MBR in Wastewater Reclamation
Highlight articles

129 Rice: Reducing arsenic content by controlling water irrigation
Ashley M. Newbigging, Rebecca E. Paliwoda and X. Chris Le

132 Apportioning aldehydes: Quantifying industrial sources of carbonyls
Sarah A. Styler

Review articles

30 Application of constructed wetlands for wastewater treatment in tropical and subtropical regions (2000-2013)
Dong-Qing Zhang, K.B.S.N. Jinadasa, Richard M. Gersberg, Yu Liu, Soon Keat Tan and Wun Jern Ng

47 Stepwise multiple regression method of greenhouse gas emission modeling in the energy sector in Poland
Alicja Kolasa-Wiecek

113 Mini-review on river eutrophication and bottom improvement techniques, with special emphasis on the Nakdong River
Andinet Tekile, Ilho Kim and Jisung Kim

Regular articles

1 Effects of temperature and composite alumina on pyrolysis of sewage sludge
Yu Sun, Baosheng Jin, Wei Wu, Wu Zuo, Ya Zhang, Yong Zhang and Yaji Huang

9 Numerical study of the effects of local atmospheric circulations on a pollution event over Beijing-Tianjin-Hebei, China
Yucong Miao, Shuhua Liu, Yijia Zheng, Shu Wang and Bicheng Chen, Hui Zheng and Jingchuan Zhao

21 Removal kinetics of phosphorus from synthetic wastewater using basic oxygen furnace slag
Chong Han, Zhen Wang, He Yang and Xiangxin Xue

55 Abatement of SO$_2$-NOx binary gas mixtures using a ferruginous active absorbent: Part I. Synergistic effects and mechanism
Yinghui Han, Xiaolei Li, Maohong Fan, Armistead G. Russell, Yi Zhao, Chunmei Cao, Ning Zhang and Genshan Jiang

65 Adsorption of benzene, cyclohexane and hexane on ordered mesoporous carbon
Gang Wang, Baojuan Dou, Zhongshen Zhang, Junhui Wang, Haier Liu and Zhengping Hao

74 Flux characteristics of total dissolved iron and its species during extreme rainfall event in the midstream of the Heilongjiang River
Jiunian Guan, Baixing Yan, Hui Zhu, Lixia Wang, Duian Lu and Long Cheng

81 Sodium fluoride induces apoptosis through reactive oxygen species-mediated endoplasmic reticulum stress pathway in Sertoli cells
Yang Yang, Xinwei Lin, Hui Huang, Demin Feng, Yue Ba, Xuemin Cheng and Liuxin Cui

90 Roles of SO$_2$ oxidation in new particle formation events
He Meng, Yujiao Zhu, Greg J. Evans, Cheol-Heon Jeong and Xiaohong Yao

102 Biological treatment of fish processing wastewater: A case study from Sfax City (Southeastern Tunisia)
Meryem Jemli, Fatma Karray, Firas Feki, Slim Loukil, Najla Mhiri, Fathi Aloui and Sami Sayadi
CONTENTS

122 Bioreduction of vanadium (V) in groundwater by autohydrogentrophic bacteria: Mechanisms and microorganisms
Xiaoyin Xu, Siqing Xia, Lijie Zhou, Zhiqiang Zhang and Bruce E. Rittmann

135 Laccase-catalyzed bisphenol A oxidation in the presence of 10-propyl sulfonic acid phenoxazine
Rūta Ivanec-Goranina, Juozas Kulyš, Irina Bachmatova, Liucija Marcinkevičienė and Rolandas Meškys

140 Spatial heterogeneity of lake eutrophication caused by physiogeographic conditions: An analysis of 143 lakes in China
Jingtao Ding, Jinling Cao, Qigong Xu, Beidou Xi, Jing Su, Rutai Gao, Shouliang Huo and Hongliang Liu

148 Anaerobic biodegradation of PAHs in mangrove sediment with amendment of NaHCO3
Chun-Hua Li, Yuk-Shan Wong, Hong-Yuan Wang and Nora Fung-Yee Tam

157 Achieving nitritation at low temperatures using free ammonia inhibition on Nitrobacter and real-time control in an SBR treating landfill leachate
Hongwei Sun, Yongzhen Peng, Shuying Wang and Juan Ma

164 Kinetics of Solvent Blue and Reactive Yellow removal using microwave radiation in combination with nanoscale zero-valent iron
Yanpeng Mao, Zhenqian Xi, Wenlong Wang, Chunyuan Ma and Qinyan Yue

173 Environmental impacts of a large-scale incinerator with mixed MSW of high water content from a LCA perspective
Ziyang Lou, Bernd Bilitewski, Nanwen Zhu, Xiaoli Chai, Bing Li and Youcai Zhao

180 Quantitative structure–biodegradability relationships for biokinetic parameter of polycyclic aromatic hydrocarbons
Peng Xu, Wencheng Ma, Hongjun Han, Shengyong Jia and Baolin Hou

191 Chemical composition and physical properties of filter fly ashes from eight grate-fired biomass combustion plants
Christof Lanzerstorfer

