high free fatty acids (FFAs) with high levels of acidity; therefore, the pretreatment of MSS lipids to remove FFAs and acids values is crucial prior to conversion into biodiesel [20]. The current study's pretreatment of MSS lipids was accompanied by the sulfuric acid (purity 96%) esterification process, utilizing methanol as a reactant. The experiment was performed using a 250 mL three-neck reactor equipped with a reflux condenser. The experiment was conducted at 60°C for 6 h with a lipid, reactant, and catalyst ratio of 20:4:41 in a weight basis. After the stipulated time period, the esterified lipids

were collected and centrifuged at 1968 g for 10 min. The methanol layer was discarded, and the esterified lipids were washed three times with deionized water. Subsequently, vacuum evaporation at 105°C was applied to remove the water content from the lipids.

The esterified MSS lipids yielded biodiesel after undergoing alkaline transesterification process, with methanol as solvent and NaOH as a catalyst. Methanol was mixed with NaOH (1 wt.%) in three-neck reactors equipped with a reflux condenser. The esterified oil was then taken into the reactor with a lipids and methanol ratio of 1 : 5 in a weight basis and heated at 60°C for 4 h. After the transesterification process had elapsed, the produced biodiesel mixture was taken in the separation funnel and allowed to settle down the glycerol in the bottom. Subsequently, the methyl ester layer was separated and the left-over methanol and water were evaporated by heating at 110°C. The percentage yield of biodiesel has been calculated using the following equation.

$$\text{Yield (\%)} = \frac{\text{Amount of Biodiesel produced}}{\text{Amount of MSS lipids}} \times 100. \quad (15)$$

2.5. Characterization. The physicochemical properties and fatty acid compositions in scCO₂ extracted lipids from MSS and sewage sludge biodiesel were determined. The percentage of moisture content in scCO₂ extracted lipids and biodiesel was identified with test method of AOAC 930.15. The density of MSS lipids and sewage sludge biodiesel was specified through the hydrometer method in accordance with the standard method of ASTM D1298. A viscometer measured kinematic viscosity in line with the standard test of ASTM D445. Acid value of both lipids and biodiesel was then determined by the AOAC 940.28 test method. The phenolphthalein indicator (3 drops) was added to a mixture of 10 mL EtOH and 1 g of sample. The mixture was then titrated using 0.1 N potassium hydroxide. Mixture turned pink in colour, and the titration process was stopped. Subsequently, acid value was identified through the use of equation (5).

$$\text{Acid value} = \frac{56.1 \times N \times V}{m}, \quad (16)$$

m denotes the mass of lipids, N and V refer to the normality of KOH and its volume. Lipids and biodiesel FFA levels' calculation is as in equation (6).

$$\text{FFAs} = \frac{\text{Acid value}}{1.989}. \quad (17)$$

The bomb calorimeter method was utilized to determine the calorific values of sewage sludge biodiesel and MSS lipids in line with ASTM D5865. The Pensky–Marten apparatus was used to gauge flashpoint of sewage sludge biodiesel and MSS lipids. ASTM D613 standard test then determined the cetane number of sewage sludge biodiesel. Biodiesel and MSS lipids saponification values were measured via AOAC920.160 test. Iodine levels of MSS biodiesel and lipids were identified with AOAC 920.159 testing. Pour points and cloud points of both biodiesel and MSS lipids were then

estimated with ASTM D2500 and ASTM D7683 standard methods. Gas chromatography (GC), equipped with the flame ionization detector (GC-FID), was used to determine the fatty acids compositions in sewage sludge biodiesel and MSS lipids. Approximately 0.05 μg of sewage sludge lipids or biodiesel had been taken into GC capillary column at a split ratio of 1 : 10. The initial oven temperature was set to 40°C, and then the temperature was raised to 100°C at a rate of 10°C/min. The oven temperature of 100°C was held for 15 min and then increased to 150°C and held for 3 min. Ultimately, the final temperature was increased to 240°C with a rising rate of 5°C per min. The injector and detector temperatures were fixed at 200°C and 220°C, respectively.

