# The potential of *Yarrowia lipolytica* in converting bioenergy resources: a preliminary review

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**Abstract.** *Yarrowia lipolytica*, a yeast species capable of producing oil or oily fatty acids, has the ability to utilize multiple carbon sources, including glycerol, acetic acid, and glucose, allows for the use of inexpensive carbon sources. Waste cooking oil can be utilized as an alternative carbon source while also there is potential in increasing the oil yield due to the presence of glycerol compounds. The study aims to explore the potential of *Yarrowia lipolytica* in producing lipid based bioenergy from by-product such waste cooking oils. One of the greatest challenges that will affect life is our continued reliance on fossil fuels, which are still derived from petroleum and fossils. Fuel is not only the primary source of energy that has a significant impact on every aspect, but its sustainability remains the primary concern as we search for alternative solutions that can circumvent these issues. Using yeast lipids, specifically *Yarrowia lipolytica*, has not been investigated, in addition to producie biodiesel, this yeast can use waste cooking oil as a growth medium and produce lipids. The third generation of biodiesel uses microorganism-produced lipids, which is new and worthy of further research to solve the problem of unsustainable and environmentally unfriendly diesel fuel. *Yarrowia lipolytica's* ability to accumulate lipids, produce wax esters synthase enzymes, and FAEE/FAME still have great potential.

## 1. Introduction

Yeast is a single-celled eukaryotic microorganism capable of converting sugar into alcohol. Yeast has high resistance to contaminants, making it easier to cultivate in various media. Some of the media that have been used in cultivation and have high resistance to inhibitors (1). Oleaginous Yeast (OY) has faster growth plus cultivation that can be done conventionally with an easier process. However, the production efficiency of biodiesel produced by OY is influenced by optimal conditions at the fermentation stage.

Fermentation with the fed-batch cultivation method, during this process the growth of yeast cells has increased in the first stage, then in the second stage there will be an accumulation of oil produced by OY (2). Growth of OY using the fed-batch method is considered to be able to increase oil production by OY because this method reduces substrate inhibition by limiting the concentration of the substrate during the fermentation process (3). Oil production by OY will accumulate in the second stage under limited nitrogen conditions, after the OY growth process has increased in the first stage.

Of all the oleaginous microorganisms, oleaginous yeast or OY was chosen as a potential microorganism in producing microbial lipids. This is because OY has advantages over other OMs, namely the characteristics of single-celled yeasts with high growth ability and coupled with high cellular lipid content. Yeast cultivation is also considered easier to improve because it can be processed in a fermenter and does not require light for the growth process (4). OY can also take advantage of cheap or low-cost substrates and even use waste like WCO. Apart from that, OY is also a microorganism that is easy to repair by involving genetic engineering in the process (5).

*Yarrowia lipolytica* which is also included as a yeast species that can produce oil or OY has been identified to produce Wax esters (WE) of 7.6 g/L WE with total lipids produced reaching 0.44 g/L.h (6). Several different types of carbon sources have been studied to find out which carbon sources can provide optimal results in OY, especially *Yarrowia lipolytica* which has the greatest potential to produce was esters in OY species. OY's ability to utilize multiple carbon sources makes it possible to use inexpensive carbon sources. In line with research conducted by (2).With the carbon sources used, namely glycerol, acetic acid and glucose, showed different yields. The carbon source in the form of sugar gave the highest yield at 0.270 gl/gs.

The utilization various types of carbon can be done by *Yarrowia lipolytica* and one of which is waste cooking oil (WCO). The use of WCO as a carbon source can increase the yield of oil produced by OY, this is due to the presence of glycerol compounds in WCO which is a carbon source that is very cheap and easy to obtain,

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moreover WCO is still a waste which has not been processed much at the moment and must be utilized immediately so that does not pollute the surrounding environment (6). So, the novelty of this study is to look deeper into the potential of *Yarrowia lipolytica* which has a high accumulation of lipids and can produce wax esters which can be converted into FAEE, a type of biodiesel. There have not been many studies on this yeast, especially the potential for wax esters produced with WCO substrates which are cooking oil waste that can be used as a substrate for the growth of this yeast.

