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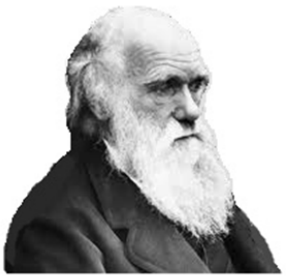

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Lignocellulosic biomass as potent feedstock resource for bioethanol production: Recent updates

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ABSTRACT

Non-renewable fossil fuels are unable to fulfil today`s requirements of the societies in terms of energy requirements. The increasing demands for energy have emphasized the researchers to search for alternative sources of energy. Among distinct alternative energy resources, bioethanol has attracted an immense attention worldwide. Currently, lignocellulosic biomasses are considered as the largest renewable resources for the production of bioethanol due to its maximum abundance on the earth. Pre-treated lignocellulosic biomasses are converted into bioethanol by both direct microbial conversion and hydrolysis process along with fermentation. Immobilization and nanotechnology have shown effective roles in the improvement of bioethanol from lignocellulosic biomasses. This review focuses on recent developments in bioethanol production from varied lignocellulosic biomasses as cheep feedstock.

***Keywords*:** Bioethanol, Fuel, Lignocellulosic biomasses, Renewable sources

1. INTRODUCTION

Since several decades, non-renewable fossil fuels, oil, natural gas, and coal have been the primary sources of energy production. Unfortunately, these sources are unable to fulfil today`s requirements of the societies. Moreover, the extensive applications of conventional energy resources are the leading causes of global warming and climate change by producing greenhouse gases (Kiran *et al*., 2014; García *et al*., 2019; Velázquez *et al*., 2020). The over-growing demands for energy due to rapid increase in world population have emphasized the researchers to search for alternative sources of energy.

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Among distinct alternative energy resources, biofuel or bioethanol has attracted an immense attention throughout the world. At present, bioethanol is the only alternative to gasoline that can be utilized without making any changes in the way fuel is distributed. Additionally, CO_2 produced during the bioethanol combustion is similar as that utilized by the plants in the atmosphere for its growth and metabolism, so it does not enhance the greenhouse production. Bioethanol has several applications in varied industries globally. Fuel for combustion processes, fuel for biofuel cells, feedstock for chemical companies, and fuel for cogeneration systems are some important applications of bioethanol. In addition, the role of bioethanol as alternative fuel to petrol in engines is one of its broad applications (Bušić *et al*., 2018). Researchers are looking different ways to improve the processes and yield of bioethanol using ideal and inexpensive feedstock for distinct purposes (Aarti *et al*., 2017; Aarti *et al*., 2018).

2. ETHANOL PRODUCTION: GLOBAL FUTURE SCENARIO

The production of ethanol in the world is projected to increase from about 120 billion (bln) L in 2017 to nearly 131 bln L by 2027. About 50% of increment is expected to originate from Brazil for filling the domestic demands. Thailand, China, India, and the Philippines are the other large contributors with 12, 10, 9, and 5% share, respectively in the global increment. The United States is expected to remain the major ethanol producer, followed by Brazil, China, and the European Union. Coarse grains and sugarcane are supposed to continue to be the prime feedstock for ethanol yield. Studies are expected to use 15 and 18% of global maize and sugarcane production, respectively for ethanol production in 2027. Biomass-based ethanol is projected to account for about 0.3% of world's ethanol yield by 2027. In the United States, ethanol production derived mainly from maize should remain around 61.6 bln L in the early years of the projection period. In the latter years, ethanol production in the United States should decrease to 60.4 Bln L with lower domestic and international needs related to decreasing gasoline demand in developed countries. Ethanol production in Brazil is projected to increase to 32.7 bln L in 2027.

China should consolidate its role as the third leading ethanol producer, with production reaching 11 bln L by 2027. Ethanol in China is expected to be produced domestically from maize using domestic stocks and from cassava. In the European Union, ethanol production using wheat, coarse grains, and sugar beet is projected to decrease to 7.1 bln L by 2027. Ethanol production using sugar beet is expected to stabilise around 1.4 bln L. In fact, ethanol production from sugar beet in the European Union should be less profitable than that of other cereal feedstock because of higher production costs. Ethanol production in Thailand is foreseen to improve by about 6% per annum. While productivity has been based particularly on molasses and cassava, sugarcane could enhance its share given the restricted accessibility of the other two feedstuffs to meet the increasing demands. Ethanol production in Thailand should reach 3.2 bln L by 2027. India is expected to increase ethanol production by 0.8 bln L, with approximately 95% of the total productivity coming from molasses.

