

Achieving partial nitrification in a novel six basins alternately operating activated sludge process treating domestic wastewater

Rusul Naseer Mohammed^{*,**}, Saad Arab^{*,**}, and Lu Xiwu^{*,†}

^{*}School of Energy and Environment, Southeast University, Sipailou Road, Nanjing 210096, P. R. China

^{**}Faculty of Engineering, University of Basrah, Basra, Iraq

(Received 6 July 2013 • accepted 26 August 2013)

Abstract—A novel technology was developed to achieve partial nitrification at moderately low DO and short HRT, which would save the aeration cost and have the capacity to treat a wide range of low-strength real wastewater. The process enables a relatively stable whereas nitrite accumulation rate (NO₂-AR) was stabilized over 94% in the last aerobic basin on average of each phase through a combination of short HRT and low DO level. Low DO did not produce sludge with poorer settleability. The morphology and internal structure of the granular sludge was observed by using a scanning electron microscope (SEM) analysis during a long-term operation. The images indicated that thick clusters of spherical cells and small rod-shaped cells (NOB and AOB are rod-shaped to spherical cells) were the dominant population structure, rather than filamentous and other bacteria under a combination of low DO and short HRT, which gives a good indication of nitrite accumulation achievement. MPN method was used to correlate AOB numbers with nutrient removal. It showed that an ammonia-oxidizing bacterium (AOB) was the dominant nitrifying bacteria, whereas high NO₂-AR was achieved at AOB number of 5.33×10^8 cell/g MLSS. Higher pollutant removal efficiency of 86.2%, 98% and 96.1%, for TN, NH₄-N, and TP, respectively, was achieved by a novel six basin activated sludge process (SBASP) at low DO level and low C/N ratio which were approximately equal to the complete nitrification-denitrification with the addition of sodium acetate (NaAc) at normal DO level of (1.5-2.5 mg/L).

Key words: SBASP, N-P Removal, Nitrite Accumulation Rate, NO₂-AR, SEM Analysis

INTRODUCTION

Achieving higher pollutant removal efficiency with less energy consumption has become a very urgent and critical task for wastewater treatment plant operation. Many biological technologies and processes have been developed for nitrogen removal from wastewater over the past few decades [1]. Nitrification-denitrification process over nitrite is a more sustainable fashion to the traditional nitrification-denitrification process [2-4]. Recently, nutrient removal via nitrite was reported to be technically feasible and economically favorable, which termed partial nitrification of NH₄⁺-N to nitrite (nitrification) and subsequently direct reduction of nitrite to N₂ gas (denitrification) [5]. This process requires less oxygen and less organic carbon in comparison with traditional nitrification-denitrification. The reduction in the oxygen demand amounts to 24% and the reduction of carbon to approximately 40%, as well as lower biomass production and increased kinetics [6,7]. The enrichment of ammonia oxidizing bacteria (AOB) and limitation-washout of nitrite oxidizing bacteria (NOB) is the critical point for stable maintaining of partial nitrification via nitrite [8,9]. Several process parameters, such as dissolved oxygen (DO) concentration, temperature, sludge retention time (SRT), substrate concentration, aeration pattern, aeration duration, and inhibitors, have been found to selectively inhibit or washout NOB [10,11]. For this reason, a better understanding of the ecology and microbiology of AOB in wastewater treatment systems is necessary to enhance treatment performance in our proposed pro-

cess technology. Until now, a significant amount of research has focused on the partial nitrification achieved by low DO [9,12,13]. Presently, most of the studies on NO₂-AR have concentrated on the SBR process [14]. There are only a few studies on partial nitrification via nitrite in a continuous flow for treatment domestic wastewater with high ammonia concentrations [15]; moreover, the short-term effect of DO on biological nitrogen removal has been discussed in many studies using batch test [16,17]. However, limited reports are available on comparisons of partial nitrification via nitrite (NO₂-AR) performance under different DO for long-term operation. It is still doubtful whether high DO levels would destroy the stable and high nitrite accumulation ratio (NO₂-AR) built by low DO or other operational factors. Additional studies are necessary to study the shift of microbial community structure as the partial nitrifying sludge exposes to different DO level over long-term runs operation.

Based on above questions, a new technology of six basins alternately operated activated sludge process (SBASP) was designed like SBR in control methodology and AA/O in spatial structure and its own property. It is composed of a rectangular box divided by baffles to form a six-basin reactor. The direction of flow in SBASP is changed automatically through changing intake location; thus our technology achieves automatic reflow without need for reflow equipment for sludge and mixed liquor. This is the main difference between our technology and other common activated sludge process technologies. Consequently, the six basin activated sludge process (SBASP) is effective for reducing energy consumption.

The objectives of this study were (1) to develop a simple start-up strategy of partial nitrification via nitrite particularly with regard to the effect of low DO concentration and short HRT in continuous

[†]To whom correspondence should be addressed.

