

# Effect of dissolved oxygen and chemical oxygen demand to nitrogen ratios on the partial nitrification/denitrification process in moving bed biofilm reactors

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## Abstract

Partial nitrification was reported to be technically feasible and economically favorable, especially for wastewater with high ammonium concentration or low C/N ratio. In this study, the effect of dissolved oxygen (DO) and influent ratio of chemical oxygen demand to nitrogen (COD/N) ratio on biological nitrogen removal from synthetic wastewater was investigated. Experiments were conducted in moving bed biofilm reactors (MBBRs) on partial nitrification process in pilot-plant configuration for 300 days. DO levels were changed from 0.04 to 0.12 and 0.42 to 3.4 mg/l in the anoxic (R1) and aerobic (R2) reactors, respectively. The optimum DO for partial nitrification was between 1-1.5 mg/l in the aerobic reactor (R2). Influent COD/N ratios between 20 and 2 g COD/g-N were tested by changing the nitrogen loading rate (NLR) supplied to the pilot plant. During operational conditions when the DO concentration in aerobic reactor was above 1 mg/l, near complete organic carbon removal occurred in the total MBBRs system. The effluent total nitrogen concentration in the operational conditions (1.7-2.1 mg O<sub>2</sub>/l and NH<sub>4</sub><sup>+</sup>-N=35.7 mg N/l) was obtained in the range of 0.85-2 mg/l. The highest nitrite accumulation (50%-52%) took place at the DO concentration of 1-1.5 mg/l and increased with decreasing COD/N ratio in aerobic reactor (R2). This study showed that the average nitrification rate at various COD/N ratios is about 0.96 gN/m<sup>2</sup> per day while the maximum nitrification rate is about 2 gN/m<sup>2</sup> per day at COD/N ratios lower than 6.

The experimental COD/N ratio for denitrification was close to complete sum of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> (NO<sub>x</sub>) removal efficiency (about 99%) at COD/N ratio equal 14 in the operational conditions in the anoxic reactor (R1).

**Keywords:** Dissolved Oxygen; COD/N; Moving bed biofilm reactors (MBBRs); Partial nitrification

## INTRODUCTION

The operational costs of the conventional nitrification-denitrification nitrogen removal process are to a great extent related to the oxygen and organic matter requirements. Some new processes have been developed recently to reduce these costs. One of these is a rapid method of biological nitrogen removal. The combination of the preceding partial nitrification and the subsequent anaerobic ammonium oxidation (Anammox) is regarded as a promising new method of removing nitrogen from wastewater with a low C/N ratio and a large quantity of ammonium (van Loosdrecht *et al.*, 1998). Nitrogen compounds are usually removed from wastewater by a combination of two processes of nitrification (NT) and denitrification (DN) (Wang and Yang, 2004). Usually, nitrite oxidation proceeds faster than ammonia oxidation, so that nitrite rarely increases in the environment. This is very likely due to a minimum substrate concentration capable of steady state biomass and relatively high substrate uptake rate of the nitrite oxidizers (Rittmann and McCarty, 2001). If the nitrite oxidation could be con-

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trolled, an important advantage can be taken in nitrogen removal by using a shortcut biological nitrogen removal process known as partial nitrification (PN). Shortcut biological nitrogen removal is a new technology that oxidizes ammonia to nitrite and reduces nitrite to nitrogen gas (Chung and Bae, 2002). New processes such as nitrification/denitrification (NT-DN) have been developed by way of nitrite accumulation (Ruiz *et al.*, 2006; Ruiz *et al.*, 2003). This process is based on the fact that, nitrite and nitrate are intermediary compounds in both processes NT-DN, a PN to nitrite and a DN from this nitrite, instead of nitrate, would be suitable (Chung and Bae, 2002). For these reasons, PN to nitrite may be attractive. Nitrite accumulation studies have been performed that focused on several factors, such as free ammonia concentration by exploit pH or temperature, dissolved oxygen concentration and heterotrophic nitrification (Antilo *et al.*, 2006; Bernet *et al.*, 2001). The first MBBR facility became operational in early 1990 in Norway and then this system developed in Europe and America. There are presently more than 400 large-scale wastewater treatment plants based on this process in operation in 22 different countries all over the world (Maurer *et al.*, 2000). More than 50 MBBR plants are in operation at commercial fish farms. The MBBR process is based on the biofilm principle that takes advantage of both the activated sludge process and conventional fixed film systems without their disadvantages. Reactor can be operated at very high load and the process is insensitive to load variations and other disturbances (Delenfort and Thulin, 1997; Odegaard *et al.*, 1994). Unlike most biofilm reactors, the reactor volume in the MBBR is totally mixed and consequently there is no dead or unused space in the reactor. In addition, this system has a small head loss and there is no need for recycling of biomass or sludge (Xiao *et al.*, 2007). The biofilm carriers (Kaldnes1), made from high-density polyethylene or polypropylene, have a large surface area and a density slightly less or heavier than  $1.0 \times 10^3 \text{ kg/m}^3$  and the length (mm), diameter (mm) and protected area ( $\text{m}^2/\text{m}^3$ ) of biofilm carriers are 7, 10 and 500, respectively (Delenfort and Thulin, 1997; Odegaard *et al.*, 1994). One important advantage of the MBBR is that the filling fraction of biofilm carriers in the reactor may be subject to preferences. In order to be able to move the carrier suspension freely, it is recommended that filling fractions should be below 70% (Rusten *et al.*, 2006). The objective of this research was to evaluate the influence of DO and COD/N ratios on removal rate of organic substances and ammonium

