Microbes for the Synthesis of Chitin from Shrimp Shell Wastes

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Abstract

Shrimp meat is consumed globally on a large scale, and their processing releases a large amount of shell waste. The major constituents of shrimp shells are chitin, proteins, calcium carbonate, and lipids. To extract chitin from the shrimp shell, it has to undergo deproteination (DP) to remove the proteins and demineralization (DM) to separate the minerals. Traditionally shrimp shell wastes were dried and directly added as a fertilizer to soil or added in animal feed or dumped in landfills. In recent years, shrimp shell wastes are valorized for producing chitin, chitosan, and other beneficial products like protein hydrolysates, carotenoids, lactic acid, etc. Industries producing chitin are employing chemicals like hydrochloric acid and sodium hydroxide for demineralization and deproteination, respectively, and the residual water is dumped into the water bodies. Considering environmentally friendly approaches, the usage of microorganisms has been tried out for chitin extraction from the shrimp shell. The recent review highlights the production of chitin using microorganisms and mentions other recent greener approaches in chitin production.

Keywords

Chitin · Biofermentation · Deproteination · Demineralization

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15.1 Introduction

The seafood industry supports the livelihood of 10–12% of the world population (FAO 2020). The proliferation of the different seafood industries across the world has enhanced the problem of waste handling and disposal. The global volume of shellfish food such as prawn, shrimp, crab, lobster, etc. reached 9.3 billion tons according to FAO (2020) reports. Since the shells or exoskeletons of the crustaceans are inedible, a significant portion of the shellfish ends up as waste and finds its way to landfills or water bodies polluting the environment and causing health hazards. Shrimp wastes are alkaline with a pH range of 7.5–8 that supports the growth of putrefying microbes that are hazardous to the environment (Bhaskar et al. 2007).

Due to the massive scale of shellfish landing and its processing, the waste generation is also huge, and the amount is increasing annually. Currently, there is no satisfactory technology for the valorization of these entire shellfish wastes to value-added products. In some Southeast Asian countries like Indonesia, Thailand, and the Philippines, the monetary value of dry shellfish wastes is very low, with prices ranging from 100 to 120 USD per ton. Considering their lack of profitability, the shellfish wastes are not utilized and eventually get disposed in water bodies or land filled causing environmental pollution. In developed countries like Australia and Canada, the shellfish waste disposal is costly, with a processing cost of up to 150 USD per ton. There are several active programs in the developed seafaring nations for valorizing this resource which includes eco-friendly waste management strategies in Canada; production of lime for construction removal of heavy metals and usage as pre-formed baits in fishery, etc. in the UK; conversion to aquaculture feed in Japan; and chitin and chitosan production in the USA and most Scandinavian countries (www.seafish.org). Interestingly, Norway has developed a technology to utilize seafood-processing waste involving enzyme treatment followed by membrane filtration at nano-level to target value-added products (The Marine Products Export Development Authority [MPEDA] 2013). However, a fully integrated process/technology for an effective total shrimp shell waste management is yet to emerge globally.

The shrimp shell composition varies from species, seasonal variation, and geographic locations. The constituents of the shrimp shell wastes include 10–25% chitin, 13–50% protein, 15–70% mineral matter (Babu et al. 2008), and low-fat content (Cira et al. 2002). The major mineral found in the shrimp shell cuticle is calcium carbonate, which helps in strengthening the exoskeleton. Depending on the tons of renewable shrimp shell waste generated annually, the potential value of these wastes is left unexplored. It is necessary to consider a greener prawn shell waste management methodology benefitting the environment and produce value-added products for economic development. The value-added products like proteins generated from the prawn shell waste are used in animal feed for livestock and aquaculture (Evers and Carroll 1998; Sumardiono and Siqhny 2018). Calcium carbonate derived from the prawn shell wastes are in greater demand due to their biological components and superior origin than limestone and marble. Chitin is the most significant component derived from the shellfish wastes with applications in

different fields varying from water purification to biomedical applications. The current commercial method for shellfish waste management uses harmful chemicals, creating environmental and economic issues. Utilization of crustacean shell wastes for the extraction of chitin and other bioactive compounds has been studied using different methods including enzymatic approaches (Hayes et al. 2008), microwave irradiation (El Knidri et al. 2016), and ultrasonication (Kjartansson et al. 2006). Strategy for chitin extraction from shrimp wastes includes demineralization (DM), deproteination (DP), and bleaching/depigmentation; and deacetylation can yield chitosan (CHS) which is an even more valuable product finding applications as surgical sutures and wound dressings (Değim et al. 2002). All these processes use acidic and basic solutions under elevated temperature and longer incubation times.

Addition of strong acids and bases for the chitin extraction affects the physiochemical properties of chitin and releases effluent wastewater containing chemicals, requiring further purification. The use of proteolytic bacteria for DP and lactic acid bacteria for DM could curtail the application of concentrated bases and acids. Therefore, biological methods using microbes or microbial enzymes are in demand due to their better reproducibility, lower processing times, easier handling, less solvent and chemical requirements, and lower energy input for producing value-added products (Hayes et al. 2008). Bio-based chitin has distinct properties like biodegradability, non-toxicity, and biocompatibility and is applied in agriculture, medicine, pharmaceutics, environmental waste management, biotechnology, and food processing (Kaur and Dhillon 2015). The protein-rich liquid fractions find applications in human and animal feed (Mizani et al. 2005). Bioprocessing of shrimp wastes for chitin production is reported using lactic acid bacteria and proteolytic bacteria/enzyme for DM and DP as single-stage fermentation (Rao and Stevens 2006), two-stage fermentation (Xu et al. 2008) and cofermentation (Francisco et al. 2015).

