Partial nitrification of piggery wastewater as pre-treatment for anammox process using flat sheet membrane bioreactor

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Received 20 August 2019; accepted 3 December 2019

Abstract:

A lab-scale flat sheet membrane bioreactor (MBR) system was used for the treatment of piggery wastewater to produce an effluent with the appropriate ratio of nitrite:ammonia (1:1 to 1:1.3) as a pre-treatment for the anammox process. The feed wastewater, which was the effluent of a biogas digester, contained 253±49 (n=60) mg.l^{-1} as COD, 231±18 mg.l^{-1} as N-ammonia, 223±19 mg.l^{-1} as total Kjeldalh nitrogen (TKN), alkalinity of 1433±153 mg.l^{-1} as $CaCO_3$, and pH=7.5±0.3. This study aimed to determine the suitable hydraulic retention time (HRT) and alkalinity to yield the appropriate influent for the annamox process. The results showed that the suitable effluent of the partial nitrification with ratio of nitrite:ammonia 1.0:1.1 at HRT of 7h30, equivalent to total nitrogen loading of 0.77 kgNm^{-3}d^{-1}. The nitrite accumulation rate (NAR) was 82% at HRT of 7h30, whereas NAR were 11 and 63% at HRT of 12h30 and 8h45, respectively, due to the high growth of nitrite oxidation bacteria (NOB) at long HRTs. As increasing alkalinity of up to 1600 mg.l^{-1} and pH of 8.0 at HRT of 8h45, NAR was increased from 63 to 73%, ratio of ammonia:nitrite reduced from 1.0:1.8 to 1.0:1.6 and free ammonia concentration reached to 20.2 mg.l^{-1} nitrogen. This shows that the increase of alkalinity inhibited strongly NOB.

Keywords: **flat-sheet membrane, partial nitrification, piggery wastewater.**

Classification numbers: **2.2, 5.1**

Introduction

Currently, nitrogen removal from wastewater using biological processes mainly follows the trend of nitrification-denitrification, which is a method from recent decades that removes nitrogen through nitrite. Conventional nitrogen removal processes require two stages, the first stage is nitrification, which requires a large amount of oxygen. The second stage demands a supply source of carbon in case that the ratio of C to N in the influent is not high enough. Therefore, the treatment cost is large and it requires a lot of technology. In 1995, Dutch scientists explored a new biological process in which nitrogen was transferred to remove a high concentration of ammonia with a low ratio of C to N [1]. This is known as the anammox process. In this process, ammonia is oxidized by nitrite under anaerobic conditions without a supply source of organic matter to form nitrogen molecules. The reaction process is described as follows [2]:

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O.$$

Compared to conventional nitrification/denitrification in activated sludge systems, nitrogen removal through anammox-based technology is an innovative method that eliminates the necessity of an organic carbon source for nitrification. Further, anammox-based technology consumes lower energy for aeration, has lower excess sludge production, and lower CO_2 emissions [3]. This technology is a combination of two processes consisting of partial nitrification followed by the anammox process. Nitrogen removal efficiency of the anammox process depends largely on partial nitrification. The suitable ratio of NH_4^+-N/NO_2^--N for the anammox process ranges from 1:1 to 1:1.3 [4]. Recent research has shown that the combination of partial nitrification and anammox could economize 20-30% of the consumed

