A pilot study on suspended activated sludge process augmented with immobilized biomass for simultaneous nitrification and denitrification

Haon-Yao Chen, Pui-Kwan Andy Hong, Ping-Yi Yang, Kok Kwang Ng, Sheng-Fu Yang, Chien-Hsien Lee and Cheng-Fang Lin

ABSTRACT

Concurrent nitrification and denitrification (CND) are natural phenomena in the soil environment that can be applied in wastewater treatment for enhanced total nitrogen removal. However, significant renovation is necessary for existing plants to equip them for nutrient removal. At a domestic wastewater treatment plant, we performed a pilot test by installing bioplates with entrapped biomass in a conventional aeration basin for CND, and investigated the effects of bioplate packing ratio (PR), hydraulic retention time (HRT), dissolved oxygen (DO) level, on/off aeration mode, and supplemental carbon and alkalinity on nitrogen removal. With the pilot aeration basin of 1.3 m^3 loaded with mixed liquor suspended solids of 1,500–2,500 mg/L and bioplates at PR of 3.2% (3.2% basin volume) operated at HRT of 6 h and DO of 4–6 mg/L without supplemental carbon or alkalinity, nitrogen in the wastewater was removed to an effluent total nitrogen (TN) of 7.3 mg/L from an influent TN of 28 mg/L, achieving a specific TN removal of 25 g $TN/m^2/d$. The bioplate, consisting of modular, robust cellulose triacetate structure carrying the biomass, shows promise in retrofitting conventional aeration basins for enhanced nutrient removal.

Key words | simmobilization, nitrification/denitrification, nutrient removal, total nitrogen

Haon-Yao Chen
Sheng-Fu Yang
Cheng-Fang Lin (corresponding author)
Graduate Institute of Environmental Engineering, National Taiwan University, Taipei 106, Taiwan
E-mail: *cflin@ntu.edu.tw*

Pui-Kwan Andy Hong
Department of Civil and Environmental Engineering, University of Utah, Salt Lake City, UT 84112, USA

Ping-Yi Yang
Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA

Kok Kwang Ng
Department of Civil and Environmental Engineering, National University of Singapore, Singapore 117576, Singapore

Chien-Hsien Lee
Sewage System Office, Construction and Planning Agency, Ministry of Interior, New Taipei City 242, Taiwan

ABBREVIATIONS

CAG	Calcium alginate gel
CND	Concurrent nitrification and denitrification
COD	Chemical oxygen demand (mg/L)
DO	Dissolved oxygen (mg/L)
EMMC	Entrapped mixed microbial cell
HRT	Hydraulic retention time (h)
MLSS	Mixed liquor suspended solids (mg/L)
NH_3-N	Ammonia nitrogen (mg/L)
NO_3-N	Nitrate nitrogen (mg/L)
NO_2-N	Nitrite nitrogen (mg/L)
PR	Packing ratio
SCOD	Soluble chemical oxygen demand (mg/L)
SND	Simultaneous nitrification/denitrification
TKN	Total Kjeldahl nitrogen (mg/L)
TN	Total nitrogen (mg/L)
WWTP	Wastewater treatment plant

doi: 10.2166/wrd.2015.087

INTRODUCTION

Engineers employ bacteria to treat human and industrial wastes to preserve environmental quality and ecosystems. Activated sludge in aeration basins of wastewater treatment plants is one example of bacteria treating organic wastes. In an aerobic environment with sufficient organic carbon, heterotrophic bacteria predominate over slow-growing autotrophic micro-organisms, including nitrifying bacteria that oxidize ammonia to nitrite and nitrate. Maintaining the desired biomass in a continuous-flow reactor can be challenging when the desired bacteria's half-life is longer than the hydraulic retention time (HRT), which would result in washing out of the bacteria and thus a shortage of desirable bacteria for waste degradation in the reactor. Recirculation aside, keeping the desirable bacteria in the flow reactor from being washed out may involve naturally attached biofilm and artificially immobilized biomass (as in bioplates or biocarriers). Ng *et al.* (2010) reported that biocarriers entrapping high concentrations of initial biomass resulted in less washout than the conventional activated sludge process. In addition, submerged membrane systems offer alternatives to retain biomass in the treatment system, enabling the desirable slow-growing bacteria to be cultivated under specific environments and keeping them from being washed out (Ng *et al.* 2014). These two techniques have been studied for two decades (Focht & Chang 1975; Pajak & Loehr 1976). Bacteria naturally attach to the disk surface of a rotating biological contactor and to the media surface of a trickling filter; both involve naturally attached biofilm. During operation, the attached biofilm grows to a certain thickness and sloughs off from the media. However, artificial immobilization techniques entrap or encapsulate the selected bacteria in a structural support, allowing the microbes to carry out desirable functions. Other artificial immobilization techniques include adsorption, cross-linkage, and embedment. As the microbes are immobilized, they do not slough off as the naturally attached biofilm does.

