Water-Energy Nexus 1 (2018) 47–55

Contents lists available at ScienceDirect

Water-Energy Nexus

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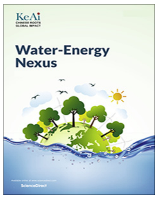

Continuous fed-batch alternating aerobic and anoxic system for biodegradation of Decontaminating Solution 2 with high nitrogen content

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ARTICLE INFO

Article history:
Received 7 March 2018
Accepted 26 May 2018
Available online 26 May 2018

ABSTRACT

A continuous fed-batch alternating aerobic and anoxic (CFBAAA) activated sludge system was developed for biodegradation of the US Army's Decontaminating Solution 2 (DS2). The high nitrogen content in DS2 (approximately 28.5% by weight) necessitated a new nitrification/denitrification process scheme for biological nitrogen removal. Nitrification, conversion of NH_4^+ from the DS2 degradation to NO_3^-, is accomplished simultaneously along with the DS2 degradation under aerobic conditions, and denitrification, conversion of accumulated NO_3^- to N_2, is achieved under anoxic conditions in which the air supply is ceased. A one-day cycle with 16 h air-on and 8 h of air-off was used.

Overall, the CFBAAA system could achieve COD removal of >97% and TN of >99% at a hydraulic retention time of 16 d, when 1% DS2 (equivalent to 17,500 mg COD L^{-1} and 2850 mg N L^{-1}) was fed. In addition, due to its cyclic operation mode, the CFBAAA system could reduce 1/3 of electricity consumption compared to the conventional activated sludge system.

Introduction

The US Army's Decontaminating Solution 2 (DS2) consists of 70% diethylenetriamine (DETA), 28% ethylene glycol monomethyl ether (EGME), and 2% NaOH, and it is used as a standard decontaminant for chemical warfare agents such as blistering mustard agent and nerve agents such as G and VX (Singh et al., 2010). The unique chemistry of DS2 solution allows DETA to sequester a sodium ion to produce a super base "OH^-" that in turn abstracts a proton from EGME to yield a strong nucleophile, i.e., ethylene glycol monomethyl ethoxide (Richardson, 1972). DS2 is extremely effective in attacking nucleophile centers in a variety of chemical agents, thereby inactivating the highly toxic properties of the chemical agents (Singh et al., 2010). However, DS2 itself is corrosive, toxic and hazardous, and it was recommended to remove from the list of the US military decontaminants (Kingsbury 1990). In particular, EGME is teratogenic (Baqchi and Waxman, 2008). The secondary amine structure in DETA like other secondary amine compounds may be chemically (Wang et al., 2011) or microbiologically (Calmels et al., 1985) converted to N-nitrosamines, many of which are potent carcinogens. An alternative chemical and/or enzyme-based decontaminant system that is less toxic and benign to the environment has been under investigation.

Two organic components of DS2, i.e., aliphatic amines and ethylene glycol ether are important functional groups of DS2. They are also important industrial chemicals; they are used in a wide range of commercial applications. Aliphatic amines are directly used as antioxidants, anti-corrosive agents, solvents, and emulsifiers and are commonly found as moieties in rubber additives, pesticides, drugs and optical brighteners (Roose and Turcotte, 2016). Recently, it has been reported that the commercial production capacity for DETA has been expanded (C&EN, 2006). As for ethylene glycol derivatives, they are used as starting materials for the production of polyethylene glycol (PEG), which ultimately finds industrial applications including antifreeze formulations, water soluble lubricants, wetting agents, pharmaceutical preparations and cosmetics (Hutanu et al., 2014). In addition, the modification of PEG generates nonionic surfactants, a very important group of industrial products with application from domestic detergents to agrochemicals and food emulsifiers (Sak-Bosnar et al., 2015). Consequently, aliphatic amines and ethylene glycol are expected to be commonly present in many industrial and domestic liquid waste discharges. The environmental fate and particularly the biodegradability of these two groups of chemicals are therefore important.

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https://doi.org/10.1016/j.wen.2018.05.002
2588-9125/

Ether glycols have been used as a carbon source by a variety of aerobic and anaerobic bacteria, and they could be readily degraded through well-documented ether cleavage reactions under either aerobic or anaerobic conditions (Huang et al., 2005; Paulo et al., 2017). Low molecular weight amines such as methyl, dimethyl, and trimethyl amine, and ethanolamine are readily degraded by various bacterial species (Boden et al., 2008; Chistoserdova et al., 2009; Chistoserdova, 2011). Also, biodegradation of nitrogen-based chelating agents that contain carboxylic groups, e.g., nitrilotriacetic acid (NTA) and ethylenediaminotetraacetic acid (EDTA) has been demonstrated (Nancharaiah et al., 2006; Wen et al., 2009). However, little is known about the biodegradability of aliphatic and/or cyclic amines lacking a carboxylic group (Emtiazi and Knapp, 1994), and information on the biodegradability of secondary amines (e.g., DETA) has been rarely reported.

In this study, a continuous fed-batch alternating aerobic and anoxic (CFBAAA) activated sludge system was applied to simultaneously remove the organic components of DS2 and nitrogen in the form of ammonia.

Materials and methods

DS2 feed preparation

The feed medium (pH adjusted to 7.2) used for the CFBAAA system consisted (per liter) of: 3.4 g KH_2PO_4; 4.36 g K_2HPO_4; 1 g $NaHCO_3$; 0.5 g NaCl; 10 mL of modified Wolin salts solution (Harvey et al., 1996); and different weight % of DS2 as the sole source of carbon, energy and nitrogen.

Bioreactor setup and operation

The CFBAAA system with mixed culture was set up with a New Brunswick BioFlow 3000 fermentation unit (GMI Inc., Boulevard Ramsey, MN, USA) fitted with a 5 L vessel (working volume: 4 L). The air was provided through a sparger connected to an external air pump (Whisper 800, Tetra, Blacksburg, VA, USA) and/or in-house air supply. The mixing was initially set at 250 rpm under aerobic cycles through a built-in impeller/motor assembly of the fermentation unit. However, to prevent dissolution of oxygen during anoxic periods, the agitation was maintained at 150 rpm during both aerobic and anoxic periods. Air on and off modes (16 h air on and 8 h air off in one-day cycle) were achieved using a three-way solenoid valve (Cole Palmer, Vernon Hills, IL, USA) that was connected to a timer (Fisher Scientific, Hampton, NH, USA). Initially, the different % DS2 was continuously fed by a built-in peristaltic pump throughout the aerobic/anoxic periods at different hydraulic retention times (HRTs), but later DS2 was fed during the anoxic periods. DO and pH probes (Mettler-Toledo Ingold, Columbus, OH, USA) were used to continuously monitor and display the DO and pH of the system. The pH of the bioreactor was controlled at 7.0 by the adding 25% NaOH solution. Samples were taken at the end of aerobic and anoxic cycles, centrifuged for 10 min at 10,000 rpm (Marathon 13 K/M, Fisher Scientific, Hampton, NH, United States), filtered through 0.2 µm membrane filter, and analyzed for NH_4^+, NO_3^-, and chemical oxygen demand (COD). The mixed liquor suspended solids (MLSS) was directly collected from the reactor at the end of an aerobic cycle and analyzed. A volume of culture equal to volume of feed over a one-day cycle was removed daily at the end of aerobic cycle, initially without a prior settling and, subsequently with settling of 15 min in order to retain biomass in the system.

