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SECTION IV: INNOVATION IN BIOTECHNOLOGY
MINI REVIEW

Recuperación biotecnológica de quitina de residuos de crustáceos

Biotechnological recovery of chitin from crustacean waste

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Resumen

Revisiones sobre recuperación de quitina a partir de residuos de crustáceos y otras fuentes usando biotecnología son reconocidas en el presente artículo. La mayoría de las revisiones concluyen que aunque se han logrado resultados importantes en la recuperación de quitina, todavía existe la necesidad de mejorar las condiciones operativas de los procesos de desproteinización y desmineralización, tales como el tiempo, la fuente de carbono, el pH (inicial y durante la fermentación), el volumen de inóculo y la temperatura, entre otros, para aplicar a nivel industrial un bioproceso que sea comercial y ambientalmente costo-beneficio viable. La presente revisión tiene como objetivo reunir la información más actualizada disponible sobre la investigación en métodos biotecnológicos para recuperar la quitina de los residuos de crustáceos, estudiada durante los últimos 10 años, centrándose en las condiciones aplicadas a la desproteinización (DP) y la desmineralización (DM), particularmente en los tiempos de bioprocesamiento y las especies microbianas involucradas.

Palabras clave:

Desechos de crustáceos; recuperación de quitina; desproteinización; desmineralización; hidrólisis enzimática microbiana; fermentación.

Abstract

Reviews on biotechnological recovery of chitin from crustacean waste and other sources are acknowledged in the present review. Most of the reviews conclude that although important results on chitin recovery have been achieved, there is still a need for better approaches to improve operational conditions of deproteinization and demineralization processes, such as time, carbon source, pH (initial and during fermentation), volume of inoculum, temperature, among others, in order to apply at industrial level, a bioprocess commercially and environmentally cost/effective viable. The present review aims to gather the most updated available information about research on biotechnological methods to recover chitin from crustacean waste, studied during the past 10 years, focussing on conditions applied to deproteinization (DP) and demineralization (DM), particularly on bioprocessing times and microbial species.

Keywords:

Crustacean waste; chitin recovery; deproteinization; demineralization; microbial enzymatic hydrolysis; fermentation.

Introduction

Chitin is one of the most abundant biopolymers in nature after cellulose and is found in crustacean exoskeletons, insects and fungal cell walls. It is a polysaccharide consisting of β-1,4-linked N-acetyl-D-glucosamine, that in natural tissues is associated mainly to proteins and minerals, but also to lipids and pigments (Dun et al. 2019; El Knidri et al. 2018). It has been observed that the content of chitin vary according to the source and the species, from which this biopolymer has been recovered. It was reported that in *Crangon crangon* shrimp waste, protein content ranges from 10 to 38%, minerals from 31 to 44% and chitin from 24 to 46% (M. Bajaj et al. 2011). Chitin and its deacetylated derivative chitosan, have a commercial value and are highly demanded due to their biocompatibility and biodegradability capacity, which makes them applicable in medicine, agriculture, environmental protection, food processing, cosmetics, pharmaceuticals, textile industries and biotechnological products (Arbia et al. 2013; Prameela et al. 2010; El Knidri et al. 2018).

The annual international trade of crustaceans was 5129421 t in 2016 (FAO 2018). The increasing amount of waste generated from industrial processing of hydrobiological resources (exoskeletons of shrimp, prawn, crab, and other crustacean) has become an environmental problem. Exoskeleton and cephalothorax of some crustacean species such as shrimp or prawn, are wasted, although they contains chitin, proteins and pigments that could have an important commercial value. The amount recovered of those components depend on processing conditions, species, seasonal variations, etc. (Duarte de Holanda and Netto, 2006; Rodde et al. 2008; Xu et al. 2008; Al Sagheer et al. 2009; Palpandi et al. 2009; Wang et al. 2011).