Immobilized biomass offers advantages over suspended activated sludge systems and naturally attached biofilms. With immobilization, a pure culture can be created and the microbes can be confined in the system regardless of the flow configuration, maintaining treatment efficiency of the system. Beginning in 1980s, researchers in the Netherlands, Japan, and USA began developing immobilized units, investigating materials suitable for immobilizing micro-organisms. Van Ginkel *et al.* (1983) used carrageenan and alginate gel to co-immobilize the nitrifier *Nitrosomonas europaea* and denitrifiers *Pseudomonas denitrificans* and *Paracoccus denitrificans* in a double-layer gel bead. They showed that oxidation of ammonia and reduction of nitrite/nitrate proceeded in the same unit, namely, via nitrification occurring at the outer layer of the beads, where dissolved oxygen (DO) was sufficient, and denitrification occurring in the anaerobic inner core, where DO was limited by transport. The polymeric gel bead containing the co-immobilized micro-organisms was given the name of 'magic bead' to recognize its ability for simultaneous nitrification/denitrification (SND). The experimental achievement was simulated in a mathematical model describing the integrated nitrification and denitrification by *Nitrosomonas europaea* and *Pseudomonas* sp., as immobilized in separate layers of the double-layer gel bead (dos Santos *et al.* 1996a, b). Although the gel beads made of k-carrageenan or Ca-alginate enabled SND, the gel supports lacked mechanical strength and durability and they lasted for 40–50 days in use. Kokufuta *et al.* (1987) reinforced the calcium alginate gel (CAG) structure with potassium polyvinyl alcohol sulfate and trimethylammonium glycol chitosan iodide (termed PEC). *Nitrosomonas europaea* and *Paracoccus denitrificans* were co-immobilized using PEC-stabilized CAG. They demonstrated that 400 mg/L of ammonia-N were removed in 100–150 h with no residual nitrite. However, the immobilized PEC-CAG sustained activities for 1 month because of the same issue, i.e., its fragile structural support. Morita *et al.* (Uemoto & Saiki 1996, 2000; Morita *et al.* 2007, 2008) developed the denitrifying packed gel envelopes for an industrial wastewater containing 150 mg N/L nitrate and total organic carbon (TOC) of 1.8–11 mg C/L. They used the photo-crosslinkable polymer PVA-SbQ (SPP-H-13, Toyo Gosei Kogyo Co., Tokyo, Japan) to immobilize and carry the nitrifier *N. europaea* and denitrifier *P. denitrificans*, and packed them to create envelopes. The internal space of the packed gel envelopes was injected with ethanol as a carbon source for denitrification of nitrate; therefore only a stochastic amount of ethanol was supplied to the denitrifier.

Based on the demonstrated immobilization techniques, it appears promising that micro-organisms especially slow-growing bacteria can be entrapped in engineered continuous-flow treatment systems to carry out desired functions if only the structural support is strong, porous for substance transport, resistant to biodegradation, water-insoluble, and readily fabricated. None of the support materials described above meet these requirements; in particular, they lack mechanical strength and durability when used in wastewater treatment systems. Yang *et al.* (1988) used cellulose triacetate as the support because of its robust nature and good mechanical strength; they referred to it as the entrapped mixed microbial cell system because of the mixed cultures in the immobilized biomass. This approach lends itself well to wastewater treatment scenarios as any initially immobilized cells are expected to vanish and be replaced by complex cultures adapted to the treatment environment (Yang & See 1991; Yang *et al*. 1997, 2003b; Yu *et al.* 2009; Ng *et al.* 2010, 2011; Wang *et al.* 2012).

As wastewater discharge standards regarding nitrogen compounds become more stringent, there is an urgent need for wastewater treatment plants (WWTPs) to upgrade existing treatment processes for total nitrogen (TN) removal. WWTPs have long used the activated sludge process in aeration basins to remove organics, by oxidation of biological oxygen demand and nitrification of ammonia nitrogen. However, the process provides inadequate removal of TN because the oxic environment in the basin is unfavorable for denitrification. Retrofitting the aeration basin with anaerobic/anoxic/oxic chambers and attached growth processes are alternatives to enable denitrification (Kremen *et al.* 2005; Gujer 2010). However, significant renovation and interruption of the treatment facility would be necessary. As a remedy, we have developed the biomass-immobilized bioplates which will provide anaerobic zones in the aeration basin with minimal retrofitting effort. The bioplate creates anaerobic condition within its core due to oxygen transport limitation (Liu *et al.* 2013). Our recent studies showed promising concurrent carbon and nitrogen removal from different wastewaters in the laboratory (Yang *et al.* 2002, 2003a; Kim & Yang 2004; Cho *et al.* 2007; Zhu *et al.* 2011; Chao *et al.* 2014a, b). We report here a pilot test of the bioplates for TN removal at a WWTP in Taipei, Taiwan. A pilot aeration basin of 1.3 m^3 with conventional activated sludge process was coupled with bioplates and tested for TN removal. The goal was to demonstrate TN removal that could be implemented in municipal WWTPs without significant interruption and renovation of existing facilities.

METHODS

Pilot system setup and operations

The bioplates were prepared by immobilizing activated sludge in cellulose triacetate as a carrier as originally described (Yang *et al.* 1997). Briefly, activated sludge (500 g, 90% water content, obtained from a conventional WWTP treating wastewater from food production) was added into 1 L of a cellulose triacetate/methylene chloride solution (1 L, 10% by w/v) to form a mixture. The mixture was transferred into a horizontally positioned wooden frame of $30 \times 20 \times 1$ cm acting as a mold to contain the mixture and shape it into a hardened bioplate as the solvent evaporated. Each bioplate contained about 30 g (dry weight) of immobilized activated sludge. The bioplates were placed in a batch reactor and acclimated for 1 week by daily replacement of the contact water with a synthetic wastewater (containing $MgSO_4 \cdot 7H_2O$, 21; $MnSO_4$, 2.7; K_2HPO_4, 290; KH_2PO_4, 140; $FeCl_3 \cdot 6H_2O$, 0.13; $CaCl_2$, 3.8; $(NH_4)_2SO_4$, 130; Sucrose, 270; all in mg/L) to establish a daily chemical oxygen demand (COD) removal of 85–90% for 2–3 consecutive days within the acclimation period. When 140 bioplates were installed in the reactor of working volume of 1.3 m^3, the system contained an equivalent mixed liquor suspended solids (MLSS) concentration of 3,500 mg/L at a packing ratio (PR; defined as total bioplate volume/reactor liquid volume) of 6.5%. The bioplate-packed reactor was then loaded with activated sludge at MLSS of 1,500–2,500 mg/L for treatment trials.

