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Review Article

A Review on Microbial Biooil Production from Biodiesel derived Crude Glycerol

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Abstract

Rapid growth in the biodiesel production naturally resulted in a redundant crude glycerol accumulation. Crude glycerol has gained considerable concern as a substrate for biooil production which replaces fossil fuel with plant oil like renewable resources. Refining processes are necessary to replace pure glycerol because of the contaminants present in crude glycerol. An integer of microorganisms applicable to the species of yeast, fungi, bacteria, and algae have capable to accumulate more than 20% of lipids of their biomass under specific conditions are called oleaginous microorganism. These organisms are breed faster than higher plants and do not necessitate land. In commercial applications such as food, fuel, pharmaceutical, and oleo chemistry, these oils can be used. This article reviewed a variety of oleaginous microorganisms for production of lipids using crude glycerol.

Keywords: Biodiesel; Crude glycerol; Oleaginous microorganisms; Biooil.

Introduction

Biooil produced from crude glycerol have the impending advantages to replace a portion of our non-renewable fossil fuel consumption with a renewable sources. These liquids have benefit in storage, combustion, retrofitting, transport, and flexibility in marketing and production [1]. Crude glycerol contains enormous amount of impurities however, it can act as a sole carbon and energy source. Single cell oils (SCO) are produced by microbial conversion of crude glycerol by oleaginous microorganisms such as yeast, fungi, algae, and bacteria [2]. Bio-oil is a heterogeneous mixture which consists of variety of different chemical compounds such as aldehydes, phenols, esters, alcohols, carboxylic acids, alkenes, syringols, furans, sugars, guaiacols, aromatic, and nitrogen. Because of that bio-fuels to replace thermal and power generation petroleum fuels. Bio-oils may be used for the production of chemical products such as biodegradable polymers, levoglucosan, hydroxyacetaldehyde, and surfactants [3].

In recent years, crude glycerol for energy production has been widely used which are by-product of biodiesel. One portion of crude glycerol is generated as byproduct for every ten portion of biodiesel production [4]. Crude glycerol is distinct from pure glycerol, which contains lots of impurities from the process of biodiesel production. Methanol, ash, soap water are the principal impurities in crude glycerol [5]. These crude glycerol can be converted into high value platform chemicals and its an excellent opportunity to produce the bio-oil production. Galvanization of crude glycerol without the need of additional treatment and purification steps is considered as a major dispute. The glycerol obtained from biodiesel production typically contains only 55-90% of purity and the remaining constitutes methanol, soap and other contamination. Consequently, direct use or fractional purification of crude glycerol is becoming favourable [6,7].

Crude glycerol are converted to microbial oil by three main process: flash pyrolysis, hydrothermal liquefaction (HTL) and microbial conversion. In the combustion process, continuous anaerobic dismantling of organic compounds involves flask pyrolysis for the processing of fluids, carbon dioxide and charcoal. HTL involved in biomass refining with or without a catalyst in water at high temperature and pressure. Unlike flash pyrolysis, HTL technological evolutions are at an early stage of development [8]. The bioconversion of crude glycerol to microbial oils by the oleaginous microorganism batch and fed-batch cultures.

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Biooil are considered as clean fuel as it creates less pollution and considered as $CO_{2/}$GHG neutral when compared with fossil fuel. There are no Sox emissions, as plant biomass contains small quantities of sulphur. Biooil fuels comprises 50% less NOx emanations in a gas turbine than diesel oil. The possibility for direct replacement of bio-oil for petroleum fuels is however limited due to their high ash and water content, high viscosity, volatility, low heating efficiency, and high corrosiveness. Therefore, it is important to upgrade the bio-oil to provide a liquid product which could be used as liquid fuel [3,8].

Oleaginous microorganisms contains triacylglycerols (TAGs) (lipids of energy reserve), glycolipids (lipids of membrane structure), phospholipids, and sterylesters. The eukaryotic microorganism can synthesize triacylglycerol (TAG), which are the glycerol trimesters with neutral fatty acids and synthesized main component inside the cell. TAG's are similar to common plant oils and vegetable oils [9]. Some lipids can be synthesized by prokaryotic bacteria, and these lipids are an efficient way to store energy. Being present with a high carbon source, oleaginous microorganisms are produced from single cell oils, and potential candidates represented a low nitrogen source. These microorganisms grow more quickly than higher plants and do not need land [4]. Oleaginous yeasts are good sources for surfactants, triglycerides, and poly unsaturated fats among a variety of microorganisms and state of cultivation. Yeast species applicable for lipid production are *Yarrowia lipolytica, Candida sp, Rhodotorulla, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces*, whose intracellular lipid accumulation levels can reach 80% of CDW. Oleaginous strains can accumulate different type of lipids according to their lipid composition[10].

