Bio-Ethanol Fuel production by White Rot Fungi for future Energy Needs and Economy

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Abstract: The energy policy of India is largely defined by the country's expanding energy deficit and increased focus on developing alternative sources of energy. The primary energy consumption in India is the third biggest after China and USA with 5.3% global share in 2015. In 2013, India's net imports are nearly 255.3 Mtone of primary energy which is equal to 42.9% of total primary energy consumption. India is largely dependent on fossil fuel imports to meet its energy demands by 2030. India's dependence on energy imports is expected to exceed 53% of the country's total energy consumption. India's growing energy demands and limited domestic fossil fuel reserves, concerns about climate change from greenhouse gas emissions and the desire to promote domestic rural economies, the country has ambitious plans to expand towards renewable energy resources. The current availability of biomass in India is estimated at about 500 million metric tones. The biomass sources contribute 14% of global energy and 38% of energy in developing countries. Ministry of New and Renewable Energy Sources (MNERS) has proposed to reach total 4324.22 MW of power generation in villages based on biomass power and gasification as well as co-generation. the cost of bio-ethanol was considerably higher than the cost of fossil gasoline supply. lignocellulosic materials are an attractive option for the production of bio-fuels. Lignocellulosic materials serve as a cheap and abundant feedstock, which is required to produce fuel ethanol from Biomass resources at reasonable costs. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials. so lignicolous fungi like *Pleurotus ostreatus, Phanerochaete sordid, Sporotrichum pulverulentum, Ceriporiopsis subvermispora, Cyathus stercoreus, Phanerochaete chrysosporium, Lenzites betulinus*, etc. were used for bioethanol production. The white rot fungi present in Andhra Pradesh also useful for bio ethanol fuel production.

Key words: Bioethanol, Bio fuel, Economy, Energy needs, white rot fungi

I. INTRODUCTION

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used for the production of fuel, electricity and other goods (Uihlein and Schbek 2009). Developing countries demand more energy in the midst of enormous economic development. The increased demand for energy has led to escalating fossil fuel prices. Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the earth's atmosphere has drastically increased (Ballesteros et al., 2006). Gross domestic product (GDP) is an indicator for a nation's socio-economic development and is correlated with energy consumption (Dincer and Dost, 1997). These countries increased their share for the world's total energy consumption to 18% in 2005 (EIA-2008). It has been projected that by 2030 the non-OECD (Organization for Economic Cooperation and Development) countries including China and India will increase their energy consumption rate to 25% of the world's energy consumption. Using energy efficiency strategies could not alone resolve the energy demands of all countries in the world. Renewable biofuel generation, application and its research & development have received greater global attention and implication. Utilization of renewable energy resources (biomass, solar, wind, hydro, geothermal) could resolve a greater portion of the energy demand problem and may replace up to 40% of the fuel demand by the middle of the 21st century (Johansson et al. 1992).

The world's total proven oil, natural gas and coal reserves are respectively, 168.6 billion tons, 177.4 trillion cubic meters, and 847.5 billion tons by the end of 2007, according to the recently released 2008 BP Statistical Review of World Energy (BPC 2008). With current consumption trends, the reserves-to-production (R/P) ratio of world proven reserves of oil is lower than that of world proven reserves of natural gas and coal — 41.6 years versus 60.3 and 133 years (BPC 2008), respectively. In 2007, world oil production was 3.90 billion tons, a decrease of 0.2% from the previous year (BPC 2008). According to International Energy Agency statistics (IEA 2008), the transportation sector accounts for about 60% of the world's total oil consumption.

Interest in the use of bio-fuels worldwide has grown strongly in recent years due to the limited oil reserves, concerns about climate change from greenhouse gas emissions and the desire to promote domestic rural economies.

