Biomass Conversion and Biorefinery (2024) 14:6111–6142
https://doi.org/10.1007/s13399-022-02791-9

REVIEW ARTICLE

Sugarcane bagasse: an important lignocellulosic substrate for production of enzymes and biofuels

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Received: 23 February 2022 / Revised: 25 April 2022 / Accepted: 6 May 2022 / Published online: 28 May 2022

Abstract

Sugarcane bagasse (SCB), a by-product of sugarcane industry, is a rich source of cellulose (45%), hemicellulose (32%), and lignin (17%) with low ash content. Being produced in large quantities by sugar industries, it is a great challenge for environment because it is mostly burnt in-open or either disposed improperly causing environmental pollution. Due to rich source of fermentable sugars, it is used as a substrate for producing microbial enzymes and biofuels. Secondly, high fuel prices, limited fossil fuel reserves, and environment pollution due to burning of fossil fuels have also highlighted the need for renewable and sustainable sources of energy such as biofuels. Sugarcane bagasse is a renewable, easily available, and cost-effective alternative for synthesis of biofuels and various microbial enzymes in submerged (SmF) as well as solid-state fermentations (SSF). However, for biofuel production, the main hindrance in utilizing bagasse is the requirement of large amount of enzymes for conversion of lignocellulosic biomass into fermentable sugars. Therefore, there is an utmost need for the production of enzymes using cost-effective and easily accessible substrates such as sugarcane bagasse followed by utilizing these enzymes for saccharification of carbohydrate polymers into fermentable sugars for biofuels. However, the presence of lignin hampers the saccharification of cellulose and hemicellulose into easily fermentable sugars. Therefore, pretreatment reduces lignin content of sugarcane bagasse and makes cellulose and hemicellulose easily accessible for enzymatic hydrolysis. Enzymatic hydrolysate can be further fermented to biofuels using aerobic and anaerobic microorganisms.

Keywords Lignocellulosic biomass · Cellulolytic enzymes · Pretreatment · Fermentation · Value-added products

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Abbreviations

SCB	Sugarcane bagasse
SSF	Solid-state fermentation
ABE	Acetone-butanol-ethanol
U/ml	Unit per ml
U/g	Unit per gram
SmF	Submerged fermentation
STB	Stirred tank bioreactor
RSM	Response surface methodology

1 Introduction

Sugarcane is one of the major cultivated crops in tropical and sub-tropical parts of the world. India is the 2nd largest producer of sugarcane after Brazil with an estimated sugarcane production of 306 million tons per year [1]. Sugarcane bagasse (SCB) is the main by-product of sugar industry, which is produced in huge amount, i.e., 100 million tons per year in India. About 50% of SB is utilized in the generation

of energy within the plant, while rest of the part remain unutilized in the environment. Most of the bagasse is burnt in open leading to increase in air pollution. Therefore, a proper strategy is needed for the management of bagasse in order to generate value-added products. Utilization of bagasse for production of enzymes and biofuels has been proven effective and valuable process. This will also solve the problem of fossil fuel crisis and problem of air pollution due to fossil fuels. Also, enzyme production can be made cost-effective by using low-cost SCB as substrate for the cultivation of microorganisms in SSF [1].

Enzymes are widely used for biofuel production and cellulases are considered industrially very important enzymes, and production of these enzymes using lignocellulosic biomass is an economical process [2–5]. The cost of enzymes is the main hindrance in biofuel production, mainly in those countries where the industrial production of enzymes is very low [6]. Ellila et al. [6] reported that carbon source which was used as a substrate for enzyme production costs more than 50% of the total amount required for enzyme production. Various approaches have been adopted to lower the cost of enzymes, such as using solid-state fermentation and utilizing lignocellulosic agricultural residues as source of carbon (e.g., SCB, rice straw, wheat bran, wheat straw, waste paper, corn cob, fruit pomace) [7, 8]. These sources are abundant, economical, and renewable source of nutrients for enzyme production [9]. The lignocellulosic biomass in addition to cost also serves as an inducer for production of enzymes [10]. An interesting alternative to high-cost carbon source for enzyme production is SCB, mainly in Brazil and India, that is abundantly available from sugar mills [11].

SCB can be used in untreated or pretreated forms [9]. The pretreatment of biomass reduces the crystallinity of structure and makes the cellulose easily accessible to microorganisms [12]. The choice of the pretreatment method has a great influence on the microbial growth because inhibitors are produced during many pretreatment methods which are inhibitory for growth of bacteria and fungi. Vasconcellos et al. [11] reported significant enhancement in production of enzymes after removal of the phenolic compounds. Various pretreatment methods are available and the choice depends on the biomass used. Combinatorial, i.e., ultrasound- and surfactant-assisted ionic liquid [13] and ultrasonic wave–assisted deep eutectic solvents [14] pretreated SCB resulted in enhanced saccharification. Currently, chemical and thermochemical methods of pretreatment are most effective and widely adopted at industrial applications.

SCB has been utilized tremendously for the production of various biofuels. It is a well-known lignocellulosic biomass for second-generation biofuel production (bioethanol, biohydrogen, biobutanol, and acetoin), xylooligosaccharide's prebiotics [1, 15]. In comparison to other biofuels, bioethanol is currently the most favorable because it has low-carbon dioxide emission, high heat of vapourization, and energy density [1]. Hydrogen is a carbon-free clean fuel which forms water as by-product after the combustion. Also in comparison to hydrocarbon fuels, it possess threefold high energy density [16]. Hydrogen as a biofuel has attracted interest of researcher's due to its distinguishing properties like odorless, colorless, and less toxicity and due to these features, it has been viewed to play an important role in future energy demands [17]. Biobutanol is formed usually by microorganisms in acetone-butanol-ethanol (ABE) fermentation. Butanol is considered a preferred fuel in industry because it has many properties similar to gasoline [1]. SCB could be utilized as economical substrate for the production of microbial enzymes at industrial scale. Therefore, huge quantity of SCB responsible for environmental pollution could be utilized for the production of microbial enzymes and biofuels for sustainable management of solid waste.

2 Background

Lignocellulosic biomass is one of the most favorable, sustainable, inexhaustible, and abundantly available resources for enzyme, biofuel, chemical, and pharmaceutical production. The complex structure of lignocellulose is composed of cellulose (35–50%), hemicellulose (25–30%), and lignin (25–30%) [18]. Cellulose is the most plentiful natural polymer present on earth; it is the chief structural component of the cell wall of plants. It is the principal agricultural waste as well as inexhaustible and economical source of energy-like biofuels. It is a polysaccharide made up of a linear chain of D-glucose units connected via β-1,4-glycosidic bonds. Hemicellulose is made up of pentoses (arabinose, xylose), hexoses (glucose and galactose), and sugar acids. These mixture of sugars are linked by β-1,4-glycosidic and β-1,3-glycosidic bonds. Lignin is a complex heteropolymer made up of phenylpropane units. It is insoluble in water, making the overall structure recalcitrant and provides the defense against microbial attack. Lignin is the major constituent which hinders in the breakdown of other lignocellulosic components. Thus, lignin removal is required to access the cellulose and hemicellulose that can be achieved by pretreatment of lignocellulosic biomass [1].

The high availability of carbohydrates in lignocellulosic biomass has attracted the interest of researchers to use this potential source as substrate for production of enzymes [1]. There are various agro-industrial wastes like SCB, sugarcane tops, peels, brans, and straws which can be used to produce enzymes; based on local agricultural practices and easy availability, they are chosen accordingly [19–21]. SCB is the waste generated from sugar industry in large quantities that serves as a suitable raw material for enzyme and biofuel production. South American and Asian countries share a

major proportion of *Saccharum officinarum* production [22]; therefore, SB offers a good raw material for enzyme and biofuel production in these regions. After juice extraction from *Saccharum officinarum*, the fibrous residue is bagasse [23], which is usually a waste and is under-utilized, that can be used to produce value-added products like biofuels, enzymes, and chip board making (Fig. 1). Similarly, SCB top is also preferred as a rich source for biorefinery due to the presence of high sugar content in it [19].

3 Global overview of sugarcane bagasse for enzyme-based bagasse biorefinery

Crop residues like SCB, rice straw, and wheat straw have the ability to replace unreplenishable resources due to their easy availability and low cost. Increasing production of lignocellulosic residues from agricultural crops and industries is presently creating environmental trouble. As a result, use of these crop residues as raw material for biorefineries is gaining interest, as it represents a potential source for lignocellulosic biomass management that integrates remediation and recycling in an eco-friendly manner [24]. Lignocellulosic biomass–based biorefineries have appeared as the most appropriate alternative for fossil fuel–based refineries. This novel concept includes a vast range of new technologies which are used to convert the structural components of lignocellulosic biomass into value-added products such as biofuels and various value-added co-products [24, 25]. Brazil is the leading producer of sugarcane in the world. Large-scale production of sugarcane results in the production of huge quantities of SB by sugar industries. Asia is also a major producer of SCB residues. India and China are the largest producers of sugarcane, after Brazil [26]. Agriculture in Oceania's is recognized by the production of large-scale, low-intensity crops due to vast fertile land [27]. The primary crops cultivated in this region include wheat, oat, and sugarcane; all of these crops produce huge amounts of crop residues. The use of these residues as feedstock for production of value-added bioproducts is a promising strategy because of their low cost and abundant availability [28].

Bioethanol production from lignocellulosic crop residues like sugarcane, corn, and wheat residues at commercial level has been practiced since 2014 by various industries across the globe, including USA, Brazil, and European countries like Italy [29]. Processing of sugarcane generates about 14% of bagasse and this residue presents great potential for use as raw material in biorefineries for production of value-added products in sugarcane-producing regions. SCB is the main residue after processing of sugarcane; it is acquired after the grinding process of sugarcane for extracting the juice [26]. Therefore, it is important to utilize bagasse which otherwise will lead to the deposition of agro-waste in large amounts [30]. SCB has been used from earlier times to produce various enzymes such as hemicellulolytic and cellulolytic enzymes, biofuels, and several other upgraded products [29]. At industrial scale, SCB is used to manufacture a particular type of paper called "megasse" which is fibrous and pulpy in nature [30].

4 Production of various enzymes using sugarcane bagasse as substrate

4.1 Cellulases

Cellulase is a complex of three enzymes which are namely, endoglucanase, exoglucanase, and β-glucosidase, which converts the cellulose polymer into free glucose units by the hydrolyzes of β-1,4 linkages [31]. Endoglucanase randomly cleaves the internal bonds of cellulose fibers and exoglucanase cleaves the end of cellulose releasing cellobiose as an end product, and finally β-glucosidase acts on cellobiose units and convert them into glucose [32]. They

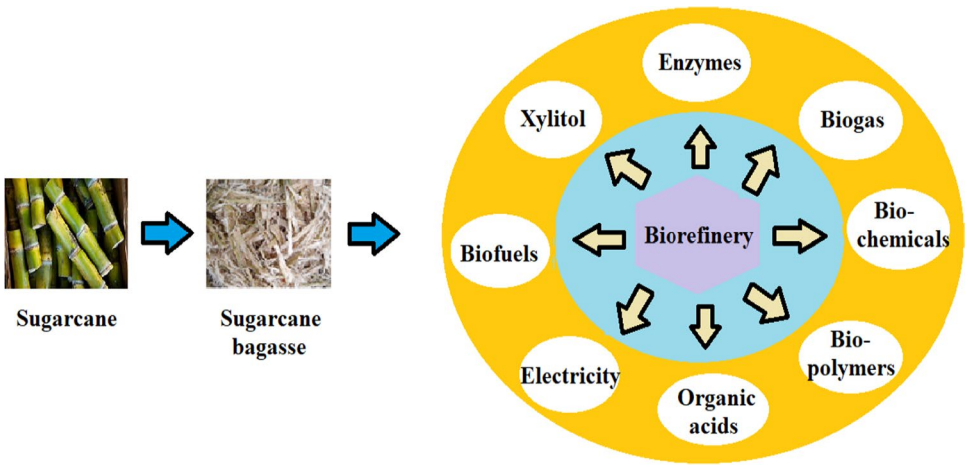

Fig. 1 Exploring the potential of sugarcane bagasse in context of biorefineries.

are inducible enzymes secreted by a diversity of microorganisms like bacteria and fungi during their growth on cellulosic materials. These cellulase-secreting microorganisms can be mesophilic, thermophilic, aerobic, or anaerobic. Genera *Aspergillus*, *Cellulomonas*, *Clostridium*, *Trichoderma*, and *Thermomonospora* are the extensively studied microbes for cellulase production [32]. Cellulases are used in various industries such as biofuel production, paper and pulp industry, food and beverage, pharmaceutical industry, textile industry, laundry detergents, and agriculture [33, 34].

4.1.1 Cellulase production using microorganisms in solid-state fermentation

Solid-state fermentation is a process in which microorganisms are allowed to grow on a solid substrate without free water. This mode of fermentation is used widely nowadays to produce industrially important enzymes, because it is more economical over submerged fermentation [35]. In SSF, solid substrates like SCB and rice bran are used which serve as a source of carbon for the growth of microorganisms [35]. High cost of substrates for enzyme production is the main problem associated with their large-scale production. With the aim to reduce the cost of enzyme production, there is a need for low-cost substrates like SCB and production via SSF [36].

Various microorganisms (bacteria and fungi) are known to hydrolyze lignocellulosic biomass. Cellulolytic bacteria belong to genera *Clostridium*, *Ruminococcus*, *Cellulomonas*, *Erwinia*, *Thermobifida*, and many others. Bacteria are able to degrade lignocellulosic biomass having low lignin content due to lack of adequate enzymatic machinery for its degradation [1–5, 37–39]. Biomass from aquatic plants is efficiently degraded by bacteria due to presence of low lignin content. Therefore, bacteria are better adapted to degrade aquatic plant biomass than terrestrial plant biomass. Furthermore, bacteria are less amenable to grow in SSF. In contrast, filamentous fungi play an important role in biomass degradation naturally. Genus *Aspergillus* is among one of the major decomposers and is the most common microbial agent for cellulolytic enzyme production [37–39]. *Aspergillus fumigatus* CWSF-7 isolated from lignocellulosic-contaminated waste was reported to produce a high level of cellulases. The endoglucanase (CMCase) activity was 1.9U/ml and exoglucanase (FPase) activity was 0.9 U/ml [40]. Maximum β-glucosidase activity of 1.16 U/ml was produced using *Aspergillus* strain on pretreated SCB supplemented with soyabean bran [41]. *Penicillium* spp. have been described as a good cellulase producer in literature [3, 42], and *Trichoderma* is commonly used fungi for commercial production of enzymes. Salomao et al. [36] reported high titre of cellulase from *Trichoderma koningii* as compared to *Rhizomucor* sp. and *Penicillium* sp. Highest cellulase activity for *Trichoderma koningii* was 8.20 U/g at 28 °C and 50% moisture using untreated SCB. The fungus *Metarhizium anisopliae* IBCB 348 was screened by Aita et al. [35] for enzyme production using different substrates, i.e., SCB, white rice, and malt bagasse. They observed that SCB without any supplement supported the highest exoglucanase (24.9 U/g) and chitinase (12.4 U/g). Gomes et al. [43] observed cellulolytic and xylanolytic activities in few yeast strains. They conducted a study on more than 350 strains of yeasts; out of these, 67 and 154 produced cellulases and xylanases, respectively. Among these, major genera are *Fellomyces*, *Cryptococcus*, *Myriangiale*, and *Ocultifer*. Only very few strains were reported to produce both cellulolytic and xylanolytic enzymes simultaneously [21]. Khan et al. [44] studied cellulase production by a thermophilic *Brevibacillus* sp. MT5 using SCB with activity of 1.776 FPU at 70 °C.

