

Inhibitory effect of 2,4-dichlorophenol on nitrogen removal in a sequencing batch reactor

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Abstract—We examined the inhibitory effect of 2,4-dichlorophenol (2,4-DCP) on nitrogen removal in the sequencing batch reactor (SBR) system. The reactor was operated with FILL, REACT (nitrification), SETTLE, DRAW and IDLE phases in the duration ratio of 2 : 12 (9 : 3) : 1 : 1 : 8 for a 24 h cycle time. The deterioration of 2,4-DCP removal efficiency from 100 to 41% was observed when the influent concentration of 2,4-DCP was increased to 30 mg/L. The residual 2,4-DCP remaining in the mixed liquor was found to inhibit the nitrification process, resulting in the decrease of nitrogen removal efficiency to 25 %. For kinetic study, the result showed that the experimental data of ammoniacal nitrogen (AN) removal at every stage fitted well to the first-order kinetics equation with high R^2 values. The rate constant of AN removal, k_{AN} , decreased with increasing influent concentration of 2,4-DCP, from 0.053 to 0.0006/min when 2,4-DCP concentration increased from 0 to 30 mg/L, respectively. However, the observed gradual recovering of AN removal with respect to the removal efficiency and kinetics during the recovery stage indicated that the inhibitory effect of 2,4-DCP on the nitrification process was reversible.

Key words: Inhibitory Effect, 2,4-Dichlorophenol, Nitrogen Removal, Sequencing Batch Reactor, Nitrification

INTRODUCTION

The presence of inorganic nitrogenous such as ammonium ions (NH_4^+) from fertilizers for land and agricultural development and untreated sewage from poultry farms, septic tanks and factories can adversely pollute the quality of the receiving water. Significant pollution concerns related to the presence of these wastes include dissolved oxygen depletion, toxicity, eutrophication and methemoglobinemia [1]. A sequencing batch reactor (SBR) system is widely used to achieve nitrogen removal due to its well reported advantages [1-7] and a SBR system is usually operated with five sequential phases of FILL, REACT, SETTLE, DRAW and IDLE [8].

Nitrification and denitrification are two principal processes for the nitrogen removal in SBR [1,9]. Nitrification, which consists of two steps, is carried out in aerobic condition. The first step involves the oxidation of ammoniacal nitrogen (AN) by *Nitrosomonas* spp. to nitrite nitrogen (NO_2^- -N). It is followed by the second step of oxidation of NO_2^- -N to nitrate nitrogen (NO_3^- -N) by *Nitrobacter* spp. [10]. For the denitrification, the process is accomplished in the presence of heterotrophic bacterium in an anoxic condition with dissolved oxygen (DO) lower than 1.0 mg/L or 2% saturation [11]. During the denitrification process, a carbon source is added to act as electron donor [12] to reduce the products of the former nitrification process, namely NO_3^- -N or NO_2^- -N to gaseous nitrogen (N_2) and released to the atmosphere [13].

The presence of phenolic compounds is not only burdening the treatment processes which remove them from the raw wastewaters but also preventing the nutrients' removals such as nitrogen and phosphorus, notably in the biological wastewaters treatment processes [14-16]. Uygur and Kargi [17] determined that the removal

efficiencies of NH_4 -N and PO_4 -P of 90 and 65%, respectively, were achieved at the phenol concentration of only 400 mg/L and these nutrient removals were adversely affected by phenol concentration above the stipulated value. Among the phenolic compounds, chlorophenols containing wastewaters are generated from various industries such as petrochemical, oil refinery, wood preservatives, herbicides, insecticides, fungicides, solvents, paints and in the glue and paper industry [18,19]. Sahinkaya and Dilek [20] showed that the unacclimated culture in biological wastewater treatment process was affected adversely, even at low concentrations of 2,4-dichlorophenol (2,4-DCP). Nalbul and Alkan [18] confirmed that the 2,4-DCP exerted higher inhibitory effect on biomass through noncompetitive inhibition force. Kargi et al. [21] proved that the percent of COD, 2,4-DCP and toxicity removals decreased with increasing feed 2,4-DCP concentrations above 150 mg/L, which led to the decrease of biomass concentration. Eker and Kargi [22] demonstrated that percent COD removal decreased with increasing feed 2,4-DCP content was due to natural selection of 2,4-DCP degrading organisms and the potential inhibitory effects of 2,4-DCP degradation products on COD removing microorganisms.

