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Employing acclimated activated sludge for biological treatment of leather manufacturing wastewater containing high dimethylamine (DMA) concentrations

Forough Mehravaran[a], Narges Fallah[a,*], Leila Davarpanah[b]

[a]*Biotechnology and Environmental Group, Faculty of Chemical Engineering, Amirkabir University of Technology, Tehran, Iran, Tel. +982164543199; emails: nfallah2001@aut.ac.ir (N. Fallah), f.mehravaran@aut.ac.ir (F. Mehravaran)*
[b]*Environmental Group, Energy Department, Materials and Energy Research Center, Tel. +982164543199; email: leiladavarpanah@gmail.com (L. Davarpanah)*

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ABSTRACT

The wastewater of artificial leather industry is contaminated with high levels of amin products that were discharged into it. The amin products can be degraded through biological methods, but no studies were performed until now on the real wastewater containing dimethylamine (DMA). In this study, the treatment of real wastewater containing high concentration of DMA by activated sludge under aerobic and anaerobic conditions was investigated. The aerobic process could reduce DMA significantly (89%), while chemical oxygen demand (COD) removal efficiency was found to be the highest under anoxic conditions (93%). Results of experiments indicated that the biodegradation efficiency of anaerobic–anoxic–aerobic (AAA) process with respect to DMA and COD removal efficiencies was the best condition. Additionally, the accumulation of ammonium and nitrate in this process would be defeated. At the end of the incubation time, a COD removal efficiency was 94%, which suggests better performance of the used sequential process. Further work was directed toward gas chromatography/mass spectrometry analysis of the Anaerobic Leather Wastewater and the AAA effluent samples. The results of the sequential AAA process showed its significant role in high DMA-containing wastewater treatment and its application using sequencing batch reactor technology is currently under investigation in the laboratory. Biodegradability of DMA-containing wastewater was evaluated applying three different redox conditions (anaerobic, anoxic, and aerobic). As well as the reduction of organic compounds in a combined AAA process was also evaluated. Using pre-. acclimated activated sludge and DMA removal followed the order aerobic > anoxic > anaerobic.

Keywords: Dimethylamine; Artificial leather manufacturing wastewater; Biodegradation; Wastewater treatment; Acclimated activated sludge

1. Introduction

The environmental problems associated with industrial wastewater have represented serious environmental challenges [1–3]. Artificial leather industry is known as a polluting industry in terms of high levels of dimethylamine (DMA) in its discharge. The concentration of DMA in artificial leather manufacturing wastewater has been reported to be as much as several hundred to thousand milligrams per liter [4].

DMA drains from different pharmaceutical, chemical, dye, pesticide, and petrochemical industries that has established as an important difficulty [5]. DMA includes of a robust, powerful, and fishy odor, which is inevitable and makes a public irritation [6]. Also, it is stated to origin headaches, dermatitis, and conjunctivitis and has been recognized as a possible neurotoxin in uremic skills [5].

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* Corresponding author.

DMA as a priority pollutant for which strict emission standard has been legislated, possesses an odorous smell. DMA is also used in chemical and pharmaceutical industries as well as in making pesticides, surfactants, dyes and explosives [7,8]. It is also formed during the industrial synthesis of certain solvents, such as tetramethylthiuram disulfide, and N,N-dimethylformamide [9].

In advanced water treatment processes, N-nitrosodimethylamine (NDMA) is formed as a byproduct being an extremely potent carcinogen [10]. As a consequence, being a potent carcinogen, NDMA formation should be avoided by separating its precursors (i.e., DMA) from wastewaters [10].

A wide study has been performed for the removal of NDMA ancestor [11]. Therefore, it is appropriate to investigate approaches for the abolition of DMA discharged from different springs. Some remediation methods were reported for the abolition of DMA [12,13]. Recently Gao et al. [14] have introduced the DMA adsorption by an adsorbent (beta-zeolite) in a chemical process. Such choices are not practical, because there is no remediation of the DMA, it just transfers the pollutant from one phase to another that further treatment is needed [14]. A significant method to remove the poisonous and dangerous contaminant from the environment is Microbial degradation. DMA has been oxidized by the bacteria and lead to monomethylamine and lastly to ammonia [15].

Different physicochemical processes such as photo catalytic degradation [7], adsorption and thermal decomposition [16], and adsorption onto ion exchange resins [17] have been used to remove DMA from synthetic wastewaters, but they suffer from high operating costs. In addition, the biological treatment is very economic in industrial scale than advanced oxidation processes [18–21].