Countries across the globe have considered and directed state policies toward the increased and economic utilization of biomass for meeting their future energy demands in order to meet carbon dioxide reduction targets as specified in the Kyoto Protocol as well as to decrease reliance and dependence on the supply of fossil fuels. Plant derived biomass energy has been reported to be 15% of world energy and 38% in developing countries. Biomass energy (bioenergy) is basically heat, electricity, and liquid and gas fuels derived from plant materials. Sustainability of biomass energy mainly depends on clean technology, government mandates, wide spread application, minimal impact on food and feed, alternative but competitive to conventional fossil fuel, and reliable and consistent supply of renewable biomass resources (Hall, 1997). Today, bio-fuels are predominantly produced from biomass resources. Biomass appears to be an attractive feedstock for three main reasons: (1) it is a renewable resource that could be sustainably developed in the future, (2) it appears to have formidably positive environmental properties resulting in no net releases of carbon dioxide and very low sulfur content, and (3) it appears to have significant economic potential provided that fossil fuel prices increase in the future. Cost is an important factor for large scale expansion of bioethanol production. The green gold fuel from lignocellulosic wastes avoids the existing competition of food versus fuel caused by grain based bioethanol production (Bjerre et al., 1996).It has been estimated that 442 billion litters of bioethanol can be produced from lignocellulosic biomass and that total crop residues and wasted crops can produce 491 billion litters of bioethanol per year, about 16 times higher than the actual world bioethanol production (Kim and Dale 2004). Lignocellulosic materials are renewable, low cost and are abundantly available. It includes crop residues, grasses, sawdust, wood chips, etc. Hence bioethanol production could be the route to the effective utilization of agricultural wastes. Rice straw, wheat straw, corn straw, and sugarcane bagasse are the major agricultural wastes in terms of quantity of biomass available (Kim and Dale 2004).

II. FUNGAL DIVERSITY

Biodiversity includes genetic diversity, species diversity, ecosystem diversity, agro diversity; 5-50 million species of living form exist on the globe and of which 1.5 million are fungi. The vast numbers of fungi that are recorded exceed 80,000 (Hawksworth, 1997, Manoharachary et al 2005). The number of fungi recorded in India exceeds 28000 species, the largest biotic community recorded after insects (Manoharachary et al 2005). The true fungi belong to kingdom eukaryota which has 4 phyla, 16 classes, 110 orders, 411 families and 5066 genera (Kirk et al 2001). The 4 phyla accepted in the 9th edition of Ainsworth & Bisby's Dictionary of the Fungi (Kirk et al., 2001) are Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota. The very high biological diversity of India is primarily due to the highly diversified ecological niches. It is obvious from the above description that India must be harboring diverse mycoflora. The marked variation in climate plays a determinate role in growth and development of a wide variety of mushrooms including coprophilous mycoflora. The Basidiomycota is the second largest phylum of kingdom Fungi, with approximately 23,000 species including many of the common macroscopic forest fungi (e.g., mushrooms, shelf fungi) (Hawksworth et al 1995). A fraction of complete fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth; one-third of fungal diversity of the globe exists in India and of this only 50% is characterized until now (Manoharachary et al 2005). About 10,000 species within the overall fungal estimates of 1.5 million belong to this group (Deshmukh, 2004). Hawksworth (1993) noted that about half the newly described fungal species come from the tropics, but that if individual countries are considered, those generating most new species are not exclusively tropical and then included the USA, France and Japan as well as India and Brazil. A comparable study of the data for 1990±99 revealed a shift with 60% discovered in tropical countries vs 40% in others, with India now generating most species (ca 913$), followed by the USA (ca 819), Australia (ca 813), China (ca 795) and France (ca 565).

Wood rotting fungi were belong to Basidiomycota; Based on degradation of wood the wood rotting fungi are differentiated into three types i.e white rot fungi, brown rot fungi, soft rot fungi (Table 1)

White rot fungi: These fungi degrade all the major wood components (cellulose, hemicellulose and lignin) more or less simultaneously, so that the wood becomes progressively more fragile, but remains white as the decays progresses. White rots are caused by two major root rot pathogens of trees, *Armillaria mellia* and *Heterobasidia annosum* and also by many saprotrophic fungi including the common colonizers of stumps *Coriolus versicolor* and the common wood rotting Ascomycetous members i.e. *Xylaria hypoxylon* and *X. polymorpha*.

