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Treatment of turtle aquaculture effluent by an improved multi-soil-layer system*

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Abstract: Concentrated turtle aquaculture effluent poses an environmental threat to water bodies, and therefore needs to be treated prior to disposal. This study was conducted to assess the effect of multi-soil-layer (MSL) systems treating turtle aquaculture effluent with adding different amounts of sludge. Four MSL systems were constructed with dry weight ratios of sludge with 0%, 5%, 10%, and 20% (MSL 1, MSL 2, MSL 3, and MSL 4, respectively). The turtle aquaculture effluent had an average chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N) and total nitrogen (TN) concentration of 288.4, 213.4, and 252.0 mg/L, respectively. The COD/TN (C/N) ratio was 1.2. The results showed that the four MSL systems could effectively treat the COD, NH_4^+-N, and TN, and MSL 4 showed significantly improved NH_4^+-N removal efficiency, suggesting the potential of sludge addition to improve the turtle aquaculture effluent treatment. The average COD, TN, and NH_4^+-N removal efficiencies of MSL 4 were 70.3%, 66.5%, and 72.7%, respectively. To further interpret the contribution of microorganisms to the removal, the microbial community compositions and diversities of the four MSL systems were measured. Comparisons of the denaturing gradient gel electrophoresis (DGGE) profiles revealed that the amount of nitrifying bacteria and diversity in MSL 4 were higher than those in the other three systems. We concluded that adding 20% of sludge improved the NH_4^+-N removal and stability of the system for nitrification, due to the enrichment of the nitrifying bacteria in MSL 4.

Key words: Turtle aquaculture effluent, Multi-soil-layer (MSL) system, Sludge, Microbial community diversity
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1 Introduction

The production of turtles has increased from 58276 t in 1999 to 230219 t in 2009 in China (Ministry of Agriculture and Fisheries Bureau of China, 2010), where turtles are traditionally used as food and traditional medicine (Shi *et al*., 2008). Demand for turtles has increased as the income of people has improved, and this has encouraged farmers to initiate more breeding farms over the past 20 years. As a lowland aquatic animal (Chen and Lue, 2010), turtles are mainly cultured in southern China, where ground water is abundant and agricultural land is limited (Shi *et al*., 2008). Farmers in this area are commonly using concentrated turtle aquaculture operations to solve the problem of low land availability. Concentrated aquatic farming of turtles requires intensive feeding of high-protein feed in aquatic systems with limited space. This breeding pattern results in a large amount of excreta and feed residuals remaining in the aquaculture water bodies, causing serious water degradation.

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Indeed, toxic substances such as ammonium and organic matter can accumulate to levels that prevent further turtle growth (da Silva *et al*., 2013). To ensure satisfactory aquaculture water quality, farmers frequently conduct water exchange with fresh water from rivers, resulting in a large quantity of wastewater containing high concentrations of ammonium, phosphorus, and organic matter being released into rivers without any treatment (Smith *et al*., 1999).

Chen *et al*. (2013) reported that wastewater from turtle aquaculture was characterized by high ammonia nitrogen (NH_4^+-N) (80–250 mg/L) and a low chemical oxygen demand (COD)/total nitrogen (TN) (C/N) ratio (1.81–2.13), which is considered to be an important parameter for nitrogen removal (Kim *et al*., 2008). Nitrification coupled with denitrification processes (Ahn, 2006) is the primary process for N removal, in which nitrate produced from NH_4^+-N through nitrification mediated by nitrifying bacteria is reduced to nitrogen gas through denitrification by denitrifying bacteria. These key processes require sufficient organic matter as the carbon source for bacterial growth (Ostace *et al*., 2011). Wastewater with a low C/N ratio is not able to supply bacteria with sufficient carbon, resulting in low nitrogen removal efficiency (Kim *et al*., 2004; Sun *et al*., 2010). Several processes have been developed to address this problem. For example, Lin *et al*. (2002) developed a planted wetland for removal of nitrogen via vegetation uptake, but this system covered a large area. Feng *et al*. (2003) reported an electrochemical treatment system that removed a high amount of nitrogen. Kim *et al*. (2008) demonstrated that a two-stage sequencing batch reactor (SBR) with independent nitrification could effectively remove nitrogen from low carbon-to-nitrogen wastewater without the supplement of any external carbon sources. Chen *et al*. (2012) showed that nitrogen was effectively removed from turtle wastewater by the anammox process in an up-flow anaerobic sludge blanket (UASB) reactor. However, this has been hampered by complicated operations and maintenance, and by a shortage of professional experts, which is a critical obstacle in turtle farms. The application of systems to turtle effluent has also been restricted by limited land space in southern China and high operational costs. Thus, an efficient, cost-effective, and easy-operating technology for low C/N ratio wastewater treatment is needed.