## 2. Characteristics of *Y. lipolytica*

The group of oleaginous yeasts that has the most potential to become OY with a large lipid producer is *Yarrowia lipolytica* (Fig. 1).The unique characteristics of this OY species is *Yarrowia lipolytica* has been used as a host cell and as an OM model used to identify lipid synthesis and also its accumulation in *Y.lipolytica* cells (7). This OY species is also included in the hemiascomycetous dimorphic yeast which belongs to the order, namely *saccharomycetales. Yarrowia lipolytica* is also included as a yeast which is generally regarded as safe (GRAS), generally it can be isolated from mediums that contain or contain lots of lipids such as oil or fat, so that *Yarrowia lipolytica* contains a high linear lipolytic with high proteolytic activity (7).

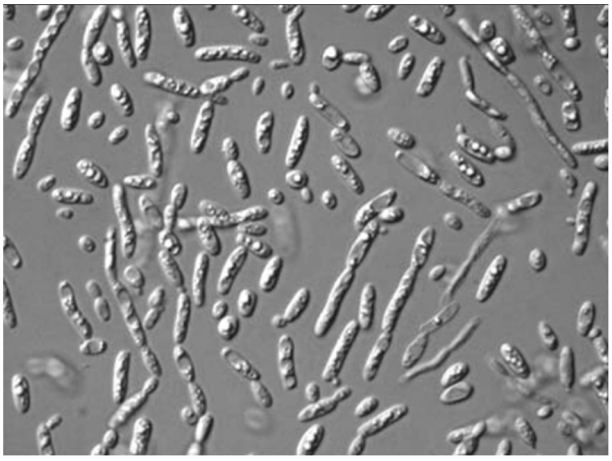

**Figure 1**. Cell morphology of *Y. lipolytica* H222 wild-type strain (8)

*Yarrowia lipolytica* has the ability to utilize various carbon sources from various types of substrates such as acetate, lignocellulose, sugar (9). for cooking, the waste is useless and underutilized. The use of *Yarrowia lipolytica* as a host cell in producing lipids as biofuels is based on the characteristics of this OY species which can produce even more than 40-90% of lipids, which are lipids stored in the form of TAGs (10). In addition, organic acids that can be produced from *Yarrowia lipolytica* are citric, isocitrate, succinic, acetic acid (9)

OY *Yarrowia lipolytica* is the main OY that has the potential to produce lipids with its ability to produce as much as 70% of lipids from dry biomass. In addition, the main characteristics found in OY are high acetyl-CoA flux and high tricarboxylic acid cycle (TCA) (11). Naturally *Yarrowia lipolytica* is an oleaginous yeast which can be found in substrates containing lipids or proteins *Yarrowia lipolytica* can also live in polluted oil or waste oil (12). *Yarrowia lipolytica* has the ability to hydrolyze lipids by secreting lipase, protease and esterase enzymes, besides that it also has the ability to form hydrocarbons and fatty acids through the terminal pathway, β-, and ω-oxidation (13). Not only that, *Yarrowia lipolytica* is an OY as a host cell that contains genes that code for 16 lipase acylglycerols, 14 fatty acid transporters, besides that there are also genes that code for 12 cytochromes P450 for oxidation of hydrocarbons and fatty acids, and encodes 38 aspartyl and 15 serine proteases (14).

*Yarrowia lipolytica* is included in the yeast *ascomycetes* which are known as yeast which have the ability to break down lipid (lipolytic) and proteolytic compounds with the secretion of high activity of the enzymes involved. Yeast *Yarrowia lipolytica* with natural or wild-type strains can be found in living environments or habitats that are rich in high lipid or protein compounds, for example from daily food products such as cheese and sausage (15), besides that it can also found in contaminated oil or water and soil. Recent research explains that *Yarrowia lipolytica* is also present in human organs and is included in the natural microbiota found in the mouth and respiratory organs in adults which are commonly found in patients with diabetes (16). *Yarrowia lipolytica* is categorized as yeast generally recognized as safe or GRAS, and is also included in the yeast group which is categorized as a microorganism with a biosafety level (BSL) 1 given by the public health service (USA)(10).

