USE OF TWO-STAGE BIOLOGICAL PROCESS IN TREATING THIN FILM TRANSISTOR LIQUID CRYSTAL DISPLAY WASTEWATER OF TETRAMETHYLAMMONIUM HYDROXIDE

Han-Lin Lin, Bo-Kuang Chen, Hui-Ping Hsia, Gen-Hao Yang, Ya-Fei Yang, Yu-Chieh Chao and Sheng-Shung Cheng*

Department of Environmental Engineering
National Cheng Kung University
Tainan 701, Taiwan

Key Words: Anaerobic hydrolysis, anaerobic ammonium oxidation (ANAMMOX), anaerobic-anoxic process, tetramethylammonium hydroxide (TMAH), total organic carbon (TOC)/NH$_4^+$-N, TFT-LCD wastewater

ABSTRACT

Anaerobic ammonium oxidation (ANAMMOX) is a newly discovered and cost-effective process to remove nitrogen compounds in wastewater treatment. The process is the anoxic oxidation of ammonium converting to nitrogen with nitrite as electron acceptor. However, many organic inhibitors and unknown niche of ANAMMOX are the challenges to apply ANAMMOX process to industrial wastewater treatment. In this study, two-stage bioprocess of anaerobic hydrolysis and anoxic ANAMMOX were performed for tetramethylammonium hydroxide (TMAH) wastewater treatment. In the first anaerobic hydrolysis process, the organic nitrogen from TMAH wastewater containing 110-250 mg L$^{-1}$ (TMAH 600-2000 mg L$^{-1}$) could be hydrolyzed into ammonium. A 95% hydrolysis efficiency of TMAH could be achieved in this process. In the second ANAMMOX process, the effluent from the first process with a total organic carbon (TOC)/NH$_4^+$-N ratio of 0.1-0.17 mg C mg$^{-1}$ N is mixed with NO$_2^-$ (50-250 mg N L$^{-1}$) as electron acceptor. The total inorganic nitrogen conversion was up to 90% under volumetric loading rate of 100-500 mg N L$^{-1}$ d$^{-1}$. Consortia in the second bioreactor were detected with molecular biomonitoring of 16S rDNA during 401 d continuous culture. ANAMMOX clusters, Brocadia anammoxidans and Kuenenia stuttgartiensis, are identified in this study. Process evaluation and ecological study were conducted on the use of two-stage biological process in treating thin film transistor liquid crystal display wastewater.

INTRODUCTION

The main organic compounds of thin film transistor liquid crystal display (TFT-LCD) wastewater are tetramethylammonium hydroxide (TMAH), monoethanolamine (MEA) and dimethylsulphoxide. Among these compounds, TMAH and MEA are the main nitrogen source, which provides a large amount of organic nitrogen. Furthermore, the total Kjeldahl nitrogen/chemical oxygen demand (TKN/COD) ratio of TFT-LCD wastewater (0.17-0.2) is much higher than the nutrient ratio of conventional activated sludge process [2]. However, previous studies also showed that TMAH could be degraded under aerobic conditions by some specific microorganism and under anaerobic condition by methanogen [3-5].

Based on the wastewater characteristics, a specific ecological consortia community provides biochemical electron transfers and biosynthetic growth. A special bioreactor combination is necessary to be conducted for the development of microbial ecology and substrate balance. In this study, an anaerobic hydroly-
sis reactor could sustain relatively high organic loading rate and toxic compounds, which is designed in the first stage of two-stage bioprocess. Nevertheless, either the low C/N ratio of the first stage effluent, or the raw TFT-LCD wastewater, is ineffective for heterotrophic denitrification. Without any organic carbon source, autotrophic anaerobic ammonium oxidation (ANAMMOX) process could be used under anoxic conditions to oxidize ammonium to N\textsubscript{2} with nitrite as electron acceptor [6]. Therefore, an anoxic ANAMMOX reactor was designed for nitrogen removal in the second stage. The overall stoichiometry of ANAMMOX process is shown below [7]:

\[
\begin{align*}
\text{NH}_4^+ + 1.32 \text{ NO}_2^- + 0.066 \text{ HCO}_3^- + 1.3 \text{ H}^+ \\
\rightarrow 1.02 \text{ N}_2 + 0.26 \text{ NO}_3^- + 0.066 \text{ CH}_2\text{O}_{0.5}\text{N}_{0.15} \\
+ 2.03 \text{ H}_2\text{O}
\end{align*}
\]

Previously, it was found that some organic inhibitory compounds caused many risks to apply ANAMMOX to industrial wastewater treatment [6,8]. Interestingly, a new species of ANAMMOX could grow mixtrophically and co-oxidize propionate and ammonium [9]. Two possible approaches could be applied to industrial wastewater treatment. One is that more mixtrophic species of ANAMMOX consortia would be found and adopted. The other is a bioprocess combination for ecological development and substrate balance. In this study, an anaerobic-anoxic bioprocess was established in treating TFT-LCD wastewater of TMAH.