Rodríguez-Zúñiga et al. [13] evaluated the effect of various pretreatment methods such as dilute acid (sulfuric acid), alkali (NaOH), liquid hot water, and combination of acid and alkali on SCB structure and cellulase enzyme production by filamentous fungi *Aspergillus niger* under SSF. The highest cellulose content (86.7%) was obtained after combined acid/alkali treatment. SCB pretreated with liquid hot water resulted in highest FPase (0.4 U/g) and CMCase (14.9 U/g) activity after 72-h incubation. Using SCB as a substrate using solid-state fermentation, 0.26 U/ml cellulase is produced with 14% (w/v) SB after 168 h of culture using *Trichoderma harzianum* Rifai strain [45]. Production of xylanolytic and cellulolytic enzymes was carried out using pretreated SCB and wheat bran by *Penicillium echinulatum* 9A02S1 under SSF. Maximum amounts of filter paper activity (FPase), β-glucosidase, and endoglucanase activity on mixtures of pretreated SCB and wheat bran were $32.89 \pm 1{\cdot}90$ U/g, 58.95 U/g, and 282.36 U/g, respectively after 96 h [46].

Membrillo et al. [47] investigated the effect of different particle sizes of SCB such as 0.92 mm, 1.68 mm, and 2.9 mm on cellulase production by *Pleurotus ostreatus*. During first days of cultivation, both maximum CMCase (250 mU/g) and FPAse activity (130 mU/g) were attained on particles size of 0.92 mm. Gutierrez-Correa et al. [48] studied cellulase enzyme production by *Trichoderma reesei* LM-UC4 and its mutant strain LM-UC4E1 when co-cultured with another fungus *Aspergillus phoenicis* QM329 on SCB by process of mixed culture SSF. Then a mutual synergism was reported between the parent *Trichoderma strain* LM-UC4 and the *Aspergillus phoenicis* QM329, resulting in overall increased FPase (13.4 U/g), endoglucanase (73.8 U/g), and β-glucosidase activities (18.1 U/g). SCB was used as a substrate for cellulase production in solid-state fermentation by *Trichoderma*

reesei RUT C30. Under optimized conditions, maximum FPAse activity of 25.6 U/g was attained at 33 °C and 67-h incubation time [49].

Cellulase production was investigated using the fungus *Trichoderma reesei* NRRL 11,460 using four different lignocellulosic biomass (both untreated and pretreated) in SSF. Highest cellulase yield attained was 154.58 U/g when SCB was used in pretreated form as substrate with initial moisture content of 66%, with pH 7.0 of medium, 28 °C incubation temperature, use of 0.075 M NH_4NO_3, and 0.005 M cellobiose at incubation time of 72 h [50]. Cellulase production by *Aspergillus nidulans* SU04 and *Aspergillus nidulans* MTCC344 under SSF using NaOH-treated SCB was studied and optimized using RSM. Maximum CMCase activity was 32.59 U/g and 28.96 U/g for *Aspergillus nidulans* SU04 and *Aspergillus nidulans* MTCC344, respectively, using 15-mm bagasse bed height, 60% moisture content at pH 5.0, and temperature 40 °C in SSF [51].

SSF was carried out using different substrates such as SCB, cassava starch, wheat bran, coconut oil cake, and rice bran, and CMCase and FPAse activity was assessed after 7 days of culture. SCB resulted in the highest activity of 3.229 U/ml CMCase and 1.009 U/ml FPAse [52]. Cellulase production using SCB, wheat bran, and soybean meal was investigated in two reference strains *Trichoderma reesei* Rut-C30 and *Trichoderma reesei* QM9414 and two strains isolated from a sugarcane growing area *Trichoderma* sp. IPT778 and *Trichoderma harzianum rifai* IPT821 and a strain of *Myceliophthora thermophile* M77 in SSF. The highest FPase was 10.6 U/g for *Myceliophthora thermophila* M77 in soybean meal and SCB (10:90) at 80% moisture, which was 4.4 times greater than yielded using pure wheat bran. SCB enhanced cellulase yield 2.5 times greater than in pure wheat bran for *Myceliophthora thermophila* M77 [53]. Cellulase productions on natural lignocelluloses such as saw dust, ground nut shells, SCB, wheat bran, rice bran, and corn cobs by *Aspergillus niger* in SSF were studied. Using SCB, 15U/g FPase, 7 U/g endoglucanase, and 13U/g β-glucosidase were attained [54], while rice bran + SCB resulted in approx. 14 U/g FPase, 6 U/g endoglucanase, and 12 U/g β-glucosidase activity.

A thermophilic strain of *Humicola insolens* TAS-13 was tested for cellulase production under SSF using SCB. Treatment of bagasse using 2.0% H_2O_2 along with 1.5% NaOH increased the production of cellulases by *Humicola insolens*. The thickness of the fermentation medium of 0.8 cm, pH of 5.5, and 50 °C supported high yields of cellulolytic enzymes after 72 h [55]. Production of fungal cellulase using raw and pretreated SCB by *Rhizopus oryzae* NS5 under SSF was investigated by Srivastava et al. [56]. A mild alkali treatment showed best results for cellulase production (FPase 14 U/g) after 96 h as compared to untreated biomass (10 U/g). Further, addition of 1.5% of graphene oxide in alkali-pretreated SCB produced maximum 25 U/g cellulase after 72 h at pH 5.0 and 40 °C [56].

4.1.2 Cellulase production using microorganisms in submerged fermentation

Submerged fermentation is the process of growing microorganisms in the presence of free-flowing liquid substrates. It is the most preferable process for enzyme production because it allows uniform availability of nutrients, and sufficient amount of oxygen supply, and less time is needed for the fermentation compared to other fermentation techniques [57]. Cuhna et al. [58] reported maximum CMCase (0.432 U/ml) activity when *Aspergillus niger* A12 was grown on SCB substrate under submerged fermentation (SmF). *Aspergillus flavus* KUB2 was grown on different substrates like rice straw, rice bran, SCB, saw dust, xylan, and pure commercial CMC under SmF, and it was observed that maximum cellulolytic and hemicellulolytic (xylanases) production was detected with SCB as substrate. They observed CMCase activity of 1.04 U/ml and FPase of 0.21 U/ml using SCB [59]. Cunha et al. and Delabona et al. [58, 60] proved that SCB can be used as an inducer for cellulolytic enzyme production in SmF.

According to several studies, *Trichoderma harzianum* is a good producer of cellulolytic enzymes [60, 61]. *Trichoderma harzianum* P49P11 strain was reported to produce FPase (0.78 FPU/ml), β-glucosidase (9.18 U/ml), and xylanase (36.96 U/ml) using steam-pretreated SCB [60]. Pinotti et al. [62] evaluated cellulase production using SCB from two strains of bacteria (*Bacillus subtilis* and *Bacillus megaterium*) and three strains of fungi (*Trichoderma koningii*, *Rhizomucor* sp., and *Penicillium* sp.). The best bacterium was *Bacillus megaterium* with cellulase production in range of 0.13–0.1567 U/ml using untreated and acid–alkaline pretreated bagasse at 28–33 °C and the best fungus was *Trichoderma koningii* with enzyme production of 3.1304 U/ml at 28 °C using untreated SCB, i.e., up to 20 times greater than that in the case of bacterium application. *Aspergillus japonicus* produced the highest FPase activity (0.14 U/ml) after 336 h using SCB as a sole source of carbon. *Fusarium oxysporum* showed (0.13 U/ml) FPase activity after 294 h. *Aspergillus japonicus* showed highest β-1,4 glucanase activity 0.10 U/ml after 168 h of culture using SCB [63].

Production of cellulase by *Penicillium funiculosum* using SCB was carried out by Castro et al. [64] that resulted in enzymatic activities of 0.25 U/ml of FPase, 1.80 U/ml of CMCase, and 0.80 U/ml of β-glucosidase. Pretreated SCB (with diluted sulfuric acid followed by sodium hydroxide) was used as a source of carbon for cellulase production by *Penicillium funiculosum* ATCC 11,797. Maximum FPase 0.015 U/ml/h, CMCase 0.1795 U/ml/h, and β-glucosidase 0.031 U/ml/h were obtained using 10% v/v inoculation

and after 60 h of incubation [65]. Aguiar et al. [66] utilized vinasse and SCB for the production of cellulases using *Trichoderma reesei*, *Pleurotus ostreatus*, *P. ostreatoroseus*, and *P. sajorcaju*. The *Pleurotus* showed a low enzymatic activity of exo- and endoglucanase while the *Trichoderma reesei* demonstrated endoglucanase activity with 3.23 and 5.88 U/ml after 504 and 576 h of cultivation, respectively. *Trichoderma reesei* also showed a high exoglucanase activity with 18.35 U/ml after 432 h of growth. Ejaz et al. [67] evaluated the effect of methyltrioctylammonium chloride (IL) and sodium hydroxide pretreatment on SCB and its subsequent utilization to produce cellulase from a thermophilic bacterium *Bacillus aestuarii* UE25. After optimization, an enhancement in endoglucanase (111.49 U/ml), β-glucosidase (120.37 U/ml), and FPase (28.05 U/ml) production was observed when alkali-pretreated bagasse was treated with ionic liquid for 30 min at 70 °C in 1:15 ratio and 10% inoculum of the strain was used in this substrate, amended with 0.5% glucose and peptone with initial pH 5.0, at 60 °C for 48 h with agitation.

Bohra et al. [68] screened cellulolytic bacteria from rumen of cattle with potential to produce cellulases. From 847 isolates, they selected *Paenibacillus polymyxa* ND24 as the most potential strain, which produced cellulase effectively by utilizing SCB, corn starch, rice straw, avicel, and CMC as a sole source of carbon. Cellulase production was carried out in lab-scale 5-L bioreactor showing maximum endoglucanase yield up to 0.72 U/ml when grown in the medium containing SCB (2% w/v) after 72 h of incubation. Rocha et al. [69] pretreated SCB and used for cellulase production by *Trichoderma harzianum* IOC 3844. Under optimized conditions in SmF, highest enzyme activity after 42 h of fermentation were CMCase (27.017 U/ml), FPase (1.225 U/ml), and β-glucosidase (0.609 U/ml).

Streptomyces misionensis PESB-25 produced high yield of endoglucanase using SCB and corn steep liquor after 72 h [70]. Cunha et al. [71] carried out endoglucanase production using steam explosion pretreated SCB as substrate in both shake flask and stirred tank bioreactor. Maximum endoglucanase production in stirred tank bioreactor was 1.59 U/ml, using steam pretreated bagasse having particle size smaller than 0.5 mm, with 700 rpm agitation, and pH 5.0. Cellulase production by *Streptomyces viridobrunneus* SCPE-09 using SCB and wheat bran as carbon source and corn steep liquid as nitrogen source was studied under SmF. The maximum value for CMCase obtained was 0.99–1.11 U/ml for 3% SCB + 1.4% corn steep liquid [72]. SCB with steam explosion pretreatment and untreated was employed for cellulase production by *Acremonium strictum*. It was observed that the maximum of CMCase activity (endoglucanase) of 0.139 U/ml was observed in bagasse treated with steam explosion pretreatment (mild) at 240 h of fermentation. FPase activities showed similar results with mild pretreated SCB and raw SCB (without treatment), with the highest activities at 192 h of fermentation, corresponding to 0.0108 U/ml and 0.00674 U/ml, respectively. In relation to cellobiase activity, the good results were attained using mild pretreated bagasse (mild pretreatment) as the substrate, followed by fermentation employing the commercial avicel; after 96 h of fermentation, the highest cellobiase activities of 0.02772 U/ml and 0.0214 U/ml were obtained, respectively [73].

Trichoderma harzianum L04 was used for cellulase production using SCB in SmF. Maximum activities of endoglucanase 4.022 U/ml at 72 h, exoglucanase 1.228 U/ml at 120 h, and β-glucosidase 1.968 U/ml at 48 h were observed [74]. SCB was biologically pretreated with the white rot fungus *Pleurotus sajorcaju* PS 2001, and this pretreated biomass was further used for the production of cellulases by the fungus *Penicillium echinulatum* in SmF. For medium supplemented with pretreated SCB, the average peak activities obtained were 0.13, 1.0, and 0.18U/ml for FPase, endoglucanase, and β-glucosidases, respectively [75]. Production of cellulase and xylanase by the cellulolytic mutant strain *Penicillium echinulatum* 9A02S1 using pretreated SCB as a carbon source was studied in SmF [76]. Cultures grown with pretreated bagasse showed similar or higher enzymatic activities than cultures grown with cellulose or untreated (raw) SCB. Maximum FPAse activity (1.253 ± 0.147 U/ml) was noticed in the medium after 144 h of incubation when SCB samples were pretreated with sodium hydroxide, hydrogen peroxide, and anthraquinone. Similarly, production of endoglucanase was also increased by pretreatment of SCB [76]. Cellulase productions on natural lignocelluloses such as saw dust, ground nut shells, SCB, wheat bran, rice bran, and corn cobs by *Aspergillus niger* were studied in SmF [54]. Using SCB approx 1.5 U/ml FPase, 0.5 U/ml endoglucanase and 1.2 U/ml β-glucosidase were attained while rice bran + SCB resulted in approx 1.7 U/ml FPase, 0.8 U/ml endoglucanase, and 1.4 U/ml β-glucosidase activity [54]. Cellulase production was studied using untreated and NaOH pretreated SCB using *Bacillus licheniformis* 2D55. The highest FPase-0.160 U/ml was produced by untreated SCB, and the mixture of untreated SCB and pretreated rice husk resulted in improved CMCase activity [77]. Cellulase production using thermophilic strain *Bacillus licheniformis* 2D55 was studied [78]. The highest CMCase 29.4 U/ml, FPase 12.9 U/ml, and β-glucosidase 0.06 U/ml were produced by untreated/raw SCB and pretreated rice husk at 60 °C, pH 3.5, and 180 rpm.

4.2 Xylanases

The second most abundant polysaccharide is xylan occupying 20–40% biomass after cellulose. It is made up of linearly linked 1,4-β-linked D-xylose units [79]. Xylanase is the enzyme which hydrolyzes the xylan [80]. They hydrolyze the bond between β-1,4D-xylans into monomeric units.

Xylanases are used widely in many industries such as biofuel production, animal feed, textile industry, food industry, pulp and paper industry, and many others [79].

