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Effects of biological treatment in reverse osmosis concentrate with non-oxidizing biocide (DBNPA) content

Jung Su Choi[a], Sang Rim Kang[b], Sang Seob Lee[c], Hyun Jong Joo[d,*]

[a]*Department of Environmental Energy Engineering, Graduate School Kyonggi University, 94 San, Iui-dong, Youngtong-ku, Suwon-si, Gyeonggi-do 442-760, Korea, email: wwtpjs@kgu.ac.kr*
[b]*Development of Biological Engineering, Graduate School Kyonggi University, 94 San, Iui-dong, Youngtong-ku, Suwon-si, Gyeonggi-do 442-760, Korea, email: rkdtkdfla@naver.com*
[c]*Development of Life Science, Kyonggi University, 94 San, Iui-dong, Youngtong-ku, Suwon-si, Gyeonggi-do 442-760, Korea, email: sslee@kgu.ac.kr*
[d]*Department of Environmental Energy Engineering, Kyonggi University, 94 San, Iui-dong, Youngtong-ku, Suwon-si, Gyeonggi-do 442-760, Korea, Tel./Fax: +82 31 257 7689; email: hjjoo@kgu.ac.kr*

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ABSTRACT

Biocide has been used in order to prevent biofouling of the membrane as a non-oxidizing disinfectant. However, there is a lack of research for its effects on the related wastewater treatment. The objective of this study is to evaluate the microbial inhibitory effect by non-oxidizing biocide containing 2,2-dibromo-3-nitrilo propionamide (DBNPA) injection. In this study, removal efficiency of $TCOD_{Cr}$ and NH_3-N was evaluated according to the DBNPA concentration. Also, the specific oxygen uptake rate (SOUR) and polysaccharides (PSs) were measured. The laboratory-scale reactor was prepared and operated with the DBNPA concentrations of 0, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/L based on concentrations frequently occurring in the reverse osmosis concentrate. The results from this study showed that as the DBNPA concentration increase, the removal efficiency of $TCOD_{Cr}$ and NH_3-N tends to decrease as expected. Also, the increase in DBNPA concentrations resulted in the decrease in SOUR. It is determined that DBNPA injection affects the microbial activity. Especially, an additional amount to above 5.0 mg/L DBNPA into reactor caused rapidly decreased removal efficiency of $TCOD_{Cr}$, NH_3-N, and SOUR due to the inhibitory effects of DBNPA. It is also shown that DBNPA destroyed the cell of the micro-organisms, thus producing the cell's PSs.

Keywords: Reverse osmosis concentrate (ROC); DBNPA; Microbial activity; Polysaccharides (PSs); Specific oxygen uptake rate (SOUR)

1. Introduction

The importance of advanced water treatment technologies has lately been highlighted as a solution to water pollution in ecosystems and water shortage due to changed summer precipitation patterns. To address water shortage problems, the Korean government accelerates its supports for development and dissemination of environmental policies and technologies in sea water desalination, wastewater reclamation and reuse system, and advanced treatment of rivers, streams, and lakes. In particular, industrial areas such

*Corresponding author.

as semiconductor, etching, and petro chemistry are recently increasing their demand for high-quality industrial water, which increases the cases of applying the reverse osmosis (RO) method, a membrane separation process. As a derivative of the sea water desalination method, the RO process has gradually extended its application to areas such as wastewater reuse and advanced treatment of rivers, streams, and lakes, and many studies on the process are underway [1].

Industrialized nations including the United States, the United Kingdom and Japan have already developed and applied technologies using the RO process to produce industrial water. Korea is also witnessing a growing use of the RO method as it is being developed for various purposes. In these circumstances, it is necessary to consider the RO process in terms of its usability in facilities, technological power, and environmental effects from various viewpoints. Key environmental concerns related to the RO process include the occurrence of reverse osmosis concentrate (ROC), which is also recently one of the most controversial issues [2]. In highly concentrated water, ROC inevitably occurs during the RO process. The increasing concentration rates of treated water, together with its higher recovery rates, further emphasize the necessity of studies on stable treatment methods and technologies of concentrated water in recent years. However, ROC contains recalcitrant organics, high-concentration nitrogen, and salt that are hard to degrade with biological reaction, which makes it difficult to satisfy legal standards for water quality [3]. Among other things, the non-oxidizing biocide (NOB), which is used to prevent separation membrane biofouling and clean up, flows into biological treatment facilities along with ROC, eventually affecting the process of biological treatment by destroying micro-organism cell membranes and damaging their cells.