Global ethanol use is projected to increase by about 12 bln L; 80% of this increment will take place in developing countries. Ethanol use in Brazil should expand by 5.4 bln L representing 42% of the global increase. Over the past few years, Thailand improved the use of ethanol by 1 bln L. Ethanol demand in India is foreseen to increase by 4.5% per annum, adding

a total of 0.7 bln L by 2027 with respect to the base period. Ethanol use in the United States is linked to mandate in place and limited by a marginally expanding blend wall as well as declining petrol use prospects. The share of ethanol (expressed in volume) in gasoline-type fuels should increase to 11.3% by 2027, but ethanol fuel use should decrease to 56 bln L. In the European Union, ethanol fuel use is expected to decrease to 5.1 bln L by 2027. This is due to decreasing gasoline use (OECD/FAO, 2018).

3. LIGNOCELLULOSIC BIOMASS AND PRE-TREATMENT

Second-generation bioethanol is playing a pivotal role globally in energy sector by utilizing inexpensive lignocellulosic wastes (Karagoz *et al*., 2019). The covalently cross-linked carbohydrate polymers (cellulose and hemicellulose) and non-carbohydrate polymers (lignin) are the major components of lignocelluloses, constituting 30–50% - cellulose, 20–40% - hemicellulose, and 10–20% - lignin. However, the concentrations of carbohydrates may vary based on the lignocellulosic biomasses used (Karagoz *et al*., 2019). Surprisingly, these lignocellulosic biomasses are recalcitrant in nature, and microorganisms are unable to degrade it (Aarti and Khusro, 2015; Ita, 2020; Debajyoti 2015; Erakhrumen, 2018; Saini, 2015).

The cross-linked fraction of lignocellulose materials is saccharified using distinct pre-treatment process. In fact, it enhances the accessibility and biodegradability of carbohydrate polymers prior to enzymes-based hydrolysis and fermentation process (Soltanian *et al*., 2020). In general, the pre-treatment process includes a variety of physical, chemical, physicochemical, and biological methods (Bilal *et al*., 2017).

4. BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASSES

Over the past few years, diversified lignocellulosic biomasses have been used as economic non-edible feedstock for the production of second-generation bioethanol (Branco *et al*., 2019). Recently, cotton stalk (Nikolić *et al*., 2016), corn stover (Dhiman *et al*., 2017), barley straw (Lara-Serrano *et al*., 2018), sugarcane bagasse (Cheng *et al*., 2019), and banana wastes (Khaliq *et al*., 2020) have been commonly used for producing bioethanol.

Gracilaria verrucosa (a red seaweed) was used for the production of bioethanol. The enzymatic hydrolysate on fermentation yielded 0.43 g of ethanol. Findings suggested that a biorefinery could be developed by maintaining the production of *G. verrucosa* (Kumar *et al*., 2013). In another study, Saha *et al*. (2014) estimated bioethanol yield of 0.333 mg/L from Pteris (a fern) biomass.

Pre-treatment of water hyacinth with dilute sulfuric acid at high temperature and pressure was used for bioethanol production. The maximum sugar yield (425.6 mg/g) through enzymatic saccharification was greatly influenced by the solid content, cellulase load, incubation time, temperature, and pH of the saccharifying medium. The statistical optimization design optimized an ethanol production of 13.6 mg/mL though a mixed fermentation process (Das *et al*., 2016).

da Silva *et al*. (2018) used carnauba straw to produce cellulosic ethanol by SSF process with three industrial yeasts. The biomass was pre-treated using hydrothermal, alkaline, and acid–alkaline methods. The alkaline pre-treatment showed maximum removal of lignin and hemicellulose. The SSF of the alkaline-pre-treated biomass produced 7.53 g/L of ethanol.

