

Chitin Extraction from Lobster Shell Waste Using Microbial Culture-based Methods

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Abstract

Background and Objective: Lobster shell waste from seafood processing industry was used as the source of raw material to produce the valuable biopolymer chitin. Chemical and biological treatments of lobster shell waste were performed and compared.

Material and Methods: The chemical method required the use of aqueous solutions of HCl and NaOH. Biological treatment included the use of co-cultures with a protease-producing bacterium, either *Bacillus megaterium* NH21 or *Serratia marcescens* db11, and an organic acid-producing bacterium *Lactobacillus plantarum*. The optimal culture conditions, including co-cultivation strategies and glucose concentrations, were identified to improve efficiency of lobster shell deproteinization and demineralization.

Results and Conclusion: Overall, the successive treatment with a combination of *Serratia marcescens* db11 and *Lactobacillus plantarum* resulted in the best co-removal of CaCO₃ and proteins and chitin yield (82.56%) from lobster shell biomass, with total deproteinization of 87.19% and total demineralization of 89.59%. The results from the proof-of-concept study described here suggest that microbial treatment may be an environmentally friendly alternative to the chemical method of chitin extraction.

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1. Introduction

The seafood processing industries generate around 6-8 million tons of crab waste, shrimp and lobster shells globally every year [1]. In the processing of crustaceans like lobster, shrimp, and crab for human consumption, about 40-50% of the total mass is waste [2]. Shellfish waste management is a huge problem faced by food industries. Only a small part of the waste is used as animal feed or fertilizers [3-5], while the rest are often dumped in landfills or into the sea and is a potential cause of pollution in coastal areas [6]. Approximately 50-60% of the total weight of the shells in lobster, shrimp, crab, is the non-edible exoskeleton, which is very rich in chitin, and is comprised of other components like calcium, proteins, and pigments [7].

Chitin or poly β -(1 \rightarrow 4)-*N*-acetyl-D-glucosamine, is a polymer that is abundantly found in a number of living organisms [8]. In the native state, chitin occurs as ordered crystalline microfibrils, which is a major component of

fungal cell walls, yeast, green algae, and the cuticles of insects [9,10]. Currently, the main commercial sources of chitin are crustacean shells [3,11]. Chitin and its derivative chitosan are biocompatible, non-toxic, biodegradable polymers [2]. These polymers have many potential applications in food and agriculture, medicine, tissue engineering, and as excipients for drug and gene delivery, wastewater treatment, as well as textile and cosmetic industries [12-21]. Chitin can also be depolymerized into its requisite monosaccharide, the amino sugar *N*-acetyl-D-glucosamine (NAG), which can be a carbon source for the growth of other microorganisms and production of value added products.

In crustaceans, chitin is present as a constituent of a complex network that includes proteins and carbonate deposits, forming the rigid shell [2]. Thus, extraction of chitin requires the removal of the two other major constituents of the shell, proteins and inorganic calcium

carbonate (CaCO_3) [22]. Conventional methods of chitin extraction from crustacean shells involve a chemical process that consists of two basic steps: (I) protein removal by alkali treatment, and (ii) CaCO_3 removal by acidic treatment [2]. Deproteinization is carried out to depolymerize the biopolymer for the removal of protein and can be achieved by using bases such as NaOH [23]. Demineralization, as the name of the step implies, consists of removing minerals, primarily CaCO_3 , and is generally easily achieved because CaCO_3 is converted into water-soluble calcium salts with the release of carbon dioxide [23]. The deproteinization step is challenging mainly due to disruption of covalent bonds between chitin and proteins [4]. The use of harsh chemicals may result in detrimental effects on the molecular mass, which will negatively affect the intrinsic properties of the purified polymer [2].

Dried shrimp shells are valued at a mere \$100–120 per ton [24]. However, the chemical method of chitin purifications from waste streams like these has limits set mainly by the production costs, including effluent treatment after acid and alkaline extraction of chitin [2]. As a result, good quality, highly purified chitin can cost up to \$200 per kilogram, even though the starting material is comparatively inexpensive [24]. The chemical method of chitin extraction is also an energy-consuming process and gives rise to a large volume of corrosive chemical wastes, which creates a disposal problem and can cause serious ecological damage and environmental pollution [25].

Due to these drawbacks of chemical chitin extraction, there has been growing interest in biological (bio-based) purification [3]. The biological method involves the use of microorganisms, either in monoculture or co-culture that produces protease enzymes and organic acids. Deproteinization can be carried out using protease-producing bacteria like *Bacillus* (*B.*) *subtilis*, *Pseudomonas* (*P.*) *aeruginosa*, *Pseudomonas maltophilia*, *Serratia* (*S.*) *marcescens* and others that break down proteins into water soluble protein hydrolysates, while the demineralization step can be carried out using organic acid-producing microorganisms like *Lactobacillus* (*L.*) *paracasei*, *Lactobacillus pentosus*, among others, that produce lactic acid that can remove calcium ions from shells [26–30]. The various biological methods of chitin extraction by microorganisms are of tremendous interest, as enzymes and organic acids can be produced by bacteria at a relatively low cost, and the process is environmental friendly, allowing for production of high quality chitin, based on degree of acetylation. The biological method also leads to a liquid fraction, rich in proteins, which potentially can be used for human and animal feed [2].

The utilization of shellfish wastes can potentially solve environmental problems, as well as providing economic

benefits. It can be an alternative to the disposal of shellfish wastes in landfills or in the oceans [24]. Since these wastes are rich sources of industrially important ingredients like chitin, there is an immense potential to bioprocess them and use chitin as a biotechnological feedstock to generate products of practical application. The sugars from seafood waste can be used as the primary feedstock to produce renewable biodiesel. It is a novel approach that would not require any current landmass in use or that could be potentially used for food crops, allowing the technology to remain out of the food vs. fuel debate, a philosophical barrier for some bio-fuel development efforts utilizing corn, palm oil, and canola oil [31].

The aim of this study is to produce crude chitin from lobster shell waste through the co-culturing of *Bacillus megaterium* NH21 or *Serratia marcescens* db11 and *Lactobacillus plantarum* in the presence shell waste. The optimal culture conditions, including co-cultivation strategies and carbon feedstock (i.e., glucose) concentrations were optimized for small-scale, batch deproteinization and demineralization of lobster shell waste. The chitin obtained was characterized and compared with those produced from using the bacterial cultures individually and the original sample. Much research has been performed on chitin extraction using different techniques and microorganisms. This work describes the use of two species of bacteria in sequential co-culture to remove mineral and protein contents from lobster shells in order to extract chitin.

2. Materials and Methods

2.1 Materials

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Lobster shell waste was a generous gift from Gloucester Seafood Processing Inc. (Gloucester, MA, USA). The shells were washed, dried, ground and sieved (1 mm sieve) and stored at room temperature until used. The protease-producing bacterium *Bacillus megaterium* strain NH21 is an environmental isolate from Lake Webster, USA. Another protease producer, *Serratia marcescens* db11 is an unpigmented isolate originally characterized by Flyg and coworkers [32]. The organic acid producer *Lactobacillus plantarum* was isolated from cellulosic waste [33]. All bacterial strains were stored in 20% glycerol at 80°C.

2.2 Inoculum preparation

L. plantarum was grown on Man Rogosa Sharpe (MRS) agar and incubated overnight at 37°C. MRS media composition used in this work is as follows (per liter): 10 g peptone, 15 g yeast extract, 20 g dextrose, 1 g Tween-80 (polysorbate 80), 2 g dipotassium hydrogen phosphate (K_2HPO_4), 5 g sodium acetate, 2 g triammonium citrate,

0.2 g magnesium sulfate, 0.05 g manganese sulfate, balance distilled H₂O. For solid media, 15 g agar was added before sterilization. Peptone, yeast extract and agar were purchased from Thermo-Fisher Scientific (Agawam, MA, USA). All other MRS media components were purchased from Sigma-Aldrich (St. Louis, MO, USA). *B. megaterium* and *S. marcescens* were grown on plates of Lysogeny Broth (LB) agar (Thermo-Fisher Scientific, Agawam, MA, USA) and incubated overnight at 30°C. To prepare starter cultures, 100 ml of sterile MRS broth was inoculated with *L. plantarum* and LB broth was inoculated with *B. megaterium* and *S. marcescens* separately and incubated with shaking (180 rpm) at 37°C and 30°C, respectively, for 48 h.

