Improving total nitrogen removal in aeration basin retrofitted with entrapped biomass
Yeong-Nan Chao, Jui-Hsien Lin, Kok-Kwang Ng, Chung-Hsin Wu, Pui-Kwan Andy Hong and Cheng-Fang Lin

ABSTRACT
This study presented a method to upgrade existing aeration tanks to remove total nitrogen (TN). Bioplates carrying entrapped biomass were installed in an aeration basin to create anoxic/anaerobic zones where denitrification can proceed. In a reactor that coupled bioplates containing entrapped biomass (equivalent to as high as 7,500 mg/L of biomass) and an activated sludge suspension (at mixed liquor suspended solids of 1,300–2,400 mg/L), nitrification efficiency exceeded 95% for an influent wastewater containing 21–54 mg/L of NH3-N. In all cases amended with alkalinity and with or without added methanol as an electron source, TN removal was between 60 and 70%. The results demonstrated anoxic/oxic or anaerobic/anoxic/oxic processes could be incorporated in a conventional aeration basin, requiring no substantial modifications of the vessel and operation, and thus providing improved treatment in terms of nitrogen removal in the conventional suspended-growth process.

Key words | activated sludge process, anaerobic, anoxic, bioplates, entrapped biomass, total nitrogen removal

INTRODUCTION
The judicious use of water resource has always been important, particularly in arid countries. In the USA and Israel, secondary effluent is routinely used in agricultural activities. In Japan, municipal wastewater (MW) is recycled for toilet flushing and other purposes. In southern Europe, reclaimed wastewater is reused mostly for agricultural irrigation (Bixio et al. 2006). Renowned NEWater, the widely accepted potable water in Singapore, originates from secondary effluent of domestic wastewater after purification by membrane processes (Qin et al. 2006). Presently, water purification technologies are capable of making ultrapure water from various wastewaters; however, treatment choices and their costs per volume of reclaimed water have been a primary concern (Shannon et al. 2008).

In Taiwan, there is a great need to reclaim domestic wastewater for industrial uses such as cooling. However, the industry sector is very concerned with residual ammonia nitrogen and total nitrogen (TN) that cause corrosion of bronze pipelines and zinc materials (Gurrappa 2005). Most traditional municipal wastewater treatment plants (WWTPs) apply activated sludge (AS) processes for organics removal and nitrification, which provide little TN removal unless the treatment system is upgraded to an anaerobic/anoxic/oxic (A2O) process or an anaerobic denitrification unit is placed after the secondary clarifier (Gujer 2010). This would incur significant construction costs that may not yield a desirable benefit/cost ratio. Major barriers to implementing nutrient (i.e. nitrogen) removal have been costs and limitation in physical expansion (USEPA 2009).

Therefore, creating an anoxic/anaerobic environment that enables simultaneous organics and nitrogen removal in the AS process of an existing aeration basin is perceived as a plausible alternative in lieu of significantly retrofitting the system hardware.

Anoxic/aerobic zones commonly occur in the soil environment where biological respiration depletes dissolved oxygen in the intra space of the soil aggregates due to advective/diffusive transport limitation, creating an anaerobic environment in the intra space that allows coupled nitrification/denitrification processes to occur (Kremen et al. 2005). Similar A2O zones are created in attached-growth biological systems including the trickling filter where the inner part of
the biological film adjacent to the support becomes anaerobic. An A2O environment can be created in the aeration basin by installation of bioplates containing entrapped biomass (Yang et al. 2003a, b), which is a cell immobilization technique for concurrent removal of carbon and nitrogen in a single pass. With dense entrapped biomass, the technique can handle high dissolved organic loads and develop slow-growing microorganisms for TN removal (Kim et al. 2011; Zhu et al. 2011). With entrapped biomass, the system can carry an equivalent sludge concentration as high as 11,000 mg/L (Ng et al. 2011) and it offers a low-permeability support that makes available an anaerobic environment in the aeration basin. Theoretically, oxic, anoxic, and anaerobic zones are formed transitioning from outside into the inner bioplate, resulting from a dissolved oxygen (DO) gradient established due to mass transfer limitation, as illustrated in Figure 1. Each bioplate acts as a small A2O unit capable of degradation of chemical oxygen demand (COD), nitrification of NH$_3$-N, and denitrification of NO$_3$-N. With installed bioplates, the AS process is upgraded into the A2O process without substantial changes of the existing aeration chamber.

