The sustained utilization of fossil fuels to meet the majority of the world’s energy demand is endangered by increasing concentrations of CO$_2$ in the atmosphere and concerns over global warming [1]. Plummeting use of fossil fuels would significantly cut the amount of CO$_2$ produced along with other pollutants [2]. To reduce the world’s dependence on petroleum-derived fuels alternatives are being sought after [3]. The only justifiable alternate to address this issue is to employ carbon based sources that are everlastingly available in enormous amounts, and which can be used without the problem of greenhouse gas emission. Plant biomass is by far the lone carbon source that can fulfill these requirements: it arises by carbon dioxide fixation during photosynthesis, and its dry weight consists mainly of three polymers (cellulose, hemicelluloses, and lignin) whose monomer constituents (hexose and pentose sugars and phenylpropan compounds) can be transformed to useful starting materials for industry by fermentation or biotransformation (the so-called biorefinery concept) [4].

However, an imperative step in the notion of employing plant biomass as “biofuels/biorefineries” is the production of the monomeric components such as hexose and pentose sugars in a sufficiently high concentration by means of technologies that do not release harmful by-products. The only process that can meet this requirement, in theory, is enzymatic hydrolysis which has been studied since the early 1960s. These studies have shown that cellulolytic, hemicellulolytic, and ligninolytic enzymes are predominantly produced by fungi, and some of these fungi have been successfully used for the production of enzymes utilized in the hydrolysis of plant cell wall material [4].

One of the biggest concerns with enzymatic hydrolysis is the price associated with the production of enzymes. The impact of enzyme costs to the economics of lignocellulosic biofuel production continues to be a hot topic for debate [5]. The cost and success of the biomass to bioethanol progression depend mainly on the inherent recalcitrance of biomass and the repertoire of enzymes involved in depolymerization of the constituent polysaccharides [6]. A solution to this problem is to find alternate ways for the production of enzymes which may be achieved by finding more potent microbial strains or by creating genetically modified strains that can excrete greater amounts of enzymes, or both [7].
For the production of lignocellulolytic enzymes, polysaccharides present in lignocellulosic materials (such as agro and forestry residues, herbaceous grasses and woody plants), including cellulose and hemicellulose are of immense importance [6]. Using lignocellulosic raw materials for enzyme production is advantageous. Another additional advantage of using lignocellulosic raw material is that it allows bioethanol production in countries with climatic conditions unsuitable for crops such as sugarcane or corn [8]. This raw material is less expensive as compared to conventional media components for the production of the enzyme. Lignocellulosic complex, the most abundant biopolymer on the earth comprises about 50% of world biomass [9]. Lignocellulose feedstocks which include agricultural and forest residues, industrial and municipal wastes, and dedicated energy crops, on account of their high carbohydrate content, hold remarkable potential for large-scale bioethanol production [10]. Lignocellulosic biomass comprises of cellulose (C₆H₁₀O₅)x, a homopolymer of glucose, in bound form along with hemicelluloses (C₅H₈O₄)m and lignin [C₉H₁₀O₃.(OCH₃)₀.9-1.7]n. Generally, lignocellulosic biomass contains about 40–60% cellulose, 20–40% hemicelluloses, and 10–25% lignin [11]. The cell wall polysaccharides can be hydrolyzed into monomeric sugars which are used for biorefining to produce a range of biomaterials.

The majority of plant biomass is available in the form pentose and hexose sugars, comprising mainly of cellulose (a glucose homopolymer); followed by hemicelluloses (a sugar hetero-polymer); and least of all lignin (a complex aromatic polymer). Both the cellulose and hemicellulose can be broken down enzymatically into the component sugars which may be then fermented to ethanol [11]. The classical model for degradation of cellulose to glucose involves the cooperative action of endocellulases (EC 3.2.1.4), exocellulases (cellobiohydrolases, CBH, EC 3.2.1.91; glucanohydrolases, EC 3.2.1.74), and beta-glucosidases (EC 3.2.1.21) [12]. Hydrolysis of hemicelluloses is brought about by enzymes like glycoside hydrolases, carbohydrate esterases, polysaccharide lyases, endo-hemicellulases and others, the concerted action of which hydrolyze glycosidic bonds, ester bonds and remove the chain’s substituents or side chains. These include endo-1, 4-β-xylanase, β-xylosidase, β-mannanase, β-mannosidase α-glucuronidase, α-L-arabinofuranosidase, acetylxylan esterase and other enzymes [13].

Cellulose, hemicellulose, and lignin are not just individual units in a plant cell wall but are intimately interlocked making it tough to deconstruct enzymatically [14]. Lignin and carbohydrates (e.g., cellulose and hemicellulose) together form the lignin–carbohydrate complexes [15]. Anchoring of lignin to plant-wall polysaccharides contributes to
recalcitrance [16, 17] by reducing the accessibility of cellulose to enzymes [18]. For a complete deconstruction of these heterogeneous structures in the plant cell wall synergistic reactions of enzymes, such as cellulases, hemicellulases, accessory enzymes and lignin-modifying enzymes is required [19].