To prepare an inoculum for fermentation, 4 ml of the starter culture was transferred into 100 ml of sterile MRS broth or LB broth (2 vol% inoculation) and incubated with 180 rpm agitation at 37°C and 30°C for 48 h, respectively.

2.3 Chemical treatment of the lobster shell waste

The method of Aytekin and Elibol [34] was adopted to chemically extract chitin from lobster shells. Briefly, the removal of protein was achieved by treating the lobster shells with 1M NaOH solution for 3 h, at elevated temperatures (80-90°C) with continuous stirring and a solids-to-solvent ratio of 1:10 (w v⁻¹). Demineralization was achieved by constant stirring of the dried, ground, deproteinized shells in 1N HCl for 2 h, at ambient room temperature and a solid-to-solvent ratio of 1:15 (w v⁻¹).

Two chemical extraction methods were followed to remove proteins and inorganic matter from shells (Fig. 1): (I) the protein was removed first, followed by removal of inorganic matter, *i.e.* deproteinization was followed by demineralization (DP-DM); ii) the reverse order was followed, *i.e.*, demineralization was followed by deproteinization (DM-DP).

For all methods, the resulting deproteinized, demineralized chitin was filtered and washed to neutrality with water. The chitin was then dried overnight at 60°C in an oven (Hi-Temp Vacuum Oven, Thermo Scientific).

2.4 Biological treatment of the lobster shell waste

In separate cultures, 10 g of lobster shells were added to 50 ml of water supplemented with 0%, 2%, 5% or 10% glucose and inoculated with 10% *B. megaterium*, *S. marcescens* and were incubated at 30°C; and with 10% *L.*

plantarum and incubated at 37°C with shaking (180 rpm) for 96 h.

Three different strategies were applied in the co-culture experiments (Fig. 1): (i) simultaneous inoculation of a protease producing bacterium (either *B. megaterium* or *S. marcescens*) and *L. plantarum*, incubated at 37°C with shaking (180 rpm); (ii) inoculation with a protease producing strain, supplemented with 5% glucose (incubated at 30°C with shaking (180 rpm)). After three days, *L. plantarum* was added to the culture, followed by incubation at 37°C with shaking (180 rpm); (iii) the reverse order of (ii). After six days incubation time for all co-culture strategies, chitin was harvested, and the culture supernatant was collected for further analysis.

2.5 Analysis of chemical properties of samples

The pH of the supernatant was determined using a potentiometer (Orion Star A211, Thermo Scientific, USA). The ash content of the shell residues was determined by combusting the shell powder in an electric furnace at 500°C for 4 h and measuring ash weight.

The protein content was determined in each sample by checking the nitrate content in the samples using the cadmium reduction method with a spectrophotometer (DR 4000⁻¹ UV-VIS, Hach, USA). The protein content in the untreated samples was considered as the basis for computing the deproteinization percentage, which was expressed by Eq.1:

$$\text{Deproteinization (\%)} = \frac{(P_{O*O}) - (P_{F*F})}{P_{O*O}} \times 100 \quad \text{Eq. 1}$$

where P_O and P_F are the protein concentrations (%) before and after the co-culture treatments, O and F represent the mass (g) of original samples and biologically treated residues, respectively on a dry weight basis [35]. The calcium content in the samples was determined by atomic absorption spectroscopy using an air-acetylene flame (AAAnalyst 300, Perkin Elmer, USA). Demineralization percentage was calculated using the Eq. 2:

$$\text{Demineralization (\%)} = \frac{(C_{O*O}) - (C_{F*F})}{C_{O*O}} \times 100 \quad \text{Eq. 2}$$

where C_O and C_F represent the calcium content in the original and in the biologically treated residues, respectively on a dry weight basis. All experiments discussed here were conducted in triplicate, and values are presented as the mean ± standard deviation.

The chitin yield was calculated with Eq. 3:

$$\text{Chitin yield (\%)} = \frac{\text{Chitin in residue (g)}}{\text{Initial chitin in shell waste (g)}} \times 100 \quad \text{Eq. 3}$$

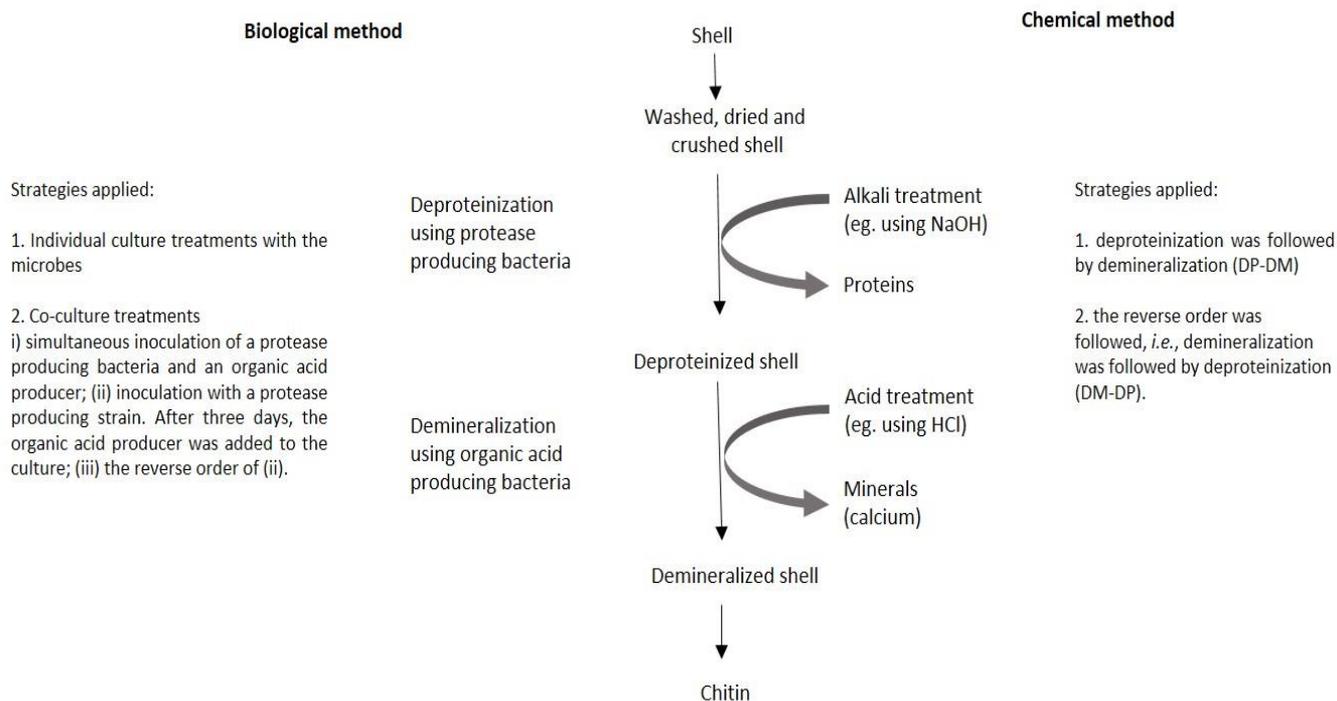


Fig. 1. Schematic description of the chitin recovery process by chemical and biological methods used in this study.

2.6 Proteolytic activity assay

The proteolytic activities were determined using the substrate casein. A 0.5 ml aliquot of culture supernatants was diluted and mixed with 0.5 ml of 1% (w v⁻¹) casein in 100 mm potassium phosphate buffer (pH 7). The mixture was incubated for 10 min at 40°C. The reaction was terminated by adding 1 ml of 0.4 M trichloroacetic acid and allowed to stand at 40°C for 20 min. The mixture was then centrifuged at 3619.1 g for 5 min to remove precipitate. A 0.5 ml aliquot of the supernatant was mixed with 2.5 ml of 0.5 M sodium carbonate and 0.5 ml of 0.5 M Folin-phenol reagent, and the mixture was then incubated for 20 min at 40°C. A standard curve was made using solutions of L-tyrosine in concentration ranges of 0–100 µg ml⁻¹.

One unit of protease activity is defined here as the amount of enzyme required to release 1 µmol of L-tyrosine min⁻¹. The protease activities were determined from the mean of at least two separate measurements carried out in multiple replicates, and the differences between the values did not exceed 5%.

2.7 Chitin characterization using Thermogravimetric analysis (TGA)

TGA monitors weight change in materials during heating as a function of time and temperature. The acquired measurements provide information about the thermal stability and composition of the material. TGA assays were conducted with a TGA Q500 (TA Instruments) by heating the samples from 10°C to 800°C by ramping temperature input of 5°C per min.

