A study on a simultaneous biological nitrification-denitrification of an aqueous solution simulating a refinery wastewater

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Abstract

A bench-scale bioreactor with a five-liter working volume was used to carry out a simultaneous biological nitrification-denitrification (SBND) of an aqueous solution simulating refinery wastewater. The bioreactor was also used to perform a similar scheme of experiments on real refinery wastewater (RRW). Despite the higher salinity of the RRW, the obtained results of the two cases demonstrated a fair trend agreement, but a lower nitrogen removal efficiency for the RRW. Anaerobic bacteria (Phenobacter) was successfully adapted to perform nitrification (aerobic) and denitrification (anaerobic) processes. Dissolved oxygen (DO), pH, chemical oxygen demand (COD), total dissolved salts (TDS), and nitrate (NO₃⁻) ions were periodically measured (each 1 hour) to determine the time for the nitrification and denitrification. The time for nitrification was around 4 hours and the time for denitrification was approximately 3 hours (75% of the nitrification time). The minimum and maximum values of the pH, COD, TDS, and NO₃⁻ ions at the start and the end of the nitrification-denitrification processes were 7.3-8.5, 318-585, 779.88-7710, and 2.4-56 respectively for the aqueous solution, while their range was 7.3-8.3, 310-660, 4736.9-8086, and 5.6-78 respectively for the RRW. The nitrogen removal efficiencies of the aqueous solution and the RRW were 95% and 87% respectively. The results prove the successful adaptation of an aerobic bacteria (Phenobacter) for nitrifying and denitrifying a refinery wastewater and reducing the investment and operational costs of the wastewater treatment plant.

Keywords: Industrial wastewater, Biological nitrification, Biological denitrification, simultaneous nitrification-denitrification processes.

1. Introduction

Forty percent of the global oil industry wastewaters are partially treated and discharged into either constructed or natural lagoons [Mustapha 2018] causing considerable soil contamination. The discharged wastewaters contain organic and non-organic pollutants [Shamkhi et al., 2021]. For high permeability soils, a higher seepage rate takes place and pollutants are transferred from points of discharge of wastewater to groundwater reservoirs [Albdiri et al., 2021]. The most prevalent treatment process of the oil industry wastewaters is the biological process [McCarty, 2018]. Conventional biological treatment processes only outperform in removing organics. However, the removal of metal ions from refinery wastewater has been successfully performed and modeled [Albdiri et al., 2020, Shamkhi et. al, 2021]. The removal of nitrogen species from refinery wastewaters called up for developing two consecutive biological processes. Nitrification of ammonia to nitrate followed by organic reduction of nitrate to diatomic nitrogen [Rittman, 2001]. The oxidation of ammonia takes place in the presence of oxygen and...
the use of aerobic bacteria, while the oxidation of nitrate takes place in the absence of oxygen and the use of an anaerobic bacteria. Many different aerobic and anaerobic biological processes are available for removing nitrogen from domestic wastewaters [McCarty, 2018]. Significant reductions in oxygen requirements for nitrogen removal are often indicated as advantageous for using the simultaneous nitrification/denitrification approach for nitrogen removal rather than the traditional nitrification/denitrification processes [Third et al., 2001]. Successful implementation of the simultaneous nitrification/denitrification process resulted in 63% saving of oxygen and 100% fewer reducing agents than conventional nitrification/denitrification process [Hendricks et al., 2014, Ali, M. and Okabe, S., 2015, Ma et al., 2016, Cao et al., 2016]. This research aimed to simultaneously nitrify and denitrify an aqueous solution simulating the wastewater of the Diwaniyah refinery. The current refinery wastewater treatment only performs a nitrification process converting ammonia into nitrite and nitrate. The presence of nitrite and nitrate in the effluent of the Diwaniyah refinery causes possible eutrophication of a neighboring groundwater reservoir. To protect the environment and avoid the pollution of nitrite and nitrate, denitrification should take place after the nitrification of the refinery wastewater. Traditional denitrification requires anaerobic bacteria to convert nitrate into diatomic nitrogen gas. The consecutive nitrification-denitrification processes result in higher costs and larger space of the refinery wastewater treatment plant, which is beyond the refinery financial priorities. Therefore, succeeding in developing simultaneous nitrification/denitrification and utilizing an aerobic bacteria could reduce cost and space. The aerobic bacteria could adapt themselves to an anaerobic environment if the denitrification time is short enough to survive. It could utilize the dissolved oxygen and the available oxygen in the NO3− ions to perform the microorganism's metabolism.

