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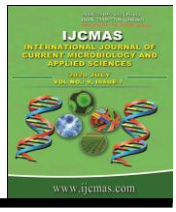

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Fermentation Technology: A Viable Tool for Bio-conversion of Lignocellulosic Biomass into Value-Added Products

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A B S T R A C T

The diminishing fossil resources over the last few decades forced us to look for alternative renewable sources to meet the increasing demand for energy and chemicals. The nature gifted agro-biomass is renewable and available in plenty and have huge potential to cater the ever growing demands of human beings. The agro-biomass is majorly made up of lignocellulose which is highly resistant to biodegradation. Some specific groups of microorganisms are capable to degrade this complex lignocellulosic structure. The agro/lignocellulosic biomass is found to be a cheap and viable substrate for fermentative production of value added products having industrial significance. The pretreatment is important for effective action of lignocellulolytic microorganisms and its enzymes on lignocellulosic substrate. Besides bio-fuel, the fermentation of lignocellulosic substrates has wide applications such as production of fine chemicals, animal feed etc. The fermentative conversion of agro-biomass into bio-manure and oyster mushroom cultivation brings additional income to the farmers.

Introduction

Microorganisms are unique creatures in the universe that they are capable of growing in diverse environmental conditions. This makes them suitable for exploration for various applications by human kind. These tiny creatures are related to human being in day to day life in several ways. In relation to human being, broadly microorganisms are classified into two groups' viz., pathogenic and beneficial microorganisms. The pathogenic microbes are the one which causes diseases or illness and beneficial microbes are those related to well being of human kind. Man makes use of microorganisms for his welfare since for many centuries. The use of microbes for bread making, wine and beer making are well known since very long. The exploitation of microorganism's for synthesis of products

and services for the well being of human kind popularly termed as "fermentation technology". Fermentation is the term derived from latin word "fervere" means "to boil" describing the action of yeast on fruits and malted grain. The anaerobic catabolism of sugars result in CO_2 production makes bubble like appearance (Stanbury *et al.,* 1995). However, now-a-days the term is broadly used as any microbiological process for the production of industrial products (Waites *et al.,* 2001). A typical fermentation process involves upstream and downstream processing as depicted in Fig. 1. The upstream processing involves the fermentation raw material, production microorganisms and the fermentation process itself while the downstream processing involves product purification and separation and the effluent treatment if any.

Fermentation products can be broadly divided into two categories viz., high volume, low value products or low volume, high value products. Examples of the first category include most food and beverage fermentation products, whereas many fine chemicals and pharmaceuticals are in the latter category. The overall economics of fermentation process are influenced by the cost of raw materials and consumables, utilities, labour and maintenance, along with fixed charges, factory overheads and operating outlay. Of these, media components may account for 60-80% of process expenditure.

The substrate plays vital role in the process yield, efficiency and commercial viability of any fermentation process. Currently researchers are focusing on viable cost effective substrate for fermentation in production of biofuel and other value-added products. Agro-biomass is abundantly and annually available in the nature and principally made up of lignin, cellulose and hemicellulose. This lignocellulosic biomass is potential source of fuel, food, feed, fine chemicals and manure. In natural process, microorganisms such as bacteria, fungi, actinomycetes etc., are grown in these materials for bioconversion into manure and biogeochemical cycling. During these process, various kinds of enzymes, antibiotics, alcohol, organic acids, polysaccharides etc. are released which have potential commercial application such as textiles, agriculture, medical etc.

Lignocellulosic biomass

Out of 4 billion tonnes of agro-biomass generated in the world, 0.7 billion tonnes are generated in India which accounts for 17.5% of world average (Phillipoussis, 2011 and Hiloidhari *et al.,* 2014). The major sources of agro-biomass are cereals, sugarcane, oilseed and pulses, cotton and jute and horticultural crops (Hiloidhari *et al.,* 2014). Billions of tonnes of this agro-biomass currently go to waste each year, which could be converted into chemical energy or other useful fermentation. The lignocellulosic composition varies with agro-biomass (Nigam *et al.,* 2009; Kumar *et al.,* 2016) and the composition of major agro-biomass is listed in Table 1.

The presence of lignocellulosic compounds in agro-biomass is complex in nature. Cellulose is a polysaccharide constitutes glucose as a monomer linked by β 1-4 glucose units. Hemicellulose constitute five carbon sugar polysaccharides such as xylan, mannan etc. Lignin is heteropolymers contains phenyl propane ring as a core component and many phenolics side chains are attached. In plant system, hemicelluloses are attached as cementing agent between cellulose and lignin. Cellulose and hemicellulose constitute about 70% of the entire biomass and highly linked to the lignin component through covalent and hydrogenic bonds that make the structure more robust and resistant to degradation

(Mielenz, 2001; Edye and Dohetry, 2008). A typical lignocellulosic structure of agro-biomass is depicted in Fig. 2.

