Enzymes are bio-active compounds or catalysts that regulate many chemical reactions in living tissues and cells (Prathyusha and Suneetha, 2011). It also catalyses various reactions involved in the preparation of different food products. Enzymes are one of the important tools in modern food industry because they simplify many intermediate processes during food processing. Bulk of the industrial enzymes fall into different groups, out of which, the most important group of enzymes is pectinase, used in fruit and vegetable processing industry. Their commercial application was first observed in 1930 for the preparation of wines and fruit juices (Oslen, 2000). Pectinases are one of the important and imminent enzymes of the commercial sector, especially, in the fruit juice industry as a pre-requisite for obtaining well clarified and stable juice with higher yields (Sandri et al., 2011). These enzymes have useful applications in paper, fruit and textile industries. Almost 75% of the estimated sale value among industrial enzymes in 1995 has been contributed by pectinases. As a result, pectinase is considered as one of the futuristically useful enzymes in commercial sector (Kashyap et al., 2001).

The plant cell wall is composed of polysaccharides and proteins. The wall polysaccharides are often classified into cellulose, hemicelluloses and pectin. Chemically, pectic substances are complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by α (1-4) linkages. The side chains of the pectin molecule consist of L-rhamnose, arabinose, galactose and xylose. The carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions. Based on the type of modifications of the backbone chain, pectic substances are classified into protopectin, pectic acid, pectinic acid and pectin (Be Miller, 1986). Protopectin is a parent pectic substance which on restricted hydrolysis yields pectin or pectinic acid. Pectic acids or pectic substances are composed of colloidal polygalacturonic acid, free of methyl ester groups. Pectinic acids are the colloidal polygalacturonic acids containing various...
amounts of methyl ester groups. Pectins are the mixture of widely differing compositions containing pectinic acid as the major component and are located in the cell wall interlinked with other structural polysaccharides and proteins to form insoluble protopectin. These are present in the plants as the major component of middle lamella in the form of calcium pectate and magnesium pectate.

Pectinases are high molecular weight, negatively charged, acidic glycosidic macromolecules that breakdown complex polysaccharides in plant tissues into simpler molecules with extraordinary specificity, catalytic power and substrate specificity (Approvi and Vuppu, 2012). Pectinases are produced during the natural ripening process of fruits where, it splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Softening of the cell wall and increase in the yield of juice extract from the fruits takes place during this process. Depending on their specificity and the type of reaction they catalyze, pectinase group of enzymes include polygalacturonase, pectin esterase, pectin lyases and pectate lyase. Protopectinases convert insoluble propectin into soluble pectin. Polygalacturonases (PGases) catalyse the hydrolytic cleavage of the polygalacturonic acid chain through the introduction of water across the oxygen bridge. They are the most extensively studied among the family of pectinolytic enzymes (Maria et al., 2006). Exo-PGases (E.C. 3.2.1.67) act on terminal monomers of polygalacturonic acid and release monogalacturonic acid. Endo-PGases (E.C. 3.2.1.15) act on polygalacturonic acid randomly and release oligogalacturonic acid. Pectin lyase (E.C. 4.2.2.10) perform non-hydrolytic breakdown of pectates or pectinates. Pectin esterase (E.C. 3.1.1.11) catalyzes the de-esterification of methyl ester linkages of the galacturonan backbone of pectic substances to release acidic pectins and methanol (Jayani et al., 2005).

Pectinases from food and food bio products processed waste, alone account for a total of one-third of the world’s food enzyme production and are one of the upcoming enzymes of the commercial sector today. By-products or waste obtained from orange, apple, grapes, pine apple, papaya, lemon juice manufacturing industries are also used as a source for the enzyme production (Prathyusha and Suneetha, 2011).
Fruit waste as substrate (Plate 1 and 2)

Fruits, the gift of nature are an important constituent of human diet and are the vital source of nutrient to human beings. Vitamins, fats, minerals and oil in the right proportion is necessary in the daily diet for growth and development of human beings. But fruits have serious challenges to their existence, like changes in climate conditions, pests, inadequate rainfall and fungal attack. It is estimated that about 20-25 per cent of the harvested fruits are decayed by pathogens during post harvesting, storage and transportation, thus causing increased amount of fruit waste. Enormous quantities of industrial waste residues are also generated throughout the world from processing of raw agricultural materials of the food and fruit processing industries. In some fruits, the discarded portion can be very high (e.g. in mango 30-40%, in banana and papaya 20% and in orange 30-50%). Thus, the utilization of renewable resources, particularly agricultural residues, has captured world-wide attention and extraction of enzymes from bio-wastes, using the technology of fermentation is one of the many ways of exploiting them profitably. The large amount of waste from agricultural and fruit processing industries became a prominent section for biological utilization of this waste (Jose et al., 2008). The agro industrial by-products can be used for the production of high value products as it prevents the environmental pollution caused by them. Recently, global trends have increased towards the efficient utilization of natural resources (Dhillon et al., 2013).