METHODS AND MATERIALS

1. Parameters Design of Anaerobic-anoxic Bioreactors

In industrial wastewater treatment, fluidized-bed-like reactors could sustain a relatively higher organic loading rate with small footprint. In the first stage, a reverse flow jet loop reactor (RFJLR) was designed as a continuous stirred tank reactor (CSTR) with high internal recirculation and dilution rate (Fig. 1). The hydrodynamic parameter of N\textsubscript{CSTR} and D/\textmu L of RFJLR were 1.26 and 1.36, respectively, which represented the CSTR mode. The well-mixed reactor is beneficial for treating highly toxic compounds in wastewater with high mass transfer, high mix-up and high bioconversion efficiency. The operating parameters of RFJLR are shown in Table 1 (Fig. 1, insert).

In the second stage, a powdered activated carbon attached biofilm expanded bed (PABEB) was initially inoculated with nitrifying sludge from a petrochemical wastewater treatment plant in southern Taiwan (Fig. 1). Due to the very low growth rate of ANAMMOX bacteria [7], a three-phase separator was located on the top of reactor for biomass retention. The feed was introduced from the bottom of the reactor and mixed with an additional recirculation flow from the effluent to maintain adequate superficial velocity and shear stress to favor granule formation. The operating parameters of PABEB are shown in Table 2 (Fig. 1, insert).

2. Start-up and Continuous Operation of Two-stage Bioprocess

The operation involved two phases. During the first 158 d, the ratio of NO\textsubscript{2}^--N: NH\textsubscript{4}^+--N of 1:1 with NO\textsubscript{2}^--N 50-100 mg L\textsuperscript{-1} at a non-inhibition level was used for the start-up strategy of ANAMMOX reactor (PABEB) using a synthetic wastewater [10,11]. During day 108-138, the anaerobic reactor (RFJLR) was start-up in batch mode with full-scale TFT-LCD wastewater of TMAH. After day 139, the first anaerobic reactor was running as a CSTR with TMAH wastewater. The TMAH wastewater provided about 99% org-N of total N, which was diluted with water for controlling org-N volumetric loading rate (VLR) (29-94 mg N L\textsuperscript{-1} d\textsuperscript{-1}). The organic nitrogen concentration of the feed was 110-270 mg L\textsuperscript{-1} (TMAH 600-2000 mg L\textsuperscript{-1}). In the second phase (159-401 d), the first anaerobic reactor was combined with the second anoxic reactor. The feed of the second reactor was the effluent from first reactor with a TOC/NH\textsubscript{4}^+-N ratio of 0.1-0.17 mg C mg\textsuperscript{-1} N and NO\textsubscript{2}^+-N (50-200 mg L\textsuperscript{-1}) was added as electron acceptor. In order to control enough electron donor and VLR of the second reactor, NH\textsubscript{4}^+-N (25-150 mg L\textsuperscript{-1}) was added for adjusting the fluctuated flow rate and N content of the full-scale wastewater. The maximum total inorganic nitrogen (TIN) VLR of second ANAMMOX reactor was then 500 mg N L\textsuperscript{-1} d\textsuperscript{-1}.

3. Genomic DNA Extraction, PCR Amplification and 16s DNA Cloning Library

Extraction of DNA from the sludge was done and amplification of the 16S rDNA gene was carried out by polymerase chain reaction (PCR) according to the previously described method [12]. Amplification of ANAMMOX-specific 16S rDNA gene was performed with the primer pair of Amx368f/1392r [13,14]. The PCR products were purified with purification kit and subsequently cloned into Escherichia coli using the TA cloning kit (TOPO TA cloning kit, Invitrogen, Carlsbad, CA). All 16S rDNA gene clones were randomly selected. The sequences of the clones were determined by dye terminator cycle sequencing with a Quick Start Kit (GenomeLab DTCS Quick Start Kit, Beckman Coulter, Fullerton, CA) and an automatic sequencer (CEQ2000XL, Beckman Coulter, Fullerton, CA). A 16S rDNA gene-based phylogenetic tree was constructed by applying the neighbor-joining method with the ARB program. Bootstrap re-sampling analysis for 1,000 replicates was performed with the
PAUP 4.0 package to estimate the confidence of tree topologies [12].

4. Other Analytical Methods

TKN, NH$_4^+$, NO$_2^-$ and NO$_3^-$ were detected according to Standard Method [15].