15.2 Economic Aspects of Chitin

The main source of raw material for synthesizing chitin is from the waste materials obtained from seafood pre-processing centers deshelling crab, shrimp, prawn, lobster, etc. (Hamdi 2017; Maruthiah and Palavesam 2017). The shrimp wastes are rich in pigments like astaxanthin, β-carotene, and other carotenoids. For several years, chitin is considered as a promising biomaterial due to its characteristic properties and has found applications in many fields like biomedical, engineering, wastewater treatment, cosmetic, food industry, and packaging. Chitin is of great economic significance as it costs 220 dollars per kilo (Jaganathan et al. 2016). The commercial value of chitin and its derivatives is accounted for 100 billion tons per year (Ioelovich 2014). The global research statistics have concluded that the chitin market is expected to rise to 53 million US dollars in 2024 (Global Chitosan Derivatives Market 2019).

15.3 Chitin Structure and their Properties

Chitin is a linear semi-crystalline polymer with high molecular weight comprising N-acetyl glucosamine units bonded by β-glycosidic bonds. They resemble cellulose polysaccharide with the C-2 position of the hydroxyl group replaced by the acetamido group. To be distinguished as a chitin, their degree of acetylation is greater than 50% (Anitha et al. 2014). Chitin is tough, inert, and insoluble in water and other organic solvents. The other characteristics of chitin are its ability to chelate metal ions and form films and polyoxy salts. Chitin is consists of three allomorphs containing α-, β-, and γ-forms. The α-chitin is abundantly found in shrimps, lobsters, and crabs with antiparallel chains with strong intra- and intermolecular bonds. The β-form consists of parallel chains bonded by intrasheet hydrogen bonding, which are of weak bonds, hence unstable, and are mainly found in squid (Ioelovich 2014), whereas γ-chitin is an amalgamation of α- and β-chitin forms comprising parallel and antiparallel chains, e.g., *Ptinus* beetles and *Loligo* squids (Ramirez-Coutino et al. 2006; Casadidio et al. 2019). The characteristics of pure chitin are dependent on their molecular weight, degree of acetylation, purity, and polydispersity index (Kaur and Dhillon 2015). The characteristics like biodegradability, bioactivity, non-toxicity, and biocompatibility have made these marine polymers useful for various versatile applications. Factors like the degree of deacetylation (DD) are used to determine the number of glucosamine units present in a chitin structure. If the degree of deacetylation exceeds 50%, it improves the solubility of chitin, by changing into chitosan. The molecular weight of chitin is based on the emergence of the source, acid and base concentration used in demineralization and deproteination, duration for incubation, and temperature required for the processes (No and Meyers 1995). The average molecular weight of chitin is reported to have a range of 0.4 to 2.5 $\times$ 10^6 (No and Meyers 1995; Ravi Kumar 2000). *Chitin portrays biological properties like antimicrobial, antiulcer, hemostatic, wound healing, fungistatic, antiacid, anticholesterolemic,* etc.*; hence, it can be used for biomedical applications* (Dutta et al. 2004; Zargar et al. 2015; Lim and Hudson 2003; Cheba 2011). Processes involved in synthesizing chitin are (a) demineralization (DM), (b) deproteination (DP), and (c) depigmentation.

15.4 Chemical Methods in the Extraction of Chitin

Traditional methods in chitin extraction from shrimp shells involved the usage of chemicals (Table 15.1 and Fig. 15.1). The usage of a strong alkali like NaOH and acids like HCl affects the ecosystem as the water obtained after processing chitin is highly acidic or basic, which are dumped into the water bodies. The process is expensive as the costs involved in neutralizing the dumped wastes are high.

Table 15.1 Shrimp shell processing with chemicals

	Deproteination			Demineralization			
Shrimp source	NaOH concentration	Temperature (°C)	Incubation (h)	HCl concentration	Temperature (°C)	Incubation (h)	References
Shrimp	1.25 M	100	0.5	1.57 M	20–22	1–3	Moorjani et al. (1975)
Metapenaeus dobsoni	0.125 M	100	0.5	1.25	Room temperature	1	Madhavan and Nair (1974)
Metapenaeus dobsoni	0.75 M	100	–	1.25	Room temperature	1	Madhavan and Nair (1974)
Shrimp	1%	65	1	0.5 M	Room temperature	–	Wu and Bough (1977)
Shrimp	3%	100	1	1 M	Room temperature	0.5	Bough et al. (1978)
Shrimp	4%	100	1	5%	Room temperature	–	Sluyanarayana Rao et al. (1987)
Penaeus monodon	4%	Room temperature	21	4%	Room temperature	2 or 12	Lertsutthiwong et al. (2002)
Litopenaeus vannamei	5%	90	12	4%	Room temperature	4	Ploydee and Chaiyanan (2014)
Shrimp shell wastes	4%	28 ± 2	20	4%, 3%, 2%	28 ± 2	16	Hossain and Iqbal (2014)
Nephrops norvegicus	150 g dm^{-3}	65	3	1 M	Room temperature	2	Beaney et al. (2005)
Seafood wastes comprising shrimps, krill, crab, and lobster	1.25 M	90	2	1 M	Room temperature	1	Kaya et al. (2015)
Shrimp shell waste	1.25	90	2	1 M	Room temperature	1	Pachapur et al. (2016)
Litopenaeus stylirostris	2 M	50	4	1	25	2.5	Díaz-Rojas et al. (2006)

