CHAPTER 2.

REVIEW OF LITERATURE
2.1 Introduction

Chicken feathers are waste products of the poultry industry. Billions of kilograms of waste feathers are generated each year by commercial poultry processing plants creating a serious solid waste problem in many developing countries (Xu et al., 2009). Feathers constitute up to 10% of the total chicken weight, reaching more than $7.7 \times 10^8$ kg/year as a by-product of the poultry industry (Grazziotin., et al., 2006). Traditional disposal strategies of chicken feathers are very expensive and more difficult. They are often burned in incineration plants, buried in landfills or recycled into low quality animal feeds. These disposal methods are restricted, generate greenhouse gases or pose danger to the environment. Several commercial applications have been explored to utilize chicken feathers as raw material. However, due to the low volume requirements of these products they had not significantly reduced the volume of feathers generated each year. In this concern, this research review highlights the biological degradation of feather and its application in agriculture as organic fertilizer.

2.2 Poultry Feather

2.2.1. Feather Structure

A chicken's wing has several flight feathers. As (Figure-1) a shows, the axial feather separates the primary feathers and secondary feathers. When a hen molts, she starts losing feathers from the axial feather out. Feather makes up about 8.5% of a chicken’s mass and function as insulation, protection, waterproofing, colouration and flight (Mokrejs, 2011) of the birds.

Feathers are made out of keratin, the same protein found in hair and nails. Feathers have a central shaft. The smooth, unpigmented base, which extends under the skin into the feather follicle, is called the calamus. The portion above the skin, from which the smaller barbs or branches extend, is termed the rachis or scapus. On each side of the rachis there is a set of filaments, called barbs, which come off at approximately a 45º angle. This portion of the feather that has barbs is called the vane. In the larger feathers, these barbs have two sets of microscopic filaments called barbules. Barbules from one barb cross the adjacent barbs at a 90º angle. Barbules, in turn, have hooklets, sometimes called hamuli or barbicels, which hook the barbules together, like a zipper, forming a
tight, smooth surface. These maintain the shape of the feather. Without these strong linkages, the feather would not be able to withstand the air resistance during flight. The barbs or hooklets may become separated from each other; if this occurs, the bird can reattach them while preening. At the base of the feathers, there are often barbs that are not hooked together. These are called downy barbs (Rupley, 1997; Bauck et al., 1997; Olsen and Orosz, 2000).


Feathers are not arranged haphazardly on the bird, but in major distinct tracts called pteryla. The featherless areas between the pteryla are called apteria (Rupley, 1997; Bauck et al., 1997; Olsen and Orosz, 2000).
2.2.2 Composition

Chicken feathers contain nutrients approximately 91% protein (keratin), 1% lipids, and 8% water (Onifade et al., 1998; Cheung, 2009). The amino acid sequence of a chicken feather is exactly same as that of other feathers and the amino acid sequence is mainly composed of cystine, glutamine, proline and serine (Mabrouk, 2008). However other amino acids such as histidine, lysine, tryptophan, glutamic acid and glycine are almost absent. Serine (16%) is the most abundant amino acid in chicken feathers (Kannappan and Bharathi, 2012).

Keratins are insoluble proteins present in feathers, wool, hooves, scales, hair, nails (hard keratins) and also in stratum corneum (soft keratins). Chicken feather fiber basically consists of α-helical and some β-sheet conformations. Its outer quill is almost entirely made up of β-sheet conformations and few α-helical conformations. Hard β-sheet keratins have higher cystine content than soft α-helix keratins and thus a much greater presence of disulphide (S-S) bonds that link adjacent keratin proteins. The presence of strong covalent bonds stabilizes the three-dimensional protein structure and is very difficult to break (Brandelli et al., 2010; Jeong et al., 2010; Chojnacka et al., 2011; Kannappan and Bharathi, 2012). These specific proteins which belong to the scleroprotein groups are compounds that are highly resistant to physical, chemical and biological actions. Mechanical stability and high resistance to proteolytic degradation of keratin is due to the presence of disulfide bonds, hydrogen bonds, salt linkages and cross linkages. The disulphide bonds of β-keratin can be reduced by the enzyme disulphide reductase (Yamamura et al., 2002) followed by proteolytic keratinases (Gupta and Ramnani, 2006). Keratin, a hard to degrade insoluble animal protein, represents 90% of this keratinous waste (Bockle et al., 1995)
2.3 Waste disposal methods