RESULTS AND DISCUSSION

1. Performances of Org-N Hydrolysis in First Stage

During the second phase, the first anaerobic reactor was combined with the second anoxic reactor. From 159-220 d, the org-N VLR was 16-29 mg L$^{-1}$ d$^{-1}$ and the hydrolysis efficiency of org-N could achieve 80-95% (Fig. 2a). It indicated that TMAH was hydrolyzed efficiently with a relatively low VLR and/or a long hydraulic retention time, HRT (4 d). Subsequently, the org-N VLR (29-60 mg L$^{-1}$ d$^{-1}$) was raised by decreasing the proportion of water during day 221-383. Unfortunately, the org-N VLR could not be controlled well because of the fluctuation in water quality of the full-scale wastewater. However, org-N was almost converted to ammonium in this period (Fig. 2b). After day 384, the org-N hydrolysis efficiency was still above 85% even when HRT was shortened from 4 to 2 d. Hence, it showed TMAH could be degraded effectively under the operation condition and function of the RFJLR was demonstrated.

2. Performances of Nitrogen Removal in Second Stage

During the first 158 d, start-up and enrichment of the second reactor with synthetic wastewater: the reactor was initially inoculated with nitrifying sludge from a full-scale petrochemical wastewater treatment plant and fed with synthetic wastewater contained a NO$_2^-$-N:NH$_4^+$-N ratio of 1:1. The NO$_2^-$ and NH$_4^+$ conversion was low during the start-up period, probably due to the slow growth rate of ANAMMOX microbes (doubling time 11 d) and low ANAMMOX biomass from the seed resulting in a long start-up period [7]. The TIN conversion rate was attained a value higher than 50 mg L$^{-1}$ d$^{-1}$ (50% of TIN VLR) only from day 97 (Fig. 3a). The NO$_2^-$ conversion was coupled with NH$_4^+$ oxidation with NO$_3^-$ production during this period (Fig. 3b). Subsequently, the TIN conversion was gradually increased as doubling VLR for intensive enrichment.

During 159-401 d, periods of two-stage biochemical process, the effluent of first reactor was introduced into the second reactor in this phase. The feed of second reactor with a low TOC/NH$_4^+$-N ratio of 0.1-0.17 mg mg$^{-1}$ indicated that electron was donated from ammonium. In previous studies, organic carbon caused ANAMMOX unable to compete with heterotrophic denitrifiers or enzyme activity was in-

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Table 2. Dimension and operating parameters of PABEB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>20 L</td>
</tr>
<tr>
<td>Superficial velocity m L$^{-1}$ h$^{-1}$</td>
<td>8-11</td>
</tr>
<tr>
<td>HRT</td>
<td>24 h</td>
</tr>
<tr>
<td>pH</td>
<td>7.5-8.0</td>
</tr>
<tr>
<td>ORP</td>
<td>-200 to +50 mV</td>
</tr>
<tr>
<td>DO</td>
<td>&lt;0.1 mg L$^{-1}$</td>
</tr>
<tr>
<td>Temperature</td>
<td>38-40 °C</td>
</tr>
</tbody>
</table>

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Fig. 1. Schematic diagram of two-stage bioprocess in treating TMAH wastewater.
The variation of (a) Organic nitrogen volumetric loading rate and hydrolysis efficiency; (b) Organic nitrogen and ammonium concentration versus operation time.

Fig. 2.

The variation of (a) Total inorganic nitrogen volumetric loading rate and conversion efficiency; (b) Nitrogen concentration versus operation time.

Fig. 3.

Hindered by intermediates of the degradation pathway [6,8]. Actually, NO$_2^-$ could be converted with NH$_4^+$ as electron donor after anaerobic-anoxic system established, due to an insufficient organic carbon source. The TIN conversion was 70-90%, which showed ANAMMOX could compete electron acceptor (NO$_3^-$) with other denitrifiers. The NO$_2^-$ production was still significant, even low TOC was present in the anoxic system. In addition, the residual TOC was not inhibitory to ANAMMOX process. The granule formation was also observed from day 203. Figure 3a shows an increase of VLR and conversion, which indicates that ANAMMOX could be applied in treating wastewater with a low TOC/NH$_4^+$-N ratio (0.1-0.17 mg mg$^{-1}$).

