Comparison of tire-sulfur mixotrophic denitrification (T-SMD) and sulfur oxidizing denitrification (SOD) for advanced nitrogen removal of slaughterhouse wastewater

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Abstract. Slaughterhouse wastewater (SWW) containing high concentrations of nitrogen and phosphorus could lead to the eutrophication of receiving water environment such as lake, river and reservoir, and even result in ground water contamination. Effluent NH₄⁺-N and COD were effectively removed through the traditional secondary anaerobic-aerobic treatment process, where NO₂⁻ and NO₃⁻ were produced. This study established two bench-scale upflow packed bed reactors, tire-sulfur mixotrophic denitrification (T-SMD) column and sulfur oxidizing denitrification (SOD) column, to compare the performance for advanced nitrogen removal from synthetic SWW. During the whole study period with a recirculating flow rate of 1 L/d, NO₃⁻ removal efficiencies were about 100% and 99.3% for T-SMD and SOD columns, respectively. Effluent TN concentrations were 6.44 and 0.73 mg/L, respectively, which were lower than the newest effluent standard for new enterprises of 20 mg/L in China. T-SMD column showed the lower SO₄²⁻ productivity due to the existence of mixotrophic denitrification. The pH in both columns remained near-neutral possibly due to the oyster shells addition. The results showed that T-SMD and SOD were the effective technologies for advanced nitrogen removal from SWW.

1. Introduction
Slaughterhouse wastewater (SWW) was generated by slaughtering of animals and cleaning of the slaughterhouse facilities[1]. SWW is characterized as a kind of high nitrogen, phosphorus and organic wastewater[2]. The discharges of untreated or partial treated SWW may lead to eutrophication of receiving water body. SWW was listed as one of the most harmful agriculture and food industrial wastewaters by the United States Environmental Protection Agency (USEPA)[3]. Per metric tons of meat produced may consume between 2.5 and 40 m³ of freshwater[4]. Large amount of SWW generated by meat processing sector need to be treated effectively and released to the environment harmlessly[5].

According to the previous pollutant discharge standard for slaughterhouse and meat processing industry in China, total nitrogen (TN) and total phosphorus (TP) were not limited. The current SWW treatment processes in China can effectively control the effluent COD and NH₄⁺-N, but not for TN, TP and produced NO₃⁻-N, NO₂⁻-N. The effluent N and P were difficult to satisfy the newest pollutant discharge standard (second draft; 000014672/2018-01201)[6]. Therefore, it is urgent to develop an effective advanced N and P removal process for SWW to satisfy the second draft.
At present, biological denitrification is the main method for NO\textsubscript{3} and NO\textsubscript{2} removal, which including heterotrophic and autotrophic denitrification. Additional organic carbon source was needed for heterotrophic denitrification, which will greatly increase treatment cost and even cause secondary pollution. Considering fewer biological available organic carbon source in secondary treated SWW effluent, autotrophic denitrification was preferred.

Sulfur oxidizing denitrification (SOD) process has attracted increasurable attention owing to its no carbon source addition, low excess biomass production and cheap and obtained easily for elemental sulfur (S\textsuperscript{0})\textsuperscript{[7]}. Sulfur oxidizing denitrifying bacteria utilizes S\textsuperscript{0} as an electron donor to reduce NO\textsubscript{3}⁻, which can be described as the following equation\textsuperscript{[8]}:

\[
1.10S^0 + 0.40CO_2 + NO_3^- + 0.76H_2O + 0.08NH_4^+ \rightarrow 0.08C_2H_7O_2N + 0.50N_2 + 1.10SO_4^{2-} + 1.28H^+ \tag{1}
\]