In this sense, it is necessary to analyze the impact of NOB flowing into the biological process on micro-organisms. In this study, 2,2-dibromo-3-nitrilo propionamide ($C_3H_2Br_2N_2O$, CAS No. 10222-01-2, DBNPA), one of the typical substances used for NOB, was injected at various concentrations to assess correlations of DBNPA injection with the characteristics of out flowing nitrogen and organic matters as well as micro-organism activity.

Among the activated sludge processes, which are basic to biological treatment, the sequencing batch reactor (SBR) process was selected for the biological process in this study. A laboratory-scale reactor was also built and operated. While different DBNPA concentrations were applied, the treatment efficiency of substrates ($TCOD_{Cr}$) and nitrogen (NH_3-N, NO_3^--N) was analyzed. At the same time, specific oxygen uptake rates (SOUR), which usually serve as an indirect indicator of micro-organism activity were used to evaluate the link between DBNPA injection and changes of micro-organisms in the reactor. In addition, the direct effect of DBNPA injection on micro-organisms was assessed using micro-organisms' polysaccharides (PSs), which are released when their cells are destroyed. How DBNPA injection affects the removal efficiency of substrates and nitrogen was also evaluated through the process of identifying which micro-organism species became dominant in the reactor.

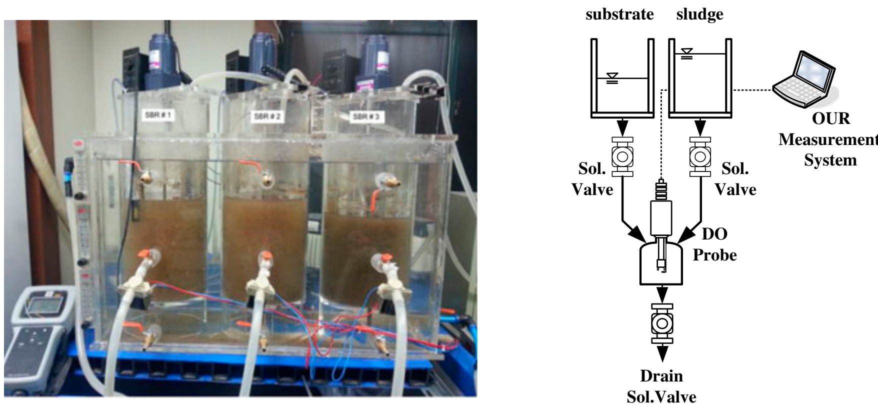
2. Materials and methods

2.1. Testing equipment

The structure and form of the reactor used in this study are shown in Fig. 1(a). This laboratory-scale SBR reactor was to evaluate DBNPA effect on nitrogen and substrate removal efficiency and micro-organism activity. The reactor volume was designed to be 6 L. The hydraulic retention time (HRT) for which the reactor would be operated was set as 8 h (influent for 30 min; oxic for 150 min; anoxic for 150 min; oxic for 30 min; settle for 60 min; effluent for 30 min; and idle for 30 min). The reactor was automatically operated by the programmable logic control. The instrumental system for measuring oxygen uptake rates (OUR) consisted of input of substrates, sludge, and washing water; the OUR measurement reactor; the dissolved oxygen (DO) measuring instrument; and the program that controls the entire system, as shown in Fig. 1(b).

2.2. Effect of DBNPA injection on substrate and nitrogen removal efficiency

The water put into the reactor in this study was a synthetic wastewater made from CH_3OH, NH_4Cl, and KNO_3 to characterize substrate and nitrogen removal at different DBNPA concentrations and identify the effect of various DBNPA concentrations on micro-organism activity. As for the concentration of influent substrates, CH_3OH was used to fix the concentration of $TCOD_{Cr}$ at 250 mg/L. Using NH_4Cl and KNO_3, the concentrations of ammonia nitrogen and nitrate nitrogen were adjusted to 25 and 50 mg/L, respectively. Based on the test settings in this study, DBNPA was injected at different concentrations. As for other substances such as minerals, the volumes only necessary for microbial synthesis were taken into account. To obtain nitrogen and substrate removal efficiencies at different DBNPA concentrations, analysis was carried out for samples at those stages of influent, aeration tank and effluent. The influent substrate concentration,



(a) Laboratory-scale reactor (b) The test equipment for OUR determination

Fig. 1. Schematic diagram of laboratory-scale reactor (a) and OUR measurement system (b).

influent flow, sludge retention time, DO concentration, pH, and temperature, all of which could affect effluent water quality, were maintained to be the same. The operational settings of the reactor during the test period are shown in Table 1.