Biological degradation is an efficient method for removing DMA from wastewater which finally converted DMA to methylamine and formaldehyde [9,22]. Using pure culture and activated sludge is common for treating wastewater-containing DMA. For example, Liao et al. [8] used activated sludge to eliminate 10 mg/L of the DMA from aqueous solutions while the concentration of DMA in real wastewater is much higher than 10 mg/L. Also they reported that glucose supplementation could enhance DMA biodegradation while, no significant impact was observed when ammonia nitrogen was supplied [8]. The literature review reveals that no study has been performed on bio-reduction of high concentrations of DMA. In the contrast of the previous research [23–25], the design of experiment was not used in the present work.

The aim of this study is the biodegradation of leather manufacturing wastewater containing high levels of DMA by activated sludge. The reduction of organic compounds during aerobic, anoxic and anaerobic conditions as well as a combined aerobic–anoxic–anaerobic (AAA) process was also evaluated.

2. Materials and methods

2.1. Microbial inoculum and wastewater

The activated sludge was obtained from sludge recycle stream from the sedimentation tank of a municipal wastewater treatment plant (Ekbatan, Tehran, Iran). The main characteristics of the artificial leather manufacturing wastewater are presented in Table 1.

The schematic diagram of the laboratory set up used at this process is presented in Fig. 1.

2.2. Adaptation of activated sludge

Phosphorus and other mineral nutrients were supplied to the feed as a phosphate-buffered medium (PBM) according to Yang et al. [26]. The composition of m containing inorganic compounds and added trace elements are listed in Table 2.

The adaptation of activated sludge was performed in two phases and by inoculating a 6 L reactor with sludge liquor having an initial concentration of mixed liquor suspended solid (MLSS) of 2,008 mg/L and using Artificial Leather Manufacturing Wastewater (ALMW) as the carbon source and subsequent incubation under aerobic conditions.

The activated sludge was aerated in the reactor for 46 h in an aerobic mode followed by a 2 h retention time and a volume of 2 L of the upper part was replaced with fresh ALMW and PBM. The enrichment process for each phase was repeated until the COD (chemical oxygen demand) removal efficiency (CRE) and MLSS reached a stable level. In the preliminary experiments, a low level of ALMW (i.e., a COD content of 530 mg/L) was supplied to the feed to promote microbial growth and the activated sludge was further exposed to higher ALMW levels (COD of 1,590 mg/L) and in these experiments glucose was added as the co-metabolic substrate having concentrations of 810 and 160 mg/L, respectively. The COD of the feed was adjusted to 1,000–1,200 mg/L during the acclimation process using two sources of carbon (i.e., ALMW and glucose).

2.3. Biodegradation experiments

Batch experiments were performed to investigate the biodegradation of leather wastewater under different redox conditions of aerobic, anoxic, and anaerobic. Biodegradation tests were conducted in a rotary shaker at 30°C and 170 rpm. The dissolved oxygen (DO) contents and definitions for each test are shown in Table 3.

The initial COD of the wastewater was 1,500 mg/L with real wastewater and a constant level of 160 mg/L glucose as the co-substrate. About 1 mL of nutrients was added

Table 1
Characteristics of real wastewater taken from Tehran Delta manufacturing plant

Parameter	Value
Total phosphorus	0 (mg/L PO_4^-–P)
TOC	585 (mg/L)
Ammonium	15.6 (mg/L NH_4^+–N)
Nitrate	0.441 (mg/L NO_3^-–N)
COD	1,590 (mg/L)
DMA	3,865 (mg/L)
pH	9.2

