Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 89 No 8, 2073 doi: 10.2166/wst.2024.089

Valorization of fat balls and primary scum from wastewater treatment: a promising renewable lipid feedstock for biodiesel production

Febrian Rizkianto, Kazuyuki Oshita* and Masaki Takaoka

Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, C-Cluster-1, Kyoto-Daigaku Katsura, Nishikyo-ku, Kyoto 615-8540, Japan
*Corresponding author. E-mail: oshita.kazuyuki.6e@kyoto-u.ac.jp

KO, 0000-0002-2193-3366

ABSTRACT

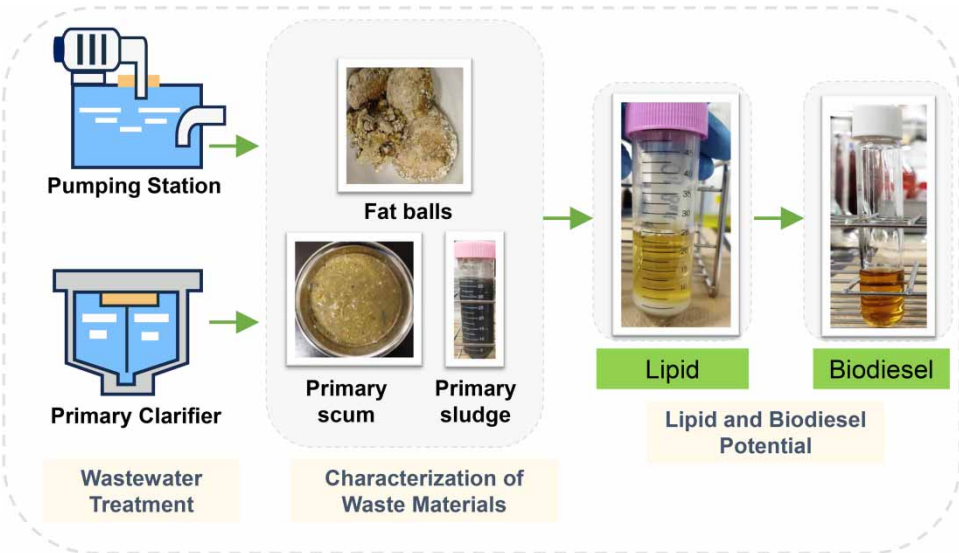
We investigated the potential of waste materials from wastewater treatment plants (WWTPs) to serve as an alternative lipid feedstock for biodiesel production. The average lipid recoveries from fat balls (46.4%) and primary scum (49.5–54.5%) were higher than the lipid recovery of primary sludge (15.8–16.4%). The yield of biodiesel produced from the extracted lipids ranged from 5.7 to 20.1%. There were considerable site- and season-dependent variations in the characteristics of the lipid waste materials. Radiocarbon analysis indicated the presence of fossil-derived carbon (26.0–42.0%) in the biodiesel obtained from wastewater lipids. Finally, we estimated the potential for biodiesel production from WWTP-derived lipids; about 333.0 metric tons of biodiesel per year could be produced from fat balls and primary scum in Japan. The results indicate that lipid-rich materials from WWTPs represent a valuable alternative feedstock for biodiesel production.

Key words: biodiesel, fat balls, lipid, primary scum, wastewater

HIGHLIGHTS

- Seasonal variation affects the characteristics of waste materials from wastewater treatment plants.
- Fat balls and primary scum had higher lipid and biodiesel yields than primary sludge.
- Conversion of wastewater-extracted lipids to biodiesel containing biogenic carbon levels of 58–74%.
- About 333 metric tons of biodiesel per year could be produced from wastewater fat balls and primary scum in Japan.

GRAPHICAL ABSTRACT



LIST OF ABBREVIATIONS

WWTP	Wastewater treatment plant
FOG	Fats, oils, and grease
FB	Fat balls
SC H	Primary scum of WWTP H
PS H	Primary sludge of WWTP H
SC S	Primary scum of WWTP S
PS S	Primary sludge of WWTP S
C	Carbon
H	Hydrogen
N	Nitrogen
TS	Total solids
VS	Volatile solids
HF	Hydrofluoric acid
HCl	Hydrogen chloride
HNO_3	Nitric acid
Ca	Calcium
Si	Silicon
Na	Sodium
P	Phosphorus
Fe	Iron
Al	Aluminum
Mg	Magnesium
K	Potassium
Zn	Zinc
Cu	Copper
Pb	Lead
Ni	Nickel
HHV	Higher heating value
LHV	Lower heating values
TG	Thermogravimetric
FTIR	Fourier transform-infrared spectroscopy
TG	Thermogravimetric
FTIR	Fourier transform-infrared spectroscopy
GC-MS	Gas chromatography–mass spectroscopy system
FAME	Fatty acid methyl esters
MUFA	Monounsaturated fatty acids
SFA	Saturated fatty acids
PUFA	Polyunsaturated fatty acids

1. INTRODUCTION

The development of renewable energies has attracted worldwide attention due to increasing energy demands and decreasing fossil fuel reserves. Moreover, the general public has become increasingly conscious of climate change, which is driven by the consumption of conventional fuels (Bora *et al.* 2020). Biodiesel is derived from lipids found in both edible and inedible sources through transesterification reactions (Helwani *et al.* 2009). It is increasingly recognized for its renewability, biodegradability, and compatibility with diesel engine systems (Ma & Hanna 1999; Carraretto *et al.* 2004; Karmakar *et al.* 2010). However, progress in the biodiesel industry is hindered by the high cost of oil feedstock (primarily vegetable oils); additionally, biodiesel prices can exceed the price of petroleum-based diesel by threefold (Demirbas 2008; Capodaglio & Callegari 2018). Hence, there has been a shift in research toward identifying affordable and easily accessible raw materials to ensure sustainable production of biodiesel.

There is increasing interest in the recovery of valuable organic components from the urban wastewater residuals, which are continuously generated during wastewater treatment (Dufreche *et al.* 2007; Zhu *et al.* 2014; Frkova *et al.* 2020). As a major organic component of wastewater, lipids are mainly derived from fats, oils, and grease (FOG) generated during cooking and food preparation in restaurants, households, and the food processing industry (Abomohra *et al.* 2020; Elsayed *et al.* 2022). Large amounts of FOG can enter sewage systems due to uncontrolled disposal of waste oil or improper management of grease traps (Husain *et al.* 2014; Kobayashi *et al.* 2014; Yau *et al.* 2018).

