Review

Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects

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ABSTRACT

During the most recent decades increased interest in fuel from biomass in the United States and worldwide has emerged each time petroleum derived gasoline registered well publicized spikes in price. The willingness of the U.S. government to face the issues of more heavily high-priced foreign oil and climate change has led to more investment on plant-derived sustainable biofuel sources. Biomass derived from corn has become one of the primary feedstocks for bioethanol production for the past several years in the U.S. However, the argument of whether to use food as biofuel has led to a search for alternative non-food sources. Consequently, industrial research efforts have become more focused on low-cost large-scale processes for lignocellulosic feedstocks originating mainly from agricultural and forest residues along with herbaceous materials and municipal wastes. Although cellulosic-derived biofuel is a promising technology, there are some obstacles that interfere with bioconversion processes reaching optimal performance associated with minimal capital investment. This review summarizes current approaches on lignocellulosic-derived biofuel bioconversion and provides an overview on the major steps involved in cellulosic-based bioethanol processes and potential issues challenging these operations. Possible solutions and recoveries that could improve bioprocessing are also addressed. This includes the development of genetically engineered strains and emerging pretreatment technologies that might be more efficient and economically feasible. Future prospects toward achieving better biofuel operational performance via systems approaches such as risk and life cycle assessment modeling are also discussed.

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1. Introduction

The agreement implemented by Policy Energy Act (PEA) [1] followed by the Energy Independence and Security Act (EISA) [2] aims to reach 36 billion gallons (136.27 L) of bioethanol by the year 2022. Rising concern over depleting fossil fuel and greenhouse gas limits has resulted in a high level of interest in non-conventional fuel originating from bio-renewable sources including sugars, starches and lignocellulosic materials [3–8]. During the last decade, the production of ethanol from biomass materials received more attention in the United States (U.S.) and worldwide. In the U.S., bioethanol is primarily produced from corn starch feedstocks while in Brazil biofuel is mainly produced from sugarcane juice and molasses. Together, these countries account for 89% of the current global bioethanol production [9].

Several countries have initiated new alternatives for gasoline from renewable feedstocks [10]. In the North American hemisphere, bioethanol has been extracted from starch sources such as corn while in the South American hemisphere, biofuel has been largely provided from sugars including sugarcane and sugar beets [11]. While European countries are deploying extensive efforts to increase their 5% worldwide bioethanol production [12], biodiesel produced in Europe primarily in France and Germany remains by far more substantial and accounts for approximately 56% of the global production mainly because of the rising importance of diesel engines and feedstock opportunity costs [13]. Although, most of the remaining countries in the world collectively account for only 5% of the global bioethanol production, China, Thailand as well as India are continuing to invest substantially in agricultural biotechnology and emerge as potential biofuel producers [14,15]. In the U.S., biofuel-derived from corn has emerged as one of the primary raw materials for bioethanol production [16]. According to the renewable fuels association [9] statistics, the production of bioethanol was historically unparalleled in the U.S. by year 2009 with nameplate capacity reaching 10.9 billion gallons (41.26 billion litres) representing 55% of the worldwide production. In the year 2010 corn-based ethanol operating productions generated a total of 12.82 billion gallons (48.52 billion litres) with the largest nameplate capacity in Iowa (28%) followed by Nebraska (13%) [17].

Although corn-based and sugar based-ethanol are promising substitutes to gasoline production mainly in the transportation sector, they are not sufficient to replace a considerable portion of the one trillion gallons of fossil fuel presently consumed worldwide each year [18]. Furthermore, the ethical concerns about the use of food as fuel raw materials have encouraged research efforts to be more focused on the potential of inedible feedstock alternatives [19–21]. Lignocellulosic biomass materials constitute a substantial renewable substrate for bioethanol production that do not compete with food production and animal feed. These cellulosic materials also contribute to environmental sustainability [22]. Additionally, lignocellulosic biomass can be supplied on a large-scale basis from different low-cost raw materials such as municipal and industrial wastes, wood and agricultural residues [23]. Currently the most promising and abundant cellulosic feedstocks derived from plant residues in the U.S., South America, Asia and Europe are from corn stover, sugarcane bagasse, rice and wheat straws, respectively [24–27].

However, lignocellulosic-based feedstock is a recalcitrant material that requires an intensive labor and high capital cost for processing [28]. Hence, these procedures currently are not economically feasible. When considering enzymatic or acidic decomposition of lignocellulosic structure, it must be taken into account that D-xylose is the second important sugar forming the hemicellulosic portion of the plant cell wall and constituting one-third of the sugars in the lignocellulosic feedstock [29]. However, the primary industrial yeast used in bioethanol production, *Saccharomyces cerevisiae* converts only hexose sugars such as glucose and is not able to co-ferment glucose and xylose [30].

There are four stages in the production of lignocellulosic-based ethanol: pretreatment, hydrolysis, fermentation and distillation. During the past decades, there have been substantial advances in genetic and enzymatic technologies that have helped to improve these steps of ethanol production and expand the capability of *S. cerevisiae* for fermenting different sugars simultaneously [31]. Although there is a wide range of fungal and recombinant bacteria that are able to ferment xylose sugar, they are not all capable of adapting to fermentation-process conditions and some of them produce only low ethanol yields. Their tolerance to ethanol and productivity still require further refinements [32,33]. Moreover, cellulosic materials contain microbial contaminants that compete with the fermenting yeast for nutrients and these contaminants can produce toxic end-products. Both of these adverse conditions can create a considerable loss in ethanol yields [34,35]. Additionally, pretreatment processes may result in the formation of toxic components including primarily, acetic acid along with furfural, hydroxymethyl furfural and phenolic components [36,37]. However, in addition to the formation of fermentation inhibitors during biofuel production, there is occurrence of lignin side effects on enzymatic hydrolysis and cellulase inhibitors including primarily phenolic-derived lignin [38,39]. Lignin and derivative effects are extensively reviewed in a later section.

This review examines what is currently known regarding recent technologies and approaches that are used in derived-lignocellulosic biofuel production. This review also provides

a summary of the current bottlenecks and barriers that interfere with the lignocellulosic based-ethanol pathway and places the emphasis on potential issues challenging biotechnological conversion and bioethanol performance. Specific focus is directed toward describing current solutions and possible systematic remedies that could be adopted to circumvent lignocellulosic-derived ethanol problems and strategies for the bioethanol industry to become more economically feasible and therefore commercially viable. Future prospects for the systematic optimization of lignocellulosic bioconversion are also addressed.

2. Historical and current trends of biofuel in the U.S.

Little attention was focused on bioethanol production in the U.S. before 1860 when Nicholas Otto initiated the use of ethanol as a fuel for engine combustion. As early as 1908, Henry Ford was already aware of the promising substitute to gasoline, ethanol. This led to the development of the Ford Model T capable of operating off of gasoline, ethanol or combinations of both [40]. At that time, the potential for fuel ethanol received only moderate consideration due to the dominance of low priced petroleum derived gasoline.

Interest in ethanol from biomass such as corn starch emerged in the 1970s when the price of fossil fuel rose and methyl tertiary butyl ether (MTBE) used in gasoline was identified as an environmental pollutant agent [41]. Moreover, the willingness of the U.S. to stay independent from high-priced foreign oil, led the federal government to implement new research programs directed toward the development of more sustainable alternative fuels originating from renewable sources. Between 1980 and 1990, there was a considerable effort from the government to boost industrial efforts toward manufacturing fuel from biomass materials by adjusting tax-exemptions and encouraging bioethanol research and development programs. Biofuel production grew exponentially from approximately 200 million gallons (757 million litres) in 1982 to 2.9 billion gallons (10.9 billion litres) in 2003 [42]. The PEA [1] implemented in 2005 followed by the EISA [2] in 2007 was accompanied by a partnership between the U.S. and Brazil, the world's largest biofuel producer at the time.

In 2009, bioethanol-based production achieved an unprecedented increase (approximately 11 billion gallons, 41 billion litres). In the year 2010, the U.S. became the world's leading biofuel producer and exporter with 13.5 billion gallons (51 billion litres) nameplate capacity. Almost 200 operational corn-based ethanol plants are currently operating in 29 states [42] most of them are located in the "corn belt" in the U.S. Midwest [12]. It was also reported in 2010 that despite the global economic-burden, bioethanol production continues to expand rapidly and to contribute significantly to the economic development of rural communities in the U.S. [42]. Although the price of most food products has increased, corn prices have not substantially been altered. However, the debate of whether to use plants as a fuel feedstock or as human food remains a controversial issue. This debate has led researchers to work on more acceptable sources containing lignocellulosic biomass that are derived mainly from agricultural residues, industrial wastes, forest biomass and other herbaceous materials [42].

3. Lignocellulosic sources and composition

3.1. Lignocellulosic sources

Lignocellulosic material constitutes the world's largest bioethanol renewable resource. In the U.S. alone the production of biomass from lignocellulosic materials is estimated to be nearly 1.4 billion dry tons per year, 30% originating from forest biomass [43].

There are several groups of raw materials that are differentiated by their origin, composition and structure. In the U.S. most cultivated land constitutes around 35% of the forestland, approximately 27% grazed land as well as herbaceous and 19% crop lands per approximately 2.25 billion acres (9.0 million km^2) [44,45]. Forestland materials include mainly woody biomass namely, hardwoods and softwoods followed by sawdust, pruning and bark thinning residues while pasture and grassland encompass primarily agricultural residues that cover food or non-food crops and grasses such as switch grass and alfalfa [46]. Municipal and industrial wastes are also potential recyclable cellulosic materials that can originate either from residential or non-residential sources such as food wastes and paper mill sludge [46,47]. Annual total tonnage available is summarized in Table 1.

3.1.1. Forest woody feedstocks

Forest woody feedstocks account for approximately 370 million tons per year (30%) of lignocellulosic biomass in the U.S. [43]. There are two types of woody materials that are classified into broad categories of either softwoods or hardwoods. Softwoods originate from conifers and gymnosperm trees [48] and unlike hardwoods, softwoods possess lower densities and grow faster. Gymnosperm trees, include mostly evergreen species such as pine, cedar, spruce, cypress, fir, hemlock and redwood [49]. Hardwoods are angiosperm trees and are mostly deciduous [50]. They are mainly found in the Northern hemisphere and include trees such as poplar, willow, oak, cottonwood and aspen. In the U.S., hardwood species account for over 40% of the trees [51]. The genus *Populus* (cottonwood) which includes 35 species is the most abundant fast-growing species suitable for bioethanol production. *Populus deltoids* species cover most of North America from the eastern to midwestern U.S., while *Populus trichocarpa* covers primarily the western U.S.[52]. Unlike agricultural biomass, woody raw materials offer flexible harvesting times and avoid long latency periods of storage [53]. Additionally, this study reported that woody feedstock possessed more lignin than agricultural residues and less ash content (close to zero). These unique characteristics of woody biomass including primarily high density and minimal ash content make woody raw material very attractive to cost-effective transportation in conjunction to its lower content in pentoses over agricultural biomass and more favorable for greater bioethanol conversion if recalcitrance is surmounted [53]. Forestry wastes such as sawdust from sawmills, slashes, wood chips and branches from dead trees have also been used as bioethanol feedstocks [43].

