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Generating alternative fuel and bioplastics from medical plastic waste and waste frying oil using microwave co-pyrolysis combined with microbial fermentation

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ABSTRACT

In the present study, microwave co-pyrolysis (MCP) was used to simultaneously convert medical plastic waste (MPW) and waste frying oil (WFO) into liquid oil products. The MCP process demonstrated a faster heating rate (24 °C/min) and shorter process time (20 min) compared to conventional pyrolysis techniques converting MPW and WFO into liquid oil (>80 wt%). The MCP reduced the oxygen content from 25.7 to 9.82 wt% in liquid oil encompassing light aliphatic hydrocarbons ranging from C₁₀ to C₂₈, generating a novel sustainable liquid fuel. The liquid having a high carbon content (approximately 77.1 wt%) and low carbon to nitrogen ratio (27.9) is a suitable energy feedstock for polyhydroxyalkanoate (PHA) bioplastic production in the form of poly-3-hydroxybutyrate [P(3HB)]. The liquid oil acted as an energy source for the growth of *Bacillus* sp. During microbial fermentation, yielding approximately 11% (w/w) P(3HB). Bioplastics are biodegradable, biocompatible with humans and non-toxic to marine organisms, representing a valuable additive in the production of cosmetics, detergents, and as medical scaffolds for tissue engineering. The results indicate the promising upcycling of waste products by this approach through pyrolytic biorefinery into value-added fuel and bioplastic products, being important for the future sustainable production of renewable resources.

1. Introduction

The global population growth and development of the healthcare

industry have increased significantly over the last few decades leading to massive production of medical waste, further aggravated by the recent coronavirus pandemic (COVID-19) [1]. The COVID-19 pandemic has amplified the use of plastic-based personal protective equipment such as

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surgical masks, gloves, medical gowns, feet and surface protectors, leading to a sudden surge in medical waste. In addition, COVID-19 has triggered the utilization of single-use plastic materials such as intrave-

cooking oil and waste plastic increased the yield of liquid oil products by 84 wt%, compared to the wastes being pyrolyzed individually [10]. Table 1 shows the current pyrolysis technologies used to convert waste

Nomenclatures			
<i>Notations</i>			
T _c	Crystallization temperature	LDPE	Low-density polyethylene
T _g	Glass transition	LPS	Lipopolysaccharides
T _m	Melting temperature	MCP	Microwave co-pyrolysis
ΔH _m	Enthalpy of fusion of the sample	MPW	Medical plastic waste
ΔH _m ⁰	Enthalpy fusion of 100% crystalline P(3HB) (142 J g ⁻¹)	MSM	Minimum salt medium
<i>Abbreviations</i>		N	Nitrogen
ATP	Adenosine triphosphate	Na ₂ HPO ₄	Disodium hydrogen phosphate
C	Carbon	NH ₄ Cl	Ammonium chloride
CDW	Cell dry weight	NO _x	Nitrogen oxides
CoASH	Coenzyme A	NR	Nutrient rich
COVID-19	Coronavirus pandemic	O	Oxygen
DSC	Differential scanning calorimetry	PHA	Polyhydroxyalkanoate
FTIR	Fourier Transform Infrared Spectroscopy	PhaA	(R)-3-hydroxybutyryl-CoA by β-ketothiolase
GC-FID	Gas Chromatography with Flame Ionization Detection	Pha B	NADPH-dependent acetoacetyl-CoA reductase
GCMS	Gas Chromatography-Mass Spectrometry	PhaC	PHA synthase
H	Hydrogen	P(3HB)	Poly3-hydroxybutyrate
KBr	Potassium bromide	S	Sulphur
KH ₂ PO ₄	Potassium dihydrogen phosphate	SDGs	Sustainable Development Goals
		TGA	Thermogravimetric analysis
		VOCs	Volatile organic compounds
		WFO	Waste frying oil

nous drips, saline bottles, and syringes. These practices significantly increase the existing challenges to disposing plastic waste. In fact, approximately 1.6 million tonnes of plastic have been produced daily worldwide since the COVID-19 outbreak, leading to an estimated 3.4 billion pieces of single-use medical plastic being discarded on a daily basis [1].

Several approaches are applied to reduce the amount of medical plastic waste including incineration, landfilling, autoclaving, and chemical disinfection. Landfilling is not a viable option as it requires huge land space and generates leachate that pollutes soil and aquatic environments, including groundwater [2]. Incineration is the most used technique as it reduces the volume of medical plastic waste by approximately 90%, while generating heat and energy for boilers and diverting waste from landfills [2,3]. Despite its benefits, incineration also poses risks to human health and the environment because of the release of hazardous compounds such as polychlorinated dibenzofurans (furans), polychlorinated dibenzo-p-dioxins (dioxins), heavy metals (i.e. cadmium and arsenic), and volatile organic compounds (VOCs), all of which are considered carcinogens [2,4].

The limitations of existing waste disposal and recovery of value-added products highlight the potential of microwave pyrolysis in converting plastic waste into energy sources. Pyrolysis is a thermochemical method operating under hypoxic conditions and high operating temperatures of approximately 400–800 °C using microwave radiation, during which waste materials decompose into energy products such as liquid oil and gases [5,6]. Microwave pyrolysis of plastic waste produces liquid oil that comprises aliphatic and aromatic hydrocarbons as potential fuel or chemical additives [7]. Nevertheless, liquid oil obtained from the microwave pyrolysis of plastic waste alone possesses high viscosity and contains waxy paraffinic components due to the unsatisfactory decomposition of long-chain paraffin wax compounds [7].

Owing to this problem, microwave co-pyrolysis may rectify the limitations of conventional microwave pyrolysis, as it involves the processing of two or more feedstock materials to generate a positive synergistic interaction, improving the oil yield and properties [8,9]. Previous studies reported that microwave co-pyrolysis of household

cooking oil and plastic into liquid fuel, including the product yield and limitations. Nevertheless, there are lack of studies investigating the capability of the liquid oil obtained as carbon source in bacterial fermentation for bioplastics production.

Liquid oil produced by microwave co-pyrolysis is an energy and carbon-rich product with potential as energy source in bacterial fermentation for the production of bioplastics, particularly polyhydroxyalkanoate (PHA). These PHAs are polymers produced by microorganisms under nutrient-limited and carbon-enriched conditions owing to their petrochemical plastic properties, biocompatibility, and biodegradability [14]. According to Yustinah et al. [15], poly-3-hydroxybutyrate [P(3HB)] is the most common PHA. Studies have shown that PHA films can naturally biodegrade in the mangrove environment [16], lakes [17] or marine environments [18] within six weeks, making PHA a suitable replacement for common non-degradable petrochemical-based plastics.

In recent studies, PHA has shown tissue biocompatibility in both humans and animals [19]. A review on the *in vivo* degradation of PHA polymers by Bhubalan et al. [20] reported that 3-hydroxybutyric acid and 4-hydroxybutyric acid were present in human and animal blood. Moreover, PHA possesses plastic characteristics, making it a viable replacement for synthetic plastics; however, PHA production is often hampered by the production costs, as the energy feedstock for microbial fermentation is expensive. An alternative, yet inexpensive continuous supply of carbon as an energy source would be suitable for PHA production. Previous studies have revealed that feedstock derived from sweetwater [21], molasses [22], and glycerol [21] are viable for PHA production. Several microorganisms including *Ralstonia eutropha* (presently known as *Cupravidus necator*), *Pseudomonas* sp., *Alcaligenes* sp., *Aeromonas* sp., *Enterococcus* sp., *Brevundimonas* sp., and *Bacillus* sp., enable the synthesis of PHA [23,24]. For example, PHA produced by gram-negative bacteria, *R. eutropha*, possesses an outer layer membrane of lipopolysaccharides (LPS), an endotoxin responsible for inflammatory reactions contaminating the PHA and rendering it unsuitable for use *in vitro* [25]. In contrast, the PHA produced by gram-positive bacteria does not possess the LPS membrane, which gives it a wider application in the

Table 1
Current research in pyrolysis of waste cooking oil and plastic waste.

