

Biofuels

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Contributor: Alessandra Procentese

As defined by the European Union, “Biofuels are liquid or gaseous transport fuels such as biodiesel and bioethanol which are made from biomass”. Bioethanol is the most common biofuel obtained by a fermentation process and can be run by using a variety of carbon sources.

Biofuels

biorefinery

circular economy

1. Introduction

Based on the starting feedstock, biofuels are classified in three categories [\[1\]](#):

- First-generation biofuels, which are those produced from sources like starch, sugar, animal fats, and vegetable oils.
- Second-generation biofuels, also known as advanced biofuels, produced from feedstocks which refer to non-food biomass (such as by-products and residues from agriculture, forestry, and related industries, as well as the non-fossilized and biodegradable organic fractions of industrial and municipal wastes).
- Third-generation biofuels. These are obtained from aquatic autotrophic organisms (such as algae, which can use light and carbon dioxide to grow).

Biofuels production from lignocellulosic biomasses takes three main steps: biomass pretreatment (to release the fermentable sugar fraction), fermentation, and recovery [\[2\]](#).

During the biomass pretreatment, the feedstock is sequentially both physico-chemically and enzymatically treated. During the physico-chemical treatment, the lignin is broken down in order to increase the enzymes accessibility (enzymatic treatment) to the polysaccharides for the fermentable sugars release [\[2\]](#). Several reviews can be found reporting the advantages and disadvantages of the physico-chemical treatments currently used [\[2\]](#)[\[3\]](#). Nowadays, the main investigated pretreatment processes are the steam explosion, the acid/alkaline hydrolysis and the autohydrolysis pretreatment [\[3\]](#). They all present several advantages with respect to the other available options, e.g., a reduced use of chemicals and less waste production compared to ionic liquid and deep eutectic solvents pretreatment [\[4\]](#), higher productivity with respect to biological pretreatment [\[5\]](#), and easier scalability compared to ultrasound pretreatment [\[6\]](#).

Once the fermentable sugar fraction has been released, different microbes can be used to convert those into biofuel through a fermentative process. The most widely known is the ethanol production by the yeast *Saccharomyces cerevisiae*. Another fermentation type is the butanol production by *Clostridia* [\[7\]](#). This anaerobic

fermentation is characterized by the production of Acetone –Ethanol–Butanol in molar ratio 3:6:1. This fermentation occurs in two steps: the acidogenic and the solventogenic phases. During the acidogenic phase, there is production of acetic and butyric acids; therefore, there is a decrease in the pH of the medium. In this condition, the microorganism reconverts the acids into solvents (solventogenic phase) [2]. The butanol yield is low, and its recovery is very energy demanding; however, butanol has several advantages with respect to ethanol (e.g., high energy content, high burning efficiency, low hygroscopicity, and low volatility). Thus, several innovative solutions for butanol production are currently being investigated [2].

The last step in the biofuel production is the recovery, which is also considered as highly energy demanding. For this reason, there are dedicated studies in the literature specifically dedicated to its optimization [7][8]. The production of biofuels by microbial fermentation goes back to the second half of the 19th Century. The first studies were focused on the characterization of the fermentation process [9][10], and more recently with the advances and application of genetic and “-omic” sciences, most of the studies have been focused on the development of engineered microorganisms able to increase production yields [11][12].

Nowadays, biofuel production through biotechnological processes has been entirely analyzed with the so-called “cradle-to-grave” approach [13]. This analysis allows to identify the main issues of a given process by considering the whole production chain: from the starting feedstock to the final product use/disposal. Thus, both the environmental benefits as well as the gas emission during the whole process are evaluated. As reported by several of these studies [13][14][15], one of the main drawbacks in the biorefinery approach is the feedstock’s selection and collection: some feedstocks are produced in a particular area, but they are available in low amounts throughout the year [16], while others are available in high amounts, but their production requires broad land use, increasing the collection/transport costs [17]. In order to overcome this problem, some studies proposed the “multi-feedstock” biorefinery concept: a biorefinery able to pretreat and convert different types of feedstocks into fermentable sugars [18]. However, based on the feedstock’s characteristics, different pretreatments can be required, and the use of different feedstock(s)/pretreatment(s) may result in the production of different types of undesired fermentation inhibitors [2]. Nevertheless, the collection and transport costs will still remain.

A possible solution in order to reduce or even eliminate these costs could be to implement the “zero miles product” approach into biofuel production. This approach aims to create a link between the final product and the environmental sustainability and thus reducing the carbon footprint of the production process [19]. Nowadays, it is still unclear whether or not the most effective way to reduce the carbon footprint of a process is only “buying local” [20].

2. Biofuel Production from Non Lignocellulosic Biomass

Nowadays, second generation biofuels are the ones attracting the most research interest according to scientific literature. As previously stated, they are defined as biofuels produced from feedstocks which refer to non-food biomass. In turn, these can be divided into two subcategories: non-lignocellulosic biomass and lignocellulosic biomass derived feedstocks. The non-lignocellulosic ones are for example vegetable or animal fats and organic

matter. In this section, the most representative biofuels obtained from non-lignocellulosic biomass through both thermochemical and fermentative processes will be presented. Biodiesel can be generated from oils or fats by transesterification. Vegetable or animal fats and oils react with short-chain alcohols such as: methanol or ethanol, ethanol is the most used because of its lower cost; however, greater conversion rates can be achieved by using methanol. Although the transesterification reaction can be catalyzed by either acids or bases, the base-catalyzed reaction is more common due to low reaction time and costs [21]. Glycerol is a major by-product of biodiesel production, with around 100 kg of crude glycerol generated per ton of biodiesel [21]. The total European biodiesel production in 2020 has been estimated at about 4.2 billion gallons [22].

Biogas is composed by methane, carbon dioxide, and may contain small amounts of hydrogen sulfide. It is produced by the breakdown of organic matter by microbes such as methanogens and sulfate-reducing bacteria by anaerobic respiration [23]. The main factors affecting the biogas production yields are temperature and pH. As reported by Issah et al. [23], these parameters strictly depend on the microorganism involved. The higher biogas yields are achieved when co-digestion involving the mixture of two or more substrates is applied. The objective of co-digestion is to balance environmental conditions such as pH or alkalinity in a digester and to maintain the optimum carbon to nitrogen ratio [23]. The biomethane plants in Europe have increased by 51% in two years, from 483 in 2018 to 729 in 2020 and there are currently 18 countries producing biomethane in Europe. Germany has the highest share of biomethane plants (232), followed by France (131) and the UK (80). By 2050, 1170 TWh of biogas are expected to be generated [24].

Biohydrogen may be produced by steam reforming of methane (biogas) produced by anaerobic digestion of organic waste. The natural gas and steam react producing hydrogen and carbon dioxide [26]. Biohydrogen can also be produced by fermentation; however, the process renders low yields; thus, there is a need to identify new strains for the process [25]. Moreover, still several hurdles need to be fixed in relation to this particular type of biofuel, such as the storage and its transportation [25].

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