3. Results and Discussion

3.1. Lipids' Extraction from Municipal Sewage Sludge.

Figure 1 shows the extraction of lipids from MSS using the scCO₂ with varying temperatures (30–80°C), pressure (10 MPa–50 MPa), treatment times (15–120 min), and addition of cosolvents. The influence of scCO₂ pressure (10 MPa–50 MPa) on the lipids' extraction from municipal sewage sludge was determined at a treatment time of 60 min and a temperature of 60°C, as shown in Figure 1(a). It was found that the percentage of lipids' extraction increased with increasing the scCO₂ pressure from 10 MPa to 30 MPa. However, the percentage of lipids' extraction was negligible with increasing pressure from 30 MPa to 50 MPa. About 8% of lipids was extracted at scCO₂ pressure of 10 MPa, and the lipids' extraction was increased to 20.34 ± 0.40% at 30 MPa. However, the lipids' extraction was further increased to 21.36 ± 0.38% at a pressure of 50 MPa. The percentage of lipids' extraction increased with increasing temperature from 32°C to 80°C at scCO₂ pressure of 30 MPa and treatment time of 60 min (Figure 1(b)). The highest lipids' extraction obtained was 21.35 ± 0.42% at 80°C for the constant pressure of 30 MPa and treatment time of 60 min. The influence of the treatment time on the percentage of lipids' extraction from municipal sewage sludge using scCO₂ was determined at a pressure of 30 MPa and a temperature of 60°C (Figure 1(c)). It was found that the percentage of lipids' extraction was increased rapidly with increasing treatment time from 15 min to 60 min and slightly increased from 60 min to 120 min. The lipids' extraction obtained was 3.67 ± 0.21% at a treatment time of 15 min, which was substantially increased to 20.34 ± 0.41% at a treatment time of 60 min, and the lipids' extraction was further increased to 22.58 ± 0.26% at the treatment time of 120 min.

Extraction efficiency from MSS with scCO₂ depends on the solvating properties of fluid CO₂ in the supercritical state [24, 25], wherein the pressure and temperature have a significant impact on the CO₂'s solvating characteristics [25]. The increase of scCO₂ pressure increases the extraction because of the increased density of the fluid CO₂ [4, 26]. The distance between the fluid CO₂ and lipids molecules decreases with increasing density of the CO₂, which increases the collision between the fluids CO₂ and lipids molecules. These substantially increase interaction between the fluid CO₂ and lipids molecules, causing more lipids' extraction.

