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Review Article

Current Trends in Lignocellulosic Bioethanol Production

Pradip Dhungana*, Bikram Prajapati, Sujeeta Maharjan, Jarina Joshi

Central Department of Biotechnology, Tribhuvan University, Nepal

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***Corresponding author**
Pradip Dhungana,
Central Department of Biotechnology, Tribhuvan University, Nepal.
Email: pradip.dhungana1993@gmail.com

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Abstract

In view of crude oil prices, and its environmental issues, utilization of sustainable renewable alternative energies such as biofuels is rapidly progressing in many countries. The increasing global energy demand and depleting fossils fuels sources has led to search alternative clean and renewable fuels. One of the best alternatives to the gasoline is lignocellulosic bioethanol. Recent researches on lignocellulosic bioethanol focuses on advancement of pretreatment techniques for improved sugar yields and decreased inhibitors production. Pretreatment technique with no or less use of chemicals and cost effectiveness is the main purpose of most of the researches. Biological pretreatment techniques produce less fermentation inhibitors than chemical pretreatments. In order to cope with fermentation inhibitors different strategies can be adopted during pretreatment processes. In the course of time, advancements in production process over separate hydrolysis and fermentation have been introduced. Simultaneous saccharification and co/fermentation; and consolidated bioprocessing for bioethanol production are gaining popularity among researchers.

Abbreviations:
SHF: separate hydrolysis and fermentation
SSF: simultaneous saccharification and fermentation
SSCF: simultaneous saccharification and co-fermentation
CBP: consolidated bioprocessing
HMF: hydroxymethyl furfural

Keywords: lignocellulosic biomass; pretreatment techniques; fermentation inhibitors; bioethanol production

Introduction

Initially automobiles were designed to work with ethanol. However, in the early 20th century, discovery of new oil fields, their abundance and low oil prices resulted engines modification giving priority to oil derivatives as fuels. Then gasoline became the basic fuel for cars all over the world. But, in Brazil since 1923, ethanol blends have been used in variable proportions in gasoline (Amorim & Lopes, 2005). The oil crisis in 1970 and 1979, contributed to produce the first car to run on ethanol only, for the Brazilian market. This resulted increase in ethanol consumption and significantly reduced oil dependence. However, factors involving oil price drops, reduction of subsidies to producers and rise of sugar prices contributed to biofuel shortage that led to a major downturn in the demand for ethanol-run cars. At the beginning of 21st century, high oil prices in the international market and the development of flex-fuel technology motivated the use of ethanol as fuel (Lopes *et al.*, 2016). At present, due to progressive diminishing of petroleum reserves, dependence of many countries on import of this raw material and negative consequences of its use; use of ethanol for fuel purposes is regaining significance (Joanna *et al.*, 2019).

Sustainable clean energy and climate change issues are the center of attraction in today's world. Many different legislations and measures are adopted at local and international level to improve the clean energy technology and reduce the dependence on fossils fuel. For this, lignocellulosic biomass is cheap and widely available substrate to generate cellulosic sugar and then to biofuels (Raud *et al.*, 2019). Bioethanol was identified as the most sustainable source of biofuel as renewable energy that can be easily maintained for which the viable feedstock resources are cassava, sugarcane, plant seed and waste materials (Adewuyi, 2019). Lignocelluloses hold considerable potential to meet the current energy demand of the modern world. It is also essential to overcome our excessive dependence on petroleum for liquid fuels that cause environmental pollution or global climate change (Rastogi & Shrivastava, 2017). Annual bioethanol production of the world is increasing per year. In 2016, total annual bioethanol production in the world was 26,000 million gallons, which increased to 29,000 million gallons by 2019. United States is the leading country in terms of bioethanol production, which alone produces 15,800 million gallons in 2019 (RFA, 2021).

