Novel A-B processes for energy-efficient municipal wastewater reclamation with minimized sludge production

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**NOVEL A-B PROCESSES FOR ENERGY-EFFICIENT
MUNICIPAL WASTEWATER RECLAMATION WITH
MINIMIZED SLUDGE PRODUCTION**

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**INTERDISCIPLINARY GRADUATE SCHOOL
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MINIMIZED SLUDGE PRODUCTION**

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ABSTRACT

The conventional activated sludge (CAS) process has been widely employed for wastewater treatment for more than one hundred years. Recently, more and more concerns have been raised on the CAS process due to its high energy consumption and production of huge amount of waste activated sludge, which are inevitably linked to the issue of environmental sustainability and global climate change. Therefore, this study aimed to develop novel A-B processes towards energy-efficient municipal wastewater treatment with minimized sludge production.

Anaerobic treatment could directly convert the COD into biogas with much less sludge production compared to aerobic processes. However, the dissolved methane in the anaerobic effluent is a hurdle for its application. Therefore, in the first phase of study, an A-B process in which an anaerobic moving bed biofilm reactor (AMBBR) served a lead A-stage for COD capture towards biogas production and an integrated fixed-biofilm and activated sludge sequencing batch reactor (IFAS-SBR) was employed as B-stage for biological nitrogen removal. Results showed that about 85% of wastewater COD was removed in the AMBBR with a total energy production rate of 0.28 kWh/m^3 wastewater treated, while 85% of N-removal was achieved when the stable nitrite shunt was established in the IFAS-SBR. Moreover, 90% of dissolved methane in the AMBBR effluent could be removed by the proposed flash chamber at the lower energy demand of 0.12 kWh/m^3 which could be offset by the potential energy harvested from produced methane. Compared to the CAS process, the production of waste sludge was

reduced by about 75% due to the efficient COD capture at the A-stage, leading to significant energy savings from aeration for COD oxidation and post-treatment of waste sludge at the B-stage. Consequently, this phase of study offers in-depth insights into A-B process which should be considered as an ideal candidate for achieving the energy-efficient operation of a municipal wastewater treatment plant.

With the improvement of A-stage performance, the nitrification-denitrification at B-stage in the first study could not be sustained due to the insufficiency of COD. To cope with this issue, in the second phase of study, a novel A-B process configuration in which a portion of influent was directed to B-stage was developed for sustainable and stable nitrogen removal via nitrite shunt with minimal impacts on energy recovery. The bypass flow was found to significantly shape microbial community structure by enriching ammonia oxidizer and denitrifiers against nitrite oxidizer. As the result, about 78% of total nitrogen was removed via nitrification-denitrification. In addition, the potential energy recovery and sludge reduction were not compromised in the proposed A-B process with the bypass of influent to B-stage. It is expected that this phase of study may offer a feasible engineering solution for concurrently achieving direct energy recovery from wastewater at A-stage, and sustainable nitrification-denitrification for nitrogen removal at B-stage.

Anammox has unique metabolic ability to oxidize ammonium to nitrogen gas using nitrite as electron acceptor without the presence of oxygen or organic carbon. The significant energy-saving potential of anammox prompted us to integrate mainstream anammox into A-B process. Therefore, the third phase of study

demonstrated the feasibility to integrate mainstream anammox into an A-2B process for municipal wastewater treatment towards energy-efficient operation with reduced sludge production. In the proposed A-2B process, an AFBR served as A-stage for COD capture, an anammox moving bed biofilm reactor (MBBR) was employed as B2-stage, which received effluent containing nitrite from a SBR at B1-stage. The results showed that under the operation conditions studied, 58% of influent COD was converted methane gas at A-stage, and 87% total inorganic nitrogen (TIN) removal was achieved with the effluent TIN concentration of 6.5 mg /L. Moreover, it was shown that at least 75% of sludge reduction was obtained due to the COD capture at A-stage. The high-throughput sequencing analysis further revealed that *Candidatus* Kuenenia was the dominant genus responsible for the observed anammox at B2-stage MBBR. This study clearly demonstrated a novel process configuration for sustaining mainstream anammox for municipal wastewater reclamation towards energy-efficient operation with minimized sludge production.

The feasibility of single-stage mainstream deammonification in an integrated A-B process was investigated in this phase of study. NOB repression strategies developed in preceding phases based on the temporal operating SBR was successfully transferred to the continuous operating step-feed reactor. Results showed that 79% of the influent COD was directly converted to methane in the A-stage AFBR, while nitrogen removal efficiency reached up to 80% via deammonification in the B-stage step-feed mainstream deammonification reactor. Sustainable and stable mainstream deammonification was achieved without bioaugmentation for more than 100 days, which significantly reduced the operation

complexity compared with other mainstream processes.

In summary, this study innovatively developed the novel integrated A-B processes towards maximized energy recovery and minimized excess sludge production. The A-B processes described in this study may lead to the paradigm shift of the WWTP operation from energy-negative to energy self-sufficient.

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LIST OF ABBREVIATIONS

The following abbreviations are used throughout this PhD thesis:

amo	Ammonia monooxygenase
AD	Anaerobic digestion
AFBR	Anaerobic fixed bed reactor
AMBBR	Anaerobic moving bed biofilm reactor
Anammox	Anoxic ammonium oxidation
AnMBR	Anaerobic membrane bioreactor
AOB	Ammonium oxidizing bacteria
CAS	Conventional activated sludge
CEPT	Chemically enhanced primary treatment
CHP	Combined heat and power
COD	Chemical oxygen demand
DO	Dissolved oxygen
DOC	Dissolved organic carbon
FA	Free ammonia
FISH	Fluorescent in situ hybridization
FNA	Free nitrous oxide
GC-TCD	Gas chromatography-thermal conductivity detector
GHG	Greenhouse gas
hao	Hydroxylamine oxidoreductase
HDH	Hydrazine dehydrogenase
HRAS	High rate activated sludge
HRT	Hydraulic retention time
HZS	Hydrazine synthase enzyme
IFAS	Integrated fixed-biofilm activated sludge
MBBR	Moving bed biofilm reactor
MLE	Modified Ludzack-Ettinger
MLVSS	Mixed liquor volatile suspended solids
nar	Nitrate reductase

LIST OF ABBREVIATIONS

NAR	Nitrite accumulation rate
nir	Nitrite reductase
NOB	Nitrite oxidizing bacteria
nor	Nitric oxide reductase
nos	Nitrous oxide reductase
ORP	Oxidation-Reduction Potential
PLC	Programmable logic control
q-PCR	Quantitative polymerase chain reaction
SHARON	Single reactor high activity ammonia removal over nitrite
SBR	Sequencing batch reactor
SFAS	Step-feed activated sludge process
SOUR	Specific oxygen uptake rate
SRT	Solids retention time
TSS	Total suspended solids
UMABR	Upflow membrane-aerated biofilm reactor
WWTP	Wastewater treatment plant

LIST OF PUBLICATIONS

1. **Gu, J.**, Xu, G. and Liu, Y., (2017) An integrated AMBBR and IFAS-SBR process for municipal wastewater treatment towards enhanced energy recovery, reduced energy consumption and sludge production. **Water Research** 110, 262-269.
2. **Gu, J.**, Yang, Q., and Liu, Y., (2018) A novel strategy towards sustainable and stable nitrification-denitrification in an A-B process for mainstream municipal wastewater treatment. **Chemosphere** 193:921-927
3. **Gu, J.**, Yang, Q., and Liu, Y. (2018). Mainstream anammox in a novel A-2B process for energy-efficient municipal wastewater treatment with minimized sludge production. **Water research**, 138, 1-6.
4. Xu, G., Wang, H., **Gu, J.**, Shen, N., Qiu, Z., Zhou, Y. and Liu, Y., (2017) A novel A-B process for enhanced biological nutrient removal in municipal wastewater reclamation. **Chemosphere** 189, 39-45.
5. Wan, J., **Gu, J.**, Zhao, Q. and Liu, Y., (2016) COD capture: a feasible option towards energy self-sufficient domestic wastewater treatment. **Scientific Reports** 6, 25054.
6. Xu, G., Zhou, Y., Yang, Q., Lee, Z.M., **Gu, J.**, Lay, W., Cao, Y. and Liu, Y., (2015) The challenges of mainstream deammonification process for municipal used water treatment. **Applied Microbiological Biotechnology** 99(6), 2485-2490.

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

The conventional activated sludge (CAS) process has been widely applied in municipal wastewater treatment. However, more and more concerns have been raised on its high energy consumption and huge excess sludge production which impair environmental sustainability and negatively affect the global climate change. In the USA, wastewater treatment consumed about 3% of the annual electrical energy (US-EPA, 2006), and produced 8 million tons of dry sludge in 2010. The current energy consumption in the conventional CAS process is in the range of 0.3 to 0.6 kWh/m³ of wastewater (McCarty et al., 2011), nearly half of which is used for aeration and the other 30% for excess sludge treatment (Henderson, 2002).

It should be noted, on the other hand, that the potential chemical energy in domestic wastewater is about five times of the energy required to drive the CAS process (Wan et al., 2016). Theoretically, it is possible to achieve energy self-sufficient wastewater treatment if energy in wastewater could be effectively recovered. It has been demonstrated that the substantial improvement in the energy efficiency might be no longer achievable through further optimization of the activated sludge process (Wan et al., 2016). Therefore, it is absolutely necessary to develop new process configurations for wastewater treatment plants (WWTPs) with considerations of enhanced energy recovery from wastewater, reduced energy consumption and less excess sludge production in a more holistic manner. To address such challenges,

attention has been paid to the processes with the ability to directly capture COD from wastewater, i.e. chemically enhanced primary treatment (CEPT), high rate activated sludge (HRAS) and anaerobic treatment coupled with energy-efficient nitrogen removal processes (e.g. nitrification-denitrification and deammonification). As conceptualized by Wan et al. (2016), A-B process configurations may offer the ultimate solution, in which COD is captured for direct energy production at A-stage, while B-stage is designed for nitrogen removal. However, little has been known about synergistic engineering integration of A and B stages towards energy-self sufficiency of wastewater treatment. Therefore, this study aimed to develop novel A-B process configurations to tackle the current challenges, and further offered a feasible engineering solution for energy-efficient municipal wastewater treatment with minimized sludge production.

1.2 OBJECTIVES AND SCOPE

The main objectives of this study are:

- (1) To develop a novel A-B process with anaerobic treatment as lead A-stage for COD capture and energy recovery, into which a newly designed degasser is integrated for dissolved methane recovery.
- (2) To optimize the A-B process in a more holistic manner with consideration of both energy recovery and nitrogen removal.
- (3) To investigate the engineering feasibility of a novel A-2B process with integrated direct COD capture and two-stage mainstream anammox.
- (4) To develop a complete mainstream deammonification process towards energy

self-sufficient municipal wastewater reclamation with minimized sludge production.

1.3 ORGANIZATION OF THE THESIS

This thesis contains the seven chapters:

- (1) Chapter 1 presented a brief introduction to the current research background and specific objectives of this study.
- (2) Chapter 2 provided a comprehensive literature review, including the conventional and novel nitrogen removal processes for municipal wastewater treatment with the focuses on the control strategies, benefits and challenges of each process; potential COD capture processes for energy recovery.
- (3) Chapter 3 developed a novel A-B process using anaerobic unit as A-stage for COD capture and energy recovery, into which an innovative flash chamber was integrated for dissolved methane recovery. A complete energy balance of the proposed process was established.
- (4) Chapter 4 further exploited the way to sustain stable nitrification-denitrification in the A-B process. A novel strategy by bypassing a portion of the influent to B-stage was successfully developed for achieving sustainable nitrification, while the effect of the bypassed flow on the energy recovery and the microbial community were also examined in detail.
- (5) Chapter 5 aimed to integrate mainstream anammox into an A-2B process, where nitrogen was removed by a two-stage anammox process, i.e.

nitritation-denitrification in B1-stage and anammox in B2-stage. The COD, and nitrogen removal and microbial community were evaluated.

- (6) Chapter 6 developed a single-stage mainstream deammonification process as B-stage towards energy self-sufficient municipal wastewater reclamation. The operation strategies developed in the preceding chapter for the effective NOB repression in the SBR were adopted in this study, i.e. from temporal control in the SBR to a spatial operation in the step-feed reactor. Sustainable deammonification was maintained at B-stage without bioaugmentation.
- (7) Chapter 7 summarized the major findings obtained in this study and recommendations for future investigation.

CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

The conventional activated sludge (CAS) process has been widely applied in municipal wastewater treatment for more than 100 years. However, this process has been challenged due to its high energy consumption and production of huge amount of excess sludge. For example, in the USA about 3% of the annual electrical energy was utilized for wastewater treatment (US-EPA, 2006), meanwhile 8 million tons of dry sludge was produced in 2010 (Paul and Liu, 2012). Currently energy consumption in the CAS process is in the range of 0.3 to 0.6 kWh/m³ of wastewater, half of which is used for aeration and the other 30% for sludge post-treatment (McCarty et al., 2011, Zhou et al., 2013). Theoretically the inherent chemical energy in domestic wastewater is approximately five times of the energy needed for wastewater treatment using the CAS as a core technology (Wan et al., 2016). Therefore, it appears necessary to develop new configurations of wastewater treatment plants (WWTPs) with considerations of enhanced energy recovery, reduced energy consumption and less excess sludge production in a more holistic manner. It has been argued that substantial improvement in the energy efficiency cannot be further achieved through optimizing the CAS process (Wan et al., 2016). Therefore, this study aimed to develop novel A-B processes which can significantly improve the energy recovery potential and minimize the excess sludge production at A-stage, while markedly reduce energy consumption for nitrogen removal at B-stage. These novel A-B processes may lead to the paradigm shift of the

wastewater treatment plant (WWTP) operation from energy negative to energy neutral or even positive.

2.2 CONVENTIONAL NITROGEN REMOVAL

2.2.1 Principle of full nitrification and denitrification

Nitrogen in municipal wastewater can be removed via nitrification and denitrification. Nitrification is a two-step biological process in which ammonium is firstly oxidized to nitrite by ammonium oxidizing bacteria (AOB), and nitrite is subsequently oxidized to nitrate by nitrite oxidizing bacteria (NOB), as shown in Equation 2.1.

