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Berita Biologi

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LIPI

Biologi

Jurnal Ilmu-ilmu Hayati

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In Memoriam

Dr Anggoro Hadi Prasetyo

Dr Anggoro Hadi Prasetyo yang merupakan staf pegawai Bidang Zoologi, Pusat Penelitian Biologi-LIPI, telah menghadap Yang Maha Kuasa pada hari Sabtu tanggal 20 Pebruari 2010, setelah dirawat selama 4 hari di RS PMI Bogor dan RS Ciptomangunkusumo, Jakarta, karena Leukaemia Akut yang dideritanya. Almarhum adalah seorang ahli taksonomi rayap yang mendapatkan gelar PhD dari Queen Mary University of London. Almarhum meninggalkan seorang istri Dr Marlina Ardiyani, yang bekerja di Herbarium Bogoriense, Bidang Botani, Pusat Penelitian Biologi-LIPI, dan dua orang anak laki laki (M Ammar Zaky dan M Zuhdi Ali) dan dua anak perempuan (Anisa Zahra dan Aisyah Zafrina Aini).

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DAFTAR ISI

NITROGEN REMOVAL BYAN ACTIVATED SLUDGE PROCESS WITH CROSS-FLOW FILTRATION[1]
[Perombakan Nitrogen Menggunakan Proses Lumpur Aktif yang Dilengkapi dengan Filtrasi]

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ABSTRACT

The simultaneous nitrification and denitrification in a single reactor using an activated sludge with cross-flow filtration was investigated. The reactor was a Sequencing Batch Reactor (SBR), operated on three phase conditions, phase I was continuous aeration, phase II was intermittent aeration and phase III was intermittent aeration with methanol addition. The microbial properties on the nitrogen removal processes were monitored by measuring the ammonia decreasing and nitrate production rate. The denitrification rate was also calculated from N_20 gas production by acetylene inhibition method. The experiment results show that the nitrification was occurred during the aeration condition, both in the phase I and II. The denitrification was occurred in the anoxic stage (phase III). The nitrate decreasing rate in the anoxic stage (phase IIIA) increased gradually reach the value of 0.19 mg-N/1/min. on the 8^{th} day operation. The nitrate decreasing rate increased to be 0.45 mg/l/min in the phase IIIB and reach up to 0.70 mg/l/min in the phase IIIC in which the methanol concentrations was increased from 762 mg/L to 1016 mg/L in the phase IIIB and phase IIIC, respectively. The increasing activity of denitrification resulted in decreasing the concentration of TOC in the reactor. More than 80% nitrogen removal occurred in phase III and TOC removal efficiency in phase III reach more than 90%.

Keywords: Nitrification, denitrification, activated-sludge, Sequencing Batch Reactor (SBR), cross flow filtration.

ABSTRAK

Telah dipelajari proses nitrifikasi dan denitrifikasi secara simultan menggunakan sistem lumpur aktif dengan reaktor tunggal yang dilengkapi dengan filtrasi. Reaktor dioperasikan dengan sistem SBR *(Sequencing Batch Reactor),* melalui tiga fase kondisi, fase I: aerasi penuh, fase II: aerasi berjangka dan fase III: aerasi berjangka yang diberi perlakuan penambahan substrat methanol. Peran mikroorganism dalam proses perombakan nitrogen dipantau dengan cara mengukur kecapatan penurunan amonia dan produksi nitrat. Proses denitrifikasi juga diamati dengan cara mengukur produksi gas N_2O menggunakan metoda penghambatan Acetylen. Hasil penelitian menunjukkan bahwa proses nitrifikasi terjadi dalam kondisi aerobik (aerasi), baik pada fase I maupun fase II. Proses denitrifikasi terjadi pada kondisi anoksik (fase III). Kecepatan reduksi nitrat pada kondisi anoksik meningkat secara bertahap hingga mencapai 0,19 mg-N/1/menit pada hari ke 8. Kecepatan reduksi nitrat meningkat menjadi 0,45 mg/l/menit pada fase IIIB dan mencapai 0,70 mg/l/menit pada fase IIIC. Peningkatan reduksi nitrat terjadi seiring dengan penambahan konsentrasi methanol dari 762 mg/L pada fase IIIB menjadi 1016 mg/L pada fase IIIC. Peningkatan aktivitas denitrifikasi mengakibatkan terjadinya pengurangan konsentrasi senyawa organik (TOC) di dalam reaktor. Pengurangan konsentrasi nitrogen yang terjadi pada fase III mencapai lebih dari 80%, sedangkan pengurangan senyawa organik (TOC) mencapai lebih dari 90%.