(continued)

Table 15.1 (continued)

	Deproteination			Demineralization			
Shrimp source	NaOH concentration	Temperature (°C)	Incubation (h)	HCl concentration	Temperature (°C)	Incubation (h)	References
Penaeus monodon	1 M	95	0.5	0.25	Room temperature	6	Charoenvuttitham et al. (2006)

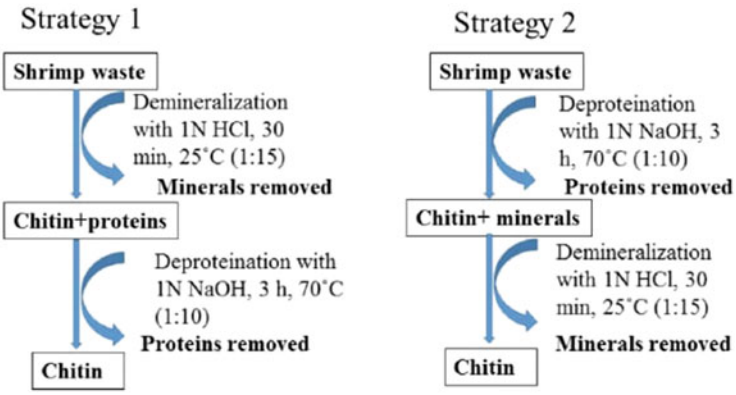

Fig. 15.1 Strategies for chitin production from shrimp wastes by chemical processes. Strategy 1: Demineralization followed by deproteination. Strategy 2: Deproteination followed by demineralization

15.4.1 Chemical Demineralization

The chitin entrapped in the shrimp exoskeleton can be extracted by the removal of the process of demineralization and deproteination. In demineralization, the inorganic minerals like calcium carbonate from the crustacean exoskeleton are removed using inorganic acids, like HCl, HNO_3, and H_2SO_4 (Younes and Rinaudo 2015; Kumar Gadgey and Bahekar 2017), and organic acids like HCOOH and CH_3COOH (Regis et al. 2015). Predominantly, hydrochloric acid is used for higher removal rate of minerals from shell wastes. HCl combines with calcium carbonate ($CaCO_3$) to form calcium chloride ($CaCl_2$) that can be removed by using activated carbon (Fadli et al. 2018) (15.1).

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2 \qquad (15.1)$$

15.4.2 Chemical Deproteination

The next step for the extraction of chitin is deproteination, which involves the removal of proteins. Proteins are removed from the shell wastes using chemicals like NaOH, KOH, $Ca(OH)_2$, $CaHSO_4$, $NaHSO_4$, $NaHCO_3$, Na_3PO4, Na_2CO_3, Na_2S, and K_2CO_3 (Younes and Rinaudo 2015). NaOH is mostly preferred for deproteination. A higher concentration of NaOH at elevated temperature causes deacetylation of chitin to chitosan (40% NaOH incubated at 100–130 °C) (Hülsey 2018).

Deproteination and demineralization can be reversed based on the quality of chitin produced with less incubation time and temperature.

15.4.3 Depigmentation

The process of demineralization and deproteination cannot completely remove the carotenoid pigments like astaxanthin, lutein, β-carotene, and astacene. In order to obtain colorless chitin, the pigments are removed using organic solvents like glacial acetone (Soon et al. 2018) and inorganic solvent like sodium hypochlorite (Srinivasan et al. 2018; Devi and Dhamodharan 2018). Duan et al. (2012) decolorized colored chitin from shrimp wastes with potassium permanganate followed by incubating in oxalic acid (1%). Through the process of decolorization, colorless chitin is obtained which improves their commercial value and utilization for various industrial applications.

15.5 Microbial Action on Shrimp Shells for Chitin Recovery

Shrimp shell waste biofermentation is probably the ideal environmentally friendly method that is cost-effective and sustainable. Although shrimp shells are insoluble and not easily degraded by natural degradation, they contain chitin, a natural polymer resembling cellulose in chemical structure. Chitin and its derivative chitosan have been used widely for commercial applications in agriculture, biomedicine, biotechnology, waste treatment, food industry, etc. Biofermentation of shrimp shell wastes is advantageous over chemical methods. The usage of chemicals release effluents into the soil and water body and are harmful that biological methods using microorganisms. Khanafari et al. (2008) found out that the quality of chitin obtained from the biological methods was better than chemical methods. Chitin with high molecular weight was produced by the deproteination of shrimp shells using proteolytic microorganisms (Bustos and Healy 1994). Shrimp shells are fermented by single-stage fermentation, cofermentation, or two-stage fermentation processes, which involve lactic acid bacteria and non-lactic acid bacteria that assist in demineralization and deproteination (Table 15.2).