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oxygen and 40% of the organic carbon source [5-7]. Besides, it could reduce the volume of the treatment tank up to 40% [8], and the nitrification rate through nitrite is 1.5 to 2 times faster than the conventional process [9]. Recent studies on partial nitrification, as well as nitrite accumulation, focus on the following affected factors [10-13]: (1) high temperature (30-40^{0}C): the growth rate of ammonia-oxidizing bacteria (AOB) is faster than nitrite-oxidizing bacteria (NOB), hence, there is a nitrite accumulation at high temperature; (2) Operation at low dissolved oxygen (DO) levels causes the cell structure of AOB to utilize oxygen more easily than the NOB's cell structure, thus, NOB is suppressed at low DO concentrations, which leads to an increase in the rate of nitrite accumulation; (3) Control of pH, free ammonia concentration (FA), and free HNO_2 (FNA); (4) Substrate concentration and ammonia loads in the influent: AOB were divided into two groups, namely, fast-growth and slow-growth. The fast-growth bacteria group has a great attraction to influent with a high concentration and load of ammonia, thus, nitrification accumulation occurs more easily than for an influent with low concentration and load of ammonia [14, 15]; (5) Sludge retention time: AOB has a shorter growth time than NOB, therefore we could determine the suitable sludge retention time to eliminate the NOB from the treatment system. A study on partial nitrification using a hollow fibre membrane with synthetic wastewater at <0.1 mg.l^{-1} DO and nitrogen load of 0.9 kgNm^{-3}d^{-1} showed that nearly 50% of the ammonia in the influent was transformed into nitrite [16]. Similar results in another study that used a tubular membrane on synthetic wastewater showed the suitable amount of alkalinity to reach the NH_4^+-N/NO_2^--N 1:1 ratio was 1500 mg.l^{-1} $CaCO_3$ at the operational conditions of <1 mg.l^{-1} DO, 510 mg.l^{-1} of influent ammonia, and a retention time of 24h [17]. Regarding livestock wastewater, another study using sequencing batch reactor (SBR) technology with an influent ammonia load of 1.47 g/l/d NH_4^+-N found the nitrite formation rate was 0.91 gl^{-1}d^{-1} NO_2^--N and the NH_4^+-N/NO_2^--N ratio was 1.38:1.00 [18]. According to research that examined the effects of COD concentration on partial nitrification with piggery wastewater with a TOC concentration of more than 2000 mg.l^{-1}, it was found that AOB was suppressed and the nitrite accumulation rate decreased [19]. Currently, most MBRs use hollow fibre membranes. In comparison with a hollow fibre MBR, the flat sheet MBR can achieve a unique advantage of high flux, longer service life, and high recovery rate. Research on flat sheet MBR for partial nitration has not been widely published.

Therefore, this study aims: (i) to evaluate the performance of partial nitritation using flat sheet MBR with control of the parameters DO, pH, and HRT to produce the suitable NO_2^--N:NH_4^+-N effluent ratio for the subsequent anammox process and (ii) to assess the effect of alkalinity on partial nitrification.

Materials and methods

Materials

The feed wastewater used in this study was collected from the effluent of a biogas tank in a pig farm with a hydraulic retention time of 20 d. The characteristics of the feed wastewater are described in Table 1.

Table 1. Characteristics of feed wastewater.

Parameter	Unit	Average value (±STD)
pH		7.5±0.3 (n=60)
Alkalinity	mg.l^{-1} $CaCO_3$	1433±153 (n=45)
TSS	mg.l^{-1}	223±21 (n=25)
BOD_5	mg.l^{-1}	131±17 (n=15)
COD	mg.l^{-1}	253±49 (n=60)
NH_4^+-N	mg.l^{-1}	231±18 (n=60)
T-N	mg.l^{-1}	256±19 (n=25)
NO_2^--N	mg.l^{-1}	<1 (n=15)
NO_3^--N	mg.l^{-1}	8±1 (n=15)
T-P	mg.l^{-1}	89±15 (n=15)

STD: standard deviation.

The feed wastewater had a low concentration of biodegradable substances, the ratio of BOD_5/COD was less than 0.5, so that the organic matter removal efficiency by biological processes may be low. However, the wastewater contained a high amount of nutrients such as T-P and T-N, and the ratio of C/N or BOD_5/TKN was less than 0.5.

Seed sludge used in this experiment was taken from the secondary sedimentation tank of a domestic wastewater treatment plant. The initial concentration of the feed sludge was introduced into the reactor at MLSS at 4000 mg.l^{-1}.

