Effects of Immobilization Time and pH Adjustment on the Immobilization of Nitrite-oxidizing Bacteria on Chitosan

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***Abstract* — Influence of immobilization time and pH adjustment on the surface of chitosan on the immobilization of nitrite-oxidizing bacteria (NOB) was investigated. The results indicated that immobilization of NOB on chitosan surface depends on immobilization time and chitosan surface modification by pH adjustment. The amount of NOB attached on chitosan surface increased with increasing immobilization time. Immobilization of chitosan with NOB for 24 hr and surface adjustment with buffer pH between 5.5 and 6.5 appeared as an optimal condition.**

Keywords: Chitosan; Immobilization; Nitrite-oxidizing bacteria; pH adjustment

I. INTRODUCTION

Outdoor-lining pond is now widely used in aquacultures because it prevents water leakage and eases of pond cleaning. A common problem associated with lining ponds is an accumulation of nitrogenous compounds especially ammonia and nitrite, which are harmful toward aquatic animals. To overcome this problem, water exchange is needed, leading to higher production cost and increasing risk of pathogen outbreak.

Biofiltration integrating with nitrification process is effective for nitrogen treatment in recirculating aquaculture systems. Nitrification is responsible by two groups of autotrophic bacteria namely ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), both of which possess slow growth rate and are sensitive to changing environment. Both bacterial populations naturally exist in culture water but are easily washed out by the frequent water exchange during the production cycle [1]. Microbial immobilization on the surface of certain media was suggested as means to retain slow-growing nitrifiers and consequently improving water quality in aquaculture systems [2]. Conventional materials for immobilized media are plastic or artificial fiber, which are difficult to degrade and may pose environmental concern during their disposal. Therefore, the use of natural materials may be a good alternative to avoid those problems.

Chitosan is a naturally occurring polymer, which can be extracted from shrimp biowaste. From the distinctive characteristics of chitosan including biodegradability, high positive charges and environmental friendly character, chitosan should be attractive for the treatment of wastewater from aquacultures [3, 4]. Literature review indicated that limited research had focused on parameters affecting the immobilization of nitrifiers on chitosan. Therefore, this study aims to evaluate the effects of immobilization time and pH adjustment on the ability of chitosan surface to mediate the nitrite removal.

II. MATERIALS AND METHODS

A. Chitosan

Chitosan with degree of deacetylation of 90% and molecular weight of 4.1×10^5 dalton was provided by A.N. Laboratory Co., Ltd., Samutprakan, Thailand. The size of chitosan was about 1-2 mm.

B. Nitrifying bacteria

Nitrifying bacteria used in this study was the mix-culture collected from indoor recirculating tanks for shrimp cultivation (30 ppt salinity) at the Center of Excellence for Marine Biotechnology, Chulalongkorn University. Nitrifying bacterial sample was incorporated with biofilm on BCN-009 polyethylene media (2H GmbH, Germany; diameter 10 mm; height 8 mm; specific surface area 864 m^2/m^3). Nitrifying bacteria are residing in biofilm, which is attached on surface of BCN-009. New biofilter media were disinfected with chlorine before acclimated with natural nitrifying bacterial population under specific condition favoring NOB growth (i.e., Sodium nitrite 10 mg NO_2-N/L; alkalinity 100 – 120 mg $CaCO_3$/L; pH = 7.5-8, Temperature = 25°C). Furthermore, oxygen was continuously supplied to the system by aeration through air stones.

C. *Immobilization of NOB on chitosan*

Twenty pieces of NOB immobilized BCN-009 media were transferred into a test tube containing 40 mL of sterilized nitrite broth and sonicated for 4 minutes. Thereafter, biofilter media were removed from the test tube and replaced with 0.60 g of sterilized chitosan flake (pH 6.5) under 150 rpm agitation in a rotary shaker at 25°C. Chitosan acclimation period was varied at 3, 6, 12 and 24 hr. Bacterial attachment on chitosan surface was confirmed using the Scanning Electron Microscope (SEM).

The effect of pH adjustment on NOB immobilization on chitosan was determined at pH 5.5, 6.5, and 7.5. It was conducted by soaking sterilized chitosan in buffer solution at the indicated pH for 2 minutes before proceeding with NOB immobilization mentioned earlier.

D. *Nitrite removal rate*

Nitrite removal experiment was conducted to determine the effect of immobilization time and pH adjustment on NOB immobilization on chitosan surface. Nitrite removal rate was determined by placing immobilized chitosan in 2.0 mg-N/L nitrite and 30 g/L NaCl solution. Nitrite concentration was measured according to Strickland and Parsons [5]. All tests was performed in triplicate. Analysis of variance (ANOVA) with further Duncan's multiple-range test was used to compare the differences between treatments.

III. Results and Discussion

The extent of nitrite reduction was clearly influenced by acclimation period of chitosan (Fig. 1). The maximum nitrite reduction rate was observed when chitosan was acclimated for 24 hr. At this condition (i.e., 24 hr acclimation), NOB immobilized on chitosan surface could remove almost 100% of nitrite within 6 hr. SEM results also indicated a larger number of bacteria on chitosan surface after 24 hr of incubation compared to other treatments (Fig. 2).