198 Assessment of the sources and transformations of nitrogen in a plain river network region using a stable isotope approach
Jingtao Ding, Beidou Xi, Qigong Xu, Jing Su, Shouliang Huo, Hongliang Liu, Yijun Yu and Yanbo Zhang

207 The performance of a combined nitritation-anammox reactor treating anaerobic digestion supernatant under various C/N ratios
Jian Zhao, Jiane Zuo, Jia Lin and Peng Li

215 Coagulation behavior and floc properties of compound biofloculant-polyaluminum chloride dual-coagulants and polymeric aluminum in low temperature surface water treatment
Xin Huang, Shenglei Sun, Baoyu Gao, Qinyan Yue, Yan Wang and Qian Li

223 Accumulation and elimination of iron oxide nanomaterials in zebrafish (Danio rerio) upon chronic aqueous exposure
Yang Zhang, Lin Zhu, Ya Zhou and Jimiao Chen

231 Impact of industrial effluent on growth and yield of rice (Oryza sativa L.) in silty clay loam soil
Mohammad Anwar Hossain, Golum Kibria Muhammad Mustafizur Rahman, Mohammad Mizanur Rahman, Abul Hossain Molla, Mohammad Mostafizur Rahman and Mohammad Khabir Uddin

241 Molecular characterization of microbial communities in bioaerosols of a coal mine by 454 pyrosequencing and real-time PCR
Min Wei, Zhisheng Yu and Hongxun Zhang

252 Risk assessment of Giardia from a full scale MBR sewage treatment plant caused by membrane integrity failure
Yu Zhang, Zhimin Chen, Wei An, Shumin Xiao, Hongying Yuan, Dongqing Zhang and Min Yang

186 Serious BTEX pollution in rural area of the North China Plain during winter season
Kankan Liu, Chenglong Zhang, Ye Cheng, Chengtang Liu, Hongxing Zhang, Gen Zhang, Xu Sun and Yujing Mu
Biological treatment of fish processing wastewater: A case study from Sfax City (Southeastern Tunisia)

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ABSTRACT

The present work presents a study of the biological treatment of fish processing wastewater at salt concentration of 55 g/L. Wastewater was treated by both continuous stirred-tank reactor (CSTR) and membrane bioreactor (MBR) during 50 and 100 days, respectively. These biological processes involved salt-tolerant bacteria from natural hypersaline environments at different organic loading rates (OLRs). The phylogenetic analysis of the corresponding excised DGGE bands has demonstrated that the taxonomic affiliation of the most dominant species includes Halomonadaceae and Flavobacteriaceae families of the Proteobacteria (Gamma-proteobacteria class) and the Bacteroidetes phyla, respectively. The results of MBR were better than those of CSTR in the removal of total organic carbon with efficiencies from 97.9% to 98.6%. Nevertheless, salinity with increasing OLR aggravates fouling that requires more cleaning for a membrane in MBR while leads to deterioration of sludge settleability and effluent quality in CSTR.

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Introduction

In fish factories, there are different steps of fish processing such as defrosting, cleaning, cooking, and canning. Large amounts of water are required in these steps, originally for washing and cleaning purposes, and also as media for storage and refrigeration of fish product before and after processing. All these processes generate high salinity wastewater streams with a high concentration of various pollutants such as, organic matter, nutrient, and fat (Méndez et al., 1992; Intrusungkha et al., 1999; Chowdhury et al., 2010).

The high salinity organic wastewater streams can be isolated and treated separately before being discharged to the environment to keep the impact on natural ecosystems at a minimum. The biological treatment depends on the application of non-halophilic microorganisms (Woolard and Irvine, 1995) which is normally able to tolerate a lower salt concentration up to 10 g/L without acclimatization. High salt concentrations (>10 g/L) cause disintegration of cells because of the loss of cellular water (plasmolysis) or recession of the cytoplasm which is induced by an osmotic difference across the cell wall and cause outward flow of intracellular water resulting in the loss of microbial activity and cell dehydration (Dinçer and Kargi, 2001). As a result, low removal performance of chemical and biological oxygen demands (chemical oxygen demand (COD), biochemical oxygen demand (BOD5)) and increases the effluent suspended solids (SS) especially when high salt concentrations (>20 g/L) occur (Dan et al., 2003; Liu et al., 2009). But in the case of a wastewater with a salt concentration higher than 30 g/L, the addition of halophilic or halotolerant microorganisms should be considered (Abou-elela et al., 2010). There are many types of biological systems for

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treated fish processing wastewater such as activated sludge, rotating biological contactor, aerated lagoon, anaerobic fixed filter and anaerobic digester (Chowdhury et al., 2010).

One of the effective and widely used biological treatment methods for industrial water treatment is the MBR technology, which is considered as a combination of the activated sludge process and membrane separation techniques (Artiga et al., 2008; Lay et al., 2010; Hoiniks et al., 2012). The advantages of membrane bioreactor (MBR) over conventional treatment have been thoroughly reviewed and include consistently high product quality, ease of operation and automation, reduced footprint, reduced sludge production due to a high biomass concentration in the bioreactor, and complete suspended solid removal from the effluent (Van Dijk and Roncken, 1997; Melin et al., 2006; Judd, 2011). Nevertheless, the main disadvantages of MBR are higher maintenance, operation costs and its limited performance due to fouling which constrains both operating flux and membrane life (Nuengjiamnomg et al., 2005; Drews, 2010).