A promising method for the treatment of turtle aquaculture wastewater, the multi-soil-layer (MSL) system created by Wakatsuki in 1990, is easy to operate and maintain, while occupying a relatively small area of land (Wakatsuki *et al*., 1993; Chen *et al*., 2009). These systems use natural soil and zeolite to facilitate wastewater treatment through a combination of physical, biological, and biochemical processes (Luanmanee *et al*., 2001). MSL systems have been widely applied to various types of wastewater treatment, such as domestic wastewater, restaurant wastewater, polluted river water, livestock wastewater, dairy effluent, and the leachate from rural unsanitary landfills (Wakatsuki *et al*., 1993; Luanmanee *et al*., 2001; Chen *et al*., 2007; Pattnaik *et al*., 2008; Guan *et al*., 2012). Guan *et al*. (2012) reported that use of an MSL system to treat leachate from rural unsanitary landfills (low C/N ratio wastewater) was feasible, but the TN removal was low.

In MSL systems, NH_4^+-N removal is dominated by the biological nitrification process, and TN is removed through nitrification and denitrification (Lahav and Green, 1998; Chen *et al*., 2009; Guan *et al*., 2012). Pattnaik *et al*. (2008) showed that increasing the microbial activity in MSL systems could increase the removal of N. Improving microbial activity and understanding the relationship between the microbial community in an MSL system and N removal are essential for the successful application of such systems to turtle aquaculture wastewater. In this study, sludge collected from sewage treatment plants was added to soil mixed blocks (SMBs), an important component of the MSL system, to alter the microbial community, and the potential feasibility of the MSL systems for treatment of turtle aquaculture effluent was evaluated.

2 Materials and methods

2.1 MSL systems and operations

An MSL system composed of acrylic plastic with a size of 80 cm×110 cm×30 cm was employed to treat turtle aquaculture effluent (Fig. 1). The system contained two parts: a permeable layer (PL) and an SMB. The SMB consisted of red soil mixed with bamboo charcoal, bamboo powder, and iron filings at the ratio of 7:1:1:1, with or without sludge collected

from a local sewage treatment plant arranged to form an alternating brick layer-like pattern (Table 1), a dimension of 20 cm×7 cm×30 cm and 10 cm×7 cm×30 cm. The sludge was mixed into the SMBs at a ratio of 0%, 5%, 10%, and 20% (dry weight basis), and the four treatments were denoted MSL 1, MSL 2, MSL 3, and MSL 4, respectively. The PL consisted of 5 cm zeolite, with a diameter of 3–5 mm. Zeolite, with a diameter of 3–5 mm, was used to fill the voids between adjacent SMBs, and the bottom of the system was filled with 10 cm of gravel with a diameter 2–3 cm. The influent emitter pipes were installed on top of the systems (Fig. 1).

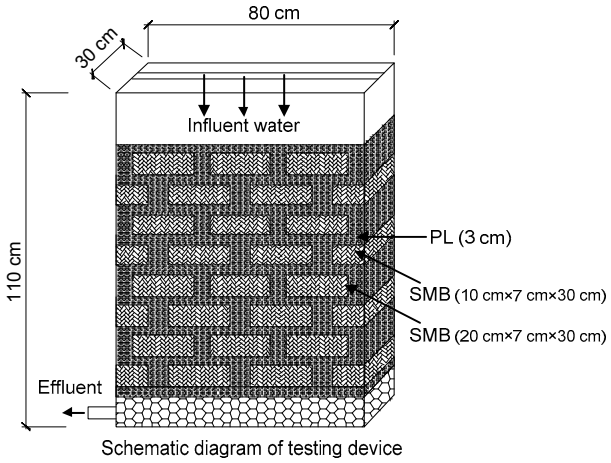

Fig. 1 Basic structure of the MSL system

The turtle aquaculture wastewater was distributed directly into the MSL systems continuously for 24 h by a peristaltic pump (Longer BT100-1J, China) after filtration through a 109-μm nylon net (140 mesh). Each system with an initial hydraulic loading rate (HLR) of 100 L/(m^2·d) was operated for 6 d, followed by 10 d at 200 L/(m^2·d) to increase sludge activity, and finally at an HLR of 400 L/(m^2·d) for 200 d with corresponding nominal hydraulic retention time (HRT) of 30 h.