It can be seen that that 4.14 g O₂ is required for oxidizing 1 g of NH₄⁺-N with about 7 g of alkalinity (as CaCO₃) and 0.1 g of inorganic carbon consumed. AOB and NOB are chemolithoautotrophic microorganisms that can derive energy from inorganic carbon sources; thereby the growth rates and biomass yields of nitrifiers are low compared with those of heterotrophic bacteria. Due to their low growth rate, sufficiently long solids retention time (SRT) should be maintained in order to avoid the washout of nitrifiers, i.e. 4-7 days at 20 °C (Metcalf and Eddy, 2014).

Denitrification is a biological process where nitrate is reduced to nitrogen gas with organic carbon as electron donor, which is mostly catalyzed by a variety of facultative heterotrophs under anoxic conditions. Denitrification may proceed in

steps as described in Eq. 2.2 involving multiple enzymes, i.e. nitrate reductase (nar), nitrite reductase (nir), nitric oxide reductase (nor) and nitrous oxide reductase (nos).

Eq. 2.3 showed the overall denitrifying reaction with methanol as the source of organic carbon (Rittmann and McCarty, 2012).

Theoretically, 2.86 grams of organic matter in terms of COD are needed for denitrifying 1 gram of NO_3^- -N. In practice, about 4.6-4.7 g methanol-COD should be provided for reducing 1 g NO_3^- -N (Bilanovic et al., 1999, Mokhayeri et al., 2006), while the actual amount of COD needed may vary, depending on the operating conditions and types of organic carbon source (Osaka et al., 2008, Rasool et al., 2014, Yang et al., 2012). Organics in the municipal wastewater can be used as electron donors for denitrification, while external organic carbon (e.g. methanol, acetic acid, etc.) are often supplemented if wastewater COD is insufficient.

2.2.2 Full nitrification and denitrification processes

Based on the CAS process, many processes have been developed for combined COD and nitrogen removal, e.g. pre-denitrification, post-denitrification, SBR, oxidation ditch (OD), etc. in which anoxic environment is created in temporal or spatial fashion for denitrifiers. (Aspegren et al., 1998, Metcalf and Eddy, 2014, Rittmann and Langeland, 1985). One of such examples is presented in Fig. 2.1 with the anoxic unit as the lead. This process indeed is known as the modified

Ludzak-Ettinger (MLE) process which is the most applied process for biological nitrogen removal worldwide. As can be seen Fig 2.1, the MLE process consists of an anoxic tank followed by an aerobic tank. In this configuration, nitrification and organic carbon oxidation take place in the aerobic tank, the effluent of which contains nitrate is then recycled back to the anoxic tank for denitrification with influent COD as organic carbon source. In this process, it should be noted that the denitrification efficiency is determined by the recycle ratio of nitrified liquor and the availability of readily biodegradable COD in influent wastewater.

Figure 2.1 Flow diagram of a typical preanoxic denitrification process (Metcalf and Eddy, 2014).

2.2.3 Challenges with the conventional process

The CAS process for municipal wastewater treatment is energy-intensive, with the electricity consumption of 0.3-0.6 kWh/m³ of wastewater treated (McCarty et al., 2011). A typical breakdown of the energy consumption in a conventional municipal wastewater treatment plant with the capability of biological nitrogen removal (Fig.

2.2) shows that aeration and excess sludge-associated treatment were the major energy consumers (Crawford, 2010). In general, the energy used for aeration and sludge treatment may account for 50% and 30% of the total in-plant energy demand, respectively (Henderson, 2002, Owen, 1982). It should be noted that the energy consumption and its breakdowns in a WWTP highly depend on the treatment capacity, effluent quality, process configuration, characteristics of influent, status of instruments and equipment etc. (Jenkins and Wanner, 2014).

Figure 2.2 Breakdown of energy consumption in a typical municipal wastewater treatment plant with biological nitrogen removal (Crawford, 2010).

In general, the aeration-associated energy consumption in WWTP with nitrification is often higher than that designed only for COD removal. For example, the

electrical energy consumption of 0.23 kWh/m^3 for both COD removal and nitrification was reported versus 0.14 kWh/m^3 only for removing COD (Goldstein and Smith, 2002). Similarly, Monteith et al. (2007) also found that the aeration-associated energy consumption was increased from 0.305 kWh/m^3 to 0.405 kWh/m^3 after nitrification was implemented. So far, extensive effort has been dedicated to reducing the aeration-associated energy consumption by improving the aeration efficiency (e.g. use of fine bubbles to replace coarse bubbles and surface aeration) (Pakenas, 1995) or implementing the sensor-based real-time control of aeration intensity (Jonasson, 2007).

In the CAS process, typically 0.3-0.5 g dry biomass is produced with 1 gram COD removed (Metcalf and Eddy, 2014). As a result, about half of the removed COD is converted to excess sludge; meanwhile intensive electrical energy is consumed for providing aeration for COD oxidation. In the European Union, more than 10 million tons of excess sludge was produced in 2010 (Stamatelatou and Tsagarakis, 2015). For the purposes of the volume reduction and energy recovery, excess sludge in the current practice is subjected to anaerobic digestion (AD) for producing biogas which is further used for energy generation through the combined heat and power (CHP) system (McCarty et al., 2011). The energy recovery efficiency in AD is about 20-40% corresponding to $0.1\text{-}0.2 \text{ kWh/m}^3$ of electricity which can offset up to 30% of the total in-plant energy expenditure (Cao et al., 2013). These suggest that the current wastewater treatment plants are still in energy-negative operation, even with anaerobic digestion of excess sludge for energy recovery. For instance, Jurong Water Reclamation Plant in Singapore achieved 35% of energy efficiency, while 31% in Gaobeidian Wastewater Treatment Plant in China (Zhou et al., 2013). In fact, it

has been argued that further optimization of the CAS process could not lead to further improved energy efficiency. Therefore, the innovative treatment configurations and emerging technologies are urgently needed globally.

2.3 NITRITATION-DENITRITATION

2.3.1 Reaction pathways

Nitrification-denitrification, also known as shortcut nitrification-denitrification, is a shortened nitrification and denitrification process. In the short-cut process, nitrite produced by AOB with ammonia monooxygenase (amo) and hydroxylamine oxidoreductase (hao) is directly denitrified to nitrogen gas, instead of being further oxidized to nitrate by NOB (Fig. 2.3).

Figure 2.3 Nitrogen removal via nitrification-denitrification.

Nitrification-denitrification may lead to 15% of reduction in aeration intensity compared to full nitrification-denitrification (van Kempen et al., 2001). According to the stoichiometry of denitrification, 2.86 gram COD is required for denitrifying 1 gram of NO_3^- -N, while 1.71 gram COD for denitrifying 1 gram of NO_2^- -N (Metcalf and Eddy, 2014). These in turn indicate that the demand on organic carbon can be reduced by 40% in nitrification-denitrification process (van Kempen et al., 2001). In

addition, the denitrification rate was reported to be about 1.5-2.0 times higher than that of denitrification on nitrate, while the sludge production was reduced by 33-35% in nitrification and about 55% in denitrification compared to full nitrification-denitrification (Turk and Mavinic, 1987).

2.3.2 Factors affecting nitrite shunt

The repression of NOB against AOB is essential towards stable nitrification or nitrite shunt as illustrated in Fig. 2.4. So far, several operation strategies have been proposed for selectively inhibiting NOB, e.g. DO concentration, SRT, control of aeration, free nitrous acid (FNA), free ammonia (FA) inhibition, real-time control, etc.

Figure 2.4 Suppression of NOB vs AOB.

DO concentration

It was reported that the half-saturation coefficient of NOB to DO was in the range of 1.2-1.5 mg/L, which was much higher than 0.2-0.4 mg/L for AOB (Picioreanu et al., 1997). As such, low DO concentration should be favorable for the growth of AOB. For example, AOB was found to outcompete NOB at the low DO concentrations of 0.4-0.7 mg/L and 0.3-0.5 mg/L, respectively (Ma et al., 2009, Zeng et al., 2010). However, the low DO-based strategy often compromises COD removal and nitrification rate, while triggers filamentous growth (Ge et al., 2014, Guo

et al., 2010). On the contrary, some studies also showed high DO was more favorable for AOB than NOB. In fact, it should be realized that DO-based NOB repression is highly difficult since the oxygen affinity varies among different genera of NOB (Regmi et al., 2014, Strous et al., 1999). For example, *Nitrospira*-like nitrite oxidizer was adapted to low DO concentration below 0.5 mg/L (Daims et al., 2001), but *Nitrobacter* was found at high DO concentration (Regmi et al., 2014). On the other hand, DO concentration higher than 2 mg/L could promote complete nitrification (Zhu et al., 2008). Therefore, it had been suggested that the DO concentration for NOB suppression should be controlled at about 1.5 mg/L (Ruiz et al., 2006).

SRT

The growth rates of AOB and NOB are governed by temperature (Fig. 2.5), e.g. AOB can grow faster than NOB at the temperature above 20 °C. This implies that NOB could be out-selected at an applied SRT longer than the SRT of AOB, but shorter than that of NOB (Xu et al., 2015). Such an operation strategy had been successfully applied in single reactor high activity ammonia removal over nitrite (SHARON) process for achieving nitrification at the temperature of 30-40 °C and SRT of 1.5-2 days (Hellinga et al., 1998).

Figure 2.5 AOB and NOB growth rates at different temperatures (Hellinga et al., 1998).

Control of aeration

Intermittent aeration had been proposed for NOB repression by creating transient anoxic condition in SBR (Fan et al., 2013, Kornaros et al., 2008, Wett et al., 2013). NOB was observed to experience longer lag time than AOB during the transition from anoxic to oxic conditions (Bournazou et al., 2013, Kornaros et al., 2010, Turk and Mavinic, 1989), likely due to the deactivation of a critical enzyme inhibiting NOB after anoxic disturbance (Bournazou et al., 2013, Wett et al., 2013) or the inhibition to NOB by intermediate products, e.g. hydroxylamine (Xu et al., 2012). However, this control strategy is difficult to be implemented in continuous treatment process (Zeng et al., 2010).

FNA and FA inhibition

FNA inhibition on nitrification was firstly reported by Anthonisen et al. (1976). For example, 50% of AOB activity was inhibited by FNA at a concentration of 0.42–1.72 mg N/L (Tan et al., 2008, Torà et al., 2010), while the NOB activity was inhibited completely at the much lower FNA concentration of 0.026–0.22 mg N/L

(Vadivelu et al., 2006, Zhang et al., 2010). It had been reported that NOB could be disadvantaged against AOB at the FNA concentration of 0.02–0.03 mg N/L (Zhou et al., 2011). In addition, FA was also found effective in NOB repression because NOB was more sensitive to FA than AOB (Painter, 1970). It was reported that the growth of *Nitrobacter* was ceased at the FA concentration of above 6 mg N/L (Vadivelu et al., 2007), but the inhibition threshold of FA to *Nitrosomonas* was in the range of about 10-150 mg N/L (Anthonisen et al., 1976). However, it should be realized that the control of NOB by FA and FNA inhibition is extremely difficult due the low ammonium concentration in municipal wastewater.

Real-time control

Real-time control of aeration had been proposed for adjusting the durations of aerobic and anoxic phases based on on-line sensing. By adopting real-time control of aeration, sustained nitrite accumulation was achieved by concurrent monitoring of oxidation-reduction potential (ORP), oxygen uptake rate (OUR), pH, etc. (Gao et al., 2009, Lemaire et al., 2008, Regmi et al., 2014, Yang et al., 2007).

2.3.3 Challenges with nitrification-denitrification

The nitrification and denitrification process has been successfully applied for treating ammonium-rich wastewater, e.g. reject liquor of anaerobic sludge digestion, landfill leachate, etc. (Aslan and Dahab, 2008, Kulikowska and Bernat, 2013). However, sustainable nitrification is still a challenge in low-strength wastewater treatment, i.e. municipal wastewater, largely because some effective control factors (e.g., FNA and FA) are no longer available in the low-strength wastewater treatment (Negulescu, 2011). Although some successful cases of nitrification-denitrification had been reported

in SBR, its application to continuous municipal wastewater treatment still remains as a great challenge. In addition, in the current development of nitrification-denitrification processes, soluble COD in wastewater has been considered as a carbon source for denitrification, but nearly no effort has been put into possible energy recovery from soluble COD in a more holistic manner.

2.4 MAINSTREAM DEAMMONIFICATION PROCESS

2.4.1 Deammonification

Anaerobic ammonium-oxidizing (anammox) bacteria have extensively explored for energy-efficient nitrogen removal (Fux et al., 2002, Kartal et al., 2010). The anammox process is a shortcut in nitrogen cycle that was discovered in the early 1990s (Mulder et al., 1995). The anammox bacteria can use inorganic carbon source for biomass synthesis and oxidation of ammonium with nitrite as the electron acceptor as illustrated in Eq. 2.4 (Kuenen, 2008).

The possible metabolic pathway of anammox is illustrated in Fig. 2.6. Briefly, the anaerobic ammonium oxidation is a three-step process with two intermediates, i.e. nitric oxide and hydrazine. Firstly, the nitrite oxidoreductase (Nir) enzyme catalyzes nitrite reduction to nitric oxide. Then the produced nitric oxide reacts with ammonium to generate hydrazine, catalyzed by hydrazine synthase (HZS) enzyme. Finally, hydrazine is converted into N_2 gas by hydrazine dehydrogenase (HDH) enzyme (Kartal et al., 2011).

Figure 2.6 Metabolic pathway of anammox (Kartal et al., 2011).

According to Eq. 2.4 and Fig. 2.7, nearly half of the ammonium needs to be partially nitrified to nitrite, which with the remaining ammonium served as the substrate of anammox bacteria. The process of partial nitrification coupled with the subsequent anammox reaction is also known as deammonification or partial nitrification and anammox.

Figure 2.7 Nitrogen cycle in deammonification process (Lackner et al., 2014).

The deammonification process has the potential to achieve significant energy savings compared with the currently used conventional nitrogen removal processes (Dai et al., 2015, Siegrist et al., 2008, Van Loosdrecht et al., 2004). The energy savings mainly result from significantly reduced aeration (about 60%) and no need of organic carbon source. Moreover, the sludge production is as low as 0.11 g VSS/g $\text{NH}_4^+\text{-N}$, owing to the extremely low growth rate of anammox bacteria

(Hendrickx et al., 2012, Strous et al., 1998). This largely reduced the cost for sludge post-treatment (Fux et al., 2002).

