ANTIBIOTIC RESISTANCE MICROBIAL INACTIVATION AND LIPID EXTRACTION FROM SEWAGE SLUDGE FOR BIODIESEL PRODUCTION USING SUPERCRITICAL CO_2 TECHNOLOGY

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by

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LIST OF SYMBOLS

kg	Kilogram
mg	Milligram
mm	Millimeter
min	Minutes
nm	Nanometer
P	Pressure
T	Temperature
λ	Wavelength
W	Watt

LIST OF ABBREVIATIONS

MSS	Municipal sewage sludge
ANOVA	Analysis of variance
EtOH	Ethanol
EU	European Union
FAME	Fatty Acid Methyl Ester
HCl	Hydrochloric acid
mag.	Magnification
CH_4	Methane gas
MUFA	Mono-unsaturated fatty acids
ppm	Part per million
PUFA	Polyunsaturated fatty acids
RSM	Response surface methodology
SFA	Saturated fatty acids
SEM-EDX	Scanning Electron Microscopy- Energy dispersive X-ray spectroscopy
NaOH	Sodium hydroxide
H_2SO_4	Sulfuric acid
wt.%	Weight percent
S/L	Sewage sludge to solvent

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PENYAHAKTIFAN MIKROB RINTANGAN ANTIBIOTIK DAN PENGEKSTRAKAN LIPID DARIPADA ENAPCEMAR KUMBAHAN UNTUK PENGHASILAN BIODIESEL MENGGUNAKAN TEKNOLOGI SUPERKRITIKAL CO_2

ABSTRAK

Pelupusan yang selamat bagi lumpur kumbahan kota (MSS) merupakan isu alam sekitar yang pelbagai di loji rawatan kumbahan di seluruh dunia disebabkan oleh jumlah yang besar dihasilkan dan mengandungi bahan berbahaya. Oleh itu, menggunakan MSS untuk pengeluaran biodiesel merupakan inisiatif positif untuk mengurangkan pencemaran alam sekitar dan menghasilkan sumber tenaga alternatif. Oleh itu, tujuan utama kajian ini adalah untuk menilai penggunaan yang mampan bagi lumpur kumbahan kota sebagai bahan mentah berpotensi untuk pengeluaran biodiesel. Sampel MSS dikumpulkan dari loji rawatan kumbahan Indah Water Konsortium Lebuh Permai di Pulau Pinang untuk penjelmaan lanjut, pengekstrakan dan sintesis. Sifat fizikokimia sampel MSS yang dikumpulkan dikenal pasti melalui analisis instrumental. $ScCO_2$ telah digunakan untuk mematikan kuman dalam MSS dan $scCO_2$ juga digunakan untuk mengekstrak hasil dari lipid. Pemisahan lipid dari MSS disiasat berdasarkan kelarutan asid lemak dan derivatifnya dalam $scCO_2$ dengan menggunakan persamaan Gompertz yang telah diubah suai untuk menjelaskan tingkah laku pemisahan lipid. Selain itu, pengekstrakan soxhlet telah digunakan sebagai kaedah perbandingan untuk mengekstrak lipid daripada MSS. Lipid yang diekstrak kemudiannya ditranseterifikasi untuk menghasilkan biodiesel. Dapatan menunjukkan bahawa pencirian mengungkapkan sampel MSS mempunyai kandungan organik keseluruhan (TOC) sebanyak 298 g/kg, kandungan karbon keseluruhan (TC) sebanyak

49.95% dan nisbah C:N sebanyak 31.82%. Selain itu, pelbagai spesies bakteria dengan ketahanan antibiotik dikenal pasti. Eksperimen pemencilan $scCO_2$ telah dioptimumkan berdasarkan pengasingan bakteria dengan ketahanan antibiotik dalam MSS menggunakan Metodologi Permukaan Tindak Balas (RSM). RSM menunjukkan bahawa pengurangan bilangan bakteria sebanyak kira-kira 7.21 log diperoleh pada tekanan 20 MPa, suhu 60 °C, dan masa 60 minit. Ekstraksi $scCO_2$ digunakan untuk mengekstrak lipid daripada MSS. Sekitar 27% lipid diekstrak dari MSS dengan $scCO_2$ pada suhu 60 °C, tekanan 30 MPa, masa rawatan 60 minit, dan 5 wt.% etanol (EtOH) sebagai ko-penyelesaian. Persamaan Gompertz yang telah diubahsuai telah berhasil disesuaikan dengan data eksperimen ekstraksi lipid dari MSS menggunakan $scCO_2$. Analisis sifat kinetik menunjukkan bahawa teknologi ekstraksi $scCO_2$ sangat bergantung pada tekanan, lebih daripada suhu dalam ekstraksi lipid. Berbanding dengan ekstraksi Soxhlet, teknologi $scCO_2$ adalah teknologi unggul dalam hal kualiti lipid. Nilai asid lipid yang diekstrak dari $scCO_2$ adalah 0.50 ± 0.80 KOH/g yang mematuhi standard ASTM yang ditetapkan iaitu 0.5 KOH/g. Lipid yang diekstrak menggunakan $scCO_2$ ditransesterifikasi untuk menghasilkan biodiesel. Oleh itu, $scCO_2$ digunakan untuk mentransesterifikasi lipid yang diekstrak untuk menghasilkan biodiesel. Nilai optimal penukaran ester metil asid lemak (FAME) adalah 84% yang diperoleh pada nisbah mol minyak: metanol 1:6, kepekatan pemangkin 5 wt.%, suhu 60 °C, dan masa 4 jam. Sifat fizikokimia biodiesel yang disintesis mematuhi standard ASTM D6751, EN 14214, dan MS 2008:2008. Hasil kajian ini menghasilkan penemuan bahawa MSS adalah bahan mentah yang tersedia untuk menghasilkan biofuel yang mampan, dan pengurusan MSS yang selamat diperlukan untuk mencapai prinsip limbah-ke-kemakmuran.

