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Review

Lipomyces starkeyi: Its current status as a potential oil producer

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ABSTRACT

Culturing of oleaginous yeasts has been studied extensively utilizing various substrates as a nutrient, such as industrial or agricultural residues. Despite many choices of oleaginous yeasts, attention should be given to specific species so that real application can be implemented, rather than on exploring new oleaginous yeasts with higher oil-producing ability. *Lipomyces starkeyi* is an oleaginous yeast that can be cultured using a wide range of feedstocks. It is worth noting that *L. starkeyi* can produce a high amount of lipids with a good proportion for biodiesel purpose and its ability to re-utilize small amount of its lipid, makes it superior compared to other oleaginous yeasts. This review offers a comprehensive summary of *L. starkeyi*, its characteristics and the type of nutrients it can assimilate, brief reviews of common fermentation modes used, and strategies for enhancing lipid accumulation will be discussed. Also, common transesterification methods, as well as possibility/future prospect of oleaginous yeast utilization to produce single cell oil will also be discussed. This review hopefully could help bridging the gap between theoretical and actual potentials of oleaginous yeasts in producing lipids as feedstock for biodiesel production.

1. Introduction

In general, crude oil price shows an increasing trend within the past 50 years. It hit the lowest point at the end of 1998, which was less than $20 per barrel, then gradually went up to $157 in June 2008 [1]. Starting in mid-2014, crude oil price started to decline to ~$62 per barrel currently [1,2]. This fluctuation was likely due to most oil-producing countries, especially Saudi Arabia, refused to cut down production due to the fear of losing market share, which resulted in a surplus of crude oil and lower oil price. On the other hand, falling crude oil price is detrimental to the development of renewable energy. Increasing consumption of crude oil inevitably leads to CO_2 accumulation and global warming. Also, world oil reserves are limited and will be depleted soon if alternative energy supplies cannot be found. In fact, as reported by EIA, global oil demand keeps increasing from 89.8 million barrels per day in 2011 to 98.5 million barrels per day by the end of 2017 [3]. World oil consumption is projected to increase to 112 million barrels per day by 2035 [4].

One way to help to reduce CO_2 emission is by using renewable fuel (biofuels). The two most prevalent biofuels nowadays are bioethanol and biodiesel. Bioethanol (C_2) mixed with gasoline (C_4-C_9) [5] can oxygenate the fuel, leading to better combustion hence reducing air pollution [6]. In the USA, 10% ethanol is used in the ethanol-gasoline blend for old vehicles [5] and 15% for new vehicles [6]. Bioethanol cannot be used at 100% to replace gasoline due to its low energy density and some other factors [5], while biodiesel consisting of C_{16}-C_{22} alkyl chains [5], can replace diesel fuel without the need to modify diesel engine.

Implementation of biofuels such as biodiesels is considered to be one of the best choices to reduce CO_2 emission. Since biodiesel is derived from plants, the CO_2 produced is not more than what the plant absorbed during growth, thus making it zero CO_2 emission [7]. Most biodiesel currently in use is derived from the so-called the first generation feedstock (edible vegetable oils or oil from other food crops). This leads to the 'food vs. fuel' debate about the use of edible oil for biodiesel production which results in food and land competition;

Abbreviations: 5-HMF, 5-hydroxymethylfurfural; ACL, ATP citrate lyase; AMP, Adenosine mono phosphate; ATP, Adenosine Tri Phosphate; ARA, arachidonic acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; GLA, Gamma linoleic acid; C/N, Carbon to Nitrogen; CoA, Coenzyme A; DAG, diacylglyceride; FAs, Fatty acids; FAME, Fatty Acid Methyl Esters; GDH, glycerol-3-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase; LCPUFA, long chain polyunsaturated fatty acids; LPA, lyosophosphatidic acid; NAD^+, Nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; PDC, pyruvate dehydrogenase complex; PGDH, 6-phosphogluconatedehydrogenase; PHB, para-hydroxy benzaldehyde; PEG, polyethylene glycol; SCO, Single cell oils; TAG, Triacylglycerol; TCA, Tricarboxylic acid

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shortage in edible oils and the higher price of foods [8]. The search for feedstock to replace edible vegetable oils led to the development of the second generation feedstock such as waste cooking oil, animal fats, rice bran oil and *Jatropha curcas* oil [9,10].

The third generation feedstock currently under development [10] aims to produce biodiesel from oleaginous microorganisms such as yeasts, microalgae, and bacteria. The term 'oleaginous' means that the microorganism can accumulate oil > 20% of its dry weight, and can reach up to 60–70% in some cases [11]. Microbial oil is recognized as one of the potential biodiesel feedstock. Yeast is the preferred microorganism for producing microbial oil to microalgae or bacteria. In spite of advantages such as it requires no land to grow, (mixotrophic) microalgae has a limitation on carbon source utilization in the sense that it cannot convert starch to oil [12], and it has lower growth rate than yeast. Only some bacteria can be used for microbial oil production since most of the bacteria only produce lipoid complex like polyhydroxyalkanoate [13] in the outer membrane thus is difficult to be extracted [14]. Certain fungus can grow in starch [15], but it produces only a small quantity of biomass [12]. On the other hand, yeast such as *Rhodoturula glutinis*, *Rhodosporidium toruloides*, *Cryptococcus curvatus*, *Trichosporon fermentans* and *Lipomyces starkeyi* are able to grow in a broad range of substrates including hydrolysate of agricultural or industrial residues, and produces a high amount of lipids. The growth of yeast is relatively fast with no seasonal limitation, and the process is easily scaled up [11]. The growth of yeast is less susceptibility to viral infection, and bacterial contamination can be controlled easily by growing at low pH [10].