There are several feather waste disposal methods available today, which can be used for synthesis of several beneficial end products such as feather meal, feed supplements, compost, nitrogen fertilizer, amino acids etc. Feather waste processing methods such as steam pressure, chemical treatment and feather milling are cost and labor intensive and may reduce the product’s nutritional value by destroying important amino acids (Shih, 1993; Wang and Parsons, 1997). Feather waste is also rendered by either steam or chemical treatment to produce feather meal (Valtcho and Zheljazkov, 2005). A current value-added use for feathers is the conversion to feather meal, a digestible dietary protein for animal feed, using physical and chemical treatments; other uses are as low cost substrates for the production of the enzymes and other value-added microbial products (Ko et al., 2010, Lateef et al., 2010, De Siqueira et al., 2010). Worldwide, commercial poultry feathers are currently converted to feather meal through steam pressure and chemical treatment (Jayalakshmi, et al., 2012).

2.4 Chicken feather Utilization

Chicken feather is the cheapest source for production of feather meal, keratin, animal feed supplement, fibers and as compost.

2.4.1 Feather meal:

Feathers are also converted to feather meal with usage as animal feed, organic fertilizers and feed supplements, as it is made up of >90% protein and are rich in hydrophobic amino acids like cystine, arginine and threonine. One of the most common methods of feather meal production is hydrothermal process where feathers are digested under high pressure at high temperature. However, hydrothermal treatment leads to destruction of essential amino acids like methionine, lysine, tyrosine, tryptophan that accounts to poor digestibility and low nutritional value (Ekta and Rani, 2012).

2.4.2 Chemical hydrolysis:

Chicken feather keratin when treated with lime (calcium hydroxide) to get a liquid product rich in amino acids and polypeptides, can be used as an animal feed supplement. At high temperatures (150°C), 80% of feather keratin is solubilised within 25 min.
However a relatively longer reaction time (300 min) is needed at moderate temperatures (100°C). After 3 h of hydrolysis at 150°C, 95% of feather keratin is digested. Under the recommended conditions (100°C, 300 min, and 0.1 g Ca(OH)2/g dry feather), after lime treatment, about 54% of calcium can be recovered by carbonating. In rumen fluid, ammonia production from soluble keratin similar to that of soybean and cottonseed meals and is greatly less than that of urea, showing that ammonia toxicity will not result from cattle being fed with soluble keratin (Coward-Kelly, 2006).

2.4.3 Bio diesel:

Slaughter house wastes like feathers, blood, and innards are being processed and utilized as high-protein animal feed sources or as fertilizer due to its high nitrogen content. It is estimated that these wastes contain up to 12 per cent fat. Scientists from the University of Nevada isolated the animal fat and successfully produced biodiesel comparable to biodiesel from other feed stock. Environmental friendly processes are developed for the production of biodiesel from feather meal. In biodiesel production, primarily fat is extracted from feather meal in boiling water (70°C) and subsequently trans-esterified into biodiesel using potassium, nitrogen and methane; 7-11% biodiesel (on a dry basis) is produced in this process. ASTM analysis confirmed that biodiesel from feather meal is of good quality when compared to other biodiesel made from other common feed stocks.

2.4.4 Technical textiles:

From the late 1970’s patent literature would suggest that poultry feathers were dry-cleaned and used for quilts, pillow filling and jacket insulations (Kruchen, 1979; Fleet and Hewinson, 1985). Printed circuit boards for the electronics industry can be made from keratin feather fibres infused with acrylated epoxidised soybean oil (AESO) (Hong and Wool, 2005). The nonwoven is prepared by using low cost chicken feathers. The advantage of application of chicken feathers in textile field are wide. The nonwoven textile materials prepared by chicken feathers are very versatile and have a wide application in the field of technical textiles (Chinta et al., 2013).
2.4.5 Biodegradable plastic:

Poultry feathers are also converted into biodegradable plastics by a process called polymerization. In this process, feathers which contain keratin protein are pulverized into fine dust. Chemicals that make keratin molecules to join together are used to form long chains (polymerization). It is further moulded into various shapes when heated at 170°C. These thermoplastics can be popularised to manufacture all kinds of products, from plastic cups and plates to furniture.

2.4.6 Fertilizer:

A slow release nitrogen fertilizer is developed from poultry feathers. In this attempt, the structure of keratin fibres are modified by steam hydrolysis for 12 weeks to break disulphide bonds, enzymatic hydrolysis by *Bacillus licheniformis* to break polypeptide bonds and steam hydrolysis (autoclaving) to improve mineralisation followed by cross linking of protein by formaldehyde reaction to minimize excess mineralisation (Jong-Myung Choi and Paul V. Nelson, 1996).