Brown rot fungi: Brown rot fungi degrade the cellulose and hemi cellulose but leave the lignin more or less intact as a brown frame work. Only about 6% of wood decay fungi cause brown rots and all these fungi are members of Basidiomycota. They include *Serpula lacrimans* dry rot fungus and common birch polypore, *Piptoporus betulinus*.

Soft rot fungi: These fungi degrade only the cellulose and hemi cellulose and typically occur in wood of high water content and high nitrogen content. They are most commonly found in rotting window frames, wet floor boards and fence posts etc., where nitrogen is recruited from the soil or from atmospheric contamination. Some of these fungi are common decomposers of cellulose in soil (e.g. *Chaetomium* species) and they are the least specialized form of the wood rot fungi.

Table 1: Generalized characteristic of white-, brown- and soft-rot fungi (Ward et al., 2004)

Organism	**Sub division**	**Examples**	**Actions**	**distribution**
White-rot fungi	Basidiomycetes	*Phanerochaete chrysosporium Trametes versicolor Phlebia radiata*	Mineralize lignin to CO_2 and H_2O; some species selectively degrade lignin; others degrade lignin and cellulose simultaneously	Predominantly degrade wood from deciduous trees (angiosperms), containing hardwood
Brown-rot fungi	Basidiomycetes	*Gloeophyllum trabeum, Serpula lacrymans,*	*Neolentinus lepidus etc* Modify lignin by demethylation, limited aromatic hydroxylation, and ring cleavage	Prefer coniferous substrates (gymnosperms), which are softwoods
Soft-rot fungi	Ascomycetes, Deuteromycetes	*Chaetomium sp, Ceratocystis sp etc*	Some lignin degradation	Active generally in wet environments and plant litter; attack hardwood and softwood

III.BIO-ETHANOL FUEL PRODUCTION BY WHITE ROT FUNGI

Bio-ethanol feedstocks can be divided into three major groups: (1) sucrose-containing feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), (2) starchy materials (e.g. corn, milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (3) lignocellulosic biomass (e.g. wood, straw, and grasses). In the short-term, the production of bio-ethanol as a vehicular fuel is almost entirely dependent on starch and sugars from existing food crops (Smith 2008). The drawback in producing bio-ethanol from sugar or starch is that the feedstock tends to be expensive and demanded by other applications as well (Enguídanos et al 2002). Any bio-ethanol project attacks seven major national issues: (1) sustainability, (2) global climate change, (3) biodegradability, (4) urban air pollution, (5) carbon sequestration, (6) national security, and (7) the farm economy. Lignocellulosic biomass is envisaged to provide a significant portion of the raw materials for bio-ethanol production in the medium and long-term due to its low cost and high availability (Gnansounou et al., 2005). For a given production line, the comparison ofthe feedstocks includes several issues (Gnansounou et al., 2005): (1) chemical composition of the biomass, (2) cultivation practices, (3) availability of land and land use practices, (4) use of resources, (5) energy balance, (6) emission of greenhouse gases, acidifying gases and ozone depletion gases, (7) absorption of minerals to water and soil, (8) injection ofpesticides, (9) soil erosion, (10) contribution to biodiversity and landscape value losses, (11) farm-gate price of the biomass, (12) logistic cost (transport and storage of the biomass), (13) direct economic value of the feedstocks taking into account the coproducts, (14) creation or maintain of employment, and (15) water requirements and water availability.