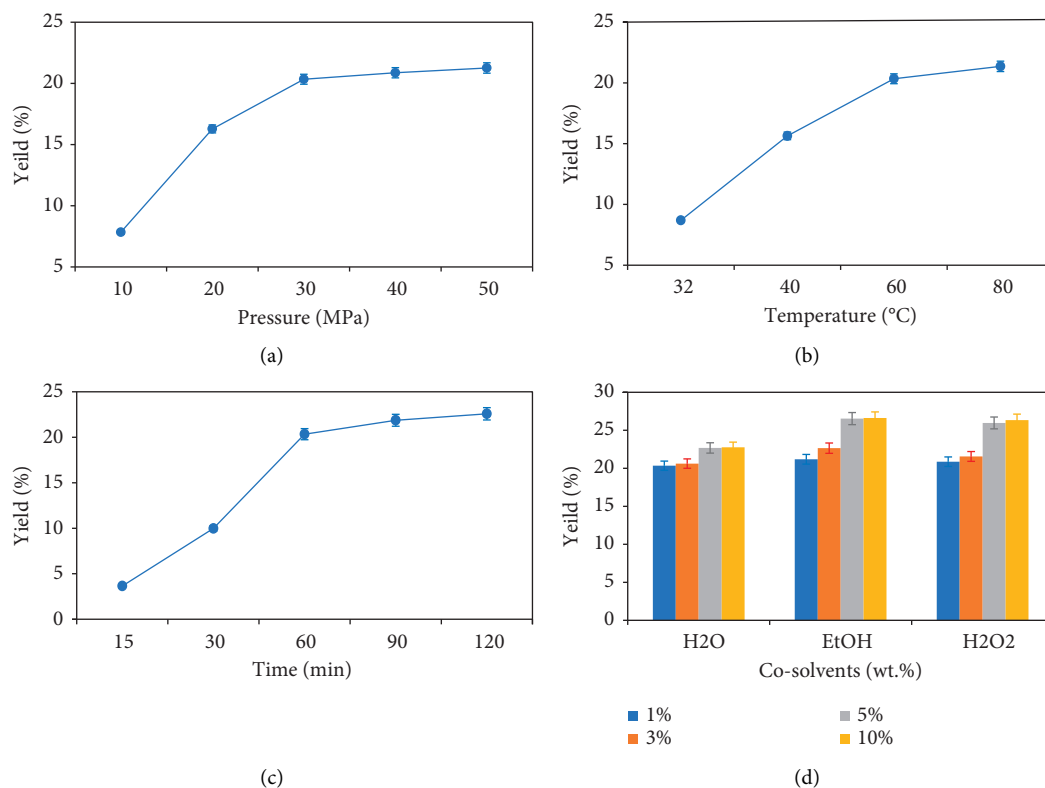


FIGURE 1: Lipids' extraction from municipal sewage sludge using scCO₂. (a) Effects of pressure, experimental conditions: temperature of 60°C and treatment time of 60 min. (b) Effects of temperature, experimental conditions: pressure of 30 MPa and treatment time of 60 min. (c) Effects of treatment times, experimental conditions: pressure of 30 MPa and temperature of 60°C. (d) Effect of cosolvents, experimental conditions: pressure of 30 MPa, temperature of 60°C, and treatment time of 60 min.

Moreover, the increase in temperature increases the fluidity of CO₂, which increases the solubility of lipids in CO₂, and therefore, lipids' extraction increases [25, 27]. Besides, the lipids' extraction increases with increasing treatment time because it requires sufficient treatment time to interact between the fluid CO₂ and lipids molecules. However, extraction percentages were negligible over scCO₂ pressure of 30 MPa, the temperature of 60°C, and treatment time of 60 min, which might be due to the saturation of solvent power of the fluid CO₂ with the further increase of scCO₂ pressure and temperature [26]. Besides, the nonpolar and lipophilic nature of fluid CO₂ might make it unable to extract polar fatty acids [17, 26]; therefore, extraction percentage declined when scCO₂ pressure increased at temperatures of over 30 MPa and 60°C, respectively.

Since CO₂ is a nonpolar solvent, it is limited in the solubility of polar fatty acid components [16, 23]. However, the inclusion of the cosolvent as a chemical modifier would enhance the lipids' extraction because of the increase in the solubility of lipids [19]. In the current study, the influence of cosolvents on MSS lipids' extraction using scCO₂ is determined by adding H₂O, EtOH, and H₂O₂ as cosolvents at cosolvents amounts of 1wt.% to 10 wt.%, the pressure of 30 MPa, temperature of 60°C, and treatment time of 60 min, as shown in Figure 1(d). It was found that the addition of cosolvents potentially increased the percentage of lipids' extraction from 1 wt.% to 5 wt.%; thereafter, the percentage

of lipids extracted was negligible with a further increase of cosolvent from 5 wt.% to 10 wt.%. However, EtOH and H₂O₂ had shown almost similar results in lipids' extraction from MSS using scCO₂. The highest lipids' extraction obtained was about 27%, 26%, and 23% using EtOH, H₂O₂, and H₂O as cosolvents, respectively. Similarly, Jafarian et al. [28] reported that the inclusion of 5% EtOH as a cosolvent increased the scCO₂ extraction of tocopherols and phytochemicals from rapeseed oil waste.