Identification and bioconversion of locally available agro-waste is advantageous as it not only leads to value addition of these residues, but also helps to keep the environment clean. Agro industrial by-products can be successfully utilized for the microbial pectinolytic enzyme production and as these are locally abundant low cost raw materials, they can be used for cost effective enzyme production. Agricultural and food processing waste such as sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace,
peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, citrus waste, and fruit pomace are the most commonly used substrate for pectinase production. Thus, fruit wastes generated from food industry can be utilized to biosynthesize pectinase enzyme at a cheaper rate which has numerous industrial applications (Patil et al., 2012).

For industrial use, pectinases can be produced from several agricultural pectin-containing wastes (apple pomace), but the main source is from citrus or orange peel. Orange peel contains soluble sugars and pectin as the main components. According to Rivas et al. (2008) the orange peel is in fact constituted by soluble sugars-16.9 % (w/w), starch- 3.75 % (w/w), fiber (cellulose)- 9.21 % (w/w), hemicelluloses-10.5 % (w/w), lignin-0.84 % (w/w) and pectins-42.5 % (w/w), ashes-3.50 % (w/w), fats -1.95 % (w/w) and proteins-6.50 % (w/w). Pomace is the solid remains of grapes or other fruits (pulp, peels, seed and stalk of the fruit) after pressing for the juice or oil. Banana waste is one of the important fruit wastes available worldwide as it is as a common table fruit. Banana waste includes mainly the leaf, pseudo stem and the banana peel, which can be utilized for the production of the enzyme.

Role of Microbes in Pectinase Production

The two major sources of the enzyme pectinase are plants and microorganisms. In nature, microorganisms have been endowed with vast potentials which can be exploited for the utilization of waste material. The main source of the microorganisms that produce pectinolytic enzymes are yeast, bacteria and large varieties of fungi, insects, nematodes and protozoas (Jayani et al., 2010). Thus, by breaking down pectin polymer for nutritional purposes, microbial pectinolytic enzymes play an important role in nature (Yadav et al., 2009). Microbes are a rich source for enzymes and the exploration of extracellular enzymatic activity from them has formed the basis of industrial enzymes of which 50 per cent is from fungi and yeast, 35 per cent from bacteria and the remaining 15 per cent are from either plant or animal origin. Fungi, with their well characterized biology, have been widely exploited as sources of
industrially important enzymes. Fungal pectinases are mainly extracellular enzymes, prominent among them being polygalacturonase, which is also most commonly assayed to determine pectinase activity. Pectinase is produced by several fungi including *Aspergillus* sp., *Botrytis cinerea*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Trichoderma* sp., *Neurospora crassa*, *Penicillium* and *Fusarium* (Joshi et al., 2006). However, *Aspergillus*, *Penicillium*, *Fusarium*, *Pythium*, *Colletotrichum*, *Aureobasidium pullulans*, *Paecilomyces clavusporus*, *Phytophthora*, *Rhizoctonia solani*, *Neurospora crassa*, *Rhizopus stolonifer*, *Thermomyces lanuginosus*, *Alternaria mali*, *Thermoascus aurantiiacus*, *Saccharomyces cerevisiae*, *Lachnospira pectinoschiza* and *Erwinia*, *Agrobacterium*, *Bacteroides thetaiotamicron*, *Ralstonia*, *Bacillus* sp., *Pseudomonas*, *Lactobacillus* have been the genera most frequently studied from the past 15 years (Jayani et al., 2005).

Separation and purification of proteins and enzymes, account for a fraction of the overall production cost. Purification of enzymes are challenging because it is important to purify enzymes that are active over a long period of time in sufficient amounts. Thus, purity and yield are the two most important parameters for selection and design of pectinases. An improved knowledge of the properties of microbial pectinases is important in commercialisation of industrial production and application of these enzymes in various potential fields. However, before advocating the usefulness of enzyme, purification of the enzyme is a pre-requisite as it is very essential to characterize the enzyme produced so that it could be made into a commercial product. Pectinases from various sources of microorganisms have been purified to homogeneity by different chromatographic procedures such as gel filtration and ion exchangers with different recovery and purification yield (Gummadi and Panda, 2003).
Purification and Characterization of Pectinase from Paecilomyces variotii and Its Effect on Bioscouring of Cotton Fabrics and Clarification of Fruit Juices

