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Application of an aquatic plant ecosystem for swine wastewater polishment: a full-scale study

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ABSTRACT

In Taiwan, swine wastewater has become one of the major causes of the deterioration of surface water quality. The objective of this study was to evaluate the efficacy of using aquatic plant ecosystem to polish the treated swine wastewater. In this study, a hog farm was selected as the case study site. The daily wastewater of the studied farm was approximately 40 m^3/d. *Eichhornia crassipes* was used as the aquatic plant for water polishment in the aquatic plant basin. Influent and effluent samples from each treatment unit were collected for water quality analyses. Results show that although the conventional three-stage system (solid separation followed by anaerobic and aerobic treatment) was effective to remove more than 97% of suspended solid (SS), ammonia nitrogen (NH_3-N), chemical oxygen demand (COD), and biochemical oxygen demand (BOD), BOD and COD in effluents still could not meet the discharge standards. The full-scale study shows that the aquatic plant ecosystem played an important role in the swine wastewater polishment and more than 27% of SS, NH_3-N, COD, and BOD could be removed further from the effluents of the three-stage system, and the treated water could comply with the discharge standards and also meet the water reuse standards for irrigation and road washing. Results of polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and nucleotide sequence analyses indicate that a variation in microbial diversity in the treatment system was observed. The system contained significant amounts of microbial ribospecies, which contributed to the carbon and nitrogen removal. DGGE results show that disappearance of *E. coli* was observed indicating that the system could remove *E. coli* and pathogens effectively. Results indicate that the aquatic plant ecosystem can be combined with the conventional three-stage treatment system to further polish the swine wastewater effluent.

Keywords: Aquatic plant ecosystem; Denaturing gradient gel electrophoresis (DGGE); *Eichhornia crassipes*; Polymerase chain reaction (PCR); Swine wastewater

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1. Introduction

In Taiwan, the main water pollution sources are livestock wastewater from hog farms, municipal wastewater, industrial wastewater, non-point source pollutants from agricultural areas, and leachate from riverbank landfills [1,2]. Hog farming is a particularly important activity in the rural and agricultural areas in most of the river basins. Some of the untreated hog farm wastes are indiscriminately discharged into the downstream rivers. Thus, swine wastewater has become one of the major causes of the deterioration of surface water quality.

Currently, most hog farms in Taiwan use the three-stage wastewater treatment system for the swine wastewater treatment to meet the wastewater discharge standards established by Taiwan Environmental Protection Administration (TEPA) [COD = 600 mg/L, biochemical oxygen demand (BOD) = 80 mg/L, and SS = 150 mg/L] [3]. The three-stage treatment system includes the solid/liquid separation process (the first stage) followed by anaerobic treatment process (the second stage) and aerobic treatment process (the third stage). The three-stage treatment facility is able to treat the swine wastewater efficiently to meet the discharge standards [2,4,5]. However, the aerobic treatment system is not properly operated to minimize the operational and maintenance cost. Therefore, effluents, which are not properly treated, could not meet the discharge standards. This causes the deterioration of the river water quality after its discharge into the surface water bodies.

Less expensive natural treatment systems such as aquatic plant ecosystem have been applied as an alternative of the aerobic treatment system to meet the discharge standards [6–9]. Concern about the deteriorating conditions of the river water quality led the local government agencies to amend the relevance legislations and strengthen the enforcement of the discharge regulations to effectively control the pollution from the hog farms. The ongoing governmental policy for hog farm waste management is to encourage hog farmers to apply natural treatment system to polish the effluent from the conventional treatment system. There has been a growing recognition of the multi-function values of natural treatment systems, which are used for treating a variety of wastewaters including industrial and domestic wastewaters [10–12]. Natural treatment ecosystem is an eco-technology, which is beneficial to small industries (such as small to mid-scale hog farms) that cannot afford high operational and maintenance cost of conventional treatment system [13–16]. Compared to the traditional wastewater treatment facilities, natural treatment systems require lower construction, operational, and maintenance costs [17–20].

