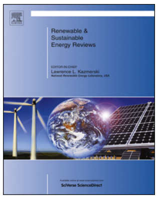
Renewable and Sustainable Energy Reviews 52 (2015) 179–188

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser



Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector

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ARTICLE INFO

Article history:
Received 23 July 2014
Received in revised form
26 May 2015
Accepted 21 July 2015
Available online 10 August 2015

Keywords:
Bioenergy
Solid-state fermentation
Enzymes
Cellulase
Biomass
Agro-industrial residues

ABSTRACT

Solid-state fermentation (SSF) processes have enormous potential for many new applications using the bioconversion of agro-industrial residues into biofuels and other high value-added products. The agricultural sector is currently undergoing global expansion, especially in relation to crops used for energy production as a strategy to reduce dependence on petroleum and mitigate the effects of climate change. Consequently, a similar expansion is expected in the amounts of agricultural and forestry residues generated. The conversion of these lignocellulosic biomasses using enzymes is likely to be a key technology in future biorefineries. However, in order to make the enzymatic conversion of biomass commercially viable, it is necessary to improve the efficiency of (hemi)cellulolytic enzymes production and reduce the costs of the enzymatic cocktails employed. The focus of this review is on recent developments in SSF processes for enzymes production, and the application of such techniques in the bioenergy sector. An overview of the enzymes required for the conversion of biomass, important SSF process variables related to the production of (hemi) cellulolytic enzymes, the bioreactors that have been used for this purpose, and novel SSF configurations is provided. It is hoped that the information gathered together here will assist in the development of SSF processes that enable efficient future production of the enzymes required for the conversion of biomass.

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http://dx.doi.org/10.1016/j.rser.2015.07.092

1. Introduction

Fermentation processes have been of great practical and economic importance to humankind for thousands of years, especially for the production of food and beverages (such as bread and wine). More recently, fermentation processes employing microorganisms for the manufacture of products of commercial interest have been successfully applied in various sectors including the food, textile, and pharmaceutical industries, amongst many others [1–3]. However, the fermentation processes have enormous potential for use in new applications. These include the bioconversion of agro-industrial residues generated by the agricultural sector into biofuels and other high value-added products. A global agricultural expansion is currently taking place, with growth in the cultivation of crops devoted specifically to energy production (agro-energy) as a strategy to reduce dependence on petroleum and mitigate the effects of climate change. In a country such as Brazil, for instance, where agriculture is the main economic activity, agricultural and forestry residues (also termed wastes or byproducts) are extremely abundant [4]. These materials are generally under-utilized; a fraction is used to generate electricity, while another large fraction is burned or remains in the field, often becoming an environmental burden. In addition to contributing to the removal of environmental pollutants, the conversion of these lignocellulosic materials into commercially valuable products could provide substantial economic dividends. One of their most promising uses involves their conversion into bioproducts, within the biorefinery concept [5–7]. A huge variety of bioproducts can be obtained from fermentation processes. The microbial production of industrial enzymes is of special interest. In the agricultural sector, important applications of microbial enzymes include the manufacture of biofuels such as cellulosic ethanol and biodiesel. In addition, fermentation processes can be used in bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, and the manufacture of other value-added products such as animal feed and biologically active secondary metabolites [2,8].

Fermentation processes for the production of enzymes can be conducted using a liquid medium (submerged fermentation, SmF) or a solid medium (solid-state fermentation, SSF). In the latter case, the cultivation employs a solid substrate with sufficient moisture only for maintenance of growth and metabolism of the microorganism (in other words, there is no free water) [2,8–11]. In SmF, on the other hand, the medium essentially consists of water containing dissolved nutrients. Submerged cultivation techniques have advantages related to instrumentation and process control, and are widely used for the production of industrial enzymes and other bioproducts. However, SSF can be particularly advantageous for the cultivation of filamentous fungi, because it simulates the natural habitat of the microorganisms [9,12,13], leading to higher enzymatic productivity, compared to SmF processes. In addition, the enzymes produced using SSF are less susceptible to problems of inhibition by the substrate, and are more stable in terms of the effects of temperature and pH [13,14]. From the environmental perspective, an important advantage of SSF is the ability to use solid substrates consisting of agro-industrial residues that serve as sources of carbon and energy for microorganism growth and enzyme production.