Kata kunci: nitrifikasi, denitrifikasi, Lumpur aktif, SBR *(Sequencing Batch Reactor)*

INTRODUCTION

The removal of nitrogen compounds from wastewater is important, since nitrogenous pollutant is responsible for promoting the eutrophication effect in ponds and lakes. Biological nitrogen removal involves two processes, i.e. nitrification and denitrification. Nitrifications transform ammonia to a more oxidized nitrogen compound such as nitrite or nitrate, which is then converted to nitrogen gas in the subsequent denitrification process (Kuenen and Robertson, 1988). Those two processes are usually carried out in the different reactors because nitrification occurs under aerobic conditions while denitrification prevails in the absence of oxygen (Hong *et al.,* 1999).However, the two processes are complementary in many ways i.e. (1) nitrification produces nitrite or nitrate, which is a reactant in denitrification, (2) nitrification reduces the pH that is raised in denitrification, and (3) denitrification generates the alkalinity that is required in nitrification (Menoud *et al.,* 1999; Chen *et al.,* 1998). Therefore, there exist obvious advantages in performing simultaneous nitrification and denitrification in a single reactor. But, usual problem in a single reactor for treating wastewater containing nitrogen and organic carbon was decreasing the number of nitrifiers, because of slower growing of

'Diterima: 02 Januari 2010 - Disetujui: 05 Februari 2010

autotrophic nitrifiers than heterotrophic microorganisms. To ensure good nitrification, a long sludge retention time is required and organic sludge loading should be maintained at a low level.

Recently, an activated sludge process using cross-flow filtration as the solid-liquid separation step has been developed. By this process, a high sludge concentration can be maintained irrespective of sludge settling ability (Suwa *et al.*, 1989). It is expected that a longer sludge retention time which is necessary for good nitrification and high volumetric loading is possible by utilizing this process. This process could be applicable to simultaneous dissolved organic carbon removal-nitrification. In this process an oxygen concentration in the reactor is created by changing the condition without and with aeration automatically, so that both aerobic and anaerobic conditions can be established inside a single reactor.

In this work, we attempt simultaneous nitrification and denitrification in a single reactor using an activated sludge with cross-flow filtration. The potential of the Sequencing Batch Reactor (SBR) for nitrification and denitrification of wastewater containing ammonium nitrate with methanol as an external carbon source were determine. The microbial properties on the nitrogen removal process (nitrification and denitrification) were monitored by measuring the ammonia decreasing and nitrate production rate on the reactor. The denitrification rate was also calculated from N_2O gas production by acetylene inhibition method.

MATERIALS AND METHODS

Apparatus

The reactor consisted of a 1000-ml reaction vessel and a membran separation apparatus which was connected with tygon tubing via a sludge circulation pump. Polysulfon ultra-filter (MW 200,000 cut-off, 38.5 cm2 of filtration area, Toyo Roshi, Tokyo, Japan) was used for cross-flow filtration. The reactor stirring rate was 180 rpm and the mixed liquor contacting the filter was agitated by magnetic stirrer at 250 rpm. The reservoir containing synthetic wastewater was stored in a refrigerator at 4° C.

