Madras Agric. J., 2020; doi: 10.29321/MAJ.S.000453

REVIEW ARTICLE

Sustainable Utilization of Tropical Plant Biomass for Bioproducts, Biocatalysts and Biorefinery

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ABSTRACT

Burgeoning population, fastest depletion of fossil fuel resources, and alarming global climate change issues are the top most challenge for a country to prosper in energy, environmental and economic security. The most significant attention has been paid to renewable biomass to generate alternative fuels, bioproducts, and fine chemicals in a sustainable manner. However, the use of lignocellulosic biomass (LCB) as a sustainable feedstock does not assure a successful transition without a cost-competitive process designed with green chemistry. In this view, the concept of integrated biorefineries encompassing robust greener technologies for production, enhanced energy, material efficiency, reduced waste generation, and toxicity, and increased reusability of the end products are gaining attention. Hence, this article reviews the challenges, biomass conversion opportunities, optimized high-efficiency biocatalysts, and derived platform chemicals and fuels as a biorefinery approach. Above all, such a bio-based process will make wealth out of waste and, more importantly, the high-value products which would pave the way for the biobased economy of the nation.

Kewords*: Biomass; Biocatalysts; Bioproducts; Fuels; Platform chemicals*

It has been estimated that every year photosynthesis alone accounts for the conversion of 100 billion metric tons of CO_2 and H_2O to cellulose and other biomass products. Hence biomass driven industry is one of the fastest-growing sectors; their share in the global economy is increasing about 5-20%. The total world annual biomass production is estimated at 2740 quadrillion BTU's. At present, only about 7% of the annual production of biomass is used. The recent trends and patterns within the energy sector predict a remarkable increase in overall energy demand (over 60%) by 2030. There exists a daunting panorama of challenges with food availability and security as well as allocation of water and other natural resources. In this context, biomass has emerged as a highly attractive renewable source of chemicals, materials, and fuels addressing the fact ' wealth from wastes'

However, for the sustainable utilization of biomass for bioproducts generation, the incredible metabolic diversity and biochemical conversion power of microbes need to be explored. With the microbes, it is possible to produce either several gaseous (hydrogen and methane) or a large variety of liquid biofuels and fine chemicals using biomass as a substrate. Currently, biofuel production is minimal, accounting for only one per cent of global production. Supporting a future bioenergy sector will likely require policy support (such as stimulus packages), community and local interest, technological breakthroughs, and cost-effective feedstock production. In this context, the Government of India (GoI) has launched the biofuel road Map for vision 2030 to promote low carbon transport (LCT) in India with the aim of achieving a target of 20% blending of bioethanol or biodiesel (Purohit *et al.*, 2015). Consequently, the biofuel energy road map also aims to generate several other benefits like employment generation for the rural poor, regeneration of wastelands, reduction of emissions resulting from energy use that can lead to positive economic and environmental change. However, biomass conversion into renewable transportation fuels as a commercial reality is still illusive because of its feedstocks availability, complexity in biomass composition, non-availability of technologies, single microorganisms, or simplified methods for obtaining simple sugars from biomass, *etc*.

However, biomass processing schemes involving enzymatic or microbial hydrolysis commonly involved four biologically mediated transformations:

- the production of saccharolytic enzymes (cellulases and hemicellulases);
- the hydrolysis of carbohydrate components present in pretreated biomass to sugars;

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- the fermentation of hexose sugars (glucose, mannose and galactose); and
- the fermentation of pentose sugars (xylose and arabinose).

These four transformations occur in a single step in a process configuration called consolidated bioprocessing (CBP), which is distinguished from other less highly integrated configurations in that it does not involve a dedicated process step for cellulase production. The three most promising microbial species that have been developed by metabolic engineering in the last two decades are *Saccharomyces cerevisiae*, *Zymononas mobilis*, and *Escherichia coli*. Significant work on the metabolic engineering of *E. coli* has been completed with the incorporation and expression of pyruvate decarboxylase and alcohol dehydrogenase genes from *Z. mobilis* into *E.coli* results in high ethanol yield due to co-fermentation of hexose and pentose sugars.

With the increased utilization of biomass and biomass components, glycoside hydrolases (GHs) undoubtedly gain increased interest, not only because of their apparent action on the biomasses but also as these biocatalysts are tools applicable at processing conditions with good potential to be environment friendly. To achieve robust enough catalysts, thermostable variants of glycoside hydrolases are of interest, not only in degradation but also for processing to obtain specific carbohydrate-containing chemicals and materials.

