

## Effect of Carbon Sources and Carbon/Nitrogen Ratio on Nitrate Removal in Aquaculture Denitrification Tank

Cholticha Playchoom, Wiboonluk Pungrasmi  
Department of Environmental Engineering, Faculty of  
Engineering, Chulalongkorn University,  
Bangkok 10330, Thailand.  
e-mail: cholticha\_ply@hotmail.com,  
wiboonluk.p@chula.ac.th

Sorawit Powtongsook<sup>\*,\*\*</sup>

<sup>\*</sup>National Center for Genetic Engineering and  
Biotechnology, Thailand Science Park, 113 Paholyothin  
Road, Klong Luang, Pathum Thani 12120, Thailand.

<sup>\*\*</sup>Center of Excellence for Marine Biotechnology,  
Department of Marine Science, Chulalongkorn University,  
Bangkok 10330, Thailand.

Corresponding author: sorawit@biotec.or.th

**Abstract**— This study evaluated effect of organic carbon sources and concentrations on nitrate removal in denitrification tanks specially designed for recirculating aquaculture system. The experimental units consisted of glass tank containing 5 cm depth of pumice rock at the bottom and 8 L of water. Effect of organic carbon sources was studied using methanol or molasses addition to treatment tanks against control tanks without organic carbon addition. The carbon to nitrogen ratio was provided by adding methanol or molasses at COD:NO<sub>3</sub><sup>-</sup>-N ratio of 5:1 and denitrification rates were calculated using Michaelis-Menten kinetics equation. The results showed that maximum denitrification rate of methanol or molasses were 4,531.3±186.1 and 4,094.8±254.4 mg-N/m<sup>2</sup>/day, respectively. However, the molasses addition tanks had higher risk of ammonia accumulation and hydrogen sulfide production, hence methanol was selected as the appropriate carbon source. Evaluation of the optimum COD:NO<sub>3</sub><sup>-</sup>-N ratio between 3:1 to 6:1 illustrated that higher denitrification rate was obtained with high methanol addition in which the maximum denitrification rate increased from 2,334 to 7,529 mg-N/m<sup>2</sup>/day.

**Keywords**- denitrification rate; organic carbon; nitrate

### I. INTRODUCTION

The advantageous of Recirculating Aquaculture Systems (RAS) is due to its capability to cultivate high fish density with minimum effluent discharge [1]. During operation, RAS must withstand large amount of waste, especially toxic nitrogenous compounds *i.e.* ammonia and nitrite which are continuously produced by fish excretion and organic waste decomposition. The most capable nitrogen treatment process in the RAS is nitrification in which ammonia is oxidized to nitrite and nitrate respectively. Although nitrification can prolong water exchange but accumulation of nitrate is generally found and water exchange is therefore recommended when nitrate reach 50 mg-N/L. Discharge of high nitrate wastewater directly to the environment can probably cause impact such as eutrophication in natural water bodies [2].

Denitrification process is an efficient process for nitrogen removal from aquaculture. Denitrification occurs in natural anaerobic sediment in the aquaculture pond but addition of organic carbon can enhance denitrification rate [3]. Methanol, ethanol and acetic acid are organic carbon sources widely used for enhancing denitrification processes in organic carbon-limited wastewaters, sludge and soil [4, 5, 6]. The mostly applied organic carbon is methanol which has high reducing power and low oxidation state. Apart from that, another consideration is the cost which is a major part of total wastewater treatment expense. With aquaculture, the nitrate removal process via anaerobic denitrification process has been implemented in [7, 8] but not yet accomplished in commercial scale.

Our previous study [9] illustrated the possibility of denitrification tank using pumice rock as filtration material coupling with organic carbon addition. It was found that pumice rock denitrification tank had high denitrification rate (3,906±36 mg-N/m<sup>2</sup>/day) and low risk of hydrogen sulfide production. Moreover pumice rock had low weight to volume than other materials such as natural aquaculture pond soil, sand, and vermiculite.

This study evaluated the effect of organic carbon sources and concentrations on nitrate removal in pumice rock denitrification tank under laboratory condition. This leads to the future development of the low-cost RAS with nitrate treatment.