2.4.2 Key factors affecting deammonification anammox process

The deammonification anammox is largely affected by the substrate availability, DO concentration, temperature, COD/N ratio, and the retention of anammox bacteria.

Substrate availability

According to Eq. 2.4, the growth of anammox bacteria requires nitrite and ammonium at a molar ratio of 1.32, while it had been widely reported that high-concentration ammonium and nitrite could inhibit anammox activity. For example, anammox activity was completely inhibited at the nitrite concentration of 100 mg N/L (Strous et al., 1999), while 50% of anammox activity was lost the nitrite concentration of 400 mg N/L (Lotti et al., 2012). These seem to suggest that the inhibition thresholds of nitrite may vary due to the differences in anammox species and experimental conditions. It had also been proposed that the effective inhibitors to anammox activity were free nitrous acid (FNA) and free ammonia (FA) which are related to solution pH (Dapena-Mora et al., 2007, Fernández et al., 2012). However, it should be realized that such inhibitions would unlikely occur in mainstream deammonification of municipal wastewater with low ammonia concentration.

DO concentration

Theoretically, anammox bacteria belong to anaerobic group which can only survive at dissolved oxygen concentration of about 0.5% air saturation (Kuenen, 2008). In

practice, mainstream deammonification can be realized in a two-stage partial nitrification and anammox process (Ma et al., 2011, Regmi et al., 2015), or a single-stage deammonification process (Gong et al., 2007, Liu et al., 2017). In two-stage process, oxygen inhibition is avoidable because aeration only takes place in the first stage for nitrification. In single-stage anammox process where partial nitrification and anammox concurrently take place in one reactor with anammox biofilm or granules as shown in Fig. 2.8. In the single-stage system, dissolved oxygen is depleted by outer aerobic layer of biofilms, whereas anammox bacteria are situated in the deeper layers of biofilms. As such, anammox activity had been found to be active even at elevated bulk DO concentration (Malovanyy et al., 2015, Wang et al., 2015). Nevertheless, the effective control of DO should be seriously considered in the design and operation of a single-stage deammonification system.

Figure 2.8 Stratification of anammox biofilm in a typical single-stage anammox reactor (Ødegaard, 2016).

Temperature

The cell growth and metabolic activity are determined by temperature. In the natural environment, anammox bacteria can survive in a wide range of temperatures, e.g. 6-43 °C (Kartal et al., 2010), with the optimal temperature of 30-40 °C (Dosta et

al., 2008, Hao et al., 2002, Strous et al., 1999). When the temperature declined from 25 °C to 15 °C, the observed anammox activity was reduced from 300-600 mg N/L day to 103 mg N/L day in a MBBR (Laureni et al., 2016). It appears that temperature is an obstacle for wide application of anammox process in all climate conditions.

COD/N ratio

Municipal wastewater has high COD/N ratio of 7-12 (Metcalf and Eddy, 2014), which creates the competition between heterotrophic denitrifiers and anammox bacteria for nitrite. Denitrifiers can readily outcompete anammox bacteria in the presence of sufficient COD due to its higher Gibbs free energy change in the respective reactions and higher growth rate than anammox bacteria (Xu et al., 2015). Table 2.1 summarizes the effects of the COD/N ratio on the anammox activity. It can be seen that high COD/N ratio negatively affect the anammox activity. In this regard, a COD/N ratio of 0.5 had been recommended for sustaining deammonification (Daigger, 2014). Such a situation becomes a technical hurdle for realizing deammonification of municipal wastewater often with a high COD/N ratio.

CHAPTER 2

Table 2.1 Effect of COD and COD/N upon anammox bacteria. (Xu et al., 2015)

COD type	COD/N	Process/reactor/biomass	Effect on anammox	Reference
Fat milk	0.9-2.0	UASB granule	Inhibition observed when COD>300 mg/L	Chamchoi et al. (2008)
Starch or peptone	1-4	SBR granule	Anammox activity sharply dropped when COD/N=4	Du et al. (2014)
Glucose	0.1-1	SBR granule	Anammox activity disappeared when COD/N increased to 1	Tang et al. (2013)
Landfill leachate	0.87	Single-stage, granule	anammox: 68% TN removal; denitrification 8% TN removal	Wang et al. (2010)
Sludge digester supernatant +Acetate/glucose	0.27-1.4	Single-stage, granule	Anammox decreased with COD/N increased to 4	Jenni et al. (2014)
N.A.	0.5-0.75	Single-stage, biofilm	TN removal efficiency decreased from 79-52%	Chen et al. (2009)

Retention of Anammox bacteria

Anammox bacteria have been known to have a very long doubling time of 11-25 days (Hendrickx et al., 2012, Strous et al., 1998). As such, in practice, a SRT longer than this doubling time should be applied for a good retention of anammox biomass in the treatment system. For this purpose, various liquid-solid separation techniques, such as hydrocyclone, physical sieving, etc., have developed for effectively retaining anammox bacteria (De Clippeleir et al., 2013, Wett et al., 2013). In addition, fixed anammox bacteria on biocarriers have also been reported (Gao et al., 2014, Gilbert et al., 2014, Persson et al., 2014, Wang et al., 2016).

2.4.3 Challenges with mainstream deammonification

So far, no successful mainstream deammonification for municipal wastewater has been reported in the literature. As summarized in Fig. 2.9, the challenges associated with mainstream deammonification may come from the following three aspects which are highly difficult to be integrated in an engineering process.

(i) Partial nitrification

Different from the nitrification-denitrification process, a partial nitrification is a pre-requisite of anammox reaction, by which the equal amount of ammonium and nitrite in a molar ration should be generated. It is rather challenging to control partial nitrification at such a stage in practice, especially for municipal wastewater with low ammonium concentration of around 40-50 mg N/L.

(ii) Effective suppression of denitrification

As illustrated in Fig. 2.9, another challenge with mainstream

deammonification is how to effectively suppress denitrification activity as nitrite produced from partial nitrification is the substrate of subsequent anammox. This appears even more challenging to manage at large-scale application simply because of the high COD/N ratio in municipal wastewater.

(iii) Effective suppression of nitrification

Nitrite produced from partial nitrification can be further oxidized to nitrate. For this purpose, NOB should be suppressed against AOB. Although extensive effort has been dedicated to this issue as discussed above, no convincing engineering solution is currently available in the literature, in particular, for municipal wastewater.

Figure 2.9 Challenges with mainstream deammonification.

2.5 COD CAPTURE

In the CAS process, COD was converted to biomass and carbon dioxide with

substantial energy consumption. As discussed above, the, COD in municipal wastewater should captured prior to the nitrification-denitrification and mainstream deammonification processes. So far, three main processes have been considered for COD capture, i.e. chemical enhanced primary treatment (CEPT), high rate activated sludge (HRAS) process and anaerobic treatment.

2.5.1 CEPT

CEPT is a physical-chemical treatment of wastewater by addition of chemical coagulants into wastewater to enhance the TSS removal. The full-scale studies showed that 80% of TSS in wastewater could be removed by CEPT (Metcalf and Eddy, 2014, Morrissey and Harleman, 1992, Wilson et al., 1975), while 60% removal for particulate COD (Parker et al., 2001, Wang et al., 2009). However, CEPT is ineffective in removing soluble COD in municipal wastewater, which accounts for about 30% of the total COD (Rossle and Pretorius, 2001). Therefore, the concentration of remaining soluble COD after CEPT is still high and needs further treatment.

2.5.2 HRAS

HRAS, originated from the A-stage of the Adsorption-Belebungsverfahren process (Böhnke, 1977), has widely explored due to its capability of capturing particulate, colloidal and dissolved organic matters with the aim to improve the primary sludge production. Briefly, the influent wastewater is mixed with the activated sludge in an aerated reactor where COD especially particulate COD is captured through aerobic biomass growth, bioflocculation, biosorption, etc. The produced biosolids are

subsequently separated in the clarifier with a portion recycled back to the main reactor and the remaining channeled to anaerobic digester for biogas production. In general, about 55-65% of the total COD could be removed (Miller et al., 2013, Wett et al., 2007). However, it is difficult to balance the wasted sludge and recycled sludge flows, i.e. more sludge is harvested for anaerobic digestion, lesser sludge would be available for subsequent COD capture (Xu et al., 2015), and this in turn would lead to the instability of the whole treatment system during the long-term operation.

2.5.3 Anaerobic treatment

In recent years, anaerobic process has gained increasing attention for municipal wastewater treatment. For the huge amount of municipal wastewater to be treated, the effective retention of slow growing anaerobic sludge at a relatively short HRT is of challenging in practice. To solve this puzzle, fixed anaerobic sludge or anaerobic granules had been extensively explored in various kinds of anaerobic reactors, e.g. upflow anaerobic sludge bed (UASB), anaerobic fluidized bed bioreactors, anaerobic membrane bioreactor (AnMBR), etc., have been developed (Alvarez et al., 2006, Martinez-Sosa et al., 2011, Urban et al., 2007, Wu et al., 2017). Typically, about 65-90% of COD in real municipal wastewater could be removed anaerobically, while the sludge production is about 3 to 20 times lower than that produced in the CAS process (Bitton, 2005, Seghezzi et al., 1998). In addition, the produced biogas is helpful for offsetting the total in-plant energy consumptions (Van Horne et al., 2012). However, it was found that a substantial amount of methane gas was dissolved in the effluent in anaerobic treatment of municipal

wastewater due to high methane solubility in water, i.e. about 18.6 g/m³ at 30 °C (Yamamoto et al., 1976). Methane has been known as a powerful greenhouse gas with a greenhouse effect power of 25 times higher than carbon dioxide (Forster et al., 2007). Therefore, dissolved methane without proper recovery in place becomes a hurdle for the wide application of anaerobic processes for treating municipal wastewater (Liu et al., 2013).

2.5.4 Summary

CEPT, HRAS and anaerobic process are able to capture COD from municipal wastewater at different capacities. Table 2.2 compared their performance, advantages and challenges. Despite the issue of dissolved methane, anaerobic process has advantages of simultaneous COD capture and biogas production, minimized sludge production, no additional chemical or aeration required etc.

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Table 2.2 Summaries of CEPT, HRAS and anaerobic process for COD capture.

	CEPT	HRAS	Anaerobic treatment
COD removal	60%	55-65%	65-90%
Advantages	Easy retrofitting in existing facility	Small foot print Better soluble COD removal	Small foot print No aeration needed High COD removal efficiency Direct conversion of organics to biogas Low sludge production
Challenges	Ineffective for soluble COD Cost of chemical Further AD for sludge treatment	Unbalanced sludge flow Further AD for sludge treatment	Dissolved methane

2.6 BNR: CHALLENGES FORWARD

As discussed above, current municipal wastewater treatment plants are all operated in an energy-negative fashion with production of huge amount of excess sludge. As pointed by Wan et al. (2016), further optimization of the CAS process could not lead to substantially improved in-plant energy efficiency. Therefore, novel process configurations should be carefully explored for turning the current WWTPs from energy negative to energy neutral or even positive operation. The ways toward such an ultimate goal is to minimize in-plant energy consumption, while maximizing energy recovery from wastewater as illustrated in Fig. 2.10. Obviously, there is a tremendous technology gap towards this end. Therefore, this thesis attempted to address these challenging issues by developing the novel biological processes which organically integrated the concepts of in-plant energy reduction and recovery as illustrated in Fig. 2.10.

Figure 2.10 Energy gain potential in A-B process (Wan et al., 2016).

CHAPTER 3 FEASIBILITY OF A-B PROCESS IN MUNICIPAL WASTEWATER TREATMENT

3.1 INTRODUCTION

The principle of the conventional activated sludge (CAS) process for wastewater treatment is to convert organic matter to biomass and carbon dioxide. After more than hundred years of successful applications, the CAS process is receiving pressing critiques due to its high energy demand and the generation of extremely huge amount of excessive activated sludge. For example, about 3% of the annual electrical energy was utilized for wastewater reclamation in USA (US-EPA, 2006). Obviously, high energy consumption and low energy recovery rates in the CAS process which is the core unit in many wastewater treatment plants (WWTPs) is posing increasing challenges on the global wastewater industry. In general, the activated sludge growth yield in the CAS process falls into the range of 0.3-0.5 g dry biomass produced per gram soluble COD removed, i.e. 1 kg COD removed will generate 0.3-0.5 kg dry biomass. As a result, huge amount of wasted activated sludge is currently generated in municipal WWTPs globally, e.g. in 2010, about 10 million tons of dry sludge in the European Union, 8 million tons in the United States, and about 11 million tons in China. The global situation of wasted activated sludge is becoming more and more pressing due to the increasing capacity of global wastewater treatment, especially in developing countries. The current practice shows that the treatment of the excess activated sludge may account for 25 to 65%

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of the total plant operation cost (Zhao and Kugel, 1996).

In order to reduce energy input and sludge disposal, anaerobic digestion of wasted activated sludge has been commonly practiced worldwide. However, only about 30–50% of the total COD or volatile solids can be degraded through anaerobic digestion, indicating a low overall energy efficiency of anaerobic digestion. This is largely because anaerobic microorganisms cannot directly utilize waste sludge prior to being hydrolyzed to soluble COD. This indeed is the major problem of the present WWTPs where soluble COD is first converted to biomass which is further subject to anaerobic digestion for energy recovery and sludge volume reduction, but at low efficiency. It has been argued that organic matter in municipal wastewater should be captured for direct anaerobic digestion prior to biological conversion to sludge (Wan et al., 2016). Therefore, this study aimed to develop an A-B process in which an anaerobic moving bed biofilm reactor (AMBBR) was employed as A-stage for COD capture, and an integrated fixed-film activated sludge-sequencing batch reactor (IFAS-SBR) as B-stage for biological nitrogen removal via nitrification-denitrification. It is expected that the results presented in this study may inspire new thinking about how future WWTP should be configured with considerations of enhanced energy recovery, reduced energy consumption and lesser production of wasted activated sludge in a more holistic manner.