The genome of *Yarrowia lipolytica* is known to be 20.5 Mb in size which consists of six chromosomes with sizes ranging from 2.6-4.9 Mb (17) with a large number of genes, namely around 6703 genes. The intron portion of the gene has a percentage of 15%, with a G/C proportion of 49% on average and 53% in the gene (18). The molecular markers used are the LEU2 and URA3 genes (19). In previous studies, genetic manipulation was carried out by expressing the WS gene, namely the gene encoding Wax ester synthases, which is an enzyme that plays a role in the synthesis of wax esters. The WS gene originates from the genome of the species Acinetobacter baylyi ADPI in host cells, namely *Yarrowia lipolytica* (20). *Yarrowia lipolytica* as a host cell in this genetic engineering in producing FAEE due to its ability to accumulate high amounts of lipids even under optimal fermentation conditions, y.lipolytica can produce lipids even up to 73-100 g/l (21) and can use variations wide substrate even waste utilization.

The WS gene and the DGAT gene are important genes that carry the code for an enzyme involved in the synthesis of FAEE. The WS gene from another species, namely M. *hydrocarbonoclasticus* DSM 8798, was not complemented by the DGAT gene allele in the gene of this species (22). The gene encoding DGAT or acyl-CoA-diacyglycerol acyltransferase, namely the DGAT1 gene, is known to be present in *Yarrowia lipolytica* species, the DGA1 gene in *Yarrowia lipolytica* is known to have a function as a role in the formation of TAG

compounds and regulates the course of the process of lipid synthesis (23,24).

## 3. The ability of *Y. lipolytica* in lipid accumulation

Several studies have been conducted to obtain high productivity oil from the OY species, namely *Yarrowia lipolytica*. *Yarrowia lipolytica* with its high ability to accumulate lipids has the potential to be applied to various products with specific structures and compositions. The application of lipids from *Yarrowia lipolytica* consists of (a) as a nutritional complement, therapeutic and plays a role in health because y.lipolytica produces about 50% linoleic acid which is an unsaturated fatty acid (17). (b) the lipids produced can play a role as a Cocoa butter substitute because the composition of the lipids produced by *Yarrowia lipolytica* is very similar to the composition of cocoa butter, (c) besides that, the lipids produced also have the potential to become renewable biofuels or biodiesel (25)

*Yarrowia lipolytica* into OY which can be used as an alternative source of biofuels. this is because *Yarrowia lipolytica* has high oleaginicity. To increase the oleaginicity, it can be done through genetic modification techniques to be able to produce the expected lipids. (9),(26). Biofuels produced from this yeast need to pay attention to the accumulation of fatty acids and their derivatives that are produced. Genetic modification is currently involved in the development of *Yarrowia lipolytica* in increasing the metabolites produced in yeast cells. Fuel production can be produced from raw materials based on fatty acids and hydrocarbon compounds which can be produced by several types of microorganisms, one of which is OY, namely *Yarrowia lipolytica*. A genetic engineering can directly identify the target cells to be modified (17).

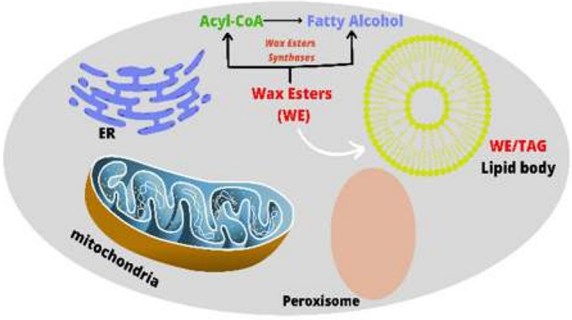


**Figure 2**. Lipid body in *Y. lipolytica* (modification from (17).

Modification of the OY *Yarrowia lipolytica* strain was focused on targeting the heterologous enzymes involved in the formation of compounds that become basic ingredients as biofuels, in the subcellular part of the enzymes in the cytoplasm, peroxisomes and ER (21). Lipid accumulation in OY *Yarrowia lipolytica* is a series of processes from the lipid metabolism pathway in *Yarrowia lipolytica* cells which is divided into a place for lipid formation, namely the cytosol which is generally the place for lipid synthesis in cells, then storage of lipids in ER organelles and lipid bodies (Figure 2), as well as lipid mobilization which is one of the roles of peroxisome organelles and lipid body (27).

