Chapter 5

Future Directions

5.1. Prospective of the isolate HA1

Firstly, the new nitrite removal bacterium (nHA1) in isolate HA1 needs to be clear in morphology. FISH technique could be used to illustrate the shape of targeting bacterium. Therefore, nHA1 specific probe called nHA1 probe will be newly designed. As fluorescent *in situ* hybridization (FISH) is a technique allowing simultaneous visualization, identification, enumeration and localization of individual microbial cells, (Motor and Gobel, 2000), so the FISH experiment using multi-probe hybridization will indicate whether there is another bacterium beside the *Alcanivorax* sp. and nHA1. Moreover, the relative quantity of them will be shown.

A comprehensive understanding of the physiology of these organisms, and of the complex environmental processes in which they engage, will undoubtedly require their cultivation. Pure culture studies is needed because it could help in microbial ecology studies, to provide the bacteria population enriched under the same competition conditions that occur in the actual ecosystem. After the shape of nHA1 is known, the symbiosis of all bacteria in HA1 will be analyzed. The nHA1 pure culture can be obtained from liquid culture enrichments by end-point dilution and from solid medium enrichments by colony picking and subculture into liquid medium (Prosser *et al.*, 2002). In some studies, end-point dilution has been combined with enumeration by most probable number (MPN) technique, with isolation and identification of organisms from cultures at the highest dilutions exhibiting growth (Belser and Schmidt, 1978; Giuliano *et al.*, 1999; Fouratt *et al.*, 2003). The micromanipulation from the dilution method is the other option. In every case, purification is based on cell morphology and confirmed by FISH using nHA1 probe to check the purity and analyze the percentage of each bacterium in the sample.

After having the pure nHA1, do the comparative study between the pure nHA1 culture and the mixed culture (nHA1 + *Alcanivorax* sp. + other?). Compare their ability to grow on HNOB agar plates and to remove nitrite and nitrate in liquid culture. This experiment will give information about the possible symbiosis phenomenon. The mass balance experiment of a pure nHA1 or mixed culture (nHA1+ Alcanivorax sp. + other?), depending on which culture having a higher the nitrite and nitrate removal efficiency, will be chosen to demonstrate how the nitrate is removed. Normally, nitrate is reduced in anoxic condition, so if nitrate removal is confirmed in aerobic conditions, the process has to be investigated. In order to confirm the nitrate removal characteristics of *Alcanivorax* sp., the pure culture *Alcanivorax* sp. will be tested by culturing in the media containing nitrate. There are two questions need to be clear; firstly, is *Alcanivorax* sp. protected from oxygen by nHA1 and other by floc formation, in case the nitrate is removed in the anoxic; and other is why nitrate is removed by mix HA1 sample but not in the reactor.

The followed parameters have to be studied in order to study the physiology of HA1 (nHA1 or mix); the efficiency to remove nitrite (and nitrate?) by varying the different concentrations of nitrite (5 to 3000 mg per liter) and analyzing the speed of the process; check the autotrophic characteristic (strictly or facultative) to give an idea about the potential of HA1 for nitrite removal in waste water treatment which composed of a lot of organic nutrients; test the growth in different salinity (0 to 60 g/l); and test the effect of oxygen on HA1. The best parameters to produce HA1 in the large scale and the process to use it as an environmental remediation need to be found out.

5.2. Optimization for ammonia and nitrite removal

There are two choices for using biomass; mixed culture or pure culture. Nonetheless, both of them need to be optimized before application to environments. Yuan and Blackall (2002) proposed to add sludge population optimization as a new dimension to the control of biological wastewater treatment systems. They stressed that optimizing the microbial community

structure and property should be an explicit aim for the design and operation of wastewater treatment plant. Therefore, the nitrifying bacteria biomass both nHA1 and mixed culture from NFSBR, HNOBSBR, and LNOBSBR which obtained from this study are needed to be optimized in order to obtain microbiological data, specifically community structure, function and kinetic data which are the major limitations to sludge population optimization reported by the same authors.

Once the dominant nitrifying bacteria are identified in the SBRs system, the optimum condition for their preferential growth and increased ammonia removal are examined. The investigations into salinity level, ammonia, nitrite concentration limitation, pH, temperature, and oxygen are also carried out to define the optimum operating conditions. In addition, the effect of heterotrophic bacteria and organic are needed to undertake research to determine the optimum density and combination of organisms to maximize the wastewater treatment.

5.3. Application for an aquaculture

Nitrification takes place by two groups of autotrophic bacteria, *Nitrosomonas* spp and *Nitrobacter* spp., both of which comprise slow-growing species. Both groups exist naturally in aquaculture water, but are readily washed out by frequent water exchange during the production cycle thereby impairing their potential beneficial effect on water quality (Shan *et al.*, 2001). Thereafter, to find out how to apply them into shrimp aquaculture to control and improve the ammonia removal is desired. Currently, biological methods to use for control ammonia level in aquaculture pond are bioaugmentation and biological filter (biofilm). The example of the successful experiment using bioaugmentation is pellet immobilization of indigenous nitrifying bacteria which represents a potentially effective TAN control system for prawn aquaculture in low-cost, but intensive tropical prawn farms (Shan and Obbard, 2001). The examples of using biological filter aquaculture were reported by Abeysinghe *et al.* (1996); Ng *et al.* (1996); Sakairi *et al.* (1996); Chin and Ong

(1997); van Benthum *et al.* (1998); Siriraksophon *et al.* (1999) and Kim *et al.*, (2002).

Bioaugmentation involves the seeding of water-purifying bacteria into aquaculture systems. Many different terms can be used for description of the process of addition of specialized bacteria to the activated sludge process: seeding, bioaugmentation, bacterial augmentation, biomass enhancement and inoculum addition. The purpose of seeding is to improve the nitrogen removal process efficiency, nitrification process stability and to decrease the volume requirements of the process. If shrimp farm activity is to be enhanced by bioaugmentation with nitrifying bacteria, it follows that inoculum must consist of species that are suited to the shrimp farm environment. The right microbes should greatly enhance the success of the future bioaugmentation attempts. The obtained nitrifying bacteria from this study could be used as bioaugmentation as a tool to protect the structure and function of the nitrification reaction and to against a shock load in aquaculture. In addition, it might be applied in the industries as the same proposes with using in the aquaculture.

5.4. ChanyF and ChanyR primers application

ChanyF and ChanyR primers (novel NOB specific primer) could be used as indicator of the good nitrification reaction in the aquaculture ponds or in the industries by polymerase chain reaction (PCR) technique. Nevertheless, first of all, presence of novel NOB in the good nitrification system is needed to prove by PCR technique using these primers set. The identification of what the bacteria in the failure or success system are, it is the indicator before system is failed. Thus, if the novel NOB is proven to be found in the good system, the reduction in the system of them will shown that the system become worse. In the case of both naturally present- novel NOB system and optimized sludge (HA1 or biomass from all SBR) - added system, ChanyF and ChanyR primers could be used for a better prediction and enhance the process performance in the system regarding to the novel NOB was found in all sources.