Yeh *et al*. (2016) produced ethanol from the fast-growing perennial grass *Miscanthus floridulus* by SSF process. The ethanol yields from 72-h SSF of *M. floridulus* biomass after different pre-treatments were 48.9±3.5, 78.4±1.0, 46.4±0.1, and 69.0±0.1% (w/w), while the ethanol concentrations after 72-h SSF were estimated to be 15.4±1.1, 27.5±0.3, 13.9±0.1, and 30.8±0.1 g/L. Overall, the highest amount of ethanol (0.124 g/g-dried raw material) was generated from dried raw material of *M. floridulus* after alkaline pre-treatment. The acid-catalyzed steam explosion pre-treatment also exhibited high ethanol yield (0.122 g/g-dried raw material).

Sudhakar *et al*. (2016) investigated the production of bioethanol from red seaweed spent biomass of *Gracilaria corticata* var *corticata*. Brown seaweed spent biomass and red seaweed spent biomass showed high content of sugar in 0.5 and 1% sulfuric acid pre-treatment, respectively. The ethanol yield from brown seaweed spent biomass and red seaweed spent biomass was observed to be 0.011 g/g and 0.02±0.003 g/g, respectively. Thus, the finding revealed the possibility of effective utilization of spent biomass from seaweed industry for ethanol production.

A multi-objective optimization of SSF process for cellulosic ethanol production was carried out to enhance the ethanol yield/cellulose conversion and reduce the enzyme consumption simultaneously by altering the initial sugar concentrations, and cellulose and enzyme loadings. Study showed significant performance enhancement in terms of ethanol yield, cellulose conversion, and enzyme loading. An overall 40% reduction of enzyme consumption per ethanol produced was attained at the same ethanol yield (32%) of a non-optimized process (Shadbahr *et al*., 2018).

In a study carried out by Fernandes *et al*. (2015), *Cynara cardunculus* (cardoon) was pre-treated by steam explosion for producing bioethanol through SHF and SSF processes. Steam explosion pre-treatment showed partial solubilisation of hemicelluloses and improved the accessibility of residual polysaccharides towards enzymatic hydrolysis. Bioethanol production in SSF mode was faster than SHF process with ethanol concentration of 18.7 g/L.

Tri *et al*. (2018) used NaOH solution (0.0–7.0% w/w) to determine its effect on bioethanol production from Japanese bamboo using the white rot fungus *Phlebia* sp. The pre-treatment showed a significant impact on the removal of lignin and xylan, causing an increased glucan composition for the pre-treated bamboo. The saccharification efficiency was increased from 41% in the initial sample to 89.5%. Bioethanol production by applying semi-SSF revealed the maximum conversion rate (58.9% in 7% NaOH pre-treated samples). However, after considering the weight loss of bamboo samples during pre-treatment, 1% NaOH pre-treated sample was observed as the maximum ethanol-producing efficiency with 38.1% conversion rate.

Keshav *et al*. (2018) focused on the effective utilization of cotton stalk for bioethanol yield. Steam exploded cotton stalk was used for the sequential acid and enzymatic saccharification. Subsequent enzymatic saccharification of steam exploded acid treated residue estimated 8.50±0.57 g/L sugar concentration with 84.20±0.34% combined saccharification efficiency after 72 h. The batch fermentation of mixed (acid-enzymatic) hydrolysate containing 45.74±1.68 g/L sugars released 19.08±0.56 g/L of ethanol with 0.47 g/g of ethanol yield.

The inulinase gene from *Kluyveromyces marxianus* (KmINU) was introduced into *S. cerevisiae* D452-2. The inulinase gene was fused to three different types of promoter (GPD, PGK1, and truncated HXT7) and secretory signal sequence (KmINU, MFa1, and SUC2) to generate nine expression cassettes. The inulin fermentation performance of the nine

transformants containing different promoter and signal sequence combinations for inulinase production were compared to select an optimized expression system for efficient inulin fermentation. Among the nine inulinase-producing transformants, *S. cerevisiae* carrying the PGK1 promoter and MFa1 signal sequence (*S. cerevisiae* D452-2/p426PM) showed maximum inulin fermentation capability. The batch fermentation of *S. cerevisiae* D452-2/p426PM in a bioreactor with 188.2 g/L of inulin produced 80.2 g/L of ethanol (Hong *et al*., 2015). In a similar kind of study, *S. cerevisiae* strain expressing β-glucosidase gene from *Humicola grisea* was utilized for bioethanol production using different cellulosic sources by SSF process. Using sugarcane bagasse pre-treated sample, crystalline cellulose, and carboxymethyl cellulose, 51.7, 41.7, and 13.8 g/L of ethanol were obtained, respectively at the end of the fermentation process (Ferreira *et al*., 2010; Kirti, 2019).

