

Full Length Research Paper

Effect of plantation pattern on the efficiency of subsurface flow constructed wetland (sfcw) for sewage treatment

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Accepted 09 September, 2018

The subsurface flow constructed wetland system (SFCW) with mono- and mixed-cultures of *Typha latifolia* and *Canna siamensis* could be applied for sewage treatment. The system efficiency was decreased with the decrease of HRT, excepted for nitrate removal. Both types of cultivated- plant did not show any difference on SS, BOD₅, ammonium-N₂, nitrate-N₂ and total phosphorus removal efficiencies. But, SFCW with mixed-cultures showed the highest phosphorus and nitrate removal efficiencies. Also, it gave the highest plant-biomass production yield. Phosphorus was highly accumulated in the plant tissue while, nitrogen was highly accumulated in the media. Number of bacteria of the system was not difference among plant species and plantation pattern, but it was decreased with the decreased of HRT. Then, the removal efficiencies of the system with both mono- and mixed-cultures were highest at the longest HRT operation of 6 days except for nitrate removal. The highest SS, BOD₅, ammonia-N₂ and total phosphorus removal efficiencies were about 91, 91, 86 and 87%, respectively were obtained in SFCW with both mono- and mixed-cultures.

Key words: Plantation pattern, hydraulic retention time (HRT), vertical-flow constructed wetland, *Typha latifolia*, *Canna siamensis*.

INTRODUCTION

Human activities have increased the input of nutrients into biogeochemical cycles, especially nitrogen and phosphorous. Nutrient enrichment or eutrophication of aquatic ecosystems can cause an increase of algae and aquatic plants, loss of component species and ecosystem function. Eutrophication is the biggest water quality problem throughout the world (Smith et al., 1999, Carpenter et al., 1998). Wetlands have been investigated as a possible solution to these global eutrophication and water quality problems. Total nitrogen and total phosphorous removal efficiencies by constructed wetlands were in the range from 3 – 98% to 31 – 99% respectively (Mitsch et al., 2001; Fraser et al., 2003, Spieles and Mitsch, 2000; Steer et al., 2002). On average, the nitrogen and phosphorous removal by above constructed wetland system were about 50%.

Plant species and climate are two factors that greatly effect to the nutrient removal efficiency of constructed wetland. Plants, such as species from the generaypha, Scirpus, and Phragmites play an important role in the biogeochemical cycle of wetlands (Brix, 1997; Wood et al., 1999; Schutes, 2001; Fraser et al., 2004). Wetlands are often highly pro-ductive systems where numerous biological transforma-tions driven by the natural energies of the sun, wind, soil, microorganisms, plants, and animals occur (Wetzel, 1993; Kadlec and Knight, 1996; Hammer, 1997; Mitsch and Gos-selink, 2000). Wetland performance is affected by solar radiation and ambient temperatures, which cycle on an annual and daily basis. These abiotic factors mediate the temperature of the wetland environment causing cyclical patterns in evapotranspiration, photosynthesis and micro-bial activity. Aquatic plant of wetland act as the filter, nutrient up take organism, to provide a substrate for microbiota (algae, bacteria, fungi, protists) and to provide a carbon source for denitrification (Greenway and Woolley, 1999; Liu et al., 2000; Kadlec and Knight, 1996; Yakushin, 1998; USEPA, 2000; Wetzel, 2000; Ingersoll and Baker, 1998; USEPA, 1999).

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In favorable climatic areas, maximum plant growth rates and biomass development are amplified by the abundant nutrients in the system. Each plant species showed the different nutrient uptake rate and specific growth pattern (Fraser et al., 2004). In this study, the experiment was designed to answer three questions:

- Are there any difference on the SFCW efficiency between *Typha latifolia* and *Canna siamensis*
- Does the mixed-culture (*T. latifolia* and *C. siamensis*) enhance the wetland efficiency, compared with mono-culture?
- Is there a relationship between hydraulic retention time and removal efficiency?

Our hypotheses, based on the research presented in the introduction are;

Difference on the plant species might affect to the nutrients (phosphorus and nitrogen) removal efficiencies,

- SFCW with mixed-culture is more effective than SFCW with mono-culture.