According to Equation (1), for every 1.0 g of NO\textsubscript{3}⁻-N consumed by SOD, 2.51 g of SO\textsubscript{4}²⁻-S and 0.07 g H\textsuperscript{+} are produced simultaneously. The main disadvantages of this process are the formation of SO\textsubscript{4}²⁻ and the consumption of alkalinity\textsuperscript{[9]}. According to these problems, scholars have studied extensively. In 2002, Soares\textsuperscript{[10]} established a sulfur-limestone autotrophic denitrification system, in which limestone dissolved continuously and neutralized H\textsuperscript{+} generated by SOD reaction and the highest rate of nitrogen removal was 0.20 kg N m\textsuperscript{-3} d\textsuperscript{-1}. In 2014, Krayzelova et al.\textsuperscript{[11]} used tire pellets (T) as solid carbon source to form a mixotrophic denitrification system based on the traditional SOD. The results showed that NO\textsubscript{3}⁻ removal efficiency was higher than 90% and average effluent SO\textsubscript{4}²⁻-S concentration was 46 mg/L. In 2015, Simard et al.\textsuperscript{[12]} inserted oyster shells (OS) to the sulfur-containing column and testified that the addition of OS could improve the alkalinity and maintain a suitable denitrifying environment. In 2017, Tong et al.\textsuperscript{[13]} found organic carbon addition possibly can improve denitrification performance by promoting mixotrophic metabolism and the addition of OS can enhance biofilm growth.

However, none of the previous studies have investigated the combination of T, S\textsuperscript{0} and OS for advanced nitrogen removal from SWW. The previous results showed that the carrier combination has good performance in denitrification process\textsuperscript{[10-13]}. This study will explore the feasibility of the carrier combination for advanced denitrification of SWW.

The column study established two up-flow packed bed reactors (PBRs), respectively: tire-sulfur mixotrophic denitrification (T-SMD) column and sulfur oxidizing denitrification (SOD) column. The overall goal of this research was to compare T-SMD and SOD performance for advanced nitrogen removal from synthetic SWW in column studies. The carriers used in these columns included T (as solid carbon source), S\textsuperscript{0} (as electronic donors), OS (as solid phase buffers and biofilm carriers) and quartz sand (S) (as additional biofilm carriers). The specific objectives were to: (1) investigate the feasibility of T-SMD and SOD for SWW treatment; (2) compare the denitrification performance of T-SMD and SOD columns; and (3) investigate by-product formation in both T-SMD and SOD columns.

2. Materials and Methods

2.1. T-SMD and SOD column study

Two 750 mL acrylic columns (Height = 38 cm; Inside diameter = 5 cm; Wall thickness = 1 cm) were used as up-flow PBRs at room temperature (25 ± 2 °C). Each column has five sample ports along the height. The T-SMD column was filled with 330 g of T, 120 g of S\textsuperscript{0} and 130 g of OS. Replaced 330 g of T with 720 g of S in the SOD column. The amount of S\textsuperscript{0} and OS was the same as in the T-SMD column. The particle size of the carriers in both columns was 2-4 mm.

The carriers purchased were washed three times with deionized water before the experiment and dried naturally for one day. The amounts of S\textsuperscript{0} and OS added to both columns were calculated according to the stoichiometry of SOD (Equation (1)), in which the amount of NO\textsubscript{3}⁻ was 50 mg N/L with a flow rate of 1 L/d for an operating period of two year. The values obtained were then multiplied by a safety factor of 1.3. The empty bed contact time in the reactor was about 8.4 h.
The mixed liquor suspended solids used in this experiment were taken from the anaerobic tank of sewage treatment plant of Beijing No. 5 Meat Factory. The concentration of the MLVSS was detected before the experiment and the result was 10,235 mg/L. The carriers in each column were fully mixed with 500 mL of mixed liquor suspended solids for one day.

This phase was 8 days and both columns were operated with a closed recirculation system at a flow rate of 1 L/d to enhance the attachment and domestication of the biofilm.

2.2. Synthetic wastewater
Experiments were carried out using synthetic SWW (treated by anaerobic aerobic phase) with the following composition (per L of deionized water): KNO$_3$ (361 mg) and KH$_2$PO$_4$ (44 mg). The corresponding composition resulted in an influent NO$_3^-$-N and PO$_4^{3-}$-P concentrations of 50 mg/L and 10 mg/L, respectively.