Analytical methods

The amino groups present in DETA showed positive interference for colorimetric analyses of NH_4^+. Therefore, for samples that contained a significant amount of both NH_4^+ and DETA (as indicated by a high COD), NH_4^+ concentration was assayed by either dabsylation (Lin and Lai, 1980) or dansylation (Henriks-Eckerman and Laijoki, 1985) followed by an HPLC (Beckman Instrument, Fullerton, CA, USA) equipped with an Ultramex 5 reverse-phase c18 (250 × 4.6 mm I.D.) and a UV detector. 1.0 or 0.5 mL of sample for dabsylation or dansylation, respectively, was placed in a 15 mL screw-capped vial, saturated with disodium carbonate or sodium bicarbonate, mixed with 1.0 mL dabsyl chloride solution (1.8 mg mL^{-1} dabsyl chloride in acetone) or 1.0 mL (10 mg mL^{-1} dansyl chloride in acetone). Then the samples were heated at 55 °C for 2 h. After cooling to room temperature, 0.5 mL (1.8 mg mL^{-1} dabsyl chloride in acetone) or 1.5 mL of deionized water was added to dabsylation or dansylation mixture, respectively, and heated one additional hour at 55 °C. After cooling to room temperature, dabsylated ammonia was extracted twice with 1 mL of n-hexane: n-butanol (1:1). The organic layers were combined, washed with twice with 2 mL of deionized water, and dehydrated over anhydrous sodium sulfate. A 25 µL aliquot of clear extract was injected into the HPLC column and monitored at 425 nm. As for the dansylated sample, 1 mL of ethyl acetate was used to extract the dansylated ammonia. After the phase separation between ethyl acetate and water for at least one hour, 0.5 mL of ethyl acetate was pipetted into a clean 3 mL vial and allowed to evaporate overnight. The solid dansylated ammonia was reconstituted with 0.5 mL of liquid mobile phase, and 50 µL was injected for HPLC analysis and monitored at 365 nm. The mobile phases consisted of 95% ethanol:acetonitrile:water (6:6:7) for dabsylated samples and acetonitrile:water containing 0.005 M 1-pentanesulphonic acid sodium salt (72%:28%) for dansylated samples. pH of the samples was adjusted to 3.5 with acetic acid. The flow rate of mobile phase was set at 1.0 and 1.5 mL min^{-1} for the analysis of dabsylated and dansylated samples, respectively.

Sample COD, NH_4^+, and NO_3^- were analyzed, following the Standard Methods (APHA, 2005). The biomass concentration was estimated as MLSS.

Results and discussion

Phase I: DS2 degradation and nitrification

The bioreactor was initially operated at a HRT of 12 d with 1% DS2 under aerobic condition. Immediate and steady increase of COD level resulted, possibly indicating an overload of DS2 (Fig. 1). Therefore, the HRT was increased to 25 d, in order to lower the loading rate. After the HRT was increased, the COD level initially decreased for 14 d with a concomitant increase of ammonia concentration, indicating degradation of DS2 – particularly DETA; DETA is the sole source of nitrogen in the DS2 feed. However, the COD level of the bioreactor eventually increased back to the level observed before the HRT was changed. It should be noted that the increase of COD occurred when the level of ammonia reached to about 1000 mg L^{-1}, at which ammonia remained almost constant, indicating that the DS2 degrading capacity of the system had been hampered by the accumulation of ammonia. On the other hand, NO_3^- concentration remained zero, indicating the absence of nitrification in the bioreactor. Incidentally, the biomass concentration drastically decreased from 1200 to 500 mg L^{-1}.

The DS2 feed was discontinued, and the bioreactor was continuously aerated in a batch mode. The COD level decreased to 1000 mg L^{-1} over a period of 25 d (Fig. 2). Meanwhile, the NH_4^+ concentration increased proportionally to the amount of COD decreased (i.e., 0.28 mg NH_4^+-N per mg COD). However, the amount of NH_4^+ generated was much higher than that expected from the theoretical nitrogen content of DS2 (i.e., 0.17 mg N per mg COD).

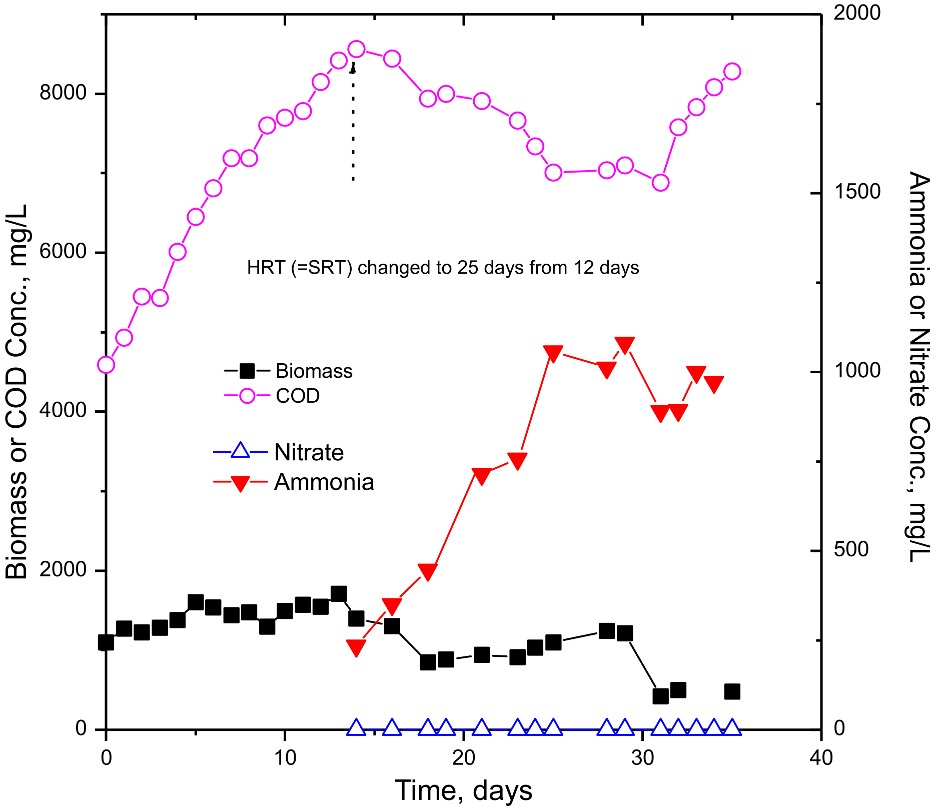

Fig. 1. Continuously fed operation with 1% DS2 under aerobic conditions.