There are a number of studies on biodegradation of phenolic laden wastewaters and nutrients removals through various biological treatment systems [23-27]. However, the information regarding the inhibitory effects of 2,4-DCP on biological nutrients removals in the SBR system is not much available. Thus, the objective of this study is to investigate the effect of 2,4-DCP on the nitrogen removal by SBR system.

EXPERIMENTAL SECTION

1. Experimental Set Up

A 10 L working volume Plexiglas reactor with a dimension of 30 cm × 20 cm × 30 cm (L × W × H) was constructed and operated with

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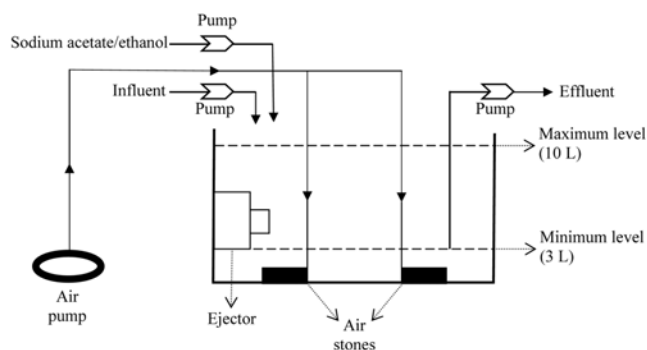


Fig. 1. Schematic diagram of the SBR system.

FILL, REACT (nitrification: denitrification), SETTLE, DRAW and IDLE at the ratio 2: 12 (9: 3): 1: 1: 8 for a cycle time of 24 h, without controlling sludge age. The reactor was equipped with peristaltic pumps for influent filling and effluent drawing, respectively, an ejector for agitation during the REACT period and two air stones for aeration during the nitrification period. The aeration system was halted during the denitrification period to create an anoxic environment within the reactor mixture. A total of 7 L of influent was filled during the FILL period and same volume of treated effluent was drawn during the DRAW period for every cycle throughout the operation period. A schematic diagram of the SBR system is shown in Fig. 1.

The reactor was inoculated with activated sludge collected from a local municipal sewage treatment plant and acclimatized to the feed solution containing carbon sources and nutrients with the following compositions (concentration in mg/L): peptone (188), sucrose (563), $(\text{NH}_4)_2\text{SO}_4$ (212), KH_2PO_4 (32), K_2HPO_4 (160), MgSO_4 (49), NaHCO_3 (354), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (18.8), CaCl_2 (40), which yielded the AN concentration of approximately 48 mg/L. Two grams of sodium acetate solution were added to the reactor at the beginning of the aerobic period as an addition buffer to maintain the pH within the range of 7.4-7.6. As soon as the aeration stopped, adequate volume of 4.6% v/v ethanol solution was added which functioned as electron donor to reduce NO_3^- -N and NO_2^- -N to N_2 gas.

2. Stages of Operation

The activated sludge was allowed to acclimatize to the feed solution without the addition of 2,4-DCP at stage I. After the SBR had attained steady state, increasing concentrations of 2,4-DCP were added during stages II to IV as shown in Table 1. The addition of 2,4-DCP was stopped at the recovery stage (Stage V).

3. SBR Performance Assessment

At various stages, the treated effluent was collected during the draw period and analyzed for nitrogen species (AN, NO_3^- -N and

Table 1. Operational stages of the reactor in increasing 2,4-DCP concentration

Stage	Period (day)	Influent of 2,4-DCP concentration (mg/L)
I	1-41	0.0
II	42-61	5.0
III	62-94	15.0
IV	95-140	30.0
V	141-222	0.0

