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REVIEW ARTICLE

Solid-state fermentation as an alternative technology for cost-effective production of bioethanol as useful renewable energy: a review

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Abstract
The ever-increasing population of the world, extended urbanization/industrialization in developing countries, improvements in quality of life, and increasing oil prices have accelerated the need for sustainable energy sources. Among different alternatives, biofuels in general and bioethanol in particular are promising sustainable and eco-friendly energy sources. However, cheap feedstocks and new production technologies are required to make bioethanol economically comparable with traditional fossil fuels. An efficient, cost-effective, and promising technology is solid-state fermentation (SSF) in which microorganisms grow on the surface of solid materials in the absence of free water resulting in elimination of sugar extraction process and less wastewater production, which in turn yields lower distillation and purification costs. Furthermore, SSF is a well-established technology for production of different enzymes. This potential of SSF makes it an appropriate process for enzymatic pretreatment and hydrolysis of substrates and subsequent bioethanol production. This review gives an overview of the applications of SSF in every step of bioethanol production; compares its efficiency and feasibility with the submerged fermentation process; and for brevity of exposition, highlights the great promise of this technology for sustainable and cost-effective bioethanol production.

Keywords Bioethanol · Solid-state fermentation · Pretreatments · Saccharification · Enzyme production

1 Introduction

Increasing population of the world, the expansion of urban life, and industrial activities along with the change in the lifestyle of the people caused a global increase in energy consumption in the world. Continuation of this upward trend in the future indicates the need for more work on new energy sources. According to the International Energy Outlook 2019 (IEO 2019), the energy consumption in the world is expected to grow by nearly 50% by 2050 [1]. Since the turn of the twentieth century, petroleum-based liquid fossil fuels have been the main energy sources and are expected to remain so over the foreseeable future. However, petroleum-based liquid fossil fuels are not sustainable and renewable energy sources. Additionally, they always have fluctuation in their price; for instance, based on the US Energy Information Administration (EIA), average European Brent crude oil prices have soared from about $25 per barrel in 2002 to $110 per barrel in 2014 and have fallen to about $60 in 2021 [2]. In the same time span, average West Texas Intermediate

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(WTI) crude oil prices have drastically increased from about $25 per barrel in 2002 to $94 per barrel in 2012, and have fallen to about $39 in 2020 [3]. Although having fallen since 2011, oil prices are expected to recover and reach $141 per barrel in 2040 [1]. Figure 1 presents average annual spot price for West Texas Intermediate (WTI) crude oil during 2005–2020 [3].

Overall, the growing population of the world, large-scale industrialization in developing countries (e.g., China, India, Brazil), betterment of quality of life, fluctuations of oil prices, and socio-political unrests in major oil-producing countries have accentuated the need for sustainable energy sources. Biofuels, due to their advantages, are the most promising alternative [4].

Biofuels are a type of fuels whose energy is derived from biological carbon fixation via different processes such as pyrolysis, gasification, liquefaction, supercritical fluid extraction, and super critical water liquefaction and biochemical processes [4]. Since the energy of biofuels comes from carbon dioxide in the atmosphere, they can prevent greenhouse gas emissions and global warming. The energy-related carbon dioxide emission in the world is projected to rise from 32.3 billion metric tons in 2012 to 35.6 billion metric tons in 2020 and to 43.2 billion metric tons in 2040 [1]. Therefore, overcoming air pollution challenges requires production of eco-friendly fuels such as bioethanol and biodiesel.

Bioethanol, biodiesel, biohydrogen, and biogas are some of the most well-known biofuels. Bioethanol is an alcohol made by fermentation of carbohydrates produced in sugar or starch crops such as corn and sugar cane, and has been widely used as fuel, especially for transportation. Brazil has utilized bioethanol since 1925 for transportation [4] and along with the USA is the largest producer of bioethanol. The global fuel ethanol production in 2016 is presented in Table 1.

Note that competitiveness of biofuels with fossil fuels depends on oil prices. For low oil prices, only the cheapest feedstocks and cost-effective technologies can compete with gasoline. The traditional technology for bioethanol production is submerged fermentation (SmF). Another interesting technology for bioethanol production is solid-state fermentation (SSF). In SSF, microorganisms can grow on the surface or interior of the solid matrix in the absence or near-absence of free water [5]. Since there is no free water in SSF, downstream processes are easier making it cost-effective and efficient in comparison with the traditional SmF. In addition, extraction of sugar content from the substrate matrix is not required in SSF. These advantages, consequently, have made researchers use SSF for bioethanol production in recent years.

In this review, we discuss the application of SSF for bioethanol production in all aspects including pretreatment and detoxification, hydrolysis, and saccharification, fermentation, and industrialization of the whole process. The advantages of the SSF process and its economic benefits over submerged fermentation and its potential to be a cost-effective process for bioethanol production have also been discussed here for the first time. To the best of our knowledge, this is the first review on using the SSF process for production of fuel bioethanol.

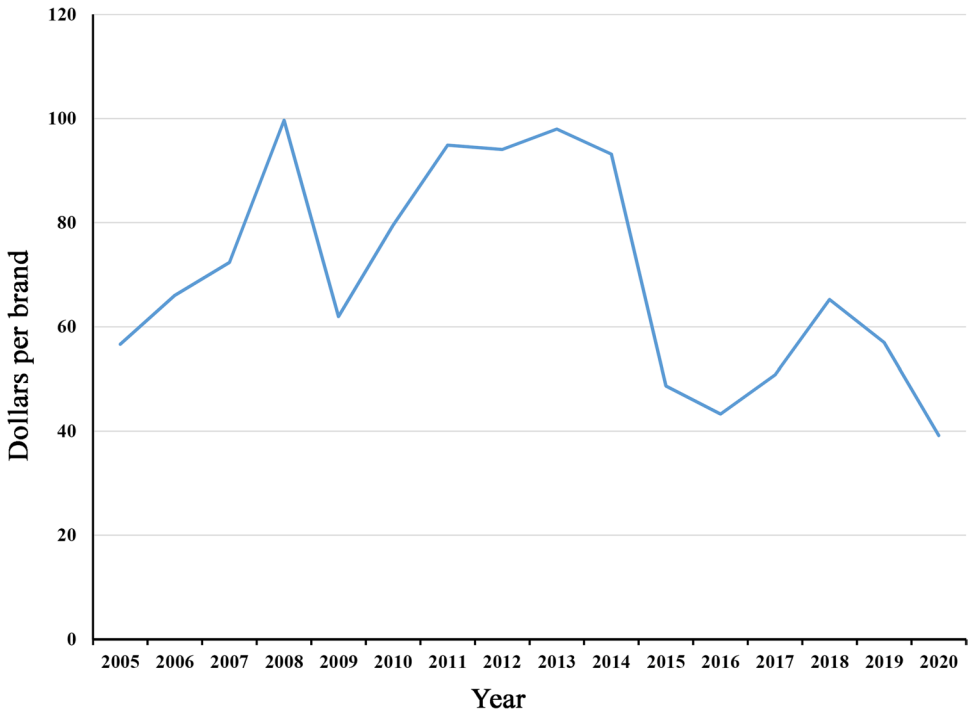

Fig. 1 Average annual spot price for West Texas Intermediate (WTI) crude oil during 2005–2020 [3]

Table 1 Global fuel ethanol production in 2016 [7]

Continent/nation	Millions of gallons
USA	15,330
Brazil	7295
European Union	1377
China	845
Canada	436
Thailand	322
Argentina	264
India	225
Rest of the world	490

The outline of the paper is as follows: in Sect. 2, bioethanol production is presented followed by general description of SSF in Sect. 3 and its application in pretreatment of substrates for bioethanol production in Sect. 4. Hydrolysis of different substrates by SSF is discussed in Sect. 5 followed by details of fermentation of substrates to ethanol in Sect. 6. The consolidated continuous solid-state fermentation (CCSSF) along with the advanced SSF is presented in Sect. 7. We concluded the article with future perspectives and concluding remarks in Sect. 8.