Synthetic wastewater and operational conditions of reactors

Mixed liquor from the Kohoku domestic sewage plant was used as inoculum for the nitrogen removal reactor. A volume of 1 liter was used to initiate the nitrogen removal reactor. The composition of the basal salt medium used for the synthetic wastewater supplied to the reactor comprised the following (per liter): 1143 mg of NH_4NO_3,16 mg of K_2HPO_4 12 mg of $CaCl_2 . 2H_2O$ and 100 mg of $NaHCO_3$. Table 1 shows the influent concentration added to the reactor for examining nitrification and denitrification. The reactor was a Sequencing Batch Reactor (SBR) with an operating volume of 1 liter. The reactor was operated on three phase conditions, phase I was continuous aeration, phase II was intermittent aeration and phase III was intermittent aeration with methanol addition. The batch time was four cycles per day, with 6 hours per cycle. There were three stages to each cycle, in the first operation: (i) feed, (ii) aerobic/oxic reaction and (iii) filtration. After the nitrification process occurred in the reactor vessel, the operational condition was changed to be four stages to each cycle: (i) feed, (ii) anoxic reaction, (iii) oxic reaction, and (iv) filtration. Table 1 shows the operation condition of the reactor. The temperature was maintained at 26 C, and pH was controlled at > 6.8 by adding 60g/l of $NaHCO_3$. During aeration period, DO was maintained at < 3 ppm and the aeration supplied was 1,000 ml/min. The activated sludge biomass was not withdrawn from the reactor

Table 1. Influent concentration added to the reactor ior examijrung ninuicauori ana aenimncation

Conditions	Days	TOC (mg/L)	NH,N (me/L)	NOrN (mg/L)	NOrN (mg/L)	Total-N (mg/L)
Phase I	**1-15**	3.0	200	0.0	200.6	400.6
Phase 11	16-33	3.0	200	0.0	200.6	400.6
Phase IIIA	34-47	146	200	0.0	200.6	400
Phase 1I1B	48-55	289	200	0.0	200.6	401
Phase niC	56-62	381	200	0.0	200.6	401

Table 2. Operation condition

Condition	Phase I	Phase H	Phase in
Aeration	Continuous	Intermittent	Intermittent
C addition	No	No	Methanol
Sequence	I	**II**	**III**
Feed	0-5 min.	0-5 min.	0-5 min.
Anoxic (denitrification)		0-180 min.	0-180 min.
Oxic (nitrification)	0-360 min.	180-360 min.	180-360 min.
Filtration (effluent)	180-360 min.	240-360 min.	240-360 min.

vessel except for MLSS determination.

Analytical methods

Sludge biomass was monitored by volatile suspended solids (VSS), which were determined as described in Gesui Shiken Ho-Hou (Japanese Sewage Works Association, 1974) using centrifuge at 3,000 rpm. The TOC was measured as dissolved organic carbon using TOC analyzers (Beckman 915B, Beckman Instruments Inc., Fullerton, cal., USA or TOC-500 Shimadzu Seisakusho, Kyoto, Japan). The concentrations of NO_2-N, and NO_3-N were determined by ion chromatography (IC7000, Yokogawa Analytical Systems, Tokyo, Japan). Ammonia-nitrogen (NH_4-N) concentration was determined an ion-chromatography (HIC6A, Shimadzu Corporation, Japan).

Denitrification assays by **acetylene inhibition technique**

Denitrification activity was determined according to acetylene inhibition technique (Yoshinari, T. and Knowles, R. 1976). Five ml portions of activated sludge (MLSS volume=875ml) were transferred to serum bottle (100 ml), and incubated for 20 minute. 0.25 ml nitrate solutions of 500 mg nitrate N/L was added to the serum bottle and then takes 0.25 ml for ion chromatography. Added a stirrer tip to the serum bottles, sealed with gastight screw caps equipped with a butyl rubber septum for gas sampling. The headspace was made anaerobic by evacuating and flushing with N_2, Acetylene of 10% was injected and 10 minute later start measuring a headspace gas. Substrate addition was made by injecting 0.25 ml organic chemical, at 15 minutes later and continued to measure. Samples were incubated and shaken at 200 rpm and continue to measure for another 45 minutes. Gas samples of 0.5 ml were withdrawn from the headspace with a gastight syringe at 15 minute intervals and transferred to glass vials (12.5 ml) sealed with a Teflon coated rubber septum, the samples were analyzed for nitrous oxide. Take sample also for ion chromatography.