ANTIBIOTIC RESISTANCE MICROBIAL INACTIVATION AND LIPID EXTRACTION FROM SEWAGE SLUDGE FOR BIODIESEL PRODUCTION USING SUPERCRITICAL CO_2 TECHNOLOGY

ABSTRACT

Safe disposal of municipal sewage sludge (MSS) is a various environmental issue in sewage treatment plants worldwide due to its enormous volume of generation and containing hazardous substances. Consequently, utilising MSS for biodiesel production would be positive initiative to reduce environmental contamination and produce alternative energy resources. Accordingly, the main aim of this study is to evaluate a sustainable utilization of municipal sewage sludge as a potential feedstock for biodiesel production. MSS samples were collected from Indah Water Konsortium Lebuh Permai sewage treatment plants in Pulau Pinang for further characterisation, extraction and synthesis. The physicochemical properties of the collected MSS samples were identified through instrumental analyses. $ScCO_2$ has been used to sterilized MSS and $scCO_2$ was employed as well to extract yield from lipids. the separation of lipids from MSS was investigate on the basis of the solubility of the fatty acids and their derivatives in $scCO_2$ by using the modified Gompertz equation to elucidate lipid separation behaviour. More over the soxhlet extraction has been used as a comparison method to extract lipids from MSS. The extracted lipids where transesterified to produce biodiesel. The finding shows that characterisation revealed that the MSS sample had a total organic content (TOC) of 298 g/kg, total carbon (TC) content of 49.95% and C:N ratio of 31.82%. In addition, multiple bacterial species with antibiotic resistance were identified. The $scCO_2$ sterilization experiments were optimized based on the inactivation of the antibiotics resistance bacteria in MSS using

Response Surface Methodology (RSM). RSM revealed that the bacterial count reduction of approximately 7.21 log was obtained at the pressure of 20 MPa, temperature of 60 °C and time of 60 min. The scCO$_2$ extraction was utilized to extract lipids from MSS. About 27% of lipids was extracted from MSS with scCO$_2$ at a temperature of 60 °C, pressure of 30 MPa, treatment time of 60 min, and 5 wt.% of ethanol (EtOH) as co-solvent. The modified Gompertz equation was satisfactorily fitted with experimental data of the lipids extraction from MSS using scCO$_2$. The kinetics properties analyses revealed that the scCO$_2$ extraction technology was highly dependent on pressure, more so than on the temperature for the extraction of the lipids. Comparing with Soxhlet extraction the scCO$_2$ was the superior technology in terms of lipid quality. since the acid value of lipids extracted from scCO$_2$ was 0.50± 0.80 KOH/g comply with the ASTM which was 0.5 KOH/g. The extracted lipids using scCO$_2$ was transesterified to produce biodiesel. Hence, scCO$_2$ used to transesterified the extracted lipids to produce biodiesel. The optimal value of the fatty acids methyl esters (FAME)conversion was 84% that obtained at the oil: methanol molar ratio of 1:6, catalyst concentration of 5 wt.%, temperature of 60 °C and time of 4 h. The physicochemical properties of the synthesised biodiesel complied with the ASTM D6751, EN 14214 and MS 2008:2008 standards. The findings of this study led to the identifying MSS as available feed stock to produce sustainable biofuels and safe MSS management needed to attain the waste-to-wealth principle.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Municipal sewage sludge (MSS) is defined as a solid, semisolid or liquid muddy residue resulting from the discharges of domestic activities (Ruj et al., 2021). It originates from sewage treatment plants as a final product (Demirbas et al., 2017). The water content of sewage sludge accounts for 95%–98% of wastewaters (Kech et al., 2018). Sewage sludge usually contains many hazards, such as heavy metals, pathogenic agents and nonbiodegradable organic and inorganic compounds, that increase environmental threats. However, sewage sludge also contains multiple valuable compounds, such as carbon, nitrogen, phosphorus, potassium, sulphur and other complex compounds (Buonocore et al., 2018). The annual generation of sewage sludge is rapidly increasing because of population explosion and urban expansion. Malaysia is expected to generate 10 million cubic metres of sewage sludge every year by the year 2035 (Mustapa et al., 2020). Sewage sludge was, and still is, an enriched material that can be utilised in research because of its benefits and to avoid potential threats.

Sewage sludge is dumped in landfills as its final fate or combusted via incineration. However, recent approaches have attempted to utilise sewage sludge in compost and the construction industry and convert it into bioenergy to minimise environmental deterioration. The improper management of organic sewage sludge can have serious environmental impacts, such as unpleasant odours, disease transmission and global warming (Ding et al., 2021). Landfilling is the classical approach for the treatment of the tremendous amounts of sewage sludge that are generated daily. It involves simply discarding sewage sludge in well-structured designed holes in the earth. In landfill systems, leached materials are drained to avoid polluting groundwater. Incineration and melting are other methods for discarding MSS; they involve burning the generated materials at 800 °C–1800 °C (Nunes et al., 2021). However, the incineration of sewage sludge raises considerable environmental concerns because it produces toxic gaseous materials, such as dioxin, furan and fly ash. The toxic metals contained by fly ash are lead, cadmium, copper and zinc (Ata et al., 2021; Rajendran et al., 2020). Composted sewage sludge can be used as a soil fertiliser in agriculture and horticulture.