Around Sfax City (Southeastern Tunisia), a tuna fish canning factory discharge is characterized by a high content of salt and organic matter, at a concentration that doesn’t fulfill the legal requirement for the environment protection. The aim of this work is the investigation of biological treatment of fish processing wastewater, with salt concentrations up to 5.5%, by two different methods such as MBR and continuous stirred-tank reactor (CSTR) involving salt-tolerant bacteria. Furthermore, we will focus on the efficiency of MBR method on the removal of total organic carbon (TOC), BOD, and nutrients at different organic loading rates (OLRs).

1. Materials and methods

1.1. Reactor design and operation

The different experimental steps were carried out for the period of 250 days. During the first 100 days (start-up period), the biomass in the bioreactor was acclimatized to saline wastewater concentration containing 5.5% and was increased by omitting sludge wastage until reaching the mixed liquor suspended solid (MLSS) concentration values, above 5 g/L. The 150 remaining days pertained to the treatment of wastewater in CSTR (50 days) and in MBR (100 days). An activated sludge process was performed in a 20 L bioreactor vessel Biolafitte (LSL Biolafitte S.A., Saint Germain en Laye, France) of a working volume of 12 L. Air was supplied using a diffuser and aeration rate was maintained constant, at 0.35 volume of air/volume of reactor/min (v.v.m), and agitation at 160 r/min. Minimum dissolved oxygen of 2–3 mg/L in the bioreactor was generally available during the experiments. Besides, the reactor temperature varied between 20 and 30°C by circulating water through the water jacket of the reactor.

The flux of wastewater influent was guaranteed by peristaltic pump (Gilson Inc., Middleton, WI, USA).

The CSTR system consists of an aerated reactor and a static decanter (Fig. 1). Following the reactor, the decanter was used to separate the activated sludge from the treated wastewater. While the excess sludge is removed, the remaining part is recycled back into the reactor, thus maintaining the sludge concentration constant. The clarified effluent was then collected and analyzed. The hydraulic retention time (HRT) was fixed at 2 days; the OLR rose from 0.5 to 0.7 kg TOC/(m³·day) during the first 10 operating days and then from day 11 onward, the OLR in the reactor was about 0.9 kg TOC/(m³·day).

For MBR system, the external tubular unit of a cross-flow ultrafiltration membrane was used instead of the decanter under the same operating conditions of CSTR. MBR system consists of a SALMSON Pump type Multi-H 206SE-T/B and KER 40 mineral membrane: 400 mm in length, 30 mm in diameter, 0.04 m² in surface and of a 0.2-μm pore size (Fig. 1). The trans-membrane pressure (TMP) of the effluent was monitored by a manometer in a bar transformed from kPa. The ultrafiltration membrane was cleaned using a solution of NaOH (pH 11, 12) for 1 hr at 45°C and then by water cycle to restore a neutral pH and to maintain a favorable flow. But when the flow is not recovered, an additional cleaning using nitric acid may be useful.

During the MBR experiment, the concentrate of biomass recovered from the membrane was sent back to the reactor. The biomass was exposed to a rising of OLR from 0.5 to 1.4 kg TOC/(m³·day) and this leads the HRT to decrease from 3.8 to 1.42 days. Before the wastewater treatment, pH was adjusted at 7 ± 1 by 2.0 mol/L of NaOH solution.

1.2. Wastewater composition

The studied industrial wastewater for biological treatment was generated from “Sultan tuna factory” located in Sfax City,
Table 1 – Composition of tuna cooking wastewater before dilution.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH</th>
<th>Salinity</th>
<th>TKN (mg/L)</th>
<th>NH₄-N (mg/L)</th>
<th>NO₂-N (mg/L)</th>
<th>NO₃-N (mg/L)</th>
<th>TP (mg/L)</th>
<th>SS (g/L)</th>
<th>VSS (g/L)</th>
<th>TOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>6.0 ± 0.2</td>
<td>110 ± 10</td>
<td>2650 ± 290</td>
<td>590 ± 150</td>
<td>0.13 ± 0.10</td>
<td>60 ± 20</td>
<td>314 ± 100</td>
<td>71 ± 20</td>
<td>26 ± 8</td>
<td>11500 ± 1000</td>
</tr>
</tbody>
</table>

BOD₅: biological oxygen demand; TKN: total kjeldahl nitrogen; NH₄-N: ammonia-nitrogen; NO₂-N: nitrite-nitrogen; NO₃-N: nitrate-nitrogen; TP: total phosphor; SS: suspended solid; VSS: volatile suspended solid; TOC: total organic carbon.

southern Tunisia. In the factory, Tuna cooking is carried out by immersion in brine tanks, then the generated wastewater was collected (Table 1) and diluted in sea water with 5–10 bypass ratios in the laboratory to reach the requested and suitable condition for the experiment.