## 4. Optimization of lipid accumulation in *Y. lipolytica*

Lipid production in yeast can be influenced by the diversity of genes from each strain which plays a role in coding enzymes that play a role in metabolic pathways to increase the production of FAEE (28). In the process of forming FAEE, lipid production in host cells must be the main thing to pay attention to to avoid any obstacles in yeast cell growth. In OY *Yarrowia lipolytica*, the most important factor in the process of metabolism is acetyl-CoA which is used as a precursor in the biosynthesis of lipids and their derivatives (20).

Previous research has provided results to increase the supply of acetyl CoA in the cytosol of *Yarrowia lipolytica* there are several ways that can be done; (a) increasing the expression of ACL (ATP-dependent citrate lyase) because this enzyme plays a role in catalyzing the biosynthesis of acetyl-CoA, this treatment is equated with providing high energy in the form of ATP as a supporting factor for the success of increasing ACL expression, either in ACL1 or ACL2. Then, (b) by increasing the enzyme encoded by the ACS2 gene from another yeast species, for example *S.cerevisiae*, which plays a role in catalyzing the conversion process from acetic acid to acetyl CoA, namely the enzyme acetyl-CoA synthetase, then (c) targeting the ACC (Acetyl Coa carboxylase) enzyme to increase acetyl CoA and increase FAEE production (29).

Lipogenesis is a process when forming lipids in microorganisms. The accumulation of lipids in microorganisms can be influenced by several factors, such as the ratio of C/N sources, temperature, pH, and incubation time (30). However, the main factor that has the most influence is a condition when nitrogen sources are limited. Lipid accumulation is closely related to the ratio of carbon and nitrogen sources, both of which have opposite effects. When lipid production increases, while the growth rate of OY and biomass decreases (7). The optimum pH for OY *Yarrowia lipolytica* is pH 6-6.5 (31). *Yarrowia* has a different increase at a certain incubation temperature, at the incubation temperature the temperature is lowered from 30-12 degrees which makes the activity of the D12-Fatty acid desaturase enzyme increase. Whereas at a temperature of 12 degrees Celsius with this low temperature it will increase unsaturation (32).

Lipids accumulated in OY can generally be in the form of triacylglycerols and small amounts of sterile esters which are stored in lipid stores in their cells. The ability to collect lipids by OY is based on metabolic pathways and enzymatic processes which also depend on each OY species (4).

In addition, the growth medium of OY also affects lipid accumulation as a carbon source for growth of OY. Limited nitrogen conditions can increase the

accumulation of lipids because the concentration of adenosine monophosphate (AMP) decreases along with the inhibition of AMP-deaminase and NADþ-isocitrate dehydrogenase (NADþICDH), so that this results in the accumulation of citric acid in the mitochondria of OY cells and then it will be secreted into the cytosol. In OY cells, the molecular precursor of fatty acid biosynthesis namely Acetyl Coa is produced from citrate by ATP citrate lyase which is an enzyme in fatty acid biosynthesis that cleaves citric acid in the lipogenic phase into oxaloacetate and acyl-CoA in peroxisomes in cells OY (33).

## 5. Isolation and extraction lipid from *Y. lipolytica*

Isolation of lipids from raw materials is one of the steps in biodiesel production. Lipid isolation is carried out using organic solvents with the aim of avoiding interference with the synthesis of methyl esters from fatty acids (34). In general, the mixture chosen for lipid extraction in microorganisms is an organic solvent combination of a mixture of chloroform and methanol as well as water (35). There are polar and non-polar phases through which the lipid layer can pass. Next is research by Bligh and Dyer (36) which suggests an extraction method by means of homogenization and solvent synthesis and then adding pure chloroform compound to the mixture. The ratio of chloroform and methanol used is 2:1 which has been described in the folch method found in research by (37) and also uses a ratio between chloroform and methanol, namely 1:1 for oleaginous yeast species *Yarrowia lipolytica* grown with WCO medium.