3.2 MATERIALS AND METHODS

3.2.1 Process configuration and operation conditions

A laboratory scale A-B process with an anaerobic moving bed biofilm reactor (AMBBR) as A-stage and an integrated fixed-biofilm and activated sludge SBR (IFAS-SBR) as B-stage was developed in this study (Fig. 3.1). In the AMBBR with the working volume of 4 L, 1 cm³ of cubic sponges (Nisshinbo Chemical Inc., Japan) were used as bio-carriers with a packing ratio of 30% by volume. The temperature was maintained at 30 ± 1 °C, while overhead mechanical mixer was run at 60 rpm. The AMBBR effluent was introduced into a flash chamber for the recovery of dissolved methane.

Figure 3.1 Schematic diagram of the proposed A-B process.

The IFAS-SBR with a working volume of 4 L was employed as B-stage for biological nitrogen removal, in which the same biocarriers as used at A-stage were packed at 25% by volume. In the IFAS-SBR, a porous stone-type diffuser was

employed for aeration at an air flow rate of 0.8 L/min. The temperature was kept at 30 °C, while the hydraulic retention time (HRT) was set at 6.5 h. The sludge retention time (SRT) of the suspended sludge was controlled by sludge wasting. The IFAS-SBR was operated in 4 sub-cycles of alternating anoxic and oxic phases with step feeding at the beginning of each anoxic phase, as shown in Fig.3.2. The A-B process was controlled by a programmable logic controller (PLC) over the whole operation period.

Figure 3.2 Operating pattern of B-stage SBR.

Anaerobic and aerobic sludges from a local wastewater treatment plant were used to inoculate the AMBBR and IFAS-SBR, respectively. Synthetic wastewater used in this study contained 400 mg COD/L as sucrose, 45 mg /L $\text{NH}_4^+\text{-N}$, 750 mg/L NaHCO_3 , 22 mg/L KH_2PO_4 , 20 mg/L $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 20 mg/L $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, 45 mg/L $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ and 1.5 mg/L $\text{FeCl}_3\cdot 7\text{H}_2\text{O}$. The pH and the alkalinity of the

synthetic wastewater were to be about 7.8 and 450 mg CaCO₃/L, respectively.

3.2.2 Recovery of dissolved methane from AMBBR effluent

A flash chamber equipped with mechanical mixer was designed for recovery of dissolved methane from AMBBR effluent, and it was operated at the predetermined hydraulic retention time (HRT) and mixing speed of 11 min and 800 rpm. Dissolved methane concentration was measured by modified headspace method (Bandara et al., 2011, Souza et al., 2011). 65 mL of sample was collected in a 120 mL serum bottle to which 1 mL of 20 mM HgCl₂ solution was dosed in order to inactivate microorganisms in the sample. The samples in sealed bottles were subject to vigorous vortex for 1 minute, and then were shaken for 30 min at 30 °C. After then, the pressure in the headspace was measured by a manometer (Dwyer 475, Dwyer Instruments, USA). 5 mL of gas sample in the headspace was further extracted by a syringe for gas composition analysis by a gas chromatography with thermal conductivity detector (GC-TCD) (Agilent Technologies 7890A GC system, USA). Lastly, the concentration of dissolved methane can be calculated according to Eq. 3.1 (Souza et al., 2011):

$$C_{DM} = \frac{\left(\frac{m_l}{k_{H,inv}^{px} \times M_l} + \frac{V_g}{RT} \right) M_m P [CH_4\%]}{V_l} \quad (3.1)$$

where C_{DM} is concentration of dissolved methane (mg/L), m_l is mass of the liquid phase (g), $k_{H,inv}^{px}$ is reciprocal value of k_H , which is 3.87×10^9 Pa at 30 °C, M_l is molecular weight of water, i.e. 18 g/mol, V_g is volume of gas phase (L), R is ideal gas constant=8.314 L·kPa/(K·mol), T is absolute temperature (K), M_m is molecular weight of methane=16 g/mol, P is pressure in the headspace of the serum bottle,

(Pa), [CH₄%] is percentage of methane in the gas phase (%) and V₁ is volume of water (L). The concentration of dissolved CO₂ in the liquid phase was also calculated according to Henry's Law in a way similar to dissolved methane.

3.2.3 Rapid mixing-associated energy demand

The mixing-associated energy demand in the flash chamber can be calculated according to Eq. 3.2:

$$P = N_p \cdot \rho \cdot n^3 \cdot d^5 \quad (3.2)$$

where P is energy dissipated into water (W), N_p is the power number of impeller, which was 0.44 according to Nagata (1975), ρ is water density (kg/m³), n is mixing speed, rps and d is impeller diameter (m).

According to Viessman et al. (2009), it was assumed that the mixer is only 70% efficient in transferring power to the water, i.e. the unit energy demand can be estimated as follows:

$$E = \frac{P}{0.7 \times Q} \quad (3.3)$$

where E is energy demand for degassing one cubic meter of AMBBR effluent (Wh/m³) and Q is wastewater flowrate (m³/h).

3.2.4 Analytical Methods

COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and volatile suspended solids (VSS) were determined according to standard methods (APHA, 2005). Dissolved organic carbon (DOC) was determined by a Total Organic Carbon Analyzer (TOC-L, Shimazu).

The volume of biogas produced from the AMBBR was measured by a gas meter (TG 05 Model 5, Ritter, Germany), while its composition was further analyzed by a gas chromatography equipped with thermal conductivity detector (GC-TCD) (Agilent Technologies 7890A GC system, USA).

3.2.5 Quantitative PCR

DNA of the suspended sludge and fixed biofilm was extracted using a Fast DNA spin kit (MP Biomedicals, LLC). The abundance of 16S rRNA gene representing AOB, *Nitrobacter*, *Nitrospira*, denitrifiers and total bacteria were determined by quantitative PCR (q-PCR) using SybrGreen assays with primers listed in Table 3.1.

Table 3.1 Primers used for q-PCR analysis

Primer	Gene	Target	Sequence (5'-3')	Annealing Temp. (°C)	References
CTO189f ^a CTO654r	16S rRNA	β-Subdivision AOB	CTAGCYTTGTAGTTTCAAACGC	57	Araki et al. (2004) Kowalchuk et al. (1997)
FGPS872 FGPS1269	16S rRNA	Nitrobacter	CTAAAACTCAAAGGAATTGA TTTTTTGAGATTTGCTAG	50	Degrange and Bardin (1995)
NSR1113f NSR1264r	16S rRNA	Nitrospira	CCTGCTTTCAGTTGCTACCG GTTTGCAGCGCTTTGTACCG	65	Dionisi et al. (2002)
nosZF nosZ1622R	nosZ	Denitrifiers	CGYTGTTCMTGACAGCCAG CGSACCTTSTTGCCSTYGCG	53	Throback et al. (2004)
341f 518r	16S rRNA	Universal	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	55	Muyzer et al. (1993)

3.3 RESULTS

3.3.1 A-stage Performance

Fig. 3.3 shows that 68% of influent COD was removed in the AMBBR as A-stage in the first 20 days. From day 21 onward, the COD removal was gradually stabilized at about 85% with an effluent COD concentration of 57 mg COD/L. It was observed that biogas production rate gradually increased to about 280 L/m³d of which 70% was methane, while 17.1 mg/L of dissolved methane was found in the effluent from the steady-state AMBBR (Fig. 3.4), which is close to the theoretical concentration of dissolved methane at 30 °C. In the AMBBR, the specific methane yield was found to be about 0.15 L/g COD removed in the gas phase and 0.08 L/g COD removed in the liquid phase, indicating a total specific methane yield of 0.23 L/g COD removed in the steady-state AMBBR. It should also be noted that dissolved methane accounted for about 35% of total methane produced in the AMBBR. Obviously, this may compromise the expectable energy yield, and also leads to a serious concern on greenhouse gas emission if dissolved methane was not properly recovered.

Figure 3.3 COD concentration profiles in the AMBBR (A-stage).

Figure 3.4 Dissolved methane in the AMBBR and flash chamber effluents.

The SRT was not controlled in the AMBBR, while the effluent suspended solids concentration was 38 ± 15 on average after 60-day operation. On the other hand, the observed biomass yield in the AMBBR gradually stabilized at 0.1 g VSS/g COD. The carbon flows in the steady state AMBBR further revealed that about 34.0% of the influent carbon was converted to carbon dioxide and about 27.7% channeled into methane, 15.2% to biomass ($C_5H_7O_2N$) and 19.2% in the AMBBR effluent (Fig. 3.5). As presented above, about 35% of the total methane produced existed in the form of dissolve methane in the AMBBR effluent. Therefore, in this study, a flash chamber was developed for recovery of dissolved methane from the AMBBR effluent. Fig. 3.4 shows that the dissolved methane in the AMBBR effluent was reduced to 1.7 mg/L after 11-min mixing at 800 rpm in the flash chamber, indicating 90% of removal efficiency.

Figure 3.5 Carbon flows in the AMBBR.

3.3.2 B-stage performance and microbial structure analysis

In this study, an IFAS-SBR was used as B-stage. The results showed that most influent ammonia was converted to nitrite with a clear nitrite shunt (Fig. 3.6), and about 87% of total nitrogen was removed via denitrification on nitrite in the period of day 10 to day 55 when the influent COD/N ratio was above 1.9 (Fig. 3.7). However, the accumulation of nitrate tended to be dominant with further decrease in the influent COD/N ratio from day 55 onward. As a result, the nitrite shunt was deteriorated when the COD concentration in the steady state AMBBR effluent which was the influent to IFAS-SBR was stabilized at 50 mg/L (Fig. 3.7).

Figure 3.6 Concentration profiles of ammonium, nitrite and nitrate in the IFAS-SBR (B-stage).

Figure 3.7 Changes in COD/N ratio, nitrite accumulation and total nitrogen (TN) removal in the IFAS-SBR.

Fig. 3.8 showed the concentration profiles of both suspended and fixed biomasses as well as corresponding microbial populations in the IFAS-SBR. Moreover, the cyclic nitrogen profiles of the IFAS-SBR were determined on day 13, 48 and 105, respectively (Fig. 3.9), which allow linking the nitrogen removal with the microbial community evolution. On day 13, both nitrite and nitrate concentrations were undetectable at the end of anoxic phase, indicating an excellent denitrification. This is probably due to the fact that the left-over COD from the A-stage effluent was sufficient for denitrification in the anoxic phase. In the oxic phase, most of ammonium was oxidized to nitrite, with 80% of nitrite accumulation. On day 48, nitrite could not be fully denitrified with about 2 mg nitrite-N/L left that was then

carried over to oxic phase, while the abundance of denitrifying population in suspended sludge dropped from 4.86×10^{11} copies/g VSS on day 20 to 2.33×10^{11} copies/g VSS on day 48. Meanwhile, the abundance of AOB remained relatively constant at 5×10^9 to 6×10^9 copies/g VSS. However, the abundances of AOB, *Nitrospira* and denitrifiers in biofilms tended to decline over this period. It should also be noted that the COD removal in the AMBBR (A-stage) was improved along the operation, and this led to a reduced COD/N ratio which in turn exerted a significant impact on the microbial community structure of the IFAS-SBR (B-stage). On day 105, nitrite and nitrate could not be totally removed via denitrification in anoxic phase of the IFAS-SBR due to insufficient COD concentration in the effluent from the AMBBR (A-stage), while nearly all ammonia was oxidized to nitrate in the oxic phase of the IFAS-SBR. Accordingly, the abundance of denitrifying population in suspended sludge of the IFAS-SBR further decreased to 5.8×10^{10} copies/g VSS, while the AOB and *Nitrospira* abundances were found to increase to 3.9×10^{10} and 1.2×10^{11} copies/g VSS, respectively. On the other hand, the abundance of *Nitrospira* in the fixed biofilms of the IFAS-SBR increased to 1.4×10^{10} copies/g SS, and the abundance of AOB and denitrifiers in biofilms remained relatively constant.

Figure 3.8 Respective abundance of 16S rRNA gene of key functional species and biomass concentration in the IFAS-SBR (a: suspended sludge; b: biofilm).

Figure 3.9 Time profiles of ammonium, nitrite and nitrate in one cycle operation on day 13 (a), day 48 (b) and day 105 (c).

Alternating anoxic and oxidic conditions has been adopted for suppressing the growth of NOB versus AOB (Ge et al., 2014, Kornaros et al., 2008, Yang and Yang, 2011). As shown above, successful nitrite accumulation was achieved under alternating anoxic and oxidic conditions in the presence of sufficient COD for denitrification in the IFAS-SBR. However, short-cut nitrification gradually disappeared when the influent COD concentration to the IFAS-SBR was lowered due to the improved performance of the AMBBR. It is reasonable to consider that undenitrified nitrite in the anoxic phase entered the oxidic phase where it promoted the growth of NOB. Consequently, the outcompetition of NOB against AOB leads to the failure of short-cut nitrification as observed. This was also supported by the abundance analysis of NOB in the presence of sufficient and insufficient COD, e.g. the

abundance of NOB on day 109 (insufficient COD for denitrification) was about 2.3 times higher than that on day 20 (sufficient COD for denitrification). These seem to suggest that alternating anoxic and oxic conditions are necessary, but not sufficient for maintaining a sustainable short-cut nitrification.

3.4. DISCUSSION

3.4.1 Reduced sludge production and dissolved methane recovery

In the CAS process, about 0.3-0.5 g dry biomass can be produced with 1 g of COD removed (Wan et al., 2016), with an average of 0.4 g dry biomass/g COD. Evidence shows that sludge production in anaerobic processes can be much less than that in the CAS process, e.g. 0.18 g VSS/g COD and 0.03 to 0.2 g SS/g COD reported by Henze and Harremoës (1983) and Lettinga et al. (1983), respectively. As discussed above, the observed growth yield of biomass in the AMBBR was estimated as 0.1 g dry biomass/g COD, which was 75% lower than that in the CAS process. As influent COD was captured in the AMBBR (A-stage) prior to biological conversion to sludge, this may lead to several advantages over the CAS process including (i) enhanced energy recovery; (ii) reduced energy consumption associated with aerobic oxidation of COD and (iii) significantly less sludge production.