3.2. Mathematical Modeling for scCO₂ Extraction of Lipids from MSS. The modified Gompertz equation was utilized to assess the scCO₂ extraction of lipids from MSS with varying pressure (10–40 MPa) and temperature (32–80°C) as a function of treatment time as presented in Figure 2. The lipids' extraction curves, obtained from the modified Gompertz mathematical model, were alienated into three distinct phases, namely, the extraction phase, lag phase (λ), and stationary phase. Figure 2(a) shows the influences of the scCO₂ pressure with a treatment time of 0–180 min, at the constant temperature of 32°C and EtOH loading of 5 wt.%. The extraction of lipids was increased with increasing pressure and reached the asymptote (time required to reach the optimal lipids' extraction) at a treatment time of 105 min, 90 min, 75 min, and 60 min for the pressure of 10 MPa, 20 MPa, 30 MPa, and 40 MPa, respectively. The estimation of

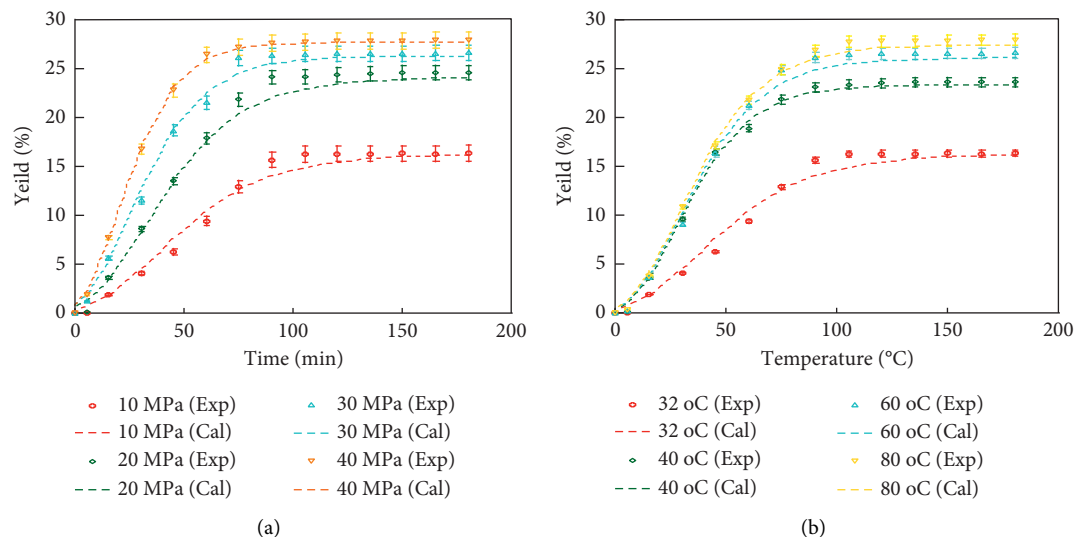


FIGURE 2: Effect of scCO_2 pressure (a) and temperature (b) on the extraction lipids from municipal sewage sludge. Exp: experimental data, Cal: calculated values by fitting the modified Gompertz equation to experimental data.

TABLE 1: Estimated the kinetics parameters of the modified Gompertz equation for the extraction of lipids from municipal sewage sludge (MSS), with varying scCO_2 pressures.

Pressure (MPa)	A (%)	k_m (min^{-1})	λ (min)	t_t (min)	R^2
10	16.32	0.184	8.7	97.39	0.9938
20	24.16	0.319	7.6	83.33	0.9921
30	26.36	0.369	4.9	76.34	0.9877
40	27.78	0.454	3.6	64.79	0.9863

kinetics parameters of the modified Gompertz equation for the extracted MSS lipids at different scCO_2 pressures and treatment times of 0–180 min at the constant temperature of 32°C and EtOH loading of 5 wt.% is shown in Table 1. It was found that the λ value was diminished by increasing pressure from 10 to 40 MPa. Conversely, the lipids' extraction rate (k_m) increased by increasing pressure from 10 to 40 MPa. The R^2 values obtained were over 0.98, demonstrating the good agreement between experimental data and estimated values for the extraction of lipids from MSS. The calculated total extraction times (t_t) for the lipids' extraction reached to the asymptote were close to the experimental values of lipids' extraction time for obtaining the asymptote.