Aquatic plant ecosystem is similar to the constructed wetland system with a shallow water depth and water flowing laterally through the system. Aquatic plant ecosystem typically consists of a basin with a subsurface barrier to prevent seepage and water at a relatively shallow depth flowing through the unit [21,22]. The shallow water depth, low flow velocity, and presence of the floating aquatic plants are the main characteristics of aquatic plant ecosystems [23]. Although the natural treatment ecosystems are well established, its application for the polishment of swine effluents has not been well documented.

Recently, molecular biological techniques [e.g. PCR-DGGE (polymerase chain reaction-denatured gradient gel electrophoresis)] have been applied to evaluate the substantial shift of microbial diversity when the biomass was exposed to the environmental pollutants and nutrients [e.g. BOD, ammonia nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N)]. These techniques are effective in determining the structure of microbial communities and trends of environmental transitions during the bioremediation processes [24]. The major objectives of this full-scale study were to (1) examine the feasibility and effectiveness of using an aquatic plant ecosystem to post-treat and polish the treated swine wastewater from the three-stage treatment scheme, and (2) determine the dominant native microorganisms at different locations of the studied treatment system through microbial identification via PCR, DGGE, and nucleotide sequence methods.

2. Materials and methods

In this study, a hog farm was selected as the case study site. The studied hog farm contained 700 hogs, and the daily wastewater was approximately 40 m^3/d. The hog farm utilized the conventional three-stage treatment system as its wastewater treatment facility. An aquatic plant unit planted with floating aquatic plants [*Eichhornia crassipes* (water hyacinth)] (collected from an adjacent natural wetland) was placed after the aerobic system for wastewater polishment. Fig. 1 presents the schematic diagram of the treatment system for swine wastewater. Table 1 shows the components and dimensions of the wastewater treatment systems.

In the full-scale study, the raw wastewater was delivered into the system (Fig. 1) continuously at a rate of approximately 40 m^3/d. Water samples of influents and effluents from four major treatment units (e.g. solid/liquid separation, anaerobic basin, sedimentation basin, aquatic plant basin) were collected and

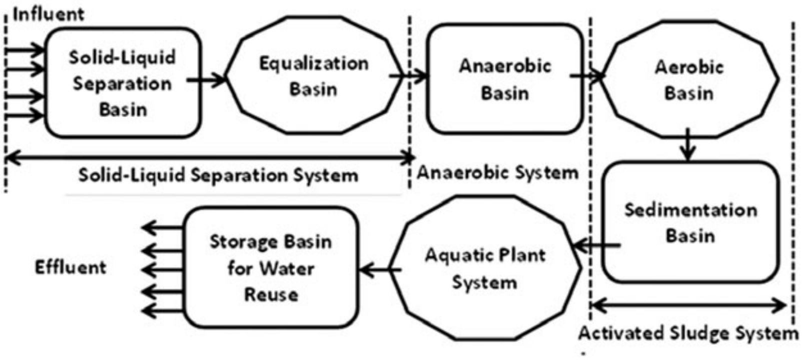

Fig. 1. Schematic diagram showing the swine wastewater system of the studied hog farm.

Table 1
Components and dimensions of the wastewater treatment and aquatic plant ecosystems

Unit	Dimensions (L × W × D) (m)	Volume (m^3)
Solid/liquid separation basin	3 × 1.5 × 1	4.5
Equalization basin	6 × 2 × 3	72
Anaerobic basin	6 × 4 × 3	216
Aerobic basin	4 × 3.5 × 3	84
Sedimentation basin	5 × 4 × 3	60
Aquatic plant ecosystem	13.5 × 4 × 3	162

analyzed monthly during the 15-month investigation period. The following major water quality indicators [e.g. chemical oxygen demand (COD), BOD, suspended solid (SS), total phosphorus (TP), NH_3-N, NO_3-N, nitrite-nitrogen (NO_2-N), total coliforms (TC), dissolved oxygen (DO), oxidation-reduction potential (ORP), electric conductivity (EC), temperature, and pH] were analyzed. Water quality analyses were conducted in accordance with the methods described in Standard Methods [25]. Perkin-Elmer Plasma II Inductively Coupled Plasma-Argon Emission Spectrometer (ICP-AES) was used for cation analyses following Standard Methods [25]. DO, ORP, pH, EC, and temperature were measured in the field. An Accumet 1003 pH/ORP meter (Fisher Scientific) was used for pH and ORP measurements, an Orion DO meter (Model 840) was used for DO and temperature measurements, and a portable conductivity meter SC-120 (Suntex) was used for EC measurements.