The SSF of steam-exploded corn stover (SECS) for ethanol production at high glucan loading and high temperature was studied by Liu *et al*. (2014). Findings suggested that high glucan loading and high temperature significantly enhanced the SSF performance of SECS using a thermal and ethanol-tolerant yeast strain. Furthermore, the inclusion of surfactant increased ethanol production in SSF process of SECS.

Dimos *et al*. (2019) investigated the impact of varied chemical and physicochemical pre-treatment methods on chemical composition of corn stalks and subsequent ethanol production by pre-hydrolysis and SSF process. Finding showed that the sequential combination of organosolv and hydrothermal pre-treatment showed maximum ethanol production (32.3 g/L) with an improvement of 32 to 50% in ethanol yield as compared to the other pre-treatments.

Nguyen *et al*. (2017) examined the pre-treatment, enzymatic saccharification, and fermentation of the red macroalgae *G. verrucosa* in order to enhance the bioethanol production. Ethanol productivity of 0.16 and 0.19 g/L with ethanol yields of 0.43 and 0.48 g were obtained using *S. cerevisiae* KCTC 1126 adapted to high concentrations of galactose and NaCl, respectively. Adaptation of *S. cerevisiae* KCTC 1126 to galactose or NaCl enhanced the ethanol yield via adaptive evolution of the yeast.

Dairy cow manure is an agricultural waste widely distributed worldwide. In view of this, Yan *et al*. (2018) investigated the induction of cellulases by cow manure and the conversion of cow manure materials into lignocellulosic ethanol. Alkaline NaOH pre-treatment increased the accessibility of cow manure lignocellulose to enzymes, followed by enzymatic hydrolysis using *Penicillium oxalicum* cellulases. The ethanol yields from pre-treated cow manure and anaerobically digested cow manure were estimated 0.19 and 0.13 g/g raw biomass, respectively using recombinant *S. cerevisiae* designed for lignocellulosic ethanol yield through SSF. Fed-batch supplementation with cellulases and substrates after initial enzymatic hydrolysis also showed ethanol yield of 25.65 g/L.

Muthuvelu *et al*. (2019) investigated the bioethanol producing ability of 4 lignocellulosic biomasses viz., *Saccharum arundinaceum* (hardy sugar cane), *Arundo donax* (giant reed), *Typha angustifolia* (narrow-leaved cattail), and *Ipomoea carnea* (pink morning glory). The maximum reducing sugar release of 185.00±1.57, 213.73±3.47, 187.57±2.14, and 294.08±3.98 mg/g and fermentation efficiency of 72.60±8.17, 82.59±7.42, 77.45±7.35, and 85.04±8.37% were estimated. Results showed that all these lignocellulosic biomasses could be utilized as an effective and sustainable source for the production of bioethanol. In another recent study, Patel *et al*. (2020) estimated 0.46 and 0.43 g/g of cellulose after 72 h of fermentation in alkali-treated and steam-exploded wheat straw, respectively.

In another study, sorghum husk was pre-treated with a white-rot fungus *Phanerochaete chrysosporium*. Enzymatic hydrolysis of untreated sorghum husk and biologically pre-treated sorghum husk produced 20.07 and 103.0 mg/g reducing sugars, respectively. Results showed a significant increment in reducing sugar yield in the pre-treated sorghum husk as compared to its untreated counterpart. The pre-treated sorghum husk hydrolysate was further fermented for 48 h using *S. cerevisiae*, *Pachysolen tannophilus*, and their co-culture which resulted in ethanol yield of 2.113, 1.095, and 2.348%, respectively (Waghmare *et al*., 2018).

Different lignocellulosic biomasses such as kans grass, sugarcane bagasse, and wheat straw were used for producing bioethanol. Results showed maximum production of bioethanol from kans grass with maximum yield of 67.28 g/L (Mishra and Ghosh, 2019). In another study, thermo-stable xylanase was produced from *Geobacillus* sp. and used to produce bioethanol from lignocellulosic biomasses (prairie cord grass and corn stover). Results showed 3.53 and 3.72 g/L of ethanol from prairie cord grass and corn stover, respectively (Bibra *et al*., 2018).