Figure 2(b) shows the influence of temperature on MSS lipids at varying treatment times of 0–180 min at scCO_2 pressure of 10 MPa and EtOH loading of 5 wt.%. The extraction of lipids increased with enhancing temperature from 32°C to 80°C . The required treatment time to reach asymptote decreases with increasing temperature and reached the asymptote at a treatment time of 105 min, 105 min, 90 min, and 90 min for the scCO_2 temperature of 32°C , 40°C , 60°C , and 80°C , respectively. Table 2 displays the estimated kinetics parameters of the modified Gompertz equation for the extraction of MSS lipids at different scCO_2 temperatures at pressure 10 MPa and EtOH loading of 5 wt.%. The k_m values were increased from 0.164 min to 1 to

TABLE 2: Estimated kinetics parameters of the modified Gompertz equation for MSS lipids' extraction at various scCO_2 temperatures.

Temperature ($^\circ\text{C}$)	A (%)	k_m (min^{-1})	λ (min)	t_t (min)	R^2
32	16.32	0.164	8.7	108.21	0.9938
40	23.42	0.213	8.1	102.05	0.9294
60	26.18	0.314	7.8	91.17	0.9573
80	27.53	0.365	7.2	82.624	0.9984

0.365 min^{-1} with increasing temperature from 32°C to 80°C . Instead, the λ value was decreased from 8.7 min to 7.2 min with increasing temperature from 32°C to 80°C . The estimation of R^2 values (0.9573–0.9984) indicated that the experimental data were fitted with estimated data for the lipids' extraction from MSS. The calculated t_t values (time required to reach optimal lipids' extraction) were close to those of experimental data.

3.3. Analyses' Dependence of Temperature Using Arrhenius Equation. The dependence of temperature on the extraction of lipids from MSS was determined using the Arrhenius equation, as shown in equations (7) and (8).

$$k_m = a \cdot e^{-E_a/RT}, \quad (18)$$

$$\ln k_m = \ln a - \frac{E_a}{RT}, \quad (19)$$

where E_a denotes the activation energy (kJ/mol), T indicates the absolute temperature, a indicates the preexponential factor (min^{-1}), and R is the ideal gas constant (8.314 J/mole. K). The dependence of temperature for the extraction of lipids from MSS using scCO_2 is shown in Figure 3. From Figure 3, the estimated E_a value was determined to be 14.82 kJ/mole for the extraction of lipids from MSS using scCO_2 . Kassim et al. [29] determined the activation energy of

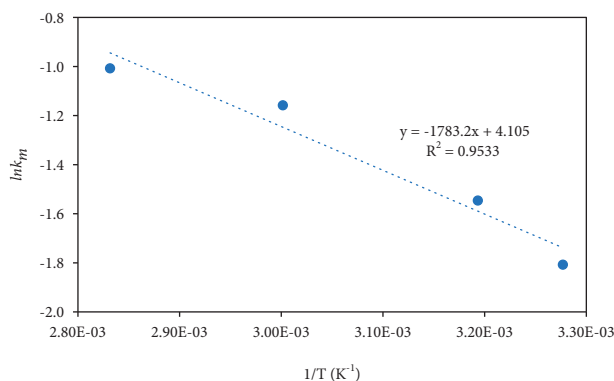


FIGURE 3: Dependence on temperature for the extraction of lipids from municipal sewage sludge (MSS) with scCO_2 at pressure of 10 MPa.

TABLE 3: Physicochemical properties of ScCO_2 extracted lipids from municipal sewage sludge (MSS) and sewage sludge biodiesel.