### II. MATERIALS AND METHODS

#### A. Denitrification tank

Glass tanks with a dimension of 20 x 20 x 35 cm<sup>3</sup> (surface area at 0.04 m<sup>2</sup>) were used as a denitrification tank in this experiment (Figure 1). Pumice rock were cleaned, dried and sieved to 1-3 mm particle size before packed at the bottom of the glass tank with 5 cm layer thickness. Continuous aeration in the denitrification tank was provided by an airstone and water in the tank was mixed using a small aquarium pump hence the dissolved oxygen (DO) in the water was maintained at higher than 2.0 mg/L. Oxidation-reduction

potential (ORP) in the water and at 2.5 cm depth of the media layer was monitored with daily basis.

At the beginning, 8 L of fresh water containing 100 mg-N/L  $\text{KNO}_3$  was added into the tanks. Thereafter, during the experiment, water was exchanged with freshwater containing 100 mg-N/L when nitrate concentration in the denitrification tanks was lower than 5 mg-N/L.

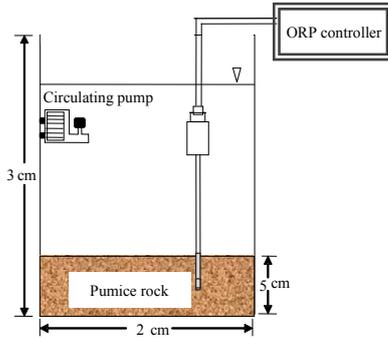


Figure 1. Side view illustration of denitrification tank in this study

### B. Experiment I: Effect of organic carbon sources

The experiment consisted of control (without carbon addition) and treatments with methanol or molasses were added at COD: $\text{NO}_3^-$ -N ratio of 5:1. To obtain physical characteristics of molasses which is the byproduct of sugar processing, Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), and pH were analyzed.

### C. Experiment II: Effect of C/N ratio

The experiment consisted of control (without carbon addition) and treatments with methanol addition various COD: $\text{NO}_3^-$ -N ratio of 3:1, 4:1, 5:1 and 6:1.

### D. Water quality analysis

Ammonia-N and nitrite-N were analyzed using the colorimetric method [10] and nitrate-N was measured with the ultraviolet spectrophotometric screening method [11]. Alkalinity was measured based on titration. Monitoring of pH, temperature and dissolved oxygen was performed using pH meter (HANNA HI98240), thermometer and DO meter (HANNA HI964400), respectively. Oxidation-reduction potential (ORP) was monitored daily with ORP probe (HANNA HI3010) at 2.5 cm depth in the pumice layer and in the middle of water column. Residual methanol in the outlet water was analyzed using the COD closed reflux method.

### E. Denitrification Rate

Denitrification rate was expressed using Michaelis-Menten kinetics (equation 1) between nitrate concentration (S) and denitrification rate (V). A linear regression between S and S/V was used for the calculation of maximum denitrification rate ( $V_{\max}$  or  $\text{DNR}_{\max}$ ) in which  $V_{\max} = 1/\text{slope}$ .

The half-maximum constant ( $K_m$ ) was the X-axis intersection on the graph [12].

$$V = \frac{V_{\max} \cdot S}{K_m + S} \quad (1)$$

For statistical analysis, analysis of variance (ANOVA) with further Duncan's multiple range test was used to compare differences among treatment groups.

## III. RESULTS AND DISCUSSION

Chemical properties analysis in Table I illustrated that molasses had high in COD and nitrogen content. With carbon content calculation, addition of carbon source into each denitrification tank was 3.4 ml or 5.61 g for methanol or molasses, respectively.

TABLE I. CHEMICAL AND PHYSICAL PROPERTIES OF METHANOL AND MOLASSES

Parameters	Methanol	Molasses
Physical characteristics	Colorless liquid	Dark brown, Viscous liquid
COD (mg/L)	1.5 g COD/g MeOH <sup>a</sup>	993,549
TKN (mg/L)	-	11,456
pH	6.84	6.70
Amount required for COD: $\text{NO}_3^-$ -N ratio of 5:1 (per tank)	3.4 ml	5.61 g

a. COD was calculated based on the theoretical values of methanol.