3.1.2. Agricultural residues, herbaceous and municipal solid wastes (MSW)

Crops residues consist of an extensive variety of types. They are mostly comprised of agricultural wastes such as corn stover, corn stalks, rice and wheat straws as well as sugarcane bagasse [54]. There are approximately 350–450 million tons per year (127 million metric tons to 317.5 million metric tons) harvested annually in the U.S. [42,43,54] with residues originating primarily from rice

Table 1
Annual total tonnages of biomass for biofuel in the U.S. (U.S. Department of Energy Biomass Program, 2009) [54].

Biomass	Million dry tons/year
Agricultural residues	428
Forest resources	370
Energy crops	377
Grains and corn	87
Municipal and industrial wastes	58
Others (i.e., oilseeds)	48
Total	1368

and wheat straws as well as corn stalks being considered the bio-ethanol feedstocks with the most potential. Crop residues contain more hemicellulosic material than woody biomass (approximately 25–35%) [55]. Aside from being an environmentally friendly process, agricultural residues help to avoid reliance on forest-woody biomass and thus reduce deforestation (non-sustainable-cutting plants). Unlike trees, crop residues are characterized by a short-harvest rotation that renders them more consistently available to bioethanol production [25,26].

Switch grass is the primary herbaceous prairie grass and energy crop that grows in the plains of the North American hemisphere, namely, Canada and the U.S. These perennial grasses are of interest due to their low-cost investment as well as abundance in the U.S., their ability to resist diseases, and their high yield of sugar substrates per acre. Moreover, switch grass is low maintenance requiring little or no fertilization. *Miscanthus giganteus* is another fast-growing grass that is a potentially optimal candidate for bioethanol production. It is native to Asia and is grown in Europe for combustible energy use [56]. In addition to cellulosic feedstocks, municipal and industrial solid wastes are also a potential raw material for biofuel production. Their utilization limits environmental problems associated with the disposal of garbage household, processing papers, food-processing by-products, black liquors and pulps [57].

Although over one billion tons of biomass per year would be potentially available to meet the 30% replacement of petroleum-derived gasoline in 2030 [43], the high cost of biomass could be a serious hindrance if potential lands and feedstocks are not managed and utilized efficiently [57]. While woody biomass and agricultural residues potential was overestimated in 2005, high-yielding energy crops including primarily *Miscanthus* have started to regain considerable interest compared to woody and agricultural residues because of their potential to cover 50–70% of the total feedstock [57]. According to this study, in addition to the possible one billion tons of various feedstocks that would be available, an additional cultivation of high yielding energy crops on Conservation Reserve Program (CRP) lands that are efficiently managed would be the key option to meet a 30% petroleum-based gasoline displacement in 2030. However, a more recent research study concluded that bioethanol production has already reached the saturation level just to cover the blending limit of 10% of bioethanol which could be a substantial obstacle for further increases to reach EISA (2007) projections [58,59].

3.1.3. Marine algae

Interest in algae as a potential biofuel feedstock has existed since 1978 in the U.S. and has recently received support by the DOE Aquatic Program [54]. Special focus was directed to assess several aspects of algae biomass including the estimation of its productivity per acre, water consumption and non-food feedstocks with respect to by- and co-products recovered during biofuel production. However, improving the efficiency of algae feedstock and thus its development as a viable and scalable source commercial enterprise remained limited during the 20th century.

More recently, marine algae biomass is regaining interest as a third generation biofuel feedstock due to the rapid biorefineries expansion leading to a shortage on current energy crops designated for bioethanol and biodiesel industries. Aside from being potential bioethanol biomass, algae would also be a feedstock for other biofuels including mainly, biodiesel and fuel for aviation in addition to other possible applications involving bio-crude oils, bio-plastics and recovered livestock co-products [60]. Furthermore, algae feedstock with its thin cellulose layer has a high carbohydrate composition making it capable of yielding 60 times more alcohol than soybeans per acre of land [61]. It also provides 10 times more ethanol than corn per growing area [62]. Unlike corn and sugar-cane, algae biomass does not compete directly with foods and does not require agricultural land or use of fresh water to be cultivated. It consumes a high level of CO_2 during its growth, which makes it environmentally attractive as a CO_2 sink [63].

3.2. Lignocellulosic biomass composition

Lignocellulosic material can generally be divided into three main components: cellulose (30–50%), hemicellulose (15–35%) and lignin (10–20%) [64–67]. Cellulose and hemicelluloses make up approximately 70% of the entire biomass and are tightly linked to the lignin component through covalent and hydrogenic bonds that make the structure highly robust and resistant to any treatment [25,66,68]. Potential lignocellulosic feedstocks and their composition are summarized in Table 2.

3.2.1. Hemicellulose

Hemicellulose is an amorphous and variable structure formed of heteropolymers including hexoses (D-glucose, D-galactose and D-mannose) as well as pentose (D-xylose and L-arabinose) and may contain sugar acids (uronic acids) namely, D-glucuronic, D-galacturonic and methylgalacturonic acids [69,70]. Its backbone chain is primarily composed of xylan β (1→4)-linkages that include D-xylose (nearly 90%) and L-arabinose (approximately 10%) [67]. Branch frequencies vary depending on the nature and the source of feedstocks. The hemicelluloses of softwood are typically glucomannans while hardwood hemicellulose is more frequently composed of xylans [69]. Although the most abundant component in hemi-cellulose, xylan composition still varies in each feedstock [71]. Because of the diversity of its sugars, hemicellulose requires a wide range of enzymes to be completely hydrolyzed into free monomers.

3.2.2. Cellulose

Cellulose is a structural linear component of a plant's cell wall consisting of a long-chain of glucose monomers linked β (1→4)-glycosidic bonds that can reach several thousand glucose units in length. The extensive hydrogen linkages among molecules lead to a crystalline and strong matrix structure [72]. This cross-linkage of numerous hydroxyl groups constitutes the microfibrils which give the molecule more strength and compactness. Although starchy materials require temperatures of only 60–70 °C to be converted

Table 2
Potential lignocellulosic biomass source and composition (% dry weight).

Raw material	Hemicelluloses	Cellulose	Lignin	Others (i.e., ash)	References
Agricultural residues	25–50	37–50	5–15	12–16	[14,54,63,189]
Hardwood	25–40	45–47	20–25	0.80	
Softwood	25–29	40–45	30–60	0.50	
Grasses	35–50	25–40	–[a]	–	
Waste papers from chemical pulps	12–20	50–70	6–10	–	
Newspaper	25–40	40–55	18–30	–	
Switch grass	30–35	40–45	12	–	

[a] Not present.

from crystalline to amorphous texture, cellulose requires 320 °C as well as a pressure of 25 MPa to shift from a rigid crystalline structure to an amorphous structure in water [73]. Cellulose is the most prevalent organic polymer and is approximately 30% of the plant composition [54]. Cotton, flax and chemical pulp represent the purest sources of cellulose (80–95% and 60–80%, respectively) while soft and hardwoods contain approximately 45% cellulose [55,56,64].

3.2.3. Lignin

Lignin is an aromatic and rigid biopolymer with a molecular weight of 10,000 Da bonded via covalent bonds to xylans (hemicellulose portion) conferring rigidity and high level of compactness to the plant cell wall [66]. Lignin is composed of three phenolic monomers of phenyl propionic alcohol namely, coumaryl, coniferyl and sinapyl alcohol. Forest woody biomass is primarily composed of cellulose and lignin polymers. Softwood barks have the highest level of lignin (30–60%) followed by the hardwood barks (30–55%) while grasses and agricultural residues contain the lowest level of lignin (10–30% and 3–15%, respectively) [55,64]. Conversely, crop residues such as corn stover, rice and wheat straws are comprised mostly of a hemicellulosic heteropolymer that includes a large number of 5-carbon pentose sugars of primarily xylose [74]. Previously, little interest has been given to lignin chemistry potential on hydrolysis. However, lignin components are gaining importance because of their dilution effect on the process once solids are added to a fed batch hydrolytic or fermentation bioreactor in addition to their structure and concentration effects that would affect potential hydrolysis [75]. For instance, the adsorption of lignin to cellulases requires a higher enzyme loading because this binding generates a non-productive enzyme attachment and limits the accessibility of cellulose to cellulase [76]. Furthermore, phenolic groups are formed from the degradation of lignin. These components substantially deactivate cellulolytic enzymes and hence influence enzymatic hydrolysis. This negative impact caused by lignin has led to interest in lowering the lignin negative effect. Chen et al. (2006) [76] demonstrated that lignin modification via genetically engineering practices targeting its biosynthetic pathways could considerably reduce lignin formation and improve ethanol yield. However, this could be somewhat problematic as lignin components serve as the major plant defense system to pathogen and insects and its modification could disrupt the plants' natural protection [77]. Retaining the lignin could have benefits as Ladisch et al. [75] have demonstrated that lignin components, once recovered from biofuel process may be a potential energy self-sustaining source to retain biorefineries financial solvency.

4. Pathways of bioethanol production from cellulosic feedstocks

Lignocellulosic biomass can be transformed into bioethanol via two different approaches, (i.e. biochemical or thermochemical conversion) [78]. Both routes involve degradation of the recalcitrant cell wall structure of lignocellulose into fragments of lignin, hemicellulose and cellulose. Each polysaccharide is hydrolyzed into sugars that are converted into bioethanol subsequent followed by a purification process [79,80]. However, these conversion routes do not fundamentally follow similar techniques or pathways. The thermochemical process includes gasification of raw material at a high temperature of 800 °C followed by a catalytic reaction. Application of high levels of heat converts raw material into synthesis gas (syngas) such as hydrogen, carbon monoxide and CO_2. In the presence of catalysts, the resulting syngas can be utilized by the microorganism *Clostridium ljungdahlii* to form ethanol and water can be further separated by distillation [81].