from synthetic wastewater by applying a lab-scale MBBR system filled with kaldnes ( $k_1$ ) for partial nitrification process with continuous operation.

## MATERIALS AND METHODS

**Experimental set-up:** The experiments were conducted using two Plexiglas laboratory scale moving bed biofilm reactors (MBBRs) in series followed by a final clarifier. Without sludge recycle was implemented. The anoxic reactor ( $R_1$ ) was constructed for study of denitrifying bacteria carry out DN utilizing nitrite and nitrate as electron acceptors. The internal recycle rate from aerobic reactor to anoxic reactor was typically 3 times the influent flow rate. The aerobic reactor ( $R_2$ ) was built to provide nitrification. MBBRs were placed into a water bath equipped with aquarium heaters in order to operate at the constant temperature of  $28.5 \pm 1^\circ\text{C}$ . A sketch of the lab-scale MBBRs is shown in Figure 1 and some key parameters listed in Table 1. Sampling ports were provided in each reactor for sample collection. Anoxic and aerobic reactor was stirred by fixed speed propellers that pushed the biofilm media downward in the center of reactors. Normal propeller speed in the anoxic and aerobic reactors was 60 and 30 rpm, respectively. Aeration was carried out with air diffuser, located at the bottom of the aerobic reactor. The airflow to the reactor was measured by a rotameter and regulated with a manual valve. Synthetic wastewater and internal recycle were continuously fed into the bioreactors using a variable speed pumps (Italian ETATRON DS model DLS-MA). The Kaldnes ( $k_1$ ) carrier elements are made of polyethylene (density  $0.95 \text{ g/cm}^3$ ) and shaped like small cylinders (about 10 mm in diameter) with a cross inside. The effective specific growth area was about 500

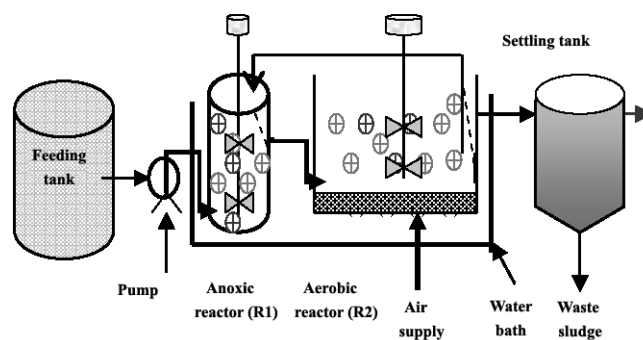


Figure 1. Schematic diagram of lab-scale MBBRs system.

**Table 1.** Technical data for the moving bed biofilm reactors.

| Parameter   | Anoxic reactor (R1) | Aerobic reactor (R2) |
|---|---------------------|----------------------|
| Volume (L)  | 3.5                 | 10                   |
| Filling ratio with bio-carriers (%)                             | 40                  | 50                   |
| Specific biofilm surface area (m <sup>2</sup> /m <sup>3</sup> ) | 200                 | 250                  |
| Total biofilm surface area (m <sup>2</sup> )                    | 0.7                 | 2.5                  |
| Flow rate (L/day)   | 16.2                | 16.2                 |
| Flow direction  | Up-flow             | Up-flow              |
| HRT (h)   | 5.2                 | 14.8                 |

m<sup>2</sup>/m<sup>3</sup> at 100% filling grade (Odegaard *et al.*, 1994). The pilot plant was operated in pre-denitrification mode with the anoxic phase preceding the aerated.

