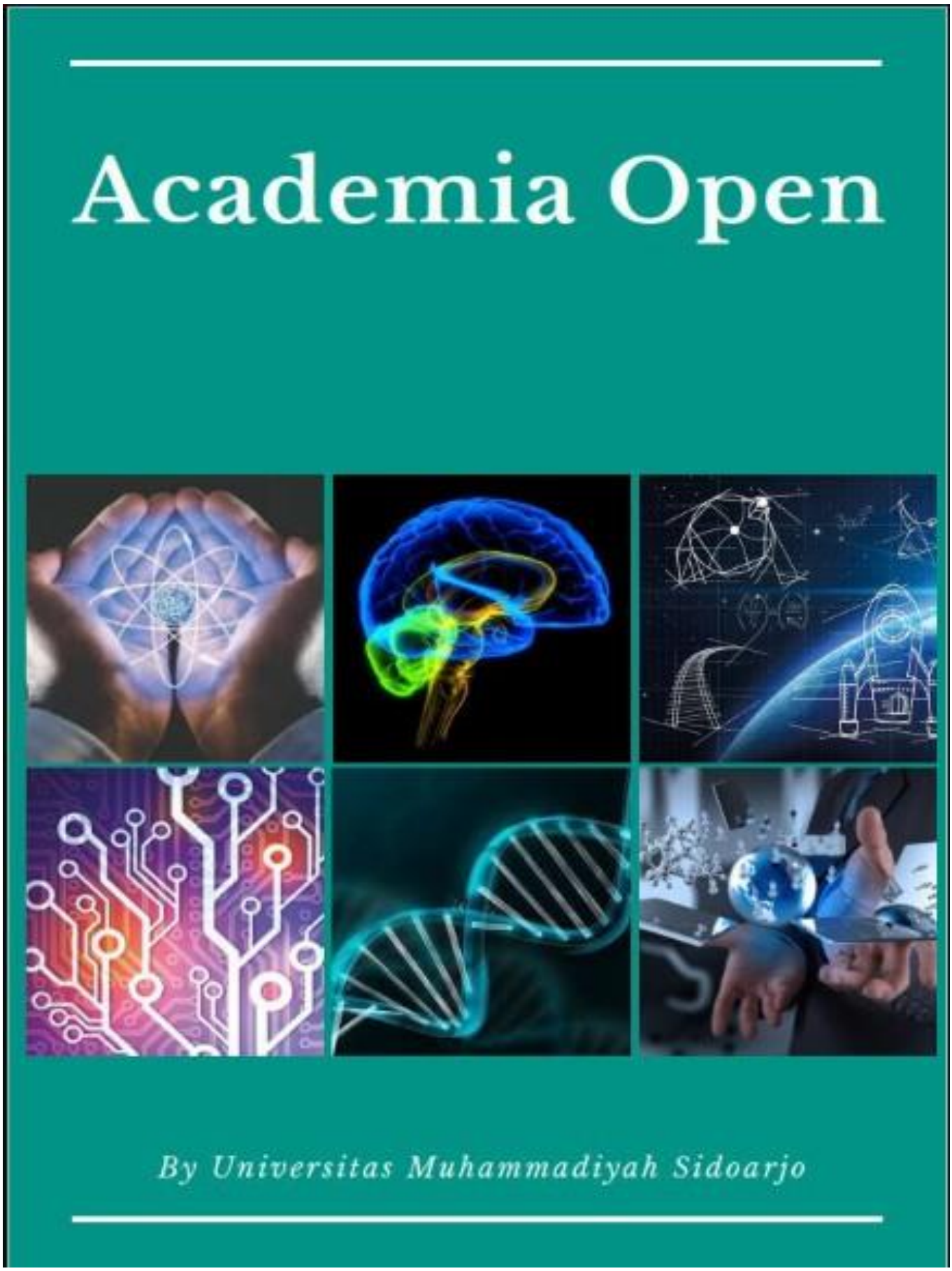
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Short Review of Chitosan and Its Diverse Applications

