1. INTRODUCTION

The enzymes, xylanase and pectinase are the upcoming enzymes of commercial sector and are widely used in paper and pulp industry, animal feed, textile industry, coffee and tea fermentation, oil extraction, waste paper recycling and in the fruit juice industries. These enzymes are the tools of nature that help us in providing everyday products in an environmentally conscious manner.

Schulze (1891) first introduced the term “Hemicellulose” for the fraction isolated or extracted from plant materials with alkali. Hemicellulose includes xylan, mannan, galactan and arabinan as the main heteropolymers. The principal monomers present in most of the hemicelluloses are D-xylose, D-mannose, D-galactose and L-arabinose.

Xylans, the substrate for xylanase, are hemicellulose compounds and are the second most abundant natural polysaccharide after cellulose (Collins et al., 2005). Xylan is the major hemicellulose in hardwood from angiosperms, but is less abundant in softwood from gymnosperms. It accounts for approximately 15-30% and 7-12% of the total dry weight in angiosperms and gymnosperms respectively (Whistler and Richards, 1970; Wong et al., 1988). It is mainly found in the secondary wall of plants and forms an interphase between lignin and cellulose. It consists of a backbone of β-1,4-xylopyranoside residues which are commonly substituted with acetyl, arabinosyl and glucuronosyl side chains. Frequency and composition of the branches are dependent on the source of xylan. The side chains determine the solubility, physical conformation and reactivity of the xylan molecule with the other hemicellulosic components and hence greatly influence the mode and extent of enzymatic cleavage (Kulkarni et al., 1999). The xylans from different sources exhibit a significant variation in composition and structure (Butt et al., 2008). The complete enzymatic hydrolysis of xylan into its constituent’s monosaccharides, requires the synergistic action of a group of xylanolytic enzymes. The most important and effective one is endo-β-1,4-xylanases (1,4-β-xylan xylanohydrolase; EC 3.2.1.8) that cleaves the β-1,4 bonds of xylan backbone and produces xylo-oligosaccharides. Complete hydrolysis of xylan to monomers involves β-xylosidases (E.C.3.2.1.37), which remove D-xylose residues from short xylo-oligosaccharides, and various debranching enzymes as acetyl xylan esterase (E.C.3.1.1.72), α-L-
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arabinofuranosidases (E.C.3.2.1.55) and α-D-glucuronidases (E.C.3.2.1.139). All of these enzymes act cooperatively to convert xylan into its constituent sugars. Among all xylanases, endoxylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and liberating short xylooligosaccharides. Xylanases are genetically single chain glycoproteins, with molecular weights ranging from 6-80 KDa.

For commercial application of industrial enzymes, microorganisms are the most important source of various enzymes which are produced by diverse genera and species of bacteria, yeast, actinomycetes and fungi. The xylanases have been reported from bacteria (Kulkarni et al., 1995; Gessesse and Gashe, 1997; Lopez et al., 1998; Marques et al., 1998; Ratanakhanokchai et al., 1998; Hamzah and Abdulrashid, 1999; Bataillon et al., 2000; Cordeiro et al., 2002; Hattori et al., 2002; Saleem et al., 2002; Tseng et al., 2002; Archana and Satyanarayana, 2003; Roy, 2004; Battan et al., 2006; Amani et al., 2007; Anuradha et al., 2007; Kapoor and Kuhad, 2007; Kiddinamoorthy et al., 2008; Sanghi et al., 2008; Yasinok et al., 2008; Dhiman et al., 2009; Prakash et al., 2009; Azeri et al., 2010; Mullai et al., 2010; Kapilan and Arasaratnam, 2011; Nagar et al., 2011; Prakash et al., 2012; Sugumaran et al., 2013; Tork et al., 2013), fungi (Tan et al., 1985; Singh et al., 1995; Christakopoulos et al., 1996a; Coral et al., 2002; Seyis and Aksoz, 2005a; Querido et al., 2006; Muthezhilan et al., 2007; Okafor et al., 2007; Cui et al., 2008; Knob and Carmona, 2008; Maciel et al., 2008; Nair et al., 2008; Xu et al., 2008; Betini et al., 2009; Gupta et al., 2009; Pal and Khanum, 2010; Amaro-Reyes et al., 2011), actinomycetes (Ball and McCarthy, 1989; Kluepfel et al., 1990; Timothy et al., 1997; Ding et al., 2004; Kumar et al., 2012) and yeast (Hrmova et al., 1984; Liu et al., 1998). Xylanases from different sources differ in their requirements for temperature, pH etc. for optimum functioning.