Properties	Unit	Lipids	Biodiesel	ASTM D6751	EN 14214
Yield	%	26.54 ± 0.21	84 ± 2	—	—
FFAs	%	0.81 ± 0.16	0.16 ± 0.06	—	—
Kinematic viscosity at 40 °C	mm^2/s	6.18 ± 0.21	4.10 ± 0.14	1.9–6.0	3.5–5.0
Density at 15 °C	kg/m^3	840 ± 6	865 ± 4	880	860–900
Saponification value	mgKOH/g	196 ± 2.54	158 ± 2.54	—	—
Acid value	mgKOH/g	0.50 ± 0.04	0.20 ± 0.02	≤ 0.5	≤ 0.5
Heating value	MJ/kg	39.20 ± 1.4	39.64 ± 2.86	—	—
Cetane number	—	56.10 ± 2.7	56 ± 2	≥ 47	≥ 51
Pour point	°C	8.5 ± 0.5	-10.50 ± 0.65	-15 to 16	—
Cloud point	°C	12.5 ± 0.5	-2.50 ± 0.14	-3 to 12	—
Heating value	MJ/kg	39.20 ± 1.4	39.64 ± 2.86	—	—
Iodine number	$\text{g I}^2/100 \text{ g}$	69.10 ± 2.80	75.9 ± 2.34	—	≤ 120
Flash point	°C	166 ± 2.10	140 ± 4	≥ 130	≥ 101

TABLE 4: Fatty acid compositions of scCO_2 extracted lipids from municipal sewage sludge (MSS) and sewage sludge biodiesel.

Fatty acids	Carbon group	Concentration (%)	
		Lipids	Biodiesel
Lauric acid	(C12: 0)	0.52 ± 0.02	0.24 ± 0.02
Myristic acid	(C14: 0)	1.22 ± 0.06	0.81 ± 0.04
Palmitic acid	(C16: 0)	37.61 ± 2.18	30.20 ± 2.14
Palmitoleic acid	(C16: 1)	0.34 ± 0.01	0.26 ± 0.01
Stearic acid	(C18: 0)	4.20 ± 0.22	4.62 ± 0.17
Oleic acid	(C18: 1)	38.55 ± 3.64	38.62 ± 2.58
Linoleic acid	(C18: 2)	14.68 ± 1.08	23.81 ± 1.56
Linolenic acid	(C18: 3)	1.92 ± 0.12	0.31 ± 0.01
Arachidic acid	(C20: 0)	0.32 ± 0.02	0.40 ± 0.02
Eicosenoic acid	(C20: 1)	0.21 ± 0.01	0.20 ± 0.01
\sum Saturated fatty acids		43.87 ± 2.58	36.27 ± 2.16
\sum Unsaturated fatty acids		55.7 ± 2.62	63.20 ± 2.38

301.70 kJ/mol to extract lipids from *Chlorella* sp. using the pyrolysis process. Ashokkumar et al. [30] obtained the activation energy of 36.72 kJ/mol for solvent lipids' extraction from microalgae with methanol/chloroform as solvent. The minimal E_a value obtained in the present study reveals that the scCO_2 is a less dependent technology for MSS lipids' extraction. Similar to this, Ahmadkelayeh et al. [26] referred to the fact that the scCO_2 is indeed a high-pressure

extraction type of technology, which is highly dependent on the pressure as compared to temperature, for the extraction of lipids.

3.4. Characterization of Municipal Sewage Sludge Lipid and Biodiesel. A biodiesel amount of about 84 ± 2 wt.% was obtained by extracting lipids from the sewage sludge, with

TABLE 5: Comparison of the physicochemical properties of sewage sludge biodiesel with other biodiesel obtained from edible and nonedible Feedstock.