GHs act on cellulose and hemicellulose components and have a great potential to degrade these materials (Li *et al*., 2006). Due to the necessity of thermal pretreatments, thermostable variants of GHs are of particular interest. The stability and reusing of the catalyst have always been major challenges in the development of biocatalytic reactions. Therefore, exploitation of thermophiles and thermostable enzymes offers several advantages owing to their robustness, better storage stability, better solubility of substrates/ products, lower viscosity, as well as a more favourable equilibrium in endothermic reactions (Uthandi *et al*., 2010; Vijayaraghavan *et al*., 2016).

The use of thermostable enzymes in the processing of biomass is highlighted, moving from the activities required to act on different types of polymers to specific examples in today's processing. Examples given involve

- monosaccharide production for food applications as well as use as a carbon source for microbial conversions (to metabolites such as fuels and chemical intermediates),
- oligosaccharide production for prebiotics applications
- treatment for plant metabolite product release, and
- production of surfactants of the alkyl glycoside class.
- Finally future possibilities in whole cell biorefining (Linares-Pastén, 2014).

Thermophilic bacteria such as *Bacillus tequilensis, Bacillus subtilis*, *Bacillus licheniformis,* and other *Bacillus* spp. were biprospected from hot springs of Manikaran (~95 °C), Kalath (~50 °C) and Vasist (~65 °C), The Himalayas, India by *in situ* enrichment method (Thankappan *et al*., 2017). The thermotolerant cellulases and xylanases identified tolerated up to 80°C and pH 7. The endoglucanase exhibited maximum relative activity of 108.2 and 112.4% in the presence of calcium and potassium ions. (Thankappan *et al*., 2018). Under the submerged condition, a thermophilic bacterial strain *B.aerius* CMCPS1 from paddy straw compost showed maximum filter paper activity (FPA) and en doglucanase activity of 4.36 IU mL^{-1} and 2.98 IU mL^{-1}, respectively, at 44 h. The saccharification efficiency of 55% was achieved with CMCPS1 multifunctional cellulases at 50 °C and pH 5.0. (Ganesan *et al*., 2020).

Similarly, thermophilic fungi are more efficient compared to bacteria, as they excrete more amount of GHs; however, maintenance of thermophilic fungi under laboratory conditions is challenging. Saranya and Sivakumar (2017) isolated thermophilic fungi *Chaetomium thermophilum* EDWF1 producing thermotolerant and alkali-tolerant endoglucanases, filter paper units, and beta-glucosidase from elephant dung. The GHs encoding genes from thermophilic fungi engineered in a suitable yeast-based vector system is a feasible technology for optimal and sustainable production of GHs from thermophilic fungi.

While bioprospecting endophytes for biomass conversion, perennial grasses are unique sources of glycosyl hydrolases. Endophytes from a C4 perennial grass *Neyraudia reynaudiana* L viz., *Bacillus tequilensis* BT5 and *Alcaligenes faecalis* B12 showed FPase, and beta glucosidase and xylanase activities (Vegnesh *et al*., 2019). A novel one-pot pretreatment technology by co-immobilizing laccase, cellulase, and β-glucosidase to act as a tri-enzyme biocatalyst, for bioethanol production potential of four sustainable lignocellulosic biomasses viz., *Typha angustifolia, Arundo donax, Saccharum arundinaceum, and Ipomoea carnea* was attempted by Muthuvelu *et al*., (2018).

For successful saccharification, the pretreatment process is primarily important. Physical, chemical and biological pretreatment processes are available based on the nature of the lingo-cellulosic biomass. Laccase, also called green catalysts, holds a critical role in the biological pretretament process. Archael laccase is thermostable, which finds application in biorefinereies, where recombinant DNA technology is implied for overproduction of laccase (Uthandi *et al*., 2010). Archaeal laccase production can be enhanced in recombinant *Escherichia coli* by modification of N-terminal propeptide and twin-arginine translocation motifs (Uthandi *et al*., 2012). In addition, a hyper laccase producing white-rot fungi, *Hexagonia hirta* MSF2 (944.44 U.ml^{-1}) delignified lignocellulosic biomass such as wood and corncob, to a level of 28.6 and 16.5%, respectively (Kandasamy *et al*., 2016). Likewise, *Pleurotus pulmonarius* BPSM10, a mushroom with enhanced laccase production also reported (Lallawmsanga *et al*., 2019). Furthermore, hydrodynamic cavitation reactor (HCR) coupled with laccase pretreatment showed 47% delignification efficiency in corn cob (Thankavelu *et al*., 2018).