NO_2^- -N) and 2,4-DCP concentrations, whereas the mixed liquor suspended solids (MLSS) concentration and sludge volume index (SVI). In addition, the concentration profiles of DO, AN, NO_2^- -N as well as NO_3^- -N were investigated at the steady MLSS concentration state from time to time during the REACT period to evaluate the performance of the reactor. The concentration of AN was distilled into boric acid and titrated with standard sulfuric acid. The concentration of NO_2^- -N was measured by colorimetric method with a UV-Vis spectrophotometer model Hitachi U-2000 at 543 nm, whereas the concentration of NO_3^- -N was determined from the reduction of oxidized nitrogen (NO_3^- -N and NO_2^- -N) concentrations by Devarda's alloy followed by subtracting with NO_2^- -N concentration. The 2,4-DCP concentration was determined by 4-aminoantipyrine method. All the samples were filtered with Whatman 1 filter paper, and the filtrates were used for the aforementioned analysis following Standard Methods [28]. The MLSS concentration and SVI, which measured the concentration of suspended solid in the SBR and settleability, respectively, were determined following standard methods [28]. The DO concentration was measured with DO meter model YSI 550A.

4. Kinetic Study

At each stage, sampling of the mixed liquor was carried out during the nitrification period at the time interval of 20 min for the determination of AN concentration. The rate of AN removal was assumed to follow the first-order kinetics and the data obtained were used to calculate the first-order rate constant of AN removal (k_{AN}) for each stage.

RESULTS AND DISCUSSION

1. Performance of the Reactor

1-1. MLSS and SVI

The MLSS concentration and SVI values during the operational stages of the reactor are shown in Fig. 2. The MLSS concentration during the acclimatization period (stage I) was around 6,700 mg/L and increased to approximately 7,700 and 9,000 mg/L at the end of stages II and III, respectively. It was reported that the average diameter of sludge flocs increased at 2,4-DCP concentration of 5 mg/L, or higher [29] because the bacterium tend to aggregate to protect themselves when they are exposed to a toxic condition [30-34]. Moreover, a higher organic loading rate facilitated the formation of larger granules [35,36]. Thus, the increase of 2,4-DCP organic loading in

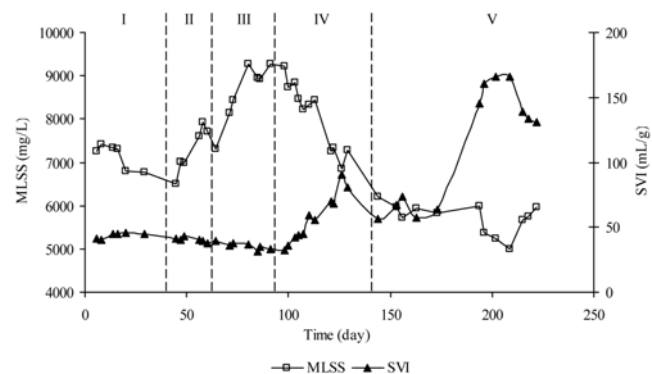


Fig. 2. MLSS concentrations and SVI values in SBR system.

the influent at stages II and III favored the formation of larger aggregate and granules. These led to better settleability, which can be observed in the lower SVI values of 37 and 33 mL/g at the end of stages II and III, respectively. Consequently, only a small amount of activated sludge was drawn out during DRAW phase, resulting in more activated sludge growth accumulating in the reactor. However, further increase in the concentration of 2,4-DCP in stage IV resulted in an increase of SVI value to around 90 mL/g towards the end of this stage. The deterioration of the settleability was attributed to excessive organic loading. This led to the formation of a light and fluffy sludge that exhibited little compacting during settling, and thus the MLSS concentration decreased to around 6,800 mg/L. During the recovery stage, the SVI value was further increased to above 150 mL/g from day-196 to day-209, indicating poor settling due to bulking, and therefore led to excessive drawing of activated sludge during the DRAW phase and decreasing MLSS concentration to approximately 5,000 mg/L at day-209. At the end of this stage, the MLSS concentration increased to approximately 6,000 mg/L, which approximates the value in stage I, indicating the MLSS concentration of the activated sludge was gradually recovered from being inhibited by 2,4-DCP.