Several reviews on SSF have focused on general applications and process conditions. The focus of this review is on recent developments in SSF processes for biomass-degrading enzymes production, and the application of these techniques in the bioenergy sector. An overview of the enzymes required for the conversion of biomass into ethanol is presented. Important SSF process variables related to the production of (hemi)cellulolytic enzymes, the bioreactors that have been used for this purpose, and new proposed SSF configurations are discussed.

2. Enzymes required for biomass conversion

Among the various technologies available for the conversion of biomass into ethanol, the biochemical route using enzymes for the saccharification step offers several advantages [15]. Although there is a great potential for bioenergy expansion, it is still necessary to improve the efficiency of the enzyme production process in order to make the enzymatic conversion of lignocellulosic biomass economically feasible. This is because the cost of the enzymatic cocktails significantly influences the viability of the overall process of biomass bioconversion into fuels and other chemicals. According to a recent report [16], the cost of enzyme production is much higher than commonly supposed, and a significant effort is still required to reduce the contribution of enzymes to the cost of biofuels production. Although literature estimates for the cost contribution of enzymes to ethanol vary significantly, the cost contribution of enzymes to ethanol produced by the conversion of corn stover was reported to be between \$0.68/gal and \$1.47/gal, depending on the saccharification and fermentation yields [16]. This significant cost contribution is due to the large scale of the processes involved in biofuel production, and the considerable quantities of enzymes that are required. The quality of enzymatic complexes, in terms of their composition, is also an important issue, since cocktails containing cellulases, hemicellulases, pectinases, and other accessory enzymes, acting in synergy in the degradation process, are often necessary due to the high recalcitrance of plant biomass [17].

Plant cell walls consist primarily of cellulose (20–50% on a dry weight basis), hemicellulose (15–35%), and lignin (10–30%) [17]. In addition to the breakdown of cellulose, deconstruction of the hemicellulose fraction to the constituent sugars is essential for the efficient production of fuels and other chemicals from plant biomass [18]. The use of hemicellulases and other auxiliary enzymes, in conjunction with cellulolytic enzymes, can improve cellulose conversion by removing hemicellulose and increasing the access of cellulases to the substrate [19].

Cellulase enzymes comprise a set of glycoside hydrolases whose action involves hydrolysis of the β-1,4-glycosidic bonds of cellulose, the major polymer present in biomass. These enzymes show synergistic action during degradation of the polymeric cellulose chain. The most widely accepted mechanism of action of cellulases involves three classes of enzymes: endoglucanases, exoglucanases, and β-glucosidases. Endoglucanases hydrolyze accessible intramolecular β-1,4-glycosidic bonds of the cellulose chains randomly, producing new chain ends; exoglucanases progressively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and β-glucosidases hydrolyze cellobiose to glucose [20]. The endo-1,4-β-xylanase (xylanase) enzymes cleave the β-1,4-glycosidic linkage between xylose residues in the backbone of xylans. This is one of the most important enzymatic activities required for depolymerization of the hemicellulosic constituent of plant cell walls [18], because xylan is the most abundant hemicellulose [21]. Furthermore, the recent discovery of the role of lytic polysaccharide monooxygenases (LPMO) and other accessory proteins in increasing the degradation of cellulose has resulted in the inclusion of a new category in the CAZy database, called “auxiliary activities” (AA), which includes a group of catalytic modules involved in the degradation of plant cell walls [22]. The discovery of these enzymes represents a revolution in the enzymatic processing of biomass and suggests a new paradigm for the enzymatic degradation of cellulose, in which the action of the classical hydrolytic cellulases is facilitated by the lytic action of the polysaccharide monooxygenases, as is schematically presented in [15].

This type of enzymatic complex is produced by a wide variety of microorganisms, including bacteria and fungi. The aerobic fungi, especially, are recognized for their high rates of growth and protein secretion [23]. Most commercial cellulase preparations are produced by filamentous fungi of the genera *Trichoderma* and