RESULTS

Performance of nitrogen removal on each phase

Performance of nitrogen removal during operated with various condition was illustrated in Fig. 1 - Fig.5. During the aeration condition (Fig.l), the

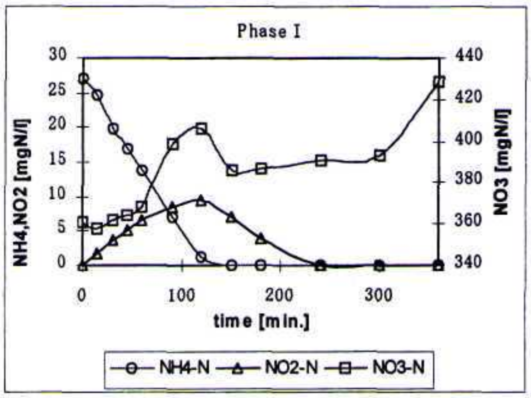

Figure 1. Time course of nitrogen removal on the Phase I

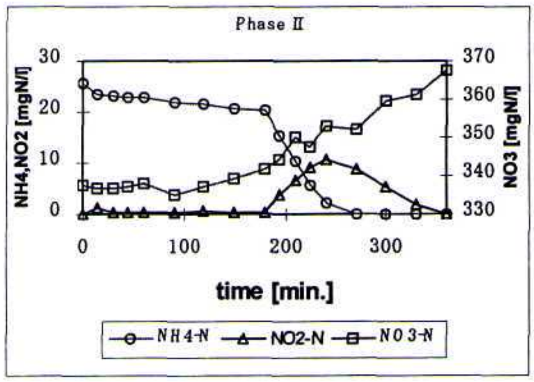

Figure 2. Time course of nitrogen removal on the Phase II

ammonium was immediately oxidized to nitrite and nitrate. Nitrification rate based on the decreasing rate of NH_4-N was increased gradually and became stable at the 14^{th} day of operation.

The concentration of nitrate in the effluent increased during operation under aeration condition (Fig.2). Until 13 days operation of the phase II, there was no significantly change on the concentration of nitrate at the anoxic stage. In the phase II, the nitrification process was occurred in the aeration stage in which the rate of nitrification was higher than that rate on the phase I.

To develop the denitrification process, methanol was added as donor electron at three step, 381 mg/L on phase IIIA (Fig.3), 762 mg/L on phase IIIB (Fig.4), and 1016 mg/L on phase IIIC (Fig.5). The nitrate decreasing rate in the anoxic stage was increased gradually reach the value of 0.1928 mg-N/1/min. on the 8^{th} day operation. The nitrate decreasing rate was increased to be 0.4540 mg/l/min in the phase MB and reach to 0.7 mg/l/min in the phase IIIC.

From the performance of effluent quality of nitrogenous compound summarized in Fig.6 show that NH_4-N was not detected in the effluent almost in each

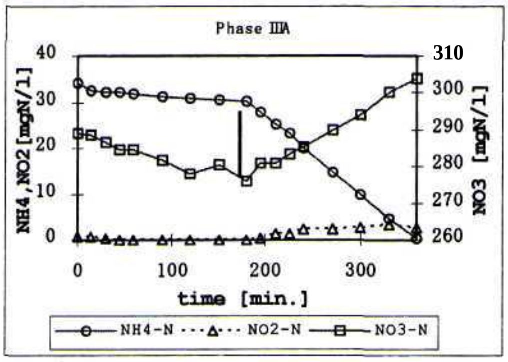

Figure 3. Time course of nitrogen removal on the Phase IIIA

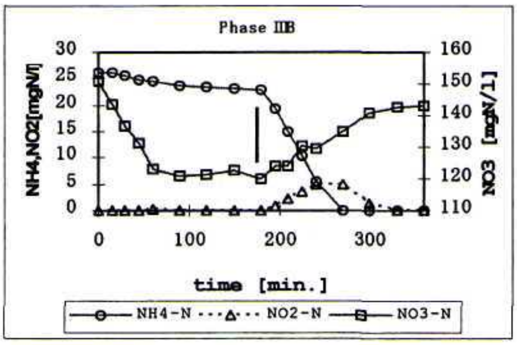

Figure 4. Time course of nitrogen removal on the Phase IIIB

run. In the phase IIIA, a much amount of NH_4-N was found in the effluent. The concentration of TOC in the reactor, both in the nitrification stage (Rnit) and denitrification stage (Rden) were high, more than 50