The pilot reactor was sited at Nafu wastewater treatment plant in Taipei, Taiwan. Figure 1 illustrates the setup, which consisted of a reservoir, the bioplate-augmented aeration basin, and a clarifier along with operation equipment, such as an air compressor, programmable logic control unit, pumps, and flow regulators. The 2-m^3 reservoir received water from the primary effluent (i.e., after primary sedimentation) and fed the aeration basin (the augmented pilot reactor) by gravity flow and gate valves. By adjusting the gate valve, the feed to the pilot reactor was kept at 0.9–3.8 L/min, encompassing a HRT range of 6–24 h. Air diffuser pipes were installed along the reactor bottom, and aeration was operated in continuous or intermittent mode of varied duration to maintain varied DO up to 8 mg/L. The clarifier provided clarified secondary effluent and returned a part of the thickened underflow (e.g., at 90 L/h) to the aeration basin to maintain the desired MLSS. The discharge of the pilot system was delivered by gravity to the full-size aeration basin of the WWTP.

Experimental parameters were varied, including HRT (6, 12, or 24 h), aeration in continuous and intermittent modes (1 h on/1 h off or 0.5 h on/0.5 h off), with or without alkalinity

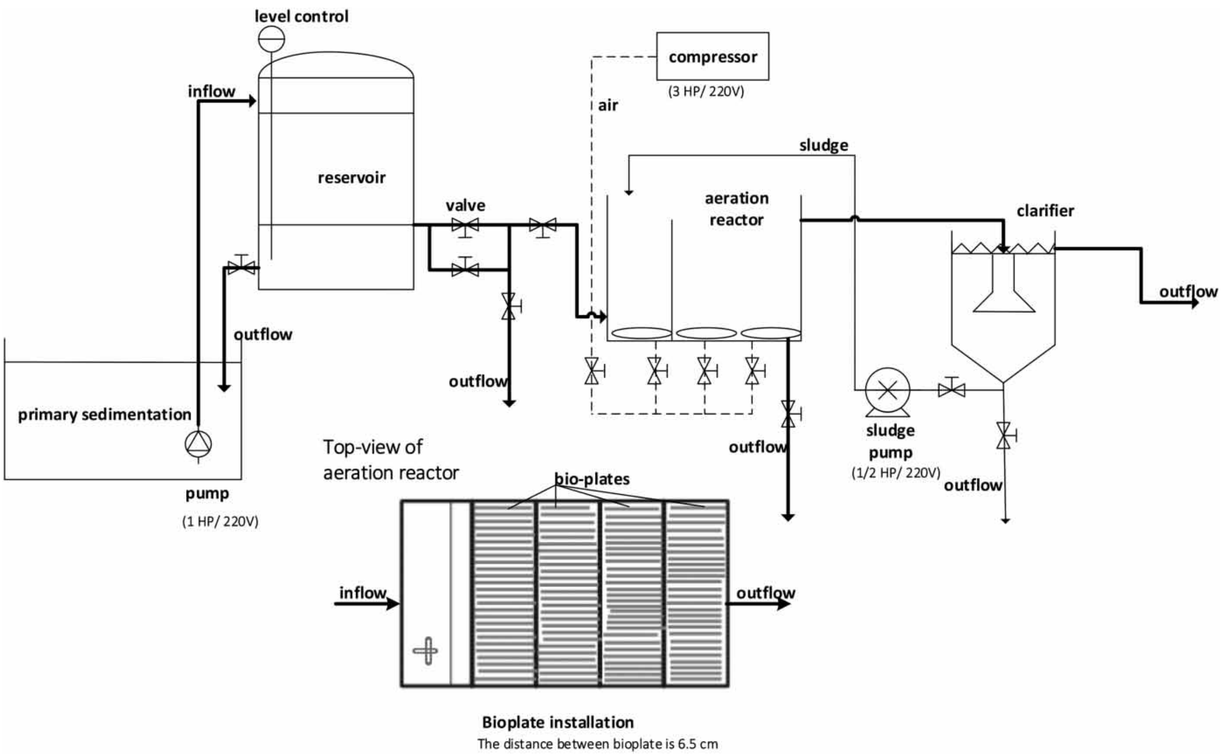

Figure 1 | Schematic diagram of pilot system with bioplates-augmented aeration basin.

amendment (Na_2CO_3, 145 mg/L), and with or without carbon amendment (CH_3OH, 115 mg/L). Each experiment was allowed to continue for 1 week to allow establishment of the steady-state prior to data collection. Control experiments without the bioplates were conducted in identical conditions for comparison of nutrient removal. The reactor was operated at ambient temperature (20–30 °C). The treatment experiments were replicated three to five times.

Analytical methods

Water flowing into and out of the aeration basin was sampled daily for analyses of soluble chemical oxygen demand (SCOD), ammonia nitrogen (NH_3-N), and nitrate nitrogen (NO_3-N) according to *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 DO/pH meter (HACH HQ30d/HACH sensION1). SCOD was analyzed by the HACH closed reflux colorimetric method (detection range of 3–150 mg/L and 20–1,500 mg/L; HACH method 8000) with a spectrophotometer (HACH DR 2800). NH_3-N was analyzed by the Nessler method (detection range 0.02–2.50 mg/L; HACH method 8038). Nitrite nitrogen (NO_2-N) was analyzed by the diazotization method (detection range 0.002–0.300 mg/L; HACH method 8507). NO_3-N was analyzed by the cadmium reduction method (detection range of 0.1–10.0 mg/L; HACH method 8171). Total Kjeldahl nitrogen (TKN) analysis was analyzed by colorimetry with preliminary digestion method. TN represented the sum of TKN, NO_2-N, and NO_3-N.