Biodiesel	Density at 15°C (kg/m ³)	Viscosity at 40°C (mm ² /s)	Acid value mg (KOH)/m ³	Cloud point (°C)	Pour point (°C)	Heating value (MJ)/Kg	Flashpoint (°C)	Iodine number g I ₂ /100 g	Cetane number	Reference
Palm	880	4.52	0.25	14.25	14.33	34.41	175	50.5		[34]
Soybean	883	6.75	0.11	N/A	-7.0	N/A	120	N/A	54.60	[35]
Sunflower	860	4.72	0.07	4.0	-5.0	N/A	183	N/A	N/A	[36]
Canola	852	4.74	0.56	—	—	—	217	N/A	N/A	[37]
<i>Jatropha curcas</i> L.	867	4.01	0.04	N/A	N/A	36.66	165	N/A	40.01	[38]
Animal fat	870	4.82	0.07	N/A	-2.0	N/A	149	77.7	N/A	[39]
Waste cooking oil	876	3.66	N/A	N/A	N/A	39.76	160	N/A	63.88	[34]
Algae	872	5.82	0.40	N/A	-16	40.80	N/A	N/A	50.54	[40]
Chicken fat	875	4.60	0.43	2.0	-5.0	N/A	168	N/A	N/A	[41]
Jojoba	868	24.84	0.71	N/A	N/A	46.47	285	50.2	58.25	[21]
MSS	875	4.10	0.50	-2.5	-10.50	39.64	140	75.9	N/A	Present study

*N/A: not available.

scCO₂ through the alkaline catalytic transesterification process, utilizing NaOH as a catalyst at lipid-to-methanol mixture of 1 : 6, catalyst loading of 1 wt %, reaction temperature 60°C, and reaction time of 4 h. However, Patiño et al. [31] obtained about 33% biodiesel from the secondary sewage sludge lipids using Amberlite IR-120 IR120 as a catalyst. Zhu et al. [21] obtained approximately 86% of biodiesel from the MSS lipids via a catalytic transesterification process. Table 3 shows the physicochemical properties of scCO₂ extracted lipids from sewage sludge and the biodiesel produced. Moreover, biodiesel physicochemical properties were compared to other standards of biodiesel, including ASTM D 6751 and EN 14214.

The kinematic viscosity of scCO₂ extracted lipids from MSS and biodiesel was 6.18 ± 0.21 mm²/sec and 4.10 ± 0.14 mm²/sec, respectively, wherein the density of lipids and biodiesel was 840 ± 6 and 865 ± 4 kg/m³, respectively. It was found that the density of sewage sludge biodiesel complied with stipulated standard limits set by EN 14214. Lipids and biodiesel acid levels are determined as 0.50 ± 0.04 mg KOH/g and 0.32 ± 0.02 mgKOH/g, respectively. However, the acid value of the MSS biodiesel sewage sludge biodiesel was below standard limits set by EN 14214 and ASTM D 6751. The percentage of FFAs content in lipids and biodiesel was $0.25 \pm 0.11\%$ and 0.16 ± 0.06 , respectively. Abdulhussein Alsaedi et al. [32] reported that the acid value for biodiesel derived from soxhlet extracted lipids from MSS was 2.60 mg KOH/g, which was higher than the ASTM standard. Thus, it can be postulated that the scCO₂ extraction method has privileged over soxhlet extraction for the extraction of lipids from MSS with lower FFAs and acid values. Saponification values of both sewage sludge lipids and biodiesel were 196 ± 2.54 mgKOH/g and 158 ± 2 mgKOH/g, respectively. Iodine numbers for sewage sludge lipids and biodiesel were found to be 76.83 ± 5.68 g I₂/100 g and 75.9 ± 2.34 g I₂/100 g, respectively. But iodine number for sewage sludge biodiesel was lower than the concentration reported by EN14214. Flashpoints, cloud points, and pour points complied with standard limitations of biodiesel reported by EN 14214 and ASTM D6751 standards. Sewage sludge biodiesel cetane number was 56 ± 2 , higher than the standard specifications of EN 14214 and ASTM D6751, indicating that biodiesel will burn more quietly and smoothly in a diesel engine than petroleum diesel.