2.8 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) analysis of chitin

The dried sample of chitin was coated with a gold layer under vacuum using a sputter coater (Denton Vacuum Sputter, Desk IV, USA). High-resolution images of surface topography of chitin samples were produced using a highly-focused JSM-5610 (JEOL Ltd., Tokyo, Japan) scanning electron microscope operated at 20 kV. Qualitative Energy Dispersive X-ray (EDX) spectra of the deproteinized and demineralized samples were recorded using an EDX detector (EDS 6587, Oxford Instruments, UK) mounted on the scanning electron microscope.

2.9 Statistical analysis

Each preparation and measurement was conducted in triplicate. The experimental data were subjected to an analysis of variance (ANOVA) for a completely random design (CRD) using a Statistical Analysis System (SAS Institute, Inc., 2000). Differences were significant at p≤0.05.

3. Results and Discussion

3.1 Chemical treatment of the lobster shell waste

Removal of proteins and inorganic matter (minerals) may be achieved by treatment of lobster shells using different strategies. Therefore, two chemical extraction methods were followed to remove proteins and inorganic matter from the shells. The chemical compositions of the lobster shells before and after the treatment are shown in Table 1.

It was seen that the protein content in the residues when the DM-DP method followed was lower than that of DP-DM, suggesting more complete deproteinization of the shells. Demineralization levels did not vary extensively between methods.

3.2. Biologically mediated chitin extraction using separate microbial cultures

Biological methods of organic and inorganic matter removal from chitin is an attractive alternative to chemical methods due the lack of toxic waste products [36]. pH and proteolytic activity were monitored throughout the experiments with respect to glucose concentration. When the concentrations of glucose varied from 0% to 10%, the changes in the pH and proteolytic activity were studied for 6 days of culture, using *B. megaterium* NH21, *S. marcescens* db11 and *L. plantarum*,

respectively, as biocatalysts (Fig. 2). The maximum protease activities of 161.3 U ml⁻¹ for *B. megaterium* and 195.13 U ml⁻¹ for *S. marcescens* were obtained from cultures initially supplemented with 5% glucose, and *L. plantarum* was found to produce negligible amounts of protease irrespective of the glucose concentration, as seen in Fig. 2. The latter result was expected, as *L. plantarum* was used in this work for its production of organic acids.

Under culture conditions that fostered maximum protease activity, the deproteinization level of lobster shells by *B. megaterium* was 70.29% and by *S. marcescens* was 76.73% (Table 2), calculated according to equation 2. The deproteinization levels coincided with the trend of proteolytic activity (Fig. 2), as the largest percentage of protein was removed from shells under culture conditions where protease activity was shown to be the strongest in culture supernatants.

Table 1. Chemical compositions of lobster shell biomass and residues after chemical treatments

Shells	Protein (%)	Calcium (%)	Deproteinization (%)	Demineralization (%)	Ash (%)	Chitin yield (%)
Before	23.54 ± 0.78	45.68 ± 0.34	-	-	12.17 ± 1.1	-
DP – DM	6.67 ± 0.98	4.26 ± 1.02	80.16 ± 0.56	92.54 ± 0.94	2.87 ± 0.83	75.4 ± 1.23
DM – DP	2.72 ± 1.2	5.09 ± 0.67	89.97 ± 0.87	91.54 ± 1.31	3.94 ± 0.48	89.8 ± 2.5

DP-DM = deproteinization was followed by demineralization. DM-DP = demineralization was followed by deproteinization. All the values are means of triplicates, the standard deviations are indicated

Table 2. Results obtained from residues after treatment with individual microbial cultures.

Microbial treatment	Glucose (% wv ⁻¹)	Protein (%)	Calcium (%)	Deproteinization (%)	Demineralization (%)	Ash (%)	Chitin yield (%)
Before	-	23.54 ± 0.7	45.68 ± 0.4	-	-	12.17 ± 0.76	-
<i>Bacillus megaterium</i> NH21	0	14.30 ± 0.9	41.32 ± 1.66	51.40 ± 0.4	27.63 ± 1.1	9.73 ± 0.43	38.73 ± 2.11
	2.5	12.58 ± 1.14	34.89 ± 0.5	58.32 ± 2.1	40.42 ± 0.94	7.21 ± 1.17	46.63 ± 0.43
	5	8.84 ± 0.67	32.72 ± 0.9	70.29 ± 1.7	43.34 ± 0.7	6.82 ± 0.3	49.73 ± 0.43
	10	18.57 ± 0.73	32.49 ± 1.3	41.62 ± 0.8	47.36 ± 0.68	8.59 ± 0.62	40.90 ± 1.05
<i>Serratia marcescens</i> db11	0	13.79 ± 1.4	43.88 ± 0.78	56.06 ± 1.03	27.95 ± 1.23	5.64 ± 0.2	39.54 ± 0.90
	2.5	16.86 ± 0.11	38.47 ± 0.84	47.51 ± 0.7	38.26 ± 1.04	6.46 ± 0.84	42.82 ± 2.76
	5	7.17 ± 0.98	35.84 ± 0.7	76.73 ± 1.3	40.05 ± 0.47	7.25 ± 0.37	50.71 ± 3.25
	10	14.21 ± 1.23	32.36 ± 0.69	51.88 ± 0.7	43.54 ± 0.75	8.62 ± 0.17	43.48 ± 2.59
<i>Lactobacillus</i> <i>plantarum</i>	0	ND	ND	ND	ND	ND	ND
	2.5	20.84 ± 0.56	19.05 ± 0.76	17.60 ± 0.17	65.64 ± 0.33	5.74 ± 1.2	29.66 ± 1.09
	5	22.79 ± 0.84	15.56 ± 1.54	21.67 ± 2.1	72.83 ± 0.26	3.16 ± 0.6	37.09 ± 2.08
	10	-	15.68 ± 1.28	-	73.02 ± 1.3	4.88 ± 0.3	-

ND = not determined. All the values are means of triplicates, the standard deviations are indicated after the ±.

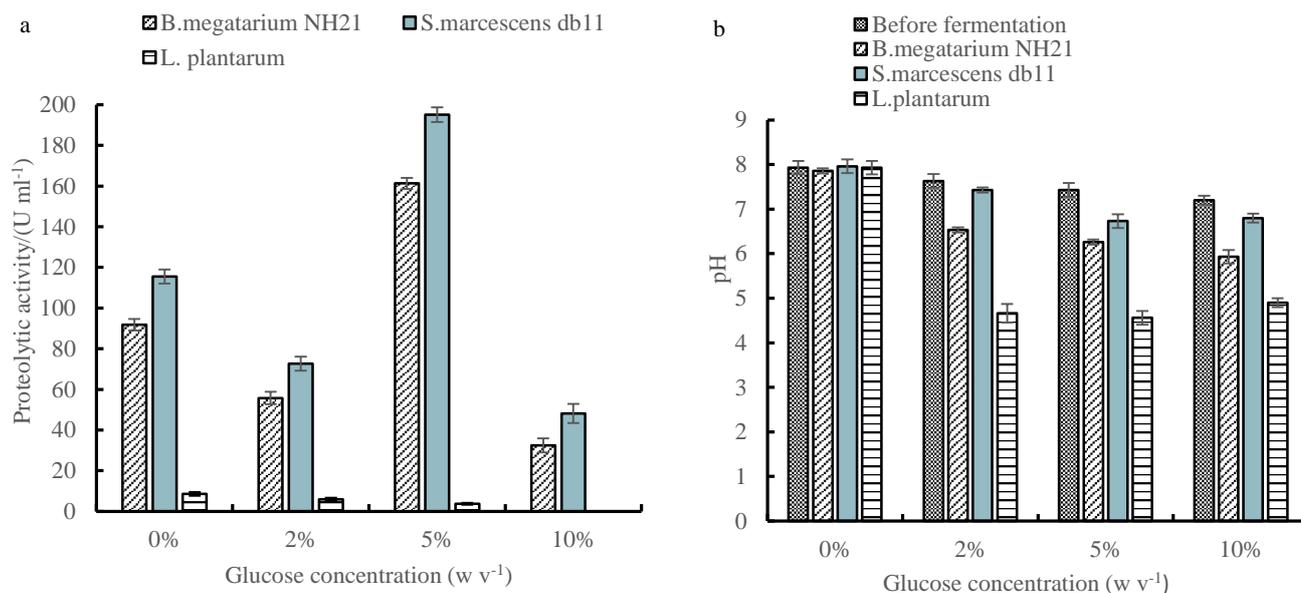


Fig. 2. Proteolytic activity amounts (a) and pH (b) of culture broths from *B. megaterium* NH21, *S. marcescens* db11 and *L. plantarum* after incubation for 120 h. Error bars represent standard deviations of triplicate tests.