RESULTS AND DISCUSSION

Although Nafu WWTP received primarily domestic wastewater without industrial sources, the plant's primary effluent that fed the pilot aeration basin contained highly varied SCOD of 40–120 mg/L, NH_3-N of 9–30 mg/L, and NO_2-N/NO_3-N of <1.5 mg/L during the project period of April–December 2013. The TKN and NH_3-N concentrations were very close to each other (88–99%), suggesting inorganic ammonium nitrogen was predominant. Table 1 shows the

Table 1 | Removal (%) of COD, TKN, and TN in the aeration basin (MLSS of 1,500–2,500 mg/L) with and without bioplates (PR of 3.2% when present)

	COD	TKN	TN
AS	76 ± 5.5	89 ± 16	49 ± 5.8
AS and bioplates	77 ± 5.0	99 ± 0.6	74 ± 4.5

Conditions: DO = 4–6 mg/L, aeration 1 h on: 1 h off, HRT = 6 h; removals determined for influent COD of 70–90 mg/L with standard deviations shown.

influence of bioplates on removals of COD, TKN, and TN by the activated sludge process. While COD removal of about 76% remained unchanged in both instances, TN removal improved from 49 to 74% and TKN removal improved from 89 to 99% when the bioplates were present.

To determine viable operation parameters when the suspended activated sludge process was coupled with bioplates for enhanced TN removal, we varied the DO level, aeration mode, HRT, and PR. Figure 2 shows removals of COD, TKN, and TN at various DO levels (2–4, 4–6, and 6–8 mg/L) and HRTs (6, 12, and 24 h) under continuous aeration. At influent COD of 75–100 mg/L, the effluent COD after treatment was 20–30 mg/L, indicating COD removal of 50–90%. Results of TKN removal (Figure 2, center) indicated that higher TKN removal occurred at higher DO levels (i.e., 4–6 or 6–8 mg/L) across various HRTs. The removal of TKN and TN at HRT of 6 h and DO of 4–6 mg/L appeared to be particularly pronounced, resulting in TN removal from 48 to 56 mg/L in the influent to 9.4–13.3 mg/L in the effluent. Although this particularly pronounced outcome of TN removal could have been a singularity, resulting from a confluence of the highly varied influent concentrations of COD, TKN, and TN, we noted that in all runs the effluent TN was typically 10–15 mg/L under all tested conditions. The nutrient concentrations as well as their ratios varied significantly as a real domestic wastewater was used in this study. Figure 2 shows that for HRT of 6 h, TKN removals at DO of 4–6 and 6–8 mg/L were higher than at DO of 2–4 mg/L, indicating improved nitrification at higher DO. For TN, removal was lower at higher DO because DO was not conducive to denitrification. TN removal was highest at HRT of 6 h and DO of 4–6 mg/L, which was attributed to the highly varied real-time nutrient concentrations in the wastewater. Furthermore, Figure 2(a) shows that COD removal was not improved at the highest HRT, and similarly neither was TN removal improved (Figure 2(c)). We considered that excessively high COD/TN at short HRT was prone to result in residual unconsumed COD in the reactor, which allowed heterotrophic denitrifying organisms to utilize a fraction of the residual COD for denitrification (Grady *et al.* 1999). Therefore, we chose to examine more closely removals under these HRT and DO conditions. Under DO of 4–6 mg/L and HRT of 6 h, TN removal of 83.1% was most significant. Without the bioplates, TN removal solely by the suspended activated sludge process was 49 ± 5.8%. At Nafu WWTP, TN removal had been 24–68% for the process. Therefore, the bioplates contributed to simultaneous nitrification and denitrification in the aerobic environment, which promoted the conversion of oxidized nitrate to nitrogen gas as reported previously (Chao *et al.* 2014a).

Figure 3 shows the effects of aeration modes on COD removal, nitrification, and TN removal at HRT of 6 h and DO of 4–6 mg/L. While aeration in intermittent mode (i.e., 1 h on/1 h off) showed similar removals of COD (77 ± 5.4%) and TKN (97 ± 0.2%) achieved by continuous aeration, the former mode achieved 12% higher TN removal

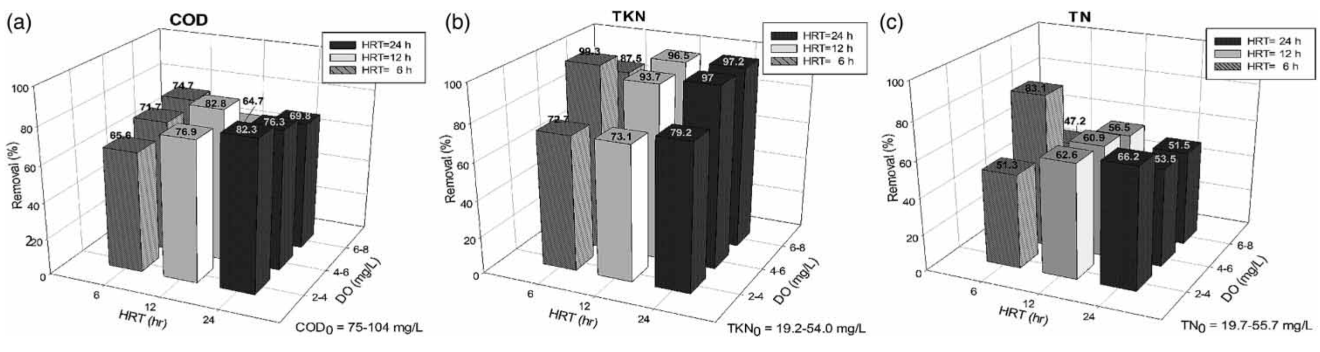

Figure 2 | Removals of COD, TKN, and TN in aeration basin with bioplates at various DO and HRT. (Conditions: MLSS = 1,500–2,500 mg/L, PR = 6.5%, continuous aeration; replicates of 3-d with standard deviations as shown.)