1.3. Consortium origin

The basic consortium (an aerobic mixed culture of salt tolerant organisms) for the treatment of fish processing saline wastewater was supplied by Aloui et al. (2009). This basic consortium was obtained from Sfax wastewater treatment plant and was acclimatized to salt concentrations of 25 g/L. The results obtained by Aloui et al. (2009) confirmed that the acclimatized culture was efficient for the treatment of saline wastewater. Additionally, in order to increase the biodiversity of halotolerant microbial population in the bioreactor, 11 samples were collected from various natural hypersaline environments (salt lake, solar saltern, sediment lagoon and fish farm at Bizerte; Kerkennah; Monastir; Sousse and Sfax) and were also used to inoculate the bioreactor during the start-up period (100 days).

1.4. Sampling

The wastewater treatment systems were operated under CSTR and MBR during 50 and 100 days, respectively. Samples were collected every 3 days from the bioreactor itself, the bioreactor-influent, and bioreactor-effluent and stored at 4°C until analysis. Then, TOC, BOD₅, pH-value, total kjeldahl nitrogen (TKN), ammonia-nitrogen (NH₄-N), nitrite-nitrogen (NO₂-N), nitrate-nitrogen (NO₃-N), total phosphor (TP) and bioreactor sludge concentration were measured regularly in a continuous mode MBR treatment.

1.5. Analytical methods

Dry weight and moisture content were determined by weighing bioreactor mixed liquor samples before and after drying overnight at 105°C and then the biomass concentration in terms of the mixed liquor volatile suspended solid (MLVSS) was analyzed, by loss on ignition at 600°C for 2 hr. The sludge volume index (SVI) is considered as the volume of mL occupied by 1 g of activated sludge, settling the aerated liquor for 30 min. The TP and TKN were determined using the Dabin and Kjeldahl methods (Kjeldahl, 1883; Dabin, 1967), respectively. The pH and the electric conductivity (EC) were determined using a Swiss multi-parameter analyzer Consort C831. The BOD₅ was determined by the manometric method with a respirometer (BSB-Controller Model 620 T (WTW), Weilheim, Germany). The TOC was determined by the use of a TOC Analyzer multi-N/C-1000 (Shimadzu) as described in APHA (Eaton et al., 2005).

1.6. Microbial community analysis (PCR-DGGE)

1.6.1. DNA extraction and PCR amplification

Fourteen sludge samples were collected from the MBR reactor at different weeks of the operational system (week1 (w1), week 2 (w2) … week 14 (w14)) and were centrifuged at 10.000 g for 5 min, then the supernatant was removed and stored at −20°C.

The extraction of the DNA from the pellets of the activated sludge samples was conducted using the Ultra Clean Soil DNA Isolation Kit (MO BIO Laboratories MO BIO ISO 9001 Certified Company, Etats-unies) according to the manufacturer’s protocol. Bacterial 16S rRNA gene fragments were amplified using the forward primer 341 F (5′-CCTACGGG AGGCAGCAG-3′) (Muyzer et al., 1995) and the reverse primer 518R (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al., 1993). A GC clamp (5′-CGCCCAGGGCCGGCCGGGCGGCGGCCGCGCCCGCGCCCGCCCG-3′ was added to the forward primer (Muyzer et al., 1993). The length of the expected amplified fragment was approximately 200 bp. PCR amplification was performed in a 50 μL total volume involving 2 mmol/L MgCl₂, 0.2 mmol/L each dNTP, 0.2 μmol/L of each primer, 0.1 mg/mL BSA, 50 ng DNA template and 2.5 U of Top-Taq DNA polymerase (BIORON) with reaction buffer supplied by the manufacturer. The thermal cycling program includes initial denaturation at 95°C for 5 min, denaturation at 94°C for 45 sec, and a touchdown primer annealing from 60 to 53°C for 45 sec (annealing temperature decreased 1°C each cycle, until a final temperature of 53°C, annealing at 53°C for 45 sec for the next 14 cycles and then a primer extension at 72°C for 45 sec. A final extension at 72°C for 10 min was then performed. Five microliters of each PCR product was visualized on 1% agarose ethidium bromide-stained gels. The concentration of the PCR products was determined with the Nanodrop ND-2000 Spectrophotometer (Thermo Scientific NanoDrop 2000c/2000 UV-Vis Spectrophotometer, Elisa Molecular Biology).

1.6.2. DGGE analysis

DGGE analysis was undertaken using the Dcode Universal Mutation Detection System (BIO-RAD Laboratories, Hercules, CA, USA). 300 ng of PCR products was loaded onto 8% (W/V) polyacrylamide gels in 1× TAE (40 mMol/L Tris, 20 mMol/L acetate, 1.0 mmol/L Na₂-EDTA) with denaturing gradient of 40%–70% (100% denaturant was 7 mol/L urea and 40% (W/V) deionized formamide). The gel electrophoresis was performed at a constant voltage of 130 V, 60°C for 6.5 hr. After electrophoresis, the gel was stained for 30 min in 1× TAE containing ethidium bromide solution and then rinsed in distilled water for 5 min and photographed under UV transillumination.
1.6.3. Sequences analysis
The target bands were excised from DGGE gels and eluted in 35 μL of MilliQ water at 4°C for one night. The eluted DNA was re-amplified with primers 341F–518R under the same previous conditions. PCR was started with initial denaturation for 5 min at 94°C. A total of 25 cycles, each including 45 sec at 94°C, 45 sec at 55°C and 1 min at 72°C, was followed by a final extension step of 10 min at 72°C. The primers used for sequencing are the same as used for the amplification. Sequence similarity was performed using the online sequence analysis resources “BLAST” (Altschul et al., 1997) and “Seqmatch” (Ribosomal Database project II; Release 10) (Cole et al., 2009). Highly similar sequences and some dominant-group reference sequences were added to the data set for MUSCLE multiple sequence alignment (Edgar, 2004) and the phylogenetic tree was constructed in MEGA 4 (Tamura et al., 2007) applying the neighbor-joining algorithm (Saitou and Nei, 1987) with Maximum Composite Likelihood corrections. Bootstrap resampling analysis (Felsenstein, 1985) for 100 replicates was performed to estimate the degrees of confidence in tree topologies.

