Evaluation of Thiosulfate-Based Autotrophic and Mixotrophic Denitrification Performances under Different Operational Conditions

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ABSTRACT

The aim of this study was to investigate the factors affecting the autotrophic and mixotrophic denitrification performances in sequencing batch reactor (SBR). Findings obtained from this study will shed light on the full-scale applicability of the autotrophic and mixotrophic denitrification processes in carbon deficient wastewaters. In this study, the effect of cycle time, varying concentrations of electron donor source and the addition of external organic carbon source was evaluated by sulfate, nitrate, nitrite, ORP and inorganic carbon parameters. Firstly, SBR was operated with thiosulfate (S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}) based autotrophic denitrification process at different cycle times (8h-48-2h). Further, autotrophic denitrification process was optimized the under varying S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} ratios (1.5-1.25-1.0) at the optimum cycle time of 2h. The maximum nitrate removal efficiency of 88% was accomplished at operational conditions containing 2h cycle time and 1.5 S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} ratio. In the rest of the study, the impact of increasing C/N ratio by methanol supplementation (0.35-0.7-1.05) was evaluated on mixotrophic denitrification performance. It was observed that nitrate was completely consumed at the C/N ratio of 1.05 and nitrate removal rate improved with increasing methanol supplementation as organic carbon source.

Keywords: Cycle time, Mixotrophic denitrification, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} ratio, Autotrophic denitrification, Thiosulfate

ÖZET

Bu çalışmanın amacı ototrofik ve miksotrofik denitrifikasyon proses performanslarının etkileyen faktörlerin arısal kesikli reaktörde araştırılmasıdır. Çalışma verileri ototrofik denitrifikasyon prosesinin karbon içeriği 다르ılık atıksular için gerçek olarak uygulanabilirliğecek tutaçaktır. Ototrofik denitrifikasyon procesinde farklı döngü süresi, elektron vericive organik karbon ilavesinin nitrat giderim performansına etkisi sütuf, nitrat, nitrit, ORP ve inorganik karbon parametreleri ile değerlendirilmiştir. Çalışmanın ilk aşamasında farklı döngü süresi olan (8saat-48-2saat) tiyosülfat (S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} bazlı ototrofik denitrifikasyon prosesine etkiği araştırılmıştır. Daha sonra 2 saatlik optimal döngü süresi içinde farklı S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} oranları (1.5-1.25-1) ile ototrofik denitrifikasyon prosesinin optimizasyon çalışmaları yürütülmüştür. Elde edilen çalışma bulguları göz önünde bulundurulduğunda maksimum %84 nitrat giderim verimi 2 saatlik döngü süresi ve 1.5 S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}NO\textsubscript{3}\textsuperscript{-} oran ile işletilen işletim koşullarından elde edilmiştir. Çalışmanın son aşamasında ise farklı C/N (0.35-0.70-1.05) oranlarının miksotrofik denitrifikasiyon performansına etkisi incelenmiştir ve C/N oranının 1.05 olduğu işletim koşulında nitratın tamamı tüketilerek organik madde kaynağı olarak metanolun artmasıyla nitrat giderim hızının arttığı gözlenmiştir.

Keywords: Döngü süresi, Miksotrofik denitrifikasiyon, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} oran, Ototrofik denitrifikasiyon, Tiyosülfat