Growth of *L. plantarum* was poor in lobster shell waste on its own could not provide enough nutrition for the growth of *L. plantarum*. Therefore, the protein, calcium, and ash content of residues from these culture conditions were not determined. However, in *L. plantarum* cultures, the initial concentration of glucose had a significant effect on the production of lactic acid. When the glucose concentration was 5%, the culture pH dropped from 7.9 to 4.6 (Fig. 2b), and the demineralization efficiency increased to 72.83%.

The pH of the medium decreased with the increase in culture time. This change can be related to the conversion of glucose to lactic acid by the microbial activity, which indicates removal of inorganic matters. Lactobacillus strains are known to produce lactic acid, which dissolves the calcium carbonate present in crustacean shells in such a way that significant demineralization had occurred in the shells by the conclusion of a co-culture treatment [33, 37].

The use of individual microorganisms in the deproteinization and demineralization of wastes for chitin production have been previously reported. *Bacillus megaterium* was used on distillery yeast biomass waste which resulted in 84% and 76% protein removal using culture and crude enzyme extracted from the isolate, respectively [38]. The protein and mineral removal from natural crab shell wastes with *S. marcescens* FS-3 was 84% and 47% respectively, after 7 days of fermentation [28]. In another study, *L. plantarum* was used to achieve demineralization and deproteinization of 54 and 45% respectively, at a low substrate concentration (2%) [12]. In this study, from Table 2, it can also be inferred that

individual cultures of *B. megaterium* and *S. marcescens* facilitated higher levels of deproteinization but were less efficient in demineralization, the reverse of which was true with cultures of *L. plantarum*. By considering all these aspects, it was hypothesized that co-culture with two distinct strains could be employed for more efficient extraction of chitin from lobster shells.

3.3. Co-cultivation experiments with *B. megaterium* NH21, *S. marcescens* db11 and *L. plantarum*

Chitin extraction was performed by incubating lobster shell biomass with co-cultures of *B. megaterium* and *L. plantarum* or *S. marcescens* and *L. plantarum*. The pH and proteolytic activity were monitored throughout the co-cultivation experiments in order to determine the optimal co-cultivation strategy. As shown in Fig. 3, the proteolytic activity of *B. megaterium* increased rapidly to a maximum level of approx. 520 U ml⁻¹ within 24 h, and remained relatively constant almost until day 3, immediately followed by a rapid decline. The pH of the culture broth decreased rapidly from 6.5 to 4.2 in 3 days, as a result of *L. plantarum* metabolic activity. For *S. marcescens*, the proteolytic activity peaked at a maximum level of 575 U ml⁻¹ at day 2 and started decreasing significantly from day 4 onwards, whereas the pH of the culture broth decreased rapidly from 6.7 to 4.3 in 3 days as a result of *L. plantarum* metabolic activity. As discussed before, the increase in acidity with time shows the production of lactic acid, which indicates removal of inorganic material. Therefore, day 3 was chosen to be the appropriate point for inoculating the lobster shell waste culture medium (already inoculated with the respective protease producers) with *L. plantarum* under the experimental conditions.

Three different strategies, as described in section 2.4, were applied in the co-cultivation experiments. The resultant changes in pH and proteolytic activity were followed throughout the culture time and are displayed in Fig. 4. When *L. plantarum* was employed in the co-cultivation, the pH of the culture supernatant (Fig. 4a) decreased gradually as culture time increased, which indicated a robust production of organic acids throughout the course of cultivation [39]. In culture supernatants where *L. plantarum* was added 3 days after inoculation with *B. megaterium*, the proteolytic activity was maximum at almost 515 U ml⁻¹ and then drastically decreased to around 13 U ml⁻¹ on day 6 when the pH decreased to 5.2. For culture supernatants where *L. plantarum* was added 3 days after inoculation with *S. marcescens*, the proteolytic activity was maximum at almost 599 U ml⁻¹ on day 3 and then drastically decreased to 20 U ml⁻¹ on day 6 when the pH decreased to 5.6 (Fig. 4 a and b).

Previously, Jung et al. [40] studied the extraction of chitin from red crab shells using a co-fermentation process. They stated that co-fermentation was more efficient than one-step extraction of crude chitin from crab shell waste. Aytekin et al. [34] had also attempted biological extraction of chitin from prawn waste using co-cultivation of *Lactococcus (L.) lactis* and *Teredinobacter (T.) turnirae*. They achieved best chitin yield with 15% glucose where *T. turnirae* was inoculated first and *L. lactis* was introduced at the end of 4 days.

In this study, for deproteinization, single cultivations with *B. megaterium* achieved a protein removal efficiency

of 70.29%, while higher deproteinization levels of 76.73% were achieved with *S. marcescens*. For demineralization, single cultivations with *L. plantarum* achieved a mineral removal efficiency of 72.83%. The co-cultivation system reached a much higher efficiency. Overall, the successive treatment with a combination of *S. marcescens* and *L. plantarum* (as in S to L) gave the best co-removal of CaCO₃ (89.59%) and proteins (87.19%) and a chitin yield of 82.56% from lobster shell biomass, as shown in Table 3.

Table 3. Results obtained from residues after 6 days of treatment with microbial co-cultures at 5% glucose

Microbial treatment	Deproteinization (%)	Demineralization (%)	Chitin yield (%)
L + B	54.54 ± 1.51	60.65 ± 1.13	52.24 ± 0.22
L to B	65.46 ± 1.22	63.68 ± 0.64	58.33 ± 1.90
B to L	78.63 ± 0.94	82.90 ± 1.01	74.85 ± 3.98
L + S	63.46 ± 1.11	66.01 ± 1.02	59.87 ± 1.45
L to S	76.34 ± 1.74	68.76 ± 1.42	63.19 ± 2.02
S to L	87.19 ± 0.78	89.59 ± 0.94	82.56 ± 2.76

L + B: simultaneous inoculation of *L. plantarum* and *B. megaterium* NH21; L to B: culture started with *L. plantarum*, *B. megaterium* NH21 was then added after 3 days of culture; B to L: culture started with *B. megaterium* NH21, *L. plantarum* was then added after 3 days of culture; L + S: simultaneous inoculation of *L. plantarum* and *S. marcescens* db11; L to S: culture started with *L. plantarum*, *S. marcescens* db11 was then added after 3 days of culture; S to L: culture started with *S. marcescens* db11, *L. plantarum* was then added after 3 days of culture;. All the values are means of triplicates, the standard deviations are indicated after the ±.

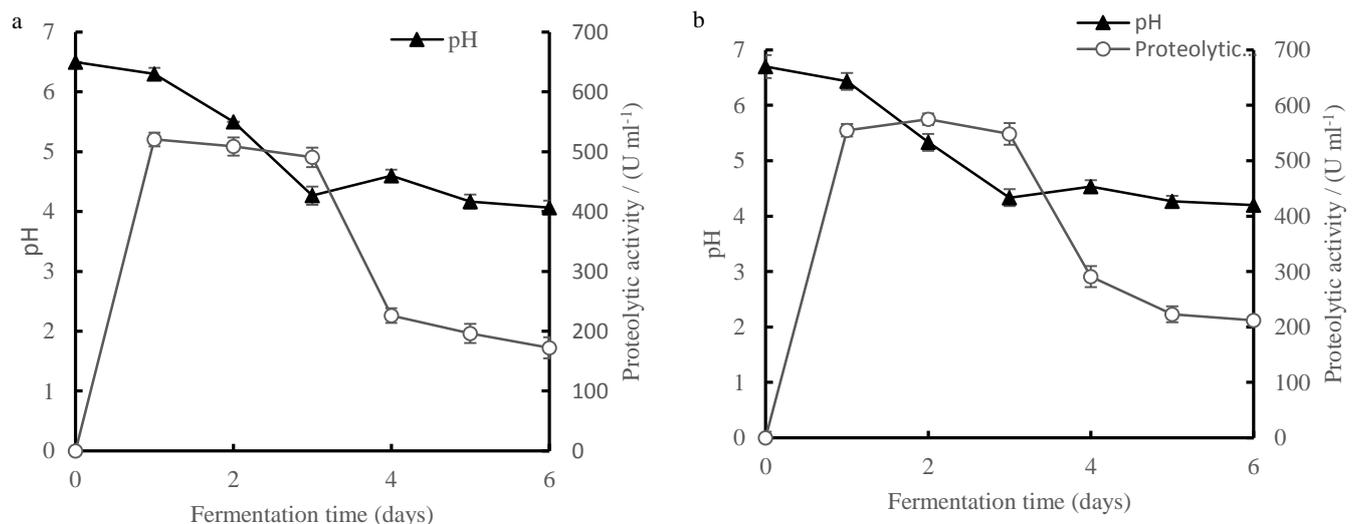


Fig. 3. Changes in pH and proteolytic activity in the culture liquor from co-cultures of: (a) *Bacillus megaterium* NH21 and *Lactobacillus plantarum* (b) *Serratia marcescens* db11 and *Lactobacillus plantarum*. Error bars represent standard deviations of triplicate tests.