In this work, we tested the AS process coupled with bioplates for TN removal from authentic domestic wastewater. Factors influencing nitrification and denitrification such as the bioplate packing ratio (PR), hydraulic retention time (HRT), alkalinity (ALK; with Na$_2$CO$_3$), electron donor (with CH$_3$OH), C/N ratio, and aeration mode were investigated. The goal was to investigate the effects of the aforementioned factors on nitrification and TN removal and the feasibility of enhancement. While studying the influence under exhaustively varied combinations of these factors was not possible, we have described below the experimental steps and decisions that led to our key findings as concluded in the Abstract.

**MATERIALS AND METHODS**

**Reactor setup**

Entrapped AS in a carrier was prepared by modification of the procedures of Yang et al. (1997). A mixture was prepared by adding 400 g (w/v) of wet AS (obtained from a WWTP for food production) into 800 mL of cellulose triacetate solution (10% w/v ratio in methylene chloride). The mixture was transferred into a wooden frame mode of 30 × 20 cm and shaped accordingly to a thickness of about 0.6 cm after the solvent evaporated and the mixture hardened. The bioplates contained about 8.2 g of immobilized AS (dry weight) each and were installed vertically in parallel to the horizontal flow of wastewater across the reactor. The reactor volume used for the experiment was 42 litres. When 8, 24, or 40 bioplates were installed in the reactor, it contained an equivalent sludge concentration of 1,500, 4,500, or 7,500 mg/L, respectively, at the PR (i.e., bioplate volume/reactor volume) of 6.3, 19, or 31%, respectively. For comparison in a separate series of experiments, the bioplates-packed reactor was further added with a suspension of AS at varied mixed liquor suspended solids (MLSS) concentrations of 1,500–2,400 mg/L. The schematic diagram of reactor setup is shown in Figure 2.

![Figure 1](https://iwaponline.com/wst/article-pdf/69/7/1558/472400/1558.pdf)

**Figure 1** | C and N removal mechanisms with entrapped biological process with oxic/anoxic/anaerobic transitions.
The entrapped biomass system consists of an aeration basin of 42 L and a clarifier with recirculation of the settled sludge backed into the aeration basin by means of a peristaltic pump. Air was delivered into the basin bottom by a compressor, and was regulated by four timer-controlled solenoid valves according to the prescribed aeration schedule such as 1 h on / 1 h off or 0.5 h on / 0.5 h off. Rising of the air bubbles provided convective mixing in the aeration basin. After fabrication, the bioplates were acclimated with synthetic wastewater (SW) for 1 week to allow COD removal efficiency to stabilize at 85–90%. Both SW and MW were tested. The compositions of SW and MW were as shown in Table 1. The MW was obtained at the exit of the primary sedimentation basin of a wastewater treatment plant in Taipei.

During the experiment, influent wastewater was transferred from a reservoir to the reactor by a peristaltic pump at a constant flow rate (38.9 mL/min (HRT, 18 h) to 116.6 mL/min (HRT, 6 h)). The reactor was operated at room temperature of 25 ± 3°C. Experimental parameters were varied: HRT of 6, 12 and 18 h, COD/NH4-N ratio of 3, 6 and 12, aeration in continuous and intermittent modes, with added Na2CO3 and without. The reactor was operated for 1 week to ensure establishment of a steady-state condition prior to data collection. Samples for analyses were withdrawn from the clarified effluent when a suspended AS was present, or directly from the aeration basin when only entrapped bioplates were present. Main experimental conditions and solids retention time (SRT) values are shown in Table 2. SRTs in the entrapped biomass system were very long (>133 d) because little sludge was generated or wasted when bioplates were used solely. SRTs in the entrapped biomass coupled with suspended AS were >75 d.