Flat sheet MBR (Fig. 1)

The experimental model had a length x width x height of 20 cm x 12 cm x 40 cm, respectively, which is equivalent to a total volume of 9.6 l and working volume of 6 l. This study used two flat membranes with total area of 0.1067 m^2. The membrane was made of polypropylene and polyester, and was put on a PVC plastic frame. The average diameter of the pores were 0.23 µm and it was a product of the GS Yuasa Corporation (Japan).

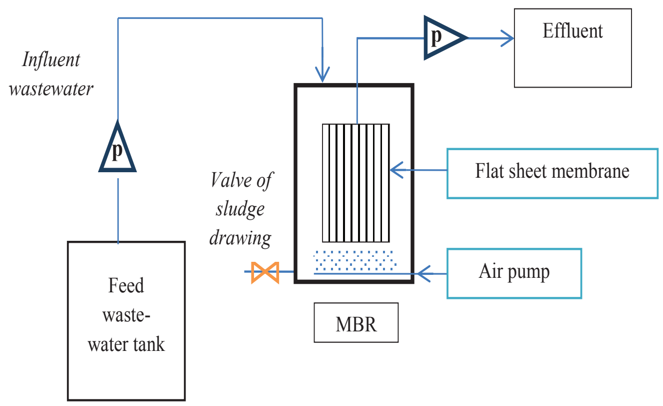

Fig. 1. Schematic diagram of the flat sheet MBR.

Experiment set-up

The feed wastewater was allowed to settle for 1 h to remove suspended solids before running the experiment. The inlet pump that introduced wastewater into the MBR tank was controlled by an automatic floating valve. This study was conducted under room temperature conditions (day: 28-32^{0}C and night: 25-27^{0}C). To inhibit NOB growth, low DO levels ranging from 1.4 to 1.8 mg.l^{-1} were maintained using an air adjusting valve [20, 21]. The running mode of the membrane was 8 min ON and 2 min OFF. The transmembrane pressure (TMP) was measured by an electrical pressure gauge. When the TMP reached 20 kPa, the membrane surface was washed manually using a brush [20].

This study was conducted under different hydraulic retention times (Table 2), namely, 12h45 (TN1), 8h45 (TN2, TN3), and 7h30 (TN4). The effect of alkalinity on the partial nitrification process was carried out at a HRT of 8h45 (TN4) by adding $NaHCO_3$ into the feed wastewater to reach an alkalinity of 1600 mg.l^{-1} as determined by the concentration of $CaCO_3$. The pH value of the influent of four experiments was adjusted in the range from 8.0 to 8.5 [21].

Table 2. Operation conditions of the experiment.

Notation	HRT h min	L_{TN} kgN.m^{-3}.d^{-1}	L_{COD} kgCOD.m^{-3}.d^{-1}	flux l.m^{-2}.h^{-1}	Note
TN1	12h45	0.47	0.43	5.5	
TN2	8h45	0.69	0.84	6.4	
TN3	8h45	0.60	0.63	6.4	Bicarbonate addition to influent
TN4	7h30	0.78	0.70	7.5	

Analysis methods

The following parameters: TKN, NH_4^+-N, NO_2^--N, NO_3^--N, COD, and alkalinity were analysed according to standard methods provided by APHA AWWA, 20th, 1998. The pH value was measured by a pH meter (pH211, Hana instrument, Italia). The DO content was measured by a DO meter (WTW 410i, Germany). The rate of nitrification accumulation was calculated as follows:

$$NAR\ (\%) = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N} \times 100\%.$$

The FA concentration was calculated by the following equations:

$$FA\ (mgN.l^{-1}) = \frac{TAN}{1 + \left(\frac{10^{-pH}}{K_{e,NH_3}}\right)}$$

$$K_{e,NH_3} = e^{\frac{-6344}{273+T}}$$

where TAN is the total ammonium as nitrogen (mgN.l^{-1}), T is the reaction temperature (^{0}C), and FA is the free ammonia concentration (mgN.l^{-1}).