E-mail: alotito@seu.edu.cn, saadaboalheel@yahoo.com

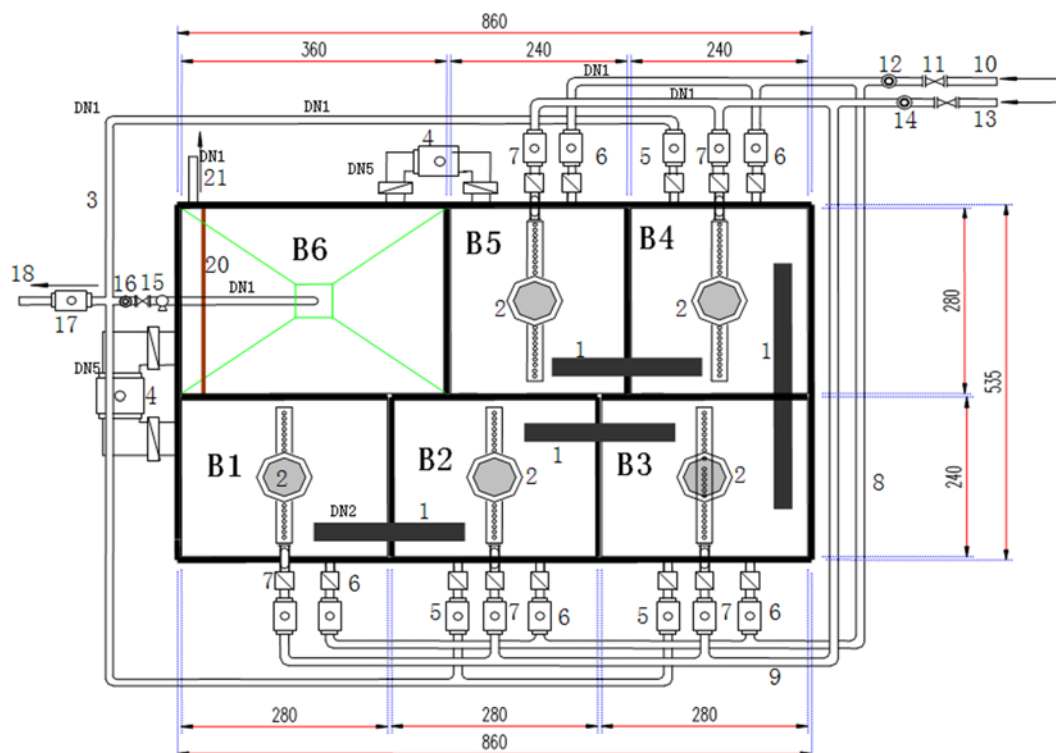


Fig. 1. Configuration of SBASP system with all main part.

B1, B2, B3, B4, B5, six basins, B6-settling tank, 1-internal pipe connection, 2-mixer, 3-electrical sludge return valves line, 4-external pipe connection, 5-electrical sludge return valve c, 10-inlet pipe, 11-inlet valve, 14-inlet aeration valve, 10-inlet aeration pipe, 6-inlet electromagnetic valves, 7-aeration electromagnetic valves, 18-excess sludge pipe, 17 excess electromagnetic sludge pipe, 15, 16-sludge discharge valves, 21-effluent pipe, 8-wastewater network, 9-aeration network

flow SBASP treating domestic wastewater with low carbon source, and (2) to find the shift of microbial community structure and the morphology of the sludge when the partial nitrifying sludge exposes to different DO level using MPN method and scanning electron microscope, respectively throughout the whole operation period.

MATERIALS AND METHODS

1. Pilot-scale Treatment System

Lab experiments were conducted in a new pilot scale SBASP which is composed of six tanks, separated by baffles with a working volume of 0.5 ton/day. The first five tank sequence in terms of any tank is typically operated under different environment state conditions (anaerobic/anoxic-aerobic) based on phase type, while the last tank is operated as a clarifier. All basins except the last one have the same rectangular plane 280×240 mm and are supplied with mechanical mixers and air diffusers for providing a suitable state condition (anaerobic-anoxic/oxic) in the same basin. The particular advantages of SBASP include compact construction, space-saving, cost-effectiveness, flexible operation and ease of maintenance. The SBASP system achieved automatic mixed liquor return due to automatic intake location changing. The main parts of the pilot plant utilized in this study are the main body, which is a rectangular box of 860×535×905 mm, pre-static pumps, mechanical agitation mixers, PLC programmable logic control, LCD display screen, inlet wastewater electromagnetic valves, outlet water electromagnetic valves, aeration electromagnetic valves, sludge discharge electromagnetic valves,

and PVC pipes. The principal diagram of the pilot plant with all major components is shown in Fig. 1. The effective water depth in the SBASP is 700 mm, while the total depth is 900 mm. Oxygen is supplied by an air compressor through an air diffuser inside the reactor. The reactor does not need equipment device for mixed liquor and sludge return. It contains an external recycle (returned sludge) without reflow device from a clarifier to other biological basins.

2. Wastewater and Sludge Characteristics

The raw wastewater was taken from Wuxi campus, southeast university. Approximately 1,000 L of wastewater was pumped from the sewer line and transported to the hall laboratory every day. To minimize the variation of wastewater characteristics from day to day, municipal wastewater was collected at approximately the same time each day. Average influent COD to nitrogen ratio (C/N) was only about 2.53, and thus the organic matter was typically limited. The characteristics of the wastewater quality are listed in Table 1.

Sludge samples were collected from the last aerobic basin (basin

Table 1. Characteristics of the raw wastewater

Contents	Range	Average
TN (mg/L)	48.5-65.62	56.4±
NH-N (mg/L)	37.6-42.8	40.6±
TP (mg/L)	3.53-4.73	3.68±
COD (mg/L)	118.4-273	125.3±
C/N	1.69-3.88	2.53±

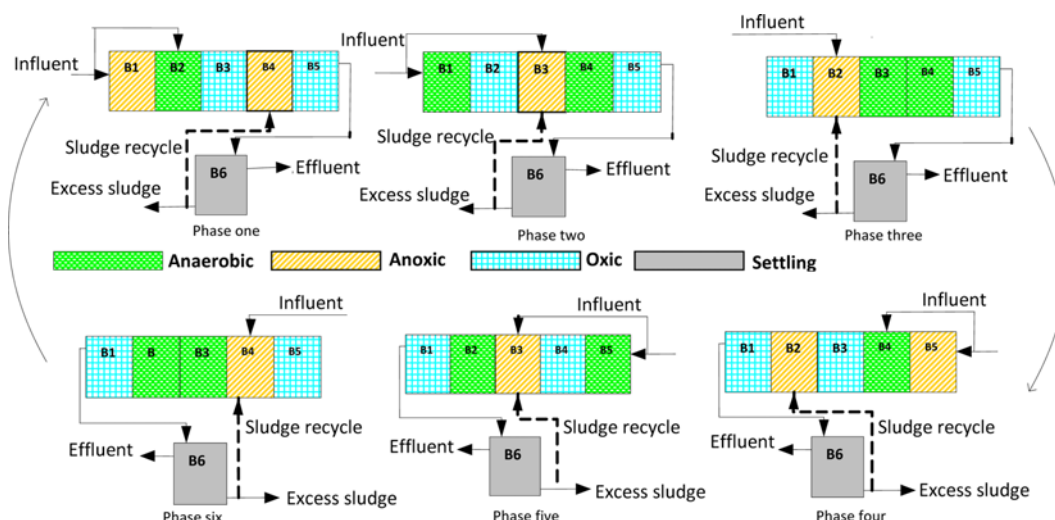


Fig. 2. Scheme of six basin alternating operating activated sludge system.

five) of each phase in a new pilot scale SBASP at 46, 76, 88, 140, 155, 158, 176, 177, 189, 210, 234, 246, 258, 270, 249 and 303 day.