Cellulose

Cellulose constitutes about 31-60 % in agro-biomass (Table 1). This linear polymer is composed of D-glucose subunits linked by β 1-4 glycosidic bonds forming cellobiose molecules. These form long chains called elemental fibrils linked together by hydrogen bonds and vander waals forces. Hemicellulose and lignin cover microfibrils which are formed by elemental fibrils. The orientation of microfibrils is different in the different wall level. Microfibrils group together to constitute the cellulose fibre (Demibras, 2005). Cellulose can appear in organized form called crystalline cellulose. In addition, there are a small percentage of non-organized cellulose chains, called amorphous cellulose.

Hemicellulose

Hemicellulose constitutes about 12-33 % of agro-biomass (Table 1). It is a polysaccharide with a lower molecular weight than cellulose. It consists of D-xylose, D- mannose, D-galactose, D-glucose, L-arbinose, 4-O-methyl glucuronic, D-galactouronic and D-glucouronic acids. Sugars are linked by β 1-4 and occasionally β 1-3 glycosidic bonds. Xylan is the major hemicellulosic polysaccharide. The principal component of hard wood hemicelluloses is glucouronoxylan, whereas glucomannan is predominant in softwood (Mc Millan, 1993). D-xylose is the second most abundant sugar in nature after D-glucose and constitute up to 25% of the dry weight of some woody trees

Lignin

Lignin constitutes about 4.5 to 22 % of agro-biomass (Table 1). It is present in cell wall, conferring structural support, impermeability and resistance against microbial attack and oxidative stress. Structurally lignin is an amorphous heteropolymer, non-water soluble and optically inactive. It consists of phenyl propane units joined together by different types of linkages. The polymer is synthesized by the generation of free radicals, which are released in the peroxidise-mediated dehydrogenation of three phenyl propionic acids viz., coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p- hyroxy phenyl propanol) and sinapyl alcohol (syringyl propanol) (Pettersen, 1984). Coniferyl alcohol is the principal component of softwood lignin while guaicyl and syringyl alcohol are the principal components of hardwood lignin (Mielenz, 2001). The final result of this polymerization is a heterogenous structure whose basic units are linked by C-C and aryl-ether linkages, with aryl glycerol β-aryl ether being the predominant structure.

Lignocellulosic biomass as fermentation substrate

The selection of suitable cost-effective carbon and energy sources and other essential nutrients, along with overall media optimization are vital aspects of industrial fermentation process development to ensure maximization of yield and profit. The media adopted also depend on the scale of the fermentation. For small-scale laboratory fermentation pure chemicals are often used in well defined media. However, this is not possible for most industrial scale fermentation process which simply account for up to 60 – 80% of process expenditure. Hence, industrial scale fermentation primarily uses cost-effective complex substrates, where many carbon and nitrogen source are almost undefinable.

In many instances, the basis of fermentation media are industrial processing wastes

notably molasses, corn steep liquor, starchy wastes, cellulosic wastes, whey, alkanes and alcohols, fats and oils. Of the different fermentation substrates, lignocellulosic materials are renewable resources, cheapest raw material and could be a potential alternative carbon source which needs to be fully exploited. Most often, these substrates varies with composition. The effects of such batch to batch variations must be determined. Small scale trials are usually performed with each new batch of substrate, particularly to examine the impact on product yield and recovery (Waites *et al.,* 2001). Madhusudhan *et al.,* 2013 reported that among different substrates viz., soil, starch, rice juice, potato juice and arrow root powder tested, arrow root powder solution (0.5%) showed maximum growth of *Bacillus laterosporus.* Lignocellulosic wastes viz., raw palm kernel cake, deffated palm kernel cake and vegetable wastes have highest cellulase activity (FPU/ml) at 2.65, 7.73 and 85.48 respectively when inoculated with *Bacillus* sp (Norsalwani and Norulaini, 2012).