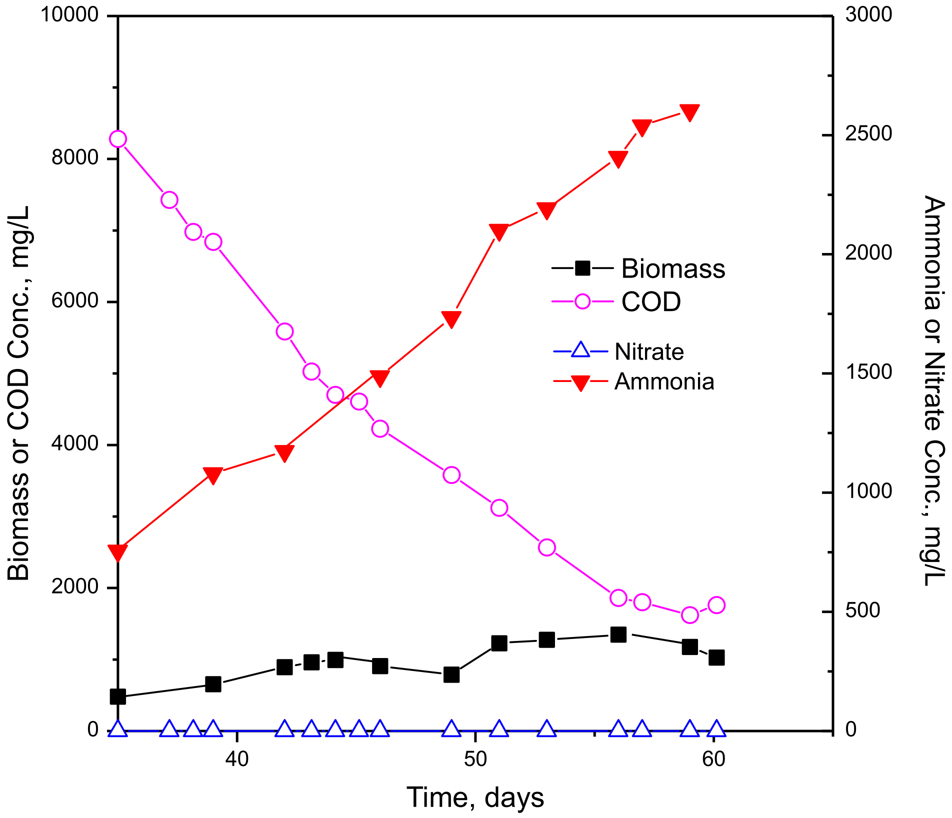

Fig. 2. Batch DS2 degradation under anaerobic conditions.

Hence, DETA, the major component of DS2 may have become the major component that contributed to the overall COD level during the previous accumulation of COD in the bioreactor. In fact, EGME which is another major component of DS2 is more easily degraded than DETA (Kim and DeFrank, 2000).

Nitrification was still not evident in the bioreactor; the NO_3^- concentration was almost zero throughout the entire batch period (Fig. 2). The apparent lack of nitrification in the bioreactor might be due to either inhibition of nitrification by the high level of ammonia or absence of nitrifiers in the mixed culture. To simultaneously remedy both of these two possible causes, the biomass of the reactor was centrifuged, washed, and resuspended in the basal medium to lower the level of accumulated ammonia. The reactor was, then, supplemented with nitrifying activated sludge from a local advanced WWTP.

The operation of the bioreactor was resumed with HRT of 25 d and with 1% DS2 feed (Fig. 3). The effluent COD kept increasing without any sign of nitrification. Therefore, the feed was diluted to 0.5% DS2, while keeping the HRT constant. However, even the 0.5% DS2 did not deter the trend of COD increase, but some limited degradation of DETA was evident, as indicated by the increased ammonia concentration. Also, during this adjustment period for nitrification, two additional attempts to affect nitrification were made by adding additional nitrifying activated sludge. Peaks in the biomass profile in Fig. 3 were observed when nitrifying activated sludge was added to the bioreactor. However, the biomass concentration gradually decreased without apparent nitrification.

The bioreactor was placed under a batch mode for the second time. Twenty days after the continuous aeration, approximately 200 mg L^{-1} of nitrate was detected, indicating that nitrification occurred in the bioreactor. Accordingly, a decrease in the ammonia level was also detected. In particular, the COD level was relatively low (about 350 mg L^{-1}) when nitrification started to occur in the bioreactor.

The high COD concentration of the bioreactor, particularly exerted by undegradable DETA might competitively inhibit nitrification. Nitrifying microorganisms, collectively denoted as nitrifiers are autotrophs that obtain their energy by the oxidations of ammonia to nitrite (e.g., *Nitrosomonas*) and nitrite to nitrate (e.g., Nitrobacter). Particularly, ammonia monooxygenase that oxidize ammonia to hydroxylamine have been shown to be broadly specific toward other substrates in addition to ammonia (Hyman and Wood, 1983; Vannelli et al. 1990), so that nitrification may be inhibited by amino groups present in DETA. To determine a possible effect of DETA inhibition on nitrification, 50 mL batches of nitrifying activated sludge were separately spiked with 120 mg L^{-1} NH_4-N in the presence and absence of 250 mg L^{-1} of DETA. In the absence of DETA, the rate of nitrification was 2.88 mg L^{-1} h^{-1}, which represented 25% substrate inhibition as compared to the nitrification rate of 3.5 mg L^{-1} h^{-1} at the initial ammonia concentration of 30 mg L^{-1}. On the other hand, the rate of nitrification was practically zero in the presence of DETA. Hence, significantly high DETA levels can inhibit nitrification, and process implications of this finding is that both COD (possibly competitive inhibition) and ammonia (substrate inhibition) concentrations in the bioreactor need to be controlled low, so that a process of DS2 degradation and nitrification could be achieved at the same time during the aerobic conditions.

