2. Review of Literature
The scientific literatures published at national and international level journals with respect to chitinases, their effective sources, versatile applications, role of streptomycetes in chitinase production, its purification, antimicrobial properties of streptomycete derived chitinases, biosorbent values of streptomycete biomass etc. were reviewed in this part of the thesis entitled, ‘Production optimization, partial purification, physio-chemical evaluation and applications of extracellular chitinase from Streptomyces sp. SJKP9’.

2.1 Chitinase

Chitinases (EC 3.2.1.14) are glycosyl hydrolases which catalyse the degradation of chitin, an insoluble linear β-1,4-linked polymer of N-acetylglucosamine (Watanabe et al., 1999). It is present in a wide range of organisms, including organisms that do not contain chitin, such as bacteria, viruses, higher plants and animals (Mukherjee and Sen, 2006).

2.1.1 Classification of chitinase

Chitinases are classified into two different families, families 18 and 19, in the classification system of glycosyl hydrolases, based on the amino acid sequence similarity of their catalytic domains (Davies & Henrissat, 1995). Family 18 contains chitinases from bacteria, fungi, viruses and animals, and some plant chitinases (classes III and V). Family 19 contains plant classes I, II and IV chitinases and the Streptomyces griseus chitinase C (Ohno et al., 1996). The chitinases of the two families do not share amino acid sequence similarity, and they have completely different three-dimensional structures and therefore thought to have different evolutionary origins (Hamid et al., 2013).

The catalytic domains of family 18 chitinases have an (α/β) 8-barrel fold as demonstrated by 3D-structural analyses of hevamine (Scheltinga et al., 1994) and Serratia marcescens chitinase A (Perrakis et al., 1994). On the other hand, the catalytic domains of family 19 chitinases have high α-helical content and have structural similarity, including a conserved core, with chitosanase and lysozyme (Monzingo et al., 1996). In addition to the difference in 3D structure, chitinases of the two families show several important differences in their biochemical properties. For example, family 18 chitinases hydrolyse the glycosidic bond with retention of the anomeric configuration (Iseli et al., 1996), whereas family 19 chitinases hydrolyse with inversion (Ohno et al., 1996). Family 18 chitinases are sensitive to allosamidin, but a family 19 chitinase from higher plants has
been shown to be insensitive (Koga et al., 1987). Family 18 chitinases hydrolyse GlcNAc-GlcNAc and GlcNAc-GlcN linkages, whereas family 19 chitinases hydrolyse GlcNAc-GlcNAc and GlcN-GlcNAc (Mitsutomi et al., 1997). These differences are probably common between all members of the two families and arise from the differences in their catalytic mechanisms. Substrate assisted catalysis is the most widely accepted model for the catalytic mechanism of family 18 chitinases (Brameld et al., 1998), whereas a general acid and base mechanism has been suggested to be the catalytic mechanism for family 19 chitinases (Watanabe et al., 1999). Typically, chitinase enzymes are composed of at least three functional domains, namely catalytic domain, chitin-binding domain, and cadherin-like domain or fibronectin type III-like domain (Han et al., 2009).

Chitinases can be classified in two major categories. Endochitinases (EC 3.2.1.14) cleave chitin randomly at internal sites, generating soluble low molecular mass multimers of GlcNAc, such as chitotetraose, chitotriose and the dimer di-acetylchitobiose. Exochitinases can be divided into two sub-categories: chitobiosidases (EC 3.2.1.29), which catalyze the progressive release of di-acetylchitobiose starting at the non-reducing end of the chitin microfibril; and 1-4-[3-N-acetylglucosaminidases (EC 3.2.1.30), which cleave the oligomeric products of endochitinases and chitobiosidases generating monomers of GlcNAc (Cohen et al., 1998).

2.1.2 Sources of chitinases

2.1.2.1 Plant chitinase

Chitinases have been reported from both monocotyledonous and dicotyledonous plant species and occur in widely different tissues, including embryos and cotyledons (Majeau et al., 1990), seeds (Yamagami and Funatsu, 1993), leaves and stems (Ishige et al., 1993), roots and flowers (Neale et al., 1990) and protoplasts (Roby et al., 1991). Few cultivated crop and non crop species where chitinases occur includes barley, bean, carrot, corn, oat, onion, rice, wheat etc and Arabidopsis, bentgrass, chestnut, petunia, poplar and rubber (Zamir et al., 1993). Plant chitinases are induced by numerous factors like infection by viruses, viroids, pathogenic fungi, mycorrhizal fungi and bacteria. Chitinases are up-regulated by a variety of stress conditions, both biotic and abiotic, and by phytohormones as ethylene, jasmonic acid, and salicylic acid (Kasprzewska, 2003).
Plant chitinases are thought to act as a part of the defence mechanism against plant-pathogenic fungi, and antifungal activity of plant chitinases from various sources, especially family 19 chitinases, has been demonstrated by many researchers (Schlumbaum et al., 1986; Broekaert et al., 1988; Roberts & Selitrennikoff, 1988; Leah et al., 1991; Iseli et al., 1993; Watanabe et al., 1999). It has been shown that overexpression of some chitinases alone or together with other antifungal proteins leads to enhanced protection against pathogen attack (Zhonga et al., 2002).

2.1.2.2 Animal chitinase

Invertebrate chitinase

Invertebrates require chitinases for partial degradation of their old exoskeletons (Kamil et al., 2007). *Anopheles gambiae* midgut chitinase seems to act in concert with a chitin synthase to modulate the thickness and permeability of the peritrophic membrane (Shen and Jacobs Lorena 1997). A fat body-specific chitinase was detected in *Glossina morsitans* milk gland tissue and could be important for the development of intrauterine larvae (Merzendorfer, 2003). *Ostrinia nubilalis* midgut-specific chitinase plays an important role in regulating chitin content of the peritrophic membrane and subsequently affecting the growth and development of the larvae (Khajuria et al., 2010).

Okada et al. (2013) performed molecular characterization and expression analysis of chitinase from the pacific oyster *Crassostrea gigas*. In insects, chitinases participate in the periodic shedding of old exoskeletons and the turnover of peritrophic membranes. Chitinase family members have been identified in dozens of species, including *Tribolium castaneum*, *Drosophila melanogaster*, and *Anopheles gambiae* (Pan et al., 2013).