2.5 Bioconversion of chicken feathers

Feather wastes are utilized on a limited basis only as a dietary protein supplement (as feather meal). Initially, the feather wastes can be cooked either with steam or chemically to make it more digestible, but such treatments are expensive. Meanwhile, microorganisms play an alternative role to increase the biovalue of feather wastes. It has already been reported that the feather-lysate produced by *Bacillus licheniformis* PWD-1 has nutritional features for feed similar to soybean protein. Even though bacterial keratinolytic proteases showed a potential for feather bioconversion, improvement of enzyme activities and higher yields are required to make these suitable for industrial applications (Kim *et al.* 2001).

2.5.1 Keratinolytic microorganisms

Microorganism is the good source of keratinases. Feather keratin is hydrolysis by microbial enzymes (Shih, 1993). Extensive studies reported that the fungi and bacteria from soil, poultry waste dumping regions and the liquid and solid waste of poultry meet processing industries. Many fungi especially that belongs to fungi imperfecti have high,
keratinolytic activity including the following genera: *Chrysosporium*, *Aspergillus*, *Alternaria*, *Trichurus*, *Curvularia*, *Cladosporium*, *Fusarium*, *Geomyces*, *Gleomastis*, *Monodictys*, *Myrothecium*, *Paecilomyces*, *Stachybotrys*, *Urocladium*, *Penicillium Doratomyces* (Santos et al., 1996; Gradisar et al., 2000; Farag and Hassan, 2004; Marcondes et al., 2008; Ismail et al., 2012). The biggest group of organisms that can utilize keratin as sole source of carbon and nitrogen are the fungus (Agarwal et al., 2008; Sakthi et al., 2012).

Burtt and Ichida, (1999) showed that the keratin hydrolyzing bacteria could also occur in the plumage of living birds. Bacillus is the dominant genera for keratinolytic activity. Different species of *Bacillus* spp. has been reported for bacterial keratinase production. *Bacillus* (Williams et al., 1990; Lin et al., 1999; Manczinger et al., 2003; Suntornsuk, 2003; Zerdani et al., 2004; Suntornsuk et al., 2005). *Bacillus cereus* (Nagal and Jain, 2010; Wei-Hsun et al., 2012), *Bacillus licheniformis* (Williams et al., 1990; Ichida, 2001; Evans, 2000; Zhao et al., 1998; Hsin-Hung and Li-Jung (2010), *Bacillus sp.* (Kao Ming-Muh and Hsing-Yao, 1995; Govarthanan et al., 2011; Ghasemi et al., 2012), *Bacillus thuringiensis* (Sivakumar et al., 2012), *Bacillus megaterium* (Anitha and Eswari 2012; Saibabu et al., 2013), *Bacillus pumilis* (Burtt and Ichida, 1999; Agrahari 2013), *Bacillus pseudofirmus* FA30-01 (Kojima et al., 2006), and *Bacillus subtilis* (Macedo et al., 2005).

*Arthrobacter* sp (Lucas et al., 2003), *Microbacterium* sp (Thys et al., 2004 and *Kocuria rosea* (Bernal et al., 2006) and gram-negative bacteria such as *Vibrio* sp (Sangali and Brandalli, 2000), *Xanthomonas maltophilia* (De Toni et al., 2002), *Chryseobacterium* sp (Riffel et al., 2003; Wang et al., 2008).
Table 2-1: Microbial source for keratinses

