



innovations from nature



# acib Project Proposal

## Valorization of shrimp shell waste Biological techniques for preparation of chitin and chitosan

### Summary

*Shrimp shells harbor several useful molecules like proteins, calcium carbonate, chitin and carotenoids. Unlike other forms of biomass, chitin and chitosan (deacetylated chitin) contains nitrogen which are excellent resources for the production of a series of valuable nitrogen-containing chemicals. Current methods for separation of these useful molecules from shrimp shell waste employ harsh conditions which are destructive, wasteful, expensive and hazardous to the environment. A sustainable biological fractionation method that avoids corrosive or hazardous reagents and minimizes waste is needed to establish profitable and sustainable shell refinery.*

### Background

Chitin, a long chain polymer of an *N*-acetylglucosamine, is the structural element in the exoskeleton of insects, crustaceans and cell wall of fungi, and the second most abundant natural polysaccharide after cellulose. Unlike most other forms of biomass such as cellulose, chitin contains nitrogen which is an excellent resource for the production of a series of valuable nitrogen-containing chemicals in a more sustainable, energy efficient and greener manner (1,2). Current manufacture of nitrogen-containing chemicals involves fossil fuels and energy-intensive processes. Chitosan (degree of acetylation <50%), the partial deacetylated and water soluble form of chitin, is the most important derivative of chitin. Chitosan is recognized as versatile biomaterials because of their diverse bioactivities, non-toxicity, biocompatibility, biodegradability and low-allergenicity.

The main commercial sources of chitin so far are crab and shrimp shells. Chitin is isolated from the shell by removal of the two major constituents of the shell, proteins by deproteinization and inorganic calcium carbonate by demineralization. Preservation of chitin structure during these two steps is important. Therefore selection of suitable methods/conditions for deproteinization and demineralization is highly important to obtain high quality chitin with high degree of acetylation and high molecular weight. Existing methods for extracting chitin are destructive, wasteful and expensive. Protein is removed with sodium hydroxide solution and the decomposition of calcium carbonate uses hydrochloric acid – both are corrosive and hazardous solvents. To make chitosan, chitin is treated with 40% concentrated sodium hydroxide solution. The production of 1 kg of chitosan from shrimp shells requires more than one tonne of water. A sustainable fractionation method to separate proteins, calcium carbonate and chitin, and deacetylation methods to generate chitosan– one that avoids corrosive or hazardous reagents and minimizes waste is needed to establish a profitable and sustainable industry from shell waste.

The application of enzymes and microorganisms for chitin extraction from shrimp shell waste and for deacetylation of chitin is gaining greater attention. It has been demonstrated that

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biological method was better than the chemical one because it preserves the structure of chitin (3). The biological extraction of chitin offers high reproducibility in shorter time, simpler manipulation, smaller solvent consumption and lower energy input. However, the biological method is still limited to laboratory scale studies (4).

## acib-Technology

### Biological separation of chitin from shrimp shell

Biological separation of chitin from shrimp shell is achieved through enzymatic/microbial deproteinization and demineralization. The demineralization is carried out by *in situ* production of lactic acid using suitable lactic acid producing organism. The lactic acid reacts with the calcium carbonate present in the shrimp shell, leading to the formation of a precipitate of calcium lactate. Once the desired demineralization efficiency and the desired quality of the product are achieved, the complete deproteinization is easily achieved with several commercially available bacterial/fungal alkaline proteases (5). After this step the chitin is separated as a precipitate. Therefore efficiency of demineralization is important and performed first to remove the minerals present in the cuticles which may decrease the accessibility of the proteases and affect the shrimp shells deproteinization efficiency. The efficiency of the demineralization process depends on many factors, mainly the organism, quantity of the inoculum, substrate concentration, initial pH and pH change during fermentation, temperature and duration of fermentation (6). Search for robust fermentation strain which grow on cheap carbohydrates, exhibit low-pH tolerance and high operational stability, higher production rates, and can easily be genetically modified is important to turn this into an elegant process. Natural lactic acid producing bacteria such as *Lactobacillus* sp. have been used in several studies for *in situ* production of lactic acid for demineralization. These natural bacteria depend on relatively expensive carbon sources such as glucose for growth and lactic acid production. On the other hand, yeasts can be considered as alternative cell factories to lactic acid bacteria for lactic acid production, despite not being natural producers, since they can better tolerate acidic environments. An engineered *Saccharomyces cerevisiae* strain has been developed by Prof. Bernd Nidetzky's research group which can convert the main lignocellulosic sugars, a cost-effective sustainable feedstock, into lactic acid (7).

### Enzymatic conversion of chitin to chitosan using chitin deacetylase

Chitin deacetylase catalyzes the hydrolysis of *N*-acetamido bonds in chitin to produce chitosan. The presence of this enzyme activity has been reported in several fungi and insect species. The fungal enzymes, which exhibit a very strong specificity for  $\beta$ -(1-4)-linked *N*-acetyl-D-glucosamine polymers, are well-studied enzymes for deacetylation of chitin. However, these enzymes are not effective on crystalline chitin because of the high degree of acetylation in chitin which limits the accessibility of the acetyl groups to the enzyme. Therefore the pretreatment of chitin substrate to modify the crystalline morphology by partial deacetylation is necessary to enhance the deacetylation yield.

## acib-Offer

- Process development for demineralization.
- Integration of demineralization (*in situ* microbial lactic acid production) and deproteinization (using commercial proteases) for high quality chitin separation from shrimp shell.
- Development of a method to reduce the crystallinity of chitin.
- Enzymatic deacetylation of chitin to make chitosan using heterologously expressed chitin deacetylase enzymes from different sources.

## References

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