Recently, interest in xylanase has markedly increased in these areas such as improving the digestibility of animal feed stocks (Selle et al. 2009), biobleaching of paper pulp (Battan et al., 2007; Kiddinamoorthy et al., 2008; Manimaran et al., 2009; Savitha et al., 2009; Kaur et al., 2010), bioscouring of textile fibres (Dhiman et al., 2008b; Garg et al., 2013), production of xylo-oligosaccharides (Maalej-Achouri et al., 2009), waste-water treatment (Rani and Nand, 1996), clarification of fruit juices (Nagar et al., 2012a), deinking of waste paper (Pala et al., 2006; Lee et al., 2007; Xu et al., 2011; Ibara et al., 2012).
Pectin, another component of the plant cell wall that forms the major component of middle lamella, is found between the primary cell walls of adjacent young plant cells. It is a polysaccharide with a backbone of galacturonic acid residues, linked by α (1-4) linkage in which 75% of the carboxyl groups of the galacturonate units are esterified with methyl alcohol and others are combined with calcium or magnesium ions. They are commonly substituted with L-rhamnose, arabinose, galactose and xylose side chains. Chemically, they are called polygalacturonides and have high molecular weight. The molecular weight of different pectins varies from 10 KDa to 1000 KDa depending upon the source of pectin. Pectic substances is the generic name used for the compounds that are acted upon by the pectinolytic enzymes. These substances account for 0.5-4.0 % of the fresh weight of plant material (Sakai et al., 1993). Pectinases comprises a heterogeneous group of enzymes that catalyze the breakdown of pectin containing substrates. Pectinolytic enzymes are classified according to their mode of action on the galacturonan part of the pectin molecule. Pectinases include depolymerizing and demethoxylating enzymes. Depolymerizing enzymes are polygalacturonase (EC 3.2.1.15) and polymethylgalacturonase, which cleaves the α-1, 4 glycosidic bonds, and pectate lyase (EC 4.2.2.2) and pectin lyase (EC 4.2.2.10), which catalyses a β-elimination reaction. De-esterifying enzymes include pectin-esterase (EC 3.1.1.11), which catalyses the demethoxylation of methylated pectin, producing methanol and pectin (Priest, 1984).

Pectinases are also produced from a wide variety of microbial sources such bacteria (Itoh et al., 1982; Dosanjh and Hoondal, 1996; Hayashi et al., 1997; Soares et al., 1999; Kapoor et al., 2000; Kashyap et al., 2000; Takao et al., 2000; Kapoor and Kuhad 2002; Soriano et al., 2005; Ahlawat et al., 2008; Bayoumi et al., 2008; Jayani et al., 2010; Nadaroglu et al., 2010; Swain and Ray, 2010; Dey et al., 2011; Janani et al., 2011; Kumar and Sharma, 2012; Kumar et al., 2012; Qureshi et al., 2012; Das et al., 2013; Kothari and Baig, 2013; Roosdiana et al., 2013), fungi (Aguilar and Huitron, 1987; Abe et al., 1988; Stratifilova et al., 1996; Huang and Mahoney 1999; Marie et al., 2002; Pereira et al., 2002; Patil and Dayanand, 2006, Pericin et al., 2007; Makky 2009; Kumar et al., 2010; Mandhania et al., 2010; Rajasekharan et al., 2010; Gomes et al., 2011; Kumar et al., 2011; Mrudula and Anitharaj, 2011; Zeni et al., 2011; Demir et al., 2012; Johnson et al., 2012; Singh and Mandal, 2012; Vasanth and Meenakshisundaram, 2012; Pasha et al., 2013; Seifollah and Khodaverdi, 2013; Demir and Tari, 2014; Heerd et al., 2014).
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Pectinases have been used in various applications such as fruit juice clarification (Alkorta et al., 1982; Semenova et al., 2006), pulp and paper industry (Reid and Ricard, 2000; Ahlawat et al., 2007), animal feed (Barreto et al., 1989), retting of plant fibers (Hoondal et al., 2002), degumming of plant bast fibers (Kapoor et al., 2001), bioscouring of plant fibers (Jayani et al., 2005; Solbak et al., 2005), haze removal from wines (Revilla et al., 2003), coffee and tea fermentation (Gar, 1985; Angayarkanni et al., 2002), oil extraction (Scott, 1978), purification of plant viruses (Salazar and Jayasinghe, 1999), protoplast fusion technology (Kashyap et al., 2001a) and waste management (Kashyap et al., 2001a).

