Studies on Utilization of Chitinous Biomass by Using Microbial Functions

Nopakarn Rattanakit

Chitin, homopolymer of *N* -acetyl-D-glucosamine (GlcNAc), is the second most abundant polysaccharide on the earth, and crustaceous waste has been expected as a renewable resource. However, because of lack of feasible method for its utilization, most of the wastes are disposed by ocean dumping, incineration and land filling, causing the secondary environmental pollutions. Such disposal methods are irrelevant in respect to recent environmental pollution and resource depletion, prompting to design an alternative method for disposal and/or utilization of the wastes. The method is ideally to be easy and simple considering chemical characteristics of the wastes as well as regional circumstances of the local areas, where the chitinous wastes occur.

The present study was undertaken to reveal fundamentals for a simple process to utilize chitinous shellfish waste. The purpose of the study is to improve and/or preserve environment quality by removing the shellfish wastes, which are transformed to new resources with additional value.

Chapter 1 revealed that the shellfish waste was usable as a substrate for the solid-state medium of a chitin-assimilating filamentous fungus, *Aspergillus* sp. S13, and that the organism formed large amounts of chitinase(s) in the solid-state culture. Solid-state cultivation has been practiced successfully for centuries in oriental countries with various agricultural materials and filamentous fungi, and has the advantages of requiring relatively simple fermentation equipment, saving energy and generating little effluent. These features meet requirements for an alternative easy method, which described above.

Chapter 2 describes saccharification of chitin in a suspension (mash) of the solid-state culture of *Aspergillus* sp. S1-13 using shellfish waste, which was treated preliminarily with lactic acid. The solid-state culture was a source of chitin and chitinase(s). Under optimum conditions, at least 33 % of hydrolysis was achieved of the initial chitin in the shellfish waste used for the solid-state medium. A notable finding was obtained on the variations of the amounts of saccharification and chitinases in the solid-state culture, which is discussed in Chapter 4 investigating chitinases formed by the organism. In this Chapter, saccharification of chitin by the solid-state culture of filamentous fungi for food processing was also investigated.

Chapter 3 deals with conversion of the saccharification of chitin to lactic acid fermentation by inoculating *N* -GlcNAc-assimilating lactic acid bacterium to the solid-state culture mash: two types of double fermentation were performed. The results suggested development of various fermentation processes with chitin as a carbon source. A notable finding in the double fermentation, an enhancement of the chitin-saccharification in the mash where the lactic acid fermentation proceeded, was discussed in Chapter 4. The enzymatic mechanism was also investigated of lactic acid fermentation with *N* -GlcNAc as a carbon source.

Chapter 4 characterized chitinases of *Aspergillus* sp. S1-13 for understanding the observations in the saccharification by the solid-state culture mash, and for improving the saccharification efficiency. The organism formed mainly an endochitinase (Endo-1) and an exochitinase (Exo). Their combined reaction could not explain all the observations in the saccharifiction in the mash. Several experiments demonstrated occurrence of another endochitinase, Endo-2, which degrade chitin in an adsorbed state to the substrate. The reaction using Endo-2 together with Endo-1 and Exo could explain the observations in the mash. The role of

the enzymes in the saccharification was discussed, and suggestions were obtained for improving the saccharification efficiency.