2.2 Sampling

Influent and effluent from the MSL systems were sampled every 10 d and stored at −20 °C until subsequent analysis. SMB in each system was sampled from Layers 1 (3–10 cm) and 8 (73–80 cm) and mixed by the X sharp method (M 1/2/3/4-1 soil and M 1/2/3/4-8 soil, respectively) at the end of the experiment (Lin *et al*., 2002). All samples were freeze-dried and stored at −20 °C until subsequent analysis.

2.3 Analytical methods

Concentrations of COD, TN, and NH_4^+-N in effluent and influent were measured according to the methods described in the Water and Wastewater Monitoring Method (Environmental Protection Agency of China, 2002). The dichromate oxidizability (COD_{Cr}) was measured by the potassium dichromate method (SEPA, 1990b), NH_4^+-N by the Nessler method (SEPA, 1987), and TN (after potassium peroxodisulfate digestion) by the cadmium reduction method (SEPA, 1990a).

2.4 Microbial analysis

The microbial community diversity in the SMB layers of all four MSL systems was assayed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Briefly, DNA was extracted from the soil samples using a FastDNA® Spin Kit for Soil (MP BIO Laboratories, CA, USA). The extracted DNA was then used as templates for PCR, which was conducted in a 22331 Master Cycler Gradient PCR machine (Eppendorf, Germany) in 50 μl reaction mixtures composed of 5 μl 10× Ex Taq buffer, 4 μl dNTP mixture (2.5 mmol/L each), 0.5 μl primer (20 μmol/L each), 0.25 μl DNA polymerase (5 U/μl) (TaKaRa, Ex Taq, Japan), and 0.5–2.0 ng of DNA. The 16S rRNA gene fragments from the V3 region were targeted using the general bacterial primers P357f-GC clamp/P518r (Muyzer *et al*., 1993). The cycling program for PCR consisted of 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 2 min at 72 °C, followed by a final extension step at 72 °C for 10 min. DGGE in this experiment was conducted using the BioRad D gene system (BioRad, USA) with poly-acrylamide gels containing denaturing gradients ranging from 30% to

Table 1 Compositions and dry weight fraction of materials in the soil mixture blocks in the four systems

MSL system	Red soil (%)	Sludge (%)	Bamboo-charcoal (%)	Bamboo powder (%)	Iron filings (%)
1	71.4	0.0	9.5	9.4	9.7
2	68.0	4.8	9.1	9.0	9.2
3	64.9	9.1	8.7	8.6	8.9
4	59.5	16.7	7.9	7.9	8.1

60% (100% denaturant is 7 mol/L urea plus 40% formamide) to separate bacteria based on their V3 regions. After electrophoresis, the gels were stained with SYBR Green I (Sigma, USA) for 30 min according to the manufacturer's instructions, and the gel images were analyzed using the Quantity One image analysis software (Version 4.62, BioRad).

2.5 Cloning and sequencing

The dominant DNA bands of interest in the DGGE gel were separated and the extracted DNA was used as the templates in PCR conducted using the primer set 357F/518R. Subsequently, the purified PCR-amplified DNA from DGGE was cloned using a pEASY™-T1 Cloning Kit (TransGene Biotech, Beijing, China) for sequencing into a pEASY™-T1 vector according to the manufacturer's instructions and then transformed into *Escherichia coli* DH5α. Ampicillin-resistant-positive clones carrying PCR products were sequenced directly by Meiji Inc. (Shanghai, China).

2.6 Data analysis

The removal efficiency for each contaminant parameter was calculated as the percentage removal (R) for each parameter, which was determined using the following equation: $R=(1-C_e/C_i)\times100\%$, where C_i and C_e are the influent and effluent concentrations in mg/L, respectively. Bacterial diversity was estimated on the basis of fingerprint densitometric measurements and the Shannon diversity index was calculated. The Shannon index was used to estimate soil bacterial diversity based on the intensity and number of bands using the following equation:

$$H=-\sum_{i=1}^{s}p_i\log p_i=-\sum_{i=1}^{s}(n_i/N)\log(n_i/N),$$

where H is the Shannon index, n_i is the height of the ith peak, and N is the sum of all peak heights in the curves (Zhao *et al*., 2010). A higher Shannon index indicates higher bacterial diversity (Boon *et al*., 2002). The nucleotide sequences were compared with those deposited in GenBank (NCBI) using the basic local alignment search tool (BLAST), after which the sequences determined in this study and obtained from the DNA database were aligned and neighbor-joining trees were constructed by pairwise deletion using MEGA Version 5.05 (Molecular Evolutionary Genetics Analysis). Tree topology was evaluated by bootstrap analysis using 1000 replicates.