Results and discussion

Change of nitrogen concentration

Figure 2 (at TN1) showed that most of the influent N-NH_4^+ was oxidized into its NO_3^--N form. The effluent nitrogen concentration had a ratio of N-NH_4^+:NO_2^--N:NO_3^--N of 1:1.9:14.7 (in terms of nitrogen). According to Ref. [22], an FA concentration higher than 3.5 mgN.l^{-1} inhibited NOB growth. However, in this experiment, a low FA concentration of 0.7 mgN.l^{-1} did not inhibit the growth of NOB, and the all nitrite was fully oxidized into nitrate. In addition, high amounts of alkalinity was consumed (90.6% of initial alkalinity). Ref. [23] has shown that the oxidation of ammonia and the oxidation of nitrite can occur at the same time. Ref. [20]

demonstrated that the optimal dissolved oxygen of an MBR should be between 0.8-0.9 mg.l^{-1}. Thus, the DO content is the key factor controlling NOB inhibition. The DO concentration was adjusted to be less than 1.0 mg.l^{-1} since the experiment TN2 was conducted. Fig. 3 showed that almost all ammonia had already been oxidized, so the nitrification accumulation reached a low level, with a NAR of 11.2%.

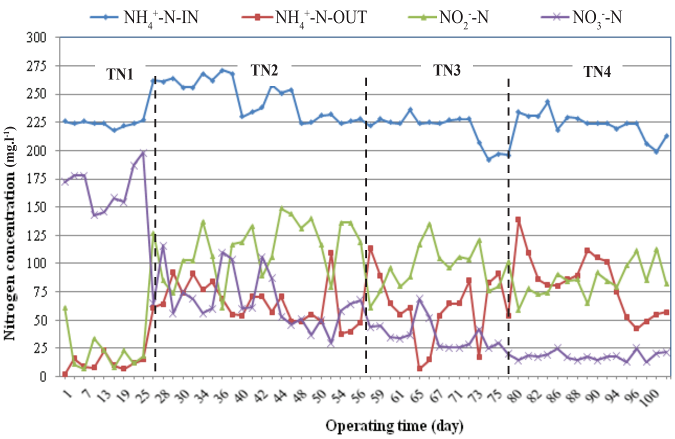

Fig. 2. Changes of nitrogen in the experiments.

In comparison with TN1, a decrease of hydraulic retention time and an increase of nitrogen load of TN2 affected a change in the nitrogen of the effluents and the ratio of N-NH_4^+:NO_2^--N:NO_3^--N was 1:1.8:1 (in term of nitrogen). As presented above, much more nitrite was formed resulting in nitrite accumulation and decreased nitrate concentration, thus an increase of NAR (63.6%) during this experiment. These outcomes could be explained by the consumed alkalinity during the ammonia oxidation in this experiment, which was smaller than that of the TN1 experiment (only 81%). Thus, the pH value in the reaction tank did not decrease rapidly (7.39), and the average FA concentration in this experiment was 6.29 mgN.l^{-1}. According to the figure, a correlation can be seen between FA, FNA, and the growth of nitrification bacteria with the influent that has a high ammonia concentration (>200 mg.l^{-1}) and an FA ranging from 1-10 mgN.l^{-1} (in the second area). The FA concentration was the main factor behind the suppression of NOB [23]. Therefore, in the TN2 experiment, the rate of nitrite oxidation was low and the NAR increased much more than in TN1. However, the NO_3^--N formed in this experiment still made up a high percent of the effluent (27.1%). The FA concentration of 6.29 mg.l^{-1} only suppressed a part of the NOB group and, thus, the ratio of NH_4^+-N:NO_2^--N, which was 1:1.8 (in term of nitrogen), was not appropriate since the requirement ranges from 1:1 to 1:1.3. To reach the appropriate ratio for the anammox process, reducing the hydraulic retention time is necessary.