15.5.1 Lactic Acid Bacteria

Conventional methods of demineralization used HCl which affected the quality of chitin altering their molecular weight and intrinsic properties (Percot et al. 2003). Lactic acid is used as an alternative instead of HCl for demineralization, and it was found that a) usage of lactic acid was less toxic to the environment due to the release of acid and alkali liquid obtained after chitin processing, b) it was also cost-effective, and c) calcium lactate ($Ca(C_3H_5O_3)_2$) formed by the action of lactic acid ($C_3H_6O_3$) (15.2) with calcium carbonate can be used as anti-icing agents (Mahmoud et al. 2007). Lactic acid is naturally produced by lactic acid-producing bacteria, which is preferred over commercial lactic acid considering their cost (Ghaffar et al. 2014). Lactic acid-fermenting bacteria can be isolated from the shrimp shell itself (Duan et al. 2012). Lactic acid fermentation converts sugars to form lactic acid, which

Table 15.2 Lactic acid and non-lactic acid bacteria in shrimp shell demineralization

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Lactic acid bacteria						
Lactobacillus sp. B2 with sucrose and whey	Single fermentation	*Penaeus* shrimp waste (2 kg)	85	87.6	13.1	Cira et al. (2002)
Lactococcus lactis with 15% glucose	Monocultivation	Shrimp wastes	78.8	66.5	49.4	Aytekin and Elibol (2009)
L. plantarum 541	Single fermentation	Shrimp waste	86			Rao et al. (2000)
L. plantarum 541 with 5% glucose and 2% salt	Single fermentation	Shrimp waste	81.4	59.8	NA	Rao and Stevens (2006)
Lactobacillus paracasei strain A3 with glucose	Single fermentation	*Nephrops norvegicus*	61	77.5	17.5	Zakaria et al. (1998)
L. helveticus cultivated with date juice	Single fermentation	*Parapenaeus longirostris*	44	91	23.6	Adour et al. (2008)
L. Plantarum	Single fermentation	Shrimp waste	87	99	NA	Neves et al. (2017)
P. acidolactici CFR2182 with 15% glucose	Single fermentation	*Penaeus monodon*	72.5 ± 1.5%			Bhaskar et al. (2007)
Pediococcus acidolactici CFR2182 (with 15% glucose)	Single fermentation	*Penaeus monodon* shrimp wastes	76	92	91.67 ± 1.86	Narayan et al. (2010)
Pediococcus sp. *L1/2* with 5% sucrose	Single fermentation	Shrimp shell wastes	83.47	NA	NA	Choorit et al. (2008)
Lactobacillus futsaii LAB06 and *L. plantarum* LAB14 (with 2% sucrose)	Cofermentation	*Litopenaeus vannamei*	88.6	84.8	15	Ximenes et al. (2019)

(continued)

Table 15.2 (continued)

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Lactobacillus strains T1 and L137	Cofermentation	Shrimp wastes	82–83	84.4	NA	Francisco et al. (2015)
First-stage fermentation with native proteolytic shrimp bacteria followed by fermentation with *L. casei* MRS1 in the presence of glucose	Two-stage fermentation	*P. monodon*	99.6	97.4	36	Xu et al. (2008)
Fermentation with bacterial enrichment cultures from ground meat and bio-yoghurt	Pilot-scale fermentation	Pre-purified shrimp shell wastes	85–90	89–91	NA	Bajaj et al. (2015)
Lactobacillus acidophilus FNCC 116 followed by *Bacillus licheniformis* F11.1	Two-stage batch fermentation process	*P. vannamei*	97.19	94.42	NA	Junianto and Setyahadi (2013)
First-stage fermentation with native proteolytic shrimp bacteria followed by fermentation with *L. casei* MRS1 in the presence of glucose	Two-stage fermentation	*C. crangon*	99.7	90.8	46	Xu et al. (2008)
Teredinobacter turnirae followed by demineralization with *Lactococcus lactis* (using 5% glucose)	Successive fermentation	Shrimp wastes	95	95	64.5	Aytekin and Elibol (2009)
Fermentation of *Lactobacillus brevis* first followed by *Rhizopus oligosporus*	Successive fermentation	Shrimp wastes	66.45 +/− 2.14%	96% +/− 0.43%	NA	Aranday-García et al. (2017)
Streptococcus thermophilus, *Lactobacillus acidophilus*, and	Cofermentation	*Penaeus vannamei*	91.3	97.7	4.42 ± 0.60	Duan et al. (2011)

Lactobacillus bulgaricus with 6.5% glucose						
Lactobacillus acidophilus FNCC116 and *Bacillus licheniformis* F11.1	Cofermentation	*Penaeus vannamei*	99.54	96.7	NA	Setyahadi et al. (2014)
Deproteination with *Serratia marcescens* B742 followed by *Lactobacillus plantarum* ATCC 8014	Two-step fermentation	Pulverized *P. vannamei* wastes	93	94.5	18.9 (chitin yield)	Zhang et al. (2012)
Lactobacillus pentosus L7 and *Bacillus thuringiensis* SA	Two-step fermentation	*Litopenaeus vannamei*	98.1 ± 0.3	96.8 ± 0.7	NA	Ploydee and Chaiyanan (2014)
SILALL 4 × 4® silage additive: *Lactobacillus salivarius*, *Enterococcus faecium*, and *Pediococcus acidilactici*	Fermentation with microbial consortia	*Nephrops norvegicus*	99.75	NA	NA	Beaney et al. (2005)
Non-lactic acid bacteria						
Bacillus cereus 8–1	Single fermentation (large-scale fermentation in 12 L)	10% shrimp shell waste	73	78.6	NA	Sorokulova et al. (2009)
Teredinobacter turnirae	Single fermentation without glucose	Shrimp wastes	23.3	77.8	40.1	Aytekin and Elibol (2009)
Bacillus subtilis with jaggery as sugar source	Single fermentation	*Metapenaeus dobsoni*	72	84	93.2 ± 0.6%	Sini et al. (2007)
Bacillus cereus	Single fermentation	3% shrimp waste	95	97.1	NA	Sorokulova et al. (2009)