2 Bioethanol

Bioethanol is derived from renewable resources such as agricultural residues (wheat, corn, sugar cane, wood, etc.). In addition to renewability, the use of bioethanol can also reduce the emission of CO_2, CO, and other greenhouse gases. Since bioethanol contains only a trace amount of sulfur, blending it with petrol can reduce the emission of sulfur oxide which is a carcinogen and a main component of acid rains [6].

The blends of bioethanol and gasoline or bioethanol itself can be used as fuel for vehicles. Bioethanol is generally blended with gasoline in concentrations of 10% bioethanol to 90% gasoline known as E10, and nicknamed "gasohol." However, utilizing bioethanol as fuel has its own disadvantages including lower energy density than gasoline, corrosiveness, low flame luminosity, lower vapor pressure, miscibility with water, toxicity to ecosystems, increase in exhaust emissions of acetaldehyde, and increase in vapor pressure when blending with gasoline [4].

Fuel bioethanol is commonly classified to first, second, and third generation. First-generation biofuels are liquid fuels generally produced from sugar-containing feedstocks. Yeasts or other ethanol-producing organisms can convert sugars (six-carbon sugars like glucose or sucrose and five-carbon sugars like xylose) directly to bioethanol. First-generation biofuel production is the least complex process, and generally, hydrolysis is not required. The most important challenge for this kind of biofuel production is their conflict with food supply, as most of the feedstocks used for this purpose exist in human diet. The competition of first-generation biofuels with food leads to increase in the production cost of bioethanol and the cost of foodstuffs. The global production of first-generation bioethanol production in 2006 was about 51 billion liters, 35% of the total [6]. The limitations of this kind of biofuels encourage using non-edible resources for biofuel production.

Second-generation biofuels are generally produced from agricultural lignocellulosic biomass. The feedstock used for second-generation biofuels is generally non-edible; the limitations associated with the first-generation biofuels, therefore, can be avoided. However, since cellulose cannot be converted to ethanol directly, some hydrolysis processes are required. This makes the production of second-generation biofuels more complex than the first generation. The main steps for production of second-generation bioethanol include (i) pretreatment (in some cases followed by detoxification) in which the separation of cellulose, hemicellulose, and lignin is facilitated, and some toxic components are removed; (ii) saccharification and hydrolysis where the fermentable sugars are released from complex carbohydrates, and cellulose along with hemicellulose is hydrolyzed to simple sugars; (iii) fermentation of sugars to ethanol by suitable microorganisms; and (iv) distillation step to purify the produced ethanol.

Second-generation bioethanol can be produced by biochemical or thermochemical methods. Although most of the second-generation biofuels are produced from thermochemical methods, this process requires extreme temperature and pressures. In contrast, biochemical methods, if developed well, are cheaper and more environmentally friendly.

Lignocellulosic materials, which have been widely used for second-generation bioethanol production, are made up of cellulose, hemicellulose, and lignin. Cellulose is a crystalline long-chain polymer of glucose molecules, and it should be broken down into its constituent sugars before microorganisms can utilize it. Cellulase enzymes, capable of hydrolyzing cellulose to glucose, have been widely used for saccharification of cellulosic materials. In contrast, hemicellulose is a polymer of 5-carbon sugars and like cellulose can be broken down into simple sugars yielding 5-carbon sugars such as xylose and pentose. Fermentation of 5-carbon sugars is more difficult than fermentation of 6-carbon sugars, and suitable microorganisms, which can metabolize pentose and xylose, should be used in the process. The third component of lignocellulosic materials, lignin, is a built up of phenols which is not fermentable. In bioethanol production processes, lignin should be removed from the substrate before fermentation. The presence of lignin and hemicellulose hinders cellulase's reach to cellulose. A

decrease in cellulose crystallinity and increase in its porosity can improve the hydrolysis process [8]. These pretreatments are usually performed before the hydrolysis process. Since solid-state fermentation is an efficient process for producing enzymes, e.g., cellulase, it could be a very suitable process for second-generation bioethanol production.

Third-generation biofuels are derived from microalgae, which can accumulate higher levels of carbohydrates such as glucose, starch, and other polysaccharides. Bacteria, yeasts, and fungi can use them as a carbon source for ethanol fermentation [9]. Microalgae have good ability for CO_2 fixation and lipid production; in result, they do not compete with food crops and can be produced on arid lands. Therefore, the potential of microalgae for producing biodiesel and bioethanol has attracted researchers. Some technological developments such as photobioreactor design, microalgal biomass harvesting, drying, and processing can affect the efficiency and cost of biofuel production process from microalgae [10].

It was reported that in the case of second-generation bioethanol production, the costs of cellulase enzymes (for hydrolysis) and ethanol distillation account for 30 to 50% and 20% of the total cost, respectively [11]. Consequently, for commercial production of cellulosic bioethanol, efficient and cost-effective technologies are required. The use of solid-state fermentation, which has been recognized as a suitable technology for enzyme production, could be a promising strategy for these challenges.

3 Solid-state fermentation

Solid-state fermentation (SSF) is a kind of fermentation in which microorganisms grow on the solid materials in the absence (or near absence) of free water; sufficient moisture, however, should be available in the solid particles in order to support the growth and metabolism of the microorganisms [5, 12]. In this process, the solid materials may act as a carbon/energy source or as an inert support. This fermentation is suitable for producing metabolites such as enzymes, secondary metabolites with high yields, and also certain kinds of enzymes which cannot be produced in traditional submerged fermentation [13]. This has been attributed to the "physiology of solid medium" which microorganisms exhibit in SSF. In other words, secondary metabolites are produced in higher yields in SSF because of the higher transcription of biosynthetic genes. However, for enzyme production, some SSF-specific genes and solid medium environmental stimuli have been identified [14]. In SSF, the natural living condition of microorganisms is simulated which allows the complete gene expression in the microbes. Accordingly, these characteristics of SSF make it suitable and efficient for producing hydrolyzing enzymes in bioethanol production process.

Generally, SSF has several advantages over submerged fermentation such as cheaper substrate (usually agricultural wastes), lower energy requirements and investment cost, better volumetric yield, and less wastewater production which makes the downstream processes easier [13, 15]. However, several challenges remain in application and scaling-up of SSF processes. For example, due to low heat conductivity of solid particles and lack of free water, heat removal is difficult in SSF processes, especially in large scales [16]. Additionally, due to the solid nature of the substrates, their mixing is not effective; as a result, significant water and temperature gradients may appear in the solid bed. Because of the heterogeneous composition of solid substrate, monitoring and controlling of the process parameters such as temperature, moisture, pH, and biomass content is difficult [16, 17]. Therefore, growth of microorganisms in SSF is non-isothermal and the effects of temperature and moisture content should be considered in the model parameters for designing bioreactors [18]. Accurate growth kinetic models combined with the mass and energy balance model of the SSF bioreactors for predicting microorganisms' growth are required for using SSF in industrial scales [19]. Table 2 highlights the advantages and disadvantages of the SSF process.