3. Experimental Procedure

An operation cycle is composed of two half-cycles with the same running schemes as shown in Fig. 2. It is divided into six phases, named as phases 1, 2 and 3 during the first half cycle and phases 4, 5 and 6 during the second half cycle. The optimum phase time was investigated previously to know the optimum fixed time of SBASP, whereas this operation parameter has a great effect on nutrient removal efficiency. It was established according to desired biochemical transformations, whereas the optimum running time was 3, 2.5, 2, 3, 2.5 and 2 hr of phase I, II, III, IV, V and VI, respectively. The optimum operating parameters were 13 d of SRT, 15 hr of a HRT, 10% of air/flow ratio and 35% of sludge recycle ratio. Step feed influent was pumped into both anoxic and anaerobic zone depending on biological reaction, whereas the influent flow distribution ratio for the two reactors was 1 : 2. Six basins alternating operating activated sludge system was operated for 10 months, including six successive runs (Table 2). Run number I was implemented to investigate the nutrient removal and partial nitrification via nitrite ($\text{NO}_2\text{-AR}$) throughout a combination of short HRT with normal DO of (1.5-2)

mg/L concentration. Based on the operation in run number I, (NaAc) was added as a carbon source for denitrification during run number II. Both phases were implemented for comparison with runs number IV and V that performed $\text{NO}_2\text{-AR}$. The aim of run number III was to determine if partial nitrification performance ($\text{NO}_2\text{-AR}$) could be achieved by controlling DO level at 0.2 mg/L and SRT at seven days. Run number IV was performed to investigate the influence of a combination of low DO concentration and low HRT control on the establishment of $\text{NO}_2\text{-AR}$. Run number V was provided to improve TN, TP and $\text{NH}_4^+\text{-N}$ removal for long-term operation in SBASP at low DO level based on a stable performance of partial nitrification performance ($\text{NO}_2\text{-AR}$). The sludge retention time (SRT) was extended in run number VI to demonstrate the effect of SRT control on the stable performance of partial nitrification via nitrite.

4. Analytical Procedure

The analytical methods for COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN were analyzed according to standard methods [18]; $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were analyzed by the IC method (Metrohm 761 compact IC equipped with Metrosep A Supp 5 column), while TN was analyzed by Analytic Jena AG multi N/C 3000. DO and pH were measured on-line using DO/pH meters. Mixed liquor volatile sus-

Table 2. Experimental schemes for partial nitrification in SBASP process treating domestic wastewater

Run no.	Days ^a	DO (mg/l)	Influent flow rate (L/h)	Flow rate of sludge recycle (L/h)	HRT (hr)	SRT(d)
I	37-67	1.5-2.5	24.4	7.3	9.1	10
II ^b	68-79	1.5-2.5	24.4	7.3	9.1	10
III	^d 113-134	0.1-0.2	15	4.71	14.2	
	135-167	0.4-0.5	24.4	7.3	9.1	10
IV	168-200	0.4-0.5	24.4	7.3	9.1	10
	201-219	0.4-0.5	20	6	11	10
V	^d 234-270	0.4-0.5	18	5.4	13	13
VI ^c	^d 285-303	0.4-0.5	24.4	7.3	9.1	16

^aDay 1-37: Inoculation for AOB, NOB, sludge by SBAS reactor

^bAdd NaAc (COD=179 mg/l)

^cAdd NaAc (COD=95 mg/l)

^d15 days operation for steady-state in a continuous-flow SBASP to regulate SRT

pended solid (MLVSS) and MLSS were measured according to the APHA standard methods [19]. The maximum probable number method (MPN) was used to find the two types of ammonia oxidizing bacteria and nitrite oxidizing bacteria. The mineral medium used for AOB was modified from [16,17] and the MPNs were calculated depending on [20-22].

5. Scanning Electron Microscope Observation

The morphology of the bacteria was examined with high resolution (JSM-6360LV, Japan). The sludge samples were pretreated by fixing with 2.5% glutaraldehyde in a 0.1 M phosphate buffer. Subsequently, the samples were washed and dehydrated in a graded series of ethanol solution (50%, 70%, 80%, 90%, and 100%) for 15 min. Ethanol in dehydrated samples was displaced by 1 : 1 (v/v) of ethanol to isoamyl acetate for 30 min with slight shaking and then by 100% isoamyl acetate for 30 min. These treated samples were dried by the CO₂ critical point drying. The treated sludge samples were observed after spray-gold treatment, using a scanning electron microscope (JSM-6360LV, Japan).

RESULTS AND DISCUSSION

1. Achieving Partial Nitrification by a Novel Technology SBASP

The temperature of the water in the SBASP reactor was maintained at 24 °C and pH varied from 7.12 to 7.43. This temperature and pH range could not be used as electron acceptor to expand the differences of specific growth rates between AOB and NOB. Therefore, temperature and pH parameters were unlikely factors contributing to partial nitrification via nitrite, in contrast to low DO concentrations and HRT control. Fig. 3 shows an overview of average NO₂-AR, nitrite-N and nitrate-N concentrations during the first half cycle. In runs I and II, six basins alternately operating activated sludge system was operated at a normal DO concentration of 1.5-2.5 mg/L and total HRT of 9.1 h. The NO₂-AR was almost not obvious during these runs. Very low DO condition (0.2 mg/L) was applied in the initiation of run III, from (113-134) day to eliminate NOB through expanding the differences of specific growth rates between AOB and NOB with limited aeration. The results showed that nitrite build-up did not occur where NO₂-AR was about 1.7% on average and NH₄⁺-N removal was only 24%, as shown in Fig. 3 and Fig. 4. It

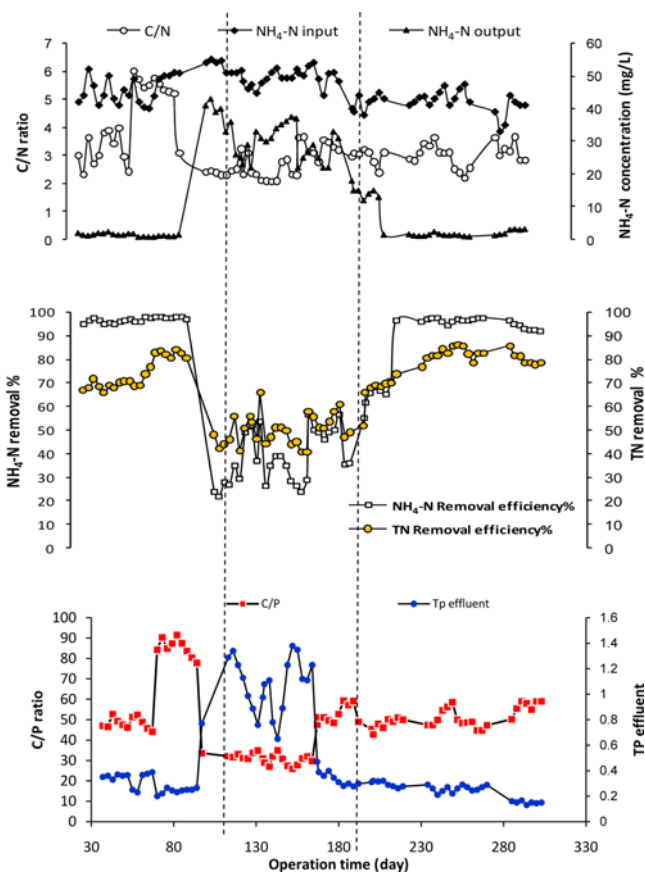


Fig. 4. Investigation results of TN, NH₄-N and phosphorus removal with nitrite and nitrate pathway.