Human chitinase

The members of mammalian chitinases and CLPs include chitinase 3-like-1 (CHI3L1) (Hakala et al., 1993), chitotriosidase (Boot et al., 1995), YKL-39 (Hu et al., 1996) Ym1 (Jin et al., 1998) acidic mammalian chitinase (AMCase) (Boot et al., 2001) oviduct-specific glycoprotein (Buhi, 2002) and stabilin-1-interacting chitinase-like Protein (Kzhyshkowska, 2006), Chitotriosidase and AMCase possess chitinase enzymatic activity, whereas other mammalian chitinases, including CLPs, do not possess this activity (Chang et al., 2001). Mammalian chitinases are found with enzymatic activity, have a chitin binding domain that contains six cysteine residues responsible for
their binding to chitin (Tjoelker, 2000). Human chitinases are related to allergies, asthma and inflammation (Kawada et al., 2007).

2.1.2.3 Microbial chitinases

2.1.2.3.1 Bacterial chitinase

Microorganisms, particularly bacteria, form one of the major sources of chitinase (Bhattacharya et al., 2007). Bacteria produce chitinases mainly to degrade chitin and utilize it as an energy source. In addition, some chitinases of chitinolytic bacteria, such as the chiA gene produced from Serratia marcescens and Enterobacter agglomerans are potential agents for the biological control of plant diseases caused by various phytopathogenic fungi (Kamil et al., 2007). Microbial chitinases have been found to hydrolyze not only chitin but also partially N-acetylated chitosan (Mitsutomi et al., 1995). Chitinases are produced by various bacterial forms such as Cellulosimicrobium cellulans FXX, Bacillus subtilis, Paenibacillus sp. CHE-N1 etc (Fleuri et al., 2009). Listeria monocytogenes produces two chitinases encoded by lmo1883 (chiA) and lmo0105 (chiB) and one chitin binding protein encoded by lmo2467 (Chaudhuri et al., 2010).

Chitinases are produced by different micro-organisms which generally present a wide multiplicity of enzymes that are mainly extracellular. They have received increased attention due to their wide range of biotechnological applications, especially in the production of chitooligosaccharides and N-acetyl D-glucosamine (Pichyangkura et al., 2002), biocontrol of pathogenic fungi (Chernin et al., 1997), preparation of sphaeroplasts and protoplasts from yeast and fungal species (Balasubramanium et al., 2003) and bioconversion of chitin waste to single cell protein (Dahiya et al., 2005). Chitinase activity largely depends on the type of chitinous substrate. Some chitinases are constitutive, while others are induced and require the provision of an appropriate chitinous substrate (Marszewska-Ziemięcka et al., 1974).

Microbial chitinases attracted the attention as one of the potential enzyme for applications in agriculture, pharmaceutical, waste management, biotechnology and industry (Gupta et al., 1995). Their high demand and wide potential uses has led to the discovery of new strains of microorganisms that are capable to produce enzymes with novel properties and the development of low cost industrial media formulations (Saito et al., 2009).
2.1.2.3.2 Fungal chitinase

The chitinase production in filamentous fungi is seen throughout its life cycle (Gooday, 1990). The main role of this enzyme is to help the fungi in releasing the spores and in hyphal branching (Muzzarelli et al., 2011). In yeast, chitinase helps in budding especially in cell separation. It also plays an important role in septa dissolution and gamete fusion (Gooday et al., 1990). Chitinase is essential not only in the various developmental stages of fungi but also in fungal nutrition (Gunaratna and Balasubramanian, 1994). It helps in utilization of chitin for its carbon and nitrogen requirements. In few it is also associated with pathogenicity of the organisms (Narayana et al., 2013). It is produced by various organisms, such as, *Trichoderma harzanium* TUBF 781, *Penicillium aculeatum*, *Lecanicillium fungicola*, etc., (Fleuri et al., 2009).

2.1.2.3.3 Chitinase from *Streptomyces* spp.

There are many reports concerning chitinolytic enzymes from *Streptomyces*. Berger and Reynolds (1958) reported about two chitinase components with similar activity produced by *S. griseus*. Jeuniax (1959) showed that chitinase preparation obtained from a culture filtrate of *S. antibioticus* was composed of three different components which had the same specific activity against colloidal chitin. Skujins et al. (1970) purified chitinase produced by an unidentified *Streptomyces* strain and found that the crystalline preparation bound four calcium atoms to one enzyme molecule for manifestation of the activity. In connection with microbial cell wall lysis, Tominaga and Tsujisaka (1976) resolved two chitinase components existing in a culture filtrate of *S. orientalis* which lysed the *Rhizopus* cell wall. Price and Storck (1975) revealed the involvement of chitosanase produced by a *Streptomyces* species in cell wall lysis of Mucolales, and purified and characterized the enzyme. Iwamoto *et al.* (1976) reported that exo-N-acetylglucosaminidase produced by a strain of *Streptomyces* was involved in lysis of *Bacillus subtilis* cell wall. Two *Streptomyces* strains J-28 and J-13-3 isolated from soil was chitinolytic in the medium containing *A. niger* cell wall, and these strains produced chitinase and glucanase in the same medium (Tagawa and Okazaki, 1991). Five extracellular chitinases of molecular weight 20.5, 30, 47, 70, and 92 kDa was purified from the culture filtrate of *Streptomyces olivaceoviridis* ATCC 11238 (Romaguera, 1992). Chitinase C from *Streptomyces griseus* HUT6037, described in 1997, is the first
<table>
<thead>
<tr>
<th>Name of the species</th>
<th>Source</th>
<th>Enzyme activity U/ml</th>
<th>Molecular weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. diasitapiticus</td>
<td>Red palm weevil</td>
<td>2.3</td>
<td>-</td>
<td>Crawford et al. (1993)</td>
</tr>
<tr>
<td>S. thermoviolaceus</td>
<td>Culture filtrate</td>
<td>1.13</td>
<td>-</td>
<td>Tsujibo et al. (1993)</td>
</tr>
<tr>
<td>S. viridificans</td>
<td>MTCC</td>
<td>0.23</td>
<td>-</td>
<td>Gupta et al. (1995)</td>
</tr>
<tr>
<td>S. griseus</td>
<td>Stock culture</td>
<td>-</td>
<td>-</td>
<td>Ohno et al. (1996)</td>
</tr>
<tr>
<td>S. lydicus</td>
<td>Rhizosphere of linseed plants</td>
<td>2.26</td>
<td>32 KDa</td>
<td>Brinda et al. (1997)</td>
</tr>
<tr>
<td>S. lydicus</td>
<td>Rhizosphere of linseed plants</td>
<td>2.26</td>
<td>-</td>
<td>Brinda et al., 1997</td>
</tr>
<tr>
<td>S. griseus</td>
<td>Saga University</td>
<td>12.56</td>
<td>-</td>
<td>Jung et al. (1999)</td>
</tr>
<tr>
<td>Streptomyces sp.</td>
<td>Cerodo soil</td>
<td>2.98</td>
<td>-</td>
<td>Gomes et al. (2000)</td>
</tr>
<tr>
<td>S. griseus</td>
<td>Stock culture</td>
<td>-</td>
<td>-</td>
<td>Ohno et al. (1996)</td>
</tr>
<tr>
<td>S. aureofaciens</td>
<td>Endophytic</td>
<td>0.0833</td>
<td>-</td>
<td>Taechowisan et al. (2003)</td>
</tr>
<tr>
<td>Streptomyces sp. M 20</td>
<td>Mongolian soil</td>
<td>137</td>
<td>20 KDa</td>
<td>Kim et al. (2003)</td>
</tr>
<tr>
<td>S. plicatus</td>
<td>Iran soil</td>
<td>-</td>
<td>-</td>
<td>Bonjar and Aghighi (2005)</td>
</tr>
<tr>
<td>S. venezuelae</td>
<td>Sea shore soil</td>
<td>8.35</td>
<td>-</td>
<td>Mukerjee and Sen (2006)</td>
</tr>
<tr>
<td>Streptomyces sp. DA11</td>
<td>Sea sponge</td>
<td>2.95</td>
<td>34 KDa</td>
<td>Han et al. (2009)</td>
</tr>
<tr>
<td>Streptomyces sp. ANU 6277</td>
<td>Soil</td>
<td>33</td>
<td>45 KDa</td>
<td>Narayana and Vijayalakshmi (2009)</td>
</tr>
<tr>
<td>Streptomyces sp. PTK19</td>
<td>Soil</td>
<td>31.62</td>
<td>33 KDa</td>
<td>Thiagarajan et al. (2011)</td>
</tr>
<tr>
<td>S. violaceusniger</td>
<td>-</td>
<td>-</td>
<td>48 kDa</td>
<td>Nagpure and Gupta (2012)</td>
</tr>
<tr>
<td>Streptomyces sp. SJKP9</td>
<td>Soil</td>
<td>-</td>
<td>-</td>
<td>Jagadeeswari &amp; Panneer Selvam (2012)</td>
</tr>
</tbody>
</table>
family 19 chitinase found in an organism other than higher plants (Watnabe et al., 1999). Few more chitinases produced by various species of *Streptomyces* is listed in table 1.