The samples of the plant materials including leaves, rhizomes, and roots were prepared according to the method described by Chen et al [6]. The plant tissues were subsequently decomposed by heating of at 300˚C for 2 h and all plant parts were wet digested in concentrated HNO_3:HCl (1:3, V:V). The digested solutions were subjected to the determination of heavy metal using either a flame atomic absorption spectrophotometer [18]. The average of three replications was considered for each sample.

The following first-order decay model for plug flow concentration evaluation was used to determine the first-order decay rates for BOD and NH_3-N in the aquatic plant ecosystem [20]:

$$C_e = C_0 \exp(-kt) \tag{1}$$

where C_e is the mean BOD or NH_3-N concentration in the effluent (mg/L), C_0 is the mean BOD or NH_3-N concentration in the influent (mg/L), k is the temperature-dependent decay rate constant (d^{-1}), and t is the hydraulic retention time (HRT) (day).

In this study, water samples collected from five different locations including raw wastewater, effluent from the equalization basin, effluent from the anaerobic basin, effluent from the aerobic basin, and effluent from the aquatic plant basin were used for the PCR/DGGE analyses to evaluate the variation in microbial diversity in water samples along the treatment system, which were responsible for the biodegradation of nutrients and carbon. Total bacterial DNAs from 2 L of collected water samples were extracted using the DNA Purification kit (GeneMark Co., Taiwan). Bacterial fragments [200 base pairs (bp)] of 16S rDNA V3 region were amplified with the primer sets [341f, forward: 5´-CCTACGGGAGGCAGCAG-3´ containing a guanine-cytosine (GC) clamp of 40-nucleotide GC-rich sequence; 534r, reversed: 5´-ATTACCGC GGCTGCTGG-3´] [26]. The mixtures of PCR contained 10 ng of DNA extract, 4 pmol of each primer, and 5 U of Taq DNA polymerase (Takara, Shiga, Japan) in final concentrations of 2.5 mM of $MgCl_2$ and 0.12 mM of deoxyribonucleoside triphosphates in PCR buffer. The PCR amplification was conducted for 35 cycles: denaturation at 94℃ for 1 min, annealing temperature was initially 65.8℃, and it was decreased by 1℃ per cycle until it was 55.8℃, after which 25 additional cycles were carried out at 55.8℃; and extension at 72℃ for 2 min. The equal concentration of each amplified PCR products (2,500 ng) was furthermore performed with DGGE using a Bio-Rad D-Code™ universal mutation detection system (Bio-Rad Lab., USA), as described by the manufacturer. The 10% polyacrylamide gel with a 30–60% denaturant gradient was used and electrophoresis was performed at 60℃ and 70 V for 14 h. The gels were then stained with SybrGreenI and photographed. This was the effective way to overcome biased amplification by Taq DNA polymerase with multi-template DNA samples for quantitative structure of the community. The gels were then stained with SybrGreen and photographed. The PCR-amplified products were electroeluted from gel and then sequenced by MdBio Inc. in Taiwan. Those sequences were compared to the GenBank database using the BLAST algorithm [27]. In this study, *E. coli* was used as the indicating parameter of fecal contamination. The existence of *E. coli* in water sample was analyzed by DGGE to evaluate the removal trend of *E. coli* in the wetland systems.