The great advantage of composting sewage sludge is that it returns carbon, nitrogen, phosphorus and essential elements to the soil. However, pathogens and heavy metals can limit the reuse of composted sewage sludge. In addition, sewage sludge can be utilised in the industry to produce cement and building bricks (Luo et al., 2020). The last approach is using sewage sludge as a feedstock to synthesise bioenergy. Different biofuels can be produced from treated sewage sludge through different methods. For example, biodiesel fuel can be synthesised from the fatty acids extracted from sewage sludge through transesterification, and the biogas methane can be produced through the anaerobic digestion of treated MSS. Therefore, MSS treatment is very crucial for reducing environmental impacts.

The use of sewage sludge for bioenergy production is attracting considerable attention because MSS offers affordable feedstock. Anaerobic digestion is a relatively simple and economical method for biogas fuel production from MSS (Sadhukhan, 2014). The biogas obtained through anaerobic digestion is mostly composed of methane (50%–70%) and contains other trace gases of hydrogen, nitrogen, water vapour and hydrogen sulphide. However, several pretreatment methods are required to increase biogas generation by sewage treatment plants. These methods include microwave irradiation, enzymatic treatment, acid/alkali treatment, ozonation, ultrasonication, wet oxidation and liquid jetting (Sun et al., 2020). Bio-oil is another biofuel that can be produced from MSS through the thermo-conversion process of pyrolysis, in which MSS feedstock is subjected to supercritical temperatures above 300 ℃–500 ℃ in a deoxygenated environment. Under such conditions, the feedstock turns into liquid oil that can be used for thermal and domestic purposes (Barry et al., 2019). The fatty acids extracted from sewage sludge can be applied as the building blocks to produce biodiesel, a biofuel, from sewage sludge feedstock.

Sewage sludge is a lipid feedstock produced in massive quantities by sewage treatment plants (Liu et al., 2021). MSS contains 5%–20% w/w lipids, which is close to the lipid content of some vegetative feedstock. Thus, it is a potential source for biodiesel production, which will solve the problem of MSS accumulation in treatment systems. However, the most difficult aspect of producing biodiesel from MSS is determining an effective lipid extraction technique (Hu et al., 2020). The oil content of sewage sludge is extracted through multiple techniques, such as the Bligh and Dyer method, acid hydrolysis, liquid–liquid extraction and Soxhlet extraction. In the Bligh and Dyer method, a mixture of chloroform/methanol/water is used as a solvent. It takes advantage of the properties of ternary mixtures. When solvents are mixed at a specific ratio, they form a monophasic solution, thus improving contact between soluble lipids and the organic solvent.

The addition of water and/or chloroform produces a biphasic system through which the separation of the lipid-containing chloroform layer is facilitated (Kech et al., 2018). The other extraction method is based on acid solvents and was thus named acid hydrolysis (Phanthong et al., 2018). Zhu et al. (2014) extracted lipids from MSS and obtained a lipid yield of only 1.3%. Similar to acid hydrolysis, liquid–liquid extraction is dependent on homogenising samples with a mixture of organic solvents except that it uses organic solvents at specific ratios instead of a sulphuric medium for lipid extraction (Kech et al., 2018). In addition to organic solvents, lipid extraction from sewage sludge through the Soxhlet method depends on temperature and time variables.

Soxhlet extraction is one of the preferable methods for extracting sewage sludge oil. A Soxhlet apparatus consists of a solvent flask containing organic solvents, an extraction chamber containing the prepared sample within a cellulose thimble and a condenser. Extraction is performed at 60 °C–80 °C. The total extraction run time ranges from 6 h to 24 h (Mustapha et al., 2017). Neat and mixed organic solvents are used in the Soxhlet extraction method. A heat source is applied to the solvent flask. Once the solvents reached the boiling point, they evaporate, hit the condenser and precipitate in the extraction chamber to meet the dried sample in the cellulose thimble. The lipids are released within the hot organic solvent to fill the whole chamber. The organic solvent with the dissolved lipids returns to the solvent flask. One extraction cycle takes 15–20 min to complete depending on the solvent volume, boiling point and applied temperature. In contrast to other extraction methods, such as acid hydrolysis and water bath shaking, the Soxhlet method can extract the maximum amount of sewage sludge lipids (Zhu et al., 2014).

The supercritical carbon dioxide (scCO_2) method has been proven to be a promising and ecofriendly technology for the sterilisation, separation and fractionation of vegetable oil and other valuable components (Vardanega et al., 2019; Yen et al., 2015). It offers numerous advantages, including the absence of the use of organic solvents, minimal environmental effects and operation at moderate temperatures of 40 °C–80 °C (Badgujar et al., 2021). The liquefied CO_2 is an ideal supercritical fluid due to its nontoxic, inflammable and lipophilic nature (Patel et al., 2020a). Fluid CO_2 has a moderate critical pressure (7.38 MPa) and temperature (31.1 °C) with tunable properties (Boock et al., 2019; Hossain et al., 2021). Many studies have reported that $scCO_2$ exhibits a faster reaction rate, easier lipid separation and higher lipid quality than solvent extraction(Patel et al., 2020; Patil et al., 2018; Fizal et al., 2022). Thus, the utilisation of $scCO_2$ for lipid extraction has attracted increased attention.

In addition to lipid extraction, $scCO_2$ technology is used for sterilisation. In the $scCO_2$ method, fluid CO_2, which is a gas at ambient temperature, is used as the solvent. Therefore, this method does not produce any residue after treatment. It offers numerous advantages as a clean sterilisation technology, such as easy separation, short processing time, environmental friendliness, waterless nature, high solubility and the damage-free treatment of heat-sensitive materials (Hossain et al., 2016). $scCO_2$ technology depends on the pressure and temperature exposed to the sample materials. Pressure has a great effect on sterilisation because it loosens the cell walls of the microorganisms present in the sample. Time is another crucial parameter for effective sterilisation (Allafi et al., 2021b). MSS samples contain millions of pathogenic bacteria that threaten human health and the surrounding environment. The existence of such agents limits the utilisation of MSS. Thus, sterilisation is highly recommended before any application of MSS in agriculture, industry and biofuel production (Ju et al., 2016).

