

การศึกษาประชาคมแบคทีเรียในถังปฏิกรณ์ไนตริฟิเคชันและดีไนตริฟิเคชันของระบบหมุนเวียนน้ำทะเลสำหรับการเพาะเลี้ยงสัตว์น้ำ

Investigation of bacterial community in nitrification and denitrification reactors of the aquaculture recirculating seawater system

สุธาสินี อ่วมจันทร์¹, สรวิต เผ่าทองสุข², เอกวัล ลือพร้อมชัย³, ประเสริฐ ภูวสันต์⁴ และเปี่ยมศักดิ์ เมนะเสวต^{2,3,5}

Sutasinee Oumchan¹, Sorawit Powtongsook², Ekawan Luepromchai³, Prasert Pavasant⁴ and Piamsak Menasveta^{2,3,5}

¹Interdepartment of Environmental Science, Graduate School, Chulalongkorn University, Bangkok 10330; ²Marine

Biotechnology Research Unit (at Chulalongkorn University), National Center for Genetic Engineering and Biotechnology;

³Environmental Research Institute and National Research Center for Environmental and Hazardous Waste Management;

⁴Department of Chemical Engineering; ⁵Department of Marine Science, Chulalongkorn University, Bangkok 10330 e-mail: sorawit@biotec.or.th

บทคัดย่อ: การศึกษาแบคทีเรียในระบบบำบัดไนตริฟิเคชันและดีไนตริฟิเคชันสำหรับใช้ในระบบหมุนเวียนน้ำทะเลแบบปิดสำหรับการเพาะเลี้ยงสัตว์น้ำ ทำในถังปฏิกรณ์ชีวภาพที่เหมือนกัน 2 ถัง โดยภายในถังมีลูกบอลพลาสติก (bioball) เป็นวัสดุสำหรับให้แบคทีเรียยึดเกาะ และจัดสภาวะให้มีการพ่นอากาศในถังปฏิกรณ์ไนตริฟิเคชันและจัดสภาวะไร้ออกซิเจน โดยการเติมแก๊สไนโตรเจนในถังปฏิกรณ์ดีไนตริฟิเคชัน ผลการศึกษาพบว่าหลังจากการจัดสภาวะอย่างเหมาะสมจะทำให้แบคทีเรียในถังปฏิกรณ์ไนตริฟิเคชันสามารถลดปริมาณแอมโมเนียในน้ำ และแบคทีเรียในถังปฏิกรณ์ดีไนตริฟิเคชันสามารถลดปริมาณไนเตรทในน้ำได้อย่างมีประสิทธิภาพ เมื่อนำตัวอย่างลูกบอลพลาสติกจากถังปฏิกรณ์ทั้งสองมาสกัดดีเอ็นเอและศึกษาโดยเทคนิค PCR-DGGE ของ 16S rDNA พบว่าองค์ประกอบชนิดของประชาคมแบคทีเรียในถังปฏิกรณ์ทั้งสองเหมือนกัน โดยมีแบคทีเรียไม่น้อยกว่า 8 ชนิดและสามารถจำแนกชนิดแบคทีเรียจากการนำแถบ DGGE ไปหาลำดับเบสเปรียบเทียบกับฐานข้อมูล 16S rDNA ได้ 2 ชนิดคือ *Methylophaga marina* และ *Marinobacter sp.* แสดงให้เห็นว่า ปฏิกริยาไนตริฟิเคชันและดีไนตริฟิเคชันในระบบบำบัดสามารถเกิดได้จากประชาคมแบคทีเรียกลุ่มเดียวกัน โดยกิจกรรมที่เกิดขึ้นอยู่กับการจัดสภาวะแวดล้อมของระบบ โดยเฉพาะอย่างยิ่งปริมาณออกซิเจน แหล่งคาร์บอน และสารอาหารในน้ำ

Abstract: Bacterial community in nitrification and denitrification reactors (NR and DNR) for the closed recirculating seawater system for aquaculture was studied with two similar bioreactors packed with plastic bioballs. For NR, the reactor was aerated to create an aerobic condition for nitrifying bacteria. On the other hand, nitrogen gas was bubbled into DNR in order to induce anoxic condition for denitrifying bacteria. After proper condition was settled up, the results showed that both nitrifying bacteria in NR and denitrifying bacteria in DNR performed an effectively nitrogen conversion. DNA extraction from bioball samples following by PCR-DGGE analysis of 16S rDNA illustrated that bacterial community in both NR and DNR was similar and consisted of at least 8 species. Bacterial identification by 16S rDNA sequencing indicated that two out of eight species were *Methylophaga marin.* and *Marinobacter sp.*. In conclusion, the study suggested that nitrification and denitrification processes in the nitrogen treatment system could be performed by similar bacterial population, in which their activities would depended on environmental factors especially oxygen concentration, carbon source and nutrients in the water.