1.6.4. Nucleotide sequence accession number
The sequences determined in this study were deposited in the GenBank database under accession numbers KJ569247–KJ569264.

2. Results and discussion
2.1. CSTR performance
The results obtained in Table 2 indicated that the TOC removal efficiency varied from 90.4% to 96.8%, while the biomass concentration gradually increased from 4.12 to 5.90 g/L and the outlet pH of the reactor was around the value 8. The specific removal of TOC with different OLR concentrations versus time is presented in Fig. 2. As it can be seen during every start-up period of OLR increase, the TOC removal efficiency was decreased, and then it was increased progressively with time. For example, when the OLR was about 0.5 kg TOC/(m³·day), the TOC outlet concentration was 116 and 42 mg/L after 4 and 10 days of treatment, respectively. Thereafter the CSTR was operated for about 30 days at OLR 0.9 kg TOC/(m³·day), the effluent TOC values were fluctuated occasionally between 39 and 98 mg/L, with a TOC removal rate of 96.8%. This indicated the performance of the treatment process using an efficient mixture of organisms, whereas it was also observed some problems associated with the increasing of OLR.

Fig. 3 reveals the evolution of the SVI in the bioreactor, where at 5.5% salt concentration, the sludge was remained around 95 ± 35 mL/g SVI at the start-up period with OLR equal to 0.5 kg TOC/(m³·day). However, the SVI versus time was gradually increased and its value 210 mL/g was reached at day 50, with OLR equal to 0.9 kg TOC/(m³·day), reflecting poor settling properties. Simultaneously, the filamentous bacteria were developed in association with OLR increase (Fig. 4). Our results were in agreement with the several reports which have pointed out the negative effects of salinity on the microbial community structure in the wastewater and on the settling performance of the activated sludge system.

<table>
<thead>
<tr>
<th>Table 2 – Summary of the CSTR performance.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLR (kg TOC/(m³·day))</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Inlet TOC (mg/L)</td>
</tr>
<tr>
<td>Outlet TOC (mg/L)</td>
</tr>
<tr>
<td>Removal TOC (%)</td>
</tr>
<tr>
<td>Inlet pH</td>
</tr>
<tr>
<td>Outlet pH</td>
</tr>
<tr>
<td>MLSS (g/L)</td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
</tr>
<tr>
<td>SVI (mL/g)</td>
</tr>
<tr>
<td>Operation period (days)</td>
</tr>
</tbody>
</table>

OLR: organic load rate; MLSS: mixed liquor suspended solid; MLVSS: mixed liquor volatile suspended solid; SVI: sludge volume index.

Fig. 2 – Performance of the CSTR system operated at three organic loading rates according to TOC-removal. Experimental conditions: salt concentration = 55 g/L, minimal [O₂] = 2–3 mg/L, initial MLVSS concentration = 3.8 ± 0.2 g/L and temperature 27 ± 2°C.

Fig. 3 – SVI evolution versus increasing organic load. Experimental conditions at decanter: salt concentration = 55 g/L, average pH = 8.1 ± 0.2, average TOC = 80 ± 38 kg/ (m³·day) and temperature 27 ± 2°C.
parameters are retained for all subsequent assays. The effect of the gradual OLR increase and HRT decrease on the MBR performance is shown in Table 3. The BOD5 was clearly reduced exceeding the removal rate of 99.9% during investigation. On the first 50 operation days, when OLR rose from 0.5 to 0.9 kg/(m³·day), the MLVSS concentration of MBR (6.24 ± 0.72 g/L) was lower than that of CSTR (4.12 ± 0.65 g/L) and the average residual TOC for MBR (26.5 ± 2.5 mg/L) before treatment and jumped to the average concentration around 65 mg/L after treatment. It is stated from previous studies that the nitrogen can only be biologically removed (Rosenberger et al., 2002) and both ammonia and nitrite oxidizer microorganisms are aerobic autotrophs and sensitive to salt stress (Lefebvre and Moletta, 2006; Dytczak et al., 2008; Yogalakshmi and Joseph, 2010). However, nitrite oxidizing bacteria is more sensitive than ammonia oxidizing bacteria in the aerobic biological treatment of saline wastewater (Ye et al., 2009). Hence, the nitrite is highly accumulated during the nitrification processing of saline wastewater as previously stated by Peng et al. (2004). Moreover, our results were consistent with the study of Villaverde et al. (1997) who revealed that the oxidation of reduced nitrogen was associated with a decrease of pH.