Chitin recovery from crustacean waste requires two main processes, demineralization (DM) and deproteinization (DP) in order to separate the biopolymer from proteins and minerals to which it is associated in natural tissues. Industrial production of chitin involves chemical methods with the use of an alkali such as sodium hydroxide (NaOH) to remove proteins and hydrochloric acid (HCl) to remove minerals (Fig. 1). Although those methods have been commercially viable, they represent an environmental cost that needs to be addressed. As an alternative, research efforts have been made to contribute to replace chemical procedures by biotechnological ones which are environmentally friendly as shown in Figure 1 (Arbia et al. 2013; Ghorbel-Bellaaj et al. 2012; Liu et al. 2014; Francisco et al. 2015; Bashandy et al. 2016; Sedaghat et al. 2017; Zhang et al. 2017; Hamdi et al. 2017; Castro et al. 2018; Dun et al. 2018; Liu et al. 2020). Comparatively high cost of their manufacturing process, has been reported as a disadvantage for the use of chitin in some industries (Sini et al. 2007), however, a biological method to recover this biopolymer seems to be low cost and feasible to scale it up to industrial level (Dun et al. 2018).

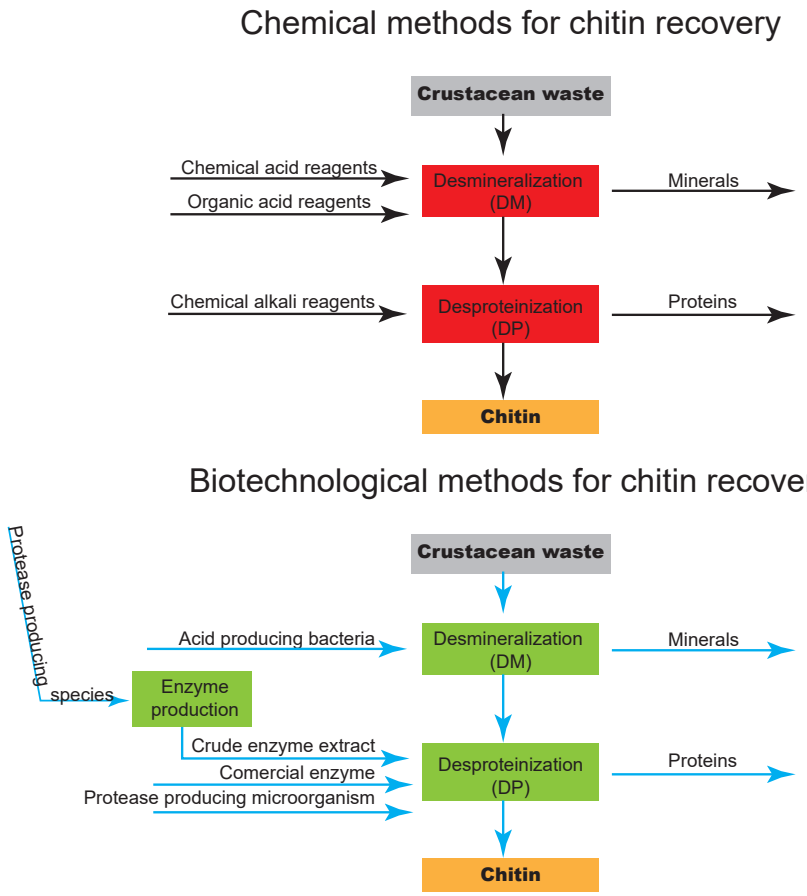

Figure 1. Chitin recovery by chemical and biotechnological methods.

Moreover, it has been detected the amino acid composition of the fermentation broth obtained after the bioprocesses, in order to give a full use of the crustacean waste (Liu et al. 2020). It also is necessary to take into consideration variations in the protein and mineral content between the exoskeletons of different species but also within the same species, particularly because they could be determinant to set up processes conditions.

This mini-review compiles some of the most updated published information on biotechnological processes applied to chitin recovery from crustacean waste.

Deproteination and demineralization of crustacean waste bioprocesses

To separate chitin from proteins and minerals to which it is associated in waste natural tissues, two steps need to be made, deproteinization (DP) and demineralization (DM). Microorganisms and proteolytic enzymes (enzymatic extracts or purified enzymes) have been used to separate proteins and minerals from the tissues. Those bioprocesses can be performed in two separate steps (to remove proteins and to remove minerals) or in one step to remove both simultaneously.