At initial, the denitrification tanks were acclimated for 20 days before starting the experiment. It was found that, after carbon addition at COD: $\text{NO}_3^-$ -N of 5:1 in day 20, nitrate removal in both treatments were found (Figure 2) in which nitrate concentration was reduced to  $6.26 \pm 0.6$  or  $28.8 \pm 4.1$  mg-N/L for methanol or molasses addition, respectively. On the other hand, it was clearly that denitrification was not occurred in control tanks without external carbon addition.

As shown in Table II, calculation of maximum denitrification rate ( $\text{DNR}_{\max}$ ) using Michaelis-Menten kinetics equation showed that  $\text{DNR}_{\max}$  of methanol and molasses supplement were  $4,531.3 \pm 186.1$  and  $4,094.8 \pm 254.4$  mg-N/ $\text{m}^2$ /day, respectively. Nitrate concentration in the outlet water of treatment tanks had low nitrate concentration but small amount of nitrite (0.1-1.8 mg-N/L) was found as nitrite is the intermediate product of the denitrification process [13, 14].

TABLE II. THE AVERAGE WATER QUALITY PARAMETERS IN CONTROL TANK AND TREATMENT TANKS WITH METHANOL OR MOLASSES ADDITION

	Control	Methanol	Molasses
<b>Max. denitrification rate</b>			
- mg-N/ $\text{m}^2$ /day	-	$4,531.3 \pm 186.1$	$4,094.8 \pm 254.4$
- mg-N/L of packing in pumice/day	-	$90.6 \pm 3.7$	$81.9 \pm 5.1$

<b>Water quality</b>			
- Amonia (mg-N/L)	0.0 <sup>a</sup>	0.0 <sup>a</sup>	5.1±0.6 <sup>b</sup>
- Nitrite (mg-N/L)	0.0 <sup>a</sup>	0.1±0.0 <sup>a</sup>	1.8±0.5 <sup>b</sup>
- Nitrate (mg-N/L)	104.5±2.4 <sup>c</sup>	6.2±0.6 <sup>a</sup>	28.8±4.1 <sup>b</sup>
- Alkalinity (mg/L as CaCO <sub>3</sub> )	116.4±35.3 <sup>a</sup>	331.3±90.2 <sup>c</sup>	248.3±73.7 <sup>b</sup>
- pH	7.6±0.2 <sup>a</sup>	8.4±0.3 <sup>b</sup>	7.82±0.5 <sup>a</sup>
- ORP in water (mV)	186.7±8.4	190.6±9.0	191.5±9.2
- ORP in pumice layer (mV)	178.1±8.8 <sup>c</sup>	-217.9±26.3 <sup>b</sup>	-274.3±37.4 <sup>a</sup>

a. to c. in the same row indicates significant different (P<0.05).

Results from Figure 2-C showed that higher ammonia concentration (5.1±0.6 mg-N/L) was found in the outlet water of molasses treatment. The higher average ammonia concentration was probably due high initial TKN value in molasses (11,456 mg/L). Furthermore, it was found that molasses addition could highly risk in oxygen depletion, as well as the occurrence of sulfate reduction to odorous and toxic hydrogen sulfide. As illustrated in Figure 3, ORP of water phase in denitrification tank was about 200 mV which indicated the sufficient amount of oxygen for aerobic condition. While in the material phase, the ORP value was below -100 mV, clearly confirmed for the anoxic or anaerobic conditions. ORP in the pumice rock media layer of molasses tanks were between -210 to -345 mV which were lower than that of methanol system which was between -160 to -260 mV. In general, complete denitrification process resulting the ORP value at less than -200 mV but hydrogen sulfide (H<sub>2</sub>S) from sulfate reduction process was detected when ORP decreased below -300 mV [15]. The occurrence of toxic hydrogen sulfide results in a lower pH and alkalinity value, which was opposite from that taken place in complete denitrification process [16]. With these reasons, methanol was hence selected for further experiment.