Primary sludge, activated sludge, and digested sludge have been investigated as potential lipid sources for biodiesel production (Mondala *et al.* 2009; Revellame *et al.* 2010; Pastore *et al.* 2013; Olkiewicz *et al.* 2015). Although sludge holds great potential as an alternative oil feedstock, a viable collection pathway must be established from wastewater treatment plants (WWTPs) because lipid extraction may impact conventional sludge treatment processes (Rizkianto *et al.* 2022). Within WWTPs, fat balls in pumping stations and primary scum in the primary clarifier contain substantial concentrations of lipids, which float on the water surface and cause operational issues such as foaming and system blockages (Long *et al.* 2012; Nieuwenhuis *et al.* 2018; Spiller *et al.* 2020). These materials must be removed and treated separately to avoid problems in WWTPs.

Fat balls and scum are both composed of mixtures of FOG and solid materials. Fat balls are formed by the agglomeration of suspended solids (e.g., FOG, metals, and debris), which create a floating layer on the water surface in pumping stations (Williams *et al.* 2012; Nieuwenhuis *et al.* 2018). In WWTPs, the primary clarifier separates settleable solids (primary sludge) and buoyant substances such as oil and grease (primary scum) from the influent after basic screening (Manara & Zabaniotou 2012; Ma *et al.* 2016; Yellapu *et al.* 2019; Cobb *et al.* 2020). Collin *et al.* (2020) performed an energy assessment of various FOG waste materials obtained from a sewage catchment, which revealed that fat balls and floating scum had lipid contents ranging from 13 to 93 wt.%. Wang *et al.* (2016) explored the potential for producing biodiesel from sewage scum and primary sludge; they found that scum had a higher biodiesel yield (28.7 wt.%) compared with primary sludge (25.4 wt.%).

According to sewage statistics in Japan, approximately 75% of the total sewage sludge was allocated for recycling purposes in 2020, but only a small proportion (9%) was used for fuel generation (MLIT 2023). In Japan, fat balls and primary scum are typically landfilled or incinerated (Japan Sewage Works Association, 2021). However, to achieve a circular economy, there must be a shift from waste disposal toward resource recovery, while minimizing potential environmental risks (Seiple *et al.* 2017; Gherghel *et al.* 2019; Salama *et al.* 2019; Glińska *et al.* 2020). Although several studies have reported that fat balls and sewage scum constitute potential biofuel feedstocks, there is limited available information regarding their characteristics and variability among seasons and collection sites, and few estimates have been published concerning their volume and potential as biodiesel feedstock (Bi *et al.* 2015; Yellapu *et al.* 2019). Additionally, there has been no attempt to study biogenic C in biodiesel derived from wastewater lipids based on radiocarbon (^{14}C) content; according to Intergovernmental Panel on Climate Change guidelines, there is a need to distinguish between the fossil and biogenic C contents in biofuel feedstocks (Lee *et al.* 2022; Sebos 2022).

The aim of this study is to conduct a comprehensive comparison of waste materials obtained from wastewater treatment plants, including fat balls, primary scum, and primary sludge, with a focus on assessing their suitability as biodiesel feedstock. The samples were analyzed for their physicochemical properties, lipid composition, and biodiesel potential across different seasons. The findings from this research are expected to make a significant contribution to the sustainable management of wastewater residuals.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Samples were collected from two WWTP and one pumping station in Kobe, a major metropolitan area in the Kansai region of Japan. WWTP Higashinada (WWTP H) and WWTP Seibu (WWTP S) treat 172,000 m^3/day and 135,000 m^3/day of residential and industrial wastewater, respectively. The pumping station Fukae-Ohashi (PS F) is located close to WWTP H and has a capacity of 37,000 m^3/day. Fig. S1 shows the schematic diagram of the pumping station and WWTP.

As summarized in Table 1, we collected fat balls from the pumping station (FB), primary scum (SC H), and primary sludge (PS H) from WWTP H, and primary scum (SC S) and primary sludge (PS S) from WWTP S. Samples were collected in three seasons (winter, summer, and autumn). Initially, the samples were characterized to assess their physicochemical characteristics, followed by lipid extraction using mechanical shaking with hexane, and subsequent biodiesel conversion via acid-catalyzed transesterification.

2.2. Physical and chemical characterization

Samples were subjected to qualitative assessments of their physical appearance, considering characteristics such as texture and color. The contents of total solids (TS) and volatile solids (VS) were determined according to the Biosolids Analytical Methods and Sampling Procedures standard 2540 method G (Rice *et al.* 2012). To ensure repeatability, each sample was measured in triplicate.

Table 1 | Summary of the sampling locations

Location	Sampling source	Sample notation
PS F		
Fat balls	Water surface of the pumping station	FB
WWTP H		
Primary scum	Water surface of the primary clarifier	SC H
Primary sludge	Collected from the primary clarifier	PS H
WWTP S		
Primary scum	Water surface of the primary clarifier	SC S
Primary sludge	Collected from the primary clarifier	PS S

2.2.1. Element analysis

Trace elements in dried samples (Ca, Si, Na, P, Fe, Al, Mg, K, Zn, Cu, Pb, and Ni) were analyzed by inductively coupled plasma atomic emission spectrometry (ICAP-7000; Thermo Fisher Scientific, Waltham, MA, USA). Before the analysis, 0.2 g of dried sample was mixed with 6 mL of HNO_3, 1 mL of HCl, and 1 mL of HF, then digested using a microwave digestion system (ETHOS One; Milestone SCI, Inc., Shelton, CT, USA). The mixtures were digested for 20 min at 220 °C and 1,000 W, then for 20 min at 200 °C and 1,000 W.

Carbon (C), hydrogen (H), and nitrogen (N) analyses were performed using an elemental analyzer (Micro Corder JM10; J-Science Lab Co., Ltd, Kyoto, Japan). The Cl and combustible S contents were determined according to the Japanese Industrial Standard (JIS) (Japan Industrial Standard 2002). The percentage of oxygen (O) was calculated using the following equation:

$$O = V - C - H - N - S \tag{1}$$

2.2.2. Thermogravimetry–differential thermal analysis

The thermogravimetric (TG) degradation of dried samples was measured using a differential thermal analysis balance (Thermo Plus EVO2, TG 8120; Rigaku, Tokyo, Japan). Each sample was heated from ambient temperature to 650 °C at a rate of 10 °C/min under an N_2 stream (50 mL/min).

2.2.3. Calorific value

The higher heating value (HHV) of dry solids was measured using an oxygen bomb calorimeter (CA-4J; Shimadzu Corp., Kyoto, Japan). The lower heating values of samples on a dry basis (LHV_d) and wet basis (LHV_w) were calculated from the measured HHV according to the following equations:

$$LHV_d = HHV - 4.186 \times 600 \times 9 \times (H/100) \tag{2}$$

$$LHV_w = (100 - w)/100 \times LHV_d - 4.186 \times 600 \times (w/100) \tag{3}$$

where LHV_d (kJ/kg) and LHV_w (kJ/kg) represent the LHVs of dried and dewatered samples, respectively; w is the moisture content of the dewatered samples; and H (% dry solids) is the H atom content of the sample.