Table 2 Advantages and disadvantages of SSF processes [12, 15, 20, 21]

Advantages	Disadvantages
Low water content	Scale-up difficulties
Lower reactor volume	Building of moisture and temperature gradients
High concentration of the product	Heat removal difficulties
High volume productivity	Mixing difficulties
Lower downstream costs	Difficulties in control of process parameters (like temperature, pH, moisture, nutrient and product concentrations, biomass concentration)
Lower sterility demands	Lack of accurate mathematical models
Simulation of the natural environment	Limited microorganisms
High interfacial surface area to liquid volume ration	
Lower energy requirement	
Easier aeration	
Cheap and simple substrates	
No antifoam chemicals	

Because of low-cost substrates such as agricultural wastes, SSF has gained attention for producing different products, especially in food industries, such as *koji*, *sake*, and *angkak* [22]. Recently, SSF is recognized as the best process for production of enzymes and other thermolabile products, especially when higher yields can be obtained in comparison with the submerged fermentation process [23].

In conclusion, according to the abovementioned advantages, SSF can be used as a cost-effective and efficient process for production of many products such as bioethanol. The potential of SSF for producing industrial enzymes, especially cellulase, has led to use of this process not only in the fermentation of sugars to ethanol but also in saccharification and pretreatment sections. By using this process for fermentation, the sugar extraction step is usually eliminated; in addition, the cost of distillation step is reduced (because of the lower water content in this fermentation type). Here, we discuss the application of SSF for the whole bioethanol production process in all steps (pretreatment and detoxification, hydrolysis and saccharification, and fermentation).

4 Application of SSF in pretreatment of substrates

Some substrates used for the production of bioethanol, especially lignocellulosic materials, require special treatment processes before the initiation of the main fermentation process. In some cases, removal of a component such as lignin is necessary. Moreover, some modifications should be made in the lignocellulosic materials to enhance the accessibility of hydrolyzing enzymes to the substrates and to disrupt the lignocellulosic structure. The most common and effective pretreatment method is steam explosion requiring high temperature and pressure and may result in by-products that adversely affect the subsequent steps [24]. Other physicochemical methods such as dilute acid, alkali, oxidant, or their combinations can also be used. These chemical and physical pretreatment methods require high energy (steam or electricity) and corrosion-resistant high-pressure reactors, which in turn increase the cost. In the bioethanol production process from lignocellulosic materials, one-third of the total production costs are related to the pretreatment section. Therefore, finding simple and effective methods with lower cost is important for the economic production of bioethanol from lignocellulosic feedstocks.

As an alternative method, biological pretreatments are cheaper, safer, less energy-consuming, and more environmentally friendly. In these methods, microorganisms capable of degrading lignin are used [25]. However, the main disadvantage of biological pretreatment methods is the extremely long pretreatment time [26].

In the pretreatment step, several basidiomycetes such as *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Phlebia subserialis*, and *Pleurotus ostreatus* have been used for delignification of different lignocellulosic biomass [27–29]. Additionally, white-rot fungi have good capability in delignification and increasing the accessibility of cell wall structure. For example, *P. chrysosporium*, a white-rot fungus, has been widely examined for pretreatment because of its high growth rate, exceptional oxidation potential, and efficiency for lignin biodegradation [30]. Like other fungi, white-rot fungi can also grow better and produce enzymes more efficiently in SSF. One of the examples for better performance of SSF in pretreatment process was reported in Shi et al.'s study [31]. They have investigated the ability of *Phanerochaete chrysosporium* for pretreatment of cotton stalks in both submerged fermentation and solid-state fermentation. Cotton stalks have a lignin content of 30.5% which is higher than other lignocellulosic feedstocks such as corn stover, wheat straws, switchgrass, and even softwoods; thus, accessibility to cellulose is more difficult in cotton stalks [31]. Delignification and pretreatment, consequently, are vital steps when cotton stalks are used for bioethanol production. Their results showed that better lignin degradation occurred in SSF in comparison to SmF (19.4% and 35.5% for submerged and SSF, respectively). Furthermore, SSF demonstrated a better selectivity of 0.82 in comparison with 0.7 in submerged pretreatment. Both delignification processes were performed during 14 days, but in the time span, the extent of delignification during SSF was significantly higher than SmF. It was also discovered that as high as 60% of hemicellulose was reduced after the SSF pretreatment process. The reason for better performance of fungus in SSF can be attributed to similarity of living conditions in SSF and natural habitat of the fungus [30]. Since having a multi-xylanase system and being able to utilize multi-carbon substrates, *P. chrysosporium* can consume hemicelluloses in the cotton stalks effectively [30].

Beside lignin, cell wall structures are also another constraint in improving the hydrolysis of lignocellulosic substrates. The insolubility of cellulose and hemicellulose polymers and their close association with insoluble matrix lead to ineffective degradation of plant cell wall [32]. Moreover, hydroxycinnamic acids, particularly ferulic and *p*-coumaric acid, are covalently bound to cell wall pectins and polysaccharides (arabinoxylans, xyloglucans) through ester linkages and to lignin, mainly by ether bonds, influencing cell wall properties and its biodegradability [33]. The ligninolytic system composed by lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase, laccase, and H_2O_2-producing oxidases is reported to be able to degrade and oxidize lignin and can be produced while growing of white-rot fungi [34, 35]. Lignin peroxidase and

versatile peroxidase are the only enzymes capable of oxidizing non-phenolic lignin parts in vitro. Enzymes cannot diffuse to some delignification sites because of their large molecular sizes. In such cases, the low molecular weight compounds may oxidize lignin [36]. Manganese peroxidase by generating reactive oxygen species, which are able to degrade lignin, from hydroperoxide compounds plays an important role in this case [37, 38]. Like other enzymes, these compounds also be produced more efficiently in the SSF process.

For example, in a recent study [33], four white-rot fungi (*Trametes versicolor*, *Bjerkandera adusta*, *Ganoderma applanatum*, and *Phlebia rufa*) which can produce MnP, LiP, laccase, carboxy methyl cellulase (CMCase), avicelase, xylanase, and feruloyl esterase were cultivated in the SSF process to modify the cell wall structure of wheat straw. Wheat straw, one of the most abundant residues in the world, can be utilized for bioethanol production, but the main constraint for hydrolysis of wheat straws is their complex cell wall structure. The high degradation of esterified hydroxycinnamic acids in the first 7–14 days of incubation was observed because of the xylanase and feruloyl esterase activities during this period. The esterified *p*-coumaric and ferulic acid content was reduced by the fungal treatment [33]. In another study, fungal pretreatment of wheat straw in SSF combined with mild alkali treatment showed good results with no generation of inhibitors for downstream process. Twenty-one days of pretreatment with *Poria subvermispora* and *Irpex lacteus* improved the ethanol production significantly [39].

According to literatures, the overall results of fungal pretreatment in SSF affirm this process as an effective, simple, cheap, and eco-friendly method for bioethanol production. Indeed, more investigations are required in this field for industrial applications, especially in scaling-up the process. Some researches on the application of SSF in pretreatment of different substrates are presented in Table 3.

5 Application of SSF in hydrolysis and saccharification of substrates

In the next step, the biomass hydrolysis step, the fermentable sugars are released in order to be converted to ethanol by microorganisms. The mature technology for this purpose is acid hydrolysis, which generates hazardous acidic wastes and involves difficulties in sugar recovery from acid. Another method is enzymatic hydrolysis, which is efficient without generation of hazardous wastes. For instance, the cellulase enzymes can catalyze hydrolysis of cellulose to its constituent sugars.

Cellulase enzymes are a group of enzymes including endoglucanases, exoglucanases, cellobiohydrolases, and β-glucosidases. Endoglucanases hydrolyze the cellulose polymer exposing reducing and non-reducing ends of the linear polymer of glucose units and attack regions of low crystallinity in the cellulose fiber and create free chain ends. Exoglucanases and cellobiohydrolases act on the free chain ends to release cellobiose and cellooligosaccharides while β-glucosidases cleaving cellobiose to release glucose. Since cellobiose is an end-product inhibitor of endo- and exoglucanases, β-glucosidases are important in the hydrolysis process [44]. The enzymatic hydrolysis process is dependent on substrate and glucose concentrations, enzyme activity, and reaction conditions (such as pH and temperature). Most cellulase enzymes show an optimum activity at temperatures and pH in the range of 45–55 °C and 4–5, respectively [45].