Fungi and bacteria both have been profoundly exploited for their ability to hydrolyze lignocellulosic materials by producing a wide variety of cellulases and hemicellulases [20]. To date, the majority of enzymes developed and being tested for lignocellulose degradation are from fungi [21] because of their ability to produce profuse amounts of cellulases and hemicellulases secreted directly into the medium for easy extraction and purification [6]. *Trichoderma reesei* was one of the first cellulolytic organisms isolated in the 1950s. By 1976, a remarkable collection of more than 14,000 fungi showing activity against cellulose and other insoluble fibers had been collected [22]. Various wood-rot fungi like white- and brown-rot, have been reported to effectively degrade lignin, cellulose, and hemicellulose. They produce extracellular enzymes like ligninase, cellulase, and hemicellulase to degrade the lignocellulosic complex [23]. The lignin-degrading enzymes secreted by white-rot fungi enable them to completely mineralize lignin to carbon dioxide and water, in turn exposing the hemicellulose and cellulose in the wood matrix [24] which are hydrolyzed by conglomerates of hemicellulase and cellulase. In contrast to the white-rot delignification process, brown-rot fungi modify the lignin structure in the wood matrix [25] enabling the access of enzymes for holocellulose degradation. Nevertheless, filamentous higher fungi primarily the basidiomycetes, which cause white rot are the major degraders [26]. Lignin modification by soft rot fungi and brown rot fungi is limited, as their growth is generally limited to the outer surfaces of wood. White rot fungi are considered as the major lignin degrader [27]. White-rot basidiomycetes such as *Pleurotus ostreatus*, *Trametes versicolor*, *Phanerochaete chrysosporium*, *Ganoderma lucidum*, *Coriolus versicolor* and *Polyporus brumalis*, constitute a crucial source of organisms with lignocellulosic machinery for the production of extracellular ligninolytic (laccase) and hydrolytic (cellulases and hemicellulases) enzymes, which are responsible for the degradation of major substrate components of lignocellulosic biomass into value-added products [28, 29].

The fauna of North-Western Himalayan region of India still needs to be explored extensively and as such, the prospective for discovering novel strains capable of producing hydrolytic enzymes of industrial potential exists [30, 31]. Isolation and screening of microbial strains for the existence of an efficient lignocellulosic enzyme machinery is a routine research being done by bioethanol industries. Research has shown that some white
rot fungi possess cellulase and xylanase activities but are devoid of laccase activity, which plays a crucial role in the pre-treatment step. Dhiman et al. (2013) utilized rice straw as a substrate for production of an endoglucanase, cellobiohydrolase, and β-glucosidase in addition to xylanase, laccase, mannanase, and lignin peroxidase from a white rot fungus, Armillaria gemina SKU2114 [32]. For the production of lignocellulolytic enzymes, industries generally focus towards the production of engineered commercial enzymes such as Accellerase® Trio™ from Genencor and Cellic CTec3 from Novozymes which act in a synergistic manner to unlock and saccharify polysaccharides contained within the lignocellulose complex to fermentable sugars. The Accellerase® Trio™ is an amalgamation of multiple enzyme activities including exoglucanase, endoglucanase, hemicellulases (including xylanases), and β-glucosidase but lacks ligninolytic activity whereas Cellic CTec3 activity is limited to only cellulosic substrates [33, 34]. Therefore, the need for developing more potent efficient enzyme preparations arises for the enzymatic saccharification process to be more economical. This necessitates the isolation and screening of novel fungi capable of efficient degradation of lignocellulosic biomass by employing a proficient lignocellulolytic enzyme system.

Apart from the role of white rot fungi in bioconversion of lignocellulosic material to bioenergy, this fungal group is an effective bioremediator for toxic textile industrial pollutants because of their dye adsorption and contaminant degradation capabilities due to their efficient enzymatic machinery. Therefore, the biodegradation abilities of fungi are given particular emphasis in management of environment through green route.

The bioconversion of lignocelluloses relies heavily on significant technological innovations focusing on efficient and low-cost enzymes, feedstocks and efficient process design. For attaining these goals, understanding the role of individual, square and interaction effects of process variables on the ultimate output will have a crucial part. Response surface methodology (RSM), which lends the principles from statistical and mathematical fundamentals is a well-practiced approach in biological sectors for getting the effect of individual, square and interaction terms of process variables on the output through developing a non-linear regression equation [35-37]. Furthermore, the response optimizer function of RSM will prove to be helpful in predicting the process variables for getting an optimum output. Several researchers have acknowledged the modeling efficiency of RSM for industrially relevant lignocellulolytic enzyme production by basidiomycetes such as Phanerochaete chrysosporium [38], Lentinula edodes [39], Agaricus arvensis [40]. Application of RSM in the development of non-linear regression models and optimization
for multiple enzymes from a cocktail is scarce. Most of the studies have targeted the optimization of production of individual enzymes and/or optimization of degradation of lignocellulosic substrates with commercial enzyme preparations [41].

Understanding the importance and the necessity of identifying novel fungal strains capable of efficient lignocellulosic biomass degradation by engaging their proficient lignocellulolytic enzyme system, the following objectives were laid down for the present study:

**Objective 1:**
Screening and identification of fungal cultures for lignocellulolytic enzyme activities

**Objective 2:**
Optimization of lignocellulolytic enzyme activities by *Cotylidia pannosa* under submerged and solid-state fermentation using one factor at a time approach

**Objective 3:**
Optimization of lignocellulolytic enzyme activities by *Cotylidia pannosa* under submerged fermentation using Response Surface Methodology (Multifactorial approach)

**Objective 4:**
Characterization of crude enzyme cocktail for lignocellulolytic enzyme activities and its application in saccharification of wheat bran for bioethanol production and dye decolorization