2.2.4. Fourier transform-infrared spectroscopy analysis

The functional group compositions of the extracted lipids were determined via Fourier transform-infrared spectroscopy (FTIR; IRSpirit-T; Shimadzu) in attenuated total reflectance mode. The detection range was 500–4,000 cm^{-1}.

2.2.5. Accelerator mass spectrometry ^{14}C analysis

The ^{14}C contents of dry solids and biodiesel were analyzed by accelerator mass spectrometry, in accordance with ASTM D6866–22, at Beta Analytic Testing Laboratory (Miami, FL, USA).

2.3. Lipid extraction

Lipids were extracted from samples using a mechanical shaker (SA300; Yamato Scientific Co., Ltd, Tokyo, Japan) with hexane (Guaranteed Reagent; Wako Co., Ltd, Tokyo, Japan). Wet samples were weighed in 50-mL tubes, and hexane was added at a 2:1 hexane:sample volumetric ratio (Dufreche *et al.* 2007; Patiño *et al.* 2018). All samples underwent three extractions. For each extraction, the sample was shaken at 200 rpm for 60 min at ambient temperature, then centrifuged at 3,000 rpm for 15 min. The supernatant phase (upper organic layer) was decanted and transferred to a pre-weighed round-bottom flask, then evaporated using a vacuum rotary evaporator at 40 °C. The organic solvent remaining in the residue was further vaporized under an N_2 stream. Finally, the extracted lipid weight was recorded after the residue had cooled in a desiccator. The lipid yield was expressed as the lipid weight per unit sludge sample weight (dry basis).

2.4. Lipid transesterification and fatty acid methyl ester analysis

Lipids were converted by acid-catalyzed transesterification into fatty acid methyl esters (FAME; i.e., biodiesel) (Dufreche *et al.* 2007; Olkiewicz *et al.* 2014). A 20-mg sample of extracted lipids was treated with 1 mL of hexane and 2 mL of 1% v/v H_2SO_4 in methanol (Guaranteed Reagent; Wako Co., Ltd). The mixture was heated overnight at 50 °C in a constant-temperature bath (MG-2200; EYELA Co., Ltd, Tokyo, Japan). After the reaction, 5 mL of 5% (w/v) NaCl (Nacalai Tesque, Inc., Kyoto, Japan) was added, followed by two extractions using 5 mL of hexane. Raw biodiesel in the hexane phase (upper layer) was then washed with 4 mL of 2% (w/v) Na_2CO_3 (Nacalai Tesque, Inc.) and dried at 60 °C.

Fatty acids were analyzed using a gas chromatography–mass spectroscopy system (GC-MS; QP2010 Plus; Shimadzu Corp.) equipped with an SP-2560 capillary column (100 m × 0.20 μm × 0.25 μm; Supelco, Bellefonte, PA, USA). Ultra-high-purity He was used as the carrier gas with a flow rate of 1.0 mL/min and a split ratio of 1:30. The sample injection volume was 1 μL. The injection and detector port temperatures were 250 °C. The GC oven temperature program was initially 100 °C, held for 5 min, then increased to 180 °C at a rate of 4 °C/min and 240 °C at a rate of 2 °C/min, and then held at 240 °C for 15 min. The calibration curve was created using a standard comprising 37 FAME (Supelco). The GC-MS concentrations were used for FAME identification and quantification of transesterifiable lipids. The biodiesel yield (dry basis) was calculated based on the mass of lipids extracted and their content of transesterifiable material.

3. RESULTS AND DISCUSSION

3.1. Physical characterization of fat balls, primary scum, and sludge

Waste samples collected from the pumping station and primary clarifiers of the two WWTPs had distinct physical appearances. FBs from the pumping station were solid, spherical, and composed of a fatty outer layer and buoyant-material core (e.g., Styrofoam); they were light yellow and had a strong, unpleasant smell (Figure S2(a)). The SC H and S samples were composed of a mixture of floating food scraps, solids, and oily waste (Figure S2(b) and (d)). The PS H and S samples were brown–black in color and composed of a slurry of suspended solids (Figure S2(c) and (e)).

We next characterized the SC, PS, and FB samples collected in winter, summer, and autumn (Table 2). Among the five samples collected across three seasons, FB samples from the pumping station generally had the highest TS content (49.9–56%). This was expected because fat balls are composed of solidified organic matter originating from discharged FOG. The SC and PS samples from the primary clarifiers had low TS contents of 0.7–6.0 and 1.5–2.5%, respectively. In comparison, reported TS for primary scum is higher, typically ranging between 8.9 and 62.5% (Anderson *et al.* 2016; Mu *et al.* 2016; Collin *et al.* 2020), whereas primary sludge was found between 1.03 and 9.09% (Tyagi & Lo 2013; Olkiewicz *et al.* 2015; Kech *et al.* 2018; Collin *et al.* 2020; di Bitonto *et al.* 2020). The large difference between the pumping station and primary clarifier samples may reflect the different stages of treatment (Villalobos-Delgado *et al.* 2023). Samples collected from primary clarifiers might have undergone initial treatment steps, whereas pumping station samples may be more representative of raw wastewater, which could have higher FOG contents.

FB and SC samples exhibited fluctuating TS contents, likely due to their origin from the separated semi-solid phase of FOG (Marie Del Mundo & Sutheerawattananonda 2017). In addition, different flows within the sewer network influence the moisture content (Gross *et al.* 2017). It is reported that FOG has a wide range of moisture levels between 0.51 and 71.4% (Abomohra *et al.* 2020).

The seasonal TS content of SC samples notably varied, whereas the TS content of PS samples remained relatively constant across seasons. This could be related to differences in the compositions of scum (i.e., floating matter) and primary sludge (i.e., settled organic and inorganic matter). Primary scum, comprised of grease, may exhibit fluctuations in TS due to its floating

Table 2 | Characteristics of pumping station (fat ball) and wastewater treatment plant (scum and sludge) samples