The operation of the bioreactor was resumed with 0.5% DS2 at the same HRT (Fig. 4). Also, at this time, the air on/off (18/6 h in one-day cycle time) was implemented to effect DS2 degradation and nitrification under aerobic conditions and denitrification where the nitrate could be used as an alternate electron acceptor under the anoxic conditions. Furthermore, 5–10 min of sludge

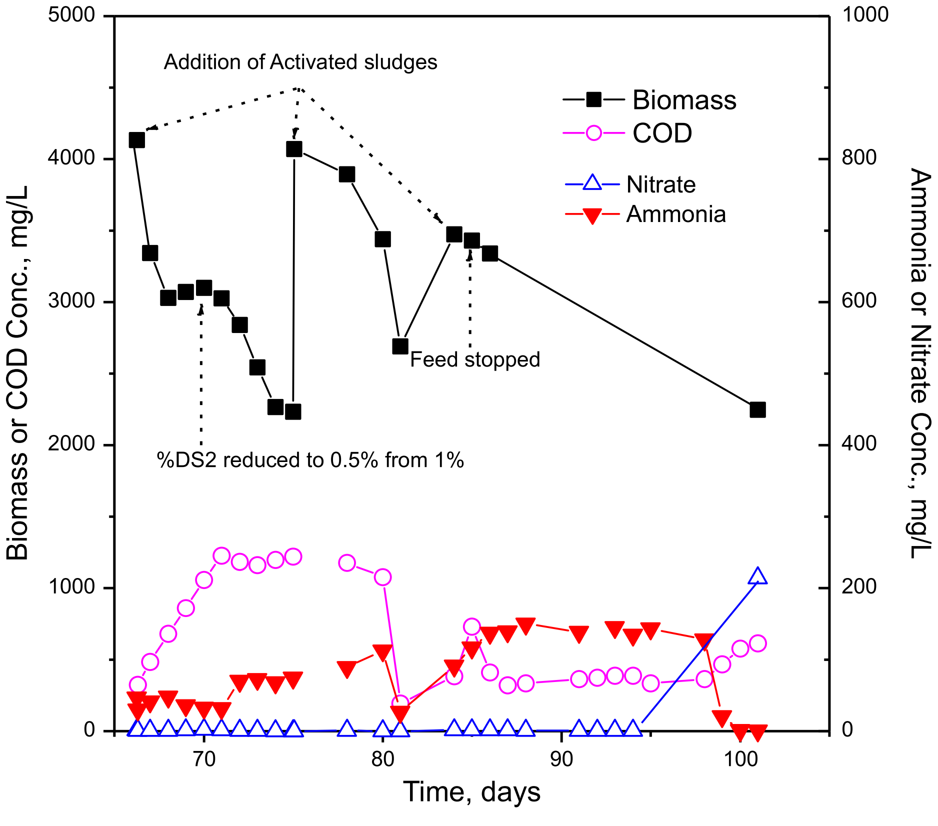

Fig. 3. Acclimation period for nitrification with nitrifying activated sludge.

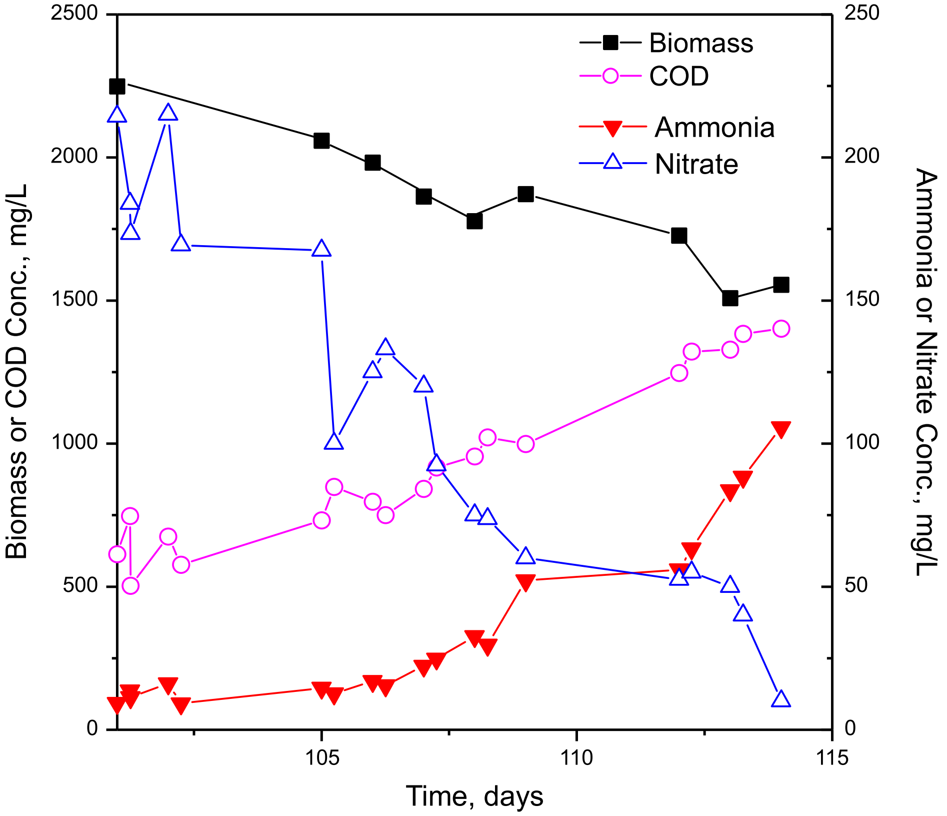

Fig. 4. Start-up period for nitrification/denitrification (HJRT = 25 d with 0.5% DS2).

settling was allowed prior to decanting at each end of the anoxic periods to retain the biomass in the system. Gradually after the start of 0.5% DS2 feeding, the levels of COD and ammonia started to increase, indicating that the DS2 loading may still have been too high. As previously discussed, both competitive and ammonia substrate inhibitions due to a high DS2 loading might be responsible for the simultaneous increases of COD and ammonia at the same time. Meanwhile, the concentrations of biomass and nitrate have been diluted to lower concentrations.

The bioreactor was placed under a batch mode for the third time. Initially after starting the third batch operation for 10 days, the level of ammonia started to increase at the expense of a decreased COD, but the level of nitrate remained low (Fig. 5). Only after the COD level was reduced to 500 mg L^{-1}, the level of nitrate started to increase with concomitant decrease in ammonia, again showing the negative impact of a high COD on nitrification.

