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# Role of Chitinases as a waste management to control global crisis

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## ABSTRACT

Crustacean waste shells contain 15-58% chitin, 20-50% calcium carbonate, and 20-40% proteins. In nature chitin is the second most abundant polysaccharide after cellulose, occurs in cell wall of several fungi, yeast, algae, bacteria, exoskeleton of insects, plants crabs, shrimps, lobsters and also in the internal structure of vertebrates. Chitin is degraded by chitinase enzymes into its oligomers and monomeric components. Chitinases play a significant role in the bioconversion of the chitin waste to biofertilizers. Chitin is a linear homopolymer of unbranched β -1, 4-N-acetylglucosamine containing 6.895% nitrogen. Chitin and its associative have broad biological applications in waste management, wound healing, biocontrol agent, drug delivery, and in the formation of food products such as sweeteners, growth factor chemicals and feed supplement for animals. Chitosan is a deacetylated product of chitin which has huge application in cosmetics, pharmaceutical industries, textile, and water treatment. The derivatives of chitin are non-toxic, non-allergic, biodegradable and biocompatible which are used in the formation of a number of artificial medical articles like contact lenses, surgical stitches artificial skin and in the preparation of single cell protein also.

## KEYWORDS

Crustacean waste | Chitinases | Biopesticides | Biofertilizers | Antimicrobial properties

## CITATION

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## Introduction

Chitin is a second most abundant renewable natural biopolymer on the earth, after cellulose. Chitin is a linear heteropolymer of β -1, 4 – N – acetyl – D- glucosamine and glucosamine and related to cellulose with the C -2 hydroxyl group(-OH) which is replaced by acetamido group (-NHCOCH3) (Fig.1) (Cohen *et al*., 1993).