Characteristics of crude glycerol

The glycerol content was identified by gas chromatography, Iodometric-periodic acid method and high pressure liquid chromatography. According to Jiaxin Chen et al, Bondoli and Bella method was reliable for determining the glycerol concentration [11,12]. The ash content was analyzed by burning glycerol in a muffle furnace was described by Mali Hunsom et al, and Jiaxin Chen et al [13,14].

The crude glycerol water content was measured by using the Karl Fisher titrator [15]. The methanol content was determined with digital evaporator was described by Jiaxin chen et al and Xiaolan Luo et al [13,15]. According to Lorenzo davino et al, methanol content was analyzed by gas chromatography [16]. Titration was used to determine the soap content described by Oscar Valerio et al and Hu et al [5,17].

Properties of crude glycerol

The organic compound glycerol with molecular formula $C_3H_8O_3$ and also popularly called as 1,2,3-propanotriol, trihydroxy propane, glyceritol and glycyl alcohol. According to IUPAC, the glycerol is the simplest form of the alcohol is known as propane-1,2,3 triol. [5,21]. Glycerol is also non-toxic to human health and also to environment[17]. Glycerol molecule has three classes of hydrophilic hydroxyl groups responsible for its viscosity and hygroscopic nature[18].

Physio chemical properties of crude glycerol

Glycerol is physically a clear, water-soluble, odorless, colorless, viscous, solubilized liquid with a high melting point and a sweet taste liquid[18]. Glycerol possess hygroscopic property and stable in normal room temperature. Glycerol is an alcohol with three hydrates which has the property of reactive molecule [5,18]. The melting point, boiling point, freezing point and flash point of glycerol is 18^0C, 290^0C, 2^0C and 177^0C respectively. Glycerol has a molecular weight of 92.02g/mol, a relative density of $1260kg/m^3$ and a viscosity of 1.41Pa s. Glycerol also have the property of heat of vaporization, heat of formation, surface tension and self ignition is 82.12 kJ/kmol, 667.8 kJ/mol, 63.4mN/m and 393°C [19,20].

Market scenario

The rising quantity of crude glycerol production during the period of 2004-2006 due to high biodiesel production[18]. Biodiesel production in the European countries has grown linearly. The European countries were the leading biodiesel manufacturer in the world with 82% of biodiesel in 2003. The growing quantity of glycerol is due to high biodiesel production from 75 liters to 250 liters during 2005-2006. The National Biodiesel

Board announced the total production of 450 million liters of biodiesel in 2007, a trend increase from under 100 million liters in 2005. Asia compensated for over 44% in 2007 and Western European countries made up for around 35% of world production[5]. Euroasia and the United states responsible for 91% of world production. Global glycerol consumption was almost 750 thousand tons in 2008. It produced 194,000 tons of glycerol in the United States of America in 2009. In 2010, biodiesel output will add 2.2 million loads of glycerol to current US and European production. According to research. Glycerol market is predicted to be stable due to its volatility in the coming years. In addition, utilization of glycerol will improve the biofuel production in future [22,23].

Various microorganisms used for bio-oil production

Yeast

Oleaginous yeasts can accumulate 20% of the dry weight lipids, grow rapidly and have a high content of oil. Most oleaginous yeast having lipids about 40% of the dry biomass and under nutrient-limiting conditions may exceed 70% of their dry weight. Yeast could grow over a range of carbon source from glucose to industrial and municipal organic waste [2]. Production of yeast oils has many advantageous over plant oils. These yeasts have a short life cycle, need less labor to grow, and are easier to scale; less seasonally and climately affected. In addition, yeast can grow well on a crude glycerol [24]. Eastering et al reported that yeast could and accumulate mor lipids in shorter time and with minimal nutrient content [25]. The best known oleaginous yeasts are found in genera *Rhodosporidium, Candida, Lipomyces, Trichosporon, Cryptococcus, Rhodotorula, Rhizpus,* and *Yarrowia* [26].