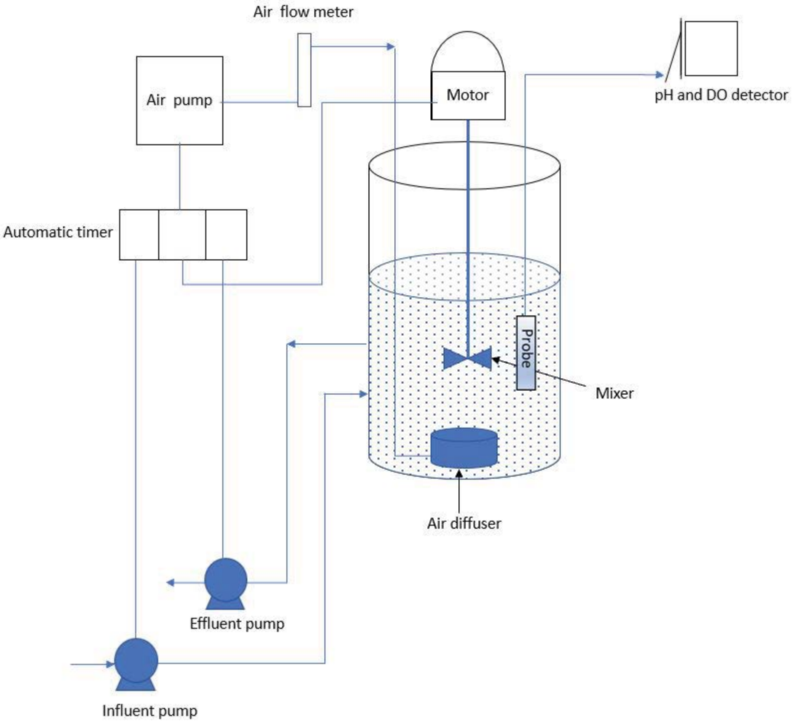

Fig. 1. Schematic diagram of the laboratory set up used in this process.

to the feed. The initial MLSS concentration of each serum bottle was 2,500 mg/mL. Samples in anaerobic and anoxic processes were sealed by a butyl rubber stopper. In order to keep the anaerobic condition, nitrogen gas was purged to the wastewater for 20 min. The treated wastewater was withdrawn and transferred to serum bottles to inspect the AAA experiments, at the end of each condition and after settlement of the sludge.

At each condition, with 48 h incubation time, parameters such as pH, COD, NO_3–N, NH_4–N, MLSS as well as DMA determination and formaldehyde concentrations were monitored throughout the treatments. All operations were done in triplicate.

2.4. Analytical methods

The COD and MLSS concentrations of the samples were determined according to Standard Methods [27]. The concentration of the remaining DMA was estimated directly using spectrophotometry in which the reaction of carbon disulfide with DMA and ammonium favors extraction of highly polar secondary aliphatic amines from aqueous media via formation of a stable complex (dialkyl dithiocarbamate) in the presence of metal ions [28]. Merck–Spectroquant analytical test kits were used to analyze nitrate (Kit no. 1.14773.0001) using a JASCO 550 UV/vis spectrophotometer (Tokyo, Japan). Ammonium concentration was determined based on the method of Fawcett and Scott [29]. Formaldehyde concentration in the samples was also measured through its spectrophotometric properties as described by Habibi and Vahabzadeh [30].

Gas chromatography/mass spectrometry (GC/MS) analysis was carried out using a GC/MS (GC-Agilent Technologies 6890 N, mass selective detector 5973 N, Tokyo, Japan) equipped with HP-5 column (30 m × 0.25 mm × 0.25 µm). The samples were prepared according to Umbreit [28] and running conditions were as follows:

The temperature program started at 50°C and held at 3 min, 10°C/min ramp to a final temperature of 300°C and held isothermally at 300°C for 10 min. The injector temperature was 270°C.

Dissolved oxygen (DO) in the samples was recorded using a HACH HQ40D digital multi-parameter with intellical LDO probe (Model LDO10101).

3. Results and discussion

3.1. Effect of different redox conditions on biodegradation of leather wastewater

3.1.1. Aerobic conditions

The profile of the time courses of COD changes as well as the growth of microorganisms during aerobic biodegradation of artificial leather manufacturing wastewater is shown in Fig. 2a. As it is shown in the Fig. 2, at 160 mg/L as the initial glucose concentration, the biomass level increased and reached to 6,148 ± 310 mg/L at the end of the logarithmic cell growth phase. COD removal efficiency under aerobic condition was found to be 77% during an incubation time of 48 h. Additionally, the DMA removal efficiency was measured to be 89% ± 0.77%. The trend of NH_4–N and NO_3–N changes is also presented in Fig. 2b.

The nitrate level increased slowly within 48 h while ammonium concentration increased. The results were indicative of the strong potential of enriched activated sludge in aerobic biodegradation of the DMA as NH_4–N concentration reached to 369.5 ± 16 mg/L which suggests high mineralization of organic materials in wastewater. The accumulation of metabolite ammonia has been reported by several researchers [8,22,31]. Liao et al. [8] reported mineralization of DMA to inorganic carbon as a result of carbon source amendment. Formation of formaldehyde during biodegradation (data not shown here) also confirmed dimethyl amine dehydrogenase catalysis in the oxidation of DMA [22].