In terms of energy production and consumption, the world is experiencing energy shortage. Total global energy usage has increased considerably. The 2021 International Energy Outlook predicted that the global energy demand will increase 50% by 2050. Global energy consumption is increasing at an accelerated rate due to economic expansion and population. Energy demand and consumption are increasing unsustainably (Gebremariam & Marchetti, 2018b).

Renewable energy is becoming an important energy resource in many countries worldwide. It is dependent on natural resources that can utilise the required energy and protect the surrounding environment. The major challenge is to increase the amount of renewable energy in the supply system. Biofuel is one of the alternative energy resources with the advantage of flexible supply systems in contrast to fossil fuels (Uğurlu, 2019). Biomass feedstocks can be used to produce a range of gaseous biofuels, such as hydrogen and methane, and liquid biofuels, such as ethanol, methanol and biodiesel. Biofuel is considered as an ecofriendly fuel because its emissions are biodegradable and have little environmental impact. Therefore, biofuel provides an alternative and sustainable clean energy resource (Adewuyi, 2020; Mat Aron et al., 2020).

In recent years, biodiesel has emerged as one of the most promising alternative fuels. In addition, it is an ecofriendly energy resource that can meet the high global energy demand, achieve sustainability and decrease environmental pollution (Mariotti et al., 2020; Singh et al., 2020). Biodiesel can be synthesised from multiple feedstock oils, including those generated from plants and animal fats. Transesterification is the main biochemical process of biodiesel synthesis, in which the alkoxy group of an ester is displaced by an alcohol group (Khan et al., 2021). In transesterification, triglycerides react with alcohol in the presence of an acid or basic catalyst to produce biodiesel and glycerol as a by-product (Rathnam et al., 2020). According to recent studies, A variety of biolipids can be used to produce biodiesel. These are virgin vegetable oil feedstock included rapeseed and soybean oils are most commonly used, though other crops such as mustard, palm oil, sunflower, hemp, and even algae show promise waste vegetable oil also animal fats including tallow, lard, and yellow grease and non-edible oils such as jatropha, neem oil, castor oil, and tall oil , the feedstock have better performance than conventional diesel (Demirbas, 2009; Fernando & Kapilan, 2020; Parniakov et al., 2015). Therefore, biodiesel is a potential alternative to petroleum diesel because it has ecofriendly properties (not environmentally harmful) and thus represents a sustainable energy resource for the coming generation. Previous studies took MSS as a feedstock to produce biodiesel. Zhu et al. (2014) found that the lipid extraction yields of acid hydrolysis, Soxhlet extraction and water-bath shaking reached 1.30%, 6.35% and 4.10%, respectively. Ivwurie and Okoro (2020), Ncube et al. (2018) and Singh and Kumar (2020) performed Soxhlet extraction by using different types of solvents. Nevertheless, studies using $scCO_2$ to extract oil from MSS are lacking. The current study intends to fill this research gap by using $scCO_2$ to extract MSS lipids for renewable biodiesel fuel production.

1.2 Problem Statement

The main option for MSS disposal is dumping in landfills. Given that MSS may contain pathogenic bacteria and antibiotic, its use as a feedstock for direct services in contact with human beings without sterilisation will effect on human health and the environment (Eid et al., 2018). Thus, MSS contains pathogenic microorganisms should be sterilised first for safe handling. Existing sterilization technologies in waste management, including steam autoclave, microwave, and ethylene oxide are not effective in denaturing bacteria and unsuitable for MSS sterilisation, the bacterial re-growth in autoclave treated clinical solid waste reveals that the steam autoclave is an ineffective method for treating clinical solid waste (Hossain et al., 2013). Wherein, the $scCO_2$, is an effective method to sterilize heat-sensitive material without degrading the quality. This technology effectively kills bacteria by dissolving cytoplasmic substances in the fluids CO_2 at a supercritical state (Allafi et al., 2021b).

On other hand, lipids are found to be rich in municipal sewage sludge it contain 10-30% (Lee et al., 2020). Lipids are usually extracted from MSS with the help of solvent extraction methods using organic solvents. In this regard, major problems associated with the conventional methods for oil extraction from inedible waste include long extraction times and low-quality and -purity oil yields; moreover, these methods require costly, high-purity toxic organic solvents and further purification and refining processes, evaporate massive amounts of solvents and have low extraction selectivity (Atabani et al., 2013; Ivwurie & Okoro, 2020). The $scCO_2$ method is a novel and ecofriendly technology. The distinct feature of the $scCO_2$ process is that it does not require further purification or lipid separation from MSS in contrast to conventional solvent extraction processes because short-chain fatty acids and glycerol have low solubility in $scCO_2$. Therefore, high-quality lipids could be separated from MSS by tuning the pressure, temperature, separation time and flow rate of the $scCO_2$ process (Obeid et al., 2018).

The literature described lipid extraction behaviour and kinetics by using linear equations. However, the determination of lipid extraction behaviour by using linear equations provides linear curves and therefore avoids the important aspects, including lag phase (contact time between the matrix and solvent) and stationary phase (the maximum lipid extraction phase). The modified Gompertz equation provides sigmoidal curves, which are effective in describing the lag, extraction and stationary phases. Therefore, utilising the modified Gompertz equation to elucidate the behaviour of lipid extraction from MSS through the $scCO_2$ method has attracted considerable interest.