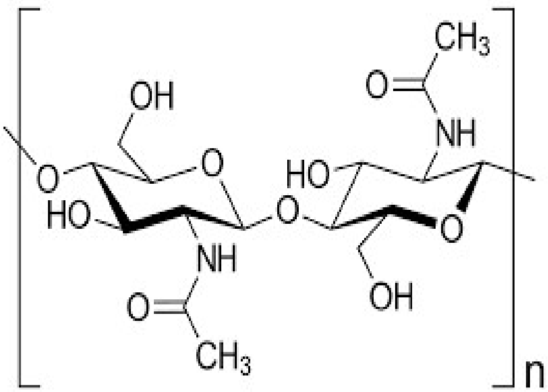


**Fig. 1:** Chemical structure of chitin

Chitin is a major structural component of several organisms including insects, crabs, shrimps, lobsters, algae, yeast, fungi, bacteria, nematodes, and also in the internal structure of a number of invertebrates (Ride *et al*., 1990). Chitin is highly crystalline, white, inelastic, and insoluble in water. Chitin is natural polysaccharides and occurs in the different allotropic forms such as α-chitin, β-chitinand γ- chitin. Due to the antiparallel arrangement of chitin chain, α–chitin is isomorphic andhighly compact crystalline form which favors strong intermolecular hydrogen bonding. The second polymeric form is β – chitin which is less stable and loosely packed as in this form of chitin, the arrangement of chitin chain occurs in parallel fashion with weaker intermolecular forces. γ-Chitin is a mixture of α and β-chitin (Ramírez-Coutiño *et al*., 2006). The percentage of chitin in various organisms is different. It has been investigated that the marine organisms such as crabs and shrimp shells contain highest percentage of chitin approximately 90% of chitinous waste. Chitin helps to form outer skeleton of several vertebrates and fungi because it associated with a major amount of proteinous and non proteinous components. Chitin contains approximately 20- 40% calcium carbonate of chitinous waste in the form of non-proteinous components, which are mainly helps to form the exoskeleton of lobsters, crabs, shrimps, and krills (Muzzarelli RA., 1999). The major distribution of chitin in the biosphere is in the form of biomass. This chitinous biomass can be degraded by chitinase into its smaller units such as chitooligomers and monomers. The degradation of chitin by microorganism was realized in early 1955, when chitin have been used as a primary source of carbon and nitrogen nutrients in an agar medium for the isolation of fungi, bacteria, and actinomycetes (Veldkamp, 1955). Now a day's interest has turned to the role of microorganism in the degradation of chitinous seafood waste such as shrimp, crabs, and lobsters shell waste (Das *et al*., 2012). Marine organism such as shrimps, crabs, lobsters, and krill contains three major components like calcium carbonate, proteins and chitin. In early 1953 it has been estimated that about 38000 tons chitinous waste was produced per year from marine invertebrates, which has been increased day by day (Sabry, 1992). In 2000 it has been increased to 80,000 tons per year (patil *et al*., 2000). Only in India approximately 60,000 – 80,000 tons of chitinous wastes are produced per year by crustacean shell waste (shrimps, lobsters, crabs, and krill). This crustacean seafood wastes are disposed either by land filling or burning or throughing in sea. Chitin is widely distributed in exoskeleton of arthopodes, the outer shell wall of nematodes and crustacean. Chitin has a broad range of application in food, biochemical, chemical industries, and medicinal application in microbial, antitumor, and anticholesterol activity. Chitin and its derivatives are also used in waste water treatment, wound healing drug delivery and as dietary fibers. The essential amount of chitin produced by marine crustacean is over several billion of ton. Chitin is highly soluble in hexafluoro acetone (CF3-CO-CF3), hexa fluoroisopropanol and chloro alcohol with aqueous solution of mineral acids and dimethyl acetamide containing 5% LiCl. The nature of chitin polymer is non- toxic, non- allergic, biodegradable, and anti-microbial. Chitosan have been used to protect crops plant from phytopathogens, in tissue and paper coating, due to their antimicrobial properties. Chitosan is soluble in dilute acids such as CH3COOH, HCOOH. The activity of chitinase can be inhibited by the presence of inhibitors by various metal ions. Chitinase also activate with the help of several metal ions. The first reported inhibitor of chitinase is allosamidin. Chitinases broadly exist in wastes from processing of marine products such as crabs, krill shell, shrimp, lobsters, as well as fish scales. wang and chio 1998 reported that approximately 10 ton of chitin is produced annually in aquatic biosphere alone.

**Chitinases, sources, and role of chitinolytic enzymes**

Bernard was the first person who observed chitinase. Chtinases are the enzymes belong to glycosyl hydrolases which hydrolyze the chitin into its smaller monomer units N–acetyl - D-glucosamine by breaking the glycosidic bonds (Fukamizo, 2000). On the basis of production sources and natural abundance chitinolytic enzymes are present in several microorganism (bacteria, algae, fungi), in higher plants, and in animals (Dean *et al*., 2012). Chitinolytic enzymes are broadly divided into two main classes first is endochitinases (E. C. 3.2.1.14) and second is exochitinases. Endochitinases act on chitin chain at an internal site whereas exochitinases (chitobiosidases and β – 1, 4 –glycosaminidases) act on the chitin chain at the non-reducing site. Chitinases are produced by three different fermentation methods such as continuous fermentation, fed-batch fermentation, and liquid batch fermentation. In recent years solid state fermentation and cell immobilization methods are also used for the production of chitinases. On the basis of amino acids sequences chitinases are classified to family 18, 19, and 20 of glycosyl hydrolases (Bhattacharya *et al*., 2007).

**Extraction of chitin, chitosan, and chito-oligosaccharides from chitinous waste**

According to the F.A.O. (food and agriculture organization) of United Nation, total world crustacean seafood waste in 2000 was 86 million tones. It was 10% of the total crustacean chitinous waste. This chitinous waste contains a very large amount of chitin which can be extracted by chemical and biochemical methods which is completed in several steps such as deproteinization, demineralization, and discoloration. Deproteinization occurs in alkaline medium at high temperature. Chemical demineralization is carried out with dil. hydrochloric acid. Coloured chitin powder is isolated from crustacean waste, thus bleaching process is required which is carried out by H2O2, oxalic acid, or potassium permanganate. By both of these methods harmful gases are released and cause global warming, so these method are harmful for environment and also having several other disadvantages due to high temperature, high concentration of acids and bases, higher energy required, time consuming, larger amount of solvent wasted, and recovery of protein and pigments are difficult.