Biodegradation of lignocelluloses occurs by exocellular enzymes. There are two types of extracellular enzymatic systems. The hydrolytic system which produces hydrolases which are responsible for cellulose and hemicelluloses degradation while unique oxidative and extracellular lignolytic system which depolymerises lignin. The complex structure of lignocelluloses is degraded majorly by fungi followed by actinomycetes while bacteria could utilize monomers and simple carbon sources at faster rate. The major fungi which are involved in biodegradation process are *Trichoderma* sp., *Ganoderma*, *Pleurotus* sp., *Phanaerochaete chrysosporium* etc. (Lee *et al.,* 1997; Wubah *et al.,* 1993) *Streptomyces* sp., are the major genera of actinomycetes involved in biodegradation process (McCarthy, 1987; Trigo and Ball, 1994). Most of the soil bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Actinobacteria* sp., are also involved in lignocelluloses biodegradation (Bayer *et al.,* 2004; Fontes and Gilbert, 2010).

Cellulose as fermentation substrate

Endoglucanases are the enzymes which are produced initially by microorganisms which acts on cellulose results in cellulosic chains with new terminal ends. These cellulosic chains produced are attacked by cellobiohydrolases (CBH) otherwise called exo β 1,4 gluconases results in cellobiose. The enzyme, β- glucosidase acts on cellobiose results in glucose units and the glucose thus produced are utilized by microbes (Mussato and Teixeira, 2010). The depiction of cellulose hydrolysis is given in Fig. 3. Mostly fungi are involved in biodegradation of cellulosic materials in nature. The well studied mesophilic fungi are *Trichoderma reesei* and *Phanaerochaete chrysosporium*. Aerobic bacteria which utilize cellulose are species from the genera *Cellulomonas*, *Pseudomonas* and *Streptomyces*. About 5 – 10% of cellulose is degraded in nature under anaerobic conditions. The well studied cellulolytic anaerobic bacteria are *Clostridium thermocellum* (Perez *et al.,* 2002). Shaheb *et al.,* 2010 reported that maximum cellulase productivity of *B. subtilis* KO strain was 35 IU by carboxymethyl cellulose (CMC) clear zone assay when molasses broth medium supplemented with cellulose.

Hemicellulose as fermentation substrate

Only a few microorganisms could ferment pentoses (Saha, 2003). The degradation of xylan occurs initially by endo-1,4-β-xylanase results in xylan oligosaccharides which in turn will be converted into xylose by 1,4-β-xylosidase. Thermophilic xylanaes have been described in actinobacteria such as *Thermomonospora* and *Actinomadura*

(George *et al.,* 2001). Xylanases active at alkaline pH have been described from *Bacillus* sp or *Streptomyces viridosporus*. Xylose can be fermented into ethanol and xylitol (sweetner). A recent breakthrough in this respect is the development of improved strains of fermentative microorganisms capable of fermenting pentose and hexose sugars into ethanol. *C. thermocellum* hydrolyze cellulose and converts glucose into ethanol. An efficient mutant strain of *Bacillus subtilis* was grown well in xylose containing medium, indicating that mutation was neither in the *xyl* nor in the *xyn* operon (Schmiedel and Ailhen, 1996). A recombinant strain of *S. cerevisiae* TMB 3130 showed increased consumption of xylose and arabinose and produced ethanol and arabitol under anaerobic conditions (Sanchez *et al.,* 2010).

Lignin as fermentation substrate

Phanerochaete chrysosporium, white rot fungus is the most predominant and extensively studied organism for lignin degradation. Lignolytic enzymes grouped into peroxidises and laccases. Peroxidases are group of enzymes includes lignin perooxidases (LiPs) which are involved in oxidation of phenolic, non-phenolic, amines, aromatic, ether and polycylic aromatics and Mn dependent peroxidises (MnPs) which converts Mn(II) to Mn(III). Mn (III) is a strong oxidant and oxidizes phenolic compounds. Laccases has been isolated from many fungi including *Aspergillus* and thermophilic fungi *Myceliophora thermophila* and *Chaetomium thermophilum* (Leunowicz *et al.,* 2001). In a batch of *Bacillus* sp. (EU978470) experimented for 6 days for the degradation of alkali lignin as a sole carbon source and achieved maximum lignin degradation at pH 6.0 (81.4 %), however the lowest lignin degradation rate was observed at pH 13.0 (34.2 %) at the end of incubation time (El-salam and El-Hanafy, 2009).