2.3. Analytical methods
NO$_3^-$, NO$_2^-$, PO$_4^{3-}$ and SO$_4^{2-}$ concentrations were measured by Metrohm Eco Compact IC Pro (Herisau, Switzerland) ion chromatography. Method detection limits (MDLs) for NO$_3^-$, NO$_2^-$, PO$_4^{3-}$ and SO$_4^{2-}$ were 0.01, 0.04, 0.07 and 0.01, respectively. NH$_4^+$, TN, TP and COD were analyzed using spectrophotometer (DR 6000, Hach, USA; 5B-3B (V8), Lian-Hua Tech, China) according to the Chinese NEPA (2002) standard methods[14]. MDLs for NH$_4^+$, TN, TP and COD were 0.025, 0.05, 0.01 and 15 mg/L, respectively. pH and ORP were measured using a pH meter with calibrated electrode (FiveEasy Plus, Mettler Toledo, Switzerland; MDL: 0-14).

3. Results and Discussion

3.1 Removal of TN and NO$_3^-$ and accumulation of NO$_2^-$ and NH$_4^+$
The column study was carried out to compare the performance of T-SMD and SOD on the advanced treatment of synthetic SWW. Biofilm formation and significant nitrogen removal were observed in both columns after a period of operation at a flow rate of 1 L/d. The concentrations of NO$_3^-$-N were almost close to MDL in the effluent of both columns (Figure 1(b)). The NO$_3^-$ removal efficiencies were about 100% and 99.3% for T-SMD and SOD columns, respectively. The corresponding effluent TN concentrations were 6.44 and 0.73 mg/L for T-SMD and SOD columns, respectively (Figure 1(a)). The effluent TN concentrations were both lower than the permitted maximum discharge concentrations for existing and new enterprises of 25 and 20 mg/L, respectively, in the second draft of “Effluent standard of pollutants for slaughter and meat processing industry” (000014672/2018-01201). It can be seen from the Figure 1(a) and (b) that the TN and NO$_3^-$ removal rates of T-SMD was higher than that of SOD.

As shown in Figure 1(c), the accumulation of NO$_2^-$ occurred during the experiment and then slowly disappeared before the end of the experiment. The accumulation peaks of T-SMD and SOD columns appeared on the 1th and 3th days, respectively. The peak values for T-SMD and SOD columns were 5.83 and 8.80 mg/L, respectively. Earlier NO$_2^-$ accumulation and lower peak value both appeared in the T-SMD column, which were most likely due to high denitrification rate and relatively lower concentration of NO$_3^-$-N at the same moment.

During the first 7 days of the operation, NH$_4^+$-N concentrations close to the MDL were observed in both columns (Figure 1(d)). However, the concentration of NH$_4^+$-N in T-SMD column increased significantly on the 8th day. The final NH$_4^+$-N effluent concentrations were 3.05 and 0.13 mg/L in T-SMD column and SOD column, respectively. Similar NH$_4^+$ accumulation had also appeared in previous studies. Zhang et al.[15] investigated the dissimilatory nitrate reduction to ammonium process (DNRA) in the bioelectrochemical system and discovered that a higher C/N might lead to more NH$_4^+$ accumulation. Hence, the accumulation of NH$_4^+$ was due to higher organic carbon concentration in T-SMD column, which was leached from the T.
Figure 1. Concentrations of TN, NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N in T-SMD and SOD columns, respectively.