Rhodosporidium toruloides

Rhodosporidium toruloides can accumulate up to 60% of its cell biomass intracellular lipid, which is mainly triacylglycerol and fatty acid composition [27]. Jingyand Xu et al revealed that *R. toruloides* had great potential for converting crude glycerol to valued lipids, although certain impurities such as soap, glycerides, methyl esters of fatty acids, inorganic salt and methanol were found in crude glycerol. Some of these impurities were found to be even more likely to increase the concentration of microbial population and lipid accumulation and the impurities did not affect the lipid composition of fatty acids. They demonstrated that crude glycerol could be directly used as a source of carbon for production of microbial oil [28].

Rhodotorula glutinis

Rhodotorula glutinis is oleaginous yeast capable of accumulating around 72% of lipid content in their dry weight and capable of activating the synthesis of triacylglycerol. In *R. glutinis*, fatty acids are activated in an ATP dependent manner prior to being used [25,29]. Hong-Wei Yen et al revealed that the crude glycerol could serve as appropriate carbon source for the growth of yeast *R. glutinis*. The scrap called thin stillage was treated with crude glycerol and was used as a cell growth medium. They reported that this combination of two components could led to a higher biomass production and higher lipid production compare to the use of crude glycerol and nutrients such as yeast extract. The consumption of cheap crude glycerol and thin stillage to cultivate oleaginous microorganisms will greatly improve the production of microbial oil [30].

Cryptococcus curvatus

The most efficient oleaginous yeast *Cryptococcus curvatus* can accumulate lipid storage at a dry weight of up to >60%, while these lipids typically consist of SOC-90% w/w triacylglycerol with a proportion of approximately 44% saturated fatty acids (%SFA) that is related to many plant seed oils when growing under N-limiting conditions [31].

Lipomyces starkeyi

Lipomyces starkeyi is an oleaginous yeast that can efficiently accumulate microbial oil using low cost substrate crude glycerol as its sole source of carbon. The lipid yield of the yeast on crude glycerol was 7.5 g/L[32]. Li-ping Liu et al demomstrated that culture conditions of *T. fermentas* and *T.cutaneum* using crude glycerol for microbial lipids were 5.2 and 5.6 g/L respectively[33].

Yarrowia lipolytica

Yarrowia lipolytica will store up to 36% of dry weight from glucose and more than 50% while hydrophobic substrates are present [4]. Elisane

Odriosolla Santos et al revealed that *Yarrowia lipolytica* was selected for cultivation using a raw glycerol based medium, mainly due to the high biomass concentration. For this yeast, it was possible to use raw glycerol without prior purification, representing an economical advantage such as no impact on generating biomass and protein content [7,41]. Adam Dobrowolski et al showed that crude soap-based glycerol was effectively converted to single- cell oil without any prior batch cultivation purification in a bioreactor resulting in increased lipid production [34].

Fungi

Fungi are capable of producing lipids, such as gamma-linolenic acid (GLA), arachidonic acid (ARA) and docosahexaeneoic acid (DHA). Several oleaginous fungi species are *Aspergillus terreus, Clavicep purpurea, Tolyposporium, Mortierella alpine, Mortierella isabellina* can also accumulate lipids [2]. Fungi could grow in the fermentor without the light energy. Due to these benefit, potentially fungal derived lipids used for lipid-based feedstocks [41]. Few filamentous fungi can accumulate lipids by consuming the waste glycerol [42,43].

Mortierella isabellina can accumulate high oil content of 60-80% of its dry biomass and can utilize variety sources of carbon like single and mixed sources of carbon. *M. isabellina* strain for bio-oil production on co-utilisation of biomass sugars and glycerol was assessed by Gui quin Cai et al[44]. In nitrogen-limited aerobic cultures, *Mortierella isabellina* shows a adequate growth with crude glycerol used as the sole source of carbon. Papanikolaoua et al found that high levels of lipid glycerol were accumulated in dry weight, which corresponds to about 51% of lipid [45].

Fakas et al studied two fungi such as *Mortierella isabellina* and *Cunninghamella echinulata* were cultured on raw glycerol under minimal nitrogen conditions. The yield reveals that *M.isabellina*transformed raw glycerol into oil more efficiently when compared with*C.echinulata* [38]. *Humicolalanuginose* can produce high concentration of lipids as much as 75% of its dry biomass. *Aspergillus oryzae* can also store lipids up to 57% of their dry weight[46]. Somashekar et al reported that *Mucor rouxii* contain 30% of lipids [47]. Lipid production on crude glycerol using various oleaginous microorganisms is shown in table 1.