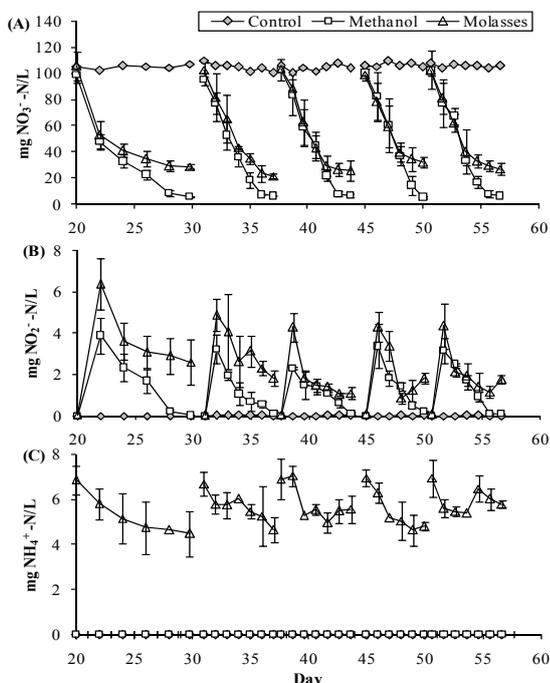


Figure 2. Nitrate (A), nitrite (B) and ammonia (C) in treatment tanks (methanol or molasses addition) and control tanks (without organic carbon addition). Nitrate addition was repeated 5 times during the experiment.

The second experiment evaluated the denitrification rate of pumice rock denitrification tank with methanol addition at COD-NO<sub>3</sub><sup>-</sup>-N ratio between 3:1, 4:1, 5:1 and 6:1. The result in Figure 4 showed that higher methanol concentration could significantly increase denitrification rate. Calculation of maximum denitrification rate in Table III revealed that DNR<sub>max</sub> was increased from 2,334 to 7,529 mg-N/m<sup>2</sup>/day when COD-NO<sub>3</sub><sup>-</sup>-N ratio was increased from 3:1 to 6:1. Nitrite and ammonia analysis showed low concentration of these two compounds compared to nitrate.

Increase of alkalinity in treatment tanks was in proportion to the denitrification rate. This was due to an accumulation of bicarbonate (HCO<sub>3</sub><sup>-</sup>) [17]. Nevertheless, organic addition at high COD:NO<sub>3</sub><sup>-</sup>-N ratio e.g. 6:1 or higher would possibly resulted in excess methanol in the water. Results from COD analysis in Table III illustrated that COD in outlet water was detected only in treatment with 6:1 methanol addition. Hence the COD:NO<sub>3</sub><sup>-</sup>-N ratio of less than 5:1 was recommended to prevent methanol residue in the outlet water because high concentration of methanol could affect growth, maturity index and fecundity of fish [18].

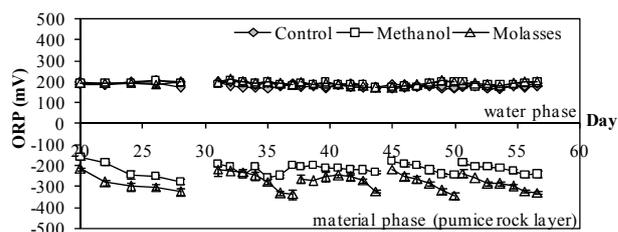


Figure 3. ORP in denitrification tanks of control (without organic carbon), and treatment tanks (with methanol and molasses addition)

TABLE III. COD OF EFFLUENT WATER AND MAXIMUM DENITRIFICATION RATE (DNR<sub>max</sub>) OF PUMICE ROCK DENITRIFICATION TANK SUPPLEMENTED WITH METHANOL AT VARIOUS COD:NO<sub>3</sub><sup>-</sup>-N RATIO

COD:NO <sub>3</sub> <sup>-</sup> -N	mg-N/m <sup>2</sup> /day DNR <sub>max</sub> -Methanol	COD (mg/L)
3:1	2,333.9±55.3	ND.
4:1	3,641.1±233.3	ND.
5:1	4,604.9±185.6	ND.
6:1	7,528.6±794.6	63.0±15.6

ND. = Not Detectable.