### Analytical methods

Influent and effluent samples were taken daily and analyzed for soluble COD (SCOD), NH4-N, and NO3-N as per...
Table 2  |  Key operation conditions and SRTs

<table>
<thead>
<tr>
<th>Run</th>
<th>Treatment Process</th>
<th>HRT</th>
<th>PR</th>
<th>SRT (Avg. d)</th>
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<td>19</td>
<td>175</td>
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<tr>
<td>3B</td>
<td>Bioplates</td>
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<td>387</td>
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<td>3D</td>
<td>Bioplates</td>
<td>6, 12, 18</td>
<td>19</td>
<td>137–384</td>
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Synthetic wastewater

Municipal wastewater

Standard Methods (APHA 1998). All samples were filtered with a 0.45 μm membrane filter. Reactor DO and pH were recorded daily with a portable dissolved oxygen/pH meter (Hach HQ30d/ Hach sensION1). SCOD was analyzed by Hach closed reflux colorimetric method (detection range of 3–150 mg/L (LR) and 20–1,500 mg/L (HR); HACH method 8000) with a spectrophotometer (Hach DR 2800). NH3-N was analyzed as per Nessler method (detection range 0.02–2.50 mg/L; Hach method 8038). NO3-N was analyzed as per the cadmium reduction method (detection range of 0.1–2.0 mg/L). Total Kjeldahl nitrogen (TKN) analysis was performed for the influent at the beginning stage of experimentation, and the TKN results were compared with NH4-N to calculate the organic nitrogen content. The influent organic N averaged 0.98 mg/L varying by 0.2–2.1 mg/L, while the effluent organic N averaged 0.94 mg/L varying by 0.3–2.0 mg/L. Thus, TKN analysis was not continued.

RESULTS AND DISCUSSION

SW as feed

The reactors with bioplates of various PR (6.3, 13, and 19%) were fed continuously with SW containing 300 mg/L of COD and 25 mg/L of NH3-N to acclimate the anchored biomass for 9 d. Effluent COD, NH3-N, and NO3-N concentrations were monitored and they reached steady states within the dynamic condition period, typically within 3 d. At steady state, COD and NH3-N in the effluent decreased but NO3-N increased with increasing PR, which suggested increased COD and NH3-N removals with increasing entrapped biomass. Thus, bioplates were shown to be active in removal of COD and NH3-N. Their activities under different conditions including HRT, C/N ratio, and added alkalinity (Na2CO3) were further investigated at PR of 19% (Figure 3).

Figure 3(a) shows the effects of added ALK of 0.2 g/L as CaCO3 (with Na2CO3 0.212 g/L) on nitrification and TN removal in continuous aeration mode. While maintaining pH at 7–8, the added Na2CO3 offered inorganic carbon supplement to autotrophic ammonia-oxidizing bacteria that could promote nitrification. Nitrification, i.e. oxidation of ammonia nitrogen, would decrease significantly at pH below 6.8 but proceed well at pH 7–8 (Tchobanoglous et al. 2005). Figure 3(a) shows that nitrification efficiency increased from 68 to 90% and from 60 to 76% at influent NH3-N of 25 and 50 mg/L, respectively, while COD removal was relatively constant around 89%. However, TN removal was only 15%, not improved under the test conditions.