Challenges and Advancements in Bioethanol Production

Production of bioethanol requires a multistep process that significantly increase the cost of biofuels produced. Finding fast, cheap, and efficient processes is the main challenge for the production of bioethanol (Carrillo *et al.*, 2019). Biomass complexity, harvesting, and transportation of biomass from farm to factory are challenges to produce bioethanol at large scale. Smooth biomass supply chain is most important for success of a biorefinery (Sultana *et al.*, 2010). The collected biomass usually has contaminants, stone and soil, which can affect the overall process in terms of sugars recovery, inhibiting the pretreatment reaction and choking the reactor lines. In addition, these issues greatly impact on operational and capital cost for the overall biomass conversion process (Antunes *et al.*, 2019). In general, majority of feedstock needs to be designed to have maximum uniformity in biomass and its smooth transfer to reactors from pipelines (Chandel *et al.*, 2018).

Besides structural modification in biomass, removal of hemicellulose and lignin is a goal of pretreatment technologies to turn the carbohydrate fraction accessible for enzymatic hydrolysis. Each pretreatment method has its own advantages, but also challenges to be overcome, as discussed in Table 1. Biological pretreatment methods are green and eco-friendly, but they are inherently slow and difficult to scale up (Antunes *et al.*, 2019). A key challenge associated with kitchen waste as substrates for bioethanol production is the varied constituents of daily wastes production, that makes difficult in appropriate choice of pretreatment (Hafid *et al.*, 2017). Other challenges in renewable energy production includes Land tenure system, high production costs, weak governmental policies and competition between biofuel feedstock and food (Adewuyi, 2019).

Developing new strategies towards maximal liberation and utilization of sugars from agricultural waste for cost effective ethanol production is an utmost requirement (Rastogi & Shrivastava, 2017). Biofuel price and production cost in China indicate that it might not be economically efficient to produce biofuels from non-food crops (Chen *et al.*, 2016).

A case study by Mamadzhanov et al (2019) on use of bioethanol among Korean public conclude consumers who are knowledgeable about renewable energy sources are more likely to purchase second-generation ethanol. In the similar manner, participants who put more value to environmental friendliness of fuel over its price are more willing to pay for the product.

Although bioethanol production cost is lower than the gasoline production cost, higher fuel consumption ratio for bioethanol fuel results in equal driving cost for both E10 and conventional gasoline but E85 provides 23% lower driving cost (Daylan & Ciliz, 2016). The production of sugarcane-based bioethanol could support transport energy demand and increases energy security by decreasing dependence on fuel imports (Gutierrez *et al.*, 2020). Use of bioethanol as a transport fuel can potentially reduce greenhouse gas emissions with respect to conventional gasoline (Daylan & Ciliz, 2016).

In spite of advancements in various pretreatment methods which results in maximum sugar yield with minimum inhibitors production, still there is a huge scope for improved biomass pretreatment and detoxification-based innovations. There is still a need for cost-effective methods that can offer maximum sugar yield with minimum inhibitory compounds, energy, and chemical consumption (Bhatia *et al.*, 2020).

Pretreatment Technologies

Major components in lignocellulosic biomass are cellulose, hemicellulose, and lignin that forms a complex structure. This complex structure results in impermeability of lignocellulosic biomass towards mechanical and biological degradation. However, to provide better accessibility to the components to be converted into useful fermentable sugars, pretreatment of biomass is done. Biomass conversion into sugars could only be achieved through the removal of hemicellulose and lignin, reduction in the crystallinity of cellulose, as well as the increase of porosity and specific surface area of biomass structure (Loow *et al.*, 2016).

Mechanical Pretreatment

The most influential factors for physical pretreatment are biomass characteristics and final particle size. Meanwhile,

high power consumption and usually low efficiency are the main drawbacks (Seidl & Goulart, 2016). There are various types of physical pretreatments such as milling, high pressure homogenization, electron beam irradiation, hot compression and photo-catalysis (Rezania *et al.*, 2020). Mechanical pretreatment of lignocellulosics is usually performed by chopping and milling. This reduces the particle size of lignocellulosic biomass by disrupting their surface structure, shear or compression forces (Tsapekos *et al.*, 2017). The milling method increases enzymatic hydrolysis and digestibility (Rezania *et al.*, 2020).