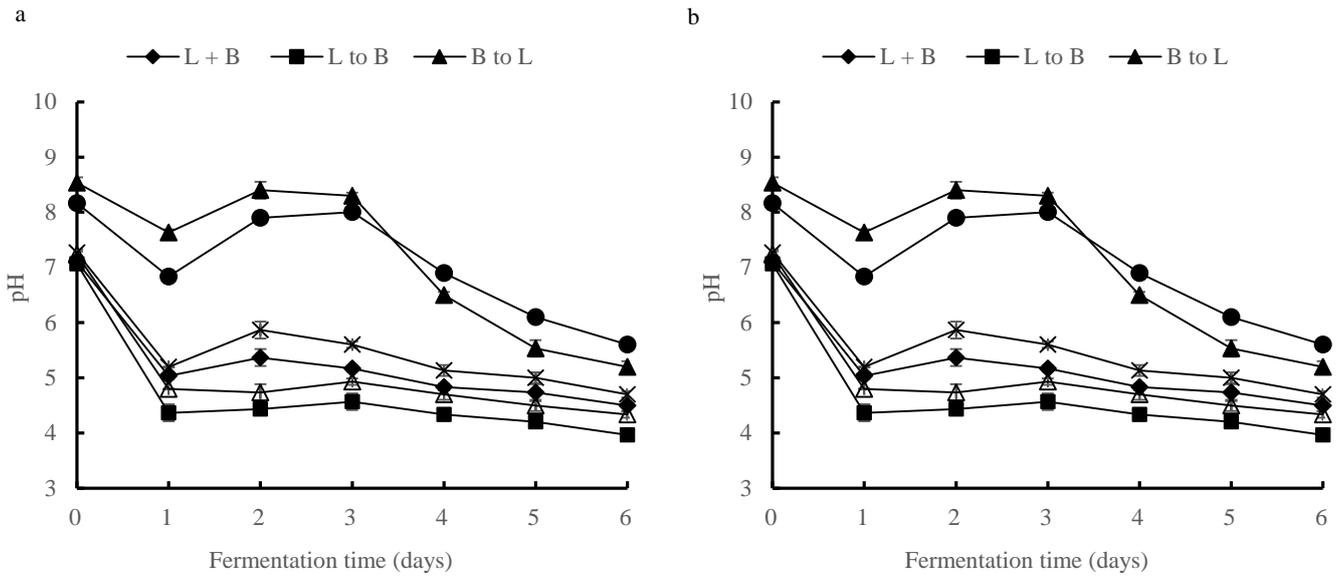


Fig. 4. Changes in pH (a) and proteolytic activity (b) of culture supernatants from the different co-cultivation experiments. L + B: simultaneous inoculation of *Lactobacillus plantarum* and *Bacillus megaterium* NH21; L to B: culture started with *Lactobacillus plantarum*, *Bacillus megaterium* NH21 was then added after 3 days of culture; B to L: culture started with *Bacillus megaterium* NH21, *Lactobacillus plantarum* was then added after 3 days of culture; L + S: simultaneous inoculation of *Lactobacillus plantarum* and *Serratia marcescens* db11; L to S: culture started with *Lactobacillus plantarum*, *Serratia marcescens* db11 was then added after 3 days of culture; S to L: culture started with *Serratia marcescens* db11 *Lactobacillus plantarum* was then added after 3 days of culture;. Error bars represent standard deviations of triplicate tests.

3.4 Chitin characterization using Thermogravimetric analysis (TGA)

Thermal decomposition was examined on chitin samples extracted after successive treatments in combination with *B. megaterium* and *L. plantarum* cultures (as in B to L, Fig. 5a) and successive treatment in combination with *S. marcescens* and *L. plantarum* cultures (as in S to L, Fig. 5b). Thermogravimetric analysis was carried out to determine the temperature and weight change of the samples, which allows quantitative composition analysis of the chitin. The thermogravimetric

curves were obtained at a heating rate of 5°C per min, in the temperature range of 10-800°C. In the chitin thermograms, mass losses were observed in two steps for all the chitin samples. The observed mass loss in the first step (between 50 and 150°C) was due to the evaporation of water. The second observed mass loss (between 300 and 500°C) can be attributed to the complete degradation of the chitin structures. It was observed that for both the samples, after 500°C, the percentage of residual mass remained constant.

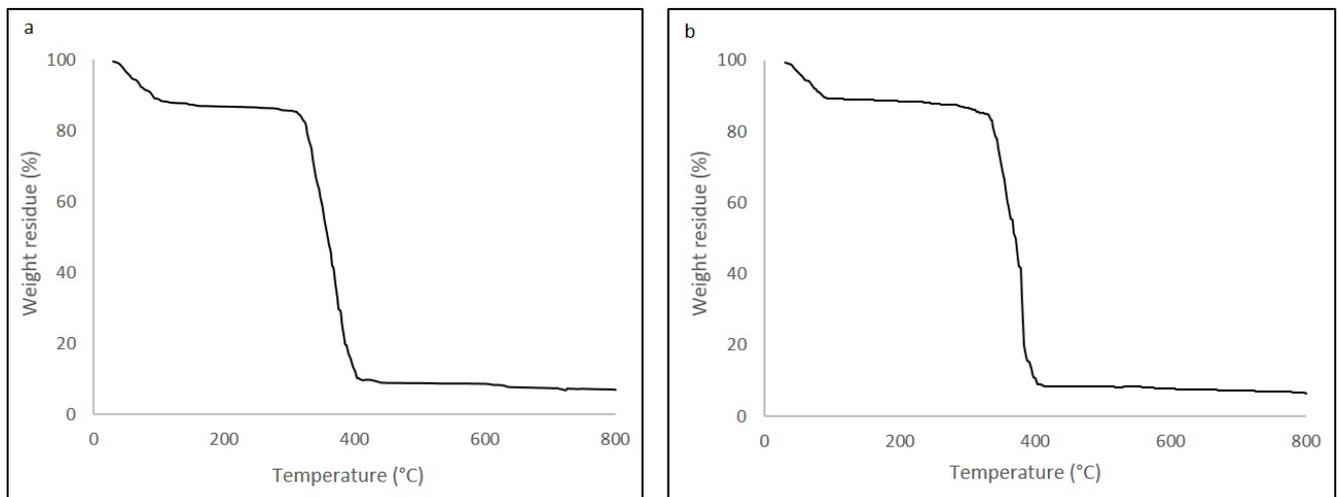


Fig. 5. Thermogravimetric analysis for chitin from lobster shell waste (a) after successive treatment with a combination of *Bacillus megaterium* NH21 and *Lactobacillus plantarum* (as in B to L), (b) after successive treatment with a combination of *Serratia marcescens* db11 and *Lactobacillus plantarum* (as in S to L).

In TGA results for chitin samples in previous studies, weight loss was observed in two steps, indicating a similarity to the analysis of the results observed here [41, 42]. Based on the findings, it can be said that this technique of using a combination of microorganisms to obtain chitin from lobster shell wastes can be commonly used in future chitin extraction studies.