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Abstract

General Background: Chitin is naturally the second most abundant biological substance found in invertebrates such as fungi, crabs, shrimps, and insects. Specific Background: The rapidly growing shrimp industry generates significant biowaste through the disposal of hard exoskeletons including skins and heads. **Knowledge Gap**: While chitosan possesses exceptional non-toxic, biocompatible, and biodegradable properties, further studies are needed to fully optimize its versatility as a chemical with diverse industrial uses. Aims: This paper summarizes production methods for obtaining chitosan from chitin and its numerous potential uses. **Results:** Chitin undergoes chemical or biological deacetylation to produce chitosan, which acts as an antioxidant and antibacterial agent applicable across agriculture, environmental protection, cancer treatment, industrial applications, food technology, regenerative medicine, biotechnology, and bionanotechnology. **Novelty:** The comprehensive integration of sustainable biological and chemical extraction pathways with broad-spectrum multi-sectoral applications of chitosan**. Implications:** Chitosan will likely have a major impact across diverse biological and industrial sectors in the near future, paving the way for advanced bio-based solutions.

Key Findings Highlights

Chitin is extracted from abundant seafood processing biowaste, specifically shrimp exoskeletons, and converted into versatile chitosan via chemical or biological deacetylation.

Chitosan exhibits powerful antioxidant properties and antibacterial activity by disrupting microbial cell membranes through its positively charged amino groups.

The biopolymer demonstrates wide-ranging applications in drug delivery, agriculture, environmental pollutant removal, regenerative medicine, and food technology.

Keywords Chitin, Chitosan, Deacetylation, Nanotechnology, Regeneration technology

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Introduction

Chitin is an incredibly ubiquitous chemical compound found in nature. The high-molecular-weight linear homopolysaccharide is formed by the repetition of N-acetyl-D-glucosamine units connected by β-(1-4) links . Chitin is present in a wide variety of invertebrates, including insects, crabs, shrimp, and mushrooms. Figure 1 shows the process of obtaining chitosan, a derivative compound of chitin, through partial deacetylation. Mixed with D-glucosamine, chitosan is a copolymer of N-acetyl-D-glucosamine and D-glucosamine. A copolymer's D-glucosamine and N-acetyl-D-glucosamine residue content is proportional to its degree of deacetylation . Aquaculture is a rapidly expanding sector of the global food industry. Asia, in particular, has seen a meteoric rise in the shrimp industry over the last several years . But shrimp farms harvest only the meat from the shrimp after removing the exoskeleton (the shrimp's head and shell) from the animal. They dispose of the exoskeleton as bio-waste. It is common practice to reject 45- 55% of raw shrimp during processing . In addition, research suggests that crustaceans, such as shrimp, may store a substantial amount of their body mass in their exoskeleton or shell. Every year, a lot of shells and chitin compounds go to waste because of this. French professor Henri Braconnot discovered chitosan in mushrooms in 1811, but it wasn't until 1951—140 years later—that a book based on the compound was published. Lots of studies on chitosan technology have been going on since then . Chitin rapidly becomes fiber-like chitosan. Plant fiber cellulose differs from chitosan. Since chitosan has a positive ionic charge, it can bind to negatively charged fats, ions, lipids, cholesterol, proteins, etc.