Fermentation kinetics of microorganisms in lignocellulosic carbon sources

The growth pattern of microorganisms in lignocellulosic or any other carbon sources is characterized by growth parameters such as specific growth rate (µ), maximum specific growth rate (µmax), oxygen uptake rate (OUR), oxygen transfer rate (OTR) and substrate utilization constant (Ks). The specific growth rate and generation time is determined based on growth using the formula 1 and 2. The OUR, OTR (dCl/dt) and Kla (oxygen mass transfer coefficient) values (formula 3) are determined by dynamic gassing out technique as described by Garcia-Ochoa and Gomez (2009). The substrate utilization constant and maximum specific growth rate is calculated using the formula 4. The detail about microbial growth and substrate utilization kinetics of environmental substrates are described in a review by Okpokwasili and Nweke (2005).

Specific growth rate, $\mu = \ln (X_1 - X_0)/ (t_1 - t_0)$.....1

(X_1 and X_0 = cell units at times t_1 and t_2 respectively)

Generation time = 0.693/ µ.....2

$Cl = -1/Kla (dCl/dt + OUR) + C^*$.....3

(Cl = oxygen concentration at time, t; C^* = initial oxygen concentration)

µ = -Ks (µ/s) + µmax.....4

(s- substrate concentration)

B. subtilis was grown in M-9 minimal medium containing glucose, sucrose, starch, cellulose, D-xylose and lignin as a sole carbon source and the growth parameters were compared. The study showed µ, µmax and Ks value of *B. subtilis* grown in cellulose, D-xylose and lignin were (0.0046, 0.049, 500), (0.077, 0.136, 132.2) and (0.034, 0.07, 660) respectively (Mageshwaran *et al.,* 2014). The recombinant strain of *Saccharomyces cerevisiae* expressing D-xylose isomerase

from *Provetella ruminicola* had a μ of 0.23 h^{-1} in D-xylose containing medium (Hector *et al.,* 2013). Similarly in another study, the cellulose degrading bacteria was screened for bioethanol production. The μmax and K_s of *Pseudomonas* sp. M1 in cellulose containing medium was 0.439 h^{-1} and 776 mg/l respectively (Chen *et al.,* 2011). Among the different substrates such as newsprint, switch grass, corn leaves, xylan, avicel, cellobiose and glucose tested, *Saccharophagus degradans* 2-40 showed higher μ in xylan (0.6 h^{-1}) than glucose (0.4 h^{-1}) (Munoz and Riley, 2008).

Bio-refinery approach of lignocellulosic biomass

Researchers are paying attention on simultaneous generation of bio-fuels, bioproducts and fine chemical from renewable biomass, so called bio-refinery. Biomass driven industry is one of the fastest growing sector, sharing global economy of 5 – 20%. It is possible to achieve sustainable utilization of biomass through bioconversion into chemicals, fuel and feed (Ramasamy, 2016). Microoganisms are biomachinery for synthesis of wide variety of lignocellulosic enzymes such as cellulases, ligninases and xylanases having applications in biofuel, composting, paper and pulping and effluent treatment. Currently, ethanol production is one of the widely studied and promising alternatives for cellulosic biomass conversion, due to the depletion of fossil fuels. Together with ethanol, bio-hydrogen and bio-diesel production from lignocellulosic biomass has shown enormous potentialities for sustainable energy production.

Besides biofuels, several organic acids including lactic, citric, acetic and succinic acids, antibiotics, microbial polysaccharides etc. are produced by bioconversion of ligno-cellulosic biomass (Mussato and Teixeira, 2010). A biorefinery approach for production of biodiesel, bio-ethanol, bio-hydrogen and bio-methane using leather solid wastes (Shanmugam, 2016). In another study, a bio-refinery of cotton stalks for fractionation of lignin and residual cellulose in the process of production of bio-ethanol was reported (Nupur *et al.,* 2020). A typical biorefinery approach of lignocellulosic biomass is illustrated in Fig. 4. In this review, the bio-refinery approach of lignocellulosic biomass for the synthesis of the products such as biomanure/biogas, bioethanol, fine chemicals and animal feed/mushroom has been discussed.

Pretreatment of lignocellulosic biomass

Pretreatment is the most important step towards bio-conversion of lignocellulosic biomass into bio-ethanol and other value-added products (Shi *et al.,* 2009). The pretreatment may be physical, chemical or biological process helps to cleave the lignin bonds and expose the cellulose and hemicelluloses microfibrils for subsequent saccharification and ethanol production. The effect of different chemical pre-treatment of cotton stalks on saccharification for bio-ethanol production was examined. The pretreatement with sulfuric acid and sodium hydroxide had significant xylan and lignin reduction (Silverstein *et al.,* 2007). The pretreatment of cotton stalks with *Phanerochaete chrysosporium* under solid state cultivation resulted in 27.6% lignin degradation, 71.1% soilds recovery and 41.6 % availability of carbohydrates over the period of 14 days. Thus pretreated cotton stalks would be amenable for efficient bioethanol production (Shi *et al.,* 2008).