Feedstock	Pyrolysis system	Main findings	Refs.
Waste cooking oil	Conventional pyrolysis using fixed-bed reactor Waste origin: Borj-Cedria Technopark refinery	Yield of liquid oil: 80 wt% Calorific value: 37 MJ/kg Main composition of liquid oil: alkanes, alkenes, cyclic hydrocarbons, carboxylic acids, aldehydes, ketones, alcohols, and esters Potential application: fuel Drawback: high amount of oxygenated compounds and low calorific value	[11]
Waste cooking oil	Catalytic microwave vacuum pyrolysis Catalysts: HZSM-5 and metal oxide Waste origin: Yihai Kerry Oils and Foodstuffs Co. Ltd. (Nanchang, Jiangxi)	Yield of liquid oil: 37.5 wt% Main composition of liquid oil: benzene, toluene, ethylbenzene, and xylene Potential application: industrial chemicals Drawback: Low yield of liquid oil	[12]
Plastic waste	Catalytic microwave pyrolysis Catalysts: ZSM-5 Waste origin: high-density polyethylene	Yield of liquid oil: 42.2 wt% Yield of wax: 17 wt% Main composition of liquid oil: aromatic, alkanes, alkenes Potential application: Fuel Drawback: low yield of liquid oil and high wax production	[13]
Plastic waste	Microwave pyrolysis Waste origin: high-density polyethylene	Yield of liquid oil and wax: 69% Main composition of liquid oil: aromatic, alkanes, alkenes Potential application: Fuel Drawback: high wax production	[7]
Waste cooking oil and plastic waste	Microwave vacuum co-pyrolysis Waste origin: Palm oil and high-density polyethylene	Yield of liquid oil: 84 wt% Main composition of liquid oil: alkanes, alkenes Potential application: Fuel	[10]

medical industry. According to Mohapatra et al. (2017) and Bhubalan et al. (2011), *Bacillus* sp., which are gram-positive, are capable of producing PHA copolymers depending on the carbon and energy source, benefitting the industrial sector, especially in agriculture, biomedicine, automobiles and packaging [19,20,26–28].

The use of biodegradable products from renewable sources represents an alternative resource that helps reduce plastic waste, which suffocates and contaminates the ecosystem. Bioplastics also decrease the carbon footprint, offer energy savings in processing, and does not use non-renewable raw materials during fermentation, while reducing the use of harmful chemicals [29]. In addition, the development of biodegradable bioplastics via upcycling of non-biodegradable plastic waste supports the United Nations Sustainable Development Goals (SDGs), especially Goal #12, to ensure sustainable consumption of waste, with the intention of minimising waste generation and maximising the production of value-added products.

To our best knowledge, no studies have reported the production of liquid oil for the synthesis of bioplastics. Previous studies have reported bioplastics production using microbial fermentation [28,30]. In contrast, this study promotes the energy application of liquid oil produced from the microwave co-pyrolysis of waste materials and use this liquid oil as an energy source for microbial fermentation to produce bioplastics. The valorisation of waste materials into value-added products, including fuel, bioplastics, and chemical additives via microwave

co-pyrolysis technology is a novel ground-breaking method to ensure sustainable consumption and production of renewable materials.

2. Materials and methods

2.1. Preparation of feedstock materials

The feedstock materials used in this study were medical plastic waste (MPW) and waste frying oil (WFO). The MPW consisted of glucose and saline bottles made of low-density polyethylene (LDPE) obtained from KPJ Penang Specialist Hospital, Malaysia. MPW used in this study was non-infectious waste that has no direct contamination from the patients. As a precaution step prior to pyrolysis, the MPW was cleaned with detergents and decon 90 cleaning solution to kill any germ or bacteria from the waste. The MPW was then dried in an oven at 30–40 °C within 1 or 2 h to remove the moisture content. After drying, it was shredded (5–10 mm) in order to fit for insertion into the pyrolysis reactor. The WFO was obtained from restaurants near the Universiti Malaysia Terengganu, Malaysia. Large solid particles and impurities in the WFO were removed using Whatman Filter Paper (grade 40) and vacuum filtration.

2.2. Microwave co-pyrolysis

The co-pyrolysis experiment was conducted in a 800 W microwave oven that has been modified. The co-pyrolysis apparatus was assimilated with several core parts: 1) a quartz reactor (150 × 100 × 100 mm) with two necks was fabricated to pyrolyse the wastes, and the reactor was enfolded within a ceramic fibre blanket to reduce heat loss; 2) a microwave oven was utilised as the source of heating; 3) a condensation system containing a liebig condenser, a vigreux column, cold traps, and liquid oil containers; 4) a temperature controller and a type K thermocouple to monitor the temperature and heating rate during microwave co-pyrolysis; 5) nitrogen was purged into the pyrolysis reactor to eliminate air and maintain an oxygen-free condition. The detailed setup of microwave co-pyrolysis apparatus is reported in our previous work [10].

In this study, various microwave powers (600, 700, and 800 W) were investigated to examine its influence on the production and characteristics of the produced liquid oil. A feedstock ratio of 1:1 (50 g of WFO and 50 g of MPW) was inserted to the pyrolysis reactor. Activated carbon (50 g) was also added to the pyrolysis reactor to act as a microwave absorber. Both WFO and MPW are poor microwave absorbers, thus activated carbon assists the heating process by absorbing the microwave radiation, efficiently converting it into heat to pyrolyse the feedstocks. The heating rate was determined according to the maximum temperature attained versus the heating time during the experiment. Microwave pyrolysis of MPW and WFO was also performed separately to compare the performance of co-pyrolysis with the pyrolysis of individual feedstock.

The pyrolysis experiment was terminated after the generation of pyrolytic volatiles discontinued in the reactor. The yield of the liquid oil product was determined by measuring the increased weight of the liquid oil container after the experiment (Eq. (1)). The experiments were performed in triplicate to obtain the average reading for each sample. The yields of char and gases were not the main focus of this study; thus, they were excluded from analysis.

$$\text{Yield of liquid oil (wt.\%)} = \frac{\text{weight of liquid oil}}{\text{weight of feedstocks}} \times 100 \quad (1)$$

2.3. Analytical method to analyse feedstock and liquid oil

The thermal behavior and proximate content of feedstocks, viz. WVO, PKS and HPW were determined using a thermogravimetric (TGA) analyser (Mettler-Toledo TGA/SDTA851e, Switzerland). The feedstocks were heated from ambient temperature to 900 °C at a heating rate of 20 °C/min. Argon was used as the carrier gas that vented through the

system at a flow rate of 0.1 L/min. The amount of volatile matters in the feedstock was obtained based on the weight loss occurred between 100 °C and 600 °C [31]. Further weight loss occurred at 600–900 °C indicated the presence of char residue [32]. Fixed carbon content was obtained by subtracting the char residue and volatile matter content and from the dry mass of the feedstock [33].

Elemental analysis of the liquid oil was executed using a Vario MACRO elemental analyser (Elementar Analyseysteme GmbH, Germany) to determine the elemental composition (*i.e.*, hydrogen, oxygen, nitrogen, carbon, sulphur) in the liquid oil. Gas chromatography-mass spectrometry (GCMS) analysis of the liquid oil was performed using a 6890 GC-MS instrument equipped with a mass spectrometer detector. The column used was 5% phenyl methyl siloxane from HP-5MS (length, 30 mm; diameter, 0.25 mm; film thickness, 0.25 µm). The initial temperature of the oven was 30 °C, with a holding time of 1 min. The temperature of the oven was subsequently set at the rate of 5 °C/min, from 30 °C to 300 °C. The concentration of each compound was calculated as a percentage of the total area of all the peaks in the analysis.

2.4. Bacterial strain isolation and preservation

Bacillus megaterium UMTKB-1 (GenBank accession number: KF991583) was previously isolated from tissue samples of the marine sponge *Callyspongia* sp., obtained from the waters near Langkawi Island, Malaysia [21]. The strain was maintained in a nutrient rich (NR) broth at 30 °C and 200 rpm, and preserved in 40% (v/v) glycerol stock at –80 °C for long-term storage.

2.5. Media and culture conditions

Media preparation and culture conditions were based on Yatim et al. (2017) [21]. The seed culture was prepared using NR medium (10 g/L peptone, 10 g/L meat extract and 2 g/L yeast extract). The PHA production was performed in minimal salt medium (MSM) which comprised 1.5 g/L KH₂PO₄, 3.6 g/L Na₂HPO₄, 0.5 g/L NH₄Cl, and 1 ml/L of MgSO₄·7H₂O (0.1 M) supplemented with 1 mL/L of trace element solution. The components of trace elements (Table 2) were dissolved in 0.1 M HCl.

2.6. Biosynthesis of polyhydroxyalkanoate with shake-flask culture

A one-stage shake-flask cultivation technique was implemented to synthesise PHA using the strain *B. megaterium* UMTKB-1, as previously reported by Yatim et al. (2017) [21] with some modifications. The effect of liquid oil produced from microwave co-pyrolysis of MPW and WFO at different microwave power on the PHA production was tested by supplementing 5 g/L of liquid oil with 0.5 g/L of ammonium chloride (NH₄Cl) as the sole supply of nitrogen. The influence of liquid oil produced from microwave pyrolysis and co-pyrolysis on PHA production was determined. The seed culture of *B. megaterium* UMTKB-1 was prepared by adding 1 mL/L of inoculum to NR broth incubated for 14 h at 200 rpm and 30 °C. The inoculum size for biosynthesis was maintained at 3% (v/v) for 48 h.

