

## Solid State Fermentation: Comprehensive Tool for Utilization of Lignocellulosic through Biotechnology

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### Abstract

Lignocellulosics are widely available natural products which are the tremendous source for the production of enzymes being used for the numerous applications in food, feed, paper, textile and agro-biotechnological industries, ethanol production, bioremediation processes and many more. Enzyme productions from microorganisms are stimulated aggressively through solid state fermentation which meets the demand of getting rid of agro-industrial waste and strengthen the consumption of renewable resources through biotechnology. Though well-developed techniques for enzyme production by submerged fermentation has been found very successful at the industrial sectors; solid state fermentation helps to overcome to the issues of production cost and high yield. Additionally the availability of the substrate with very much economical rate can compensate the overall economic expenses which promote the application of solid state fermentation at industrial level. However several reports regarding fermentation techniques and their pre-treatments are available, the present review will discuss about utilization of lignocellulosics through solid state fermentation for production of enzymes and their enhanced applications in different sectors in recent years.

**Keywords:** Solid state fermentation; Lignocellulosics; Enzymes

### Introduction

Our biosphere is under the constant threat due to intense consumption of natural resources and selfish interest of mankind, which is consequently leading towards depletion of the environment. The continuous exploitation of nature to meet the demands of increasing population and greed is the major reason for the rapid industrialization. These developments are directly linked to the environmental issues global. In the developing countries where there is a race to become fully developed and economically stronger, constantly utilizing the natural resources and disposing various pollutants directly or indirectly in the atmosphere. The major challenge of maintaining the nature and persisting against the shortage of necessary resources made it obligatory to look for the possible solutions/alternatives to non-renewable resources and harmful chemicals being used widely to save the environment. One of such proposed, realistic and extensively developed alternative is the biological approach for consumption of renewable resources like lignocellulosic and its utilization through biotechnology.

Ligno-cellulosic biomass is widely available as the residue from the agricultural, forestry and alimentary industries whose elimination is always an issue. The ultimate solution adopted by most of the farmers/foresters is to get rid of the wastes by burning them in the field itself. If this carbohydrate rich biomass is utilized as a resource for displacing some of the non-renewable resources and hazardous chemicals, it will assuage the economic and environmental issues in addition to the waste management concerns. Ligno-cellulosic substrates are generally composed of three different polymers i.e., lignin, cellulose and hemicellulose. Cellulose is the major constituent of the plant materials and it forms about half to one-third of plant tissues [1] and present in the plants as crystalline and amorphous structure [2]. It is available as D-glucose subunits linked together by  $\beta$ -1,4-glucosidic bonds [3] whereas hemicelluloses are heteropolysaccharides and hence contain many different sugar monomers. However, xylan and glucomannan are the dominant components of hardwood/agricultural waste and softwood respectively [3,4]. Like cellulose, the most hemicelluloses function as supporting materials in the cell walls and relatively easily hydrolysed by acids [5]. In contrast, lignin is one of the most complex

and widely distributed renewable aromatic polymers on the terrestrial earth. After cellulose and hemicellulose, lignin is the second most abundant biopolymeric material synthesized every year by the plants in the nature. It acts as a binding agent and holds the cellulose together and fills the space in the cell wall among cellulose, hemicellulose and pectin components. It is one of the major structural components of wood tissue, which binds the wood fibres together and imparts the desired strength, rigidity and elasticity to the secondary xylem and provides resistance against microbial attack and under stress conditions.

Ligno-cellulosic material is widely used as a supportive substrate during the solid state fermentation and is a very commonly used technique for the production of different microbial enzymes. These enzymes contribute to the inclusive applications in the paper pulping industries, biofuel production, for animal feedstock, degradation of xenobiotic compounds and many other commercially important inputs [6-10].

Few reviews on the solid state fermentation have been reported [2,11,12] which were based on the general aspects of fermentation or pre-treatment to the ligno-cellulosic for solid state fermentation. However the present article represents the application of solid state fermentation for utilization of ligno-cellulosics through biotechnology in different sectors.

### Solid State Fermentation (SSF)

Solid State Fermentation (SSF) is the fermentation process taking place in the absence of the free flowing water where any solid material

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is the substrate/support [13]. SSF is a notable technique used in Asian continent from the ancient time (approximately 2600 BC) [14]. Though, it was discontinued because of its disadvantages to control the process parameters and higher impurities at the end product, development of technologies met the solution of these problems too. Additionally, some reports of transformation studies using SSF gave kick to this arena and helped SSF to attain another landmark. With continuous extension, it has been widely accepted and gained attention of the researchers for different applications over other fermentation techniques because of abundant availability of waste material, its cost effective availability and management issues.