Constructed wetlands in King Mongkut's University of Technology Thonburi (KMUTT), Thailand supplied with municipal wastewater often provide ideal environmental conditions for growth of emergent wetland plant species. According to our previous wetland system which the soil was used as a media, both *T. latifolia* and *C. siamensis* could be applied to SFCW for treatment of domestic wastewater with high removal efficiency (Sirianuntapiboon and Jitmaikesem, 2007). But the clogging problem was occurred rapidly after 6 weeks of operation. Then, in this paper, we use gravel as a media to avoid or prolong the clogging problem.

METHODS AND MATERIALS

Study site

The subsurface flow constructed wetland system (SFCW) employed was located in King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand and is schematically illustrated in Figure 1. The dimension of each microcosm was 0.7 x 0.82 x 0.82 m³. The empty-bed volume of each microcosm was approximately 0.47m³. The SFCW was made from concrete and inner wall was painted with waterproofing paint to prevent potential leakage. The total empty bed was filled with gravel (diameter of 0.73 ± 0.14 cm) with 70 cm height. The volume of wastewater that was filled in wetland system was 0.297 m³. [A pump transferred the influent wastewater from a storage tank to the wetland cell and passed through the first wetland to the second wetland. This system was

Wetland operations

The experiment was carried out in designed-SFCW with two plant species (*T. latifolia* and *C. siamensis* under mono-culture and mixed-culture plantation conditions. The system was operated under various hydraulic retention times (HRTs) of 6, 3 and 1.5 days (organic loading of 7.85, 15.41 and 31.45 g/ m³-d) to observe the system efficiency and performance during 90 days cultivation. One month aged rhizomatous cuttings of each species

from the nearby swamp were collected. Each microcosm was planted with 9 cutting (approximately 20 cm in length) of each species in mono and mixed-cultured microcosm. There were five shoots of *T. latifolia* and four shoots of *C. siamensis* in mixed-cultured microcosm. Wastewater from KMUTT dormitory was used as the influent for the system. Each condition was repeated three times.

Sample collection and analysis

Wastewater

100-mL wastewater samples were taken twice a week from in-let and out-let port of each microcosm cell. Sampling was usually performed at around 10 a.m. on each sampling date. The samples were immediately transferred to the laboratory and measured for suspended solids (SS), biochemical oxygen demand (BOD₅), ammonia nitrogen (ammonia-N₂), nitrate nitrogen (nitrate-N₂, NO₃-N), and total phosphate (TP), were analyzed according to standard methods for water and wastewater examination (APHA AWWA WPCF, 1998).

Cultivated plant

All plants were observed throughout the experimental period for color, plant height and healthiness. Plant tissues were sampled following completion of each experiment (each HRT operation). Whole cultivated-plant tissue of each experiment was harvested and then chopped. Five 100 g sub-samples were sampled randomly and oven dried for approximately 48 h at 80°C and weighted. To estimate nitrogen and phosphorus content of each species, 50 g of dried material were crushed using a grinder with 0.5 mm trapezoidal-perforation sieve. The plant powder was mineralized as follows; a 0.25 g-sample of plant powder was treated with 4 mL of concentrated sulfuric acid in a 100 ml flask which was placed on a Hach Digestdahl apparatus at 440°C. 5 ml of hydrogen peroxide was added and the mixture was chilled. Then, the volume was brought to 100 ml with demineralized water. To quantify nitrogen content, 0.4 ml of mineralized solution and 1 ml of Nessler reagent were put in a 50 mL flask, and filled up to 25 mL with polyvinyl alcohol (0.1 g/l) Spectrophotometric reading was carried out at 420 nm; and nitrogen content (in percentage of dry weight) was deduced from a standard curved. Atomic absorption spectrophotometry with PK DDL program was used for phosphorus content-estimation from the mineralized solution (Radojevic and Bashkin, 1999).

Gravel media and sediment

Gravel media was weighted and 100 mL of de-ionized water was then added. This sample was then shake (120 rpm) horizontally for 30 min to separate bio-film from the media. The suspension was next analyzed for total nitrogen and total phosphorus using the same method as that employed for plant tissue analysis. The wetland sediment was dried at 105°C for two hours and weighted before total nitrogen and total phosphorus analysis. The same method as used for plant tissue analysis was employed.