2.7 Nucleotide accession numbers

The 16 nucleotide sequences identified in this study were deposited in the GenBank database under accession numbers JF988302.1, JX040355.1, AB635 918.1, HE801216.1, CU923526.1, AY114341.1, EF 636477.1, AM934744.1, CU926694.1, AB240495.1, HQ133204.1, JQ177476.1, JQ012289.1, HQ003474.1, AB604822.1, and JQ278964.1, respectively.

3 Results and discussion

3.1 C/N ratio in turtle aquaculture wastewater

The turtle wastewater is typical of low C/N ratio wastewater, of which the C/N ratio is 1.2±0.5 (Table 2). When the influent C/N ratio is lower than 3.4, extra carbon should be added to the system to remove redundant N (Kuba *et al*., 1996). Influent with a low C/N ratio is deficient in organic carbon, and a low carbon source can limit biological denitrification (Kim *et al*., 2008; Sun *et al*., 2010). Chu and Wang (2011) reported that, under the average influent of TN 52.6 mg/L, TN removal was only 1%–20% when using a moving bed biofilm reactor to treat low C/N ratio wastewater (3.7–4.2). Due to insufficient carbon levels, TN removal could be inhibited when the influent C/N ratio is low when sequencing batch membrane bioreactors (SBMBRs) (Zhang *et al*., 2006), SBR (Kim *et al*., 2008), and wetlands (Wen *et al*., 2010) are used for treatment. Therefore, the wastewater with a low C/N ratio in the present study would not be able to supply enough degradable carbon to act as an electronic donor for denitrification if conventional methods were used.

3.2 Performance of the MSL system in wastewater treatment

3.2.1 COD removal

MSL systems could effectively remove COD from turtle wastewater. As shown in Table 2 and Fig. 2, during the initial operation period (in Days 0–80), the COD removal efficiencies of the four treatments were over 70.0%, and the corresponding effluent CODs of MSLs 1–4 were (53.6±12.1), (48.5±15.2), (48.9±12.9),

Table 2 Comparison of the average pollutant concentrations of turtle wastewater with the discharge concentration limit

Sourcre	Concentration (mg/L)			Removal (%)			C/N ratio
	COD	TN	NH_4^+-N	COD	TN	NH_4^+-N	
Influent	288.4±72.1	252.1±80.7	213.4±95.7				1.2±0.5
MSL 1	96.7±43.3	119.2±58.8	93.3±59.6	66.3a	47.7a	55.2a	
MSL 2	91.9±44.4	131.2±62.9	101.9±65.5	67.9a	52.9a	52.7a	
MSL 3	87.5±39.0	118.8±69.2	85.1±66.0	69.1a	53.1a	60.4a	
MSL 4	84.9±48.1	87.7±55.2	59.5±49.9	70.3a	66.5a	72.7b	
Legislation (GB 18918-2002)	100		30				

Data are expressed as mean±standard deviation (SD) (n=3). Different letters in the same column indicate significant differences (P<0.05). COD: chemical oxygen demand; TN: total nitrogen; C/N ratio: COD/TN ratio

and (40.0±11.4) mg/L, respectively. The average effluent COD of all systems was lower than the limits, except on Day 40. These efficiencies then decreased gradually to 50%–60% until Day 140 in all systems, which might have been due to the low temperatures (−3 °C to 8 °C) at this time. Following this period, the COD removal efficiencies of all MSL systems subsequently increased to 50%–80% as the average temperature increased to 12 °C, where they remained until the end of the experiment, except on Day 180. These results are similar to a study conducted by Chen *et al.* (2007), who found that a decrease in temperature was associated with a decrease in the efficiency of COD and vice versa.