There are several essential factors which reflect immense impact on the triumph of solid state fermentation such as the substrate, optimum process parameters and acting agent being used for the manufacturing particular product. Looking to the suitable habitat for the growth, SSF is proved to be the most appropriate inoculation media for fungi and yeast because of the presence of anonymous natural base which are utilized very widely for the production of extracellular enzymes and bioethanol from lignocellulosic wastes. In contrast, it is considered incongruous for bacteria due to theoretical concept of water activity [15]. Production of chemicals, enzymes, value added products, secondary metabolites, organic acids and pharmaceuticals etc. through SSF is very promising and preferred choice in the recent years. SSF is also demonstrated as an active alternative to the other techniques for the production of metabolites with importance to the food industry [16]. Some of such examples for the production of the various compounds by SSF are shown in Table 1.

Beside production of traditional bulk of chemicals, food, fuel and feed, it has attracted an attention in areas such as solid waste management, biomass energy conservation and its exploitation in production of highly valued products such as biologically active secondary metabolites [17]. As biomass is the only foreseeable energy source, bio-refineries have added more value to SSF to meet the demand and needs of the future generation, which adds to the significance of agro-residual waste [18,19].

### Submerged Fermentation vs. Solid State Fermentation

Submerged fermentation is the technique where microorganisms are grown in the liquid media which is vigorously aerated and mostly agitated in contrast to the use of solid media. This technique received overnight fame with the invention of marvel drug Penicillin through the same, and dominated the world of fermentation. Perhaps Solid State Fermentation was continued and had enormous significance in Asia because of the largest agricultural producing countries. Though, both fermentation techniques have advantages and disadvantages over each other, SSF is widely accepted because it mimics natural living conditions and it reproduces the processes similar to the composting and ensiling. Exploitation of agro-industrial waste provides favourable environment that is similar to the natural habitat of fungi and offers the opportunity of recycling and managing the agro-industrial waste successfully. Additionally, it includes simplicity of the fermentation media, with fewer requirements of complex machinery, equipment and control systems, greater product yield; reduced energy demand, lower capital and low recurring expenditures in industrial operation [20]. The major drawback of SSF is its complexity in product recovery and their purification. However, abundant and easily available substrates, low production cost and high yield can compensate the overall economic expenses. Castilho et al. [21] estimated detailed economic analysis for the production of lipase in both SSF and SmF (Submerged fermentation), it is found that the capital investment for SSF was 78%

lower than that of SmF and there is about 47% of the profit on the product cost which directly indicates the advantages of using cheaper substrates.

### Application of Solid State Fermentation

Solid state fermentation has always found elevated applications in the production of antibiotics, surfactants, and other value added products like enzymes, secondary metabolites, biopesticides, aroma compounds etc. at industrial and commercial level. The latter, due to its role in enzyme production by fungi attended as special attraction. Although, numbers of studies on enzyme production have been carried out using different fermentation methods, SSF has incredible potentials in the production of enzymes due to resemblance to the natural habitat of microorganisms. Therefore, it is an ideal choice for microbes to grow and produce value-added products cost effectively. It can be of exceptional interest in processes where crude fermented products are used directly as enzyme sources. However, the lignin present in the lignocellulosics wastes cannot be easily degraded by microbial flora because of its intricate composition. Since white rot fungi is the only organisms acknowledged for possessing the potential of lignin degradation [22,23] due to their unique extracellular enzymatic system. On the other hand, non-ligninolytic fungi are also credited for the extraction of very essential commercially important enzymes with wide applications in industrial segments like detergents, food, feed, pharmaceutical, and biofuels. Fungi would be more capable of producing certain enzymes with high productivity during SSF as compared to SmF [24]. Additionally, some bacteria are also known for the enzyme production through SSF. The production of biologically active secondary metabolites in SSF represents another incredible aspect in the recent years. Several studies carried out since many decades also emphasize the importance of working in solid state condition [25-30].

### Enzyme Production

Enzymes are among the essential microbial products utilized by human beings. Beside bacteria, fungi are considered to be the best sources for the same. The plentiful applications of enzymes in industrial and non-industrial sectors demand for different enzymes being employed to the different segments. The rapidly growing world enzyme market which was \$5.1 billion in 2009 [31] is forecasted to climb the height of \$6.9 billion by 2017 [32] which reflects its necessity to meet the demands of the recent world. The main reason behind this trend stimulation is its significant cost reduction and broad range of applications. The implementation of this biotechnological process in the industrial sector is very successful and reported to reduce 9-90% of the production cost [33,34]. The enzymes are produced at large scales using microbial resources or many of them are commercially available and employed to different industries like food, feed, paper, textile, pharma etc. Necessity of the sustainable and energy saving production of the enzymes requires better fermentation techniques, where SSF emerged as the pollution free and clean system for the same.