Chitinase genes have been cloned from *Streptomyces* species. Extensive analysis of these genes revealed an extraordinary high multiplicity of chitinase genes in *Streptomyces*. In *S. coelicolor* A3(2), seven distinct genes belonging to family 18 and 19 chitinases were dispersed on the chromosomal DNA. Genes for family 19 chitinases, which had been considered to be present only in higher plants, were found to be widely spread in *Streptomyces* (Saito et al., 1999).

2.1.3 Applications of chitinase

Chitinases have received increased attention because of their wide range of applications (Rani Gupta et al., 1995). Potential application of chitinolytic enzymes (chitinases) in biotechnology is wide. Traditionally, these hydrolases could find uses in chitin hydrolysis, production of chitin derivatives, protoplast formation and biocontrol of pathogenic organisms. Some unconventional applications of chitinases in food and wine industries have been successfully tested at laboratory level (Jimenez et al., 2008).

Chitinases can be used in controlling pathogenic fungi in plants and insects, production of biologically active chitin- oligosaccharides, production of single cell protein, preparation of mycolytic enzymes and in the formation of fungal protoplasts (Fleuri et al., 2009). Chitinases are of great biotechnological interest because of their potential use as food and seed preservative agents and for engineering plants for resistance to phytopathogenic fungi (Kamil et al., 2007). It is used in the preparation of pharmaceutically important chitooligosaccharides and N-acetyl D-glucosamines (Han et al., 2009), treatment of chitinous waste, and control of malaria transmission (Dahiya et al., 2006). Chitinases also show biofunctional potential such as lytic activity (Patil et al., 2000).

Some chitinases function as pathogenesis-related (PR) proteins. It accumulates upon induction of Systemic Acquired Resistance (SAR) in plant tissues as response to parasitic attack such as fungi (Kastner et al., 1998), bacteria, (Gerhardt et al., 1997), viruses (Van Loon and Van Kammen, 1970), nematodes (Rahimi et al., 1998) and insects (Van der Westhuizen et al., 1998). The enzyme can also be induced by wounding (Cabello et al., 1994), heat shock (Margispinheiro et al., 1994) and the application of
plant disease resistance activators (Narusaka et al., 1999) or ethylene (Boller et al., 1983). The induction of chitinase after pathogen or pest attack provided further correlative evidence for the role of this enzyme in pathogen resistance (Velasquez and Hammerschmidt, 2004).

The numerous applications of chitinase includes the biocontrol of fungal diseases in plants (Chang et al., 2003), biopesticides (Mendonza et al., 1996), production of single cell protein from chitin wastes (Vyas and Deshpande, 1991), production of chitooligosaccharides, glucosamine and GLcNAc by the hydrolysis of chitin (Pichyangkura et al., 2002). The ability of chitinase for digesting insect chitin raises the idea of using it for controlling insects (Zarei et al., 2012). The medical applications of chitinase comprise antibacterial, antifungal, hypocholesterolemic and antihypertensive activity (Dahiya et al., 2006).

### 2.2 Chitin

Chitin, a linear β-1, 4-linked homopolymer of N-acetylglucosamine, is one of the three most abundant polysaccharides in nature besides cellulose and starch (Han et al., 2009). The annual global yield of chitin is assumed to be 1 to 100 billion metric tons, making chitin the second most abundant polysaccharide on the earth (Kamil et al., 2007). It has a highly ordered crystalline structure, as shown by X-ray diffraction studies, and is insoluble in water and generally bound to other polysaccharides and proteins. Chitin chains present three forms of arrangement, denominated as α, β and γ. The α form is dominant and more stable and consists of alternating parallel and anti-parallel chains; it occurs mainly in crustaceans, insects and fungi. The β form consists of parallel chains and occurs only in marine organisms. The γ form is still being elucidated (Fleuri et al., 2009). In nature chitin is not found alone but forms a part of very complex system, such as chitin- protein complex, calcium carbonate in addition to protein and organic substances (Mane and Deshmukh, 2009).

#### 2.2.1 Sources of chitin

Chitin is insoluble in water, dilute and concentrated alkalis, alcohol and other organic solvents. The major contribution of chitin to nature is in the form of animal biomass (Nawani and Kapadnis, 2005). It is the major constituent of the fungal cell wall (22% to 44%), insect exoskeletons and crustacean shells (25% to 58%). Worldwide
annual recovery of chitin from the processing of marine invertebrates is $3.7 \times 10^4$ metric tons (Mukherjee and Sen, 2006).