The efficiency of both bioprocesses, depends on the species, carbon source, pH (initial and during fermentation), volume of inoculum, temperature, among others (Prameela et al. 2010; Gortari & Hours 2013; Liu et al. 2020). Biotechnological bioprocesses have shown advantages and disadvantages; it has been remarked the need for the development of new methods to produce high quality chitosan with an improved degree of deacetylation (El Knidri et al. 2018).

Table 1 shows the results reported by some authors between years 2009 and 2020, to achieve chitin recovery with different operational conditions, most of which are similar, however the table also shows observed differences, which are relevant if the bioprocesses are to be scaled to pilot and industrial level; those factors are species used and bioreaction times achieved, among others.

The studies discussed in the present review can only be partially compared among them due to the use of crustacean waste that comes from different species, therefore they have different protein and minerals content, which may have an impact on the processing time.

Table 1: Overview for chitin recovery by biotechnological methods.

	Deproteinization (DP)	Demineralization (DM)	Species	Bioprocess Time (hours/day)	Results	Authors	Country
1	4% glucose concentration, 37 °C, initial pH 6.5, inoculum level 6%	5% glucose concentration, 37 °C, initial pH 6.5, final pH 3.4, inoculum level 4%.	*Lactobacillus rhamnoides, Bacillus amyloliquefaciens* (BA01)	48 h/84 h	DP 96.8% DM 97.5%	Liu et al. 2020	China
2	3% (w/v) shrimp waste, 37°C, 150 rpm	-	*Brevibacillus parabrevis* TKU046	4 d	DP 95%	Doan et al. 2019	Taiwan Vietnam
3	50 °C, 5% (w/v) crayfish shell waste, 5% (w/v) glucose, proteinase K, 10% inoculum.		*Bacillus coagulans*	48 h	DP 93% DM 91%	Dun et al. 2018	China
4	15% sucrose and 85% crab biomass.		*Lactobacillus plantarum* sp.	60 h	DP 95.3% DM 99.6%	Castro et al. 2018	Mexico
5	5% glucose, 180 rpm, 30 /37 °C		*Serratia marcescens* db11 *Lactobacillus plantarum*	6 d	DP 87.19% DM 89.59%	Chakravarty et al. 2018	USA
6	Sucrose (10% w/w), 30 °C		*Lactobacillus brevis Rhizopus oligosporus*	120 h/72 h	DP 96% DM 66.5%	Aranday-García et al. 2017	Mexico Japan
7	50°C, E/S ratio of 5U/mg, shrimp shells, crab shells and pH 8.	-	*Portunus segnis*	3 h	DP 84.7%, 91.06%	Hamdi et al. 2017	Tunisia
8	Shrimp shell waste 5% (w/v), 20% glucose, 50 °C and 100 rpm.		*Pseudomonas aeruginosa*	6 d	DP 92% DM 82%	Sedaghat et al. 2017	Iran
9	pH10, 60°C and E/S ratio of 10 U/mg	-	Bacillus safensis S406	3 h	DP 93%	Mhamdi et al. 2017	Tunisia
10	33% w/v shrimp shell waste, 50% (v/v), pH 6.2, 125 rpm, 35 °C.		*Lactobacillus plantarum*	72 h	DP 99% DM 87%	Neves et al. 2017	Brazil
11	Sucrose 5% (w/v), shrimp shell waste (12.5%, w/v),		*Bacillus subtilis*	7 d	DP 97% DM 82%	Gamal et al. 2016	Egypt
12	2% shrimp shell powders, 15 % glucose, 35 °C.		*Serratia marcescens* B742, *Lactobacillus plantarum* ATCC 8014	6 d	DP 94.5% DM 93.0%	Zhang et al. 2016	China USA
13	7.75 U/mg A21, 60 °C; 10 U/mg *S. scrofa*, 50 °C.	-	*Bacillus mojavensis* A21 *Scorpaena scrofa*	9 h	DP 96%	Younes et al. 2016	Tunisia France