(continued)

Table 15.2 (continued)

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Exiguobacterium acetylicum	Single fermentation	3% shrimp waste	92	92.8	NA	Sorokulova et al. (2009)
Pseudomonas aeruginosa 2 cultured with 5% glucose	Single fermentation	*Metapenaeus monoceros* shrimp wastes	92	90	19	Ghorbel-Bellaaj et al. (2012b)
Pseudomonas aeruginosa	Single fermentation	*Penaeus merguiensis*	82	92	47	Sedaghat et al. (2017)
Kurthia gibsonii and *Aspergillus* sp.	Two-stage fermentation	*Fenneropenaeus semisulcatus* (1:25 shell to bacterial broth)	NA	NA	16.06	Bahasan et al. (2017)
Kurthia gibsonii and *Aspergillus* sp.	Two-stage fermentation	*Fenneropenaeus indicus* (1:25 shell to bacterial broth)	NA	NA	13.87	Bahasan et al. (2017)
Bacillus licheniformis 21,886 and *Gluconobacter oxydans* DSM-2003	Successive cofermentation	*Litopenaeus vannamei*	93.5	87	90.8	Liu et al. (2014)

reduces the pH of the fermentation broth, reducing the growth of unwanted bacteria (Vandenbergh 1993).

$$2C_3H_6O_3 + CaCO_3 \rightarrow Ca(C_3H_5O_3)_2 + H_2O + CO_2 \quad (15.2)$$

For lactic acid fermentation using shrimp shells, different parameters have been considered; these are various sugar sources and their optimal concentrations, the concentration of inoculum used, and incubation time to produce lactic acid (Mathew and Nair 2006; Healy et al. 2003; Rao et al. 2000; Bhaskar et al. 2007). Lactic acid fermentation of shrimp wastes is optimized using different parameters like type of lactic acid bacteria used, sugar concentration, incubation time, etc. using response surface methodology (RSM), a statistical method that uses a sequence of designed experiments with different variables to obtain an optimal condition (Bhaskar et al. 2007). The addition of glucose in shrimp shell fermentation leads to the formation of lactic acid that lowers the pH causing demineralization (Khanafari et al. 2008). Different concentrations of glucose were added to test the demineralization efficiency. It was observed that the presence of glucose inhibited the protease activity of non-lactic acid bacteria; hence, other sugar sources were also considered (Aytekin and Elibol 2009). Some of the commonly used sugar sources that were added along with shrimp waste to enhance lactic acid production included sucrose (Cira et al. 2002), molasses (Fagbenro 1996; Evers and Carroll 1998), date juice (Khorrami et al. 2011), cassava starch (Francisco et al. 2015), fruit peels, etc. (Tan et al. 2020).

LAB can undergo single fermentation or cofermentation for shrimp shell degradation. Shrimp shells were fermented with *Lactobacillus plantarum* 541 resulting in a demineralization value of 90% (Rao et al. 2000). Natural curd containing lactic acid bacteria (LAB) was used for shrimp biofermentation having a demineralization value of 69% and deproteination of 89% (Prameela et al. 2010). Pacheco et al. (2011) isolated *Lactobacillus* strain B2 from the shellfish waste, and through fermentation, it resulted in 92% demineralization and 94% deproteination, respectively. Lactic acid bacteria can be combined with other non-lactic acid-producing bacteria that aid in protease activity causing deproteination. Some LAB organisms can carry both demineralization and deproteination and hence be used as a single strain for the biofermentation of shrimp shells. Chitin was obtained using *Lactobacillus plantarum* from fresh shrimp shell wastes by batch fermentations adjusting the pH, incubation time, and inoculum to obtain a deproteination of 99% and demineralization of 87% (Neves et al. 2017). The chitin produced by biological fermentation was observed to be 40% better than the chemical produced chitin. Lactic acid bacteria are used for deproteination of shrimp shells (Woods 1998).

Lactic acid bacteria were co-cultured with other lactic acid bacteria/non-lactic acid bacteria to enhance the demineralization and deproteination efficiency in shrimp shells. Co-culturing of *Lactobacillus* isolates T1 and L137 in the presence of sugar sources like glucose and cassava starch led to DM efficiency of 82–83% and deproteination value of 84.4% (Francisco et al. 2015). Evers and Carroll (1998) co-cultured *Lactobacillus plantarum* and *Enterococcus faecium* for shrimp shell biofermentation using dry molasses. Ploydee and Chaiyanan (2014) co-cultured