Bio-ethanol

In the present scenario, India imports nearly 70 % of its annual crude petroleum

requirements which is approximately 110 million tones. The price are in the range of US $ 50 – 70 per barrel and the expenditure on crude purchase is in the range of Rs. 1600 billion per year impacting a bigway the country's foreign exchange reserves. The petroleum industry now looks committed to the use of ethanol as fuel. Ethanol is used as an automotive fuel by itself and also mixed with petrol, popularly called "Gasohol". The use of ethanol reduces the particulate emission in the environment. Ethanol is being produced from wheat, corn beet, sweet sorghum etc. Since, ethanol production competes with food crops, the concept of second generation biofuels came into existence where renewable lignocellulosic biomass are used for ethanol production. Limayem and Ricke, 2012 reviewed elaborately on current approaches on lignocellulosic bioconversion for ethanol production. The potential organisms in lignocellulosic-based bioethanol fermentation are *S. cerevisiae* (Mc Millan, 1993), *C. shehatae* (Ligthelm *et al.,* 1988; Zaldivar *et al.,* 2001), *Zymomonas mobilis* (Herrero, 1983; Balat & Balat, 2008) and thermophilic bacteria (Zeikus *et al.*, 1981). In a study, the bio-ethanol production from cotton stalks and corn stover were compared. The results showed that the economic performance of bio-ethanol from cotton stalks is higher than corn stalks (Petrou and Pappis, 2014)

Fine chemicals

Mostly, the cellulose obtained from lignocellulosic biomass is used for bio-ethanol production. The cellulosic residue recovered after bio-ethanol fermentation has industrical applications as fine chemicals. Cellulose powder is widely used in pharmaceutical industry as excipient, binder, disintegrant and antiadherent (Useu *et al.,* 2000). Moreover, the derivatives of cellulose such as cellulose acetate, cellulose nitrate, carboxy methyl cellulose and lignin derivatives such as vanillin, quinones, benzene etc. have wider industrial applications. Lignin recovered from agro-biomass has been used as natural adhesives replacing phenol-formaldehyde based synthetic adhesives in polywood industries. Low cost natural lignin was prepared from agro-biomass viz., ground nut shell, baggase and pulp waste for replacing synthetic phenol-formaldelhyde resins and found that Lignin phenol-formaldehyde (LPF) could substitute up to 50 % of phenol as wood adhesive (Gothwal *et al.,* 2010).

Organic acids such as lactic, citric, acetic and succinic acids may be produced by cellulose and hemicelluloses bioconversion. Lactic acid was obtained from cellulose by *Lactobacillus* sp. (Mussato *et al.,* 2008) and from hemicelluloses by *L. pentosus* (Moldes *et al.,* 2006). A mixed culture of *L. brevis* and *L. pentosus* yielded 95% of lactic acid in hemicellulosic hydrolysate of wet-oxidized wheat straw (Garde *et al.,* 2002). Citric acid was successfully produced by *Aspergillus niger* from the substrates such as cellulosic hydrolysate (Watanabe *et al.,* 1998) and hemicellulosic hydrolysate (Santos and Prata, 2009). Corn stalk and cotton stalk hydrolysates produced by steam explosion and enzymatic hydrolysis were fermented to succinic acid by *Actinobacillus succinogenes* (Li *et al.,* 2010). Xylitol, a five carbon sugar alcohol that can be used as natural food sweetener, dental caries reducer and as a sugar substitute for diabetics was efficiently produced by *Candida guilliermondii* from hemicellulosic hydrolysates (Mussatto and Roberto, 2004)

Oligomeric or polymeric tannins can be covalently bonded on the surface of wood or other lignocellulosic materials by enzymatically catalyzed oxidation. The modified lignocellulosic surfaces had shown

improved antibacterial properties (Heathcote, 2010). Solid state fermentation was used for the production of various bioactive compounds such as giberellic acid from corb cobs, cyclodepsipeptides from rice husk, ellagic acid from pomegranate peel etc. (Aguilar *et al.,* 2008; Pandey *et al.,* 2000; Martins *et al.,* 2011).