Phase II: DS2 degradation, nitrification and denitrification

A step-up strategy in %DS2 in the bioreactor's feed was initiated from 0.1% at a HRT of 16 days to alleviate the unfavorable impact of a high DS2 loading on DS2 degradation and nitrification. By allowing the low COD level at the reduced DS2 loading, a simultaneous DS2 degradation and nitrification could be achieved. At 0.1% DS2 in the feed, both COD and NO_3^- concentrations decrease to pseudo-steady values of 140 and 240 mg L^{-1}, respectively, while the NH_4^+ concentration remained below 15 mg N L^{-1} (Fig. 6). Based on the theoretical 1760 mg COD L^{-1} (data not shown but the actual COD measured at various DETA concentration matched the theoretical COD values) and 285 mg N L^{-1} in 0.1% DS2, pseudo steady values of 140 mg COD L^{-1} and 15 mg NH_4^+-N mg L^{-1} represented approximately 92 and 95% of DS2 degradation and nitrification, respectively. However, despite the imposed anoxic condition, practically no denitrification was evident during this period except a slight decrease in the nitrate concentration due to a dilution. Also, the decrease in nitrate concentration to 240 mg L^{-1} from 260 mg L^{-1} at the start of 0.1% DS2 feed could be attributed to a cycle-to-cycle dilution rather than denitrification, i.e., amount of organic nitrogen mineralized and nitrified being diluted out by the effluent removed per cycle. Since the theoretical nitrogen content of 0.1% DS2 is 285 mg L^{-1}, it can be estimated that approximately 30 mg L^{-1} (285-240-15) of nitrogen is being used for the nitrogen requirement of microbial growth in the bioreactor. Similarly, when the DS2 feed was increased to 0.25% DS2 (Fig. 6), the nitrate concentration started to accumulate without denitrification, eventually reaching a higher pseudo-steady state concentration of approximately 530 mg L^{-1}. The concentrations of effluent COD and NH_4^+-N were below 150 and 10 mg L^{-1}, respectively, yielding approximately 96% and 98% removal efficiencies of COD and NH_4^+, respectively, at 0.25% DS2 feed.

The lack of denitrification during anoxic periods suggested that the two organic components of DS2 could not be used as a carbon source for denitrification. Incidentally, there was always an increase in COD, perhaps due to the undegraded DS2 feed at each end of anoxic periods (data not shown). Consequently, the DS2 feeding during the anoxic periods was paused, and the bioreactor was operated with the same 0.25% DS2 feed only during the aerobic periods to find out if an endogenous nitrate respiration (ENR) of the biomass and/or residual COD from the aerobic periods could potentially reduce the nitrate concentration. As shown in Fig. 7, the continued build-up of nitrate even after the discontinuation of DS2 feed during the anoxic periods suggested that limited ENR capacity of the biomass in the system.

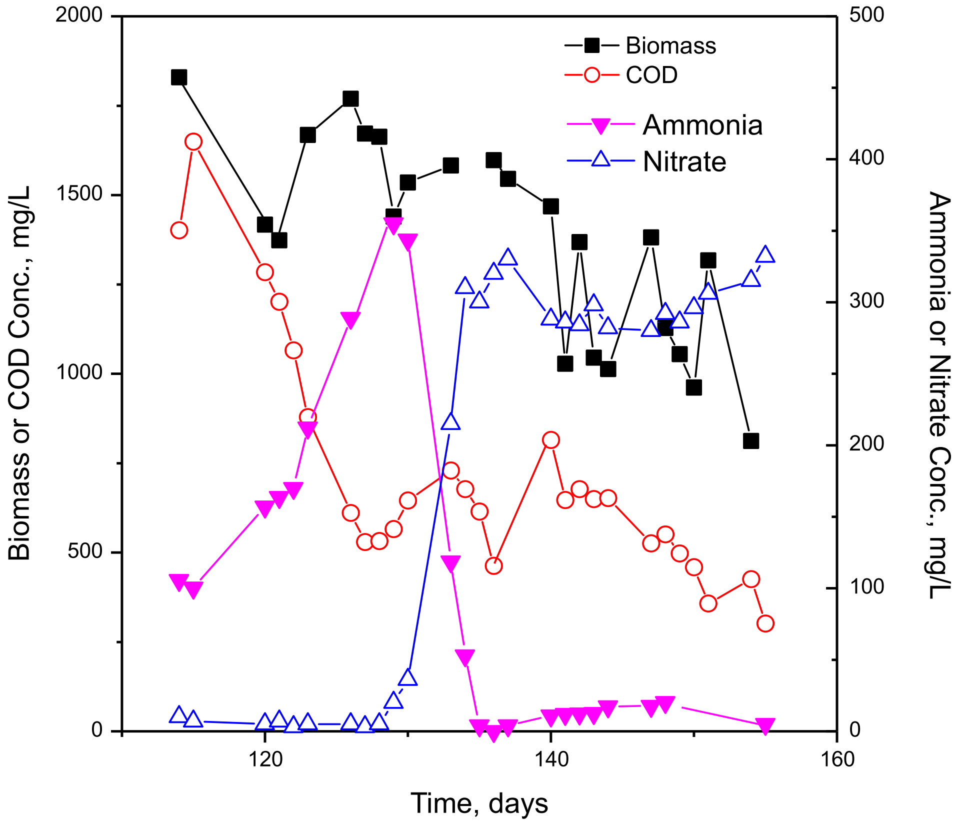

Fig. 5. Third batch DS2 degradation under aerobic conditions.