4.2.1 Xylanase production in solid-state fermentation

Production of xylanase has been studied under SSF and SmF using various microorganisms [81]. Production of xylanase is influenced by both physical (temperature, pH, incubation time, inoculation size) and chemical (carbon source, nitrogen source, vitamins, agitation, and trace elements) factors [81]. Optimum conditions for production of maximum xylanase were observed at 48 h, inoculum size of 5%, and an agitation speed of 200 rpm [82, 83]. Virupakshi et al. [84] evaluated the production of xylanase in SSF by *Bacillus* sp. JB-99 using various lignocellulosic substrates including SCB. The xylanase production using bagasse was 1845 U/g. *Trichoderma harzianum* P49P11 strain was reported to produce about 67 U/ml xylanase using glucose as initial substrate followed by steam-exploded SCB after 120 h [61]. *Paenibacillus* sp. AR247 was used to produce xylanase by Marco et al. [85] using raw SCB, and the maximum yield of 2.99 U/ml was attained after 48 h. Apart from cellulases, *Aspergillus* sp. have been reported for hemicellulase production. Some strains like *Aspergillus niger* and *Aspergillus flavus* were reported by De Alencar Guimaraes et al. [86] for their ability to produce xylanases using agrowaste as substrate. The highest activity of xylanase (3.55 U/ml) was observed with *Aspergillus japonicas* using SCB as substrate [63].

Pretreatment with milling and then fungal hydrolysis using *Trichoderma* sp. at 30 °C resulted in 380 U/g xylanase [87]. Irfan et al. [88] observed 72.4 U/g xylanase by *Trichoderma viride*-IR05 using SCB pretreated with KOH (2.5%). Rodríguez-Zúñiga et al. [13] evaluated the effect of various pretreatment methods on SCB structure and hydrolytic enzyme production by *Aspergillus niger* under SSF. The highest cellulose content (86.7%) was obtained after combined acid/alkali treatment. SCB pretreated with liquid hot water resulted in highest xylanase (26.1 U/g) activity after 72 h.

SCB was used as substrate for xylanase production by *Trichoderma harzianum* Rifai strain [45]. The highest enzyme titres for xylanase were 27.6U/ml obtained with 14% (w/v) SCB after 168 h of cultivation. SCB was chemically pretreated and evaluated for the production xylanase by *Penicillium janthinellum* NCIM 1171 and *Trichoderma viride* NCIM 1051 [89]. Higher xylanase activity of 130 U/ml was detected in the case of *Penicillium janthinellum.* Xylanase production using SCB and soyabean meal by *Aspergillus niger* LPB 326 was carried out in SSF [90]. Under optimized conditions, the maximum xylanase activity was obtained using SCB and soybean meal in the ratio of 65:35, respectively, and the medium was moistened to 85% of initial water content with a nutrient salt solution and incubated at 30 °C for 96 h. Under these conditions, xylanase activity of 3099 U/g was obtained.

Milagres et al. [91] demonstrates that high levels of thermostable xylanase were produced by *Thermoascus aurantiacus* ATCC 204,492 using SCB as a substrate. Maximum xylanase activity of 1597 U/g was obtained after 240 h of using 8 g substrate. Production of xylanolytic and cellulolytic enzymes was carried out using pretreated SCB and wheat bran by *Penicillium echinulatum* 9A02S1 under SSF [46]. Maximum amount of xylanase activity was around 10U/g from 48 to 96 h. A mixture of ionic liquid pretreated and untreated SCB was utilized to obtain bacterial multienzyme under SSF using thermophilic strain, *Bacillus aestuarii* UE25 [92]. Optimization of fermentation conditions was done by adopting a central composite design. Volumetric and specific productivity of xylanase was 4580 U/ml/ h and 244.25 U/mg substrate under optimized conditions.

Production of xylanase was investigated by Padmavathi et al. [93] by *Streptomyces coelicolor* using 4 different agro residue–based substrates such as SCB, pineapple, orange peels, and pomegranate peels, and results showed that SCB was good substrate for xylanase production. Highest xylanase yield of (423.9 U/g) by *Streptomyces viridosporus* T7A was reached in the medium having 10% soybean meal, 20% Napier grass, and 70% SCB [94]. High activity of β-xylosidase and xylanase was observed using *Aspergillus tamarii* in solid-state fermentation using wheat bran, corn cob, and SCB as substrate. Xylanase activity using SCB is approx 700 U/g and β-xylosidase is about 200 U/g using untreated samples [95].

Xylanase production using SCB with different particle size such as 0.92 mm, 1.68 mm, and 2.9 mm by *Pleurotus ostreatus* was investigated by Membrillo et al. [47]. A peak of xylanase was observed after 72 h with enzyme activity of 11 U/g and then declined afterwards followed by a peak after 360 h with xylanase activity of 20 U/g dry wt with particle size of 2.9 mm. Kumar et al. [38] observed that the highest xylanase activity of 205.98 U/g bagasse was observed after 240 h by *Pandoraea* sp. ISTKB.

4.2.2 Xylanase production in submerged fermentation

In submerged fermentation, *Bacillus subtilis* subsp. *subtilis* JJBS250 has been observed for xylanase production using SCB. Maximum xylanase activity (20.35 U/g) was observed by Singh et al. [79] using SCB in SmF. Lee et al. [96] reported maximum xylanase production of 452.3 U/ml after 72 h using SCB and palm kernel cake as carbon source in SmF from a fungus isolated from decayed wood. From *Aspergillus japonicus*, highest endo-β-1,4-xylanase activity of 3.55 U/ml was observed after 14 day and β-xylosidase of 9.74 U/ml was observed after 504 h [63]. The β-xylosidase

was higher than endo-β-1,4-xylanase when SCB was used. This is due to the fact that xylooligosaccharides which are inhibitors of xylanase are removed by β-xylosidase, thus allowing more efficient hydrolysis of xylan [63]. Xylanase production was studied by Ifran et al. [57] using *Bacillus subtilis* and *Bacillus megaterium.* Maximum xylanase production (51.60 U/ml) was observed at 35 °C after 48 h incubation at pH 8.0 by *Bacillus subtilis* and 41.66 U/ml xylanase by *Bacillus megaterium* at 40 °C at pH 8.0 after 48 h. Use of SCB and grass as cost-effective substrates for production of xylanase by *Bacillus circulans* D1 in SmF was studied by [97]. *Bacillus circulans* D1 was grown in a mineral medium containing bagasse or grass hydrolysate as carbon source. Maximum production of xylanase was attained during growth in media containing bagasse hydrolysates (8.4 U/ml) as compared to grass hydrolysates (7.5 U/ml). Bhosale et al. [98] screened 25 actinomycetes isolated for xylanase production on xylan agar plates. Based on zone of clearance, 7 isolates were further studied using several agro-industrial by-products such as SCB, wheat bran, rice bran, and corn cobs in SmF. The highest level of xylanase activity was obtained at 30–50 °C, at pH 8.5, and SCB concentration of 20 g/l after 168 h of incubation using isolate *Streptomyces rameus*. Further addition of 2% glucose resulted in 54.28% increase in xylanase production and 1.5% peptone as nitrogen source showed 73.87% increase in the level of xylanase production. Cunha et al. [71] carried out endoglucanase production using steam explosion–pretreated SCB as substrate in both shake flask and stirred tank bioreactor (STB). Maximum xylanase production in stirred tank bioreactor was 4.212 ± 0.133 U/ml, under sequential cultivation using steam-pretreated bagasse particles smaller than 0.5 mm, agitation speed of 700 rpm, and pH 5.0. Oliveira et al. [99] reported xylanolytic activity of 23.0 U/ml in SCB by *Penicillium janthinellum.* Dhillon et al. [100] found that SCB and oat husk were the most efficient substrates for xylanase production by *Bacillus circulans* as compared to corn cob. SCB and soybean meal were used for xylanase synthesis in SmF by *Aspergillus niger* [101]. The fermentation process was studied in Erlenmeyer flasks, a stirred tank bioreactor, and a bubble column reactor, with the xylanase activities of 52.9, 33.7, and 60.5 U/ml, respectively [101]. Kumar et al. [38] studied comparison of SmF and SSF of SCB by *Pandoraea* sp. ISTKB on lignolytic enzyme production. During SmF, production of xylanase was the highest after 264 h with activity of 359.4 U/g bagasse. Mostly single strain of microorganism is used for enzyme production; however, use of two or more strains in co-culture has been proved an effective approach for conversion of agro-industrial waste biomass into upgraded products like enzymes and biofuels. An investigation was conducted by Qadir et al. [102] to study the production of cellulase and xylanase by co-culture of two strains namely *Saccharomyces cerevisiae* MK-157 and *Candida tropicalis* MK-118 using untreated and pretreated SCB under SSF. Initially, they studied the enzyme production separately by these two strains on untreated SCB and observed that MK-118 produced high titres of endoglucanase (9.66 U/ml) as compared to MK-157 (6.22 U/ml). On the other hand, MK-157 produced high levels of xylanase in comparison to MK-118. Further co-culture results showed increase in overall enzyme yields of both cellulase and xylanase. Biological pretreatment of SCB with the white rot fungus *Pleurotus sajorcaju* PS 2001 was carried out, and the resulting biomass was further utilized in the production of xylanase by *Penicillium echinulatum* in SmF. For medium supplemented with pretreated SCB, the average peak activity obtained was 0.33 U/ml xylanases [75].

4.3 Laccases

Laccases are distributed widely in bacteria, fungi, insects, and higher plants. In fungi, laccases have been isolated from *Basidiomycetes*, *Deuteromycetes*, and *Ascomycetes*. *Basidomycetes* also known as white rot fungi are the efficient degrader of lignin as compared to *Deuteromycetes and Ascomycetes*. Laccases are used widely in textile industry, food industry, bioremediation (degradation of phenolic pollutants), paper and pulp industry, cosmetics, delignification of wood pulp, biosensors, waste detoxification, and many others. Bacterial sources of laccases are *Streptomyces cyaneus*, *Streptomyces lavendulae*, and *Marinomonas mediterranea* and *Bacillus* spp. Fungal sources of laccase are Basidiomycetes (*Theiophora terrestris*, *Phanerochaete chrysosporium*), white rot fungi (*Pleurotus ostreatus*, *Phlebia radiate*, *Trametes versicolor*), Ascomycetes (*Monocillium indicum*), and *Trichoderma* species (*T. harzianum* and *T. longibrachiatum*). In plants, laccases take part in lignification whereas in fungi they are involved in sporulation, delignification, production of pigments, plant pathogenesis, and formation of fruiting bodies [103].

Laccases, lignin peroxidases, and manganese peroxidases oxidize the lignin present in the cell wall of plants, which is an aromatic recalcitrant polymer. These enzymes produce aromatic radicals by catalyzing one-electron oxidation of lignin units. Depending upon the environmental conditions and fungal species, these enzymes are secreted in multiple isoforms [104].

4.3.1 Laccase production in solid-state fermentation

Laccases are oxidase enzymes containing copper usually found in many fungi. Karp et al. [105] observed maximum laccase production (151.6 U/g) using *Pleurotus ostreatus* after 120 h under SSF using SCB. Using SCB as a substrate and $CuSO_4$ as an inducer, laccase activity of 86.8 U/g was obtained from *Pleurotus ostreatus*. However, when ferulic

acid was used as an inducer, laccase production was almost doubled [105]. Generally, laccases are produced in low concentrations by fungi but addition of various xenobiotic compounds into media resulted in enhanced production of these enzymes. Aromatic compounds such as veratryl alcohol, 2,5-xylidine, and lignin are known to induce laccase production/activity [103]. Another aromatic inducer veratryl alcohol resulted in increase in concentration of laccase after addition in culture medium. An increase in ninefold laccase activity was reported by addition of 2,5-xylidine in culture medium after 24 h of cultivation but at much higher concentration it also reduced the laccase activity due to toxic effect [103]. This increase in production of laccase enzyme after addition of inducers is because of the presence of various recognition sites for xenobiotic compounds on promoters region of laccase encoding genes; they bind to these sites and induce laccase synthesis/production [103].

The highest laccase activity (167 U/g) by *Pleurotus ostreatus* was achieved on the 5th day in SSF using SCB supplemented with yeast extract, $CuSO_4$, and ferulic acid [104]. The expression of laccases and manganese peroxidase was observed in the *Ganoderma lucidum* secretome cultivated on SCB [106]. They also identified that *Ganoderma lucidum* secreted five laccase isoforms and one manganese peroxidase form by zymogram and plate assay [106]. Meza et al. [107] investigated the effect of gaseous ethanol addition as inducer of laccase produced by *Pycnoporus cinnabarinus* ss3 using SCB. A 45-times-higher laccase production of 90 U/g dry support was attained when supplemented with 31 g/l ethanol, than without ethanol. Meza et al. [108] reported 80-fold higher laccase (80 U/g) production using pretreated SCB after induction with ethanol vapours after 672 h by *Pycnoporus cinnabarinus* ss3.

Hernandez et al. [109] explored the use of synthetic dyes for lacasse induction using SCB as substrate by *Pycnoporus sanguineus*. Their study found that carminic acid can induce 722% and alizarin yellow 317% more laccase production than controls, and they promoted good fungal growth in liquid cultures. Based on these results, carminic acid was used as an inducer for laccase production under SSF using SCB. Increase in production of laccase was observed when carminic acid was added as an inducer. With addition of carminic acid, maximum laccase activity of 1.09 ± 0.40 U/g than control (0.75 ± 0.31 U/g) after 192 h of culture was attained. Meza et al. [110] tested various chemical compounds as laccase inducers by *Pycnoporus cinnabarinus* grown on SCB. They observed that laccase activity was 5- to 8.5-fold higher in the presence of aliphatic alcohols, like methanol and ethanol and phenolic compounds, like ferulic acid and veratryl alcohol. Laccase activity by *Pleurotus ostreatus* grown on SCB with different particle sizes (0.92–2.9 mm) was investigated by Membrillo et al. [47] and they observed high laccase activity on the 3rd day using 2.9-mm particle size. Guerra et al. [111] studied laccase production using SCB by three fungi *Earliella scabrosa*, *Trametes maxima*, and *Ganoderma zonatum* on SSF. *Earliella scabrosa* showed maximum laccase activity after 264 h (44.3 U/g), while *Ganoderma zonatum*on 216 h (4.7 U/g). *Trametes maxima* showed negligible laccase activity. Enhanced production of laccase by *Trichoderma harzianum* HZN 10 under SSF was attained with SCB (53 U/g) [112]. High laccase production by *Neurospora sitophila* was observed after 96 h with SCB among all the residues tested [113].