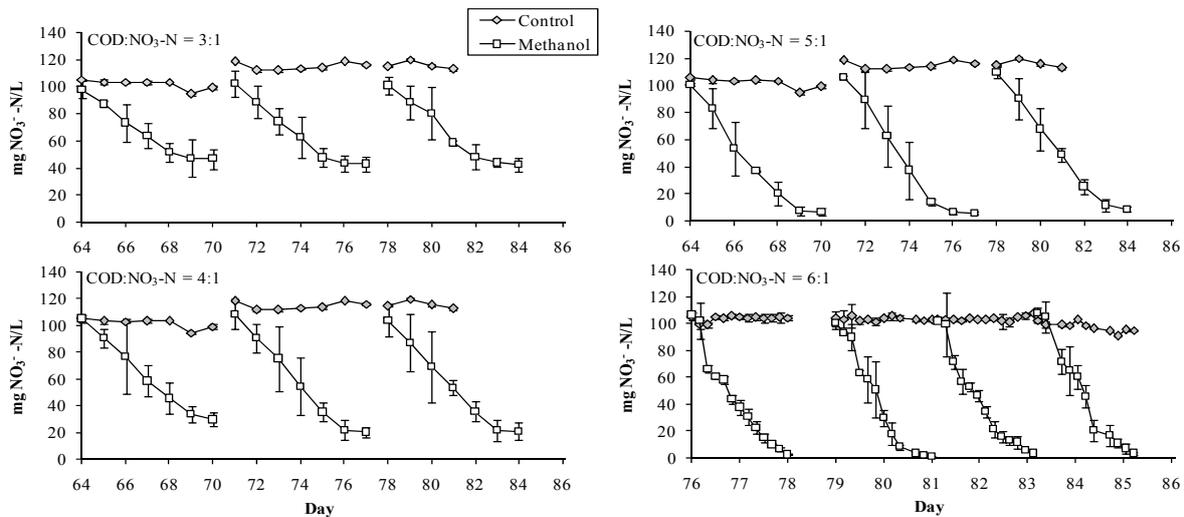


Figure 4. Concentrations of nitrate in the denitrification tanks of control (without carbon addition) and methanol addition treatment tanks at various COD:NO<sub>3</sub><sup>-</sup>-N ratio from 3:1 to 6:1.

#### IV. CONCLUSIONS

Organic carbon addition in either methanol or molasses could accelerate denitrification rate in the pumice rock denitrification tanks under laboratory condition. However, methanol is more suitable for aquaculture application since molasses could cause problems regarding darken water color, ammonia accumulation, as well as the risk of toxic hydrogen sulfide production. With methanol, addition at COD:NO<sub>3</sub><sup>-</sup>-N ratio between 3:1 to 6:1, denitrification rate would increased from 2,334 to 7,529 mg-N/m<sup>2</sup>/day.

#### ACKNOWLEDGMENT

This research was supported by The National Research Universities Fund, received a partially financial supported by the graduate thesis grant, Chulalongkorn University and National Research Council of Thailand. All the equipments and facilities in this research were provided by the Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University, Thailand.