**Operating procedure:** The study was carried out using synthetic wastewater which contained glucose as the main organic constituent and provided balanced macro- and micro- nutrients and alkalinity. The wastewater was enriched with the macro-nutrients by adding NH<sub>4</sub>HCO<sub>3</sub> as nitrogen source and KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub> as phosphorus sources. The micro-nutrients were added to correct growth conditions for microorganisms according to Dulkadiroglu *et al.*, 2005; Hem *et al.*, 1994. KOH and NaHCO<sub>3</sub> were used for pH and alkalinity adjustment. Seeding sludge was obtained from Isfahan Municipal Wastewater Treatment Plant. Prior to the experimental phase, the carrier elements were acclimated for at least four weeks in the batch reactors to allow biofilm development. The aerobic reactor was operated under dissolved oxygen (DO) concentrations in the range of 0.5 to 3.1 mg/l. The wastewater was prepared with COD, NH<sub>4</sub>-N and orthophosphate phosphorus (PO<sub>4</sub>-P) concentrations of 300 to 2000 mg/l, 25 to 250 mg/l and 5 to 50 mg/l, respectively and was used to feed the system.

**Sampling and analysis:** Samples were collected from influent and sampling port of each reactor. Temperature, dissolved oxygen and pH were measured in each reactor immediately before sampling twice a day and were controlled manually. DO and pH levels were measured with an oxygen electrode (YSI-55, YSI) and a pH meter (model CG-824), respectively. The samples were analysed immediately after filtered through 0.45 µm filter paper. Soluble COD, ammonium (NH<sub>4</sub>-N), nitrate (NO<sub>3</sub>-N) and nitrite (NO<sub>2</sub>-N) was measured in accordance with standard methods

(APHA, 2005) and all measurements were made in duplicate. Concentrations of total suspended solids (TSS) on the fixed biomass elements were analysed as follows. The ten bio-carriers were put in a flask with demineralised water and placed in an ultrasound bath for 45 min to remove attached biomass. The bio-carriers were then rinsed with demineralised water, and the mixed liquid was filtered through a 0.45 µm fiber filter, dried at 105°C and weighed. Due to variability in the dimensions of the carriers, the obtained value was referenced to the total measured surface area. TSS was assessed for the total surface area in a cubic meter of the reactor (Helness, 2007; Jahren *et al.*, 2002; Andreottola *et al.*, 2000).

## RESULTS

Active denitrification occurs under anoxic conditions that does not involve molecular oxygen, but rather chemical forms (e.g., nitrite, nitrate, sulfate, etc) with combined oxygen atoms. DO can inhibit the denitrification reaction because oxygen functions as the electron acceptor for microorganisms over nitrate, and aerobic conditions repress enzymes involved in denitrification (Zumft, 1997). Although high DO concentrations in the biofilm reactor are necessary to enhance the activity of nitrifying bacteria, denitrification is inhibited by oxygen (Hagedorn-Olsen *et al.*, 1994; Lie and Welander, 1994). Figure 2, shows the maximum and minimum distribution of DO levels 0.04-0.12 and 0.42-3.4 mg/l under the steady-state conditions in the anoxic (R1) and aerobic (R2) reactor, respectively. Different concentrations of DO were supplied to the aerobic reactor to investigate the effect of DO on partial nitrification. Figure 3, shows the profiles of soluble COD (SCOD) removal (SCOD effluent) under var-

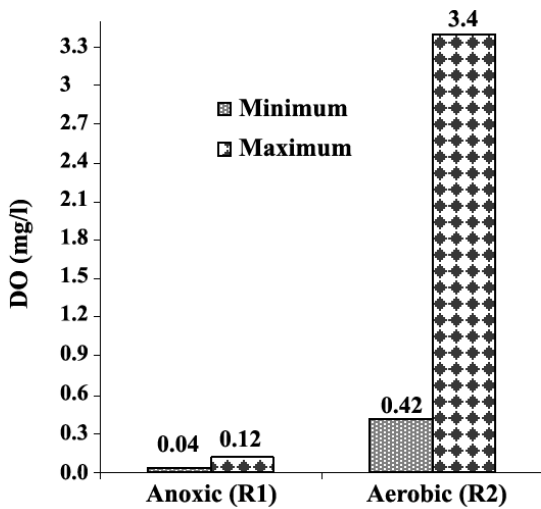


Figure 2. Do concentration in anoxic reactor and aerobic reactor.