Statistical analysis showed that the percentage removal of COD did not differ significantly among the four MSL systems with different amounts of sludge (P>0.1), indicating that the addition of sludge to the MSL systems was not closely related to COD removal. The average COD in influent was (288.4±72.1) mg/L, whereas the average CODs in effluent of MSL 1, MSL 2, MSL 3, and MSL 4 were (96.7±43.3), (91.9±44.4), (87.5±39.0), and (84.9±48.1) mg/L with corresponding average removal rates of 66.3%, 67.9%, 69.1%, and 70.3%, respectively (Table 2). Although the effluent COD did not meet the discharge standards, the average removal efficiency of all MSL systems was more than 65.0%, indicating that the MSL system could efficiently remove COD from turtle wastewater with greater efficiency than soil cultivating systems (Lao, 2001), which show an average COD removal rate of 44.7% under an influent COD of 159.2 mg/L.

3.2.2 NH_4^+-N removal

The efficiencies of the MSL systems in removing NH_4^+-N from turtle wastewater varied significantly.

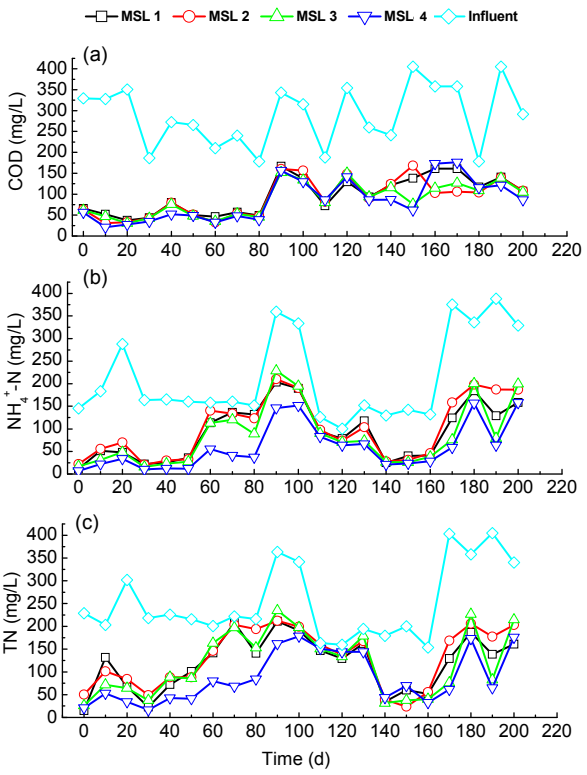

Fig. 2 Influent and effluent concentrations of COD (a), NH_4^+-N (b), and TN (c) of the four MSL systems for turtle aquaculture wastewater

When the average influent NH_4^+-N was (213.4±95.7) mg/L, the average effluent NH_4^+-N values were (93.3±59.6), (101.9±65.5), (85.1±66.0), and (59.5±49.9) mg/L and the corresponding average NH_4^+-N removal efficiencies were 55.2%, 52.7%, 60.4%, and 72.7% for MSLs 1–4, respectively (Fig. 2b and Table 2). The NH_4^+-N removal efficiency of MSL 4 was significantly higher than those of the other MSL systems, and also was higher than that after the treatment of water-hyacinths (71.5%) when concentrations varied from 11.27 to 39.68 mg/L (Yuan and Liu, 2001).

Good nitrification is known to be an advantage of MSL systems, which is dependent on biological nitrification to remove NH_4^+-N (Guan *et al*., 2012). NH_4^+-N from the influent is concentrated in the zeolite, which has no nitrate adsorption capacity, but provides a good habitat for nitrifiers, and the adsorption capacity of zeolite could be regenerated (Jung *et al*., 2004). The concentrated NH_4^+-N is then consumed as the substrate of microorganisms and converted to NO_3^--N through nitrification (Lahav and Green, 1998; Guan *et al*., 2012). Since the sludge possessed a variety of functional microorganisms including nitrifying bacteria (Juretschko *et al*., 2002), the highest NH_4^+-N removal efficiency was observed in the 20% sludge MSL system, indicating that a 20% sludge addition into the MSL system could improve NH_4^+-N removal for increasing biological nitrification.

3.2.3 TN removal

The MSL system could efficiently remove TN from wastewater with low C/N ratio 1.2±0.5. As shown in Fig. 2c and Table 2, under the average influent TN of (252.1±80.7) mg/L, the average TN removal efficiencies were 47.7%, 52.9%, 53.1%, and 66.5% for MSLs 1–4, respectively. The TN purification efficiency was higher than that of the constructed wetlands to treat this low C/N ratio wastewater (Wu *et al*., 2009). Luanmanee *et al*. (2001) suggested that the low C/N ratio (1–3) of the pre-treated wastewater tended to result in low TN removal owing to insufficient carbon available for denitrification. In the present study, the MSL systems could effectively remove TN from turtle wastewater (Fig. 2). This was likely because, although the C/N ratio of turtle wastewater was low, the organic matter in the wastewater was biodegradable and the bamboo charcoal powder was added to SMB. Isaacs and Henze (1995) and Meinhold *et al*. (1998) suggested that the addition of organic materials (10% litter and 15% pelletized jute) to the SMB might provide a sufficient carbon source for microorganisms and contribute to the efficient TN removal for low C/N wastewater treatment.