In the plant kingdom, chitin is present in the algae, commonly known as marine diatoms. Chitin from the diatom spines such as Cyclotella cryptica and Thalassiosira fluviatilis are the only form reported to be 100% poly-N-acetyl-glucosamine that is not associated with proteins and is termed chitan (Khor, 2001).

Chitin in fungal cell walls is normally present in a highly rigid, crystalline state. In the hyphal apex, however, the chitin is sensitive to treatments with dilute HCl or chitinase. The sensitivity of the fungal cell wall to lytic enzymes has been exploited by using chitinase-producing bacteria to control plant-pathogenic fungi in the rhizosphere (Mahadevan and Crawford, 1997).

Chitin is particularly abundant in the marine environment being the principle component of the exoskeletons of crustaceans and many molluscs. The high proportion heterotrophic marine bacteria that have evolved to use chitin as a sole carbon and nitrogen resource suggests that this polymer plays as important role in marine food webs (Metcalfe et al., 2002). Degradation of chitin in the aquatic biosphere is a very diffused and efficient process mainly carried out by bacteria. In spite of the enormous quantity of chitin produced annually (approximately $10^{11}$ metric tonnes), marine sediments contain only trace of this polysaccharide (Jimenez et al., 2008).

The biodegradation of chitin requires the synergistic action of several hydrolytic enzymes for efficient and complete breakdown. The combined action of endochitinases (EC 3.2.1.14) and exochitinases [(chitobiosidases and β-N-acetyl hexosaminidase (EC
3.2.1.82]) results in the degradation of chitin polymer into the soluble $N$-acetyl D-glucosamine (Gkargkas et al., 2004).

### 2.2.4 Chitosan

Chitosan is the low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2- amino-2-deoxy-b-d-glucose, known as (1,4)-2-amino- 2-deoxy-(d-glucose). Chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively. Chemical modifications of these groups have provided numerous useful materials in different fields of application. These biopolymers offer a wide range of unique applications including bioconversion for the production of value-added food products (Shahidi and Synowiecki, 1991) preservation of foods from microbial deterioration (Chen et al., 1998), formation of biodegradable films (Kittur et al., 1998), recovery of waste material from food processing discards (Sun and Payne, 1996), purification of water (Deans and Dixon, 1992) and clarification and deacidification of fruit juices (Chen and Li, 1996). Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth (Cuero et al., 1991). It also activates several defense processes in the host tissue (Ghaouth et al., 1992), acts as a water binding agent and inhibits various enzymes (Young et al., 1982). Binding of chitosan with DNA and inhibition of mRNA synthesis occurs via chitosan penetrating the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins (Shahidi, 1990).
2.3 Actinomycetes

Actinomycetes are the dominant group of soil population together with bacteria and fungi. They are Gram-positive bacteria having high G+C (>55%) content in their DNA and they are originally considered as an intermediate group between bacteria and fungi. They are free living, saprophytic bacteria, and a major source for production of antibiotics. They play a major role in recycling of organic matter, production of novel pharmaceuticals, nutritional materials, cosmetics, enzymes, antitumour agents, enzyme inhibitors, immune-modifiers and vitamins (Remya and Vijayakumar, 2008). In the most of the cases the ability to produce one or another bioproduct is taxonomically determined, which makes taxonomic identification of the isolates so important (Hirsch and Christensen, 1983). They are abundant in terrestrial soils and contribute significantly to the turnover of complex biopolymers, such as lignocellulose, hemicellulose, pectin, keratin, and chitin (Okasaki, 2006).

Actinomycetes not only inhabit soils, but are also present in aquatic environment. Jensen et al. (1991) and Burns et al. (2003) found actinomycetes in seawater, and Terkina et al. (2002) and Klaus et al. (2004) in fresh water. Actinomycetes were found in association with cat-tail (Typha latifolia) and bulrush (Scirpus acutus) roots in constructed wetland (Hatano et al., 1994) and in sewage treatment scum (Jenkins et al., 1993).

Ability to decompose chitin is a characteristic property of actinomycetes. Paul and Clark (2000) found that 90% of all actinomycetes obtained from soil belonged to Streptomyces genus and nearly all were able to decompose chitin, however, El-Fiky et al. (2003) reported that only 7% actinomycetes from the rhizosphere of tomato and green pepper were capable of decomposing chitin (Ziemiecka et al., 2009). Actinomycetes are very sturdy group of microorganisms found in myriad of environments including the extreme environments. The chitinases produced from such actinomycetes are reported to have high applicability in their uses (Loni and Bajekal, 2009). Thus, expanding the isolation sources beyond just terrestrial soils may be one approach to discover novel natural products.
Endophytic actinomycetes have been isolated from various plant tissues and some of them have potential as biocontrol agents against phytopathogenic fungi (Shimizu et al., 2000). Some strains of endophytic actinomycetes that produced chitinase might protect against phytopathogenic fungi in plant tissue by this property. Thus the role of chitinase of endophytic actinomycetes in antifungal activity should be studied as they may endow their host plant with some natural protection against infections (Nakashima et al., 2009). Actinomycetes, particularly *Streptomyces* spp. have been a widely exploited group of microorganisms in the production of secondary metabolites and enzymes of commercial importance in medical and agricultural applications (Narayana and Vijayalakshmi, 2009).

### 2.4 Streptomycetes

*Streptomyces*, which are Gram-positive bacteria present in soil, have a high GC content and are able to grow under a wide variety of environmental conditions. Soil streptomycetes are the major contributors to the biological buffering of soils and have roles in decomposition of organic matter conductive to crop production (Bafti et al., 2005). *Streptomyces* produce a large number of extracellular enzymes as part of their saprophytic mode of life. Their ability to synthesize enzymes as products of their primary metabolism could lead to the production of many proteins of industrial importance (Gilbert et al., 1995). They also produce a variety of antibacterial and antifungal substances.