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Source</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><em>Burkholderia</em>, <em>Chryseobacterium</em>, <em>Microbacterium</em> and <em>Pseudomonas</em> spp.</td>
<td>Feather waste</td>
<td>Riffe and Brandelli (2006)</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp.</td>
<td>Poultry plant waste water</td>
<td>Tapia and Simoes (2008)</td>
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<tr>
<td><em>Bacillus subtilis</em> KD-N2</td>
<td>Soil</td>
<td>Chen <em>et al</em>., 2008</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Poultry industry wastes, soil, water, fodder and feather</td>
<td>Tork <em>et al</em>., 2008; Sharma and Gupta 2010</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> F7-1</td>
<td>Feather waste</td>
<td>Geun-Tae and Hong-Jooson (2009)</td>
</tr>
<tr>
<td><em>Thermoanaerobacter</em>, <em>Chryseobacterium</em> sp</td>
<td>Feather waste</td>
<td>Riffel and Brandelli, 2002</td>
</tr>
<tr>
<td><em>Flavobacterium</em></td>
<td>Feather waste</td>
<td>Riffel and Brandelli, 2002</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em> YJ4</td>
<td>Feather waste</td>
<td>Hsin-Hung and Li-Jung, 2010 Nam <em>et al</em>., 2002</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>Feather waste</td>
<td>Sangali and Brandelli, 2000</td>
</tr>
<tr>
<td><em>Xanthomonas maltophilia</em></td>
<td>Feather waste</td>
<td>De Toni <em>et al</em>, 2002</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> Wu2</td>
<td>Poultry farm</td>
<td>Wei-Hsun <em>et al</em>., 2012</td>
</tr>
<tr>
<td><em>Streptomyces fradiae</em></td>
<td>Feather waste</td>
<td>Pugh, 1965; Hubalek, 1976; Hubalek, 1978</td>
</tr>
<tr>
<td><em>Streptomyces pactum</em></td>
<td>Feather waste soil</td>
<td>Bokle <em>et al</em>., 1995</td>
</tr>
<tr>
<td><em>Streptomyces fradiae</em></td>
<td>Feather waste soil</td>
<td>Kunert, 1989; Sinha <em>et al</em>., 1991</td>
</tr>
<tr>
<td><em>Streptomyces pactum</em> and <em>Streptomyces albidoflavus</em></td>
<td>Feather waste soil</td>
<td>Ignatova <em>et al</em>., 1999; Bressollier <em>et al</em>., 1999</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. <em>Saccharothrix xinjiangensis</em></td>
<td>Feather waste</td>
<td>Saha and Dhanasekaran 2010; Jani <em>et al</em>., 2014</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> C11</td>
<td>Feather dumping soil</td>
<td>Han <em>et al</em>., 2012</td>
</tr>
<tr>
<td><em>Bacillus Subtilis</em>, <em>Bacillus amyloliquefaciens</em> and <em>Bacillus velesensis</em></td>
<td>Amazon basin</td>
<td>Brandelli &amp; Riffel, 2005</td>
</tr>
</tbody>
</table>
2.5.2 Keratinase enzyme production

Bacterial keratinases are of particular interest because of their action on insoluble keratin substrates, and generally on a broad range of protein substrates (Lin et al., 1995). Bacterial extracellular keratinase is a metalloprotease with great potential for Biotechnological applications (Riffel et al., 2003). These enzymes have been studied for de-hairing processes in the leather industry (Raju et al., 1996) and hydrolysis of feather keratin (Lin et al. 1995). Gram-positive and Gram-negative bacteria are reported to be the keratinase producers, *Vibrio* (Sangali and Brandelli, 2000), *Xanthomonas* (De Toni et al., 2002), *Flavobacterium* (Riffel and Brandelli, 2002) and *Chryseobacterium* (Lucas et al., 2003), *Bacillus licheniformis* (Williams et al., 1990), *Bacillus subtilis* (Macedo et al., 2005), *Bacillus pumilus* FA30-01 (Kojima et al., 2006) *Bacillus megaterium* F7-1 (Geun-Tae and Hong-Jooson2009) etc. Brandelli and Riffel, (2005) reported that, isolates of novel *Bacillus* sp. produced keratinase with remarkable dehairing activity of bovine pelts. Keratinase could reduce the high degree of cross-linking in keratinous proteins and make the proteolysis with protease to easily occur on the wool surface (Selvam and Vishnupriya, 2012).

2.5.3 Optimization of enzyme production

Submerged fermentation of poultry waste by microorganism producing keratinase helps in the conversion of non-soluble keratin (feather) into soluble protein or polypeptide (Suntornsuk and Suntornsuk, 2003). For the optimum enzyme production various factors such as substrate, pH, temperature, carbon and nitrogen sources, could regulate the enzyme biosynthesis. *Bacillus subtilis* produced keratinolytic protease in the presence of various proteins such as casein, feather and Bovine Serum Albumin.

In most of the reports on constitutive keratinases, the nature of the enzyme is based on their caseinolytic rather than keratinolytic activity. Keratinase is an inducible enzyme produced by the microorganism in the presence of keratin as the substrate. Various microorganisms have produced wide range of protease enzyme with keratinolytic activities. *B. pumilis, B. cereus* produces keratinase enzyme in feather keratin as the substrate, but *B. subtilis* produces keratinase enzyme constitutively at casein, feather meal, or any of the protein sources. Keratinase production for soy meal was used as
substrate Joo et al. (2002). Keratin serves as the inducer; however, soy meal is also known to induce enzyme production (Gradisar et al., 2000). Further, simple sugars such as glucose have been reported to suppress the synthesis of keratinase due to catabolic repression (Wang and Shih, 1999; Yamamura et al., 2002; Bernal et al., 2003; Sun torsuk and Sun torsuk, 2003; Thys et al., 2004), which is a well known phenomenon for microbial proteases (Gupta et al., 2002).