Xylanase and Pectinase have been produced in combination from a thermophilic fungus belongs to Deuteromyces (Gomes et al., 1992), Streptomyces sp. QG-11-3 (Beg et al., 2000c), Thermomyces lanuginosus (Mendoza et al. 2006), Aspergillus awamori (Botella et al., 2007), Bacillus pumilus (Kaur et al., 2011) and Aspergillus niger F3 (Rodríguez-Fernández et al., 2011). There are only few such reports of production of these enzymes in combination from the same isolate.

The major problem encountered by the industries is the availability of the enzyme at low cost. Production of enzymes using the commercial substrates is expensive. The high cost of enzyme production reduces the economic viability of the process. Hence, there is a need to increase its cost-effective production for industrial application purpose. The cost of carbon sources play major role in the economics of enzymes production. Hence, an approach to reduce the cost of enzymes production is the use of lignocellulosic materials as substrate rather than opting for the expensive substrate (Haltrich et al., 1996; Beg et al., 2000c). Several microorganisms are capable of using these agricultural residues as carbon and energy sources by producing a vast array of enzymes in different environmental conditions (Kaur et al., 2004; Geetha and Unasekaran, 2010; Bajaj and Wani, 2011).

Efforts on cost reduction have been directed towards increasing the enzyme production by isolating or developing better microbial strains, efficient fermentation and
recovery systems (Park et al., 2002; Xu et al., 2005). Enzyme production can be increased by introducing more potent strains and by generating mutants secreting higher levels of the enzyme. Random mutagenesis can enhance enzyme productivity several fold than the parental strain. Mutagenesis is a well recognized approach to enhance the expression of inducible enzymes. Modification and improvement of the strain through mutation are typically achieved by a variety of physical or chemical agents called mutagens. Although most mutations that change protein sequences are neutral or harmful, some mutations have a positive effect on an organism. In this case, the mutation may enable the mutant organism to withstand particular environmental stresses better than wild-type organisms, or reproduce more quickly.

Reducing the cost of enzyme production by optimizing the fermentation medium and the process is the goal of basic research for industrial applications (Park et al., 2002). In general, medium optimization by varying the traditional “one factor at a time” technique was used. This method is although simple, often requires a considerable amount of work and time. Recently statistical design methods for optimization of fermentation conditions have been successfully employed for enzyme production. These statistical experimental designs have been used for searching optimal enzyme production conditions.

Immobilization of enzymes on an inert carrier offers the prospect of significant cost savings by facilitating enzyme recycling through multiple cycles of batch-wise hydrolysis (Hudson et al., 2005). Moreover, enzyme immobilization results in improved thermal stability or resistance to shear inactivation (Tu et al., 2006). Immobilization of the enzymes provides the advantage of reusability and stabilization (Tischer and Kasche, 1999). Various carriers have been used for xylanase immobilization, such as chitosan (Naby, 1993), magnetic latex beads (Tyagi and Gupta, 1995), chitosan-xanthan hydrogel (Dumitriu and Chornet, 1997), Duolite A147 pretreated with glutaraldehyde (Gouda and Naby, 2002), cellulose acetate membrane grafted with acrylamide (Sarbu et al., 2006), chitosan and agarose (Manrich et al., 2010), and Dialdehyde starch linked chitosan (CS-DAS) (Chen et al., 2010). Pectinase has also been immobilized on various supports including nylon (Lozano et al., 1987), ion-exchange resin (Kminkova and Kucera, 1983), Silk (Zhu et al., 1988), chitin (Iwasaki et al., 1998), alginate (Busto et al., 2006; Ipsita et al., 2003).
Today the growing concern about the environment is giving rise to technological changes towards the environmentally acceptable and friendly processes. Major areas of this concern are the paper and textile industry.

Recycling of waste paper is an alternative process which can preserve the green plants and saves the environment in terms of pollution, water and energy (Imamoglu, 2006). Current deinking processes depend upon the use of large amount of environment damaging chemicals such as NaOH, Na$_2$SiO$_3$, Na$_2$CO$_3$, H$_2$O$_2$, chelating agent and surfactants (Zhang et al., 2008; Pathak et al., 2010). Use of these chemical based deinking methods produce toxic effluents which increase the COD values of water and hence resulting in costly waste water treatment (Zhang et al., 2008). Deinking using enzymes is less polluting, energy saving and also results in lower disposal problems. Enzymes which are being used in deinking are pectinases, hemicellulases, cellulases and lignolytic enzymes. These enzymes alter the fibre surface or bonds near the ink particle and remove the ink from the fibre surface. The released ink is then removed by washing or floatation (Jeffries et al., 1994; Xu et al., 2009, 2011; Pathak et al., 2010). Use of enzymes in deinking resulted in lower COD values thus reducing the cost of waste water treatment in an environment friendly manner (Gubitz et al., 1998).