Fatty acid properties analyses of scCO₂ extracted lipids from MSS and sewage sludge biodiesel were conducted using GC-FID, as shown in Table 4. Both scCO₂ extracted lipids and sewage sludge biodiesel contain more quantity of unsaturated fatty acids than saturated fatty acids. The major fatty acids determined in scCO₂ extracted lipids and sewage sludge biodiesel were palmitic acid (C16 : 0), oleic acid (C18 : 1), linoleic acid (C18 : 2), and stearic acid (C18 : 0). Similarly, D'Ambrosio and others [33] found that stearic acid, palmitic acid, linoleic acid, and oleic acid were the major fatty acids detected in lipids extracted from MSS using the solvent of ethyl butyrate. The total identified fatty acids for lipid extracted by scCO₂ was 99.57 compared with Abdulhussein Alsaedi et al. [32] that showed total identified fatty acids for

sewage sludge from soxhlet extraction at 98.78. This result proves the superiority and efficiency of scCO₂ to extract lipids from MSS, which is higher than the soxhlet extraction method by 0.80%. Those findings were confirmed by Pradhan et al. [33] found that the scCO₂ extraction method was better with a total identified component of fatty acids at 96.8%, compared to solvent extraction and screw press of 92.5% and 95%, respectively.

Table 5 compares the physicochemical properties of sewage sludge biodiesel with biodiesel obtained from edible and nonedible feedstocks, such as animal fat, palm oil, soybean oil, canola oil, waste cooking oil, algal oil, and jojoba oil. It was observed that the MSS biodiesel physicochemical properties, such as density, acid value, pour point, heating value, iodine number, and cetane number, were almost similar to biodiesel obtained from edible and nonedible feedstocks. The current study findings reveal that MSS has the possibility to be used as a lipids feedstock for the production of biodiesel. Implementation of scCO₂ is a green approach for lipids' extraction that does not generate wastewater and residual waste. Besides, using MSS lipids for biodiesel production would be employed for the beneficial purposes of (i) obtaining a cheap source of biodiesel feedstock, (ii) defining an alternative and sustainable feedstock for biodiesel, (iii) minimizing the volume of solid waste disposed of in landfills, (iv) minimizing pollution of the environment, and (v) enhancing the sustainable usage of environmental solid waste materials.

4. Conclusions

In the present study, lipids were extracted from MSS using scCO₂ with varying temperature (32–80°C), pressure (10–50 MPa), treatment time (15–120 min), and addition of 1–10 wt.% of cosolvents. Maximum lipids' extraction obtained was about 27% at the scCO₂ pressure of 30 MPa, temperature of 60°C, treatment time of 60 min, and the addition of 5 wt.% of EtOH as a cosolvent. The modified Gompertz mathematical model equation was adequately fitted with experimental data for the extraction of lipids from MSS using scCO₂. The estimated E_a value was 14.82 kJ/mole, indicating that the scCO₂ extraction technology for the extraction of lipids from MSS is highly dependent on pressure than temperature. About 84% biodiesel was isolated from scCO₂ extracted lipids using methanol as a solvent and NaOH as a catalyst at lipids-to-methanol molar ratio of 1 : 6, temperature of 60°C, and reaction time of 4 h. It was found that the physicochemical properties of sewage sludge biodiesel comply with EN 14214 and ASTM D6751 standards. Oleic acid, palmitic acid, linoleic acid, and stearic acid were the major fatty acids determined in scCO₂ extracted lipids and sewage sludge biodiesel. Moreover, the physicochemical properties of sewage sludge biodiesel were almost similar to the biodiesel obtained from edible and nonedible feedstocks. The present study findings revealed that MSS has the potential to be used as a promising lipids feedstock for biodiesel production. The implication of the sewage sludge

lipids for biodiesel would determine an alternative and low-cost lipids feedstock for biodiesel, which will substantially minimize environmental pollution, minimize solid waste disposal in landfills, and enhance sustainable utilization of environmental solid waste materials.

Data Availability

The data used to support the findings of this study can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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