Single cell oil (SCO) has been known since the 1980s for use as a substitute for cocoa butter [16]. Also, researchers also focused on producing other beneficial FAs for human health such as γ-linoleic acid (GLA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) [17,18]. But utilization of SCO as a potential feedstock for biodiesel production just gained interests in recent years. However, high producing cost hinders its commercial application [11]. But hurdles are expected to be tackled with improving and maturing technologies [19], and in the future, it is possible that petroleum diesel price will be higher than what is now due to scarcity; allowing better chances of massive biodiesel utilization. This situation gives opportunities for researchers to explore SCO for cost-effective biodiesel production. This review focuses on oleaginous yeast *L. starkeyi*, about its classification, various carbon sources it could consume, and to a lesser extent about its other marginal products [20]. Various substrates-hydrolysis processes, lipid regulation, lipid extraction and conversion will be briefly explained. Many reviews have been published about lipid regulation mechanism and utilization of lignocellulosic or agro-industrial by-products for fermentation feedstock, however, specific yeast and overall practices needed for fermentation were seldom discussed. Thus, this review provides summaries and knowledge on producing SCO as biodiesel feedstock from *L. starkeyi* fermentation with the focus mainly on low-cost feedstock utilization, particularly agricultural biomass residues.

2. Taxonomy of *L. starkeyi*

Genus *Lipomyces* is in *Lipomycetaceae* family, order *Sacharomycetales*, *Saccharomycetes* class (subclass *Saccharomycetidae*, subdivision *Saccharomycotina* [21]), phylum *Ascomycota* in kingdom Fungi [22]. To date, there are 16 species accepted as genus *Lipomyces* [23]. Among these species, *L. starkeyi* (together with *L. lipofer* to a lesser extent) is the most extensively studied yeast, chosen for its excellent ability to produce lipid. *L. starkeyi* (scientific name: *L. starkeyi* Lodder & Kregger van Rij) is a unicellular eukaryotic yeast-which has a nucleus and other organelles. This strain was originated from the soil in the USA and was isolated by R.L Starkey (Starkey's strain number 74) [24]. It reproduces sexually by developing 4–20 ellipsoidal [23] or round [21] ascospores (contains an oil droplet) per ascus. The light amber to brown ascopores then germinate and divide by budding [23]. Macromorphology of the colony shows that it is smooth and white-creamy in appearance with a mucoid texture [21]. This species reproduces asexually by multilateral budding resulting in round or oval-shaped cells [21]. The optimum temperature for *L. starkeyi* to accumulate lipid was found to be 25.5–29.5 °C [25].

The original strain of this yeast is CBS 1807; while mutation or modification results in CBS 1809, CBS 2512, CBS 6047, CBS 7536, CBS 7537, CBS 7544, CBS 7545, CBS 8064 [24] and other 10 strains [21]. *L. starkeyi* CBS 1807 has different collection number in each research center (The Netherlands-CBS 1807, USA-ATCC 58680, NRRL Y-11557, NRRL Y-1388, Germany-DSM 70295, Japan-IFO 1289 and JCM 5995, Taiwan-CCRC 21522 and BCRC 23408, Belgium-MUCL 39418, Italy-DBPVG 6193).

3. Physiological properties of *L. starkeyi*

Yeast is composed of organic compounds, inorganic compounds and water [26]. After removing water which is the major part of yeasts, what left are organic and inorganic compounds. Organic compounds of yeasts are polysaccharides, lipids, and proteins, while inorganic compounds include cofactor, and trace metals [26]. The organelles in yeast cells are explained briefly [26]:

- Cell wall, of which 80–90% is rigid polysaccharides (usually composed of glucan, mannan, and chitin)
- Cell (selective) membrane, which is composed of the lipid bilayer (phospholipid and sterols) with membrane proteins (ATPase, transport proteins, etc.), cholesterol and glycolipid attached to it
- Cytosol, liquid where other organelles are suspended inside the cell
- Nucleus contains genetic material of the yeast
- Ribosome, place where proteins are biosynthesized
- Mitochondria converts food to form energy
- Endoplasmic reticulum synthesizes (together with ribosome) and packages protein; transports molecules around the cells
- Golgi apparatus, a place for protein modification and distribution
- Vacuole, as a food storage

Some important factors affecting lipid accumulation in yeasts include temperature, pH, dissolved oxygen and C/N ratio of the medium. An early report on *L. starkeyi* IAM 4753 grown aerobically in simple defined medium at 30 °C showed that lipid accumulation was low during cell growth (logarithmic phase) and increased significantly when the yeast was in early stationary phase, lipid accumulation was probably caused by a change in metabolism due to limited amount of dissolved oxygen left [27]. Suutari et al. [28] observed a similar phenomenon in which yeast started to accumulate lipid during the growth phase, and 28 °C was the optimum temperature for both biomass and lipid productivity.

Investigation on *L. starkeyi* IAM 4753 showed that it could grow well in glucose mineral medium with pH 5 [29]. pH above 5 may inhibit the enzyme activity to produce biotin that promotes cell growth. Thus additional biotin in the medium was needed for pH 5.5–6.5 [29]. It was found that cations and anions were important substance for cells; Mg^{2+}, Mn^{2+}, and Zn^{2+} were important for fermentation, cell growth, and metabolism; Cu^{2+} and Fe^{2+} were needed as a cofactor; while phosphate and sulfate were essential for structural molecules and cell physiology, respectively [26]. Sufficient amount of Mn^{2+} was needed to increase biomass growth by 1.6 fold, while the low amount of Zn^{2+} was needed to obtain higher lipid content [25]. A subsequent study reported that the simultaneous addition of Mn^{2+}, Zn^{2+} and mono-potassium phosphate at stationary phase was able to induce biomass growth but not lipid production [30].