#### 2.4.1 Isolation of *Streptomyces*

The use of microorganisms to produce natural products and processes that benefit and improve our socioeconomic life-styles has been a part of human history since the days of early civilization. Isolating microorganisms from the environment is the microbiologist’s first step in screening for natural products such as secondary metabolites and enzymes (Biswas et al., 2011). Isolation of rare *Streptomyces* is important to exploit the various novel products they synthesize. To enhance the isolation rate of rare species, the soil samples are subjected to pretreatments before processing. Addition of CaCO₃, powdered chitin or pollen membranes can result in 100 fold increase in *Streptomyces* colonies. Rare thermophilic species in soil may be isolated after heating at 120° C for 1 hour (Lim et al., 2000). Because many streptomycetes can use chitin as sole source of carbon, chitin can be used as an enrichment medium for the isolation of *Streptomyces* from soil (Mukherjee and Sen, 2006). The pretreatment of soil sample compensates the
poor competitive ability of *Streptomyces* under laboratory conditions (El Nakeeb and Lechevalier, 1963). Many recipes for media, designed to encourage the growth of soil actinomycetes rather than other soil micro-organisms, have been suggested. The nutritional factors such as carbon and nitrogen source, ammonia, inorganic phosphate, and metal ions such as manganese, cobalt, zinc, calcium, and magnesium in the media may also affect the production of secondary metabolites during the cultivation of the microorganisms (Xu et al., 1996).

### 2.4.2 Identification and Classification of *Streptomyces*

Numerous classifications were devised to accommodate the increasing number of *Streptomyces* species, most of them based on a few subjectively chosen morphological and pigmentation properties which were rarely studied under standardized growth conditions (Atalan et al., 2000). Biochemical, nutritional and physiological characters had also been used in streptomycetes taxonomy, but usually had been applied to only selected species (Kutzner et al., 1989). Similar actinomycetes tend to have a similar colour. This is exploited mainly in the classification of streptomycetes, where one can recognize: the production of diffusible pigments, the color of the aerial mycelium, and the colour of substrate mycelium (Shirling and Gottlieb, 1970). However, variants differing in color from their parents can be obtained rather easily both as the result of natural variation and as the result of irradiation with ultraviolet light (Krassilnikov, 1970). Thus colour is of little value as a basic criterion in the systematics of actinomycetes, although it is of great value in the presumptive identification of many actinomycetic species.

The genus *Streptomyces* was classified in the family streptomycetaceae that includes also a number of other taxa (Waksman, 1961). The cell wall of actinomycetes, like that of bacteria contains a murein (mucopeptide) layer. Boone and Pine (1968) proposed a rapid method for characterization of actinomycetes by cell wall composition. Lipid patterns can be used to separate streptomycetes from nocardiae (Mordarska, 1968) confirming the separation made on the basis of the isomer of DAP present in their cell walls. The spores of the *Streptomyces* are classified into various groups such as smooth, warty, spiny, and hairy based on its surface (Dietz and Mathews, 1971).
2.4.3 16S rDNA studies and molecular identification of *Streptomyces* species

Traditionally, identification of bacteria, including actinomycetes, has been very time consuming and laborious, except for some pathogenic species for which simple identification methods have been established (Laidi *et al.*, 2006). The sequence of the 16S rRNA gene is highly conserved for different organisms and has been widely used as a molecular clock to estimate relationships among bacteria (phylogeny) and to identify an unknown bacterium up to the genus or species level as biochemical identification is not accurate for determining the genotypic differences of microorganisms (Sacchi *et al.*, 2002). The 16S rDNA sequencing data are being used to establish the boundaries and internal taxonomic structure of the genus *Streptomyces* (Kim *et al.*, 2000).

2.5 *Streptomyces*

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and classified in the family *Streptomycetaceae* on the basis of morphology and subsequently cell wall chemotype. Among the genera of actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology, and biochemical activities. Members of this genus are aerobic, Gram-positive soil saprophytes with extensive branching substrate and aerial mycelia. Characteristic long chains of arthrospores are formed within the aerial mycelia at a mature stage in their life cycle (Laidi *et al.*, 2006). Many novel bioactive compounds with several unique structures have been isolated from *Streptomyces* (Han *et al.*, 2008).

Interestingly, the majority of the antibiotic-producing actinomycetes are found among these species, which led to a growing economic importance for this group of organisms (Remya and Vijayakumar, 2008). This resulted in isolation and description of numerous new species, requiring new divisions into subgenera or series. In an attempt to make such separations, some authors have considered certain important characters such as the morphology, including the structure of the substrate mycelium, the nature and formation of aerial mycelium, the structure and branching of pseudohyphae containing spores, and the spore surface. Cultural criteria, such as growth on different media, colour of aerial and substrate mycelia, and formation of soluble pigments, among others, were also taken into account. Biochemical criteria like utilization of carbon sources, proteolytic properties, usage of nitrogenous compounds, presence of oxidases and reductases as well as sensitivity to antibiotics and phages, serological reactions, ecological properties and
genetic relationships were also used for the classification of *Streptomyces* spp (Laidi *et al.*, 2006).

Several *Streptomyces* species have been reported to possess the ability to degrade various polysaccharides such as chitin, cellulose, xylan and starch by the secretion of extracellular enzymes (Tsujibo *et al.*, 1999). Chitin is an excellent carbon and nitrogen source for many *Streptomyces* strains (Han *et al.*, 2009). The ability to hydrolyze chitin to utilize as a carbon source is an important characteristic of *Streptomyces*, which are considered to be major decomposers of chitin in soil (Saito *et al.*, 1999).

### 2.5.1 Endophytic *Streptomyces*

Endophytic microbes including bacteria, actinomycetes and fungi are ubiquitous in most plant species, especially in field-grown plants. Although some of the endophytes are pathogenic to host plants and can locally or systemically colonize plant tissues, others latently reside in the internal tissues of non symptomatic plants without causing any adverse effects to the plants (Hasegawa *et al.*, 2006). Endophytes have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses (Hallman *et al.*, 1997). Kado (1992) defined endophytes as “bacteria that reside within living plant tissues without doing substantive harm or gaining benefit other than securing residency.” Endophytes are synergistic to their host, at least some of them are thought to be making returns for the nutrition from the plant by producing special substances such as secondary metabolites to prevent the host form successfully attacking fungi and pests (Taechowisan and Lumyong, 2003). Endophytic *Streptomyces* inhabiting the cortical tissues of root system was isolated by Quaroni *et al.* (1989). Their involvement in the enhancement of plant growth was reported (Quaroni and Saracchi, 1994).

### 2.6 Screening for chitinase production

Screening and isolation of organisms capable of producing chitinase is usually done on a medium containing chitin (Kamil *et al.*, 2007). Chitinase activity can be qualitatively assayed by determining the clearance zone developed around the colonies growing on the colloidal chitin agar medium (Wirth and Wolf, 1990). The potency of the isolates for chitinase production is determined on the basis of ratio of zone of clearance (CZ) to colony size (CS) (Gohel *et al.*, 2006).
2.7 Chitinase production and optimization

Biologically active enzymes may be extracted from any living organism. The naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use. However, by isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained (Oyeleke et al., 2011). Of the hundred enzymes being used industrially, over a half is from fungi, over a third is from bacteria with the remainder divided between animal (8%) and plant (4%) sources (Ryan et al., 2006). Microbes are preferred to plants and animals as sources of enzymes because they are generally cheaper to produce and their enzyme contents are more predictable and controllable (Burhan et al., 2003).