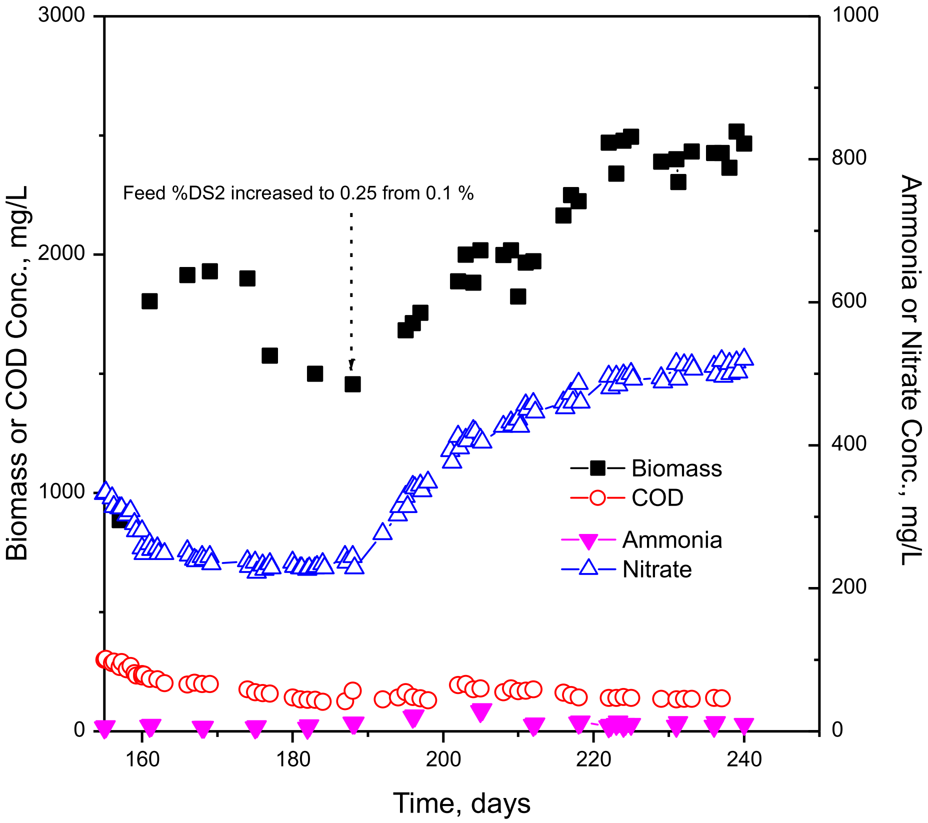

Fig. 6. Continuously fed-batch operation for nitrification/denitrificatiopon with biomass recycle (HRT = 16 d; SRT = 80 d).

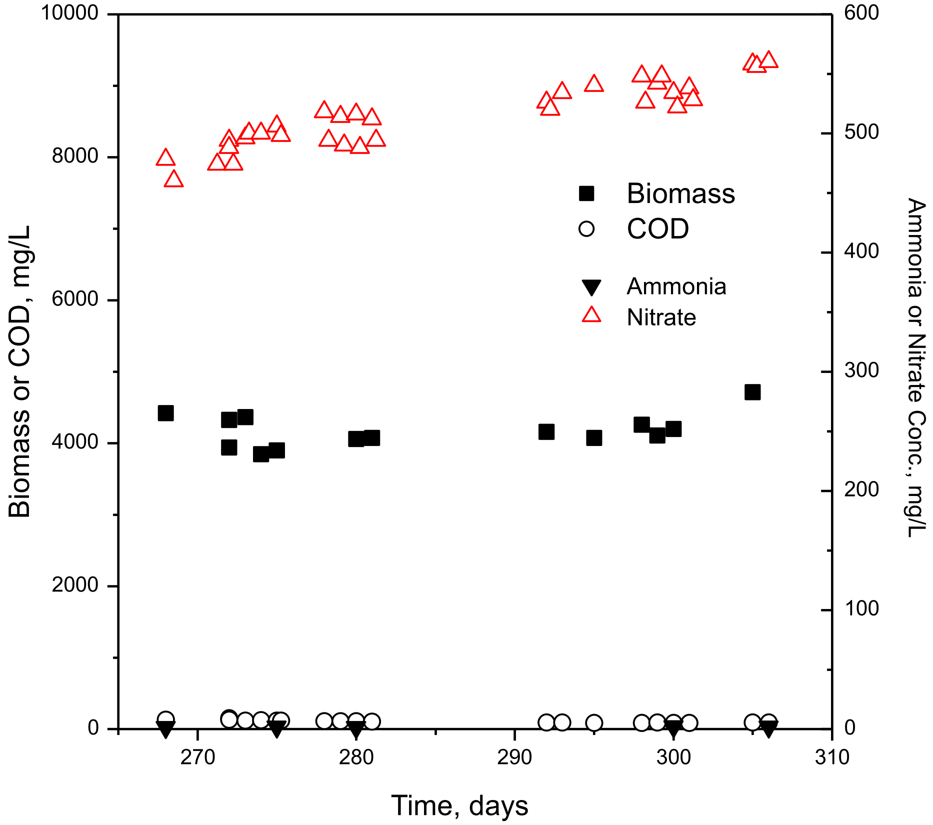

Fig. 7. Build-up of nitrate during discintinuation of DS2 feed under anoxic conditions.

Therefore, methanol, which has been applied as an alternate carbon source during wastewater denitrification processes (Swinarski et al., 2009) was added at the beginning of each anoxic period. Upon the addition of methanol in the beginning of each anoxic period, the concentration of nitrate decreased immediately without any detrimental effect, i.e., both COD and ammonia levels remained low, and, as a result, the % DS2 in the feed was stepwisely increased to 1% DS2 at the same HRT of 16 d (Fig. 8). For the 14 cycles at 0.5% DS2 feed, an average of 101 mg N L^{-1} of NO_3^- was produced over each aerobic period, and an average of 112 mg N L^{-1} of NO_3^- was consumed for denitrification, resulting in the overall decrease in the nitrate concentration. Meanwhile, average concentrations of COD and NH_4^+ at the ends of aerobic cycles during this period were 126 and 1 mg N L^{-1}, respectively, yielding 98% of DS2 degradation and almost 100% nitrification of NH_4^+. For the subsequent eight cycles at 0.75% DS2 feed, the average NO_3^- consumption during each anoxic period was 144 mg N L^{-1}, offsetting the average NO_3^- production of 140 mg N L^{-1} over each aerobic period. The nitrate concentration at the end of each anoxic period remained below 5 mg N L^{-1}. The average concentrations of COD and NH_4^+ at the ends of aerobic periods were 194 and 1.8 mg N L^{-1}, respectively, yielding >98% of DS2 degradation and essentially 99% nitrification. For the subsequent twenty-seven cycles at 1% DS2 feed (equivalent to 17,600 mg L^{-1} of COD and 2850 mg L^{-1} of nitrogen), the average NO_3^- consumption during each anoxic cycle was 130 mg N L^{-1} while the NO_3^- accumulation per cycle was 130 mg N L^{-1}, resulting in no net accumulation of NO_3^-. A slight but steady increase in the effluent COD was noticed as the %DS2 was increased: from 100 mg L^{-1} at 0.1% DS2 to 430 mg L^{-1} at 1% DS2. However, the NH_4^+ concentration remained below 2 mg N L^{-1}.