Chemical Pretreatment

Alkaline Pretreatment:

Alkaline solutions such as sulfite, sodium hydroxide, ammonium hydroxide, and lime have been used. Based on the literature, sodium hydroxide is the most used alkaline solution as it is effective for delignification and works in various conditions (Rezania *et al.*, 2020). Alkaline treatment disrupts the ester and glycosidic side chains causing alteration in lignin structure, cellulose swelling and its partial decrystallization and partial solubilization of hemicelluloses (Rastogi & Shrivastava, 2017).

Acid Pretreatment

Various concentrated or dilute inorganic and organic acids were assessed to hydrolyze lignocelluloses. These aids are used as catalysts (Rabemanolontsoa & Saka, 2016). Dilute rather than concentrated acid is used either at high temperature for short time period or lower temperature for longer retention time to solubilize hemicelluloses and lignin (Rastogi & Shrivastava, 2017). Dilute-acid processes require high temperatures to achieve acceptable cellulose to sugar conversion rates. The high temperatures increase the rates of sugar decomposition and equipment corrosion (Jones & Semrau, 1984). Acid pretreatment results formation of inhibitory compounds like furfural, 5- HMF, phenolic acids and aldehydes (Rastogi & Shrivastava, 2017).

Physico-Chemical Pretreatment

Physico-chemical processes are improvements of the established chemical processes, to diminish reaction time and improve efficiency (Rabemanolontsoa & Saka, 2016).

Physico-Chemical Alkaline Pretreatment:

Physical parameters were added to the chemicals alkaline pretreatment in order to enhance lignin removal and increase efficiency. Alkali agent with high pressure creates a physico-chemical alteration in biomass structure. As like ordinary alkaline pretreatment, there is almost no sugar loss and results in decrystallization cellulose, partial depolymerization of and removal of acetyl group from hemicellulose, cleavage of lignin-carbohydrate complex linkages and the C–O–C bonds in lignin. The pressurization increases the surface area and the wettability as compared to regular alkaline hydrolysis (Rabemanolontsoa & Saka, 2016).

Ammonia Fiber Explosion:

Ammonia fiber explosion (AFEX) is similar to steam explosion being liquid ammonia at high temperature (90–100 ^{o}C) for several minutes. High pressure and temperature allow for sudden expansion of ammonia that causes swelling and physical breakdown of biomass structure and partial reduces the decrystallization of cellulose and lignin (Laureano-Perez *et al.*, 2005). The ammonia can be recycled and nitrogen source is not required for subsequent microbial fermentation. But only slight amounts of hemicellulose and lignin are dissolved during the pretreatment (Kim, 2018).

Ionic Liquid:

Ionic liquid has gained more attention due to high solubility of biomass in it resulting high yield of sugars and carbohydrates. It enhances enzymatic delignification by maintaining cellulase activity and stability. Temperature and biomass loading affect the hydrolysis rate (Elgharbawy *et al.*, 2016). Ionic liquids are expensive and require tedious recycling, and their toxicity and biodegradability are not yet well understood (Rabemanolontsoa & Saka, 2016).

Hydrothermal Pretreatment

Hydrothermal treatment is the reaction occurring under the conditions of high temperature and high pressure in aqueous solutions in a closed system. It includes steam explosion, supercritical/subcritical water and hot-compressed water treatments depending on the conditions of temperature and pressure (Rabemanolontsoa & Saka, 2016).

Stem Explosion:

It is the most commonly used method for hydrothermal pretreatment of woody biomass. Biomass exposed to hot steam under high pressure followed by sudden release in pressure cause auto hydrolysis of acetyl group of hemicellulose. This disrupts the cell wall structure and individual fibers get separated (Rastogi & Shrivastava, 2017).

Supercritical and Subcritical Water:

Supercritical water treatment provides high yield of hydrolyzed products but part of the sugar is lost due to cellulose fragmentation. On the other hand, cellulose under subcritical water is likely to be less decrystallized. A combined supercritical and subcritical treatments were therefore developed and increased the yields with fewer degradation products (Saka & Ehara, 2004).