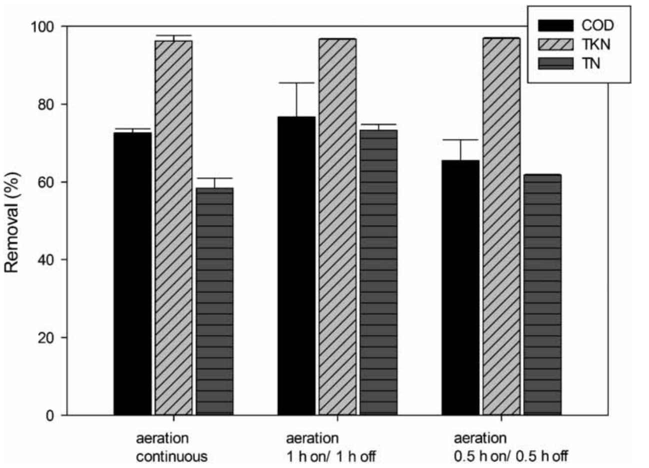

Figure 3 | Removal of COD, TKN, and TN with bioplates according to aeration modes. (Condition: MLSS = 1500–2500 mg/L, DO = 4–6 mg/L, HRT = 6 hour, PR = 6.5%; replicates of 3 d with standard deviations shown in error bars.)

than the latter. The merit of bioplates lies in the inherent mass transfer limitation through the bioplates that limits DO transport into the inner zone, creating an anoxic environment for the denitrifiers to convert nitrate into nitrogen gas. Therefore, the DO level at the water-bioplate interface dictated a DO concentration gradient across into the bioplate medium, resulting in an anoxic zone within the bioplates. As factors such as microbial activity, DO, substrate availability, and other environmental parameters could affect the overall nitrification and denitrification reactions of the system, experimental runs using an online real wastewater would provide an apparent system performance under complex, real conditions. While it might be difficult to fundamentally explain the influence of each factor, it would more clearly show the effects of bioplates when they were coupled to the conventional suspended growth process under complex settings at the WWTP.

The amendment of carbon and alkalinity was evaluated for the bioplate-augmented aeration basin under varied PR of 3.2, 6.5, 9.1, and 11.4% while holding HRT constant at 6 h and DO at 4–6 mg/L via intermittent aeration of 1 h on/1 h off. Table 2 presents the removal of COD, TKN, and TN under varied PR with and without methanol and sodium carbonate addition, i.e., carbon and alkalinity, respectively. For example, at PR of 6.5%, COD removal was above 80% under all conditions. With methanol addition, TN was reduced from 28 ± 4.0 to 4.3 ± 0.4 mg/L, corresponding to a TN removal of 84 ± 2.8%. Without methanol (carbon) amendment, residual TN was 9.2 ± 1.5 mg/L, indicating insufficient electron donor in the original wastewater for use by nitrifying bacteria in the bioplates. The results showed enhanced TN removal by entrapped nitrifying and denitrifying bacteria in the bioplates, which were enhanced when carbon and/or alkalinity were amended.

When increasing presence of bioplates to a PR of 9.1% without supplemental carbon or alkalinity, the residual TN remained at about 11 ± 0.3 mg/L and the residual TKN at about 3.9 ± 0.5 mg/L (reduced from an influent TN of 25 ± 1.9 mg/L). COD removal was 72 ± 3.0% and TN removal was 57 ± 4.5% with 84 ± 2.7% nitrification. When supplemented with carbon or with both carbon and alkalinity, the effluent TN was reduced to 4.7 ± 1.5 and 3.6 ± 1.9 mg/L, respectively. At the same time, the effluent TKN was reduced to 2.9 ± 1.2 mg/L, indicating excellent nitrification performance. The results show that increasing the PR of bioplates has not led to proportionally increased TN removal. Further increasing bioplate PR to 11.4% did not result in TN removal beyond those with PR of 6.5 and 9.1%. Without supplemental carbon or alkalinity, the effluent TN was 8.5 ± 1.0 mg/L from an influent TN of 27 ± 0.1 mg/L. With supplemental carbon or with both supplemental carbon and alkalinity, the effluent TN lowered to 5.2 ± 2.3 mg/L.

The results in Table 2 showed no enhanced TN removal with increased packing of bioplates. Although improved treatment using entrapped biomass had been studied since 1985, much work had focused on the performance and mechanisms of simultaneous nitrification and denitrification with little attention on the extent of immobilized biomass. Morita *et al.* (2007, 2008) developed the packed gel envelopes system using PVA-SbQ gel that occupied 25% of the reactor volume, and they achieved 95% TN removal with supplemental carbon. In the present case, extensive bioplates at PR of 25 or 30% might significantly interfere with the hydraulics and mass transport of pollutants in the aeration basin, causing incomplete mixing and shortened flow path in the basin.