4.3.2 Laccase production in submerged fermentation

Pycnoporus cinnabarinus, among the basidiomycetes, is observed to produce laccase predominantly. This white rot fungus has been also reported for bioremediation and decolorization of dyes. Laccase production using *Pycnoporus cinnabarinus* and *Trametes versicolor* was stimulated by inducers such as alcohols (methanol and ethanol), phenols (2,5-xylidine, ferulic acid), and sulfones in submerged cultivation using SCB as carbon source [107]. Téllez-Téllez et al. [114] compared the laccase production in SSF and SmF by *Pleurotus ostreatus* using SCB as substrate. In SmF, cultures showed lacasse activity of 13 U/ml with biomass production of 5.6 g/l and four isoforms of laccase were observed. On the other hand, cultures grown by SSF showed laccase activity of 2.430 U/ml with biomass production of 4.5 g/l and observed three laccase isoforms. Laccase activity of (0.395 U/ml) was obtained from *Aspergillus japonicas* after 168 h using SCB [63]. *Aspergillus heteromorphus* using SCB and 5% anaerobically treated distillery spent wash resulted in 2.9 (U/ml) laccase production [115]. Kumar et al. [38] studied the comparison of SmF and SSF of SCB using *Pandoraea* sp. ISTKB for lignolytic enzyme production. In SmF, maximum laccase activity of 132.87 U/g bagasse was attained after 240 h. Both extra and intracellular laccase production was observed using *Pandoraea* sp. ISTKB. Extracellular and intracellular levels of laccase produced in SmF were 44.29 and 132.16 U/g bagasse, respectively. Using SCB, *Pleurotus sajorcaju* showed 0.0105 U/ml laccase activity after 144 h, while *Pleurotus ostreatoroseus* and *Trichoderma reesei* did not produce laccase [66]. *Pleurotus ostreatus* produced 0.325 U/ml laccase activity. SCB resulted in 16.68 ± 0.53 U/mg protein laccase specific activity using *P. martensii* NRC 345 [116]. Abdelgalil et al. [117] investigated various agro residues for laccase production by *Lysinibacillus macrolides* LSO. Among them, SB supplemented with NH_4Cl, $MnSO_4$, and $CuSO_4$ supported high laccase production. Maximum laccase activity of 7.65 U/ml/min was attained after 28 h in a 10-l stirred tank bioreactor.

5 Other enzymes produced by microorganisms using SCB as substrate

5.1 Lipases

Lipases (E.C. 3.1.1.3) catalyze the hydrolysis of oil and fats into free fatty acid and glycerol at oil–water interface. Lipases are secreted extracellularly by several bacteria, fungi, and yeasts during growth on renewable low-cost substrates in SSF and SmF [118, 119]. Among microbes, filamentous fungi are widely used for production of lipase in SSF. Different fungal strains such as *Rhizopus*, *Aspergillus*, *Rhizomucor*, *Penicillium*, and *Geotrichum* have been used for commercial lipase production. *Rhizopus oryzae* is a potential well-known producer of lipase in SSF [120]. Microbial lipases are industrially very useful enzymes in transesterification [121] and other applications such as synthesis of biosurfactants, ester synthesis, laundry detergent, pulp and paper industry [122], and are also used in pharmaceutical industry [123].

In a study carried out by Naqvi et al. [124], maximum lipase 40.0 U/ml was produced after 48 h, when grown on NH_4OH-pretreated SCB hydrolysate. According to a study by Vaseghi et al. [125], maximum lipase production 215.16 U/g was achieved at 45 °C and 80% humidity after 72 h. Production of maximal lipase by *Rhizomucor pusillus* and *Rhizopus rhizopodiformis* using SCB in solid-state fermentation was 1.73 U/ml and 0.97 U/ml, respectively. However, when the mixture of SCB (50%) and olive oil cake (50%) was used, the lipase activity increased as high as 43.04 U/ml and 10.83 U/ml obtained by *Rhizopus rhizopodiformis* and *Rhizomucor pusillus*, respectively [126].

Rhizopus homothallicus IRD13a was used for lipase production using SCB as substrate and soaked with liquid medium. A maximum of 826 U/g lipase production was reached after 12 h [127]. Production of fungal lipase using mixed substrates wheat bran, soyabean bran, and SCB was carried out by *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. [128]. Lipase activities of about 164.2 U/g, 130.1 U/g, and 189.5 U/g were obtained using SCB, soyabean meal, and rice meal as substrates, respectively, at 30 °C and 60% moisture content by *Sporobolomyces ruberrimus* [129].

Extracellular lipase production was observed from solid state and SmF using thermotolerant fungus *Rhizopus homothallicus* and purified to homogeneity. Lipase activity of 8600 U/mg in SmF at 30 °C and 10,700 U/mg in SSF at 40 °C was obtained [130]. Vashegi et al. [125] carried out SSF of two kinds of agricultural residues (SCB and soyabean meal) for lipase production using *Rhizopus oryzae* as a potential fungus strain. The highest activity of lipase under optimum conditions was 199.66 and 235.79U/g for SCB and soybean meal, respectively, after 72 h.

5.2 Phytases

Phytases are used to mitigate the anti-nutritional effect of phytate; thus, they act as a green feed additive to increase the availability of phosphorus. Environmental pollution caused by phosphorus can also be reduced by phytases [131, 132]. In a study by Sato et al. [133], extracellular thermostable phytase was produced using SCB as carbon source by *Rhizopus microsporus.* Singh et al. [134] employed different substrates for phytase production by *Aspergillus niger*. The best substrate for phytase production was selected as SCB as the mold secreted high (8.0 U/g) phytase using SCB as compared to wheat bran, sesame oil cake, and rice bran. *Aspergillus niger* secreted high phytase enzyme in solid state (SSF) using SCB [134]. High enzyme titres were secreted by mold using SCB with a substrate to moisture ratio of 1:2.5, at 32 °C after an incubation period of 48 h. Supplementation of SCB with starch and ammonium nitrate resulted in further increase in phytase production as reported by Singh et al. [134]. In order to increase production of phytase, mixed substrate (SCB and wheat bran) fermentation was employed by Kumari et al. [135] using *Sporotrichum thermophile*. Under unoptimized conditions, the mold produced 164 U/g. After optimization, an overall 11.6-fold increment in production of phytase was recorded with decrease in fermentation time from 120 to 48 h. Different media components were optimized for enhanced phytase production using Placket-Burman design and response surface methodology (RSM). It was revealed that yeast extract, Tween 80, and incubation period significantly affected phytase production. Interaction between Tween 80 and yeast extract revealed that enhanced phytase production was observed using 2.5% Tween 80 and 1% yeast extract. Under optimized conditions, phytase production of 1868.56 U/g was observed [135]. Phytase production by twenty thermophilic filamentous fungi belonging to *Rhizomucor* sp., *Myceliophthora thermophila*, *Thermoascus aurantiacus*, and *Paecilomyces variotii* were screened in SSF. SCB was used as support material for evaluating the effect of different nitrogen, carbon sources, and mineral salts. *Myceliophthora thermophile* produced high phytase after 36 h in synthetic medium. Maximum phytase production was 165 U/ml at optimized conditions such as pH 6.0; moisture content, 75%; and aeration rate, 25 ml/min/column [136]. Various agricultural by-products such as rice husk, SCB, wheat bran, wheat straw, wood powder, and starch (0.5%, w/v) were used as sole source of carbon for phytase production under shaking conditions using *Nocardia* sp. MB 36. The highest phytase yield of 0.25 U/ml was attained using wheat bran followed by SCB 0.22 U/ml, rice husk 0.21 U/ml, and wheat straw 0.18 U/ml. However, for phytase production, wood powder and starch proved inadequate substrates [137].

Sandhya et al. [138] investigated various agro-residues such as rice bran, groundnut oil cake, wheat bran, mango seeds, citrus peel, and SCB for phytase production by *Aspergillus niger*. All the above substrates supported growth of *Aspergillus niger* and phytase production was detected in each sample. Among those, SCB showed about 7 U/ml in SmF and 12 U/ml in SSF. Suresh et al. [139] evaluated various agro-residues such as SCB, wheat bran, mustard oil cake, rice bran, and corn oats substrates for phytase production under SSF by *Rhizopus oligosporus* MTCC 556. SCB resulted in 1.2 ± 0.09 (U/g) phytase activity. Phytase production by *Sporotrichum thermophile* TLR50 in various agroresidues such as sesame oil cake, SCB, wheat bran, wheat straw, mustard oil cake, rice straw, cotton oil cake, and corn cob in SSF was carried out. Phytase production using SCB resulted in approx 20 U/g enzyme activity [140]. Phytase production by *Humicola nigrescens* was optimized in SSF using various agro residues such as SCB, wheat bran, mustard oil cake, rice bran, and citrus peel. SCB resulted in approx 5 U/g phytase activity [141]. Mittal et al. [142] investigated the production of phytase by *Klebsiella* sp. DB-3 FJ711774.1 using various agro-residues. Phytase activity of 0.67 U/ml was observed using SCB.

5.3 Chitinases

Chitinases have importance to control pests in agriculture. Chitin is an insoluble polymer which forms the cuticle of insects and is made up of N-acetylglucosamine units. Enzymes which can convert N-acetylglucosamine polymer into individual monomer units are divided into chitinase (GH family 18 and 19) and N-acetylglucosaminidases (glycoside hydrolase (GH) family 20) [143]. Apart from their use in agricultural pest control, they are also used widely in other biotechnological applications such as medicine and waste management [144]. A limiting factor in the use of chitinase for pest control in agriculture is lack of commercial large-scale production due to production cost [145]. Use of SCB as a carbon source is environmental friendly because it is a waste by-product which can cause environmental pollution if disposed improperly [146].

dos Reis et al. [145] produced chitinase of 6.78 U/g at 33 °C using SB as a substrate under solid-state fermentation by fungus *Metarhizium anisopliae*. It is a well-known fungus for biological control of disease vectors and to control pests in agriculture and livestock [145]. Sudhakar et al. [147] reported that *Trichoderma harzianum* gave the highest chitinase activity of 34 U/ml at 30 °C temperature, initial pH of 6.0, inoculum size of 2.4%, and 2 g/l substrate concentration using SCB after incubation for144 h.

Aita et al. [35] reported that SCB was the most potent substrate for chitinase production. The highest activity was 12.07 ± 0.50 U/g for chitinase at moisture content of 40 wt%, 10% (v/w) of inoculum, at 28 °C after incubation of 192 h using *Metarhizium anisopliae* IBCB 348 in SSF. Sudhakar et al. [147] recorded 37.2 U/ml chitinase activity from *Serratia marcescens* at 30 °C, inoculum size 2.4 g/l, pH 6.0, and substrate concentration 2% using SCB. Chitinase activity of the conidia of *Verticillium lecanii* was as follows: wheat bran (3.0 U/ mg), polyurethane foam (2.7 U/ mg), and SCB (3.3 U/ mg) [148]. Agro-industrial residues were investigated for exo- and endochitinase activity by *Streptomyces champavatii* AZ-1 using various residues such as wheat bran, SCB, and colloidal chitin in combination. Mixture of 5 g wheat bran, 0.05 g colloidal chitin, and 0.05 g SCB resulted in 12.5 U/g exochitinase and 74.3 U/g endochitinase activity [149]. Production of chitinase using wheat bran and SCB by *Alternaria* sp. strain Sha was carried out in SSF. A maximum exochitinase activity of 28.931 U/g was attained in the optimized medium containing wheat bran, SCB, and colloidal chitin after 384 h at 25 °C [150].

5.4 Amylases

Amylases catalyze the hydrolysis of starch into glucose, maltose, and malto-oligosccharides. They are utilized in various industries for different purposes such as in sugar syrup synthesis, alcohol fermentation, food processing industries, textile, and detergent industries. Commercially, 25–30% of amylase in world enzyme market is produced by microorganisms [151]. Synthesis of amylase can be made economical by using cost-effective substrates such as agricultural wastes like SCB. SCB can be utilized in solid state without any pretreatment in raw fiber form or it can be used in SmF after acid hydrolysis of bagasse [152]. Rajagopalan et al. [153] reported maxiumum amylase 68.8 U/ml after 30-h incubation in SCB hydrolysate using *Bacillus subtilis* strain KCC103. Amylase production from SCB under SSF was investigated by Roses et al. [154] using *Aspergillus niger* UO-01. They reported maximum amylase 457.82 U/g of dry support at 30.2 °C, pH 6.0, and particle size of bagasse, 6–8 mm.

Aspergillus japonicus produced maximum amylase with activity of 0.35 U/ml after 336 h using SCB as sole source of carbon [63]. Vijayabaskar et al. [155] studied various substrates such as wheat bran, rice bran, black gram husk, greengram husk, and SCB using *Bacillus cereus* in SSF. Among them, SCB came out to be the best substrate for amylase production (232.65 ± 2.74 U/ml). Higher amylase production was attained at pH 7.0 and 40 °C after 48 h; the highest amylase activity was attained when *Aspergillus flavus* was grown on SCB. Among various carbon and nitrogen sources, soluble starch as carbon source and yeast extract as nitrogen source (1% w/w) resulted in production of the highest yield at 30 °C and pH 6.0 after 120 h [156]. Production of alpha amylase from *Streptomyces erumpens* MTCC 7317

in SSF was investigated for various agrobased residues such as wheat bran, SCB, and cassava bagasse. SCB resulted in 2821 U/g amylase production after incubation of 48 h at pH 7.0 and 50 °C [157].

5.5 Proteases

Proteases are important industrial enzymes with applications in food, detergent, pharmaceutical, and leather industries. They hydrolyze the peptide bonds and help to control many metabolic processes like fibrinolysis, blood coagulation, phagocytosis, blood pressure control, and activation of complement.

A strategy for protease production was developed by Rathakrishnan et al. [158] using SCB as a substrate under SSF using *Bacillus licheniformis*. By adopting Plackett–Burman design, optimization of various process parameters affecting protease production was done. Under optimized conditions of fermentation, 146.28 U/g activity of protease was reported. SCB and soybean meal were used for xylanase and protease production in SmF by *Aspergillus niger.* Higher protease production of 7 U/ml was attained [101]. Xylanase, β-xylosidase, and protease production by *Aspergillus tamarii* in SSF were carried out using different untreated and pretreated lignocellulosic materials. Protease level of about 140–200 U/g was observed in corn cob and SCB cultures [95].

5.6 Invertases

Sucrose hydrolysis into glucose and fructose is catalyzed by invertases. For the synthesis of artificial sweetener, invertase is the key enzyme in the food industry. It helps in improving intestinal microflora and prevents colon cancer, cardiovascular disease, and osteoporosis [159]. Utilization of low-cost substrate like SCB and molasses was investigated for the production of fungal invertase using *Aspergillus niger* GH1 in SSF [160]. Invertase level of 5.231 U/ml was produced after fermentation with *Aspergillus niger* GH1.

Invertase production using different carbon sources such as wheat bran, lemon pomace, orange pomace, and SCB was carried out by *Saccharomyces cerevisiae* under solid-state fermentation. SCB resulted in 49.7 U/mg enzyme [161]. Invertase production by *Aspergillus ochraceus* was screened using various agroresidues. The highest level of extracellular invertase activity was obtained in Khanna medium supplemented by SCB (108 Total U) by *Aspergillus ochraceus* with optimum activity at 40 °C and pH 4.5 after 96 h [162]. Alegre et al. [163] investigated the effect of various agroresidues as carbon sources on the production of extracellular and intracellular invertases in SmF by *Aspergillus caespitosus*. Among them, SCB resulted in 2.2 U/ml extracellular and 2.9 intracellular invertase activity.