SSF is considered as the most appropriate method for the cultivation of fungi to scale up the production of enzymes i.e., laccase, manganese independent peroxidase and manganese peroxidase, xylanase, cellulase, amylase etc. using inexpensive and easily available lignocellulosic substrates such as natural, agricultural and agro-industrial wastes [10,35-41]. Lignocellulosics biomass promotes the excellent growth of fungi and boosts the enzyme activity by means of providing the nutrients to the fungi. Ligno-cellulosic material may contain specific compounds which stimulate the ligninolytic enzyme synthesis, for instance; the presence of extractive substances, derived from straw was

Product	Substrate	Organism	Reference
Rifamycin SV	Ragi bran	<i>Amycolatopsis mediterranei</i>	[77]
Rifamycin B	Coconut oil cake and ground nut shell Corn husk	<i>Amycolatopsis Mediterranean</i> <i>Amycolatopsis sp.</i>	[78] [79]
Cephalosporin C	Wheat rawa Barley	<i>Acremoniumchrysogenum</i> <i>Cephalosporium armonium</i>	[80] [81]
Cyclosporine A	Wheat bran	<i>Tolyocladium inflatum</i>	[82]
Iturin A	Rice bran, Wheat bran	<i>Bacillus subtilis</i>	[83]
Neomycin	Wheat rawa	<i>Streptomyces marinensis</i>	[84]
Oxalic acid	Wheat kernels	<i>Aspergillus oryzae</i>	[85]
Gluconic acid	Tea waste and Sugarcane molasses	<i>Aspergillus niger</i>	[86]
Aroma compounds (esters)	Cassava bagasse, Giant palm bran, Apple pomace, Sugarcane bagasse and Sunflower seeds	<i>Kluyveromyces marxianus</i>	[87]
Biopesticides	Coffee husks and Sugarcane bagasse	<i>Beauveria bassiana</i>	[88]
Xanthan	Potato peels	<i>Xanthomonas citri</i>	[89]
Methylketones	Coconut fat	<i>Aspergillus niger</i>	[90,91]
Acetaldehyde methylbutanol	Rice koji	<i>Aspergillus oryzae</i>	[92]
Vanillin	Beet pulp and cereal bran	<i>Pycnoporus cinnabarinus</i>	[93]
Gallic acid	Cashew husk	<i>Aspergillus oryzae</i>	[94]
Phenolic compounds	Rice bran	<i>Rizhopus oryzae</i>	[95]
Pectinase	Lemon peel pomace	<i>Aspergillus niger</i>	[96]
Ferulic acid	Agro industrial waste	<i>Streptomyces setonii</i>	[97]
Tannin acyl hydrolase	Coffee husk	<i>Lactobacillus ntarum</i>	[98]
Chitosan	Soybean meal and hulls	<i>Mucor rouxii</i>	[99]
Docosahexaenoic acid (DHA)	Rapeseed meal and Waste molasses	<i>Cryptocodium cohnii</i>	[100]
Antioxidant protein hydrolysates	<i>Acanthogobius hasta</i> processing by products	<i>Aspergillus oryzae</i>	[101]
Bioactive metabolites	Coffee husk, Sugar cane bagasse and Mango seeds	<i>Monascus purpureus</i>	[102]
Biosurfactants	Sunflower seed shell	<i>Pleurotus ostreatus</i>	[103]
Natural pigment	Corn meal	<i>Monascus purpureus</i>	[104]
Pigments and Monacolin Kp	Sorghum	<i>Monascus purpureus</i>	[105]

Table 1: Products produced through solid state fermentation.

essential for the production of manganese peroxidase by *Phanerochaete chrysosporium* [42]. It was also demonstrated by Elisashvili et al. [43], that the presence of lignocellulosic substrate is mandatory for manganese peroxidase production by *Pleurotus dryinus* IBB 903, since there was no enzyme production when the fungus was grown in the synthetic medium with different carbon sources. Not only the organism but the medium composition or supporting substrate is also crucial factors for the growth of the organism and production of particular enzyme or isoenzymes, otherwise they behave differently in presence of different compounds. There are few compounds which can stimulate while many can suppress the growth and enzyme production. Production of manganese peroxidase and lignin peroxidase by *Phanerochaete chrysosporium* is strongly affected by medium composition [44] and encourages the production of peroxidases if lingo-cellulosic are used as substrate [42]. Survey of literature reveals that much of the evaluations are carried out on the production of ligninolytic enzymes using SSF system and few of recent ones are mentioned in Table 2. Over the years, productions of industrially important non-ligninolytic enzymes such as xylanase, pectinase, cellulase, insulinase, amylase, lipase, phytase etc. have also been published and employed at commercial level (Table 3). Another notifying advantage of using SSF is its unique possibility of using mixed cultures to exploit metabolic synergisms among various fungi due to their characteristics of associating with the solid substrates in the natural habitat [11]. However, the interpretation of the investigation and comparison of the results is relatively complicated, but considerable amount of work has been done in recent years [11]. Enhanced enzyme production through co-culturing can also help to understand the biochemical engineering aspects of SSF. Application of

this technique is the most suitable when we concern about demand of enzymes, energy, environment and availability of raw materials.