3. Results and discussion

Table 2 shows the averaged water quality monitoring data. Fig. 2 shows the distributions of the influent and effluent concentrations for SS, BOD, COD, and TC for the treatment system. Fig. 3 presents the distributions of the influent and effluent concentrations for TN, TP, NH_3-N, NO_3-N, and NO_2-N for the treatment system. The BOD, COD, SS, TP, NH_3-N, and TC in the raw wastewater were about 20,286, 25,167, 16,198, 222, 1,522 mg/L, and 2.15×10^8 CFU/100 mL, respectively. The high concentrations of BOD, COD, SS, NH_3-N, and TP indicate that swine wastewater contained significant amount of solid organics and nutrients. Thus, the solid/liquid separation unit is a necessity for the wastewater treatment system. Results show that the effluent of solid/liquid separation unit contained 7,400 mg/L of COD, 2,430 mg/L of BOD, 3,670 mg/L of SS, 114 mg/L of TP, and 753 mg/L of NH_3-N. After the treatment by the three-stage system, the averaged BOD, COD, SS, TP, NH_3-N, and TC were about 81, 708, 123, 46, 44 mg/L, and 1.65×10^4 CFU/100 mL. The conventional three-stage system was effective to improve the effluent quality, which reached 99.2% of SS, 79.2% of TP, 97.1% of NH_3-N, 99.6% of BOD, and 97.2% of COD removal.

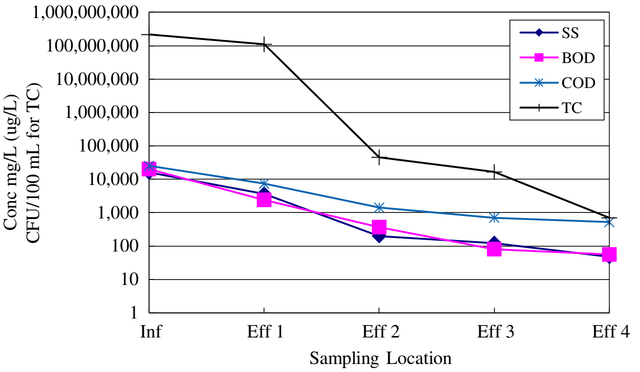

Fig. 2. The distributions of the influent (C_0) and effluent (C_e) concentrations for SS, BOD, COD, and TC for the treatment system.

Table 3 presents the pollutant removal efficiencies for each treatment unit. Results show that solid/liquid separation, anaerobic unit, and aerobic unit played the important roles in pollutant removal. Most TP and TC

Table 2
Averaged concentrations for the major water quality parameters

		NO_2-N (mg/L)	NO_3-N	NH_3-N	TKN	TN	BOD	COD	SS	TP	TC (CFU/100 mL)
Influent		11.7	1.6	1,522	2,404	2,417	20,286	25,167	16,198	222	2.15×10^8
Effluent	(1)	1.0	2.4	753	1,011	1,014	2,430	7,395	3,670	114	1.1×10^8
	(2)	0.9	0.4	596	707	708	370	1,419	201	55	4.55×10^4
	(3)	121	275	44	75	471	81	708	123	46	1.65×10^4
	(4)	50	165	19	47	262	56	518	48	36	700

Notes: (1) Solid–Liquid separation system; (2) Anaerobic basin; (3) Sedimentation basin; (4) Aquatic plant ecosystem.

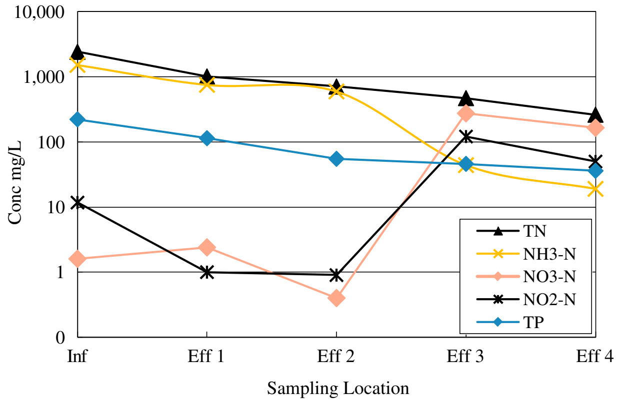

Fig. 3. The distributions of the influent and effluent concentrations for TN, TP, NH_3-N, NO_3-N, and NO_2-N for the treatment system.

were removal by solid/liquid separation and anaerobic unit. Higher NO_3-N and NO_2-N were observed after the anaerobic unit due to the denitrification process. The results indicate that the conventional three-stage system (solid/liquid separation, anaerobic unit, and aerobic unit) was effective to improve the effluent quality. Although the SS of the effluents from three-stage system could meet the swine wastewater discharge standard of 150 mg/L of SS, COD and BOD of the effluents still could not meet the wastewater discharge standards (80 mg/L for BOD and 600 mg/L for COD), and thus, a further treatment scheme was required.