1-2. Removal of 2,4-Dichlorophenol (2,4-DCP)

The biosorption of 2,4-DCP onto dried biomass was found to be negligible, based on a preliminary test. Thus, the removal of 2,4-DCP in the SBR was achieved by biodegradation by activated sludge. The effluent concentrations of 2,4-DCP and the removal efficiencies in stages II to IV are stipulated in Fig. 3. When the concentration of 2,4-DCP added was 5 mg/L in stage II, 100% of removal efficiency was observed throughout this stage. This was due to the low concentration of 2,4-DCP and the activated sludge was able to tolerate and remove it completely. However, when the concentration of 2,4-DCP was increased to 15 and 30 mg/L in stages III and IV, the removal efficiency dropped to about 91 and 41%, respectively, at the end of each stage. The reason was that the activated sludge was unacclimated to high concentration of 2,4-DCP and thus less resistant to the inhibitory effect of 2,4-DCP. Sahinkaya and Dilek [37] reported that 2,4-DCP began to inhibit its own degradation at 25 mg/L in the batch reactor experiment.

1-3. Removal of Nitrogen Species

Fig. 4 shows the concentration of AN, NO_3^- -N and NO_2^- -N in the effluent and nitrogen removal efficiency. It was found that the NO_3^- -N and NO_2^- -N concentrations in the effluent were less than 1.5 and 0 mg/L, respectively, throughout the operational period.

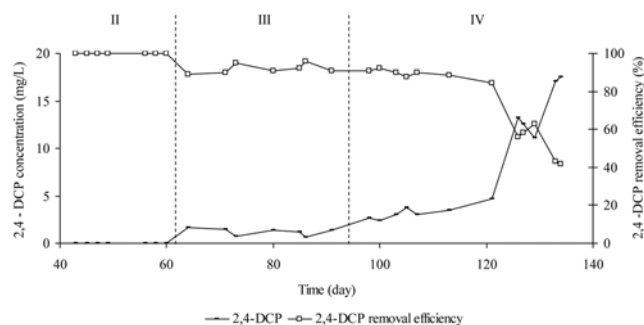


Fig. 3. Concentrations and removal efficiencies of 2,4-DCP during different operational stages in SBR system.

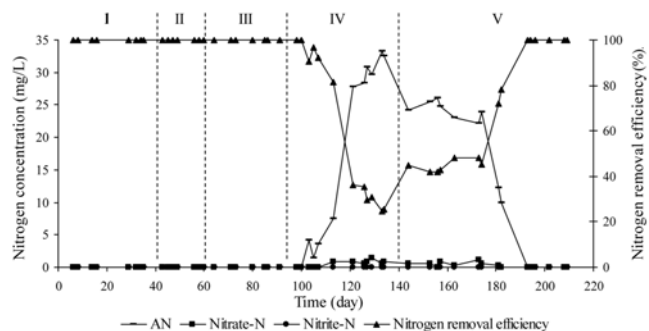


Fig. 4. Nitrogen species concentrations and nitrogen removal efficiency in SBR system.

Nitrogen removal efficiency of 100% was observed from stage I until stage III. The high sludge age in the reactor had partially overcome the adverse effects of 2,4-DCP and resulted in high nitrogen removal efficiency. This was in parallel with the result obtained by Kargi et al. [15], who reported that the percent of nutrient removals increased with increasing sludge age in the presence of the inhibitory compound of 4-CP. When the influent concentration of 2,4-DCP was 30 mg/L in stage IV, the nitrogen removal efficiency decreased to approximately 25% at the end of this stage. The presence of the residual 2,4-DCP in the mixed liquor, which was caused by incomplete biodegradation, would lead to the inhibition of the activity of the nitrifying bacteria. However, the activity of the nitrifying bacteria was observed to recover gradually when the addition of 2,4-DCP was stopped during the recovery stage. Complete nitrogen removal was achieved after 52 days in this stage. This can be deduced that the inhibitory effect of 2,4-DCP was reversible for the nitrification process, although Nalbur and Alkan [18] had documented that the 2,4-DCP exhibited noncompetitive inhibition in the SBR system.

2. Profile Studies of Nitrogen Species

The inhibitory effect of 2,4-DCP on the nitrogen species removal could not be revealed clearly, especially for the first three stages as the nitrogen removal was almost completed at the end of REACT period. Thus, profile studies of nitrogen species were conducted to gain more understanding on the removal of nitrogen species in the presence of various concentrations of 2,4-DCP.

