Oleaginous Yeasts

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The production of lipids from oleaginous yeasts involves several stages starting from cultivation and lipid accumulation, biomass harvesting and finally lipids extraction. However, the complex and relatively resistant cell wall of yeasts limits the full recovery of intracellular lipids and usually solvent extraction is not sufficient to effectively extract the lipid bodies. A pretreatment or cell disruption method is hence a prerequisite prior to solvent extraction. In general, there are no recovery methods that are equally efficient for different species of oleaginous yeasts.

Keywords: oleaginous yeasts ; single cell oil ; lipid extraction

1. Introduction

Numerous microorganisms that belong to the genera fungi, bacteria, yeast and algae have been reported to have capability to produce oils under certain cultivation conditions [1][2]. Oleaginous microorganisms are good alternative sources for industrial used oil. Depending on the fatty acid composition, the oil produced can be exploited for human consumption and in certain valuable industrial applications such as paints and coatings, detergents, cleaning products and cosmetics [3]. Moreover, the oils from oleaginous microorganisms have been increasingly explored as a substitute for high added value lipids and biodiesel feedstock due to their high lipid content, short production cycle and similar fatty acid composition to triacylglyceride from vegetable oils[4][5]. Among oleaginous microorganisms, yeast has advantages over bacteria, molds and algae. This is due to its unicellular relatively high growth rate and high lipids production ability in discrete lipid bodies [6]. Furthermore, yeast has the ability to produce “specialty-type” lipids such as cocoa butter (saturated lipids) as well as saturated exotic fats such as shea butter and sal fat that may replace the high value and expensive lipids rarely found in the plant and animal kingdom [7][8]. To date, various strategies have been developed to increase the content of intracellular saturated lipids in oleaginous yeasts [9]. Nonetheless, an efficient extraction of the intracellular lipids is one of the bottlenecks in ensuring that the use of microbial oil is commercially feasible [10].

Yeast cells are composed with thick and rigid cell walls that make the extraction of its intracellular lipid products challenging. There are no universal downstream strategies that are applicable for all yeast species and the best methods must be established and optimized for each species [11]. This is due to the fact that different yeast species may present different physical properties and cell wall structures as well as different lipid compositions [12][13][14]. A pretreatment method involving cell wall disruption after yeast cultivation is often required to make the intracellular lipids available to be extracted in the subsequent steps. For example, for the biodiesel processing that involved extraction of lipid from yeast starting from growth, harvesting, lipid extraction and lipid transesterification, the common essential step is the disruption of the cell [11][15]. Most of the work reported for lipid extraction from oleaginous yeasts describe on the dry biomass (dry route) as it allows higher recovery of lipid in comparison to the wet biomass (wet route). The extraction of lipids from the dry route has been extensively reported for analysis and small-scale research [7]. Despite the high recovery of lipids, one of the major obstacles of dry route are the high costs arising from energy and labor expenditure for the drying process prior to extraction [16]. Besides, the drying treatment may cause degradation and lipid peroxidation due to long-term exposure to high temperatures. The presence of water in wet biomass hinders the efficiency of a solvent-based lipid extraction process that may be due to limited lipid accessibility, reduced mass transfer [17] and the formation of emulsions [18]. Hence, intensive research and development is important to fully understand the mechanisms involved in the lipid extraction via wet route to allow practicality of the method at industrial scale in particular.

In general, cell disruption methods are classified according to the mechanisms they act on the cells, either by mechanical disintegration of the cells or by nonmechanical treatments via cell wall digestion or desiccation/drying at an optimized temperature [19]. The mechanical methods are further categorized as liquid (i.e., ultrasonication, microwaving, high-pressure homogenization) and solid shear methods (i.e., bead mill, grinding and freeze press) [20][21]. Meanwhile nonmechanical methods are further categorized as physical (i.e., osmotic shock, desiccation), chemical (i.e., acid, base, solvent, detergent) and biological (such as enzyme, autolysis) treatments. Practically, cell disruption is very important not only for enhancing the capability of lipid extraction, but also to evaluate the lipid content for oleaginous yeast. In general,

the research trend on cell lysis of oleaginous yeast has mostly targeted for low cost with high lipid recovery to make it commercially feasible. Following cell disruption, lipids are typically extracted via liquid–liquid extraction by partitioning into organic solvents, mostly by using a combination of polar and nonpolar solvents for extraction of a greater amount of lipids. The extraction with the combination of chloroform and methanol as developed by Folch and by Bligh and Dyer, these methods are the most cited methods for lipid extractions [22][23]. To further improve the extraction of lipids, several studies have also reported on the adaptation and modifications of both methods, whereas several authors have started to investigate the usage of “green” solvents that are considered environmentally friendly in comparison to the conventional solvents such as chloroform, methanol and hexane [24].