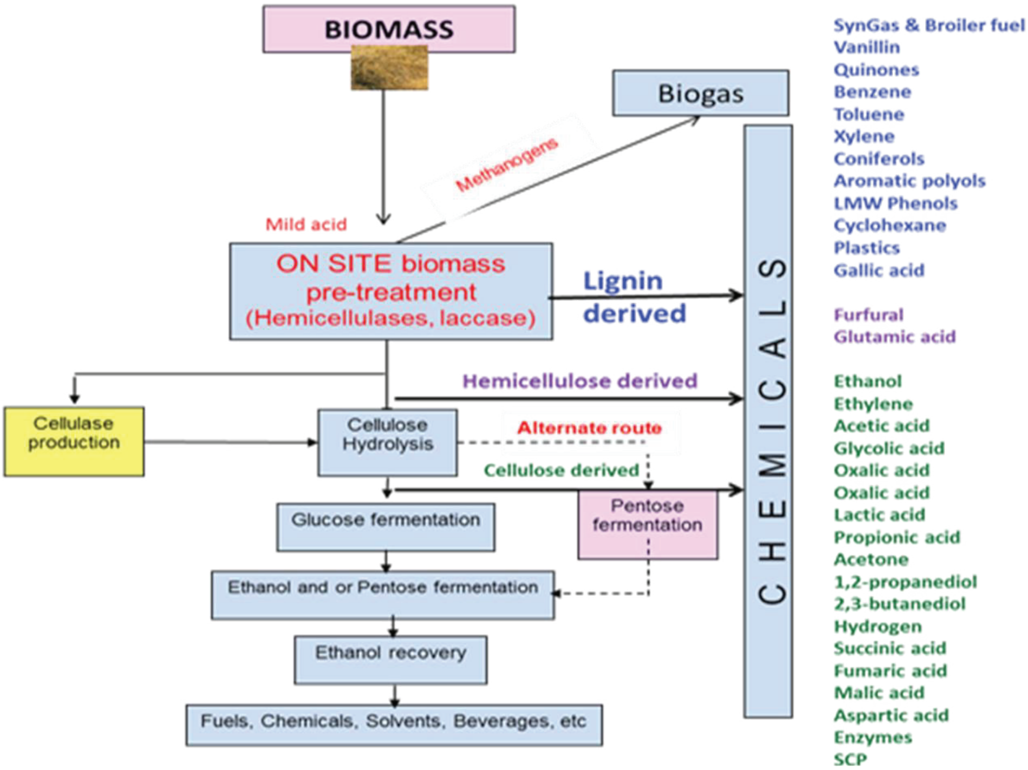

Figure 1. Lignocellulose biomass derived bioproducts, fuels, and chemicals

A part from major cell wall degrading biocatalysts (cellulases, hemicellulases and ligninases) a new arena of enzymes are being explored for efficient conversion of plant biomass towards the production of commodity chemicals. Lytic polysaccharide monooxygenases, a new class of oxidative cellulases with higher catalytic functions, are being tested. Similarly, in the depolymerization of xylan, xylanases, ferulyl oxidases and xylosidases are recently excavated for their potential. Biotechnological synthesis of the valuable chemicals derived from the lignocellulosic biomass, including ethanol, *n*-butanol, isobutanol, 2,3-butanediol, and lactic and succinic acids, are possible through different approaches, including SHF, SSF, SSCF, and CBP (Sorokina *et al*. (2018).

Depolymerization of the most recalcitrant polymer in plant cell wall i.e. lignin, *via* ß-etherases are reported to have multifunctional depolymerizing and higher catalytic efficiencies over laccase, lignin peroxidase, and manganese peroxidases. Biocatalysts with such wide diversity and novel catalytic properties are the need of the hour for bio-based economy. Nishanth and Uthandi (2018) succeeded in the enzyme and catalyst mediated depolymerization process. High-value Low weight monomeric (LWM) green chemicals such as eugenol, cinnamic acids, guiacol, etc are observed in the GC-MS spectrum of the depolymerized corn cob and birch wood biomass. Finally, at the industrial level, extremozymes are a towering impact in reduction of enzyme cost and pinnacling conversion efficiencies. We can call the biomass industry is going to be at its peak and continuously growing towards sustainability.