3.5. Scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy (EDX) analysis of chitin

High-resolution scanning electron micrographs of lobster shells were examined before and after different chitin extraction treatments. Fig. 6a shows the characteristic microstructure of raw lobster shells (without any treatments). The lobster shell fragment appears homogenous and solid as many inorganic components are tightly embedded in the gaps in the chitin structure and flexible protein molecules. Therefore, the inherently arranged and ordered chitin fibers, i.e. the major component of the shell, could not easily be detected. Fig. 6b shows the image of the shell after demineralization with *L. plantarum*. Individual chitin fiber bundles were observed in contact with each other in certain areas. Fig. 6c and 6d shows the shell after deproteinization with *B. megaterium* and *S. marcescens*, respectively. These samples have visible shallow depressions; however, single fibers cannot be discerned. The SEM images of the shell after deproteinization showed less fractured chitin than after demineralization. Fig. 6e and 6f shows the shell after successive treatment with a co-culture of *B. megaterium* and *L. plantarum* (as in B to L) and of *S. marcescens* and *L. plantarum* (as in S to L), respectively. After these treatments, the shell fragments became highly porous, have several visible depressions with a densely-fractured structure and “swollen” look. The results showed that the treatments markedly altered the lobster cuticle microstructure and the results are in accordance with previous studies [25, 43]. The qualitative EDX analysis of the demineralized shells (Fig. 7a) shows that calcium has been removed considerably, whereas qualitative EDX analysis of deproteinized shells (Fig. 7b) shows strong signals for calcium and magnesium. The smoothness in the structure after the demineralization process in contrast to the blocky or rough appearance of the untreated samples suggests that the minerals were perhaps located between and around chitin-protein fibers, as has been demonstrated in previous studies [44].

3.6 Comparison of chemical and biological methods of chitin recovery

Chemical and biological treatments of lobster shell waste have been achieved for recovery of chitin by

applying several strategies. For the chemical method DM-DP, deproteinization level was about 89.97%, which was much higher than the DP-DM method. This could be attributed to the ability of NaOH to penetrate the matrix for breaking the protein bonds, since deproteinization was performed after mineral removal. However, previous studies have reported that the use of harsh acid treatments can cause hydrolysis of the polymer, inconsistent physical properties in chitin can be a source of pollution [40]. Other studies have also shown that high NaOH concentrations, high deproteinization temperatures and use of acids such as HCl can cause potentially undesirable depolymerization of chitin [2]. Thus, the optimal conditions for biological demineralization and deproteinization using organic acid and protease producing microorganisms were also investigated. Previously, Fagberno [45] used *L. plantarum* to extract chitin from the heads of African river prawn. Lactic acid bacteria have been reported to extract chitin from various sources, such as crayfish, scampi waste, and prawn waste, etc [29,34,42]. Crab shell waste was treated using *S. marcescens* FS-3, a strain isolated from environmental samples that exhibited strong protease activity [28]. *B. megaterium* PB 4 has been used for deproteinization of distillery yeast biomass waste [38].

3.7 Comparison of microbiological culture-based chitin recovery methods

In this study, three microorganisms, *B. megaterium* NH21, *S. marcescens* db11 and *L. plantarum*, have been studied, individually and as co-cultures, as biocatalysts for chitin purification. *L. plantarum* was more effective in removal of minerals as compared to *B. megaterium* and *S. marcescens*, while the reverse was true for the protein removal (Table 2). Therefore, it was concluded that the individual treatment with either of these strains individually was not sufficient for chitin extraction. Yet, if the aim is to obtain protein hydrolysate from lobster shell waste, the use of *B. megaterium* or *S. marcescens* (or similar bacterial strains) may be a good choice due to higher protease activity. The deproteinization levels coincided with the trend of proteolytic activity (Fig. 2), which indicates that strong proteolytic activity is necessary for achieving efficient protein removal from the shells. An attempt was made to cultivate both the organic acid and protease producing microorganisms together. Previously, the extraction of chitin from red crab shell waste was carried out by co-culture of two different microorganisms, and it was observed that co-culture of the waste using two strains was efficient and applicable for a one-step extraction of crude chitin from the waste shells [40].

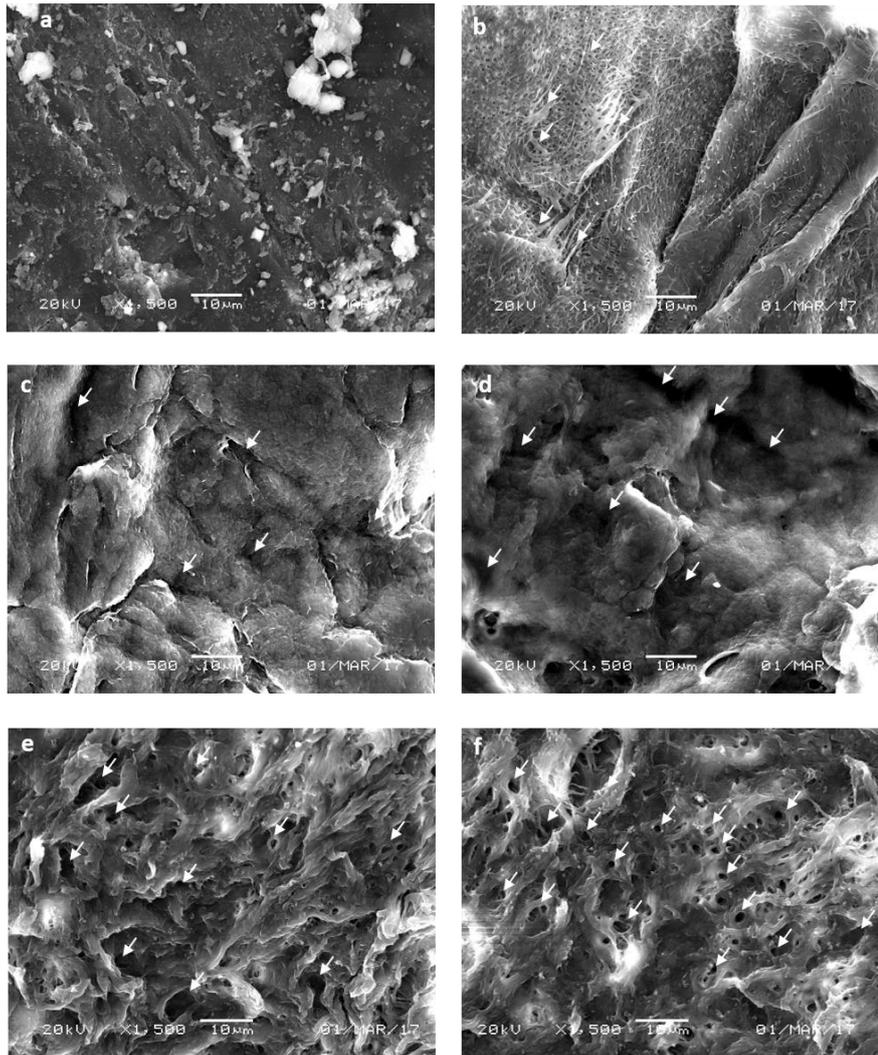


Fig. 6. SEM micrographs for (a) raw lobster shell showing its characteristic microstructure, (b) shells after treatment with *Lactobacillus plantarum*, (c) shells after treatment with *Bacillus megaterium* NH21, (d) shells after treatment with *Serratia marcescens* db11, (e) shells after successive treatment with a combination of *B. megaterium* NH21 and *L. plantarum* (as in B to L, according to Fig. 5 legend), (f) shells after successive treatment with a combination of *S. marcescens* db11 and *L. plantarum* (as in S to L, according to Fig. 5 legend). In (c) and (d), arrows point to shallow depressions on the surface of the shell that are likely the result of protease treatment. The shell fragments became highly porous, and (e) and (f) display several visible depressions with a densely-fractured structure (arrows).