Lactobacillus pentosus and *Bacillus thuringiensis* for shrimp shell processing resulting in calcium carbonate removal efficiency of 98.1 ± 0.3% with a protein removal efficiency of 96.8 ± 0.7% (w/w). Junianto and Setyahadi (2013) demonstrated three different strategies for the pretreatment of shrimp shells using *Lactobacillus acidophilus* FNCC 116 and *Bacillus licheniformis* F11.1 by two-stage fermentation processes. 99.6% of minerals were removed when 100% of the medium was replaced by fresh media after 24 h of incubation with *Lactobacillus acidophilus* FNCC 116. 95.37% of protein was removed after subsequent fermentation and 100% media removal and replaced with fresh media after 24 h. Co-culturing of *L. plantarum* subsp. *plantarum* ATCC14917 and *B. subtilis* subsp. *subtilis* ATCC 6051 in the presence of fruit peels enhanced the shrimp biofermentation to produce good-quality chitin (Tan et al. 2020). Zhang et al. (2012) demonstrated two-stage fermentation of shrimp shells using *Lactobacillus plantarum* and *Serratia marcescens*. For the deproteination, *S. marcescens* was cultured with the shrimp shells at 30 °C for 4 days. The solid mass obtained after drying was further demineralized at 37 °C for 2 days. Their deproteination efficiency was 93% and demineralization 94.5% resulting in a chitin yield of 18.9% (Zhang et al. 2012). Similarly, heterofermenting *Lactobacillus brevis* was cultured with *Rhizopus oligosporus* for the biological shrimp shell processing (Aranday-García et al. 2017). In this study, *L. brevis* was cultured first followed by *R. oligosporus* to yield 66.45 ± 2.14% demineralization and 96 ± 0.43% of deproteination efficiency. Aytekin and Elibol (2009) studied the fermentative action of *Lactococcus lactis* and *Teredinobacter turnirae* on shrimp shell wastes for demineralization and deproteination. From their studies, co-culturing of *Lactococcus lactis* and *Teredinobacter turnirae* showed the best results, especially when proteolytic *T. turnirae* was cultured first followed by the demineralization with *L. lactis* displaying a DP and a DM value of 95%.

15.5.2 Non-lactic Acid Bacteria

Non-lactic acid bacteria produce proteases responsible for the deproteination process. The non-lactic acid bacteria produce protein hydrolysates, which help in the growth of lactic acid bacteria that help in demineralization. The proteolytic activities of the microorganisms are responsible for the deproteination of the shrimp shells (Table 15.3). Wang and Chio (1998) observed that the deproteination efficiency of *Pseudomonas aeruginosa* K-187 grown with shrimp and crab shell wastes was 82%. Shimahara et al. (1984) used *P. maltophilia* LC 102 for the protein removal of shrimp shells of *Penaeus japonicus* supplemented with EDTA. Paul et al. (2015) deproteinized the shrimp shells of *P. monodon* with *Paenibacillus woosongensis* TKB2 containing NaCl and chicken feather leading to 80% deproteination efficiency.

Bacillus species were used in shrimp shell deproteination. The proteolytic activities of six *Bacillus* species namely, *B. amyloliquefaciens*, *B. subtilis* A26, *Bacillus pumilus* A1, *B. licheniformis* RP1, and *B. cereus* SV1 strain, were studied

Table 15.3 Microorganisms involved in deproteination (that produce proteases)

Microorganisms	Shrimp species	Proteolytic activity	Deproteination (%)	Reference
Teredinobacter turnirae	Shrimp wastes	1139 l g/mL h	77.8	Aytekin and Elibol (2009)
Serratia marcescens	Shrimp waste	0.043 U/mL	90	Damodarasamy et al. (2012)
Paenibacillus woosongensis TKB2 with NaCl and chicken feather	*Penaeus monodon*	1.57 mg/mL of 71.4 U/mL	80	Paul et al. (2015)
Brevibacillus parabrevis TKU046	Cooked tiger shrimp shell	NA	96.44 ± 0.72	Doan et al. (2019a)
Rhizopus oligosporus	Shrimp waste	NA	96 ± 0.43	Aranday-García et al. (2017)
B. subtilis	Shrimp waste	137.5 U/mL	74	Pachapur et al. (2016)
B. Licheniformis	Shrimp wastes	178.7 U/mL	84	Pachapur et al. (2016)
Pseudomonas aeruginosa	*Penaeus merguiensis*	NA	92	Sedaghat et al. (2017)
Bacillus mojavensis A21	*Metapenaeus monoceros*	7.75 U/mg	88 ± 5%	Younes et al. (2012)
Pseudomonas aeruginosa K-187	Shrimp shell waste	21.2 U/mL	78	Oh et al. (2000)
Bacillus cereus SV1 (without adding glucose)	*Metapenaeus monoceros*	1152 ± 53 U/mL	95	Ghorbel-Bellaaj et al. (2012a)
Bacillus subtilis A26 (without adding glucose)	*Metapenaeus monoceros*	193 ± 90 U/mL	79.9	Ghorbel-Bellaaj et al. (2012a)
Paenibacillus sp. TKU047	0.5% shrimp head powder	2.98 U/mL	NA	Doan et al. (2019b)

for deproteination (Ghorbel-Bellaaj et al. 2012a). The deproteination of shrimp shells enzymatically was optimized by Box-Behnken design using *Bacillus mojavensis* A21 crude protease resulting in 88% deproteination (Younes et al. 2012). A chitinase-free extracellular protease was isolated from *Brevibacillus parabrevis* TKU046 which was used for the deproteination study against shrimp shell wastes (Doan et al. 2019a). It was observed that maximum deproteination of 96.44 ± 0.72% was observed on cooked tiger shrimp shell by liquid fermentation.