Hot-Compressed Water or Liquid Hot Water:

Liquid hot water is a milder condition compared to sub and supercritical water. Liquid hot water process primarily solubilizes hemicellulose and lignin, and exposes internal cellulose contents, increasing enzyme-cellulose accessibility (Rezania *et al.*, 2020).

Table 1: Advantages and disadvantages of common pretreatment methods

Pretreatment strategy	Advantage	Disadvantage	References
Mechanical	Increases enzymatic hydrolysis and digestibility	High power consumption and low efficiency	(Rezania *et al.*, 2020; Seidl & Goulart, 2016)
Alkaline	Selective lignin removal, no reducing sugar loss, improve enzymatic hydrolysis and, increases surface area and wettability	Longer reaction time	(Bali *et al.*, 2014; Rabemanolontsoa & Saka, 2016; Seok *et al.*, 2015)
Acid	Lignocellulosic matrix disruption and amorphous cellulose conversion	Problems with acid recovery and equipment corrosion, formation of inhibitory compounds	(Jones & Semrau, 1984; Rastogi & Shrivastava, 2017; Rezania *et al.*, 2020)
Stem explosion	Cost effectiveness, decreased environmental influence, lower energy requirement,little to no chemical usage	Formation of inhibitory products, less effective for softwoods	(Kim, 2018; Rabemanolontsoa & Saka, 2016; Rastogi & Shrivastava, 2017)
Supercritical and subcritical water	High yield of hydrolyzed products, fewer degradation products	Low cellulose decrystallization.	(Saka & Ehara, 2004)
Liquid hot water	Increase enzyme-cellulose accessibility		(Rezania *et al.*, 2020)
Ammonia fiber explosion	High sugar recovery with no inhibitors production, does not require additional steps for reduction of particle size before pretreatment.	High energy required for ammonia recovery.	(Kim, 2018; Rabemanolontsoa & Saka, 2016)
Ionic liquid	Enhances enzymatic delignification and high sugar yield	Expensive and require tedious recycling	(Elgharbawy *et al.*, 2016; Rabemanolontsoa & Saka, 2016)
Biological	Selective, with no chemical addition, less energy required, low severity	Slow process, extremely low hydrolysis rate and partial hemicellulose hydrolysis.	(Maurya *et al.*, 2015; Sharma *et al.*, 2019; Sindhu *et al.*, 2015)
CBP	Effective for simultaneous saccharification and fermentation, improve process economics and ethanol yield.	Difficulty in finding suitable microbial consortium, common growth condition and in genetic manipulation.	(Farias & Filho, 2019; Sharma *et al.*, 2019)
Combined	Improve lignin degradation, reduction in sugar loss, improve ethanol yield.	Tedious process	(Ummalyma *et al.*, 2019)

Biological Pretreatment

Biological pretreatments are generally carried out by growing microorganisms directly on the feedstocks or by use of enzyme cocktails. This process is selective, with no chemical addition, less energy required, and low severity. It does not release any hazardous or toxic compounds to the environment and the byproduct does not inhibit subsequent hydrolysis as well as fermentation since it is carried out at mild conditions (Sindhu *et al.*, 2015). But extremely low rate of hydrolysis (Maurya *et al.*, 2015), partial hydrolysis of hemicellulose and chances of health hazards (Sharma *et al.*, 2019) are the major drawbacks. It mostly utilizes fungal and bacterial strains or their enzymes. This method is drawing more attention due to its ability to operate in the relatively short reaction time and low nutrition requirement for the enzymatic reactions (Rezania *et al.*, 2020). Important process parameters affecting biological pretreatment include the nature as well as composition of biomass, type of microorganism involved, incubation temperature, pH, incubation time, inoculums concentration, moisture content and aeration rate (Sindhu *et al.*, 2015).