Several factors such as chitin, yeast extract, ammonium sulphate, trace elements, tween-20, magnesium sulphate, ammonium chloride, potassium nitrate, diammonium hydrogen phosphate, sodium nitrate, L-glutamine, L-asparagine, peptone and urea have been reported to influence chitinase production by bacteria (Gohel et al., 2006). Medium components greatly influence the microbial production of extracellular chitinase and their interaction play an important role in the synthesis of this enzyme. On the other hand media optimization is very important not only to maximize the yield and productivity, but also to minimize the production cost (Park et al., 2005). Chitinase activity has been assayed variously by viscosimetry, turbidimetry, and calorimetry, using colloidal chitin and glycol chitin as substrates.

2.8 Purification of chitinase

The purification of a particular enzyme involves removal of other substances (proteins as well as non-proteins) present in the preparation. It is generally a multi-step process exploiting a range of biophysical and biochemical characteristics such as its relative concentration in the source, solubility, charge, size (molecular weight), hydrophobicity/hydrophilicity of the target protein. In general, design of the purification technique is focused on high recovery, highly purified enzyme protein, reproducibility of the methods, economical use of the chemicals and reagents and shorter time for complete purification (Kumar and Garg, 2006).
Chitinases have been purified by means of multistep procedures including affinity chromatography on chitin or cross-linked chitin followed by gel and/or ion-exchange chromatographic purification of chitin-retained proteins (Yanai et al., 1992). Chitinases from Serratia marcescens were isolated from culture supernatant by one-step affinity sorption on chitin as an insoluble substrate (Robert and Cabib, 1982). An enzyme system produced by S. kurssanovii was found to be one of the most effective sources of chitinolytic enzymes. It consists at least of four chitinases, chitobiase, some proteinases, and several unidentified proteins. Two of these chitinases with molecular masses of 42 kDa (Chi 42) and 26 kDa (Chi 26) are the main ones. They were selectively isolated and obtained in homogeneous forms using a one-step chromatographic procedure on N-cross-linked fully N-acetylated chitin or partially deacetylated chitin sorbents, respectively (Stoyachenko et al., 1994).

2.9 Crude sources of substrates for chitinase production

The production of inexpensive chitinolytic enzymes is an important element in the use of chitinous wastes, which not only solve environmental problems but do with added value in certain cases (Mukherjee and Sen, 2006). The main commercial sources for chitin production are crustacean wastes due to abundance and disponibility. The limitations encountered when using crustacean wastes is its perishability and seasonal production (Matsumoto, 2006). Shrimp and crab shell contain chitin, protein and inorganic compounds mainly composed of calcium carbonate (Cosio et al., 1982). Traditional methods for the preparation of chitin include demineralization and deproteinization of the waste material with strong acids and bases (e.g., HCl and NaOH) (Wang et al., 1982). The production of chitin and its hydrolyzed derivatives, such as acetylglucosamine and chitooligosaccharide, from waste of the shellfish industry has been limited due to the high cost of chitinase and the shrimp and crab shell pretreatment process (Wang and Chang, 1997). The utilization of this waste for the purpose of chitinase production seems to be of highly considerable interest. This is due to the promising application of this enzyme in many industries and agriculture (Uria et al., 2006).

In recent years, chitin obtained by extraction from fungal mycelia is gaining importance. Fungal mycelia can be cultivated throughout the year by fermentation that is rapid, synchronized and can be organized in a closed or semi-closed technological circuit.
to comply with modern ecological requirements. In addition, fungal mycelia are relatively consistent in composition and are not associated with inorganic materials; therefore, no demineralization treatment is required to recover fungal chitin (Badawy and Rabea, 2011). In the recovery of chitin from shellfish sources, the removal of associated minerals and proteins normally requires the use of HCl and NaOH. These reagents can cause deacetylation and depolymerization of chitin. An alternative to these harsh chemical treatments is the use of proteolytic microorganisms, in particular extracellular proteases secreted from fungi (Teng et al., 2001).

2.10 Antimicrobial properties of chitinases

2.10.1 Antifungal role of chitinase

Lysis of the host structure by secretion of extracellular lytic enzymes is one of the important mechanisms that are involved in the antagonistic activity of biocontrol agents (Kim et al., 2001). Chitinase plays a vital role in the biological control of many plant diseases by degrading the chitin polymer in the cell walls of fungal pathogens (Haran et al., 1993). It affects fungal growth through the lysis of cell wall (Kunz et al., 1992), hypal tips, and germ tubes (Gunarantna and Balasubramanian, 1994). Fungal phytopathogens cause serious problems worldwide in agriculture and food industry by destroying crops and economically important plants in the field and during storage, especially in the subtropical and tropical regions (Pohanka, 2006). In addition, many also produce mycotoxins, which are harmful to humans and livestock. To a certain extent, chemical fungicides but also antifungal antibiotics and compounds based on natural products can control fungal pathogens. Many of synthetic compounds can keep fungal infections at an acceptable level. Nevertheless, they are associated with several drawbacks such as their lack of specificity, accumulation if biodegradation is slow or even missing, and others are toxic not only to fungi but also to other beneficial life forms as well, including humans. They also have led to environmental pollution and development of pathogen resistance (Pohanka, 2006). Against these worsening problems in fungal disease control, biodegradable antifungal agents are preferred as they are free from polluting residues and represent a reduced likelihood of developing resistant fungal strains. Therefore, microbe-based biocontrol methods are one alternative way to control diseases in place of agrochemicals (Oskay, 2009).
Fungal cell walls are rich in chitin, the potential application of chitinase in biocontrol of fungal phytopathogens is promising (Mahadevan and Crawford, 1997). Elegant demonstrations of the involvement of chitinase in the control of *Sclerotium rolfsii* by *Serratia marcescens* using genetic engineering techniques have provided evidence about the role chitinase plays in disease control (Chet et al., 1990). Chitinases have been purified from various fungi, including *Trichoderma harzianum* (Ulhoa & Peberdy 1992) and *Phanocladium album* (Kunz et al., 1992). Culture filtrates of *Acremonium obclavatum* inhibited uredospore germination and germ-tube growth of *P. arachidis*, possibly because of secondary metabolites or lytic enzymes present (Jayapal and Balasubramanian, 1993).