Cellulases are now the third largest industrial enzyme in the world (by dollar volume) and have the potential to become the largest volume industrial enzyme if ethanol from lignocellulosic feedstocks through enzymatic hydrolysis becomes the major fuel for transportation in the world [46].

Many microorganisms such as white-rot and soft-rot fungi, aerobic and anaerobic bacteria, and anaerobic fungi have the ability of cellulase production. Filamentous fungi like *Trichoderma*, *Penicillium*, *Fusarium*, *Humicola*, and *Phanerochaete* produce most of the cellulase for industrial

Table 3 Some applications of SSF for pretreatment of different substrates

Substrate	Methods	Reference
Wheat straw	Fungal pretreatment with mild alkali treatment	[39]
Wheat straw	White-rot fungi (*Pleurotus ostreatus*)	[26]
Wheat straw	Fungal pretreatment (Euc-1)	[40]
Wheat straw	Hot water extraction with white-rot fungal pretreatment	[25]
Corn fiber	Fungal pretreatment (*Phanerochaete chrysosporium*)	[41]
Corn stover	Fungal pretreatment (*Irpex lacteus*)	[42]
Corn stover	Fungal pretreatment (*Ceriporiopsis subvermispora*)	[25]
Water hyacinth	White-rot fungal pretreatment with diluted acid	[43]
Cotton stalks	Fungal pretreatment (*Phanerochaete chrysosporium*)*	[30]
Spruce wood	Various species of white-rot fungi	[38]

*Lignin degradation: 19.4% for SmF and 35.5% for SSF.

Table 4 Some applications of SSF for production of cellulolytic enzymes

Microorganisms	Substrates	Reference
Penicillium citrinum	Wheat bran	[52]
Aspergillus niger NRRL3	Wheat bran + corn cob	[53]
Bacillus subtilis	Banana waste	[54]
Trichoderma reesei RUT C30 and *Aspergillus niger* MTCC 7956	Wheat bran	[47]
Bacillus subtilis	Soybean industry residue	[55]
Trichoderma reesei, *Aspergillus niger*	Rice chaff + wheat bran (9:1)	[56]
Trichoderma reesei, *Aspergillus niger*	Wheat bran	[47]
Penicillium decumbans	Wheat straw + bran (8:2)	[57]
Thermoascus auranticus	Wheat straw	[58]
Trichoderma reesei ZU 02	Corn cob residue	[59]
Trichoderma reesei RUT C30	Wheat bran	[60]
Aspergillus terreus	Corn stover	[61]
Fusarium oxysporum	Corn stover	[62]
Trichoderma harzianum	Oil palm empty fruit bunches	[63]
Thermoascus aurantiacus	Wheat straw	[64]
Trichoderma reesei, *Aspergillus niger*	Rice straw + pea pods wastes + cauliflower waste + kinnow pulp	[65]
Penicillium decumbens L-06	Bagasse	[66]
Aspergillus ellipticus	Distillery spent wash + wheat bran	[67]
Penicillium decumbens Mutant	Rice bran	[68]
Trichoderma reesei RUT C30	Sugar cane bagasse	[69]
Aspergillus terreus	Rice straw	[70]
Trichoderma reesei	Kinnow pulp + wheat bran	[71]
Fomitopsis sp. RCK2010	Wheat bran + urea	[72]
Neurospora crassa	Wheat bran + wheat straw	[73]
Irpex lacteus CD2	Corn stover	[42]
Fusarium oxysporum	Brewer's spent grain + corn cob	[74]
Aspergillus oryzae and *Aspergillus awamori*	White waste bread	[75]
Trichoderma reesei RUT C30 and *Aspergillus niger* MTCC 7956	Water hyacinth	[76]

applications. These filamentous fungi produce cellulase complexes with all the three classes of enzymes at different proportions needed for the complete hydrolysis of cellulose [46]. However, most of the commercial cellulases are produced from *Trichoderma reesei* and *Aspergillus niger*. Table 4 presents some of the microorganisms used for production of cellulase in SSF.

The commercial production of ethanol from lignocellulosic materials is highly dependent to the cost of cellulase production. To reduce the cost, cheap raw materials and cost-effective technologies like SSF should be used [47]. It was estimated that cellulase production cost by submerged fermentation accounts for almost 20% of the total production cost of bioethanol, whereas, the cost of enzyme production by SSF corresponds to 8% of total costs [48]. In another study, it was observed that cellulase production in SSF resulted in about a tenfold reduction in the cost of production compared to SmF [49].

Additionally, as mentioned before, SSF is a very efficient process for production of such enzymes with high yield and it was widely used in many studies for this purpose. As proof, the list of some cellulases produced in the SSF process is provided in Table 4. Furthermore, in comparison with SmF, SSF is simpler, low-grade lignocellulosic substrate can be utilized in it, the risk of contamination is lower [50], and a higher yield of cellulase can be achieved [51]. The stability of produced cellulase in SSF with respect to temperature, pH, metal ions, and alkali has also been reported to be remarkable [46, 52]. Although cellulolytic fungi can produce cellulase enzymes in both submerged and SSF, it is believed that with appropriate technology, better bioreactor design, and improvements in the control of operational parameters, SSF may become a commercial process for industrial cellulase production.

5.1 Review of some studies on cellulase production in the SSF process

Lever et al. produced cellulase by SSF in the way that they could remove some steps of the process and reported the

possibility of producing a crude unprocessed cellulase extract by SSF. They produced the enzyme in SSF by using fermented substrates (like *koji*) or by mixing the fermented substrate with water and making a liquid extract. Using this crude extract can eliminate several steps in cellulase production such as purification, concentration, addition of buffers, stabilizers and preservatives, freeze-drying, and packaging. Ground wheat straw and *Trichoderma reesei* were used to produce the crude unprocessed cellulase extracts. Afterwards, the diluted crude cellulase extract obtained from SSF was used in the simultaneous saccharification and fermentation of ground wheat straw to ethanol. The results suggested that a crude unprocessed cellulase extract produced at the site of ethanol production may be used instead of commercial preparations [50].

In another study, SSF was found to be capable of producing cellulase enzyme being able to convert the biomass to reducing sugars with the yield of 85%. Crude cellulase and a relatively glucose-tolerant β-glucosidase were produced from wheat bran, by *Trichoderma reesei* and *Aspergillus niger* in SSF, respectively. The hydrolysate produced in this study did not contain inhibitors for the subsequent ethanol fermentation step [47]. The results of another study also showed the mixtures of enzyme solutions produced by SSF of *Aspergillus oryzae* and *Aspergillus awamori* could increase the hydrolysis of major wheat components (starch, protein, and sources of phosphorus) into glucose, free amino nitrogen, and free phosphorus, respectively [75].

Mutant strains, in some cases, may result in better enzyme production in SSF process. Cellulase production from bagasse using *Penicillium decumbens* L-06 strain in the SSF process showed the maximum cellulase production of 3.9 FPu g^{-1} under optimized conditions [66], while a mutant strain of *Penicillium decumbens* ML-017 in SSF of rice bran showed more efficient cellulase production. The maximum cellulase production obtained under the optimized condition using the mutant strain was reported to be 5.7 IU g^{-1} which is 44.1% higher than that of the original strain [68].

In addition to using mutants, some inducers can also be used to increase the production of cellulase in SSF. For example, it was known that cellobiose is an inducer at low concentrations for the cellulase system of *T. reesei* [77], and SSF of sugar cane bagasse by *T. reesei* for cellulase production demonstrated that the addition of a crude mixture of inducers can highly improve the cellulase production [69].