Measurement of DMA in the gas phase of serum bottles showed that despite the volatility of DMA, due to high concentration of biomass, no DMA was observed in the head space of serum bottles.

3.1.2. Anoxic conditions

The plots of COD and cell growth vs. incubation time under anoxic condition (DO = 0.5 mg/L) are presented in Fig. 3a. As it is shown in that figure, dry cell weight slightly changed within the first 24 h and a maximum biomass concentration of 3,310 ± 61 mg/L was observed in the late log phase (i.e., 36 h) and the growth was stopped afterwards. The cell growth under anoxic condition in the present study was much lower (more than half) than that observed in the presence of oxygen as the electron accepter agent. The CRE under anoxic conditions during 12 h was 72% and changed slightly afterwards until reaching to 86% at the end of the incubation time, which was higher than that observed under aerobic conditions (The CRE changed from 9% to 77% under aerobic condition during the last 36 h). Although the CRE was found to be higher under anoxic condition, the DMA reduction percentage was much lower than that of under oxic condition (47% ± 1.3% vs. 89% ± 0.77%) and the quantity of CRE was higher compared to the value of DMA removal efficiency. This phenomenon might be due to the low value of COD to theoretical oxygen demand (ThOD) ratio for DMA (COD/THOD = 0.03) [32] therefore, the contribution of changes in DMA concentration on COD variations is low. Additionally, the CRE is not only affected by DMA changes via biodegradation, but also other unknown organic compounds possibly present in the Anaerobic Leather Wastewater (ALW) play role. The results suggest that CRE was positively influenced at lower redox conditions while it adversely affected DMA reduction. A similar result has been reported by Wang and Li [10] on DMA biodegradation by activated sludge isolated from municipal wastewater treatment plant and it was shown that DMA biodegradation was higher under anoxic condition.

Table 2
Characteristics of nutrients used for microbial growth

Material	Concentration (mg/L)
$MgSO_4{\cdot}7H_2O$	200
K_2HPO_4	1,500
KH_2PO_4	1,500
$CaCl_2{\cdot}2H_2O$	20
$FeSO_4{\cdot}7H_2O$	3
$MgCl_2{\cdot}2H_2O$	1.8
$CoCl_2{\cdot}6H_2O$	1.06
$Na_2MoO_4{\cdot}2H_2O$	0.34

The ammonium nitrogen level during incubation time reached to a maximum value of 129 ± 3.6 mg/L after 36 h and decreased to 45.5 ± 4.3 mg/L at the end of the process while the nitrate–nitrogen level was almost unchanged (Fig. 3b).

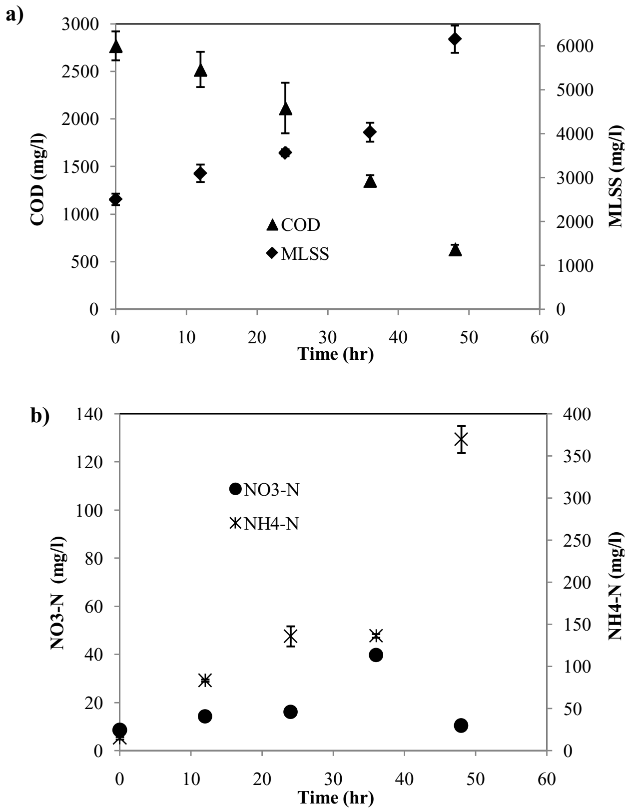

Fig. 2. Time course of the concentrations of COD and MLSS (a) and nitrate and ammonia levels (b) during ALMW biodegradation under aerobic condition.