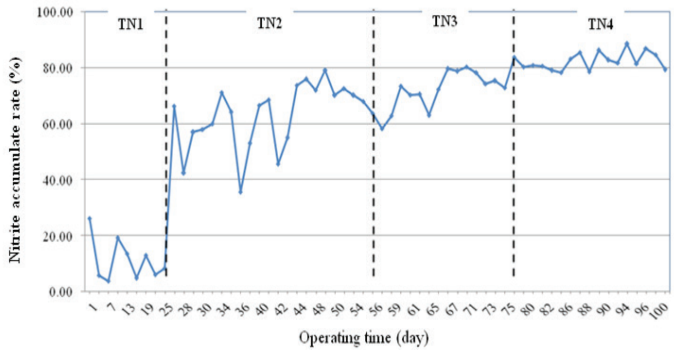

Fig. 3. Rate of nitrite accumulation in the experiments.

In the TN4 experiment, the HRT was adjusted from 8h45 to 7h30. The experimental results in Fig. 3 showed that NH_4^+-N was oxidized and the NO_3^--N concentration in the effluent was very low. The change of nitrogen in the effluent had a ratio of N-NH_4^+:NO_2^--N:NO_3^--N of 1:1.1:0.2 (in term of nitrogen) and a NAR of 82.3%. Therefore, the NH_4^+-N:NO_2^--N ratio at this hydraulic retention time was in a suitable range for the anammox process. This could be explained by the decrease of ammonia oxidation time, as the consumed alkalinity was very low (only 44.4% of the initial alkalinity), so the pH value in the reaction tank was maintained at a high level (7.59). The FA concentration in this experiment continuously increased to reach 9.1 mgN.l^{-1}, thus a strong suppression of NOB occurred. Besides, according to Refs. [14, 15], when the nitrogen load increases up to a suitable value, it is easy for nitrite accumulation or partial nitrification to occur.

Effects of Bicarbonate (HCO_3^-)

The nitrification process consumed a large amount of alkalinity (1 g NH_4^+-N requires 7.07 g alkalinity in terms of $CaCO_3$), so the pH value and FA concentration also decreased rapidly [24]. This reduced the ability of NOB suppression. Therefore, to raise nitrite accumulation, it is necessary to amend the alkalinity. The TN4 experiment (Fig. 4), after an alkalinity amendment of the influent, the nitrogen concentration had a ratio of NH_4^+-N:NO_2^--N:NO_2^--N of 1:1.6:0.6 (in terms of nitrogen) and a NAR of 72.8%. The alkalinity used in the TN3 experiment was 54%. The nitrite accumulation process increased, and the NOB growth was much more suppressed. The pH value of the reactor in this experiment was always kept at a high level (7.99) and the FA concentration significantly rose to reach the value of 20.2 mgN.l^{-1}, same as the results reported by Refs. [20, 25].

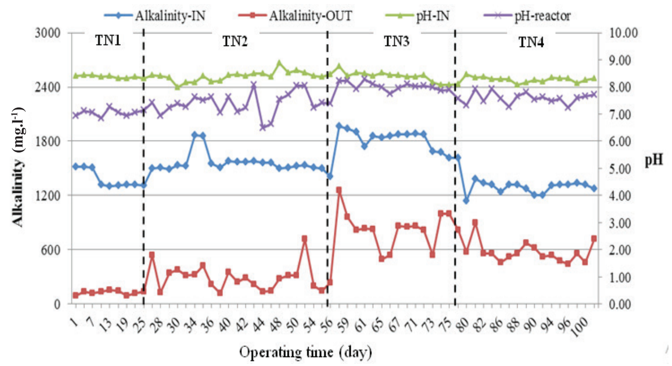

Fig. 4. Change of pH value and alkalinity in the experiments.

Assessment of organic matter removal efficiency

In the TN1 experiment (Fig. 5), the average COD removal efficiency was 41.6%, which was pretty low in comparison to other regular MBR tanks. A reason for this is that the oxygen supply (DO 1.4-1.8 mg.l^{-1}) was much lower than the required amount of oxygen to oxidize organic matter and the influent ratio of BOD_5/COD was less than 0.5, thus the amount of refractory organic compounds was large and the COD removal efficiency by biological process was low. Similarly, for the TN2, TN3, and TN4 experiments, the COD removal efficiencies were low 18.1, 10.5, and 14.29%, respectively.