### 2.10.2 Antibacterial role of chitinase

Chitinases has activity against both Gram-positive and Gram-negative bacteria (Wang and Chang, 1997). The enzyme produced by *Monascus purpureus* CCRC31499 inhibited the growth of *Bacillus cereus* CCRC10603, *Bacillus subtilis* CCRC10255, *Escherichia coli* CCRC 10239, *Pseudomonas aeruginosa* K-187, *Staphylococcus aureus* CCRC10780, and *Streptomyces actuosus* A-151 (Wang et al., 2002). The antimicrobial activity of chitinase produced from *Bacillus* sp. strain showed activity against some microbial strains viz. *E. coli*, *S. pyogenes*, *Klebsiella pneumoniae* and *Lactococcus* sp., by well diffusion method (Mathur et al., 2011).

### 2.11 N acetyl glucosamine and its Production

N-Acetylglucosamine and glucosamine are amino sugars with therapeutic potential for the treatment of a variety of diseases, including arthritis (Hochberg et al., 2001), inflammatory bowel disease (Salvatore et al., 2000) and general inflammatory damage (Kamel, 1991). These sugars can be produced from chitin substrates. The most common source of chitin for producing GlcNAc is shellfish biomass. However, the supply of shrimp and crab shells is highly dependent on seasonal and environmental factors, leading to unpredictable limitations on production capacity. Another option to stabilize the chitin source for the production of GlcNAc is to utilize a non-shellfish chitin source. In several typical fungal species, at least 15% of chitin exists in their cell walls. Chen et al. (2001) established a method to produce chitin by culturing the fungus *Actinomucor taiwanensis* and GlcNAc using it as a substrate. Bohlman et al. (2004) obtained GlcNAc of high purity by degrading fungal chitin using either acid (6.42
mg/mL) or enzymes (4.04 mg/mL). Raetz et al. (1998) compared several chitins from different sources and tested their susceptibility to enzymes isolated from Penicillium janthinellum P9. Their results also confirm that fungal chitin has a higher susceptibility to hydrolysis than arthropod chitin.

2.11 Chemical synthesis of N-acetylglucosamine

Commercial production of these amino sugars currently relies upon acid hydrolysis of de-proteinized and demineralised crustacean shells (Ferrer et al., 1996). Acid hydrolysis is relatively efficient but involves strong acids (4 - 8 M HCl) at high temperatures and results in production of toxic wastes. Moreover, the extreme conditions used in the process may result in unwanted modifications to the hydrolysis products.

Although GlcNAc is produced by acid hydrolysis of chitin, this procedure has some problems such as high cost, low yield (Sakai, 1995), and acidic wastes by use of conc. HCl, etc. N-acetylation of GlcN is also possible to produce GlcNAc. This product, however, is not approved as a natural type material owing to its chemical process.

GlcNAc produced by chemical methods is not widely commercialized, not only due to technical reasons but also because of environmental concerns. The large quantities of chemical waste resulting from chemical processes are not environmentally friendly. However, the enzymatic hydrolysis of chitin can produce GlcNAc under mild conditions.

Acid hydrolysis of chitin using hydrochloric acid is an industrial process for the production of glucosamine hydrochloride salt (GlcNHCl), one of the most popular food supplements and drugs prescribed for osteoarthritis patients (Hitoshi et al., 2002). To avoid the several side effects of inorganic salt and its salty taste, a non-salt form of GlcNAc which has pure sweet taste has been proposed to be used as an alternative for the same applications (Rath et al., 2002).

2.11.2 Enzymatic synthesis of N-acetylglucosamine

Conversely, enzymatic degradation of crustacean shells is environmentally friendly but is more complex since it involves both production of the enzyme and the digestion of the substrate (Donzelli et al., 2003). The conversion of chitin to NAG through microbial action requires the sequential action of the chitinolytic system comprising of an endochitinase, chitobiase and an exochitinase whose actions may be synergistic and consecutive in the degradation of chitin to free GlcNAc (N-acetyl
glucosamine) (Gohel et al., 2006). Chitin degradation is generally started by random cleavage within the chain of chitin by chitinases to release oligomers of N-acetyl glucosamine chains (GlcNAc). The oligomers are subsequently degraded to monomeric GlcNAc by β-N-acetylhexosaminidase which progressively breaks down chitin and chitooligosaccharides from the non-reducing end of the molecule (Paraman et al., 2005).

Reports on preparation of GlcNAc using a mild reaction condition involving the use of bacterial enzymes have emerged in literature in recent year (Li et al., 2006; Seok et al., 2007; Yuji et al., 2008). Various isolates of Bacillus thuringiensis produce chitinases that are able to hydrolyse chitin molecules into N-acetylglucosamine (GlcNAc) monomers (Thamthiankul et al., 2001). Preparation of GlcNAc from chitin with a yield of 65% was done by utilization of the crude enzyme from Aspergillus (Setthakaset et al., 2008). Trichoderma chitinolytic preparations have a high ratio of exochitinase to endochitinase activity and release almost exclusively monomeric GlcNAc from chitin (Donzelli et al., 2003). The reports of Sashiwa et al. (2001) emphasizes on the good yield of GlcNAc from α-chitin or β-chitin by use of crude enzymes. The quantitative production of GlcNAc could be achieved from β-chitin by the mixing of two crude enzymes such as cellulases derived from Trichoderma viride (T) and Acremonium cellulolyticus (A) (Sashiwaa et al., 2002). For the industrial production of GlcNAc, a large quantity of enzymes can be purified from the mass production of microbes or genetically engineered microorganisms (Pan et al., 1996).

2.11.3 Applications of N acetyl glucosamine

GlcNAc is an important component of biomacromolecular synthesis in the body (Shoji et al., 1999). It has been widely utilized as a nutritional supplement for therapeutic usage (Lee et al., 2004) and to treat joint damage (Creamer, 2000). GlcNAc is a potential candidate to treat inflammatory bowel disease (IBD) by enhancing the release of acid mucopolysaccharides by fibroblasts and restoring the formation of the protective structure of the gastrointestinal tract (Karzed et al., 1971). GlcNAc promotes the proliferation of keratinocytes and fibroblasts and increases the production of hyaluronic acid in the skin which maintains the moisture and heals wounds (Minami and Okamoto, 2007). GlcNAc is used as a substrate in sialic acid production. Sialic acids are found at the distal ends of cell surface glycoconjugates and are major determinants of cellular recognition serving as receptors for influenza viruses (Tanner, 2005).
Chitooligosaccharides and their N-acetylated analogues are useful for applications in various fields because they have specific biological activities such as antimicrobial activity, antitumor activity, immuno-enhancing effects (Gohel et al., 2006). Some chitooligosaccharides such as (GlcNAc) and (GlcNAc) have been reported to possess antitumor activity (Liang et al., 2007). Topically applied N-acetyl-D-glucosamine or N-acetylgalactosamine containing compositions exfoliate the skin. Combinations of N-acetyl-D-glucosamine and N-acetylgalactosamine can also be used. These compositions which exfoliate the skin can also include chitin to enhance the process of exfoliation (David and Thomas, 2000). N-Acetyl-D-glucosamine (GlcNAc) has been a focusing material for the improvement of osteoarthritis (Vaz, 1982).