Table 3
Specification of different redox conditions applied during biodegradation experiments

No.	Abbreviation	Redox condition	Sample volume (mL)	DO (mg/L)	Incubation time (h)
1	A-1	Aerobic	200	7–8	48
2	AN-1	Anoxic	300	0.5	48
3	ANA-1	Anaerobic	350	ND	48

* ND – Not detected

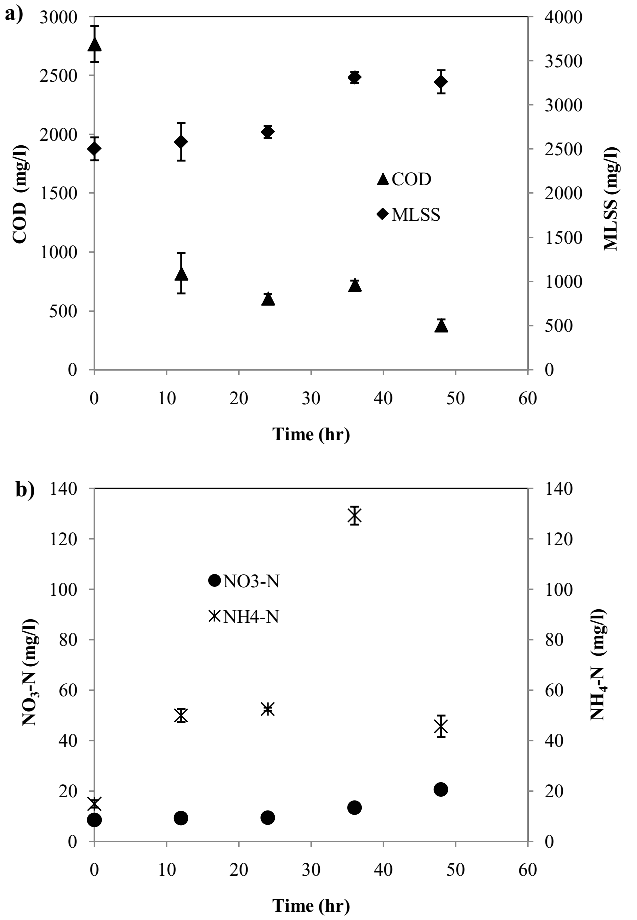

Fig. 3. Time course of the concentrations of COD and MLSS (a) and nitrate and ammonia levels (b) during ALMW biodegradation under anoxic condition.

Accumulation of ammonium–nitrogen under anoxic condition is much lower than that of under oxic condition. This reduction of ammonium accumulation could be attributed to less DMA removal efficiency under lower redox condition. Higher values of CRE observed under anoxic condition were due to lower ammonium accumulation which had a less inhibitory effect on both nitrifying and heterotrophic bacteria [10].

Ammonium could also be reduced via partial denitrification through NO_2^- [33]. The SHARON[1] process in which nitrification and denitrification occur via nitrite pathway has attracted attention and can be shown as follows:

$$\begin{tikzcd} NH_4^+ \arrow[r] & NO_2^- \arrow[r] \arrow[rr, bend right] & NO_3^- \arrow[r] & NO_2^- \arrow[r] & NO \arrow[r] & N_2O \arrow[r] & N_2 \end{tikzcd} \quad (1)$$

The reduction of oxygen and organic carbon requirements in this partial denitrification process makes it suitable for treatment of high ammonium containing wastewaters [34,35]. In the last 12 h of incubation time, it can be suggested that the decreasing trend of ammonium–nitrogen concentration was due to nitrite production through the SHARON process while nitrate–nitrogen level slightly increased and the activity of nitrite–oxidizing bacteria was inhibited by ammoina–oxidizing bacteria under anoxic conditions [34]. The nitrite production in the late incubation time was further confirmed using Merck-Spectroquant analytical test kits (Kit no. 114776) spectrophotometrically.

The constant level of COD in the second day of incubation can be explained by the low requirement of organic carbon source in the SHARON process as reported by Verstraete and Philips [35].

3.1.3. Anaerobic conditions

Further investigation was carried out in the present work considering the anaerobic redox condition (DO < 0.06) and Fig. 4a shows the time course of both COD and dry cell weight within 48 h. As can be seen in that figure, biomass growth on ALW was low which is typical of anaerobic processes and the specific growth rate was measured to be 0.012 h^{-1}. During the first 24 h, the cell concentration was almost unchanged and a maximum amount of 3,081 ± 62 mg/L was attained at the late log phase. On the other hand, COD decreased 60% during the first 12 h and reached to an amount of 657 ± 98 mg/L at the end of the incubation process (total reduction of 76%). Although no acclimation process was performed for anaerobic process, no lag phase was observed in the early stages of incubation. Biomass growth as well as COD removal efficiency was under influence of the applied redox condition and here in the present study a lower value for yield was observed in an anaerobic process (i.e., 0.54 vs. 1.88 for aerobic processing).