The concentration profiles of AN, NO_3^- -N, NO_2^- -N and DO are shown in Fig. 5 for stages I to V. For all the profile studies, it was found that the oxidation of AN by *Nitrosomonas* spp. to NO_2^- -N could only be observed after the end of the lag phase. Simultaneous nitrification and denitrification processes (SND) would reduce the AN concentration [38], and it may occur during the lag phase period with the presence of insufficient DO concentration (DO less than 1.0 mg/L). The production of NO_3^- -N and NO_2^- -N was not observed during SND.

In stage I, in the absence of the 2,4-DCP, the oxidation of NO_2^- -N to NO_3^- -N by *Nitrobacter* spp. can be observed from the decrease of NO_2^- -N concentration from the maximum point at min-180, together with the increase of NO_3^- -N concentration. A plateau value of the concentration NO_3^- -N and coupled with the concentration of NO_2^- -N at 0 mg/L indicates the completion of nitrification process.

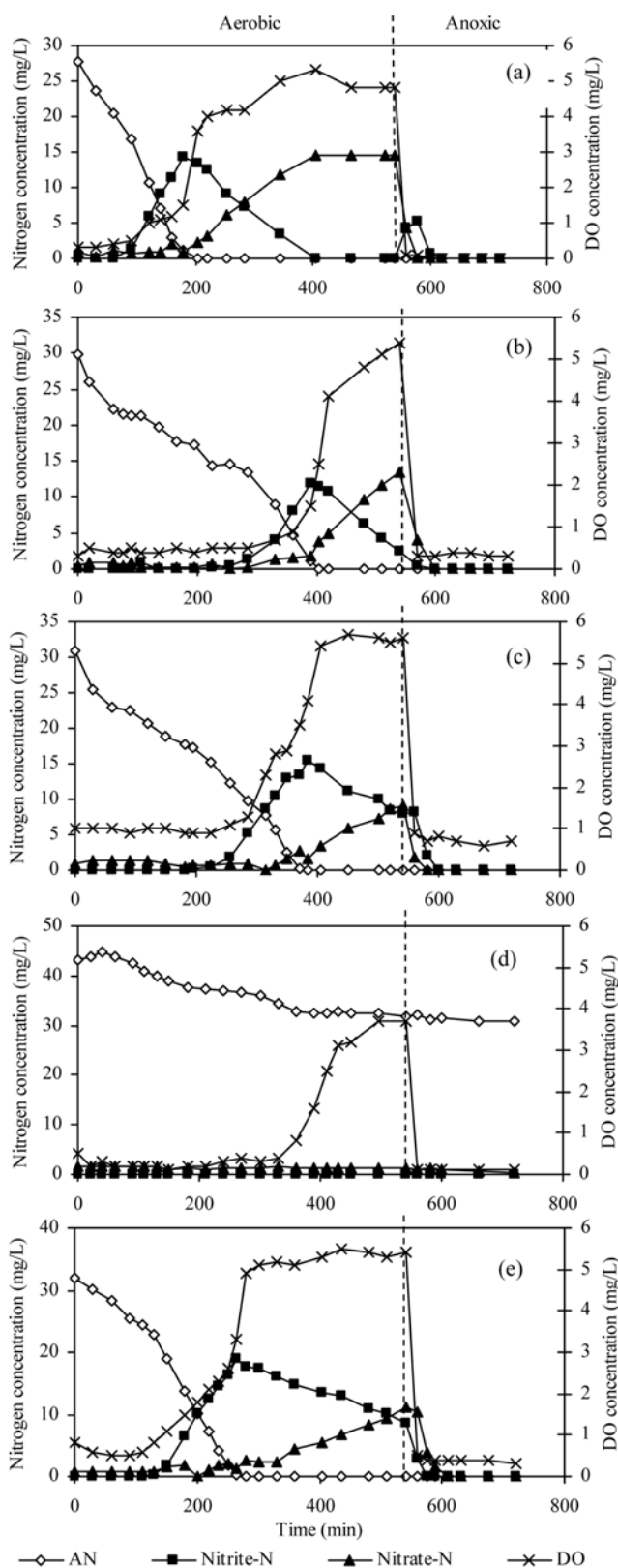


Fig. 5. The concentration profiles of nitrogen species and DO of stage I (a), stage II (b), stage III (c), stage IV (d), and stage V (e) in SBR system.