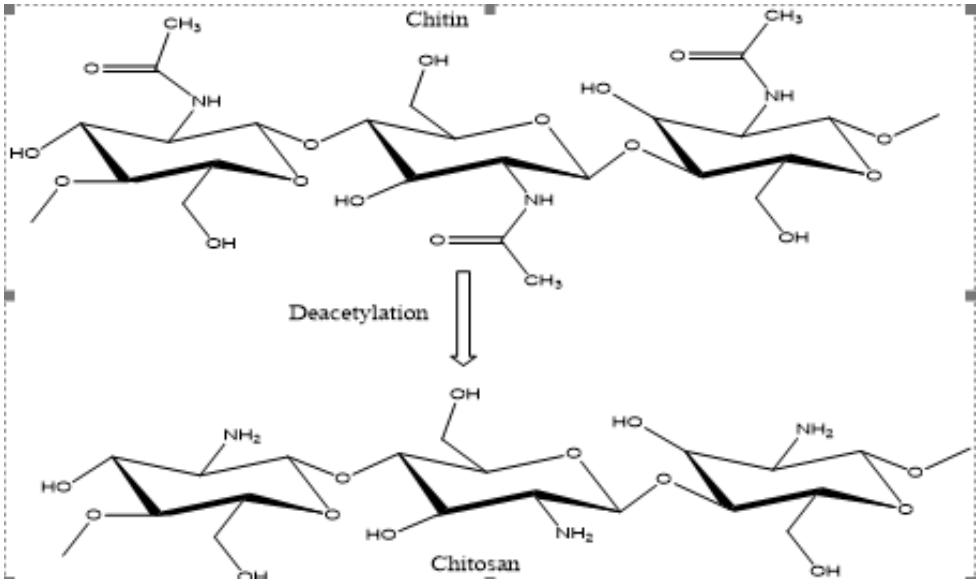


Figure 1.

Figure 1. Chitosan is produced by deacetylating chitin.

Chitosan is nontoxic, bioavailable, biodegradable, and adsorbent. These qualities make it useful for wound treatment, medicine transportation, food container design, dietary supplements, medicines, and biomaterials . The exoskeleton is processed chemically and biologically to get chitin and chitosan . Biological chitin production is more ecologically friendly, cost-effective (depending on the microbe), more effective at decalcification (up to 86%), and more viscous than chemical manufacturing. The natural method reduces shrimp shell leftover proteins. Studies show that biological techniques produce better chitosan than chemical methods . The FDA approved chitosan as a feed ingredient in 1983. In recent years, chitosan has found numerous uses in biotechnology, environmental protection, and functional foods . This paper provides a concise overview of chitosan production processes and the many uses of this versatile polymer. Two main methods make chitosan: chemical and biological .

A Chemical Approach to Chitosan Production

Chitosan manufacture boils down to two basic processes: first, extracting, and second, purifying chitin from shrimp exoskeleton. Chitosan production entails demineralization, deproteinization, and deacetylation as its three main steps. When making chitosan from shrimp shells, the first step is to collect shrimp waste from processing plants, then wash and dry it to make shrimp waste powder . Next, salicylic acid pre-treatment lasts for hours. Demineralization follows pre-treatment. Demineralization uses hydrochloric acid. However, shrimp concentration and demineralization must be considered. This step turns insoluble calcium carbonate into soluble calcium chloride, which water may easily remove later. Use NaOH for deproteinization after demineralization. Varying shrimp species affect NaOH concentrations. Filter shrimp waste to produce protein hydrolysate. Take this protein hydrolysate with you when you run. After deproteinization and demineralization, only chitin and a few of proteins remain. Chitosan may currently be produced by deacetylating chitin. For the deacetylation step, you'll need some extreme chemistry, like 70-90% heat and 50% NaOH. To make chitosan, you must deacetylate 70–90% of the chitin **(Figure 2)**. However, its environmental impact limits the use of this chemical technique .

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Figure 2. Process for chemically producing chitin and chitosan. Chemically producing chitin and chitosan involves demineralization and deproteinization with HCL, NaOH, etc.