Media pH is an important physiological factor that can regulate the enzyme production. Each organism has their own pH optima for its growth and enzyme production. Optimum pH values were reported to be 6 to 10 (Lin et al., 1992; Bockle et al., 1995; Cheng et al., 1995; Friedrich and Antranikian 1996; Bressollier et al., 1999). B. subtilis has the pH range of 5–9, B. cereus and B. pumilis 5–6, B. subtilis with pH of 10.0 (Onuoha and Chukwura 2011). Mukesh Kumar et al. (2012) reported the Bacillus sp MPTK 6 showed maximum keratinase production at pH 10.0 at 72 h of fermentation. Keratinases from Fervidobacterium, Stenotrophomonas, Chryseobacterium, Thermoanaerobacter, Nesterenkonia, Lysobacter, Xanthomonas, Kocuria, Clostridium, and Serratia are optimally active in alkaline pH ranging from pH 8 to 11. Keratinase from various Streptomyces sp. are also active in alkaline pH range (pH 7.5–11), while highly alkaline keratinase with optimal pH >12.5 was reported in case of Nocardiopsis sp. TOA-1 in which keratin serves as the substrate (Mitsuiki, et al., 2004). Brevibacillus thermoruber T1E produced neutrophilic keratinase with a pH optimum at 6.5 and strong inhibition in the alkaline pH range (Biharia et al., 2010).

Alkaline pH from 6 to 9 supports keratinase production and feather degradation in many cases. Temperature for keratinase production ranges from 28 to 50°C for most bacteria, actinomycetes and fungi to as high as 70°C for Thermoanaerobacter and Fervidobacterium spp. (Friedrichand Antranikian, 1996; Rissen and Antranikian, 2001; Nam et al., 2002). Psychrotrophic production of keratinase has also been reported for Stenotrophomonas sp. (Yamamura et al., 2002). Keratinase has been produced under submerged shaking conditions, except for a few thermophilic bacteria (Friedrich and Antranikian, 1996; Nam et al., 2002; Rissen and Antranikian, 2001) and fungi (Kaul and Sumbali 1999; Singh 1999) where static submerged fermentation has been reported.
Temperature is the next important physiological factor influence on the growth and enzyme production of microorganisms. Bacteria have wide range of growth temperature, based on its growth temperature, and classified the bacteria into mesophiles, thermophiles and psychrophiles. Most of the enzyme producers are mesophiles and thermophiles. The optimal conditions required for the specific enzyme production by *B. subtilis* are 40°C, *B. pumilis* for 40°C, *B. cereus* for 30°C. Onuoha and Chukwura. (2011) studied the optimization of keratinase production by bacillus was at the temperature 50 and 60°C. *Bacillus* spp. usually shows optimum keratinase production at temperatures ranging from 30 to 50°C (Cai et al., 2008; Suntornsuk and Suntornsuk 2003; Williams et al., 1990). *Fervidobacterium pennavorans* (Friedrich and Antranikian, 1996), *F. islandicum* AW-1 (Nam et al., 2002), and *Thermoanaerobacter keratinophilus* 2KXI (Riessen and Antranikian, 2001) exhibit a high feather decomposition rate, at hyperthermophilic (70°C) culture conditions. Among the moderately thermophilic, aerobic bacteria, *Bacillus licheniformis* PWD-1 (Williams et al., 1990), *Streptomyces thermonitrificans* MG104 (Mohamedin, 1999), and *Meiothermus ruber* H328 (Matsui et al., 2009) isolates with temperature optima of 50 – 55°C have proved to possess keratinolytic activity.

The enzyme from *Chrysosporium keratinophilum* (Dozie et al., 1994) and thermophile *Fervidobacterium islandicum* (Nam et al., 2002) showed exceptionally high temperature optima of 90 and 100°C, respectively, with a half-life of 30 and 90 min, respectively. Thermal stabilization of the enzyme in the presence of divalent cations such as calcium has also been reported (Mukhopadhyay and Chandra, 1990; Dozie et al., 1994; Bressollier et al., 1999; Chitte et al., 1999; Ignatova et al., 1999; Rissen and Antranikian, 2001; Nam et al., 2002; Riffel et al., 2003; Farag and Hassan, 2004). Keratinases have a broad substrate specificity and are active against both soluble and insoluble proteinaceous substrates. Among soluble proteins, they possess the ability to hydrolyze casein, gelatin, bovine serum albumin and hemoglobin, whereas among insoluble proteins, they hydrolyze feathers, wool, silk, collagen, elastin, horn, stratum corneum, hair, azokeratin and nail. Many keratinases have not been sequenced but
sequence homologies of some known keratinases indicate that they belong to subtilisin family of serine proteases.