The results in Tables 2 and 3 revealed that at the same or at different OLR, MBR showed better performance, mainly due to the higher biomass production. As shown, by a comparison of the two processes (MBR and CSTR) at the same condition (OLR from 0.5 to 0.9 kg TOC/(m³·day)), the MLVSS concentration of MBR (6.24–7.37 g/L) was higher than that of CSTR (4.12–5.90 g/L) and the average residual TOC for MBR (26.5–30.0 mg/L) was lower than that of CSTR (56–93 mg/L). These observations indicated that at high salt concentration, the OLR increase leads to the poor sludge settling and the loss of biomass in CSTR system. On the other hand, in MBR system, ultrafiltration unit may be useful in maintaining biomass.

2.2.1. Analysis of DGGE banding patterns

The evolution of the bacterial community during MBR operation was investigated through PCR-DGGE analyses. Actually, 14 samples (w1 to w14) were analyzed and bacterial community profiles elucidated by DGGE are shown in Fig. 5. It appears that numerous dominant bands are presented in all profiles indicating diverse microbial community in the MBR. In addition, the majority of DGGE bands were present...
Table 3 – MBR performance.

<table>
<thead>
<tr>
<th>OLR (kg TOC/(m³·day))</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1</th>
<th>1.2</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation period (days)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>HRT (days)</td>
<td>3.8</td>
<td>3.2</td>
<td>2.7</td>
<td>2.4</td>
<td>2.2</td>
<td>2.0</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Inlet TOC (mg/L)</td>
<td>1900 ± 220</td>
<td>1900 ± 31</td>
<td>1900 ± 23</td>
<td>1900 ± 52</td>
<td>1960 ± 58</td>
<td>2000 ± 47</td>
<td>2000 ± 17</td>
<td>2000 ± 38</td>
</tr>
<tr>
<td>Outlet TOC (mg/L)</td>
<td>33 ± 9</td>
<td>30 ± 2</td>
<td>28 ± 1</td>
<td>26.5 ± 2.5</td>
<td>29.5 ± 2.0</td>
<td>34 ± 4</td>
<td>39.0 ± 8.5</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>Rem. TOC (%)</td>
<td>98.26 ± 0.46</td>
<td>98.42 ± 0.10</td>
<td>98.52 ± 0.04</td>
<td>98.63 ± 0.14</td>
<td>98.50 ± 0.13</td>
<td>98.3 ± 0.2</td>
<td>98.05 ± 0.41</td>
<td>97.90 ± 0.27</td>
</tr>
<tr>
<td>Inlet BOD₅ (mg O₂/L)</td>
<td>3066 ± 378</td>
<td>3033 ± 152</td>
<td>267 ± 208</td>
<td>3000 ± 173</td>
<td>3200 ± 300</td>
<td>3066 ± 152</td>
<td>3033 ± 125</td>
<td>3100 ± 120</td>
</tr>
<tr>
<td>Outlet BOD₅ (mg O₂/L)</td>
<td>2.66 ± 1.41</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>3.6 ± 0.4</td>
<td>2.66 ± 1.50</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
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<tr>
<td>Rem. BOD₅ (%)</td>
<td>99.90 ± 0.01</td>
<td>99.90 ± 0.03</td>
<td>99.92 ± 0.05</td>
<td>99.93 ± 0.02</td>
<td>99.9 ± 0.02</td>
<td>99.91 ± 0.04</td>
<td>99.93 ± 0.03</td>
<td>99.93 ± 0.03</td>
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<tr>
<td>Inlet TKN (mg/L)</td>
<td>532 ± 124</td>
<td>587 ± 17</td>
<td>492 ± 25</td>
<td>526 ± 26</td>
<td>628 ± 99</td>
<td>595 ± 74</td>
<td>472 ± 105</td>
<td>512 ± 85</td>
</tr>
<tr>
<td>Outlet TKN (mg/L)</td>
<td>29.8 ± 7.2</td>
<td>36.0 ± 3.2</td>
<td>49 ± 8</td>
<td>32 ± 5</td>
<td>61 ± 13</td>
<td>75 ± 9</td>
<td>33 ± 6</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Rem. TKN (%)</td>
<td>94 ± 0.2</td>
<td>93.8 ± 0.8</td>
<td>90 ± 2</td>
<td>94.0 ± 0.8</td>
<td>90.2 ± 2.0</td>
<td>88.5 ± 0.9</td>
<td>93.0 ± 0.7</td>
<td>90.0 ± 2.2</td>
</tr>
<tr>
<td>Inlet NH₄-N (mg/L)</td>
<td>151 ± 10</td>
<td>80.0 ± 4.5</td>
<td>119 ± 22</td>
<td>104 ± 10</td>
<td>116 ± 14</td>
<td>129 ± 18</td>
<td>109 ± 17</td>
<td>115 ± 18</td>
</tr>
<tr>
<td>Outlet NH₄-N (mg/L)</td>
<td>50 ± 4</td>
<td>35 ± 5</td>
<td>54 ± 12</td>
<td>48.0 ± 4.5</td>
<td>57 ± 9</td>
<td>53 ± 8</td>
<td>48.0 ± 3.5</td>
<td>63 ± 14</td>
</tr>
<tr>
<td>Rem. NH₄-N (%)</td>
<td>66.9 ± 0.4</td>
<td>56 ± 4</td>
<td>55 ± 1</td>
<td>53.8 ± 1.6</td>
<td>50 ± 3</td>
<td>59 ± 2</td>
<td>55.0 ± 3.7</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>Inlet NO₂-N (mg/L)</td>
<td>200 ± 1.5</td>
<td>10.0 ± 2.5</td>
<td>14 ± 1</td>
<td>11 ± 3</td>
<td>22.0 ± 1.7</td>
<td>14 ± 2</td>
<td>10 ± 3</td>
<td>9.0 ± 2.5</td>
</tr>