5. BIOETHANOL PRODUCTION FROM AQUATIC WEEDS

High cellulose, starch, and lipid content of aquatic weeds make them an auspicious feedstock for bioenergy production. Over the past few years, diversified research activities are being undertaken globally in order to produce biofuel from disparate aquatic biomasses.

E. crassipes (water hyacinth) is a well known aquatic weed due to its abundant availability, remarkable adaptive potential, and massive growth rate (Venkata Mohan *et al*., 2010). Unfortunately, suppressing the growth of water hyacinth is difficult. It is considered as a potent feedstock for both liquid and gaseous biofuel productions (Sindhu *et al*., 2017). Huang (2015) analyzed the net energy input in bioethanol production and CH_4 emission from switchgrass and water hyacinth through fermentation and anaerobic digestion, respectively. Results showed that the total energy input in CH_4 production from water hyacinth (1685.42 MJ/t of biomass) was lower than ethanol production from switchgrass (2034.92 MJ/t of biomass). This is because of the higher yield of water hyacinth (60-100 ton/ha/yr) with respect to switchgrass (12.9 ton/ha/yr) without the usage of chemical fertilizers. Water hyacinth fulfils the prerequisite as a potent raw material for biofuel production due to its easy availability and high lingocellulosic content (Rezania *et al*., 2015). Magdum *et al*. (2012) produced ethanol (19.2 g/L) from water hyacinth using *Pichia stipitis* NCIM 3497. In another study, the ethanol concentrations of 10.44, 8.24, and 6.76 g/L were obtained by the fermentation of its hydrolysate using *P. stipitis*, *Candida shehatae*, and *S. cerevisiae*, respectively (Das *et al*., 2015). Malveaux (1995) estimated 20.2 kg of sugar yield from 9.62 metric tons of water hyacinth dry biomass per day that can produce 1131.3 L of ethanol per day.

Duckweed is regarded as a potent feedstock for bioenergy production. It is one of the most abundant and smallest plants in the world with a total of 37 species (Landolt, 1986). It has also been widely used in the treatment of industrial and municipal waste water due to its resistivity towards high nutritional level (Venkata Mohan *et al*., 2010). It has comparatively higher specific growth rates than other larger aquatic plants. Perniel *et al*. (1998) estimated an ethanol yield of 30.8±0.8 g/L from *Landoltia punctuate* biomass. Study also showed that duckweed is an ideal starch resource for bioethanol production (Cheng and Stomp, 2009). Zhao *et al*. (2015) demonstrated the influence of high loading concentrations of duckweed biomass

on bioethanol production and observed that high biomass loading (20% w/v) reduced the ethanol yield to 18.8% as compared to ethanol yield of 80% at low biomass loading.

Azolla (water fern) is a genus constituting 7 species and found in wetlands, ponds, and ditches. It is one of the fastest growing weeds that double its biomass every 5–7 days (Kollah *et al*., 2016). *Azolla* spp. are considered a potent substrate for biofuel production due to its lignocellulosic composition. Miranda *et al*. (2016) reported ethanol yield of 0.09 g/g from *Azolla*. The ethanol yield of 2.0 g/L was estimated from the fermentation of salvinia using *S. cerevisiae* and *S. carlsbergensis* (Muhammad *et al*., 2013).

Typha constitutes several carbohydrates in its leaf, stem, and root, which is considered a good source for ethanol production. Study reported the highest glucose yield (97% of the cellulose) after pre-treatment and enzymatic hydrolysis of *Typha* which estimated a theoretical ethanol yield of about 90% (Zhang *et al*., 2011). Rahman *et al*. (2015) adopted a green refinery method and used *Typha* sp. biomass for ethanol production.

Pistia stratiotes or water lettuce is a noxious perennial aquatic plant whose growth rate is similar to the water hyacinth (Mishima *et al*., 2008). The water lettuce contains carbohydrate - 49.45%, protein - 16.47%, fat - 3.56%, and crude fiber 17.81% which indicated its utilization for bioenergy production. Mishima *et al*. (2008) reported 14.9 g/L of ethanol using water lettuce as feedstock.