Optimization of enzyme production can be carried out using the response surface methodology (RSM). RSM is a collection of statistical techniques for designing experiments and searching optimum conditions of factors for desirable responses (Oh et al., 1995; Sunitha et al., 1999) either in a full- or a fractional-factorial design that allows the testing of multiple independent process variables within a set of experiments. It has been successfully applied to the optimization of culture media for the production of primary and secondary metabolites in many fermentation processes (Li et al., 2002).

2.5.4 Characterization

Keratinase properties depend upon its producers. It is usually a serine protease (Gradisar et al., 2000; Riessen & Antranikian 2001). Infrequently, it has been found to be a serine protease with a cysteine protease (Sangali and Brandelli 2000) and a metallo protease (Lee et al., 2002). For the characterization of keratinase proteins, enzymes are purified from the fermented medium. Various strategies are now being used for purification of proteins, in which salt precipitation followed by column chromatography methods are most suitable. Poopathi et al. (2014) reported the protein precipitate obtained at ammonium sulphate saturation at 60% level and Sephacryl S-200 column chromatography resulted in 2.3 and 11.68 fold purification of the enzyme respectively. Tamilmani et al. (2008) extracted the keratinase and purified by salt precipitation. A new alkaline keratinase extracted from a Bacillus sp (Zhang et al., 2009), and it was purified by ammonium sulfate precipitation, which increases 17.7-fold activity, followed by DEAE-Sephadex-A50 column chromatography with a yield of 46.5%. Han et al. (2012) reported the keratinolytic protease from P. aeruginosa C11 was purified 17.4-fold through ammonium sulphate precipitation, a sephadex G-75 gel filtration column and a DEAE sepharose fast-flow column. In purification steps 2.69 fold purification was achieved after 80% ammonium precipitation in which 34.42% recovery was found. In further purification steps, a 5.69 fold purified keratinase was recovered by Sephadex G-75 chromatography with 21.23% of recovery (Sivakumar et al., 2012).
The purified enzyme revealed a molecular weight ranged from 35 – 70 kDa. 35 kDa keratinase protein was obtained from *B. licheniformis* (Ana Maria *et al.*, 2011). Hsin-Hung and Li-Jung, (2010) purified two keratinases (keratinase I and II) with molecular masses of 35.5 and 32.8 kDa, isoelectric point (pI) of 6.63 and 6.50, respectively from *Bacillus licheniformis* YJ4. Han, *et al.* (2012) showed relative molecular mass of the protease was estimated to be 33 kDa by SDS-PAGE. Sivakumar *et al.* (2012) studied the zymogram of crude enzyme on native-PAGE presented a band with keratinase activity of 41 kDa. Sinoy *et al.*, 2011 isolated a *Pseudomonas* sp, the molecular weight of the enzyme was determined as 30 KDa. Rajput *et al.*, (2010) studied the purified an extracellular keratinase from *Bacillus pumilus* KS12 with a molecular weight of 45 kDa. Sinoy *et al.* (2011) reported the molecular weight of the enzyme was determined as 30 KDa. Chen *et al.* (2008) studied the molecular mass of purified keratinase was 30.5 kDa.

Purified form of protease enzyme has wide range of pH and temperature stability. Microbial keratinase showed optimum temperatures and pH values were ranged to be 40–80ºC and 6–10, respectively (Lin *et al.*, 1992; Bockle *et al.*, 1995; Cheng *et al.*, 1995; Friedrich & Antranikian 1996; Bressollier *et al.*, 1999). *B. licheniformis* ER-15 keratinase exhibited maximum activity at pH 11 and 70ºC (Gupta and Ramnani 2006). *B. subtilis* keratinase had the highest enzyme activity at pH 11(Mousavi *et al.*, 2013). Although, alkaline pH and high temperature would facilitate rapid feather degradation by reducing disulfide bonds (Gupta and Ramnani 2006) but is not often recommended for the direct use of feather meal in feed due to loss of some essential amino acids (Brandelli 2008; Hood and Healy 1994). The enzyme was stable at 30-40ºC and active around wide pH range (6-8). Ghasemi *et al.*, 2012 reported the strain produced a thermostable keratinase with optimum activity at 70ºC and pH 8.0. Agrahari, (2013) studied the best enzyme activity of the strain was observed at pH 3 and caesinolytic activity and keratinolytic activity was observed at 60ºC and 70ºC respectively. Hsin-Hung and Li-Jung, (2010) obtained the enzyme has stable at pH 6.0-10.0 and 10-50ºC. The optimal pH and temperature were similar (i.e) at 9.0 and 60ºC, respectively (Sinoy *et al.*, 2011). The optimum pH at 8.5 and the optimum temperature was 55ºC (Chen *et al.*, 2008). The
protease was stable in pH range 5 to 10 and at temperature below 50°C with optimum pH of 7.5 and optimum temperature of 60°C (Han, et al., 2012). Optimum temperature and pH were determined to be 35°C and pH 8.0, respectively (Sivakumar et al., 2012).