Biological (microbial) method is used to decomposition of chitinous waste by using chitinase producing bacteria, which degrade chitin to its oligomers (chitooligomers) and monomers (N-acetyl glucosamine). Chito- oligosaccharides (COS) are partially hydrolised product of chitin as acid hydrolysis (HF, HCl, HNO2, H3PO3) and oxidative reductive depolymerization by peroxides, persulphate, ozone and enzymatic and microbial approach for the synthesis of COS. Due to its lower molecular weight COS have been used several interesting bioactivities and bio-applications. Microbial degradation of chitin is environmentally friendly, safer, low cost, and also gives good yield (Kuk*et al*., 2005). Microbial production of N – acetyl-glucosamine (NAG) and COS from crustacean chitinous waste play a significant role in the agricultural, medicinal, food and biotechnology sectors. Microbial extraction occurs in two different steps such as enzymatic deproteinization and microorganism mediated fermentation. In enzymatic deproteinization, proteases are used for the deproteinization of crustacean shell waste. Several other proteolytic enzymes such as chymotripsin, papain, pepsinand trypsin are used to extract and separate the protein and chitin residue from crustacean shell waste. Extraction of chitin from crustacean shell waste consists of two different methods of fermentation such as fermentation with lactic acid bacteria, and fermentation without lactic acid bacteria. Fig. 2: provide a scheme of extraction of chitin, chitosan and COS from marine shell waste following the chemical and microbial process.

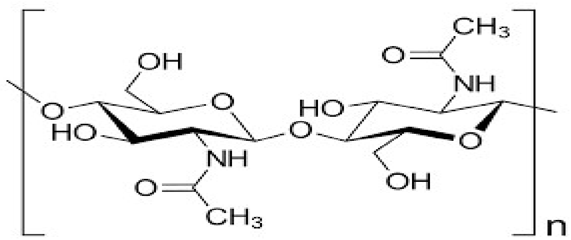


**Fig. 2**

Chitosan (fig. 3) is a deacetylated form of chitin which has been used in the production of several value-added products.

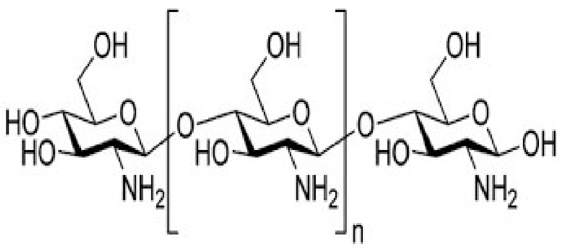
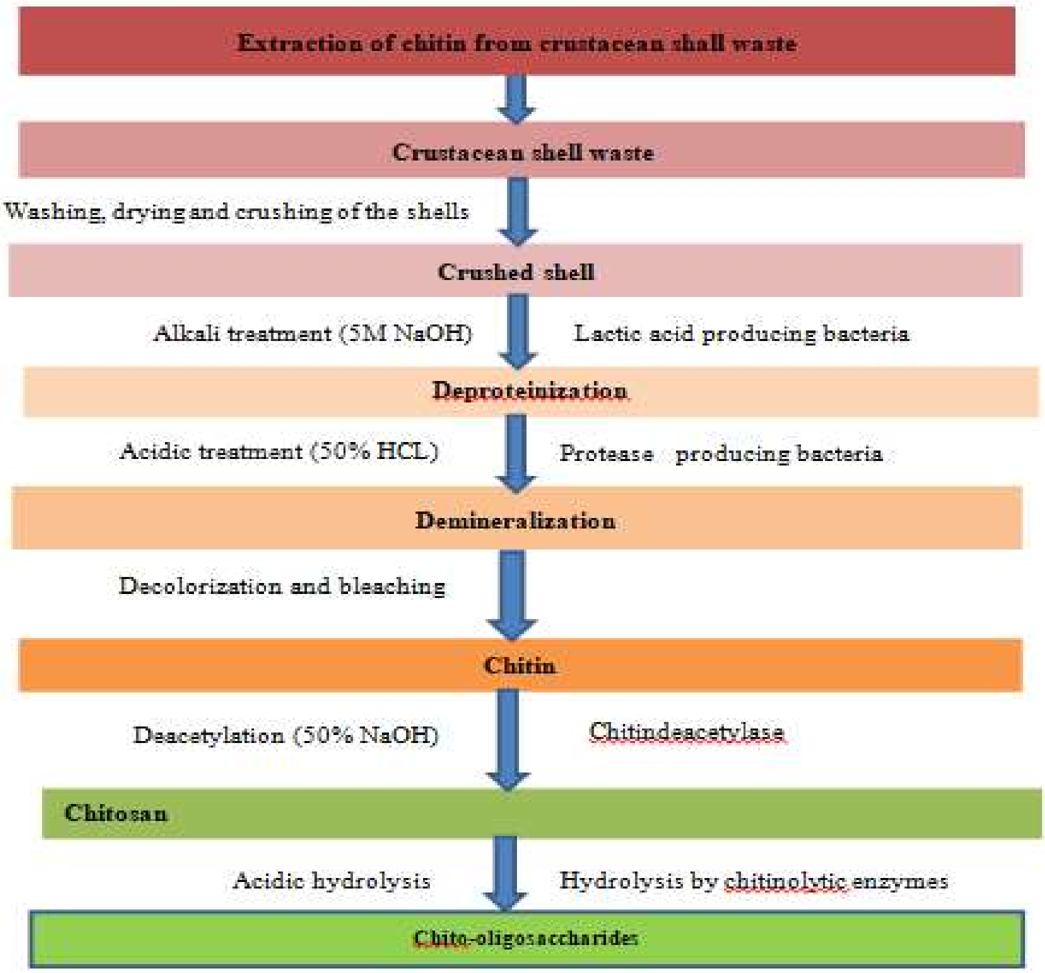