can be seen readily that very low DO concentration (0.2 mg/L) limited AOB to oxidize NH₄⁺-N to NO₂⁻-N to some extent, whereas a little HRT prolonged of 14.2 h with 7 d of sludge retention time was enough for NOB to oxidize nitrite to nitrate, which resulted in a lower efficiency of NH₄⁺-N removal and nitrite build-up. In the period (135-167) d of run III, at DO level of 0.4-0.5 mg/L, nitrite build-up was not observed and NO₂-AR was almost below 2.5% on average. Partial nitrification via nitrite could not be achieved by control

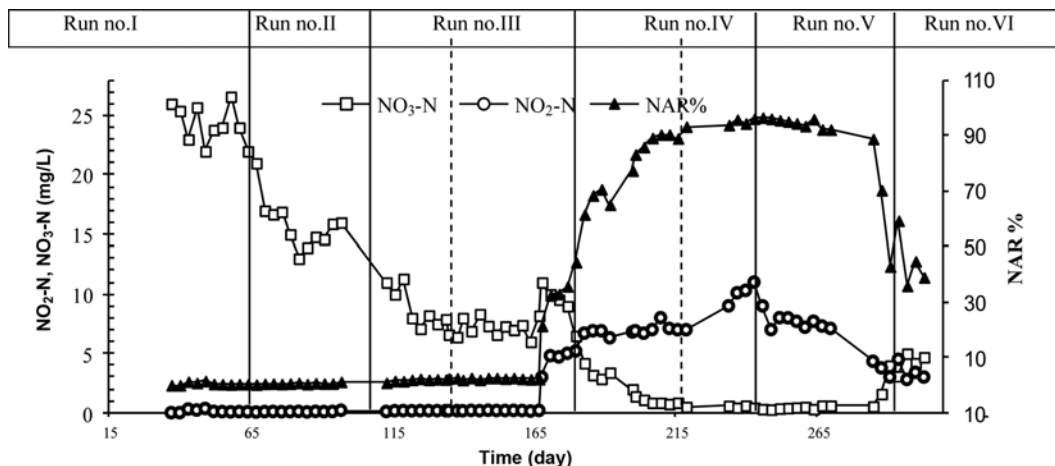


Fig. 3. Variation of NO₂-AR in SBASP system during six runs.

of low DO level, whereas total HRT was used as an electron acceptor for AOB growth. Thus, DO concentration was kept constant at 0.4–0.5 mg/L in run IV and V. During initiation of run IV (168–200) days, partial nitrification via nitrite was successfully achieved through increasing the flow rate of sludge recycles to 9 L/h. Results showed that $\text{NO}_2\text{-AR}$ reached 83% at 201 d, which illustrated that NOB activities were successfully eliminated. It was interesting that DO concentration had no distinct influence on $\text{NO}_2\text{-AR}$, which was 95% in run IV and V. Many studies achieved partial nitrification via nitrite by using low DO to enrich AOB or washout NOB due to their different oxygen affinity constants [11,12]. Additionally, some studies reported that low DO would destroy or decrease stable nitrite accumulation ratio after long-term operation [17,23]. However, the results from this study did not support this point, which was mainly attributed to limited (even negligible) number of NOB in the reactor during runs (IV and V). Furthermore, the short HRT resulted in poor $\text{NH}_4^+\text{-N}$ and TN removal of 50% and 56%, respectively. Thus, total HRT was increased to 11 h in the period (201–219) d of run IV for improvement $\text{NH}_4^+\text{-N}$ and TN removal. During this period, $\text{NO}_2\text{-AR}$ remained about 90% (Fig. 5). HRT was increased to 13 h and SRT also was prolonged to 13 d to enhance TN removal at the period (234–270) d of run V, whereas TN and $\text{NH}_4^+\text{-N}$ removal was stabilized over 83% and 97%, respectively (Fig. 4). In addition, $\text{NO}_2\text{-AR}$ was stabilized at over 94% as shown Fig. 3; this was observed probably due to inhibition of NOB at low DO concentration com-

bined with short HRT parameter. Run number VI (285–303) d was implemented to investigate the impact of extension SRT on nitrification process. Result showed that $\text{NO}_2\text{-AR}$ declined obviously to less than 44%. These observations demonstrated that the control of SRT was necessary to stabilize partial nitrification via nitrite.

2. Overall Performance of the Partial Nitrification on Phosphorus Removal

Fig. 4 showed that the average phosphorus removal during all operation runs in SBASP. In run I, influent COD concentration fluctuated with a low concentration 209 mg/L on average, which resulted in unsteady fluctuation in C/P ratio. However, phosphorus in the effluent of basin five was low 0.35 mg/L on average. In run II, NaAc was added (COD 179 mg/L), which resulted in increasing in C/P ratio to 84.3 on average; thus, good phosphorus removal was obtained during run II where the effluent phosphorus was below 0.23 mg/L. In run III, C/P ratio was fluctuating and poor phosphorus removal was achieved due to low DO level during period 113–134 d. In run IV, nitrite build-up in the aerobic zone was gradually increased while influent C/P ratio was reduced as shown in Fig. 3. Thus, effluent phosphorus concentration was increased obviously to 0.288 mg/L during this period. In run V, good phosphorus removal (95.8%) was achieved via nitrite pathway. NaAc was added in run VI where C/P ratio reached 57% on average and effluent phosphorus concentration became 0.16 mg/L which met the Chinese discharge standard (GB18918-2002) level A. In comparison of runs I and II with