Keratinases are extracellular enzymes, and they are inhibited by certain metal ions. Tapia and Simoes, (2008) reported that keratinolytic activity was not inhibited by EDTA, DMSO and Tween 80. On the other hand, CaCl$_2$, ZnCl$_2$, and BaCl$_2$ slightly inhibited the keratinolytic activity. Vigneshwaran et al, (2010) showed, zinc and magnesium were found to enhance the enzyme activity, where as mercury, copper, cadmium, 1, 10 phenanthroline and EDTA completely inhibited the enzyme activity. Ghasemi et al. (2012) found enzyme activity was increased significantly by using 2-mercaptoethanol as a reducing agent and the keratinase was activated substantially in the presence of Co$^{2+}$, Mg$^{2+}$, TritonX-100, Tween-80 and EDTA, whereas SDS had a negative effect on enzyme activity. Mn$^{2+}$ and Ba$^{2+}$ strongly activated caesinolytic activity and keratinolytic activity respectively (Agrahari 2013). Mn$^{2+}$ stimulated the keratinolytic activity by 21% at 2 mM, while Co$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Hg$^{2+}$, Pb$^{2+}$ and Fe$^{2+}$ inhibited the keratinolytic activity (Han, et al., 2012). Rajput et al., 2010 studied the purified an extracellular keratinase from *Bacillus pumilus* was found to be an alkaline, protease. It was thiol activated with two- and eight-fold enhancement in presence of 10 mM DTT and $\beta$- mercaptoethanol, respectively. In addition, its activity was stimulated in the presence of various surfactants, detergents, and oxidizing agents where a nearly 2- to 3-fold enhancement was observed in presence of H$_2$O$_2$ and NaHClO$_3$. Saibabu et al., 2013 studied the partially purified keratinase has slightly inhibited by K$^+$ and Co$^{2+}$ whereas Hg$^{2+}$ exhibited a complete inhibition.

2.6 Compost fertilizers

Compost is decomposed organic matter that helps build healthy soil by stimulating and feeding the many beneficial soil microorganisms. Along with adding nutrients, compost helps soil hold water (and nutrients) and air. Compost works on any soil; it helps sandy soils hold nutrients and water, and it loosens clay soils. Balakrishnan et al, (2010), studied that biocompost is an alternative to chemical fertilizer, has been increase the yield
characteristics of *Arachis hypogaea* Linn. For sustainable agriculture, to evaluate the effect of three different types of halophytic composites in combination with farmyard manure (FYM) and phosphate solubilising bacteria (*Bacillus megaterium*) on yield characteristics such as number of pods per plant, fresh pod weight, dry pod weight, pod yield, haulm yield, shelling percentage and hundred kernel weight (Hatim Abdalla Sulfab, 2013).

The groundnut plant growth, application of FYM increased the pod and haulm yields and improved the yield parameters (Sriramachandragekaran 2001; Manikandan 2003). Jagdev Singh, (2000) have reported application FYM increased the 10% shelling percentage, 100 kernel weight 32%, numbers of pods and pod yield per plant in groundnut crop. Dharma (1996) found that FYM might have stimulated the activities of microorganisms that make the plant nutrients readily available to the crops. Balasubramanian and Palaniappan (1994) reported that use of microbial inoculants in combination with FYM favored groundnut production. Asmus (1993) reported that application of FYM increased the nitrogen supply to soil. Das *et al.* (1992) reported that application of FYM and poultry manure to groundnut crop increase post harvest soil organic C and available Ca contents.

### 2.7 Feather compost as substrate for biofertilizers

Composting has been advocated and promoted as an effective and economical answer for dealing with solid organic wastes (Hubbe *et al.*, 2010). Environmental friendly biofertilizer with keratin degradation is the best choice of compost preparation (Pettett and Kurtboke, 2004). The nature and population size of microorganism in feather composting is depending on number of factors, one of which is temperature, temperature level was elevated during keratin degradation (Lyndall *et al.*, 2004). The eco-friendly bio-fertilizer and plant growth using feather compost (Sreenivasa Nayaka and Vidyasagar, 2013).