The Folch method of lipid extraction technique has been tested and modified a lot, with the aim of producing the best method for extracting lipids. There are two extraction techniques that are modified, the first is a modification by reducing the use of organic solutions but is carried out with increased shaking time. The second was the acidification and boiling technique before adding organic solvents (38), but it was found that the technique with acidification resulted in a lipid yield of only 2.5 g/L while without acidification the amount of lipids was higher, namely 3 g/L. In addition, the Bligh and dyer method with a mixture ratio of 1:2 chloroform and methanol using freeze drying and bead milling can produce maximum lipids (39).

The use of a mixed ratio between chloroform and methanol as a solvent that is generally widely used from several studies above with yeasts *Yarrowia lipolytica*. Solvent extraction is also a solvent commonly used in the extraction of plant oils which are usually used for biodiesel. The use of a mixed ratio between chloroform and methanol as a solvent that is generally widely used from several studies above with yeasts *Yarrowia lipolytica*. Solvent extraction is also a solvent commonly used in the extraction of plant oils which are usually used for biodiesel.

However, the use of chloroform is considered dangerous for the environment. Chloroform is a chemical that is carcinogenic which is very toxic and dangerous. so that the use of this solution for extraction is considered not feasible on an industrial scale (40). Although the extraction of chloroform and methanol compounds is a conventional extraction solvent which is considered cheaper if used on an industrial scale. However, the use of chloroform tends to be dangerous with its chemical properties, so it is recommended to look for other alternative extraction solvents for lipid extraction in *Yarrowia lipolytica.*

**Table 1**. Extraction of Lipid, Wax esters and FAEE/FAME in *Yarrowia lipolytica*

| Yeasts | Modification | Lipid extraction | Wax Esters extraction | FAEE/FAME extraction | References |
|---|---|---|---|---|---|
| *Yarrowia lipolytica* | Metabolic engineering | chloroform/methanol (2:1 v/v) | - | chloroform/methanol (2:1 v/v)(FAEE) | (41) |
| *Yarrowia lipolytica* | Manipulation extensive metabolism and addition of exogenous ethanol | - | - | Dodecane (FAME) | (20) |
| *Yarrowia lipolytica* Po1g | FAME production of vegetable cooking oil as an engineered medium | Chloroform/methanol (2:1 v/v) | sulfuric acid and hexane (1:12.5 v/v) | - | (24) |
| *Yarrowia lipolytica* ATCC 201249 | FAR heterologous expression | Ethyl acetate | - | ethyl acetate (FAEE) | (42) |
| *Yarrowia lipolytica* | Increased FAME production by changing lipid biosynthetic precursors | - | - | Hexane (FAME) | (43), (21) |