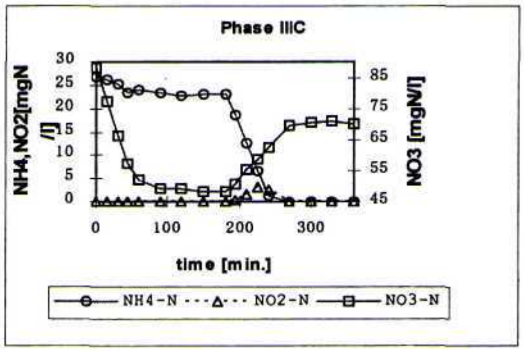

Figure 5. Time course of nitrogen removal on the Phase IIIC

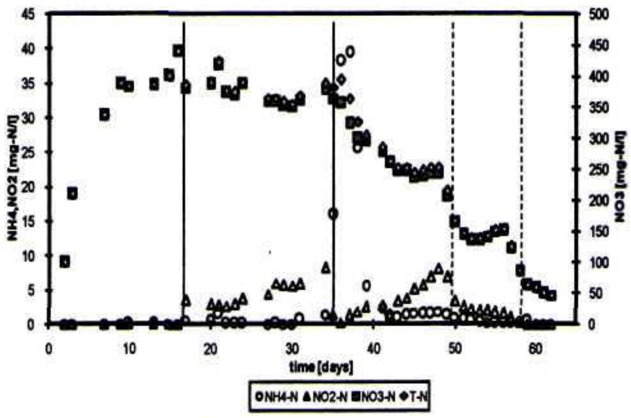

Figure 6. Effluent quality of nitrogenous compounds

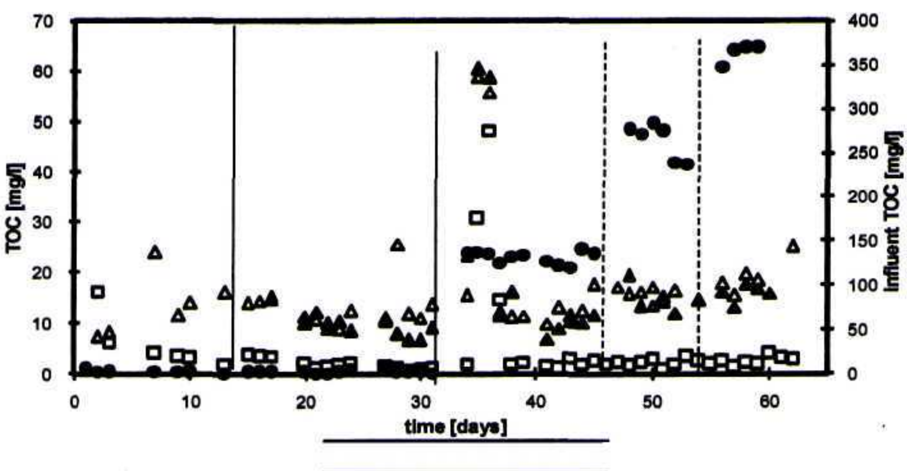

Figure 7. Performance of TOC concentration in the reactor

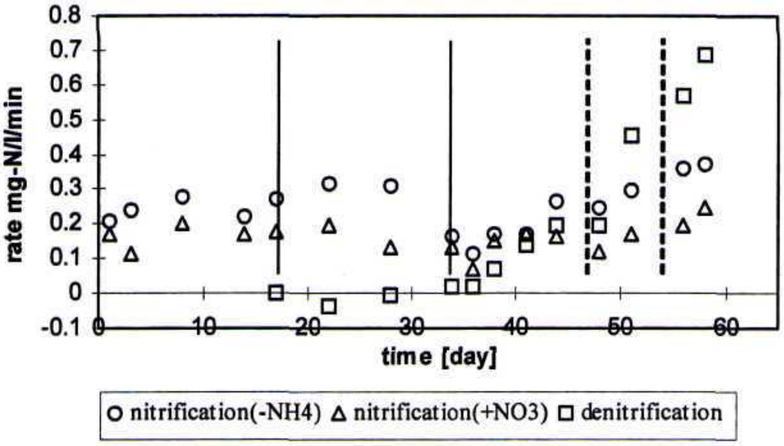

Figure 8. Performance of nitrification and denitrification rate based on nitrate utilization