## Agrobiotechnological Process

Production of bio-fertilizers, bioprocessing of crops and crop residues, soil detoxification, feed production, fibre processing etc. are the processes widely assisted by SSF. The fertilizers containing living organisms are generally referred as bio-fertilizers and their activities are expected to influence the soil ecosystem and to produce supplementary substances for plant growth [45]. Market for the production of these agro-waste based bio-fertilizers and their utilization is expanding rapidly since last two decades because of cheap and easily available substrate. Moreover, it also solves the problems of the agricultural wastes disposal and even farmers can produce their own without any investment. Recently coir pith from coconut husk treated with *Aspergillus nidulans* through solid state fermentation is reported to be a carrier material for preparation of bio-fertilizer [46]. Chen et al. [47] reported that agro-industrial wastes of cattle dung; residues after vinegar-production and rice straw were solid-state fermented with *Trichoderma harzianum* which is used as bio-fertilizers to control the *Fusarium* wilt of cucumber in a continuously cropped soil. Available literature indicates that bio-fertilizers are also prepared from the agricultural waste using thermo tolerant and thermophilic organism to enhance the rate of maturity and improve the quality of the resulting biofertilizer [48]. Bio-fertilizers produced from agro-waste using SSF are found to be more economical in its production and most potent in improving the soil quality and significantly enhance the crop yield. Different wastes from the fruits like, banana, watermelon, papaya, pineapple, citrus orange

can be very good substrates for the production of biofertilizer, which are applied to the vegetable plantation [45]. Health consciousness in human beings navigated them towards organic agricultural products and this rapid expansion demanded for inexpensive phosphate source which imposed farmers to apply insoluble rock phosphate, the direct source of soil pollution and eutrophication. Instead some of the fungi have been accepted as excellent phosphate solubilizers [49] which are better substitute for the rock phosphate processing [50]. Vassilev et al. [51] developed the biotechnological technique for solubilizing rock phosphate by fungi grown on agro-industrial waste and the resultant fermented products employed to the plants demonstrated significantly enhanced growth, higher level of mycorrhization and increased soil enzyme activity [52-54]. Therefore, further techniques can be formulated and sustainable agriculture can be inextricably linked to the SSF. Formulation of such techniques will not only save from excessive application of synthetic fertilisers and soil pollution but will also make farmers independent from the issues related with black marketing of fertilizers due to demand vs. supply and more profit to farmers.

Similar approach has also been adapted for the production of better feed for pet animals such as cattle, pigs, goat and poultry feed. For over 20 years, feed enzymes have been available for their use in poultry to improve performance and production efficiency [55]. Agriculture products that are used as a cattle feed is also a good source of substrate for SSF to grow fungi which excrete the extracellular enzymes on the substrates in order to modify the cell wall structure and enhance the nutrition value of the substrate as a feed. Fermentation of sweet sorghum stalk using *Candida tropicalis* and *Lactobacillus rhamnosus* has been successfully applied in China by which around 200 tons of the feed was produced from two tons of dry sweet sorghum stalk, which is of high quality and low price [56]. Two forage grasses, Napiergrass and pangolagrass used as cow feed were treated with cellulolytic microbes to enrich protein content and improve *in vitro* digestibility of herbage using SSF technique for chicken feed [57]. Utilization of apple pomace for the value added production and animal feed through SSF can become model for developing the technology from laboratory to the pilot scale [58]. SSF residue of whole rice crop can also be used

Product	Substrate	Organism	Reference
Manganese Peroxidase and Laccase	Wheat bran	<i>Agaricus bisporus</i>	[106]
	Wheat straw	<i>Pleurotus ostreatus</i>	[107]
Laccase	Wheat straw and Rice straw	<i>Ganoderma sp.</i>	[108]
	Agricultural residue	<i>Pleurotus florida</i>	[109]
	Tamarind shell	<i>Ganoderma lucidum</i>	[110]
	Rice husk	<i>P. sajor-caju</i>	[111]
	Wheat straw	<i>Pleurotus eryngii</i> <i>Pleurotus ostreatus</i>	[112]
	Wheat straw and sugarcane bagasse	<i>Schizophyllum sp.</i> , <i>Polyporus sp.</i>	[113]
	Sugarcane bagasse	<i>Phanerochaete sp.</i> <i>Trametes sp.</i>	[114]
	Black gram husk (BGH) and Green gram husk	<i>Pleurotus ostreatus-IE8</i>	[115]
	Wheat bran	<i>Corioloopsis caperata</i>	[116]
	Sugarcane bagasse	<i>Pleurotus ostreatus</i>	[117]
	Orange waste	<i>Pleurotus ostreatus</i>	[118]
Manganese Peroxidase And Lignin Peroxidase	Vegetable leaf and Rice straw	<i>Pleurotus pulmonarius</i>	[119]
	Rice straw	<i>P. chrysosporium</i>	[120]
	Wheat straw	<i>P. chrysosporium</i> , <i>Fusarium moniliforme</i>  <i>Irpex lacteus</i>	[121]  [122]

