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Zygomycetes-based biorefinery: Present status and future prospects

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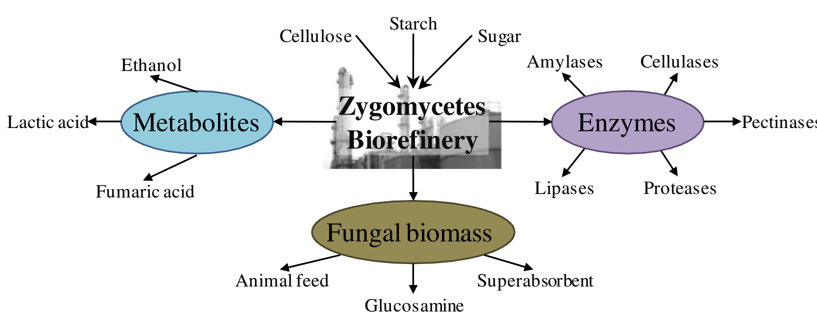
HIGHLIGHTS

- Zygomycetes meet all requirements for being utilized as catalysts in biorefineries.
- Metabolites such as lactic acid, fumaric acid, and ethanol can be produced.
- Starch, cellulose, phytic acid, and proteins can be assimilated.
- Zygomycetes fungi are the source of a great diversity of enzymes.
- Potential source of single-cell protein with high nutritional value.

GRAPHICAL ABSTRACT



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ABSTRACT

Fungi of the phylum Zygomycetes fulfil all requirements for being utilized as core catalysts in biorefineries, and would be useful in creating new sustainable products. Apart from the extended use of Zygomycetes in preparing fermented foods, industrial metabolites such as lactic acid, fumaric acid, and ethanol are produced from a vast array of feedstocks with the aid of Zygomycetes. These fungi produce enzymes that facilitate their assimilation of various complex substrates, e.g., starch, cellulose, phytic acid, and proteins, which is relevant from an industrial point of view. The enzymes produced are capable of catalyzing various reactions involved in biodiesel production, preparation of corticosteroid drugs, etc. Biomass produced with the aid of Zygomycetes consists of proteins with superior amino acid composition, but also lipids and chitosan. The biomass is presently being tested for animal feed purposes, such as fish feed, as well as for lipid extraction and chitosan production. Complete or partial employment of Zygomycetes in biorefining procedures is consequently attractive, and is expected to be implemented within a near future.

1. Introduction

Microbial fermentation has been used since ancient times. During the last century, however, petroleum-based refineries have prevailed over microbial routes for production of fine chemicals, due to the abundance and low cost of crude oil. Due to oil depletion forecasts, global warming, and the steadily increasing amount of waste products, the situation is now changed. Sustainable development is presently on the agenda, and great efforts have been made by seeking to replace oil-based refineries with biorefineries based on renewable feedstocks. Using filamentous fungi, with diverse and expanding products, constitute an important contribution to this development. Fermentation processes for the production of organic acids, antibiotics, enzymes, food components, and other miscellaneous products have already been applied (Gibbs et al., 2000).

Given their structural and physiological properties, Zygomycetes are receiving increased attention within the biotechnological field. They are already well known due to their extended use in China and Southeast Asia, for the production of fermented foods such as tempe and tofu (Lennartsson et al., 2012). The fungi have in recent times been investigated and used for production of a wide range of metabolic products. Examples include organic acids,

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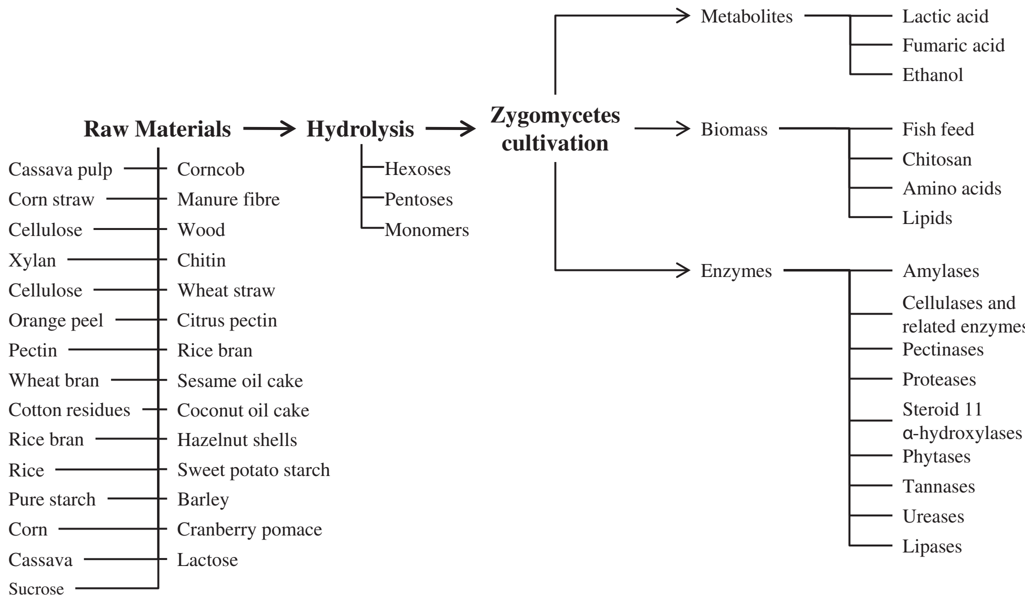