5.7 Inulinases

High-fructose syrups from inulin are produced by inulinase; therefore, they are important enzymes. Widely used food additives such as fructose and fructo-oligosaccharides are produced by inulinase by the hydrolysis of inulin. Inulin hydrolysis with the help of inulinase can result in products with 95% of fructose. Inulinase production by *Kluveromyces marxianus* NRRL Y-7571 was optimized by Mazutti et al. [164] using SCB as substrate for SSF. Highest yield for inulinase was 390 U/g under optimized conditions. Thus, SCB seems as best substrate for growth of *Kluveromyces marxianus* NRRL Y-7571 and inulinase production. Assessment of inulinase production by SSF using *Kluveromyces marxianus* NRRL Y-7571 was carried out. The solid medium consisted of SCB added with molasses and corn steep liquor. The enzymatic activity reached a maximum of 445 U/g under optimized conditions [165]. Inulinase production using sugarcane bagasse under SSF was carried out and maximum inulinase activity of 250 U/g was achieved at 20% (w/w) of **CSL**, 5% (w/w) of soybean bran, at pH 5.0 and 55 °C. Soybean bran decreased the fermentation time from 96 to 24 h to reach maximum activity [166].

Bender et al. [167] investigated inulinase production by SSF of SCB by *Kluveromyces marxianus* NRRL Y-7571. Results showed that maximum yield of 396.6 U/g was obtained at pH 4.8. Inulinase production by SSF using SCB and soybean bran as substrates was carried out by the yeast *Kluyveromyces marxianus* NRRL Y-7571. The highest inulinase activity (436.7 ± 36.3 U/g) was observed after 24 h at an inlet air temperature of 30 °C [168]. Inulinase production using solid-state fermentation in a fixed-bed reactor with dimensions (34 cm diameter and 50 cm height) was carried out by *Kluyveromyces marxianus* NRRL Y-7571 using SCB and soybean meal as substrate that resulted in 586 U/g [169]. Cell growth and inulinase production was investigated by Mazutti et al. [170] using *Kluyveromyces marxianus* NRRL Y-7571 in a packed-bed bioreactor using SCB, cane molasses, and soybean bran. The best conditions for enzyme production was 30 °C inlet air temperature, reaching an activity of around 463 U/g. Abd El Aty et al. [171] carried out inulinase production by *Aspergillus parasiticus* using various substrates and found that SCB resulted in maximum enzyme activity of 1.773 ± 0.627 U/g.

5.8 Pectinases

Hydrolysis of pectin is catalyzed by pectinases, which have a wide variety of applications in food and beverage industries. It is used widely for fruit juice clarification, coffee and tea fermentation, pulp and paper industry, protoplast isolation, waste management, and haze removal from wines. Production of pectinase using SCB and rice bran by *Aspergillus*

awamori was reported by Baladhandayutham and Thangavelu [172]. The highest yield of pectinase 103.33 U/ml was reported at 35 °C when a mixture of rice bran and of SCB at 85:15 ratio was used. *Aspergillus japonicus* produced maximum pectinase with activity of 0.53 U/ml after 168 h using SCB as sole carbon source [63]. Ejaz et al. [173] carried out a study to observe the effect of chemical pretreatment of SCB for its utilization by *Geotrichum candidum* AA15 for enhanced pectinase production. It was concluded that lignin removal by chemical pretreatment negatively affected the capacity of SCB to support cell adhesion. Pectinase production utilizing *Aspergillus niger* in SSF was studied using wheat bran and SCB as substrates. Medium consisting of 90% of wheat bran and 10% of SCB gives the highest pectinase yield of 169.50 U/ml after 96 h at 40 °C and pH 5.0 [174]. Silva et al. [175] investigated various substrates for pectinase production by *Penicillium vidiricatum* Rfc3 in SSF and found that a combination of SCB and wheat bran resulted in 27 U/g polygalaturonase after 8 days and 1500 U/g pectin lyase after 240 h. The combination of SCB and orange bagasse resulted in 9 U/g polygalacturonase after 288 h cultivation and 2500 U/g pectin lyase after a 12-day cultivation.

6 Major parameters affecting the production of various enzymes by microorganisms utilizing sugarcane bagasse

6.1 Physical factors

6.1.1 Effect of pH and temperature on production of enzymes

Outcomes of temperature on production of enzymes and microbial growth vary from species to species. Many microorganisms grow on wide range of temperatures while some are limited to narrow range. Due to metabolic activities of microbes, they produce some amount of heat naturally. Similarly, the pH of culture medium plays a key role in metabolic activities and growth of microorganisms. Generally, after a few days of microbial incubation, the pH of the medium drops which influences the production of hydrolytic enzymes. It is reported that lignolytic fungi displayed higher enzyme activity under acidic conditions [19]. Solubility of medium components and transport of many enzymes across the cell membrane is very much affected by the pH.

In production of enzymes, the role of temperature is a major factor that affects the cell viability, growth of microorganisms, enzyme activity, and protein denaturation. Therefore, temperature of fermentation needs to favor both enzyme production and microbial growth [36]. The highest xylanase production (26.53 U/g) using SCB in SSF was noticed with *Bacillus subtilis* subsp. *subtilis* JJBS250 at 40 °C [176]. A study was conducted by Namnuch et al. [59] using *Aspergillus flavus* KUB2 for cellulolytic enzyme production to compare the effect of pH and temperature on enzyme yield. *Aspergillus flavus* can grow on all temperatures and pH, but maximum yield was only at 30 °C and pH 7.0 [177]. Salomao et al. [36] conducted a study on three fungal species, *Trichoderma koningii*, *Penicillium* sp., and *Rhizomucor* sp., using SCB; the maximum yield was observed in the range of 28–30 °C. High xylanase activity (19 U/g) by bacterial culture *Bacillus subtilis* subsp. *subtilis* JJBS250 was observed at pH 7.0 [176]. Namnuch et al. [59] observed that *Aspergillus flavus* KUB2 produced maximum enzyme activity at pH 7.0. Pectinase production using wheat bran and SCB as substrate was studied by Suresh et al. [174] utilizing *Aspergillus niger* in SSF. Maximum pectinase was produced at pH 5.0 and 40 °C.

Vaseghi et al. [120] investigated the effect of temperature on lipase production using SCB as carbon source and attained high activity at 45 °C. *Bacillus subtilis* resulted in the highest enzyme production at 35 °C and *Bacillus megaterium* at 40 °C [57]. Both strains showed maximum activity at pH 8.0 [57]. Vijayabaskar et al. [155] attained maximum amylase production at 40 °C and pH 7.0. Ferriera et al. [63] studied the outcomes of temperature and pH on endo-β-1,4-xylanase and β-xylosidase activities of *Aspergillus japonicas* on SCB as the sole source of carbon for168 h. The highest production of enzyme was achieved at 50 °C at pH 5.5.

6.1.2 Effect of incubation period on enzyme production

For optimum yield of enzymes, fermentation time should be taken into consideration because it is a very crucial factor [120]. Incubation time depends upon the kind of substrate and microorganism used for enzyme production. Also, it depends on whether substrate is pretreated or natural [19]. Maximum activities of cellulolytic (CMCase and FPase) and hemicellulolytic (xylanases) enzymes were observed after 336-h incubation using *Aspergillus flavus* KUB2 in SmF on SCB as substrate. Further, increase in incubation time resulted in decreased enzyme activity [59]. Decrease in enzyme yield with increasing fermentation time is due to formation of protease in medium and depletion of nutrients [178, 179]. Singh et al. [176] observed high titres of xylanase (32.56 U/g) from *Bacillus subtilis* subsp. *subtilis* JJBS250 cultured on SCB in SSF after 48 h.

The highest xylanase titre was obtained after 72 h from *Trichoderma harzianum* P49P11 [180]. Okeke et al. [181] found maximum xylanase production after 120 h by *Penicillium janthinellum* and *Trichoderma virens*. Vaseghi et al. [120] investigated the effect of incubation time on lipase production using SCB and reported maximum yield after

72 h. Irfan et al. [57] showed that fermentation time of 48 h was optimum for xylanase production by *Bacillus subtilis* BS04, while 72 h is the optimum for *Bacillus megaterium* BM07. Maximum pectinase activity by *Aspergillus niger* in SSF was achieved after 96 h [174]. Enhanced production of α-amylase using *Streptomyces erumpens* MTCC 7317 in SSF was reported after 48 h [157]. Vijayabaskar et al. [155] reported maximum amylase using *Bacillus cereus* in SSF after 48 h.

6.1.3 Effect of moisture content

The moisture content of the nutrient medium plays a significant role in development of microbial growth and enzyme production. Optimum moisture content range for growth of most of the bacteria and fungi is from 40 to 70% [19]. Singh et al. [176] reported maximum xylanase (19.98 U/g) production by *Bacillus subtilis* subsp. *subtilis* JJBS250 in SmF using SCB at a moisture ratio of 1:4. It was observed by Salomao et al. [36] that maximum enzymes titres from *Trichoderma koningii*, *Penicillium* sp., and *Rhizomucor* sp. were obtained at 50–60% moisture content at 30 °C. Vaseghi et al. [120] observed maximum lipase production at 80% moisture level. Qadir et al. [102] studied the effect of moisture content on endoglucanase production and found that this parameter played a significant role. One of the critical parameter for growth of microorganisms is moisture content of media in SSF [182].

Feluri et al. [128] studied the effect of various physical parameters including moisture content on lipase production using wheat bran, soybean bran, and SCB by three fungal strains, *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp., with different combinations of residue and moisture content. Out of these, 40% moisture content resulted in higher lipase activity. Lipase production by a newly isolated *Sporobolomyces ruberrimus* strain was studied using different substrates such as SCB, soyabean meal, and rice meal. For SCB, maximum activity was achieved at 60% moisture content [129]. Optimal moisture contents to wheat bran, corn cob, and SCB cultures were 86%, 80%, and 75%, respectively, for the production of xylanase, β-xylosidase, and protease by *Aspergillus tamarii* [95].

6.2 Chemical factors

6.2.1 Carbon sources

For enzyme production with low cost and enhanced yield, there is a requirement of low-cost carbon source such as agricultural residues; the choice of a suitable substrate is very essential for efficient growth of microorganisms and production of enzymes [183]. A large number of low-cost lignocellulosic biomasses are available such as SCB, wheat bran, rice bran, cane molasses, and wheat straw which may act as a suitable substrate for enzyme production. Using SCB as carbon source, maximum production of xylanase was reported by *Penicillium citrinum* [184], *Trichoderma viride*-IR05 [88], and *Aspergillus* sp. [185]. SCB being a complex substrate requires the addition of easily assimilable carbon and nitrogen sources along with certain micronutrients. Several carbon sources such as glucose, sugar, dextrose, fructose, maltose, and SCB were used by Pramanik et al. [186] and they observed that maximum cellulase enzyme was produced using SCB only. Another important factor is the supplementation of substrate with carbon source which affects the enzyme production. According to a study by Vaseghi et al. [120], addition of olive oil 8% (w/v) with SCB substrate leads to maximum lipase production after 72 h.

Irfan et al. [57] showed that xylose and sucrose (0.5%) are the best additives to SCB for the induction of xylanase production by *Bacillus subtilis* and *B. megaterium* in SmF, respectively. Vijayabaskar et al. [155] studied the effect of different carbon sources such as glucose, galactose, maltose, sucrose, lactose, fructose, and dextrose on *B. cereus* amylase production in SSF using SCB. Maximum amylase production was observed using maltose supplementation in SCB. Hassouni et al. [136] investigated the influence of various carbon sources such as glucose, sucrose, starch, phytic acid, and myo-inositol on phytase production by thermophilic filamentous fungi belonging to *Rhizomucor* sp., *Myceliophthora thermophila*, *Thermoascus aurantiacus*, and *Paecilomyces variotii* in SSF using SCB. Of the various carbon sources tested, the addition of glucose led to maximum phytase activity followed by starch and sucrose. Invertase production by *Aspergillus ochraceus* was not enhanced after addition of carbon source to SCB medium [162].

6.2.2 Nitrogen sources

Various nitrogen sources such as peptone, yeast extract, urea, sodium nitrate, and ammonium sulfate were used by Pramanik et al. [186], for enhanced enzyme production. Yeast extract supplementation showed maximum cellulase activity using SCB by *Bacillus pseudomycoides*. However, tryptone supported the highest xylanase activity (33.03 U/g) by *B. subtilis* subsp. *subtilis* JJBS250 in SmF using SCB [176]. Various sources of nitrogen such as peptone, yeast extract, tryptone, soymeal, and beef extract also increased the xylanase production [81]. Using yeast extract as a source of nitrogen by *B. pumilus* VLK-1, maximum xylanase activity was observed [187]. Similar results were obtained by Singh et al. [83] using *B. subtilis* subsp. *subtilis* JJBS250.

Maximum production of xylanase was observed after the combination of yeast extract and tryptone using *Gracilibacillus* ap. TSCPVG [188] and *Bacillus mojavensis* AG137 [189]. Laccase production by *Pleurotus ostreatus* was improved

by yeast extract and peptone as compared to other nitrogen sources. An increase in lacasse production (23U/g) using yeast extract as nitrogen source was also observed by Mishra and Kumar [190], from *Pleurotus ostreatus* under SSF. Irfan et al. [57] tested several organic and inorganic nitrogen sources for xylanase production using SCB in SmF. Among them, the best sources are tryptone and $(NH_4)_2SO_4$ for *B. subtilis* while for *B. megaterium* KNO_3 malt extract showed better response. Hassouni et al. [136] observed that maximum phytase production was achieved by $(NH_2)_2HPO_4$ followed by KNO_3 addition to SCB. Vijayabaskar et al. [155] reported maximum amylase production by *Bacillus cereus* using yeast extract addition to SCB. Hadri et al. [113] investigated the effect of peptone and yeast extract on laccase production by *Neurospora sitophila* using agro-wastes such as corn cobs, SCB, and rice straw. They observed that peptone had a negative effect while 0.4% yeast extract enhanced the laccase production.