**Table 1**. (Continued)

| Yeasts | Modification | Lipid extraction | Wax Esters extraction | FAEE/FAME extraction | References |
|---|---|---|---|---|---|
| *Yarrowia lipolytica* Po1f | Production of WE from various types of growth media (sugar, FFA, soybean oil and WCO) | Hexane | Ethanol, hexane, deionized water (5:1:16 v/v/v) | - | (6) |
| *Yarrowia lipolytica* ACA-DC50109 | Under double limitation of nitrogen and magnesium | Chloroform-Methanol-(2:1 v/v) | - | - | (44) |
| *Yarrowia lipolytica* Po1g | Chromosome-based co-overexpression of two heterologous genes | N-hexane | - | N-hexane (FAEE) | (45), (13) |
| *Yarrowia lipolytica* | Construction plasmids | Chloroform-Methanol-(2:1) | Hexane | - | (46) |
| *Yarrowia lipolytica* E26E1 | Efficient lipid extraction | Dimethylcyclohexylamine Ethylbutylamine Dipropylamine (2:1 v/v) | - | N-hexane (FAME) | (47) |
| *Yarrowia lipolytica* MUCL 28849 | Optimization of Solvent Extraction | Chloroform/methanol (1:2 v/v) | - | Hexane (FAME) | (35) |
| *Yarrowia lipolytica* W29 (ATCC20460) | Engineering biosynthesis in lipid | Chloroform-Methanol-(2:1) | - | Hexane (FAME) | (48) |
| *Yarrowia lipolytica IFP29* (ATCC 20460) | Intensification techniques for extraction of lipids | Chloroform-Methanol-(1:2) | - | Methanolic sulfuric acid (FAME) | (39) |
| *Yarrowia lipolytica* LANGIT-7 | Detergent assisted lipid extraction | Chloroform-Methanol-(2:1) | - | Hexane (FAME) | (49) |
| *Yarrowia lipolytica* | Variation of preatreatment | hexane: isopropanol 5:3 | - | Hexane (FAME) | (50) |
| *Yarrowia lipolytica* ACA-DC 5033 *dan* LFMB Y19) | Biotechnological valorization | Chloroform-Methanol-(2:1) | - | - | (38) |
| *Yarrowia lipolytica* (JMY 5289) | Lipid extraction high-pressure homogenization | Chloroform-Methanol-(2:1) | - | N-heptane (FAME) | (51) |
| *Yarrowia lipolytica* NCIM 3589 | Determined extraction conditions | Chloroform-Methanol-(1:1) | - | - | (52) |

## 6. WCO as growth media for *Y. lipolytica*

The wide variety of carbon sources that OY can utilize, one of which is Waste Cooking Oil (WCO) which can be a carbon source for OY and an energy source for the transesterification process to form FAEE from oleaginous yeasts, so that the benefits are; (i) establishment of FAEE which is safer and environmentally friendly from OY species, (ii) can utilize waste oil and reduce waste, (iii) overcome

unsustainability and competition from FAEE produced by vegetable oils and animal fats (53).

*Yarrowia lipolytica* is currently an OY that has the potential to produce high-production FAEE due to its ability to produce high-fat acyl-CoA due to its excellent lipid accumulation capability (money), *Yarrowia lipolytica* can also utilize many carbon sources, moreover utilizing waste oil such as waste cooking oil (WCO) or what is also called used cooking oil which is currently not widely used (54), so that WCO can be used as a cheap and easy-to-obtain raw material for the growth of *Yarrowia lipolytica* which can process WCO into a source of food for life Y.lipolytica and to become an energy source OY carries out a transesterification process (45).

Used cooking oil or what is called Waste Cooking Oil (WCO) is used oil that has been used for cooking and has been used many times, causing changes in its physical and chemical (55). WCO is disposed of and becomes as much as 15 tons of waste annually worldwide (56). WCO is routinely produced as a waste product from households, restaurants and industries, which until now has not done much processing of WCO waste which basically can cause many negative effects to the environment such as pollution, pollution (27).

Utilizing WCO as a raw material for biodiesel production, can reduce problems regarding environmental damage caused because WCO waste has not been properly utilized and can produce biodiesel production costs that are cheaper when compared to other raw materials, and do not compete with the food market (57). WCO can be converted into biodiesel with a percentage of 80%, to glycerol around 10% and the other proportion is left unused. Although, the amount of energy produced from WCO is still relatively low, with only 0.5% using WCO as biodiesel (58).

The use of WCO as a medium can be used as a substitute for glycerol. WCO is superior to be used as a substitute for glycerol compared to vegetable oil. This is because vegetable oil requires the addition of lipase enzymes to be able to decompose TAGs into FFA forms before they can be used, while WCO does not require the addition of lipase enzymes because WCO already contains FFA compounds (6).

In a previous study conducted by (6) stated that WE produced from various types of substrates in *Yarrowia lipolytica* would be able to produce WE types with different chain lengths. On the fat substrate, the resulting WE consisted of C32, C34 and C36 WE. While the WE produced with glucose and glycerol as substrates were WE C32 and C34. In addition, the WE produced was formed from fatty acids and alcohol chains C16:0, C18:0, C18:1 and C18:2 were found to be the most dominant in the formation of WE.