The lignolytic microorganisms and its enzymes are employed for bio-pulping and colour removal in paper industry. Biopulping of non-woody plants or agricultural residues with *C. subvermispora*, *Pleurotus* and other basidiomycetes reduces the amount of electrical power used for the refining stage as much as 30% and improves paper properties (Berrocal *et al.,* 1997; Dorado *et al.,* 1999). Vikarri *et al.,* (1986) showed bleaching of kraft pulp with fungal hemicellulases reduces subsequent chlorine bleaching requirements. Color removal from paper and pulp industry was achieved by employing FPL/ NCSU Mycor method, which uses *P. chrysosporium* in rotating biological contractors (Eaton *et al.,* 1980). The lignolytic fungi, *Phlebia radiate* and *Poria subvermispora* reduces the energy and chemicals needed for pitching and deinking in paper and pulping industries which uses recycling of used papers (Gutierrez *et al.,* 2001).

Bio-manure/Biogas production

Most of the lignocellulosic agro-residues produced, are burnt in the field after harvest and thus increases the greenhouse gases and causes environmental pollution. It is estimated that the organic wastes available in India can supply about 7.1, 3.0 and 7.6 million tonnes of N, P_2O5 and K_2O respectively (Veeraraghavan *et al.,* 1983).Composting is a method of solid waste management where by the organic component of the organic waste is biologically decomposed and stabilized under controlled conditions to a state where it can be handled, stored and applied to the land to supply essential nutrients without adversely affecting the environment (Cooper and Golueke, 1977; Nagarajan *et al.,* 1985 and Sumermerell and Burges, 1989). Tuomela *et al.,* 2000 reported that thermophilic and thermotolerant bacteria, actinomycetes and fungi are essential for the lignolytic and cellulolytic activities during the process of composting.

During the composting process, besides the final product in the form of humus; heat, compounds of nitrogen, phosphorus, CO_2, H_2O, a significant amount of microbial biomass is also created. Many variables like temperature, moisture content, oxygen concentration and nutrient availability affect the rate of decomposition of organic matter. These factors, in turn, strongly influence the structure and diversity of the microbial community, microbial activities and the physical and chemical characteristics of the substrate (Miller, 1993). The composting of high lignin containing agro-biomass like cotton stalks within shorter period is still a challenge. Using efficient microbial consortia, the bio-enriched compost with high NPK content was prepared from cotton stalks within sixty days (Mageshwaran *et al.,* 2013). The NPK content (%) of bio-enriched cotton compost was 1.4, 0.8 and 1.5 respectively, while the traditional farm yard manure (FYM) yielded 0.5, 0.2, and 0.5 % respectively. Considering the less availability of FYM in the present conditions, compost from cotton stalks is a viable *on site* solution for soil fertility management. As an entrepreneurial activity, a farmer can earn additional income of Rs. 1000/- per acre through preparation of bio-enriched compost from cotton stalks (Mageshwaran *et al.,* 2017). The compost prepared from cotton stalks is depicted in Fig. 5a.

Biogas and biomanure was produced from willow dust, a cottony dust material generated

in textile mill. About 50 m^3 of biogas containing 55-60 % methane in 45 days was produced from 100 kg of willow dust. The capital investment required is Rs. 15 lakhs for the installation of 3 digesters and 1 gas holder. Annually 50 thousand m^3 biogas and 30 tonnes of manure were produced by processing of 100 tonnes of willow dust. The running cost was Rs. 5 lakhs/ annum including the cost of raw material, alkali, water and labour. The payback period was two years (Balasubramanya and Mageshwaran, 2013).

Table.1 Composition of major agro-biomass

Agro-biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Barley straw	31-34	24-29	14-15
Corn cobs	33-35	31-33	4.5-6.6
Corn stalks	40-43	22-24	17-18
Cotton stalks	55-60	12-15	19-22
Rice straw	31-35	23-25	17-18
Rye straw	33-35	27-30	16-19
Soya stalks	34-36	24-26	18-20
Sugarcane baggase	38-43	25-27	18-20
Sunflower stalks	40-45	28-30	12-15
Wheat straw	33-36	23-25	15-17