Recently, the wastewater treatment-energy-greenhouse gas nexus in municipal wastewater treatment plant has drawn more and more attention due to its potential impact on global climate change and in-plant energy recovery. As shown above, about 35% of methane produced was found to be dissolved in the AMMBR effluent. In fact, the theoretical solubility of methane in water is about 18.6 mg/L at 30 °C

(Yamamoto et al., 1976), while dissolved methane may account for 50% of total methane generated in an anaerobic MBR at 15 °C (Smith et al., 2013). Obviously, dissolved methane has become a serious hurdle limiting wide application of various anaerobic processes for low-strength municipal wastewater treatment (Liu et al., 2014). On the other hand, methane has been known to have a greenhouse power of 25 times higher than carbon dioxide (Forster et al., 2007). These all point to the fact that without proper recover/removal of dissolved methane, anaerobic processes for municipal wastewater treatment is seriously challenged by its economic viability and environmental sustainability for their large-scale applications.

In this study, a flash chamber was developed for high-efficiency removal of dissolved methane from the AMBBR effluent. 90% of dissolved methane could be recovered from the AMBBR effluent when the flash chamber was operated at the HRT of 11 min and 800 rpm, leading to a residual dissolved methane concentration of 1.7 mg/L on average. It should also be realized that few methods so far have been investigated for dissolved methane recovery from anaerobic effluents. Bandara et al. (2011) reported a degassing membrane for separation of dissolved methane from anaerobic effluent, with a recovery efficiency of about 76%, while a highly spacious two-stage down-flow hanging sponge system was also reported, in which most of dissolved methane was removed in the first stage and the residue was then oxidized in the second stage (Matsuura et al., 2010, Matsuura et al., 2015). Obviously, compared to the methods reported in the literature, the flash chamber developed in this study for dissolved methane recovery from anaerobic effluent is easily scalable, simple in operation and maintenance, smaller footprint, high efficiency, low capital and operation cost.

3.4.2 Potential energy recovery at A-stage

In the CAS process, excessive activated sludge produced from biological oxidation of COD is subjected to anaerobic digestion for energy recovery. It had been shown that about 26% of primary sludge-COD and 7% of secondary sludge-COD would be converted to methane equivalent (Wan et al., 2016). In contrast, a total 55% of COD was channeled into methane in the A-stage AMBBR (Fig. 3.5), i.e. such overall methane yield is significantly higher than that currently achieved in the CAS process coupled with anaerobic digestion of waste activated sludge. In general, the combustion value of methane is 40 MJ/m³ (Gupta et al., 2015), 35% of which can be converted to electrical energy (McCarty et al., 2011). In the steady-state AMBBR from day 90 onwards, the potential electricity potentially generated from methane produced including recovered dissolved methane can be estimated as (21.6 L dissolved CH₄ recovered/m³ wastewater + 49.1 L CH₄ gas/m³ wastewater) × 40 MJ/m³ methane gas × 35% / 3600 J/Wh ≈ 0.28 kWh/m³. On the other hand, the mixing-associated energy demand for removing dissolved methane from the AMBBR effluent at the HRT of 11 min and 800 rpm was calculated by Eqs. 3.1 and 3.2, which was about 0.12 kWh/m³. Although the energy consumption associated with dissolved methane recovery was higher than the energy recovered from dissolved methane (i.e. 0.08 kWh/m³), it should be pointed out that the total energy harvested from the A-stage was more than sufficient to offset the degassing-associated energy consumption.

As discussed above, the proposed A-B process can significantly enhance the energy recovery at A-stage, while markedly reduces aeration-associated energy demand at

B-stage with a substantially less production of waste activated sludge. The energy gain potential (E_g) of this A-B process against the CAS process can be estimated in a way such that:

$$E_g = E_a + E_b + E_c - E_d \quad (3.4)$$

where E_a is energy recovered from methane, E_b is energy saved from reduced aeration for COD oxidation, E_c is energy saved from post-treatment of wasted activated sludge and E_d is energy associated with mixing for recovery of dissolved methane. As shown above, E_a and E_d were estimated to be 0.28 kWh/m^3 and 0.12 kWh/m^3 respectively. In the current practice, aeration and sludge treatment may account respectively for 50% and 30% of the total in-plant energy consumption in the CAS process (Henderson, 2002). Generally, the energy consumption in the CAS process is in the range of 0.3 to 0.6 kWh/m^3 of domestic wastewater treated with an average of 0.45 kWh/m^3 (McCarty et al., 2011, Wan et al., 2016). For the purpose of discussion, these suggest that E_b and E_c could be respectively calculated in a simple fashion, i.e. 0.23 kWh/m^3 and 0.1 kWh/m^3 with 75% sludge reduction in the proposed A-B process as compare to the CAS process. Therefore, the energy gain potential in the proposed A-B process against the CAS process was calculated to be $(0.28+0.23+0.1-0.12) = 0.49 \text{ kWh/m}^3$, which is greater than the averaged energy consumption of 0.45 kWh/m^3 in the CAS process. Therefore, it appears possible and feasible to achieve energy-neutral or even positive operation of the proposed A-B process versus the CAS process.

Theoretically, to maximize the energy recovery, influent COD should be captured as much as possible at A-stage. However, such ideal strategy would not be feasible

with consideration of COD eventually needed for biological nitrogen removal at B-stage. Obviously, more COD captured at A-stage leads to lesser COD entering B-stage for biological nitrogen removal. According to the denitrification stoichiometry, about 2.86 gram and 1.71 gram COD should be required for completely denitrifying 1 gram NO_3^- -N and NO_2^- -N respectively. In this study, the ammonia concentration to the IFAS-SBR was about 38 mg/L, i.e. at least 109 mg/L and 65 mg/L COD should be needed for denitrification via nitrate and nitrite, which represented about 27% and 16% of COD in the influent wastewater. In fact, these in turn determine to what degree wastewater COD should be captured at A-stage. As shown above, when more COD was captured in the AMBBR (A-stage), the COD entering the IFAS-SBR (B-stage) was insufficient for complete biological nitrogen removal, leading to lower nitrogen removal efficiency. Thus, the conventional nitrification-denitrification process could not be considered as a reasonable choice of B-stage in the A-B process. In fact, Wan et al. (2016) pointed out that only shortcut nitrification-denitrification and partial nitrification combined with anammox processes would be the ideal candidates for B-stage. However, it should be realized that mainstream deammonification of municipal wastewater has not yet been fully demonstrated at full scale, e.g. its operation largely relies on frequent bioaugmentation of anammox and AOB sludges from sidestream (O'Shaughnessy, 2016). On the contrary, nitrogen removal via nitrification-denitrification is more mature and reliable. Consequently, the operation of A-B process should be optimized in a more holistic manner with consideration of both energy recovery and nitrogen removal.

3.5 CONCLUSIONS

This study demonstrates that the integrated AMBBR and IFAS-SBR is a feasible A-B process for low-strength wastewater treatment with the benefits of energy recovery, reduced energy consumption and production of waste activated sludge. It was also shown that the proposed flash chamber was able to recover about 90% of dissolved methane in the AMBBR effluent with the lower energy demand of 0.12 kWh/m³. With the recovery of dissolved methane, a total of 0.28 kWh/m³ of energy could be harvested at the A-stage (i.e. AMBBR), which was more than sufficient to offset the mixing-associated energy consumption in the flash chamber for degassing of dissolved methane. Compared to the CAS process, the overall sludge production could be cut by about 75% in the proposed A-B process due to the COD capture in the AMBBR, which in turn significantly relieves energy demand on aeration for COD oxidation and post-treatment of waste activated sludge at the B-stage (i.e. IFAS-SBR). It appears highly possible and feasible to achieve the energy-neutral or even energy positive operation of a municipal wastewater treatment plant by adopting A-B process as its core unit. Given the complex situation in A-B process, future study is still needed to look into the system optimization with the focus on the overall performance of the whole system.

CHAPTER 4 A NOVEL STRATEGY TOWARDS SUSTAINABLE NITRITATION-DENITRITATION IN AN A-B PROCESS FOR MAINSTREAM MUNICIPAL WASTEWATER TREATMENT

4.1 INTRODUCTION

In order to achieve energy-neutral or even energy-positive operation of WWTPs, logically organic matter in municipal wastewater should be captured for direct anaerobic treatment prior to biological conversion to activated sludge (Wan et al., 2016). In the preceding chapter, an A-B process with anaerobic unit as A-stage and biological nitrogen removal unit as B-stage via nitrification and denitrification was proposed towards enhanced energy recovery, reduced energy consumption and minimized sludge production. However, in this process it was difficult to maintain a stable nitrification-denitrification at B-stage due to the excessive COD capture at A-stage.

So far, several different operation strategies have been proposed for single-stage mainstream nitrification, e.g. alternating anoxic and oxic conditions (Ge et al., 2014, Kornaros et al., 2008, Yang and Yang, 2011), aggressive SRT (Peng and Zhu, 2006, Regmi et al., 2014, Yuan et al., 2008), step feeding (Dytczak et al., 2008, Ge et al., 2014, Lemaire et al., 2008), intermittent aeration (Kornaros et al., 2010, 2008) and real-time control of pH, oxygen uptake rate, and ORP (Blackburne et al., 2008,

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Gao et al., 2009, Gu et al., 2012, Lee et al., 2013). However, it should be realized that these operational options may not be applicable for A-B process where most influent COD is captured at A-stage, and the influent to B-stage often has much lower COD/N ratio (Gu et al., 2017), and such a situation may introduce complex kinetic competitions among ammonia-oxidizer (AOB), nitrite oxidizer (NOB) and denitrifiers in A-B process. These suggest that the energy recovery at A-stage and nitrogen removal at B-stage should need to be synergized.

Given the situation elaborated above, this study aimed to develop a novel strategy towards sustainable and stable nitrification-denitrification in A-B process for mainstream municipal wastewater treatment, where an anaerobic fixed bed reactor (AFBR) served as A-stage for COD capture, a sequencing batch reactor (SBR) as B-stage for nitrogen removal via nitrification and denitrification. Moreover, the proposed novel A-B process was also characterized in terms of energy consumption, energy recovery, and sludge reduction against existing biological processes for municipal wastewater treatment. It is expected that this study may trigger new thinking about possible process configuration for future wastewater reclamation.

4.2. MATERIALS AND METHODS

4.2.1 Process design and operation

A laboratory scale A-B process with an anaerobic fixed bed reactor (AFBR) as A-stage, a sequencing batch reactor (SBR) as B-stage was developed in this study (Fig. 4.1). The AFBR with a working volume of 5 L was packed with bio-ropes as bio-carriers, while the temperature was maintained at 30 ± 1 °C. The hydraulic

retention time (HRT) of the AFBR was controlled at 6.4 hours, and the effluent was firstly introduced into a flash chamber for the recovery of dissolved methane. The SBR with a working volume of 4 L was employed as B-stage for biological nitrogen removal, in which aeration was supplied through a porous stone-type diffuser with an air flow rate of 0.8 L/min giving a dissolved oxygen (DO) concentration of 1.5 mg/L. The temperature in the SBR was kept at 30 °C, while the HRT was set at 5.5 h. The sludge retention time (SRT) was controlled between 4 and 6 days with a volume exchange ratio of 50%. The SBR was operated in 4 alternating anoxic and oxic sub-cycles of with step feeding of 0.5 L at the beginning of each anoxic phase. pH was not specifically controlled, while varied in the range of 7.3 and 8 along with the alternating anoxic and oxic phases. The whole process was controlled by a programmable logic controller (PLC). For the purpose of comparison, the whole study was structured into two phases as illustrated in Fig. 1, i.e. Phase 1 (from day 1 to 39) and Phase 2 (from day 40 onward) without and with bypass flow, respectively. 19% of influent wastewater was directly bypassed to the B-stage SBR, resulting in a targeted COD/N ratio of about 2.

Figure 4.1 Schematic diagram of the A-B processes operated in Phases 1 and 2. (a): without bypass flow (Phase 1); (b): with bypass flow (Phase 2)

Anaerobic and aerobic sludge from a local WWTP were used to inoculate the AFBR and SBR, respectively. Synthetic wastewater used in this study was similar to that presented in the preceding chapter. A flash chamber as described in the preceding chapter was used for recovery of dissolved methane from the AFBR effluent.

4.2.2 Analytical Methods

Concentrations of COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and volatile suspended solids (VSS) were determined according to standard methods (APHA, 2005). The volume of biogas produced from the AFBR was measured by a gas meter (TG 05 Model 5, Ritter, Germany), while its composition was further analyzed by a gas chromatography equipped with thermal conductivity detector (GC-TCD) (Agilent Technologies 7890A GC system, USA).

4.2.3 Specific activities of AOB and NOB

In this study, the activities of AOB and NOB were represented by their respective specific ammonia and nitrite oxidation rates (i.e. $q_{NH_4^+}$ and $q_{NO_2^-}$) which were determined by batch experiments with freshly discharged sludge and effluent from B-stage. The initial biomass concentration was fixed at about 500 mg/L MLVSS. Samples were taken at time intervals of 15 min and were filtrated immediately by 0.45 μ m filters for ammonia, nitrite and nitrate analyses. The $q_{NH_4^+}$ and $q_{NO_2^-}$ could be calculated by the ammonium and nitrite oxidation rates further divided by the biomass concentration.

4.2.4 Quantitative PCR and FISH

DNA of activated sludge taken from the B-stage was extracted using a Fast DNA spin kit (MP Biomedicals, LLC). The abundance of 16S rRNA gene representing ammonia oxidizing bacteria (AOB), *Nitrobacter spp.*, *Nitrospira spp.*, denitrifiers and total bacteria were determined by quantitative PCR (q-PCR) using SybrGreen assays with primers previously described (Gu et al., 2017). To visually observe the relative abundance AOB and nitrite oxidizing bacteria (NOB) in the activated sludge, sludge sample was observed by fluorescence in situ hybridization (FISH) method. For this, the sludge sample was treated according to Manz et al. (1992), and the probes used for hybridization were EUBmix (equimolar of EUB338, EUB338 II and EUB338 III) for staining all bacteria (Daims et al., 1999), NEU for AOB (Wagner et al., 1995), NIT 3 for *Nitrobacter spp.* (Wagner et al., 1996), and NTSPA 714 for *Nitrospira spp.* (Loy et al., 2002). The FISH samples were visualized with a Zeiss LSM 880 (Carl Zeiss, Jena, Germany) confocal laser scanning microscope.