Materials and Methods

Plate 1
Fruit Waste

Plate 2
Soil containing Decomposed Fruit Waste
Fungal Inoculant (Paecilomyces variotii) (Plate 3 and 4)

Paecilomyces is a cosmopolitan filamentous fungus, which is found in the soil, decaying plants, and food products. It belongs to the Phylum Ascomycota of Class Euascomycetes, Paecilomyces variotii are thermophilic and can grow well at temperatures as high as 35°C and possibly at 50°C. The colonies are flat, powdery or velvety in texture and the colour is initially white but becomes yellow and then yellow-green. Mycelium is septate hyaline with conidiophores bearing dense verticillatally arranged branches bearing cylindrical or ellipsoidal conidia in basipetal succession. Conidiophores are often branched at their tips and carry phialides which are swollen at their bases and gradually tapering towards their apices.

Plate 3
Plate Culture of Paecilomyces variotii

Plate 4
Mycelium with conidia of Paecilomyces variotii
Applications of pectinase

In recent years, there has been a great increase in industrial applications of enzymes owing to their significant biotechnological potential. However, pectinase was put into commercial use for the first time in 1930 for the preparation of wines and fruit juices. But the chemical nature was apparent only in the 1960s and with this knowledge, scientists began to make greater use of this wide range of enzymes more efficiently. Pectinases have attracted attention globally as biological catalysts in numerous industrial processes. These enzymes are used in processing agricultural and agro-industrial waste (Patil and Dayanand, 2006b) for the production and clarification of fruit juices to improve the cloud stability of fruit and vegetable juices and nectars, for depectinization in order to produce high density fruit juice concentrates and for haze removal from wines. As a result, today pectinases are one of the promising enzymes of the commercial sector. These enzymes are primarily responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary cell walls of young plant cells (Kashyap et al., 2001). Other industrial applications of pectinase include scouring of cotton, degumming of plant fibres, waste water treatment, vegetable oil extraction, tea and coffee fermentations, bleaching of paper, and as feed additives in poultry (Rangarajan et al., 2010).

Alkaline microbial pectinase reveals a great significance in the current biotechnological arena with wide ranging applications in textile processing, degumming of plant bast fibers, treatment of pectic waste waters, paper making, and coffee and tea fermentations (Pasha et al., 2013). The largest industrial application of pectinases is in fruit juice extraction and clarification. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. Treatment of fruit pulps with pectinase also showed an increase in fruit juice volume from banana, grapes and apples (Kaur et al., 2004). With the addition of pectinases, the viscosity of the fruit juice drops, the pressability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained with higher yields.
Materials and Methods

In textile industry, bio-scouring is a novel process for removal of non-cellulosic impurities from the fibre with specific enzymes. Pectinases have been used for this purpose without any negative side effect on cellulose degradation (Hoondal et al., 2000). The chemical degumming treatment is toxic and non-biodegradable but using pectinases in combination with xylanases presents an eco-friendly and economic alternative to the problem (Kapoor et al., 2001). Pectins are responsible for the hydrophobic properties of raw cotton and its degradation by pectinolytic enzymes was also suggested to facilitate the removal of waxes and could thus, lead to a considerable reduction in the rate of water and chemicals consumption.

Pectinase treatment accelerates tea fermentation and also destroys the foaming property of instant tea powders by destroying pectins (Carr, 1985). They are also used in coffee fermentation to remove mucilaginous coat from coffee beans. During paper making, pectinase can depolymerise pectins and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching (Reid and Richard, 2004).

The present investigation therefore, aims to advance the state of the art, production of industrially valuable pectinase from Paecilomyces variotii isolated from soil containing decomposed fruit waste with further exploration of new and innovative applications. With this background, the present investigation entitled “Purification and Characterization of Pectinase from Paecilomyces variotii and its effect on Bioscouring of Cotton Fabrics and Clarification of Fruit Juices” was undertaken with the following objectives.

- To isolate native mycoflora from soil containing decomposed fruit waste.
- To determine the pectinolytic activity of isolated native mycoflora.
- To assess the enhancement of enzyme production by Paecilomyces variotii in different carbon and nitrogen sources and fruit waste as substrates
Material and Methods

- To optimize the pectinase production by *P. variotii* at different pH and temperature.

- To purify pectinase obtained from *Paecilomyces variotii*.

- To characterize the purified enzyme.

- To analyze the inhibitory activity of purified pectinase on metal ions.

- To assess the efficacy of the purified pectinase in bioscouring of cotton fabrics and clarification of fruit juices.