Different volumes of DBNPA were applied to the reactor to evaluate the substrate and nitrogen removal efficiencies at various DBNPA concentrations. According to the material safety data sheet (MSDS) on DBNPA provided by Dow Chemical, the results of toxicity evaluation of DBNPA on micro-organisms in activated sludge showed the effective concentration of 50% (EC50) for DBNPA is 3.1 mg/L, and even injection with the slightest amount had a great impact. Therefore, in this study, the concentration ranges were confirmed as shown in Table 2 based on the results from Dow Chemical.

2.3. SOUR at various DBNPA concentrations

The indirect effects of various DBNPA concentrations on micro-organisms were analyzed with the OUR measuring instrument. Sludge and influent were collected during the time for reaeration reaction and measured every day. Fig. 2 displays an example of calculation that provides information on OUR during

Table 2
Experimental conditions with concentrations of DBNPA

Item	Mode (DBNPA injection concentration, mg/L) 1	2	3	4	5	6
DBNPA	0	1	1.5	2	3	5

the implementation of this program. The OUR values transmitted from the measuring instrument were obtained through a real-time analysis of DO concentrations from the measurement cycle of sludge influent, substrate influent, agitation, effluent, and washing. For information on OUR, the changed values as a result of micro-organisms' uptake of substrates were used among the values of DO concentrations measured at an interval of 5 min [4]. Fig. 2(a) shows the change in DO concentration measured with a polarographic-type DO probe (YSI 5905/5010/200) in the OUR measurement reactor every 5 min during the processes of sludge influent, substrate influent, agitation, effluent, and washing. Fig. 2(b) displays the process of obtaining valid values among the measured DO concentrations. The values in the range from the location where the DO concentration began to drop

Table 1
Experimental conditions of SBR process

Experimental conditions							
					DO, mg/L		Methanol required, mg NO_3^--N/mg CH_3OH
Volume, L	Influent flow rate, L/d	pH	SRT, d	HRT, h	Anoxic	Aeration	
6	10.5 (3 cycle)	7.0–8.0	20	8/1 cycle	0.1–0.2	1.5–3.0	3.3

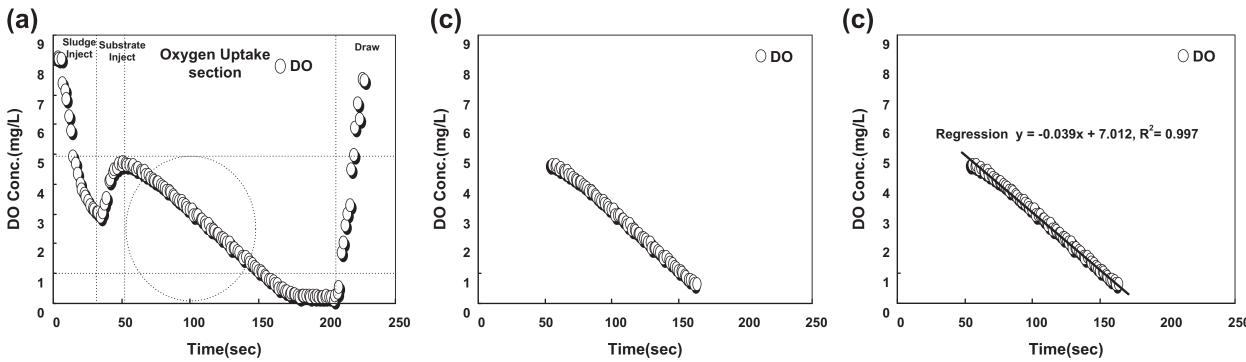


Fig. 2. Measurement of OUR (a), data screening (b), and data regression (c).

along with substrate injection to the location where the concentration reached 1 mg/L were set as valid values in this test. Fig. 2(c) shows the process of finding OUR values using regression analysis.