Table 2
Components of trace element solution.

Constituents	Concentration (g/L)
Cobalt (II) sulfate heptahydrate (CoSO ₄ ·7H ₂ O)	2.81
Manganese (II) chloride tetrahydrate (MnCl ₂ ·4H ₂ O)	1.98
Iron (II) sulfate heptahydrate (FeSO ₄ ·7H ₂ O)	2.78
Calcium chloride dihydrate (CaCl ₂ ·2H ₂ O)	1.67
Zinc sulfate heptahydrate (ZnSO ₄ ·7H ₂ O)	0.29
Copper (II) chloride dihydrate (CuCl ₂ ·2H ₂ O)	0.17

2.7. Polyhydroxyalkanoate content determination

The amount of PHA produced by *B. megaterium* UMTKB-1 was determined using GC 2010 (Shimadzu, Kyoto, Japan). This method was adapted from the work of Yatim et al. (2017) [21]. Freeze-dried bacterial cells (15 mg) were added to sulphuric acid and methanol (15:85, v/v %). The mixture was incubated for 2 h and 20 min at 100 °C to transform PHA monomers into methyl esters. The mixture was left to cool to room temperature, followed by the addition of 1 mL distilled water. The mixture was vortexed to encourage the phase separation. The bottom layer with the reaction product was extracted and dehydrated using anhydrous sodium sulfate. The moisture-free product was analysed by GC-FID with a Supelco SPB™-1 fused silica capillary column of dimensions 30 m × 0.25 mm × 0.25 µm (Sigma Aldrich, USA).

2.8. Polyhydroxyalkanoate characterisation

2.8.1. Fourier Transform Infrared analysis

Approximately 5 mg of PHA and 100 mg of potassium bromide (KBr) were weighed and pounded into a fine powder. The mixture was pelletized using a KBr pellet and inserted into a Fourier Transform Infrared (FTIR) spectrometer IRTracer-100, Shimadzu. The PHA was scanned 50 times to determine the functional groups at a resolution of 4 cm⁻¹ and a spectral range of 4000–400 cm⁻¹. This method was adapted from Vignesswari et al. (2015), with some modifications [34].

2.8.2. Differential scanning calorimetry analysis

The thermal properties of the PHA materials were analysed using differential scanning calorimetry (DSC), which was performed using a Diamond PerkinElmer Pyris 1 thermal analyser that was paired with a liquid nitrogen cooling accessory. An estimated 5–8 mg of polymer was encapsulated within an aluminium pan. The sample was heated from –50 to 200 °C at a rate of 10 °C/min under a N₂ flow [35]. The sample was maintained at 200 °C for 2 min and then rapidly quenched to –50 °C for 5 min. The sample was then reheated from –50 to 200 °C at a rate of 10 °C/min. The DSC curve of the second heating was employed for PHA thermal property analysis. The glass transition temperature (*T*_g) was derived from the heat capacity change midpoint, whereas the melting point (*T*_m) and enthalpy of fusion (ΔH_m) were derived from the melting endotherm peak. The percentage of crystallinity was calculated based on the study by Gumel et al. (2012) [36] and the formula is as follows (Eq. (2)):

$$\text{Crystallinity (\%)} = \frac{\Delta H_m}{\Delta H_m^0} \quad (2)$$

ΔH_m : enthalpy fusion of the sample.

ΔH_m^0 : enthalpy fusion of 100% crystalline P(3HB) (142 J g⁻¹).

3. Results and discussion

3.1. Characteristics of waste frying oil and medical plastic waste

Fig. 1 shows the thermogravimetric analysis of WFO and MPW. The first stage of thermal decomposition occurs from ambient temperature (28 °C) to 110 °C, which is also known as the dehydration stage [32]. As illustrated in Fig. 1, both WFO and MPW show negligible mass reduction prior to reaching 110 °C, indicating low moisture content in both samples. Interestingly, MPW and WFO started to decompose at 390 °C and 285 °C, respectively, indicating that MPW has a higher decomposition temperature than the WFO. MPW is composed of long-chain hydrocarbons with a high degree of crystallinity and higher bond strength that require higher energy and temperature to break the bond. In contrast, WFO consists of low molecular-weight hydrocarbons (*e.g.* fatty acid chains) that can be broken down at lower temperature [32].

Most volatile matter was released prior to 600 °C, followed by a

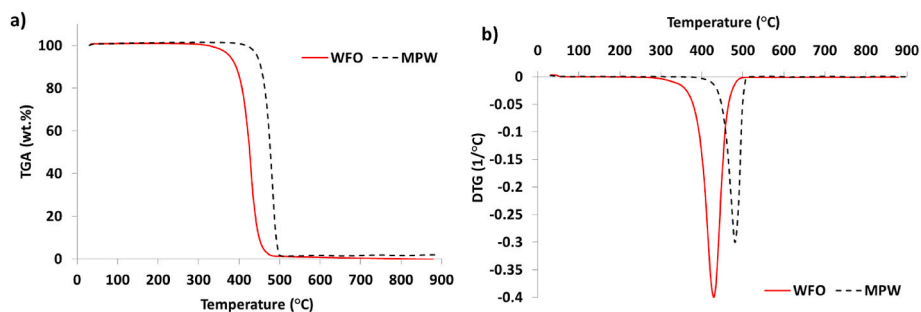


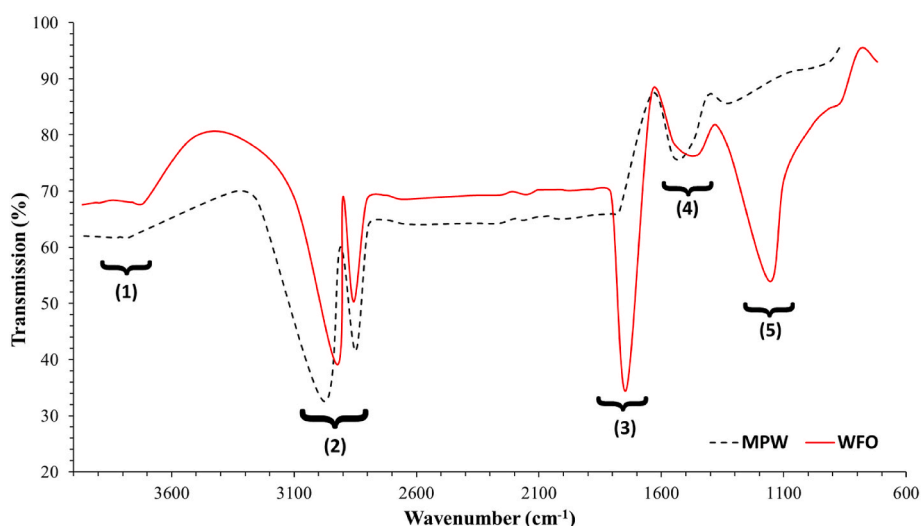
Fig. 1. Thermogravimetric analysis (a) and derivative thermogravimetry (b) of waste frying oil and medical plastic waste.

slight weight reduction observed at 600–900 °C, suggesting that limited charring occurred for WFO and MPW, producing less than 0.5 wt% of fixed carbon in both samples. Approximately 99 wt% of the content are released as volatile matter, hence limiting the char production during the decomposition process. Both MPW and WFO showed high weight loss between 200 and 600 °C, indicating the presence of high volatile matter content (~99 wt%) in both feedstocks. This signifies the desired characteristic of MPW and WFO to decompose and convert into mainly volatiles that condense to form potentially useful liquid oil product for use as fuel [10,37].

As aforementioned, MPW is a polymer consisting of long-chain hydrocarbons that is rich in carbon (86.2 wt%) and hydrogen (13.3 wt%), with a potential to be converted into aliphatic hydrocarbons that can be used as a source of fuel or energy for bacterial growth. Additionally, WFO contains saturated, unsaturated, and short-fatty acid chains composed of carboxylic acid groups, thus containing a high carbon

(52.6 wt%), oxygen (26.3 wt%) and hydrogen (17.8 wt%) content [38]. The high carbon content in WFO and MPW demonstrates their potential as energy and carbon sources for the selected bioplastics producing bacteria [39]. The carbon content of feedstocks could act as an energy source in the metabolic activities of bacteria, which influence bacterial growth, redox potential of cell metabolism, composition, and yield of bioplastic polymers [40]. This implies that WFO and MPW are potentially suitable for enhancing bacterial growth and accumulating PHA for use as bioplastics. Moreover, WFO and MPW show low nitrogen content (0.02–0.33 wt%) with no sulphur, which validates their potential as a green feedstock for bioplastics production. This is due to the limited nitrogen content with potential to promote and boost homogeneous bioplastics production [39,41].

