Review

CellPress

Microbial advanced biofuels production: overcoming emulsification challenges for large-scale operation

Arjan S. Heeres[1], Carolina S.F. Picone[2], Luuk A.M. van der Wielen[1,3], Rosiane L. Cunha[2], and Maria C. Cuellar[1]

[1] Department of Biotechnology, Delft University of Technology, Delft, The Netherlands
[2] Department of Food Engineering, University of Campinas (UNICAMP), Campinas, Brazil
[3] BE-Basic Foundation, Delft, The Netherlands

Isoprenoids and alkanes produced and secreted by microorganisms are emerging as an alternative biofuel for diesel and jet fuel replacements. In a similar way as for other bioprocesses comprising an organic liquid phase, the presence of microorganisms, medium composition, and process conditions may result in emulsion formation during fermentation, hindering product recovery. At the same time, a low-cost production process overcoming this challenge is required to make these advanced biofuels a feasible alternative. We review the main mechanisms and causes of emulsion formation during fermentation, because a better understanding on the microscale can give insights into how to improve large-scale processes and the process technology options that can address these challenges.

Microbial production of advanced biofuels

Increases in the world population and global prosperity cause an increased global energy demand. To reduce the dependence on fossil energy, targets for the incorporation of renewable energy are being set worldwide. Biofuels are expected to make a significant contribution in achieving these goals. Commercial production of bioethanol is well established in the United States and Brazil and biodiesel produced from vegetable oils is also emerging [1]. However, their fuel properties and feedstock requirements have some inherent drawbacks.

Currently, microorganisms are being developed that produce and secrete molecules similar to fossil fuels, so called advanced or drop-in biofuels. The focus is on engineering of well-known industrial microorganisms (mostly *Escherichia coli* and *Saccharomyces cerevisiae*) and photosynthetic organisms (mostly cyanobacteria), enabling the production of isoprenoid-derived compounds or fatty acid (FA)-derived alkanes and alkenes.

Isoprenoids are molecules composed of multiple isoprene blocks and are abundant throughout nature. By the mevalonate (MEV) pathway or the deoxy-D-xylulose 5-phosphate (DXP) pathway, the two building blocks are formed: isopentenyl pyrophosphate and dimethylallyl pyrophosphate. By linking these blocks, a variety of molecules can be formed, with varying applications, for instance, artemisinic acid, a precursor for an antimalarial therapeutic [2], and different sesquiterpenes, which are applied in flavors and fragrances [3]. With metabolic yields increasing, isoprenoids become interesting for biofuel application. Farnesenol [4], farnesene [5,6] and bisabolene [7] have good fuel properties, but these molecules have multiple double bonds and require a hydrogenation step to improve their fuel quality. For farnesene, this route has been demonstrated at pilot and production scale (www.amyris.com).

Alkanes and alkenes can be produced microbially by the FA pathway. In cyanobacteria, intermediates in the FA synthesis chain (acyl protein carriers) are reduced and decarboxylated, forming hydrocarbons [8]. This pathway was implemented in *E. coli* to further tailor alkane productivity. By directly using the free FAs in the cell, product composition can directly be adapted by adjusting the type of free FAs present in the cell [9,10]. This approach has led to the formation of C13–C17 hydrocarbons up to pilot scale [11].

In both these routes, the product is secreted by the cells, resulting in a multiphase mixture consisting of cells, aqueous fermentation medium, oil droplets, and fermentation gas bubbles (Figure 1). Product secretion eliminates the need for cell disruption in product recovery, potentially simplifying downstream processing and lowering production costs, which is a key factor for making advanced biofuels a feasible alternative for fossil fuels (Box 1). However, not much is known about the secretion mechanism, but considering the size of the cells producing the biofuel, the initial droplet size after secretion can be assumed to be smaller than the cell size, so in the order of micrometers.

The presence of the product as a second liquid phase may result in unconventional process configurations for biotechnology, where most products are water soluble or

Corresponding author: Cuellar, M.C. (m.c.cuellar@tudelft.nl).
Keywords: advanced biofuels; multiphase fermentation; oil recovery; biosurfactants; bioemulsifiers; Pickering stabilization.

0167-7799/$ – see front matter
 http://dx.doi.org/10.1016/j.tibtech.2014.02.002

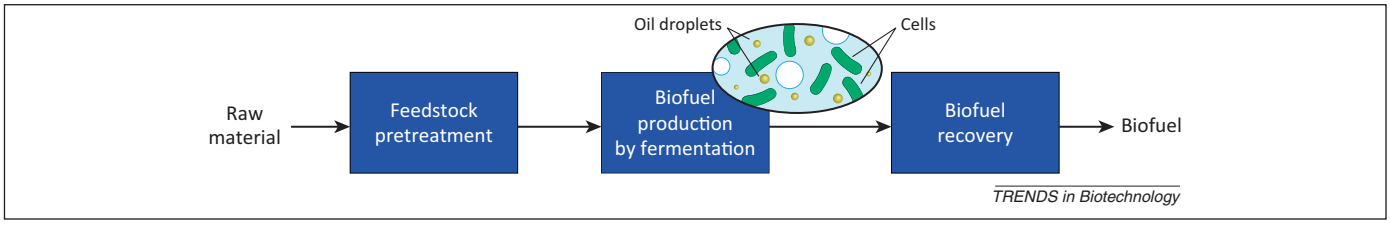

Figure 1. A simplified block diagram of the production of advanced biofuels. In the inset is a sketch of the multiphase mixture obtained during fermentation (not to scale).

solids. To go from the product droplets – dispersed in the broth under fermentation conditions – to the formation of a continuous oil phase, several steps are required (Box 2). When any of these steps is impeded, a stable emulsion if formed, as has already been described in advanced biofuel production [12]. Emulsion formation hinders product recovery; therefore, it is a key aspect for the large-scale implementation of advanced biofuels. In this review, we first discuss the potential causes of emulsion formation during fermentation, followed by process technology solutions that might be applied at a large scale to overcome emulsification challenges.