2.Theoretical background

SBND processes are sequential processes used in the removal of ammonium ions from domestic and industrial wastewaters with a high C/N ratio [Bernet et al. 2000; Guo et al. 2013; Moya et al. 2012]. Nitrification is a two steps process in which an oxidative reaction of ammonium takes place by ammonia-oxidizing bacteria (AOB) to produce nitrite, followed by another oxidative reaction to convert nitrite into nitrate by autotrophic bacteria [Peng and Zhu 2006; Shijian et al. 2014]. Both steps take place under aerobic conditions as shown in equations 1 and 2. Denitrification is also an oxidative process converting nitrate into nitrogen gas (N2) utilizing organic matter as an electron donor and heterotrophic microorganisms as a reducing agent [Pepper et al. 2006]. Denitrification takes place under anaerobic conditions and exogenous sources (such as methanol and acetate) as shown in equations 3 and 4[Pepper et al. 2006].

\[
2 \text{NH}_4^+ + 3 \text{O}_2 \rightarrow 2 \text{NO}_2^- + 4 \text{H}_2\text{O} + \text{H}^+ \]  
(1)

\[
2 \text{NO}^- + \text{O}_2 \rightarrow 2 \text{NO}_3^- \]  
(2)

\[
6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} \rightarrow 3\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} + 6\text{OH}^- \]  
(3)

\[
8\text{NO}_3^- + 5 \text{CH}_3\text{COOH} \rightarrow 4\text{N}_2 + 10\text{CO}_2 + 6\text{H}_2\text{O} + 8\text{OH}^- \]  
(4)

Facultative anaerobes make up approximately 80% of the bacteria within an activated sludge. These organisms have an enzymatic ability to use free molecular oxygen, nitrite, and nitrate ions for cellular activity, growth, and reproduction [Gerardi, 2002].
Experimental Work

- **Materials**
  
  All materials used in this study were of analytical grade. An aqueous solution of nutrient and ammonium was prepared using glucose (C₆H₁₂O₆), magnesium sulfate heptahydrate (MgSO₄·7H₂O), sodium bicarbonate (NaHCO₃), Calcium chloride dihydrate (CaCl₂·2H₂O), and ammonium chloride (NH₄Cl). All materials were of Indian origin. The aqueous solution was prepared to simulate the refinery wastewater.

- **Equipment and procedures**
  
  The experimental setup is shown in Figure 1. The setup consists of a five-liter beaker, overhead mixer (HS50A, 0 - 3000 min⁻¹, China), two aeration devices (Rs-348 air-pump, 5W, 2*3.5 L/min, China), pH meter (HI-98107 pHep pH Tester, Hanna instrument, China), Dissolved Oxygen meter (HI9142 DO meter, Hanna instrument, China), conductivity and total dissolved salts meter (HM DIGITAL, Korea), COD and BOD testing devices.

  The 5 L beaker was inoculated with 4 liters of Phenobacter bacteria and the aqueous solution. The Phenobacter bacteria were collected from the biological tank of the Diwaniyah refinery which is used for the removal of organics from the refinery wastewater. The system was operated in cyclic mode (Nitrification/ Denitrification processes). The nitrification cycle was operated aerobically using two aeration devices (air pumps) to keep the DO concentration of the inoculum not less than 2 mg/L. The nitrification cycle was set to 4 hours. pH, COD, TDS, DO, turbidity, and NO₃⁻ ions concentrations were measured every hour up to the end of the nitrification cycle (4 hours) to monitor the conversion of ammonium ions to NO₃⁻ ions. The denitrification cycle starts immediately after the end of the nitrification cycle. During the denitrification cycle, aerators were stopped supplying air to the inoculum. The microorganisms (bacteria) strived to grow utilizing the oxygen of NO₃⁻ ions to survive and digest organics available in the wastewater. The utilization of the oxygen of NO₃⁻ ions converted the NO₃⁻ ions to N₂ gas. The denitrification continued for 3 hours. pH, COD, TDS, DO, turbidity, and NO₃⁻ ions concentrations were measured every hour up to the end of the denitrification cycle (3 hours) to monitor the conversion of NO₃⁻ to N₂.