Bacteria cells in gravel and water

Gravel samples (about 5 g each) were resuspended in 50 mL sodium sodium pyrophosphate solution (0.2 %, wt/vol) and sonicated for 10 min. After sedimentation for 30 min at room temperature, the samples were immediately diluted in sterile saline solution (0.85% NaCl solution, wt/vol) and inoculated in triplicate onto plate count agar (pour plate method). These were incubated at 35°C for 48 h and then the numbers of forming colony were counted (APHA AWWA WPCF, 1998). The forming colony from the gravel samples were counted as

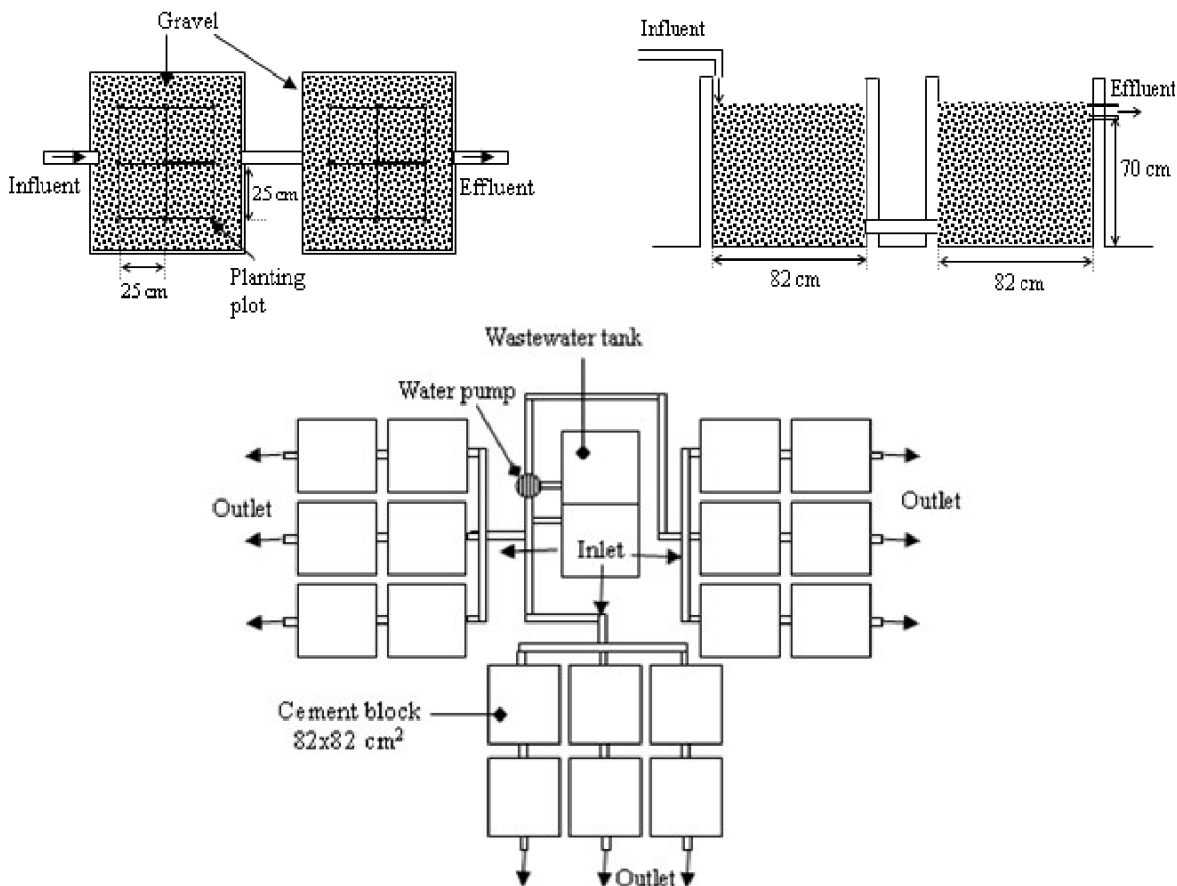


Figure 1. Flow diagram of the complete set of subsurface flow constructed wetland system

Table 1. Chemical properties of KMUTT dormitory's wastewater.