<tr>
<td>Outlet NO₂-N (mg/L)</td>
<td>96 ± 2</td>
<td>49.0 ± 2.5</td>
<td>60 ± 7</td>
<td>71 ± 8</td>
<td>86 ± 4</td>
<td>80.0 ± 5.5</td>
<td>69 ± 2</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>Inlet NO₃-N (mg/L)</td>
<td>0.08 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.08 ± 0.03</td>
<td>0.16 ± 0.08</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Outlet NO₃-N (mg/L)</td>
<td>64 ± 6</td>
<td>70 ± 3</td>
<td>64 ± 3</td>
<td>65 ± 4</td>
<td>88 ± 1</td>
<td>60 ± 4</td>
<td>67 ± 4</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Inlet TP (mg/L)</td>
<td>98 ± 7</td>
<td>108 ± 18</td>
<td>92 ± 7</td>
<td>100 ± 15</td>
<td>101 ± 14</td>
<td>98 ± 7</td>
<td>104 ± 6</td>
<td>87 ± 11</td>
</tr>
<tr>
<td>Outlet TP (mg/L)</td>
<td>69 ± 4</td>
<td>49 ± 5</td>
<td>30 ± 4</td>
<td>31 ± 3</td>
<td>36 ± 3</td>
<td>49 ± 9</td>
<td>48 ± 9</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>Rem. TP (%)</td>
<td>30 ± 3</td>
<td>55 ± 3.5</td>
<td>65 ± 5</td>
<td>69 ± 4</td>
<td>64 ± 2</td>
<td>50 ± 4</td>
<td>54 ± 5</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Inlet pH</td>
<td>7.37 ± 0.47</td>
<td>7.66 ± 0.20</td>
<td>7.13 ± 0.07</td>
<td>7.1 ± 0.1</td>
<td>6.75 ± 0.40</td>
<td>6.81 ± 0.21</td>
<td>6.78 ± 0.35</td>
<td>6.24 ± 0.34</td>
</tr>
<tr>
<td>Outlet pH</td>
<td>6.11 ± 0.29</td>
<td>6.57 ± 0.30</td>
<td>6.77 ± 0.09</td>
<td>6.43 ± 0.28</td>
<td>6.38 ± 0.14</td>
<td>6.30 ± 0.18</td>
<td>6.36 ± 0.24</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
<td>6.24 ± 0.72</td>
<td>6.57 ± 0.23</td>
<td>6.88 ± 0.43</td>
<td>7.37 ± 1.00</td>
<td>6.41 ± 0.36</td>
<td>6.88 ± 0.30</td>
<td>6.82 ± 0.40</td>
<td>5.99 ± 0.20</td>
</tr>
</tbody>
</table>
in the beginning of the process, after that some of them disappeared (bands 9, 11, 12, 13, 14, 15, 16 and 18), remained visible thereafter (bands 6, 8 and 17) or increased in intensity and become as major bands (bands 1, 2, 3, 4, 5, 7 and 10). These results indicate that some change occurred within the microbial community structures in MBR. This was partly due to influent wastewater characteristics, treatment conditions and/or interactions between community members (e.g., predation). In fact, a total of 18 bands were excised from the DGGE gel and sequenced. Nucleotide sequences were compared to the GenBank and RDP databases using BLASTN and SeqMatch, respectively. All sequences exhibited 91% and 100% of similarity with the previously-identified 16S rRNA gene sequences.

Fig. 6 illustrates a phylogenetic tree-based on partial sequences of the 16S rRNA gene and shows that the found microorganisms belong to Proteobacteria (Alpha, Gamma, Delta and Epsilon-proteobacteria class) and Bacteroidetes (Flavobacteriaceae family) phyla.

Bands 1, 4, 5, 8, 9, 10 and 12 are clustered with the Flavobacteriaceae family bacteria belonging to one of the major branches of the phenotypically diverse phylum Bacteroidetes. Recently, phylogenetic analyses have revealed that many marine species of this family cluster together into a well-defined ‘marine clade’ (Bowman and Nichols, 2005). These marine Flavobacteria are known to be important members of the bacterial community in many aquatic environments and have a special role in using high-molecular-mass dissolved organic matter (Kirchman, 2002), while the sequences from bands 2, 3, 6, 7 and 15 are affiliated to the Halomonadaceae family (Oceanospirillales, Gammaproteobacteria) which contains halophilic and halotolerant genera. Some of the Halomonas species are known by their use in biotechnology owing to their producing potential of exopolysaccharides (EPS), biosurfactants, polyhydroxyalcanoates (PHA), compatible solutes and enzymes and by their active role in the process of denitrification and degradation of aromatic compounds (Oren, 2010). Halomonas alimentaria (Yoon et al., 2008) and Halomonas halodenitrificans (Miller et al., 1994) use nitrate and nitrite and are therefore denitrifiers. Cobetia marina (formerly Halomonas marina) (Arahala et al., 2002) is a hydrophilic bacterium that produces large quantities of exopolysaccharides mainly composed of uronic acids (Shea et al., 1991).