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	Deproteinization (DP)	Demineralization (DM)	Species	Bioprocess Time (hours/day)	Results	Authors	Country
14	E/S ratio of 55U/g, pH7 and 37°C.	25°C and shells-lactic acid ratio of 1:11 (w/w)	*Streptomyces griseus*	3 h/ 20 min	DP 91.1% DM 98.6%	Hongkulsup et al. 2016	UK
15	Crustacean waste 18g/L, 10g/L glucose, initial pH 7, 40°C and 150 rpm.		*Bacillus subtilis* and *Bacillus licheniformis*	24h	DP 84%, 74.2% DM 55%, 60%	Pachapur et al.2015	Canada
16	5% glucose and 5% cassava starch		*Lactobacillus plantarum strains* T1 and L137	7 d	DP 84.4% DM 83%	Francisco et al. 2015	Philippines
17	5% (w/v) shrimp shell waste, 10% (w/v) glucose, 10% (v/v) inoculum, 37 °C and 100 rpm.		*Pseudomonas aeruginosa, Serratia marcescens, Bacillus pumilus*	6 d	DP 74.76%, DM 76.46%,	Sedaghat et al. 2015	Iran
18	5% (w/v) shrimp shell waste, 5% (w/v) glucose, and initial pH 7, 37°C.		*Bacillus pumilus* A1, *B. mojavencis* A21, *B. licheniformis* NH1, *B. cereus* BG1, *B. amyloliquefaciens* An6 and *B. subtilis* A26	5 d	DP 94 % DM 80%	Hajji et al.2015	Tunisia
19	Shrimp waste 15g, 45°C, E/S ratio of 5 U/mg	-	*Bacillus mojavensis* A21 and *Balistes capriscus*	3h	DP 77%, 78%	Younes et al. 2014	Tunisia France
20	30 °C, 180 rpm.		*Bacillus licheniformis* 21886 *Gluconobacter oxydans* DSM-2003	168h	DP 87% DM 93.5%	Liu et al. 2014	China
21	171.37 g/L sugars, 32°C, 4.84 g shell, 100 mL of fermentation medium.		*Lactobacillus helveticus*	254.38h	DP 78% DM98%	Arbia et al. 2013	Algeria France
22	Shrimp shell concentration of 70 g/L, glucose 50 g/L, pH of 5.0, 35 °C.		*Bacillus pumilus* A1	6 d	DP 94% DM 88%	Ghorbel-Bellaaj et al. 2013	Tunisia
23	55°C, pH 7.8-8, aeration 2.3 vvm, 275 rpm.	30 °C, 50 rpm	*Lactobacillus acidophilus* FNCC 116 *Bacillus licheniformis* F11.1	96,60 h	DP 95.37% DM 97.69%	Junianto et al. 2013	Indonesia
24	Inoculum 5%, shrimp head waste 10g/80mL, 30°C, 180 rpm, initial pH 10.	-	*Bacillus licheniformis* OPL-007	2 d	DP 85.3%	Mao et al. 2013	China
25	10% (w/w) shrimp head, 5% glucose, 1.2% (v/v) inoculum size, 42 °C, initial pH of 5.0.		*Streptococcus thermophilus*	64 h	DP 93.59% DM 92%	Mao et al. 2013	China
26	2% shrimp shell powders.	2% shrimp shell powders, 15% glucose.	*Serratia marcescens* B742 *Lactobacillus plantarum* ATCC 8014	4,2 d	DP 94.5% DM 93%	Zhang et al. 2012	China USA
27	30°C, 120 rpm, 50g/L sugar cane molasses, 66.7 g/L crustacean wastes from crab.		*Lactobacillus* sp. B2	120 h	DM 88% DP 56%	Flores-Albino et al. 2012	México
28	30°C,180 rpm, 20g/L date syrup, 5% of inoculum		*Lactobacillus plantarum*	6 d	DM 45% DP 54%	Khorrami et al. 2012	Iran
29	E/S rate of 7.75 U/mg, 60 °C and pH 9.	-	*Bacillus mojavensis* A21. *B. subtilis* A26 *B. licheniformis* NH1 B. *licheniformis* MP1, *Vibrio metschnikovii* J1 and *Aspergillus clavatus* ES1	6 h	DP 88.5 %	Younes et al. 2012	Tunisia France
30	15% glucose, 37°C.		*Lactobacillus acidophilus* SW01	168 h	DP 97.4%, DM 97.7%	Duan et al. 2012	China
31	5% (w⁄v) shrimp shell waste, 5% (w⁄v) glucose, initial pH 7.0, inoculum 1.5% (v/v), 30 °C, 200 rpm.		*Pseudomonas aeruginosa* A2	7 d	DP 90% DM 92%	Ghorbel-Bellaaj et al. 2012	Tunisia
32	10% (w/v), bacterial starter 5%, 35 °C.		*Lactobacillus plantarum*	96 h	DP 94% DM 92%,	Pacheco et al. 2011	Mexico
33	55 °C, 250 rpm, 2.5 vvm aeration.	30 °C, 50 rpm.	*Bacillus licheniformis* F11.1, *Lactobacillus acidophilus* FNCC116	60, 48h	DP 79.61% DM 88.65%	Wahyuntari et al. 2011	Indonesia
34	37 °C.	HCl	*Erwinia chrysanthemi* mutant	16h	DP 95% DM 99%	Giyose et al. 2010	South Africa
35	5% Inoculum, 15% glucose.		*Natural probiotic (milk curd).*	72h	DP 89% DM 69%	Prameela et al. 2010	India