In this study, *E. crassipes* was selected as the plant species in the aquatic plant basin for the following polishment study. Results show that significant amounts of pollutants were removed by the aquatic plant ecosystem. The observed efficiencies for COD, BOD_5, NO_3-N, NO_2-N, NH_3-N, and TP removal were 27, 30, 40, 59, 57, and 23%, respectively. Moreover, concentrations of BOD and COD were reduced to below the discharge standards after the aquatic plant ecosystem. This indicates that the aquatic plant ecosystem is an economically and technically acceptable treatment unit for the polishment of swine wastewater to meet the discharge standards. Because the aquatic plant ecosystem provides various pollutant removal mechanisms including microbial biodegradation, plant uptake, and sedimentation, this treatment scheme is effective on nutrient removal compared with conventional treatment systems. Results also show that the overall removal efficiencies for COD, BOD, NH_3-N, and TP were approximately 98, 99.7, 99, and 84%, respectively (Table 3).

In the anaerobic biodegradation process, a significant variation of the wastewater characteristics was observed. Results show that the BOD removal efficiency was higher than that of COD indicating that most of readily biodegradable organic matter had been removed during the anaerobic treatment process. The TN removal efficiency reached 89.2% after the three-stage and aquatic plant system. The removal efficiency of TN via the solid–liquid separation system was approximately 58% indicating that a significant amount of nitrogen was adsorbed onto the solids, which was removed through the first stage of the treatment system. Approximately 30% of TN could be removed in the anaerobic system indicating that the wastewater contained significant organic nitrogen, which could be digested under anaerobic conditions

Table 3
Averaged treatment efficiency for each treatment unit

	BOD_5 (%)	COD	NO_2-N	NO_3-N	TP	NH_3-N	TKN	TN	SS
(1)	88.0	70.6	91.5	–(a)	48.4	50.5	58.0	58.1	77.3
(2)	84.8	80.8	6.6	84.7	52.0	20.8	30.1	30.2	94.5
(3)	98.2	94.4	92.1	76.3	75.2	60.8	70.6	70.7	98.8
(4)	78.2	50.1	–	–	16.1	92.6	89.5	50.3	38.9
(5)	99.6	97.2	–	–	79.2	97.1	96.9	80.5	99.2
(6)	30.1	26.9	58.5	40	22.6	57.1	36.3	44.4	61.0
(7)	99.7	97.9	–	–	83.8	98.8	98.0	89.2	99.7

Notes: (1) (Influent of Solid–liquid separation system—effluent of solid–liquid separation system) × 100/influent of solid–liquid separation system; (2) (Influent of anaerobic system—effluent of anaerobic system) × 100/influent of anaerobic system; (3) (Raw wastewater—effluent of anaerobic system) × 100/raw wastewater; (4) (Influent of aerobic system—effluent of aerobic system) × 100/influent of aerobic system; (5) (Raw wastewater—effluent of aerobic system) × 100/raw wastewater; (6) (Influent of aquatic plant ecosystem—effluent of Aquatic plant ecosystem) × 100/Influent of Aquatic plant ecosystem; (7) (Raw wastewater—effluent of each unit) × 100/raw wastewater.

[a] Not available.

[2]. Results also show that approximately 10% of TN could be further removed by the aquatic plant ecosystem. This indicates that the aquatic plants played a key role for further nitrogen removal via the plant uptake, biodegradation, and sorption mechanisms [20]. Up to 83.8% of TP removal efficiency was observed in the system. The TP removal mechanisms included the solid settlement, chemical precipitation with iron, alum, calcium or magnesium, and formation of phosphine in anaerobic conditions [20,28].

Fig. 4 shows the distributions of the influent and effluent average DO, ORP, and pH results for the treatment system. Low DO and ORP values were observed in influent and effluents from solid–liquid separation basin and anaerobic basin. Higher DO and ORP were observed from the effluents of sedimentation basin and aquatic plant unit. This indicate that the high BOD and nutrients contained swine wastewater was under the anaerobic conditions before treatment and the water remained under anaerobic conditions until it flowed into the aerobic basin. The aeration in the aerobic basin caused the increase in DO and ORP in the aerobic basin and aquatic plant unit. The decomposition and biodegradation of the organic materials and occurrence of nitrification process would cause the variation in pH values. Anaerobic biodegradation produced the organic acids, which caused the decrease in pH values. During the denitrification process in the aquatic plant ecosystem, the pH started increasing due to the production of ammonia.