In a single reactor, the concurrent production of chitin was initiated by adding shrimp shell with *Aspergillus niger*. The proteases produced from *A. niger* caused deproteination releasing protein hydrolysates that were of low pH. Lower pH of the supernatant facilitated the demineralization process aiding in chitin separation (Teng et al. 2001). Cofermentation of non-lactic acid-producing microorganisms also helped in shrimp shell degradation. Successive cofermentation of proteolytic

B. licheniformis and *Gluconobacter oxydans* produced a DP efficiency of 87% followed by a DM value of 93.5%, and the chitin content was 90.8%.

15.6 Other Green Methods for Chitin Synthesis

Biological fermentation can be combined with other greener approaches to extract chitin. Some methods are ionic liquid extraction, the usage of protease enzymes for deproteination, micro-irradiation, and ultrasonication before or after the demineralization and deproteination in shrimp shell biofermentation (Qin et al. 2010; Mao et al. 2017; Suryawanshi et al. 2020; El Knidri et al. 2016). Extraction of chitin using ionic liquids is a one-pot method using ionic liquids (ILs) like hydroxyl ammonium acetate that has low inflammability, low vapor pressure, and highly soluble nature (Shamshina et al. 2016). Apart from using ionic liquids in chitin extraction, deep eutectic solvents (DESs) are preferred over ionic liquids in chitin extraction for their better solubility and economical and simple extraction process. In a two-step chitin extraction process, shrimp shells were pretreated first using citric acid leading to a DM value of 98% followed by the addition of DESs with the microwave irradiation causing deproteination with an efficiency of above 88% (Zhao et al. 2019). High-quality chitin (DESs-chitin) was produced in this method and matched the standards of chemically produced chitin. Huang et al. (2018a, b) devised a chitin extraction method from shrimp shells with Natural Deep Eutectic Solvent (NADES) along with microwave irradiation. Demineralization was attained by the adding malic acid, which removed 99% calcium chloride. The deproteination efficiency was dependent on the microwave radiation, the incubation time, and the shrimp shell-to-NADES ratio. Maximum deproteination efficiency was obtained at 93.8% with a shrimp shell-to-NADES ratio of 1:20 and microwave irradiation for 9 min. The chitin obtained through this process had a high crystallinity index of 71%. Devi and Dhamodharan (2018) developed a green and facile process to obtain chitin nanofibers from prawn shell wastes. The prawn shells were pretreated in hot glycerol (at 200 °C, for 4 min) that caused deproteination leading to the release of low molecular weight water-soluble proteins. The deproteinated shells were demineralized using citric acid forming calcium citrate salt and chitin of high crystallinity index (80.9%). From this process, the glycerol could be reused by using charcoal. Ultrasonication is another method for enhancing the pretreatment processes involved in deproteination and demineralization (Suryawanshi et al. 2019). In an ultrasonication-assisted method, a mild concentration of HCl (0.6 M HCl) and NaOH (O.6 M NaOH) was employed for demineralization and deproteination of shellfish wastes (Suryawanshi et al. 2020). Through ultrasonication, microbubbles are generated leading to an increase in the reaction rate with temperatures of 5000 K and 1000 atmospheric pressure.

For the deproteination of shrimp shells, commercial enzymes like pepsin, papain, bluefin trypsin, Alcalase®, and protease are used. Shrimp shell wastes of *Penaeus indicus* were demineralized with 1.75 N glacial acetic acid and papain (1:100 papain to shrimp shells) incubated at 72 h room temperature to obtain a deproteination value

of 73.1%, and the degree of acetylation (DA) of the chitin produced was 19.37% (Gopalakannan et al. 2000). Pepsin enzyme was incubated with white shrimp shells for 16 h at 40 °C, and it resulted in 92% deproteination efficiency (Duong and Nghia 2014).

Hongkulsup et al. (2016) used commercial protease enzyme from *Streptomyces griseus* for deproteination of *L. vannamei* shells and effectively removed 91.1% proteins, and the chitin produced had a DA of 90.83% with a crystallinity index of 82.56%, with lactic acid as the demineralization agent. Another enzyme like Alcalase® was used in the removal of proteins from shrimp heads to recover chitin (Valdez-Peña et al. 2010). Hence, commercial proteolytic enzymes can be used in shrimp shell degradation to obtain chitin, but are expensive compared to using proteolytic microorganisms.

15.7 Functional Aspects of Chitin

Due to the insoluble nature of chitin, chitin is deacetylated to chitosan, which has pleiotropic applications in the field of agriculture, food, waste management, and biomedical sectors (Table 15.4). In the wastewater management, green chitin nanoadsorbents were developed for the removal of carmine dyes (Meshkat et al. 2019). Adsorption of anionic dyes was initiated using a chitin biopolymer (Longhinotti et al. 1998). Chitin derivatives are used in heavy metal removal of lead (Zhou et al. 2005), chromium (Baran et al. 2007), cadmium (Benguella and Benaissa 2002), copper, and arsenic (Kartal and Imamura 2005). Biological denitrification and sulfate reduction in groundwater were initiated using crab shell chitin (CS-20) (Robinson-Lora and Brennan 2009). Chitin is also used for coagulating and flocculating activated sludge (Kurita 2006).

In the biomedical application, chitin fabrics (non-woven) and chitin threads are used in the development of artificial skin and sutures for wound dressing because of their biocompatibility and degradability (Nishimura 2001). The mechanical strength of pure chitin sutures can be improved by incorporating graphene oxide with chitin monofilament (Zhang et al. 2019).