Parameter	Average	S.D.
pH	7.69	0.72
SS (mg/L)	33.14	5.27
BOD ₅ (mg/L)	105.3	7.8
Total nitrogen (mg/L)	18.27	3.09
Ammonium (mg/L)	16.64	3.31
Nitrate (mg/L)	0.1903	0.0730
Total phosphorus (mg/L)	5.03	0.46

Attached growth bacteria. Water samples (10 mL each) were diluted and inoculate as the method above. The forming colony of water samples were count as the suspended growth bacteria.

Statistical analysis

All statistical tests were performed using SPSS 14.0 by SPSS Inc. In all cases, significance was defined by $P < 0.05$. Test for significant difference in water quality between hydraulic retention times and plant species of the treatment wetland were tested using a completely randomized design (CRD) analysis of variance (ANOVA) with a posteriori LSD.

RESULTS

Suspended Solids (SS) removal performance

Influent SS of this experiment was quite low (Table 1). Both species showed similar removal performance at each HRT. *C. siamensis* showed highest SS removal efficiency ($P < 0.05$) at HRT of 6 days (Figure 2). But, the effluent SS of the system with mono-and mixed-cultures were lower than 10 mg/L under all HRTs operation tested (6, 3, 1.5 days). And the SS removal efficiencies of the system with both mono-and mixed-cultures were about 90 - 93%, 87 - 91% and 84 - 87% under HRT of 6, 3 and 1.5 days, respectively (Table 2). Even, the highest SS removal efficiency was obtained in the system with monoculture (*T. latifolia* or *C. siamensis*) under longest the system with mixed-cultures. Throughout this study, no clogging problem was observed during 3 months operation.

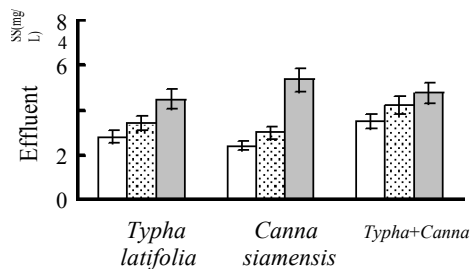
BOD₅ removal performance

There was no significant difference ($P < 0.05$) in BOD₅ reduction among the plant species and plantation pattern

Table 2. Pollutants removal efficiency in each condition.

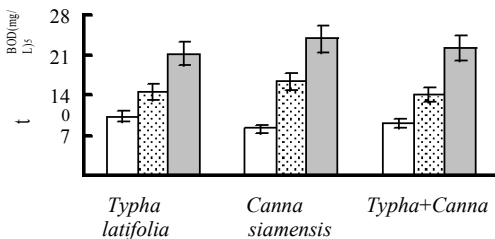
HRT (days)	<i>Typha latifolia</i>	<i>Canna siamensis</i>	<i>Typha latifolia</i> + <i>Canna siamensis</i>
SS removal efficiency (%)			
6	91.6	92.8	89.5
3	89.7	90.9	87.4
1.5	86.6	83.9	85.7
BOD₅ removal efficiency (%)			
6	90.3	92.3	91.3
3	86.2	84.4	86.6
1.5	79.8	77.4	78.9
Ammonia removal efficiency (%)			
6	85.8	84.3	88.3
3	69.2	67.8	71.5
1.5	58.8	55.6	63.4
Nitrate removal efficiency (%)			
6	-2,469	-1,366	-2,864
3	-1,778	-920	-2,326
1.5	-700	-562	-958
Phosphorus removal efficiency (%)			
6	85.5	85.9	90.3
3	77.1	78.9	81.5
1.5	52.9	58.4	64.4

Remark: HRT 6 days: Organic loading of 7.85 g/m³-d; HRT 3 days: Organic loading of 15.71 g/m³-d; HRT 1.5 days: Organic loading of 31.45 g/m³-d.



Symbol: □ 6 days; ▨ 3 days; ▩ 1.5 days

Figure 2. SS concentration in effluent with SD bar in each plantation pattern.