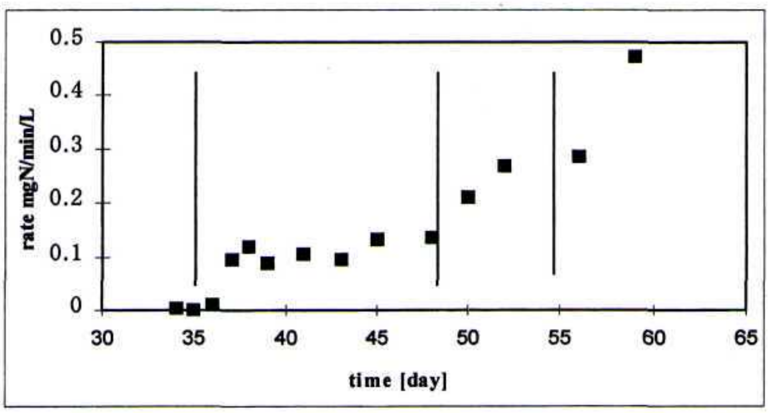

Figure 9. Performance of denitrification rate based on the N_2O production

mg/1 (Fig.7). After 5 days operation under methanol addition, only small concentration of NH_4-N was detected in the effluent. In the same time the denitrification process has been observed successfully. The concentration of TOC in the effluent was observed less than 10 mg/1 (Fig.7).

Comparison of denitrification rate by nitrate utilization and N_2O production

In this experiment both of nitrate utilization and production ofN_2O by acetylene inhibition method were used to characterize the denitrification in the reactor. Fig. 8 showed the result of denitrification rate based on the nitrate utilization. The denitrification process was initially detected in the phase III, when methanol was added to the influent. The nitrate decreasing rate were increased from 0.19 mg/1/minute in the phase IIIA to 0.45 mg/1/minute in the phase IIIB and finally reach to be around 0.7 mg N/1/minute in the phase IIIC. Comparing to the denitrification rate based on the production of N_2O gas, the denitrification rate base on nitrate utilization was higher. The denitrification rate based on N_2O peroduction was 0.13 mg N/l/min in the phase IIIA, 0.26 mg N/l/min in the phase IIIB and 0.47 mg N/l/min in the phase IIIC (Fig.9).

Efficiency of **nitrogen removal**

Comparing the total nitrogen in the influent and in the effluent, nitrogen removal was lower in the phase I and II that were not more than 10%, in which no addition of methanol in the influent. The decreasing concentration of total nitrogen in the phase I was suggested due to synthesis of bacteria. When methanol was added to the influent, the nitrogen removal occurred in the reactor was increased from 27% to 60% in which the methanol addition was

Table 3. Efficiency of nitrogen and organic removal

Condition	Concentration of total N			Concentration of TOC		
	Influent	Effluent	N removal	Influent	Effluent	TOC removal (%)
Phase I	392.7	378.8	3.5	2.7	3.1	0
Phase II	393.5	361.0	8.3	3.7	1.1	70
Phase IIIA	404	294	27.2	131	9	93
Phase IIIB	405	156	55.3	264	2	99
Phase IIIC	402	71	82.3	361	3	99

increased from 381mg/l to 762 mg/1. More than 80% nitrogen removal occurred in phase IIIC, in which methanol addition was 1016 mg/1 (Table 4). Almost all of methanol added in the influent was used as donor electron for denitrifier, resulted in TOC removal efficiency in phase III reach more than 90% (Table 3).