Introduction: Biological nitrate removal (BNR) with bacterial biofilter is one of the main component in the closed recirculating seawater system for aquaculture. Unlike industrial wastewater treatment with high nitrogen concentrations, aquaculture BNR must withstand a low nitrogen concentration (less than 10 mg-N/L) which is suitable for BNR rather than chemical treatment. Recently, most studies on BNR in aquaculture tanks have focused on the nitrifying consortia while very few reports were published with denitrifying bacteria. Previous study of Tal *et al.* (2003) indicated that the nitrifying bacteria (*Nitrospira* and *Nitrosomonas*) and the denitrifying bacterium (*Pseudomonas*) were associate with nitrogen transformation process in the closed mariculture systems. On the other hand, PCR-DGGE study of bacterial consortia found in a marine methanol-fed denitrification reactor at the Montreal Biodome were

related with the genus *Methylophaga* (Labbe *et al.*, 2003). This study aims to investigate the bacterial community in the nitrification and denitrification biofilter used in shrimp culture tanks under laboratory condition. Dominant bacterial species were identified using PCR-DGGE analysis of 16S rDNA.

Methodology: Photographs of nitrification reactor (NR) and denitrification reactor (DNR) are shown in



Fig. 1 Denitrification reactor (left) and nitrification reactor (right)

Figure 1. The reactors were made of acrylic plastic tube packed with 700 plastic bioballs (Super Bioball™) and 5L of 30 psu seawater from shrimp culture tank. For NR, aerobic condition was provided by continuous aeration and 2-6 mgNH₄-N/L (as NH₄Cl) was added as a sole nitrogen source. For DNR, nitrogen gas was bubbled into an enclosed reactor in order to produce an anaerobic condition and 50 mgN/L (as NaNO₃) was used as the nitrogen source. Carbon and other nutrients source for DNR were methanol and sterilized shrimp feed extracted. During the experiments, water in the reactors was changed after ammonium (NR) or nitrate (DNR) was completely removed. Decrease in ammonia (NH₄⁺) or

nitrate (NO₃⁻) concentrations was used as an indicator for nitrification and denitrification processes in the reactors. Water samples were collected from the sampling ports of each reactor every day and kept refrigerated prior to analysis. Ammonia was determined by the phenol hypochlorite reaction, nitrite was determined by reaction with sulfanilamide according to Strickland and Parsons (1972). Nitrate was measured using UV screening method according to Standard Methods for the Examination of Water and Wastewater (APHA, 1992). Bacterial cells were collected from NR and DNR at day 57 in which the decrease of ammonia in NR and the decrease of nitrate in DNR were detected. This confirmed that, at the time of bioball sampling, NR was performing nitrification and DNR was performing denitrification processes. Biofilm from ten bioballs were stripped by 5 minutes sonication and resuspended in sterilized seawater. After centrifugation, DNA in the bacterial pellet was extracted with Fast SPIN KIT (Bio system). 16S rDNA was amplified by PCR using GC clamp primer PRBA 338f and primer PRUN 518r (Øvreås *et al.*, 1997) with PCR program applied from Tal *et al.* (2003). Denaturing gradient gel electrophoresis (DGGE) was performed with D-Code Universal Mutation Detection System (Biorad) using 8% polyacrylamide gel and 25-60% gradient of urea denaturant. After electrophoresis, two of the high intensity DGGE bands were excised, reamplified, and sequenced. Bacterial identification was analyzed using BLASTIN program (National Center for Biotechnology Information). In addition, scanning electron microscope (SEM) was used for bacteria morphological study on the surface of plastic bioball.

Results: Figures 2 and 3 illustrate inorganic nitrogen concentrations in NR and DNR respectively. It was found that ammonium added into NR through water exchange was rapidly converted into nitrate (Figure 2). This indicated the activity of nitrification process in the reactor. During day 20 to 30 that was 10 days after the first water exchanged, total inorganic nitrogen was found slightly decrease. This might due to nitrogen accumulation in bacterial cells. For DNR (Figure 3), denitrification was detected after day 42 when nitrogen gas bubbling, methanol and sterilized shrimp feed extract were added into the DNR. This made anoxic condition and nutrients supply for denitrifiers therefore the complete denitrification process without nitrite accumulation was finally achieved. Nitrate concentration in day 54, just after the second water exchange, was rapidly decreased from 50 mg-N/L to less than 10 mg-N/L within one day.