While the installed bioplates were intended to create the A2O environment in the aeration basin, excessive DO in the basin would accelerate DO transport into the inner anoxic/anaerobic zones of the bioplates possibly sequestering the O2-deficient environment that was favorable to desired denitrification. In addition, energy cost for aeration accounts for more than 70% of the total expenditure of biological WWTPs (Cornel et al. 2003). Excessive aeration is not desirable. Therefore, we investigated the effects of aeration patterns on COD and N removals, including continuously on, repeating 1 h on and 1 h off, and repeating 0.5 h on and 0.5 h off (Figure 3(b)). Both COD and NH3-N removals were over 90% and stably so under different aeration patterns. However, TN removal was 8.5% with continuous aeration, but increased to 36% with 0.5 h intermittent pattern and to 43% with 1 h intermittent pattern. The results support our hypothesis that high DO abolishes the O2-deficient zones of bioplates favorable for denitrification. In general, DO needs to be >6 mg/L for complete nitrification (Hanaki et al. 1990); at DO <2 mg/L, it becomes inhibitive to nitrification (Goreau et al. 1980).

Organic carbon is crucial in denitrification as it supplies electrons for NO3-N to be reduced to N2. The effect of organic carbon supplement in the form of methanol addition on denitrification was examined. Figure 3(c) shows the effects of varied COD/NH4-N ratios on COD and N removals under the specified conditions. Both COD removal and nitrification efficiencies were >90%. TN removal was significantly increased from 42 to 56 and 83% when the COD/NH4-N ratio was increased from 3 to 6 and 12, respectively. The benefits of organic carbon supplement were clear comparing TN removal of 16% during...
the dynamic condition period to 83% at augmented COD/NH₄⁺-N ratio of 12. These results were consistent with the trend of increasing NO₃-N removal with increasing COD/NH₄⁺-N ratio, as observed by Alves et al. (2002). However, increased COD/NH₄⁺-N ratio may favor heterogeneous bacteria to become the predominant species that may suppress nitrifying bacteria (Okabe et al. 1996). Our results thus far indicated that the selected aeration mode and COD/NH₄⁺-N ratio with adequate alkalinity accomplished extensive, concurrent nitrification and denitrification.

We subsequently examined the effect of HRT under selected conditions of COD/NH₄⁺-N of 12 and 1 h intermittent aeration with supplemented ALK. Figure 3(d) shows removal efficiencies at HRT of 6, 12, and 18 h. An HRT of 12 h provided COD, NH₃-N, and TN removals of 95, 89, and 80%, respectively. At HRT of 6 h, while COD removal remained comparable to others, both nitrification and denitrification fell short at 64%. The limiting step was likely nitrification in that nitrifying bacteria might have been overwhelmed by heterogeneous bacteria stimulated by organic carbon augmented at COD/NH₄⁺-N of 12.

MW as feed

Results above have shown the A2O environment created by bioplates in an aeration chamber is capable of concurrent nitrification and denitrification, the extents of which are subject to operation conditions including HRT, aeration mode, COD/NH₄⁺-N, and alkalinity. For balance in this study, we turn our focus to the treatment of authentic MW. Figure 4(a) shows SCOD, NH₃-N, and TN removals at HRT of 6 h (approximating those of typical WWTPs) and continuous aeration (DO 6–7 mg/L) under conditions: (1) no addition of alkalinity or electron donor, (2) ALK of 0.2 g/L as CaCO₃ added, and (3) ALK of 0.2 g/L as CaCO₃ and 63 mg/L of CH₃OH added. SCOD removals were 70, 78, and 80% under these three sets of conditions, respectively. Likewise, nitrification efficiencies were 88, 96 and 96% for the three sets, respectively, which showed an 8% increase in nitrification by adding alkalinity. TN removals in all three sets were comparable at 42%. Apparently, additions of alkalinity and organic carbon did not result in increased TN removal from the MW, albeit with extensive occurrence of nitrification.

The lack of TN removal might have been due to insufficient development of anoxic/anaerobic zones needed for denitrification. Thus, bioplates in the reactor were increased to a PR of 51% (Figure 4(b)). Under various conditions, SCOD, NH₃-N, and TN removals were 64–80%, 90–98%, and 52–58%, respectively, with apparent increases in TN removals.