Fig. 2 shows the FTIR spectra, functional groups, and classification of components obtained from WFO and MPW. A strong peak was discovered at wavenumbers ranging from 3000 to 2800 cm^{-1} for both samples,



Frequency range (cm^{-1})	Wavenumber (cm^{-1})		Functional group	Classification of compounds
	WFO	MPW		
(1) 3700-3600	3713.0	-	O-H stretching	Alcohol
(2) 3000-2600	2922.2,	2912.5,	C-H stretching	Aliphatic CH_2 (e.g. alkane, alkene)
	2856.6	2848.9		
(3) 1800-1700	1743.7	-	C=O stretching	Carboxylic acid
(4) 1600-1400	1452.4	1548.8	C-H bending	Aliphatic CH_2 and CH_3
(5) 1200-1100	1157.3	-	C-O stretching	Carboxylic acid

Fig. 2. FTIR spectra, functional group and classification of compounds content in waste frying oil and medical plastic waste.

signifying the existence of aliphatic hydrocarbons such as alkanes with C–H stretching vibrations. The presence of aliphatic hydrocarbons was further confirmed by the peak at 1600–1400 cm^{-1} for both WFO and MPW [32]. There was a difference in FTIR spectrum observed between WFO and MPW at 3713.0 cm^{-1} , 1743.7 cm^{-1} and 1157.3 cm^{-1} which can be attributed to O–H stretching, C=O stretching and C–O stretching, respectively, resulting from oxygenated compounds such as carboxylic group in WFO [42]. Additionally, MPW consists of polymers with repeating units and only C and H; thus, no peaks were observed at wavenumbers within the range of 3700–3600 cm^{-1} , 1800–1700 cm^{-1} , and 1200–1100 cm^{-1} . The FTIR results reveal that WFO and MPW consist of aliphatic hydrocarbons such as alkanes and alkenes, exhibiting their potential as carbon sources for the production of bioplastics and biofuels.

3.2. Temperature behaviour and heating rate

Fig. 3 shows the temperature profile versus heating time during microwave co-pyrolysis of the combined WFO and MPW, and WFO and MPW, individually. The microwave power significantly affects the temperature behaviour and heating rate during co-pyrolysis and microwave pyrolysis of all samples. A lower microwave power (600 W) decelerates the heating rate during the pyrolysis process, with the maximum temperature achieved after 30 min. The main decomposition of samples into volatiles and active pyrolysis cracking occurs at maximum temperatures of 300–480 °C [43,44]. This is followed by passive pyrolysis, where the remaining feedstock is transformed into volatiles during declining temperatures, until no more volatiles are formed. The long heating time required to achieve maximum temperature results in a low heating rate (10–15 °C/min) for all samples operated at 600 W. Conversely, a higher microwave power resulted in a higher heating rate, and a maximum temperature of approximately 486 °C for 20 min at 800 W, with the highest heating rate (24 °C/min). This is likely because a high microwave power with a higher energy input results in a faster heating rate [45].

During microwave pyrolysis with only WFO, pyrolytic volatiles were produced at 200–300 °C and 8–10 min after the initiation of the experiments. The maximum temperature of 306–364 °C was observed at 20–30 min of heating time with a slower heating rate (10–12 °C/min) compared to both microwave pyrolysis of only MPW and co-pyrolysis. Microwave pyrolysis of MPW alone generated pyrolytic volatiles at 150–200 °C after 10–15 min, with extended volatiles generation as compared to WFO. MPW is a polymer that possesses high thermal stability and can withstand high temperatures; thus, extended heating times and higher temperatures are needed to break the long MPW polymer chains, especially the C–C bonds, compared to WFO [46].

Interestingly, during microwave co-pyrolysis of WFO and MPW, pyrolytic volatiles generated at 300–350 °C within 4–7 min after initiation of the experiments. Shorter time was needed to heat and pyrolyse WFO and MPW into volatiles, and the maximum temperature was achieved at 438–486 °C. This suggests that microwave co-pyrolysis has a positive synergistic effect on waste materials to reach a high pyrolysis temperature (*i.e.* showing a higher heating rate) compared to microwave pyrolysis of WFO and MPW, individually.

3.3. Production of liquid oil

The liquid oil yield from microwave pyrolysis of WFO, MPW, and co-pyrolysis is shown in Fig. 4. As the production of pyrolytic gases and char is not the focus of this study, these are not discussed. The liquid oil generated from the co-pyrolysis of WFO and MPW was higher than that generated by WFO and MPW, individually. This is attributed to the positive synergistic radical interactions [47] that occur during the co-pyrolysis of WFO and MPW. The interaction of free radicals with other polymers or long hydrocarbon chains in MPW and WFO may stimulate chain breaking reactions (*e.g.* fragmentation and

depolymerisation) to produce pyrolytic volatiles containing smaller compounds (*e.g.* light hydrocarbons) that additionally condense into liquid oil products [48]. Furthermore, previous research reported that MPW contained a higher volatile matter content (97–98 wt%) compared to WFO (~90 wt%) [49,50]. The high content of volatile matter likely increases the formation of pyrolytic volatiles, and consecutively enhances condensation to generate more liquid oil [51]. This explains the high yield of liquid oil attained using microwave co-pyrolysis of WFO and MPW.

As the microwave power increased from 600 to 700 W, the liquid oil produced from all the samples increased significantly. Nevertheless, as the microwave power increased to 800 W, the liquid oil produced individually from WFO and MPW decreased likely because of secondary cracking reactions that normally occur at high microwave power, leading to high process temperatures [52–54]. As discussed in Section 3.2, the process temperature increases with microwave power, which promotes secondary cracking reactions (*e.g.* decarboxylation, decarbonylation, depolymerisation, and carbonisation). These reactions convert pyrolytic volatiles into gases or char [55,56], thus reducing liquid oil production. Surprisingly, the liquid oil produced from the microwave co-pyrolysis of WFO and MPW increased to approximately 81.1 wt% with an increase in microwave power up to 800 W. Perhaps, the increased production and interactions between free radicals generated at higher microwave power during the co-pyrolysis of WFO and MPW stimulated chain-breaking reactions and volatile formation that condensed into liquid oil.

3.4. Elemental and chemical composition of liquid oils

The elemental compositions of liquid oils are shown in Table 4. Liquid oils are mainly composed of carbon, oxygen, and hydrogen produced at a lower microwave power (600–700 W) with a higher carbon content (72.4–77.1 wt%) compared to those obtained at a higher microwave power of 800 W (68.2 wt%). The carbon content is likely derived from aliphatic hydrocarbon compounds found in liquid oil, which corroborates with the GCMS analysis of the liquid oil, indicating a higher concentration of aliphatic hydrocarbons in liquid oil obtained at 600 and 700 W (~96%) as compared to 800 W (~90%). In addition, the higher temperature at higher microwave power increases the occurrence of intensive secondary cracking reactions, such as decarbonylation, polymerisation, and decarboxylation, which convert hydrocarbon compounds into gases (*e.g.* CO, CO₂, C₂H₂, C₂H₄). Conversely, a lower temperature at a lower microwave power may reduce the extent of secondary cracking reactions, thereby producing liquid oil with higher amounts of carbon and hydrogen compounds.

Sulphur was absent in the original feedstock and liquid oils, while lower nitrogen was detected in the liquid oils compared to the original WFO. Similarly, a lower oxygen content was observed in liquid oils compared to the original WFO, indicating that the interaction between MPW (containing low oxygen with almost no nitrogen content) and WFO reduced the nitrogen and oxygen-containing compounds in the oils. The presence of free radicals during co-pyrolysis reactions may convert nitrogen-containing compounds into nitrogen oxides (NO_x) or nitrogen gas that can be released into the atmosphere. Nevertheless, the composition of the gases needs to be verified in future studies. Additionally, the role of MPW as a hydrogen-rich material stimulates the formation of hydrogen radicals, thereby increasing decarbonylation and decarboxylation. The oxygen-containing compounds in the liquid oil are likely reduced because of the elimination of compounds with carbonyl group (C=O) and carboxyl group (COOH) in MPW and WFO, while oxygen-containing compounds are converted into aliphatic hydrocarbons and gases such as CO and CO₂ [10].