Manganese Peroxidase, Lignin Peroxidase, Laccase	Wheat straw	<i>Trametes versicolor, Bjerkandera adusta, Ganoderma applanatum and Phlebia rufa</i>	[123]
	Wheat straw and oak saw dust	<i>Trametes pubescens and Trametes multicolour</i>	[124]
	Sugarcane baggase	<i>Pleurotus florida, Corioloopsis caperata RCK 2011 and Ganoderma sp. rckk-02</i>	[125]
	Rice straw	<i>Fusarium moniliforme, Phanerochaete chrysosporium</i>	[121]
	Grape waste	<i>Pleurotus eryngii</i>	[126]
	Pineapple leaf	<i>Ganoderma lucidum</i>	[127]
	Banana stalk	<i>Schizophyllum commune</i>	[128]
	Wheat straw	<i>Pleurotus ostreatus</i>	[129]
Lignin Peroxidase	Wheat straw	<i>Irpex lacteus</i>	[130]
	Corn cob	<i>Ganoderma lucidum</i>	[131]
	Wheat straw		[132]
Manganese Peroxidase	Wheat straw	<i>P. chrysosporium</i>	[10,133]
		<i>Fomitopsis pinicola BEOFB 600 and L. betulinus</i>	[112]
	Pine sawdust and Rice straw	<i>Schizophyllum sp. F17</i>	[134]
	Sugarcane baggase	<i>Trametes villosa</i>	[135]
	Arecanut husk	<i>Phanerochaete chrysosporium</i>	[136]
	Eucalyptus residue	<i>Lentinula edodes</i>	[137]
	Pine sawdust, Rice straw, and Soybean powder	<i>Irpex lacteus</i>	[138]
Polyphenol Oxidase (PPO) and Manganese Peroxidase	Sugarcane bagasse	<i>Phanerochaete chrysosporium PC2, Lentinula edode LE16 and Pleurotus ostreatus</i>	[139]

**Table 2:** Ligninolytic enzyme production using different substrates by solid state fermentation technique (Recent reports).

as the cattle feed [34] while soybean fermented with three different fungi demonstrated as having potential for nonruminant feed improvement [59]. Enzyme productions by SSF provide the great benefit of producing different enzyme combinations with alteration of the substrates being used while addition of promoters helps to encourage the enhanced secretion of particular enzymes, to be used as a target protein.

Exploitation of agricultural waste as the biomass for SSF is a successful tool for the enzyme production commercially. According to the estimation of Royal Dutch/Shell group renewable resources could supply 30% of the worldwide chemical and fuel needs, resulting in a biomass market of \$150 billion by the year 2050 [60,61].

## Bioremediation

The pilling up of the complex xenobiotic compounds introduced to the nature worsening the ecosystem at an alarming rate and its dispersion back to the nature is challenging for the environmental scientists. The fungal enzymes having prowess of degrading the most complex and highly recalcitrant lignin would have definite potential to mineralize intricate chemical structures, gave rise to the new era for biodegradation. Lignolytic enzymes have been paid particular attentions because of their endowed environmental friendly technologies of remediating xenobiotic compounds. Utilization of enzymes produced through SSF in remediation of chemicals as pollutants is linked directly to the energy consumption when employed at industrial level. Aromatic compounds containing different groups and links make them stronger

Product	Substrate	Organism	Reference
β-Glucosidase	Rice straw and compost	<i>Talaromyces Pinophilus</i>	[140]
	Corn cob	<i>Aspergillus aculeatus</i>	[141]
Xylanase	Corn cob/Pineapple peel powder	<i>Trichoderma koeningi</i>	[142]
	Wheat straw and Rice straw	<i>Bacillus pumilus</i>	[108]
	Wheat straw	<i>Bacillus sp.</i>	[143]
Cellulase	Wheat straw and Rice straw	<i>Fomitopsis sp</i>	[108]
	Pangolagrass	<i>Digitaria decumbens</i>	[60]
	Banana	<i>Bacterial consotia</i>	[114]
Xylanase and Cellulase	Mustard stalk and straw	<i>Termitomyces clypeatus</i>	[144]
	Soybean	<i>Aspergillus oryzae, Trichoderma reesei, and Phanerochaete chrysosporium</i>	[145]
	Sugarcane bagasse	<i>Pleurotus ostreatus-IE8</i>	[146]
Lipase	Rice hulls		[147]
	Cassava peel	<i>Colletotrichum gloesporioides</i>	[148]
	Groundnut oil cake	<i>Aspergillus niger</i>	[149]
	Jatropha Seed Cake	<i>Pseudomonas sp.</i>	[150]
	Agroindustrial residue	<i>Bacillus subtilis</i>	[151]
	Sugarcane bagasse, Wheat bran, Corn meal, Barely bran	<i>Pseudomonas aeruginosa</i>	[152]
	Soybean meal and Sugarcane bagasse	<i>Yarrowia lipolytica</i>	[153]
		<i>Rhizopus oryzae</i>	[154]