Pretreatment especially using white rot fungi can improve the hydrolysis efficiency with the advantage of limited energy consumption (Shi *et al.*, 2009). White rot fungi belong to Basidiomycetes are selectively lignin degrading and enhance hydrolysis efficiency (Suhara *et al.*, 2012). *Irpex lacteus* a white rot fungus produced varieties of extracellular hydrolytic and oxidative enzymes with hydrolysis yield of 82% after 28 days of biological pretreatment (Du *et al.*, 2011). Treatment of *Eucalyptus grandis* saw dust with *Pleurotus ostreatus* and *Pleurotus pulmonarius* resulted in selective degradation of lignin (Castoldi *et al.*, 2014).

Combined Strategy

A single pretreatment method does not provide the expected results as it is limited due to its functioning modes. For this, combined pretreatment process incorporating two or more pretreatments from different categories is used. For example: biological pretreatment incorporated with different methods like stem explosion, liquid hot water, mild acid/alkali, ultrasonic, organosolvents, ammonia fiber expansion, alkaline/oxidative have been reported (Ummalyma *et al.*, 2019).

Hydrolysis and Fermentation

Cellulose is enzymatically hydrolysable by three different cellulolytic enzymes: endoglucanases, exoglucanases and β-glucosidases. This enzymatic complex system is inhibited by its final hydrolysis products, particularly by glucose. The simultaneous saccharification and fermentation processes, combines enzymatic hydrolysis of cellulose with simultaneous fermentation of glucose to ethanol. In this process, the stages are virtually the same as in the separate hydrolysis and fermentation system, except that both are performed in the same bioreactor. Thus, the presence of yeast together with the cellulolytic enzyme complex reduces the accumulation of the inhibiting sugars within the reactor, thereby increasing the yield and the saccharification rates. Also there is use of single bioreactor for the entire process, therefore reduces the investment costs (Ferreira *et al.*, 2010). The SSF scheme minimizes the inhibition by end-product on the enzyme activity and prevents microbial contamination (Azhar *et al.*, 2017).

A comparative study of separate hydrolysis and fermentation versus simultaneous saccharification and fermentation by Althuri & Banerjee, (2019) illustrated that higher ethanol productivity was obtained in SSF under optimized conditions. Similar study by Tulcan & Hadaruga, (2011) demonstrated a higher productivity of SSF process comparing with SHF process which can be explained by inhibition of cellulase activity in SHF process due to glucose accumulation. This inhibition can be avoided in SSF process fermenting the glucose simultaneously with its production. They also demonstrate that yeasts and cellulases can work in the same medium conditions and at common temperature of 35-40 ^{o}C.

Consolidated bioprocessing of dilute acid pretreated wheat straw using a consortium of *Trichoderma reesei*, *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* by Brethauer & Studer, (2014) resulted maximum ethanol concentration was about 9.1 g/L. Similarly, a co-culture of *Clostridium phytofermentans* and *Saccharomyces. cerevisiae* with added endoglucanase produced approximately 22 g/L ethanol. However, *C. phytofermentans* and *S. cerevisiae* mono-cultures produced approximately 6g/L and 9 g/L, respectively (Zuroff *et al.*, 2013).

Bioethanol Production Process

To make bioethanol production steps more efficient, extensive research on: various pretreatment methods, enzyme development for enhanced hydrolysis, more tolerant yeasts capable of fermenting different sugars, has been carried out. Different combined processing technologies such as simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) can be applied to achieve value addition and perspective, cost saving measures. Residues from bioethanol process can be used to produce additional products like bio-chemicals, fertilizer, heat and energy by applying integrated biorefinery approach (Raud *et al.*, 2019).

Currently separate hydrolysis and fermentation (SHF) is the main process in bioethanol production. In this process the pretreated lignocellulosic biomass is hydrolyzed by enzyme or chemicals like, HCl in a reactor and then the hydrolysate is fermented to ethanol in different reactor. Usually, hexoses and pentoses fermentation are carried out in different independent reactors. The major advantage of this

method is the saccharification and fermentation can be carried out at its own optimal conditions. However, high production cost due to longer time and equipment costs and the chance of contamination are the major pitfalls of this method (Verardi *et al.*, 2020).