Several track studies of nitrate reduction were performed during selected anoxic periods at different biomass concentrations (Fig. S1). In each nitrate track study, the denitrification rate was initially zero-order until the concentration of nitrate became a limiting factor to make the rate of denitrification progressively lower. The denitrification rate calculated in each track study was divided by the respective biomass concentration during each track study, yielding the specific denitrification rate (Fig. 9). Over the wide range of biomass concentrations (4000–12,000 mg SS L^{-1}), the specific denitrification rates remained at a relatively constant value (i.e., 4 mg N g^{-1} SS h^{-1}), suggesting that a stable population of denitrifiers was established in the mixed culture system for DS2 degradation, nitrification and denitrification. The specific denitrification rate of 4 mg N g^{-1} SS h^{-1} is one order of magnitude higher than those specific rates encountered in wastewater denitrification (Kim and Hao, 2001) and aerobic/anoxic sludge digestion (Al-Ghusain et al., 1994).

The amount of COD degraded during the nitrate track studies was also used to determine the amount of methanol required for denitrification (Fig. S2). The calculated amount of methanol requirement was 2.78 mg COD mg^{-1} N with a standard deviation of 0.3. Despite the higher specific denitrification rates, the methanol requirement of 2.78 mg COD mg^{-1} N is comparable to those methanol requirements for wastewater denitrification.

Conclusion

In this study, a CFBAAA system was applied for treating DS2, which has a high nitrogen content (approximately 28.5% by weight). Since an alternating aerobic and anoxic cyclic operation (air-on cycle of 16 h and air-off of 8 h) was applied, nitrification and denitrification could be accomplished to result in outstanding nitrogen removal. Overall, the CFBAAA system could achieve COD and nitrogen removal efficiencies of >97% and >99%, respectively,

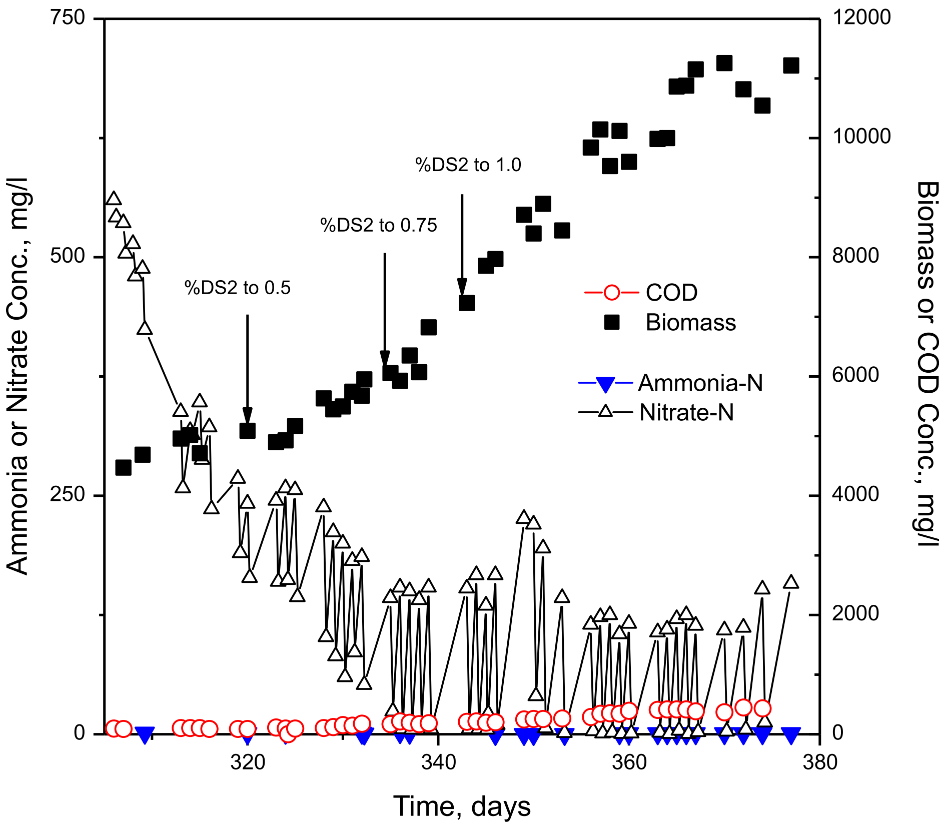

Fig. 8. DS2 degradation with nitrification/denitrification.

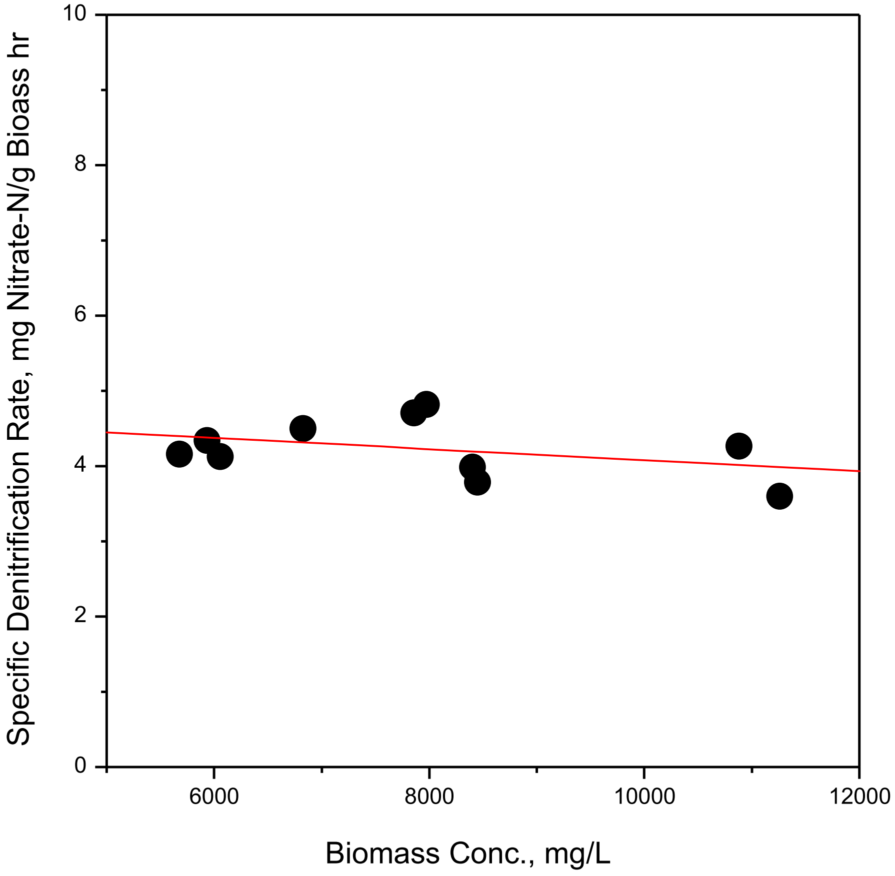

Fig. 9. Specific denitrification rates at different biomass concentrations.

at a HRT of 16 d, when 1% DS2 (equivalent to 17,500 mg COD L^{-1} and 2850 mg N L^{-1}) was fed.