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	Deproteinization (DP)	Demineralization (DM)	Species	Bioprocess Time (hours/day)	Results	Authors	Country
36	Shell waste (10%, w/v), 15% glucose (w/v), and initial pH 8.8.		*Lactococcus lactis Teredinobacter turnerae*	7 d	DP 90.2% DM 98.3%	Aytekin and Elibol 2010	Japan Turkey
37	Ratio shells /water 1:2 (w/v), 40 °C.	Acid treatment, 25 °C.	*Bacillus cereus SV1*	9 h	DP 88.8% DM 99%	Manni et al. 2010	Tunisia
38	55 °C, 2 vvm, 500 rpm.		*Bacillus licheniformis strains* F11.1, F11.2, F11.3 and F11.4	60 h	DP 84% DM 96.4%	Hoffmann et al. 2010	Germany
39	3% shell waste, pH 7.0, 37 °C, and 200 rpm.		*Bacillus cereus and Exiguobacterium acetylicum*	7 d	DP 97.1%, 92.8% DM 95.0%, 92.0%	Sorokulova et al. 2009	USA

Biotechnological methods to extract chitin from crustaceans are effective and environmentally friendly. However, one of the most relevant requirements still to be achieved, when compared with chemical methods, is reduction of processing time for total DP and DM. Some authors have reported that DP and DM takes altogether between **2 to 7 days** (Sorokulova et al. 2009; Aytekin & Elibol 2010; Ghorbel-Bellaaj et al. 2012; Khorrami et al. 2012; Zhang et al. 2012; Mao et al. 2013; Ghorbel-Bellaaj et al. 2013; Hajji et al*.* 2015; Sedaghat et al. 2015; Francisco et al. 2015; Zhang et al. 2016; Rawia et al. 2016; Sedaghat et al. 2017; Chakravarty et al. 2018; Doan et al. 2019). Some other authors have reported processing time between **24 to 254 hours** (Hoffmann et al. 2010; Prameela et al. 2010; Wahyuntari et al. 2011; Pacheco et al. 2011; Duan et al. 2012; Flores-Albino et al. 2012; Mao et al. 2013; Junianto et al. 2013; Arbia et al. 2013; Liu et al. 2014; Pachapur et al. 2015; Mhamdi et al. 2017; Aranday-Garcia et al. 2017; Neves et al. 2017; Liu et al. 2020).

It is remarkable to have reduced bioprocessing time to **3 to 16 hours** (Manni et al. 2010; Giyose et al. 2010; Younes et al. 2012; Younes et al. 2014; Hongkulsup et al. 2016), bearing in mind that some conditions such as the use of commercial enzymes, processing times, and the possibility of applying different microorganisms from those already studied (Table 1), need to be evaluated before attempting to scale the bioprocess up.

