CHAPTER - I

GENERAL INTRODUCTION

Waste management is one of the big challenges facing human society today. Agricultural, industrial and household waste contaminates the environment, disrupts the food chain and spreads infectious diseases. But, waste is no longer considered to be a nuisance as recovery of raw materials and energy is feasible on an industrial scale. Energy recovery from waste can be established in different ways. Research has been focussed on production of not only RDF (refuse derived fuel), but also other potential fuels, such as ethanol and methane obtained by bioconversion of organic wastes (Moolenaar, 1981).

Bioconversion Technology is an ideal solution for managing industrial and municipal wastes. Bioconversion is the conversion of organic materials, such as plant or animal wastes, into usable products or energy sources by biological processes or agents, such as certain microorganisms, some detritivores or enzymes. (https://en.wikipedia.org/wiki/).

Fish and meat waste management has been one of the problems having the greatest impact on the environment. Research has been focussed to develop methods to convert these wastes into useful products (Arvanitoyannis and Kassaveti, 2008; Arvanitoyannis and Ladas, 2008). Bioconversion of Marine Trash Fish (MTF) to organic liquid fertilizer for effective solid waste management has been envisaged (Aranganathan and Radhika Rajasree, 2016). The recovery of chemical components from seafood waste materials for use in other segments of the food industry is a promising area of research. Researchers have shown that a number of useful
compounds can be isolated from seafood wastes including chitin, enzymes, gelatin and proteins.

Shrimp industry is a rapidly growing industry in India and all over the world. But major concern of the seafood industry is the large amount of waste materials. They are highly perishable in nature as they are quickly colonised by spoilage organisms and can rapidly transform into a public health hazard (Sini et al., 2005). The shrimp waste contains useful components such as chitin, protein, lipid and astaxanthin pigment, thus making the commercial shrimp waste as an attractive material for extraction of the above mentioned components (Arvanitoyannis and Kassaveti, 2008).

Shell waste produced by the seafood industry is one of the most important problems contributing to significant environmental and health hazards. The most frequent method employed for its disposal is burning which becomes environmental costly due to low burning capacity of shells. In such a scenario, conversion of shrimp shell waste to chitosan a commercially valuable product with a myriad of uses, could serve as an effective mode of shell remediation (Divya et al., 2014). Alternatively, this waste can be utilized as an economic source of chitin and its derivative chitosan. There has been a glorious opportunity to utilize these shrimp wastes in the manufacture of value added human food, animal and fish feed and valuable medicinal products like chitin and chitosan. Chitin and chitosan are considerably versatile and promising biomaterials (Tarafdar and Biswas, 2013).

Crustacean shell waste consists mainly of 30-40% protein, 30-50% calcium carbonate and 20-30% chitin (Hobel, 2004; Crini et al., 2009; Jo et al., 2011) with species and seasonal variations (Cho et al., 1998). Chitin makes an insoluble linear
beta 1-4 polymer of N-acetylglucosamine, one of most common polysaccharides occurring in nature after cellulose (Tsujibo et al., 1998; Arbia et al., 2013). It is approximately produced at levels more than $10^{11}$ tonnes annually in the aquatic biosphere. For example, copepods, a single subclass of marine zooplankton alone produce billions of tonnes of chitin annually (Jung et al., 2008). More than $1\times10^{10}$ to $1\times10^{12}$ tonnes of chitin is produced annually in terrestrial and marine habitats (Kumar et al., 2004). The major contribution of chitin to soil is in the form of animal biomass. Similarly, in the marine environment more than $10^{11}$ metric tonnes of chitin is produced annually (Kehyani and Roseman, 1999).

Chitin and its derivatives are increasingly finding use in diverse fields such as biomedicine (Yadav et al., 2005), agriculture and even in cosmetics (Keyhani and Roseman, 1999). They have antifungal and nematicidal properties and hence used in agricultural biocontrol methods (Gohel et al., 2004). Chito-oligosaccharides and their N-acetylated analogues are useful for applications in various fields because they have specific biological activities such as antimicrobial activity, antitumor activity and immune enhancing effects (Liang et al., 2007a). It has a wide range of applications in areas such as medicines, fine chemicals for water treatment, pulp and paper industries, biomedical devices, therapies, cosmetics, membrane technology, biotechnology, food applications and textiles (Mohmoud et al., 2008; Park et al., 2005; Boutistat et al., 2001; Yang et al., 2000; Wang et al., 2006, 2011).

The commercial method for the preparation of chitin from shrimp shells involves strong acid and alkali treatments to remove the minerals and proteins (Bautistat et al., 2001). However, the use of these chemicals cause depolymerisation of the product and therefore affects its properties. These chemical treatment methods
bring about hazardous environmental problems like disposal of wastewater. The cost of the chemicals is another drawback of this approach. To overcome the problems of chemical treatments, different microorganisms were used (Sini et al., 2007; Ghorbel Bellaaj et al., 2011). During fermentation with microorganisms, deproteinisation takes place through the activity of proteases and demineralisation through the acid produced by the microorganisms (Rao et al., 2000).