Result from the metal extract show that Zn, Cu, Ni, and Pb concentrations in plants varied from 25 to 168 mg/kg (168 mg/kg for Zn, 143 mg/kg for Cu, 25 mg/kg for Ni, and 48 mg/kg for Pb). Metal accumulation in aquatic plants were observed in other studies accumulated [15,21]. The metal accumulation also indicates that the aquatic plants were able to remove pollutants via the uptake and biological processes [5]. Although the accumulated metal concentrations in aquatic plants were not high, the metal accumulation might cause the plant disposal problems after harvest after a long period of operation. Thus, frequent metal monitoring in both influent water and plant is a necessity to evaluate the potential environmental risk resulted from the heavy-metal contained aquatic plants.

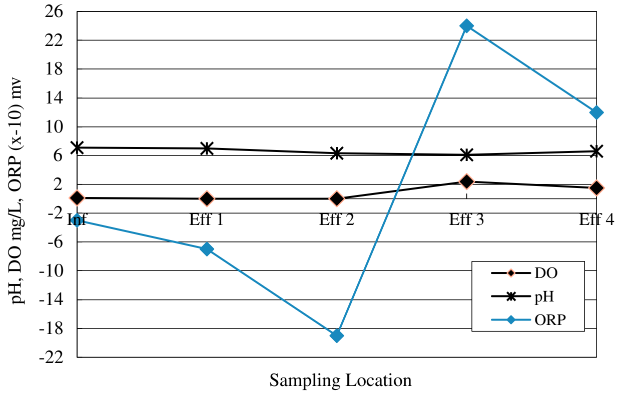

Fig. 4. The distributions of the influent and effluent average DO, ORP, and pH results for the treatment system.

Table 4 shows the variations in the BOD to COD and BOD to N to P ratios in each treatment unit. The different pollutant removal rates resulted in the change of ratios among the pollutants. Higher biodegradability of raw swine wastewater was obtained at BOD/COD ratio of 0.81. After the processes of solid–liquid separation, anaerobic and aerobic biodegradation, and aquatic plant treatment, decreased wastewater biodegradability was observed with decreased BOD to COD ratio because readily biodegradable compounds were removed.

The first-order decay model was applied to determine the decay rate (k) for BOD and NH_3-N in the aquatic ecosystem. The calculated k values were 0.1 1/d for BOD and 0.21 1/d for NH_3-N, which were close to those reported in literatures [16,20,23,29]. Thus, the removal rates for BOD and NH_3-N were in the acceptable ranges. Because higher temperature favors the biodegradation rate, the aquatic plant ecosystem is more suitable for treating wastewater in Taiwan or other countries located in subtropical or tropical regions. Results show that more than 80% of all pollutants were removed by the three-stage system followed by the aquatic plant ecosystem. The effluent also meets the requirements of water reuse set by local government ($EC < 4$ ms/cm, $SS < 50$ mg/L, $TC < 1{,}000$ CFU/100 mL) for irrigation and limited cleaning usage (e.g. road washing).

Fig. 5 presents the DGGE profiles of the PCR-amplified 16S rDNA of those five water samples collected from different effluent locations of the treatment facility including equalization basin, anaerobic basin, sedimentation basin, and aquatic plant basin. Results from the DGGE analyses indicate that all water samples contained significant amounts of microbial ribospecies. Because the raw swine wastewater contained high organic and nutrient concentrations, high microbial diversities were observed. Compared with the microbial diversity in raw water sample, no significant variation in microbial diversity was observed for the water sample collected from the effluent of the equalization basin. This was due to the fact that the solid/liquid separation and equalization units were mainly physical processes, and no chemical and biological reactions occurred. Thus, the microbial diversity would not be significantly affected.