Fig. 5 shows the chemical components of the liquid oil produced from microwave co-pyrolysis of MPW and WFO. The liquid oils primarily comprise of aliphatic hydrocarbons (*e.g.* alkanes and alkenes), carboxylic acids (*e.g.* fatty acids), aromatics (*e.g.* benzenes), and other

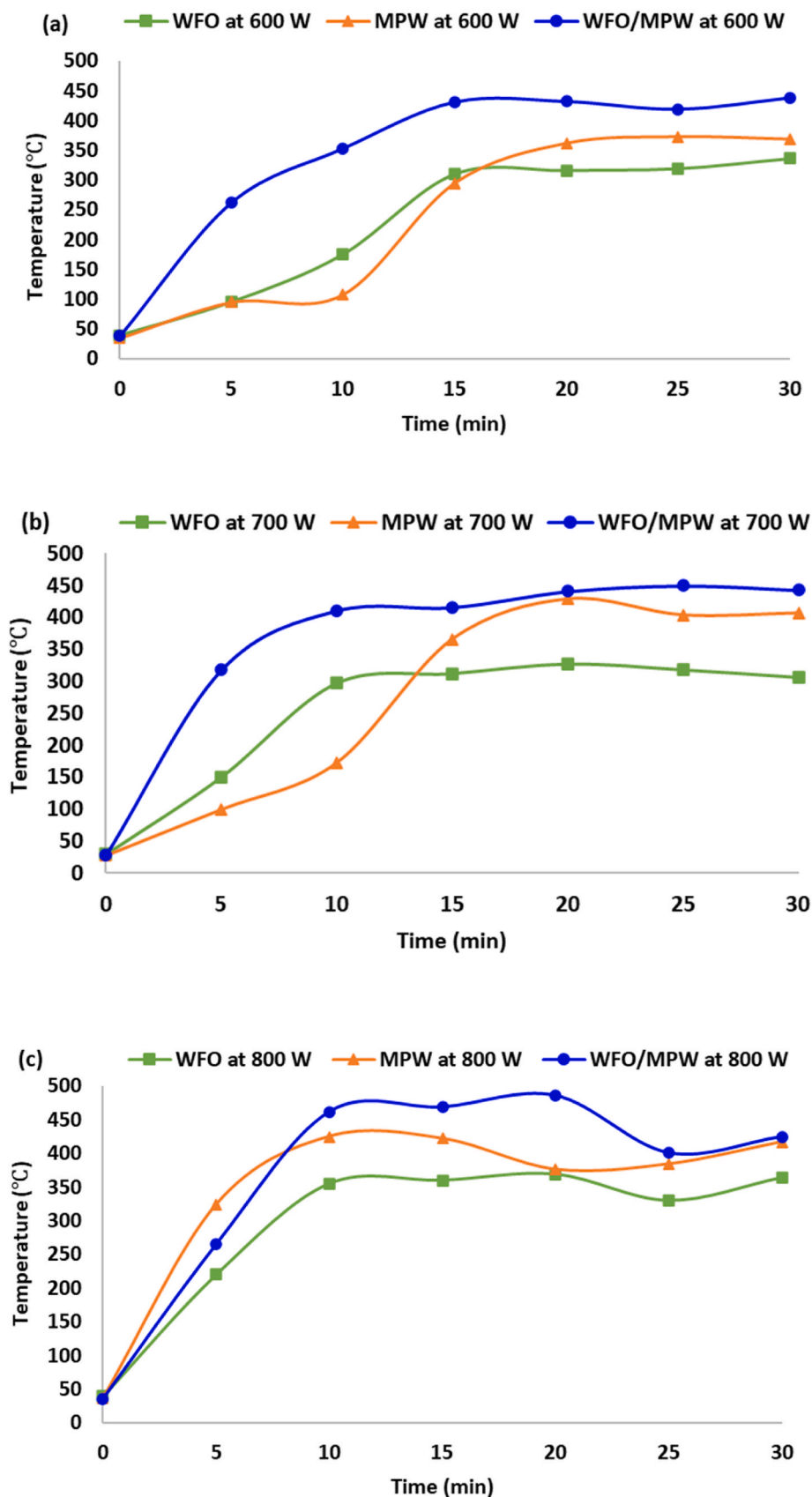


Fig. 3. Temperature behavior versus heating time. (a) pyrolysis performed at microwave power of 600 W, (b) pyrolysis performed at microwave power of 700 W, (c) pyrolysis performed at microwave power of 800 W.

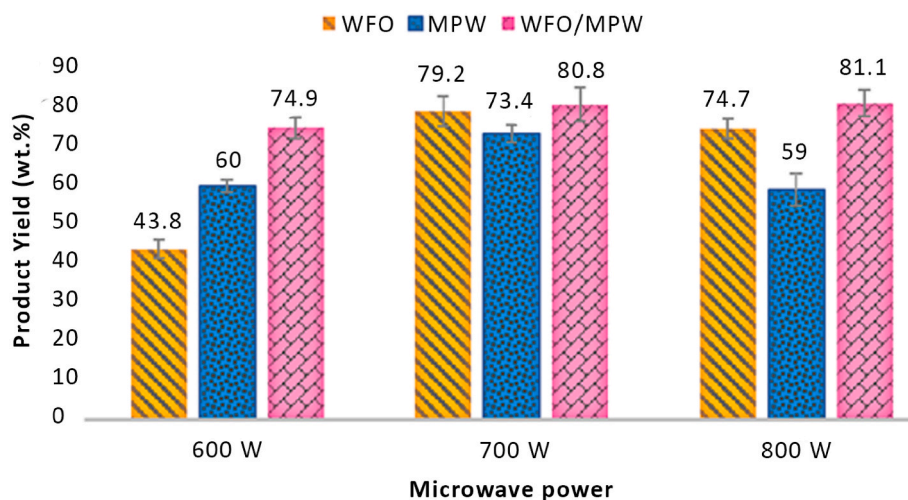


Fig. 4. Yield of liquid oil obtained at various microwave power.

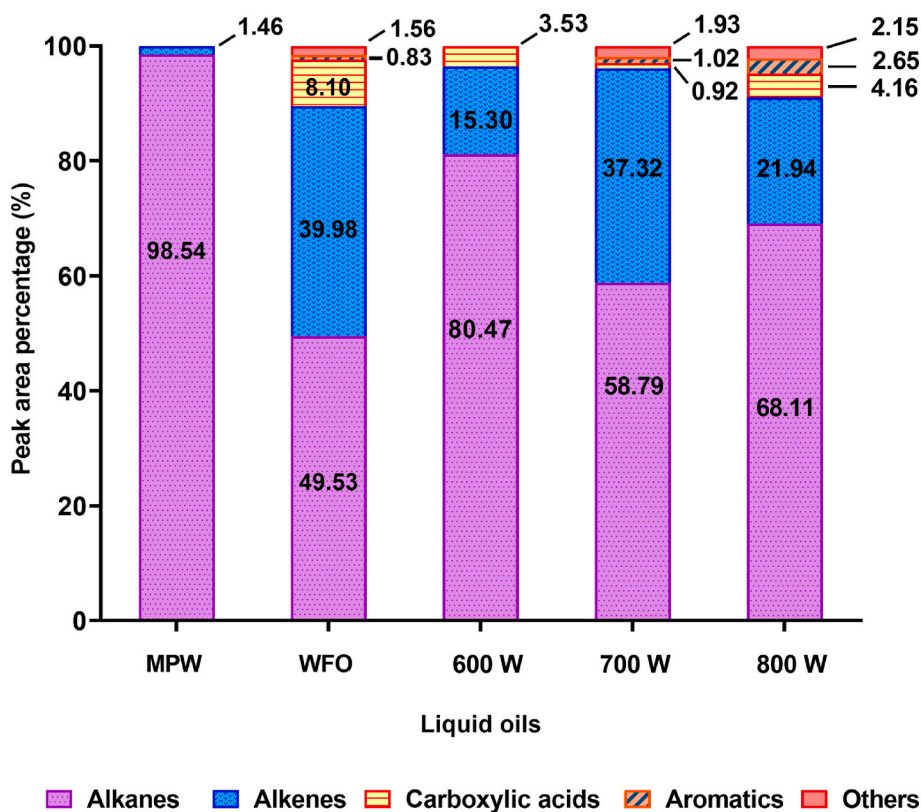


Fig. 5. Chemical composition of liquid oil obtained from microwave pyrolysis of MPW alone, WFO alone and microwave co-pyrolysis of MPW and WFO at 600 W, 700 W, and 800 W of microwave power.

unidentified compounds. Liquid oil produced at 700 W had the highest aliphatic hydrocarbons (96.1%), followed by liquid oil produced at 600 W (95.8%) and 800 W (90.1%). The aliphatic hydrocarbons are mainly decane ($C_{10}H_{22}$) to octacosane ($C_{28}H_{58}$). Octadecane ($C_{18}H_{38}$) and pentacosane ($C_{25}H_{52}$) were the major compounds found in the liquid oil produced at 600 W, whereas heptadecane ($C_{17}H_{34}$) and heneicosane ($C_{21}H_{44}$) were the major compounds found in liquid oil produced at 700 and 800 W (Table S1). This suggests that high process temperatures enhance the thermal cracking reactions of hydrocarbon molecules in MPW and triglyceride molecules in WFO, subsequently cracking heavier hydrocarbon compounds into lighter ones. In addition, higher microwave power may stimulate the production of hydrogen radicals during

the random scission polymer degradation reaction, which normally occurs during co-pyrolysis. Other polymer chains may interact with free radicals to break the chains of the polymer into lighter hydrocarbon components.