In view of performance with PR of 6.5, 9.1, and 11.4% and potential cost implication, experiments deploying bioplates at PR of 3.2% were studied in detail. The aeration basin with bioplates at PR of 3.2% removed 74 ± 6.7% of COD, 96 ± 2.1% of TKN, and 74 ± 1.3% of TN without supplemental carbon or alkalinity. From an influent TN of 28 ± 0.5 mg/L, the effluent TN was lowered to 7.3 ± 0.3 mg/L, including 1.1 ± 0.5 mg/L

Table 2 | COD, TKN, and TN removals in bioplates-augmented aeration basin

		COD removal (%)		TKN removal (%)		TN removal (%)	
PR	**Additive**	**Inflow COD mg/L**	**Outflow COD mg/L**	**Inflow TKN mg/L**	**Outflow TKN mg/L**	**Inflow TN mg/L**	**Outflow TN mg/L**
3.2%	None	74 ± 6.7%		96 ± 2.1%		74 ± 1.3%	
		82 ± 9.2	22 ± 7.8	27 ± 0.4	1.1 ± 0.5	28 ± 0.5	7.3 ± 0.3
	Methanol	60 ± 15%		85 ± 11%		76 ± 20%	
		180 ± 5.7	75 ± 20.5	29 ± 4.1	4.0 ± 2.5	29 ± 3.9	6.5 ± 4.9
	Methanol and sodium	75 ± 9.1%		96 ± 3.2%		91 ± 5.0%	
	carbonate	180 ± 0.7	46 ± 17	27 ± 1.1	1.1 ± 0.9	27 ± 1.0	2.5 ± 1.5
6.5%	None	85 ± 6.4%		81 ± 7.2%		59 ± 3.5%	
		84 ± 8.2	13 ± 5.8	22 ± 2.0	4.1 ± 1.4	22 ± 2.0	9.2 ± 1.5
	Methanol	92 ± 2.2%		91 ± 2.2%		84 ± 2.8%	
		180 ± 2.2	14 ± 3.9	27 ± 4.1	2.3 ± 0.4	28 ± 4.0	4.3 ± 0.4
	Methanol and sodium	89 ± 1.2%		93 ± 1.4%		86 ± 3.1%	
	carbonate	200 ± 3.6	22 ± 2.6	24 ± 2.4	1.8 ± 0.6	25 ± 2.4	3.4 ± 0.6
9.1%	None	72 ± 3.0%		84 ± 2.7%		57 ± 4.5%	
		78 ± 5.1	22 ± 2.5	24 ± 1.9	3.9 ± 0.5	25 ± 1.9	11 ± 0.3
	Methanol	89 ± 2.1%		88 ± 5.0%		81 ± 5.8%	
		180 ± 3.0	19 ± 3.2	23 ± 0.7	2.9 ± 1.2	24 ± 0.71	4.7 ± 1.5
	Methanol and sodium	72 ± 5.1%		92 ± 10%		85 ± 8.3%	
	carbonate	180 ± 3.5	51 ± 8.7	23 ± 0.3	1.8 ± 2.2	23 ± 0.3	3.6 ± 1.9
11.4%	None	80 ± 4.2%		85 ± 0.0%		69 ± 3.5%	
		96 ± 1.4	19 ± 4.2	27 ± 0.0	4.0 ± 0.0	27 ± 0.1	8.5 ± 1.0
	Methanol	79 ± 12%		89 ± 0.0%		84 ± 2.5%	
		18 ± 11	40 ± 24	28 ± 0.0	3.0 ± 0.0	29 ± 0.1	4.5 ± 0.7
	Methanol and sodium	79 ± 4.1%		82 ± 8.1%		78 ± 5.7%	
	carbonate	190 ± 13	39 ± 5.0	23 ± 1.6	4.1 ± 1.6	24 ± 1.7	5.2 ± 2.3

Conditions: MLSS = 1,500–2,500 mg/L, DO = 4–6 mg/L, aeration 1 h on: 1 h off, HRT = 6 h.

as TKN. The removal of TN by 74% to <10 mg/L in the effluent was remarkable for a conventional activated sludge process augmented with entrapped biomass at a PR of only 3.2%. With supplemental carbon, TKN and TN removal were 85 ± 11% and 76 ± 20%, respectively. The effluent TN was reduced to 6.5 ± 4.9 mg/L including 4 ± 2.5 mg/L in TKN from an influent TN of 29 ± 3.9 mg/L. TN removal reached 91 ± 5.0% when supplemental carbon and alkalinity were provided; the effluent TN was reduced to 2.5 ± 1.5 mg/L including 1.1 ± 0.9 mg/L of TKN from an influent TN of 27 ± 1.0 mg/L. These results suggested the pivotal role of the augmenting bioplates at PR of 3.2% in enabling the denitrifier in an otherwise conventional activated sludge process for the removal of TN, particularly when supplemental carbon was available as the electron donor.

The TN removal results were used to calculate the 'specific TN removal' defined as mass of TN (g TN) removed per unit area (m^2) of bioplates per day, g TN/m^2/d. This parameter assesses the process performance and efficiency of the bioplates and can be used as a reference in engineering design. Specific TN removals are shown in Table 3 for PR of 3.2, 6.5, 9.1, and 11.4%. The specific TN removal for PR of 3.2% was

Table 3 | TN removal performance according to PR of bioplates and supplemental carbon and alkalinity

	TN removal performance (TN g/m^2/d)			
PR addition	**3.2% EMMC**	**6.5% EMMC**	**9.1% EMMC**	**11.4% EMMC**
No	25 ± 0.9	8.2 ± 1.7	6.3 ± 1.0	6.5 ± 0.3
Methanol	32 ± 5.8	15 ± 2.1	8.4 ± 0.4	8.4 ± 0.2
Methanol and Sodium carbonate	31 ± 0.6	13 ± 1.5	8.5 ± 0.9	6.5 ± 1.4

Conditions: MLSS = 1,500–2,500 mg/L, DO = 4–6 mg/L, aeration by alternate 1 h on/off, HRT = 6 h.