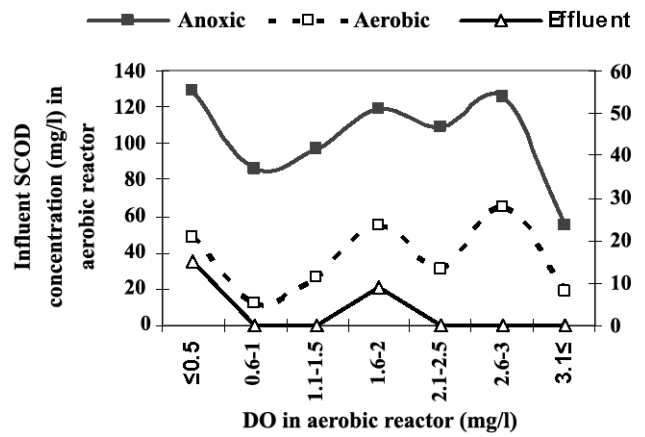


Figure 3. The effect of DO on SCOD removal rate under optimum conditions in the anoxic and aerobic reactors (influent COD in the anoxic reactor =500 mg/l).

ious DO concentrations. SCOD removal increased along with DO concentration in the aerobic reactor under optimum conditions (COD=500 mg/l,  $\text{NH}_4\text{-N}$ =35.7 mg-N/l, hydraulic retention time (HRT) =20 h). When influent DO concentration in the aerobic reactor was above 1 mg/l, complete soluble organic carbon removal (> 99%) occurred for optimum conditions, fluctuations in DO concentration did not negatively influence the COD removal rate in the aerobic reactor (R2) and complete soluble organic carbon removal occurred in the total MBBRs system. Most (about 80%) of the COD in the influent was used to reduce sum of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  ( $\text{NO}_x$ ) in the anoxic reactor (R1) (influent COD= 500 mg/l), and the remaining COD was removed in the aerobic reactor

(R2) by a combination of denitrification and aerobic oxidation.

Figure 4, shows the profiles of total nitrogen (TN) removal under various DO concentrations. Nitrate removal decreased in the anoxic reactor (R1), as DO concentrations in the aerobic reactor (R2) increased (above 2.7 mg  $\text{O}_2$ /l). While, in the optimum conditions (1.7-2.1 mg  $\text{O}_2$ /l and  $\text{NH}_4^+\text{-N}$ =35.7 mg N/l) the effluent TN concentration was obtained in the range of 0.85-2 mg/l under partial nitrification in the MBBRs system.

Figures 5 and 6, present the aerobic reactor (R2) and anoxic reactor (R1) behavior during consecutive increased in DO concentration in the aerobic reactor. The results show that nitrification or  $\text{NO}_x$  production

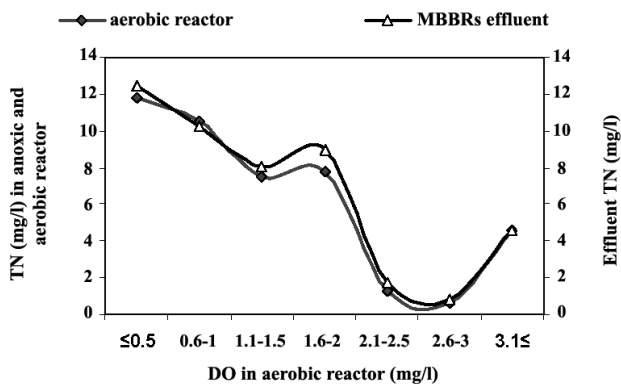


Figure 4. The effect of DO on TN removal rate in the aerobic reactor MBBR system under partial nitrification.