Source	Season	TS (%)	VS (%TS)	C (%)	H (%)	N (%)	HHV (MJ/kg)	LHV_w (MJ/kg)
FB	Winter	56.0 ± 5.0	97.1 ± 0.5	71.1 ± 0.6	11.2 ± 0.2	0.23 ± 0.03	40.2 ± 9.6	20.0 ± 5.4
	Summer	49.9 ± 4.0	92.5 ± 1.3	76.5 ± 1.4	13.2 ± 0.3	0.57 ± 0.3	29.9 ± 15	12.2 ± 7.6
	Autumn	55.0 ± 5.0	92.4 ± 0.5	68.4 ± 0.6	11.4 ± 0.2	0.16 ± 0.03	39.2 ± 1.0	19.0 ± 0.5
Average		53.6	94.0	72.0	11.9	0.32	36.4	17.1
SC H	Winter	3.50 ± 2.0	86.5 ± 2.5	71.7 ± 0.6	11.3 ± 0.1	1.21 ± 0.04	37.4 ± 0.8	−1.20 ± 0.03
	Summer	1.30 ± 0.6	90.1 ± 3.0	47.5 ± 1.9	7.83 ± 0.4	2.38 ± 0.1	31.7 ± 2.1	−2.10 ± 0.14
	Autumn	0.70 ± 0.1	86.0 ± 3.0	56.9 ± 19	7.60 ± 2.7	2.66 ± 0.6	29.8 ± 3.4	−2.30 ± 0.02
Average		1.83	87.5	58.7	8.91	2.08	32.4	− 1.87
SC S	Winter	2.10 ± 0.8	91.0 ± 3.0	59.5 ± 1.0	9.30 ± 0.3	1.67 ± 0.1	31.9 ± 5.6	−1.90 ± 0.14
	Summer	1.40 ± 0.5	92.3 ± 5.0	54.1 ± 1.0	9.00 ± 0.2	3.41 ± 0.1	22.6 ± 6.4	−2.10 ± 0.01
	Autumn	6.00 ± 1.0	96.2 ± 0.6	65.0 ± 3.4	9.50 ± 0.5	1.85 ± 0.2	38.1 ± 4.3	−2.10 ± 0.1
Average		3.17	93.2	59.5	9.26	2.31	30.9	− 2.03
PS H	Winter	2.50 ± 0.1	82.9 ± 5.0	44.7 ± 4.0	6.90 ± 0.6	4.90 ± 0.5	24.4 ± 5.5	−1.80 ± 0.1
	Summer	2.20 ± 0.1	83 ± 0.3	42.1 ± 0.8	6.90 ± 0.1	4.40 ± 0.05	20.0 ± 0.6	−2.20 ± 0.1
	Autumn	1.70 ± 0.02	83.4 ± 1.0	45.2 ± 9.0	5.90 ± 1.1	5.60 ± 2.3	25.2 ± 4.3	−0.20 ± 0.3
Average		2.13	83.1	47.5	7.15	4.95	23.2	− 1.40
PS S	Winter	1.60 ± 0.1	91.6 ± 0.3	46.5 ± 0.3	7.30 ± 0.1	2.41 ± 0.1	20.0 ± 0.1	−2.20 ± 0.01
	Summer	1.70 ± 0.1	87.0 ± 3.0	44.9 ± 1.2	7.40 ± 0.2	3.18 ± 0.03	20.1 ± 0.9	−2.20 ± 0.02
	Autumn	1.50 ± 0.1	88.5 ± 2.0	51.2 ± 13	6.80 ± 1.8	3.00 ± 0.8	18.3 ± 0.8	−2.20 ± 0.01
Average		1.60	89.0	44.0	6.59	2.87	19.5	− 2.20

Samples are abbreviated as shown in Table 1.

nature (Marufuzzaman *et al.* 2014; Urrutia *et al.* 2016). Furthermore, scum is often skimmed out from the surface of the primary clarifier. In contrast, primary sludge tends to maintain a more consistent TS level as it is generated after the primary treatment process (Qi *et al.* 2016).

The VS contents were generally comparable across samples, ranging from 82.9 to 97.1% (dry basis), slightly higher than that reported in the literature for primary sludge, which typically falls between 60 and 80% (Tyagi & Lo 2013). These results indicate that the waste samples contain rich organics. Among all samples, the average elemental composition fell within the range of 42.1–76.5 wt.% C, 5.9–13.2 wt.% H, and 0.16–5.6 wt.% N.

3.2. Calorific value of dry samples

The heating value represents the energy released when a specific volume of material is combusted. The composition of fuel impacts its energy content, and a larger HHV corresponds to a greater energy content (Collin *et al.* 2020). The HHV was highest for FB (36.4 MJ/kg), followed by SC (23.2–32.4 MJ/kg) and PS (19.5–30.9 MJ/kg) samples (Table 2).

Among the three seasons, calorific values tended to be lower in warmer seasons because of the higher sample moisture contents. Accordingly, samples collected from the primary sedimentation tank had comparable LHV_w. The heating values tend to increase with higher levels of VS and ratios of C in samples (Parikh *et al.* 2005; Folayan *et al.* 2019). FB samples had higher VS (92.4–97.1) and C fractions (68.4–71.7%). Additionally, they exhibited lower moisture contents ranging from 44.0 to 50.1%. The comparison of calorific value of the waste samples evaluated in this study has been compared to the literature, as summarized in Table S1. The variation in the calorific value of waste samples is likely due to contact with sewage and other materials in the sewer.

3.3. TG–differential thermal analysis of dried samples

The TG and differential thermogram curves of the dried samples based on average measurements in the three seasons are shown in Fig. S3 of the supplementary material. The thermal decomposition of sewage sludge can be divided into three stages: dehydration (up to 180 °C), organic matter degradation (180–580 °C), and inorganic matter decomposition (580–1,000 °C) (Wang *et al.* 2013; Magdziarz & Werle 2014). Smaller molecules are thermally degraded at lower temperatures, whereas larger molecules require higher temperatures (Chen *et al.* 2020).

We observed considerable thermal weight loss in the second stage, although there were differences in the specific temperature at which organic matter exhibited thermal decomposition among the samples. FB and SC have higher C content, indicating a greater proportion of organic matter. In contrast, PS decomposes earlier in the second stage of TGA, suggesting a distinct thermal decomposition profile likely due to its lower C content. The differential thermogram curves of the PS H, PS S, and SC S samples showed similar trends, with maximum weight loss around 330–340 °C. The SC H and FB samples required a higher temperature (442–460 °C) to completely decompose the organic substances.

3.4. Trace metal contents in dried samples

Table S2 of supplementary material displays the trace element concentrations measured in the solid samples. The samples contained relatively high contents of some major elements, including Ca, Si, Na, P, Fe, Al, and Mg, but lower contents of elements such as Zn, Cu, Ni, Pb, Cl, and S. Some elements presumably underwent natural release into the water from rock, soil, or concrete corrosion (e.g., Ca, Si, P, and S) (He *et al.* 2013, 2017). Effluent from human activities also likely increased the concentrations of some elements, such as household products (Na, K, and Cl), agricultural runoff (P), and industrial effluent (Cu, Fe, Zn, Ni, Al, Mg, and Pb) (Nitayapat & Chitprasert 2014; Gurd *et al.* 2019; Chen *et al.* 2021).