2.4. Fluctuation in PSs at various DBNPA concentrations

The PSs of micro-organisms were measured to analyze the direct effects of different DBNPA concentrations on micro-organisms. To conduct evaluation, sludge was disposed off in SBR #1 where DBNPA was not injected. A digital jar-tester was used to change the concentration of DBNPA injection, while PSs that occurs when micro-organisms are destroyed were measured. The jar-test settings for PSs measurement are shown in Table 3.

2.5. Analysis methods

Collecting and analyzing water samples were conducted at the same hour every day. Each item for the samples was analyzed in accordance with the Standard Methods [5]. The phenol–sulfuric acid method [6] was applied to measure PSs. The pour plate method was used to cultivate sludge sampled from each reactor to identify micro-organism species when DBNPA was injected. Each single colony was identified and inoculated into the culture medium at 25℃ for 3 d. The 16S rRNA sequencing analysis was carried out to identify purely separated strains. The sequencing analysis identified each strain through the National Center for Biotechnology Information's Basic Local Alignment Search Tool.

3. Results and discussion

3.1. Characteristics of influent

The characteristics of influent observed during the reactor operation are shown in Table 4. Under the same conditions of source water, DBNPA was injected at different concentrations while the reactor was being operated.

3.2. Characteristics of substrate removal at various DBNPA concentrations

The characteristics of substrate removal observed while different concentrations of DBNPA were injected into the bioreactor are shown in Table 5. The correlations between the characteristics and DBNPA concentrations were obtained through regression

Table 3
Experimental conditions of PSs measurement with concentrations of DBNPA

	DBNPA injection concentrations and sampling time					
Item	0[a]	1	1.5	2	3	5
PSs	0–6 h[b]	0–6 h	0–6 h	0–6 h	0–6 h	0–6 h

[a]DBNPA injection concentrations.
[b]Sampling time: 0, 5, 10, 15, 30, 60, 120, 180, 240, 300, 360 min.

Table 4
Characteristics of influent in the laboratory-scale reactor

	Influent concentrations (mg/L)		
Constituent	Min.	Max.	Ave.
$TCOD_{Cr}$	247.3	254.7	251.7
TN	72.5	78.4	76.4
NH_3-N	24.5	26.2	25.5
NO_3^--N	48.4	55	51.5

analysis, as displayed in Fig. 3. The result of substrate removal in each DBNPA condition was as follows: when DBNPA was injected at concentrations of 0, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/L, the influent $TCOD_{Cr}$ was 252.3, 254.8, 252.3, 254.8, 257.8, and 257.8 mg/L on average, respectively, while the effluent $TCOD_{Cr}$ was each 12.0, 29.5, 25.9, 32.6, 56.1, and 110.4 mg/L. This represented the mean treatment efficiency of 95.2, 88.4, 89.7, 87.2, 78.2, and 57.2%, respectively. The $TCOD_{Cr}$ removal efficiency consistently dropped as the DBNPA concentration was rising. At a concentration of 5.0 mg/L, in particular, the mean effluent water quality and removal efficiency were 110.4 mg/L and 57.2%, respectively. This indicates that the injection of DBNPA has significant impact on substrate removal.

The correlations between DBNPA and $TCOD_{Cr}$ effluent as a result of regression analysis were expressed as a quadric function: $f(x) = 5.8391 + 18.8605x$ ($R^2 = 0.8614$). Whenever the DBNPA concentration rose by 0.1 mg/L, the effluent water quality was found about 1.91 mg/L higher on average. According to a study of disinfectant effect on micro-organism growth rates [7], DBNPA was classified into a disinfectant. In that study, the growth rates of micro-organisms turned out 4.8, 3.5, and 3.0 h^{-1} when DBNPA was injected at 0, 12.5, and 25.0 mg/L, respectively, thereby showing the maximum growth inhibition rate of around 37.5%. The same study also reported that the cell walls of micro-organisms were destroyed when DBNPA was injected.