Unlike the thermochemical route, biochemical conversion involves physical (i.e. size reduction) or/and thermo-chemical with possible biological pretreatment [82]. Biochemical pretreatment is mainly used to overcome recalcitrant material and increase surface area to optimize cellulose accessibility to cellulases [53,82,83]. The upstream operation is followed by enzymatic or acidic hydrolysis of cellulosic materials (cellulolysis) and conversion of hemicellulose into monomeric free sugars (saccharification) subsequent to biological fermentation where sugars are fermented into ethanol and then purified via distillation [79,81]. Concurrently, lignin, the most recalcitrant material of cell walls is combusted and converted into electricity and heat [80]. Overall, biochemical approaches include four unit-operations namely, pretreatment, hydrolysis, fermentation and distillation [84,85]. Currently the biochemical route is the most commonly used process [86]. Fig. 1 adopted from Ladisch et al. [75] provides a flow diagram illustrating the major steps involved in biochemical process with lignin co-product recovery for a self-sufficient energy system.

4.1. Pretreatment overview

Effective pretreatment is fundamental for optimal successful hydrolysis and downstream operations [87]. Pretreatment upstream operations include mainly physical, (i.e., biomass size-reduction) and thermochemical processes that involve the disruption of the recalcitrant material of the biomass. This upstream operation increases substrate porosity with lignin redistribution. Therefore, it enables maximal exposure of cellulases to cellulose surface area to reach an effective hydrolysis with minimal energy consumption and a maximal sugar recovery [53,82,83,88]. Fig. 2 illustrates the major outcomes from pretreatment upstream processes subsequent to hydrolysis and fermentation operations. Zhu and Pan [53] concluded that the pretreatment process of woody biomass differs substantially from the agricultural biomass due to differences in their chemical composition and physical properties. Unlike woody biomass, agricultural residues pretreatment does not require as much energy as recalcitrant woody material to reach size reduction for further enzymatic saccharification. This study placed emphasis on the importance of the energy consumption from the mechanical operation (size-reduction) primarily based on the estimation of woody biomass pretreatment energy efficiency ($\eta_{Pretreatment}$ = Total sugar recovery (kg)/Total energy consumption (MJ)). In addition to sugar recovery and ethanol yield, this energy efficiency ratio and mass balance was deemed crucial for the complete estimation of pretreatment efficiency [53,89–91]. Toxic inhibitory level estimation has also been considered important for evaluating pretreatment cost-effectiveness primarily when dilute acid is added. Costly detoxification steps could be a major hindrance to reach high-performance pretreatment [36,92]. Overall, the ratio including energy consumption versus sugar yield with regard to feed stock versatility [53,89] as well as toxic inhibitors formed per level of sugars recovered are of prime consideration on the estimation of the pretreatment efficiency and cost effectiveness of the operation in an effort to reach optimal conditions [93].

Several pretreatment methods, namely, mechanical, chemical or microbiological have been used to remove the recalcitrant cell wall material of lignocellulosic biomass depending on the raw material being extracted [93,94]. More recently, there has been considerable advancement in development of pretreatment processes [19,23,94–96]. Table 3 illustrates some of the pretreatment methods that have been examined over the years. Although most of these treatments can liberate hemicellulose and cellulose from the cell wall, some of them remain economically unfeasible due to key technical issues. Furthermore, they are not all able to
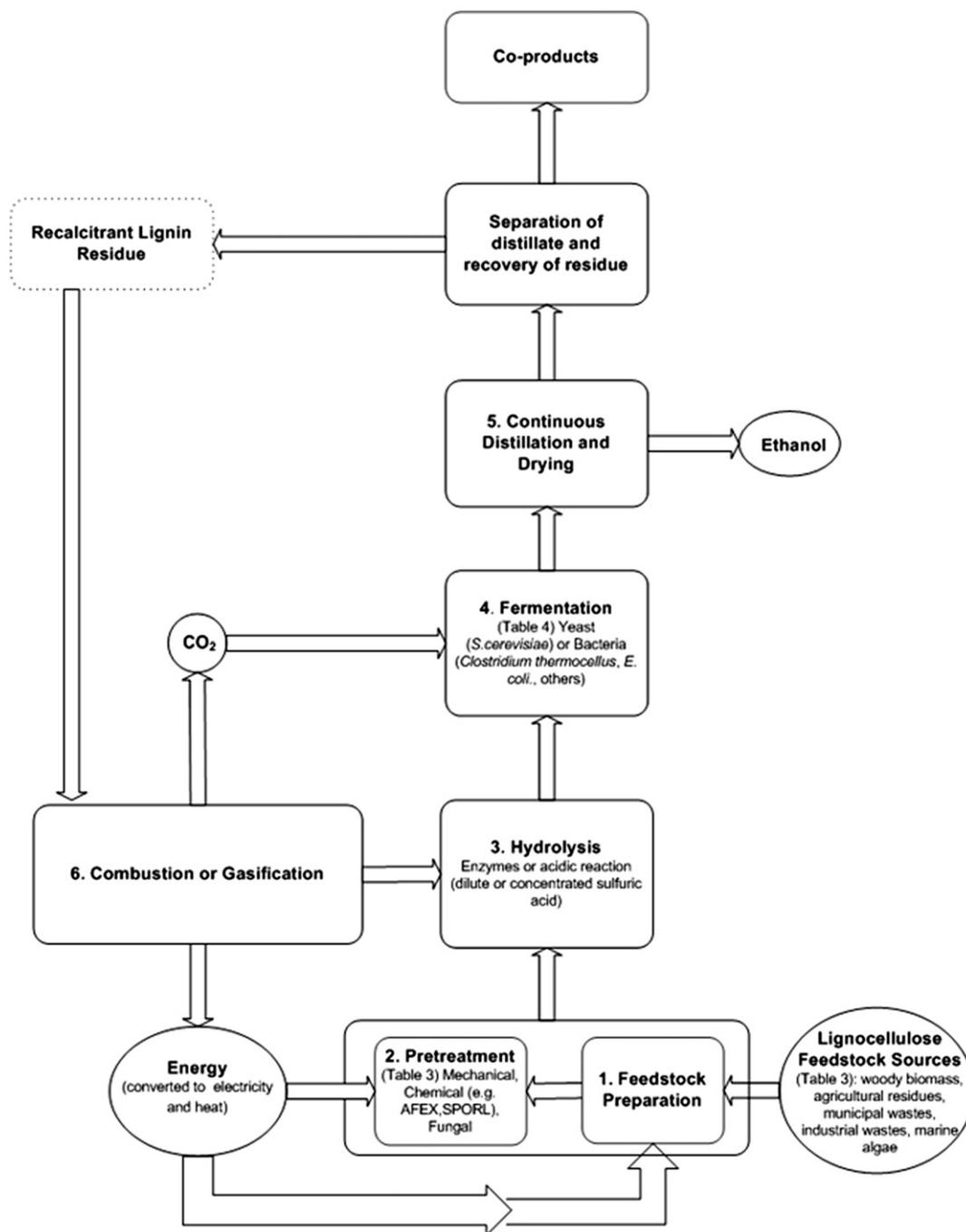
Fig. 1. Lignocellulose substrate conversion steps for ethanol and coproducts generation. Lignin coproduct is returned for a self-energy sufficient system (adopted from Refs. ([75,113])).

overcome the recalcitrant material found mainly in wood-based feedstocks. Typically, few treatments are endowed with ability to overcome feedstock versatility [97,98]. Unlike agriculture residues, forest and wood materials are high in lignin (approximately 29%) and cellulose (approximately 44%) [55] which renders them more recalcitrant. Agricultural residues such as corn stover, rice and wheat straws are mostly composed of hemicellulose (32%) and low levels of lignin (3–13%) conferring to them a less resistant texture but a higher level of pentose sugars rendering them less practical than woody recalcitrant material.

The most prevalent treatments include acid hydrolysis, hot water, dilute acid pretreatment and lime [92,93,99–108]. However, the conventional methods using acidic treatments (usually dilute sulfuric acid with concentrations below 4 wt% and temperatures greater than 160 °C) [109] are always accompanied by formation of toxic inhibitors such as furfural from xylose and hydroxymethyl furfural (HMF) from glucose in addition to phenolics and acetic acid [20,36,93,110]. Acetic acid resulting from dilute acid pretreatment of agricultural residues as well as herbaceous and hardwoods is pH dependent and can reach a high concentration of approximately 10 g/L [20,36] that is more difficult to separate and detoxify than HMF and furfural. Unlike dilute acid pretreatment, ammonia fiber explosion (AFEX) treatments are sufficient to hydrolyze primarily agricultural residues such as corn stover and have not been associated with the formation of toxic products including HMF [97]. Given that woody feedstock is gaining increasing attention for its
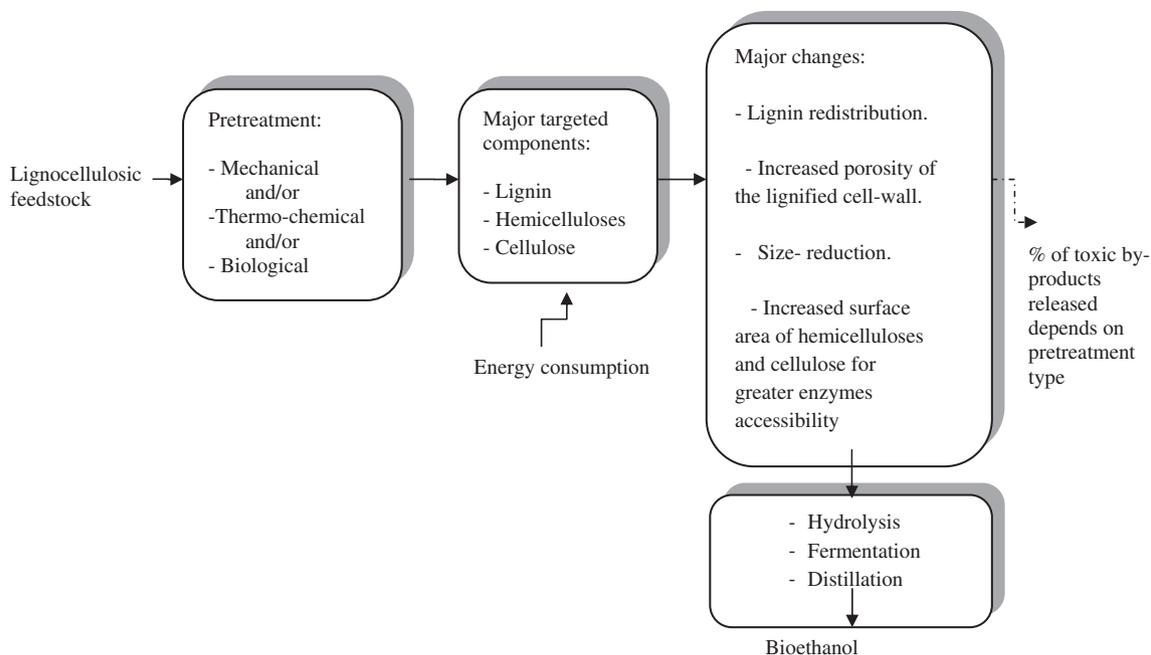
Fig. 2. Pretreatment upstream process: Major effects.