The ratio of NO$_2^-$ conversion and NO$_3^-$ production rates of NH$_4^+$ conversion was calculated to compare with TIN conversion rate. Both ratios were fluctuated considerably when TIN conversion rate was below 250 mg L$^{-1}$ d$^{-1}$, but gradually approached constant when TIN conversion rate was above 250 mg L$^{-1}$ d$^{-1}$. The average ratio of NO$_3^-$-N conversion to NH$_4^+$-N conversion (Y$_{NO3-NH4}$) at TIN conversion rate above 250 mg L$^{-1}$ d$^{-1}$ was 1.2 ± 0.2 and the ratio of NO$_3^-$-N production to NH$_4^+$-N conversion (Y$_{NO3-NH4}$) was 0.08 ± 0.04 (Fig. 4). Comparing the average value with the theoretical ANAMMOX stoichiometry, the Y$_{NO2-NH4}$ of 1.2 was closed to theoretical value 1.3, but the Y$_{NO3-NH4}$ of 0.08 is much lower than theoretical value 0.26. The lower NO$_3^-$ production maybe caused by heterotrophic denitrification under low TOC residuals from the effluent of the first reactor. However, it still showed ANAMMOX could compete electron acceptor with heterotrophic denitrifiers when a low TOC/NH$_4^+$-N ratio wastewater was adopted.

3. Application of Molecular Biomonitoring Technology

Molecular BioMonitoring technique was applied
Fig. 4. Conversion ratio between nitrite conversion rate of ammonium conversion rate ($Y_{\text{NO}_2^-/\text{NH}_4^+}$); ratio between nitrate production rate of ammonium conversion rate ($Y_{\text{NO}_3^-/\text{NH}_4^+}$).

Fig. 5. Anammox-specific 16S rDNA-based population identified in the second reactor was constructed by the neighbor-joining method based on 16S rDNA sequences.

Table 3. Phylogenetic distribution of 16s rDNA clone sequence retrieved from the second reactor clone library

<table>
<thead>
<tr>
<th>Clone number</th>
<th>Sequence length</th>
<th>Phylogenetic relationship</th>
<th>Closest species in Genebank</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LU24</td>
<td>994/1019</td>
<td>Candidatus Brocadia anammoxidans</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>LU16</td>
<td>993/1019</td>
<td>Candidatus Brocadia anammoxidans</td>
<td></td>
<td>97</td>
</tr>
<tr>
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<td>994/1019</td>
<td>Candidatus Brocadia anammoxidans</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>LU37</td>
<td>1016/1020</td>
<td>Candidatus Kuenenia stuttgartiensis</td>
<td></td>
<td>99</td>
</tr>
<tr>
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<td>1016/1017</td>
<td>Candidatus Kuenenia stuttgartiensis</td>
<td></td>
<td>99</td>
</tr>
</tbody>
</table>

CONCLUSIONS

With the first anaerobic reactor of high bioconversion efficiency, the conversion from organic nitrogen (TMAH) to ammonium could be achieved at least 80-100%. It showed that TMAH wastewater could be degraded effectively by acclimated anaerobes in RFJLR. In addition, the HRT of RFJLR could be decreased from 4 to 2 d without significant impact on the hydrolysis efficiency. Degrading TMAH is a very uncommon case with a relatively short HRT in the anaerobic hydrolysis process. Consequently, a relatively high dilution rate and more flexible parameters (e.g., dilution rate) are required for practical application. With two-stage bioprocess operation, the maximum volumetric liquid reaction rate (max VLR) could be used in the full-scale industrial wastewater treatment. The nitrogen compound conversion reached 90% within TIN VLR of 200-500 mg L$^{-1}$ d$^{-1}$ in the second ANAMMOX process. It indicated that ANAMMOX bacteria could be applied to degrade TFT-LCD wastewater with a low TOC/NH$_4^+$ ratio of 0.1-0.17 mg C mg$^{-1}$ N. Both undegraded TMAH and/or intermediates of the degrading pathway contributed to the TOC compounds in effluent of the first reactor. Although, it appears that the residual TOC is not inhibitory to ANAMMOX, the inhibition level and TOC/NH$_4^+$ threshold should be determined in the future.

With molecular biomonitoring technology of 16s rDNA cloning library, ANAMMOX microorganisms of the second anoxic reactor were detected (clusters, B. anammoxidans and K. stuttgartiensis). The similarity of each species was 97 and 99%, matched with database on NCBI. With such low TOC/NH$_4^+$ ratio, it is not possible for heterotrophic denitrifiers converting nitrite. Furthermore, a low anaerobic oxidation rate of nitrifiers could not remove the major part of nitrogen under the anoxic condition [11]. There the role of ANAMMOX is apparent. However, the dominant species still need to be evaluated in the future. In short, two-stage anaerobic-anoxic bioprocess could be used in treating TFT–LCD wastewater.

REFERENCES


Discussions of this paper may appear in the discussion section of a future issue. All discussions should be submitted to the Editor-in-Chief within six months of publication.

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