Simultaneous Saccharification and Fermentation/Co-Fermentation

In order to overcome the limitations of SHF, other technologies have been developed including SSF, SSCF and CBP. These technologies combine the enzyme hydrolysis and fermentation in a single reactor (Verardi *et al.*, 2020).

Since SSF is a shorter process than SHF, it reduces the time of a technological process leading to low production costs. The research trend in biofuels is cost reduction in order to be competitive on the fuel market, where cellulosic ethanol should replace gasoline and ethanol obtained from sugar, food or feed crops (Tulcan & Hadaruga, 2011). The advantage of saccharification and fermentation simultaneously in a single reactor is the possibility to rapidly convert the newly formed sugar into ethanol, which help to decrease sugar buildup in the medium and alleviate feedback inhibition of cellulose (Azhar *et al.*, 2017; Pinaki *et al.*, 2015). The risk of contamination in SSF is less due to presence of ethanol in the broth. Ethanol makes the reaction mixture less susceptible to the undesired microbial action. However the main drawback is the different optimum temperatures of the hydrolysis and fermentation processes (Verardi *et al.*, 2020).

In SSF hexoses and pentoses are fermented in two different bioreactors with respective fermenting microorganism. To overcome this problem a new concept simultaneous saccharification and co-fermentation (SSCF) has been introduced and performed in a single reactor (Azhar *et al.*, 2017). Co-culture of different microorganisms can be employed for mixed saccharides fermentation (Paulova *et al.*, 2014). Co-culture of xylose and hexoses fermenting yeasts consume all reducing sugars in a mixed sugar concentration, where hexose content was utilized first. Xylose is utilized slowly in co-culture fermentation compared to single culture. This might be due to oxygen competition, faster ethanol accumulation by hexose fermenting species and due to diauxic growth of microorganisms. Co-fermentation strategy could improve process economics by shortening fermentation times, and ethanol yield through complete sugar utilization from biomass (Farias & Filho, 2019).

Consolidated Bioprocessing

In SSF and SSCF, saccharification is done by enzyme provided externally or produced in separate unit operation. A key challenge to cost-competitiveness in cellulosic bioethanol is the cost of the enzyme that converts polymeric cellulose into single molecules of sugar. Recent researches focused on the appropriate strategy to overcome this challenge. A significant effort is still required to lower the contribution of enzymes to biofuel production costs (Marcuschamer et al., 2012). A new approach CBP have been introduced in which a special microorganism or microbial consortium are used to convert the feed stock into bioethanol without pre-treatment. The raw material for CBP does not require any special pre-treatment and particle size should be reduced sufficiently (Paulova et al., 2014). This can reduce operational costs and capital investment for purchasing enzyme or its production. However, selection or design of a suitable microorganism/microbial consortium is a daunting task (Verardi et al., 2020). In microbial consortium one microorganism may favor the growth of second microorganism producing growth factors, removing inhibitors or ensuring optimal environmental conditions (Rago et al., 2019). There exist some technical challenges with microbial consortium, as low ethanol yields associated with different optimal oxygen transfer rate demanded by each microorganism. One of the basic requirements is the absence of any inhibitory effect of one microbial species over each other (Ashoor et al., 2015). Besides that, there is also mandatory that each strains share similar culture characteristics in order to provide the maximum activity (Farias & Filho, 2019). The most challenging task with CBP is selection or design of a suitable microorganism or microbial consortium that must express appropriate hydrolytic enzymes matching the lignocellulosic feedstock, and produce ethanol (Paulova et al., 2014).

Fermentation Inhibitors

Pretreatment processes allow enzyme accessibilities to the exposed cellulose and result in the enhancement of conversion yield. However, undesired lignocellulose-derived compounds such as furans, organic acids, phenolic compounds, lignocellulose extractives and other soluble mono/oligomeric sugars can also be released during the pretreatment. These inhibitory molecules present in the pretreated hydrolysates could be categorized into four groups as phenolic compounds, furan aldehydes, carboxylic acids and soluble sugars (Kim, 2018). Formation of degradation molecules from lignocellulosic materials strongly depends on the type of raw material, pretreatment method, and pretreatment conditions (Almeida et al., 2007).