Amylase	Groundnut oil cake	<i>Aspergillus niger</i>	[155]
	Mustard Oil seed cake	<i>Bacillus sp</i>	[156]
	Millet	<i>Bacillus sp</i>	[157]
	Tapioca	<i>Aspergillus niger</i> <i>Trichothecium roseum</i>	[158]
	Wheat bran	<i>Candida parapsilosis</i> , <i>Rhodotorula mucilaginosa</i> , <i>Candida glabrata</i>	[159] [160]
	Rice straw	<i>Bacillus subtilis</i>	[161]
Pectinase	Orange peel powder	<i>Aspergillus niger</i>	[162]
	Wheat bran, Orange and Lemon peel		[163]
	Wheat bran		[164]
	Apple pomace		[165]
	Wheat bran and Sugarcane bagasse		[166]
	Pine apple peel	<i>Aspergillus flavus</i>	[167]
Proteases	Wheat bran	<i>Aspergillus oryzae</i>	[168]
	Coffee by products		[169]
	Canola cake		[170]
	Rice bran		[171]
	Soybean meal		[172]
	Lentil husk	<i>Bacillus subtilis</i>	[173]
	Punica granatum peel	<i>Aspergillus niger</i>	[174]
	Rice bran	<i>Fusarium oxysporum</i>	[175]
	Chickpea (CF) and Faba bean	<i>Bacillus mojavensis</i>	[176]
Endogluconase	Sugarcane bagasse (SCB) and Wheat bran	<i>Myceliophthora thermophila I-1</i>	[177]
Phytase and Protease	Wheat ban and Soybean bran	<i>Aspergillus niger</i> and <i>Aspergillus oryzae</i>	[178]
$\alpha$ -L-Arabinofuranosidase	Maize stover	<i>Aspergillus niger</i>	[179]
Polygalacturonases	Cashew apple bagasse	<i>Aspergillus niger</i>	[180]

Glucoamylase and Proteas	Waste bread	<i>Aspergillus awamori</i>	[181]
Tannase	Wood chips	<i>Aspergillus niger</i>	[182,183]
Endogluconase and Xylanase	Wheat bran	<i>Coniophora puteana</i>	[184]
Cellulase	Wheat bran and Rice Bran	<i>Aspergillus niger</i>	[185]
Inulinase	Corn steep/ Sugarcane bagasse	<i>Kluyveromyces marxianus</i>	[185]
Polygalacturonase	Apple bagasse and Wheat bran	<i>Aspergillus niger</i> and <i>Penicillium sp</i>	[186]

**Table 3:** Non-lignolytic enzyme production using different substrates through solid state fermentation (Recent reports).