1. INTRODUCTION

Nitrogen compounds are the most common pollutants in groundwater. Serious aquatic problems will occur if these compounds are discharged into the environment, including the eutrophication of rivers, the degradation of water sources, and hazards to human health (Guo et al., 2017). Therefore, nitrogen removal from wastewater is one of the most important issues that cause concern about water pollution control. Biological processes are generally used for nitrogen removal from wastewater. Nowadays, nitrification and denitrification are the most commonly used biological nitrogen removal processes. However, the nitrification process usually requires high oxygen concentration and high sludge age while the denitrification process is often need for external carbon source. Hence, reducing the operating cost of the process and increasing the efficiency of nitrogen removal system is very important in terms of sustainability of this process. Recent studies have been carried out on innovative solutions that can provide nitrogen removal and autotrophic denitrification may be a useful approach for nitrogen removal from the nitrate-contaminated water.
Autotrophic denitrification process is accomplished by a group of autotrophic denitrifying bacteria which utilize the hydrogen, iron, thiosulfate or sulfur compounds as energy source, with inorganic carbon such as carbon dioxide or bicarbonate as carbon source (Beller, 2005; Manconi et al., 2007; Sierra-Alvarez et al. 2007). The most important advantages of this autotrophic denitrification over conventional heterotrophic denitrification processes are that no external organic material is needed, low sludge formation, absence of pathogenic organic byproducts and chlorinated organic substances do not form during chlorine disinfection of treated wastewater (Sahinkaya and Dursun, 2012). However, problems resulted from the low growth rates of microorganisms responsible for autotrophic denitrification and the toxic effect of the resulting sulfate according to the carbon source, form the disadvantages of this process in real-scale applications (Sahinkaya and Kilic, 2014). The combination of heterotrophic and autotrophic denitrification processes (mixotrophic denitrification) can be a good strategy in the treatment of wastewaters containing high nitrate content due to the minimization of the output sulfate concentration, limitation of external organic carbon requirement and use of alkalinity produced by heterotrophic denitrification in autotrophic denitrification process (control of alkalinity requirement) (Oh et al., 2001; Soares, 2002; Moon et al., 2008; Liu et al., 2009; Sahinkaya et al., 2011). The studies on the mixotrophic process in which autotrophic and heterotrophic denitrification processes are combined are very limited at the present time. Oh et al. (2011) have studied for about 1 year in sulfur-based autotrophic, heterotrophic and mixotrophic conditions in order to investigate the effect of organic matter using methanol and leachate. It has been observed that the formation of sulfate based on reaction time was decreased in low hydraulic retention times in autotrophic denitrification process. Denitrification performance was achieved at 80-90% even when the methanol dose was below the theoretical level and no dissolved organic carbon was found at the effluent. The mixotrophic denitrification process was performed by the addition of organic matter and alkalinity produced in heterotrophic denitrification neutralized the acidity produced during autotrophic denitrification. In addition, organic matter supplementation under mixotrophic conditions reduced sulfate production compared to autotrophic conditions.

The purpose of this study was to investigate the factors affecting the autotrophic and mixotrophic denitrification process performances in a SBR. Initially, thiosulfate-based autotrophic denitrification process was optimized at operational conditions including different cycle times and influent S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}/NO\textsubscript{3}\textsuperscript{-} ratios. Further, the optimum C/N ratio at which the mixotrophic conditions were achieved was determined. The purpose was to improve the biological nitrogen removal plants and decrease the operating costs with the obtained data.

2. MATERIALS AND METHODS

2.1. Microbial Culture and Synthetic Wastewater

The inoculum culture was inoculated with heterotrophic denitrification sludge taken from a full-scale domestic wastewater treatment plant located at Kayseri, Turkey. The reactor was operated for approximately two months for adaptation to synthetic wastewater composition and operating conditions of sludge and thiosulfate-based autotrophic denitrification microorganisms have been enriched. In the mixotrophic denitrification process, organic and inorganic carbon-energy sources were added to the system to enrich the mixotrophic microorganisms that can exhibit metabolic activity under different environmental conditions.

Thiosulfate was used as the electron donor source of the autotrophic denitrification process while methanol was used as an organic carbon and energy source in mixotrophic denitrification. Additionally, sodium bicarbonate was used as carbon source for autotrophic microorganisms, corresponding to influent inorganic carbon of 110 mgC/L. The SBR was operated in batch mode and fed with synthetic wastewater containing per liter of distilled water: 78.6 mg NaNO\textsubscript{3}, 162.5-81.25 mg Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}, 10-30 mg CH\textsubscript{3}OH, 56 mg K\textsubscript{2}HPO\textsubscript{4}, 50 mg yeast extract and 11 mg ascorbic acid. The synthetic wastewater was prepared daily. The reactor was operated during about 30 days before each study period and experimental data were collected when reactors reached steady conditions.