Nitrification coupled with denitrification is the major N removal process. As the dominant form of N, NH_4^+-N from influent was concentrated in zeolite and then decomposed by microorganisms (Wakatsuki *et al*., 1993; Lahav and Green, 1998). Under aerobic conditions, NH_4^+-N is converted to NO_3^--N through biological nitrification, after which influent is translocated to the SMB, where nitrogen gas is formed via biological denitrification under anaerobic conditions (Chen *et al*., 2009).

3.3 Analysis of bacterial community diversity

As shown in Table 3 and Fig. 3, the diversity in SMB Layer 1 (3–10 cm) was higher than that in SMB Layer 8 (73–80 cm) in all four MSL systems. This phenomenon may be attributed to inadequate oxygen or decreased microbial available nutrients in Layer 8. The influent was distributed into MSL systems from Layer 1 to Layer 8 one by one. Thus, the nutrients from the influent in tested Layer 1 were higher than those in tested Layer 8. Similarly, Iasur-Kruh *et al*. (2010) showed that physicochemical characteristics (oxygen and nutrients) had a strong effect on the microbial community of the biofilm. In addition, the Shannon index of the tested layers in MSL 4 was the lowest, while the NH_4^+-N removal of MSL 4 was the highest. This observation is possibly owing to the fact that the microbial community diversity was not closely related to the NH_4^+-N removal in MSL 4.

Table 3 Shannon-Wiener diversity index for different layers of the four MSL systems

Layer	Number of bands	Shannon index (*H*)
$M_{1\text{-}1}$	20	1.13
$M_{2\text{-}1}$	20	1.19
$M_{3\text{-}1}$	15	1.14
$M_{4\text{-}1}$	8	0.88
$M_{1\text{-}8}$	14	1.09
$M_{2\text{-}8}$	9	1.02
$M_{3\text{-}8}$	9	1.03
$M_{4\text{-}8}$	7	0.59

$M_{1\text{-}1}$, $M_{2\text{-}1}$, $M_{3\text{-}1}$, and $M_{4\text{-}1}$ represent the 1st layer (3–10 cm) of soil in MSL 1, MSL 2, MSL 3, and MSL 4, respectively. $M_{1\text{-}8}$, $M_{2\text{-}8}$, $M_{3\text{-}8}$, and $M_{4\text{-}8}$ represent the 8th layer (73–80 cm) of soil in MSL 1, MSL 2, MSL 3, and MSL 4, respectively

3.4 Bacterial community composition and NH_4^+-N removal

The phylogenetic positions of the 16S rRNA gene sequences are illustrated in the neighbor-joining tree shown in Fig. 4. The microbial community profiles revealed that nitrifying bacteria were richer in the first layer of MSL 4, which had higher NH_4^+-N removal than others. All identified sequences matched

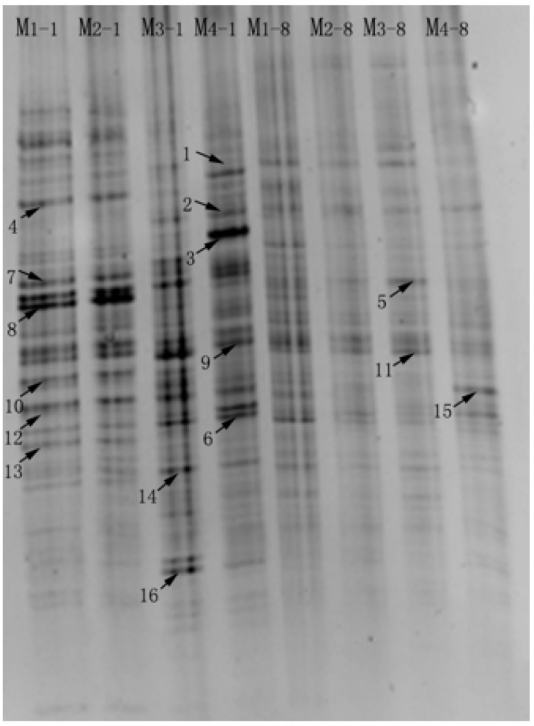

Fig. 3 DGGE analysis of the V3 region bacterial fragments retrieved from SMB layers in the four MSL systems