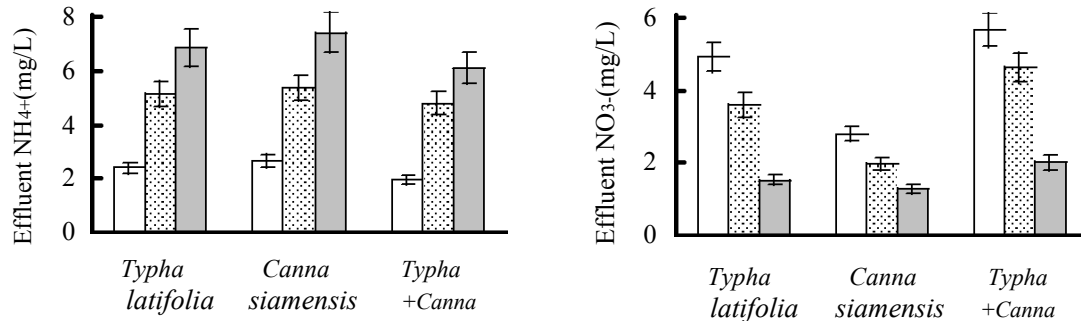
Symbol: □ ; 6 days, ▨ ; 3 days, ▩ ; 1.5 days

Figure 3. BOD₅ concentrations in effluent with SD bar in each plantation pattern.

at each HRT operation tested as shown in Figure 3. The system showed high and stable BOD₅ removal efficiency of higher than 84% under high BOD₅ loading rate of 15.71 g/m²-day which was equivalent to the nominal retention time of 3 days (Table 2) which was the lowest HRT that could give the satisfy efficiency.

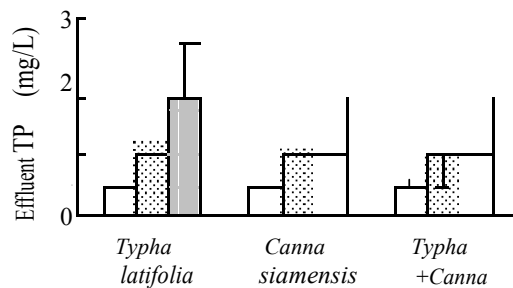
Removal of nitrogen species and phosphorus

Effluent Ammonium and nitrate of the SFCW system with mono- and mixed-cultures were illustrated Figure 4. Plant species and plantation pattern did not show any effects on ammonium removal efficiency, but they affected nitrate removal efficiency (Figure 5 and Table 2). The SFCW with *T. latifolia* and mixed-cultures showed quite low nitrate removal efficiency while *C. siamensis* showed high nitrate removal efficiency ($P < 0.05$). The effluent nitrate of the system with *C. siamensis* was lower than 3 mg/L in all HRT operation tested. The reduction of HRT would increase influent ammonium, but it was opposite to the nitrate removal efficiency and HRT relationship. Ammonium-N removal efficiency of the system under HRT of 6, 3 and 1.5 days were 85 - 88, 68 - 72 and 56 - 63%, respectively as shown in Table 2. Phosphorus removal efficiency of the system with mono- or mixed-culture was in the range of 85 - 90, 77 - 81 and 52 - 63% under the HRTs of 6, 3 and 1.5 days, respectively Table 2. Different among species did not affect



Symbol: □; 6 days, ▨; 3 days, ▩; 1.5 days

Figure 4. Ammonium and nitrate concentrations with SD bar in effluent in each plantation pattern.



Symbol: □; 6days, ▨; 3 days ▩; 1.5 days

Figure 5. Total phosphorus concentration with SD bar in effluent in each plantation pattern.

phosphorus concentration in effluent.

Plant growth and nutrient assimilation

Both cultivated plant could grow healthy under all HRT operation tested. The plant biomass production yield was increased with the increase of HRT (Figure 6a). And bio-mass production yield of *T. latifolia* in SFCW with mono-culture was highest under HRT of 6 days ($P < 0.05$). However, the average plant biomass production yields of both mono- and mixed-cultures were in the range of 5.6-6.8, 4.4-6.6 and 3.9- 6.6 kg/m² under HRT of 3, 6 and 1.5 days, respectively. For the difference on the root pattern under HRT of 1.5, 3 and 6 days in each plant species. But, the different plant species showed difference in root pattern (root depth) as shown in Figure 7b ($P < 0.05$).