The element concentrations were highly variable among samples and across seasons, although the SC and PS samples generally contained much higher trace element contents than the FB samples. Numerous studies have reported high levels of heavy metals and sulfur in lipids derived from grease trap waste (Ward 2012; Nitayapat & Chitprasert 2014; Hums *et al.* 2016; Ma *et al.* 2016). Hence, further treatment is necessary before wastewater lipids can be utilized for biodiesel production.

Among the trace ions, Ca was the most prominent (9,080.0–24,900.0 mg/kg), consistent with the literature (Table S3) on the supplementary material) (Keener *et al.* 2008; Williams *et al.* 2012; Yellapu *et al.* 2019). FB samples had the highest Ca concentrations, which may have contributed to the solidification process (Keener *et al.* 2008; Williams *et al.* 2012; Iasmin *et al.* 2014). FOG agglomerates via saponification when a large concentration of Ca reacts with liquid fat (as a precursor of free fatty acids) (Pastore *et al.* 2013; Iasmin *et al.* 2016; He *et al.* 2017). We also observed that Styrofoam acted as a nucleus in the FB samples (Figure 2(a) on the supplementary material); FOG likely adheres to Styrofoam, leading to the formation of fat balls.

3.5. Lipid content

We used a mechanical shaking method to extract lipids from waste samples, with hexane as the solvent. The average lipid contents extracted from SC and FB samples were generally comparable, ranging from 34.9 to 65.1% (Table 3). This finding may have occurred because chemically, FB and SC originate from discharged FOG that mixes with water and other contaminants in the sewage network (Williams *et al.* 2012; Yellapu *et al.* 2019). Consequently, these samples are primarily composed of lipids. Such a relationship would explain the higher lipid concentrations in both SC and FB samples. In contrast, lipids in primary sludge mainly consist of settled organic compounds from wastewater influent (Zhu *et al.* 2019). Moreover, during the precipitation process in the primary clarifier, some organic matter may be microbially decomposed or transformed (Collin *et al.* 2020). Therefore, the PS samples had relatively low lipid contents (6.7–19.9%).

The lipid yields of the different samples exhibited seasonal variation. FB and SC samples showed similar trends, with lower lipid yields in summer. Conversely, the lipid yield of PS was slightly higher in summer, possibly because FOG accumulates more readily in cold temperatures. FOG tends to form a layer on the surface of the water; in colder temperatures, it may more readily accumulate and combine with other sewage debris, whereas in warmer temperatures, it may oxidize and undergo more rapid decomposition (Bi *et al.* 2015; Mu *et al.* 2016).

3.6. FTIR analysis of the lipid fraction

We used FTIR analysis to qualitatively characterize the functional groups of the lipid samples. Fig. S4 of the supplementary material presents the seasonally averaged FTIR spectra of the samples. All samples shared common peaks, indicating that the lipids had several similar functional groups across all samples. The absorption bands at 2,848 and 2,915 cm^{-1} represented $-CH_2-$ stretching, indicating the presence of fatty acids. The band around 1,700 cm^{-1} represented axial deformation of the C = O group in esters (Da Silva Almeida *et al.* 2016); the dominance of this peak indicated the presence of fats (e.g., free fatty acids and triglycerides) (He *et al.* 2011; Williams *et al.* 2012). The band at 1,010–1,260 cm^{-1} represented the C–O stretching of aliphatic chains in crude soap mixtures (e.g., triglyceride, soap, and residue alkali) (Iasmin *et al.* 2014; Kumar *et al.* 2016). Finally, the absorption peaks between 900 and 650 cm^{-1} indicated the presence of aromatic compounds (Chen *et al.* 2020).

Table 3 | Lipid, transesterifiable, and biodiesel yields of the pumping station and wastewater treatment plant samples

Sample	Season	Lipid yield[a] (%)	Transesterifiable yield[b] (%)	Biodiesel yield[c] (%)
FB	Winter	56.6	36.9	20.9
	Summer	36.2	37.6	13.6
	Autumn	51.7	44.1	22.8
Average		46.4	37.3	17.3
SC H	Winter	50.6	44.0	22.3
	Summer	48.4	36.9	17.9
	Autumn	64.6	27.1	17.5
Average		49.5	40.4	20.1
SC S	Winter	74.1	29.2	21.6
	Summer	34.9	49.2	17.2
	Autumn	65.1	44.9	29.3
Average		54.5	39.2	19.4
PS H	Winter	12.9	46.5	5.99
	Summer	19.9	41.0	8.15
	Autumn	8.00	58.4	4.67
Average		16.4	43.7	7.10
PS S	Winter	14.9	40.3	6.00
	Summer	16.7	32.7	5.45
	Autumn	6.70	38.8	2.60
Average		15.8	36.5	5.70

Samples are abbreviated as shown in Table 1.
[a]Based on the sample dry weight.
[b]Transesterifiable lipids (convertible into biodiesel) based on GC-MS analysis.
[c]Based on lipid and transesterifiable contents on a dry weight basis.

3.7. Biodiesel potential and methyl ester analysis

Extracted raw lipids are composed of acylglycerol and free fatty acids (i.e., transesterifiable fraction), which can be transformed into FAME/biodiesel, along with hydrocarbons, wax esters, steroids, terpenoids, and other non-polar compounds, which cannot be converted into FAME (Pastore *et al.* 2013; Olkiewicz *et al.* 2015; Patiño *et al.* 2018). The average proportion of transesterifiable compounds considerably varied from 27.1 to 49.2% (Table 3); these yields were greater than the yields obtained by Dufreche *et al.* (2007), who converted FAME from activated sludge lipids. However, the present findings are generally consistent with other research concerning biodiesel conversion from different types of sludge, in which transesterifiable yields ranged from 36 to 76% (Willson *et al.* 2010; Olkiewicz *et al.* 2015; Villalobos-Delgado *et al.* 2023).

In the present study, the transesterifiable contents in the samples slightly fluctuated among seasons. Kobayashi *et al.* (2014) investigated the effects of seasonal variation in lipids extracted from restaurant grease trap waste. Their findings reported that lipid characteristics were influenced by oil sources, rather than seasons. This suggests that seasonal changes likely influence the utilization of different cooking oils in restaurants.

Total biodiesel yields were calculated based on the lipid and transesterifiable contents of the samples. The biodiesel yield varied from 2.6 to 29.3% (Table 3). The winter SC S sample had the maximum biodiesel yield (29.3%), whereas the autumn PS S sample had the lowest biodiesel yield (2.6%). The biodiesel yields of the FB and SC samples were higher than the biodiesel yields of PS, regardless of season. Our results are similar to a previous study, which showed that the total biodiesel yield declined as the wastewater treatment stage progressed from physical treatment to biological treatment stages (Frkova *et al.* 2020). This concluded that FOG-rich substances (fat balls and scum) are preferred for lipid feedstocks compared to primary sludge.