25 ± 0.92 g TN/m^2/d without supplemental carbon or alkalinity, and for PR of 6.5%, 9.1%, and 11.4% were 8.2 ± 1.7, 6.3 ± 1.0, and 6.5 ± 0.3 g TN/m^2/d, respectively. The specific TN removal of 25 ± 0.9 g TN/m^2/d is equivalent to a volumetric TN removal rate of 0.083 ± 0.004 kg TN/m^3/d.

Other efforts concerning specific TN removal abound. Kokufuta *et al.* (1987) reported removal of 0.61 kg TN/m^3/d using calcium alginate stabilized by polyelectrolyte complexes. dos Santos *et al.* (1996a, b) reported removal of 1.54 kg TN/m^3/d using calcium alginate and k-carrageenan. Mori *et al.* (1993) reported removal of 0.066 kg TN/m^3/d using polyethylene glycol resin. Uemoto & Saiki (2000) reported removal of 1.60 kg TN/m^3/d using PVA-SbQ. Morita *et al.* (2008) reported removal of 0.252 kg TN/m^3/d using PVA-SbQ. Many of these high specific TN removal rates, such as 0.61, 1.54, and 1.60 kg TN/m^3/day, were achieved with bench top reactors of <0.25 L, and must be assessed at the pilot scale. Further, the specific TN removal of 0.252 kg TN/m^3/d was obtained via packed gel envelopes occupying 25% of the reactor space with supplemental carbon source (Morita *et al.* 2008). While the anaerobic ammonium oxidation (ANAMMOX) process offers several advantages such as high nitrogen removal rates at low-operational costs and smaller carbon footprint than conventional nutrient removal plants, the application of ANAMMOX has been limited by its long start-up period due to the very low growth rate of ANAMMOX bacteria and its incompatibility with an aerobic system (Kuenen 2008). While the high specific TN removal rate of ANAMMOX at >1 kg TN/m^3/d much exceeded that of the bioplate system of this study at 0.083 ± 0.004 kg TN/m^3/d, the ANAMMOX system is a separate system with its own operation requirements and it does not provide complete COD removal. The present bioplate system is readily retrofitted to existing aeration basins for concurrent removal of organic carbon and nitrogen, and is particularly suitable for aerobic conditions. Unlike systems with entrapped biomass that seemed structurally fragile, the present system using cellulose triacetate as support provided a strong structure that lasted over several years of experimental use.

CONCLUSIONS

This pilot study showed that incorporating bioplates of entrapped biomass into a conventional aeration basin added the capability of simultaneous nitrification and denitrification to the aerobic environment. Occupying only 3.2% of the basin space, the bioplates achieved TN removal of 74% from domestic wastewater reducing the TN to 7.3 mg/L in the effluent. With HRT of 6 h and DO of 4–6 mg/L via intermittent aeration of alternate 1 h on and off, the pilot system achieved a specific TN removal of 25 ± 0.9 g/m^2/d, equivalent to a volumetric TN removal of 0.083 ± 0.004 kg TN/m^3/d, without a supplemental carbon source. The present system accomplished this with bioplates occupying only 3.2% of the reactor space and without supplemental carbon source. The PR of 3.2%, which was at the low end of the test range in this study, was found most efficient given its lowest occupied reactor volume, although it might be possible that a lower PR could achieve similar satisfactory removals and thereby reduce the quantity of bioplates (i.e., the occupied reactor space) in the system. The cellulose triacetate provided strong structural support for the entrapped biomass, which had already been used for years. The immobilized biomass technology in the form of bioplates can be implemented in WWTPs without significant interruption and renovation of the facility.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Science and Technology of the Republic of China (Under Contract No. 101-2221-E-002-209-MY3) for financially supporting this research.

REFERENCES

American Public Health Association 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association, Washington, DC.

Chao, Y. N., Lin, J. H., Ng, K. K., Wu, C. H., Hong, P. K. A. & Lin, C. F. 2014a Improving total nitrogen removal in aeration basin retrofitted with entrapped biomass. *Water Sci. Technol.* **69**, 1558–1564.

Chao, Y. N., Ng, K. K., Wu, C. H., Hong, P. K. A. & Lin, C. F. 2014b Enhancing total nitrogen removal from wastewater of a science and industrial park using entrapped biomass. *Environ. Technol.* **35**, 1401–1408.

Cho, E. S., Zhu, J. & Yang, P. Y. 2007 Intermittently aerated EMMC-Biobarrel (entrapped mixed microbial cell with

Bio-barrel) process for concurrent organic and nitrogen removal. *J. Environ. Manage.* **84**, 257–265.

dos Santos, V. A. P. M., Bruijnse, M., Tramper, J. & Wijffels, R. H. 1996a The magic-bead concept: an integrated approach to nitrogen removal with co-immobilized micro-organisms. *Appl. Environ. Microbiol.* **45**, 447–453.

dos Santos, V. A. P. M., Marchal, L. M., Tramper, J. & Wijffels, R. H. 1996b Modeling and evaluation of an integrated nitrogen removal system with microorganisms co-immobilized in double-layer gel beads. *Biotechnol. Progr.* **12**, 240–248.

Focht, D. D. & Chang, A. C. 1975 Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol.* **9**, 153–186.

Grady, J., Daigger, C. P. L. & Lim, H. C. 1999 *Biological Wastewater Treatment*. Marcel Dekker, New York.

Gujer, W. 2010 Nitrification and me – a subjective review. *Water Res.* **44**, 1–19.