With promising removal results for authentic MW using bioplates, we proceeded to couple the bioplates with

Figure 3 | Effects of (a) added ALK, (b) aeration patterns, (c) C/N ratios and (d) HRT on C and N removals from SW. (a): HRT, 8 h; COD₀, 300 mg/L; PR, 19%; DO, ≥ 7 mg/L. (b): HRT, 18 h; COD₀, 75 mg/L; NH₃-N₀, 25 mg/L; PR, 19%; COD/NH₄⁺-N ratio, 3. (c): HRT, 18 h; NH₃-N₀, 25 mg/L; PR, 19%; aeration (on/off), 1 h/1 h; pH, 7–8; DO, 2–8 mg/L. (d): COD₀, 300 mg/L; NH₃-N₀, 25 mg/L; PR, 19%; aeration (on/off pattern), 1 h/1 h; pH, 7–8; DO, 2–8 mg/L; COD/NH₄⁺-N, 12.
conventional AS in an aeration chamber. The experiments were carried in five sets of conditions: (1) DO of 4–6 mg/L, PR of 31%, HRT of 12 h, ALK of 0.2 g as CaCO₃/L, and methanol of 63 mg/L; (2) DO of 2–4.2 mg/L, PR of 16%, HRT of 12 h, ALK of 0.2 g as CaCO₃/L, and methanol of 63 mg/L; (3) DO of 6–7.4 mg/L, PR of 16%, HRT of 12 h, ALK of 0.2 g as CaCO₃/L, and methanol of 63 mg/L; (4) DO of 2.4–4.5 mg/L, PR of 16%, HRT of 6 h, ALK of 0.2 g as CaCO₃/L, and methanol of 63 mg/L; and (5) DO of 2.8–4.7 mg/L, PR of 16%, HRT of 6 h, ALK of 0.2 g as CaCO₃/L, and no methanol addition. MLSS concentrations were maintained between 1,300 and 2,400 mg/L via recirculation of sludge from a clarifier after the aeration chamber. The influent SCOD and NH₃-N concentrations were 42–172 and 16–54 mg/L, respectively. The results of all runs show over 80% removal of SCOD, over 95% nitrification of NH₃-N, and near 70% removal of TN (Figure 5(a)). These results show removal of SCOD alongside concurrent nitrification and denitrification processes in the coupled aeration chamber.

The effects of adding methanol as an electron donor to the coupled MLSS and bioplates were further explored (Figure 5(b)). The addition of methanol resulted in little changes in SCOD, NH₃-N, and NO₃-N removals, which remained at high levels of 80, 98, and 60%, respectively. The presence of AS coupled with bioplates enhanced all removals, albeit necessitating ALK supply for the autotrophic nitrifying bacteria. These results have demonstrated the potential of coupled bioplates and AS creating the AO or A2O process for enhancing TN removal. While the AS is responsible for most nitrification, the bioplates follow through with denitrification. The effective removal of TN in the entrapped biomass system was attributed to its extended SRT. When coupled with bioplates at PR of 16%, the conventional aeration basin with AS is upgraded...
to an AO or A2O reactor with minimal retrofitting undertaking.

**CONCLUSIONS**

Increasing stringent TN effluent standards call for an upgraded capacity of WWTPs to remove TN, which would make the treated water more amenable to beneficial uses such as for cooling towers. This study shows that bioplates with entrapped biomass create anoxic/anaerobic zones that are capable of carrying out denitrification and that, when coupled with a conventional AS process, they empower the conventional aeration basin for concurrent removals of TN and SCOD. The coupled AS and bioplates system enabled over 95% of nitrification and promoted denitrification, which led to 60–70% removal of TN. The coupled process readily upgrades an AS aeration basin to an AO or A2O reactor.

**REFERENCES**


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