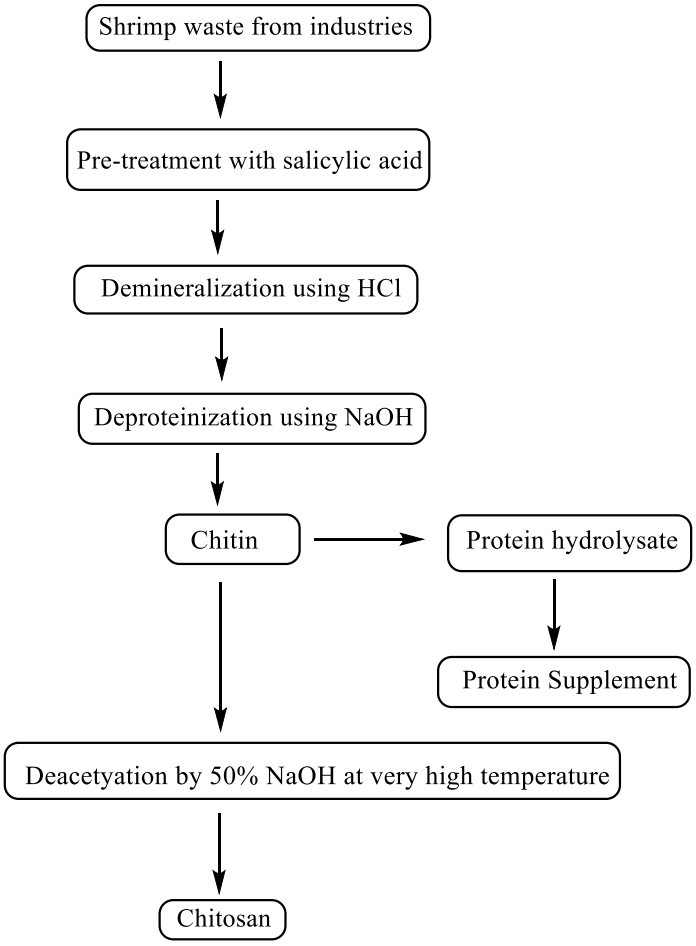


Figure 2. Process for chemically producing chitin and chitosan. Chemically producing chitin and chitosan involves demineralization and deproteinization with HCL, NaOH, etc.

Biological Method for Chitosan Production

Chitosan manufacturing via biological processes is more advantageous than chemical processes because it is environmentally sustainable, cost-efficient, and better suited than chemical methods [8]. The biological perspective can employ two methodologies. One approach is the enzymatic technique, while the alternative is the fermentation technique. The enzymatic procedure resembles the chemical technique mentioned above [19]. The only variation is the substitution of enzymes like pepsin, alcalase, papain, and trypsin with other compounds that were previously used for deproteinization and demineralization [20]. However, enzyme inactivation and centrifugation are necessary. After centrifugation, the supernatant contains protein and the pellet contains chitin. Sequentially washing the pellet with water, ethanol, and acetone yields pure chitin. However, charcoal decolorizes the supernatant, followed by filtration, NaOH neutralization, and lyophilization to produce protein hydrolysate [21]. Protein hydrolysate produces valuable peptides [22] **(Figure 3)**.

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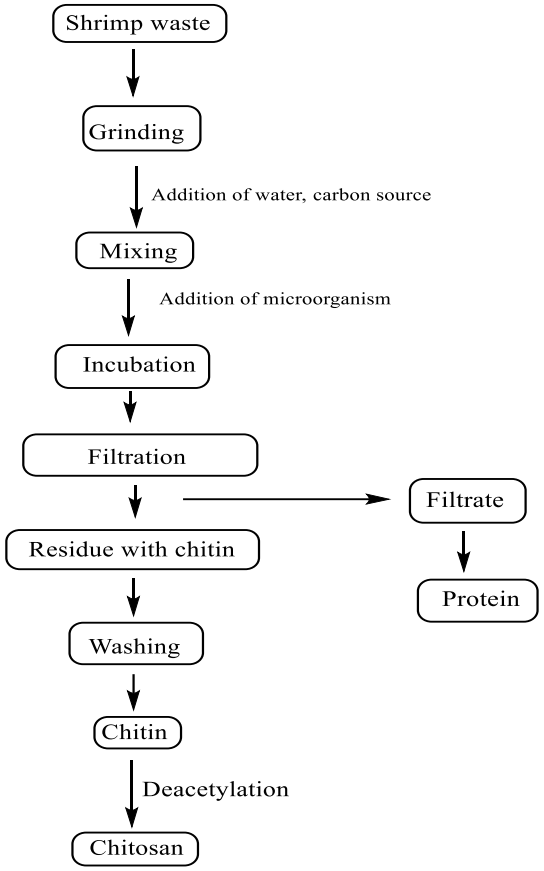


Figure 3. Chitosan and chitin enzymatic production flowchart. Enzymes such as trypsin, alcalase,d pepsin demineralize and deproteinize chemically produced chitin and chitosan.