Microorganisms produce the chitinase enzyme primarily for the assimilation of chitin as carbon and nitrogen source (Flach et al., 1992). Chitinases have been isolated from variety of bacteria including Bacillus sp. and some of them are reported to produce multiple forms of chitinases with different molecular masses (Vaidya et al., 2001). Almost all the chitinase producing strains use chitin (or colloidal chitin) as a major carbon source (Wang et al., 2006). However, the utilization of chitinous shellfish waste not only solves environmental problems but also decreases the production costs of microbial chitinases. The production of inexpensive chitinolytic enzymes is an important element in the process (Wang et al., 2008b; Wang and Yeh, 2008). There is an increasing interest in the use of chitinases for the control of moulds, insects, nematodes and production of different chitin oligomers (Sakai et al., 1991). Chitinases also play a role in the utilization of crustacean waste (Vyas and Deeshpand, 1991). The ability of chitinase to hydrolyze chitin makes it very useful for the production of value added products such as sweeteners, growth factors and single cell protein (Asadpour et al., 2003).

Shrimp shell powder was used as a substrate for the coproduction of chitinase and protease by Bacillus cereus SV1 (Ghorbel Bellaaj et al., 2012). Several species of bacteria such as Bacillus pabuli (Frandsberg and Schnurer, 1994), Bacillus licheniformis (Takayanga et al., 1991) and mainly Serratia marcescens have shown a
chitinase producing ability. Chitinolytic enzyme producing *Aspergillus* species have long been recognized as an agent for biowaste management. Chitinase is an enzyme responsible for metabolizing chitin. *Aspergillus terreus* CBNRKR KF529976 has been isolated from marine soils and its growth conditions were characterised for the maximum biodegradation of marine waste in favour of the production of highly active chitinase (Krishnaveni and Ragunathan, 2014).

Chitinases have received attention because of their wide applications in the medicine, biotechnology, agriculture, waste management and industrial applications such as food quality enhancer and biopesticide. Excessive use of insecticides has led to several problems related to pollution and environmental degradation (Zarei et al., 2012). Recently, the different applications for chitinase have been envisaged, such as biocontrol of fungal diseases in plants (Demarco et al., 2000; Chang et al., 2003), biopesticides (Mendonsa et al., 1996), in production of single cell protein from shellfish waste (Raveh and Carrod, 1981; Vyas and Deshpande, 1991), isolation of protoplast from fungi (Dahiya et al., 2005), production of chito-oligosaccharides, glucosamine and N-acetyl glucosamine (GLcNAc) by chitinase extracted from *Burkholderia cepacia* TU09 for the hydrolysis of chitin (Kuk et al., 2005; Pichyangkura et al., 2002), for treatment of chitinous waste (Wang and Hwang, 2001) and in medical application (Dahiya et al., 2006). The chitinase has been extracted from number of microorganisms such as *Trichoderma harzianum* 8 (Seyedasli et al., 2004), *Bacillus subtilis* SG2 (Khorramzadeh et al., 2005) and *Trichoderma atroviride* PTCC5220 (Harighi et al., 2006) in Iran.

Chitinases can also be added to common fungicides and insecticides, not only to increase their strength and activity, but also to minimize the concentration of their chemically synthesized components, which pose serious threats to the environment
and human health. Dahiya et al. (2006) showed that chitinases from Bacillus sp. BG - 11 were highly compatible with common fungicides and insecticides. It is also used along with antifungal agents and also in skin lotions and creams for fungal infections. Due to its broad range of applications in agricultural and pollution degradation, there exists a strong interest to enhance the chitinase production for industrial purposes (Felse and Panda, 2000a).

Chitin present in the solid waste from shell fish was converted to single cell protein by chitinolytic enzymes. Some of the fungal source for the production of SCP is Saccharomyces cerevisiae, Candida tropicalis, Hansenula polymorpha and Myrothecium verrucaria. Best reports for the production of chitinases are from S. cerevisiae where more than 60% SCP was produced with less nucleic acid content (1 to 3%) (Dahiya et al., 2006). Streptomyces thermoviolaceus, Bacillus sp. BG 11 and Bacillus licheniformis X-7u are thermophilic microorganisms which are major sources for chitinase enzyme. Exochitinase which is thermo stable was recovered from Bacillus stearothermophilus CH-4 from organic solid waste compost (Haki and Rakshit, 2003). Chitinolytic enzymes are used in different combinations to get the desired oligomers. The oligomers GlcNAc, chito-oligosaccharides and glucosamine have an essential role in pharmaceuticals. Chito-oligosaccharides is used even in human medicines (Dahiya et al., 2006). The chitinase activity in bioconversion of shell fish waste to NAG (N-Acetyl Glucosamine) (Tom and Carroad, 1981), which is a monomer of chitin is used in the manufacture of food products such as sweeteners, growth factors, chemicals and pharmaceutical intermediates (Felse and Panda, 2000a).