2.2. SBR Design and Operation

Laboratory scale Modular BioFlo 115 Fermenter (New Brunswick, BiofloCelligen 115, ABD) was used for the treatability studies of synthetic wastewater containing nitrate and thiosulfate. The working volume of the reactor was selected as 4L. The system was mixed by a single shaft impeller system at a speed of 250 rpm. The pH of the reactor liquid was adjusted at 7.75 using 0.1 N H\textsubscript{2}SO\textsubscript{4} and 0.1 N NaOH. The reactor temperature was maintained at 30°C with a water jacket around the reactor. Sludge age was controlled by withdrawing a certain volume of biomass/liquid mixture at each SBR cycle. The nitrogen gas was passed through the reactor to remove possible oxygen leaks from the outside and provide anoxic environmental conditions. The performance of the SBR was investigated in two parts containing nine different periods (Table 1).
Table 1. Experimental plan

<table>
<thead>
<tr>
<th>Parts</th>
<th>Period</th>
<th>Cycle time (hour)</th>
<th>Carbon source</th>
<th>$S_2O_3^{2-}$ (mg/L)</th>
<th>$NO_3^-$ (mg/L)</th>
<th>$S_2O_3^{2-}$/NO$_3^-$ ratio</th>
<th>Organic carbon (mgCOD/L)</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I: Optimization of thiosulfate-based autotrophic denitrification process</td>
<td>I</td>
<td>8</td>
<td>NaHCO$_3$</td>
<td>100</td>
<td>50</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>II</td>
<td>4</td>
<td>NaHCO$_3$</td>
<td>100</td>
<td>50</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>III</td>
<td>2</td>
<td>NaHCO$_3$</td>
<td>100</td>
<td>50</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>2</td>
<td>NaHCO$_3$</td>
<td>75</td>
<td>50</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>2</td>
<td>NaHCO$_3$</td>
<td>62.5</td>
<td>50</td>
<td>1.25</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>VI</td>
<td>2</td>
<td>NaHCO$_3$</td>
<td>50</td>
<td>50</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Part II: Effect of C/N ratio on mixotrophic denitrification performance</td>
<td>VII</td>
<td>2</td>
<td>CH$_3$OH, NaHCO$_3$</td>
<td>75</td>
<td>50</td>
<td>1.5</td>
<td>15</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>VIII</td>
<td>2</td>
<td>CH$_3$OH, NaHCO$_3$</td>
<td>75</td>
<td>50</td>
<td>1.5</td>
<td>30</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>IX</td>
<td>2</td>
<td>CH$_3$OH, NaHCO$_3$</td>
<td>75</td>
<td>50</td>
<td>1.5</td>
<td>45</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Optimization of thiosulfate-based autotrophic denitrification process was performed in the first study part, while the last part mainly related to the nitrate reduction studies under mixotrophic conditions. In the three first periods of part I, cycle time was decreased stepwise from 8h to 4h, and 2h. Then, the effect of different $S_2O_3^{2-}$/NO$_3^-$ ratios on autotrophic denitrification performance was investigated in decreasing thiosulfate concentrations at the constant nitrate concentration during the 2 hour optimum cycle time (period IV, V and VI). In the last part of the study (period VII, VIII and IX), methanol was included to the feed solution to provide mixotrophic condition and the optimization of the mixotrophic denitrification process was ensured in operational conditions including different C/N ratios.

2.3. Analysis

Liquid samples were filtered through sterile syringe filter, having pore sizes of 0.45 μm to separate the bacteria from the liquid phase. Nitrate, nitrite, and sulfate were determined by ion chromatography with suppressed conductivity detection using a Dionex ICS-5000 device (Dionex, Sunnydale, CA, USA) equipped with ASRS-300 (4mm) suppressor, IonPac® AG9-HC (4x50mm) guard column and AS9HC (4x250mm) analytical column. The eluent (20 mM methanesulfonic acid, 9 mM sodium carbonate) used in the analyzes was continuously passed through the device at 1 mL/min. Inorganic carbon(IC) concentrations of samples were measured by a TOC-TN analyzer (Shimadzu TOC-VPN, Kyoto, Japan). Changes in oxidation-reduction potential (ORP) and pH parameters during the reaction were observed with ORP (M 300, Mettler Toledo, Greifensee, Switzerland) and pH (Mettler Toledo, Switzerland) probes in the reactor. Total sulfur formation, which is the result of sulfate removal under anoxic environmental conditions Total sulfide was analyzed spectrometrically using a Chebios Optimum-One UV–VIS Spectrometer following the method described by Cord-Ruwisch (1985). All assays were run in triplicate and mean values were presented.

3. RESULT AND DISCUSSION

Optimization studies of autotrophic, mixotrophic denitrification processes under different operational conditions were carried out using SBR in anoxic conditions, and the obtained data were presented and discussed in this section.