Moreover, the DMA removal percentage in this redox condition was found to be 27%, which is much lower than that obtained by the two other redox conditions (i.e., aerobic and anoxic).

Meiberg and Harder [22] investigated DMA biodegradation by *Hyphomicrobium* X under aerobic and anaerobic conditions. They concluded that DMA removal efficiency was higher under aerobic condition and formation of formaldehyde and methylamine is involved in the biotransformation of DMA by dimethylamine dehydrogenase. On the other hand, NDMA was formed as an intermediate metabolite during anaerobic biodegradation of DMA which was further degraded to formaldehyde and methylamine [22]. This additional step extended the biodegradation process for attaining the same amount of DMA removal efficiency as obtained by aerobic process.

Fig. 4b also shows the changes of NH_4–N and NO_3–N as a function of time under anaerobic condition within 48 h.

As it is shown, nitrate production during biodegradation was almost low. On the other hand, NH_4–N reached a maximum value of 60 ± 1.3 mg/L and showed a decreasing trend after the first 12 h along with no significant reduction of COD. Ammonium reduction under anaerobic conditions in the present study suggests occurrence of ANAMMOX process with nitrite as the electron acceptor. In the ANAMMOX process, there is no need for COD in autotrophic ammonium oxidation to dinitrogen gas, which

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[1] Single reactor system for high rate ammonium removal over nitrate .

is in agreement with the unchanged trend of COD as well as reduction of nitrite (data not shown here) in the 2nd day of biodegradation experiments [36]. In DMA biodegradation process, the possible pathway is the production of ammonium and formaldehyde by diemethylamine dehydrogenase [31] and here in the present work, the lower ammonium accumulation in the anaerobic process could be due to the lower amount of biodegraded DMA which is in agreement with the value of 27% ± 1.6% observed as DMA removal efficiency.

3.1.4. AAA process

Sequential biological treatment strategies using activated sludge have been used as a cost-effective technology in the area of wastewater treatment. Through this process azo dye containing wastewater has been subjected to decolorization under anaerobic conditions while, the recalcitrant aromatic amines released from this step were further degraded aerobically [37]. The three step process of AAA has been also known for nutrient removal [38] while, pre-denitrification of wastewaters has been performed in many treatment processes shortening the aerobic stage [33]. Removal of NDMA and its precursors in six wastewater treatment plants with the use of different treatment technologies, including the AAA process was investigated by Wang et al. [39]. The results of their study revealed that DMA removal efficiencies in all applied processes including, AAA were greater than 97%. In the present study, the performance of the AAA process in treating ALW wastewater was also evaluated and Fig. 5a shows the changes in COD and microbial biomass growth during 6 d. In the first stage of the process (i.e., anaerobic phase), the trend of changes was the same. In the second phase, the cell growth increased and remained constant afterwards. The contribution of the following stages (i.e., anoxic–aerobic stages) in total COD reduction was found to be 18%. At the end of the incubation time, a COD removal efficiency of 94% was obtained which suggests the better performance of the used sequential process. The trend of NH_4–N and NO_3–N as a function of time is presented in Fig. 5b.

As it is shown, slight increase in ammonium concentration at the end of the second stage was observed indicative of organic carbon removal which is in agreement with the change in nitrate level. The accumulated ammonia was defeated and the AAA process efficiency in treating ALW wastewater was established.