6. BIOETHANOL PRODUCTION USING IMMOBILIZED ENZYMES AND CELLS

Immobilization technique is widely used in biotechnology for the physical and chemical fixation of cells and enzymes (Ahmed *et al*., 2008). The technique uses various natural support materials for cross-linking of cells and enzymes (Osho *et al*., 2014). Reusability, non-toxicity, mechanical strength for necessary support, and open spaces within the matrix for growing of cells are some of the major benefits of this technique (Akhtar *et al*., 2004). Several methods such as entrapment, encapsulation, cross linking, and adsorption are used for immobilization (Martins *et al*., 2013).

Immobilization techniques are beings extensively used for the production of bioethanol. Various yeast cells and hydrolytic enzymes are immobilized on solid supports and non-porous materials. In facts, hydrolytic enzymes play pivotal roles in distinct areas (Khusro and Aarti, 2015; Khusro, 2015; Khusro, 2016). Researchers demonstrated that when enzyme is attached on non-porous materials, the diffusion of enzyme is less. On the other hand, when enzyme is immobilized on the porous materials, loading of enzyme is higher, but diffusion is very slow (Hartmann and Kostrov, 2013). Immobilization of enzymes and cells can improve the stability, storage, and reusability. It can also enhance the production of sugar by degrading cellulosic content (Husain, 2017; Aarti *et al*., 2020).

Bioethanol was produced from corn straw by immobilizing cellulolytic yeasts on *Mucuna urens*. As per the results obtained, bioethanol production was maximum with 4 mm bead size, 10% substrate concentration, pH 4.5, and 10% inoculum load. Maximum ethanol production (55.27 g/L) was obtained by immobilized *Saccharomyces diaststicus* (Adelabu *et al*., 2019). In another study, *S. cerevisiae* var. *ellipsoideus* yeast cells were immobilized for producing bioethanol from corn meal hydrolysate. The maximum ethanol concentration of 10.05% (w/w) was obtained in the fermentation of corn meal hydrolyzates by 5% (v/v) of inoculum concentration of the yeast immobilized in calcium alginate (Rakin *et al*., 2019).

Duarte *et al*. (2013) immobilized *S. cerevisiae* cells in calcium alginate and chitosan-covered calcium alginate beads and investigated their roles in the production of bioethanol by fermenting glucose and sucrose. The final amount of ethanol using free cells was 40 g/L and the yields using glucose and sucrose as carbon sources were obtained 78 and 74.3%, respectively. For immobilized cells in calcium alginate beads, the final ethanol content from glucose was estimated 32.9±1.7 g/L with 64.5±3.4% yield, while the final ethanol level from sucrose was 33.5±4.6 g/L with 64.5±8.6% yield. For immobilized cells in chitosan-covered calcium alginate beads, the ethanol content from glucose was estimated 30.7±1.4 g/L with 61.1±2.8% yield, while the final ethanol content from sucrose was estimated 31.8±6.9 g/L with 62.1±12.8% yield. Eiadpum *et al*. (2012) reported that a co-culture of *K. marxianus* and *S. cerevisiae* immobilized on thin-shell silk cocoon was effective for ethanol production at high temperature, releasing ethanol content of 81.4 and 77.3 g/L with an initial sugar quantity of 220 g/L.

The SHF and SSF processes were implemented for producing bioethanol from microalgal biomass. SSF was selected to improve the bioethanol productivity using immobilized yeast cells. A bioethanol yield of 0.5 g/g and volumetric productivity of 0.22 g/L/h was obtained after 48 h of SSF. Immobilized yeast cells enabled repetitive production of ethanol for 7 cycles. The maximum ethanol yield of 9.7 g/L was estimated in 2^{nd}-4^{th} cycles (El-Dalatony *et al*., 2016).

The dried sugar beet pulp-immobilized biocatalyst was utilized for repeated ethanol fermentation. Repeated batch ethanol fermentation of thick juice by yeast immobilized on hydrated dried sugar beet pulp was successfully carried out for 7 successive cycles. A maximum ethanol content of 52.26±2.0 g/L and ethanol yield of 0.446±0.017 g/g was obtained in the 7^{th} fermentation batch (Vučurović and Razmovski, 2012).