**Fig. 3**: Biological degradation of chitin into chitosan

Processing of large amount of crustacean shell; insects and other organism produced a corresponding large amount of byproducts and waste. A large bulk of these waste are used for the formation of various value added products. Soil and water pollution from sea food industries by organic and inorganic contaminants is a growing concern due to their potential detrimental effects and human health and the environment, as environmental protection is an important global problems. Several industries pay attention to the development of technology which reduces the environmental problems.

**Applications of chitinases**

**In waste management:** Crustacean seafood (shrimps, crabs, lobsters wastes are disposed either by land filling or burning. This chitinous waste caused soil contamination and water pollution. Chitinous biomass of marine organism can be degraded by chitinase into useful depolymerized components, therefore water and soil pollution can be reduced by the bioconversion of chitinous waste into biofertilizers (Sakai *et al*., 1998). Chitinases also used in the production of single cell protein (SCP) in which chitin used as nutrients sources (Revah Moiseev *et al*., 1981). S. cerevisiae, M. vrrucaria, Candida tropicalis, have been widely used to produce SCP. Chitosan, a derivative of chitin used in medicinal, agricultural, cosmetic, textile, and biotechnology for the formation of value-added products (Hamed *et al*., 2015). COS have been used as a food additives, dietary supplements, and product enhancers. NAG can also be used in the manufacture of several food products such as growth factors, sweeteners and pharmaceutical intermediates (Felse *et al*., 2000). Due to the burning of chitinous waste CO and CO2 released in the environment, which increases global warming. Dumping of chitinous waste into the environment increase global warming also due to the generation of methane gas which has 21 times higher global warming potential than CO2. Green processing of chitinous waste is low cost and produced organic biofertilizers (Sahu *et al*., 2017). Chitosan can bind to heavy metals and used as a waste water treatment. Proteins, obtained by chitinolytic degradation of chitinous marine seafood waste have been used as a feed supplement for animals. Chitin and its derivatives have been used for several different environmental applications such as biocompatibility nature of chitin and its derivatives making them suitable for immobilizing sensing elements like chitin nanoparticle and enzymes for the sensing of environmental hazardous chemicals. Removal of toxic metals from water is a matter of great interest in the field of water pollution control. In recent years chitin and its derivatives has been used for the removal of heavy metal such as Cd(II), Zn(II), Hg(II), Cu(II), and Pb(II) from water (Yong *et al*., 2015). Chitin and its derivatives have been used for enhancing the metal sorption capacity with the help of protonated chitin(PC), graft chitin (GC) and carboxylated chitin (CC)by using metal ion such as Fe(III) and Cu(II) (Kousalya *et al*., 2011) for this purpose chitin microfibers and nano particles of chitin and its derivatives have been used for examples chitin nanofibers (CNF) have been used for the removal of Cd(II), Zn(II), Ni(II), Cr(II) and Pb(II) in aqous solution (Liu *et al*., 2013). Chitin have been modified for the removal of metals from water for example- functionalization of chitin by using polypyrrole, acitophenon derivatives of nano chitosan, thio-functionalization of chitin nanofibers, crosslinking chitosan into poly (alginic acid) nanohydrogel have been used for the removal of toxic metals such as Pb(II), Cd(II), As(III), and Hg(II) etc. (Karthik *et al*., 2014).