3.1. Effect of Different Cycle Times on Autotrophic Denitrification Process

The electron accepetor, electron donor and carbon source were nitrate/nitrite, thiosulfate, bicarbonate in the autotrophic denitrification process used in the study, respectively. This biological nitrate removal occurred by reduction-oxidation...
reactions. The electrons generated by the oxidation of electron donor source react with the nitrate, which acts as the electron acceptor in the system, and thus nitrogen was removed by microorganisms. In the conventional denitrification process, microorganisms are exposed to shock nitrate loads. Therefore, cycle time is an important parameter in terms of the treatability of wastewater, operational feasibility, energy requirements and system performance. High cycle times generally lead to greater investment costs.

In the first three periods of the study (period I, II and III), the impact of different cycle times was initially evaluated on the autotrophic denitrification performance in terms of nitrate, sulfate, ORP and IC profiles (Fig. 1). Cycle time was gradually reduced from 8h to 2h. Nitrate, thiosulfate and IC concentrations were kept constant at 50 mg/L, 100 mg/L and 110 mg/L during this study part, respectively.

![Figure 1](image.png)

**Figure 1.** Effect of different cycle times on autotrophic denitrification process

In Fig. 1A, it was observed that the nitrate removal capacities of microorganisms was not adversely affected by decreasing cycle times and nitrate was completely consumed (100% nitrate removal efficiency) at the end of the reaction time of about 1 hour. A decrease in IC profile was observed during the reaction because the inorganic carbon source was used in cell synthesis. The amount of IC consumed increased with increasing reaction times (Fig. 1B).

The concentration of sulfate formed as a result of the denitrification process in which thiosulfate was used as an electron donor is shown in Fig. 1C. The reduction of the reaction time to 2h resulted in stress conditions for the microorganisms and the effluent sulfate concentration increased to 243 mg/L (period III) while the maximum effluent sulfate concentration was 227 mg/L at the end of the 8h cycle (period I). However, no significant differences in sulfate formation rates were observed during the first two-hour reaction of each period. The steady state of the sulfate concentration at the end of the average reaction time of 2.5 h per 3 periods can be explained by the depletion of the electron donor source (thiosulfate).

Fig. 1D represents the effect of different cycle times on the ORP profile in the autotrophic denitrification process. In the first hours of the anoxic reaction, the ORP values were rapidly reduced to negative values. In this phase, the added thiosulfate was used as electron donor source by microorganisms and nitrate was reduced. It was observed that the bio-oxidation rate of microorganisms decreased and the ORP value increased simultaneously with the depletion of the electron donor source in the system. The low ORP values observed in period I can be explained by an increase in the rate of thiosulfate consumption during the first hours of the reaction. Since the electron donor source was depleted in the first hours of the 8h long reaction time and the microorganisms exposed to the starvation phase for a longer time.

The shortest cycle time of 2h was considered optimal for use in subsequent study periods, due to the complete removal of nitrate and the stabilization of the amount of sulfate formed by the changing cycle time in all operating conditions.
3.2. Effect of Different $S_2O_3^{2-}$ / $NO_3^-$ Ratio on Autotrophic Partial Denitrification Process

This part of the study (period IV, V, VI), the thiosulfate concentration used as the electron donor and energy source was gradually reduced to 75 mg/L to 50 mg/L, while the input nitrate concentration was kept constant at 50 mg/L, corresponding to $S_2O_3^{2-}$/$NO_3^-$ ratio between 1.5 and 1.

With the supplementation of thiosulfate, nitrate is biologically reduced under anaerobic conditions and five electrons are added to nitrate to produce sulfate as shown in the following reactions (Eqs. (1)–(3)) (Manconi et al., 2007; Sierra-Alvarez et al., 2007):

$$ S_2O_3^{2-} + 5H_2O \rightarrow 2SO_4^{2-} + 10H^+ + 8e^- \quad (1) $$

$$ NO_3^- + 6H^+ + 5e^- \rightarrow 0.5N_2 + 3H_2O \quad (2) $$

$$ S_2O_3^{2-} + 1.6NO_3^- + 0.2H_2O \rightarrow SO_4^{2-} + 0.8N_2 + 0.4H^+ \quad (3) $$

According to the above reactions, the stoichiometric $S_2O_3^{2-}$ / $NO_3^-$ ratio required to reduce nitrate to nitrogen gas was 0.62, which was lower than $S_2O_3^{2-}$ / $NO_3^-$ ratios used in this study. The lowest rate used in this study was 1. Because some of the electrons will go to the electron acceptor and some will be used for cell synthesis. The nitrate and IC removal performances with sulfate production profile of SBR system are shown in Fig. 2.