Besides disinfectants, the effects of heavy metals on micro-organism substrates were also looked up in the existing research data. A study on characteristics of TOC removal with nickel injection [8] found that when the injected amount of nickel was 10 mg/L or higher, the removal efficiency was 88% or lower, down more than 10% compared with no nickel injection. In addition, Seo [9] reported that the inflow of

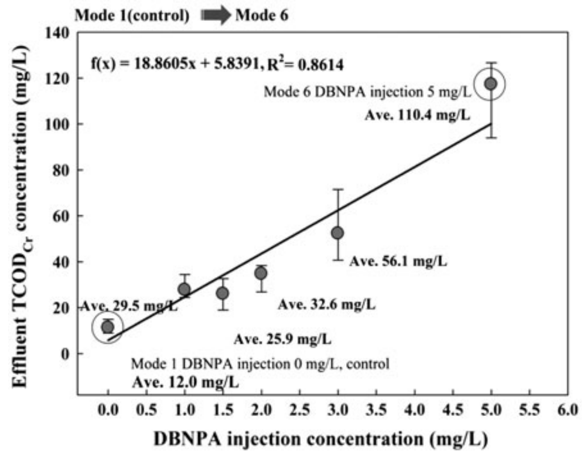


Fig. 3. $TCOD_{Cr}$ effluent concentration with injection of DBNPA.

heavy metals such as Cu and Zn has adverse impact on substrate degradation and OUR values.

This study also found that the removal efficiency fell by more than 38% compared with no DBNPA injection, as it was 57.2% at a DBNPA concentration of 5.0 mg/L. This indicates that DBNPA included in ROC is a cause of substrate removal efficiency reduction in the bioreactor.

3.3. Effect of DBNPA on nitrogen removal efficiency

The characteristics of nitrogen removal at various DBNPA concentrations in the bioreactor were analyzed as follows: when DBNPA was injected at 0, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/L, the influent NH_3-N was 25.2, 25.9, 25.6, 25.4, 25.9, and 25.8 mg/L on average, respectively, while the effluent NH_3-N was each 0.53, 0.61, 0.31, 7.22, 17.6, and 23.1 mg/L, thereby showing that the mean treatment efficiency was 98.0, 97.7, 97.7,

Table 5
Substrate removal efficiencies in the laboratory-scale reactor

Substrate[a] conc. Mode[b]		Influent			Effluent			Removal efficiency (%)		
		Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Mode 1	0, control	247.3	257.3	252.3	7.8	17.3	12.0	93.3	96.8	95.2
Mode 2	1.0	249.8	259.8	254.8	21.3	37.3	29.5	85.6	91.5	88.4
Mode 3	1.5	244.8	257.3	252.3	17.3	37.3	25.9	85.5	92.9	89.7
Mode 4	2.0	249.8	257.3	254.8	21.8	39.8	32.6	84.5	91.3	87.2
Mode 5	3.0	254.8	262.3	257.8	37.3	82.3	56.1	68.6	85.4	78.2
Mode 6	5.0	252.3	259.8	257.8	77.3	124.8	110.4	52.0	69.4	57.2

[a]Substrate: $TCOD_{Cr}$, mg/L.
[b]Mode: DBNPA injection concentrations, mg/L.

71.6, 32.0, and 10.5%, respectively. The removal efficiency of NH_3-N proportionally decreased along with the increasing concentrations of injected DBNPA. Particularly, NH_3-N removal efficiency was only 10.5% when the effluent water quality was 23.1 mg/L on average at a DBNPA concentration of 5.0 mg/L. This indicates that DBNPA injection has a serious effect on NH3-N removal efficiency. The removal efficiency of NO_3^--N was also analyzed: when the concentration of injected DBNPA was 0, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/L, the influent NO_3^--N was on average 51.2, 49.5, 52.3, 52.1, and 51.4 mg/L, respectively, while the effluent NO_3^--N was each 5.2, 4.5, 5.3, 6.5, and 6.9 mg/L, showing the mean removal efficiency of 89.8, 91.0, 89.7, 87.6, and 86.6%, respectively. The removal efficiency of NO_3^--N declined as DBNPA concentrations was raised, but the decrease rates were very low, compared with those of NH_3-N. This may be attributable to the domination of heterotrophic bacteria (substrate-oxidized and denitrified), rather than autotrophic bacteria (nitrified), among those bacteria used for biological treatment. In this view, 16S rRNA sequencing was used to identify Mode 6 sludge into which DBNPA was constantly injected at 5 mg/L. As a result, such bacteria as *Paracoccus*, *Flavobacteriaceae*, *Sphingomonas*, *Acidovorax*, and *Bosea* were identified. Previous studies [10] suggest *Paracoccus* and *Flavobacteriaceae* as typical heterotrophic micro-organisms. Given that autotrophic bacteria such as *Nitorosomonas*, *Nitrobacter*, and *Nitrococcus* were not identified, it is assumed that DBNPA has more impact on nitrogen oxidation, especially ammoniacal nitrogen than substrate uptake. The result of identifying single colonies using 16S rRNA sequencing is shown in Table 6.