Tray bioreactors are one of the traditional reactors in SSF processes and are widely used in SSF for production of many metabolites, even in large scales. Tray solid-state fermentation of low-cost agricultural wastes (rice straw, pea pod wastes, cauliflower waste, kinnow pulp) using *A. niger* and *T. reesei* showed cheap and feasible enzyme production. The mixed cultures of *A. niger* and *T. reesei* produced higher amounts of extracellular enzymes than either of the monocultures. Higher enzyme activity was obtained when rice straw–supplemented wheat bran in the ratio of 3:2 was used as substrates, in comparison with the activity obtained when rice straw was used alone [65]. By optimization of media, provision of aeration during static tray SSF, and controlled conditions during SSF in trays, the higher enzyme activities can be produced. It was concluded that SSF is an efficient and valuable process for complete production of the cellulolytic enzyme system with balanced activities, capable of hydrolyzing complex biomass [65].

Prévot et al. conducted another study that can prove better performance of SSF for enzyme production than SmF. Results of their study showed a significant difference in the laboratory enzymatic complex produced by SSF compared to SmF [78]. This observation exhibits a greater efficiency of cellobiohydrolase on cellulose and better conversion capacity on wheat bran, probably due to the presence of side activities. A comparative economic analysis of the entire process, from biocatalyst production to their use and between the crude unprocessed SSF complex and the best enzymatic complexes produced by SmF proved that the SSF is a promising technology to overcome the biomass recalcitrance and lower the cost of the conversion step [78].

Using cheap raw materials can strongly decrease the cost of enzyme production, and many such materials can be used in SSF. For example, using distillery spent wash (the residual liquid waste generated during alcohol production) for production of high-value compounds can prevent the pollution caused by this waste. Cellulase production using anaerobically treated distillery spent wash with *Aspergillus ellipticus* and wheat bran as substrate under SSF resulted in production of β-glucosidase and endo-β-1,4-glucanase with the activities of 26.7 and 130.9 U per g of substrate, respectively [67]. Cellulase produced under SSF of rice straw with *Aspergillus terreus* was reported to cause 74.2% efficiency in generating fermentable sugars from rice straw, which can be further used for ethanol production [70]. Another ideal substrate for cellulase production is kinnow pulp, which does not have any significant commercial use. The mixture of dried kinnow pulp and wheat bran as substrate, and *Trichoderma reesei* as the producing microorganism, was used for cellulase production in the SSF process. It was observed that the ratio of 3:2 kinnow pulp to wheat bran without any mineral solutions resulted in an optimum cellulase and β-glucosidase ratio which is ideal for saccharification of lignocellulosic biomass [71]. Brewer's spent grain, the most abundant brewing by-product, can also be used as a cheap feedstock for ethanol production. Brewer's grain has high pentose content; therefore, microorganisms capable of fermenting xyloses such as *Fusarium oxysporum* can be used for this substrate. The mixture of brewer's spent grain and corn cobs in a ratio of 7:3 could be a suitable substrate for simultaneous production of cellulolytic and hemicellulolytic

enzymes under the SSF process by the use of the mesophilic fungus *Fusarium oxysporum* [74].

Instead of the conventional cellulase-producing fungi, a newly isolated brown-rot fungus, *Fomitopsis* sp. RCK2010, was used for enhanced cellulase production under the SSF process. It was found that wheat bran as a carbon source and urea as a nitrogen source are the best conditions for cellulase production with this strain. Hydrolysis of wheat bran and rice straw with cellulase produced by *Fomitopsis* sp. RCK2010 resulted in release of 157.16 and 214.04 mg per g of reducing sugar, respectively [72]. The ability of mesophilic fungus *Neurospora crassa* for production of cellulolytic and hemicellulolytic system was also determined in SSF. Using the mixture of wheat bran and wheat straw as carbon sources in this process resulted in the highest endoglucanase, β-glucosidase, and β-xylosidase activities ever reported for ethanol-producing fungi. The produced cellulolytic enzyme in this system was used for saccharification of sweet sorghum bagasse which showed good ability for releasing fermentable sugars [73]. Among the 140 strains of wild white-rot fungi, *Irpex lacteus* CD2 displayed the ability of enzymatic hydrolysis of corn stover in the SSF process [42].

6 Application of SSF in fermentation of sugars to ethanol

The main step in the production of bioethanol from biomass is the fermentation step, in which fermentable sugars are converted to ethanol by appropriate microorganisms. These fermentable sugars may exist in the substrate initially (such as sweet sorghum, carob, grape and sugar beet, sugar cane) or be generated during the hydrolysis step from lignocellulosic feedstocks. Substrate cost makes up to 55–75% of the final cost of the produced ethanol [79]. Therefore, using cheap and abundant feedstocks as substrate is an important factor. In addition, choosing the suitable microorganisms capable of metabolizing the sugars should be done according to the sugar profile of the substrate.

To reduce the cost and time of the whole process, simultaneous saccharification and fermentation can be used instead of the traditional saccharification and subsequent fermentation. In simultaneous saccharification and fermentation process, the yield of ethanol is increased by minimizing product inhibition, where the need for separate saccharification and fermentation reactors is eliminated and the sugars produced in saccharification can be rapidly utilized by the microorganisms [80, 81]. Kádár et al. studied the use of simultaneous saccharification and fermentation for bioethanol production from some lignocellulosic substrates like Solka Floc, old corrugated cardboard wastes, and paper sludge. By employing *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, they reported the ethanol yield in the range of 0.31–0.34 g/g for both strains [80]. Canabarro et al. evaluated the production of ethanol by solid-state saccharification and fermentation of rice bran. The hydrolyzing enzymes of amylase and cellulase along with *Saccharomyces cerevisiae* cells were simultaneously added to the solid substrate. They also employed a packed bed column for scaling-up the process (10 times scaling-up from Erlenmeyer to packed bed bioreactor). The ethanol yield of 138.7 g kg^{-1} solid was obtained from this process [82]. Their results demonstrated that it is possible to perform the whole process of simultaneous saccharification and fermentation in solid-state process and obtain comparable yield with liquid fermentation.

The organic fraction of municipal solid waste is promising biomass for bioethanol production. The increase in the production of such organic wastes in the world and the increasing demand of bioethanol attract the researchers to study this type of substrate. The organic fraction of municipal wastes has large amounts of fruit and vegetable residues (about 70%) which can be easily fermented to bioethanol [83]. For further releasing of fermentable sugars, pre-treatment process is required to break down the fiber structures. For economic production of bioethanol from this substrate, it is required that both hexoses and pentoses in the hydrolysates are utilized and converted to ethanol. Estrada-Martínez et al. used a mild thermal pretreatment method for allowing bioethanol production from this type of solid materials. Then, mixed cultures of *S. cerevisiae*, *Scheffersomyces stipitis*, and *Schwanniomyces occidentalis* were used in solid-state fermentation for bioethanol production. The maximum ethanol concentration of 282.6 ± 13.1 l ethanol per ton of dry matter was reported [83].

To convert the sugars of agricultural products to ethanol in liquid fermentation, an extraction step is required for leaching the sugars from solid particles. Note that this process is time and energy consuming and can affect the final cost of the produced ethanol. One of the most important advantages of SSF is that the extraction step is not required and microorganisms can grow and metabolize the sugars on the surface of the solid particles. Apart from the characteristic benefits of SSF, such as high concentration of product, reduction in distillation plant investment, lower energy and operating costs, less water requirement (which is crucial in regions with water shortage), and less capital investment and pollution problems, the elimination of the extraction step can also help the SSF process to become an efficient and time/energy/cost-effective process for bioethanol production. Table 5 summarizes recent applications of different substrates for bioethanol production via SSF and SmF, types of fermentation, microorganisms involved, and corresponding results.