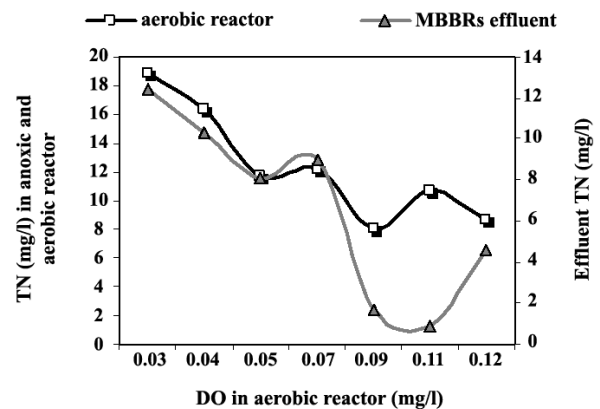
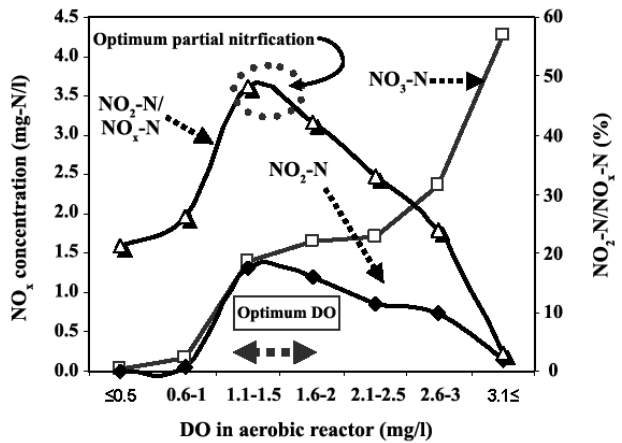
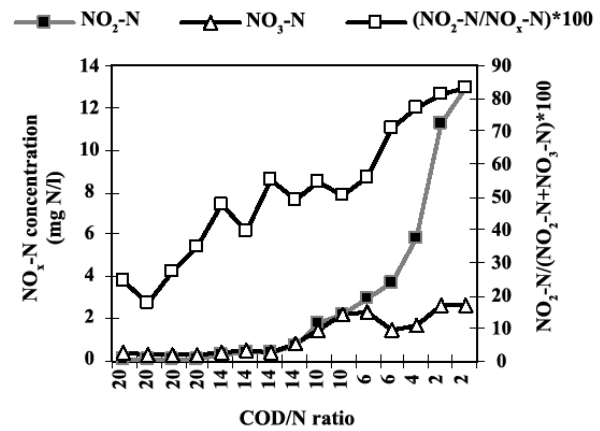


Figure 5. The effect of DO on TN removal rate in the anoxic reactor MBBR system under partial nitrification.



**Figure 6.** The effect of DO level on nitrogen species concentration in the aerobic reactor under optimum conditions (COD=500 mg/l, NH<sub>4</sub>-N= 35.7 mg-N/l, HRT=20 h, internal recycle ratio (IR) =3Q).



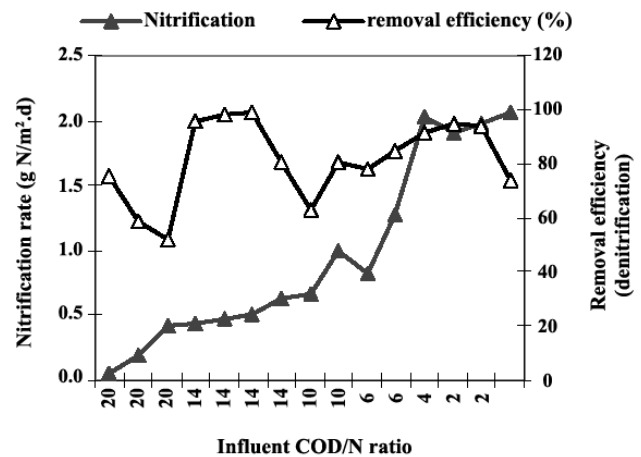
**Figure 7.** Effect of influent COD/N ratio on nitrogen species concentrations in the aerobic reactor (R2) under partial nitrification: (DO concentration=1.1-1.5 mg/l).

NO<sub>x</sub>-N=Σ(NO<sub>2</sub> + NO<sub>3</sub>) was affected by DO concentration in the range ≤0.5-3.1 mg/l. It can be observed that the nitrification rate increased when DO level in aerobic reactor (R2) increased. A higher nitrate ions around 4.3 mg/l was observed in the case of DO concentration above 3.1 mg/l (influent ammonium concentration =35.7 mg/l) but concentration of nitrite ions was the lowest (by 0.14 mg NO<sub>2</sub>-N/l) in R2 (complete nitrification occurred and dominated). The highest nitrite accumulation took place at the DO concentration of 1-1.5 mg/l because partial nitrification occurred and dominated. In addition, in the aerobic reactor (R2), nitrite accumulated ratios (NO<sub>2</sub>-N/NO<sub>x</sub>-N) in the optimum conditions (HRT=20 h, Q<sub>r</sub>=3Q and T=28.5±1°C) and different DO concentrations were from 50% to 52%. Generally, the nitrite accumulation rate in the aerobic reactor (R2) decreased with increasing DO concentration above 1.5 mg/l (because complete nitrification dominated).