White-rot, brown-rot and soft-rot fungi were used to biologically break down cellulosic and hemicellulosic components of lignocellulosic material into fermentable sugars. Efficient conversion of lignocellulosic biomass to biofuel requires three sequential steps: Pretreatment, Hydrolysis and Fermentation (Karimi et al 2006, Singh et al 2011). The high crystallinity of cellulose and the presence of lignin matrix make the lignocellulosic complex fairly resistant to enzymatic hydrolysis processes without pretreatment (Chahal and Chahal, 1999, Gong et al., 1999). Chemical pretreatments with either acid or base have serious disadvantages in terms of the requirement of specialized corrosion resistant equipments, extensive washing, proper disposal of chemical wastes and glucose degradation (Taniguchi et al., 2005). Physical pretreatments like Liquid hot water pretreatment, Microwave pretreatment, subcritical and supercritical watertreatment processes require expensive special instruments that have substantial energy requirements depending on the complexity of the process (Taniguchi et al., 2005). Hydrolysis with commercial enzymes is the more favourable pretreatment method compared to costly and environmentally unfriendly chemical methods. Biological pretreatment is a safe and an environmental friendly method. The most promising microorganisms for biological pretreatment are white rot fungi, especially (Taniguchi et al., 2005). The most promising organisms for alcoholic fermentation of lignocellulosic sugars are yeasts.

IV.BIOLOGICAL PRETREATMENT

Degradation of the lignocellulosic complex to liberate cellulose can be brought about with the help of microorganisms like white rot, brown rot, and soft rot fungi. Biological pretreatment renders the degradation of lignin and hemicellulose (Schurz, 1978, Sun and Cheng 2002, Talebnia et al., 2010, Prasad et al., 2007) and white rot fungi seem to be the most effective microorganism. Brown rot attacks cellulose while white and soft rots attack both cellulose and lignin (Fan et al., 1987, Prasad et al., 2007). Cellulase-less mutant was developed for the selectivedegradation of lignin and to prevent the loss of cellulose but in most cases of biological pretreatment the rate of hydrolysis is very low. This method is safe and energy saving due to less mechanical support (Sun and Cheng 2002, Talebnia et al., 2010). It needs no chemicals but low hydrolysis rates and low yields impede its implementation (Balat et al., 2008, Hamelinck et al 2005). From literarure the White rot fungi like *Bjerkandera adusta, Daedalea dickinsii, Flammulina velutipes Ganoderma lucidum Irpex lacteus Panellus serotinus Phanerothaete sordida Pleurotus pulmonarius Pleurotus cystidiosus Pleurotus ostreatus Schizophyllum commune Trametes hirsutus Trametes versicolor Peniophora cinerea and Trametes suaveolens Phanerochaete chrysosporium Gloeophyllum trabeum* were used for bioethanol production (Figure 2 A to H). Hatakka (1983) studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in four weeks. In order to prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips (Ander and Eriksson, 1977). Akin et al. (1995) also reported the delignification of Bermuda grass by white-rot fungi. The biodegradation of Bermuda grass stems was improved by 29–32% using *Ceriporiopsis subvermispora* and 63–77% using *Cyathus stercoreus* after 6 weeks. Biological pretreatment of bamboo culms with white rot fungi has been performed at low temperature (25 C) (Zhang et al 2007). In the case of a marine microorganism *Phlebia* sp. MG-60, it was seen that when the substrate was supplemented with a nutrient medium such as Kirk's Medium, better delignification was observed compared to sterilized water (Cardona et al., 2009). Bio-delignification generally needs long periods of time. In a biological pretreatment study with an aim to release the sugars from the lignocellulosic matrix of sugarcane trash using a number of microorganisms it was observed that both cellulose and lignin contents of the raw material can be drastically reduced. Reduction in the cellulose content by *Aspergillus terreus* was about 55.2% while delignification was found to be about 92% (Singh et al., 2008).