Table 6
Bacteria identification in sludge of SBR with DBNPA content

Bacteria identification
Paracoccus sp.
Flavobacteriaceae
Sphingomonas sp.
Acidovorax sp.
Bosea sp.

Tchobanoglous and Burton [10] reported that the ratios of nitrifying micro-organisms ranged from 0.029 to 0.350 based on the influent C/N ratios, presenting that a very small population of nitrifying micro-organisms exists. This suggests that the inflow of toxic substances has more effect on the removal of NH_3-N than that of NO_3^--N. The characteristics and correlations of NH_3-N removal with different DBNPA concentrations in the bioreactor are displayed in Table 7 and Fig. 4.

3.4. Fluctuation of micro-organism activity at various DBNPA concentrations

This study analyzed the effects of DBNPA injection into the bioreactor on substrate and nitrogen removal. At the same time, the cause of such DBNPA effect on substrate and nitrogen removal was also searched in association with micro-organism activity. To this end, evaluation was performed based on SOUR values, which are used as an indirect indicator of micro-organism activity. When DBNPA was injected into the bioreactor at concentrations of 0, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/L, the mean SOUR value was measured to be 71.2, 63.1, 58.6, 55.5, 40.9, and 37.6 mg O_2/g MLVSS h, respectively. The values decreased along with higher DBNPA concentrations (Table 8, Fig. 5). When the reactor was not injected with DBNPA, the SOUR reached 71.1 mg O_2/g MLVSS h, the highest level. With higher DBNPA concentrations injected, the SOUR went down to 37.6 mg O_2/g MLVSS h, the lowest level. The DBNPA inhibition rates were found to range from 11.0 to 46.5%.

According to the MSDS provided by Dow Chemical, DBNPA has acute toxic effects (lethal concentration of 50% or LC50 0.1–1.0 mg/L) on

Table 7
NH_3-N removal efficiencies in the laboratory-scale reactor

NH_3^+-N conc. Mode[a]		Influent (mg/L)			Effluent (mg/L)			Removal efficiency (%)		
		Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Mode 1	0, control	24.5	26.5	25.5	0.23	0.82	0.53	97.0	99.2	98.0
Mode 2	1.0	25.3	26.5	25.9	0.22	1.03	0.61	96.2	99.2	97.7
Mode 3	1.5	25.1	25.8	25.6	0.74	1.22	0.31	97.2	95.3	97.7
Mode 4	2.0	25.2	25.9	25.4	6.85	7.54	7.22	70.9	72.8	71.6
Mode 5	3.0	25.4	26.3	25.9	10.7	22.0	17.6	16.3	57.9	32.0
Mode 6	5.0	25.3	26.3	25.8	19.4	25.0	23.1	4.9	23.3	10.5

[a]Mode: DBNPA injection concentration, mg/L.

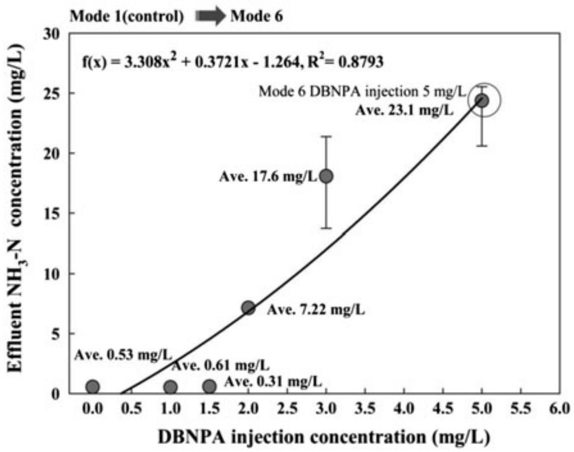


Fig. 4. NH_3-N effluent concentration with injection of DBNPA.