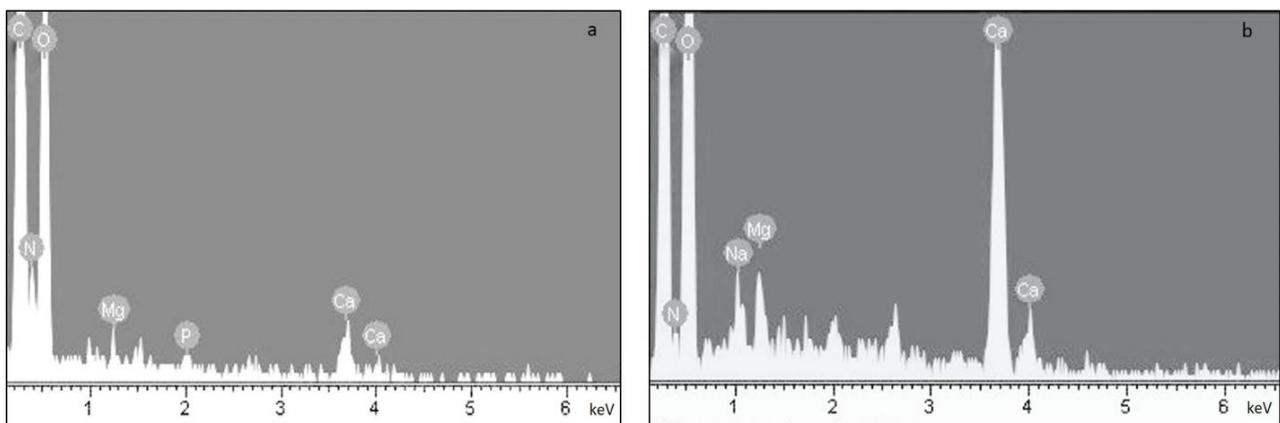


Fig. 7. Qualitative energy dispersive X-ray (EDX) analysis of (a) decalcified and (b) deproteinized samples.

In the present work, the co-cultivation system produced isolated chitin with a greater purity. Overall, the best chitin yield and co-removal of proteins and CaCO_3 from lobster shell biomass was achieved by successive treatment with a combination of *S. marcescens* and *L. plantarum* (as in S to L) (Table 3). The SEM results also show that the deproteinization and demineralization treatments had a noticeable impact on the lobster shell biomass. The calcium peak (Fig. 7a) area decreased in intensity according to the qualitative EDX analysis, suggesting that the structure is significantly depleted of mineral after removal of calcium chloride. The smoothness of the structure (Fig. 6c and d) without the majority of the calcium carbonate is in contrast to the more “blocky” appearance of untreated and deproteinized samples, indicating that the biominerals were indeed located between and around the chitin–protein fibers, as has been shown from previous studies [43, 45]. The strong signals of calcium obtained by qualitative EDX analysis for deproteinized (but not demineralized) samples confirm the presence Ca^{2+} in the structure (Fig. 7b). The biological treatment resulted in lower deproteinization and demineralization efficiencies as compared to the conventional chemical extraction method. Finally, it should be noted that this work is only a small, flask-scale study.

4. Conclusion

In conclusion, organic acid and protease producing microorganisms can be used as biocatalysts to treat lobster shell waste to reduce the presence of inorganic and proteinaceous matter. The lobster shells used in this study were demineralized and deproteinized to a considerable degree. The optimization of lobster shell degradation by the microorganisms in this study is essentially a proof-of-concept and offers a preliminary glimpse of an industrial scale process for biological chitin extraction. This extraction process is complicated and is affected by many parameters such as the microorganisms used, concentration of carbon source provided, specific strategy applied, etc. The results obtained here suggest that the microbial co-culture has the potential to be a “green” alternative to the chemical method. Thus, for reduction of harsh, corrosive chemicals and also to reduce the costs involved in waste management, the biological method of chitin extraction is a more environmentally friendly option for extraction of chitin. This is a small-scale study under laboratory conditions. Studies are being undertaken to make this method more suitable for large-scale chitin extraction operations. More research must be done in order to overcome the problems that are commonly related to up-scaling any biological process such as mass transfer challenges (O_2 transfer to cells, nutrient transfer, etc.), stirred-tank fermenter power requirements with larger

amounts of chitin and cellular biomass, the effect of increased metabolic load on the culture’s yield and output, maintaining steady supply of nutrients and removal of wastes, stream recycling, solid-liquid separation. Fundamental studies on lobster shell surface interactions of the microorganisms used here could also be performed in order to explore other frontiers of bioreactors, thus providing stimulating research in this area. This study could potentially be the first step in the process of utilizing sea food wastes to serve as a carbon feedstock for microbial bioconversions to value added products.

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6. Conflict of Interest

The authors declare that they have no conflicts of interest.

References

- Silva JG. The state of fisheries and aquaculture. FAO, Rome. 2014.
- Arbia W, Arbia L, Adour L, Amrane A. Chitin extraction from crustacean shells using biological methods-a review. Food Technol Biotechnol. 2013; 51(1): 12-25.
- Xu Y, Gallert C, Winter J. Chitin purification from shrimp wastes by microbial deproteination and decalcification. Appl Microbiol Biotechnol. 2008; 79(4): 687-697. doi: 10.1007/s00253-008-1471-9
- Manni L, Ghorbel-Bellaaj O, Jellouli K, Younes I, Nasri M. Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from *Bacillus cereus* SV1. Appl Biochem Biotechnol. 2010; 162(2): 345-357. doi: 10.1007/s12010-009-8846-y
- Linden JC, Stoner RJ. Pre-harvest Application of Proprietary Elicitor Delays Fruit senescence. In: Ramina A, Chang C, Giovannoni J, Klee H, Perata P, Woltering E. (eds) Advances in Plant Ethylene Research. Springer, Dordrecht. 2007; 301-302. doi: 10.1007/978-1-4020-6014-4_65
- Xuemei Z, Hawkins SJ. Interactions of aquaculture and waste disposal in the coastal zone. J Ocean Univ Qingdao. 2002; 1(1), 8-12. doi: 10.1007/s11802-002-0023-7
- Wang X, Xing B. Importance of structural makeup of biopolymers for organic contaminant sorption. Environ Sci Technol. 2007; 41(10): 3559-3565. doi: 10.1021/es062589t
- Rinaudo M. Chitin and chitosan: Properties and applications. Prog Polym Sci. 2006; 31(7): 603-632.