Table 5 Some of the substrates and microorganisms used in both SSF and SmF processes for bioethanol production and comparison of the results

Substrate	Type of fermentation	Microorganism	Results	Reference
Apple pomaces	SSF	*S. cerevisiae*	Ethanol yield of 29–40 g per kg of apple pomaces	[84]
Arrowroot	SSF	Angel® thermal-tolerant alcohol active dry yeast	Ethanol yield of more than 0.28 ton per 1 ton feedstock	[79]
Grape pomaces	SSF	*S. cerevisiae*	Theoretical ethanol yield of more than 80%	[85]
Grape pomaces	SSF	*S. cerevisiae*	0.42 g of ethanol produced/grams of sugar consumed	[86]
Sugar beet pomaces	SSF	*S. cerevisiae*	0.70 g of ethanol produced/grams of sugar consumed	[86]
Sugar beets	SSF	*Z. mobilis*	Theoretical ethanol yield of 95%	[87]
Sugar beet juice	SmF	*S. cerevisiae*	0.4 g of ethanol produced/grams of sugar consumed	[86]
Mahula flowers	SmF	Free *S. cerevisiae*		
Immobilized *S. cerevisiae*	Ethanol yield of 193 g/kg			
Ethanol yield of 205 g/kg	[88]			
Mahula flowers	SSF	*S. cerevisiae*	Ethanol yield of 58.4 g/100 g sugar consumed	[89]
Sweet sorghum	SSF	Thermotolerant baker yeast	Ethanol yield of 7.9 g per 100 g fresh stalks	[90]
Sweet sorghum	SSF in rotary drum	Ethanol-tolerant yeast	9.6 g EtOH/100 g mash	[91]
Sweet sorghum	Deep bed SSF	*Issatchenkia orientalis* IPE 100	Ethanol yield of 0.25 g ethanol/g dry stalk	[92]
Sweet sorghum juice and sorghum grain	SmF	*S. cerevisiae* N96	Ethanol level of 16.8% (v:v)	[93]
Sweet sorghum juice	SmF	Immobilized *S. cerevisiae*	Ethanol average yield of 84.8%	[94]
Carob pod	SmF	*S. cerevisiae*	Ethanol concentration of 75 g/l	[95]
Carob pod	SmF	Immobilized *S. cerevisiae*	Theoretical ethanol yield of 58.8%	[96]
Carob pod	SmF	*S. cerevisiae*	Ethanol concentration of 95 g/l	[97]
Carob pod	SmF	*S. cerevisiae*	Ethanol concentration of 44.5%	[98]
Carob pod	SmF	*Z. mobilis*	0.34 g ethanol g^{-1} of initial sugars	[99]
Carob pod	SSF	*S. cerevisiae*	160 g ethanol kg^{-1} dry pods	[100]
Carob pod	SSF	*Z. mobilis*	Ethanol yield of 0.30 g ethanol g^{-1} initial sugar	[101]
Cassava flour	Sequential SSF and SmF	*A. awamori* and *S. cerevisiae*	Ethanol yield of 0.31 g ethanol g^{-1} cassava flour	[102]
Liquefied cassava	SmF	Co-immobilized *S. diastaticus* and *Z. mobilis*	Ethanol concentration of 53.5 g/l	[103]
Potato peel	SmF	*Z. mobilis*	Ethanol concentration of 23.3 g/l	[104]
Pomegranate peel	SmF	*S. cerevisiae*	Ethanol concentration of 12.9 g/l	[81]

6.1 Grape and sugar beet

Grape pomaces, the residue from musts and wine elaboration, and sugar beet are suggested to be appropriate materials for bioethanol production by direct fermentation without previous hydrolysis. Direct fermentation processes for ethanol production have lower production costs compared to processes that use starch or cellulose as raw materials. Rodriguez et al. [86] used sugar beet and grape pomaces for bioethanol production under the SSF process by *Saccharomyces cerevisiae* and reported an ethanol yield of 82%. They also examined the ethanol production from sugar beet juice in liquid fermentation using the same strain. The results of ethanol production yield in SSF of grape and sugar beet pomaces were more than those in liquid fermentation. In this process, lower waste mass and more concentrated ethanol were produced in SSF, which made the ethanol recovery less expensive. These results show the great promise of SSF for ethanol production and encourage further studies in this regard [86]. The theoretical ethanol yield of more than

80% was also obtained from grape pomaces by Hang et al. in SSF [85].

The nature and amount of by-product formation during SSF and SmF could also be different. During the fermentation of sugar beet to ethanol by *Zymomonas mobilis* in both SSF and SmF processes, it was found that *Z. mobilis* formed fewer by-products in SSF than in the SmF process. In the SmF process, the bacterium converted more than 19% of the utilized sugars to by-products, while in the SSF process, this value was only 8%. Final ethanol concentration of 142 g l^{-1} was reportedly obtained in SSF of sugar beet by *Z. mobilis* [87].

6.2 Sweet sorghum

Sweet sorghum belongs to the genus *Sorghum bicolor* L. Moench which also includes grain and fiber sorghum. It is a C4 crop in the grass family and has a high photosynthetic efficiency. Sweet sorghum is one of the most drought-resistant agricultural crops and can grow to a height of 120–400 cm, depending on the condition [90]. According to its sugar content, this crop could be an interesting raw material for ethanol production without hydrolysis process.

There are some studies on bioethanol production from sorghum juice in SmF [93, 105]. In SSF, by using an ethanol-tolerant yeast, Kargi et al. [106] investigated the production of ethanol from sweet sorghum. Their successful results in static flasks motivated them to continue the study of this system in a rotating drum SSF bioreactor. The rate of ethanol formation decreased with increasing rotational speed of drum. They reported the ethanol production of 9.6 g ethanol per 100 g mash at 1 rpm [91]. The potential of a thermotolerant mutant strain of baker yeast AF37X for bioethanol production from sweet sorghum was evaluated in the SSF process. With the reducing agent of 30 mg H_2SO_3 per 100 g fresh sorghum stalks, the maximum ethanol yield was 7.9 g ethanol per 100 g fresh stalks or 0.46 g ethanol per g total sugar after 40 ± 2 h, which is 91% of the theoretical yield [90]. Adding sulfurous acid can help in providing anaerobic conditions to enhance the ethanol fermentation. Apart from *S. cerevisiae*, another microorganism, *Issatchenkia orientalis* IPE 100, exhibited the ability to produce ethanol from sweet sorghum in the SSF process [92]. After scaling-up this SSF process from flasks to a 10-l bioreactor, temperature gradient in the substrate bed was observed due to heat accumulation in the bioreactor. The temperature gradient was dependent on both substrate depth and operation temperature. However, the IPE 100 strain used in this process was thermotolerant and could tolerate this condition in the bioreactor. The highest ethanol yield of 0.25 g ethanol per g dry stalk was obtained at 37 °C with 15–20-cm substrate depth in the bioreactor. These results indicate the potential of SSF for ethanol production even in large-scale bioreactors [92].

6.3 Arrowroot

Canna edulis Ker is an agricultural product in subtropical highlands. It contains 12–19% starch and is now used in local starch industries and could be a potential substrate for bioethanol production [79]. Wu et al. reported the production of 10.1 (%, v/v) of ethanol concentration by using 40 g corn cob and 10 g rice bran per 100 g arrowroot powder. The simultaneous saccharification-fermentation process was used for this purpose and no shortage of fermentable sugars was observed during the SSF process. They also reported that the whole process time was reduced by using the simultaneous saccharification-fermentation process and the energy required for saccharification decreased by performing the saccharification at lower temperatures. Using solid-state saccharification and fermentation, no wastewater was produced in the process. The yield of ethanol produced in this process was more than 0.28 ton per ton of feedstock [79].