In the field of agriculture, chitin is used for developing resistance against plant diseases and develops elicitor activity in fruits and vegetables (Parada et al. 2018; Pusztahelyi 2018). Nanochitin, derived from shrimp shells, is used to improve the quality and quantity of winter wheat: multi-spike wheat and large spike wheat, respectively (Xue et al. 2018). To improve soil fertility, chitin can be used as a fertilizer due to their rich nitrogen content (Malerba and Cerana 2019).

In the food sector, chitin derivatives are utilized as a food preservative (Hu and Gänzle 2019). They are also used as thickener mixed with vegetable oil for developing bio-lubricants (Sánchez et al. 2011). As a stabilizer/emulsifier, chitin is used in food, cosmetics, and biomedical applications (Casadidio et al. 2019; İlyasoğlu et al. 2018). Lipophilized chitin as chitin fatty esters (chitin laurate, chitin palmate, chitin stearate, chitin octanoate) is used for developing novel stabilizers with oil in water emulsions (İlyasoğlu et al. 2018). Chitin materials are replacing petroleum-based

Table 15.4 Functional aspects of chitin

Areas	Functions	References
Agriculture	Used as coatings in seeds, vegetables, and fruits; mixed as an anti-nematode agent along with fertilizer; soil improvement; as elicitors to enhance plant immunity against pests	Malerba and Cerana (2019), Shamshina et al. (2019), Parada et al. (2018), Sahu et al. (2017)
Aquaculture	Act as protective coating for raw shrimp and shellfish spat (juvenile stage) in the hatcheries; shrimp canning; formulated fish feed	Abdel-Ghany and Salem (2020)
Animal husbandry	Poultry feed	Khempaka et al. (2006)
Food and nutrition	Emulsifiers; stabilizers; thickeners; dietary fiber in tempeh; antioxidants; in food packaging	Harkin et al. (2019), Elhussieny et al. (2020)
Biomedicine	Wound dressings and sutures; anticoagulants; gene therapy; as scaffolds for drug delivery; in tissue engineering; regenerative medicine	Değim et al. (2002), Zhang et al. (2019), Anitha et al. (2014)
Cosmetics	Moisturizers; thickening agents; skin smoothener; anti-static agents; oral healthcare	Aranaz et al. (2018)
Biotechnology	Support material for immobilization and encapsulation of enzymes and cells.	Verma et al. (2020)
Nanotechnology	Development of chitin nanocrystals, chitin nanofibers, and composite materials	Salaberria et al. (2015), Aranday-García et al. (2019)
Waste management	Adsorbents for the removal of dyes, heavy metals, and petroleum derivatives	Akkaya et al. (2009), Meshkat et al. (2019), Anastopoulos et al. (2017), Jaafarzadeh et al. (2015), Barros et al. (2014)

packaging materials as they are eco-friendly and biodegradable (Srinivasa and Tharanathan 2007). Chitin-based packaging materials, in the form of antimicrobial films and composite materials, are used in preserving fruits and vegetables after post-harvest to maintain their freshness and enhance the shelf life (Srinivasa and Tharanathan 2007; Suryawanshi et al. 2019). In paper finishing, hydroxyl methyl chitin is added to improve the wet strength characteristics of paper (Allan et al. 1980; Song et al. 2018). For cosmeceutical applications, chitin was used as a skin conditioner, moisturizer, emollient, and surfactant, shows antimicrobial activity against skin acne, was used as an ingredient in hair care products, and in oral health-care acts as a carrier for herbal extracts in toothpaste, mouthwash, and chewing gums (Aranaz et al. 2018). In the field of nanotechnology, chitin nanoparticles developed from shrimp wastes of *P. semisulcatus* are used in developing iron/chitin nanocomposite with aqueous leaf extract of *Corchorus olitorius* that were analyzed for their

antimicrobial activity and heavy metal and dye adsorption (Gomaa 2018). In the textile industry, chitin can be used to prevent the wear and tear of fabrics while weaving and can be used to improve properties like water resistance and antimicrobial resistance to the fabric (Hahn et al. 2019). Chitin is used in textile dyeing as anti-wrinkle, anti-static, and anti-bacterial finishing by blending chitosan with cotton, silk, wool, etc., thus enhancing the value of the fabric and utilizing the natural polymers (Huang et al. 2018a, b). Hence, chitin can be used for various pleiotropic applications that can benefit humankind.

15.8 Conclusion

The production of chitin from shrimp wastes involving microorganisms is beneficial over other chemical methods. Although there are several reports on microbial shrimp shell degradation, the usage of the environmentally safe microorganisms (GRAS status) for shrimp shell biofermentation is beneficial, as the byproducts like protein hydrolysate derived from them can be used in animal, fish, and poultry feed, without causing risk of any infection. The derived protein hydrolysates from such GRAS organisms can be attempted to cultivate beneficial fungi that produce SCP and other enzymes like chitinases, cellulases, etc. Lactic acid bacteria, being GRAS microorganisms, can be used directly in the demineralization process in shrimp shell processing, producing beneficial products like calcium lactate and lactic acid. Thus, the chitin derived by microbial action of shrimp shell wastes is a safer approach that can resolve the problem of environmental pollution and be beneficial for innumerable applications in various industries.

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