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Contributions of emergent vegetation acting as a substrate for biofilms in a free water surface constructed wetland

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Abstract. This study assessed the contribution of emergent vegetation (Phragmites australis, Typha latifolia, and Nelumbo nucifera) to the submerged surface area, the amount of biofilms attached to the submerged portions of the plants, and the treatment performance of a free water surface (FWS) constructed wetland. Results showed that a 1% increase (31 m^2) in the vegetative area resulted in an increase of 220 m^2 of submerged surface area, and 0.48 kg Volatile Suspended Solids (VSS) of attached biofilm. As the vegetation coverage increased, effluent organic matter and total Kjeldahl nitrogen decreased. Conversely, a higher nitrate concentration was found in the effluent as a result of increased nitrification and incomplete denitrification, which was limited by the availability of a carbon source. In addition, a larger vegetation coverage resulted in a higher phosphorus in the effluent, most likely released from senescent biofilms and sediments, which resulted from the partial suppression of algal growth. Based on the results, it was recommended that constructed wetlands should be operated with a vegetation coverage of just under 50% to maximize pollutant removal.

Keywords: biofilm; emergent plant; free water surface wetland; submerged surface area; treatment performance; vegetation coverage

1. Introduction

Free water surface (FWS) constructed wetlands, which mimic the structure of natural wetlands, have been widely used as an alternative to conventional treatment of a variety of wastewaters (Maine *et al.* 2017, Vymazal 2013). Pollutants in wastewater are removed by sedimentation, filtration, oxidation, reduction, adsorption, and precipitation processes as the water flows through the wetland (Kadlec and Wallace 2009).

In FWS wetlands with emergent macrophytes, plants serve a number of different functions (USEPA 2004). Submerged plant parts provide attachment sites for a variety of microbial species that facilitate many biochemical transformations including decomposition of organic matter, periphyton fixation, nitrification-denitrification, and sulfate reduction. Plants also assist in the filtration of pollutants and help trap and settle particulate matter suspended in the water. The stems and leaves above the surface of the water provide shade that reduces penetration by solar radiation, therefore suppressing further algal growth. Emergent parts of the plant also reduce wind action on the water surface, thus providing excellent conditions for particles to settle (Kim and Kim 2000), while gas exchange is diminished by slow air movement, reducing dissolved oxygen (DO) concentrations, a condition which may not be desirable for wastewater treatment.

Plants can also directly uptake dissolved pollutants, such as nutrients, metals and salts, and some release oxygen from their roots. However, the amount of nutrients that can be removed by plant uptake is generally insignificant relative to the load from the incoming water (Bendoricchio *et al.* 2000), and the nutrients may be quickly recycled into the water column or may be deposited in the sediments as soon as the plants die (Gottschall *et al.* 2007). Oxygen release plays a lesser role in FWS wetlands since most treatment processes occur in the water column and within the litter layer bottom sediments (Vymazal 2013). Plants may play a crucial role in removing pollutants firstly, by working as a physical filter for biofilms to attach (since the activity of the biofilms is responsible for most of the biological processes within the wetland) and secondly, by influencing the efficiency with which pollutants are removed by altering the surrounding water chemistry, e.g., pH and DO.

An adequate provision of open water/emergent vegetation ratio is important but is often overlooked in the design and implementation of FWS constructed wetlands (USEPA 1999). Vegetative areas are necessary in order to properly filter water, abate the concentration of nutrients, and improve sedimentation. On the other hand, open water allows for oxygenation, increases pathogen mortality from exposure to ultraviolet (UV) radiation and unfavorable temperatures, increases in detention time, and it improves the wetland's appearance. In terms of habitat diversity, open water areas are required to provide a landing for waterfowl

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and to enhance fish habitat, while the vegetative areas provide habitat for the macroinvertebrate. In most cases, it is recommended that an FWS constructed wetland incorporate a mix of shallow vegetated and deep open water areas that should result in a more complex, dynamic, and self-sustaining wetland ecosystem. The ratio of open water area to vegetative area is determined not only by defining objectives but also by the importance of each objective relative to one other. According to guidelines for FWS wetland design (Bendoricchio *et al.* 2000), a high open water/vegetative area ratio, e.g. 1:5, should be selected if the most important objective is water quality. The high proportion of vegetative areas could encourage the filtration process and nutrient transformation. On the other hand, if habitat diversity is considered to be an important objective, a lower ratio, e.g. 1:1, may be desired. The balance of open water areas for waterfowls and vegetative areas for macroinvertebrates creates habitat diversity. However, in order to achieve multiple objectives of improving water quality, protecting habitat diversity, and enhancing aesthetic beauty, a moderate ratio (e.g. 1:3) is usually suggested.