The dominant bands were numbered 1–16. $M_{1\text{-}1}$, $M_{2\text{-}1}$, $M_{3\text{-}1}$, and $M_{4\text{-}1}$ represent the 1st layer (3–10 cm) of soil in MSL 1, MSL 2, MSL 3, and MSL 4, respectively. $M_{1\text{-}8}$, $M_{2\text{-}8}$, $M_{3\text{-}8}$, and $M_{4\text{-}8}$ represent the 8th layer (73–80 cm) of soil in MSL 1, MSL 2, MSL 3, and MSL 4, respectively

those present in the NCBI database with 93% to 100% similarity. Phylogenetic analysis of the excised DGGE bands revealed that the dominant members included α, β, γ, and δ subdivisions of Proteobacteria (Bands 4, 8, 13, 14, and 15), Bacteroidetes (Bands 1, 5, and 7), Spirochaetes (Bands 11 and 12), and Actinobacteria (Band 9). Sequence analyses against the GenBank revealed similarities to bacteria performing specific activities expected to be relevant in an MSL system. For example, the sequence recovered from Band 3 most closely matched uncultured Nitrospirae bacterium AB635918.1 (100% homology), which was only observed in SMB Layer 1 of MSL 4, while the sequence of Band 6 most closely matched uncultured *Nitrosospira* sp. (97% DNA identity) and appeared in SMB Layer 1 of all four systems, and the sequence of Band 16 was closest to *Nitrospira* sp. JQ278964.1 (99% similarity), which appeared in SMB Layer 1 of MSL 3 and MSL 4. These microorganisms are nitrifying bacteria, which correspond well with the relatively high nitrification potential (Siripong and Rittmann, 2007; Iasur-Kruh *et al*., 2010). Taken together, these results indicated that nitrifying bacteria were ubiquitous in SMB Layer 1 of all four MSL systems, especially MSL 4, and that they played an important role in NH_4^+-N reduction. The coexistence of various nitrifiers may help maintain the stability of nitrification systems (Siripong and Rittmann, 2007). Furthermore, Bands 3 and 6 were expressed at higher intensity in MSL 4, indicating that the nitrifying bacteria were more abundant in MSL 4. Indeed, a high abundance of nitrifying bacteria facilitates NH_4^+-N removal (Juretschko *et al*., 2002; Li *et al*., 2011). Overall, these findings indicated that the MSL system with 20% added sludge was enriched with nitrifying bacteria, which are regarded to confer performance stability and improve NH_4^+-N removal. Other dominant members in the systems (Proteobacteria, Bacteroidetes, Spirochaetes, and Actinobacteria) might also actively participate in wastewater treatment; therefore, further studies are also needed to evaluate the functions of these strains in wastewater treatment.

4 Conclusions

Under an HLR of 400 L/(m^2·d), the four MSL systems could effectively remove COD, NH_4^+-N, and TN from turtle wastewater, which was characterized by a low C/N ratio 1.2±0.5, indicating that the MSL system has the feasibility to treat low C/N wastewater. The addition of 20% sludge to the MSL system could significantly improve the removal efficiency of NH_4^+-N. The average COD, TN, and NH_4^+-N removal efficiencies of MSL 4 were 70.3%, 66.5%, and 72.7%, respectively. Furthermore, after the addition of 20% sludge, nitrifying bacteria were richer and became more diverse in the first SMB layer, which may be valuable for maintaining the stability of nitrifying performance and improving NH_4^+-N removal efficiency.

Compliance with ethics guidelines

Ying SONG, Yu-ting HUANG, Hong-fang JI, Xin-jun NIE, Zhi-yuan ZHANG, Chuan GE, An-cheng LUO, and Xin CHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