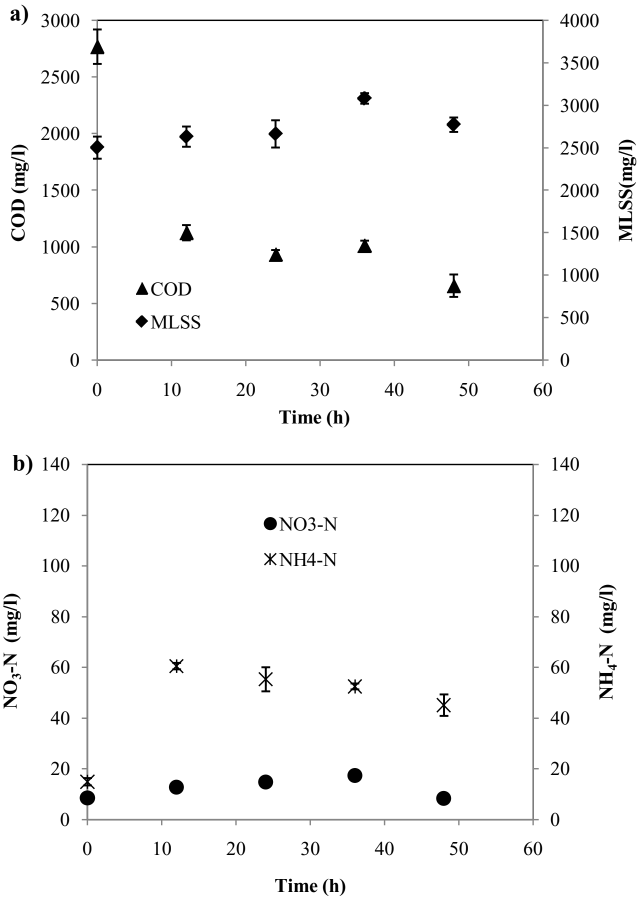

Fig. 4. Time course of the concentrations of COD and MLSS (a) and nitrate and ammonia levels (b) during ALMW biodegradation under anaerobic condition.

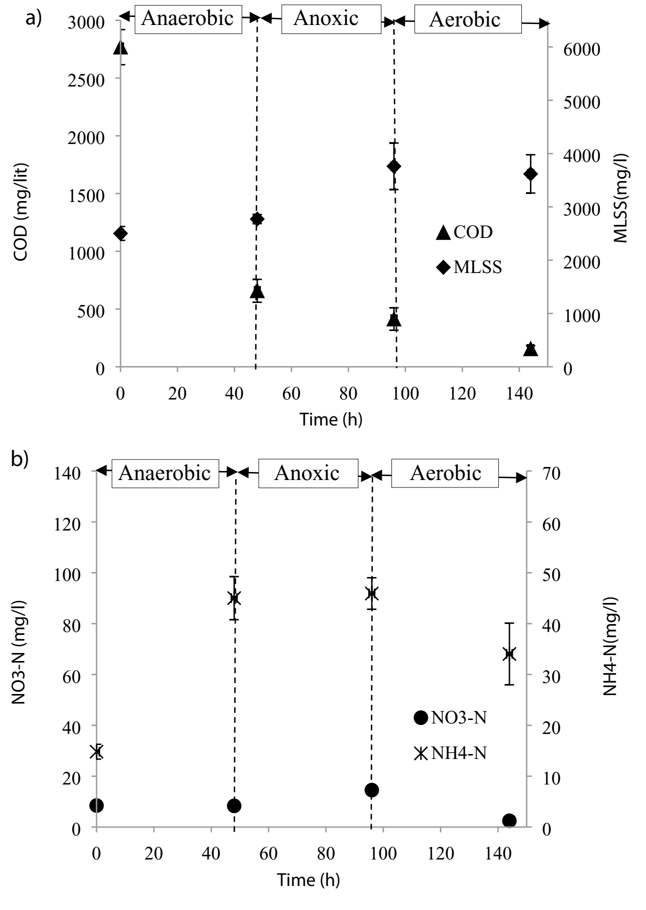

Fig. 5. Time course of the concentrations of COD and MLSS (a) and nitrate and ammonia levels (b) during ALMW biodegradation under combined AAA process.