6.2.3 Effect of metal ions

Metal ions have been reported to affect the production of hydrolytic enzymes by various microorganisms using SCB in SmF and SSF. Metal ions are known to act as inducers for the production of hydrolases by microbes. Pramanik et al. [186] observed that maximum cellulase activity (2.84U/mg) was obtained using $MgSO_4$ in growth media. Marco et al. [85] observed an improvement in extracellular xylanase activity from *Paenibacillus* sp AR489 when Zn^{2+} and Ba^{2+} salts were added to the reaction mixture, but the activity was reduced by 12% with addition of Mg^{2+} and by 31% with addition of Fe^{2+} salts. Karp et al. [105] reported that addition of $(NH_4)_2SO_4$ and $CuSO_4$ significantly increased laccase production. Replacement of ammonium sulfate by yeast extract and addition of feluric acid (as inducer) resulted in 5.7- and twofold increase in laccase production, respectively [105]. A 30-fold increase in laccase production by *Pleurotus ostreatus* was observed after addition of 150 µM copper sulfate in culture medium [191]. Similarly, Hou et al. [192] observed 4.5-fold increase in laccase activity when copper ions (1 mM) were added in culture medium of *Pleurotus ostreatus*. Phytase production by twenty thermophilic filamentous fungi was enhanced after the addition of $CaCl_2$, and $MgSO_4$ in SCB medium [136]. Vijayabaskar et al. [155] reported that maximum amylase production was achieved by *Bacillus cereus* using calcium chloride.

7 Effect of pretreatment strategies on the production of various enzymes

SCB being a source of polymeric carbohydrates like cellulose and hemicellulose acts as a suitable medium for microbial growth and enzyme production [9]. But, due to recalcitrant nature, it may not be properly utilized by microbes as a source of nutrients. Therefore, various pretreatment strategies have been employed for the pretreatment of SCB followed by its utility in the generation of reducing sugars after enzymatic hydrolysis. There are many pretreatment methods such as physical, biological, chemical, and physicochemical for SCB. Choice of pretreatment usually depends on the substrate used, and pretreatment methods must be chosen carefully because many pretreatments can generate toxic compounds which are inhibitors for microbial growth and fermentation [36]. Detoxification is an important step for efficient bioconversion into useful products [1–5, 36]. Various physical, chemical, and biological methods (membrane filtration, neutralization, adsorption using activated charcoal, cell biomass, etc.) have been employed to reduce the effect of these inhibitors [1–5, 36]. Salomao et al. [36] used untreated and pretreated (acid–alkali and hydrogen peroxide) SCB for enzyme production in SSF by *Trichoderma koningii*, *Penicillium* sp., and *Rhizomucor* sp. Approx 47.3% lignin and 90.6% hemicellulose were removed after acidic and alkaline pretreatment. However, 32% lignin and 22.3% hemicellulose removal was observed with hydrogen peroxide pretreatment. Rezende et al. [193] also found similar observations after acid–alkali pretreatment of SCB. Guilherme et al. [194] observed 55% lignin removal after hydrogen peroxide and acid-alkaline pretreatment on SCB. The above results showed that acid–alkali pretreatment of SCB was more efficient in lignin removal, thus reducing the recalcitrant structure of biomass (Table 1).

Salomao et al. [36] observed the highest titre of endoglucanase 8.20 U/g using untreated SCB at 28 °C and 50% moisture by *Trichoderma koningii*. These results showed that pretreatment of SCB resulted in poor production of endoglucanase. Presumably, during the pretreatment, amorphous structure of the cellulose was removed, which was still present in the untreated bagasse that was better utilized by the fungus. Kumar et al. [198] also reported that pretreatment removes the cellulosic amorphous fraction leaving behind crystalline fraction. It is verified by some studies that amorphous fraction of cellulose was a better inducer for production of cellulase by microorganisms [199–201]. Another reason for low yield of cellulase after pretreatment is the generation of inhibitors. Phenolic compounds generated as a result of lignin hydrolysis during pretreatment are one of those inhibitors [202]. During alkaline pretreatment, inhibitory products such as organic acids and phenols are formed at high concentration [203]. Endoglucanase activity of 13.188 U/ml was obtained by co-culture of yeasts *Saccharomyces cerevisiae* MK-157 and *Candida tropicalis* MK-118 on acid pretreated SCB in SSF by Qadir et al. [102]. Similarly, an increase in β-glucosidase activity from 1.184 (untreated) to 14.45 U/ml (acid pretreated) and xylanase

Table 1 Production of various enzymes by microorganisms using sugarcane bagasse

Enzyme	Micro-organism	Bioprocessing conditions	Enzyme production	References
Cellulase	*Trichoderma koningii*	SSF with 5g substrate and 2.0×10^7spores/$g_{substrate}$; Temp- 28 °C; moisture content- 50%,	8.20 U/g	[36]
Cellulase	*Aspergillus niger*	SSF, highest cellulose content (86.7%) was obtained after combined acid/ alkali treatment. Sugarcane bagasse pretreated with liquid hot water resulted in highest CMCase activity after 72 h incubation.	14.9 U/g	[13]
Cellulase	*Penicillium* sp.	SSF with 5g substrate and inoculated with 2.0×10^7 spores/$g_{substrate}$; Temp- 28 °C; moisture content- 70%.	1.71 U/g	[36]
Cellulase	*Trichoderma harzianum*	Using sugarcane bagasse as a substrate under SSF, cellulase is produced with 14% (w/v) SCB after 168 h of culture.	0.26 U/ml	[45]
Endoglucanase	*Saccharomyces cerevisiae* MK-157 and *Candida tropicalis* MK-118	SSF; Temp-35 °C; incubation period- 94 h; alkali pretreated SB was inoculated with 0.5 ml of the mono- or co-culture and was moistened to 50 or 80% using 1% (w/v) peptone or yeast extract medium as a nitrogen source.	9.81 U/ml	[102]
Cellulase	*Trichoderma koningii*	SSF; Temp- 28 °C; moisture content- 60%, using hydrogen peroxide pretreated sugarcane bagasse.	3.19 U/g	[36]
Cellulase	*Aspergillus flavus* KUB2	SmF containing fermentation medium (1 g/l KH_2PO_4, 0.5 g/l $MgSO_4.7H_2O$, 5 g/l peptone and 5 g/l yeast extract) supplemented with 20 g/l of sugarcane bagasse; incubation period- 14 days; temp- 30 °C	CMCase (1.27 U/ml), FPase (0.72 U/ml)	[59]
Cellulase	*P. funiculosum* ATCC 11797	SmF; Pretreated sugarcane bagasse (with diluted sulphuric acid followed by sodium hydroxide) was used. The temperature, agitation and pH were maintained at 30 °C, 200–350 rpm and 5.0, respectively with 60 h of incubation.	179.5 U/1 h	[65]
Endoglucanase	*Bacillus aestuarii* UE25	SmF; Maximum endoglucanase was observed when alkali pretreated (NaOH) bagasse was treated with Ionic liquid (Methyltrioctylammonium Chloride) for 30 min at 70 °C in 1:15 ratio and 10% inoculum of the strain was used in this substrate, amended with 0.5% glucose and peptone with initial pH 5.0, at 60 °C for 48 h with agitation.	111.49 U/ml	[67]

Table 1 (continued)

Enzyme	Micro-organism	Bioprocessing conditions	Enzyme production	References
Cellulase	*T. harzianum* IOC 3844	Pretreatment of sugarcane bagasse with two subsequent pretreatments with acid (with sulphuric acid 1% v/v at solid liquid ratio of 1:2 at 121 °C for 45 min) and further pretreated with alkali (with sodium hydroxide 4% w/v with solid to liquid ratio of 1:20 at 121 °C for 30 min. Maximum cellulase activity was obtained after 42 h incubation.	27.02 U/ml	[69]
Cellulase	*Rhizomucor* sp.	SSF; Temp- 33 °C; moisture content- 70%, using Natural Sugarcane bagasse as solid substrate	0.41 U/g	[36]
Endoglucanase	*Aspergillus niger* A12	SmF; cultivation time -30 h, Supplemented with glucose.	0.432 U/ml	[58]
Cellulase	*Penicillium* sp.	SSF; Temp- 33 °C; moisture content- 50%, using acid-alkali pretreated bagasse.	0.08 U/g	[36]
Cellulase	*Trichoderma Koningii*	SmF; Temp-28 °C using 2.7% w/v natural bagasse.	3.13 U/ml	[62]
Cellulase	*Penicillium* sp.	SmF; Temp-33 °C using 1.6% w/v acid-alkaline pretreated.	0.111 U/ml	[62]
Cellulase	*Rhizomucor* spp	SmF; Temp-28 °C using 1.6% w/v natural bagasse.	0.064 U/ml	[62]
Endoglucanase	*Bacillus megaterium*	SmF; Temp- 33 °C using acid alkaline pretreated sugarcane bagasse.	0.156 U/ml	[62]
Cellulase	*Bacillus pseudomycoides*	Sugarcane bagasse 1% (carbon source), yeast extract 1.5% (nitrogen source); at pH 7.0; temp-40°C in 72 h of incubation	3.5 U/mg	[186]
β-1,3-glucanase	*Metarhizium anisopliae* IBCB 348	SSF during 8 days of fermentation; Moisture content of 40 wt%, 10% (v/w) of inoculum, temp- 28 °C.	13.73 ± 0.11 U/g	[35]
Endocellulase and exocellulase	*Metarhizium anisopliae* IBCB 348	SSF; temp- 28°C; Moisture content of 40 wt%, 10% (v/w) of inoculum, during 192 h of fermentation.	17.90 ± 0.45 U/g and 24.90 ± 1.20 U/g	[35]
Xylanase	*Aspergillus flavus* KUB2	SmF containing fermentation medium (1 g/l KH_2PO_4, 0.5 g/l $MgSO_4 \bullet 7H_2O$, 5 g/l peptone and 5 g/l yeast extract) supplemented with 20 g/l of sugarcane bagasse; incubation period-336 h, temp- °C	376.81 U/ml	[59]
Xylanase	*Aspergillus niger* LPB 326	SSF, using 10g sugarcane bagasse and soyabean meal in the ratio of 65 and 35 %, respectively, medium was moistened to 85 % of initial water content with a nutrient salt solution composed of: $CuSO_4$ - 0.4 g/L, KH_2PO_4 - 1.5 g/L and $CoSO_4$ - 0.0012 g/L, and incubated at 30 °C for 96 h.	3099 U/g	[90]
Xylanase	*Thermoascus aurantiacus* ATCC 204492	SSF; Fermentations were performed in a glass-column reactor with forced aeration. Maximum xylanase activity was attained after 240 h of SSF with 6 l/(hg) airflow rate and 8 g substrate.	1597 U/g	[91]

Table 1 (continued)

Enzyme	Micro-organism	Bioprocessing conditions	Enzyme production	References
Xylanase	*Aspergillus japonicas* Saito	Temp-50 °C, pH- 5.5; Incubation period- 168 h	3.55 U/ml	[63]
Xylanase	*Aspergillus niger* A12	SmF, cultivation time -30 h	0.714 U/ml	[58]
Xylanase	*Aspergilus awamori*	Temp- 30 °C, pH-6; incubation period- 60 h using sugarcane bagasse as carbon source and sodium nitrate as nitrogen source	45 U/ml	[195]
Xylanase	*Trichoderma harzianum*	Temp-30 °C, pH-5; incubation time- 168 h	288 U/ml	[45]
Xylanase	*Bacillus substilis* subsp. *subtilis* JJBS250	SSF with cultivation medium containing 5 g of sugarcane bagasse substrate at a ratio of 4:1 with moistening medium [(%w/v) $(NH_4)_2SO_4$ 0.50, $MgSO_4{\cdot}7H_2O$ 0.10, K_2HPO_4 0.10 and NaCl 0.10]; at pH 7.0; temp- 40 °C after 48 h incubation.	20.35 U/g	[176]
Xylanase	*Chromohalo-Bacter* sp. TPSV 101	Temp-40 °C, pH- 9; incubation time- 140 h using sugarcane bagasse as carbon source and feather hydrolysate as nitrogen source	250 U/ml	[196]
Amylase	*Aspergillus japonicas*	Fermentation media containing 50 mL of the following medium (gL^{-1}): (6) $NaNO_3$, (1.5) KH_2PO_4, (0.5) KCl, (0.5) $MgSO_4.7H_2O$, (0.01) $FeSO_4$, (0.01) $ZnSO_4$, and 1% sugarcane bagasse. Incubation time 336 h.	0.35 U/ml	[63]
Laccase	*Aspergillus japonicas* Saito	Fermentation media containing 50 mL of the following medium (gL^{-1}): (6) $NaNO_3$, (1.5) KH_2PO_4, (0.5) KCl, (0.5) $MgSO_4.7H_2O$, (0.01) $FeSO_4$, (0.01) $ZnSO_4$, and 1% sugarcane bagasse. Incubation period- 168 h	395.73 U/l	[63]
Laccase	*Pleurotus ostreatus*	SSF, Using sugarcane bagasse as support and yeast extract and $(NH_4)_2SO_4$ as nitrogen source after 120 h.	151.6 U/g	[105]
Laccase	*Pycnoporus cinnabarinus* SS3	SSF, Laccase production in a biofilter packed with sugar-cane bagasse as a solid substrate. Maximum laccase production was obtained from a concentration of about 7 $g_{ethanol}$ m^{-3} (ethanol concentration in the liquid phase of 31 g L^{-1}).	90 U/g	[107]
Chitinase	*Metarhizium anisopliae* IBCB 348	SSF, Moisture content of 40 wt%, 10% (v/w) of inoculum, temp- 28 °C during 192 h of fermentation.	12.07 ± 0.50 U/g	[35]
Lipase	*Rhizopus homothallicus* IRD13a	*SSF,* lipase production using sugarcane bagasse as support and soaked with liquid medium. Nutrients that mainly impact growth and lipase production were olive oil, urea and oligo-elements. Maximum lipase production was reached in only 12 h of incubation.	826 U/g	[127]

Table 1 (continued)

Enzyme	Micro-organism	Bioprocessing conditions	Enzyme production	References
Lipase	*Aspergillus* sp.	7.5 g of wheat bran + 2.5 g of sugarcane bagasse with 40% moisture content.	10.82 U/ml	[128]
Phytase	*Sporotrichum thermophile*	Mixed substrate (sugarcane bagasse and wheat bran) with 2.5% Tween 80 and 1% yeast extract; 48 h fermentation time.	1868.56 U/g	[135]
Phytase	*Aspergillus niger*	SSF using sugarcane bagasse with substrate to moisture ratio of 1:2.5, at 32 °C after an incubation of 48 h.	8.0 U/g	[134]
Phytase	*Myceliophthora thermophila*	Sugarcane bagasse supplemented with mineral salts such as $(NH_4)_2HPO_4$, $CaCl_2$, and $MgSO_4$ favoured high phytase activity at pH- 6.0; moisture- 75%; aeration rate- 25 ml/min/column, after 36 h of fermentation.	165 U/g	[136]
Amylase	*Aspergillus niger* UO-01	SSF; using sugarcane bagasse at 30.2 oC, pH-6.0 and particle size of bagasse 6-8 mm.	457.82 U/g	[154]
Amylase	*Bacillus cereus*	SSF; using sugarcane bagasse at 40 °C, pH- 7.0, after 48 hr of fermentation.	232.65 ± 2.74 U/ml	[155]
Amylase	*Streptomyces erumpens* MTCC 7317	SSF, using sugarcane bagasse at 50 °C, pH- 7.0, after 48 h of fermentation.	2821.9 U/g	[157]
Invertase	*Aspergillus ochraceus*	Sugarcane bagasse with optimal activity at 40 oC, at pH- 4.5 after 96 h of incubation.	108 Total U	[162]
Inulinase	*Kluyveromyces marxianus* NRRL Y-7571	SSF, using sugarcane bagasse with sodium acetate buffer 0.1 M pH 4.8 was used, using a solid/liquid ratio of 1:10, at 53°C and 150 rpm for 40 min.	396.6 U/g	[167]
Inulinase	*Kluyveromyces marxianus* NRRL Y-7571	SSF, using sugarcane bagasse and soybean bran as substrates in a 3-kg (dry basis) packed-bed bioreactor after 24 h at an inlet air temperature of 30°C, air flow rate 2.2 $m^3\ h^{-1}$	436.7 ± 36.3 U/g	[168]
Pectinase	*Aspergillus niger*	SSF, mixed substrates consisting of 90% of wheat bran and 10% of sugarcane bagasse after the fermentation period of 96 h at 40°C at pH 5.0.	169.50 U/ml	[174]
Pectinase	*Aspergillus oryzae*	Using 15 kg of a substrate containing 48.4% sugarcane bagasse and 51.6% citrus pulp (w/w, dry basis) in packed bed bioreactor, bed temperatures were controlled to 1°C.	33–41 U/g	[197]

activity from 3.87 (untreated) to 20.24 U/ml (acid pretreated) was also observed. In a yeast co-culture study for cellulase and xylanase enzyme production conducted by Qadir et al. [102], the alkali-pretreated SCB showed better results than acid-pretreated SCB. Alkali pretreatment resulted in overall increase in enzyme activity compared to untreated, i.e., endoglucanase from 2.07 to 21.37 U/ml, xylanase from 2.9 to 14.9 U/ml, and β-glucosidase from 3.9 to 18.12 U/ml [102]. For cellulase production, the importance of temperature was also observed by [204].