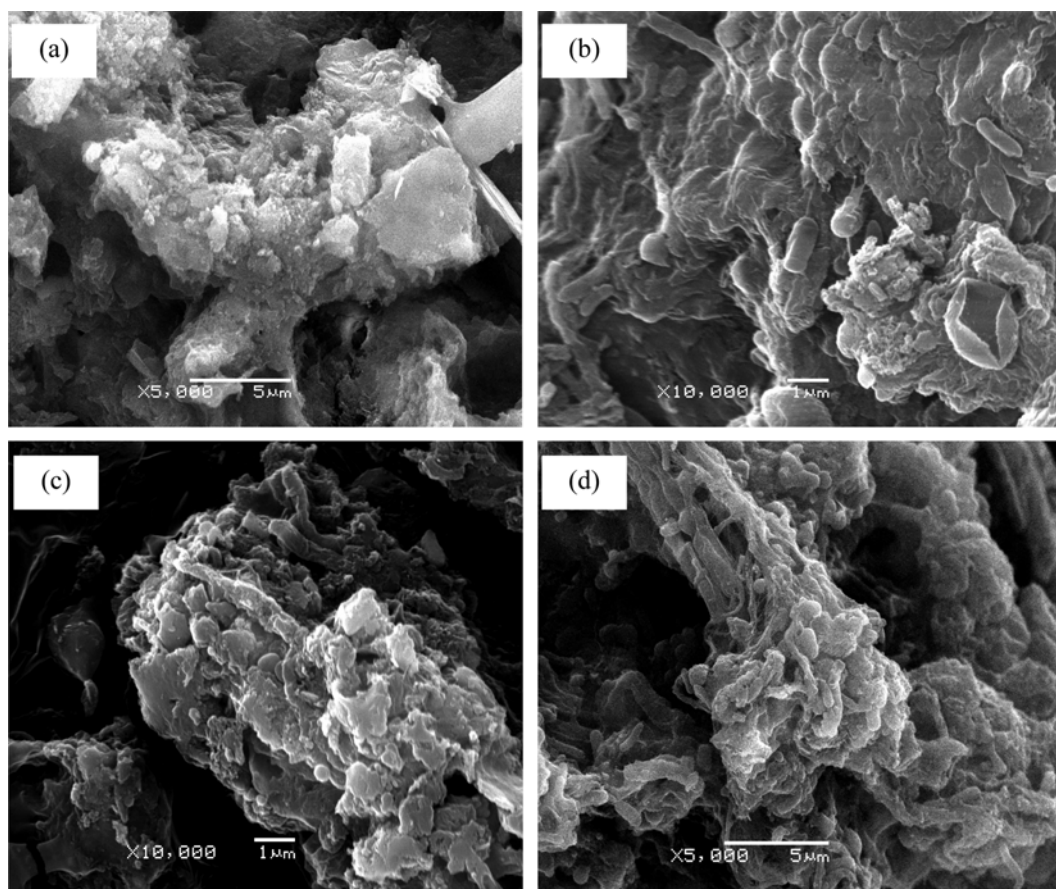


Fig. 5. SEM pictures taken from SBASP system during different runs: a, seed sludge; b, run I (73 day); c, run IV (217 day); d, run V (266 day).

run VI, phosphorus removal via nitrite pathway during run VI was 96%, which shows the actual reason for enhancing biological phosphorus removal in comparison with runs I and II.

3. SEM Analysis

The morphology and inner structure of the granules sludge in the new SBASP system were observed in more detail using SEM analysis. Sludge samples were taken from the last aerobic basins of a system for scanning electron microscope examination to observe the sludge morphology. Images taken from the seed sludge showed that the biomass was exposed with some substance and the shape of the bacterial cluster could not be identified properly (Fig. 5(a)). Sludge images showed that clusters of different sizes were observed clearly and distributed all over on the day 60 as shown in Fig. 5(b). It can be seen readily from Fig. 5(c) and d that thick clusters of spherical cells and small rod-shaped cells (NOB and AOB) were the dominant population structure rather than filamentous and other bacteria during a long-term operation of runs IV and V. This similar event was found in a nitrification reactor treating high $\text{NH}_4^+\text{-N}$ concentration in wastewater [9]. However, it was not easy to identify these clusters of bacteria by scanning electron microscope presentation alone. To investigate whether DO would influence the bacteria community structure, the composition of the microbial population was characterized using MBN method.

4. Variations of Ammonia Oxidizing Bacteria with $\text{NO}_2\text{-AR}$ in the Reactor

Fig. 6 shows the variation of ammonia oxidizing bacteria with $\text{NO}_2\text{-AR}$ in all investigated runs. It illustrates that AOB population had a clear correlation with $\text{NO}_2\text{-AR}$. In runs I and II, at normal DO levels, the SBASP system showed very good complete nitrification, whereas the AOB population increased obviously from 4.7×10^7 cell/g MLSS on day 46 to 7.7×10^7 cell/g MLSS on day 88. Over long-term operation under different DO levels, the MPN method showed that higher nitrification efficiency in run II was not only caused by high DO, but also might be due to more AOB percent. According to MPN results, it can be speculated that the competition mechanism between microbial species was different in the reactor. To carry out stable partial nitrification via nitrite, it is critical to achieve the enrichment of AOB and inhibition of NOB. Generally, high DO might destroy high $\text{NO}_2\text{-AR}$, and it is difficult to get the AOB accumulation under high DO. Therefore, the further transformation from nitrite to nitrate could be avoided effectively after 80 day of aeration period. Differently, the main competition relationship existed between AOB and filamentous bacteria at limited DO condition. Compared with NOB, AOB with high oxygen affinity would be outcompete

with NOB under low DO, thereby resulting in partial nitrification via nitrite. The conditions of low DO and insufficient organic matter would make filamentous bacteria get competition dominance. However, filamentous bulking did not occur in run IV although some limited amount of filamentous bacteria could be found by microscopic examination, which was attributed to controlling DO concentration. Sludge population optimization, as an emerging concept and a new dimension to the control of biological wastewater treatment systems, was first proposed by [24]. Recently, many studies have shown that control system and process design have significant effects on microbial community [10]. Some control systems have been designed and exploited to achieve an optimized microbial community. In a new SBASP, the combination of low DO and short HRT was not only favorable for a novel biological nitrogen removal technology, such as partial nitrification via nitrite, but also beneficial for achieving sludge population optimization based on scanning electron microscope and MPN analysis. In run III, nitrification deteriorated in the beginning of this run due to low DO of (0.2 mg/L), leading to a decrease in AOB by 36.36% on day 140 as shown in Fig. 6. At the second period of run III, $\text{NO}_2\text{-AR}$ increased gradually and AOB population increased to 1.25×10^8 cell/g MLSS on day 176 due to increased DO level of (0.4-0.5) mg/L. In Run number IV, $\text{NO}_2\text{-AR}$ increased gradually to 90% as the HRT was reduced to 9.1 h; the AOB population reached 4.46×10^8 cell/g MLSS. During run V, the AOB population stabilized at about 5.33×10^8 cell/g MLSS, of which a maximum $\text{NO}_2\text{-AR}$ level was over 95%. These observations indicated that the AOB population tended to be stable during steady partial nitrification performance. Finally, in run VI, $\text{NO}_2\text{-AR}$ was decreased from 93% on day 270 to 37% on 303 day due to the increasing of SRT. Furthermore, the stability of the SBASP system deteriorates if the SRT is too short (7 days); thus it is not recommended in this system. As the SRT of 16 day, prolonged time is required to achieve higher nitrification rate, whereas $\text{NO}_2\text{-AR}$ is low during the operation of run VI. The proposed explanation for this observation is that (a) removal of AOB throughout sludge wastage is effectively slow when applying prolonged SRT, and (b) both nitrifying bacteria of NOB and AOB are grown during the aerated period of the operation. Thus SRT of 10 close to 13 days is more appropriate in this study where AOB population is stabilized at about 5.33×10^8 cell/g MLSS.