1.3 Research Objectives

The main aim of this study is to evaluate a sustainable utilization from MSS as a potential feedstock for biodiesel production. The detailed objectives are listed as:

i. To determine the physicochemical properties and the presence of microbes in MSS.

ii. To evaluate the $scCO_2$ sterilisation efficiency on the inactivation of microbes in MSS.

iii. To determine the influence of the $scCO_2$ pressure, temperature and treatment time on the lipids yield from MSS.

iv. To elucidate lipids extraction behaviour from MSS under $scCO_2$ using modified Gompertz equation.

v. To determine physicochemical properties of biodiesel synthesized from the $scCO_2$ extracted lipids using catalytic transesterification process.

1.4 Scope of Study

This study is based on a lab-work approach. MSS samples were taken from a sewage treatment plant of Indah Water Konsortium Lebuh Permai in Pulau Pinang for further characterisation, extraction and synthesis. The physicochemical properties of the collected MSS samples were identified through instrumental analyses to determine the existence of heavy metals; total organic nitrogen, carbon, hydrogen, ash, moisture, potassium and phosphorus contents; pH and EC. The bacterial load and presence of antibiotic resistance were also determined. The $scCO_2$ method was chosen for sterilisation and lipid extraction from MSS. Waterless sterilisation was conducted to inactivate the microbial load of MSS. This method depends completely on high pressure, temperature and time. The modified Gompertz model was used to adjust temperature, pressure, flow rate and extraction time. The extracted lipids were characterised on the basis of fatty acid composition by using a GC-fid and lab instruments (such as viscometer, hydrometer, Calorimeter, ignition tester, etc.) to determine the physicochemical properties of the $scCO_2$ extracted lipids from MSS. Catalytic transesterification was used to synthesise the extracted oil for renewable biodiesel fuel production. The oil: methanol molar ratio, catalyst ratio and reaction temperature and reaction time were adjusted to obtain the highest possible yield of biodiesel from the MSS lipids. The synthesised biodiesel was subjected to characterisations to determine its physicochemical properties, namely, density, viscosity, CP, pour point (PP), flash point, heating value, acid value, iodine number and cetane number.

1.5 Thesis Organisation

This thesis consists of five chapters: the Introduction, Literature Review, Methodology, Results and Discussion and Conclusion. Chapter 1 introduces the background of MSS, $scCO_2$ Gompertiz modelling and biodiesel. The problem statement was formulated on the basis of the existing theoretical and practical gaps. The objectives of this research project were designed to advance a new area of research. However, the scope of this study was restricted by temporal and spatial limitations. In Chapter 2, the concepts and backgrounds are expanded by reviewing the literature on MSS, lipid extraction and biodiesel production. MSS generation, characterisation, application and treatment methods are also discussed in this section. Different approaches for extracting lipids from MSS are described. This section presents the $scCO_2$ method as the most advanced technology and the novelty of the present project. Finally, biodiesel synthesis, characterisation and effects are included in the discussion. The experimental materials and methods are reported in Chapter 3. This chapter describes the initial steps for conducting the laboratory work, instrumental analyses and calculations. Computer-based modelling approaches were implemented to derive the optimal values for sterilisation, extraction and synthesis methods. The experimental results are reported in eight sections in Chapter 4. Discussion and critical analysis are applied to interpret findings. Similarities and differences are introduced to enhance the explanation of the current results. The results obtained in this research work are summarised in Chapter 5. Recommendations are given for future studies related to this research project. The references and appendices are in the final sections of this thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 Municipal sewage sludge (MSS)

Municipal sewage sludge (MSS) is a semi-solid residue discharged from municipal buildings into underground sewer pipelines and transferred to treatment plants (Collivignarelli et al., 2019; Thatai et al., 2019). In STP, the MSS is separated from the wastewater as the by-product. The major components of the MSS are human faecal waste, other organic and inorganic contaminants (i.e. heavy metals), and other contaminants from different sources (Haynes et al., 2009; Poblete et al., 2022). Municipal sewage sludge has extensively sourced every year throughout the world. MSS is generated from several sources, such as residential and institutional buildings, healthcare facilities, streets, and commercial buildings (Demirbas et al., 2017; Thatai et al., 2019), as shown in Figure 2.1. MSS is commonly separated from wastewater during physicochemical treatment, in the primary stage of wastewater treatment (Poblete et al., 2022; Saleh et al., 2020). sewage sludge and wastewater are generated from local community buildings.