Kim, S. J. & Yang, P. Y. 2004 Two-stage entrapped mixed microbial cell process for simultaneous removal of organics and nitrogen for rural domestic sewage application. *Water Sci. Technol.* **49**, 281–288.

Kokufuta, E., Yukishige, M. & Nakamura, I. 1987 Coimmobilization of *Nitrosomonas europaea* and *Paracoccus denitrificans* cells using polyelectrolyte complex-stabilized calcium alginate gel. *J. Ferment. Technol.* **65**, 659–664.

Kremen, A., Bear, J., Shavit, U. & Shaviv, A. 2005 Model demonstrating the potential for coupled nitrification denitrification in soil aggregates. *Environ. Sci. Technol.* **39**, 4180–4188.

Kuenen, J. G. 2008 Anammox bacteria: from discovery to application. *Nature Reviews Microbiol.* **6**, 320–326.

Liu, C. C., Ng, K. K., Wu, C. J., Lin, C. F., Hong, P. K. A. & Yang, P. Y. 2013 Organics and nitrogen removal from wastewater across a plate of entrapped mixed microbial cells. *J. Environ. Sci. Manage.* **16**, 29–35.

Mori, N., Sumino, T. & Shirai, M. 1993 Advanced waste water treatment using immobilized nitrifiers in synthetic resin. *J. Resour. Environ.* **29**, 1057–1062.

Morita, M., Uemoto, H. & Watanabe, A. 2007 An additional simple denitrification bioreactor using packed gel envelops applicable to industrial wastewater treatment. *Biotechnol. Bioeng.* **97**, 1439–1447.

Morita, M., Uemoto, H. & Watanabe, A. 2008 Nitrogen-removal bioreactor capable of simultaneous nitrification and denitrification for application to industrial wastewater treatment. *Biochem. Eng. J.* **41**, 59–66.

Ng, K. K., Lin, C. F., Lateef, S. K., Panchangam, S. C., Hong, P. K. A. & Yang, P. Y. 2010 The effect of soluble microbial products on membrane fouling in a fixed carrier biological system. *Sep. Purif. Technol.* **72**, 98–104.

Ng, K. K., Lin, C. F., Panchangam, S. C., Hong, P. K. A. & Yang, P. Y. 2011 Reduced membrane fouling in a novel bio-entrapped membrane reactor for treatment of food and beverage processing wastewater. *Water Res.* **45**, 4269–4278.

Ng, K. K., Shi, X., Tang, K. Y. M. & Ng, H. Y. 2014 A novel application of anaerobic bio-entrapped membrane reactor for the treatment of chemical synthetic-based pharmaceutical wastewater. *Sep. Purif. Technol.* **132**, 634–643.

Pajak, A. P. & Loehr, R. C. 1976 Treatment of poultry manure wastewater using a rotating biological contactor. *Water Res.* **10**, 399–406.

Uemoto, H. & Saiki, H. 1996 Nitrogen removal by tubular gel containing *Nitrosomonas europaea* and *Paracoccus denitrificans*. *Appl. Environ. Microbiol.* **62**, 4224–4228.

Uemoto, H. & Saiki, H. 2000 Nitrogen removal reactor using packed gel envelopes containing *Nitrosomonas europaea* and *Paracoccus denitrificans*. *Biotechnol. Bioeng.* **67**, 80–86.

Van Ginkel, C. G., Tramper, J., Luyben, K. C. A. M. & Klapwijk, A. 1983 Characterization of *Nitrosomonas europaea* immobilized in calcium alginate. *Enzyme Microbial. Technol.* **5**, 297–303.

Wang, C. H., Liu, J. C. W., Ng, K. K., Lin, C. F., Hong, P. K. A. & Yang, P. Y. 2012 Immobilized bioprocess for organic carbon and nitrogen removal. *Desal. Water Treat.* **37**, 296–301.

Yang, P. Y. & See, T. S. 1991 Packed entrapped mixed microbial cell process for removal of phenol and its compounds. *J. Environ. Sci. Health A.* **26**, 1491–1512.

Yang, P. Y., Chi, T. & Wang, M. L. 1988 Immobilized mixed microbial cells for wastewater treatment. *Biol. Wast.* **23**, 295–312.

Yang, P. Y., Zhang, Z. Q. & Jeong, B. G. 1997 Simultaneous removal of carbon and nitrogen using an entrapped-mixed-microbial-cell process. *Water Res.* **31**, 2617–2625.

Yang, P. Y., Cao, K. & Kim, S. J. 2002 Entrapped mixed microbial cell process for combined secondary and tertiary wastewater treatment. *Water Environ. Res.* **74**, 226–234.

Yang, P. Y., Chen, H. J. & Kim, S. J. 2003a Integrating entrapped mixed microbial cell (EMMC) process for biological removal of carbon and nitrogen from dilute swine wastewater. *Bioresour. Technol.* **86**, 245–252.

Yang, P. Y., Su, R. & Kim, S. J. 2003b EMMC Process for combined removal of organics, nitrogen and an odor producing substance. *J. Environ. Manage.* **69**, 381–389.

Yu, T. H., Lin, A. Y. C., Lateef, S. K., Lin, C. F. & Yang, P. Y. 2009 Removal of antibiotics and non-steroidal anti-inflammatory drugs by extended sludge age biological process. *Chemosphere* **77**, 175–181.

Zhu, J., Lin, C. F., Kao, J. C. M. & Yang, P. Y. 2011 Evaluation of potential integration of entrapped mixed microbial cell and membrane bioreactor processes for biological wastewater treatment/reuse. *Clean Technol. Environ. Policy* **13**, 153–160.

First received 2 October 2014; accepted in revised form 11 November 2014. Available online 4 February 2015