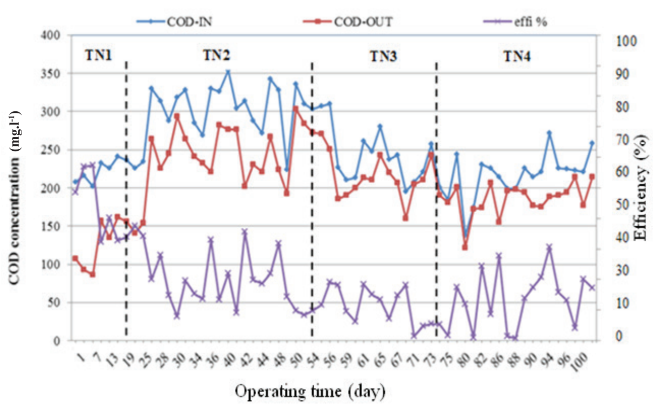

Fig. 5. COD removal efficiency versus operation time.

Conclusions

The appropriate hydraulic retention time for partial nitrification of piggery wastewater using a flat membrane is 7h30 at DO levels ranging between 0.8-1.0 mg.l^{-1}. When the alkalinity was amended in the influent to over 1600 mg.l^{-1}, the pH of the reactor was maintained at 8.0 and the FA concentration reached 20.2 mgN.l^{-1}. This increased the ability of NOB suppression and thus nitrite accumulation increased. The organic removal efficiency (COD) was low in this experimental model, as the highest efficiency was only 41.6%.

ACKNOWLEDGEMENTS

The authors deeply thank Japanese International Cooperative Association JICA-Supreme for financial aids and the technical support of Faculty of Environment and Natural Resources - University of Technology, Vietnam National University, Ho Chi Minh city for this study.

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] A.A. Van de Graaf, P. de Bruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen (1996), "Autotrophic growth of anaerobic ammonium oxidizing micro-organisms in a fluidized bed reactor", *Microbiology*, **142(8)**, pp.2187-2196.

[2] M. Strous, J.G. Kuenen, M.S.M. Jetten (1999), "Key physiology of anaerobic ammonium oxidation", *Appl. Environ. Microbiol.*, **65**, pp.3248-3250.

[3] A. Bertino (2010), *Study on one-stage partial nitritation-anammox process in moving bed biofilm reactors: a sustainable nitrogen removal*, Master Thesis, Stockholm, Royal Institute of Technology.

[4] I. Schmidt, O. Sliekers, M. Schmid, E. Bock, J. Fuerst, J.G Kuenen, M.S.M. Jetten, M. Strous (2003), "New concepts of microbial treatment processes for the nitrogen removal in wastewater", *FEMS Microbiol. Rev.*, **27**, pp.481-492.

[5] G. Ciudad, O. Rubilar, P. Munoz, G. Ruiz, R. Chamy, C. Vergara, D. Jeison (2005), "Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process", *Process Biochemistry*, **40**, pp.1715-1719.

[6] W. Jianlong, Y. Ning (2004), "Partial nitrification under limited dissolved oxygen conditions", *Process Biochemistry*, **39**, pp.1223-1229.

[7] N.H. Johansen, N. Suksawad, P. Balslev (2004), "Energy saving processes for nitrogen removal in organic wastewater from food processing industries in Thailand", *Water Science and Technology*, **50**, pp.345-351.

[8] Y.Z. Peng, Y. Chen, C.Y. Peng, M. Liu, S.Y. Wang, X.Q. Song, Y.W. Cui (2004), "Nitrite accumulation by aeration controlled in sequencing batch reactors treating domestic wastewater", *Water Science and Technology*, **50**, pp.35-43.