#### REFERENCES

- [1] H.J. Hamlin, J.T. Michaels, C.M. Beaulaton, W.F. Graham, W. Dutt, P. Steinbach, T.M. Losordo, K.K. Schrader and K.L. Main, "Comparing denitrification rates and carbon sources in commercial scale upflow denitrification biological filters in aquaculture," *Aquacultural Engineering*, vol. 38, April 2008, pp. 79-22, doi:10.1016/j.aquaeng.2007.11.003.
- [2] M.T. Gutierrez-Wing, and R.F. Malone, "Biological filters in aquaculture: trends and research direction for fresh water and marine applications," *Aquaculture Engineering*, vol. 34, Issue 3, May 2006, pp. 163-171, doi:10.1016/j.aquaeng.2005.08.003.
- [3] R. Crab, Y. Avnimelech, T. Defoirdt, P. Bossier and W. Verstraete, "Nitrogen removal techniques in aquaculture for a sustainable production," *Aquaculture*, vol. 270, Issue 1-4, Sep. 2007, pp. 1-14, doi:10.1016/j.aquaculture.2007.05.006.
- [4] J.J. Her and J.S. Huang, "Influences of carbon source and C/N ratio on nitrate/nitrite denitrification and carbon breakthrough," *Bioresource Technology*, vol. 54, Issue 1, 1995, pp.45-51, doi:10.1016/0960-8524(95)00113-1.
- [5] K. Bernat and I. Wojnowska-Baryła, "Carbon source in aerobic denitrification," *Biochemical Engineering Journal*, vol. 36, Issue 2, Sep. 2007, pp.116-122, doi:10.1016/j.bej.2007.02.007.
- [6] P.J. Murray, D.J. Hatcher, E.R. Dixon, R.J. Stevens, R.J. Laughlin and S.C. Jarvis, "Denitrification potential in a grassland subsoil: effect of carbon substrates," *Soil Biology and Biochemistry*, vol. 36, Issue 3, Mar. 2004, pp. 545-547, doi:10.1016/j.soilbio.2003.10.020.
- [7] M.J. McCarthy and W.S. Gardner, "An application of membrane inlet mass spectrometry to measure denitrification in a recirculating mariculture system," *Aquaculture*, vol. 218, Issue 1-4, Mar. 2003, pp. 341-355, doi:10.1016/S0044-8486(02)00581-1.
- [8] W.B. Sailing, P.W. Westerman and T.M. Losordo, "Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations," *Aquacultural Engineering*, vol. 37, Nov. 2007, pp. 222-233. doi:10.1016/j.aquaeng.2007.06.003.
- [9] C. Playchoom, W. Pungrasmi and S. Powtongsook, "Nitrate Removal in Recirculating Aquaculture System Using Denitrification Tank with Artificial Soil Layer," *Proc. 16th AAS and 1st ISAT Symp. Sufficiency Agriculture*, Bangkok, Thailand, Aug. 25-27, 2010, pp. 160-163.
- [10] J.D.H. Strickland and T.R. Parsons. *A practical handbook of seawater analysis*, 2nd ed., Ottawa: Fisheries research board of Canada, 1972.
- [11] APHA. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. APHA, AWWA and WPCF. Washington, D.C. 2005.
- [12] P.J. Harrison. *Determining phosphate uptake rate of phytoplankton*. In Lobban C.S. et al. *Experimental Phycology a Laboratory Manual*: Cambridge University Press. 1988.
- [13] J. van Rijn, Y. Tal and H.J. Schreier, "Denitrification in recirculating systems: Theory and applications," *Aquacultural Engineering*, vol. 34, Issue 3, May 2006, pp. 364-376, doi:10.1016/j.aquaeng.2005.04.004.
- [14] Y.C. Chiu and M.S. Chung, "Determination of optimal COD/nitrate ratio for biological denitrification," *International Biodeterioration and*

Biodegradation, vol. 51, Issue 1, Jan. 2003, pp. 43-49, doi:10.1016/S0964-8305(02)00074-4.

- [15] P. Menasveta, T. Panritdam, P. Sihanonth, S. Powtongsook, B. Chuntapa and P. Lee, "Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock," *Aquacultural Engineering*, vol. 25, Issue 1, Aug. 2001, pp. 35-49, doi:10.1016/S0144-8609(01)00069-3.
- [16] P.G. Lee , R.N. Lea, E. Dohmann, W. Prebilsky, P.E. Turk, H. Ying and J.L. Whitson, "Denitrification in aquaculture systems: an example of a fuzzy logic control problem," *Aquacultural Engineering*, vol. 23, Issue 1-3, Sep. 2000, pp. 37-59, doi:10.1016/S0144-8609(00)00046-7.
- [17] S. Ghafari, M. Hasan and M.K. Aroua, "A kinetic study of autohydrogenotrophic denitrification at the optimum pH and sodium bicarbonate dose," *Bioresource Technology*, vol. 101, Issue 7, April 2010, pp. 2236-2242, doi:10.1016/j.biortech.2009.11.068.
- [18] A. Kaviraj, F. Bhunia and N. C. Saha, "Toxicity of Methanol to Fish, Crustacean, Oligochaete Worm, and Aquatic Ecosystem," *International Journal Toxicity*, vol. 23, no.1, Jan. 2004, pp. 55-63, doi: 10.1080/10915810490265469.