Box 1. The cost of producing advanced biofuel

Currently, first-generation ethanol and biodiesel supply 2% of the global transport energy demand. The biofuel contribution is expected to increase up to 27% by 2050 (www.eia.gov), given the need for fuel replacement in transport vehicles that are not suited for other renewable energy resources (e.g., electricity), as is the case of planes. For this, however, cost- and energy-efficient processes are required.

Little information is available in literature on the overall process for extracellular production of diesel, plane and jet fuel replacements. However, such processes seem to be performed aerobically, making use of conventional fermentation equipment under monoseptic conditions and relying on centrifugation for product recovery [6,76]. Companies claim target production costs around $0.60 per liter with matured technology [6], which would make them competitive in the diesel and jet fuel market. However, according to Westfall *et al.* [6], most of these technologies are not yet mature and most companies are not producing at full scale yet. A selling price as high as $7.7 per liter was recently reported [77]. As noted by Cuellar *et al.* [78], in order to reach competitive production costs a combination of process improvements will be required: low-cost feedstock, maximized product yield on substrate under anaerobic conditions, cell reuse, and low-cost fermentation and recovery technology [78]. To further illustrate this, let us consider the general stoichiometric reaction for alkane production from glucose:

$$\left(\frac{1}{4}n+\frac{1}{12}\right)\cdot C_6H_{12}O_6 \rightarrow C_nH_{2n+2}+\left(\frac{1}{2}n+\frac{1}{2}\right)\cdot CO_2 + \left(\frac{1}{2}n-\frac{1}{2}\right)\cdot H_2O$$

Assuming that the maximal theoretical yield from this equation can be achieved and considering the production of a long-chain alkane such as octadecane ($C_{18}H_{38}$), about 3 kg of sugars are required per kg of product. At a sugar price of $0.4 per kg (http://ers.usda.gov), this results in $1.2 per kg or $0.9 per liter only on feedstock cost. In mature biofuel processes, 80% of production cost corresponds to the feedstock and 20% is equally distributed among other operating costs and capital charges [79]. This results in capital charges of $0.15 per kg, which for a production capacity of 100×10^3 ton/year, 10% interest and 10-year plant life leads to a maximal investment of $150 million. This is comparable to the investment for a mature sugar cane/ethanol mill with similar production capacity ($100–300 million) [79]. This rough calculation clearly shows that the process technology must be competitive for these processes to be feasible on a large scale.

Emulsion stabilization in bioprocesses

An emulsion is a thermodynamic unstable system formed by one immiscible liquid dispersed in another. With no stabilizing components present, the emulsion would phase separate into the two liquids (Box 2). However, in bioprocessing that involves microorganisms, a wide range of stabilizing components could be present. The next sections discuss potential causes of emulsion stabilization in bioprocessing: which components specific for bioprocesses stabilize the droplet interface and which decrease the droplet mobility (Table 1).

Stabilization of the interface

The interface can be stabilized through several mechanisms (Box 3). The interface stability is enhanced by the presence of surface active components (SACs), originating from the substrate or produced by the microorganisms (biosurfactants/bioemulsifiers), or by the presence of surface active particles (e.g., cells).

Biosurfactants and bioemulsifiers. Depending on the microorganism type and metabolism, different SACs are produced that change the interfacial properties of the emulsion. The SACs are produced as a protection mechanism or to increase the substrate availability when there is a second liquid phase present [13,14]. Due to their high biodegradability, lower toxicity, and emulsifying properties at specific conditions (at extreme temperatures, pH, and salinity), SACs offer an alternative for the traditional surfactants and their isolation has been extensively studied [15–17]. These compounds are divided into two main classes: biosurfactants and bioemulsifiers. The biosurfactants are compounds with high surface active properties and usually low molecular weight [18]. The bioemulsifiers also show surface active properties, but they commonly do not decrease the surface and interfacial tension appreciably [15]. They are usually high-molecular-weight compounds, such as polymers, polysaccharides, lipopolysaccharides, or lipoproteins [13,19]. However, some compounds show intermediate characteristics and may act as both biosurfactant and bioemulsifier. Both biosurfactants and bioemulsifiers can be produced intracellularly, secreted, or attached to the cell membrane [20]. The most common biosurfactants and bioemulsifiers found in bioprocesses are glycolipids, lipopeptides/lipoproteins, phospholipids, and polymeric surfactants [21].

Glycolipids are combinations of carbohydrates and long-chain aliphatic acids or hydroxy aliphatic acids [22]. Microorganisms secrete glycolipids to make hydrocarbons or other hydrophobic substrates available for cell metabolism [23]. Rhamnolipids are the best known glycolipids,