5. Nutrient Removal by the Pathway of Nitrite and Nitrate

In this study, the SBASP system was performed for treating domestic wastewater with low (C/N) ratio. Fig. 7 shows the concentration profiles of TP, TN and $\text{NH}_4^+\text{-N}$ during the first half cycle. $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations along the system during different phases and runs were also tracked to gain a better insight into nitrogen removal.

In run I, short HRT of 9.1 hr was applied with normal DO concentration of 1.5-2.5 mg/L. The total removal efficiency of TP, and $\text{NH}_4^+\text{-N}$ was over 95% and 92.4%, respectively, while TN removal was only 70% due to low C/N ratio on average with an effluent of $\text{NO}_3\text{-N}$ concentration reaching 23 mg/L (Fig. 6(a)).

In run II, TN, TP and ammonia-N removal were improved as shown in Fig. 6(b) by adding NaAc as a carbon source to increase C/N ratio of influent to six. Thus, TN removal reached 84% with an effluent of $\text{NO}_3\text{-N}$ concentration below 14 mg/L on average, even though the nutrient removal of SBASP met the Chinese require-

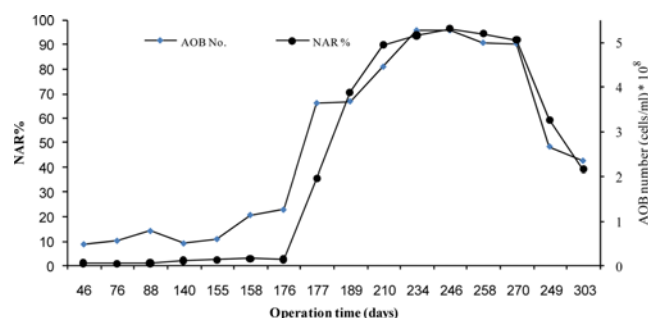


Fig. 6. AOB quantification by MPN analysis.

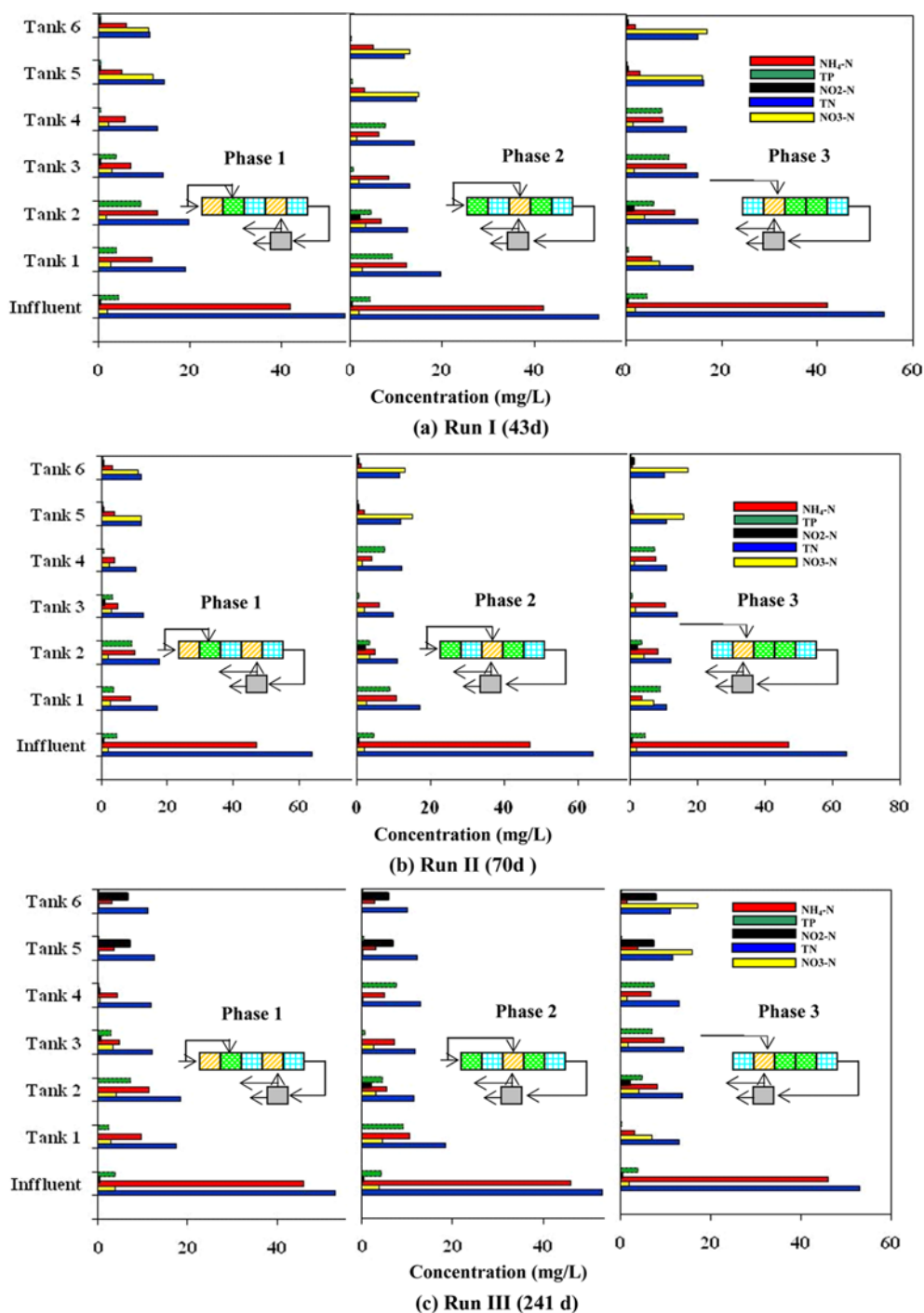


Fig. 7. Average dynamic pollutant concentrations in SBASP of phase 1, 2, and 3 during run I (a) run II (b) run IV (c).

ment of discharge wastewater level A (GB-18918) during run I.