attractive attributes over low-lignin materials, organosolv along with steam explosion [111] and sulfite pretreatment to overcome recalcitrance (SPORL) [112] have become of prime interest for their ability to degrade high-lignin forest materials [53,112]. A recent study reported that steam explosion consumed the highest level of energy yielding the lowest pretreatment energy efficiency ratio of 0.26 kg sugar/MJ when compared to organosolv (0.31–0.40 kg sugar/MJ) and SPORL (0.35–043 kg sugar/MJ) [53]. While the organosolv treatments degrade high-lignin woody biomass including both softwood and hardwood, they produce considerable quantities of inhibitors namely furfural and HMF, yield a low hemicellulosic sugar concentration and are also associated with a high capital investment [113]. Consequently, SPORL remains the most attractive candidate for its flexibility and ability to overcome both hardwood and softwood recalcitrance with the highest sugar recovery and lowest energy consumption [53].

4.2. Hydrolysis

The success of the hydrolysis step is essential to the effectiveness of a pretreatment operation [80]. During this reaction, the released polymer sugars, cellulose and hemicellulose are hydrolyzed into free monomer molecules readily available for fermentation conversion to bioethanol [79]. There are two different types of hydrolysis processes that involve either acidic (sulfuric acid) or enzymatic reactions [114]. The acidic reaction can be divided into dilute or concentrated acid hydrolysis. Dilute hydrolysis (1–3%) requires a high temperature of 200–240 °C to disrupt cellulose crystals [115]. It is followed by hexose and pentose degradation and formation of high concentrations of toxic compounds including HMF and phenolics detrimental to an effective saccharification [19]. The Madison wood-sugar process was developed in the 1940s to optimize alcohol yield and reduce inhibitory and toxic byproducts. This process uses sulfuric acid H_2SO_4 (0.5 wt%) that flows continuously to the biomass at a high temperature of 150–180 °C in a short period of time allowing for a greater sugar recovery [116]. Concentrated acid hydrolysis, the more prevalent method, has been considered to be the most practical approach [102]. Unlike dilute acid hydrolysis, concentrated acid hydrolysis is not followed by high concentrations of inhibitors and produces a high yield of free sugars (90%); however, it requires large quantities of acid as well as costly acid recycling, which makes it commercially less attractive [117].

While acid pretreatment results in a formation of reactive substrates when acid is used as a catalyst, acid hydrolysis causes significant chemical dehydration of the monosaccharides formed such that aldehydes and other types of degradation products are generated [19]. This particular issue has driven development of research to improve cellulolytic-enzymes and enzymatic hydrolysis. Effective pretreatment is fundamental to a successful enzymatic hydrolysis [118]. During the pretreatment process, the lignocellulosic substrate enzymatic digestibility is improved with the increased porosity of the substrate and cellulose accessibility to cellulases. *Trichoderma reesei* is one of the most efficient and productive fungi used to produce industrial grade cellulolytic enzymes. The most common cellulase groups produced by *T. reesei* that cleave the $\beta \rightarrow 1,4$ glycosidic bonds are β-glucosidase, endoglucanases and exoglucanases [113]. However, cellulase enzymes exposed to lignin and phenolic-derived lignin are subjected to adverse effects [36,37,119] and have demonstrated that phenolic-derived lignin have the most inhibitory effects on cellulases. This study reported that a ratio of 4 mg to 1 mg peptides, reduced by half the concentration of cellulases (i.e. β-glucosidases) from *T. reesei*. This strain was also shown to be 10 to 10 fold more sensitive to phenolics than *Aspergillus niger*. In addition to phenolic components effect on cellulases, lignin has also an adverse effect on cellulases. As mentioned previously, the lignin adverse effect has two aspects including non-productive adsorption and the limitation of the accessibility of cellulose to cellulase. Although considerable genetic modifications (GMs) have been deployed to transform lignin effects, lignin has been shown to be a potential source of self sustaining-energy and added-value components. Consequently, several research studies have determined practical approaches in eliminating inhibition of cellulases without involving GM approaches. Lui et al. [120] have demonstrated that the application of metal components namely, Ca(II) and Mg(II) via lignin–metal complexation substantially enhanced

Table 3
Pretreatment methods and key characteristics.

Pretreatments	Key characteristics	References
Dilute acid (H_2SO_4, HCL (0.5–5%)	- Practical and simple technique. Does not require thermal energy. - Effective hydrolyze of hemicelluloses with high sugar yield. - Generates toxic inhibitors - Requires recovery steps	[79,93,103,105,106,194]
Hot water	- The majority of hemicelluloses can be dissolved. - No chemicals and toxic inhibitors. - Average solid load. - Not successful with softwood.	[46,92,94–96,108,195,196]
Lime	- High total sugar yield including pentose and hexose sugars. - Effective against hardwood and agricultural residues. - High pressure and temperature hinder chemical operation. - Commercial scalability problem	[53,107,196]
Ammonia fiber expansion (AFEX)	- Effective against agricultural residues mainly corn stover without formation of toxic end-products. - Not suitable for high-lignin materials. - Ammonia recovery - No wastewaters	[19,118,122,147,149,183]
Ammonia recycle percolation (ARP)	- High redistribution of lignin (85%) - Recycling ammonia - Theoretical yield is attained	[26,199,200]
Steam explosion with catalyst	- Effective against agricultural residues and hardwood. - High hemicelluloses fractions removal - Not really effective with softwood	[106,122,201–203]
Organosolv	- High yield is enhanced by acid combination. - Effective against both hardwood and softwood. - Low hemicellulosic sugar concentration - Formation of toxic inhibitors - Organic solvent requires recycling - High capital investment	[202,204]
Sulfite pretreatment top overcome recalcitrance (SPORL)	- Effective against high-lignin materials, both softwood and hardwood. - Highest pretreatment energy efficiency - Minimum of inhibitors formation - Accommodate feedstocks versatility. - Steam explosion combined to SPORL in presence of catalyst becomes effective against softwood materials - Cost-effective.	[53,89,90,112,132,133,184]
Ozone	- Effectively remove lignin from a wide range of cellulosic material without generating inhibitors. - Expensive	[19]
Alkaline wet oxidation	- The combination of oxygen, water, high temperature and alkali reduce toxic inhibitors. - High delignification and solubilization of cellulosic material - Low hydrolysis of oligomers	[97,202]
Fungal bioconversion	- Environmentally friendly - Low use of energy and chemical - Slow bioconversion	[181,206]

enzymatic hydrolysis. Additionally, Erickson et al. [121] have reported the importance of additives namely, surfactants and bovine serum albumin (BSA) in blocking lignin interaction with cellulases. Sewalt et al. [119] have reported that the adverse effect of lignin on cellulases can be surmounted by ammoniation and various N compounds. Moreover, the enzymatic treatment can be accomplished simultaneously with the engineered co-fermentation microbial process known as simultaneous saccharification and fermentation (SSF) [31,122]. This process has been of interest since the late 1970s for its effectiveness to minimize cellulolytic product inhibition and subsequently increase alcohol production [122]. Typically, separate hydrolysis and fermentation (SHF) processes involve the inhibition of the hydrolytic enzymes (cellulases) by saccharide products such as glucose and cellobiose. Unlike SHF, the SSF process combines hydrolysis and fermentation activities simultaneously and hence

keeps the concentration of saccharides too low to cause any considerable cellulase inhibition [109].

4.3. Fermentation

Pretreatment and hydrolysis processes are designed to optimize the fermentation process [80]. This natural, biological pathway depending on the conditions and raw material used requires the presence of microorganisms to ferment sugar into alcohol, lactic acid or other end products [11,79]. Moreover, industrial yeasts such as *S. cerevisiae* have been used in alcohol production mostly in the brewery and wine industries for thousands of years. *S. cerevisiae* has also been utilized for corn-based and sugar-based biofuel industries as the primary fermentative strain. Once becoming accessible for enzymatic or acidic hydrolysis, the pretreated cellulosic slurry is subsequently converted into fermentable

free sugars. The sugars are mixed with water to form a broth. Typically, during batch fermentation *S. cerevisiae* ferments hexose sugars, mainly glucose, into ethanol in a large tank via the Embden–Meyerhof pathway under anaerobic conditions and controlled temperature. Yeast-based fermentation is always accompanied by formation of CO_2 by-products and supplemented by nitrogen to enhance the reaction. This conventional strain is optimal at a temperature of approximately 30 °C and resists a high osmotic pressure in addition to its tolerance to low pH levels of 4.0 as well as inhibitory products [123]. *S. cerevisiae* can generate a high yield of ethanol (12.0–17.0% w/v; 90% of the theoretical) from hexose sugars [34,124].

Traditionally, separate hydrolysis and fermentation (SHF) sequential steps are used in bioethanol production. However, there is particular interest in targeting bioethanol production that can be derived from lignocellulosic biomass materials where both hexose and pentose sugars are available from the hemicellulose fraction. Despite its broad tolerance to stressful bioethanol process conditions, *S. cerevisiae* is not able to ferment sugars other than hexose. Unfortunately, lignocellulosic material includes a large proportion of hemicellulosic biomass that contains mainly pentose sugars such as D-xylose [125]. Moreover, an optimal fermentative microorganism should be tolerant to a high ethanol concentration and to chemical inhibitors formed during pretreatment and hydrolysis process. In response to this inability of *S. cerevisiae* to ferment pentose sugars, extensive efforts have been employed to develop genetically engineered microorganisms that are capable of fermenting pentose and hexose sugars simultaneously. An optimal fermentative microorganism should be able to utilize both hexose and pentose simultaneously with minimal toxic end-products formation. Different techniques including SSF and consolidated bioprocessing (CBP) have been developed to ensure the combination of hydrolysis (step 3) and fermentation (step 4) in one single reactor and thus, reduce product inhibition and operation costs. In addition to continuing downstream steps, CBP processing integrates both fermentation and cellulase formation in one fermentative/cellulolytic microorganism [75]. However, despite the extensive range of prokaryotic and eukaryotic microorganisms that have been shown to be able to produce ethanol from sugars, most of them remain limited in terms of sugars co-fermentation, ethanol yield and tolerance to chemical inhibitors, high temperature and ethanol.