Fig.1 Outline of a typical fermentation process

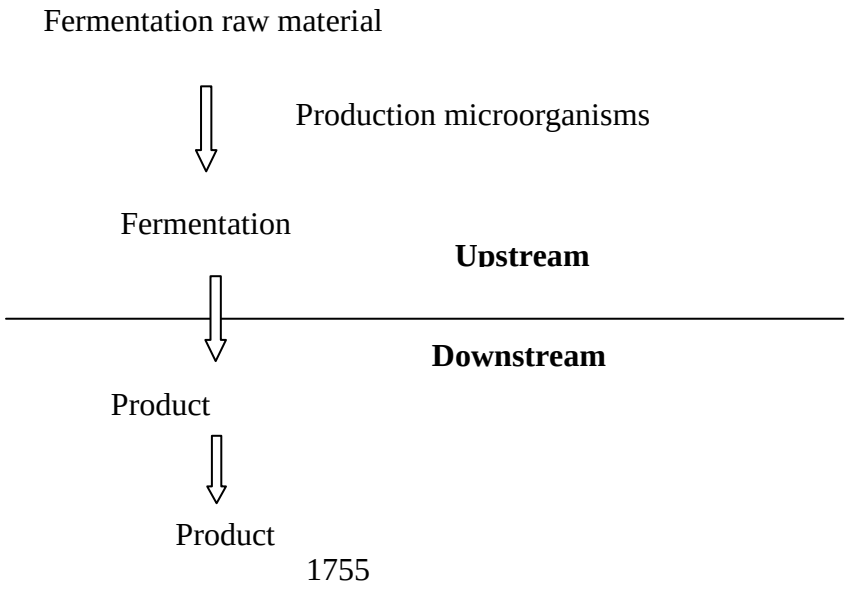


Fig.2 A typical ligno-cellulosic structure

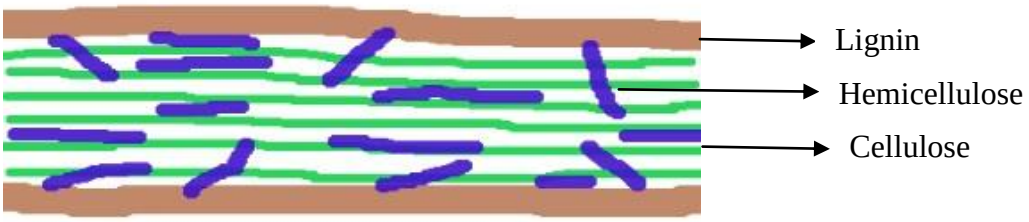


Fig.3 Biodegradation of cellulose by microorganisms

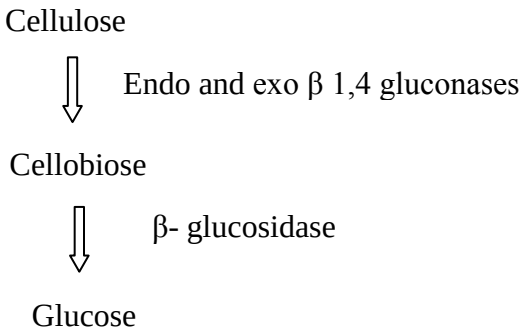


Fig.4 A typical bio-refinery approach

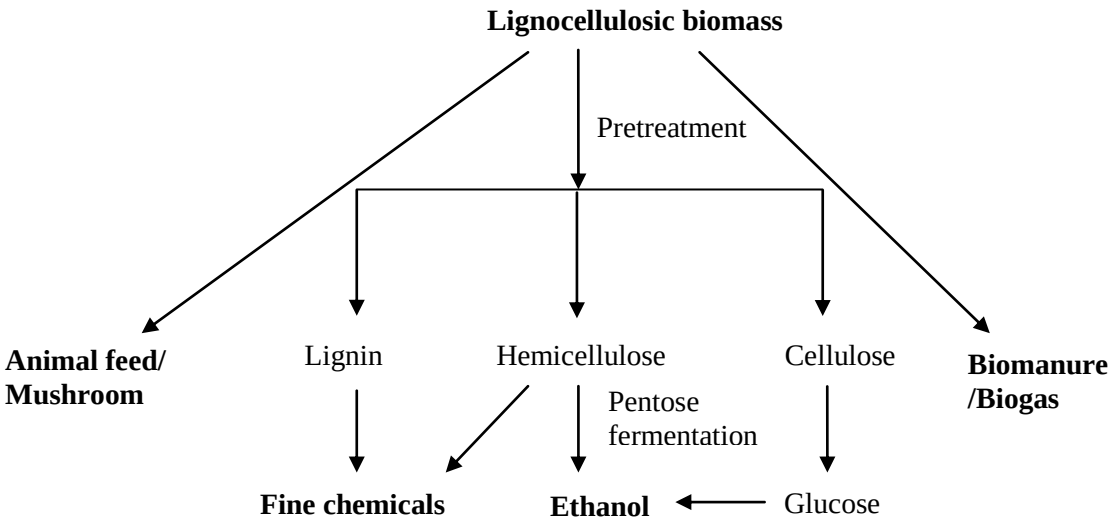
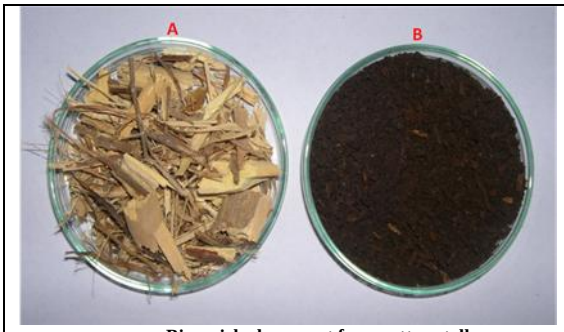