The antifungal activity and highly biocompatible quality make chitinase and its derivatives particularly useful for biomedical applications, such as wound healing, drug delivery, cartilage tissue engineering and nerve generation (Manivasagan et al.,
2014). The enzyme produced by immobilized cells seems to be valuable in biotechnological applications and can be used against fungal pathogens as an alternative to chemical pesticides. The ability of chitinase in digesting insect chitin raises the idea of using it for controlling insects (Mendonsa et al., 1996).

_Bacillus sp., Enterobacter sp., Aeromonas sp._ and _Serratia_ sp. with chitinolytic activity were isolated from soil and water which can be used to control plant pathogenic fungi and biopesticides (Dahiya et al., 2006). A chitinase produced by _Trichoderma viride_ N9 isolated from a soil sample and its purified chitinase showed antifungal activity against phytopathogenic fungi (Jenifer et al., 2014). The _Bacillus subtilis_ chitinase has antifungal activity against plant pathogens _viz_, _Aspergillus niger_, _A. flavus_ and _Penicillium chrysogenum_ (KaviKarunya et al., 2011).

An extracellular serine protease with novel surfactant and solvent stable alkaliphilic properties was purified from the culture supernatant of _Bacillus subtilis_ TKU007 with shrimp shell wastes as the sole carbon/nitrogen source. The unique properties which include high stability to the solvents, surfactants and alkali, make it an ideal choice for application in detergent formulations and enzymatic peptide synthesis (Wang and Yeh, 2006).

The shrimp wastes have been proven to be an excellent source of carotenoids such as astaxanthin, chitin, proteins and endozymes (Cao et al., 2008). The pigments such as astaxanthin has been widely used in aquaculture feeds (Chien and Shiau, 2005), food industries, pharmaceutical, cosmetics (Seki et al., 2001) and medical studies (Bhuvaneswari et al., 2010).

The chitin structure can be modified by removing the acetyl groups, which are bonded to amine radicals in the C2 position on the glucan ring, by means of a
chemical hydrolysis in concentrated alkaline solution at elevated temperature to produce a deacetylated form. When the fraction of acetylated amine groups is reduced to 40-35%, the resultant copolymer \( (1 \rightarrow 4)-2\text{-amine}-2\text{-deoxy-}\beta\text{-D-glucan} \) and \( (1 \rightarrow 4)-2\text{-acetamide}-2\text{-deoxy-}\beta\text{-D-glucan} \), is then referred to as chitosan (Goy et al., 2009). The non toxic, biodegradable and biocompatible properties of chitin and chitosan provide much potential for many of the food, pharmaceutical and biotechnological applications (Li et al., 1992).

There is some evidence on the effect of chitosan on lowering cholesterol and body weight, but the effect is unlikely to be of clinical importance. To some extent, chitosan is used in the emergency setting to control bleeding. Chitosan has been used in various drug delivery systems. Antimicrobial and other effects are being evaluated for use in dentistry (http://www.drugs.com/npp/chitosan.html).

Positively charged amino groups in chitosan bind to negatively charged lipid and bile components, preventing their absorption by the body. Chitosan dental chewing gum, mouthwashes and gels have been investigated for antibacterial action. Chitosan adheres to salivary pellicles (negatively charged protein film), reduces plaque, increases salivary secretion and exerts antibacterial action effective in managing chronic periodontitis. Chitosan also exerts action against Candida and Chlamydia (Knapczyk et al., 1992; Petronio et al., 1997). Rheology, flocculation and film formation testing have been performed with chitosan, demonstrating its usefulness in medical and analytical applications (Shepherd et al., 1997). As already mentioned, the deacetylation (DA) is determinant in the solubility and charge development, where the \(-\text{NH}_2, -\text{OH}\) groups in the molecule of chitosan are considered as the dominating reactive sites. Hence, as the DA is reduced, higher will be the free amino groups present in chitosan and higher will be the antimicrobial
effect (Andres et al., 2007). Similarly to bacteria, the chitosan activity against fungus is assumed to be fungistatic rather than fungicidal with a potential to communicate regulatory changes in both the host and fungus (Assis, 2008). Generally, chitosan has been reported as being very effective in inhibiting spore germination, germ tube elongation and radial growth (El Ghaouth et al., 1992b; Sashai and Manocha, 1993).

Millner et al. (2009) reported bandages impregnated with chitosan and chitosan granules have been approved by the FDA for use in emergency settings to control blood loss (Hemostasis). Additionally, good film forming properties are valuable for wound dressing, artificial skin or packaging etc. (Younes and Rinaudo, 2015). Its application in these areas has been investigated (Rao and Sharma, 1997; Tsipouras et al., 1997). Chitosan has been used in water purification plants to absorb greases, oils, metals and toxic substances. Chitosan has been used in the cosmetic and fabric industry (Budavari et al., 1989; Skaugrud, 1989). Chitosan film is regarded as biofunctional material, well tolerated by living tissues, particularly applicable as edible coatings to prolong shelf life and preserve quality of fresh foods (Assis and Pessoa, 2004).