2.12 Biosorption and effluent treatment using microbial biomass

Comprising over 70% of the earth's surface, water is undeniably the most valuable natural resource existing on our planet, without which, the life on the earth would be non-existent. Although this fact is widely recognized, pollution of water resources is a common occurrence. The potable water has become greatly affected, and in many instances has lost its original purpose. Industrialization is a hallmark of civilization, however, the fact remains that industrial emissions have been adversely affecting the environment leading to destruction of many agricultural lands and water bodies, thus becoming a matter of great concern. There are many sources of water pollution, but two main general categories include direct and indirect contaminant sources. Direct sources include effluent outfalls from industries, refineries and waste treatment plants; whereas, indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water (Vijayaraghavan and Yun, 2008).

Environmental pollution from hazardous metals and minerals can arise from natural as well as anthropogenic sources. Heavy metals are commonly defined as those having a specific density of more than 5 g/cm³. Unlike other pollutants, these metals in the environment may accumulate unnoticed to toxic levels (Lars, 2003). The impact of heavy metals on the environment and their accretion through the food chain have promoted research aimed at developing alternative, efficient and low cost waste water purification systems before discharging them into streams and rivers. (Saurav and Kannabiran, 2011).
Among all heavy metals, copper, chromium and zinc ingestion beyond permissible quantities causes various chronic disorders in human beings (Beszedots, 1983). Chromium, an essential micronutrient required for the growth of many organisms in its trivalent form is relatively insoluble in water and 100 times less toxic than the hexavalent form (Ganguli and Tripathi, 1999). It has been designated as the priority pollutant by US EPA (Srinath et al., 2002). It is released into the environment by a large number of industrial operations such as electroplating, chromate manufacturing, dyes and pigment manufacturing, wood preservation, leather tanning industry, manufacture of alloys and as corrosion inhibitor in conventional and nuclear power plants (Poopal and Lakshman, 2009) at concentrations ranging from tenths to hundreds of mg/l (Park et al., 2005).

Though chromium exists in nine valence states ranging from -2 to +6, Cr (III) and Cr (VI) are of major environmental significance because of their stability in the natural environment. The chromate anion is highly soluble and therefore can overcome the cellular permeability barrier, entering via sulphate transport pathways since it bears structural similarity with SO$_4^{2-}$ (Mabbet et al., 2001). Unless it is rapidly reduced it can oxidatively damage the DNA via the production of free radicals. It has been reported that hexavalent chromium causes lung cancer, chromate ulcer, perforation of nasal septum and kidney damage in humans and it is also toxic to other organisms as well (Smith et al., 2002).

The leather industry that deals with lot of pre-tanning, tanning and post-tanning chemicals faces very challenging task because of the regulations enforced by the various pollution control bodies to maintain the discharged norms of the chemical in the treated water. Chromium tanning also falls under this category because of the considerable amount of chromium discharged in the effluent. Potable waters containing more than 0.05 mg/l of chromium is considered to be toxic (Vishwanatham, 1997). The discharge norm for chromium in the effluent is 2 ppm and this level can only be achieved, if chromium from the spent liquor is recovered and reused in the tanning process (Kanagaraj et al., 2008). Though there are different methods such as chemical precipitation, coagulation, solvent extraction and membrane process, ion exchange and adsorption methods available to treat the chromium waste generated from chrome tanning process (Minghua et al., 2006), these conventional methods are not completely feasible to reduce the chromium
concentration to levels as low as required by environmental legislation (Cossich et al., 2002). In this context, the biosorption process has been recently evaluated.

Biosorption is a process in which solids of natural origin are employed for binding heavy metals. It is a promising alternative method to treat industrial effluents, mainly because of its low cost and high metal binding capacity. The mechanism of metal biosorption depends upon status of biomass (living or non-living), types of biomaterials, properties of metal solution chemistry, ambient/environmental conditions such as $pH$, influence the mechanism of metal biosorption. The major factors that affect the biosorption processes are initial metal ion concentration, temperature, $pH$, and biomass concentration in solution.

Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents, due to metal sequestering properties and can decrease the concentration of heavy metal ions in solution. The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the later is attracted and removed by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases (Volesky, 1986).

Potent bacterial metal biosorbents includes Bacillus (Akar and Tunali, 2006) and Pseudomonas (Uslu and Tanyol, 2006) biomass. The Streptomyces biomass such as S. pimprina (Puranik et al., 1995), S. rimosus (Chergui et al., 2007), Streptomyces sp. (MB2) (Sharma and Goel., 2009) and S. griseus (Ashwini et al., 2009), originating as by products from industrial bioprocesses have been tested for biosorption of heavy metal ions. Important fungal biosorbents include Aspergillus (Binupriya et al., 2006), Rhizopus (Park et al., 2005) and Penicillium (Tan and Cheng, 2003), etc. These microorganisms are used widely in different food and pharmaceutical industries. As they are generated as waste, they can be attained free or at low cost from these industries. Sea weeds are also excellent biosorbents due to the presence of alginate in their cell wall (Davis et al., 2003). However, it should be noted that seaweeds are not regarded as wastes; in fact they are the only source for the production of agar, alginate and carrageenan. Therefore, utmost care should be taken while selecting seaweeds for a biosorption process (Vieira and Volesky,
Thus the production of cheap biosorbent materials based on natural and waste biomasses constitutes the basis for a new cost-effective technology that can find its largest application in the removal of metal contaminated industrial effluents (Nilanjana et al., 2008).

Though biosorption is a process of treating pollutant-bearing solutions to make it contaminant free, it is also necessary to be able to regenerate the biosorbent which is possible with the help of appropriate elutants, that must be non damaging to the biomass, less costly, environmental friendly and effective resulting in a concentrated pollutant solution (Kuyucak and Volesky, 1989). Therefore, the overall achievement of a biosorption process is to concentrate the solute, i.e., sorption followed by desorption. Thus desorption is of utmost importance when the biomass preparation/generation is costly, as it is possible to decrease the process cost and also the dependency of the process on a continuous supply of biosorbent.