DISCUSSION

Performance of nitrogen removal during operated with various condition was illustrated in Fig. 1-5. During the aeration condition (Fig. 1.), the ammonium was immediately oxidized to nitrite and nitrate, indicating successful nitrification under this condition. Nitrification rate was increased gradually, resulted in increasing concentration of nitrate in the effluent (Fig.2). To decrease the level of nitrate, we change the condition from fully aeration to be intermittent aeration (phase II). We supposed that the denitrification will be occurred in the anoxic stage with internal carbon source as donor electron. Until 13 days operation of the phase II, there was no significantly change on the concentration of nitrate at the anoxic stage, indicating that the denitrification has not been observed yet. While, the nitrification process was occurred in the aeration stage in which the rate of nitrification was higher than that rate on the phase I. To develop the denitrification process, methanol was added as donor electron at three step, 381 mg/L (Fig.3), 762 mg/L (Fig.4), and 1016 mg/L (Fig.5). The nitrate decreasing rate was increased to be 0.4540 mg/l/min in the phase IIIB and reach to 0.7 mg/l/min in the phase IIIC (Fig.5). From the performance of effluent quality of nitrogenous compound summarized in Fig.6 show that NH_4-N was not detected in the effluent almost in each run, indicating that nitrification was successfully achieved. In the phase IIIA, a much amount of NH_4-N was found in the effluent, indicating the inhibition of nitrification. Inhibition in nitrification might causes by the higher concentration of carbon (TOC) on the reactor. As shown in Fig. 7, the concentration of TOC in the reactor, both in the nitrification stage (Rnit) and denitrification stage (Rden) were high, more than 50 mg/1. From this result also indicating that the denitrification process has not successfully occurred, therefore the concentration of organic carbon has still high in the reactor. After 5 days operation under methanol addition, only small concentration of NH_4-N was detected in the effluent, in the same time the denitrification process has been observed successfully indicating by the decreasing nitrate concentration in the effluent (Fig.6). The concentration of NO_3-N was decreased gradually from the phase IIIA to the IIIB, and IIIC, indicating the increase denitrification activity in the reactor. The increasing activity of denitrification resulted in decreasing the concentration of TOC in the reactor, although the TOC concentration in the influent increased, the concentration TOC in the effluent was observed less than 10 mg/1 (Fig.7). Under anoxic conditions, nitrate is transformed into nitrite and finally nitrogen gas, corresponding to the utilization of organic carbon sources.

The most widely used technique to characterize denitrification in activated sludge is to measure nitrate utilization. However, by only measuring the consumption of nitrate, assimilative nitrate reduction and dissimilative nitrate reduction to nitrite or ammonium cannot be excluded. Measurements of formation of gaseous product might therefore be an appropriate way of determining denitrification rates in waste water. The acetylene inhibition technique (Yoshinari and Knowles, 1976) is based on the inhibition of the nitrous oxide reductase by acetylene, thereby resulting in an accumulation of the obligate intermediate nitrous oxide (N_2O) in the denitriflcation pathway. In this experiment both of nitrate utilization and production of N_2O by acetylene inhibition method were used to characterize the denitriflcation in the reactor. Comparing to the denitrification rate based on the production of N_2O gas, the denitriflcation rate base on nitrate utilization was higher. The difference value of denitrification rate of two measurement method as shown in the Fig. 8 and Fig. 9 might be due to the difference measurement condition. The denitrification rate shown in the Fig 8 was coming from *insitu* measurement method, while denitrification rate shown in the Fig. 9 was coming from *exsitu* measurement.

The nitrogen removal was lower in the phase I and II that were not more than 10%, in which no addition of methanol in the influent. The decreasing concentration of total nitrogen in the phase I was suggested due to synthesis of bacteria. The nitrogen removal increased from 27% to 60% in which the methanol addition was increased from 381mg/l to 762 mg/1. More than 80% nitrogen removal occurred in phase Hie (Fig.5), in which methanol addition was 1016 mg/1 (Table 3). Almost all of methanol added was used as donor electron for denitrifiers, resulted in TOC removal efficiency in phase III reach more than 90% (Table 3).

CONCLUTIONS

The nitrification and denitrification was occurred simultaneously in the reactor. The nitrification was occurred during the aeration condition, both in the phase 1 and II, while the denitrification was occurred in the anoxic stage (phase III). The nitrate decreasing rate in the anoxic stage (phase III) increased gradually from 0.19 mg-N/1/min to 0.45 mg/l/min and reach up to 0.70 mg/l/min in which the methanol concentrations was increased from 381 mg/L (phase III A) to 762 mg/L (phase IHB) and 1016 mg/L (phase IIIC), respectively. The increasing activity of denitrification resulted in decreasing the concentration of TOC in the reactor. More than 80% nitrogen removal occurred in phase III and TOC removal efficiency in phase III reach more than 90%

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