Figure 1. Bench Scale Bioreactor for SBND
3. Results and discussions

**NO$_3^-$ profile of the aqueous solution (SWW) and the real refinery wastewater (RRW)**

Figure 2 demonstrates the concentration profile of NO$_3^-$ at room temperature 23°C and an initial value of pH 7.3. It is clearly shown that NO$_3^-$ concentration increases for the first 4 hours (during the nitrification), and rapidly declines during the denitrification process (during the final 3 hours). The figure reflects the trend agreement of both the SWW and the RRW. It was observed that the nitrification of the RRW resulted in a higher concentration of NO$_3^-$ in comparison with the SWW. It was attributed to the higher initial concentration of nitrous organics within the refinery effluents. The obtained results emphasize the survivability of the Phenobacter bacteria through both aerobic and anaerobic conditions (nitrification and denitrification processes). The Phenobacter bacteria were efficiently adapted itself to the anoxic environment and sustained their growth utilizing the oxygen of the nitrate ions. The sequential occurrence of nitrification-denitrification resulted in simultaneous oxidation of ammonium ions and reduction of NO$_3^-$ ions converting the NO$_3^-$ ions to diatomic nitrogen gas (N$_2$).

![Figure 2. NO$_3^-$ concentration profile](image)

**pH profile of the SWW and the RRW**

Figure 3 shows that pH is slightly increased during the initial phase of the nitrification period. It was observed that the pH of the SWW exceeded the pH of the RRW during the initial phase of nitrification. This could be due to the fast dissociation of ammonium chloride, which was used in preparing the SWW. However, the pH of the SWW and RRW tends to decline to neutral conditions (pH 7.2) upon the nitrification process. The pH was kept constant during the denitrification process as the concentration of the H$^+$ ions was constant and only NO$_3^-$ ions were reduced to N$_2$. The decline of the pH during the nitrification time could be attributed to the alkalinity consumption [Ali et. al, 2017]. The obtained results of this research agree with the findings concluded by Ali et. al, 2017.
The accumulated DO in the SWW and RRW through the nitrification period is elucidated in Figure 4. During the initial time of the nitrification, the DO was partially consumed by the bacteria to degrade organics. Therefore, the DO concentration could be related to bacteria activity [Ali et. al, 2017]. The DO concentration attained the maximum when the ammonia was depleted. This agrees with the conclusion of Ali et. al, 2017 in which they reported that the bacterial respiration decreased with the decrease of the COD, and consequently the DO accumulate to attain the saturation concentration. It is clearly shown that the trend of DO accumulation and consumption is similar for both SWW and RRW. The initial concentration of the DO of the RRW was higher than the initial concentration of the SWW by 50%. The DO concentration of the RRW reached a maximum in three hours suggesting that the time for the degradation of organics and the conversion of the Ammonium-N to nitrite and nitrate became low. This reduction in the nitrification time by 25% could lead to better survival and growth of the Phenobacter bacteria, as it reduces the total time of the nitrification-denitrification process. The DO was rapidly consumed through the denitrification time, to sustain the growth of the bacteria. The higher rate of consumption of DO during the denitrification, the better survival of the bacteria. The DO profile could be utilized to determine the minimum time of denitrification.

Figure 3. pH profile

**DO profile**

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Figure 4. Dissolved oxygen profile

**Total dissolved salts (TDS) profile**

Figure 5 shows the progressive salt formation of the SWW due to the chemical reactions through the nitrification and denitrification processes, while the dissolved salts of the RRW exhibited inconsiderable formation during the early stage of the nitrification process and then kept constant indicating no reactions take place. These results are consistent with the observations of Ju et. al, 2007.

Figure 5. TDS profile
**Turbidity profile**

The turbidity of wastewaters indirectly indicates the COD concentration. Figure 6 indicates the reduction in the turbidity of the SWW and RRW as nitrification and denitrification processes take place. It is clearly shown that the turbidity decreases as a function of the COD removal. During the nitrification time, the rate of decrease of the turbidity is very low, while it exhibits a progressive decrease during the denitrification time. This could be attributed to the slow growth rate of the Phenobacter bacteria under the low level of the dissolved oxygen.

![Figure 6. Turbidity profile](image)

4. **Conclusions**

Successful removal of ammonium ions from an aqueous solution (SWW) through an SBND process has been demonstrated and proven. The obtained results were compared to an RRW nitrification-denitrification treatment. The comparison shows fair agreement in trend and values. The traditional procedure of the nitrification-denitrification processes is to sequentially carry out these two processes using two types of bacteria (aerobic and anaerobic bacteria). These two types require separated processing tanks. Therefore, the successful adaptation of intermittent aeration reduces the investment and operational costs of the simultaneous nitrification-denitrification processes and consequently the cost of the treatment of the wastewater.
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6. References

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