3.2 Comparing T-SMD with SOD
According to the Equation (1), SOD process consumed NO$_3^-$ and was accompanied by the production of SO$_4^{2-}$. It can be seen from the Figure 1(b), T-SMD column had a higher denitrification rate, which should be has a higher SO$_4^{2-}$-S concentration. But SO$_4^{2-}$-S concentration in T-SMD column was well lower than SOD (Figure 2(a)). The highest SO$_4^{2-}$-S concentration in T-SMD was only 19.87 mg/L, which was far below the Standards for Drinking Water Quality (GB5749-2006) (83 mg/L). The SO$_4^{2-}$-S concentration in SOD column accumulated gradually over time and reached 192 mg/L at the end of the experiment. The final effluent COD concentration was 133.15 mg/L in T-SMD column, which was lower than the newest effluent standard of 80 mg/L in China. Higher COD concentration was observed in T-SMD column, which was leached from the T (Figure 2(b)). Compared with Figure 1(b), we can see that higher COD concentration resulted higher denitrification rate. T can provide biologically available organic carbon, which facilitate the process of heterotrophic denitrification and thus improve the denitrification rate. Lower COD concentration was observed in SOD column (without tires addition) (Figure 2(b)), which could come from the OS or sloughed biofilm. Compared with SOD column, T-SMD column had higher removal efficiencies for NO$_3^-$ and TN (Figure 1(a) and (b)). The concentration of SO$_4^{2-}$-S in T-SMD column was maintained at a low level and was well lower than SOD column (Figure 2(a)). Higher NO$_3^-$ and TN removal efficiencies and lower SO$_4^{2-}$-S concentration were likely due to the coexistence of autotrophic and heterotrophic denitrification processes in T-SMD column. The bioavailable organic carbon leached from T could promote the process of mixotrophic denitrification[10]. Mixotrophic denitrification can not only preserve the advantages of the above two denitrification, but also improve the disadvantages of both denitrifications.

Relatively higher TN concentration in T-SMD column at the end of experiment may be due to the residual organic carbon in heterotrophic denitrification. The organic carbon leached from T was not
completely utilized by heterotrophic denitrification. Hence, this proved that heterotrophic denitrification has the problem of incomplete utilization of organic carbon.

**Figure 2.** Concentrations of $\text{SO}_4^{2-}$ and COD in T-SMD and SOD columns, respectively.

### 3.3 pH and OPR in both columns

Based on Equation (1), for every $1.0 \text{ g}$ of $\text{NO}_3^{-}-\text{N}$ removed, $0.07 \text{ g H}^+$ are produced by the process of SOD. As the reaction proceeds, the pH in both columns should gradually decrease following the above equation. But the pH remained near-neutral in both columns (Figure 3(a)), which was likely due to the addition of the OS. Sengupta et al. [16] investigated that the OS were an effective pH buffer in the process of SOD. According to the Figure 3(b), ORP concentration in T-SMD column was lower than SOD, indicating that $\text{NO}_3^{-}$ in the T-SMD column was more easily converted to $\text{N}_2$. Therefore, there was a higher denitrification rate in the T-SMD column.

**Figure 3.** The pH and ORP in T-SMD and SOD columns, respectively.

### 3.4 Removal of TP and PO$_4^{3-}$

High removal rates of TP and PO$_4^{3-}$ was observed in both columns (Figure 4(a) and (b)). Lower TP and PO$_4^{3-}$-P concentrations in the effluent mainly due to the existence of denitrifying phosphorus removal process and biosynthesis in both systems. TP and PO$_4^{3-}$-P concentrations in the T-SMD columns were relatively low, indicating mixotrophic denitrification system may had relatively higher denitrifying phosphorus removal efficiency.
4. Conclusions

Column studies were carried out to compare the performance of T-SMD and SOD for advanced nitrogen removal from synthetic SWW. At a recirculating flow rate of 1 L/d, NO$_3^-$ removal efficiencies were about 100% and 99.3% for T-SMD and SOD columns, respectively. Effluent TN concentrations in both columns were 6.44 and 0.73 mg/L, respectively. Effluent SO$_4^{2-}$ concentration was always below 20 mg/L in T-SMD column, which was much lower than that in SOD column. The results showed that the organic carbon source provided by T can support mixotrophic metabolism of denitrifying microorganisms in T-SMD, leading to lower SO$_4^{2-}$ production and higher TN and NO$_3^-$ removal efficiencies. However, higher final effluent COD and TN concentrations were showed in T-SMD. The combination carriers (consist of T, S$^0$ and OS simultaneously) were used for advanced nitrogen removal from SWW firstly in this study. The following work need to investigate the adaptability of the reactor at different load rates and the application in practical SWW treatment.

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