Variation of nitrite and nitrate ions in relation to influent COD/N ratio in the aerobic reactor is shown in Figure 7. It shows the ratio of nitrite to total nitrogenous oxide in the optimum conditions and various COD/N ratios were from 20% to 83% and average by 52%. As indicated, the nitrite accumulation rate increased with decreasing COD/N ratio in the aerobic reactor (R2) in the optimum conditions because partial nitrification occurred and completed (500 mg COD/l, HRT=20hr, Q<sub>r</sub>=3Q, DO= 1-1.5 mg/l and T=28.5±1°C).

The average TN effluent during the steady state and in optimum conditions (data not shown) was 3.5±1 mg/l in the aerobic reactor for the partial

nitrification/denitrification (PND) process in MBBR system. Figure 8, shows the relationships between nitrification and denitrification (removal efficiency percentage) and NO<sub>2</sub>-N/NO<sub>x</sub>-N (%) versus the COD/N influent ratio in the anoxic reactor (R1) in the MBBRs system. Influent COD/N ratios strongly affected the nitrate and nitrite ions in the reactor (R1). At the low COD/N ratio, limited denitrification was achieved in the anoxic reactor. This study showed that the average nitrification rate at various COD/N ratios is about 0.96 g-N/m<sup>2</sup> per day while the maximum nitrification rate is about 2 g-N/m<sup>2</sup> per day at COD/N ratios lower than 6 in the MBBRs system. Denitrification percentage was defined as the nitrogen



**Figure 8.** Relationship between the partial nitrification and denitrification capacity versus the COD/N ratio in the anoxic reactor (R1).

removed from oxidized ammonium nitrogen in anoxic reactor (R1). NO<sub>x</sub> (NO<sub>x</sub>-N=Σ(NO<sub>2</sub><sup>-</sup>+NO<sub>3</sub><sup>-</sup>)) removal efficiencies in all phases were excellent, despite decreasing from 76% to 52% at COD/N=20 with nitrite accumulation because of DO concentration restricted in aerobic reactor (R2) and partial nitrification occurred (date not shown). These results demonstrated close to complete NO<sub>x</sub> removal efficiency (about 99%) at COD/N=14 in the optimum conditions (500 mg COD/l, 35.7 mg NH<sub>4</sub>-N/l, HRT=20 h, Q<sub>r</sub>=3Q, DO= 1-1.5 mg/l and T=28.5±1°C) in anoxic reactor (R1).

## DISCUSSION

**Influence of DO on partial nitrification:** Partial nitrification (ammonium oxidation to nitrite) has gained a lot of interest among researchers in the last years especially in the field of high-strength ammonium wastewater treatment. Turk and Mavinic, (1987) demonstrated that this shortcut in the biological ammonium removal process has several advantages with respect to the complete nitrification: (1) a 40% reduction of COD demand during denitrification; (2) 63% higher rate of denitrification; and (3) 300% lower biomass production during anoxic growth. Furthermore, partial nitrification can save up to 25% of the oxygen demand for nitrification due to the suppression of the nitrification (oxidation of nitrite to nitrate) and reduce the CO<sub>2</sub> emissions by 20% due to the denitrification from nitrite instead of nitrate (Peng and Zhu, 2006).

The oxygen concentration is also mentioned as a very important limiting factor and could be used as a tool for partial nitrification (Ruiz *et al.*, 2003; Bernet *et al.*, 2001; Bae *et al.*, 2001). Low DO concentrations can affect the specific growth rate of both ammonium-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB), depending on its oxygen saturation constant; however, its influence on the NOB, (1.1 mgO<sub>2</sub>/l) is significantly greater than on the AOB (0.3 mgO<sub>2</sub>/l) (Wiesmann, 1994). At low oxygen concentrations, changes in the population structure were observed by Park and Noguera (2004), which could affect nitrite accumulation rates. Oxygen level for nitrite accumulation is in the range of 0.5-1.5 mg O<sub>2</sub>/l for suspended cultures (Ciudad *et al.*, 2005; Botrous *et al.*, 2004; Bernet *et al.*, 2001). Otherwise, the transport mechanisms in immobilized systems might even enhance the nitrite accumulation, since the oxygen is normally con-