2. Oleaginous Yeast as Single Cell Oil

Single cell oils (SCO) are defined as edible oils obtainable from single-celled microorganisms that are primarily yeast, fungi and algae [25]. The term SCO is used in parallel to single cell protein (SCP) to represent oils of microbial origin. A small percentage of microorganisms have the ability to synthesize and accumulate 20–87% of their total biomass as intracellular lipids, which are defined as oleaginous microorganisms [26][27][28]. Furthermore, microorganisms are considered to be oleaginous if they are able to provide supply of acetyl-CoA into the cytosol, which acts as an important compound preceding for fatty acid synthetase (FAS), and as a source of NADPH, a reducing agent for fatty acid biosynthesis, respectively [29]. Throughout the fermentation process, nitrogen deprivation will trigger the lipid synthesis in oleaginous microorganisms by converting substrates such as carbon dioxide, sugars and organic acids to SCO [30]. Oleaginous microorganisms produce a wide range of lipid classes including acylglycerides, free fatty acids (FFA), phospholipids, glycolipids, lipoprotein, sterols and hydrocarbons.

De novo synthesis and ex novo syntheses are the two available routes for lipid accumulation in yeast cells [31]. Basically, for de novo lipid biosynthesis, fatty acid precursors, such as acetyl-CoA and malonyl-CoA, are generated and incorporated in the lipid storage biosynthesis. It occurs only in oleaginous microorganisms and is stimulated by nitrogen deprivation [32][33]. Hydrophilic substrates are the preferable carbon source for lipid accumulation via the de novo pathway. Carbon sources such as glucose, fructose, lactose, sucrose, whey, glucose-enriched wastes and molasses (that are categorized as sugar-based media) can be used as the substrates for de novo lipid biosynthesis [3]. During cell growth, nitrogen is consumed for cell propagation and synthesis of proteins and nucleic acid. The decrease of nitrogen content in the medium will inhibit the metabolic pathways, causing a decrease in growth rate while initiates the synthesis of fatty acids and triglycerides. Extra carbon is then directed for the synthesis of lipids. It is then accompanied by the accumulation of triglycerides in lipid bodies and within this storage period, the requirements for lipid synthesis include precursors such as acetyl-CoA, malonyl-CoA and glycerol, and energy such as ATP and NADPH. For both oleaginous and nonoleaginous microorganisms, growth rate is reduced in accordance to nitrogen content, however, for nonoleaginous microorganisms, the conversion of carbon into polysaccharides (i.e., glycogen, glucans and mananes) occurs [32]. In the meantime, several oleaginous yeasts such as *Yarrowia lipolytica*, *Geotrichum*, *Rhodosporidium*, *Cryptococcus* and *Trichosporon* are reported to survive in the hydrophobic environment and show lipid biosynthesis via ex novo synthesis [34]. In ex novo lipid biosynthesis, it is started by the introduction of fatty acid from the digestion of hydrophobic substrates into the internal environment of the cell via active transport [31]. Another way for obtaining fatty acid is by breaking down triacyglycerols or fatty esters, which is catalyzed by lipase enzyme. Then, a modification of fatty acid occurs purposely for the cell growth. The fatty acid is broken down into chain of acyl-CoAs and acetyl-CoA by enzyme acyl-CoA oxidases. Throughout this reaction, energy requirements for cell maintenance and growth are fulfilled, accompanied by organic substances that are produced as precursors for cellular materials synthesis [3]. In general, ex novo lipid biosynthesis is different from de novo lipid biosynthesis in term of nitrogen dependency. For ex novo synthesis, lipid accumulation is initiated independently from nitrogen availability in the hydrophobic medium (i.e., waste cooking oils, effluent from dairy and butter industries and industrial waste stream) and it is generated simultaneously with the cell growth [35][36].

The SCO from oleaginous microorganisms have been increasingly explored as substitutes for high added value lipids and biodiesel feedstocks [37][38][39]. SCO production by oleaginous yeasts has many advantages over bacteria, molds and algae due to their unicellular relatively fast and high growth rate, high oil content in discrete lipid bodies and the resemblance of their triacylglycerol fraction to plant oil [40]. In comparison to vegetable oils, cultivation of oleaginous yeast is not affected by climate change, seasonal production or environmental conditions, in addition to their easiness for further scaling up [14]. *Yarrowia lipolytica*, *Lipomyces lipofera, Lipomyces starkeyi, Rhodosporidium toruloides, Rhodotorula glutinis* and *Trichosporon oleaginosus* are among the oleaginous yeast strains that have been extensively used to accumulate oils in their cells from glucose, xylose, arabinose, mannose and glycerol [41][42][43][44]. At present, the high cost of oil production by microbial oil extraction is the major barrier to its commercialization [45]. Thus, a suitable strategy must be implemented to cater the problems from the oil production processes whilst minimizing the cost [46][47]. Among others, total production cost can be reduced by utilizing renewable feedstocks such as low-cost lignocellulosic materials and other