Aromatic compounds were absent in the liquid oil produced at a lower microwave power (600 W). At a higher microwave power (700 W), 1.02% of aromatics was produced, while oil produced at 800 W had the highest content of aromatics. This signifies the occurrence of secondary cracking reactions, such as the depolymerisation of polyethylene, along with increased microwave power, converting aliphatic hydrocarbons into aromatics such as benzenes [31]. This is also corroborated by the lower aliphatic hydrocarbons in the liquid oil

produced at 800 W (90.1%) compared to other liquid oils produced at a lower microwave power.

Carboxylic acids in liquid oils include hexadecanoic acid, octadecanoic acid, and 9-octadecenoic acid, which is derived from the original WFO, with the highest content of carboxylic acids in the liquid oil produced at 800 W, followed by 600 W and 700 W. At 700 W, intensive deoxygenation of oxygenated compounds (e.g. aldehydes, ketones, and fatty acids) enhances its conversion into aliphatic hydrocarbons, especially alkanes and alkenes. Low carboxylic acids detected in the liquid oil following 700 W treatment are desirable for use as alternative fuels, because the characteristics of carboxylic acids degrade liquid oil quality during storage, thus reducing the stability of the oil [10]. Nevertheless, the higher carboxylic acids detected in the liquid oil produced at 800 W is desirable as a supplement for microbial fermentation to produce bioplastics, because the fatty acids can serve as a bacterial food source, creating the ester bond during PHA formation.

The results show the strong potential of the liquid oil produced from the microwave co-pyrolysis of plastic waste and waste frying oil for its use as an alternative fuel, as reflected in our previous studies [10,32,51]. In the subsequent section, we emphasize the use of liquid oil as an energy source and growth substrate to produce bioplastics.

3.5. Production of polyhydroxyalkanoate using liquid oil produced from microwave co-pyrolysis of medical plastic waste and waste frying oil

A negligible growth of *B. megaterium* UMTKB-1 was observed with limited PHA accumulation when the liquid oil generated from microwave pyrolysis of MPW and WFO, individually, was used as the energy source and carbon substrate for bacterial growth (Table 5). Probably, the deficiency of WFO carbon (52.6 wt%, Table 4) inhibits bacterial growth and PHA accumulation. Additionally, WFO also contains the antimicrobial compound, *i*-Propyl 11,12-methylene-octadecanoate, which can inhibit bacterial growth [57]. The non-existence of nitrogen content in the liquid oil produced from MPW likely contributes to the absence of bacterial growth and PHA. In addition, excessive carbon content can inhibit the pathway of PHA synthesis, revealing that extreme conditions, such as excessive and deficient nutrient content (e.g. carbon and nitrogen), inhibit bacterial growth and PHA accumulation [58].

Interestingly, *B. megaterium* UMTKB-1 strain accumulated PHA in the form of P(3HB) using the liquid oil obtained from the microwave co-pyrolysis of MPW and WFO. The liquid oil served as carbon substrate and energy source for the bacteria to grow and accumulate PHA simultaneously (Table 5). This implies that the encouraging synergistic effect between MPW and WFO increases the carbon and nitrogen content in liquid oil, resulting in a higher carbon to nitrogen ratio that facilitates the growth of *B. megaterium*, and subsequently, the accumulation of PHA. More specifically, the highest PHA content was observed while using liquid oil produced at 600 W, followed by liquid oil produced at 800 and 700 W (Table 4). This was likely due to the desirable properties of liquid oil produced at 600 W, such as higher carbon content (77.1 wt%), lower carbon to nitrogen ratio content (27.9), and slightly higher carboxylic acid (3.52%) content compared to other liquid oils. PHA is a linear polyester comprising monomers of hydroxy acid linked to an ester bond produced by linking the carboxylic group of a monomer to the hydroxyl group of a compound [59].

The levels of PHA accumulation observed using the liquid oils obtained from 600 W to 800 W of microwave power was expected because

the C/N ratio of liquid oil obtained at 700 W was the highest compared to the C/N ratio obtained at 600 and 800 W (Table 3). Previous studies reported that PHA accumulation occurred in a limited nitrogen-excess carbon environment, which agrees with our current finding [60–62]. Notably, higher cell dry weight (CDW) was observed when the liquid oil obtained at both 700 and 800 W were used as the energy source compared to the liquid oil obtained at 600 W. Sufficient amounts of nitrogen and carbon facilitate bacterial growth, rather than accumulate PHA [41]. However, the limitation of nitrogen in the culture medium is an important factor for PHA production; therefore, the liquid oil obtained at 600 W is the best carbon source, because of its high carbon and low nitrogen content, which fits the criteria for PHA accumulation.

The fatty acids in liquid oil serve as an energy source for bacterial propagation mainly through the following biosynthesis pathways. The adenosine triphosphate (ATP) molecules, coenzyme A (CoASH), and acyl-CoA synthetase activate fatty acids into acyl-CoA before entering the β -oxidation cycle [63]. Although the acetyl-CoA produced from the β -oxidation cycle usually enters the Krebs cycle for ATP production, nutrient-deficiency decrease the electron transport activity and hence generating lesser ATP and triggering PHA accumulation as an alternative energy storage approach [64]. The metabolic pathway of P(3HB) accumulation in *B. megaterium* UMTKB-1 naturally competes for the β -oxidation of fatty acids to produce acetyl-CoA that is not only used in development of growth but also vital for P(3HB) production, which may explain the reasons of the low PHA accumulation (Fig. 6) [65]. Two acetyl-CoA molecules are formed into acetoacetyl-CoA and subsequently (*R*)-3-hydroxybutyryl-CoA by β -ketothiolase (PhaA) and NADPH-dependent acetoacetyl-CoA reductase (PhaB), then polymerized by PHA synthase (PhaC), thus releasing CoA to be used for P(3HB) synthesis replacing the Krebs cycle. However, during normal conditions without malnourishment, CoA prevents the generation of PhaA in the Krebs cycle.

In liquid oil, these fatty acids help to promote bacterial growth by utilising the readily available alkanes. The alkane is degraded by oxygenases that introduce oxygen atoms into alkane substrates and target the terminal methyl group of alkanes to produce fatty alcohols. Subsequently, alcohol dehydrogenases oxidise alcohols to form fatty acids that then enter the β -oxidation pathway, hence allowing liquid oils to be utilised with other substrates, such as reducing and non-reducing sugars [66]. Although there have been reports of PHA conversion from waste oils, not all PHA-producing bacteria produce the necessary enzymes to perform the biological syntheses and conversions from waste oils and plastic wastes needed for PHA synthesis and ATP production, thus making the mentioned conversion ability observed in this study considered as valuable [9,11,15]. Furthermore, microwave-pyrolyzed waste oils and plastics have altered properties and compositions compared to non-pyrolyzed wastes [40,42,47]. To date and to our knowledge, this is the first report of PHA production from medical plastic waste and waste frying oil using *B. megaterium*. Further studies are needed to further optimize the approach in producing higher yield of PHA.

FTIR analysis provides further insights into the chemical structure of the P(3HB) homopolymer. Fig. 7 shows the functional groups of P(3HB) produced by *B. megaterium* UMTKB-1. The IR spectra recorded peaks at 2978.09 (-CH group), 1737.86 (C=O stretch), and 1286.52–1068.56 (C–O stretch) cm^{-1} , which corresponded with the previous studies [15, 28,70]. Absorption bands detected at 2735.06, 2384.02, and

Table 3
Properties of waste frying oil and medical plastic waste.

Feedstock	Elemental analyses (wt.%)					Proximate analyses (wt.%)			
	C	H	N	S	O	Moisture	Volatile matter	Fixed carbon	Char residue
WFO	52.6	17.8	3.33	0	26.3	0	99.8	0.2	0
MPW	86.2	13.3	0.02	0	0.48	0	99.5	0.5	0

Table 4
Elemental composition of the liquid oils.

Elemental Analysis	Carbon (wt%)	Hydrogen (wt%)	Nitrogen (wt%)	Sulphur (wt%)	Oxygen (wt%)	H/C	C/N
Original WFO	52.6	17.8	3.33	0	26.3	4.06	18.4
Original MPW	86.2	13.3	0.02	0	0.48	1.81	–
600 W (1:1)	77.1	10.3	3.18	0	9.82	1.60	27.9
700 W (1:1)	72.4	7.22	2.19	0	18.8	1.19	38.5
800 W (1:1)	68.2	9.14	2.28	0	20.7	1.61	34.9

(–) not available.