In runs III and IV, TN and $\text{NH}_4^+\text{-N}$ removal was reduced to 66% and 51%, respectively, due to a low DO level and short HRT.

In run V, due to the stable performance of partial nitrification via nitrite, $\text{NO}_2\text{-AR}$ average was above 95% during the first half cycle (Fig. 7(c)). Thus, TN was removed by the nitrite pathway in this run, whereas TN removal reached 79% at 247 d, even though the average C/N ratio was only 2.39. In addition, $\text{NH}_4^+\text{-N}$ removal was also increased to above 97% through slightly extending of HRT to 13 hr (Fig. 7(c)). In comparison to run I at a low C/N ratio, with

TN removal by the nitrate pathway, TN removal during run V was 9% higher than TN removal by nitrite pathway, and was essentially equal to that of run II with the addition of external carbon sources.

In run VI, the total removal efficiency of $\text{NH}_4^+\text{-N}$, TP and TN was observed as 96.6, 86.3 and 96.1, respectively, where the system was changed from a partial nitrification to complete nitrification owing to the extension of SRT to 16 d. The addition of external carbon source during run VI increased TN removal to 85% with an effluent of TN concentration 8.51 mg/L during the first half cycle.

Consequently, the nutrient removal along the six basin activated

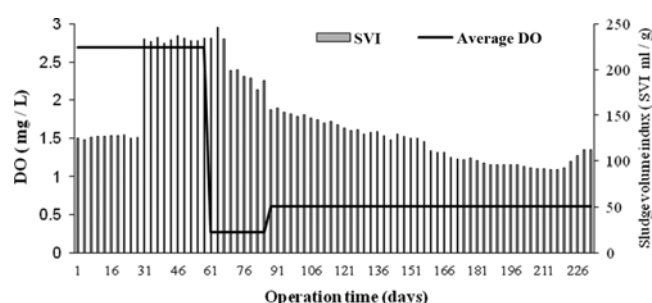
Table 3. Selected Suspended biomass of TP and TN removal processes^(Day, 1992)

Process	Influent (mg/l)	Effluent (mg/l)	
	TP	TN	TP
A/O	<7	-	2
Phostrip	<7	-	2
A/O	<4	-	1
Phostrip	<4	-	1
A ² O	<7	10 or 15	2
Phostrip+anoxic reactor Bardenpho, VIP, Bio Denipho, SBR	<4	10 or 15	1
A ² O Bardenpho, Bardenpho, VIP, Bio Denipho, SBR, phostrip+anoxic reactor	Matel salt supplement/or SCFA addition	10 or 15	1 or 2
SBASP/this study	<4	8-10	<0.5

sludge reactor via nitrite pathway at low DO concentration of 0.4–0.5 mg/L was approximately equal to the complete nitrification-denitrification with the addition of external carbon sources at DO of 1.5–2.5 mg/L. Hence, TN removal via nitrite pathway is highly beneficial for domestic wastewater treatment with limited carbon. Table 3 shows the average performance for total P and Total N removal for a number of biological P-removal processes [25]. Results showed that average effluent concentrations of less than mg/L are only achieved when the influent concentration is low. To meet the 1 mg/L standard requires an influent of less than 4 mg/L, and to meet a 2 mg/L standard needs an influent concentration less than 7 mg/L. In AA/O process, the last decay study showed that effluent concentrations were 15 mg/L of TN and 2 mg/L of TP, which were only achieved when the influent concentration was higher than 7 mg/L. In this study, TN and TP removal was achieved via both nitrite and nitrite pathway with effluent concentration less than 0.5 mg/L of TP and less than 8 mg/L of TN by a new process of SBASP.

6. Effect of Low DO Level on Sludge Volume Index (SVI) in SBASP

In this study, the sludge volume index was measured during a long-term operation to evaluate and monitor settling characteristics of activated sludge and other biological suspensions in SBASP. In a continuous-flow system, sludge bulking readily occurs during long-term operation with low DO concentrations [26] which it has accrued by the excessive hydration of activated sludge. It may be caused by sludge overloading, lack of nutrients in wastewater, a deficit or too high dissolved oxygen concentration, low pH value or finally also for technical reasons. As a result, unwanted activated sludge microorganisms appear, especially the filamentous bacteria. These bacteria cause a distinct increase of the active surface of flocs which in turn significantly slows down the sludge settling. This occurrence influences sludge settling in the secondary clarifier and is the main cause of unsatisfactory effluent quality [26]. However, sludge bulking was not observed in this study, whereas sludge volume index was (93–109) ml·g⁻¹ during the performance of partial nitrification at low DO level (Fig. 8). In run I, at normal DO concentrations of (1.5–2.5) mg/L, sludge volume index was maintained at about 100 ml/g, indicating a well-settled sludge. In Run number II, with the NaAc addition as external carbon source, sludge bulking occurred with sludge volume index was more than 230 ml/g (Fig. 8(a)). High amounts of filamentous organisms were observed that extended from the flocs into the bulk solution, interfering with compaction and set-

**Fig. 8. Variations of sludge volume index during long-term operation.**

ting. Thus, sludge volume index increased rapidly above 230 ml·g⁻¹. We hypothesized that the addition of a readily biodegradable carbon source (NaAc) was the main cause of sludge bulking. In the initiation of Run number III, at DO levels of 0.1–0.2 mg/L, sludge volume index was still in the high range of 179–233 ml·g⁻¹; however, in the initiation of the middle of run III (113–134) it was found that sludge settleability was obviously improved and SVI gradually decreased with DO increased to 0.4–0.5 mg/L. Especially in run V, the result indicated that sludge volume index stabilized below 97 ml·g⁻¹ as the partial nitrification via nitrite occurred and NO₂-AR stabilized over 94%. Lower DO of (0.4–0.5) mg/L produced clearer effluent but did not lead to poorer settling properties. Therefore, this study revealed very good settling properties of the sludge at low DO of (0.4–0.5) operation and the sludge bulking phenomena did not appear. However, low DO is an essential parameter to achieve NO₂-AR and favorable to higher oxygen transfer rate, so it is recommended to be employed in SBASP.