In order to reduce processing times and improve the effectiveness of biotechnological methods to recover chitin from crustacean waste, microbial species employed in DP and DM, have taken a relevant role. Some species of bacteria have been studied, as *Bacillus* spp. which have a high proteolytic capacity (Liu et al. 2020; Doan et al. 2019; Dun et al. 2018; Mhamdi et al. 2017; Gamal et al. 2016; Younes et al. 2016; Pachapur et al.2015; Sedaghat et al. 2015; Hajji et al. 2015; Younes et al. 2014; Liu et al. 2014; Ghorbel-Bellaaj et al. 2013; Junianto et al. 2013; Mao et al. 2013; Younes et al. *2012*; Wahyuntari et al. 2011; Manni et al. 2010; Hoffmann et al. 2010; Sorokulova et al. 2009). Similarly, another genus highly used is *Lactobacillus*, not only due to its high proteolytic activity, but also to its capacity to produce lactic acid, which has allowed to perform DP and DM in a single step. The acid produced by *Lactobacillus* spp. inhibits the growth of undesirable competitive bacteria (Castro et al. 2018; Chakravarty et al. 2018; Aranday-García et al. 2017; Neves et al. 2017; Zhang et al. 2016; Francisco et al. 2015; Arbia et al. 2013; Junianto et al. 2013; Zhang et al. 2012; Flores-Albino et al. 2012; Khorrami et al. 2012; Duan et al. 2012; Wahyuntari et al. 2011; Pacheco et al*.* 2011; Aytekin & Elibol 2010). *Lactobacillus* has also been used to demineralize crustacean waste only (Liu et al. 2020).

Additionally, other proteolytic bacterial species with relevant results on crustacean waste DP and DM have been studied, such as *Pseudomonas, Serratia, Streptomyces,* beside others (Doan et al. 2019; Chakravarty et al. 2018; Sedaghat et al. 2017; Zhang et al. 2016; Hongkulsup et al. 2016; Sedaghat et al. 2015; Liu et al. 2014; Mao et al. 2013; Zhang et al. 2012; Ghorbel-Bellaaj et al. 2012; Giyose et al. 2010; Aytekin and Elibol 2010; Prameela et al*.* 2010; Sorokulova et al*.* 2009).

Furthermore, in the search of improving DP and DM, the fungi *Rhizopus oligosporus* has also been considered (Aranday-García et al. 2017); and crude extracts from eukaryotes tissues as *Portunus segnis* (blue crab), *Balistes capriscus* (gray triggerfish) and *Scorpaena scrofa (red scorpionfish)* (Hamdi et al. 2017; Younes et al. 2014; Younes et al. 2016).

The price of crustacean waste is comparable to that of wood waste (Mao et al. 2016). On the other hand, conventional exoskeletons treatment based on the use of acid and base, although convenient and effective, is expensive and damaging to the environment. Biotechnological methods seem to be encouraging, but still in need of innovation that allows the technology to move into large-scale production, having to improve long processing times in order to obtain pure chitin.

At the moment, the challenge is to innovate towards environmentally sustainable technologies to transform crustacean waste into products such as biopolymers, pigments (astaxanthin), calcium, peptides, and protein hydrolysates, among others that have a potential high market value.

Conclusions

- Biotechnological extraction of chitin from crustaceans waste has been achieved by different strategies. Some of them are, the treatment with microbial acid fermentation for demineralization (DM)

and deproteinization (DP); microbial proteases fermentation for DP; and direct use of proteolytic enzymes for DP.

- Treatments of crustacean waste with crude enzyme extracts, have achieved the fastest processing times, however, previous steps to produce the enzyme extracts may represent an increase in operational costs that needs to be taken into account before scaling the process up.
- Microbial fermentation has shown a potential for deproteinization and demineralization, keeping in mind that processing times still need to be reduced, in order to scale the bioprocess up.
- The utilization of crustacean waste needs to be further investigated to the aim of adapting laboratory-scale biotechnological methods for industrial scale extraction of chitin from crustacean waste and its derivative products. The challenge now is to develop a bioprocess that is commercially and environmentally cost/effective viable.

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