The methyl ester compositions according to sample and season are presented in Figure 1. The methyl ester contents considerably varied among samples and seasons. Methyl ester concentrations and compositions may be affected by the source oil (Abomohra *et al.* 2020). Additionally, the composition of lipids in wastewater varies depending on the wastewater treatment plant and the collection site where various lipid precursors accumulate (Tran *et al.* 2021). Environmental factors can induce

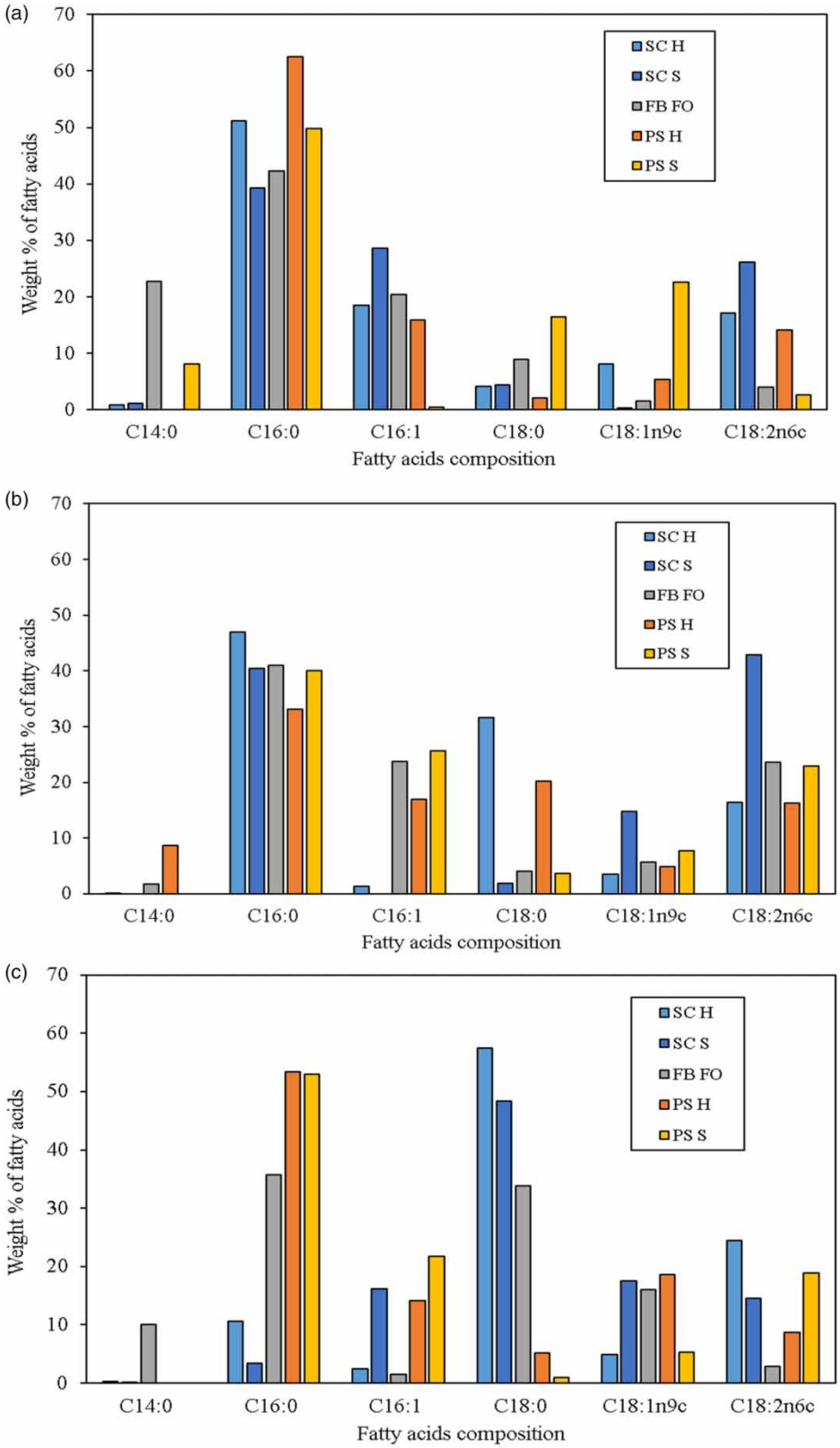


Figure 1 | Profiles of the fatty acids myristic acid (C14:0), palmitic acid (CC16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) in biodiesel obtained from different samples during (a) winter, (b) summer, and (c) autumn. Samples are abbreviated as shown in Table 1.

changes in the characteristics of fatty acids (Chipasa & Mędrzycka 2006; Liu *et al.* 2020). Despite variations in ester profiles, palmitic acid (C16:0) was predominant in most samples, followed by stearic acid (C18:0) and linoleic acid (C18:2).

The proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in biodiesel from wastewater lipids significantly varied among samples. However, all biodiesel samples mainly consisted of SFA (42.3–79.7%), followed by MUFA (4.90–33.6%) and PUFA (2.85–43.0%) (Figure 2). The saturation trends were consistent with previous reports (Mondala *et al.* 2009; Revellame *et al.* 2010; Patiño *et al.* 2018). The levels and composition of SFA vary, possibly due to the utilization of different types of fats and oils in warmer versus colder months (Tran *et al.* 2021). Furthermore, the seasonal consumption of food might still be influenced by cultural or ritual connections, such as Thanksgiving, Christmas, and New Year celebrations (Spence 2021).

The methyl ester composition impacts biodiesel characteristics, including viscosity, stability, cold flow properties, and ignition quality. Biodiesel containing higher levels of SFA and MUFA is preferable due to its oxidative stability and improved combustion properties (Dufreche *et al.* 2007; Magalhães-Ghiotto *et al.* 2022). Our findings confirm that the FB and SC samples had desirable properties as potential lipid sources for biodiesel production. However, considering the high moisture content in the SC samples, initial concentration steps may be required before lipid extraction, which could increase production costs. Therefore, we recommend fat balls as the most promising feedstock for biodiesel production.

3.8. Contributions of biogenic and fossil C in wastewater lipid-derived biodiesel

Wastewater contains large amounts of organic C, which can originate from biogenic or fossil sources (Law *et al.* 2013). The fossil C fraction is typically derived from anthropogenic particulate matter (e.g., surfactants, pharmaceuticals, personal health care products, and industrial effluent) in wastewater (Paxéus 1996; Tseng *et al.* 2016). The biogenic C fraction is derived from biomass (e.g., plants or animal byproducts). The use of lipids from wastewater as a potential biofuel feedstock requires assessing the proportion of biogenic C according to ^{14}C content. Therefore, we analyzed the biogenic and fossil C fractions of dry solids and biodiesel from summer samples by accelerator mass spectrometry.