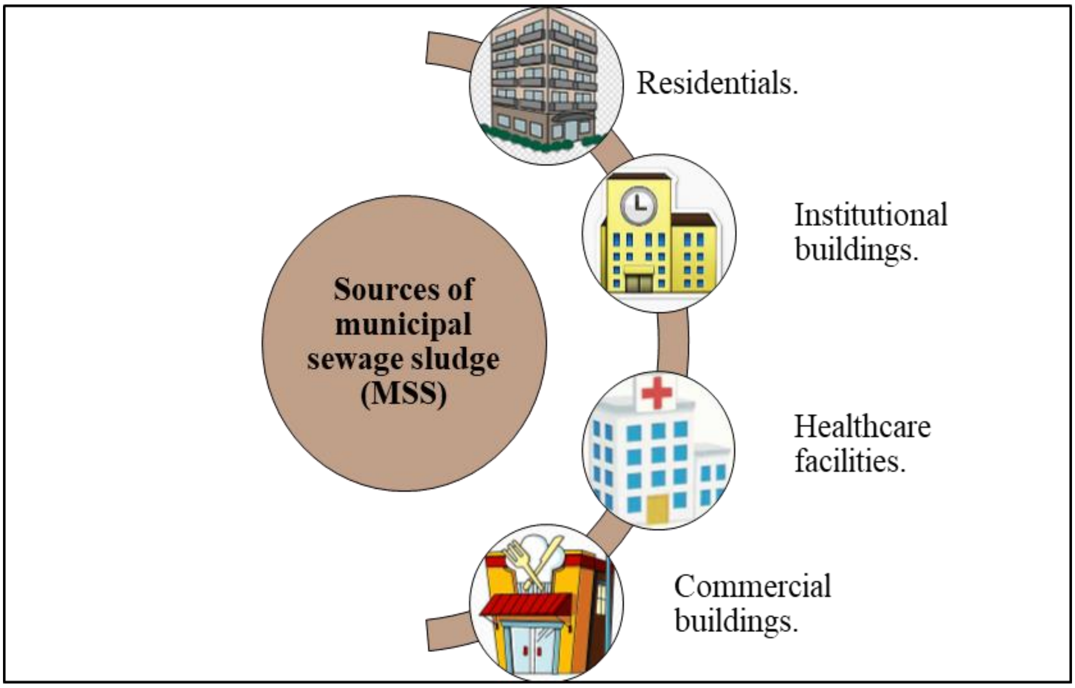

Figure 2.1 Municipal sewage sludge generated from local community buildings (Demirbas et al., 2017; Thatai et al., 2019).

The sewage sludge and wastewater are transferred through the pipeline in the sewer to wastewater treatment (Demirbas et al., 2017; Kim & Phae, 2022; Thatai et al., 2019). Wherein the MSS is separated from wastewater in the form of a thick slurry (Han et al., 2018). Several approaches can be utilized for sewage sludge production during wastewater treatment, such as anaerobic digestion, activated sludge process, and oxidation process (Kim & Phae, 2022; Wang et al., 2019). During wastewater treatment, most contaminants in the wastewater are concentrated into the sludge (Alisawi, 2020; Gao et al., 2020). Therefore, it can be said that sewage sludge as a by-product of wastewater treatment may reflect the actual raw wastewater composition and anthropogenic activities (Bondarczuk et al., 2016; Bubalo et al., 2021). On the other hand, MSS has an abundant amount and sustainable sources from its generators (Bratina et al., 2016; Đurđević et al., 2022). Therefore, a huge amount of sewage sludge is regularly discharged during primary wastewater treatment. The direct disposal of untreated sewage sludge may lead to serious problems such as environmental hazards, expensive treatment costs, and increased waste loads (Bubalo et al., 2021; Lu et al., 2018).

During wastewater treatment, most of the contaminants that are selectively removed from the water concentrate into sludges (Rout et al., 2021; Zhang et al., 2020). Table 2.1 shows the municipal sewage sludge generation per annum and generation rate from 2018 to 2021. It has been found that the countries with the highest municipal sewage sludge from 2018 to 2021 are India (35 mil. tonnes), Europe (13.5 mil. tonnes), and USA (12.6 mil. tonnes), with generation rate (in kg/capita.day) of 2.7, 1.9, and 3.6, respectively. It is followed by China, Malaysia, Japan, Germany, and UK with municipal sewage sludge generation (in mil. tonnes) of 6.3, 3.0, 2.4, 1.8, and 1.5, and generation rate (in kg/capita/day) of 1.09, 2.70, 1.48, 1.81, and 1.80, respectively (Hanum et al., 2019b). The generation of municipal sewage sludge has increased significantly over the past decades due to population increase and anthropogenic activities. Wherein it is estimated more than 20 million tonnes of municipal sewage sludge is generated per annum throughout the world. Meanwhile, researchers estimated that MSS would double by 2022 (Hanum et al., 2019).

Table 2.1 Estimation of municipal sewage sludge generation per annum and the generation rate in 2018-2021 (Hanum et al., 2019; Kiselev et al., 2019)

Countries	**Sewage sludge generation per annum (mil. tonnes)**[1]	**Generation rate (kg/capita.day)**[1]
India	35.0	2.70
Europe	13.5	1.90
USA	12.6	3.63
China	6.3	1.09
Malaysia	3.0	4.16
Japan	2.4	1.48
German	1.8	1.81
UK	1.5	1.80

[1]Based on a wet weight basis.

2.1.1 Characterisation of municipal sewage sludge

Sewage sludge as a complex biosolid originally contained in raw wastewater, contains also various pollutants such as toxic heavy metals, persistent organic pollutants, emerging contaminants, detergents, and pathogens. This can pose potential risks to human health and the environment (Pöykiö et al., 2019; Zoghlami et al., 2016). In this regard, MSS can exhibit different characterisations depending on its source and the process that gave rise to it. Therefore, the current section provides an explanation of the physical, chemical and biological properties of sewage sludge as follows:

2.1.1(a) Physicochemical properties

The characterisation of physicochemical properties is essential to determine the composition of the sewage sludge, including organic compounds and heavy metals. The characterisation is commonly carried out during primary and secondary treatment before the sewage sludge being disposed in the landfill site (Collivignarelli et al., 2019; Singh & Kumar, 2020). Depending on the source, geographical location, and country, MSS's physical and chemical characteristics show different variations. MSS can be a solid, semisolid, or liquid muddy residue.

The nutrient content of Sewage sludge (including N, P, organic matter, & trace elements) is considered high, which is important for plant development (Mtshali et al., 2014). Sewage sludge can also be considered a suitable alternative to chemical fertilizers as its use will reduce total dependence on commercial fertilizers (Rodríguez et al., 2010). Sewage sludge also contains various nutrients especially (H. Yuan et al., 2016), potassium (Kirchmann et al., 2017), phosphorous (Cieślik & Konieczka, 2017), which are a potent source of fertilizers (Kahiluoto et al., 2015). The pH of sewage sludge normally ranges from neutral and alkaline to slight acidic based on the treatment process applied and the presence of sludge conditioners (Mtshali et al., 2014).