4.3. RESULTS AND DISCUSSION

It had been demonstrated in the preceding chapter that the excessive COD capture for energy production at A-stage led to unsustainable nitrification-denitrification at B-stage. To address this challenging issue, an innovative A-B process was developed in which an AFBR was used as A-stage and a SBR as B stage, while a portion of influent wastewater was bypassed from A-stage and directed to B-stage as illustrated in Fig. 1b. For the purpose of comparison, this study was conducted in two phases, i.e. a normal A-B process without bypassing of influent wastewater in Phase 1 (Fig. 4.1a) and a novel A-B process with a bypassed influent wastewater to B-stage in Phase 2 (Fig. 4.1b).

4.3.1 General performance of A-stage

Fig. 4.2 shows the COD concentration profiles in the influent and effluent of AFBR throughout the whole operation period (i.e. Phases 1 and 2), while the performances of the steady-state AFBR are summarized in Table 4.1. It can be seen that the COD removal was gradually stabilized at about 80% with an effluent COD concentration of 74 mg COD/L and the biogas production of 277 L/m³d of which 67% was methane in the steady-state AFBR. Meanwhile, 29.6 mg/L of dissolved methane was found in the AFBR effluent. The specific methane yield was determined as 0.17 L/g COD in the gas phase and 0.14 L/g COD in the liquid phase, i.e. a total methane yield of 0.31 L/g COD was achieved at A-stage. However, it should be realized that dissolved methane accounted for about 45% of total methane produced in the AFBR, which may seriously compromise energy recovery. In this study, 89% of dissolved methane was recovered by using the flash chamber. In addition, the observed

biomass yield in the steady state AFBR was as low as 0.09 g VSS/g COD, which was about 78% lower than that of the conventional activated sludge, e.g. 0.4 g VSS/g COD (Wan et al., 2016). Based on these results, it is reasonable to consider that the bypassed influent wastewater to the B-stage had no impact on the performance of the A-stage AFBR.

Table 4.1 The performances of steady-state AFBR

Parameter	Value
COD	
Influent (mg/L)	375
Effluent (mg/L)	74
Removal efficiency	80%
Sludge	
Effluent MLVSS (mg/L)	33.7
Sludge yield (g biomass/g COD)	0.09
Methane production	
Methane yield in gas phase (L/g COD)	0.17
Methane yield in liquid phase (L/g COD)	0.14
Total methane yield (L/g COD)	0.31
Dissolved methane recovery rate	89%

Figure 4.2 COD profile in the A-stage AFBR and the B-stage SBR.

4.3.2 Effect of bypassed influent flow on B-stage performance

The soluble COD concentration in the steady stage B-stage effluent was found to be about 10 mg/L in both Phases 1 and 2 (Fig. 4.2), showing that the bypassed influent had no negative effect on the COD removal at the B-stage. In Phase 1, most influent ammonia was initially converted to nitrite with a clear nitrite shunt indicated by a NAR of above 86% (Figs. 4.3 and 4.4). In fact, about 78% of TN was removed via denitrification in the period of day 12 to day 30 of Phase 1 when the influent COD/N ratio was above 2.9 (Figs. 4.3 and 4.4). However, the accumulation of nitrate gradually became dominant with further decrease of the influent COD/N ratio from day 30 onward in Phase 1. As the consequence, the NAR and TN removal efficiency

at B-stage were reduced to 28% and 42% at the end of Phase 1, i.e. both nitrite shunt and denitrification were deteriorated due to the insufficient supply of COD by the end of Phase 1. These clearly suggest that the A-B process with the configuration as illustrated in Fig. 4.1a would not be sustainable toward the long-term stability of nitrification-denitrification in treating low-strength municipal wastewater.

Figure 4.3 Nitrogen profiles in the A-stage AFBR and the B-stage SBR.

Figure 4.4 Nitrogen accumulation rate (NAR), TN removal efficiency and COD/N ratio in SBR.

In Phase 2 of this study, the A-B process (Fig. 4.1a) was modified by bypassing 19% of influent wastewater from the A-stage, and the bypassed flow was further mixed with the A-stage AFBR effluent. The mixed wastewater stream was then fed to the B-stage SBR as illustrated in Fig. 4.1b. It can be seen in Fig. 4.3 that the excellent ammonium removal was achieved throughout the entire Phase 2. After 20 days of operation with the bypassed influent to the B-stage, the nitrate concentration in the SBR effluent gradually decreased to below 1.5 mg NO_3^- -N/L, while the NAR was increased to 97%. The nitrogen removal at the B-stage was significantly improved from 50% to 85% after the bypass flow was practiced in phase 2.

The ammonium and nitrite oxidation rates ($q_{\text{NH}_4^+}$, $q_{\text{NO}_2^-}$) were also determined at the steady states of Phases 1 and 2, which indeed reflect the respective specific activities of AOB and NOB. It was found that the $q_{\text{NH}_4^+}$ and $q_{\text{NO}_2^-}$ values were comparable in Phase 1 without bypassing of influent (Fig. 4.5a), indicating nitrite produced by AOB should be largely oxidized by NOB without expected accumulation. As the result, it is difficult to maintain sustainable nitrification as shown in Figs. 4.3 and 4.4. However, the situation in Phase 2 was completely different. As shown in Fig. 5b, $q_{\text{NH}_4^+}$ (i.e. 0.65 g N/g VSS d) was almost 4-times higher than $q_{\text{NO}_2^-}$ (i.e. 0.17 g N/g VSS d) in Phase 2. Consequently, it is reasonable to consider that the bypassed-influent flow had no effect on the activity of AOB, but significantly suppressed the NOB activity, leading to a stable nitrification as observed in Fig. 4. It was found that 8% of influent ammonium was removed at the A-stage AFBR, thus about 92% of influent ammonium was fed to the B-stage SBR (Fig. 4.5). The nitrogen mass balance on the B-stage showed that 78% of incoming ammonium-N was removed in Phase 2 with the bypassed flow against 42% in Phase 1 without bypassed flow.

Figure 4.5 Nitrogen distribution in the steady-state B-stage SBR. (a): Phases 1; (b): Phase 2

The profiles of key functional microbial species responsible for N-removal in the B-stage SBR were determined for both Phases 1 and 2 (Fig. 4.6a). Similar to the $q_{\text{NH}_4^+}$ values (Fig. 4.5), the abundances of AOB in the steady state Phases 1 and 2 were found to be comparable, while the abundances of denitrifiers in the stable Phase 2 was nearly 4-fold higher than that in Phase 1. In fact, these microbiological analyses are consistent with the $q_{\text{NO}_2^-}$ values determined in Phases 1 and 2 (Fig. 4.5). However, it should be noted that the abundance of NOB was significantly decreased when the bypassed influent flow was applied in Phase 2. In fact, the FISH image provided visual evidence showing that NOB could not be detected at the

steady-state Phase 2 (Fig. 4.6b). These indeed provide strong evidence from the aspect of molecular microbiology supporting the observed stable nitritation in Phase 2.

Figure 4.6 Abundances of key functional species in B-stage SBR. (a) 16S rRNA genes of key functional species; (b) FISH image of microorganisms hybridized with EUBmix (FAM, green), NIT 3 and NTSPA 714 (Cy 3, red), and NEU (Cy 5, pink) at steady state (Phase 2)

Fig. 4.7 further shows a typical cyclic nitrogen profile of the SBR in phase 2. Obviously, almost all ammonium was oxidized to nitrite at the end of the oxic phase, with extremely low nitrate concentration of below 0.5 mg N/L. Moreover, at the end of each anoxic phase, nitrate was undetectable. These provide additional support for the excellent denitrification-denitrification established in the steady-state Phase 2 with the bypassed influent flow.

Figure 4.7 Cyclic profiles of ammonium, nitrite, nitrate and DO in steady state Phase 2

4.3.3 Strategy to stabilize nitrification-denitrification in A-B process

Although many different operation strategies (e.g. aggressive SRT, intermittent aeration, alternating anoxic-oxic conditions, etc.) have been reported for establishment of nitrite shunt in the mainstream municipal wastewater treatment process, they are not readily applicable to A-B process where majority of COD is captured at A-stage (Fig. 4.1a). In fact, it should be realized that the central point of having a stable nitrite shunt is all about how to suppress NOB versus AOB. For stable nitrification-denitrification, nitrite produced by AOB should be timely denitrified by denitrifying bacteria without any accumulation. This is simply because accumulated nitrite may promote the growth as well as stimulate the activity of NOB, leading to further oxidation of nitrite to nitrate as observed in Phase 1. As a consequence, it is highly difficult to maintain sustainable and stable nitrification-denitrification in the A-B process illustrated in Fig. 4.1a. It should be noted that sustainable nitrification-denitrification indeed is largely determined by the kinetic competitions among AOB, NOB and denitrifying bacteria in the mainstream wastewater treatment process.

In order to promote denitrification instead of nitrite oxidation at the B-stage, the COD concentration should be sufficient for rapid denitrification. As presented above, a novel strategy for stabilizing nitrification-denitrification at the B-stage was proposed and verified in this study, i.e. bypassing a portion of influent wastewater to the B-stage (Fig. 4.1b). It was demonstrated that deteriorated nitrification-denitrification in Phase 1 was gradually restored after the bypassing of influent was implemented in Phase 2. For example, compared to Phase 1, the bypassed flow in Phase 2

significantly re-shaped the microbial community structure at the steady state B-stage, in which NOB was largely suppressed in terms of both abundance and activity against AOB and denitrifying bacteria (Figs. 4.6). This study probably for the first time shows a feasible engineering strategy for achieving a sustainable and stable nitrification-denitrification in A-B process for mainstream municipal wastewater treatment, without compromising energy recovery, while having significantly reduced waste activated sludge and cost-associated with aeration.

4.4. CONCLUSIONS

This study presents a novel A-B process configuration in which a portion of influent wastewater is bypassed from A-stage AFBR, while directed to B-stage SBR. The results clearly show that sustainable nitrification and denitrification could be achieved and maintained stably in the A-B process with the bypassed flow, e.g. 97% of nitrite accumulation was observed with 78% of nitrogen removal via nitrification-denitrification. Furthermore, it was found that the specific activity of AOB was not affected by the bypass flow, whereas NOB was significantly inhibited. It is expected that this study may foster further development of sustainable and stable A-B process for nitrogen removal in mainstream municipal wastewater treatment with the ultimate aims of energy-neutral or even positive.

CHAPTER 5 MAINSTREAM ANAMMOX IN A NOVEL A-2B PROCESS FOR ENERGY-EFFICIENT MUNICIPAL WASTEWATER TREATMENT WITH MINIMIZED SLUDGE PRODUCTION

5.1 INTRODUCTION

Anaerobic ammonia oxidizing bacteria (anammox), which is capable of anaerobically oxidize ammonium with nitrite as the electron acceptor (Strous et al., 1998), has been considered as an alternative for energy-efficient nitrogen removal. Although anammox process has been successfully applied for treating high-concentration ammonia wastewater, e.g. reject liquor from anaerobic sludge digestion (van der Star et al., 2007, Wett, 2007), landfill leachate (Anfruns et al., 2013, Sun et al., 2016), etc. However, mainstream anammox for low-strength municipal wastewater still remains challenging. In fact, it is very difficult to sustain short-cut nitrification which is essential for anammox in municipal wastewater with low ammonium-N concentration of about 40 mg/L, and anammox bacteria were outcompeted by denitrifiers at high COD/N ratio (Xu et al., 2015). In addition, the effective retention of anammox bacteria is crucial due to the extremely slow growth rate (Hendrickx et al., 2012, Strous et al., 1998). Although two-stage partial nitrification and anammox process had been reported, it appeared difficult to

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effectively control a proper ratio of ammonia to nitrite for anammox (Ma et al., 2011, Regmi et al., 2015, Regmi et al., 2016). In order to tackle such a situation, highly complex control strategies relying on online monitoring of ammonia, nitrite and nitrate had been developed (Regmi et al., 2015).

Therefore, this study aimed to develop an integrated A-2B process where most of COD was captured in the form of biomethane at A-stage, while nitrogen was removed through nitrification and denitrification at B1-stage and anammox at B2-stage. It was demonstrated that the proposed process had high operation stability and may lead to significant energy recovery, reduced energy consumption and sludge production.

5.2 MATERIALS AND METHODS

5.2.1 Process design and operation

In this study, a novel integrated A-2B process was developed for concurrently achieving mainstream deammonification and energy recovery from municipal wastewater. In this system, an anaerobic fixed bed reactor (AFBR) was used as A-stage for COD capture, while a sequencing batch reactor (SBR) and a moving bed biofilm reactor (MBBR) were employed to serve as B1-stage and B2-stage, respectively (Fig. 5.1) for nitrogen removal. The operation temperature of the integrated system was maintained at 30 ± 1 °C, while the hydraulic retention time (HRT) of the AFBR was controlled at 6.4 hours, and the effluent was introduced into a flash chamber for the recovery of dissolved methane. As shown in Fig. 1, a portion of influent was bypassed via Bypass 1, and mixed with A-stage effluent in the

holding tank 1, and the mixed stream was then fed into B1-stage SBR for nitrite production. The SBR was operated in 4 alternating sub-cycles of anoxic and oxic phase with step feed prior each anoxic phase, followed by settling phase and effluent discharge phase. Subsequently, mixed B1-stage effluent and anaerobic effluent via Bypass 2 was fed to B2-stage MBBR for nitrogen removal through anammox. The MBBR with a working volume of 4 L was packed with 1 cm³ cubic sponges at a packing ratio of 50% by volume (Nisshinbo Chemical Inc. Japan) and was inoculated with enriched anammox bacteria. Mechanical mixing at 120 rpm was provided in the MBBR. Synthetic wastewater used in this study was similar to that presented in the preceding chapters.

Figure 5.1 Schematic diagram of the A-2B process.

5.2.2 Analytical Methods

COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and volatile suspended solids (VSS) were determined according to standard methods (APHA, 2005). To measure the dry biomass in biofilms, the biocarriers suspended in PBS buffer were subjected to sonication (E100H Elmasonic, Elma, Germany) for 50-60 min. The collected pellets were further used for determination of fixed VSS. The composition of biogas

produced in AFBR was analyzed by a gas chromatography equipped with thermal conductivity detector (GC-TCD) (Agilent Technologies 7890A GC system, USA), while its volume was measured by a gas meter (TG 05 Model 5, Ritter, Germany).