Table 4 compares the contents of biogenic C between the present study and previous studies. The FB samples did not contain fossil C, indicating that they were primarily derived from natural sources. The SC samples had higher fossil C contents (4.0–8.0%), compared with the PS samples (1.0–5.0%). Our findings also reflected the impacts of different catchments; samples from WWTP H had higher fossil C contents (5.0–8.0%), compared with samples from WWTP S (1.0–4.0%). This difference was likely due to the distinct treatment capacities and operating conditions of the primary treatment systems (Law *et al.* 2013).

Conversion of the extracted lipid samples into biodiesel enhanced the fossil C proportions, from 0 to 8.0% (dry solids) to 26.0 to 42.0% (biodiesel). This enhancement could be due to the inclusion of methanol (a fossil-derived reagent), which chemically bonded with methyl esters ($ROOCH_3$), contributing to 5.0–10.0% of fossil C (Lee *et al.* 2022; Sebos 2022). Previous studies reported that commercial 100% biodiesel (B100) had a biogenic C content of 92.4–94.6% due to the use of methanol during biodiesel production (Lee *et al.* 2022; Sebos 2022). Based on our initial feedstock (<10% fossil C), the biogenic fractions of our biodiesel samples should have been comparable with B100, assuming that most of the fossil C was derived from methanol. However, our biodiesel samples contained lower biogenic C contents. The reason for this discrepancy is unclear, but it may have resulted from variations in C fractions driven by the fatty acid composition. Lee *et al.* (2022) noted that the ^{14}C content of biofuel can vary according to factors such as biomass age, type, and geographical source. Additionally, we cannot rule out the possibility that other fossil-derived chemicals remained in the biodiesel after processing, thus impacting the fossil C fraction. Nevertheless, our findings highlight the successful conversion of wastewater-extracted lipids into biodiesel containing biogenic C levels of 58.0–74.0%, representing substantial improvements relative to the biogenic C contents of B20 (22.0%) and petroleum diesel (2.0%).

3.9. Estimation of biodiesel potential and CO_2 emissions

Based on information obtained from the wastewater facilities in Kobe, Japan, where we collected samples, the facilities generated 8.3 kg (pumping station FBs), 77.2 kg (SC H), and 47.7 kg (SC S) of materials per day (wet basis). Using these estimates and our experimental data, we calculated that a total of 97.5 kg of lipids per day could be extracted from the facilities (pumping station and WWTP H and S); these lipids could produce 39.7 kg of biodiesel per day (Table 5).

According to the Japan Sewage Works Association (2023), Japan has over 2,100 WWTPs and 3,700 pumping stations, which can process ~40,100,000 m^3 of wastewater per day. Extrapolating from our above-described calculations, we estimated that 333.0 metric tons of biodiesel per year could be produced from wastewater fat balls and primary scum waste

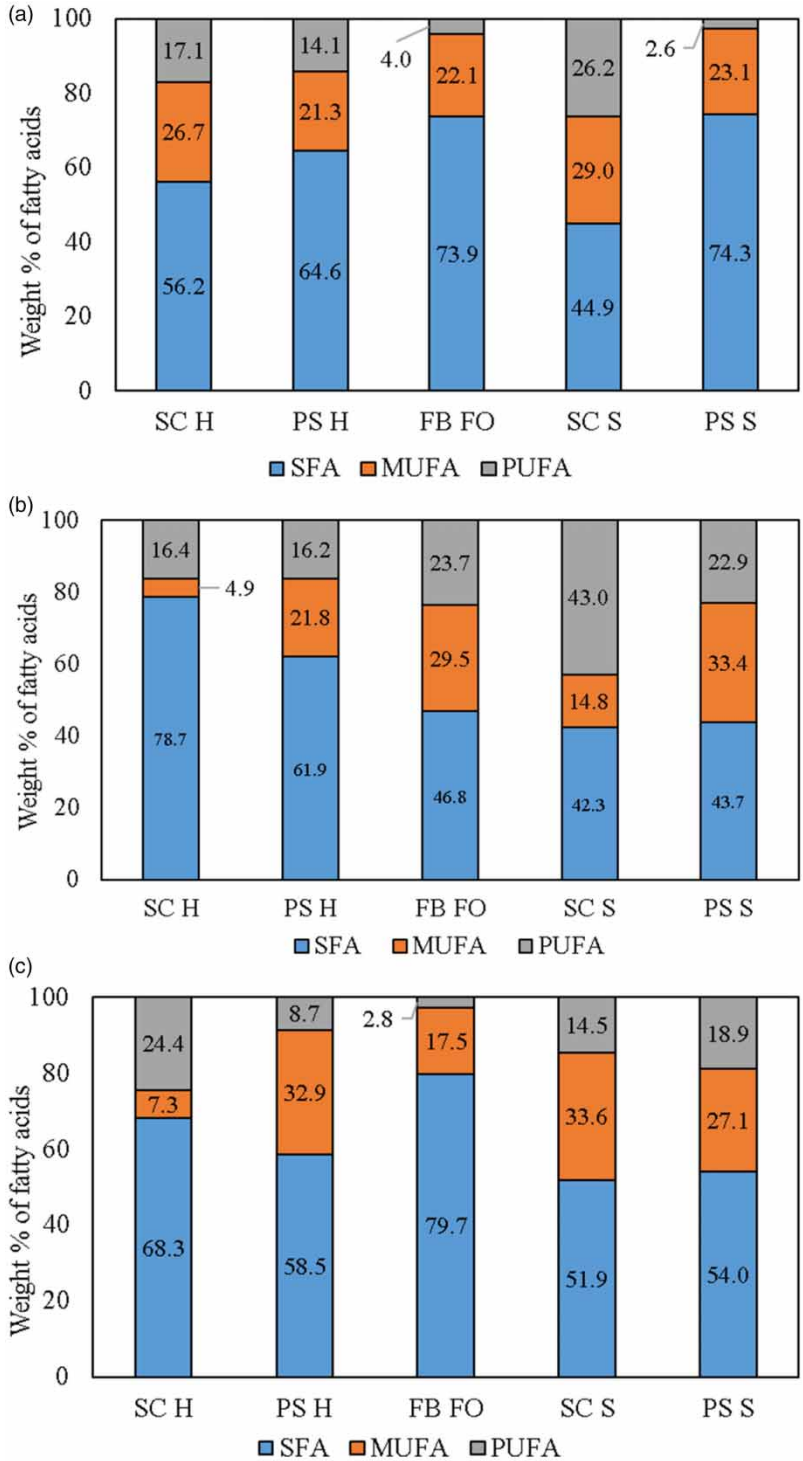


Figure 2 | Profiles of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in biodiesel obtained from different samples during (a) winter, (b) summer, and (c) autumn. Samples are abbreviated as shown in Table 1.

in Japan (Table 6). Importantly, this value may be an overestimation because fat balls and primary scum likely are not frequently skimmed at treatment facilities. Nevertheless, this value suggests that efficient management of wastewater-derived lipids could make them a valuable resource for biodiesel production, which would match the goals of achieving a circular economy.