Schematic diagram of extraction of chitin derivative from crustacean waste by chemical and biological process

Chitin and its derivatives have been used for the remediation of organic contaminants from waste water. Chitosan microparticles (CMS) and silver complexes chitosan micro particles (SCMs) have been used for the removal of a pesticide methyl parathion (MP) from waste water. (Yoshizuka*et al*., 2000; Dolpher and Thiravetyan *et al*., 2011) have been synthesized chitin nanofibers from shrimp shell and studied the absorption of a food aditives melonadin, which causes some carcinogenic, mutagenic and cytotoxic effects. They show a maximum absorption capacity of melanodins 131, 331, and 353 mg/g at 20˚C, 40˚C and 60˚C respectively (Dolphen *et al*., 2011). Lu *et al*., 2011have been synthesized chitosan beads porous crab shell powder from waste shrimp shell and used for the removal of 17 organochlorene particles (OCPs) from the polluted water. The result of the study concluded that the surface morphology of chitosan bead having a rough surface and pores, an enhance of the absorption site for pesticides (Lu *et al*., 2011). Chitosan-carbon based bio compounds are used for the removal of phenols from aqueous solution (Soni*et al*., 2017).

**Medicinal functions**

Chitinases play a significant role to control chitinous biomass into useful depolymerized components. The utilization of catabolic products of chitin, COS has a broad medicinal potential. In medicinal and pharmaceutical field COS has been used as an antioxident, hypoallergic and in drug delivery system. Chitohexaose and chitoheptaose shows antitumor activity. NAG synthesized from glucose, act as an anti-inflammatory drug which is useful in the treatment of ulcerative colitis and other gastrointestinal Inflammation disorders (Aloise *et al*., 1996). It has been reported that chitin and chitin binding proteins have been used for the recognition of fungal infection in humans (Laine *et al*., 1996). Chitinases play a significant role in human health care. Due to their non-toxic, biodegradable, biocompatible, and non-allergic nature of chitin derivatives have been used in antifungal lotions and creams, and in the formation of a several medical articles like artificial skin, contact lenses, and surgical stitches (Muzzarelli *et al*.,1997). Acidic mammalian chitinases (AMCs) have been used in the pathogenesis of asthma. It has been investigated that mammalian chitinases does not have chitinase activity, while AMCs and chitotrisidases have chitinase activity due to its chitin binding domains (Chang*et al*., 2001). Several derivatives of chitin have a potential in various medicinal field such as immune-enhancing effects, anti-tumor, anticancer activity, antihypertensive activity, and hypocholesterolenic. Chitinases play o major role as a vector for gene delivery, as an antifungal, antibacterial agent (Liang *et al*., 2007), as an anti-malaria, as a bone strengthener in osteoporosis and as a haemostatic agent in wound dressing (Aam *et al*., 2010). COS is an anticancer agent with the highest degree of deacetylation and lowest molecular weight has been reported in human myloid leukemia HL-(Kim*et al*., 2012). Huang *et al.,* 2006 have been studied that the highly charged cos shows cell specific anticancer activity against Hep3B, HeLa and SW480 cell lines by reducing cancer cell viability regardless for the positive or negative charges (Huang*et al*., 2012). Salah *et al*., 2013 reported the active mechanism of chemically prepared low molecular weight chitin against human monocyte leukemia cells (THP-1) and human monocytic cells (MRC-5). It has been studied that the antibacterial properties of chitin its derivatives such as chitosan isolated from shrimp shell by chemical method were tested against E. coli strains. Chitosan and its derivatives has high antibacterial activity than chitin (Abdel-Rahman *et al*., 2015). Enzymatically deproteinized chitin, chitosan and other byproducts of chitin have been isolated from Norway lobster shows good antimicrobial activity against fungal and bacterial strains (Sayari *et al*., 2016). Similarly the sulphonated chitosan shows antimicrobial activity against fungal and bacterial strain and the result of the study further confirmed that the microbial inhibition was depend on the type of microorganism and on the type of chitosan used (Sahraee *et al*., 2016; Sun *et al*., 2017). Chitosan used in tissue and paper coating and wound dressing for scar- free wound healing due to its antimicrobial properties.