sumed in the first 50-100 μm of biofilms due to the deficient oxygen transfer into biofilms (Okabe and Watanabe, 2000) and an outward diffusion of the accumulated nitrite from the inside of the biofilm to the liquid bulk also occurs. Therefore, biofilm reactors with low mass transfer coefficient at the interphase biofilm/liquid, such as the rotating disk reactor (RDR) or biological aerobic filter (Lindemann and Wiesmann, 2000), and moving bed biofilm reactors (MBBRs) (this article) may be at an advantage for nitrite accumulation. Figure 2 shows the minimum and maximum DO in both anoxic and aerobic reactors. The amount of DO fed to the anoxic reactor due to the internal recycle flow from the aerobic reactor was influenced because DO in anoxic reactor was found increased when DO in aerobic reactor increased. Generally, DO levels in anoxic reactor (R1) was lower than 0.1 mg/l because at low DO level, ammonia-oxidizers use nitrite as an artificial electron acceptor and generate nitrous oxide (N<sub>2</sub>O) gas (Ritichie and Nicholas, 1972). Nitric oxide (NO) gas is similarly produced by ammonia-oxidizers, but this activity is less sensitive to DO (Anderson and Levine, 1986). It is important to note that these gases result from partial denitrification, rather than incomplete nitrification using oxygen (Remde and Conrad, 1990). Nitrite-oxidizers can also reduce nitrite to nitric oxide (Bock *et al.*, 1991), but apparently not to nitrous oxide (Martikainen, 1985). However, in an anaerobic and heterotrophic medium, nitrite-oxidizers can reduce nitrate to nitrite (i.e. nitrate respiration) as well as to ammonia (i.e. dissimilatory nitrate reduction) (Sundermeyer-Klinger *et al.*, 1984). Under the steady-state conditions DO concentration in the aerobic reactor (R2) was between 1 and 1.5 mg/l, which resulted in a lower nitrate ion production (partial nitrification dominated in R2) because this study was intended to evaluate partial nitrification of the MBBRs system in continuous flow mode.

As indicated, the soluble COD removal efficiency versus variation DO concentration at different reactors of MBBRs system is shown in Figure 3. The results showed that during operational conditions (500 mg COD/l, 35.7 mg NH<sub>4</sub>-N/l, 7.14 mg PO<sub>4</sub>-P/l, HRT=20 h, Q<sub>r</sub>/Q=3) fluctuations in the concentration of DO had no negative influence on COD removal rate in R2 and complete soluble organic carbon removal more than 99% efficiency occurred in the total MBBRs system. Most (about 80%) of the COD was used to reduce NO<sub>x</sub> in the anoxic reactor (R1) (influent COD= 500 mg/l), and the remaining COD was removed in the aerobic reactor (R2) by a combination of denitrification

and aerobic oxidation. Chung *et al.* obtained the same results, which are reconfirmed in this study (Chung *et al.*, 2006).

As shown in Figure 4 during optimum conditions, effluent total nitrogen (TN) concentration was affected by DO concentrations in the range 0.5–3.1 mg/l. TN removal rate increased with the increase in DO concentration; however, it decreased when DO concentration increased more than 2.7 mg/l. because when the dissolved oxygen concentration in the reactor (R2) exceeded 2.5 mg/l, ammonia was fully converted to nitrate and ammonia conversion was limited by the incoming ammonia load to the reactor (complete nitrification occurred). The average DO value seemed to be optimal at the range of 1.7–2.1 mg/l and the maximum DO value should be at the range from 1.8 to 3 mg/l. It is generally known that DO concentration above 1 mg/l is essential for complete dominated nitrification; if the DO level is lower, oxygen becomes the limiting factor and nitrification slows or ceases. On the contrary in denitrification, high DO levels will suppress the necessary enzyme systems. Thus, when partial nitrification-denitrification via nitrite becomes the focus, controlling the DO level is critical to balance the degrees of nitrification and denitrification, and the resulting levels of nitrogen compounds in the effluent (Ruiz *et al.*, 2006; Yoo *et al.*, 1999). Because the partial nitrification process requires nitrite accumulation, the second step must be restrained so as to accumulate AOB and wash out NOB (Laanbroek and Gerards., 1993). The dissolved oxygen half-saturation coefficients of AOB and NOB are 0.2 to 0.4 mg/l and 1.2 to 1.5 mg/l, respectively (Picioreanu *et al.*, 1997). Therefore, low DO concentrations restrict NOB growth more strongly than AOB growth, resulting in nitrite accumulation (Peng *et al.*, 2004). Garrido *et al.* (1997) suggested that the specific growth rate of ammonium oxidizers is superior to nitrite oxidizers at DO concentrations below 1.5 mg O<sub>2</sub>/l. This result was reconfirmed in the present study.