Compound	Enzyme	Organism	Reference
Textile dyes	Manganese peroxidase	<i>Phanerochaete chrysosporium</i>	[10,133]
Dye effluent	Manganese peroxidase	<i>Musa acuminata</i>	[187]
Azo dyes	Manganese peroxidase	<i>Pleurotus ostreatus</i>	[188]
Textile effluent	Laccase	<i>Curvularia lunata</i> <i>Phanerochaete chrysosporium</i>	[189]
Polymeric model dye Poly R-478	Manganese peroxidase	<i>Irpex lacteus</i>	[141]
Nonylphenol	Laccase	<i>P. ostreatus</i>	[190]
2,4-dinitrophenol	Laccase	<i>T. versicolor</i>	[191]
Phenol	Laccase	<i>P. simplicissimum</i>	[192]
Naphthalene, Anthracene and Benzo[a]anthracene	Laccase	<i>Lentinula edodes</i>	[193]
Fluorene	Laccase	<i>Coprinus plicatilis</i>	[194]
Malachite green	Laccase	<i>Bacillus thuringiensis</i>	[195]
Bisphenol A	Laccase	<i>Funalia trogii</i>	[196]
Anthroquinone	Laccase	<i>Lentinus sp</i>	[197]
Salicylic acid, Naproxen, Ibuprofen, Gemfibrozil, Diclofenac and Triclosan	Laccase	<i>Trametes versicolor</i>	[198]
Bisphenol A and Diclofenac	Laccase	<i>Aspergillus oryzae.</i>	[199]
Endocrine Disrupters	Laccase	<i>Cerrena unicolor</i>	[200]
Textile effluent	Laccase	<i>Pleurotus ostreatus</i> IBL-02 and <i>Coriolus versicolor</i>	[201]
Dyes	Peroxidase	<i>P.ostreatus</i>	[202]
Olive Mill Wastewater	Laccase, Manganese peroxidase, Manganese Independent peroxidase	<i>Hapalopilus croceus, Irpex lacteus, Phanerochaete chrysosporium</i>	[203]
Olive Mill Wastewater	Peoxidases	<i>Agrocybe cylindracea, Inonotus andersonii, Pleurotus ostreatus and Trametes versicolor</i>	[204]
Atrazine	Ligninolytic enzymes	<i>Pleurotus ostreatus</i>	[205]
2,4 Dichlorophenol	Ligninolytic enzymes	<i>Phanerochaete chrysosporium</i>	[206]
Bentazon	Laccase and Manganese peroxidase	<i>Ganoderma lucidum</i>	[207]
Heptaclor	Ligninolytic enzymes	<i>Phlebia acanthocystis, P. brevispora, Phlebia lindtneri and Phlebia aurea</i>	[208]
Methylene blue	Manganese peroxidase	<i>Phanerochaete chrysosporium</i>	[209]
	Versatile peroxidase and laccase	<i>Pleurotus ostreatus</i>	[210]
coracryl brilliant blue,	Ligninolytic enzymes	<i>Phanerochaete chrysosporium, Phlebia brevispora and Phlebia floridensis</i>	[211]
graphene	Lignin peroxidase	<i>White rot fungi</i>	[212]

**Table 4:** Involvement of ligninolytic enzymes in bioremediation.

Substrate	Organism	Reference
Carob pod, Wheat bran	<i>Zymomonas mobilis</i>	[213]
Sweet Sorghum Bagasse	<i>Z. mobilis</i>	[214,215]
	<i>Neurospora crassa, Saccharomyces cerevisiae</i>	
Sweet Sorghum stalks	<i>Saccharomyces cerevisiae</i>	[216,217]
	<i>Trichoderma reesei, Saccharomyces cerevisiae</i>	
Sugarcane stalks	<i>Saccharomyces cerevisiae</i>	[218]
Lignocellulosic biomass	<i>Aspergillus aculeatus, Trichoderma reesei</i>	[219,220]
Sugarcane baggase		
Soybean meal	<i>Saccharomyces cerevisiae and Zymomonas mobilis</i>	[221]
Sweet Sorghum stalks	<i>Saccharomyces cerevisiae</i>	[222-224]
	<i>Issatchenkia orientalis</i>	
Sweet Sorghum juice	<i>Saccharomyces cerevisiae</i>	[225]
Paddy straw	<i>Trichoderma ressei</i>	[226]
Sugarcane baggase	<i>Trichoderma and Penicillium Saccharomyces cerevisiae</i>	[227]
<i>Ulva fasciata</i>	<i>Cladosporium sphaerospermum</i>	[228]
Food waste	<i>Myceliophthora thermophila Saccharomyces cerevisiae</i>	[229]
<i>Ziziphus jujuba</i>	<i>Saccharomyces bayanus</i>	[230]

Table 5: Ethanol production through Solid state fermentation.

to disassociation of each group and make the compound resist for long or sometimes almost as undegradable compound. Though, the application of enzymes to waste treatment was proposed in 1930s [62], was first illustrated in the late 1970s through degradation of parathion using enzyme [63]. Followed by hundreds of studies have been reported for the transformation of pollutants using enzymes replacing traditional conventional chemical treatments. However, enzyme production through SSF is the key driving force for the development of eco-friendly enzyme technology. Lignin modifying enzymes like copper containing laccase and heme containing peroxidases belonging to oxidoreductases group are investigated widely for their involvement in bioremediation and well represented in Table 4. Oxidoreductases catalyse the electron transfer through oxidation and reduction of the substrate. It is more convenient than using chemicals for the removal of other harmful chemicals which may yield other unhealthy products. Engineering inputs for the modification of the catalytic properties also pave the way of using these enzymes at the harsh industrial conditions, which are being systematically explored.