Enzymes active at high temperature and alkaline pH values have great potential as they can be introduced at different stages of the bleaching process without requiring change in pH or temperature (Shohan et al., 1992). In biobleaching of kraft pulp, it increases the access of bleaching chemicals to the lignin layer by opening the pulp structure (Paice et al., 1988). Hemicellulases are also known to release the chromophores associated with carbohydrates (Patel et al., 1993). Furthermore, the enzyme treatment of kraft pulp releases the lignin-carbohydrate complexes (Yang and Eriksson, 1992). The enzyme aided bleaching results in less requirement of bleaching chemicals to attain the same extent of brightness as obtained by conventional bleaching and enhanced physical properties (Damiano et al., 2003; Sanghi et al., 2009). Reduction in bleaching chemicals would result in reduction of organochlorine compounds in the effluent.

Similarly in the textile industry, bioscouring is an alternative and more environment-friendly method to remove noncellulosic impurities from raw fiber to make the surface more hydrophilic (Li and Hardin, 1998). Xylanases and pectinases appeared to be the efficient enzymes for optimal scouring, being capable of depolymerising the
xylan and pectin, respectively, breaking it down to low molecular weight water-soluble oligomers, and thereby improving the absorbency and whiteness of the textile material (Csiszar et al., 2004; Dhiman et al., 2008b; Ahlawat et al., 2009). Conventional scouring is carried out at higher temperature with caustic soda solution, which has disadvantages such as fiber deterioration, high-energy consumption, and large amount of strongly basic waste water pollution (Karmakar, 1999; Lenting and Warmoeskerken, 2004). In contrast to drastic alkaline conditions conventionally used, treatment of fibers with xylan and pectin degrading enzymes does not affect the cellulose backbone and thus avoid fiber damage (Rouette, 2001). Strength of the fibers is not affected using enzymes in pretreatment processing, which makes the process viable. Thus, the enzymatic treatment is an efficient, ecofriendly and energy saving method, as it is done under mild reaction conditions without damaging the cellulose fibers and reduces the use of toxic chemicals used in scouring or bleaching. Some workers have proved bioscouring as an efficient alternative of scouring (Ossola and Galante, 2004; Sharma et al., 2005). Xylanases in conjugation with pectinases have been used in the degumming of bast fibres such as Jute, Flax, Ramie and Hemp (Beg et al., 2001a).

Xylanases are being used extensively in wheat based diets to counteract the effects of non starch polysaccharides (NSP) in broiler (Bedford and Schulze, 1998). Several researchers have used xylanase for treatment of poultry feed (Dusel et al., 1998; Danicke et al., 1999; Wu et al., 2004; Cowieson et al., 2005). Kocher et al. (2002) investigated the effect of enzymes (CMCase, hemicellulase and pectinase) treated feed on growth performance and metabolism in broiler chicks. Saleh et al. (2005) studied the effect of a mixture of pure enzymes (CMCase, hemicellulase and pectinase) and a commercial enzyme Energex on growth performance and metabolism in broiler chicks, given a maize-soybean meal diet. Babalola et al. (2006) observed improved apparent nitrogen and fiber absorption as well as feed transit time by the application of xylanase in poultry feed. The use of pectinase enzymes have also been reported by several workers (Igbasan et al., 1997).

Enhanced and cost-effective production of these enzymes is the focus of our research, which is essential for making the process commercially viable. Enzyme production may be enhanced by mutagenesis and statistical design methods. The production of these enzymes using agricultural wastes can be effective since they contain large amount of hemicellulose and pectin, which could serve as an inducer for the
simultaneous production of xylanase and pectinase respectively. Enhanced production of both the valuable enzymes from a bacterial isolate simultaneously in the same production medium using agro-wastes will reduce the production cost and ultimately help in making the process commercially viable. Use of this xylano-pectinolytic synergism for different applications will ultimately make the process cost effective and environmentally safe with drastic reduction in the requirements of conventional chemicals. Immobilization of enzymes will further reduce the cost for industrial applications due to their reusability. Keeping in mind the various industrial applications of xylanase and pectinase in combination, the present investigation has been carried out.

Important criteria for industrial implementation of enzyme technology include the availability of inexpensive, highly active enzyme preparations, with good characteristics and that can be easily obtained in bulk quantities.

- So, keeping in mind the above approaches, the research has been planned with the following objectives:

  - To increase the potency of the strain by mutagenesis.
  - Optimization of production conditions for xylanase and pectinase by statistical design methods.
  - Purification and Characterization of Acidic and Alkaline forms of xylanase.
  - Co-immobilization of xylanase and pectinase.
  - Industrial applications of xylanase and pectinase.