According to Figure 5 and 6, when the average DO value was high in the aerobic reactor, NO<sub>x</sub> concentration was high and TN was mostly removed from the effluent and the opposite trend occurred when the DO value was low. At a DO concentration of 0.6 mg/l nitrite accumulation took place due to partial nitrification. The higher nitrite accumulation (by 50%) occurred at DO concentrations in the range 1.1–1.5 mg/l in R2. At a DO below 0.65 mg/l ammonia conversion was affected and higher than 2 mgO<sub>2</sub>/l nitrite, accumulation was decreased. Ruiz *et al.* (2006) and

Jainlong and Ning, (2004) have observed that nitrite accumulation occurred at DO concentration of 1.4 mg/l and 0.7–1.4 mg/l, respectively. This result was reconfirmed in the present study.

**Effect of influent COD/N ratio:** Carrera *et al.* (2004) reported that influent the COD/N ratio is one of the most critical parameters for wastewater nitrogen removal process, because it directly effects on functional microorganism populations, including autotrophic ammonium (NH<sub>4</sub><sup>+</sup>-N) oxidizer bacteria, nitrite (NO<sub>2</sub><sup>-</sup>-N) oxidizer bacteria and heterotrophic denitrifies. In a nitrogen removal system, different microorganism populations compete for substrate, which causes fluctuations in the effectiveness of organic matter and nitrogen removal. According to Figure 7 and 8, as indicated, the nitrite accumulation rate increased with decreasing COD/N ratio in the aerobic reactor (R2) in the optimum conditions (500 mg COD/l, HRT=20 h, Q<sub>r</sub> =3Q, DO= 1–1.5 mg/l and T=28.5±1°C). The average TN effluent during the steady state and in optimum conditions (data not shown) was 3.5±1 mg/l in the aerobic reactor for the partial nitrification/denitrification process in MBBR system. The decrease of nitrification rate was observed when the COD/N ratio increased. Wiesmann, (1994); Satoh *et al.* (2000) and Okabe and Watanabe, (2000) obtained the same results, which are reconfirmed in the present study.

Figure 8 showed that the experimental COD/N ratio for maximum denitrification was 14 g COD/g-N while the stoichiometric ratio was 4.2 g COD/g-N. This difference is attributable to the oxidation of organic matter in the anoxic reactor with the oxygen of the internal recycle (Carrera *et al.*, 2004).

Park *et al.* (2000) reported that nitrous oxide formation during nitrification is favoured under lower DO concentrations (0.2 to 0.5 mg/l). Nitrous oxide has a relatively high toxicity for microorganisms and is also a potent green-house gas with an activity 200 to 300 fold higher than that of carbon dioxide (CO<sub>2</sub>). During partial nitrification, nitrous oxide could be formed in three possible ways (Park *et al.*, 2000): the oxidation of hydroxylamine over nitrification (Goreau *et al.*, 1980), the possible reduction of accumulated nitrite to nitrous oxide in anoxic zones inside the bio-carriers, and the production of a small amount of nitrate during denitrification in the anoxic region (Wrage *et al.*, 2001) (a small amount of nitrate is also produced during the process of partial nitrification). It is commonly agreed that low oxygen concentrations and high NO<sub>2</sub>-

N concentrations favour higher N<sub>2</sub>O emissions.

Ultimately, the results indicated that the lab-scale MBBR system under partial nitrification/denitrification (pre-denitrification) has acceptable performance for removal of nitrogenous compounds and organic matter. It can significantly enhance the economical balance of the treatments through a reduction in the organic matter's needs for denitrification step, a decrease in aeration requirements in nitrification, without sludge recycle and decrease in the surplus sludge generation. Hence it can be suggested that under partial nitrification/denitrification (PND) process (pre-denitrification), the MBBRs system could be used as an ideal and efficient option for the total nutrient removal from municipal and industrial wastewater.

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