## Pulp and Paper Industry

Cellulosic fibres, directly from the wood or any other cellulose rich resources are converted to pulp and used to produce different quality of papers. While using wood as a source of paper making, lignin the main hampering compound must be separated from cellulose which requires strong acids and other harsh chemicals that generate heavy soil and water pollution. Biopulping of the wood chips by SSF using white rot fungi for the delignification process is substantiated economical and environment friendly alternative. The demonstration by Scott et al. [64] using the large scale biopulping experiment proved its potential for improving paper quality, brightness and low energy consumption. Michel Boudet in 2011 also noticed 30% of energy savings in the studies. Akhtar [65] reported about 37% saving of energy within four weeks of incubation with *Ceriporiopsis subvermisporea*. Initially, application of fungal enzymes in biopulping was not much appreciated due to time required for the delignification by fungal enzymes is much more than

mechanical or chemical biopulping. However, further researches not only enhanced the process of biopulping but also resulted in patents [66-69], which indicates widely acceptance of delignification of wood chips through SSF technique by using white rot fungi. Application of ligninolytic enzymes to the paper industry for the preferential delignification of the substrate is very important for such benefits. Furthermore they can also be applicable for the elimination of heavy metals flushing out through recycling paper mills. Falling amount of lignin in the substrate using ligninolytic enzymes and replacing the bleaching agents to enzymes like xylanase supports reduced production of aromatic by products throughout the paper making process. Use of the xylanase in the bleaching and processing can eliminate the main pollution cause created by the chlorine implementation to the major part of the process and also helps in managing the cost. Production of the xylanase through solid state fermentation process and its efficient utilization to the paper industry is contributing widely to the green revolution in industrial sector. Other xylan-debranching enzymes like acetyxylan esterase and feruloyl esterase may encourage the lignin-carbohydrate solubilisation process through linkage removal from polymers during pulping process [70]. Feruloyl esterase is also known for synthesis of organic solvents and value added products through bioconversion of ligno-cellulosic wastes [71].

Huge amount of residual solid wastes of the paper pulp called the sludge is produced every year and their disposal through landfill cause severe financial burden and enhances the overall cost worryingly. However, the commercial application of technology for transforming high carbohydrate content of the sludge into the value added products through SSF can support to meet the environmental and economic concerns. Using the sludge which generally contains low lignin content has been proven to be extremely proficient for its bioconversion into ethanol [72,73]. Moreover, it was also found quite successful for the ethanol production at commercial scale as they get the sludge as a waste free of cost since the sludge have no market value, pre-treatment can be eliminated and simultaneously the issue of the sludge disposal is also being compromised with no cost.

## Bioethanol Production

High consumption of non-renewable resources such as petrol, diesel and coal, leading to unavoidable increase in prices of fossil fuels, diminishing fossil fuel reservoir and emission of CO<sub>2</sub> that contributed to the high global warming effects and consequently strengthened the thought for the alternative fuel and promoted the sustainable production of biofuels. Biomass hydrolysis with well adopted microorganisms converts cellulose and hemicellulose into sugars and ultimately leads to the biofuel production. Therefore, the demand of cellulase production by SSF using agro-industrial residues is enhancing rapidly. To make the bioethanol production and other sugar based fermentation economically viable, the US Department of Energy awarded \$32 million to Genencor and Novozymes to reduce the price of cellulase by a factor of ten [34,74]. Promoting the consumption of renewable resources as the source of biofuel production, US government approved the Energy Independence and Security Act of 2007 (EISA) which mandates the production of 21 billion gallons of advanced biofuels by 2022, of which 16 billion gallons must derive from lignocellulosics feedstock's [75]. Different sources of biomass i.e., crop and crop residues, woody biomass, grasses, agro-industrial wastes etc. have been reported to be fermented using well known fermentation pathways and modified techniques. Substrates like molasses, maize starch, sugarcane, sugar beet, tapioca etc. are commonly being used for the Industrial alcohol production but traditional technologies for use of grains (e.g., from corn and wheat) and some sugar (e.g., cane and beet sugar) are considered to be responsible for immediate expansion of ethanol production [76]. Recently Horita et al. [59] also reported the production of ethanol from SSF of whole crop forage rice and demonstrated on-site ethanol production system. Several reports for the ethanol production through SSF have been listed in Table 5.

## Conclusion and Future Perspectives

Application of the submerged fermentation was taken over by SSF before decades yet is more successful only with fungal cultivation. However production of bacterial enzymes and metabolites with submerged fermentation is more frequently preferred technique. Though perusal of literature reveals SSF as an advantageous process for the production of enzymes, secondary metabolites and other value added products, grater optimization, standardization and automation of SSF process is mandatory for enhancement of its industrial exploitation. However application of bioengineered microorganisms, biotechnologically modified enzymes, development of bioreactors and potentialities of synthetic biology increase the possibilities of its practical application in many sectors which are to be encouraged essentially SSF in a whole represents environmental, industrial and economical feasibility for utilization of lignocellulosics through biotechnology and therefore, would be promoted for their optimum exploitation in an eco-friendly way without any conflicts to the nature.

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