![Figure 2](image_url)

**Figure 2.** Effect of different $S_2O_3^{2-}$ / $NO_3^-$ ratio on autotrophic denitrification process

In the first period (period IV), SBR was operated at $S_2O_3^{2-}$/$NO_3^-$ ratio of 1.5. Nitrate and IC removal efficiencies were about 84.6% and 10%, and sulfate production was 145 mg/L (Fig. 2). The limitation of influent thiosulfate concentration to 62.5mg/L (Period V) and 50mg/L (Period VI), corresponding to $S_2O_3^{2-}$ / $NO_3^-$ ratio to 1.25 and 1, respectively; had a negative effect on nitrate removal efficiency which was approached to 78.9% and 67.2%, respectively (Fig. 2A). Furthermore, the nitrite was not reduced to nitrogen gas and nitrite accumulation was observed in the system due to the limitation of the electron donor source (thiosulfate) with decreasing $S_2O_3^{2-}$/$NO_3^-$ ratio (Fig. 2B). The nitrite accumulation was 17 mg/L at the end of the reaction in effluent and the maximum nitrogen removal efficiency was determined as 49.2% under the operating conditions with the ratio of $S_2O_3^{2-}$/$NO_3^-$ 1.5 (Period IV). Campos et al. (2008) examined the effect of different S/N ratios on specific thiosulfate-based autotrophic denitrification activity. At low nitrate concentrations (S/N ratio 6.67-3.70 g/g), it was stated that the nitrate completely converted to nitrogen gas via nitrite and nitrite accumulation was not observed. Additionally, they emphasized that
thiosulfate was the limiting factor for denitrification and nitrite was observed in the effluent wastewater composition in was operating conditions (S/N ratio 1.16-2.44 g/g) where the S/N ratio was lower than the stoichiometric ratio.

The influent inorganic carbon concentration was kept constant at 110 mg C/L during the study and the IC concentration consumed by microorganisms was not affected by the changing $\text{S}_2\text{O}_3^{2-}/\text{NO}_3^-$ ratio, corresponding to consumed IC amount of average 10% (Fig. 2D). Because autotrophic microorganisms used the available IC to perform their vital activities during the 2h reaction and the reactor was operated with constant microorganism concentration throughout the study.

The ORP profile gives very important information about the biochemical environment that occurs under different operating conditions. The increase in ORP indicates that the tendency of oxidation was very high and the electron acceptor was very strong. In general, high positive ORP values states aerobic phase, while negative values are more representative of anaerobic environmental conditions.

![Figure 3. Effect of different S$_2$O$_3^{2-}$/NO$_3^-$ ratio on ORP profile](image)

The use of electrons generated by the oxidation of thiosulfate by the nitrate acting as electron acceptor in the system, caused a sudden ORP decline in the first hours of the reaction (Fig. 3). No significant change in ORP data was observed after the substrate was consumed in the system. The operating condition, which maximum nitrate removal with minimum nitrite and sulfate accumulation were observed and $\text{S}_2\text{O}_3^{2-}/\text{NO}_3^-$ ratio was 1.5, was determined to be optimum.

### 3.3. Effect of Different C/N Ratios on Mixotrophic Denitrification Process

In this part of the study, the mixotrophic denitrification process was carried out by addition of methanol as an external organic carbon source to the autotrophic denitrification process at constant influent nitrate concentration of 50 mg/L. The effect of variable C/N ratios with increasing methanol concentration was determined on mixotrophic denitrification performance. This study consisted of three different periods and the influent methanol concentrations were 15, 30 and 45 mg COD/L in Period VII, VIII and IX, respectively. The profiles of nitrate, nitrite, IC and sulfate were shown in Fig. 4.
In Fig. 4A, it was observed that the rate of nitrate consumption improved by increasing methanol concentration although nitrate was totally consumed by mixotrophic microorganisms at the end of the reaction in all three periods. Similar findings obtained by Oh et al. (2001) and Sahinkaya et al. (2013) supported these results. An increase in nitrate removal rate was observed due to the predominance of heterotrophic species by increasing of organic carbon amount in the system and the heterotrophs having higher denitrification performance than autotrophs (Liu et al., 2009; Zhao et al., 2011). Liu et al. (2009) used a combined two-step process of heterotrophic denitrification and sulfur autotrophic denitrification processes for nitrate removal in drinking water. The nitrate removal efficiency was nearly 100% (30 mg NO$_3$-N/L) and there was no accumulated nitrite or residual methanol in the effluent at a higher C/N ratio (C/N ratio: 2.0) than that used in this study (methanol as carbon source).