Phenolic Compounds

Wide range of phenolics compounds are produced due to lignin breakdown and acid hydrolysis. A mixture of phenolics such as syringaldehyde, 4-hydroxybenzaldehyde, catechol, vanillin, 4-hydroxybenzoic acid, dihydro coniferyl alcohol, coniferyl aldehyde, and syringic acid was reported as lignin degradation products from hardwoods and agricultural residue (Taherzadeh & Karimi, 2011).

The phenolic compounds can decrease the rate of ethanol production, microbial growth rate and yields by affecting the cell membrane integrity (Heipieper *et al.*, 1994) as well

as can cause breakdown of DNA, resulting in the inhibition of RNA and protein synthesis (Klinke *et al.*, 2004). Some researches indicated that the phenolic compounds are more toxic than other potent inhibitory molecules even at lower concentrations, since their low molecular weight allow them able to penetrate cell membranes and damage internal structures, as well as causing changes in the morphology of cells (Kim, 2018; Klinke *et al.*, 2004; Palmqvist & Hagerdal, 2000).

Qin et al (2016), investigated, Vanillin significantly decreases enzyme activity and concentration during enzymatic hydrolysis which cannot be significantly mitigated by pH, temperature, and the addition of calcium chloride, BSA and Tween 80. Similarly it is concluded that phenolic compounds despite their low concentrations strongly inhibit cellulase; precipitating and deactivating β-glucosidase (Kim *et al.*, 2011; Michelin *et al.*, 2015).

Furan Derivatives

Furfural and hydroxymethyl furfural (HMF) are furan derivative degradation products of lignocellulosic biomass. Five carbon sugars such as xylose form furfural and hexose sugars and carbohydrates form HMF during acid pretreatment and hydrolysis at high temperature (Taherzadeh & Karimi, 2011). Furfural is usually found in lower levels than HMF. However, it is often still in enough concentration, around 1 g/L to be inhibitory (Almeida *et al.*, 2007). Furfural and HMF concentration in the order of 1.0 g/L and above has clear negative effects on many bacteria, yeasts, and filamentous fungi on the vitality, viability, specific growth rate, lag phase, ethanol yield, and ethanol productivity (Nilvebrant *et al.*, 2001). Several intercellular enzymes such as dehydrogenases (Modig *et al.*, 2002) and hexokinase (Taherzadeh & Karimi, 2011) have shown to be sensitive to furfural and HMF.

Small Organic Acids

Organic acids such as acetic, formic, lactic, and levulinic acids can hinder the microbial growth due to improper ion transportation (Pampulha & Loureiro, 1989). Formation of these kinds of acids is highly dependent on pretreatment conditions and usually generated from acetyl groups linked to the sugars or from the hemicellulose backbones. Minor weak acids such as gallic acid, caproic acid, furoic acid, benzoic acid, and vanillic acid, have also been identified in pretreated hydrolysates (Kim, 2018).

Soluble Sugars

Soluble hydrolysis intermediates and end products of cellulose digestions, inhibits enzyme activity. For instance, glucose, cellobiose, and cello-oligomers accumulation inhibit cellulase activity (Holtzapple *et al.*, 1990), glucose inhibit β-glucosidase and cellobiose inhibit cellobiohydrolase (Philippidis *et al.*, 1993).

Strategies to Minimize the Effect of Inhibitors

The inhibition of furan, phenolics and acids is the main hurdle in large scale production of lignocellulosic bioethanol. Several studies reported that *S. cerevisiae* has innate tolerance to some extent (Almeida *et al.*, 2007) and convert these inhibitors to less harmful compounds (Palmqvist & Hagerdal, 2000). For instance, HMF is reduced to 2,5- bis-hydroxymethyl furan under aerobic and anaerobic conditions (Liu *et al.*, 2004).Whereas, furfural can be reduced to furfuryl alcohol under both aerobic as well as anaerobic conditions and also can be oxidized to formic acid under aerobic conditions (Palmqvist *et al.*, 1999). However, tolerance to HMF and furfural is clearly strain dependent (Nilsson *et al.*, 2005). Similarly presence of phenylacrylic acid decarboxylase (PAD) in *S. cerevisiae* metabolizes some phenolic compounds present in lignocellulose hydrolysate (Klinke *et al.*, 2003).

