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Microbial lipid-based lignocellulosic biorefinery: feasibility and challenges

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Although single-cell oil (SCO) has been studied for decades, lipid production from lignocellulosic biomass has received substantial attention only in recent years as biofuel research moves toward producing drop-in fuels. This review gives an overview of the feasibility and challenges that exist in realizing microbial lipid production from lignocellulosic biomass in a biorefinery. The aspects covered here include biorefinery technologies, the microbial oil market, oleaginous microbes, lipid accumulation metabolism, strain development, process configurations, lignocellulosic lipid production, technical hurdles, lipid recovery, and technoeconomics. The lignocellulosic SCO-based biorefinery will be feasible only if a combination of low- and high-value lipids are coproduced, while lignin and protein are upgraded to high-value products.

Lignocellulosic SCO biorefinery

Second-generation biorefineries

Conversion of food materials to fuels in first-generation biorefineries has sparked intensive debate and motivated the development of second-generation biorefineries, which use abundant non-edible lignocellulosic biomass as feedstock [1]. Life-cycle assessments (LCAs) of second-generation biorefineries showed greater greenhouse gas (GHG) emission reduction than in first-generation biorefineries [2]. However, lignocellulosic biomass is highly recalcitrant due to its complex composition of cellulose, hemicellulose, and lignin [3]. Biorefineries that go with the biochemical route apply pretreatment to disrupt the plant cell-wall structure of lignocellulosic biomass, thus facilitating subsequent enzymatic hydrolysis to obtain fermentable sugars [4]. The fermentable sugars can then be fermented by various microorganisms into fuels and chemicals [5]. Fuel molecules that are undergoing or have undergone pilot scale trials include ethanol, butanol, isobutanol, biofene, bisbolene, organic acids, long-chain alcohols [6], and lipids from oleaginous microorganisms (SCO) [7]. Although the process cost of second-generation biorefineries is high, it is expected to decrease as the technology matures. SCO production in a second-generation biorefinery is the focus of this review.

Potential markets for microbial lipids

The lipids produced by oleaginous organisms are mostly in the form of Triacylglycerols (TAGs), with some in the form of free fatty acids (FAs). Lipids are attractive feedstocks for production of renewable fuels due to their high carbon-to-heteroatom ratios. The two most prevalent biofuels produced from lipids are biodiesel (via transesterification of TAGs) and renewable diesel (via hydrotreatment) [8]. The major process for transforming lipids into oleochemicals is the hydrolysis of TAGs into glycerol and FAs under the influence of water, temperature, and pressure (Figure 1A) [9–11]. Various products can be formed from FAs, such as oleic acid [10–13] (Figure 1B). Microbial lipids containing essential FAs (EFAs) are especially valuable in food applications [14]. Important EFAs include gamma linoleic acid (GLA), eicosapentaenoic acid (EPA), arachidonic acid (ARA), and docosahexaenoic acid (DHA). Glycerol, produced as a byproduct during lipid processing, is an important feedstock for various C3 commodity chemicals that are currently produced in petrochemical processes from propylene [10–12] and has many possible applications and transformations (Figure 1C) [9,15–20].

SCO microbes and culture conditions

Oleaginous microbes

An ‘oleaginous’ microorganism is one that is able to accumulate greater than 20% of its dry body mass as ‘oil’ in the form of lipids, primarily as TAGs and FAs [7,21]. A literature survey by Subramaniam *et al.* [22] included: 14 genera of microalgae with the highest reported oil contents ranging from 20% to 77% of dry weight, most notably 50–77% for *Schizochytrium* sp.; four genera of bacteria with oil accumulations ranging from 24% to 78% of dry weight, the highest achieved by *Arthrobacter* sp. at >40%, and up to 78% from glucose [23]; four genera of yeast with oil contents ranging from 58% to 72% of cell dry weight, with a *Rhodotorula glutinis* strain accumulating the highest level; and four genera of molds with oil contents ranging from 57% to 86%, with a strain of *Mortierella isabellina* accumulating the highest level in the range.