Inhibition of nitrification was observed by the longer lag phase in stage II and III in the presence of 2,4-DCP with the concentra-

Table 2. The first-order rate constants of AN removal, k_{AN} , for various operational stages in the reactor

Stage	$k_{AN} \times 10^2$ (1/min)	R^2
I	5.32 ± 0.36	0.92
II	2.16 ± 0.07	0.94
III	1.78 ± 0.32	0.93
IV	0.06 ± 0.02	0.94
V	2.58 ± 0.27	0.91

tion of 5 and 15 mg/L, respectively. No NO_3^- -N plateau level was observed in these stages. In addition, oxidation of NO_2^- -N to NO_3^- -N was found to be slower in stage III than that in stage II. This indicated that the inhibitory effect of 2,4-DCP was more significant on the activity of *Nitrobacter* spp. compared to *Nitrosomonas* spp. *Nitrosomonas* spp. was reported to be able to reproduce faster than *Nitrobacter* spp. due to its higher energy gained during the oxidation process [1]. This was also supported the findings by Yang and Alleman [39] that the formation of NO_3^- -N is normally regarded as the rate determining step in nitrification process as the *Nitrobacter* spp. is weak in oxidizing NO_2^- -N to NO_3^- -N.

From Fig. 5(d), there is no observable peak of NO_2^- -N or NO_3^- -N, indicating the absence of nitrification process. This could be explained by the complete inhibited activity of the nitrifying bacteria in the presence of the high concentration of 2,4-DCP at 30 mg/L. Gradual decrease of AN concentration was not due to assimilation process as the MLSS concentration was decreasing during this stage (Fig. 2), but was due to high maintenance requirements of the organism at inhibitory 2,4-DCP concentration [21].

During the recovery stage, the nitrifying bacteria was gradually recovered as evidenced by the reducing of the AN concentration to 0 mg/L at min-280. However, the concentration of NO_3^- -N produced from the oxidation of NO_2^- -N did not reach a plateau value, indicating that the activity of *Nitrobacter* spp. was not fully recovered.

3. Kinetics of AN Removal

The k_{AN} values for all the steady stages are shown in Table 2, with high R^2 values ($R^2 > 0.91$). The k_{AN} values decreased with increasing 2,4-DCP concentration. This is due to the higher inhibitory effect on the nitrifying bacteria at higher concentration of 2,4-DCP as described earlier. When 30 mg/L of 2,4-DCP was spiked in stage IV, k_{AN} was drastically reduced by 99% to only 0.0006/min compared to stage I. The tremendous drop of k_{AN} value was attributed to the absence of the nitrification process and the gradual removal of AN was plausibly being utilized to repair or replace the enzyme damaged by the inhibitory compound of 2,4-DCP. In the recovery stage, the k_{AN} value increased to 0.0258/min, which was nearly half of the k_{AN} value in the stage I. Extending the recovery period would further increase the k_{AN} value to the approximate value of that in stage I.

CONCLUSIONS

Based on the results obtained, it was observed that without the addition of 2,4-DCP, complete nitrification and nitrogen removal can be achieved. At stages II and III, the inhibitory effect of 2,4-DCP on nitrification process was observed in the profile studies despite the 100% nitrogen removal at the end of the REACT period. The performance of the reactor was further decreased at 30 mg/L of

influent 2,4-DCP, as observed in the decreased 2,4-DCP and nitrogen removal efficiencies of 41 and 25%, respectively. At this stage, the nitrification process totally inhibited by the residual 2,4-DCP in the mixed liquor and the reduction of AN could be explained by its utilization for cell maintenance. The inhibitory effect of 2,4-DCP on nitrogen removal can also be observed in the kinetics of AN removal. The rate constant of AN removal, k_{AN} , decreased with increasing concentration of 2,4-DCP. However, it is deduced that the inhibitory effect of 2,4-DCP on the activity of nitrifying bacteria was reversible, with increasing nitrogen removal efficiency and k_{AN} value during the recovery stage in which the addition of 2,4-DCP was ceased.

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