Table 4
Performance of the BOD/COD and BOD:N:P in each treatment unit

	COD	BOD	TN	TP	BOD/COD	BOD:N:P
Raw water	25,167	20,286	2,417	222	0.81	91.4:10.9:1
Solid–Liquid separation system	7,395	2,430	1,014	114	0.33	21.3:8.9:1
Anaerobic system	1,419	370	708	55	0.26	6.7:12.9:1
Aerobic system	708	81	471	46	0.11	1.8:10.2:1
Aquatic plant ecosystem	518	56	262	36	0.11	1.6:7.3:1

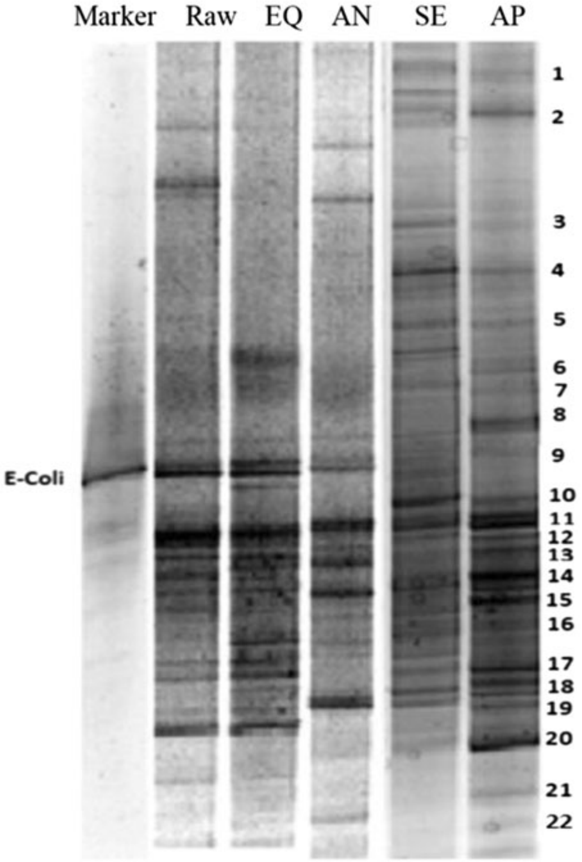

Fig. 5. DGGE profiles of the PCR-amplified 16S rDNA of those four water samples collected from raw wastewater (Raw), effluents of equalization basin (EQ), anaerobic basin (AN), sedimentation basin (SE), and aquatic plant basin (AP).

Slightly decreased DNA bands were observed in water samples collected from the anaerobic basin effluent (Fig. 5). This indicates that the anaerobic environmental conditions resulted in the inhibition of the growth of some aerobic microorganisms. Compared to the DGGE results for anaerobic basin effluent, increased microbial diversities were observed in water samples collected from the effluents of aerobic basin and aquatic plant basin. Although no obvious variations in microbial diversity were observed in water samples collected from aerobic basin and aquatic plant basin, dominant microbial species were different. This might be due to the major pollutant removal mechanisms and the environmental conditions were different in these two basins, which resulted in the variations in dominant microorganisms.

To annotate the meaning of representatives for bacterial species, 22 prominent bands of DGGE profiles in four sediment samples were eluted and then amplified and sequenced for their nucleotide sequences of the 16S rDNA variable V3 regions (Fig. 5). Table 5 shows the predominant microbial species of the 22 bands with the highest

Each band appeared on the DGGE fingerprints represented one microbial ribospecies. The PCR amplification of 16S rDNA and DGGE analysis was performed to determine the dominant microorganisms on carbon and nitrogen biodegradation. Results show that some microbial ribospecies were related to the carbon-degrading strains in NCBI database (with more than 95% of similarity), which included *Alcaligenes* sp., *Bacillus pumilus*, *Bellilinea caldifiatula*, *Guggenheimella bovis*, *Hydrocarboniphaga effuse*, and *Pseudomonas* sp. [30–36]. These bacteria could be responsible for the carbon removal in the treatment system. Results also indicate that some microbial ribospecies were related to nitrifying and denitrifying bacteria in the NCBI database (with more than 95% of similarity), which included *Anaerovorax* sp., *Denitratisoma oestradiolicum*, *Nitrosospira* sp., *Nitrosovibrio* sp., and *Steroidobacter denitrificans* [37–42]. Fig. 5 also presents gradual decay of *E. coli* from the equalization basin to the aquatic plant basin along the flow courses of the treatment system indicating that the biological mechanisms caused the decrease in *E. coli* population. Results reveal that the treatment system might be able to cause the reduction of *E. coli* and pathogens.