Nitrite profile formed as a result of the activity of heterotrophic and autotrophic microorganisms in anoxic environment conditions is shown in Fig. 4B. In the period VII, the added methanol concentration (15 mg COD/L) was insufficient to reduce nitrite to nitrogen gas and nitrite accumulation of 5.3 mg/L was observed at the end of the reaction. However, the nitrite accumulation observed in the autotrophic denitrification process in which organic carbon was not added (Part I: period IV, Fig. 2B) was reduced by 68% with the methanol concentration added to the system. In Period VIII and Period IX, no nitrite accumulation resulting from reduction of nitrate (<0.1 mg NO$_2$-L) was observed with an increase of external methanol from 15 mg COD/L to 30 mg COD/L and 45 mg COD/L. Nitrate was rapidly reduced to nitrogen gas with mixotrophic microorganisms under present operational conditions.

Inorganic carbon source was only used as a carbon source by autotrophic microorganisms in the mixotrophic denitrification process. In this study, heterotrophic microorganisms become dominant with increasing organic load and C/N ratio. Thus, the amount of inorganic carbon used by autotrophic microorganisms decreased 70% with the increase of heterotrophic microorganism population compared to data obtained from part I (Fig. 2D and 4D).

In Figure 4C, the effluent sulfate concentrations were similar because the influent inorganic donor source (thiosulfate) was kept constant in this last part of study. However, the sulfate concentration produced at the end of the reaction could be controlled in the mixotrophic process which heterotrophic and autotrophic denitrification processes were used simultaneously. The amount of effluent sulfate was reduced by 18.6% in mixotrophic process formed by methanol supplementation (Figs. 2C
and 4C). Similarly, Sahinkaya et al., (2013) aimed the simultaneous nitrate and Cr(VI) reduction using sulfur-based mixotrophic denitrification process and in activated carbon packed column bioreactor. The mixotrophic denitrification process allowed controlling effluent sulfate concentration.

![Figure 5. Effect of different C/N ratio on ORP profile](image)

Fig. 5 shows the ORP changes during the mixotrophic denitrification process. Period VII and VIII, no significant ORP change was observed. However, the rate of electron donor utilization increased with the increase of the organic carbon load proportional to the increased C/N ratio and the ORP data decreased to negative values in the last period.

4. CONCLUSIONS

The following conclusions from the present study can be drawn:

- It was observed that the changing cycle time had no significant effect on autotrophic partial denitrification process performance. However, a successful denitrification process was carried out under mixotrophic conditions and the shortest cycle time of 2 hours was determined to be optimum operational conditions.
- The reduction of nitrate to nitrogen gas was not completed until the end of the reaction in the autotrophic denitrification process due to the limitation of the electron donor source (thiosulfate) with decreasing $S_2O_3^{2-}/NO_3^{-}$ ratios. Additionally, the nitrite accumulation in the system increased.
- The $S_2O_3^{2-}/NO_3^{-}$ ratio of 1.5 was found optimum for the thiosulfate-based autotrophic partial denitrification process and maximum nitrogen removal efficiency was 49.2%. However, in this operational conditions, the formed sulfate could be controlled using the mixotrophic process, in which heterotrophic and autotrophic denitrification processes were used simultaneously, in order to minimize the high effluent sulfate concentration and increase the nitrogen removal efficiency.
- The effluent sulfate concentration was decreased 18.6% in the mixotrophic process formed by methanol supplementation to the same operating conditions. The 0.70 C/N ratio generated with 30 mg/L additional methanol concentration was determined as the limit value for a successful mixotrophic denitrification process.

5. ACKNOWLEDGMENTS

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6. REFERENCES