Dussán *et al*. (2019) demonstrated bioethanol production from sugarcane bagasse hydrolysate using immobilized *Scheffersomyces shehatae* on magnetic bio-supports in a fluidized bed bioreactor. The ethanol/substrate yield and ethanol productivity were estimated 0.15±0.8E-3 g/g and 0.055 ± 0.3E-3 g/g, respectively.

Elbashiti *et al*. (2017) produced ethanol from tomato waste and wheat straw using free and immobilized yeast cells in calcium alginate beads with microwave-assisted acidic pre-treatment of the lignocellulosic materials. The maximum amount of ethanol of 641 mg/g was produced by free cells when pre-treated straw was used. On the other hand, 543.5 mg/g of ethanol was estimated from pre-treated tomato waste using immobilized cells.

7. NANOTECHNOLOGY IN BIOETHANOL PRODUCTION

At present, the high production costs and other technical barriers are the major concern of the biofuel industries. In this regard, nanotechnology is gaining interest considering the environmental and economic issues. Nanotechnology is one of the most important fields in modern science because it works at molecular and cellular levels. Over the past few years, the applications of nanobiotechnology in bioenergy sector have increased. Nanoparticles show unique traits with respect to the bulk materials as they are small enough to confine their electrons and produce quantum effects (Verma *et al*., 2013; Khusro *et al*., 2020a; Khusro *et al*., 2020b).

Application of nanoparticles helps in enhancing the efficacy of pre-treatment, enzymatic hydrolysis, and the fermentation process. The particle size, morphology, surface area, nature of

nanoparticles, and type of biomass utilized are important parameters to generate end products and manage the effectual control of reaction rate (Chaturvedi *et al*. 2012). Metal nanoparticles penetrate the cell wall of biomass because of their small structure. Razack *et al*. (2016) obtained about 15.26% of the total carbohydrate yield from *Chlorella vulgaris* biomass [150.l g/g of silver nanoparticles prepared through biological method]. Pena *et al*. (2012) used acid-functionalized (perfluoroalkylsulfonic and alkylsulfonic) magnetic nanoparticles for the pre-treatment of wheat straw at different temperatures.

Several studies demonstrated the application of metallic nanoparticles as co-factor for improving the enzymatic stability and immobilization of enzymes onto a support material for improved enzymatic activities (Srivastava *et al*., 2016). Enzyme immobilization over the nanomaterials reduces the processing cost because of their easy recovery process and reusability (Mohamad *et al*., 2015). Process efficiency is improved using varied nanoparticles as these particles provide large immobilization surface to enzymes, prolong self-life, and stability (Kim *et al*., 2006). Srivastava *et al*. (2015) reported that iron nanoparticles (Fe_3O_4) and nanocomposites (Fe_3O_4/alginate), both efficiently enhanced the enzyme activity and stability by providing ideal support for enzyme immobilization. Fe_3O_4 nanoparticles and uniquely structured nanocomposites were prepared by co-precipitation method for its use in bioethanol yield.

Jordan *et al*. (2011) showed the immobilization of cellulase onto magnetic nanoparticles for bioethanol production. Hermanova *et al*. (2015) covalently immobilized *Rhizopus oryzae* associated lipase onto graphene oxide nanoparticles and showed that it possessed high solvent resistivity and temperature stability and an improved activity. In fact, the covalent binding of certain enzyme onto the support matrix improves the self-life of the enzyme, and its reusability decreases the processing cost. Graphene-based nanomaterials provide large surface area for immobilization of enzymes and catalytic site for ethanol oxidation (Kakaei *et al*., 2016). Enzyme immobilization onto the graphene oxide surface could take place without using any cross-linking reagent and surface modification, and does not affect the thermal and solvent reseistance traits of enzymes (Hermanova *et al*., 2015).

Different metallic nanoparticles viz. oxides of iron, cobalt, copper, manganese, etc. act as promising catalytic materials for the production of renewable energy. Kim *et al*. (2014) estimated improved bioethanol production (166.1%) with methyl-functionalized silica nanoparticles in syngas fermentation. In this investigation, authors used varied nanoparticles (palladium on carbon, palladium on alumina, silica, hydroxyl-functionalized single-walled carbon nanotubes, alumina, and iron oxide). Among these, silica nanoparticles proved their effectiveness for increased gas–liquid mass transfer that was further modified with hydrophobic functional groups (methyl and isopropyl) to enhance the activity. Kim and Lee (2016) utilized methyl-functionalized magnetic nanoparticles to enhance bioethanol production during syngas fermentation, and about 213.5% higher production was achieved using methyl-functionalized cobalt-ferrite-silica ($CoFe_2O_4$@SiO_2-CH_3) nanoparticles.