In an effort to summarize relevant advantages and major limitations of microbial fermentative species, Table 4 compares potential microorganisms for lignocellulosic-based biofuel fermentation including bacteria, yeasts and fungi that could be optimized and become potential avenues to enhance alcohol yield and productivity in large-scale lignocellulosic-based ethanol fermentation.

4.4. Separation/distillation

Bioethanol obtained from a fermentation conversion requires further separation and purification of ethanol from water through a distillation process. Fractional distillation is a process implemented to separate ethanol from water based on their different volatilities. This process consists simply of boiling the ethanol–water mixture. Because the boiling point of water (100 °C) is higher than the ethanol-boiling point (78.3 °C), ethanol will be converted to steam before water. Thus, water can be separated via a condensation procedure and ethanol distillate recaptured at a concentration of 95% [23]. Typically, most large-scale industries and biorefineries use a continuous distillation column system with multiple effects [126]. Liquid mixtures are heated and allowed to flow continuously all along the column. At the top of the column, volatiles are separated as a distillate and residue is recovered at the bottom of the column.

5. Current issues and challenges of lignocellulosic bioethanol production

5.1. Overcoming recalcitrance of lignocellulosic materials

Although lignocellulosic biomass is a potential feedstock for biorefineries, its recalcitrant structure and complexity remain a major economic and technical obstacle to lignocellulosic-based biofuel production [127]. The resilience of lignocellulosic materials is due to their composition and physicochemical matrix. The organization of vascular, epicuticular waxes as well as the amount of sclerenchymatous and the complexity of matrix molecules, contribute to the compactness and strength of the cellulosic material [87].

Furthermore, lignocellulosic materials as discussed previously are composed principally of three components namely, cellulose, hemicellulose and lignin. Together the polysaccharides, cellulose and hemicelluloses serve as initial substrates for subsequent saccharification and fermentation. However, these components are encapsulated via a tight covalent and hydrogen link to the lignin seal [96]. These tight bonds not only give the cell wall its compact structure but limit enzyme access to the surface area. Moreover, cellulose, a polymer of glucose molecules linked via β (1→4)-glycosidic bonds confers to cellulose a crystalline and compact structure [66].

Hemicellulose, the amorphous part of the cell wall, is composed of different hexoses and pentose sugars including xylose and arabinose bonded through xylans β (1→4)-linkages. These varieties of sugars polymers and linkages between molecules impose more complexities to the cell wall and therefore the hydrolysis process necessitates numerous cost-prohibitive enzymes to cleave polysaccharides entirely into fermentable sugar fragments. Additionally, components including primarily xylo-oligosaccharides produced from hemicelluloses hydrolysis have been shown to be inhibitory to cellulase enzymes [128]. Although xylose causes a higher level of inhibition to cellulase enzymes than xylan, soluble xylo-oligomers are considered the most inhibitory to cellulase and substantially influence enzymatic hydrolysis [129,130]. Hence, the removal of these components in addition to organic acids and phenolics is desired in an attempt to achieve an efficient cellulose conversion via enzymatic hydrolysis [75]. Thus, a successful and low-cost ethanol bioconversion is closely related to the efficiency of the pretreatment step. Pretreatment which is mechanical and/ or thermo-chemical, and/or a biological agent primarily involves redistribution of lignin and improving cellulose accessibility to enzymes by increasing the surface area that will be subjected to further hydrolysis. An effective pretreatment also requires a reduction of energy consumption with minimum toxic inhibitory products formation [53,80]. However, in addition to these complexities and differences between components within the lignocellulosic material, lignocellulose composition from each type of biomass varies depending on the origin and geographical location. Not all types of lignocellulosic feedstocks require the same pretreatment strategy. These heterogeneities have an important impact on the choice of pretreatments and the downstream processes [131]. Currently, the SPORL treatment is of interest for its broad spectrum ability on acting in both softwood and strong hardwood materials [115,132]. This pretreatment degrades high-lignin forest material with a limited formation of hydrolysis inhibitors [133]. Wang et al. (2009) [132] have demonstrated that lignin redistribution and increased porosity and surface area were achieved in only 30 min and was followed by 10 h of enzymatic

Table 4
Advantages and drawbacks of potential organisms in lignocellulosic-based bioethanol fermentation.

Species	Characteristics	Advantages	Drawbacks	References
Saccharomyces cerevisiae	Facultative anaerobic yeast	- Naturally adapted to ethanol fermentation. - High alcohol yield (90%). - High tolerance to ethanol (up to 10% v/v) and chemical inhibitors. - Amenability to genetic modifications	- Not able to ferment xylose and arabinose sugars. - Not able to survive high temperature of enzyme hydrolysis.	[69] [143] [207] [80] [123] [208]
Candida shehatae	Micro-aerophilic yeast	- Ferment xylose	- Low tolerance to ethanol - Low yield of ethanol. - Require micro-aerophilic conditions - Does not ferment xylose at low pH	[69] [209] [94] [210]
Zymomonas mobilis	Ethanologenic Gram-negative bacteria	- Ethanol yield surpasses S. cervesiae (97% of the theoretical), - High ethanol tolerance (up to 14% v/v) - High ethanol productivity (five-fold more than *S. cerevisiae* volumetric productivity) - Amenability to genetic modification. - Does not require additional oxygen	- Not able to ferment xylose sugars. - Low tolerance to inhibitors - Neutral pH range	[211] [212] [69]
Pichia stiplis	Facultative anaerobic yeast	- Best performance xylose fermentation. - Ethanol yield (82%). - Able to ferment most of cellulosic-material sugars including glucose, galactose and cellobiose. - Possess cellulase enzymes favorable to SSF process.	- Intolerant to a high concentration of ethanol above 40 g/L - Does not ferment xylose at low pH - Sensitive to chemical inhibitors. - Requires micro-aerophilic conditions to reach peak performance - Re-assimilates formed ethanol	[69] [213] [209] [214]
Pachysolen tannophilus	Aerobic fungus	- Ferment xylose	- Low yield of ethanol. - Require micro-aerophilic conditions - Does not ferment xylose at low pH	[209] [215]
Esherichia coli	Mesophilic Gram-negative bacteria.	- Ability to use both pentose and hexose sugars. - Amenability for genetic modifications	- Repression catabolism interfere to co-fermentation - Limited ethanol tolerance - Narrow pH and temperature growth range - Production of organic acids - Genetic stability not proven yet - Low tolerance to inhibitors and ethanol	[80] [215] [33]
Kluveromyces marxianus	Thermophilc yeast	- Able to grow at a high temperature above 52 °C - Suitable for SSF/CBP process - Reduces cooling cost - Reduces contamination - Ferments a broad spectrum of sugars. - Amenability to genetic modifications	- Excess of sugars affect its alcohol yield - Low ethanol tolerance - Fermentation of xylose is poor and leads mainly to the formation of xylitol	[153] [109] [180]
Thermophilic bacteria: *Thermoanaerobacterium saccharolyticum* *Thermoanaerobacter ethanolicus* *Clostridium thermocellum*	Extreme anaerobic bacteria	- Resistance to an extremely high temperature of 70 °C. - Suitable for SSCombF/CBP Processing - Ferment a variety of sugars - Display cellulolytic activity - Amenability to genetic modification.	- Low tolerance to ethanol	[217] [109,154,155] [95]

hydrolysis. A small amount of 4% sodium bisulfate was added to the solution under pH level of 2.0–4.5 and at a temperature of 180 °C. The entire conversion of cellulose to glucose sugar was accompanied by generation of low concentrations of inhibitors (less than 20 mg/g).

5.2. Potential water availability challenges for the biofuel system

Although biofuel water use is an important component to consider for the sustainability of biorefineries, limited information is available worldwide and in the U.S. on water requirements for the emerging agricultural practices and technologies that could impact water supplies and quality [134]. While water availability does not pose a serious constraint in several countries such as Brazil, Canada, Russia and some African nations, other countries including China, India, South Africa and Turkey are already encountering scarce water issues before even considering estimates of additional water consumption associated with biofuel production [135]. In the U.S., water availability could become an issue in the near future if appropriate and more effective agricultural water sustainability practices are not implemented. To date, U.S. lignocellulosic-based ethanol is only produced at a pilot scale level and is not yet commercially available [134]. However, this study also reported that energy corn-derived biofuel has already achieved an exponential growth requiring an increasing availability of water in the Great Plains and other arid regions of

the country. Moreover, biofuel water availability is a very complex issue because it varies by regions and type of crops [136]. With the increasing awareness toward the adverse effects of biofuel system on the quality and availability of water, there has been a series of investigations led by the U.S. National Academy of Science (NAS) to determine current agricultural practices and their impact on water resources and quality [136]. NAS has reported that the most important factors that cause substantial water stress due to biofuel production is the expansion of energy crops such as corn in those areas of the U.S. Midwest that are already susceptible to drought and hence require intensive irrigation. Although biofuel processing utilizes a significant level of water, it does not consume as much water as biofuel crops. Furthermore, biofuel crops involve a substantial use of pesticides and herbicides in addition to fertilizers resulting in a surplus of nutrients including, nitrogen and phosphorus. This excess of nutrients used for corn and other energy crops was demonstrated to lead to an expansion of the "dead zone" in the Gulf of Mexico caused by oxygen depletion [137]. NAS envisions a solution that places the emphasis on increasing irrigation-efficiency used by farmers as well as plant water recycling. However, Huffaker [138] suggests that efforts should be directed toward improving water quality impact rather than water recycling and irrigation efficiency. While further expansion of cellulosic feedstock sources would be an attractive alternative within the next decade to mitigate water supplies and reduce fertilizer use geared toward intensive crop cultivation, a shortage of water resulting from inefficient water utilization during biofuel processing could also jeopardize biofuel water sustainability [134].