In the fermentation process, strains of microbes such as Lactobacillus plantarum, Pseudomonas aeruginosa, Bacillus subtilis, and others produce chitosan [23]. The first step in fermenting shrimp waste is to gather and grind the shells. For fermentation, instal the ground material in an incubator with distilled water, an appropriate carbon source, and a microbe set at 37 °C. Filter the filtrate after two or three days of incubation [24]. The filtrate contains proteins. Conversely, chitin makes up the majority of the collected residue. The next step in making chitosan is to deacetylate the chitin after washing (Figure 4). Demineralization of shrimp waste occurs during fermentation because of chemicals produced by fermenting microbes [25]. Lactic acid fermentation is a reaction between the calcium carbonate in shrimp excrement and the lactic acid produced by bacteria that ferment sugars. This reaction results in calcium lactate. An easy way to eliminate the calcium lactate is to wash it away once it precipitates [26]. However, autolysis, which is prevalent in fish and shrimp waste, is the main mechanism by which chitin waste is deproteinized during fermentation [27].

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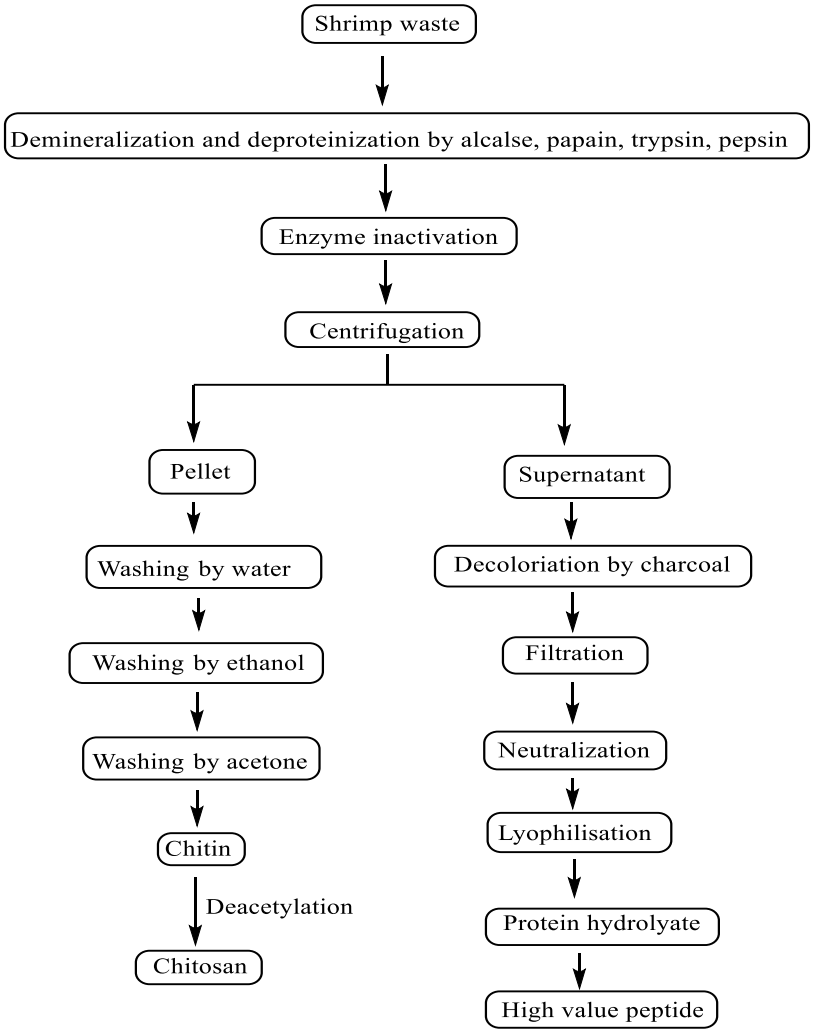


Figure 4. Chitin and chitosan fermentation flowchart. Chemically producing chitin and chitosan involves fermentation by various bacteria to demineralize and deproteinize.