Table 4 | Biogenic and fossil C contributions in wastewater treatment plant samples and biodiesel from this study and diesel in previous studies

Source		C fraction	
		Biogenic (%)	Fossil (%)
Dry solids	FB	100	0.0
	SC H	92.0	8.0
	PS H	95.0	5.0
	SC S	96.0	4.0
	PS S	99.0	1.0
Biodiesel	FB	74.0	26.0
	SC H	58.0	42.0
	PS H	67.0	33.0
	SC S	72.0	28.0
	PS S	64.0	36.0
Biodiesel 100% (B100)[a]		92.4	7.6
Biodiesel 100% (B100)[b]		94.6	5.4
Biodiesel 20% (B20)[a]		22.0	78.0
Petroleum diesel[a]		2.0	98.0

Samples are abbreviated as shown in Table 1.
[a]Lee *et al.* (2022).
[b]Sebos (2022).

Table 5 | Estimated daily biodiesel potential of materials from a pumping station and two wastewater treatment plants (WWTPs) in Kobe, Japan

Source	Sample	Wastewater flow rate (m^3/day)	Dry matter (kg/day)	Lipids (kg/day)	Biodiesel (kg/day)
Pumping station	FB	37,000.0	4.4	2.1	0.8
WWTP H	SC H	172,000.0	1.4	0.7	0.3
	PS H		366.0	60.0	26.0
WWTP S	SC S	135,000.0	1.5	0.8	0.3
	PS S		215.0	34.0	12.3

Samples are abbreviated as shown in Table 1.

Table 6 | Biodiesel potential and CO_2 emissions based on pumping station (fat balls) and wastewater treatment plant (primary scum and sludge) samples

Source	Wet quantity (metric tons/year)	Dry matter (metric tons/year)	Lipids (metric tons/year)	Biodiesel (metric tons/year)	CO_2 emissions from biodiesel (metric tons CO_2eq/year)		CO_2 emissions from petrodiesel[a] (metric tons CO_2eq/year)
					Biogenic C fraction	Fossil C fraction	
Fat balls	3,300.0	1,800.0	820.0	300.0	690.0	240.0	926.0
Primary scum	6,600.0	165.0	90.0	33.0	64.3	34.6	102.0
Primary sludge	251,000,000.0	1,510,000.0	242,000.0	96,300.0	192,000.0	101,000.0	303,000.0

[a]CO_2 emissions of petrodiesel were estimated at 3.15 kg CO_2 per kg (Usman *et al.* 2023).

Teixeira *et al.* (2018) noted that over 0.5 million metric tons of used cooking oil is generated in Japan and could potentially be used for biodiesel feedstock; however, the establishment of a cost-effective and efficient collection network remains a challenge in optimizing the valorization of used cooking oil (Maddikeri *et al.* 2012). Japan has been importing used cooking oil to meet the demand for biodiesel production (Sasatani 2023). The biodiesel production capacity in Japan was reported to be between 18,000.0 and 20,000.0 metric tons (Iijima 2018; Statista 2024). Therefore, biodiesel derived from fat balls and primary scum could be a viable option to supplement used cooking oil, which has been the main biodiesel source in Japan.

The biogenic and fossil CO_2 emissions associated with biodiesel can be calculated according to the following equations (Sebos 2022):

$$\text{Biogenic } CO_2 \text{ in biodiesel} = \text{biodiesel quantity} \times \text{C content} \times \text{biogenic fraction} \times (44/12) \quad (4)$$

$$\text{Fossil } CO_2 \text{ in biodiesel} = \text{biodiesel quantity} \times \text{C content} \times \text{fossil fraction} \times (44/12) \quad (5)$$

By assuming a biodiesel C content of 0.83 g C/g (Lee *et al.* 2022), we estimated that the potential maximum amount of biodiesel produced annually in Japan from fat balls and primary scum (333.0 metric tons/year) would release 274.6 metric tons of fossil CO_2 emissions per year; petroleum-based diesel production would generate a threefold greater amount of CO_2 emissions (Table 6). Unlike the emissions from petroleum-based fuels, biogenic C is presumed to contribute zero additional atmospheric CO_2 emissions (Hums *et al.* 2016). Therefore, the use of wastewater lipids as an alternative feedstock for biodiesel production is expected to reduce greenhouse gas emissions.

4. CONCLUSION

We investigated the characteristics of and potential for biodiesel production using lipid sources from a pumping station (i.e., fat balls) and WWTP primary clarifier (i.e., primary scum and primary sludge) in Kobe, Japan. FB samples from the pumping station had higher TS and C contents, along with greater calorific values, than SC and PS samples collected at the primary clarifier. FB and SC samples had higher lipid contents (46.4–54.5%) and biodiesel yields (17.3–20.1%), compared with PS samples (lipid yield: 15.8–16.4%; biodiesel yield: 5.7–7.1%). Considering that primary scum has a higher moisture content, our findings suggest that fat balls are the most promising biodiesel feedstock from wastewater facilities. Analyses of methyl ester composition revealed that FAME (i.e., biodiesel) produced from the samples contained high SFA and MUFA contents. Moreover, ^{14}C analysis revealed that the produced biodiesel contained 58.0–74.0% of biogenic C. Taken together, our findings highlight the potential for alternative resource management strategies at wastewater facilities to recover valuable resources for the production of value-added products. Future research efforts should focus on optimizing lipid recovery to further enhance biodiesel yields.

ACKNOWLEDGEMENTS

We extend our gratitude to the staff of the wastewater treatment plants in Kobe City for their valuable cooperation in collecting samples for this study. Additionally, the author expresses appreciation to the Japanese Government (MEXT) Scholarship for providing financial support.

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories: https://gbr01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoi.org%2F10.6084%2Fm9.figshare.25539490.v1&data=05%7C02%7Cjournalproduction%40iwap.co.uk%7Cadccb26629294e2d2cee08dc5a15f014%7Cf17b0bf345ed46b3bcfbc0bd2ae47303%7C0%7C0%7C638484297461694579%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=oNVIQKLqHkHFbg8%2BkDeEh7EXurVk9FcDpdWiMdwvf4w%3D&reserved=0.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 3 January 2024; accepted in revised form 17 February 2024. Available online 19 March 2024