The physicochemical properties of the municipal sewage sludge have been reported in previous studies, as shown in Table 2.2. The physical composition of the municipal sewage sludge comprises moisture content (55-60%), organic matter (25-30%) and volatile matter (10-15%), respectively (Bubalo et al., 2021; Hanum et al., 2019a; Khnaijer et al., 2020). The rich composition of organic matter and nutrient content in MSS are useful for fertilizer and soil enhancers. Besides, the high moisture content may support the life of pathogenic bacteria, fungi, and viruses (Babola, 2019). Meanwhile, the chemical properties of the municipal sewage sludge include ammonia (11-12%), phosphorus (6-15%), nitrogen (5-8%), calcium (4-8%), aluminium (1-4%), and others (0.5-1.5%), respectively (Hanum et al., 2019).

Table 2.2 Physicochemical composition of municipal sewage sludge (Hanum et al., 2019)

Physicochemical properties	**Composition (wt.%)**
Physical properties	-
Moisture content	55-60
Organic matter	25-30
Volatile solids	10-15
Chemical properties	
Ammonia	11-12
Phosphorus	6-15
Nitrogen	5-8
Calcium	4-8
Aluminium	1-4
Others (i.e., magnesium, potassium, sodium)	0.5-1.5

Heavy metals are toxic to the environment, including human health and the aquatic ecosystem (Poblete et al., 2022). Besides, several heavy metals, such as chromium, are highly toxic and carcinogenic to human health and aquatic life. Table 2.3 shows the permissible limit for heavy metals in municipal sewage sludge, according to the Malaysia Department of Environment. The standard A permissible limit for cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), manganese (Mn), nickel (Ni), zinc (Zn) are 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.2 mg/L, 0.2 mg/L, 0.2 mg/L, and 2.0 mg/L, while for standard B, 0.02 mg/L, 0.05 mg/L, 0.5 mg/L, 1.0 mg/L, 1.0 mg/L, 1.0 mg/L, 2.0 mg/L, respectively.

Table 2.3 Permissible limit for heavy metals in municipal sewage sludge according to Department of Environmental Malaysia (2009).

Heavy metals	**Unit (mg/L)**	
	Standard A	**Standard B**
Cadmium (Cd)	0.01	0.02
Chromium (Cr)	0.05	0.05
Lead (Pb)	0.1	0.5
Copper (Cu)	0.2	1.0
Manganese (Mn)	0.2	1.0
Nickel (Ni)	0.2	1.0
Zinc (Zn)	2.0	2.0

Previous studies reported that municipal sewage sludge contains various toxic substances and other inorganic contaminants, such as heavy metals and pesticides (Kim & Phae, 2022; Rodríguez et al., 2018). The heavy metals contaminant commonly detected in municipal sewage sludge throughout the world are summarized in Table 2.4. It was found that copper, chromium, lead, manganese, zinc, nickel, and cadmium are the most common heavy metals found in municipal wastewater. The concentration of these heavy metals in municipal sewage sludge was found to be exceeded the standard permissible limits acquired by the government (Hanum et al., 2019; Khnaijer et al., 2020). In Malaysia, the permissible limit for heavy metals in municipal sewage sludge follows standard (B) applicable to discharge into any other inland waters or Malaysian waters (Discharge downstream of water supply sources), rather than standard (A) is applicable to discharge into any water within any catchment areas (Discharge upstream of water supply sources) (Noor et al., 2019).

Wherein, the concentration of heavy metals of Cd, Cr, Pb, Cu, Mn, Ni, and Zn in the municipal sewage sludge discharged must not exceed 0.02 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.0 mg/kg, 1.0 mg/kg, and 1.0 mg/kg, respectively. However, Rosenani et al. (2004) reported the concentration of heavy metals in the municipal sewage sludge in Malaysia exceeded the permissible limit. Wherein, the concentration of Cd, Cr, Pb, Cu, Mn, Ni, and Zn analysed were found to be 3.4 mg/kg, 269 mg/kg, 100 mg/kg, 257 mg/kg, 189 mg/kg, 32 mg/kg, and 1986 mg/kg, respectively. Thus, it can be concluded that municipal sewage sludge contains a high concentration of heavy metals. Wherein direct usage of municipal sewage sludge for composting may affect plant growth and pollutes the soil and groundwater (Paz-Ferreiro et al., 2013). Therefore, a proper recycling method is required to recycle municipal sewage sludge into a value-added product.

Table 2.4 Heavy metals concentrations in municipal sewage sludge (Fang et al., 2019).

Countries	**Cadmium (mg/kg)**	**Chromium (mg/kg)**	**Lead (mg/kg)**	**Copper (mg/kg)**	**Manganese (mg/kg)**	**Nickel (mg/kg)**	**Zinc (mg/kg)**	**References**
USA	16	240	240	850	260	400	1,740	(Barros et al., 2007)
China	0.8	435	79	194	435	108.5	458	(Hanum et al., 2019)
India	4.6	2,400	2,400	37	240	88	182	(Khnaijer et al., 2020)
Ireland	14	120	75	979	386	47	1,268	(Rodríguez et al., 2018)
Italy	20	62	26	195	100	193	290	(Lakhdar et al., 2012)
Spain	0.01	75	144	205	105	23	1,808	(Paz-Ferreiro et al., 2013)
Saudi Arabia	4.2	500	500	800	260	80	1,700	(Al-Malack et al., 2008)
Malaysia	3.4	269	100	257	189	32	1986	(Rosenani et al., 2004)

2.1.1(b) Microorganisms in sewage sludge

Sewage sludge consists of a high amount of organic matter and is rich in nutrients, which support the life of pathogenic microorganisms such as viruses, bacteria, fungi and parasites (Fijalkowski et al., 2017; Rorat et al., 2019). The characterisation of biological properties is essential to determine the pathogens, microorganisms, and antibiotic residue composition of the sewage sludge (Markowicz et al., 2021). However, the type and concentration of pathogens in sewage sludge are a function of the type of the wastewater source and other environmental parameters (Rorat et al., 2019). Sewage sludge can be seen as a mixture of different biological organisms, both pathogenic and saprophytic. However, the most diverse pathogens in sewage sludge are the chemoautotrophic and heterotrophic types (Delibacak et al., 2020; Pepper & Gentry, 2015). Enteric bacteria are the major pathogenic bacterial component of sewage sludge; hence, sewage sludge can be a reservoir of virulent bacterial strains and depending on the level of antibiotics in an environment, there could be cases of antibiotic resistance among such pathogenic bacterial strains, such as multi-resistant *E. coli* strains (Reinthaler et al., 2013).