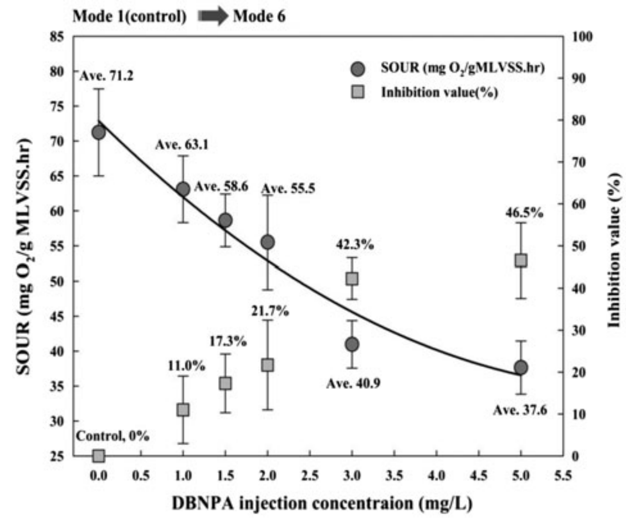


Fig. 5. SOUR data and inhibition value with injection of DBNPA.

aquatic species [11]. The result of assessing the impact of DBNPA toxicity on micro-organisms in activated sludge showed EC50 (Effective Concentration of 50%) at a DBNPA concentration of 3.1 mg/L, which indicates that even a small amount of DBNPA injection could have serious impact. In a batch OUR measurement test, Pabitra [12] injected aminosidine, a widely used aminoglycoside antibiotic, at 20, 40, and 60 mg/L, and found the inhibition rate 44.8, 45.9, and 53.8%, respectively. When cetrimide, which is used as an antiseptic or detergent, was injected at 10, 15, and 20 mg/L in the OUR measurement test, the inhibition rate turned out 33.2, 22.7, and 58%, each. Studies on the effects of toxic substances besides disinfectants on micro-organism substrates were also reviewed.

Herrera et al. [13] evaluated nitrification removal efficiency based on IC_{50} (half maximal inhibitory concentration) to identify the toxic effect of fluorine injection on micro-organisms. As a result, the inhibition rate reached 50% at a fluorine concentration of 148.8 mg/L.

This study also found that along with higher concentrations of injected DBNPA, the concentrations of substrates and NH_3-N increased while the SOUR values decreased. Thus, DBNPA injection into the bioreactor may affect micro-organism activity. In addition, the SOUR values obtained at various DBNPA concentrations may possibly be used as an indirect indicator of DBNPA effect on micro-organism activity.

3.5. Fluctuation in PSs at various DBNPA concentrations

The characteristics of PSs at different DBNPA concentrations in the reactor are shown in Table 9. With no DBNPA injection, PSs were found to be at 4.484, 4.406, 4.563, 4.641, 4.563, 4.875, 5.656, 5.734, 5.656, 5.813, and 5.891 mg/L, respectively, for different lengths of reaction time. When DBNPA was injected at the highest concentration (5.0 mg/L), on the other

Table 8
SOUR data and inhibition value with injection of DBNPA

Mode		SOUR[a] data			Inhibition value[b]		
		Min.	Max.	Ave.	Min.	Max.	Ave.
Mode 1	0, control	63.0	79.8	71.2	–	–	–
Mode 2	1.0	57.0	70.3	63.1	0.8	24.0	11.0
Mode 3	1.5	53.1	63.6	58.6	2.4	24.8	17.3
Mode 4	2.0	45.8	63.7	55.5	6.3	34.0	21.7
Mode 5	3.0	38.0	48.5	40.9	32.4	47.2	42.3
Mode 6	5.0	31.6	44.6	37.6	31.9	60.4	46.5

[a]SOUR: mg O_2/g MLVSS h = dO_2/dt/MLSS.
[b]Inhibition value: 100 – [100 × (SOUR at the tested conc./SOUR of the control)], %.