Nitrogen and phosphorus accumulation in plant tissue was increase with the increase of HRT in both mono- and mixed-culture system (Figure 7). Nitrogen contents in the plant tissue were accounted 0.44, 0.54 and 0.82% for *T. latifolia*, *C. siamensis* and mixed-culture, respectively. Phosphorus contents in the plant tissue

were about 0.08, 0.12 and 0.17 percent for *T. latifolia*, *C. siamensis* and mix-ed culture, respectively.

Nitrogen and phosphorus content in media and sediment

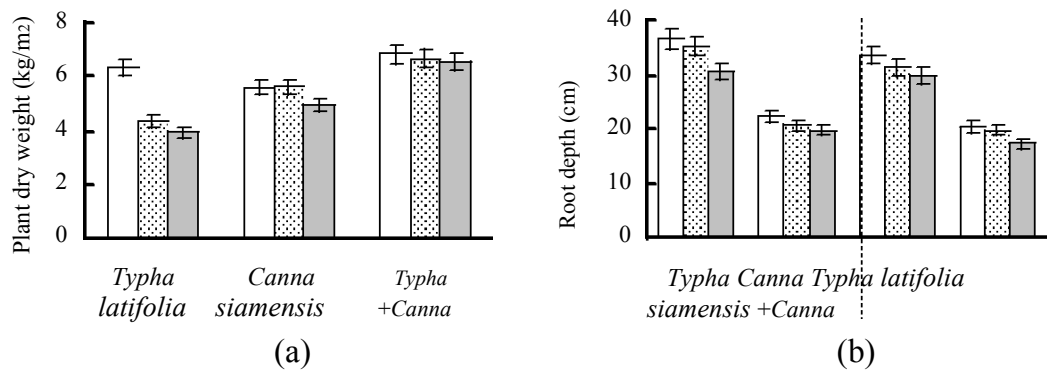
Plant species and HRT significantly effected to nitrogen content in media (Figure 8a). Nitrogen content in media was highest under HRT of 6 days. Also, the system with *T. latifolia* under HRT of 6 days showed the highest nitrogen content in media. However, the system with mixed-culture accumulated nitrogen content higher in the media than that with mono-culture. In the case of nitrogen content in the sediment, it showed the similar result as the nitrogen content in the media (Figure 9a). For the phosphorus determination, the phosphorus content in the media was decreased with reduction of HRT (Figure 8b), but HRT, plant species and plantation pattern did not effect to the phosphorus accumulation in the sediment (Figure 9b). For the nitrogen and phosphorus accumulation in each component of SFCW system, it could calculate that they were in the range of 0.189-0.822 and 0.026-0.144 g-P/ m²- d in plant tissue, 0.016-0.042 and 0.013-0.021 g-P/ m²-d in media 0.016 - 0.020 and 0.157 - 0.214 g-P/ m²-din sediment, respectively (data not shown) root performance, the system did not show any

Number of bacterial cell on media

Plant species and plantation pattern did not give any effect to the number of bacteria as shown in Figure 10. But the number of bacteria of the bio-film was increased with the increase of HRT. At the highest HRT of 6 days, the number of bacteria was increased up to 10¹⁷⁻¹⁸ cells/mL.