Biological Detoxification

Jonsson et al (1998) investigated detoxification of wood hydrolysate using laccase and peroxidase extracted from the white rot fungus *Trametes versicolor*. They found that both enzymes were effective and laccase was more effective in eliminating phenolics and other acidic compounds to increase glucose consumption and ethanol productivity. Similarly, Martin et al (2002) found that approximately 80% of phenolic compounds were eliminated by the laccase treatment of sugarcane bagasse.

In-Situ Microbial Detoxification (ISMD)

In-situ microbial detoxification is a process where microorganisms are involved directly in the detoxification of lignocellulosic hydrolysates (Chandel *et al.*, 2013). Selective lignin degrading microorganisms, particularly white rot fungi, are grown on raw lignocellulosic biomass. This is then hydrolyzed to obtain fermentable sugars and contains fewer fermentation inhibitors (Gupta *et al.*, 2011). Detoxification and adaptation procedures are potential routes to overcome hydrolysate toxicity and microorganism sensitivity (Nouri *et al.*, 2020).

Several researches have been conducted in order to detoxify lignocellulose hydrolysates using a variety of microorganisms along with genetically engineered recombinants expressing laccase or peroxidases. Simultaneous detoxification using *Trichoderma reesei* shows sharp decrease in phenolics as well as decrease in concentrations of furans, and weak acids (Palmqvist *et al.*, 1997). The treated hydrolysates showed a threefold improvement in ethanol productivity and a fourfold improvement in yield (Chandel *et al.*, 2013).

Many researchers and efforts have been employed in order to cope with these inhibitors. Some strategies to counteract lignocellulose derived inhibitors are summarized in Table 2.

Table 2: Strategies to counteract lignocellulose derived inhibitors

Strategy	Effect/process	Consideration	References
Biomass selection and modification	Screening of biomass that produce less undesirable products	Time consuming	(Kim, 2018)
Fermentation strategy	Fed-batch or Continuous operation, high cell density, encapsulation	Cell viability	(Taherzadeh & Karimi, 2011)
Vaccum or high-pressure evaporation	Sugar concentration, part of toxic components removal	High evaporation may make the hydrolyzates unfermentable.	(Dehkhoda *et al.*, 2009; Walton *et al.*, 2010)
Chemical detoxification	Chemical supplementation	Require chemical	(Kim, 2018)
Enzymatic detoxification	Specific enzymes (i.e. laccase and peroxidases)	High cost of enzyme	(Chandel *et al.*, 2013)
In situ microbial detoxification	Microbial innate tolerance to inhibitors	Selective screening of microorganism	(Palmqvist *et al.*, 1999)
Co-culture	Use of microbial consortium	Screening of suitable consortia	(Farias & Filho, 2019)
Genetic engineering	Fermenting organisms that produce chimeric enzymes with altered properties of detoxification	Require time for development	(Chandel *et al.*, 2013)

Conclusion

This article is meant to contribute to researches on alternative fuel production. For improvement in sustainable and clean energy, many researches dealing with lignocellulosic ethanol production primarily focuses on pretreatment and production process. So far, advancements in bioethanol production: SSF, SSCF and CBP have been introduced. Despites many researches deal with lignocellulosic ethanol production, including CBP, still the yield is less. CBP technique can be an alternative, to eliminate chemicals and extensive pretreatment strategy in production process. Extensive researches need to be performed to make bioethanol as a competitor in fuel market.

Authors' Contribution

Conception and design: Pradip Dhungana and Jarina Joshi.

Drafting of the manuscript: Pradip Dhungana, Bikram Prajapati and Sujeta Maharjan.

Critical revision of the manuscript as for important intellectual content: Jarina Joshi.

Revise the article for the final approval of the version to be published: Pradip Dhungana.

Final form of the manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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