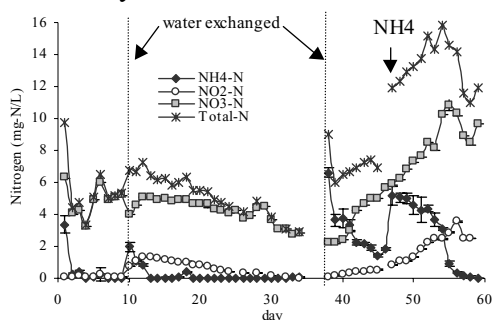


Fig 2 Inorganic nitrogen concentrations in NR

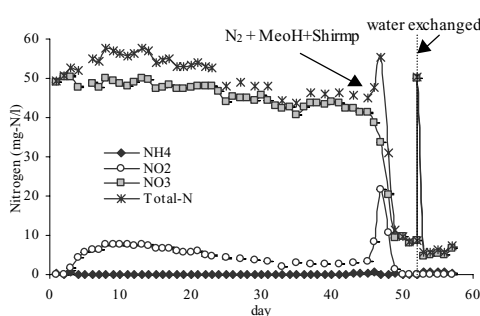


Fig. 3 Inorganic nitrogen concentrations in DNR

PCR-DGGE profile of 16S rDNA collected in day 57 (Figure 4) showed that bacterial community in biofilm from NR and DNR were similar. This was confirmed by SEM in Figure 5 in which the dominant bacterial species in both reactors composed of a mix population of filamentous and rod shape bacteria. There was at least 8 bacterial species as estimated by 8 distinct DGGE bands on the gel. DNA sequencing of two DGGE bands (a and b in Figure 4) showed that band a was possibly *Methylophaga marina* (98% similarity) and band b was possibly *Marinobacter* sp. (Proteobacteria).

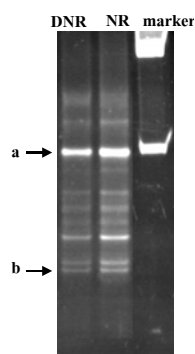


Fig 4 DGGE profile illustrates bacterial community of NR and DNR.

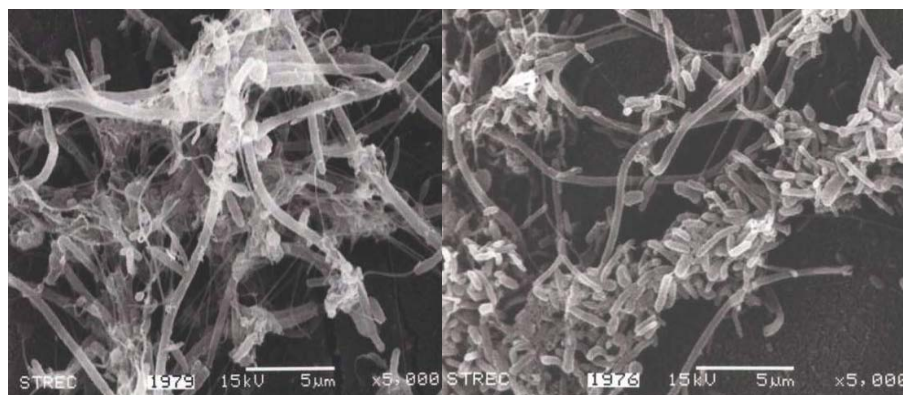


Fig. 5 SEM of bacterial biofilm attached on the plastic bioball in NR (left) and DNR (right)

Discussion: PCR-DGGE analysis showed the similar bacterial population in both NR and DNR. The *Methylophaga marina* (obligate aerobic bacteria) and *Marinobacter* sp. (denitrifying bacteria) were dominant in both reactors. In spite of the fact that only two bacterial species were identified in this study, these two dominant species were not the common bacteria that could be isolated, and identified using the classical bacterial identification technique. Hovanec *et al.* (1998) suggested that the nitrifying bacteria in nitrification bioreactor might not relate with the well-known *Nitrosomonas/Nitrobacter* species. They found that *Nitrospira* was the primary species for nitrite conversion rather than *Nitrobacter*. Our results agreed with Hovanec *et al.* (1998), therefore, more investigation is needed to understand the role of bacterial community in biological nitrogen removal systems, especially in seawater environment. Moreover, although the reactors were operated for more than 50 days in different conditions (*e.g.* aerobic for NR and anoxic for DNR), the bacterial community was unchanged and the same bacterial population are suggested to perform both nitrification and denitrification processes depended on oxygen concentration, carbon source and nutrients availability.

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Keywords: nitrification, denitrification, bacterial community, DGGE, aquaculture recirculating seawater