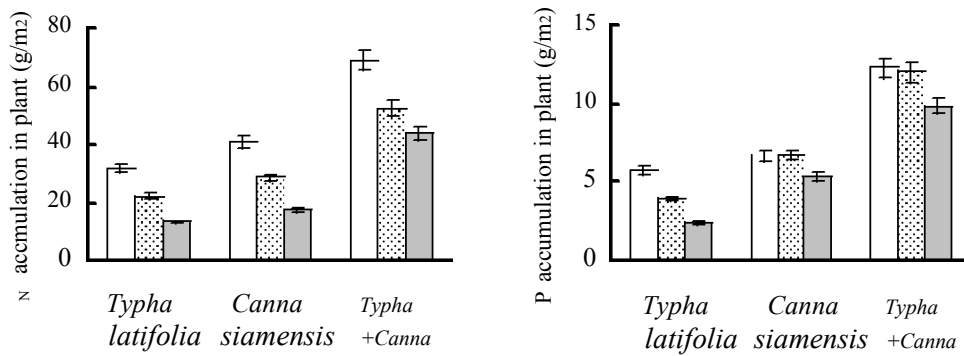
DISCUSSION

The SFCW system with both mono- and mixed-cultures



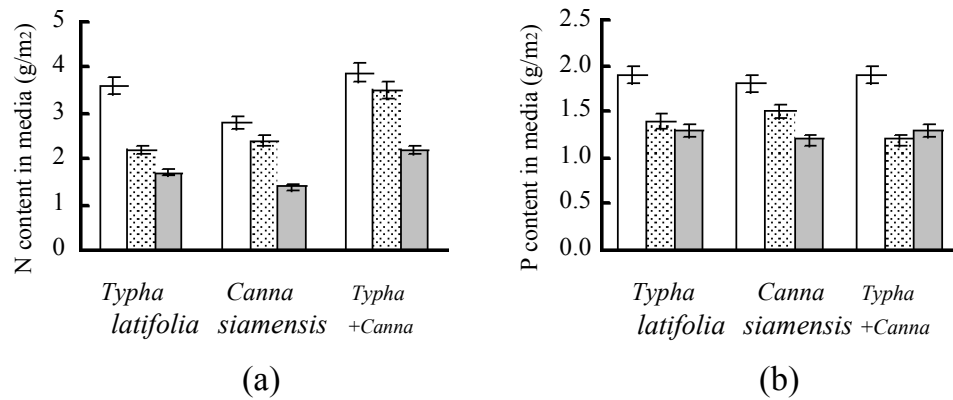
Symbol: □ ; 6 days, ▨ ; 3 days, ▩ ; 1.5 days

Figure 6. Plant dry weight (kg/m²) and root depth (cm) with SD bar in each plantation pattern



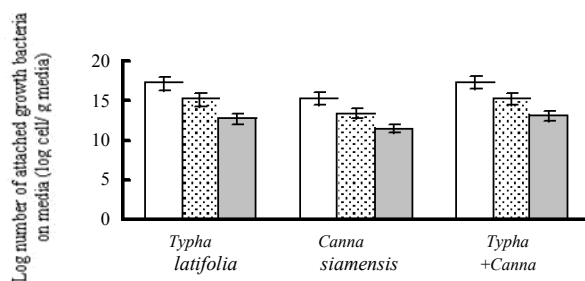
Symbol: □ ; 6 days, ▨ ; 3 days, ▩ ; 1.5 days

Figure 7. Nitrogen and phosphorus accumulation in plant tissue (g/m²) with SD bar in each plantation pattern.



Symbol: □ ; 6 days, ▨ ; 3 days, ▩ ; 1.5 days

Figure 8. Nitrogen and phosphorus content in media (g/m²) with SD bar in each plantation.



Symbol: □; 6 days, ▨; 3 days, ▩; 1.5 days

Figure 10. Log numbers of attached growth bacteria (log cell/g media) with SD bar in each plantation pattern.

of *T. latifolia* and *C. siamensis* could be applied to treat domestic wastewater with high SS, BOD₅ nitrogen and phosphorus removal efficiencies. Also, the system showed the other advantage on stability of infiltration rate. The system could be operated without clogging problem for 3 months. However, this may due to the short term operation and low influent SS concentration. A long term operation with high SS concentration wastewater was suggested for future work to observe infiltration rate performance after long term operation.

The plant plantation pattern and HRT of the system did not affect the BOD₅, ammonium-N and phosphorus removal efficiencies. The results obtained in this study were consistent with the known mechanism of BOD₅ removal in which microbial degradation in the attached bio-film played the dominant role (Vymazal, 1998). The wetland vegetations were confined to the roles of providing a support medium for microbial degradation to take place and of conveying oxygen to the rhizosphere for aerobic biodegradation. The other advantage of this SFCW system was that the system could be operated with high BOD₅ removal efficiency of more than 84% under high organic loading rate of 15.71 g/m².day, even the report by Tchobanoglous and Burton (1991) suggested that the maximum BOD₅ loading rate for subsurface flow system should be limited to 13.3 g/m².day. However, SS and nitrate removal efficiency was affected by the HRT, plant species and plantation pattern. This might be due to root growth pattern among species. *T. latifolia* root grew vertically and deeply to the lower level of media while *C. siamensis* root grew on the upper level of the media. And the root growth pattern might effect to physical properties of the media especially, infiltration rate (Stottmeister et al., 2003). And SS of the wastewater was settled and filtered during passing through media (Kadlac and Knight, 1996).