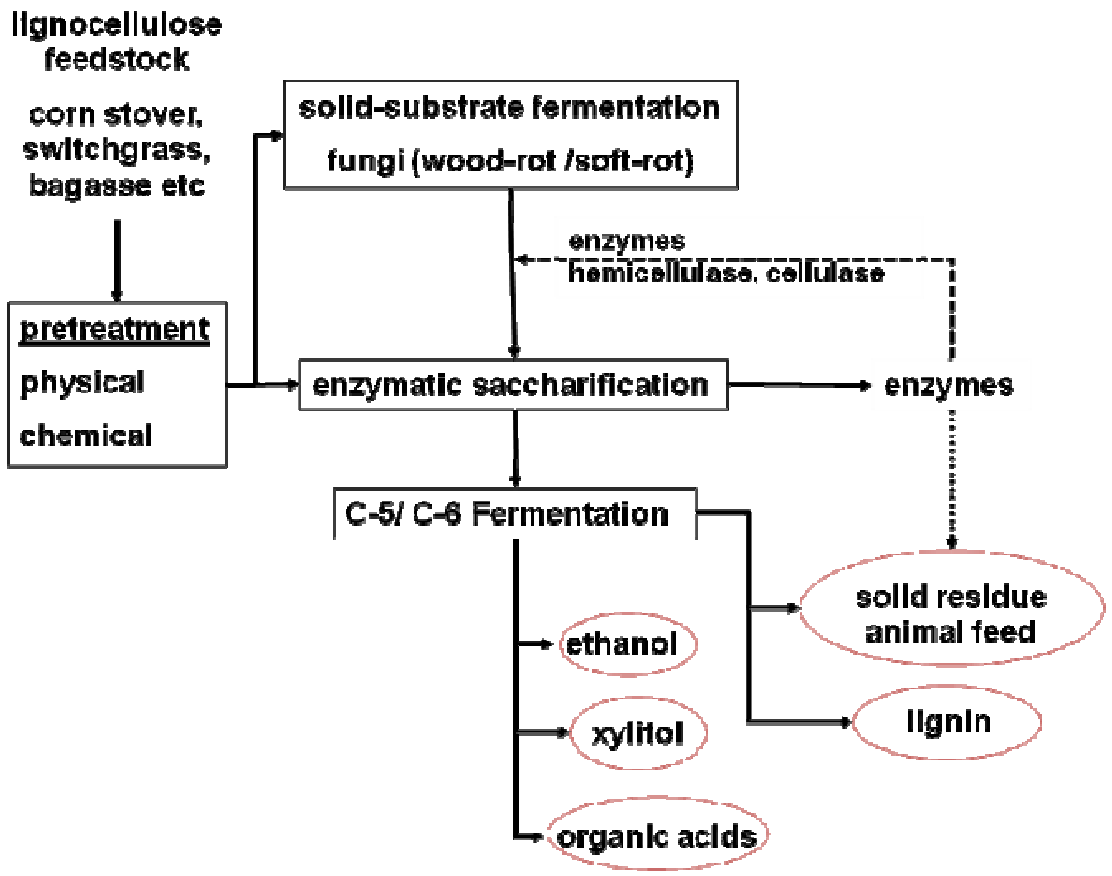

Fig1. Flow chart of Bioethanol production and other products

Wiegel (1982) reported the two steps process of converting cellulose to ethanol (1) hydrolysis of the polysaccharide and (2) fermentation of glucose to ethanol using yeast, Saccharomyces cerevisiae. Consolidated bioprocessing (CBP), which is the simultaneous biological hydrolysis and fermentation of biomass in a single process step by microorganisms that express cellulolytic (and hemicellulolytic) enzymes, (Lynd, L.R. et al. 2002, Lynd, L.R. et al. 2005, Demain, A.L. et al. 2005, and Carere C.R. et al. 2008). Wood rot fungi are the one of filamentous fungi and produce both of ligninase and cellulase. Ethanol production from lignocellulosic material requires a delignification step and a saccharification step and wood rot fungi are capable to participate as degrader. Additionally, some fungi can ferment ethanol like yeast. Therefore, wood rot fungi are suitable for ethanol production by CBP. Shrestha et al. (2008a) reported application of the white-rot fungus *Phanerochaete chrysosporium* in solid-substrate fermentation of lignocellulose material like corn fiber (co-product from wet milling plant) and subsequent simultaneous saccharification and fermentation to ethanol. Similar work was also examined by Rasmussen et al. (2008) using the brown-rot fungus *Gloeophyllum trabeum*. These studies on woodrot fungi opened a new frontier for biological saccharification and fermentation of lignocellulosic biomass to ethanol. These fungi were also reported to produce ethanol without yeast co-culture. White rot fungi had been studied mainly for degradation of lignocelluosic substrates (Cowling, 1961; Highley and Dashek, 1998) while cellulase activities have been extensively studied for *Trichoderma reesei* (Shulein, 1988). White rot fungi were incubated in the liquid media including 2% glucose as sugar under aerobic condition for 3 weeks and aliquot media were taken from the flasks on the 10th and 21st day. *Schizophyllum commune* and *Daedalea dickinsiii* showed better fermentation ability. *Flammulina veltipes* also showed ethanol fermentation as previous studies reported (Mizuno et al. 2009 and Maehara et al. 2013). Ethanol concentration on the 21st day was lower than that on the 10th day in some strains (Figure 1).

V. BIO-ETHANOL ECONOMY

The cost of bio-fuels is also an important consideration; biofuels must be competitive with each other and with mineral fuels such as petrol and diesel. This competitiveness ensures a market for the bio-fuel is available, as people will have an incentive to convert to a renewable source of energy. Thus when analyzing crop rotations cost optimization must also be considered (Murphy and Power 2009). The price of the raw materials is highly volatile, which can highly affect the production costs of the bio-ethanol (Yoosin and Sorapipatana 2007). Feedstock represents 60–75% of the total bio-ethanol production cost. Production technology from sugar/starch containing crops is relatively mature and most likely