Table 9
Results of PSs measurement with concentrations of DBNPA

Item		DBNPA injection concentrations (mg/L)[a] and react time (min)[b]										
		0[b]	5	10	15	30	60	120	180	240	300	360
0[a]	PSs ave. conc. (mg/L)	4.484	4.406	4.563	4.641	4.563	4.875	5.656	5.734	5.656	5.813	5.891
1.0		4.424	6.828	7.063	8.547	8.859	9.094	10.34	11.20	11.28	11.05	11.13
1.5		4.215	6.984	6.906	7.766	8.313	9.875	14.33	15.81	16.05	15.66	15.03
2.0		4.324	9.250	8.781	9.250	10.66	11.36	15.73	15.27	15.03	15.81	17.38
3.0		4.214	7.297	5.891	6.594	8.313	9.406	14.02	16.13	19.95	20.42	21.05
5.0		4.235	6.672	7.453	8.313	8.781	10.11	16.91	20.34	21.44	22.69	23.55

[a]DBNPA injection concentrations.
[b]Sampling time: 0, 5, 10, 15, 30, 60, 120, 180, 240, 300, 360 min.

hand, PSs were each measured to be 4.235, 6.672, 7.453, 8.313, 8.781, 10.11, 16.91, 20.34, 21.44, 22.69, and 23.55 mg/L. The leached PSs concentrations increased at a higher DBNPA concentration or for a longer reaction time. This helped directly check that DBNPA has toxic effect on micro-organisms. It is also assumed that DBNPA affects substrate and nitrogen removal efficiency by destroying PSs. Morton et al. [14] found that when NOB went into biological treatment facilities, the first reaction came from PSs which exist on the outer walls of micro-organism cells. Xu et al. [15] reported that when quaternary ammonium chloride, which is used as a disinfectant, was reacted with micro-organisms, it was absorbed to PSs while the specific growth rates of micro-organisms dropped.

Substrate and nitrogen removal efficiency were comprehensively evaluated and the results showed that the removal efficiency decreased when more than 2.0 mg/L of DBNPA was injected. Therefore, an optimum dosing amount to minimize the effect on micro-organism is considered below 2.0 mg/L.

4. Conclusion

This study was conducted to identify correlations that DBNPA-injected bioreactor has with substrate and nitrogen removal and micro-organism activity, as well as the effects of DBNPA on micro-organisms. To this end, the characteristics of substrate and nitrogen removal at different DBNPA concentrations were analyzed and SOUR values were measured. In addition, the degree of cell destruction and domination of micro-organisms at different DBNPA concentrations were directly evaluated by isolating and identifying bacteria as well as analyzing PSs. This study drew the following conclusion:

(1) As DBNPA was injected at a higher concentration, $TCOD_{Cr}$ and NH_3-N effluent concentrations were found rising. This indicates that the biocide contained in ROC may affect substrate and nitrogen removal efficiency in the bioreactor. It is considered that the $TCOD_{Cr}$ removal efficiency decreased with the injection of DBNPA, because DBNPA, as a disinfectant, affected cell walls and PSs.

(2) The effect of DBNPA injection on PSs was analyzed: the more the DBNPA was injected or the longer the reaction time lasted, the higher the leached PSs concentrations became. This suggests that DBNPA has direct impact on micro-organisms, thereby affecting biological treatment efficiency.

(3) As with the link between DBNPA injection and SOUR values, it was found that SOUR values decreased at higher DBNPA concentrations. In the reactor, DBNPA reduced substrate and nitrogen removal efficiency and affected micro-organism growth. The reason for DBNPA effect on substrate removal was determined through the SOUR measurement, an indirect indicator of micro-organism activity. The fluctuations in SOUR values along with various DBNPA concentrations may possibly be used as an indicator to evaluate effects of the biocide contained in ROC on micro-organism activity objectively. DBNPA also directly affects micro-organism cell walls and PSs, and is considered to reduce substrate and nitrogen removal efficiency.

(4) It was revealed that even a very small amount of DBNPA injection could seriously affect micro-organism cell walls. Thus, before selecting the type of biocide for adjusting

bio-fouling, it may be necessary to consider biocide impact on membranes in order to minimize the effect of DBNPA included in the biocide on micro-organisms in the bioreactor. The selection of biocide should also come after thorough analyses and tests of possible biocide effects on biological treatment in advance.

(5) This study focused on how biological treatment and micro-organisms are affected by DBNPA, which is one of the various non-oxidizing substances typically included in biocides. Further studies may need to investigate the effects of other NOBs and closely look into their impact on not only PSs but also micro-organisms including protein.

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