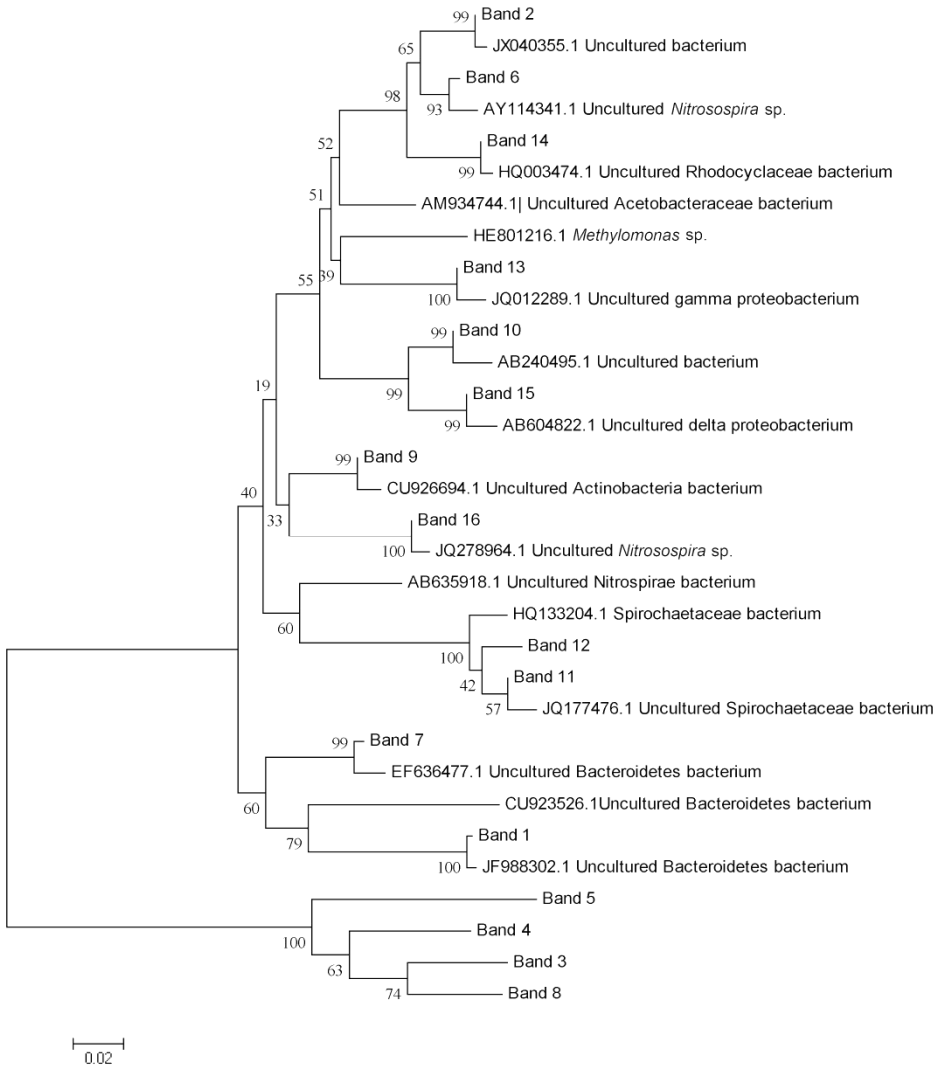

Fig. 4 Phylogenetic tree based on neighbor-joining analysis of gene sequences from the V3 region of bacteria
Bands 1–16 represent the sample sequences. Numbers at the branches represent bootstrap values, and the scale bar indicates 2 changes per 100 amino acid positions

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中文概要

题　目：改良型多介质土壤渗滤系统对甲鱼养殖废水的净化效果研究

目　的：通过向多介质土壤渗滤系统（MSL）的土壤层添加污泥，研究改进后的系统对工厂化甲鱼养殖废水的处理效果，分析系统内微生物群落的变化，以期部分揭示其可能的作用机理。

创新点：工厂化甲鱼养殖废水排放量日益增大，且水体氨氮含量较高。目前市场上缺乏针对甲鱼养殖废水的处理技术，MSL 系统对该废水的处理也未有报道。本文在 MSL 系统的基础上进行改良，并将其应用于甲鱼养殖废水处理上，提出一套有效的甲鱼养殖废水处理技术，并对 MSL 系统内微生物群落结构进行了分析。

方　法：向 4 套 MSL 小试装置中分别添加 0%、5%、10%和 20%污泥，研究其对工厂化甲鱼养殖废水的净化效果。试验中水质指标测定均按国家标准方法进行，系统内微生物群落结构采用聚合酶链反应–变性梯度凝胶电泳（PCR-DGGE）法测定。

结　论：MSL 系统可有效地处理工厂化甲鱼养殖废水，向系统中添加 20%污泥后处理效果更佳。添加 20%污泥的系统内具有较高的硝化类细菌多样性和都较多的生物量。

关键词：甲鱼养殖废水；多介质土壤渗滤系统；污泥；微生物群落结构