CHITOSAN'S POTENTIAL USES

Chitosan is used in several areas, from agriculture to advanced sciences like biotechnology and nanotechnology (Table 1).

Table 1. Possible utilizations of chitosan and its derivatives

No	application	Mechanism of action / Used as	References
1.	Antioxidant	Protects against free radicals, including hydroxyl and superoxide, as well as DPPH	[28]
2.	Antimicrobial agent	Chitosan molecules, which are positively charged, can rip apart the negatively charged membranes of microbes. This is how chitosan destroys germs.	[29]
3.	Drug delivery	Administration of pharmaceuticals by mouth, intranasally, intramuscularly, etc.	[30]
4.	Food technology	Chitosan-coated foods have antibacterial properties and can act as flocculants and adsorbents.	[31]
5.	Gene therapy	Gene therapy, cancer treatment, and siRNA technology all rely on the delivery of specific genes.	[32]
6.	Bio-nanotechnology	Micro-electrochemical systems and 3D networks use materials such as graphitic carbon nanocapsules/composites and tungsten carbide chitin whiskers.	[33]
7.	Regenerative technology	Used in bone, neural, ocular, cardiac, and skin regeneration.	[34]
8.	Electrolyte	Sulfuric acid and chitosan discharge high voltage.	[35]

Antimicrobial Agent

Several investigations show that chitosan has potent antibacterial properties. Several studies have shown strong antibacterial activity against B. cereus, Staphylococcus aureus, L. bulgaris, Salmonella typhimurium, E. coli, Pseudomonas fluorescens, Vibrio parahaemolyticus, and Lactobacillus plantarum [36]. Chitosan and chitin's antibacterial mechanisms remain largely unknown; however, several theories point to different pathways [37]. One of the most widely accepted theories is that chitosan's antibacterial activity is driven by its positively charged amino group. The microbial cell membrane is negatively charged, and the positively charged amino group interacts with it. When microbes come into contact with one another, they release several proteins and other biological components [38]. With a higher degree of quaternary ammonium substitution, quaternized chitosan outperformed chitosan in many

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tests comparing the two antibacterial agents [39]. As a result, its antibacterial activity may be more pronounced and its interactions with cell membranes more robust. Numerous fungi, including Botrytis cinerea, Piricularia oryzae, Fusarium oxysporum, and Trichophyton equinum, are susceptible to chitosan's antifungal activity. Chitosan has several antifungal effects. By inhibiting Saccharomyces cerevisiae protein production and Candida albicans intracellular ultrastructure and membrane integrity, chitosan may reduce both fungi [40]. pH and molecular weight regulate antimicrobial activity. pH inversely influences the antibacterial activity of chitosan [41]. Studies have also shown that chitosan with a molecular weight below 300 kDa enhances antibacterial action against S. aureus but decreases its efficacy against *E. coli* [42].

Drug Delivery

Chitosan's medicine delivery effectiveness is promising. Drug delivery uses nanotechnology to safely transfer a pharmaceutical chemical's therapeutic impact in the body [43]. This technology uses nanoparticles and other approaches to target body sites and provide systemic pharmacokinetics [44]. Scientists are considering chitosan to enhance drug delivery because of its unique features [45]. Among pharmaceutically biodegradable biopolymers, chitosan is the only cationic one [46]. Its positively charged amine group makes it bioadhesive to negatively charged biological surfaces (mucosal glycoproteins) [47]. The amino polysaccharide is biocompatible, biodegradable, bioactive, and non-toxic. It is also soluble in aqueous solutions at pH < 6.5 [48]. Because of its mucoadhesive properties, it may be a viable drug-delivery medium [49].