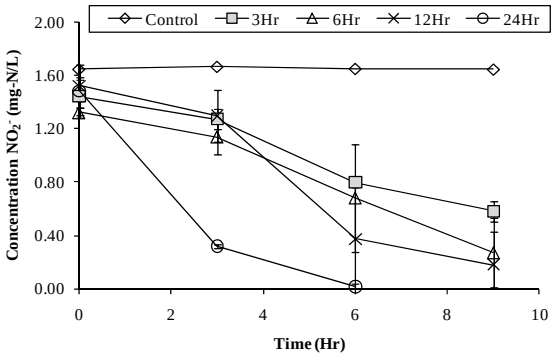
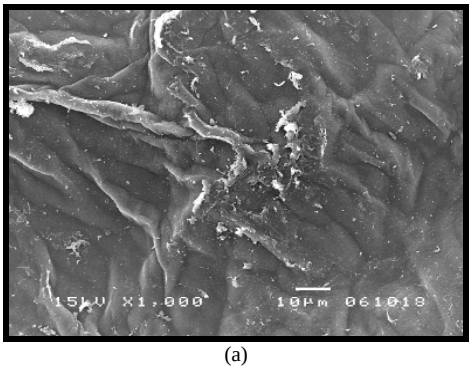
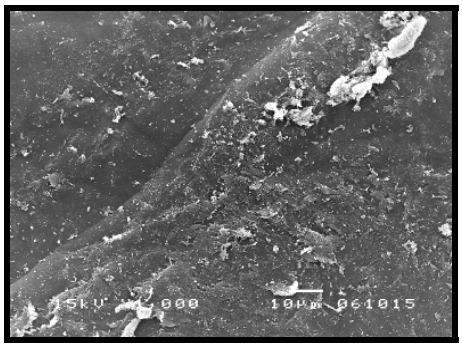


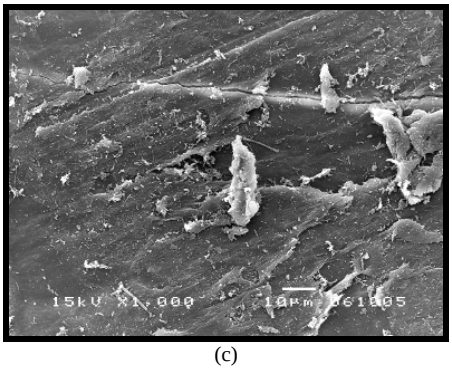
Figure 1. Nitrite concentrations after being treated with immobilized NOB on chitosan surface.



(a)



(b)



(c)

Figure 2. Scanning electron micrographs of bacteria immobilized on the surface of chitosan after incubation for (a) control, (b) 3 hr and (c) 24 hr.

The nitrite removal rate by NOB immobilized on chitosan surface increased significantly with incubation time (Fig 3).

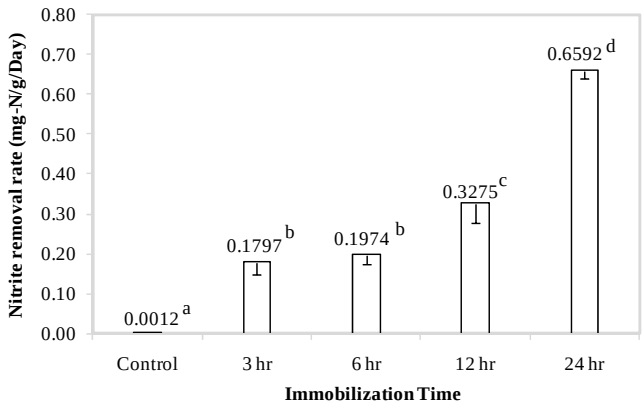


Figure 3. Nitrite removal rates of NOB immobilized chitosan as a function of immobilization time, the letter a, b and c indicate significant differences in statistics (P>0.05) of the data.

Fig. 4 indicates the extent of nitrite reduction as a function of pH adjustment of chitosan surface. The pH adjustment between 5.5 and 6.5 yielded a comparable nitrite reduction rate ranged from 0.3177 - 0.3498 mg-N/L/hr while increasing the pH to 7.5 reduced the nitrite reduction rate to 0.2382 mg-N/L/hr. This observation was probably due to the charge density of chitosan after pH adjustment [6]. With the pH less than 7.0 chitosan contains high cationic charges, which make an ionic bonding to anionic cell wall of bacteria.

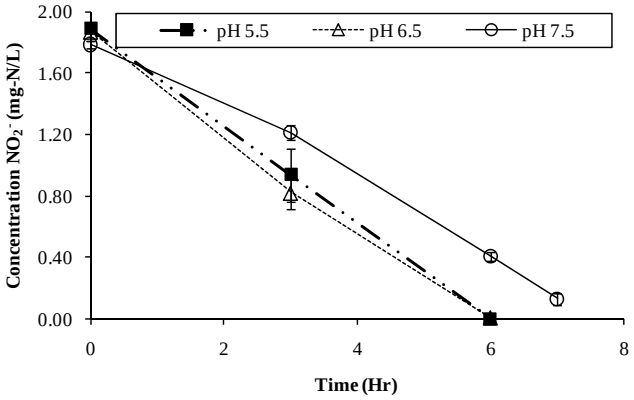


Figure 4. Nitrite concentration after being treated with immobilized NOB on chitosan surface at pH 5.5, 6.5 and 7.5.

IV. CONCLUSIONS

Chitosan can be employed to immobilize NOB. The extent of NOB immobilization on chitosan surface depends on immobilization time and pH adjustment on the surface of chitosan. In this experiment, immobilization time of 24 hr and surface adjustment with the pH between 5.5 and 6.5 yielded the maximum nitrite reduction rate.

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