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Review

Thermotolerant fermenting yeasts for simultaneous saccharification fermentation of lignocellulosic biomass

Jairam Choudhary, Surender Singh *, Lata Nain

Division of Microbiology, ICAR — Indian Agricultural Research Institute, New Delhi 110012, India

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ABSTRACT

Lignocellulosic biomass is the most abundant renewable source of energy that has been widely explored as second-generation biofuel feedstock. Despite more than four decades of research, the process of ethanol production from lignocellulosic (LC) biomass remains economically unfeasible. This is due to the high cost of enzymes, end-product inhibition of enzymes, and the need for cost-intensive inputs associated with a separate hydrolysis and fermentation (SHF) process. Thermotolerant yeast strains that can undergo fermentation at temperatures above 40°C are suitable alternatives for developing the simultaneous saccharification and fermentation (SSF) process to overcome the limitations of SHF. This review describes the various approaches to screen and develop thermotolerant yeasts via genetic and metabolic engineering. The advantages and limitations of SSF at high temperatures are also discussed. A critical insight into the effect of high temperatures on yeast morphology and physiology is also included. This can improve our understanding of the development of thermotolerant yeast amenable to the SSF process to make LC ethanol production commercially viable.

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* Corresponding author.
E-mail address: ssriari@gmail.com (S. Singh).
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1. Introduction

Driven primarily by the global increase in energy consumption, depletion of fossil fuel reserves and concerns about climate change, new renewable and environment-friendly sources of energy are being explored. Plant biomass is the most abundant renewable source of energy. It can be used to produce second-generation biofuels; however, it is largely wasted either by burning or by disposal in landfill sites. This leads to the release of greenhouse gases, which has a harmful effect on the environment. The process of ethanol production from lignocellulosic (LC) biomass requires four main steps: i) pretreatment of LC biomass; ii) enzymatic saccharification of pretreated biomass to yield sugar monomers; iii) fermentation of hydrolyzed sugars to ethanol, butanol, etc. by fermenting organisms; and iv) distillation. The process is termed as separate hydrolysis and fermentation (SHF) when saccharification and fermentation are performed separately. Due to the high cost of the biomass-hydrolyzing enzymes (cellulases and hemicellulases) and pretreatment methods, the production of LC ethanol by SHF is not economically viable.

During enzymatic saccharification, the hydrolytic enzymes are subject to feedback inhibition due to the accumulation of sugar monomers and cellobiose in the medium. This in turn reduces the efficiency of these enzymes. This limitation can be overcome by a process known as simultaneous saccharification and fermentation (SSF). Here, saccharification and fermentation are performed simultaneously; thus, the hydrolyzed sugars are continuously converted into ethanol, thereby enhancing the efficiency of enzymatic saccharification in the absence of feedback inhibition. However, the major limitations of SSF are the different temperature optima of biomass-hydrolyzing enzymes (45–50°C) and the fermenting organisms (30°C). Therefore, there is a need to discover cold-adaptive hydrolytic enzymes and thermotolerant fermenting yeasts to develop economically viable SSF technology. In practice, it is very difficult to reduce the optimum temperature of cellulases via protein engineering. Therefore, identifying thermotolerant yeast with higher ethanol production efficiency can be a key breakthrough for the SSF process. The SSF by thermotolerant yeasts offer the following advantages in bioethanol production:

- Reduction in total number of steps, thereby lowering the utility requirement and reducing capital investment including equipment costs
- Reduction in contamination possibility by decreasing glucose concentration and ethanol production
- Improvement in efficiency of saccharification by alleviating feedback inhibition of cellulase
- Reduction in cooling cost, as chiller unit is not required
- Continuous ethanol evaporation from broth under reduced pressure
- Suitability for use in tropical regions with high temperatures

2. LC biomass as substrate for ethanol production

LC biomass refers to plant dry matter, which is mainly composed of carbohydrate polymer, cellulose (38–50%), hemicellulose (23–32%), and the aromatic polymer lignin (15–25%) [1]. It is the most abundant raw material for the production of ethanol. Every year, 2×10^{11} mt of LC biomass is produced globally, 8–20 $\times$ 10^9 mt of which is potentially accessible for processing. Structurally, cellulose and hemicellulose are closely linked to lignin, making the polysaccharides inaccessible for hydrolysis by cellulases and hemicellulases. Cellulose is a linear polymer of D-glucose joined by β(1,4) glycosidic linkages with reducing and nonreducing ends. Cellulose fibrils are arranged in parallel stacks with hydrogen bonding and weak van der Waals forces, forming cellulose microfibrils. These cellulose microfibrils have both crystalline and amorphous regions that are bound together by hemicellulose and lignin to form macrofibrils. The second most important fraction of LC biomass is hemicellulose, a heteropolymer of pentoses (xylose and arabinose) and hexoses (glucose, galactose, and mannose). Xylan, a β(1,4) linked xylose homopolymer, is a major hemicellulosic component of hardwood trees, whereas softwood primarily contains mannans and glucomannans [2]. Lignin, which provides rigidity to plants, is a heteropolymer of *p*-hydroxyphenyl, syringyl, guaiacyl, and syringyl monolignol units, which form a complex network around cellulosic microfibrils. LC biomass can be grouped under three different categories: virgin biomass, energy crops, and waste biomass. All terrestrial plants such as trees, bushes, grasses, and crop plants are collectively termed as virgin biomass. Waste biomass is the low-value by-product of virgin biomass such as corn stover, sugarcane bagasse, and saw mill and paper mill wastes. Energy crops such as switchgrass, elephant grass, cassava, and sweet sorghum, which produce more biomass, are cultivated for use as raw materials in ethanol production.

Cellulose and hemicellulose can be hydrolyzed by holocellulases to sugars. These in turn can be fermented to produce biofuel. However, lignin is a polyphenolic compound that cannot be fermented. Agricultural crop residues, industrial and urban waste, forestry residues, and dedicated energy crops such as switchgrass, giant reed, *Miscanthus*, poplar, and willow are the most widely used and abundant LC feedstocks. The proportion of constituents of LC feedstocks varies with the type of feedstock used. The residue from cultivable land can include straw from agricultural crops such as paddy and wheat; groundnut shells; corn stover; sunflower stalks; cotton stalks; grass fibers; and agricultural by-products such as corn cobs, sugarcane bagasse, palm mesocarp fibers, sunflower, and barley hulls. Rice husks and wheat bran arising from the processing of agricultural commodities can also be used as a substrate for LC ethanol production [3,4,5,6]. Forestry waste includes wood chips, slashes, branches of dead trees, hardwood, softwood, and tree prunings [7]. Processing papers, household wastes, cotton linters, pulps, food processing waste, and wastes from fruit and vegetable processing are categorized as industrial and urban waste [8,9].

3. Processes of second-generation bioethanol production

Scientists across the globe have developed different processes for ethanol production from LC biomass (Table 1). These processes include SHF; SSF: simultaneous saccharification and co-fermentation (SSCF); consolidated bioprocessing (CBP); and simultaneous saccharification, filtration, and fermentation (SSFF). Every process has its own advantages and limitations, which are listed in Table 2.

3.1. SHF process

The SHF process is the oldest method used to produce LC ethanol. In this process, externally produced enzyme cocktails are used to hydrolyze pretreated LC biomass to yield sugar monomers. The