It also appears that fragments 13 and 14 show 100% of similarity with uncultured bacterium clones of the class Alphaproteobacteria, described in the microbialites of the alkaline Lake Alchichica (Couradeau et al., 2011) and in contaminated coastal sediment with oil pollution (Païssé et al., 2010). Whereas, band 11 presents 95% similarity with sulfate-reducing bacteria Desulfopila aestuarii (Suzuki et al., 2007) and Desulfurohupalus singaporensis (Lie et al., 1999). Detecting sulfate-reducing bacteria in aerobic reactor which is inoculated with sediment and seawater is not surprising.

Band 16 is affiliated to Arcobacter bivalviorum isolated from shellfish (Levican et al., 2012) and band 17 is related to a nitrogen-fixing bacterium Arcobacter nitrofigilis (Wesley et al., 1995). A. nitrofigilis (McClung et al., 1983) is the species type of the genus Arcobacter of the Campylobacteraceae family among the Epsilonproteobacteria. This strain couples the oxidation of sulfide to sulfur with the reduction of nitrate to nitrite (Gevertz et al., 2000). Finally, the sequence from band 18 shows similarity to the uncultured organism belonging to the SR1 division candidate, described in hypersaline microbial mat, (Kirk Harris et al., 2013) or in activated sludge for wastewater treatment (Kwon et al., 2010; Wan et al., 2011).

2.2.2. Membrane fouling
Notably, previous studies confirmed that high salt concentration in mixed liquor increases the osmotic pressure and viscosity and this has an effect on increasing the fouling by forming a more densely packed cake layer on the membrane surface (Lefebvre et al., 2004). In our case, when MBR filtration performance dropped to 60%, the chemical cleaning was proceeded to remove foulants from the membrane. Moreover, the permeate flux declined in 11 days from 130 to 59 L/(m²•hr) under the condition of HRT 3.8 days and OLR 0.5 kg TOC/(m³•day) (Fig. 7a). On the other condition, at HRT 1.4 days and OLR 1.4 kg TOC/(m³•day), the permeate flux dropped to 60% after 7 days (Fig. 7b). Therefore, it noted that there is quick gradual reductions of permeate flux versus time and there is a need to a progressive cleaning of the membrane to improve the flow rate.

Cleaning at day 11 (HRT 3.8 days) increases the permeate flow rate of the membrane to that reached during the start-up period. But cleaning at day 7 (HRT 1.4 days) mentioned that the cleaned membrane did not recover its properties as the new one. Indeed, the start-up permeates flow decreased from 3.8 to 1.4 days. These results are similar to that obtained by Chae et al. (2006) and Meng et al. (2007) who reported that a decrease in HRT leads to an increase in both EPS concentration and mixed liquor viscosity, which in turn results in a significant increase in membrane fouling. It was also demonstrated from previous studies that the decline in permeate flow can be attributed not
only to the organic remaining of pollutants as irreversible fouling, but also to common scalants including salts. High salt concentration in mixed liquor increases the osmotic pressure and the viscosity which can increase the fouling by forming a more densely packed cake layer on the membrane surface (Lefebvre and Moletta, 2006). Both effects of elevated salt environment and high total dissolved solids can lead to supersaturation condition that causes scaling.

Fig. 6 – Phylogenetic analysis of representative partial 16S rRNA gene sequences. The tree is based on the Maximum Composite Likelihood distance model and the neighbor-joining method. The bold names correspond to the sequence types isolated from the DGGE analysis, while the other sequences used in the analysis were obtained from GenBank. The scale bar represents 5% estimated sequence divergence. Palaeococcus ferrilphilus was used as outgroup.
membrane and exert detrimental influence on membrane performance, causing its flux decline (Le-Clech et al., 2006; Wang and Tarabara, 2007).

3. Conclusions

The MBR process proved that it was powerful for the salt wastewater treatment, as it achieved higher organic removal efficiencies more than CSTR, it was also shown to significantly reduce C, N, P and BOD₅. Nevertheless, salinity with increasing OLR aggravates membrane fouling in MBR process and leads to deterioration of sludge settleability and effluent quality in CSTR.

The structure and the dynamics of bacterial communities within MBR were investigated using the PCR-DGGE method. Some change occurred within the bacterial community structures in MBR during the treatment and the identified bacterial sequences belonged to halotolerant or halophilic bacteria and biofouling communities in MBR system.

This study implied that the results from MBR treatment are preliminary and full development with stable operation is yet to be reached. The optimization of operational variables, including the requirements for denitrification, needs to be carried out.

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