The activity of AOB and NOB in the B1-stage SBR were quantified by the specific oxygen uptake rate (SOUR) which was determined in a DO meter (YSI 5100, USA) according to the method by Surmacz-Gorska et al. (1996). The allylthiourea (ATU) (125 mg/L) and NaClO₃ (0.5 M) solutions were used as selective inhibitors to inhibit the activity of AOB and NOB, respectively. Briefly, the COD-free mixed liquor with sufficient NH₄⁺-N and NO₂⁻-N was aerated to a DO concentration above 7 mg/L, after which the total OUR (OUR_{total}) is determined. After the DO concentration was decreased by about 2 mg/L, NaClO₃ was dosed to the mixed liquor for NOB inhibition and then the OUR was determined as OUR_{total-NOB}. Subsequently, after the DO was further decreased by another 2 mg/L, ATU was dosed to the mixed liquor for AOB inhibition and then the OUR was determined as OUR_{endogenous}. Thereby, the SOUR_{NOB} could be calculated by (OUR_{total}-OUR_{total-NOB})/MLVSS, while the OUR_{AOB} was determined by (OUR_{total-NOB}-OUR_{endogenous})/MLVSS.

5.2.3 In-situ reaction kinetics of Anammox

The in-situ batch experiment was carried out in the anammox MBBR. The water samples were taken at different time intervals over a period of 4 hours. The specific anammox reaction rate (q_{AMX}, g N₂-N/g VSS/d) was calculated as follows (van Loosdrecht et al., 2016):

$$q_{AMX} = \frac{R_{NH_4^+} + R_{NO_2^-} - R_{NO_3^-}}{X_{VSS}} \quad (5.1)$$

where $R_{NH_4^+}$ and $R_{NO_2^-}$ are respective ammonium and nitrite removal rates (g N/L d), $R_{NO_3^-}$ is nitrate production rate (g N/L d) and X_{VSS} is fixed biomass concentration (g/L).

5.2.4 Mass balance on COD and nitrogen

In the A-2B process described in Fig. 5.1, the overall mass balance on COD can be written as follows:

$$\begin{aligned} \text{Total influent COD (M}_{COD,inf}, \text{ g/d)} &= \text{COD in the effluent (M}_{COD,eff}, \text{ g/d)} \\ &+ \text{COD converted to biomass (M}_{COD,sludge}, \text{ g/d)} \\ &+ \text{COD converted to methane (M}_{COD,CH_4}, \text{ g/d)} \\ &+ \text{carbonaceous COD oxidized (M}_{COD,O_2}, \text{ g/d)} \\ &+ \text{COD utilized for denitritation (M}_{COD,DN}, \text{ g/d)} \end{aligned} \quad (5.2)$$

The COD converted to biomass could be estimated by Eq. 4.3:

$$M_{COD,sludge} = Q_{sludge} \times X_{sludge} \times f \quad (5.3)$$

where Q_{sludge} is the flow rate of wasted sludge liquor (L/d), X_{sludge} is the concentration of produced biomass (mg VSS/L) and f is the stoichiometric COD/biomass coefficient which is equal to 1.42 g COD/g for biomass with an empirical formula of $C_5H_7NO_2$.

M_{COD,CH_4} can be determined according to Eq. 4.4:

$$M_{\text{COD,CH}_4} = \frac{Q_{\text{biogas}} \times P_{\text{CH}_4}}{22.4} \times 64 + 4 \times Q_{\text{inf}} \times C_{\text{dCH}_4} \quad (5.4)$$

where Q_{biogas} is the biogas generation rate (L/d), P_{CH_4} is the percentage of CH_4 in the biogas (%) and C_{dCH_4} is the concentration of produced dissolved CH_4 (mg/L).

$M_{\text{COD,DN}}$ was estimated via denitrification in B1-stage SBR:

$$M_{\text{COD,DN}} = 1.71 \times C_{\text{N,B1,RE}} \times Q_{\text{B1}} \quad (5.5)$$

where $C_{\text{N,B1,RE}}$ is the concentration of nitrogen removed via denitrification in B1-stage SBR (mg/L) and Q_{B1} is the flow rate in B1-stage SBR (L/d).

As for $M_{\text{COD,O}_2}$, it is negligible since nearly all the COD in B1-stage SBR was used for denitrification. The mass balance on nitrogen can be established in a similar manner.

5.2.5 Fluorescence in situ hybridization

Biofilm harvested from the B2-stage MBBR was observed by fluorescence in situ hybridization (FISH) method. Biocarriers were fixed with 4% (w/v) paraformaldehyde in PBS solution at 4 °C for 6 h, and were then washed 3 times with PBS solution. The washed biocarriers were embedded in tissue freezing medium (Jung Tissue Freezing Medium, Leica Microsystems Nussloch GmbH, Germany) and were further sectioned into 20 µm slices (Leica CM1950; Leica Biosystems Nussloch GmbH, Germany) which could be fixed onto slides pretreated according to the method by Nielsen et al. (2009). For the observation of anammox biofilms, the probes for hybridization included EUBmix (equimolar of EUB338, EUB338 II and EUB338 III) (Daims et al., 1999) for staining all bacteria and

Amx368 for staining all anammox bacteria (Schmid et al., 2005). The FISH samples were finally visualized with a Zeiss LSM 880 confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

5.2.6 DNA extraction, PCR amplification and 16S rRNA gene sequencing

The microbial community in the stable anammox MBBR was analyzed by high-throughput sequencing technique. Briefly, DNA was extracted with a Fast DNA spin kit (MP Biomedicals, LLC), while bacterial universal primers Nobar 341F (CCTACGGGNGGCWGCAG) and Nobar 805R (GACTACHVGGGTATCTAATCC) were used to amplify V3-V4 region of 16S rRNA genes. Afterwards, high-throughput sequencing analysis was performed with Illumina Miseq platform (Sangon Biotech, Shanghai, China).

5.3. RESULTS AND DISCUSSION

5.3.1 Performance of A-stage AFBR

The performances of the steady-state AFBR were summarized in Table 5.1. It can be seen that the effluent COD concentration was stabilized at the level of about 29 mg/L on average, with the direct biogas production of about 0.27 L methane/COD removed. However, for the low-strength organic wastewater studied, it was found that about 41% of the produced methane was eventually dissolved in the AFBR effluent, about 90% of which was thereafter recovered in the subsequent flash chamber. It should be also realized that due to the direct COD capture, the sludge yield in the AFBR was as low as 0.06 g biomass/g COD removed, which was 85% smaller than that of activated sludge with a typical growth yield of 0.4 g biomass/g COD removed in CAS process

(Wan et al., 2016). Similar to other anaerobic systems, influent ammonium was almost not removed in the AFBR, which would be handled in the subsequent B-stages.

Table 5.1 Performances of steady-state A-stage AFBR

	AFBR	
	Influent	Effluent
COD (mg/L)	384	29
NH ₄ ⁺ -N (mg/L)	45	41
Sludge yield (g biomass/g COD removed)	0.06	
Methane yield (L/COD removed)		0.27
Dissolved methane recovery rate		90%

5.3.2 Performance of B1-stage SBR

In this study, the B1-stage SBR was designed for producing nitrite for anammox process in B2-stage MBBR. This is because the alternating anoxic and oxic conditions coupled with the control of SRT had been shown to be effective in repression of NOB against AOB (Xu et al., 2017). Fig. 5.2 showed that most of the influent ammonium was converted into nitrite with a clear nitrification. For example, the nitrite concentration in the SBR effluent was gradually increased to around 15 mg N/L due to the reduced COD concentration coming from the A-stage AFBR. Since then the effluent nitrite concentration stabilized at about 15 mg N/L, while nitrate concentration remained low. These clearly indicated the establishment of stable nitrite shunt, i.e. NOB was successfully repressed against AOB, further evidenced by the specific AOB and NOB activities presented in Fig. 5.3. As illustrated in Fig. 5.1, 16% of influent wastewater was bypassed to the B1-stage SBR. With the COD in the bypassed flow, it was found that about 33% of the total inorganic nitrogen was

removed through nitritation and denitritation.

Figure 5.2 Nitrogen profiles in the B1-stage SBR.

Figure 5.3 SOUR of AOB and NOB in the B1-stage SBR.

5.3.3 Performance of B2-stage anammox MBBR

Figs. 5.4 and 5.5 showed the $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentration profiles in the anammox MBBR throughout the whole operation period. It can be seen that in Phase 1 obvious removal of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentration were observed after 25-day operation when influent $\text{NO}_2^-\text{-N}$ concentration started to build up. After fixing the bypass 2 ratio at 22% in Phase 2, the ratio between $\text{NO}_2^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ concentration was about 1.1 which was ideal for anammox process. Accordingly, the averaged effluent $\text{NO}_2^-\text{-N}$ concentration was reduced to below 1 mg N/L, while $\text{NH}_4^+\text{-N}$ concentration in the effluent was below 2 mg N/L. Concurrently, very

limited NO_3^- -N was produced (Fig. 5.6) with no COD consumption (Fig. 5.7), indicating that the removal of NH_4^+ -N and NO_2^- -N in the anammox MBBR were attributed to the anammox activity. The nitrogen loading rate reached 0.12 kg N/m³/d, with 74% nitrogen removal efficiency inside the anammox MBBR.

Figure 5.4 Ammonium profiles in the anammox MBBR.

Figure 5.5 Nitrite profiles in the anammox MBBR.

Figure 5.6 Nitrate profiles in the anammox MBBR.

Figure 5.7 COD profiles in the anammox MBBR.

The results of in-situ batch tests at around day 100 (Table 5.2) showed that the $\text{NO}_2^-/\text{NH}_4^+$ and $\text{NO}_3^-/\text{NH}_4^+$ ratios were 1.27 ± 0.05 and 0.28 ± 0.03 , respectively, which are comparable with those reported by Strous et al. (1998), i.e. 1.32 and 0.26, respectively, and later reported at 1.2 and 0.21, respectively by Lotti et al. (2014). The maximum specific anammox reaction rate was measured at 0.14 ± 0.01 g $\text{N}_2\text{-N/g}$ VSS/d. The routine measurements throughout the operation period revealed that from day 45 the ratios gradually stabilized and approached the theoretical value as previously reported (Fig. 5.8), indicating fast start-up of anammox process. This might be attributed to the biocarriers' unique sponge structure, which was capable for effectively trap the anammox granules. After the physical trapping effect, the

anammox biofilm would eventually grow on the surface of the biocarriers.

Table 5.2 Stoichiometric coefficient ratios, $\text{NO}_2^-/\text{NH}_4^+$ and $\text{NO}_3^-/\text{NH}_4^+$, and maximum specific anammox reaction rate, q_{AMX} , of the anammox MBBR.

$\text{NO}_2^-/\text{NH}_4^+$ ratio	$\text{NO}_3^-/\text{NH}_4^+$ ratio	q_{AMX} g $\text{N}_2\text{-N/g VSS/d}$
1.27 ± 0.05	0.28 ± 0.03	0.14 ± 0.01

Figure 5.8 $\text{NO}_2^-/\text{NH}_4^+$ ratio and $\text{NO}_3^-/\text{NH}_4^+$ ratio throughout the operating period.

Compared with one-stage processes, two-stage partial nitrification/anammox processes segregated the anammox process and the partial nitrification process, which firstly could avoid the inhibition on anammox bacteria due to oxygen exposure (Li et al., 2011), and achieve higher AOB activity in partial nitrification reactor because

higher DO concentration could be applied (Regmi et al., 2015, Regmi et al., 2014). In order to provide equimolar ratio of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ for subsequent anammox process, the normal strategy was to biologically oxidize about half of the ammonium to nitrite (Ma et al., 2011, Wyffels et al., 2004), which often rely on NO_3^- , NO_2^- , and NH_4^+ sensors for aeration control (Regmi et al., 2014). Therefore in such a configuration of A-2B process in this study, the control could be simplified.

5.3.4 Microbial community analysis

Fig. 5.9 presented the main functional bacteria at genus level with the relative abundance greater than 1%. Three genera affiliated with anammox, i.e. *Candidatus Kuenenia*, *Candidatus Brocadia* and *Candidatus Anammoxoglobus*, were identified in the B2-stage biofilms, among which *Candidatus Kuenenia* appeared to be dominant with the abundance of 14.19%. The presence of anammox bacteria in the B2-stage biofilms were also affirmed by the FISH images (Fig. 5.10).

Figure 5.9 Microorganisms with the abundance greater than 1% in the biofilms from the steady-state B2-stage anammox MBBR.

Figure 5.10 FISH-stained anammox bacteria (AMX368, pink) in the biofilms from the steady-state B2-stage anammox MBBR.

Nitrolancea with the abundance of 1.52% was found to be the dominant NOB, which could tolerate high-concentration nitrite, while *Nitrospira* and *Nitrobacter*, which were often reported as ubiquitously dominant NOB in WWTPs (Whang et al., 2009), were observed at the abundance below 0.94%. On the other hand, *Nitrosomonas* was only present at the abundance of 0.14%, and other ammonia oxidizing bacteria (i.e. *Nitrosococcus* and *Nitrosospira*), were almost not detectable. These further confirmed a culture environment primarily dominated by anammox bacteria in the B2-stage MBBR.

5.3.5 Engineering implications

For the comparison purpose, the mass flows of COD and nitrogen were determined over the proposed A-2B process (Fig. 5.11). As can be seen, 58% of the influent COD was directly converted to methane in A-stage AFBR, with only 11% channeled into wasted sludge. It should be realized that about 30-50% of the influent COD ended up in excess sludge in the conventional activated sludge process. In fact, the total sludge production yield in the A-2B process was only about 0.1 g VSS/g COD removed, which was 75% less than that in the conventional activated sludge process with a typical value of 0.4 g VSS/g COD removed. In fact, huge amount of excess sludge is currently produced in WWTPs adopting conventional activated sludge process, i.e. in 2010, 8 million tons of dry sludge produced in the United States and about 11 million tons in China. To tackle such a challenging situation, anaerobic digestion of waste sludge has been widely applied for both volume reduction and biogas generation. However, the overall energy efficiency of anaerobic digestion was found to be very low, e.g. only about 30-50% of VSS could be degraded and digested solid residue still needs further disposal. Ideally, soluble COD in wastewater should be captured for direct energy production as in the proposed A-2B process. On the other hand, 87% of total nitrogen removal was achieved in the A-2B process, of which 33% and 34% were removed through nitrification-denitrification and anammox, respectively. As such, the energy associated with nitrogen removal could be reduced by about 47%.