Results from this study indicate that a more reliable effluent quality could be received with the application of the aquatic plant ecosystem for the further treatment of the swine wastewater discharged

Table 5
The predominant microbial species of the 21 bands with the highest identities of the nucleotide sequences as compared to database of GeneBank

DGGE band number	Related bacterial sequence (NCBI Accession No.)	Similarity (%)
1	*Caldilinea aerophila* (AB067647.1)	96
2	*Anaerovorax* sp. (EU498370.1)	99
3	*Denitratisoma oestradiolicum* (AY879297.1)	95
4	*Bellilinea caldifistulae* (AB243672.1)	98
5	Uncultured *Pseudomonas* sp. (AM778223.1)	98
6	*Eubacterium angustum* (L34612.1)	97
8	*Eubacterium* sp. (AY134903.1)	96
9	Uncultured *Alcaligenes* sp. (EF173341.1)	96
10	*Nitrosovibrio* sp. (AY631269.1)	97
11	*Hydrocarboniphaga effuse* (AY363244.1)	95
12	Uncultured *Clostridium* sp. (AB288647.1)	96
13	*Desulfuromonas thiophila* sp. nov. (Y11560.1)	96
14	*Azorhizophilus paspali* (EF100148.1)	96
15	*Chlamydia murdarum* (AE002160.2)	98
16	*Stenotrophomonas maltophilia* (EU627172.1)	95
17	Uncultured *Hydrocarboniphaga* sp. (AY755409.1)	97
18	*Dethiosulfatibacter aminovorans* (AB218661.1)	98
19	*Steroidobacter denitrificans* (EF605262.1)	98
20	*Bacillus pumilus* (EU622830.1)	99
21	*Guggenheimella bovis* (AY272039.1)	98
22	*Nitrosospira* sp. (EF175101.1)	95

from the conventional three-stage treatment facility. Results also imply that the knowledge obtained from this study is useful in designing a scale-up and costly treatment system for other practical application.

4. Conclusions

In this study, the aquatic plant unit planted with floating aquatic plant *E. crassipes* has been applied to polish the swine wastewater treated by traditional wastewater treatment facility. The floating aquatic plant could survive well in the full-scale system containing high concentrations of organic compounds and nutrients from a hog farm. Conclusions of this study include the following:

(1) The conventional three-stage system was effective to improve the effluent quality, which reached 99.2% of SS, 79.2% of TP, 97.1% of NH_3-N, 99.6% of BOD, and 97.2% of COD removal. Although the SS of the effluents from three-stage system can meet the swine wastewater discharge standard of 150 mg/L of SS, COD and BOD of the effluents could not meet the wastewater discharge standards (80 mg/L for BOD_5 and 600 mg/L for COD).

(2) The aquatic plant treatment system played an important role in the swine wastewater treatment and more than 27% of SS, NH_3-N, COD, and BOD could be further removed from the effluents of the three-stage system. The application of the aquatic plant treatment system could effectively further polish the effluents from the conventional three-stage system and the treated water could comply with the discharge standards and the water reuse standards for irrigation and cleaning.

(3) Results indicate that the treatment units caused the variations in the dominant microbial ribospecies. The observed nitrogen and carbon degrading bacteria might be responsible for nitrogen and carbon removal. According to the results from GenBank, some microorganisms, which can biodegrade nitrogen and carbon might exist in the system. Results also reveal that DGGE and nucleotide sequence techniques provide a guide for microbial ecology evaluation, which can be used as an indication of the trend of biological treatment.

(4) Results show that a stable effluent and treatment efficiency can be obtained using the aquatic plant unit for the polishment of treated

swine wastewater. Thus, the aquatic plant ecosystem has the potential to be developed into an environmentally and economically acceptable technology for the treatment of swine wastewater.

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