In Run number VI, owing to the extension of sludge retention time, SVI increased to 106 ml·g⁻¹ (Fig. 8) and sludge bulking did not occur, even though external carbon sources (NaAc) were added. NaAc dosage of COD (95) mg/L was depleted in the anaerobic and anoxic basins, and thus the substrate was not available to filamentous organisms in the aerobic basin. These experimental results are different from other previous studies, in which SVI variations showed a clear correlation with nitrite accumulation rates [27].

CONCLUSION

A new technology of six basins alternately operating activated

sludge process in treating domestic wastewater was designed and investigated for a long-term period of 303 days for effective removing of nutrients and organics from domestic wastewater. It can be strongly recommended for advanced treatment of domestic wastewater due to the following reasons:

SBASP can be operated safely with limited carbon source in terms of low carbon requirement and aeration cost, whereas the total nutrient removal efficiencies of TN, $\text{NH}_4^+\text{-N}$, and TP with low C/N ratio and DO concentration of 0.4-0.5 mg/L were 86.2, 97 and 96.1%, respectively, which was approximately equal to the complete nitrification-denitrification with the addition of external carbon sources at a DO level of 1.5-2.5 mg/L.

As a final engineering observation, the proposed system is regarded as an effective process because nitrite accumulation rate ($\text{NO}_2\text{-AR}$) in the last aerobic basin was stabilized over 95% throughout a combination of low DO of 0.4 mg/L and short HRT of 9.1. The achievement of nutrient removal via nitrite in SBASP process is highly beneficial for the treatment of domestic wastewater with low C/N. Moreover, energy saving by low DO would be technically feasible if sludge settleability did not become too weak to affect separation of sludge and effluent quality.

The morphology and internal structure of the granular sludge was observed by using a scanning electron microscope, and the image indicated that thick clusters of spherical cells and small rod-shaped cells were the dominant population structure than filamentous and other bacteria during the operation period. MPN method was also used to correlate AOB numbers with nutrient removal. It showed that an ammonia-oxidizing bacterium (AOB) was the dominant nitrifying bacteria and nitrite-oxidizing bacteria (NOB) was not recovered, whereas high $\text{NO}_2\text{-AR}$ was achieved at AOB number of 5.33×10^8 cell/g MLSS.

The SBASP achieves automatic recirculation without equipment for sludge and mixed liquor return because the direction of flow is changed automatically via changing intake location. Therefore, the SBASP system is also effective for reducing energy consumption.

ACKNOWLEDGEMENTS

This research was financially supported by the Major science and Technology program for water pollution control and treatment (No. 2012ZX07101-005) and Key Grant Project of National natural science foundation of China (Grant No.51078074).

REFERENCES

1. C. Y. Chang, D. S. Kim, J.-H. Cho, S. W. Choi and I. B. Lee, *J. Korean J. Chem. Eng.*, **18**(4), 408 (2001).

2. T. Yamamoto, K. Takaki, T. Koyama and K. Furukawa, *Bioresour. Technol.*, **99**(3), 6419 (2008).
3. G. Zhu, Y. Peng, B. Li, J. Guo, Q. Yang and S. Wang, *Rev. Environ. Contam. Toxicol.*, **192**(4), 159 (2008).
4. S. C. Eui and S. Ho, *Korean J. Chem. Eng.*, **13**(4), 364 (1996).
5. O. Turk and D. Mavinic, *Water Res.*, **23**, 1383 (2004).
6. T. Tokutomi, *Water Sci. Technol.*, **49**(11), 81 (2004).
7. R. Van Kempen, J. Mulder, C. Uijterlinde and M. Loosdrecht, *Water Sci. Technol.*, **44**, 145 (2001).
8. R. Blackburne, Z. Yuan and J. Keller, *Water Res.*, **42**(6), 2166 (2008).
9. S. Aslan, L. Miller and M. Dahab, *Bioresour. Technol.*, **100**, 659 (2009).
10. Z. Yuan, Z. Oehmen, Y. Peng, Y. Ma and J. Keller, *Environ. Sci. Biotechnol.*, **7**(8), 243 (2008).
11. R. Blackburne and Z. Yuan, *J. Biodegradation*, **19**, 303 (2008).
12. J. Wang and N. Yang, *Process Biochem.*, **39**, 1223 (2004).
13. D.-J. Kim, D. H. Ahn and D.-I. Lee, *Korean J. Chem. Eng.*, **22**(1), 85 (2005).
14. Q. Yang, Y. Peng, X. Liu, W. Zeng, T. Mino and H. Satoh, *Environ. Sci. Technol.*, **41**(7), 8159 (2007).
15. Y. Peng and G. Zhu, *Appl. Microbiol. Biotechnol.*, **73**, 15 (2006).
16. W. Bae, S. Baek and Y. Chung, *J. Biodegradation*, **12**, 359 (2001).
17. G. Ciudad, O. Rubilar, P. Muñoz, G. Ruiz, L. Chamy, C. Vergara and D. Jeison, *Process Biochem.*, **40**, 1715 (2005).
18. S. E. P. A. Chinese, *Water and wastewater monitoring methods*, 4th Ed., Chinese Environmental Science Publishing House, Beijing, China (2002).
19. APHA, *Standard methods for the examination of water and wastewater*, 20th ed., American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, USA (1998).
20. R. Rowe and T. R. Waide, *Appl. Environ. Microbiol.*, **33**, 675 (1977).
21. L. Aanbroek and H. J. Pfennig, *Arch Microbiological.*, **128**, 330 (1989).
22. M. Alexander and F. E. Clark, *Nitrifying bacteria*, In: Black CA, Ed. *Methods of soil analysis*, Part 2. Madison, WI: 1965; *American Society of Agronomy*, 1477-1483.
23. J. Garrido, W. van Benthum, M. van Loosdrecht and J. Heijnen, *Bio-technol. Bioeng.*, **53**, 168 (1997).
24. Z. Yuan and L. Blackall, *Water Res.*, **36**, 482 (2001).
25. M. Day and P. Cooper, *Guide on nutrient removal processes*, Report FR 0253, Foundation for water Research, Marlow, Bucks, UK, June (1992).
26. Y. Ma, Y. Peng, S. Wang and Z. Yuan, *Water Res. J.*, **43**, 563 (2009).
27. T. Casey, M. Wentzel, G. Ekama, R. Loewenthal and G. R. Marais, *Water Sci. Technol.*, **29**(7), 203 (1994).