[9] U. Abeling, C.F. Seyfried (1992), "Anaerobic-aerobic

treatment of high-strength ammonium wastewater - nitrogen removal via nitrite", *Water Science and Technology*, **26(5-6)**, pp.1007-1015.

[10] O. Turk, D.S. Mavinic (1989), "Maintaining nitrite build-up in a system acclimated to free ammonia", *Water Res.*, **23**, pp.1383-1388.

[11] L. Kuai and W. Verstraete (1998), "Ammonium removal by the oxygenlimited autotrophic nitrification-denitrification system", *Appl. Environ. Microbiol.*, **64**, pp.4500-4506.

[12] S.K. Rhee, J.J. Lee, S.T. Lee (1997), "Nitrite accumulation in a sequencing batch reactor during the aerobic phase of biological removal", *Biotechnol. Lett.*, **19**, pp.195-198.

[13] A. Pollice, V. Tandoi, C. Lestingi (2002), "Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate", *Water Res.*, **36(10)**, pp.2541-2546.

[14] H. Sliegris, S. Reithaar, P. Lais (1998), "Nitrogen loss in a nitrifying rotating contactor treating ammonium rich leachate without organic carbon", *Water Sci. Technol.*, **37(4-5)**, pp.589-591.

[15] V.N. Dongen, M.S.M Jetten, M.C.M. Van Loosdrecht (2001), "The SHARON- Anammox process for treatment of ammonium rich wastewater", *Water Sci. Technol.*, **44(1)**, pp.153-160.

[16] S. Wyffels, S.W.H. van Hulle, P. Boeckx, E.I.P. Volcke, O. van Cleemput, P.A. van Rolleghem, W. Verstraete (2004), "Modelling and simulation of oxygen-limited partial nitritafition in a membrane-assisted bioreactor (MBR)", *Biotechnol. Bioengineering*, **86**, pp.531-542.

[17] Y.J. Feng, S.K. Tseng, T.H. Hsia, C.M. Ho, W.P. Chou (2007), "Partial nitrification of ammonium-rich wastewater as pretreatment for anaerobic ammonium oxidation (Anammox) using membrane aeration bioreactor", *Journal of Bioscience and Bioengineering*, **104(3)**, pp.182-187.

[18] M. Albert, B. Matias Vanotti, A.S. Ariel (2011), "Partial nitritation of swine wastewater in view of its coupling with the Anammox process", *Proceedings of First International Anammox Symposium*, pp.9-16.

[19] C.D.P. Marina, K. Airton, G. Caroline, Casagrande, M.S. Hugo (2011), "Influence of COD concentration on the partial nitritation", *Proceedings of First International Anammox Symposium*, pp.25-30.

[20] H. Xiaowu, U. Kohie, W. Qiaoyan, Y. Yuki (2016), "Fast start-up of partial nitritation as pre-treatment for Anammox in membrane bioreactor", *Biochemical Engineering Journal*, **105**, pp.371-378.

[21] L. Michele, P. Falas, R. Orlane, W. Arne, A.T. Thomas, et al. (2016), "Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures", *Water Research*, **101,** pp.628-639.

[22] G.M. Wong-Chong, R.C. Loehr (1978), "Kinetics of microbial nitrite nitrogen oxidation", *Water Res.*, **12**, pp.605-609.

[23] A.C. Anthonisen, R.C. Loehr, T.B.S. Prakasam, E.G. Srinath (1976), "Inhibition of nitrification by ammonia and nitrous acid", *J. Water Pollut. Control Fed.*, **48(5)**, pp.835-852.

[24] M. Henze, P. Harremoes, J.L.C. Jansen, E. Arvin (2002), *Wastewater treatment: biological and chemical processes*, Springer.

[25] S. Uemura, S. Suzuki, K. Abe, A. Ohashi, H. Harada, M. Ito, H. Imachi, T. Tokutomi (2011), "Partial nitrification in an airlift activated sludge reactor with experimental and theoretical assessments of the pH gradient inside the sponge support medium", *Int. J. Environ. Res.*, **5(1)**, pp.33-40.