6.4 Mahula flowers

Mahula (*Madhuca latifolia* L.) is a tropical forest tree, which can be found in tropical rain forests of Asia and Australia. Its flower is a cheap source for ethanol fermentation and tribal people in India and Pakistan have used it for production of alcoholic beverages [88]. Mahula trees contain high fermentable sugar ranging from 28.1 to 36.3 g per 100 g [88], which can be directly fermented to ethanol without hydrolysis [107]. However, it was not commercialized due to difficulties in collection, storage, and marketing. Ethanol production from mahula flowers was studied using *S. cerevisiae* in both SmF (using free and immobilized yeast) [88] and SSF processes [89]. The results showed that maximum ethanol concentration in SSF (225 ± 4 g per kg flower) was obtained at 72 h, while the same concentration was achieved in SmF after 96 h [89], which prove the better performance of SSF.

6.5 Carob pods

Carob (*Ceratonia siliqua* L.) is an evergreen shrub or tree native to the Mediterranean area, southwest Asia, and many areas of North America whose drought resistance enables it to grow in dry lands. Carob is really a very attractive crop for bioethanol production due to its high content of fermentable sugars (about 50% w/w). Analysis of some Turkish carob pods yielded 102–115 g kg^{-1} of fructose, 33.0–36.8 of glucose, and 299–384 of sucrose [108].

Due to its low cost and high content of easy fermentable sugars, carob has attracted many researchers to study the production of bioethanol in both SSF and SmF processes. Roukas has published some papers regarding bioethanol production from carob pods [95, 96, 109, 110]. Recently, the global process of ethanol production from carob pod by *S. cerevisiae* was also studied by Sánchez et al. [97], where a maximum of 95 g l^{-1} of ethanol was obtained after 24 h. *Zymomonas mobilis*, another candidate for efficient ethanol production, can be a suitable organism for fermentation of carob pod. *Z. mobilis* is a Gram-negative, facultative anaerobic bacterium, which can assimilate the sugars in carob pods (glucose, fructose, and sucrose). For this reason, Vaheed et al. [99] used this bacterium for bioethanol production from carob extract in SmF process and reported the production of 0.34 g ethanol per g initial sugars. In all of these processes, a time- and energy-consuming extraction process is required for preparing sugar extract for ethanol fermentation.

The results of SSF of carob pods also indicated the good potential of using this crop for bioethanol production. Cultivation of *S. cerevisiae* in SSF using carob pods as a substrate for ethanol production resulted in maximum ethanol production of 160 g kg^{-1} dry pods [100]. The results also revealed that the same ethanol concentration, productivity, yield, biomass concentration, and fermentation efficiency (sugars consumed during fermentation divided by the initial sugars) were obtained in sterilized and non-sterilized medium, indicating that this process can be done under non-sterilized conditions in SSF where equipment and energy can be saved [100]. This is another advantage of the SSF process, which is less sensitive to contamination.

The good potential of *Z. mobilis* for converting the carob sugars to ethanol and the advantages of the SSF process make the SSF of carob pod by *Z. mobilis* an interesting process. Recently, a maximum of 0.3 g ethanol per g initial sugar was produced by SSF of carob particles and wheat bran using *Z. mobilis* [101]. Mazaheri et al. reported that *Z. mobilis* could not grow when carob alone was used as a solid substrate. This may be attributed to the high sugar content of carob pods, especially in the liquid film-coated carob particles, which inhibits bacterial growth and ethanol production. Therefore, a mixture of carob pods and wheat bran (as a support for bacterial growth) was used as solid substrate [101]. They also performed this process in a packed bed column, and realized that the entrapped CO_2 in the bed inhibits the production of ethanol. To overcome this problem, they suggest an intermittent aeration method to remove the entrapped CO_2 from the solid bed and simultaneously maintain the anaerobic condition in the bed. It was found that low rate aeration for 15 min each hour in the packed bed column during the exponential growth phase can result in the maximum amount of bioethanol production [111].

In general, using carob pod in the SSF process does not require any pretreatment, hydrolysis, or sugar extraction process, which can strongly affect the final cost of ethanol production.

6.6 Cassava

Cassava (*Manihot esculenta*), a woody shrub native to South America, is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Cassava is a starchy raw material (contains about 70% starch) and requires hydrolysis before the ethanol fermentation.

A sequential solid-state and submerged fermentation was used for ethanol production from cassava flour, and the process was reported to be suitable in rural areas, where cassava farms are located [102]. In this process, cassava flour was first converted to *koji* using *Aspergillus awamori* in the SSF process for 2 days. During the SSF process, hydrolytic enzymes were produced. Subsequently, water and yeast pre-culture (*S. cerevisiae*) were added to produce ethanol in the SmF process (simultaneous saccharification and fermentation). It was observed that the ethanol concentration obtained with *koji* prepared with cassava flour alone was very low, but after adding appropriate amounts of rice bran, the solid texture was improved and resulted in better mixing and aeration. The process yielded a maximum production of 0.44 g ethanol per g cassava starch. The use of cassava for *koji* preparation in the SSF process is particularly important, because the same cassava *koji* is directly fermented to ethanol, and there is no need for additional enzymes, thereby reducing the cost of production [102].

7 Advanced SSF processes for bioethanol production

The price of bioethanol still cannot compete with the traditional petroleum-based fuels. Aside from the use of cheap or low-value feedstocks, some modifications should be performed in the process and technology of the production. The reduction of energy requirement, for example, is an important factor for reducing the ethanol cost.

The whole process of ethanol production includes delignification, saccharification, fermentation, recovery, and final purification of ethanol. Any technology that can remove one of these steps or integrate some steps can strongly affect the production cost. The total energy consumption in the process is not only for the fermentation step but also for transportation of biomass and wastewater treatment. In addition, nitrogen, phosphorus, potassium, and other elements in wastewater and solid wastes must be recycled in the land where the biomass is harvested [112]. In liquid fermentation, due to the high content of water in fermentation, a large amount of energy is required for treatment and recycling. The SSF is an efficient

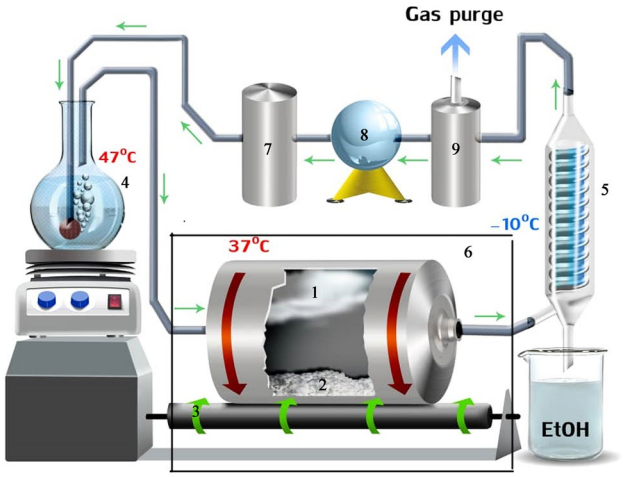

Fig. 2 Schematic representation of the CCSSF system: 1, rotating drum reactor; 2, mixture of biomass, saccharifying enzymes, and yeast; 3, rotor; 4, humidifier; 5, condenser; 6, air incubator; 7, mass flow control valve; 8, pump; 9, gas purge [112]

and cost-effective alternative for reducing the water content and energy requirement. However, controlling the concentration of sugar and ethanol in SSF is challenging, as the activity of yeast may adversely be affected by these factors.