In addition to mucoadhesivity, Chitosan offers additional drug delivery benefits [50]. It controls medication release well. Sustained release has a set drug-release strategy and duration [51]. It may also be controlled by drug dissolution or diffusion, or by a membrane-regulated osmotic mechanism that creates ionic contact. A cationic drug's release may be controlled by polyacrylates, sodium carboxymethylcellulose, or alginate [52]. Its cationic nature makes Chitosan mucoadhesive. The mucus gel layer contains sialic acid and sulfonic acid, forming the anionic substructure [53]. Chitosan's cationic amino groups interact with the anionic gel layer, enabling mucoadhesion. These unique properties of Chitosan enable it to be used to deliver drugs intravenously, orally, nasally, and ocularly [54]. Ionotropic gelation, microemulsion, polyelectrolyte complexation, and other methods can produce chitosan nanoparticles [55].

Regenerative Therapy

Chitosan in bone tissue engineering

Bone grafting treats various bone disorders and defects. In bone grafting, bone is harvested from another source and transplanted into the damaged area [56]. The classifications of bone grafts include autograft and allograft. In an autograft, the bone is harvested from a different site within the patient's body; hence, it is histocompatible and nonimmunogenic. Autografts additionally deliver essential signals for osteoinduction, including bone morphogenetic proteins (BMPs) [57][58]. Conversely, allografts are sourced from the body of a different individual. Consequently, allografts might provoke various types of infections and immune reactions while exhibiting diminished osteoinductive effectiveness [59]. Researchers examined chitosan-silica xerogel for guided bone regeneration. The amalgamation of chitosan and silica xerogel has demonstrated promising outcomes in bone regeneration with few adverse effects, including inflammation. Conversely, a novel chitosan nanofibre membrane and a chitosan-nanocrystalline calcium phosphate composite scaffold have demonstrated advantages for bone regeneration. Chitosan is applicable in the processes of bone regeneration—the effectiveness of chitosan hybrid [60][61].

Chitosan in cardiac regenerative technology

Chitosan rejuvenation technology can heal several heart problems [62]. Because the heart can't repair itself, transplants and artificial devices are now the most common ways to treat these conditions [63]. However, this approach is limited by the lack of donor hearts [64]. Because of this, scientists have been very interested in finding new regenerative treatments for heart problems. One promising application is using chitosan hydrogels for heart regeneration [65].

Regenerative technique based on chitosan for skin

Skin is the body's largest organ and one of its most complex organs. Because skin is constantly exposed to the external environment, the risk of injury from hazards such as trauma, injury, and especially fire is significant [66]. If it burns, biomolecule homeostasis and tissue architecture are severely modified. The healing process of wounds is particularly tough due to continuous creation of fluid and attack of germs [67]. Conventionally, autologous transplantation is done to treat burnt, wounded areas. However, because there is not enough skin to transplant from an autologous source, researchers are investigating novel biological chemicals for implantation. In this respect, chitosan technology can play a major role. Recently, The researchers created a chitosan scaffold studded with silver nanoparticles. This scaffold shows promise for application in tissue regeneration and wound healing [68]. Such technologies will help treat burn wounds sooner [69].

Agriculture

Chitosan's antibacterial, anti-insecticidal, non-toxic, and biodegradable properties make it useful in agriculture [70]. Plants can get critical agrochemicals and genetic elements via chitosan-based nanoparticles [71]. Chitosan nanoparticles can protect crops from pesticides and herbicides, deliver fertilizers, monitor crops using nanosensors, and improve soil health [72]. Moreover, studies have shown that chitosan nanoparticles can improve soil auxin and urea release. For instance, chitosan stops the growth of numerous plant-harming microbes and fungi, including Botrytis cinerea, Fusarium oxysporum, Micronectriella nivalis, Rhizoctonia solani, Erwinia carotovora, and Agrobacterium tumefaciens [73]. In several ways, chitosan biopolymers help plants defend against pathogen attacks. Chitosan can activate some plant cell pumps that release anti-pathogenic chemicals [74]. Chitosan activates phenylpropanoid pathway enzymes [75]. Each reaction has antiviral and innate immune-stimulating effects. These reactions also increase secondary metabolite production and plant resistance to subsequent infections [76]. Additionally, chitosan can increase seedling height, root length and activity, and germination ability. Using its film-forming capabilities, chitosan can help keep plant seeds moist by forming a semi-permeable film on seed surfaces, according to studies [73, 77]. In addition, the chitosan coating supports root development and increases water absorption capacity, which in turn helps plants develop drought tolerance [78]. Further research showed that chitosan increases abscisic acid production, reducing transpiration and maintaining stomatal closure under drought [79].