8 Pretreatment and saccharification of sugarcane bagasse

The process of converting lignocellulosic substrates into biofuels and value-added products needs three steps: (1) delignification/pretreatment of biomass, (2) saccharification/hydrolysis of polymers into fermentable sugars, (3) fermentation of resulting sugars by microbes [19, 205]. Out of these three, pretreatment is a crucial step in the conversion of lignocellulosic biomass to sugars because the presence of crystalline cellulose, lignin, and high degree of polymerization hinders the action of hydrolytic enzymes (Table 2). Pretreatment aims to reduce the crystallinity and remove the lignin content, and increase the porosity and available surface area of lignocellulosic biomass. It is performed by various methods such as physical, chemical, physicochemical and biological [104]. After pretreatment, carbohydrate polymers need to be saccharified into fermentable sugars, which can be done by chemicals (acids) and enzymes (cellulolytic and xylanolytic enzymes) (Fig. 2). Hydrolysis without pretreatment yields low amount of sugars because enzymes cannot penetrate the crystalline structure of cellulose bound with hemicellulose and lignin. Therefore, pretreatment is a necessary step to expose cellulose to enzymes, to remove the lignin and increase the porosity [206]. Enzymatic hydrolysis is preferred over acid because it offers many advantages such as low energy requirements, higher yield, lesser by-product formation, and low cost of chemical disposal.

9 Sugarcane bagasse liquor—a rich source of sugars for enzyme production

After pretreatment of bagasse, liquor generation is common process. These liquors contain sugars from depolymerization of cellulose and xylan into glucose and xylose, respectively. These sugar-rich liquors are good substrates for enzyme production but various highly toxic by-products are also produced during pretreatment which inhibit the growth of microorganisms. Due to these toxic compounds, the use of liquor becomes limited [180]. Main components in the liquor after pretreatment of SCB are carbohydrate monomers (glucose and xylose), carbohydrate by-products (acetic acid, formic acid, furfural, and hydroxymethylfurfural), and lignin by-products (phenolics) [180]. Different detoxification methods for transformation of toxic compounds into inactivated counterparts have been devised. These methods are physical, chemical, and biological ones [207]. Biological methods include the use of microbes and their enzymes to reduce the inhibitory compounds [14]. Fillat et al. [208] observed that among microorganisms, *Aspergillus* and *Trichoderma* have shown the best results to remove inhibitory compounds. Liquor from the hydrothermal pretreatment of SCB was used as a substrate for growth and induction of *Trichoderma harzianum* [61]. Similarly, liquor from hydrothermal pretreatment of SCB was used by Robl et al. [41] to produce xylanase from *Aspergillus niger*. The chemical analysis of liquor revealed that it contains adequate concentration of sugars to support the growth of microbes for enzyme production. One hundred percent of liquor did not allow growth of microorganisms due to inhibitors present in the crude liquor. To overcome this effect, a two-step cultivation strategy was adopted; in the first step, only glucose is used as a carbon source followed by sequential addition of liquor at 50% (v/v) in the second step. This approach was successful for the growth of six fungi including *Trichoderma harzianum* [180]. Endophytic fungus was studied by Robl et al. [41] using the liquor of SCB pretreated with hydrothermal pretreatment. They reported that *Aspergillus niger* DR02 produced 458 U/ml xylanase in fed-batch cultivation. Therefore, the side products generated at each step of SCB can be utilized in the development of biorefinery approach.

10 Sugarcane bagasse hydrolysate for the production of biofuels

Cane molasses, a by-product of sugarcane industry, has been utilized as a medium for the production of various enzymes and biofuel production. SCB is a right choice for the production of second-generation biofuels. Therefore, enzymatic hydrolysate of pretreated and untreated SCB is a potential source of nutrients for the cultivation of fermenting microbes for the synthesis of biofuels, sugar syrups, organic acids, biopolymers, and many other products of biotechnological importance across the world (Table 3). Few biofuels are listed below.

10.1 Bioethanol

An alcoholic beverage, ethanol is used as a biofuel and it is also used as a base material for many industrial products such as ethyl esters, acetic acid, diethyl ether, and ethyl amines [15]. Presently, bioethanol is the most preferred biofuel compared to other biofuels. Bioethanol produced from the lignocellulosic biomass such as agricultural waste (SCB, rice straw, wheat bran, etc.) is considered one of the best alternative to fossil fuels [209]. It is also used as a solvent for medical purposes and acts as a good source for various chemical manufacturing that are produced from unsustainable sources of energy [210]. As a biofuel,

Table 2 Various pretreatment methods for sugarcane bagasse

Method	Description	Toxic by-products	Cost	Reference
Steam explosion	Steam injected up to a pressure of almost 1.3 MPa equivalent to 190 ºC and was maintained for 15 min. The treatment solubilized an average of 82.7±4.3% of the hemicelluloses and Lignin was solubilized at the proportion of 7.9±9.1%.	High	High	[246]
	Phosphoric acid-catalyzed steam pretreatment at different temperature ranging from 180–220 °C for different reaction time (5–7.5 min) and 180 °C for 5 min was reported as optimum pretreatment conditions.			[247]
	H_2O_2 assisted steam pretreatment of sugarcane bagasse at 25°C for 60 min for enhanced saccharification and bioethanol production			[248]
Alkaline pretreatment	1.0% NaOH solution (w/v), using a solid-liquid ratio of 1:10 (w/v) was carried out at 100ºC for 1 h and 92.7±3.9% removal of lignin from biomass. This process hydrolyzed 31.1±3.5% of the cellulose and 94.7±0.9% hemicellulose.			[246]
	0.5 M aqueous KOH solution with ratio 50 g/1.5L under nitrogen at 35 °C for 3.5 hr. The purity of bagasse lignin obtained with this method is 95% related to soluble lignin.			[249]
	NaOH pre-treatment (i.e., 110 °C for 1 h and 0.18% of NaOH) has resulted in 77.3% sugar release after 72 h of enzymatic hydrolysis.			[250]
	Extractions of lignin fraction was done using sequential pretreatment of 96% dioxane, 50% dioxane, and 80% dioxane containing 1% NaOH at boiling temperature.			[251]
	2% H_2O_2 together with 1.5% NaOH at 121ºC for 15 min. Increase of up to 1.2 times cellulose is observed after this treatment and decreased of 8.5 times in hemicellulose content.			[66]
Acid pretreatment	Microwave-alkali (1% NaOH), followed by acid pretreatment (1% H_2SO_4) and enzymatic hydrolysis gave an overall reducing sugar yield of 0.83 g/g dry sugarcane bagasse.	High	Low	[252]
	Pre-treatment using sulfuric acid with 0.5% concentration at 121°C for 60 mins resulted in 24.5 g/L of total sugars after cellulose hydrolysis.			[253]
	Pretreatment with HCl at 130°C, 1.25% HCl concentration for 10 min resulting in 76.6% sugar release after 72 h of enzymatic hydrolysis.			[250]
	Dry powder treated with a dioxane solution and 0.1 N aqueous HCl (8.5:1.5, v:v) with ratio (100 g/1L) and heated under nitrogen at 100°C for 2 h. The purity of bagasse lignin is 90% related to soluble lignin.			[249]
Ammonia fiber expansion	2 kg ammonia + 1.5 kg water kg dry bagasse, at 140°C, for 30 min resulted in 85% glucan conversion by cellulases and 95-98% xylan conversion by hemicellulases in bagasse and cane leaf residue.	Low	High	[254]
	Pretreatment with 15% ammonia at 170 °C for 60 min, resulted in enhanced delignification as well as saccharification			[255]
Organosolv	Combination of a dilute-acid pretreatment followed by NaOH under (60 min, 195 ºC, 30% v/v ethanol), yielding a residual solid material containing 67.3% (w/w) glucose, which was easily recovered by the enzymatic hydrolysis.	High	High	[256]
	Bagasse was mixed with 60:40 EtOH/water with 5% dosage of acetic acid at 190 °C for 45 min, resulted in 85.4% sugar yield after 72 h of enzymatic hydrolysis.			[257]
	Glycerol (80%) - water mixtures for 150 min, at 198.3°C and obtained a pulp with 81.4% delignification.			[258]
Wet oxidation	Alkaline wet oxidation at 195 ºC for 15 min yielded solids with nearly 70% cellulose, with a solubilization of approximately 50% of lignin and 93% of hemicelluloses.	Low	High	[259]
Ionic liquid pretreatment	Treatment with 1-butyl-3-methylimidazolium chloride ([C4mim]Cl) at 110 °C for 15 min under a nitrogen atmosphere with agitation resulted in 54.62% lignin removal.	Low	High	[260]
	1-(4-sulfobutyl)-3-methylimidazolium hydrosulfate ([BMIM][SO3] [HSO4]) (ethanol/water) at 200 °C for 30 min resulted in 100% lignin removal.			[261]
	1-butyl-3-methylimidazolium methyl sulfate ([BMIM][MeSO4]) (7% H2SO4) at 125°C for 2 h resulted in 26% lignin removal.			[262]
	1:20 solution of bagasse to 1,3-dimethylimidazolium dimethyl phosphate ([Mmim][DMP]) at 120 °C for 120 min resulted in 70.38% of sugar conversion after 48 h of enzymatic hydrolysis.			[263]

Table 2 (continued)

Method	Description	Toxic by-products	Cost	Reference
Ultrasound pretreatment	Ultrasound-assisted alkaline pretreatment of sugarcane bagasse at 24 kHz, 400 W at 30–50 °C for 5–50 min, resulted in 99.6% delignification of biomass	Low	High	[264]
	Slurry of bagasse (10%) followed by addition of H_2SO_4 and exposed the slurry to ultrasound pretreatment at 120 W for 180 sec resulted in efficient lignin removal.			[265]
Hot water pretreatment	Liquid hot water pretreatment of bagasse at solid to liquid ratio of 1:20 at 180°C for 30 min resulted in effective removal of hemicellulose with enhanced saccharification.	Low	Low	[266]
Biological pretreatment	Laccase from *Pycnoporus cinnabarinus* was used for effective delignification of sugarcane bagasse	Low	Low	[267]
	Pretreatment with *Ceriporiopsis submervispora* at 27 °C for 144 h resulted in high sugar recovery			[243]

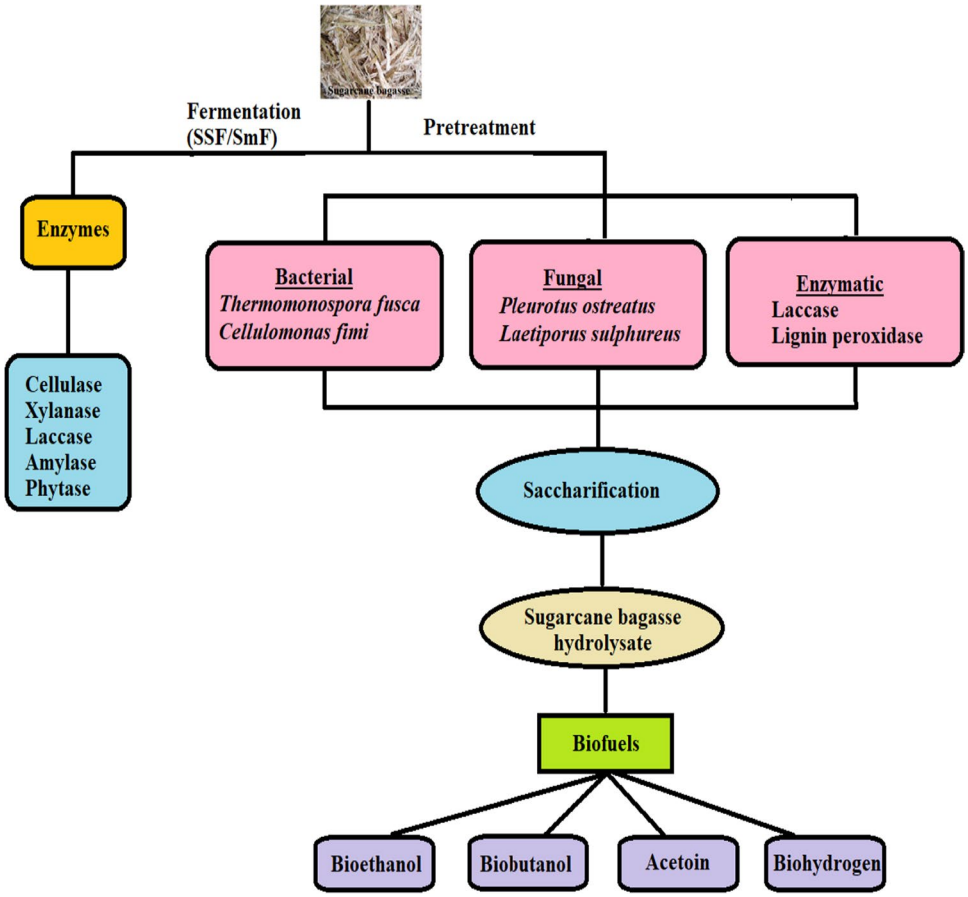

Fig. 2 Schematic diagram representing enzyme and biofuel production from sugarcane bagasse.

ethanol has various advantages such as low carbon dioxide emission, high energy density, and high heat of vaporization which makes it a favorable biofuel for various purposes [211]. Several microorganisms produce ethanol by fermenting sugars generated from various lignocellulosic biomasses such as SCB. The hydrolysate of SCB is rich in fermentable sugars which act as a good source for ethanol production.