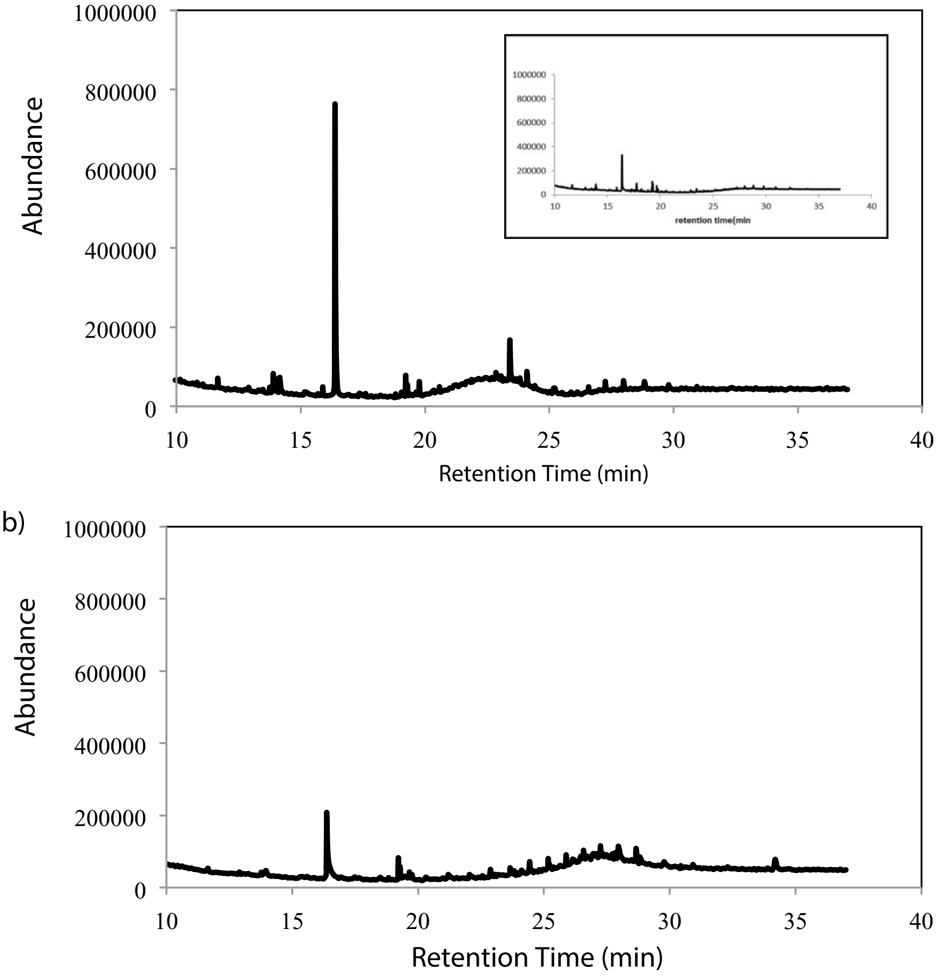

Fig. 6. GC/MS chromatogram of untreated ALMW (a) and treated ALMW in the AAA effluent (b). The insert shows the chromatogram of DMA aqueous solution having concentration of 2,000 mg/L for comparison.

In the study of Wang and Li [10], the biodegradation of DMA in AAA process was found to be higher than those of each individual redox conditions. Here in the present work and considering the DMA removal under AAA condition (i.e., 93% ± 1.7%), the better efficiency of this combined process is confirmed compared to that of each single redox conditions. Additionally, the high DMA biodegradation efficiency of 89% ± 0.77% observed under aerobic condition coincided with accumulation of high levels of ammonium which would be undesirable for discharge of wastewaters and requires subsequent nitrification–denitrification processes [40]. The limitations of high consumption of electrical energy for aeration as well as the requirement of high sludge treatment cost are driving forces to develop low-cost biological processes [41], and considering the economic balance of the treatment technologies, this process is of significant importance.

Further work was directed toward GC/MS analysis of the ALW and the AAA effluent samples and Fig. 6 shows the corresponding chromatograms.

As can be shown, high removal efficiency of DMA (the peak at 16.38 min which is validated by GC/MS analysis of pure DMA solution shown as the insert in Fig. 6a) was observed, which compares favorably with DMA removal result.

The results of the sequential AAA process showed its significant role in high DMA-containing wastewater treatment and its application using sequencing batch reactor (SBR) technology is currently under investigation in our laboratory.

4. Conclusion

Biodegradability of DMA-containing wastewater was evaluated applying three different redox conditions (anaerobic, anoxic, and aerobic) using pre-acclimated activated sludge and DMA removal followed the order aerobic > anoxic > anaerobic. At the end of the incubation time, a COD removal efficiency was 94%, which suggests the better performance of the used sequential process. Further work was directed toward GC/MS analysis of the ALW and the AAA effluent samples. The results of the sequential AAA process showed its significant role in high DMA-containing wastewater treatment and its application using SBR technology is currently under investigation in the laboratory. The accumulation of ammonium formed from partial mineralization of DMA also followed the same order while this trend was not observed considering the effluent COD removal efficiency due to low ratio of COD to THOD for DMA and little contribution of decomposed DMA in observed quantity for COD removal. Other organic materials possibly existed in ALMW may have role in lower COD removal efficiency of aerobic biodegradation compared to that observed under anoxic condition. In the AAA system, the removal of DMA and COD was found to be higher than each individual redox conditions and accumulation of ammonium was reduced. These aspects draw attention to the potential of using the AAA process to treat high DMA-containing ALMW in wastewater treatment technologies.

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