Ivanova *et al*. (2011) demonstrated enhanced bioethanol production using alginate/magnetic nanoparticles (with entrapped yeast cells) covalently immobilized on chitosan-magnetite microparticles and cellulose-coated magnetic nanoparticles. Results showed approximately 91% of ethanol yield with the yeast cells entrapped in matrix of alginate/magnetic nanoparticles and immobilized on magnetite-containing chitosan. Santos *et al*. (2016) used glassy carbon electrode modified with graphene oxide constituting copper nanoparticles for the determining sugars production and obtained better accuracy and

reusability. Lin *et al*. (2016) synthesized ultrathin two-dimensional polycrystalline zinc oxide nanosheets with uniformly dispersed silver nanoparticles to enhance the surface reaction for ethanol production.

Abraham *et al*. (2014) immobilized cellulase on magnetic nanoparticles which enhanced the enzymatic saccharification of pre-treated hemp biomass. Miao *et al*. (2016) synthesized magnetic chitosan microsphere by anchoring iron oxide nanoparticles on the surface of chitosan nanoparticles. *Aspergillus niger* associated cellulase was immobilized on β-cyclodextrin conjugated magnetic nanoparticles (Huang *et al*., 2015). The immobilized cellulose released higher amount of glucose as compared to the free cellulose. Sulfonated magnetic carbonaceous acid nanoparticles were used for the hydrolysis of jatropha, bagasse, and plukenetia hulls and revealed significant rate of bioconversion (Su *et al*., 2015). In another study, cellulase immobilized on magnetic nanoparticles was utilized as a nanobiocatalyst to hydrolyze *Sesbania aculeate* biomass which estimated 5.31 g/L of bioethanol (Baskar *et al*., 2016). Iron oxide nanoparticles were synthesised using cell filtrate of *Alternaria alternate* and cellulase was immobilized on it which showed high rate of cellulose conversion (Ingle *et al*., 2017).

Cherian *et al*. (2015) immobilized cellulase onto manganese oxide (MnO_2) nanoparticles, which improved the activity of cellulase and offered a better support. Cellulase immobilized on MnO_2 nanoparticles hydrolyzed cellulosic substances over a broad range of temperature and pH. Results confirmed that cellulase immobilized on MnO_2 nanoparticles exhibited pronounced cellulolytic activity. Mishra and Sardar (2015) synthesized of silver and gold nanoparticles using cellulase. Cellulase-assisted synthesized nanoparticles were further exploited as immobilization matrix for cellulase.

Thermal stability analysis revealed that the immobilized cellulase on synthesized gold nanoparticles retained about 80% activity as compared to free enzyme. In a different study, Sanusi *et al*. (2019) investigated the effect of different metallic oxide nanoparticles on ethanol production by *S. cerevisiae*. Ethanol concentrations decreased at higher concentrations of nanoparticles used. Ethanol production was mainly enhanced by Fe_3O_4 nanoparticles with a maximum ethanol yield of 0.26 g/g. Further, the addition of NiO and Fe_3O_4 nanoparticles in SSF process improved ethanol production from potato peels by 1.6 and 1.13-fold, respectively. Findings suggested the appropriate uses of NiO and Fe_3O_4 nanoparticles in bioethanol production from agro wastes. In a recent study, Sanusi *et al*. (2020) optimized ethanol yield using nickel oxide nanoparticles as a biocatalyst. The optimized process showed a biomass concentration and ethanol yield of 2.04 g/L and 0.26 g/g, respectively.

8. CONCLUSION

Lignocellulosic biomasses are considered as the largest renewable resources for the production of bioethanol due to its maximum abundance on the earth. Pre-treated biomasses are converted into bioethanol by both direct microbial conversion and hydrolysis process along with fermentation. The immobilization process enhances the productivity of the biocatalysts and improves their features for varied applications in bioethanol production. Roles of nanomaterials in the biofuel industries have provided new directions to the energy sector. However, a lot more investigations are required in this era to evaluate the effective role of different nanoparticles in biofuel industries.

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