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Metabolic engineering and enzyme-mediated processing: A biotechnological venture towards biofuel production – A review

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ABSTRACT

Increasing concerns over the unstable and uncertain petro-based reserves, global warming and energy insecurity have led to a resurgence in the development of an environmentally-responsive greener approach for biofuel production. Exploration of renewable bio-resources for producing biofuels as well as industrially-commodity chemicals has become a universal research theme and attracted massive scientist's impetus over the last decade. Inarguably, lignocellulosic biofuel represents a renewable, sustainable, and the sole foreseeable alternative energy source to transportation fuels. Nevertheless, the lignocellulose recalcitrance postures technical hurdles to develop a cost-competitive bio-refinery process. To do this, the fuel-producing microbial hosts and their metabolic pathways must be engineered/tailored and optimized. Protein engineering has been used to amplify the biocatalytic performance of lignocellulose-modifying enzymes, but only a modest outcome has seen in bio-transforming the lignocellulose-to-biofuels than that of its great success in other industrial applications. Recent advancements in metabolic engineering and synthetic biology will provide novel tools for biotechnologists and metabolic engineers to create an efficient and well-performing biocatalyst with desired phenotypes for producing economically viable biofuels. The present review article describes various technical obstacles, advances, and bio-economic aspects in the research arena of lignocellulose to biofuel which is crucial in the future biofuel industry to respond inevitably accelerating oil prices and depleting oil assets.

1. Statement of problem and potential opportunities

The inclination for bio-based fuel ethanol production is increasing exponentially due to extensive urbanization, dwindling fossil resources, and accelerating public awareness for environmental threats caused by petro-fuel based greenhouse gas emissions [1]. Comprehending the necessity of modern world, the exploration of renewable resources abundantly available in the contemporary geographical location could be a state-of-the-art biotechnological landmark contributing to national bio-economy. Bio-based economy necessitated the consolidation of sustainability, obtainability, effective utilization, superior technological facets of bioenergy in an environmentally-friendlier manner to make the overall process economically-applicable [2,3]. Amid the existing fuels, lignocellulosic-derived ethanol is acquiring burgeoning researcher's impetus across the globe for its economic production from lignocellulosic biomass [4–9]. Inarguably, the theoretical bioethanol productivity from sugar and starch is superior to that produced from lignocellulosic feedstocks; these current feed-based resources are inadequate to satisfy the global fuel challenge. To harness the energy stored in the lignocellulosic biomasses, it is crucial to scrutinize biochemical composition of the biomass and implicate fewest operating steps for commercial product retrieval. The principle biochemical constituents of lignocellulosic feedstocks are cellulose, hemicellulose, and lignin. The long chain cellulose polymers along with hemicellulose and lignin are connected through hydrogen bonding and Van der Waals forces building a compacted micro-fibril [10]. Therefore, the disruption of lignin is indispensable for approaching the cellulose and hemicellulose modules, which in turn entails an appropriate pretreatment strategy.

Till to date, a significant number of physical, chemical or physiochemical biomass pretreatment approaches have been attempted. These techniques include but not limited to acid, alkali, ammonia fiber explosion, ionic liquid treatment, organosolv, ozonolysis, steam treatment, wet oxidation, and microwave irradiation. However, the high operating costs, the formation of inhibitors, fluctuated biomass heating upon irradiation, washing of the treated biomass, bio-abatement for inhibitors removal, loss of hemicelluloses content, and unreliable ethanol yield have compelled the scientific community to explore an

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alternative technology circumventing these disadvantages. Much sadly, the conventional biomass to ethanol transforming processes typically targets hexose sugars rendering an enormous amount of pentoses unutilized by the microorganisms. Worth to mention is that the lignocelluloses fluctuate in the proportion of celluloses to hemicelluloses and some predominantly encompass pentoses. In this avenue, the pentoses must also be assimilated in combination with celluloses to transform into commodity bioproducts including ethanol; this can intensify the thrust of the biotechnologist and scientific community for economic biofuels production [11]. The utilization of hemicellulosic sugar can considerably upgrade the overall productivity and yield of fuel ethanol.

In this perspective, enzymatic pretreatment "a green and environmentally-friendlier technology" is demonstrated to be valuable for recovering industrial biofuels production by effectively exploiting both hexoses and pentoses. Given lignin specific action without impeding the nearby carbohydrates (i.e., cellulose and hemicellulose), the application of ligninolytic consortia including lignin peroxidases (LiPs), manganese peroxidases (MnPs), laccase, and versatile peroxidase (VPs) have attracted the particular attention of researchers for bio-based ethanol production.

Besides the high sugar recovery, the generation of harmful by-products including furfurals, citric acid, formic acid, and succinic acid are also ignored through enzymatic delignification. This is advantageous for the following saccharification and fermentation steps since these inhibitors are unfavorable to the growth of yeasts used as fermentative organisms during fermentation. Moreover, the extremely mild working temperature and non-corrosiveness to the bioreactors on continuous operation additionally favor the implementation of ligninolytic pretreatment over non-enzymatic pretreatment practices [12]. Fig. 1 illustrates a comparative evaluation of bioethanol production strategies and a fermentative pathway following pre-treatments. The depiction of single step bioprocessing – "one-pot" pre-treatment, hydrolysis and fermentation" and multistep bioprocessing – hydrolysis and fermentation accomplished by engineered consolidated bioprocessing.