- doi: 10.1016/j.progpolymsci.2006.06.001
9. Kikkawa Y, Tokuhisa H, Shingai H, Hiraishi T, Kaneshato M, Imanaka T, Tanaka T. Interaction Force of Chitin-Binding Domains onto Chitin Surface. *Biomacromolecules*. 2008; 9(8): 2126-2131. doi: 10.1021/bm800162x
 10. Einbu A, Varum KM. Characterization of chitin and Its hydrolysis to GlcNAc and GlcN. *Biomacromolecules*. 2008; 9(7): 1870-1875. doi: 10.1021/bm8001123
 11. Ruocco N, Costantini S, Guariniello S, Costantini M. Polysaccharides from the marine environment with pharmacological, cosmeceutical and nutraceutical potential. *Molecules*. 2016; 21(5): 551. doi: 10.3390/molecules21050551
 12. Khorrami M, Najafpour GD, Younesi H, Hosseinpour MN. Production of chitin and chitosan from shrimp shell in batch culture of *Lactobacillus plantarum*. *Chem Biochem Eng Quart*. 2012; 26(3): 217-223.
 13. Dai M, Zheng X, Xu X, Kong X, Li X, Gang G, Luo F, Zhao X, Wei YQ, Qian Z. Chitosan-Alginate sponge: Preparation and application in curcumin delivery for dermal wound healing in Rat. *J Biomed Res Int*. 2009; 595126: 1-8. doi: 10.1155/2009/595126
 14. Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydr Polym*. 2009; 76(2): 167-182. doi: 10.1016/j.carbpol.2008.11.002
 15. Nagahama H, Maeda H, Kashiki T, Jayakumar R, Furuike T, Tamura H. Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. *Carbohydr Polym*. 2009; 76(2): 255-260. doi: 10.1016/j.carbpol.2008.10.015
 16. Ragetly GR, Slavik GJ, Cunningham BT, Schaeffer DJ, Griffon DJ. Cartilage tissue engineering on fibrous chitosan scaffolds produced by a replica molding technique. *J Biomed Mater Res A*. 2010; 93(1): 46-55. doi: 10.1002/jbm.a.32514
 17. Foster LJR, Ho S, Hook J, Basuki M, Marçal H. Chitosan as a biomaterial: Influence of degree of deacetylation on Its physicochemical, material and biological properties. *Plos One*. 2015; 10(8): 1-22. doi: 10.1371/journal.pone.0135153
 18. Anitha A, Sowmya S, Kumar PTS, Deepthi S, Chennazhi KP, Ehrlich H, Tsurkan M, Jayakumar R. Chitin and chitosan in selected biomedical applications. *Prog Polym Sci*. 2014; 39(9): 1644-1667. doi: 10.1016/j.progpolymsci.2014.02.008
 19. Zhou D, Zhang L, Guo S. Mechanisms of lead biosorption on cellulose/chitin beads. *Water Res*. 2005; 39(16): 3755-3762. doi: 10.1016/j.watres.2005.06.033
 20. Bhatnagar A, Sillanpaa M. Applications of chitin- and chitosan-derivatives for the detoxification of water and wastewater-a short review. *Adv Colloid Interface Sci*. 2009; 152(1-2): 26-38. doi: 10.1016/j.cis.2009.09.003
 21. Muxika A, Etxabide A, Uranga J, Guerrero P, Caba K. Chitosan as a bioactive polymer: Processing, properties and applications. *Int J Biol Macromol*. 2017; 105(Pt2): 1358-1368. doi: 10.1016/j.ijbiomac.2017.07.087.
 22. Kurita K. Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar Biotechnol*. 2006; 8(3): 203-226. doi: 10.1007/s10126-005-0097-5
 23. Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar Drugs*. 2015; 13(3):1133-1174. doi: 10.3390/md13031133
 24. Yan N, Chen X. Sustainability: Don't waste seafood waste. *Nature*. 2015; 524(7564):155-157. doi: 10.1038/524155a.
 25. Francisco FC, Simora RMC, Nuñal SN. Deproteinization and demineralization of shrimp waste using lactic acid bacteria for the production of crude chitin and chitosan. *AACL Bioflux*. 2015; 8(1): 107-115.
 26. Yang JK, Shih IL, Tzeng YM, Wang SL. Production and purification of protease from a *Bacillus subtilis* that can deproteinize crustacean wastes. *Enzyme Microb Technol*. 2000; 26(5-6): 406-413. doi: 10.1016/S0141-0229(99)00164-7
 27. Wang SL, Chio SH. Deproteinization of shrimp and crab shell with the protease of *Pseudomonas aeruginosa* K-187. *Enzyme Microb Tech*. 1998; 22 (7): 629-633.
 28. Jo GH, Jung WJ, Kuk JH, Oh KT, Kim YJ, Park RD. Screening of protease-producing *Serratia marcescens* FS-3 and its application to deproteinization of crab shell waste for chitin extraction. *Carbohydr Polym*. 2008; 74(3): 504-508. doi: 10.1016/j.carbpol.2008.04.019
 29. Zakaria Z, Hall GM, Shama G. Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. *Process Biochem*. 1998; 33(1): 1-6. doi: 10.1016/S0032-9592(97)00069-1
 30. Bautista J, Jover M, Gutierrez JF, Corpas R, Cremades O, Fontiveros E, Iglesias F, Vega J. Preparation of crayfish chitin by in situ lactic acid production. *Process Biochem*. 2001; 37(3): 229-234. doi: 10.1016/S0032-9592(01)00202-3
 31. Tilman D, Socolow R, Foley JA, Hill J, Larson E, Lynd L, Pacala S, Reilly J, Searchinger T, Somerville C, Williams R. Energy Beneficial biofuels-the food, energy, and environment trilemma. *Sci*. 2009; 325(5938): 270-271. doi: 10.1126/science.1177970.
 32. Flyg C, Kenne K, Boman HG. Insect pathogenic properties of *Serratia marcescens*: Phage-resistant mutants with a decreased resistance to *Cecropia* immunity and a decreased virulence to *Drosophila*. *J Gen Microbiol*. 1980; 120(1): 173-181. doi: 10.1099/00221287-120-1-173.
 33. Bringel F, Castioni A, Olukoya DK, Felis GE, Torriani S, Dellaglio F. *Lactobacillus plantarum* subsp. *Argentoratensis* subsp. nov., isolated from vegetable matrices. *Int. J. Syst. Evol. Micr*. 2005; 55: 1629-1634. doi: 10.1099/ijs.0.63333-0
 34. Aytakin O, Elilob M. Cocultivation of *Lactococcus lactis* and *Teredinobacter turnirae* for biological chitin extraction from prawn waste. *Bioprocess Biosyst Eng*. 2010; 33(3): 393-399. doi: 10.1007/s00449-009-0337-6
 35. Rao MS, Muñoz J, Stevens WF. Critical factors in chitin production by fermentation of shrimp biowaste. *Appl Microbiol Biotechnol*. 2000; 54(6): 808-813. doi: 10.1007/s002530000449
 36. Jung W, Park R. Bioproduction of chitooligosaccharides: Present and perspectives. *Mar Drugs*. 2014; 12(11):5328-5356. doi: 10.3390/md12115328

37. Hammes WP, Vogel RF. The genus *Lactobacillus*. In: Wood BJB, Holzapfel WH (Eds.). The genera of lactic acid bacteria. Glasgow: Blackie Academic, Professional, 1995: 19-54.
38. Kandaiah R, Ramasamy M. Deproteinization of Distillery Yeast Biomass Waste by Protease-producing *Bacillus megaterium* PB 4.J Bioremediation Biodegrad. 2015; 6(6): 4-11.
39. Moon GS, Narbad A. Monitoring of bioluminescent *Lactobacillus plantarum* in a complex food matrix. Korean J Food Sci Anim Resour. 2017; 37(1): 147-152. doi: 10.5851/kosfa.2017.37.1.147
40. Jung WJ, Jo GH, Kuk JH, Kim KY, Park RD. Extraction of chitin from red crab shell waste by cofermentation with *Lactobacillus paracasei* subsp. Tolerans KCTC-3074 and *Serratia marcescens* FS-3. Appl Microbiol Biotechnol. 2006; 71(2): 234-237. doi: 10.1007/s00253-005-0126-3
41. Romano P, Fabritius H, Raabe D. The exoskeleton of the lobster *Homarus americanus* as an example of a smart anisotropic biological material. Acta Biomater. 2007; 3(3): 301-309. doi: 10.1016/j.actbio.2006.10.003
42. Khor E, Wu H, Lim LY, Guo CM. Chitin-Methacrylate: Preparation, characterization and hydrogel Formation. Mater. 2011; 4(10): 1728-1746. doi: 10.3390/ma4101728
43. Guiraud-Guille MM. Plywood structures in nature. Curr Opin Solid State Mater Sci. 1998; 3(3): 221-227. doi: 10.1016/S1359-0286(98)80094-6
44. Beaney P, Lizardi-Mendoza J, Healy M. Comparison of chitins produced by chemical and bioprocessing methods. J Chem Technol Biotechnol. 2004; 80(2): 145-150. doi: 10.1002/jctb.1164
45. Fagbenro OA. Preparation, properties and preservation of lactic acid fermented shrimp heads. Food Res Int. 1996; 29(7): 595-599. doi: 10.1016/S0963-9969(96)00077-4

استخراج کیتین از ضایعات پوست خرچنگ با استفاده از روش‌های بر پایه کشت میکروبی

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چکیده

سابقه و هدف: ضایعات پوست خرچنگ حاصل از صنعت فراوری غذاهای دریایی به عنوان ماده خام برای تولید زیست-بسیار با ارزش کیتین مورد استفاده قرار گرفت. تیمارهای شیمیایی و زیستی ضایعات پوست خرچنگ انجام و مورد مقایسه قرار گرفت.

مواد و روش‌ها: روش شیمیایی نیازمند استفاده از محلول‌های کلریدریک اسید و هیدروکسید سدیم بود. تیمار زیستی شامل استفاده از کشت‌های همزمان به همراه پروتئاز باکتریایی *باسیلوس مگاتریوم NH21* یا *سراتیا مارسنس db1* و یک اسید آلی باکتریایی تولید شده توسط *لاکتوباسیلوس پلانتروم* بود. به منظور بهبود کارایی پروتئین زدایی و کانی-زدایی^۱، شرایط بهینه کشت شامل راهکارهای کشت همزمان و تعیین غلظت گلوکز بررسی شد.

یافته‌ها و نتیجه‌گیری: به‌طور کلی، تیمار با *سراتیا مارسنس db11* و پس از آن با *لاکتوباسیلوس پلانتروم*، بهترین نتیجه را در حذف همزمان کلسیم کربنات و پروتئین‌ها، راندمان کیتین (۸۲/۵۶٪) از توده زیستی پوست خرچنگ موجب شد و پروتئین‌زدایی کلی ۸۷/۱۹ و کانی‌زدایی کلی ۸۹/۵۹ درصد بود. براساس مطالعه اثبات-مفهوم تیمار میکروبی می‌تواند جایگزین دوستدار محیط زیست برای روش شیمیایی استخراج کیتین باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافی وجود ندارد.

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- *لاکتوباسیلوس پلانتروم*
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