Another means of effective biomass feedstock utilization in large-scale applications will evolve from innovative research aimed at the development and implementation of biorefineries – multi-step, multi-product facilities established for specific bio-sourced feedstocks. The bioconversion platform can serve as the basis for full-fledged biomass-based biorefining operations, generating value-added bioproducts as well as fuel and energy. Development of novel routes to high-value aromatic chemicals and fine commodity chemicals will have wide applications in plastics manufacture, food industry and personal care industries. More importantly, converting surplus commodities to biofuels and/or bioproducts will create new jobs, increase commodity prices, increase farm income, improve the balance of trade, and reduce the country's dependency on imported fuel and chemicals paves the way for sustainable development of the nation.

Chemicals will have wide applications in plastics manufacture, food industry and personal care industries. More importantly, converting surplus commodities to biofuels and/or bioproducts will create new jobs, increase commodity prices, increase farm income, improve the balance of trade, and reduce country's dependency on imported fuel and chemicals thereby pave the way for sustainable development of the nation. On the other hand, Industrial biotechnology processes aim to be cost-competitive, eco-friendly, and self-sustaining compared to their petrochemical equivalents. Common to all processes for the production of energy, commodity, added value, or fine chemicals is that raw materials comprise the most significant cost fraction, significantly as operating efficiencies increase through practice and improving technologies. Specifically, bio-based ethanol as an alternative biofuel has emerged as the single largest biotechnology commodity and is a leading example of how systems biology tools have significantly enhanced metabolic engineering, inverse metabolic engineering, and protein engineering strategies. Ariyan and Uthandi (2019) engineered *E.coli* cells with a xylose reductase gene, converting xylose to xylitol from a mesophilic yeast *Candida tropicalis*. This enhancement stems from the method development for measurement, analysis and data integration of functional genomics, including the transcriptome, proteome and metabolome.

Algae for biofuels have been studied for many years for the production of hydrogen, methane, vegetable oils (triglycerides, for biodiesel), hydrocarbons and ethanol. The cultivation of microalgae for biofuels in general and oil production, in particular, is not yet a commercial reality and, outside some niche but significant, applications in wastewater treatment, still require relatively long-term R&D. In addition to biomass productivity and high oil content, one short-cut to the goal of algae biofuels development would be to co-produce algal biofuels, specifically, vegetable oils, with higher value products, or in wastewater treatment. This pathway of development would allow this technology to develop and mature to the point where the algae biofuels could become an ever more important component of such processes, and eventually even the main outputs. More recently, biodiesel production from industrial wastes is fascinating to researchers.

Eventually, the bioconversion platform should have the ability to serve as the basis for full-fledged biomass-based biorefining operations, generating value-added bio-products as well as fuel and energy. In addition, the development of novel routes to high-value aromatic chemicals will have wide applications in the cyclic economy and a conceptual model of using biomass substrate for various derived products are outlined in Figure 1. More importantly, success in bio-based economy endeavor could provide a leap forward concerning the low-cost conversion of renewable biomass into fuels as well as a variety of industrial chemicals with multiple applications, thereby realizing societal benefits.

CONCLUSION

The fore mosts constraints impeding the biomass conversion are robust, scalable technologies for a smooth transition from laboratory to commercial scale, including biocatalysts for biomass hydrolysis, biomass deconstruction technologies, and fermentation strategies that work on a biorefinery approach. Thermostable and pH stable cellulases isolated from these organisms from extreme environments have shown their potential under conditions that are appropriate for bioconversion processes, which have a role in industries. Their importance stems from the fact that cellulose swells at higher temperatures, thereby becoming easier to break down. GHs originated from microbes of extreme environments have unique characteristics such as temperature, chemical, and pH stability. They can be used to replace mesophilic enzymes or chemicals. The main advantages of performing processes at higher temperatures are reduced risk of microbial contamination, lower viscosity, improved transfer rates, and improved solubility of substrates, reduce the viscosity of the reaction mixture allowing the use of higher solids loadings, decreasing water demand. Thermophilic enzymatic combo with multifarious functions is a future demand for sustaining bio-refineries and bio-economy.

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