6. Current prospects for systems approaches to biomass conversion

Current research is continuing to deploy individual and specific efforts toward achieving optimal solutions via improving lignocellulosic-based ethanol performance with a minimum capital investment on energy consumption and water supplies. Future prospects for the optimization of lignocellulosic bioconversion must embrace a more systematic enhancement of bioethanol for all four major steps in bioethanol production. Pretreatment as a first step is the most costly operation and accounts for approximately 33% of the total cost [139] with respect to the economic feasibility of each step as well as the consideration of microbial and chemical contaminations that can potentially reduce yields. Developing genetically modified fermentative and cellulolytic microorganisms enhanced by co-culture systems is desirable to increase ethanol yield and productivity under the stressful conditions associated with high production bioethanol-processes [140]. SSF as well as simultaneous saccharification and combined fermentation (SSCombF) of the enzymatic hydrolyzate, glucose with the hemicelluloses-derived sugars [120] and CBP are also considered to be cost-effective and offer promise in reducing end-product inhibition and operation numbers [122,141]. However, an overall analysis of performance would provide a clear vision of the system conditions and allow implementation of feasible preventive interventions aimed at enhancing biofuel production efficiency.

6.1. Overall analysis of performance: life cycle assessment (LCA) comparisons

As technologies emerge that improve various stages of biofuel production from biological sources, there is increasing need to compare overall performance with current operational systems to verify their validity in terms of water use and energy performance on biofuel systems as well as the environmental impact. LCA methodologies are considered to be the analysis model of choice for quantitatively comparing the environmental impacts of each biomass-based energy generating system. This approach primarily focuses on the estimation of direct impacts along with indirect and co-products credits including the carbon cycle as well as gas emission, fossil fuel consumption, water consumption and generation of wastes involving energy utilization.

Recent studies conducted by Mu et al. [81] have analyzed and compared biochemical and thermochemical conversion pathways based on LCA studies. They concluded that despite the equivalent alcohol productivity and energy efficiency performance between the two routes, in the short run biochemical conversion is considered to have a more favorable environmental performance than the thermochemical route. LCA approaches rely on quantitative estimations of direct (chemical pollutant agents) and indirect (greenhouse gas emissions (GHG), fossil fuel intake, water consumption) impacts along with biomass contribution and co-product credits (electricity, mixed alcohol and heat). Assessments performed by legislators on the validity of the biomass-based energy, stipulated that a satisfactory alternative to petroleum gasoline should achieve at least 20% reduction in GHG. Biochemical conversion of cellulosic materials was able to achieve 50% reduction of GHG emission compared to a non-renewable fuel. The biochemical route also saved consumption of fossil fuel resources (1.13 MJ/L) but generated chemical releases including phosphorus and nitrogen to the atmosphere causing additional eutrophication and acidification. While the biochemical route exhibited higher water consumption than the thermochemical process, it did yield a better short-term environmental performance on parameters such as GHG emissions and fossil fuel consumption. This in turn leads to a lower impact on the environment as it uses components such as lime, sulfuric acid and nutrients that can considerably influence LCA estimates of fossil oil, water consumption and greenhouse gas emission. Much more detailed LCA comparisons between thermochemical and biochemical operations have been discussed elsewhere [81].

6.2. Optimization of the biofuel process main steps

To date, various approaches have been advanced to improve the four-steps of the bioethanol process. Pretreatment is considered the most costly operation and a major constraint toward achieving high-yield via low-cost capital [93]. Therefore, an initial step for improvement is crucial to the success of downstream operations. There has been considerable advancement in pretreatment technology and several approaches are already available and successful depending on the characteristics of the respective lignocellulose biomass source. Feedstocks richer in lignin exhibit a high recalcitrance and resistance, thus requiring different treatment approaches from raw materials that have a higher quantity of amorphous hemicelluloses rich in pentose sugars [142]. Hence, the inevitable feedstock versatility and variability has become a potential issue for bioethanol investors. Given that ethanol is a commodity product, bioethanol plants would have limited choices for available feedstock. This key issue has led researchers to look for a pretreatment process able to deal with a variety of raw materials [53]. Moreover, the appropriate treatment is also correlated to the manufacturing economics as well as lay-out and possible investments. The selection of a suitable pretreatment relies primarily on environmental, economical and technological factors including energy savings, wastewater, recycling issues, substrate recovery along with a maximal solid loading yield and minimal use of chemicals [143].

Traditionally, dilute acidic pretreatment is the most commonly used method in the bioethanol process. This upstream treatment is considered to be the most practical due to its effectiveness at a low-cost [102,144]. However, the formation of high levels of toxic inhibitors namely, acetic acid, HMF and phenolic components requiring an additional detoxification step have led researchers to focus on better alternatives. Phenolic components particularly phenolic hydroxyl groups can influence cellulase enzyme activities [53]. Consequently, it is important to remove phenolics if enzymatic hydrolysis is to be improved. Furthermore, according to Ladisch et al. [75], since toxic inhibitors such as aldehyde components considerably influence microbial growth rate and volumetric productivity, selecting a fermentative culture from metabolically modified microorganisms would improve microbial resistance to inhibitors.

Steam explosion in the presence of catalyst has gained considerable interest and researchers are examining the potentially high correlation between catalyst concentration and ethanol yield. Of the numerous techniques tested, Öhgren et al. [145] confirmed the effectiveness of catalyzed steam-explosion by 3% (w/w) sulfur dioxide (SO_2) pretreatment accompanied by a cellulase and xylanase hydrolysis step at 45 °C during 72 h. These operations yielded approximately 96% glucose and 86% xylose from residue corn stover feedstocks. The Consortium for Applied Fundamentals and Innovation [145] have also demonstrated the efficiency of SO_2 steam explosion against poplar hardwoods (*P. deltoids*) as it produced an 86.2% xylose yield with a final ethanol concentration of 25.9 g/L. Although SO_2 could be toxic to the environment and sulfur alone could pose potential harmful effects to some cellulolytic enzymes and distillation, a SO_2 catalyst has been demonstrated to increase enzymes accessibility to the biomass owing to a more complete and rapid hemicellulose release [145,146]. Additionally, information is still lacking to confirm residual SO_2 side effects once ethanol is used in motor vehicles. Moreover, Hu et al. (2008) [46] reported that the acetic or uronic acid associated to autocatalysis effects from wood pretreatment could be a better alternative to sulfuric acid or SO_2 catalysts. According to this study, despite optimal cellulases pH levels of 4.5–5, an impregnation of the biomass at room temperature with an appropriate dosage of acetic acid of 1 mM corresponding to a pH level of 3.9 is feasible. This acid impregnation followed by a pretreatment temperature at 200 °C for 10 min would not require substantial toxic compound removal or adverse effects to cellulolytic enzymes. Thus, acetic acid could be a potential alternative to dissociate the biomass. However, further investigations need to be performed to validate these assumptions.

AFEX has also been developed as another emerging economical pretreatment that limits inhibitor formation for agricultural residues such as corn stover [19,147,148]. Moreover, extensive research continues to improve steam explosion with catalyst effectiveness against recalcitrant softwood materials. Zhu et al. [112] developed a potential pretreatment SPORL to overcome the high recalcitrance of woody biomass such as softwood material. This approach produced readily hydrolyzed sugars and achieved excellent recovery of the hemicelluloses with minimal generation of inhibitors. Interestingly, 87.9% of the hexose and pentose sugars were recovered with the SPORL method when compared with overall saccharides recovered from dilute acid (56.7%) [133]. The short pretreatment time period associated with this approach permitted a low liquid-to-wood-ratio leading to a greater pretreatment energy efficiency [53]. Moreover, SPORL appears to be complementary to steam-explosion when using a catalyst and thus improves its effectiveness against softwood biomass [133].

Different strategies including SHF, SSF as well as SSCombF have been extensively evaluated and subsequently implemented to initiate hydrolysis of released sugar polymers. There is some evidence that while these treatments have advantages there are disadvantages as well. Since optimal enzymatic hydrolysis is initiated at approximately 50 °C while an optimal fermentation is enhanced at 35 °C, the SHF operation appears to be more cost effective than SSF [148]. However, the SSF pathway has the advantage of saving one step-costs in addition to its potential to prevent cellulase inhibition by end-products such as glucose and cellobiose. From another perspective, SSCombF improves the SSF technique by adding the co-fermentation process as it allows saccharification along with simultaneous sugar co-fermentations in a single reactor.

6.3. Cellulolytic/fermentative microbial ecology − identification of indigenous candidates

Although extensive research has been devoted to lignocellulosic-based biofuel conversion [147], less information has been provided on the microbial ecology and natural occurrence of viable microflora in cellulosic biomaterial as well as its derived residues. Typically, an in-depth knowledge and understanding of the ecology of the indigenous candidates could yield potential microorganisms useful for microbially-based fermentation and cellulolytic hydrolysis in biofuel production. However, most research efforts have focused on forestry and agricultural soil microbial characteristics reflecting microbial diversity associated with these ecosystems, since there is a mutual and close relationship between the soil-microflora and plant roots [150]. Cellulosic-containing soil consists of a wide range of microorganisms including bacteria, filamentous fungi and wild yeasts. Synergism among these microorganisms is fundamental to the ecological balance constituting the biomass ecosystem [151]. The nature of microorganisms as well as the frequency and abundance vary depending on the ecological factors such as geographical location, climate, soil and viable forms. Bacterial populations in normal fertile agricultural soil can reach 10−100 million colony-forming units (CFU)/g [150]. Yeasts in soil can range from a few to greater than a 1000 cells per gram. In southwestern Slovakia, 111 yeast strains were isolated from 60 different agricultural soil samples. Among the wide range of collected strains 4 genera namely, *Cryptococcus*, *Candida*, *Metschnikowia* and *Sporobolomyces* were considered to be the most predominant [151]. This study revealed that the number of yeasts collected from agricultural soil was ten times lower than yeasts isolated from forest soil since less fungicide and tillage were used in the nearby forest.

Of the numerous microorganisms collected from biomass ecosystems, only a few strains have proven to be of interest for their ethanologenic or cellulolytic abilities in bioethanol bioconversion. In northeastern Brazil, genera such as *Candida*, *Pichia* and *Dekkera* were isolated from sugarcane molasses. Despite their overall fermentative ability, these genera yielded low ethanol concentrations in comparison to *S. cerevisiae* and produced acetic acid which was inhibitory to the fermentative yeast [152]. However, some natural ethanologenic yeast species such as *Pichia stipilis*, *Pachysolen tannophilius*, *Kluyveromyces marxianus* and *Candida shehatate* appeared to have promise in replacing *S. cerevisiae* in lignocellulosic-based ethanol fermentation [140]. Nevertheless, these wild yeasts still require further development to survive bioethanol fermentation conditions and yield an optimal ethanol concentration. The competitive exclusion as well as repression catabolism (competitive inhibition of hexose and pentose sugar transport) among these microorganisms in the bioethanolic ecosystem render addition of a selective agent to not be of particular value for improving yield performance [131]. However, selective temperatures with thermophilic yeasts including *K. marxianus*

or bacteria such as *Clostridium cellulolyticum* and *Thermoanaerobacterium saccharolyticum* may serve as alternatives if these microorganisms are used as the major fermentative and cellulolytic agents at high temperature operations (approximately 50 °C) [153–156]. Furthermore, indigenous groups of mesophilic and thermophilic-ethanologenic bacteria such as *Zymomonas mobilis* and *Bacillus stearothermophilus* have proven to be promising candidates to convert sugars into ethanol [140]; however, they remain deficient as optimal ethanol producers in comparison with *S. cerevisiae* in terms of resistance to high alcohol concentration and chemical inhibitors.