Figure 5.11 The mass flows of COD and nitrogen in stable A-2B process.

It had been reported that the overall energy efficiency of the current municipal wastewater treatment plants was only about of 20–50% (Zhou et al., 2013). Indeed, the design of the present wastewater treatment plants has a serious drawback of that soluble COD in wastewater is converted to biomass at high energy cost, from which energy is subsequently recovered through anaerobic digestion, but at low energy efficiency. As demonstrated in this study, COD in influent wastewater should be anaerobically captured for direct methane production with minimized waste sludge production. In this sense, the proposed A-2B process provides a feasible engineering solution towards energy-neutral or even energy-positive operation of wastewater treatment, i.e. significantly improved energy recovery at A-stage, together with markedly reduced energy consumption at B-stage.

5.4. CONCLUSIONS

This study demonstrated the engineering feasibility of a novel A-2B process with integrated direct COD capture and mainstream anammox. In this process, about 58% of influent COD was directly converted to biomethane in the A-stage anaerobic fixed-film reactor, while stable and sustainable anammox was achieved with 74% of nitrogen removal. The detailed mass balance clearly revealed that 33% of inorganic nitrogen was removed in the B1-stage SBR via nitrification-denitrification, together with 34% removal in the B2-stage MBBR via anammox. *Candidatus* Kuenenia known as anammox bacteria accounted 14.19% of total microbial population in the B2-stage MBBR. Compared to the conventional activated sludge process, the excess sludge production was reduced by 75%, while aeration-associated energy consumption for COD oxidation could be completely waived in addition to 47% cut

in energy demand for nitrogen removal in the proposed A-2B process. The A-2B process developed in this study opens a new window towards the sustainable energy self-sufficient wastewater reclamation, which is obviously of significance, especially in the scenario where global climate change is of a serious concern.

CHAPTER 6 SINGLE-STAGE MAINSTREAM DEAMMONIFICATION IN A NOVEL A-B PROCESS

6.1 INTRODUCTION

Increasing concerns have been raised on the conventional activated sludge (CAS) process for municipal wastewater treatment due to its high energy demand and production of huge amount of excess sludge. To cope with this challenging situation, extensive efforts are being dedicated to exploring new process configurations towards improved energy efficiency and reduced sludge production.

In the recent years, anaerobic ammonia oxidizing (anammox) bacteria have attracted great attention due to their capability of anaerobically oxidizing ammonium to nitrogen gas with nitrite as the electron acceptor (Strous et al., 1998). Compared with the conventional nitrification and denitrification, the anammox process is considered as an alternative approach for energy-efficient nitrogen removal from municipal wastewater (Kartal et al., 2010), which is known as mainstream partial nitritation coupled with anammox or mainstream deammonification. However, mainstream deammonification is facing many challenges, i.e. high COD/N ratio, sustainable nitrite shunt, effective retention of anammox bacteria, etc., which have not yet been properly addressed (Xu et al., 2015).

Based on the research progresses made in the preceding chapters, this study aimed

to synergistically integrate single-stage deammonification into an A-B process configuration in which COD was effectively captured for direct energy recovery at A-stage, while nitrogen was removed through deammonification in a single continuous step-feed reactor at B-stage. The proposed process was demonstrated to be feasible for achieving sustainable and stable mainstream deammonification for the cost-effective removal of ammonium from municipal wastewater.

6.2 MATERIALS AND METHODS

6.2.1 Process design and operation

In this study, a novel A-B process coupled with mainstream deammonification was developed and its feasibility was successfully demonstrated. An anaerobic fixed bed reactor (AFBR) was employed as A-stage for direct COD capture towards biogas production, while a step-feed reactor at B-stage was specifically designed for deammonification (Fig. 6.1). The hydraulic retention time (HRT) of the AFBR was controlled at 6.2 hours, and the effluent of the AFBR was introduced into a flash chamber for the recovery of dissolved methane as described in Chapter 3. The influent to the step-feed reactor was equally fed into the 4 anoxic chambers (i.e. A1, A2, A3 and A4) which were packed with anammox bacteria-attached biocarriers at the packing ratio of 50%. The volumetric ratio of the anoxic chamber and the oxic chamber was designed to be 4:3, while dissolved oxygen concentrations in the oxic chambers were maintained at 1.2-1.5 mg/L. The biocarriers in the anoxic chambers were gently expanded by nitrogen gas sparging. The whole system was operated at 30 ± 1 °C and synthetic wastewater used in this study was similar to that presented

in the preceding chapters.

Figure 6.1 Schematic diagram of the A-B process.

6.2.2 Analytical Methods

COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and volatile suspended solids (VSS) were determined according to standard methods (APHA, 2005). To measure the dry biomass of the attached biofilm, biofilms were scraped off from the biocarriers and the collected pellets were further used for determination of fixed VSS. The composition of biogas produced in the AFBR was analyzed by a gas chromatography equipped with thermal conductivity detector (GC-TCD) (Agilent Technologies 7890A GC system, USA), while its volume was measured by a gas meter (TG 05 Model 5, Ritter, Germany). The ratio of $\text{NO}_3^-\text{-N}_{\text{eff}}/\text{NH}_4^+\text{-N}_{\text{removed}}$ was calculated to reflect the effectiveness of NOB repression. The activities of AOB and NOB in the step-feed reactor were quantified by the respective specific oxygen uptake rates (SOUR) which were determined according to the method by Surmacz-Gorska et al. (1996).

6.2.3 Quantitative PCR

DNA of the suspended sludge and harvested anammox biofilm were extracted by a Fast DNA spin kit (MP Biomedicals, LLC). The copy numbers of 16S rRNA genes representing key functional species were determined by quantitative PCR using SybrGreen assays with primers listed in Table 6.1.

CHAPTER 6

Table 6.1 Primers used for q-PCR analysis

Primer	Gene	Target	Sequence (5'-3')	Annealing Temp. (°C)	References
CTO189f ^a CTO654r	16S rRNA	β-Subdivision AOB	CTAGCYTTGTAGTTTCAAACGC	57	Araki et al. (2004) Kowalchuk et al. (1997)
FGPS872 FGPS1269	16S rRNA	Nitrobacter	CTAAAACTCAAAGGAATTGA TTTTTTGAGATTGCTAG	50	Degrange and Bardin (1995)
NSR1113f NSR1264r	16S rRNA	Nitrospira	CCTGCTTTCAGTTGCTACCG GTTTGCAGCGCTTTGTACCG	65	Dionisi et al. (2002)
341f 518r	16S rRNA	Universal	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	55	Muyzer et al. (1993)
A438f A684r	16S rRNA	Anammox	GTCRGGAGTTADGAAATG ACCAGAAGTTCCACTCTC	55	Humbert et al. (2012)

^aMix of CTO189fA-B (GGAGRAAAGCAGGGGATCG) and CTO189fC (GGAGGAAAGTAGGGGATCG) at ratio of 2:1

6.3 RESULTS AND DISCUSSION

6.3.1 Process performance

Fig. 6.2 shows that the influent COD concentration was significantly reduced from 387 mg/L to about 19 mg/L in the A-stage AFBR, 79% of which was directly channeled to methane. However, 38% of methane produced was found to be in its dissolved form with a concentration of 29 mg/L. It was demonstrated once again that the flash chamber developed in Chapter 3 was able to recover 91% of dissolved methane. In addition, the observed sludge production in the AFBR was as low as about 0.05 g VSS/g COD, which was much lower than that of the activated sludge with a typical value of 0.4-0.5 g VSS/g COD. These suggested that the sludge production in the proposed system would be 89% lower than that in the conventional activated sludge process. The detail COD flows were presented in Fig. 6.2. Obviously, most of influent COD was removed at the A-stage and the final effluent COD concentration was as low as 15 mg/L.

Figure 6.2 COD mass flow in the A-B process.

As most COD in the wastewater was removed at the A-stage (Fig. 6.2), the COD/N ratio in the feed water to the B-stage was found to be about 0.44, which was favorable for anammox. As shown in Fig. 6.3, the $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentrations gradually stabilized at 3.2 and 3.0 mg/L, while the $\text{NO}_3^-\text{-N}$ concentration remained below 5 mg/L over the 110-day operation. Moreover, it appeared from Fig. 6.4 that the nitrogen removal indeed could be quickly stabilized within 10 days.

Figure 6.3 Nitrogen profiles in B-stage step-feed deammonification reactor.

Figure 6.4 TN removal efficiency and the ratio of $\text{NO}_3^- \text{-N}_{\text{eff}} / \text{NH}_4^+ \text{-N}_{\text{removed}}$ in the course of operation.

6.3.2 Suppression of NOB against AOB at the B-stage

Effective NOB repression is essential for sustainable short-cut nitrification which is a prerequisite for anammox. However, stable short-cut remains a big challenge in mainstream deammonification. The operation strategies developed in Chapter 4 for the effective NOB repression in the SBR were adopted in this study, i.e. from temporal operation in the SBR to a spatial operation in the step-feed reactor. In practice, the ratio of $\text{NO}_3^- \text{-N}_{\text{eff}} / \text{NH}_4^+ \text{-N}_{\text{removed}}$ has been used to indicate the effectiveness of NOB repression. It appeared from Fig. 6.4 that this ratio determined in this study was very close its theoretical value of 0.11, i.e. the NOB

activity was effectively repressed. Moreover, the repressed NOB activity was further confirmed by the results from the SOUR tests. It was found that the SOUR of AOB (i.e. 1.07 ± 0.02 g O₂/gVSS·day) was about 6 times higher than that of NOB (i.e. 0.17 ± 0.07 gO₂/gVSS·day). Meanwhile, Fig. 6.5a further revealed that the copy number of AOB was increased by more than 8 times from day 1 to the end of the operation. On the contrary, the copy number of *Nitrospira* stayed relatively stable, while *Nitrobacter* experienced a clear declining trend. These in turn may explain the successful NOB repression against AOB achieved in the step-feed reactor. This can be attributed to the the alternating shift of anoxic and oxic conditions in the step-feed recator as described in Fig. 6.1.

Figure 6.5 Respective abundances of N removal-associated species in the B-stage step-feed reactor. a: Suspended sludge; b: Anammox biofilm.

6.3.3 Engineering implications

Although some mainstream deammonification processes have been reported, the

process stability remains unresolved. For example, it is extremely difficult to maintain stable nitrite shunt due to reduced AOB activity, while anammox bacteria are often outcompeted by denitrifying bacteria. As such, the operation of the reported deammonification processes largely depends on the process bioaugmentation with AOB and anammox bacteria harvested from sidestream processes (Lemaire et al., 2014, Wett et al., 2013). On the contrary, as discussed above, bioaugmentation was not required in the process developed in this study and the SOUR and qPCR results clearly showed that AOB activity could be maintained at the high level against NOB activity.

6.4 CONCLUSION

This study demonstrated the feasibility of achieving sustainable and stable mainstream deammonification in the A-B process for most cost-effective removal of ammonium from municipal wastewater. In this process, 79% of the influent COD was directly converted to methane in the A-stage AFBR, while nitrogen removal efficiency reached up to 80% via deammonification in the B-stage step-feed mainstream deammonification reactor. The SOUR and qPCR results clearly showed successful NOB repression throughout the whole operating period, indicating that the unique configuration of step-feed reactor was favorable for NOB repression. Sustainable and stable mainstream deammonification was achieved without bioaugmentation for more than 100 days, which significantly reduced the operation complexity compared with other mainstream processes on bioaugmentation. The A-B process developed in this study may lead to the paradigm shift of the WWTP operation from energy-negative to energy self-sufficient.

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

In this study, the novel A-B processes were developed towards energy efficient municipal wastewater reclamation with minimized sludge production. In these innovative process configurations, the anaerobic treatment was adopted as A-stage for direct COD capture and energy production, while nitrification-denitrification, two-stage mainstream deammonification and single-stage mainstream deammonification were developed as B-stage for energy-efficient nitrogen removal, respectively. The following conclusions can be drawn.

- (1) Using anaerobic treatment as the leading stage led to significant energy gains from reduced sludge production and direct methane gas production. The recoverable energy of methane gas was estimated at 0.28 kWh/m^3 of treated wastewater which was determined by the influent COD concentration, while the sludge production was reduced by 75%. The COD capture at A-stage in turn significantly relieved energy demand on aeration for COD oxidation.
- (2) In this study, a patented degasser was developed, by which 90% of the dissolved methane was recovered from the anaerobic effluent. The energy consumption associated with the degasser could be offset by the potential energy harvested from the produced methane.

- (3) A novel operation strategy was developed towards sustainable nitrification-denitrification at B-stage. The nitrite accumulation reached 97% with 78% nitrogen removal via nitrification-denitrification. It was found that the specific activity of AOB was not affected, whereas NOB was significantly inhibited due to the rapid denitrification with the COD from the bypassed flow.
- (4) Mainstream anammox in a MBBR was successfully integrated into a novel A-2B process treating municipal wastewater. About 67% of the total nitrogen was removed via nitrification-denitrification in the B1-stage SBR and anammox in B2-stage anammox MBBR. Microbial community analysis revealed that *Ca. Kuenenia* with the abundance of 14.19% of the total was dominant in the MBBR biofilm.
- (5) Sustainable and stable mainstream deammonification was achieved in a continuous step-feed reactor without bioaugmentation. NOB was effectively repressed in the unique configuration of the step-feed reactor. In this A-B process integrated with complete mainstream deammonification, 79% of the total COD was channeled to methane gas at A-stage with nitrogen removal up to 80% at B-stage.

7.2 RECOMMENDATIONS

The A-B processes developed in this study shed light on the sustainable energy self-sufficient municipal wastewater reclamation. Future study may look into the following aspects.

Dissolved methane is ubiquitous in anaerobic process for municipal wastewater

treatment. The effective recovery of dissolved methane is essential for improving the overall energy recovery. Hence, the flash chamber developed in this work should be demonstrated at large-scale for better evaluation of its performance and energy consumption.

Although anammox biofilms as demonstrated in this study are of an excellent choice for effective retention of anammox bacteria, the development of anammox biofilm indeed is a very slow process due to their extremely low growth rate. This may become a bottleneck of the future large-scale application. Therefore, a fast and effective method for mass production of anammox biofilms should be investigated.

Although the mainstream deammonification was successfully integrated into the A-B process as shown in this study, further process optimization is still needed for a better system design.

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