Table 5
Biosynthesis of P(3HB) by *B. megaterium* UMTKB-1 using pyrolysis liquid oil as growth substrate.

Liquid oil	PHA content [% (w/w)]	Cell dry weight, CDW (g/L)
MPW	Trace amount ^a	Trace amount ^b
WFO	Trace amount	Trace amount
MPW + WFO (600 W)	11.03	0.13 ± 0.04
MPW + WFO (700 W)	0.80	0.16 ± 0.04
MPW + WFO (800 W)	1.54 ± 0.13	0.16 ± 0.07

CDW = cell dry weight; MPW = medical plastic waste; WFO = waste frying oil.

^a PHA content <5 wt%.

^b Cell dry weight <0.05 g/L.

964.41–509.21 cm^{-1} correspond to $-\text{CH}$ stretching, $\text{C}\equiv\text{C}$ stretching, and $-\text{OH}$ groups, respectively. The detection of the $\text{C}=\text{O}$ bond at 1737.86 cm^{-1} represents the character of P(3HB). Additionally, DSC was conducted to investigate the melting temperature (T_m), glass transition (T_g), crystallization temperature (T_c), and degree of crystallinity of P(3HB). The T_g of the P(3HB) obtained was 2.00 °C, which was comparable with previous studies by Wellen et al. [71] and Gunaratne et al. [72]. The T_m and T_c of the P(3HB) obtained was 172.65 °C and 48.20 °C, respectively. The T_m was comparable to that reported in previous studies by Mohanrasu et al. [73] and Trakunjae et al. [74] while T_c was in agreement with Wellen et al. [71]. The degree of crystallinity of P(3HB) was 55.75% in the present study, which exhibited results approximately similar to the previous studies [75,76]. These analyses provided sufficient data to confirm the identity of the synthesised polymer, P(3HB).

Numerous studies have been performed on the valorisation of waste

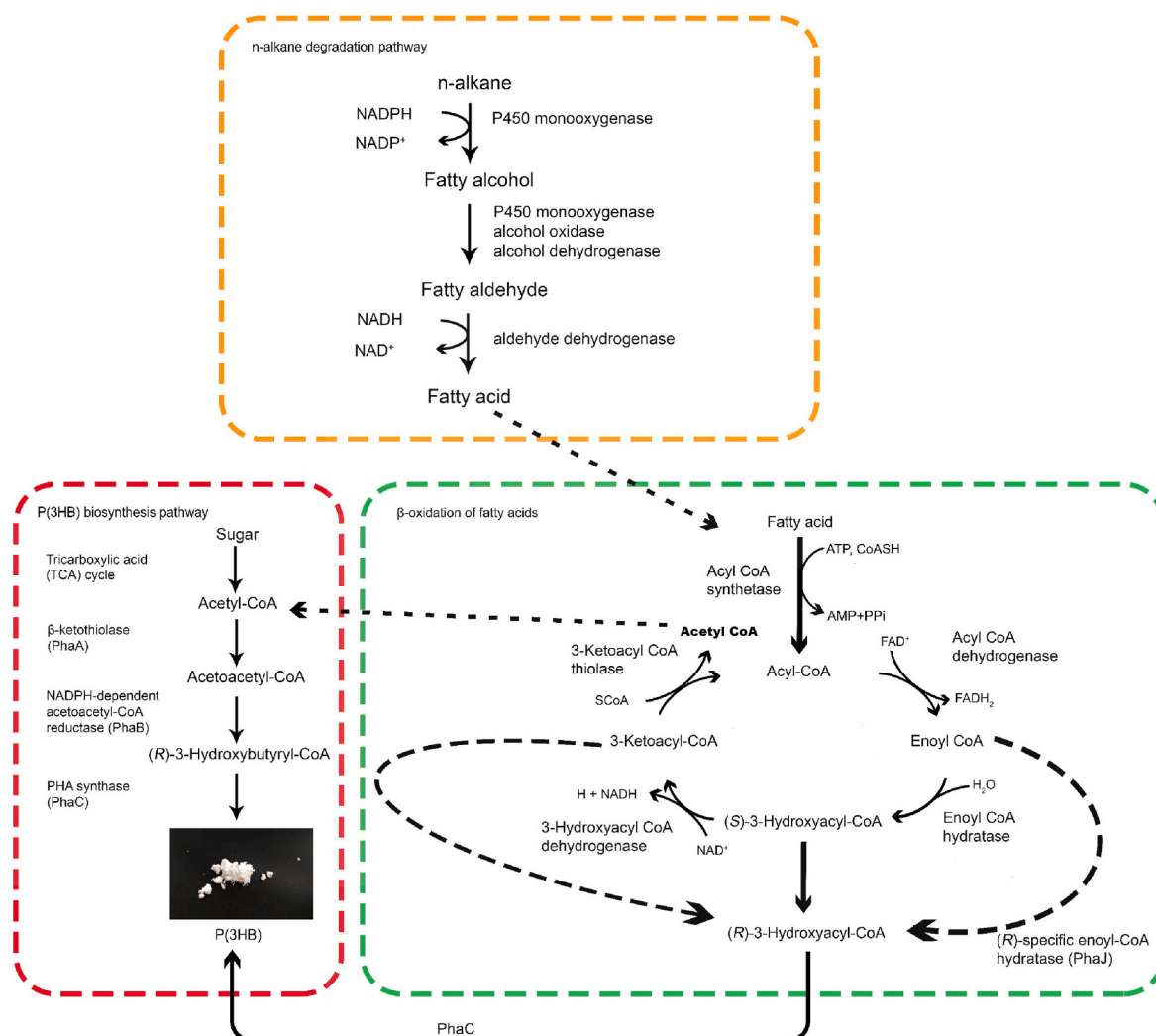


Fig. 6. n-alkane degradation pathway, β -oxidation of fatty acids pathway and P(3HB) biosynthesis pathway. Reproduced from previous studies [67–69].

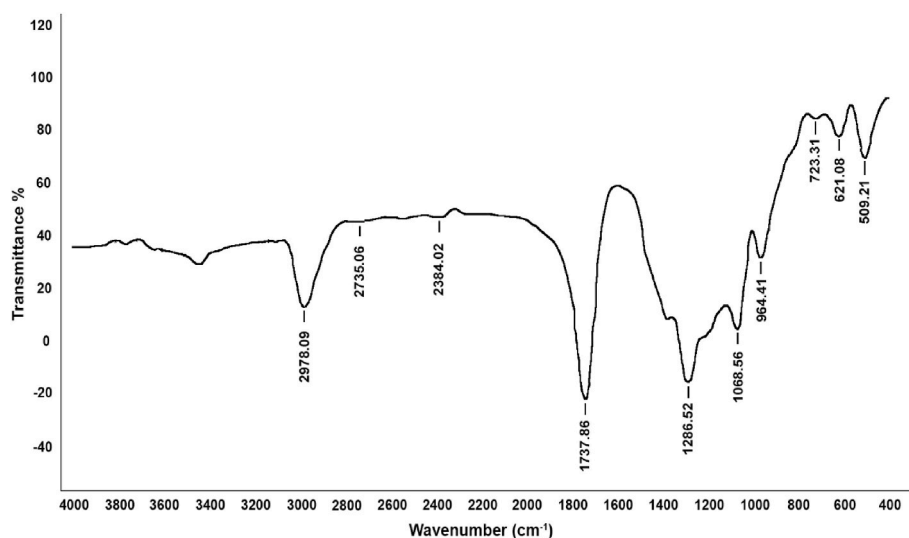


Fig. 7. FTIR spectrum of P(3HB) homopolymer generated by *B. megaterium* UMTKB-1. Resolution = 4 cm⁻¹; 50 scans.

as the sole energy and carbon source for PHA production. Table 5 shows the various bacteria, and wastes used as carbon sources and growth substrates for PHA production. Although previous studies (Table 6) had similar purpose of valorising wastes to produce PHA, the carbon was mainly sourced from organic wastes. The novel aspect that sets our study apart from the previous studies is that P(3HB) was produced from the liquid oil generated by the microwave co-pyrolysis of medical plastic wastes and waste frying oil. However, the P(3HB) yield [11% (w/w)]

Table 6

Previous studies of polyhydroxyalkanoate production using various bacteria and different energy feedstocks.