This study was carried out to investigate the contribution of the emergent plants *Phragmites australis* (common reed), *Typha latifolia* (cattail), and *Nelumbo nucifera* (lotus) as a substrate for biofilms in an FWS wetland that receive wastewater from cattle feedlots and runoff from agricultural lands. The objectives of this study were: 1) to quantify the surface area provided for biofilm growth by the submerged plant parts; 2) to evaluate the amount and the significance of the attached biofilms; and 3) to determine the effects of the change in vegetation coverage on the treatment performance of the wetland.

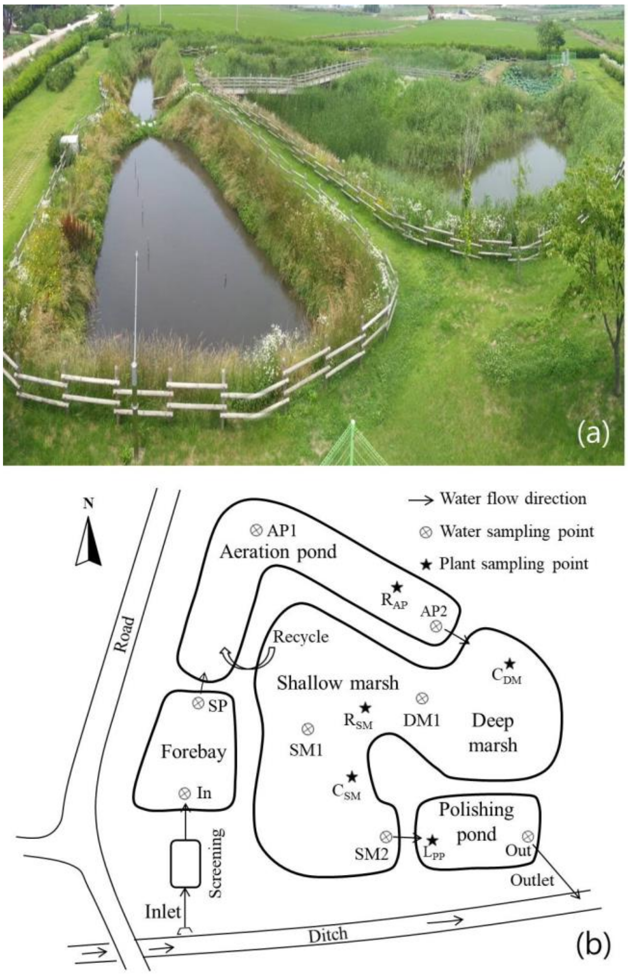

Fig. 1 (a) Photo and (b) sketch showing the water and plant sampling points in the wetland

2. Materials and methods

2.1 Study site description

The wetland studied is an FWS constructed wetland located near Jeongeup City, Jeollabuk-do, South Korea (35° 38'11" N, 126° 48'47" E). It was commissioned in 2009, and it has a catchment area of 64 ha and a water surface area of 3085 m^2. The purpose of the wetland is to control nonpoint source pollution received from effluents produced by residential areas, fields, and rice paddies. However, the majority of the wastewater is produced in cattle feedlots. This wetland consists of a forebay, aeration pond, deep marsh, shallow marsh, and polishing pond (Fig. 1). Wastewater is collected in a channel and is then transported to the wetland system by gravity. Water flows sequentially through five treatment cells, before being discharged back to the channel downstream. In order to improve the degradation of organic matter and to improve nitrification, oxygen is supplied in an aeration pond through mechanical aeration with an aeration cycle of 3 h on and 3 h off. The wetland has an internal recycling capability, by which water from the shallow marsh is pumped back to the aeration pond for further treatment.