Fig. 1. Overall scheme of a Zygomycetes-based biorefinery, illustrating the alternative feedstock inputs as well as the product outputs, including metabolites, enzymes, and potential cell mass applications.

enzymes, and biofuels such as bioethanol and biodiesel. In addition, the Zygomycetes biomass contains beneficial quantities of proteins, lipids, amino acids, chitosan, and chitin. Thus, the biomass is envisaged for production of animal feed, human food, and chitosan. Zygomycetes are able to grow on a large variety of carbon sources at different temperatures, oxygenation rates, and pH-values. They are amylolytic, and able to consume pentose sugars. They are also able to perform simultaneous saccharification and fermentation (SSF) of starch materials. Contemplating the minimal requirements of growth, the easy recovery of fungal mycelium, and the variety of feasible co-products, Zygomycetes biomass holds the capacity of playing an important role in the future establishment of economically attractive Zygomycetes-based biorefineries, and may well be exploited in already established industrial processes to enhance profitability.

The aim of the present review is to gather the different biotechnological applications of Zygomycetes holding the highest potential for a biorefinery (Fig. 1), which mainly refers to flexibility in terms of the raw materials to be used, and their capability of producing metabolites, enzymes, and biomass.

2. Zygomycetes as core catalysts in biorefineries

The kingdom *Fungi* is considered to comprise four phyla; *Chytridiomycota*, *Zygomycota, Ascomycota*, and *Basidiomycota*. *Zygomycota* is divided into two classes, Trichomycetes and Zygomycetes, which are found worldwide as saprophytes growing on dead organic matter. Zygomycetes have long been used in food production. However, they have also been observed to be pathogens of plants, animals, and other fungi, and suitable strains must hence be selected (Lennartsson et al., 2012).

Zygomycetes are versatile filamentous fungi with well demonstrated growth ability in a vast array of culture media. The medium requirements are generally characterized as inexpensive and unspecific, and include, apart from some salts, a variety of nitrogen and carbon sources (Zhang et al., 2007), the range of the latter being wide and expanding, mostly due to the variety of enzymes carbon sources produce (Fig. 1). Glucose has been the first choice as carbon and energy source. However, some strains are able to consume more complex sugars, such as the disaccharides sucrose and lactose (Guo et al., 2010; Vamvakaki et al., 2010). Valorization of cheese whey, a lactose-containing dairy waste, as a renewable substrate for Zygomycetes has also been investigated (Vamvakaki et al., 2010). Furthermore, these fungi have, as amylase producers, the prerequisites for simultaneous saccharification and fermentation (SSF) of starchy materials. SSF has been widely examined due to its capacity to enhance process productivity, reduce the reactor volume required, and hence also capital costs. Starchy crops, such as barley, cassava, corn, oats, and rice, have been used as carbon sources for Zygomycetes in the production of lactic acid (Zhang et al., 2007). Koutinas et al. (2007) designed an oat-based biorefinery for production of lactic acid and various value-added co-products, including β-glucan and an anti-irritant solution. Due to their renewable character, abundance, and cheap price, lignocellulosic materials are also promising feedstocks for biotechnological processes (Zhang et al., 2007). A wide variety of lignocellulosic materials, providing cellulosic and hemicellulosic feedstocks, have been evaluated for production of value-added products, using Zygomycetes (Fig. 1). Corn straw and wood hydrolysates have for instance been examined for production of fumaric acid and ethanol, respectively, while wheat bran, cellulose, and xylan have been analyzed for production of cellulases and xylanases (Lennartsson et al., 2012; Xu et al., 2010). Valorization of spent sulphite liquor, a waste hemicellulosic product from paper pulp mills, for producing high-value Zygomycetes biomass for fish feed as well as chitosan, has also been carried out (Bankefors et al., 2011; Lennartsson et al., 2012; Mydland et al., 2007). Although lignocellulosic materials have been scrutinized for ethanol production for decades, developing a feasible commercial process has not yet been accomplished. In a facility using lignocellulosic materials, the total value might be raised with Zygomycetes producing biomass as a second product (Lennartsson et al., 2012). At present, agricultural crop-based facilities are being used for ethanol production. The process results in large volumes of low value by-products, thin stillage being a major one. Thin stillage may however be utilized by Zygomycetes for cell mass production. The process results in a product enriched with protein (including amino acid composition), lipids, and chitosan (van Leeuwen et al., 2012). Zygomycetes are also capable of utilizing lipids and fatty acids. Being lipase producers,