will not be improved to decrease production costs. Bio-ethanol from sugar cane in Brazil costs US$0.23–0.29 per litter (Kojima and Johnson 2005), while in the EU and the United States sugar and corn-derived bio-ethanol cost US$0.29 per litter (Mitchell 2008) and US$0.53 per litter (Christensen and Smith 2008), respectively. Estimates of the costs of bio-ethanol production from different feedstock are shown that bio-diesel production costs are generally lower than bio-ethanol production costs (The Royal Society 2008). The cost of ethanol production from lignocellulose is very high because additional processes, delignification and cellulose saccharification, are required. Reduction of manufacturing cost is serious issue in the biofuel production. Consolidated bioprocessing (CBP), which is the simultaneous biological hydrolysis and fermentation of biomass in a single process step by microorganisms that express cellulolytic (and hemicellulolytic) enzymes, is one of the solution for lower biofuel production costs (Lynd, L.R. et al. 2002, Lynd, L.R. et al. 2005, Demain, A.L. et al. 2005, and Carere C.R. et al. 2008). Wood rot fungi are the one of filamentous fungi and produce both of ligninase and cellulase. Ethanol production from lignocellulosic material requires a delignification step and a saccharification step and wood rot fungi are capable to participate as degrader. Additionally, some fungi can ferment ethanol like yeast. Therefore, wood rot fungi are suitable for ethanol production by CBP. Up to now the cost of bio-ethanol was considerably higher than the cost of fossil gasoline supply; national governments had to enact special policies in order to encourage production and use of bio-ethanol in the transportation sector. In general, the following three main approaches can be distinguished in the implementation of bio-fuels supporting policies and regulation :(1) taxation-based policies, (2) agriculture-based policies/ subsidies, and (3) fuel mandates (Smith 2008). At present, the development and promotion of bio-fuels are mainly driven by the agricultural sector and green lobbies rather than the energy sector. In fact, most bio-fuel programs depend on subsidies and government programs, which can lead to market distortion and is costly for governments. Nevertheless, at sustained high oil prices and with a steady progression of more efficient and cheaper technology, bio-fuels could be a cost-effective alternative in the near future in many countries (De Fraiture et al., 2008). The practicality and process validation of such processes would also be very beneficial to implement such technological concepts into long-term and sustainable transition to utilize abundant lignocellulosic biomass to fuel and other biobased products. Progressive exploration for wood-rot fungal enzymes should lead to techno-economic development of biological conversion of lignocellulosic feedstock to renewable biofuels and biobased products. This could well be the key to sustainable biofuel production to meet the growing need for transportation fuels.