The vegetation types used in the wetland were the emergent plant species: *Phragmites australis*, *Typha latifolia*, and *Nelumbo nucifera* (Fig. 2). The forebay was kept open, while the aeration pond was planted solely with common reed. The deep marsh and the shallow marsh were partly planted with common reed and cattail, and the polishing pond was covered with lotus. The average depths of each zone in the wetland are as follows: forebay, 1.2m; aeration pond, 1.1 m; deep marsh, 1.6 m; shallow marsh, 1.3 m; and polishing pond, 1.5 m.

2.2 Water quality sampling and analysis

The water was monitored by taking samples approximately twice a month from late April to mid-November in 2011, from September to early November in 2012, and again from late April to mid-November in 2013. Samples were taken a total of 35 times at the inlet and outlet of each treatment cell, in the middle of the aeration pond, and in the shallow marsh (Fig. 1 and 2).

The parameters selected to assess the water quality included temperature, pH, DO, total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), total nitrogen (TN), ammonia-nitrogen (NH_3-N), total Kjeldahl nitrogen (TKN), nitrate-nitrogen (NO_3-N), total phosphorus (TP), and Chlorophyll *a*. Temperature, pH and DO were measured *in situ* using a YSI 556 portable instrument and YSI 5000 DO Meter. The

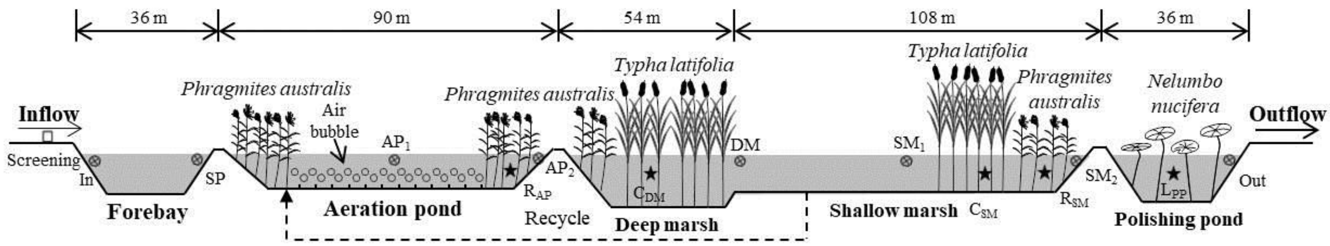

Fig. 2 Sketch showing the primary treatment elements and the dominant plant species in each zone

others were analyzed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA 2005).

2.3 Plant monitoring

The plants were monitored on a monthly basis from June to October in 2013 by measuring the vegetation coverage, surface area provided by submerged plant parts, and the amount of attached biofilms (Figs. 1 and 2).

The vegetation coverage in each treatment cell is the ratio of the vegetative area to the area of the treatment cell. Measurements of the vegetative areas in each treatment cell were obtained from direct surveying. The overall vegetation coverage in the wetland was defined as the percentage of the water surface that was covered by all kinds of vegetation (i.e., the water surface covered by either common reed, cattail, or lotus) in the entire wetland water surface (Equation 1).

$$\text{Vegetation coverage (\%)} = \frac{Vegetative\ area}{Wetland\ water\ surface\ area} \times 100\% \quad (1)$$

A total of five quadrats were used to determine the surface area provided by the submerged plant parts for biofilm attachment (see Fig.1(b), Fig. 2 and Fig. 3). Among them, two quadrats were separately installed in the aeration pond and shallow marsh to monitor the growth of common reed, another two quadrats were placed in the deep marsh and shallow marsh to monitor the growth of cattail, and the last one was located in the polishing pond for the measurement of lotus. The quadrat had a cross-sectional area of 0.5×0.5 m for common reed and cattail and 1×1 m for lotus. Plants were surveyed in each quadrat to assess their individual density, and samples were taken outside of the quadrats to determine the perimeter and the length of submerged parts and the amount of attached biofilm.