#### 2.7.1 Carrier

These biofertilizers are to play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone productivity, ammonia
excretion, siderophore formation and to control various plant diseases (Pankhurst and Lynch, 1995; Pathak et al., 1997; Dadarwal et al., 1997; Hedge et al., 1999). Bioinoculants always prepared with suitable carrier materials. It should also be non polluting, biodegradable, non toxic and cost effective which can maintain high viable count for a longer period (Tilak and Singh, 1994). The lignite and wood charcoal were the most commonly used carrier material in India (Dadarwal et al., 1997). Peat, lignite and charcoal as the efficient carrier for *Trichoderma* and *Rhizobium* (Gaur and Gaind, 1984), which can maintain the microorganisms in a viable state (Yardin et al., 2000) improve survival / shelf-life, product bio-efficacy etc of bio-fertilizers (Gaind and Gaur, 2004). The, commercially available carrier materials are typically based on milled peat, clays, rice, bran, seeds, or other complex organic matrices. Graham-Weiss et al. (1987) reported that when supplemented with nutrient source, sterile finely ground vermiculite can be used as a carrier material. Muthukumarasamy et al. (1996) suggested that the vermicompost could be used as an alternative carrier material to lignite. The survival of *Azospirillum* and phosphate solubilizing bacteria was better in press mud compared to peat, lignite, and composted press mud (Rajannan et al., 1996).

Besides, types of carrier and storage temperatures are important factors determining shelf life of bioinoculants (Kremer and Peterson, 1983), and acceptance of agricultural products (Bashan, 1998), storing such inoculants in a warehouse without refrigerator in the range of 5 to 30ºC often causes reduction in microbial longevity. Number of researchers studied the effect of carrier temperature on shelf life of the biofertilizer (Thungtrakul, 1987; Rajakumar and Lakshmanan, 1995; Saleh Nassar and Yassen, 2001).

Tilak et al. (1979) found that soil plus farmyard manure (1:1 ratio) had higher *Azospirillum* count followed by soil plus FYM plus vermiculite (5:3:2). Kundu and Gaur (1981) suggested that soil could serve as a carrier for spore formers like *Bacillus polymyxa*. Tilak and Subba Rao, (1978) established that lignite, press mud and farmyard manure after suitable amendment with charcoal or coconut shell powder could substitute peat under Indian condition. Peat formulations have been the carriers of choice, and are the most commonly used in the rhizobia inoculation industry (Bashan, 1998, Abd -Alla
and Omar, 2001, Temprano et al., 2002). Bacterial multiplication continues during the storage period, as long as sufficient nutrients, moisture, and the correct temperature are maintained (Bashan, 1998).

Development of compatible carrier materials for formulations of efficient decomposer inoculants was felt (Subba Rao, 1984), resulting in search and recognition of some classic carrier materials (Smith, 1992). Carrier based formulations of efficient microorganisms for rapid composting (Rasal and Patil, 2001) are generally based on cereals; jowar grains being the material of choice as carrier (Nakasone et al., 2004). Recently, Subba Rao, (1999) mentioned that biofertilizers are carried based preparations containing beneficial microorganisms in available state intended for seed or soil application and designed to improve soil fertility and help plant growth. Carrier based starter culture formulations of effective composting microorganisms are generally based on food grains (Rasal and Patil, 2001). The lignite and wood charcoal were the most commonly used carrier material in India (Dadarwal et al., 1997). Lakshmi et al., (1977). Graham-Weiss et al., (1987) reported that when supplemented with nutrient source, sterile finely ground vermiculite can be used as a carrier material. Use of fly-ash in soil as bio-formulation is useful because it increases pH of soil, converts the nutrients in available form, supplies nutrients to soil and it solves disposal problem of fly-ash (Dwivedi, 2007).

Poultry manure is excellent organic manure, as contains high nitrogen, phosphorus, potassium and other essential nutrients. In contrast to mineral fertilizer, it adds organic matter to soil which improves soil structure, nutrient retention, aeration, soil moisture holding capacity, and water infiltration (Deksissa et al., 2008). Poultry manure is relatively resistant to microbial degradation. However, it is essential for establishing and maintaining optimum soil physical condition and important for plant growth (Rahman, 2004). Ismail et al. (1998) reported significant increase in organic C, available N and P content of the soil with application of FYM possibly due to the increase in decomposition product of organic matter. So present work planned to investigate the role of feather waste in compost preparation and application.