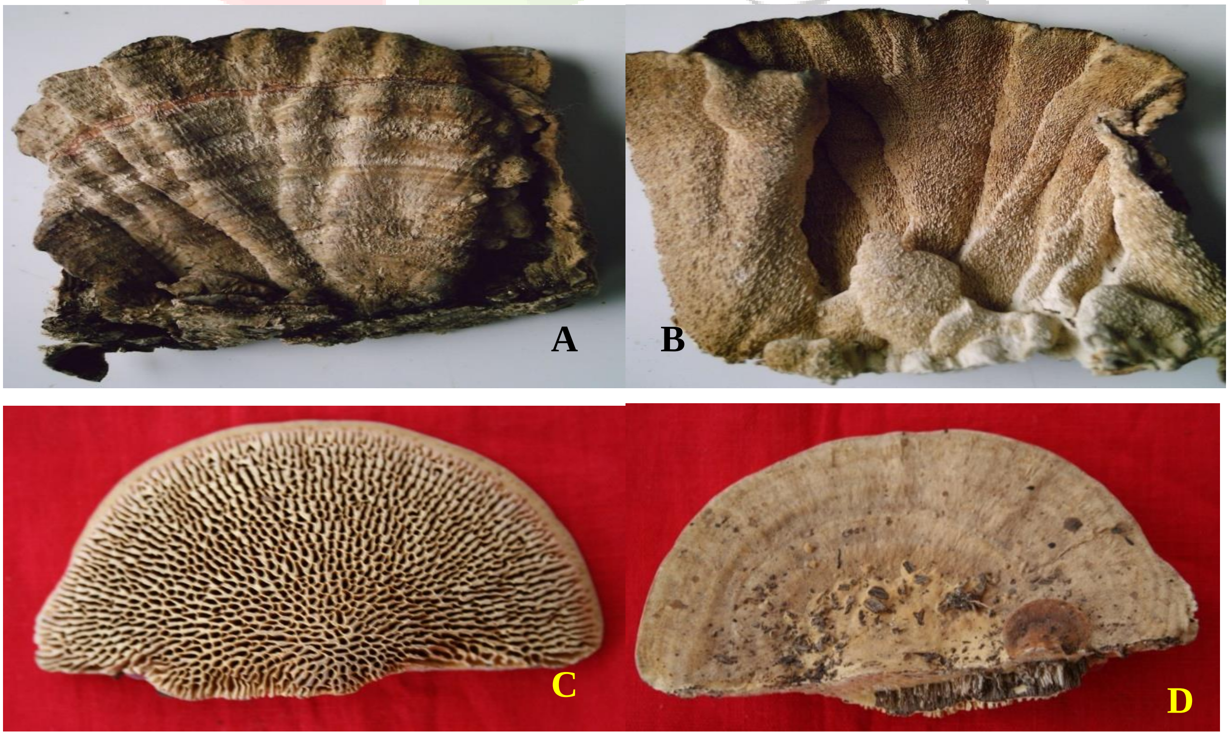

Figure 2. A. Upper surface of *Tremates versicolor* B. Lower surface of *Tremates versicolor* C. Upper surface of *Daedalea adamani* D. Lower surface of *Daedalea adamani* E. basidiocarp of *Pleurotus pulmonarius* F. basidiocarp of *Schizophyllum commune* G. Upper surface of *Ganoderma lucidum* H, Lower surface of *Ganoderma lucidum*

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