However, the presence of bacteria in sewage sludge is a source of concern as they can multiply rapidly in the environment, especially those that do not require a host cell for replication (Larsson & Flach, 2022; Li et al., 2022). A summary of the microorganisms found in MSS in various countries has been presented in Table 2.5. Laura et al. (2020) reported bacteria found in the MSS, including *V. chlorella, C. jejuni, E. coli, Salmonella sp., and Y. enterocolitica*. Khnaijer et al. (2020) found *E. coli, S.aureus, P. vulgaris, and Salmonella sp*. in municipal sewage sludge. Meanwhile, pathogenic bacteria such as *Leptospira sp. and Enterocolitica sp*. were also found in municipal sewage sludge (Al-Gheethi et al., 2018).

Table 2.5 Microorganisms contains in municipal sewage sludge in various countries.

Source	**Country**	**Microorganisms**	**References**
Sludge	USA	*Proteus, Citrobacter, V. cholerae, Y. enterocolitica, Enterobacter sp., Salmonella sp., Mycobacterium sp., B. antracis, Streptococcus sp., Campylobacter sp., Faecal coliforms, Faecal streptococci.*	(Laura et al., 2020)
Activated sludge	Morocco	*E. coli, S. aureus, P. vulgaris, Salmonella sp.,* *E. faecalis, Enterococci staphylococci.*	(Khnaijer et al., 2020)
Sewage sludge	Malaysia	*Salmonella Enterica, Salmonella Bongori,* E. Enterococci	(Selambakkannu et al., 2022)
Treated sludge	Egypt	*E. coli, V. cholera,* *Leptospira sp., Salmonella sp., Enterocolitica sp.*	(Al-Gheethi et al., 2018)
Urban wastewater	Italy	*Escherichia coli, Klebsiella pneumoniae, and Enterococci*	(Triggiano et al., 2020)
Activated sludge	China	*Proteobacteria, Bacteroidetes, Acidobacteria, and Firmicutes*	(Gao et al., 2016)

2.1.1(c) Antibiotics in sewage sludge

Antibiotics are essential to treat infectious diseases caused by pathogens and viruses in humans and livestock. Antibiotics can be consumed orally or by injection into the body parts (Thiang et al., 2021). They may enter the environment directly along with the faeces or urine. The antibiotic residue discharged from these generators will enter the pipeline and sewer to be treated at the sewage treatment plant. Municipal sewage sludge is being separated and discharged at primary treatment, suggesting that there is a high possibility for antibiotic residue still contained in the municipal sewage sludge (Praveena et al., 2018). The antibiotic residue in sewage sludge is mainly discharged from healthcare facilities, residentials, markets and meat processing factories, and agriculture farms, as shown in Figure 2.2. Meanwhile, meat processing factories and agriculture farms have used antibiotics on livestock such as cows, chickens, and sheep. Antibiotics have been extensively utilized in farms to fight infectious diseases among livestock and as growth promoters to increase the livestock's size and weight (Ilias et al., 2022; Samreen et al., 2021).

In addition, several antibiotic types are known to be toxic to the environment, including human health and the aquatic ecosystem. It is well established that sewage sludge contains significant amounts of diverse antibiotics representing nearly all major classes (excluding labile β-lactams). The concentrations assessed in sewage sludge vary between ng to mg per kg of dried weight (Bondarczuk et al., 2016; Jelić et al., 2012). Thiang et al. (2021) and Praveena et al. (2018) reported a high concentration of antibiotics residue of ciprofloxacin (299 µg/kg), tetracyclines (830 µg/kg), sulphonamides (720 µg/kg), quinolones (690 µg/kg), and sulfamethoxazole (114 µg/kg) were found in municipal sewage sludge in Malaysia respectively. Wherein it has been revealed that the antibiotics exceeded the permissible limit. These findings show high possibilities that sewage sludge may contain antibiotic residue.

Figure 2.2 Sources of antibiotics in municipal sewage sludge (Arun et al., 2020; Dinh et al., 2017; Samreen et al., 2021; Zou et al., 2022)

2.1.1(d) Antibiotic resistance bacteria in municipal sewage sludge

Antimicrobial resistance is a serious threat in veterinary medicine and human healthcare. Resistance genes can spread from animals, through the food chain, and back to humans. Sewage sludge may act as the link back from humans to animals (Dadgostar, 2019; Fijalkowski et al., 2017). Generally, Antimicrobial resistance is a term that refers to the ability of certain bacteria to thrive and protect themselves despite being subjected to the antibiotic. When an antibiotic is used, it kills only susceptible bacteria; other bacteria can survive and grow into resistant bacteria because of genetic mutation. Then, resistant bacteria multiply, and some can transmit their drug resistance DNA to other bacteria (Pang et al., 2019; Subedi et al., 2018). When these antibiotic-resistant bacteria were treated again, only a tiny number of bacteria were killed, while others developed resistance.