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Review article

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Lipid Accumulation Bioprocess of Oils and Fats: From the State of the Art to the Challenges

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Introduction

Microorganisms, including yeasts and bacteria, have long been studied as alternative sources of oils and fats [1, 2]. Microorganisms synthesize lipids as a part of their metabolism, and as a source of energy. Some species have been reported to accumulate more than 20% of their dry cell mass in the form of lipids, and have been classified as "oleaginous" microorganisms [3]. Moreover, some oleaginous yeast species are particularly promising in this respect, as they can accumulate more than 70% of their dry cell weight as lipids [3]. In addition to this considerable capacity for lipid accumulation, oleaginous yeasts present various fatty acid profiles. In particular, they synthesize valuable polyunsaturated fatty acids, and are, therefore, a target of choice for potential applications as a renewable raw material for energetic and chemical production or as nutritional supplements. The analysis of the international state of the art revealed that oleaginous microorganisms have been studied over decades.

Yarrowia lipolytica

Is one of the most widely studied "nonconventional" oleaginous yeast species [4, 5]. It has been isolated from various food-related environments (e.g. cheese, sausage), but also from sewage, soils and oil fields [6]. Its classification by the American food and drug administration as "Generally Recognized as Safe" (GRAS) paved the way for the development of various biotechnological applications, including (i) heterologous protein production, (ii) organic acids production and (iii) single-cell oil productions from agro industrial by-products or wastes [7-9]. Under specific growth conditions, Y. lipolytica accumulates large amounts of lipid, sometimes accounting for more than 50% of its dry cell weight [10]. One of the major advantages of this yeast is its ability to use hydrophobic substrates (e.g. alkanes, oils, fatty acids...) efficiently as a sole carbon source [6, 11]. Y. lipolytica cells accumulate large amounts of lipids on these substrates, using specialized protrusions formed on their cell surface to facilitate the uptake of hydrophobic compounds [12]. These characteristics, together with the availability of the complete genome sequence, render Y. lipolytica a model of choice for investigations of lipid accumulation in oleaginous yeast species. Various studies have already made use of the genome sequence to decipher aspects of lipid metabolism in Y. lipolytica, and some of the genes involved in the bioconversion, synthesis and mobilization of lipids have been described [13]

Rhodotorula glutinis

Is another relevant microorganism for lipid accumulation studies, as it is able to accumulate up to 70% lipid (w/w) of dry cell mass. The lipids accumulated are mainly triacylglycerol (TAG) with fatty acids having aliphatic tails of 16–18 carbons, saturated and unsaturated (up to 2 unsaturations). R. glutinis is able to metabolize xylose, glucose and glycerol [14-16]. However, few experiments were done in bioreactors with co-substrates, under perfectly controlled conditions to quantify and manage yeast metabolism [17].

Streptomyces lividans

A filamentous soil bacteria well known for its ability to produce antibiotics, has the natural ability to degrade plant polymers, including lignocellulose, as well as to accumulate large reserves of Triacylglycerols (over 25% of its dry weight) when grown in a medium with a high C/N ratio and P limitation [18, 19]. However, the genetic basis of these abilities remains to be established and very few works in the world have been published on these topics [20-22].

Lipid accumulation bioprocess

Lipid accumulation is induced by nutrient limitation or deficiency with a carbon excess [23, 3]. The carbon to nitrogen ratio C/N is a key parameter to monitor fatty acid accumulation and profile with an optimum value depending on the strain. For higher values, nutritional deficiency becomes lethal. Fatty acid composition is also dependent on culture temperature, as the degree of saturation generally decreases with decreasing temperature in order to maintain the cell membrane integrity [24]. Most of the processes described in previous publications relate to batch and fed batch cultures [25-27]. In batch cultures, minerals and carbon substrates are initially mixed in the bioreactor, with a high initial C/N ratio. As nutrients are consumed from the start of culture, C/N ratio continually increases and lipid production occurs [28]. Nevertheless, in batch mode, by-product production led to decreasing carbon conversion yield into lipids. In fed batch culture, nitrogen and carbon flows are monitored to monitor specific growth and lipid production rates with minimization of by-product production to perform the highest performances [29].

The challenges

High importance is given in the substrate choice to ensure microbial and process requirements, economic and environmental criteria as cheap and sustainable; carbohydrate substrate resources from industrial by-products (from starch industry or sugar refinery, biodiesel production industry, and food industries), lignocellulosic substrates, CO_2 and its derivatives within a recycling by-product strategy are under consideration. Microorganisms have the best natural characteristics to convert a large range of renewable carbon substrates into lipids. Promising raw materials are lignocellulosic resources: lipids production from lignocellulose sources requires enzymatic hydrolysis of cellulose and hemicellulose (respectively by cellulases and hemicellulases) to release sugars (saccharification) that can subsequently be fermented by yeasts or bacteria to lipids. To be economically and environmentally viable on an industrial scale, this requires operating at high dry mass to achieve sufficiently high cellulose or hemicellulose levels. However, high substrate concentration in the form of fibrous, solid materials poses two principal problems that need to be investigated: (1) the increased concentrations of potential inhibitors hamper the performance of yeast and enzymes and (2) high viscosity results in more power consumption in the fermenter and lowered mixing and heat transfer efficiency [29-32]. Moreover, in order to reduce the cost of the conversion of lignocellulose to lipids, biomass-to-products conversion in one step could be of major interest: this strategy called an integrated or consolidated bioprocess strategy is highly attractive [33-36].

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