industrial residues (containing high percentages of assimilable sugars) as fermentation substrates [48][49]. However, in an absence of cellulolytic activity in yeasts, free sugars are required to be released out from lignocellulosic materials through a pretreatment method (i.e., acid, base [50]or enzymatic hydrolysis [51]) before they can be converted into lipids by oleaginous yeasts. Furthermore, in ensuring economic viability and to minimize environmental pollution, a biorefinery approach in which intracelullar and extracellular coproducts (i.e., proteins, amino acids, carbohydrates, carotenoids, alcohols, fragrance chemicals and energy products) are valorized alongside the SCOs by oleaginous yeasts has also been suggested [48][52].

3. Yeast Cell Wall and Lipid Composition

Most of the lipids in oleaginous yeasts are intracellular that is stored as lipid bodies [53]. Besides lipid bodies, it is also consists of lipophilic compounds, specifically aromatic compounds that are difficult to be removed during lipid purification. The evaluation of the amount of lipid productivity can be determined by using an extraction method with organic solvents such as petroleum ether, methanol or chloroform, following the disruption of the biomass by either cellular breakage, chemical or enzymatic hydrolysis. However, many studies revealed that there is no effective method for extraction that can yield 100% of lipid from biomass [14]. Furthermore, the method would be different between yeast, algae, fungi or bacteria due to differ forms of physical properties, including the differences in cell wall and lipid compositions by different oleaginous microorganisms [21].

The composition of the yeast cell wall and its functions vary according to the species [12]. The overall structure of yeast is thicker than in Gram positive bacteria. Approximately one fourth of the dry cell weight is attributed to the thickness of the yeast cell wall, which is within the range of 100 to 200 nm [54]. Generally, yeast cell wall is mainly made up of a polysaccharide that provides strength, known as glucans, with β-(1,3) and β-(1,6) linkages. These linkages provide the yeast with a solid structure [55]. Beside glucans, it is also composed of mannans, which is a polysaccharide made up of mannose monomer linked by α-(1,6) linkage with some short oligosaccharide side chain [56]. Other constituents are also present in minimal amounts, such as chitin, proteins, lipids and inorganic phosphates [54]. Numerous bonds presented in the cell wall, especially disulphide bond, together with other components contribute to the thickness and uniqueness of the cell wall structure of yeast [12][56]. A significant change in the thickness of the cell walls can also be observed after oleaginous yeasts start to synthesize lipid droplets in their compartments [57]. The complete disruption of the wall and the release of all intracellular components requires the destruction of glucan, the main strength-providing component of the cell wall in yeast.

In general, yeast can be further divided into two categories, which are Crabtree positive yeast, and Crabtree negative yeast [30]. Under high glucose concentration, Crabtree positive yeast produces a lesser amount of lipid than that of Crabtree negative yeast due to catabolite repression. *S. cerevisiae* and *Y. lipolytica* are examples of Crabtree positive yeast, known also as respiratory deficient yeast, while respiratory sufficient yeasts such as *R. glutinis*, *C. utilis* and *Pichia stipitis* are examples of Crabtree negative yeast. Nitrogen deprivation in the medium will lead to the termination of cell division as well as the synthesis of protein and nucleic acid by yeast [1]. For nonoleaginous yeast, excess carbon is transformed into polysaccharides such as glycogen, glucan and mannan, with minimal level of lipid synthesis. However, lipid synthesis in most of the oleaginous yeast species will be stimulated during nitrogen deprivation and the product will be stored as triacylglycerides (TAG) in lipid bodies, except for *Cryptococcus terricola,* in which the lipid accumulation starts during logarithm growth, before nitrogen depletion [30].

The fatty acid composition of microbial oil is significantly affected by the types of microorganisms, substrates and culture conditions employed. Based on the fatty acid profile, microbial oils could have various applications to produce biodiesel, surfactants, waxes, lubricants and chemical feedstocks [58][59]. Oleaginous yeast has been proven to contribute to the sustainable biodiesel industry as studies showed that it can successfully accumulate microbial oil with similar composition to the conventional plant oil, which is composed mainly of oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0), and low amounts of linoleic, linolenic and palmitoleic acid on substrates such as industrial glycerol, solid waste, sewage sludge or sugar cane molasses[30][60][61][62].

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