**In the formation of biopesticides**

Pesticides are chemical compounds used in agriculture fields for protecting crops from damage by insects, fungi, weeds, and disease. Chemical pesticides are harmful for environment and human being also, as these are toxic. Chemical pesticides are caused for several diseases such as respiratory problems, asthma, and cancer. An alternative method has been used to control crop pests and disease by chitinase from microorganism (Sharp, 2013). It has been reported that the strawberry plants was free from insects or pathogenic fungi by direct spray of chitinases from Bacillus species (Koga, 2005).chitin and chitosan can also be directly used as a biopesticides as chitin has a major role in insects metamorphosis and also in guts lining of insects, a pseudo-trisacharide allosamidin is an inhibitor which inhibits the growth of housefly larva and mites can be potentially used as a biopesticides (Muzzarelli, 1999).

**In agriculture**

Chattiness plays a significant role against phytopathogens. Phytopathogens are reducing crop yield. Chitinolytic enzymes such as chitinases has been used to maintenance several plants diseases, as chitinases are able to hydrolyze chitin which is commonly exist in plant fungal pathogens (Knowles *et al*., 1987). Utilization of chitinolytic enzymes not only enhance plant immunity, but also enhance plant growth and crop development by improving their passiveness for various biotic and abiotic stresses that reduce crop yield (Nagpure *et al*., 2014). The development of chitinase encoding gene are play a major role in the development of diseases resistant transgenic plants such as resistant against Sclerotinia sclerotorum in canola by an endochitinase gene, chit33 from Trichoderma atroviride has been reported (Solgi *et al*., 2015). Now day, cloned chitinase genes are manifested into various plant species, as a result diseases resistance has been improved in the developed transgenic plants (Fahmy *et al*., 2018). Utilization of COS has been studied for plant protection from pathogens as plant growth regulators, anti-bactericidal and anti-fungicidal (Winkler *et al*., 2017). Cloned chitinases into different plant species improved disease resistance in the developed transgenic plants (Fahmy *et al*., 2018; Jalil *et al*., 2015). Fungal phytopathogens create most serious problems for economically important plants. In general chemical fungicides are used to reduce fungal pathogen worldwide,but the excessive use of fungicide led to contamination and degradation problems for natural environment and also fatal for beneficial insects and microorganism present in the soil (Bedit *et al*., 2000). Use of microorganism to control phytopathbogens gives a significant approach for sustainable agriculture. Recently recombinant technique can be used to increase the production and isolation, biocontrol efficiency of gene encoding for the chitinases against fungal pathogens (viterbo *et al*., 2001). N-acetylhexosaminidase act as a target for the design of low molecular weight antifungals (Harschet *et al*., 1997). In insecticides and fungicides chitinase enzyme can be added as a supliment to make them more potent, and minimize the chemical ingredients. (Chang *et al*., 2003) has been reported that the chitinases from B. cereus YQ 308 inhibited the growth of plant pathogenic fungi such as F. oxysporum, F. solani, P. ultimum (De las Mercedes Dana *et al*., 2006).

**Conclusion**

Large amount of crustacean shell waste resources from several countries are waiting to be converted to useful and commercial value- added products. The decomposition of crustacean shell waste by chitinase play a major role for reduces environmental pollutions. In this context chitinolytic enzyme plays an important role as a biocontrol agents and waste treatment. The conversion of chitinous waste into value-added products not only has economic values but also environmental friendly. The biological extraction method of chitin and its derivatives from shell fish processing industries is a green process and reduce global warming. Chtin is a noval compound from shell fish processing industries because it has a huge application ranging from agriculture, food processing, biotechnology, textile, tissue engineering, medical, pharmaceutical to cosmetics.

**Future prospects**

Due to huge waste waste produced by the shell fish processing industries , the extraction of chitin from crustacean sea food waste may be a good solution to minimize or reduce the waste an produce several valuable compounds which have been potential for biological, pharmaceutical, agriculture medicinal area and also play a significant role to reduce global warming. Recovery of pigments, proteins and polysaccharides from crustacean sea food waste industries is a green solution to maintain ecological balance. Recombinant techniques can be used to increase the production of chitin and its derivatives for industrial purposes. Researchers are trying to find out new functions of chitinase enzyme. Chitosan is one of the novel compounds for many researchers in the field of biomedical, pharmaceutical sciences and also in various fields of biotechnology. In global scenario, the chitosan and its derivatives have greater scope in biomedical industry.

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