Fig.5 Bio-enriched compost and oyster mushroom from cotton stalks



a. Bioenriched compost from cotton stalks
A- Cotton stalks B- Cotton stalks compost

b. Oyster mushroom on cotton stalks

Animal feed/ oyster mushroom cultivation

The preferential delignification of lignocelluloses materials by white-rot fungi increases the nutritional value of forages (Akin *et al.,* 1995; Chen *et al.,* 1995). The detoxification of toxic compounds by microbial fermentation in oil cakes improves the nutritional quality of animal feed. Solid-state fermentation (SSF) is an ideal tool to produce industrial products including enzymes (Deschamps *et al.,* 1985) as well as microbial biomass and to improve the nutritional quality of animal feed due to its low capital investment and operating expenses (Pandey, 1992). SSF of cottonseed cake using fungal cultures, *Diplodia* sp. (Baugher and Campbell, 1969), *Pleurotus* sp. (Mageshwaran and Kathe, 2013) and *Candida tropicalis* (Weng and Sun, 2006) resulted in detoxification of gossypol and improvement of protein quality.

The mushrooms are rich in protein and excellent source of minerals and vitamins. The cultivating species are *Agaricus bisporus* (button mushroom), *Lentinus edodes* (Japanese mushroom), *Volvariella* spp. (Paddy straw mushroom) and *Pleurotus* spp. (Oyster mushroom). The button mushroom requires low temperature (20° C) and fermented substrates for its growth. The paddy straw and oyster mushrooms grow in elevated temperature of 30 - 35° C in fermented and un-fermented agro substrates (Satankar *et al.,* 2018). The degradation of lignin and hemicelluloses during cultivation of oyster mushroom in makes the spent mushroom suitable for animal feed. The cotton stalks fermented by *Pleurotus* had significantly lower lignin content and ruminants consumed fermented cotton stalks up to a level of 40% of their diet (Hadar *et al.,* 1992).

The oyster mushroom was grown in cotton stalks pretreated with anaerobic microbial consortium (Balasubramanya and Khandeparkar, 1989; Sundaram *et al.,* 1989). The novel method developed was ecofriendly and alternative to hot water pretreatment. Considerably higher yield was obtained in cotton stalks compared to other agro substrates. *P. sajor-caju* was grown in pretreated cotton stalks and the yield of oyster mushroom recorded was 250-400 g /kg of dry cotton stalks (Balasubramanya and Kathe, 1996). In another study, the effect of different levels of cottonseed hulls and cotton stalks substrates on growth, yield and nutritional composition of tow oyster mushroom was tested. The results showed the substrate composition of 75: 25 (cotton stalks and cottonseed hulls) had higher yield ($P<0.05$) with biological efficiency of 23.2 and 22.5 % in *Pleurotus ostreatus* and *P. florida*

respectively (Sardar *et al.*, 2020). As an entrepreneurial activity, a farmer can earn an additional income of Rs. 10,000 by cultivation of oyster mushroom using cotton stalks generated from an acre of land (Mageshwaran *et al.*, 2017). The oyster mushroom cultivated in cotton stalks is depicted in Fig. 5b.

In conclusion, this review summarizes the use of fermentative bio-conversion of lignocellulosic biomass into bio-ethanol and other value-added products. Lignocellulosic biomass is predominantly made up of cellulose, hemicelluloses and lignin. Microorganisms use specific enzymatic mechanism for the biodegradation of lignocellulosic substrates. The bio-refinery approach of lignocellulosic biomass result into industrially and agriculturally important products such as bio-ethanol, fine chemicals, animal feed/oyster mushroom and biomanure/biogas. The *on-farm* preparation of bio-enriched compost and oyster mushroom cultivation using agro-biomass provides additional income to the farmers. Besides, it restores soil fertility and reduces the problem that rose due of burning of agro-biomass in the field.

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