Bacteria

All type of bacteria can store lipid lower than microalgae, an average volume of oil is approximately 20-40% of dry biomass. The high growth can be aheived in bacteria within 12-14h by normal fermentation process. The high concentration accumulation of triacylglycerols (TAG) is achieved by many bacterial species like *Rhodococcus, Mycobacterium, Streptomyces,* and *Nocardia*etc. Alternately, storage lipid accumulating bacteria are a group of actinomycete that can synthesize high amounts of fatty acids from simple carbon sources of about 70% of its cellular dry weight under growing conditions and accumulate intracellularly as TAGs[49]. The poly hydroxyl alkanoates can be accumulated only by few species of bacteria not by all [46]. These lipids are produced in the external membrane and difficult to extract.

Rhodococcus opacus can store up to 87% and *Strpetomyces* can store up to 60% of its dry weight [52]. The TAG bodies of these bacteria are mainly comprised of TAGs (87%), diacylglycerols (5%), free fatty acids (5%), phospholipids (1.2%) and proteins (0.8%). There is a possibility of metabolically engineered *E. coli* to increase their fatty acid production of 0.7g/l to 3.8g/l by using renewable carbon source under fed-batch [2,50]. *Acinetobacter calcoaceticus* and *Arthrobacter sp*. reached the highest growth rate and lipid content is approximately 40% and 38% of its dry biomass with simple culture method[46].

Application of bio-oil

Microbial oils are expansively used for the biodiesel production that has been extremely attracted as a renewable, biodegradable, and non-toxic fuel. In near future, single cell oils could acts as second feedstocks for large scale biodiesel production because of the short life span of microbes and labor requirement is less [51]. Unpurified crude glycerol may be used in *Yarrowia lipolytica* to effectively accumulate lipids and does not require any additional nutrients [34].

Microbial oils are not only used for biomass feeding and replacing plant oils and lipids like palm oil, cocoa butter, and different fatty acids. Single cell proteins, oils, polysaccharides or other metabolites could be used for food processing operations such as thickening agents, stabilizing agents, emulsifiers and demulsifiers. It may decrease the essential for chemical preservatives, detoxification of assured food products as well as abolition of specific unwanted ingredients [52].

Table 1. Lipid content of selected oleaginous microorganisms

Microorganisms	Lipid content (%, w/w)	Reference
YEAST		
Trichosporonoides spathulata	41.9	[24,40]
Kodamaea ohmeri	53	[24,40]
Trichosporon cutaneum	32.2	[33]
Trichosporon fermentans	32.4	[33]
Rhodotorula glutinis	72	[31,35]
Candida curvata	58	[35,36,37]
Cryptococcus albidus	65	[35,36,37]
Lipomyces starkeyi	68	[31,35]
Cryptococcus curvatus	58	[31]
Rhodosporidium toruloides	58	[31]
Yarrowia lipolytica	31	[39]
FUNGI		
Mortierella isabellina	53	[48,38]
Mucor mucedo	62	[48,37]
Aspergillus oryzae	57	[46]
Cunninghamella echinulata	35	[31]
Humocola lanuginose	75	[46]

Microbial oils can also offer a pharmaceutical objective. Biooil may be used for dental cements, demulcents, laxatives, liniments, emollients, and other product formulations. Oils can also add to the ingredients parental and enteral nutrition treatment as balanced dietary supplements. PUFAs and additives are blended in pharmaceutical products have additional benefit to the prevention of circulatory diseases, cancer and inflammatory diseases. TAGs mightserve as substrates for the production of PHA by lipid utilizing bacteria. The main purpose of PUFAs produced from yeast was skin care and promising hair growth [53]. Biooil is also used in industries like construction, food flavorings, resins, adhesives, and agrichemicals. It contains phenolic compound which is used to replace adhesive, also known as glue. Resin can be produced from synthetic or plant is used to make polymer.

Conclusions

It is critical to handpick the superlative microorganism to convert crude glycerol into bio-oil which could act as feasible substitute to fossil fuels. Oleaginous microorganisms such as yeast, fungi, algae, and bacteria are the finest to accomplish this goal. This could be inferred from the literature that yeast has achieved a massive amount of lipid relative to other microorganisms as it produces cast amount in consistency. Microbial oil production via oleaginous yeast using crude glycerol without any refining process yields higher amount of bio-oil because of their high biomass concentration. In addition, yeast can replace crude glycerol by raw glycerol, since there is effect on the production of biomass and protein content. This study argues that the primary target of renewable fuels is accomplished with high yield of bio-oil derived from oleaginous yeast.

Conflict of interest

Authors declared no conflict of interests.

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