The basics of lipid biosynthetic metabolism is well understood (Figure 2 and Box 1) [24–27]. The theoretical yield of lipid production is 0.32 g/g sugar from glucose and

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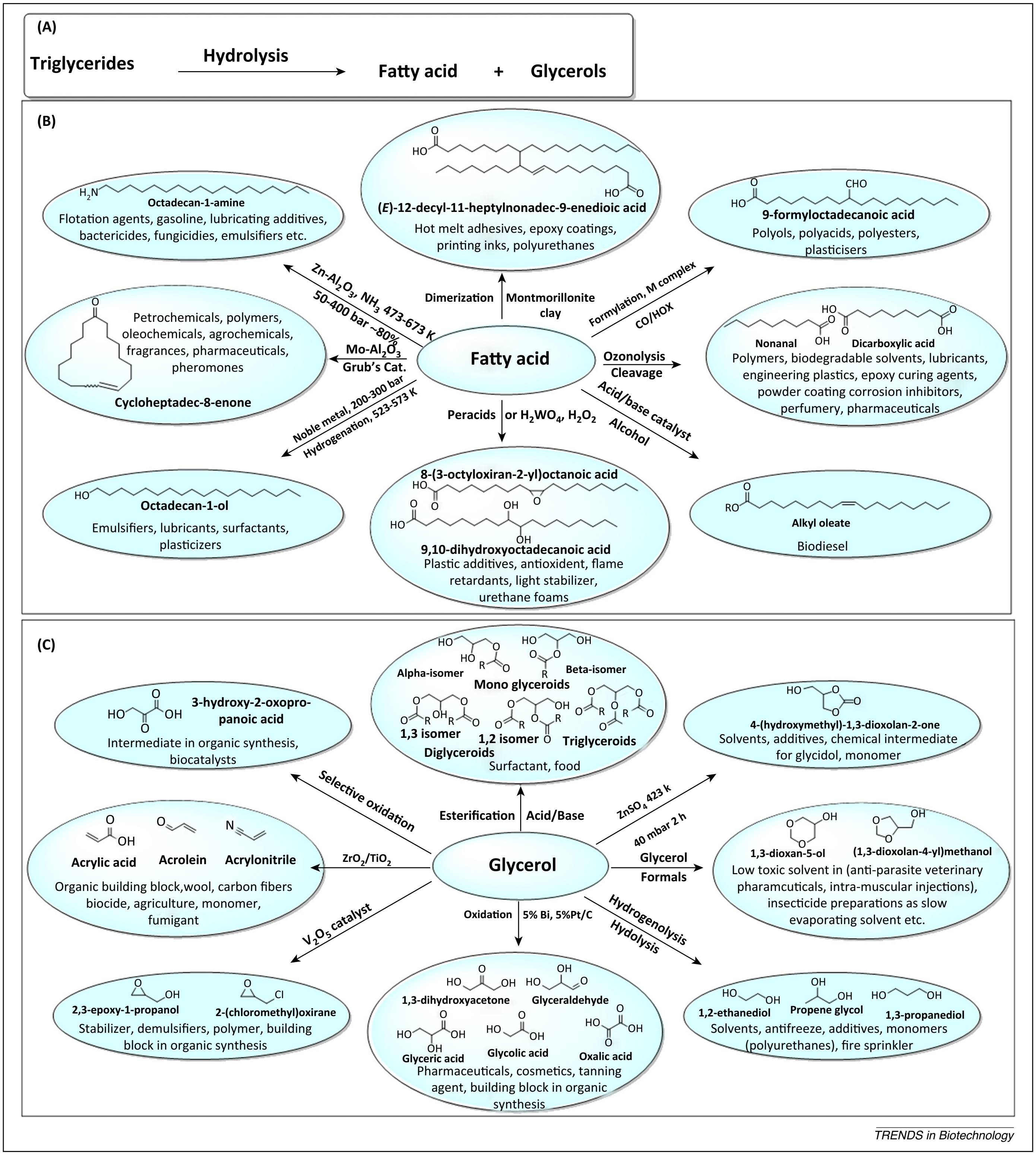

Figure 1. Possible applications and transformations of glycerol and fatty acids derived from microbial lipids.

0.34 g/g sugar from xylose [28]. However, the practical yield after production of biomass and other products is generally considered to be approximately 0.22 g lipid/g glucose [29,30]. The review of Kosa and Ragauskas [23] lists the comparative productivities (g/l/h) of the top heterotrophic yeast, molds, and bacteria on glucose in synthetic media by decreasing rate as follows: the yeast *Rhodosporidium toruloides* (0.54); *Cryptococcus curvatus* (0.42); *Candida* sp. 107 (0.40); the bacterium *Rhodococcus opacus* PD630 (0.38); the mold *Mortierella ramanniana* (0.17); the yeast *Trichosporon cutaneum* (0.14); *Trichosporon fermentans* (0.10); and the mold *Cunninghamella echinulata* (0.07).

Culture conditions

An optimally high carbon-to-nitrogen (C:N) ratio is key to allowing cells to reach their maximal lipid storage capacity