While a selection of indigenous bacteria and yeasts that possess fermentative abilities is possible, fungi isolated from agricultural residues and forest woods also possess attractive lignocellulolytic properties for initiation of the pretreatment step. In 1976, almost 14,000 cellulolytic fungi were collected from plant cell walls [157]. Only a few fungal isolates were selected for additional research and further categorized into three groups, namely white-, soft- and brown-rot fungi. Brown-rot fungi primarily hydrolyze the cellulose polymer, while white- and soft-rot fungi are able to degrade most of the lignin, hemicellulose and cellulose. White rot fungi such as *Basidomycetes* (e.g. *Phanerochaete chrysosporium* RP78) are indigenous to the northern part of the world. *P. chrysosporium* is considered among the most attractive alternative fungi for biomass processing due to their physico-chemical abilities to non-selectively break down lignin recalcitrant material from the cell wall while liberating cellulose and hemicellulose. These fungi are thermo-tolerant and can survive a temperature of 40 °C [158]. *Chrysosporium* is also known as a wood-decaying fungus for its unique oxidative system and has been shown to be effective on the pre-treatment of cotton stalks [159]. *Phlebia radiata*, as well as *Phlebia floridensis* and *Daedalea flavida* belong to *Basidomycetes* species and are capable of selectively degrading lignin in wheat straws and cellulosic residues [160]. *Trichoderma viride*, *Trichoderma emersoni* along with *T. reesei* (*Ascomyctes*) and *A. niger* are also attractive for their cellulolytic properties, tolerance to low pH and high temperature in addition to their ability to release large-scale cellulase enzymes [158]. *T. viride* grows rapidly at a wide pH range of 2.5–5.0 reducing potential contamination from other microorganisms [129,162].

Mushrooms including *Volvariella* species also possess hydrolytic capabilities. They have been isolated mostly from rice straws in Asian or African countries. *Lentinus edodes* has also been used in Japan and China to digest lignified residues. Aside from their ability to degrade lignocellulosic biomaterial, some white-rot fungi belonging to the genus *Pleurotus* are able to convert waste into protein for human and animal consumption [163,164].

Clostridium thermocellum, an anaerobic thermophilic microorganism, is among the rare bacteria that possess cellulolytic properties in addition to its ability to ferment sugar polymers into ethanol [162]. Several physiological attributes make this microorganism a promising candidate. It has a selective growth temperature of 50 °C during the fermentation process and can convert cellulose polymer directly into ethanol yielding 0.3 g/g ethanol per converted cellulose at a high temperature of approximately 60 °C [165,166]. *C. thermocellum* has been considered among the more promising thermophilic microorganisms suitable for SSF and CBP [141].

6.4. Fermentation optimization – potential genetically modified organisms (GMO)

Advances in genetic engineering have been made to alter the conventional yeast, *S. cerevisiae*'s capability to ferment glucose and pentose sugars simultaneously [167,168]. A *S. cerevisiae* TMB3400

modified stain, designed on the basis of expressing the same gene for *P. stipilis* xylose reductase (Ps-XR) is not only capable of co-fermenting saccharides but can also generate less HMF products (3 times less than the initial industrial strain) [169]. As mentioned previously, CBP is also a promising approach in combining both hydrolysis and fermentation operations in one single vessel. Additionally, CBP bioprocessing enables genetically-modified microorganisms that are able to produce cellulase enzyme to ferment sugars in one step and thus prevent further investment in costly cellulolytic enzymes [141]. Furthermore, Ladisch et al. [75] have reported that CBP could be combined with the pretreatment operation to generate lignin that could be used as a boiler fuel and provide sufficient energy to run the process (see Fig. 1).

However, fermentative microorganisms must be thermotolerant to survive the high temperatures of SSF/SSCombF/CBP processes. These processes can also be accompanied by a biological treatment step that utilizes cellulolytic fungi which require high temperature and low pH. Furthermore, Kumar et al. [109] suggested examining thermophilic anaerobic bacteria and yeasts such as *T. saccharolyticum*, *Thermoanaerobacter ethanolicus*, *C. thermocellum* and *K. marxianus* IMB3 for their potential to utilize a wide range of feedstocks at high temperatures above 65 °C. These thermophilic bacteria are able to ferment both hexose and pentose sugars in addition to their ability to produce cellulase enzymes and avoid the addition of commercial enzymes. Kumar et al. [109] have also reported that *Thermoanaerobacter* BG1L1 had the potential to ferment corn stove feedstocks at 70 °C within an undetoxified biomass in a continuous reactor system. This thermophilic fermentation yielded 0.39–0.42 g/g (ethanol per sugar consumed) and nearly 89–98% xylose was utilized despite the low tolerance to ethanol reported by Claassen et al. [124]. Ethanol fermentation at high temperature continues to be an emerging technology as it allows selection for microorganisms by temperature and does not require cooling costs and cellulase addition [170]. Recently, the thermo-tolerant yeast, *K. marxianus* has been documented as an attractive candidate due to its ability to co-ferment both hexose and pentose sugars and survive high incubation temperatures of 42–45 °C [171]. Moreover, *K. marxianus* was genetically modified to exhibit *T. reesei* and *Aspergillus aculeatus* cellulolytic activities allowing direct conversion of cellulosic β-glucan into ethanol at 48 °C under continuous conditions, yielding 0.47 g/g ethanol; 92.2% from the theoretical yield and making it an ideal GMO for CBP processing [171].

The industrial potential for *S. cerevisiae* fermentation has already been proven for first generation large-scale bioethanol production. The genetic improvement of the conventional fermentative strain is gaining increasing research interest since this strain is already the most optimally adapted to bioethanol fermentation conditions. To date, CBP for biofuel fermentation using genetically modified *S. cerevisiae* is an emerging technology that has been developed in several studies [172–174]. These studies demonstrate that in addition to its co-fermentative genetic flexibility, *S. cerevisiae* can also be genetically engineered to express cellulolytic and hemicelluloytic heterologous enzymes. van Zyl et al. [173] demonstrated this type of modification of *S. cerevisiae* by reassembling all existing components of a minicellulosome on its membrane surface from the thermophilic microorganism *C. cellulolyticum* via heterologous expression of a chimeric protein scaffold under phosphoglycerate kinase 1 (PGK 1) regulation. The successful functionality of cohesin and dockerin from *C. cellulolyticum* cellulosomein *S. cerevisiae* proved that this genetic modification based on a minicellulosome model may be an attractive option to the CBP process in hydrolyzing and fermenting substrates in a single step. Unlike *T. reesei*, recombinant *S. cerevisiae* is not able to simultaneously control cellulolytic enzyme expression to effectively

hydrolyze cellulose. Yamada et al. [175] reported the effectiveness of a cocktail δ-integration approach that consists of the insertion of a high cellulase activities based cassette into the yeast chromosome to optimize its cellulase expression ratio.

Z. mobilis is also among the more attractive ethalonogenic bacteria candidates due to its high ethanol yield production and resistance to temperatures in the range of 40 °C (2.5 fold higher than *S. cerevisiae*) [176]. Numerous genes have been introduced and heterologous expression has been incorporated into *Z. mobilis* to extend its effectiveness toward other substrates namely, xylose and arabinose since this strain is only able to ferment glucose [177]. Furthermore, the insertion of β-glucosidase gene into *Z. mobilis* to also convert cellobiose can be used in the SSF process [176,178,179]. Currently, commercial companies (DuPont Danisco Cellulosic Ethanol (DDCE) and Butalco) have assayed genetically engineered *Z. mobilis* and *S. cerevisiae* potential for their high ethanol yield performance and adaptability [180].

Enhancing large-scale low-cost ethanol bioprocessing by biological pretreatment involving fungi (e.g. *T. reesei* and a *Basidiomyctes*) that exhibit lignocellulolytic properties at low pH levels and high temperatures is also a promising added-value treatment to SSF ethanol bioconversion. While fungi bioconversion activities have been demonstrated to be slow, optimization of potential lignocellulolytic fungi has been demonstrated possible via mutagenesis, heterologous gene expression and co-culturing [181].

Although some of the emerging strategies and methods have proven to be promising under different circumstances, some of these technologies remain biomaterial-type and cost dependent. For example, Talebnia et al. [143] have concluded that the most suitable pretreatment for wheat straw material was steam explosion since it required a shorter reaction time, lower chemicals and high solid solubilization. However, this study also demonstrated that steam explosion operation exhibited a high level of influence on the downstream operations and its success depended on the framework of the entire process. Thus far, Binod et al. [182] hypothesized that an environmentally friendly biological conversion approach using thermo-tolerant stains such as *Clostridium phytofermentums* and *Basidomyctes* in SSF/CBP processings would be the future method of choice for rice straw feedstock if slow bioconversion is to be overcome.