Preserving the Environment

Chitosan and its derivatives can help eliminate several environmental contaminants. Both organic and inorganic contaminants can fall under this category. For instance, chitosan immobilized with Cu(II) may remove phosphates, while polyethylene glycol (PEG)- and polyvinyl alcohol (PVA)-chitosan can eliminate nitrate pollutants [80, 81]. Additionally, chitosan can eliminate methylene blue and methylene violet, two dyes that contribute to pollution. The chitosan-alginate complex can remove glyphosate and other pesticides from the environment [82]. Studies show chitosan can remove heavy metal ions from the environment [83].

Chitosan Restrictions

Chitosan has numerous possible uses in the biopharmaceutical and biomedical industries, among others, but it does have some restrictions [7]. Due to its unique macromolecular characteristics, it has garnered more citations in scholarly literature than any other polymer [84]. The biomedical community holds it in high esteem as a potential game-changer in the fight against cancer, gene therapy, and controlled drug release [85]. To use chitosan effectively, it must be washed and extensively modified. The marine polysaccharide chitosan is derived from the tough exoskeleton of crustaceans like shrimp, crab, and lobster [86]. Therefore, this is

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seen as a substance that is easily accessible from either food or marine debris. The chitosan samples are actually unprocessed materials [87]. The identification of chitosan interacting with certain mutual solutes or other biomolecules is supported by research into its use in drug delivery, which is facilitated by its precise absorption, customized release, distribution, and lack of toxicity [88]. Also, in classical solution thermodynamics and biological applications, these could have subtle effects. These unforeseen effects, which aren't always obvious or easy to talk about, make people question the whole process [89]. Chitosan has attracted interest for siRNA transport because of its strong buffering activity in the endosomal pH range [90]. Its ionization, colloidal stability, solubility, and weak binding to nucleic acids at physiological pH are all areas where researchers are aiming to improve it. Problems such as inadequate transfection, premature nucleic acid release, and poor cell uptake are caused by these concerns. Regardless, its sector-specific applicability is still low. Despite promising biological and physicochemical characteristics, it has not seen widespread clinical use because of its poor solubility and weak mechanical properties [91]. For drug delivery, the preparation method and the medication to be administered must be modified because chitosan has poor solubility at neutral and alkaline pH [92].

Conclusion

The biological compound chitosan is derived from chitin. Its of toxicity, compatibility with living organisms, inherent biodegradability, and environmental friendliness make it highly desirable—deacetylation of chitin to varying degrees yields chitosan. Chitin can be synthesized using either chemical or biological methods. However, chitosan is a highly adaptable molecule with many potential uses. The biological applications of chitosan are practically endless. Because of its potent antibacterial properties, chitosan has several uses as an antimicrobial agent in the food, agriculture, and healthcare sectors. Among the many regenerative technologies chitosan can impact are those that address the cornea, skin, heart, and so on. Moreover, chitosan possesses a multitude of possible uses in fields as diverse as farming, ecological preservation, the lumber industry, personal care, energy generation, and the management of obesity, immunological therapy, genetics, and cancer. Research into chitosan's potential use in nanotechnology is another emerging field. While not all of those businesses will use chitosan just yet, it is expected to have a significant impact on those sectors in the near future. More research is needed to explore chitosan's potential as a versatile molecule.

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