The cultivated plant which was adapted to waterlogged areas had the anatomical and physiological attributes necessary for their long-term survival (Vertapetian and Jackson, 1997). In fact, the cultivated plant could transport oxygen to the rhizosphere zone (root system)

from the atmosphere. However, gas transport and oxygen release into the rhizosphere were different in each plant species. The oxygen that needed for nitrification and other aerobic microbial processes, might diffuse into plant tissue from the atmosphere, or enter the rhizosphere through the porous stems and roots of emergent plant (Armstrong et al., 1990).

This also effected on nitrate removal efficiency. The result also showed that a long root pattern of *T. latifolia* promoted nitrification yield and this also showed the similar result in mixed-culture system. Mixed-culture showed better nitrification yield than mono-culture. The reduction of HRT was effected to removal efficiency of the system, plant biomass production yield and numbers of bacteria (bio-film) in both mono- and mixed- cultures. The results obtained above were consistent with the known mechanism of oxygen demand for microbial degradation of organic matters by biofilm bacteria played the dominant role. The wetland vegetations were confined to the roles of providing a support medium for microbial degradation to take place and of conveying oxygen to the rhizosphere for aerobic biodegradation. Nitrification/denitrification was one of the three main mechanisms for nitrogen removal in wetland systems, the other two mechanisms were ammonia volatilization and plant uptake (Drizo et al., 1997; Billore et al., 1998; Huang et al., 2000). Then, the nitrogen transformation could be occurred in the oxidized and reduced layers of the media, the root system and the submerged portion of the emergent plants (Metcalf and Eddy, 2004; Drizo et al., 1997). At the root matrix, atmospheric oxygen was transferred to the root zone through the stem of the cultivated plants thus creating an aerobic layer similar to that existed at the media-water or media/air interface. Nitrification was occurred in the aerobic rhizosphere where ammonium was oxidized to be nitrate which was either taken up by the plants or diffuses into the reduced zone to be converted to N₂ or N₂O by denitrification (Billore et al., 1998; Drizo et al., 1997; Huang, et al., 2000). The various form of nitrogen involved in chemical transformation of nitrogen compound from inorganic to organic compounds and back from organic to inorganic. Some of these processes required energy (typically derived from an organic carbon source) to proceed, and others release energy, which was used by organisms for growth and survival (Kedlac and Knight, 1996). The microorganisms, which grow on submerged portions of plant, on litter and other detritus, were a major contributor to wastewater treatment efficiency in the attached growth system. As the microorganisms attach on different parts of plants and media, they form a bio-film layer, which played an important role to removal nitrogen from the wastewater (Polprasert, 1997). These processes are influenced by temperature, pH and alkalinity of the water, inorganic carbon source, microbial population and concentrations of dissolved oxygen (Vymazal et al., 1998, Metcalf and Eddy, 2004). This indicated that plant uptake is one of the major nutrients removal pathways. Plantation pattern influenced the nutrient accumulation on plant tissue, media

except for sediment.

Conclusions

It could suggest that the SFCW system with Mono- and mixed-culture of *T. latifolia* and *C. siamensis* could be applied to treat domestic wastewater with high SS, BOD₅ nitrogen and phosphorus removal efficiencies of about 90, 90, 85 - 88 and 85 - 90%, respectively under HRT of 6 days (organic loading of 7.85 g/m².day) without clogging problem during 3 months operation. However, the SFCW with mixed culture was most suitable to apply for the treatment of wastewater under high organic loading of 15.71 g/m²-day according to the ammonium -N₂ and total phosphorus removal efficiencies of 88.3 % and 90.0%, respectively.

ACKNOWLEDGEMENT

The authors wish to express deep thanks to Department of Environmental Technology, King Mongkut's University of Technology Thonburi and Ministry of Education for providing the research materials, equipment and funds.

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