Approximately, 300 l of ethanol is produced from 1 ton of SCB [212]. However, the yield of ethanol production depends upon many parameters such as SCB quality, microbial strain, and process used for ethanol production [212]. In a study conducted by Santos et al. [213], SCB was first treated with H_2SO_4 and the solid biomass is separated which is again treated with NaOH at 121 °C for 30 min. They reported maximum conversion of cellulose to ethanol

Table 3 Sugarcane bagasse and its hydrolysate as the substrates for the production of various biofuels

BIOFUELS	MICROORGANISM	DESCRIPTION	REFERENCE
Bioethanol	*Zymomonas mobilis*	After acid treatment with H_2SO_4 (1% v/v), bioethanol yield of 60g/L was obtainted.	[210]
	Klebsiella pneumoniae CGMCC 1.9131	NaOH (10%) treatment for 1.5 hr at 90 oC followed by 15% PAA treatment for 3 hr at 75 °C resulted in 50.6 g/L yield.	[214]
	Saccharomyces cerevisiae	56.3 g/L yield was obtained after steam explosion treatment for 5 min. at 215 °C.	[215]
	Saccharomyces cerevisiae	Treatment with NaOH for 2 hr resulted in 11.81 g/L bioethanol production.	[216]
	Pichia stipitis	3.70 g/L yield was obtained using acid preatment with 4% H_2SO_4(v/v) for 90 min at 120 °C.	[217]
	Saccharomyces cerevisiae, Pichia stipitis	Ammonia pretreated sugarcane bagasse hydrolysate resulted in 31.56 g/L bioethanol	[202]
	Candida shehatae NCIM 3501	8.67 g/g ethanol was produced at temp-30 °C, 150 rpm after 24 h	[244]
	Saccharomyces cerevisiae	4.88 g/L ethanol was produced after Salt-alkali pretreatment, first by $ZnCl_2$ (1.73 M) and then followed by NaOH (1.36 M) both for 30 min at 121°C.	[268]
	Pachysolen tannophilus DW06	0.34 g/g ethanol was produced at temp-30 °C, pH- 5.0 and 150 rpm	[244]
	Candida tropicalis	20% Ammonium hydroxide pretreatment for 48 h at 50 °C resulted in 57.2 g/L yield.	[269]
	Escherichia coli	29.0 g/L ethanol was produced at temp- 37 °C, pH: 6.5, 150 rpm, 24 h	[244]
	Saccharomyces cerevisiae	Acid pretreatment with H_2SO_4 (2% v/v) followed by alkali treatment with NaOH (4% w/w), both at 121 °C, 30 min resulted in 29.83 g/L bioethanol.	[270]
Biohydrogen	*Clostridium butyricum* TISTR 1032	Immobilization using delignified sugarcane bagasse (7 g) in 63 ml of tryptone sucrose yeast extract which was autoclaved for 15 min at 121°C was done followed by addition of 10% inoculum of *Clostridium butyricum*resulted in 1.52 mol H_2/mol hexose consumed.	[230]
	Thermophilic microbial consortium	Steam explosion at 200°C for 7 min followed by alkaline pretreatment with NaOH at 121°C for 30 min resulted in 1.2 mol H_2/g substrate.	[231]
	Thermoanaerobacterium aotearoense	Sulphuric acid (2.3%) pretreatment for 114.2 min at 115°C resulted in 520 g/L biohydrogen.	[232]
	Clostridium butyrium	Two-step dilute acid hydrolysis with sulphuric acid solution (2 wt%) with 1:10 (g/g) solid-liquid ratio for 1 h at 130°C followed by sulphuric acid solution (4 wt%) with solid liquid ratio of 1:10 (g/g) at 150 °C for 1 h resulted in 15.66 g/L biohydrogen.	[233]
Biobutanol	*Clostridium beijerinckii* NCIMB 8052	Different sequential pretreatments were done like milling, hot water pretreatment for 1 h at 200 °C, microwave pretreatment at 120 °C for 7 min and then ammonia immersion (2:1; v/w) at 90 °C for 30 min and last enzymatic hydrolysis, the resultant yield of biobutanol was 6.86 g/L.	[220]
	Clostridium acetobutylicum	Combined dilute acid with oxidate ammonolysis pretreatment was performed by 1% sulphuric acid at 140 °C for 1 h and then bagasse was washed, dried and pretreated using 5% NH_3H_2O with 6% H_2O_2 at 80 °C for 6 h resulted in 4.62 g/L biobutanol.	[11]

Table 3 (continued)

BIOFUELS	MICROORGANISM		DESCRIPTION	REFERENCE
2,3-Butanediol	*Klebsiella pneumoniae*		Alkali-PAA pretreatment was performed using NaOH (10%) at (3:1 liquor to solid ratio) at 90 °C for 1.5 h followed by PAA (15%) treatment for 3 h at 75°C, and the 2,3-Butanediol yield obtained after fermentation was 17.35 g/L.	[226]
	Klebsiella pneumoniae	CGMCC 1.9131	Xylose syrup obtained in SSF of alkali–PAA (first treated with 10% NaOH at 90 °C for 1.5 h with 3:1 liquor to solid ratio (L/kg). The pretreated solid was washed with water till neutrality. The solid was further treated with PAA loading of 15% at 75 °C for 3 hr) was converted to 2,3-butanediol with yield of 0.35–0.50 g 2,3-BDO/g consumed xylose.	[214]

with yield of 0.29–0.30 g/l/h using acid pretreated (H_2SO_4) SCB in SSF. Recombinant *Saccharomyces cerevisiae* with β-glucosidase gene was used by Ferreira et al. [214] for ethanol production from SCB in SSF; they reported 60 g/l ethanol production.

Biofuels have been extensively produced from the hydrolysate of SCB [215]. Kondo et al. [216] reported that 6.16 g/l ethanol was produced which is equivalent to 340 mg of ethanol for each gram of pretreated bagasse using *Phlebia* sp. MG-60 grown on NaOH (5%)-pretreated SCB. Fermentation of ammonia-pretreated SCB hydrolysate (20%) was carried out by *Saccharomyces cerevisiae* and *Pichia stipitis* separately followed by fermentation in combination using both spp. at 30 °C, for 72 h and 150 rpm that resulted in 24.56 g/l, 23.56 g/l, and 31.56 g/l production of bioethanol, respectively [205]. NaOH (10%) treatment for 1.5 h at 90 °C followed by 15% PAA treatment for 3 h at 75 °C resulted in 50.6 g/l yield of bioethanol by *Klebsiella pneumoniae* CGMCC1.9131 [217]. Bioethanol yield of 56.3 g/l was obtained after steam explosion treatment for 5 min at 215 °C using SCB by *Saccharomyces cerevisiae* [218]. Wahono et al. [219] reported 11.81 g/l bioethanol production using *Saccharomyces cerevisiae* from SCB pretreated with NaOH for 2 h. Bioethanol yield of 3.70 g/l was obtained using acid pretreatment with 4% H_2SO_4 (v/v) for 90 min at 120 °C by *Pichia stipitis* [220].

10.2 Biobutanol

The properties of biobutanol are almost similar to gasoline, a next-generation biofuel. It is a better fuel than bioethanol due to high energy density and similarity to gasoline [1]. It is produced by various microorganisms by fermentation of sugar-rich hydrolysates. In butanol production, various *Clostridium* sp. are involved such as *C. beijerinckii*, *C. acetobutylicum*, and *C. saccharobutylicum* [221]. Various lignocellulosic biomass, agro-industrial waste, and forest products are used for biobutanol production. Efforts are currently going on to produce biobutanol on a large scale. Various characteristics such as high mixing capacity with gasoline and diesel, low corrosiveness, high calorific value, and hydrophobicity make it a better fuel over ethanol [222].

Su et al. [223] used *Clostridium beijerinckii* NCIMB 8052 for biobutanol production using SCB pretreated with different sequential pretreatments such as milling, hot water pretreatment for 1 h at 200 °C, microwave pretreatment at 120 °C for 7 min and then ammonia immersion (2:1; v/w) at 90 °C for 30 min, and last enzymatic hydrolysis; the resultant yield of biobutanol was 6.86 g/l. Similarly, Li et al. [11] used SCB pretreated with 1% sulfuric acid at 140 °C for 1 h and then bagasse was washed, dried, and pretreated using 5% NH_3H_2O with 6% H_2O_2 at 80 °C for 6 h and then inoculated with *Clostridium acetobutylicum*, which resulted in 4.62 g/l biobutanol production.

10.3 Acetoin and 2,3-butanediol

It is naturally found as a flavoring agent in many foods. Acetoin is also a valuable precursor for a wide variety of chemical synthesis and value-added products [224]. Chemical synthesis of acetoin is usually done by using fossil substrates. Efforts for natural production of acetoin using microorganisms such as bacteria have been made in the last few years [1]. For acetoin production, commonly used microorganisms are *Bacillus subtilis*, *Enterobacter cloacae*, *Serratia marcescens*, and *Paenibacillus polymyxa* [225]. 2,3-Butanediol which is widely used in industries is produced from acetoin [226]. For 2,3-butanediol production, commonly used microorganisms are *Klebsiella aerogenes*, *Bacillus subtilis*, and *Enterobacter aerogens* [227, 228]. Alkali-PAA pretreatment was performed on SCB using NaOH (10%) at 3:1 liquor-to-solid ratio at 90 °C for 1.5 h followed by **PAA** (15%) treatment for 3 h at 75 °C, and then inoculated with *Klebsiella pneumonia*, which resulted in 17.35 g/l of 2,3-butanediol after fermentation [229].

10.4 Biohydrogen

For future prospects, biohydrogen is a great fuel because its use results in pollutant-free by-products [1]. It is an odorless, colorless, non-toxic, and tasteless gas, which is a higher energy-producing fuel than other fuels [230]. It can be produced by three methods namely dark fermentation, bio photolysis of water, and photo-fermentation [231]. Biohydrogen can be synthesized by utilizing various lignocellulosic biomass, industrial waste, and municipal solid waste [232]. But above all, biohydrogen production from agro-industrial residues like SCB is considered the best source which is present abundantly in nature.

Immobilization using delignified SCB in tryptone sucrose yeast extract was done followed by addition of 10% inoculum of *Clostridium butyricum* and resulted in 1.52 mol H_2/mol hexose consumed [233]. Ratti et al. [234] produced 1.2 mol H_2/g substrate using SCB, pretreated with steam explosion at 200 °C for 7 min followed by alkaline pretreatment with NaOH at 121 °C for 30 min using themophilic microbial consortium. Lai et al. [235] observed 520 g/l biohydrogen using SCB pretreated with sulfuric acid (2.3%) for 114.2 min at 115 °C by *Thermoanaerobacterium aotearoense*. Two-step dilute acid hydrolysis with sulfuric acid solution (2 wt%) with 1:10 (g/g) solid–liquid ratio for 1 h at 130 °C followed by sulfuric acid solution (4 wt%) with solid–liquid ratio of 1:10 (g/g) at 150 °C for 1 h resulted in 15.66 g/l biohydrogen using *Clostridium butyrium* [236].

Peroxyformic acid pretreatment of SCB resulted in enhanced saccharification with 59.0% delignification [195]. Pretreated biomass under dark fermentation increased hydrogen production by 195.5% using anaerobic cellulolytic microbial consortium MC1. Supplementation of *Thermoanaerobacterium thermosaccharolyticum* MJ2 and biochar improved hydrogen production from SCB by 95.31 and 158.10%, respectively [196]. Biochar addition improved substrate degradation, activity of hydrogenase, and microbial growth and metabolism. SCB after hydrogen peroxide-acetic acid pretreatment resulted in hydrogen production of 226 mL/g substrate using co-culture fermentation of *T. thermosaccharolyticum* and *C. thermocellum* [197]. Enhanced biohydrogen production (277.4 mM) was attained from non-detoxified SCB in a novel two-stage anaerobic fermentation [237].The hydrogen production of the second-stage fermentation (167.8 mM) was significantly higher than that of the first-stage fermentation (108.6 mM).

11 Conclusions and future perspectives

Sugarcane is an important crop used for the production of sugar and other by-products (cane molasses), which are accounting for major contribution to the growing economy of a country like India and Brazil. During sugarcane processing, large quantities of bagasse are generated by sugar industries. Only a small fraction of this bagasse is used by the industry for energy generation and left is burnt in open or either disposed improperly. The burning of SCB causes environmental pollution and other health hazards. Use of bagasse for production of enzymes, biofuels, and other value-added products will be helpful to overcome the environmental pollution. On the other hand, burning of fossil fuels causes global warming due to particulate pollutants such as CO, CO_2, SO_2, and nitrogen oxides released by their burning. SCB is a potential source for biofuel production which acts as an alternative to traditional fossil fuels. Use of low-cost substrate such as SCB to produce value-added products is a sustainable, ecofriendly, and economical approach. Apart from biofuel production, SCB has been used to produce microbial enzymes useful for different industries such as biofuel, textile, pharmaceutical, food, and beverages industries. Also, SCB serves as a cost-effective substrate for production of organic acids (lactic acid, citric acid etc.), single-cell protein, and animal feed, as a biosorbent for heavy metals. Leftover biomass after fermentation by microorganisms can be used as an organic fertilizer for improving crop production. SCB is an ideal lignocellulosic biomass for developing biorefinery integrated with sugar industries. However, the lack of a suitable, economical, and environment-benign pretreatment process is the major bottleneck in this direction. Future research should be directed toward the use of biological pretreatment alone or in combination with other methods for SCB, making it more prone for enzymatic hydrolysis. Thermostable enzymes (including cellulases and xylanases) from thermophilic microorganisms could be the best choice for efficient, faster, and complete saccharification of biomass. Future research should be focused for efficient utilization of bagasse to produce cleaner energy and value-added products for a sustainable future.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13399-022-02791-9.

Author contribution All authors contributed to the study conception and design. The idea was initially drafted by Pratiksha Yadav, Bijender Singh, Anu, and S.K. Tiwari. Literature search was performed by Pratiksha Yadav, Bijender Singh, Vinay Malik, and Sandeep Kumar. Initial draft was written by Pratiksha Yadav, Anu, and Bijender Singh. Data analysis and design of the article was performed by Pratiksha Yadav, Bijender Singh, S.K. Tiwari, and Manisha. The draft was critically revised and improved by Davender Singh, Vinod Kumar, Bijender Singh, and Vinay Malik. Figures and tables were designed and compiled by Pratiksha Yadav, Bijender Singh, Manisha, and Davender Singh. Final list of contents was revised by all the authors. All authors read and approved the final manuscript.

Funding Pratiksha Yadav acknowledges the financial support (Award no. 09/1152(0026)/2021-EMR-I) by the Council of Scientific and

Industrial Research (CSIR), New Delhi, during the course of writing this review.

Declarations

Conflict of interest The authors declare no competing interests.

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