From the result, it is concluded that the currently developed system can be applied for treatment of used detoxification solutions for warfare agents like DS2 and other wastewater containing a high amount of nitrogen.

Acknowledgments

This research was supported in part by an appointment to the Research Participation Program at the U.S. Army Edgewood Chemical Biological Center (ECBC – United State) administered by the Oak Ridge Institute for Science and Education Program through an interagency agreement between the U.S. Department of Energy and ECBC. H. Kim was financially supported by the Korea Institute of Energy Technology Evaluation and Planning (KETEP – South Korea) (Grand # 2017010000780).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.wen.2018.05.002.

References

Al-Ghusain, I., Huang, J., Hao, O.J., Lim, B.S., 1994. Using pH as a real-time control parameter for wastewater treatment and sludge digestion processes. Water Sci. Technol. 30, 159–168.

Baqchi, G., Waxman, D.J., 2008. Toxicity of ethylene glycol monomethyl ether: impact on testicular gene expression. Int. J. Androl. 31, 269–274.

Boden, R., Thomas, E., Savani, P., Kelly, D.P., Wood, A.P., 2008. Novel methylotrophic bacteria isolated from the River Thames (London, UK). Environ. Microbiol. 10, 3225–3236.

Calmels, S., Ohshima, H., Vincent, P., Gounot, A.-M., Bartsch, H., 1985. Screening of microorganisms for nitrosation catalysis at pH 7 and kinetic studies on nitrosamine formation by *E. coli* strains. Carcinogenesis 6, 911–915.

Anon, 2006. Dow to expand ethyleneamines. Chem. Eng. News 84 (22), 16.

Chistoserdova, L., 2011. Modularity of methylotrophy, revisited. Environ. Microbiol. 13 (10), 2603–2622.

Chistoserdova, L., Kalyuzhnaya, M.G., Lidstrom, M.E., 2009. The expanding world of methylotrophic metabolism. Ann. Rev Microbiol. 63, 477–499.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Franson, M.A.H., 2005. APHA: Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF, Washington, DC.

Emtiazi, G., Knapp, J.S., 1994. The biodegradation of piperazine and structurally-related linear and cyclic amines. Biodegradation 5, 83–92.

Harvey, S.P., Szafraniec, L.L., Beaudry, W.T., Haley, M.V., Rosso, T.E., 1996. HD Hydrolysis/Biodegradation Toxicology and Kinetics (No. ERDEC-TR-382). Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD, USA.

Henriks-Eckerman, M.-L., Laijoki, T., 1985. Ion-pair high-performance liquid chromatographic determination of dansylated aliphatic polyamines with fluorescence and ultraviolet detection. J. Chromatogr. 333, 220–224.

Huang, Y.-L., Li, Q.-B., Deng, X., Lu, Y.-H., Liao, X.-K., Hong, M.-Y., Wang, Y., 2005. Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes. Process Biochem. 40, 207–211.

Hutanu, D., Frishberg, M.D., Guo, L., Darie, C.C., 2014. Recent applications of polyethylene glycols (PEGs) and PEG derivatives. Mod. Chem. Appl. 2 (2), 132.

Hyman, M.R., Wood, P.M., 1983. Methane oxidation by *Nitrosomonas europaea*. Biochem. J. 212, 31–37.

Kim, H., Hao, O.J., 2001. pH and oxidation–reduction potential control strategy for optimization of nitrogen removal in an alternating aerobic–anoxic system. Water Environ. Res. 73 (1), 95–102.

Kim, M.H., DeFrank, J.J., 2000. U.S. Patent No. 6,054,310. Washington, DC: U.S. Patent and Trademark Office.

Kingsbury, N.R., 1990. DOD Should Eliminate DS2 from Its Inventory of Decontaminants, U.S. General accounting office, UNCLASSIFIED Report.

Lin, J.-K., Lai, C.-C., 1980. High performance liquid chromatographic determination of naturally occurring primary and secondary amines with dabsyl chloride. Anal. Chem., 53, 630–635. Manual, Washington, D.C..

Nancharaiah, Y.V., Schwarzenbeck, N., Mohan, T.V.K., Narasimhan, S.V., Wilderer, P. A., Venugopalan, V.P., 2006. Biodegradation of nitrilotriacetic acid (NTA) and ferric-NTA complex by aerobic microbial granules. Water Res. 40 (8), 1539–1546.

Paulo, A.M.S., Aydin, R., Dimitrov, M.R., Vreeling, H., Cavaleiro, A.J., García-Encina, P. A., Stams, A.J.M., Plugge, C.M., 2017. Sodium lauryl ether sulfate (SLES) degradation by nitrate-reducing bacteria. Appl. Microbiol. Biotechnol. 101 (12), 5163–5173.

Richardson, G.A., 1972. Development of a Package Decontaminating System, EACR-1310-17, Edgewood Arsenal, Aberdeen Proving Ground, MD, Unclassified Report (AD-B905475L).

Roose, P., Turcotte, M.G., 2016. Amines, lower aliphatic. Kirk-Othmer Encylopedia Chem. Technol., 1–20

Sak-Bosnar, M., Madunić-Čačić, D., Grabarić, Z., Grabarić, B., 2015. Potentiometric determination of anionic and nonionic surfactants in surface waters and wastewaters. In: Milačič, R., Ščančar, J., Paunović, M. (Eds.), The Sava River. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 157–176.

Singh, B., Prasad, G.K., Pandey, K.S., Danikhel, R.K., Vijayaraghavan, R., 2010. Decontamination of chemical warfare agents. Defence Sci. J. 60 (4), 428–441.

Swinarski, M., Makinia, J., Czerwionka, K., Chrzanowska, M., Drewnowski, J., 2009. Comparison of the effects of conventional and alternative external carbon sources on enhancing the denitrification process. Water Environ. Res. 81, 896–906.

Vannelli, T., Logan, M., Arciero, D.M., Hooper, A.B., 1990. Degradation of halogenated aliphatic compounds by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. Appl. Environ. Microbiol. 56, 1169–1171.

Wang, W., Ren, S., Zhang, H., Yu, J., An, W., Hu, J., Yang, M., 2011. Occurrence of nine nitrosamines and secondary amines in source water and drinking water: potential of secondary amines as nitrosamine precursors. Water Res. 45 (16), 4930–4938.

Wen, J., Stacey, S.P., McLaughlin, M.J., Kirby, J.K., 2009. Biodegradation of rhamnolipid, EDTA and citric acid in cadmium and zinc contaminated soils. Soil Biol. Biochem. 41 (10), 2214–2221.