Recently, a new alternative technology based on SSF was developed by Moukamnerd et al. to maintain yeast activity and decrease the amount of wastewater, the number of process steps, and the energy input [112]. In this system, a consolidated continuous solid-state fermentation (CCSSF) composed of a rotating drum reactor, a humidifier, and a condenser was developed by combination of simultaneous saccharification and fermentation with continuous recovery of ethanol in solid-state fermentation. A schematic diagram of this system is depicted in Fig. 2. Biomass, saccharifying enzymes, and yeast were mixed in the rotary drum, where simultaneous saccharification and fermentation occurred. In this way, saccharification and fermentation steps were consolidated and performed in one reactor. During the SSF process, the produced ethanol may inhibit the activity of yeast and even the saccharifying enzymes, and since the concentration of the product is higher in SSF processes than that in liquid fermentation, the inhibitory effects of ethanol become very serious. In this system, the produced ethanol was continuously recovered as vapor from the headspace of the reactor and condensed in the humidifier. A pump performed the circulation of the headspace gas in the reactor to the condenser (which enables the continuous recovery of ethanol). In most SSF processes, the product was extracted from the solid substrate by adding water, which makes the downstream process difficult. This method of ethanol recovery is also an energy-saving method, which removes the ethanol extraction step. Using raw corn starch as a substrate, ethanol solutions with maximum concentration of 509 ± 64 g l^{-1} were recovered continuously [112]. By using this new system, the cost and energy for wastewater treatment can be reduced and the activity of yeast and enzymes can be preserved.

Like other fermentation processes, bacterial contamination is a challenging issue in bioethanol production systems. Lactic acid bacteria such as *Lactobacillus plantarum*, *L. paracasei*, and *L. fermentum* have been reported to be the major contaminants of ethanol fermentations [113, 114]. The conventional methods of adding antibiotics and antiseptics for repressing the contamination are costly and not environmentally friendly. Katakura et al. [115] observed that the addition of exogenous ethanol to the fermentation mixture at the start of the fermentation can prevent microbial contamination and reported that the ethanol yield in the CCSSF system can reach 0.5 g g^{-1}. In this way, the contamination can be avoided without the additional cost and damage to the environment. These results combined with the CCSSF system encourage the use of the SSF process as a cost-effective and eco-friendly process for ethanol production in the future. However, the lack of engineering data, accurate mathematical models, knowledge on the scale-up of the SSF process, and difficulties in the design of suitable bioreactors have hindered the use of SSF in large scales.

However, some engineering efforts and robust bioreactor designs have emerged for large-scale use of SSF. For example, to overcome the heat accumulations in the solid bed, a pilot-scale Zymotis bioreactor was recently developed [116] in which cooling plates help remove generated heat from the solid bed. The short space between the cooling plates and suitable materials can improve the heat conduction in the bioreactor. Zymotis is a suitable SSF bioreactor, which minimizes the problems of heat removal from the solid bed and has an excellent potential for use in industrial scale. Although the Zymotis bioreactor has not yet been used for bioethanol production, the successful results of this bioreactor in removing the generated heat may nominate the Zymotis bioreactor for production of bioethanol in the SSF process in large scales.

In the last century, there was a special effort for the production of fuel ethanol and protein feed from fodder beets, reaching a farm scale in an SSF semi-continuous process [117–119]. It was reported that in conventional submerged fermentation of fodder beets, mixing problems (resulted from the high viscosity of the medium) prevented the better extraction of sugars [117]. So, the maximum ethanol concentration in the medium was limited which increases the cost of distillation. To solve the problem, the SSF process (continuous) was employed and ethanol yield of 87 l/metric ton was achieved [117]. The production costs of this SSF process was $0.47/l. As reported in this research, by using the SSF process, fodder beets are competitive with corn for fuel ethanol production. In this regard, the semi-continuous diffusion fermenter was used to produced bioethanol and cubed protein feed from fodder beets [119]. In this system, the cubes of fodder beets were

augered diagonally upward against a flow of H_2SO_4 and yeast in a tubular bioreactor. Protein feed exited from one end of the bioreactor and ethanol from the other end. For a scaled-up conceptual version of this system, production costs were calculated to be \$0.529/l for 95% ethanol [119].

In addition to heat removal difficulties in SSF bioreactors, challenging agitation of solid substrate, resulting in physicochemical heterogeneity in the solid bed, is another major disadvantage of the SSF process. To overcome these drawbacks, a new technology was developed by Li et al. using a rotary drum bioreactor [120]. In this study, a process design for bioethanol production was performed based on advanced solid-state fermentation technology. The main considerable features of this advanced process are the rotary drum bioreactor and the distiller. The rotary drum is a continuous SSF bioreactor, which rotates at a speed of 0.02–0.25 rpm to homogeneously mix the solid substrate and drive them forward from inlet to the outlet of the fermenter. The novel distiller in this technology allows the recovery of ethanol without adding water to the substrate. The continuous steam distiller has ten layers of fixed tray and rotating baffles. Each tray has a fan-shaped hole on the radial direction, which allows the fermented substrate to pass through from the upper tray to the lower tray. When the fermented substrate is fed onto the top of the distiller, it falls on one side of the hole and then moves around to the other side without falling into the hole directly because of the rotating baffles with speed of 0.2–1.1 rpm. Heat and mass exchange begins when the fermented substrate moves around the surface of the tray and encounters the steam coming from the bottom of the distiller. Afterwards, the substrate moves to the other side of the hole and then begins to fall into the next tray. The entire process is then repeated. Using sweet sorghum as the substrate, ethanol yield during continuous fermentation is reported to be 90.46% of the theoretical yield. The cost of fuel ethanol production using this technology is \$615.4 per ton [120]. The results indicate the great potential of advanced SSF technology for bioethanol production. Some problems such as heat transfer control still exist in the system and for commercialization, the cooling system must be improved and fermentation parameters should be optimized for better ethanol yields.

8 Perspectives and concluding remarks

Due to the world's growing need for more energy sources and the non-renewability of fossil fuel resources, it seems that the future world should look for new energy sources to maintain global energy security. Also, due to severe environmental problems such as air pollution, water shortages, and global warming, it seems that bioethanol as an environmentally friendly energy source can play an important role in the future energy of the world. However, fossil fuels will remain a serious competitor to this type of energy. Therefore, by creating new technologies and using low-cost methods, the production volume should be increased and at the same time the production cost of this type of energy should be reduced.

Based on the results of studies on SSF, this process has a promising potential for use in all steps of bioethanol production. The ability of this process for enzyme production makes SSF an appropriate process for pretreatment and hydrolysis of substrates for bioethanol production. Since the cost of hydrolytic enzyme production can strongly affect the final cost of ethanol, using efficient processes such as SSF may reduce the final cost. In addition, less water is required in SSF in comparison with SmF, which reduces the cost of distillation and purification steps and wastewater production. Another special feature of SSF for bioethanol production is elimination of the sugar extraction process, and less contamination risks. These advantages along with the high yield and concentration of product, lower energy consumptions, and using cheap and available substrates make SSF a cost-effective and efficient technology for bioethanol production in the future.

For industrial scale applications of SSF, some modifications should be made, especially, in the bioreactor design, mathematical modeling, and controlling the process parameters. Using advanced bioreactor designs such as Zymotis, rotary drum, and CCSSF system may solve some of the technical problems of the SSF system, especially the heat accumulation and mixing the solid matrix. More investigations are required in modeling, design, and other engineering aspects of SSF before it can be used for economic production of bioethanol.

Abbreviations SSF: Solid-state fermentation; SmF: Submerged fermentation; IEO: International Energy Outlook; EIA: Energy Information Administration; WTI: West Texas Intermediate; CCSSF: Consolidated continuous solid-state fermentation; LiP: Lignin peroxidase; MnP: Manganese peroxidase; CMCase: Carboxy methyl cellulase; FPu: Filter paper unit; IU: International unit

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