Furthermore, Lau and Dale [183] have demonstrated the effectiveness of AFEX against corn stover feedstock via SSF process, using the 424 A (LN-ST) strain of *S. cerevisiae*, designed by Ho et al. [168]. This pretreatment achieved an ethanol concentration of 40.0 g/L (5.1 vol/vol%) without adding nutrients or requiring washing and detoxification steps. The Consortium for Applied and Innovation [173] team selected by the Department of Energy (DOE) office of the Biomass program has demonstrated a higher recalcitrance of poplar wood in comparison with corn stover. Optimal performance was achieved by a more severe treatment involving mainly SO_2 steam explosion or lime associated with the co-fermenting yeast strain 424 A (LN-ST) of *S. cerevisiae*. However, a large portion of these studies focused more on sugar yield with minimal attention given to mass balance and energy estimates crucial for a complete evaluation of pretreatment efficiency. Zhu and Pan [53] conducted an in depth study on the impact of the energy consumption from woody feedstock on estimating the effectiveness of potential pretreatments. They established the benchmark based primarily on the energy consumption for comparing the performance of the more attractive lignocellulosic biomass pretreatments including, SPORL, organosolv and steam explosion with catalyst. They demonstrated that SPORL pretreatment overall was the most advantageous and commercially scalable to sugar recovery along with total energy consumption (physical and thermo-chemical) in addition to the returned lignin

co-product potential from softwood. Zhu et al. [89] confirmed the effectiveness of SPORL pretreatment prior to a disc-milling operation on Lodgepole pine softwood in terms of pretreatment energy efficiency of 0.26 kg of sugar/MJ, an ethanol yield of 276 L/ton softwood (using thermo-tolerant, *S. cerevisiae* D5A), and an energy output of 4.55 GJ/ton wood correlated to the mass balance. Recent studies published by Tian et al. [184] identified the benefits from SPORL technique over dilute acid (DA) pretreatment used for the least resistant woody biomass, aspen (*Populus tremuloides*). This study revealed that SPORL pretreatment exhibited a higher substrate enzymatic digestibility (SED) than DA and was favorable to the high ethanol yield SSF process. Tian et al. [184] also concluded that SPORL pretreatment with 10% higher sugar and bioethanol yield as well as a higher ethanol and sugar production energy efficiency 395 kg/GJ over 339 kg/GJ for DA, remained one of the most attractive alternatives for low and high recalcitrant woody material. Olofsson et al. [131] used raw spruce material to demonstrate the importance of adopting a controlled feeding of cellulase enzymes to prevent the competitive inhibition of sugars transport (glucose over xylose). This study demonstrated that controlled-cellulase addition increased the total xylose uptake from 40 to 80%. Overall, sustained efforts are still required to improve bioconversion technology toward reaching the best performance possible to deal with lignocellulosic feedstock variability.

Improvement in each of these prospects represents individual steps toward implementing successful cost-effective lignocellulosic-based bioethanol operations. However, to accomplish substantial improvement will require more of a comprehensive systems approach that simultaneously accounts for all inputs and outputs during the entire operation regardless of changes in any of these individual steps.

6.5. Microbial risk assessment (MRA) modeling

6.5.1. Concepts

The use of GMOs presents another challenge to the bioethanol industry. Introduction of such organisms into large-scale fermentation operations opens up the possibility of environmental dissemination and potential exposure risks to public health. Likewise, industrial operations using antibiotics to control microbial contaminants in industrial scale fermenters or as strain markers would generate and release antibiotic resistant organisms and offer another potential environmental public health risk [35,185]. MRA is a comprehensive approach that can provide guidance for reducing potential microbial public health exposure by estimating the risk of microbial dissemination over all steps in a microbial-based process such as bioethanol formation. MRA is an emerging systematic and science-based method generally used to provide a qualitative and quantitative evaluation of the probability of occurrence of adverse health effects originating from microbial hazard contamination in food products [186]. It is based on four major steps namely, hazard identification, hazard characterization (response–dose assessment) followed by exposure assessment and risk characterization [186]. Currently MRA is the primary science-based tool of Codex Alimentarius on which the World Trade Organization (WTO) uses to describe food safety and risk estimation of food products [187].

6.5.2. Application of risk assessment in large-scale fermentation systems

Applications using MRA to certify the safety and equivalence of food products in today's global market are still early in development. For biofermenters, MRA would be a useful tool in assessing the exposure risk of using antibiotics to control large-scale microbial contamination by evaluating major steps from the plant source to the distillation final process for potential generation and
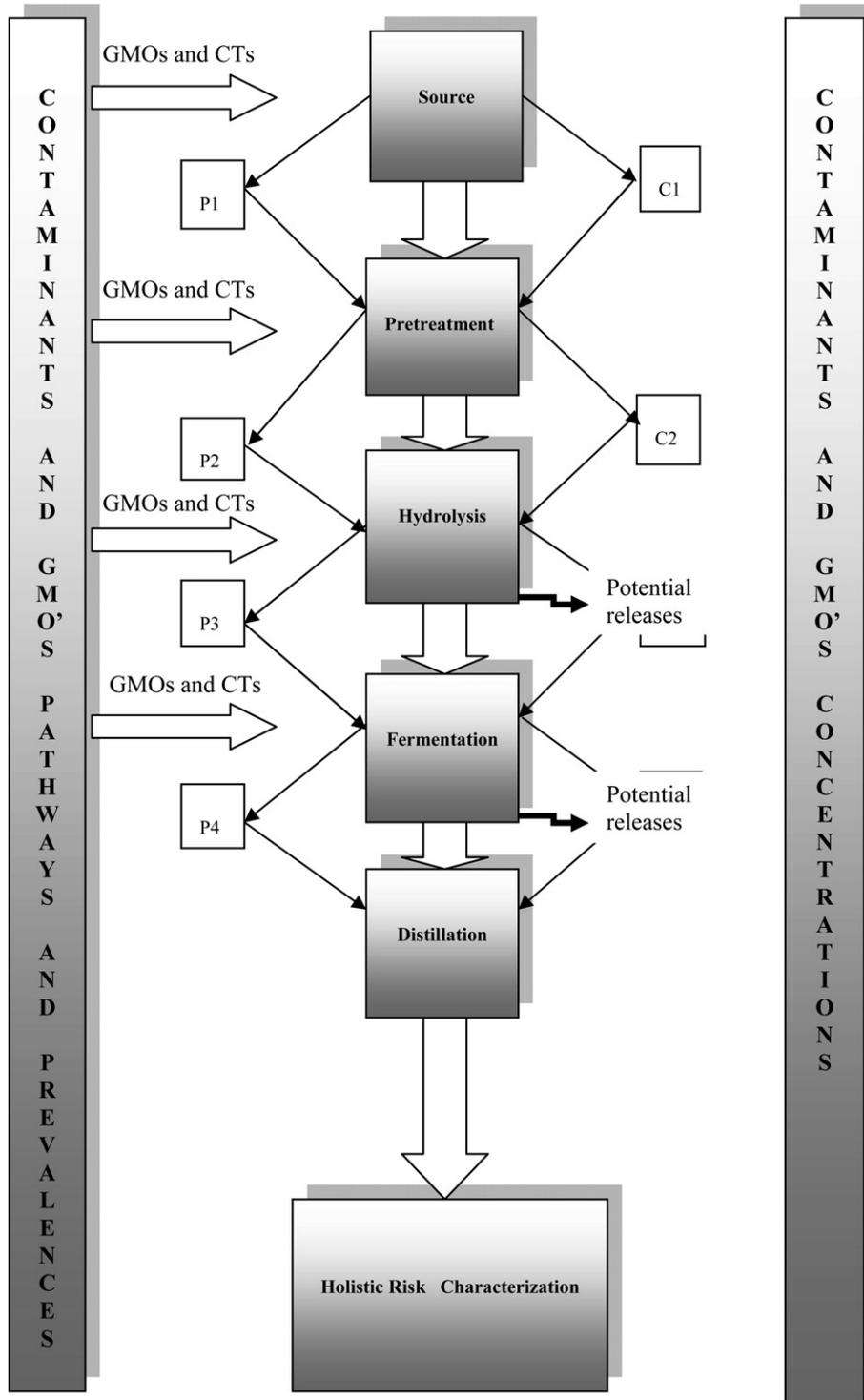
Fig. 3. Hypothetical MRA Model of Biofuel Source-to-distillation System (FAO, 2005) [190]. GMOs: Genetically modified organisms; CTs: Contaminants including antibiotic resistance organisms.

dissemination of antibiotic resistant organisms [188]. Fig. 3 illustrates a hypothetical model system of MRA for biomass processing based on the methodology adopted by Food and Agriculture Organization [189] of the United Nations. In this representation, the MRA concept was applied to the lignocellulosic-based biofuel operation from harvest-to-distillation in an attempt to design a model describing transparently dynamic microbial contamination. Detecting microbial problems at an early stage and

suppressing microbial dissemination via selective cost-effective control measures that does not cause damage to the ecosystem is of primary concern [185].

Rapid development of agricultural biotechnology in the early 1980s has led to the emergence of GMOs. Therefore, it has increased public concern on their potential hazards including pathogenic microbial mutations and the long-term proliferation of harmful genes in the environment that could have a serious

consequence on public health and the respective environments [190]. The awareness of the possible impact that could originate from large scale GMO applications has encouraged work primarily from the Toxic Substances Control Act (TSCA) on a pragmatic science-based methods such as MRA combined to biotechnology risk assessment (BRA) to predict the probability of occurrence of adverse outcomes in the environment from large scale GMOs based applications[191]. Thus, greater control could be performed to improve public health and ensure comprehensive environmental safety.

7. Conclusions – future prospects

Cellulosic-based biofuel is a potential alternative over food-derived bioethanol originating mainly from cornstarch and sugarcane provided by the world's large producers U.S. and Brazil, respectively. Pretreatment, the most costly step is of particular concern due to the high recalcitrance of lignocellulosic raw materials. Given that lignocellulosic feedstock is a versatile material and bioethanol is a commodity product, it has been deemed imperative to design a general pretreatment combination that would be effective against a wide range of cellulosic material and hence deal with feedstock variability. For instance, researchers have shown that pretreatments involving steam explosion with either catalyst or lime are potential candidates to agricultural residues, herbaceous materials and hardwoods. The inability of steam explosion combined with catalyst to degrade softwood materials can be compensated by the low-cost and the energy efficient SPORL pretreatment approach. Emerging technologies including SSCombF and CBP represent potential improvements as they reduce operation steps as well as chemical inhibitors and can be enhanced by lignin, energy-self-sustaining co-products. These processes are typically associated with thermophilic and cellulo-lytic microorganisms including organsisms such as *T. reesei* along with *P. chrysosporium*, *K. marxianus* and *C. cellulolyticum* with some of them possessing fermentative abilities in addition to their hydrolytic properties. However, some companies such as DDCE (DuPont Danisco Cellulosic Ethanol) and Butalco prefer using genetically engineered conventional strains, *S. cerevisiae* and ethanologenic *Z. mobilis* for their higher alcohol tolerance and yield.

In conjunction to rapid molecular biology techniques, mathematical modeling including MRA and biotechnology risk assessment (BRA) can be used to ensure greater predictability for limiting antibiotic resistant microflora and GMO dissemination during operation. While technological accomplishments and multiple research coalition efforts are still progressing, an efficient combination of the most advanced systems analysis and economical techniques designed to cope with feedstock versatility and commodity should emerge as the option of choice in an attempt to achieve optimal second-generation biofuel performance.

Acknowledgments

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