2. Lignocellulose – source with bioethanol potential

Lignocellulosic material (LCMs) manifests an attractive option as biomass feedstock for biorefineries given their obtainability, low cost, output/input energy ratio, and high ethanol productivities [2,5–8,13]. Exploitation of renewable biomass resources has received considerable attention for second-generation biofuel production in the today's world. Conversion of biomass to bioethanol process could help in alleviating global climate scenario by reducing CO_2 emissions along with decreasing fossil fuels dependency. Therefore, employments of bio-based resources are believed to play a fundamental role in the sustainable production of second-generation biofuels explicitly hydrogen, natural biogas, bio-oils, bioethanol, and biodiesel. In developing countries like Pakistan, massive amounts of agricultural, forests and agro-industrial residues are generated annually as wastes which do not find any alternative consumption and are either left in the fields or burned. Hence, these leftovers could be harnessed as potential alternative resources to produce a variety of industrially-relevant bio-products, in particular biofuels in an environmentally-friendlier way. Moreover, the agricultural crop residues have short harvesting period that presents them more consistently available resource to bio-based biofuel production [14]. A schematic illustration of lignocellulose processing strategy – from waste collection to market value is shown in Fig. 2 [3].

The chemical composition of lignocellulosic materials (LCMs) is a key factor affecting the efficiency of bioethanol production. Chemically, the cellulose, hemicellulose, and lignin are the main constituents of lignocelluloses that constitute up to 90% of dry matter in lignocelluloses [15]. Cellulose and hemicellulose are biological macromolecules composed of different sugars, whereas lignin is an aromatic

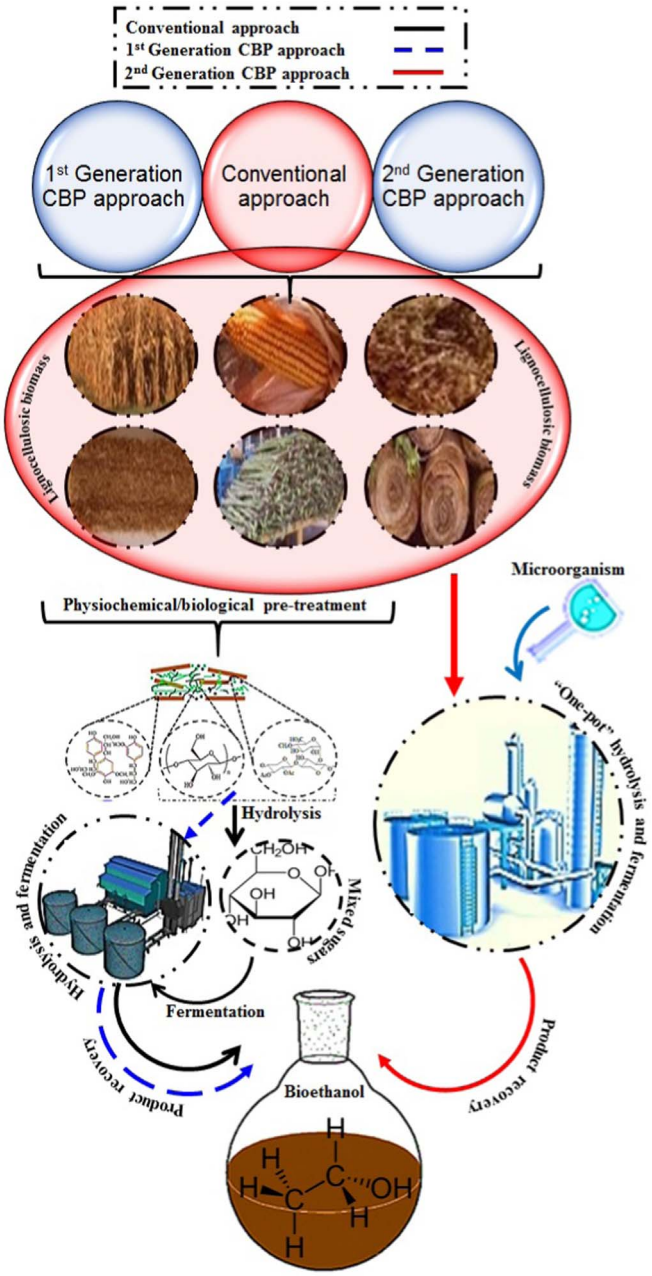

Fig. 1. Comparison of bioethanol production strategies and a predicted fermentative pathway following pre-treatments. Depiction of single step bioprocessing – "one-pot" pre-treatment, hydrolysis and fermentation" and multistep bioprocessing – hydrolysis and fermentation accomplished by engineered consolidated bioprocessing.

polymer containing three major phenolic components: p-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S). It is worth noting that bioethanol production is directly related to cellulose, hemicellulose, and individual sugar concentrations in the feedstock hydrolysate, while the lignin cannot be used for bioethanol production. A detailed compositional profile of various previously reported LCMs is summarized in Table 1 [8].

Its compositional complexity renders it recalcitrance to chemicals as well as microbial degradation and its tight association with cellulose and hemicelluloses also contribute degradation preventing sustenance to these biopolymers [46,47]. In the last decade, many significant efforts have been made to convert lignin-based substrates to value-added products including fine chemical, animal feed, pulp and paper, biofuels and enzymes. However, the conversion is hindered by the structural