## 7. Wax esters potential in *Y. lipolytica*

One of the yeasts that has a lipid precursor isolated in its cells is *Yarrowia lipolytica* which can produce FAEE by utilizing the production of Wax Esters Synthases (WS) which are very important in the production of FAEEs in a strain of yeasts (21). OY is a potential microorganism for producing biodiesel because of its ability to accumulate single sex oils or SCOs and OY's ability to utilize a wide variety of carbon sources (59). Currently, research on WS continues to be carried out because WS production in microorganism cells can produce FAEE directly through the fermentation process of various host cells or host cells (20), (60).

However, genetic engineering still seems to be a way to increase FAEE production in several ways, for example by carrying out a genetic manipulation of gene expression for acetyl-CoA accumulation and also eliminating the competitive pathway, namely peroxisome β-oxidation) and TAG biosynthesis (61).

Wax esters are neutral lipid compounds formed from fatty acids that are esterified into long chain fatty alcohols, so the properties of WE will vary and depend on the number of carbon atoms that make up WE from fatty acids and alcohols. This can also affect the properties of WE in the form of unsaturation, melting temperature, oxidation and pressure stability which can make WE a source of different applications. WE can be produced from petroleum, chemical synthesis or natural sources such as plants, animals or microorganisms.

WE is one of the potential chemical compounds in the future due to its many functions applied in various fields, namely in WE from chemical synthesis production, it can function as a wax, lubricant, coating or adhesive for rubber and plastic (62). In WE which is produced from living things or bio, WE plays a role in pharmaceutical and cosmetic products such as skin care, hair and other products (63). So that the market size for WE can reach $9.9 billion in 2019 and is expected to increase over time (6). The potential for WE produced by living things, WE has its own role as a carbon and energy storage, acting as a protective layer against ultraviolet rays or pathogens (64,65).

WE in plants such as that produced by *Simmondsia chinensis,* only a few species produce WE and it is still a rare natural source. This also limits the use of WE in everyday products because production is difficult in plants and animals so that WE is expensive for applications such as cosmetics and basic ingredients for pharmaceutical products (66,67). WE production can also be carried out by means of chemical synthesis, through a biotechnological process using immobilized lipase (68). However, this has many negative effects such as the use of corrosive acids, the use of high energy and the degradation of ester compounds and inconsistent performance. Not only that, the chemical synthesis process of WE also requires fatty alcohols as substrates (6).

WE produced by microorganisms is the newest potential that can produce large quantities of WE through metabolism in microorganism cells. *E.coli* is a bacterial species that has been involved in the formation of WE and is studied intensively, due to its fast growth with high protein recombinant rates (69,70). However, WE is still produced in low quantity. So that several previous studies used yeast as a host in the genetic engineering process to produce WE. Yeast has advantages over bacteria in terms of WE production, namely having the ability to withstand phage

contamination, a high degree of tolerance for by-products, and has a unique metabolic pathway and high ability to accumulate lipids as in y.lipolytica (12).

## 8. Future prospects

Dependence on fossil fuels which are still based on fossils and petroleum is one of the big problems that will have an impact on life, not only that fuel is also the main energy which is very influential in any aspect whose sustainability is still the main topic in looking for other alternatives that can overcome these problems. Utilizing lipids produced by the yeast group, especially *Yarrowia lipolytica*, is still a topic that has not been studied more deeply, especially on the effect of Wax esters synthases (WS) as an enzyme that plays a role in the production of FAEE/FAME in this yeast. not only that, the use of this yeast can also utilize waste such as waste cooking oil (WCO) which is a good prospect in the future because the use of this yeast is not only for producing biodiesel, but can reduce WCO waste as a growth medium for this yeast which can later be used for production resulting lipids. The utilization of lipids produced by microorganisms is included in the third generation of biodiesel which is very new and still very worthy of further research related to its aim to overcome the problem of unsustainable and environmentally unfriendly diesel fuel, so that the potential utilization of this *Yarrowia lipolytica* ability to accumulate lipids, producing WS enzymes and wax esters as well as lipid extraction, WS or Wax esters and FAEE/FAME still have very good potential for the future.

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