Bacteria	Carbon feedstock	PHA type	PHA yield [% (w/w)]	Refs.
<i>Bacillus megaterium</i> UMTKB-1	Microwave co-pyrolyzed MPW and WFO	P(3HB)	11	This study
<i>B. megaterium</i> R11	Oil palm empty fruit bunch hydrolysate	P(3HB)	58	[78]
<i>B. megaterium</i> NCIM 5472	Cheese whey permeate	P(3HB-co-3HV)	87	[79]
<i>B. megaterium</i> NCIM 5472	Ultrafiltered cheese whey	P(3HB)	75	[80]
<i>B. megaterium</i>	Glucose	P(3HB)	59	[81]
<i>B. megaterium</i>	Glycerol	P(3HB)	62	[81]
<i>B. megaterium</i> UMTKB-1	Sugarcane molasses	P(3HB)	12	[21]
	Sweetwater	P(3HB)	7	
	Glycerol	P(3HB)	49	
<i>Deftuivococcus varus</i>	Sugarcane molasses	PHA	37	[82]
<i>Burkholderia sacchari</i> DSM17165	Wheat straw hydrolysate	P(3HB)	57	[83]
	Glucose	P(3HB-co-4HB)	27	
<i>Cupravidus necator</i> DSM 428	Used cooking oil	P(3HB)	37	[84]
<i>Haloferax mediterranei</i> DSM1411	Whey	PHA	70	[25]
<i>Bacillus cereus suaeda</i> B-001	Glucose	P(3HB)	43	[15]
	Oil palm empty fruit bunch	P(3HB)	40	
<i>Bacillus subtilis</i> RS1	Pre-treated sugarcane molasses	P(3HB)	45	[23]
Recombinant <i>C. necator</i> RE2058/pCB113	Sludge palm oil	P(3HB-co-3HHx)	74	[85]
<i>Pseudomonas aeruginosa</i> STN-10	Frying oil	P(3HB)	53	[86]

obtained in our study was lower than that obtained in other studies, which suggests the need for further optimisation of the biosynthesis processes using the feedstock obtained in this study.

The liquid oil produced from the pyrolysis of MPW and WFO has potential and is beneficial as an energy source and carbon substrate for P(3HB) production. This is the first initiative where plastic waste combined with waste cooking oil can be converted into biodegradable P(3HB). One of the main challenges in a circular economy is reducing the generation of solid wastes [77]. Studies on PHA production utilising different organic wastes as an energy source and carbon substrate have been performed, but none to date on synthetic wastes or a combination of organic and synthetic wastes. The potential of waste has yet to be uncovered, and the combination of MPW and WFO can be one of the alternatives to reduce plastic waste and carbon footprint (Table 6).

4. Practical implications of the study

This work was initiated due to massive accumulation of MPW at hospitals in Malaysia, which require high cost for disposal via incineration and specific sanitary landfill. The COVID-19 pandemic has led to sudden upsurge of MPW and this exacerbates the problem for its disposal. The current disposal approach such as incineration and sanitary landfill could escalate environmental pollution due to the release of harmful gases including dioxins, furans and CO₂ [87]. Therefore, the present work demonstrates the practicality of applying microwave co-pyrolysis to simultaneously reduce waste materials (MPW and WFO) while recovering energy from these wastes.

The addition of WFO into MPW during microwave co-pyrolysis is effective to reduce the production of undesirable wax and increase the yield of liquid oil compared to pyrolysis of plastic waste alone (Fig. 4). The MPW also represents other types of plastic waste produced from household and industry sectors. This study also demonstrated the capability of MPW to facilitate the pyrolysis of WFO, leading to the reduction of oxygenated compounds and increase the carbon, hydrogen and aliphatic hydrocarbons composition in the liquid oil, thereby showing potential to be upgraded into fuel. The high carbon content in the liquid oil is also desirable to be used as energy source during bacterial fermentation for producing bioplastics. This suggests that microwave co-pyrolysis is a desirable approach to enhance the quantity and quality of the liquid oil product, thereby showing support to the United Nations Sustainable Development Goals (SDGs) in producing affordable and clean energy (Goal#7) and ensuring sustainable consumption and production (Goal #12). The feedstocks used during microwave co-pyrolysis are not limited to MPW and WFO only, but other feedstocks

that exhibit similar properties could be used to generate a liquid oil product with desirable properties. This study could be a suitable reference for researchers or industrial operators who seek for a sustainable approach in using waste materials as feedstocks to produce energy.

Further research is needed to optimize and scale up the technology for industrial application. A microwave co-pyrolysis system can be developed and constructed at hospital so that the MPW can be converted into energy on-site, thus reducing transportation cost for waste disposal. In addition, the liquid oil may be further converted into fuel or ignited in boilers to generate power and electricity. Nevertheless, more research is needed to assess the environmental sustainability of the developed technology via exergetic, exergoenvironmental and exergoeconomic assessments.

5. Concluding remarks and future perspectives

Microwave co-pyrolysis of MPW and WFO produced liquid oil significantly higher than the microwave pyrolysis of MPW and WFO individually, and at higher heating rates and reduced process time. Liquid oil consists of light aliphatic hydrocarbons ranging from C₁₀ to C₂₈ and contains low oxygen, thus exhibiting potential for use as an alternative fuel. The liquid oil produced from the microwave co-pyrolysis at 600 W with a C/N ratio of 27.9, acts as an energy source for microbial fermentation and produces higher P(3HB) content compared to the range of microwave power considered. The production of bioplastics from synthetic plastic waste is possible using the microwave co-pyrolysis technique. Since this finding is relatively new, the future direction of related research includes optimisation of microwave pyrolysis parameters such as microwave temperature, retention time, presence of catalyst, physical and chemical fermentation parameters, and bioreactor production scale-up for increased yields of P(3HB). Moreover, future research on co-polymer production should also be considered.

This study showed that microwave co-pyrolysis reduced the content of heteroatoms (e.g. oxygen and nitrogen) and increased the composition of aliphatic hydrocarbons compounds in the liquid oil, which can potentially be applied as fuel. Nevertheless, it should be mentioned that the presence of heteroatoms such as oxygen and nitrogen could generate nitrogen-containing and oxygen-containing compounds, which could deteriorate the quality of the liquid oil. For instance, the oxygen-containing compounds (e.g. carboxylic acids) could cause corrosiveness to the engine if the liquid oil is applied as fuel. In addition, the nitrogen-containing compounds could emit harmful NO_x during the combustion of the liquid oil as fuel. Therefore, future studies should be performed to further improve this pyrolysis technique and the composition of the liquid oil:

1. Upgrading techniques to reduce or eliminate the content of heteroatoms in the liquid oil. For example, ionic liquids can be applied as extraction solvents for the reduction of nitrogen compounds from the liquid fuel [88].
2. Examination of microwave co-pyrolysis models using uncertainty analyses that identify and quantify possible faults of this approach. By performing this analysis, the range of possible outputs can be determined based on the uncertainty of the inputs applied to scrutinize the impact of the errors or lack of knowledge of the model.
3. This study showed that *B. megaterium* UMTKB-1 was able to accumulate PHA when fed with microwave co-pyrolyzed MPW and WFO liquid oil. However, the yield is relatively low when compared to previous studies. The liquid oil contains oxygen which can be oxidized and polymerized to generate unfavourable compounds for bacteria growth. Post-treatment can be considered to further stabilize the oil. The bioavailability of carbon from the liquid oil also affects its utilization by the bacteria to accumulate PHA. It was observed from the biosynthesis that solidified liquid oils still present after 48 h. The bacteria were likely not able to fully utilize the liquid

oil due to low total surface area. This phenomenon was common when oily substrates with high melting point compared to the bacteria fermentation temperature were used as carbon source. To 'break' the oil higher agitation speed, addition of biosurfactant or biological or physicochemical treatments can be considered to increase the bioavailability of the carbon from the liquid oil. Nonetheless the effect of this factors on the PHA yield is yet to be fully understood.

4. Find other factors that could affect the PHA yield such as the type of strains, genes and enzyme produced [89]. The *B. megaterium* UMTKB-1 strain may not be the most suitable candidate to accumulate PHA when fed with liquid oil produced from microwave pyrolysis. It is important to emphasize that the carbon source utilization is strain dependent. Hence, a quest to search for a more suitable wild-type strain or a robust genetically engineered strain can be considered that can withstand the impurities of the liquid oil is necessary and accustomed to utilising liquid oil for PHA accumulation purposes [30]. Additionally, the gaps in the physiology and complete metabolic pathways of bacterial PHA-producers using liquid oil as carbon source still pose question regarding other competing pathways or inhibitors. Therefore, the findings here show the possible conversion of pyrolyzed bio-oil into PHA but this requires future research in the optimisation of culture conditions and treatment of feedstocks for higher PHA yield.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rser.2021.111790>.

Credit author statement

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