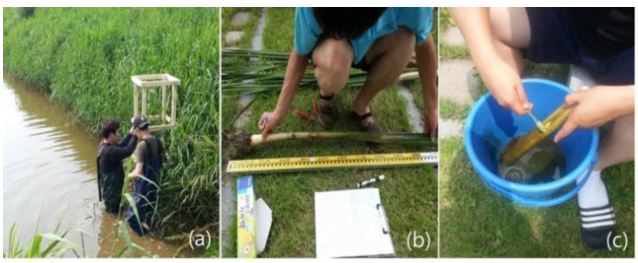

Fig. 3. Photos showing (a) the installation of a quadrat, (b) the measurement of the length and perimeter of sampled plants, and (c) the collection of biofilms

The surface area of the submerged parts of a plant species in a treatment cell was calculated according to Equation (2). Firstly, five plant samples around a quadrat were randomly selected and drawn out from the mud. The lengths and perimeters of submerged parts of these plants were subsequently measured by using a tape, and thus the average surface area of submerged part for each plant was calculated. The total submerged surface area of the plants in a quadrat and in a treatment cell was obtained by multiplying the average value with the quantity of the plants in the quadrat and in the treatment cell, respectively. The total submerged surface area of the vegetation in the wetland was obtained by summating those in each treatment cell.

$$A = \left[\frac{1}{n}\sum_{i=1}^{n}(P_i \times L_i)\right] \times N \times A_p/A_q \quad (2)$$

where A is the submerged surface area provided by a plant species in a treatment cell (m^2), n is the number of sampled plants, P_i and L_i are the average perimeter and length of the submerged part of one sampled plant, N is the number of the plants in a quadrat that was investigated, A_p is the area covered by this plant species in the treatment cell, and A_q is the area of the quadrat.

Biofilms attached to the submerged parts of the plants were carefully collected using a brush, and the amount of the biofilms attached on the submerged parts of a plant species in a treatment unit was evaluated using Equation (3). The total mass of the attached biofilms in the wetland was calculated by summating those in each treatment unit.

$$M_B = \frac{M_{sp}}{A_{sp}} \times A \quad (3)$$

where M_B is the mass of the biofilms (VSS, g) attached on the submerged parts of a plant species in a treatment unit, M_{sp} is the mass of the biofilm (VSS, g) attached on the submerged parts of the sampled plant, A_{sp} is the submerged surface area of the sampled plant (m^2), and A is the total submerged surface area of this plant species in the treatment unit (m^2).

2.4 Calculation of the vegetative area

The results of the plant sampling conducted in 2013 were used to fit the saturation curve model (Bartlett 1960), which was used to assess changes in the vegetative area covered by common reed or cattail during the growth seasons over the three years of the study with the equation (4):

$$A_v = \frac{K}{1 + e^{-a \times \theta^{(T-20)} \times t}} \tag{4}$$

where A_v is the vegetative area (m^2) covered by the common reed or cattail in a treatment cell on a specific date, K is the final (or maximum) area (m^2) covered by common reed or cattail during one year, a is the intrinsic expansion rate of the common reed or cattail, θ is the temperature compensation coefficient (1.024), T is the water temperate (°C), and t is the growth period (in days) of the plants.

To apply the S-curve growth equation, the following two assumptions were made: (1) the expansion of the vegetative area during one year presents an S-shaped increase (Logistic Growth); and (2) the annual expansion rates of the vegetative area were 3 and 5% for common reed and cattail, respectively as compiled from Wilcox *et al.* (1984), Warren *et al.* (2001), Próchnicki (2005) and Toth and Galloway (2009).

3. Results and discussion

3.1 Change in vegetation coverage

The variations in the percentage of vegetative cover in each of the treatment elements of this wetland are presented in Fig. 4. Rhizome growth, rather than seed, was the primary method used to expand common reed and cattail (Toth and Galloway 2009, Juneau and Tarasoff 2013). Since rhizome growth begins in the spring, is active in the summer, and ceases in the fall, the expansion rates of areas covered by common reed and cattail were not linear. They gradually increased in the summer, reached a maximum in early September, and subsequently remained stable (Fig. 4a, b, and c). During the monitoring period, the maximum percent cover in the aeration pond was of 41%, while those in the deep and shallow marshes were 87% and 56%, respectively.

The polishing pond was originally fully seeded with Lotus at a high density, based upon the wetland design. Thus, when the lotus sprouted in the spring, the shoots always filled the pond. As the Lotus grew, although some plants died due to intraspecific competition, the living parts became stronger and developed broad leaves that shaded the water surface completely. Therefore, the vegetation coverage in this pond was considered to be 100% throughout the growing seasons (Fig. 4d).

The overall change in the vegetation coverage in the wetland and the contribution of each plant species are shown in Fig. 5. As seen in this figure, the total vegetative area continuously increased until early September, reaching a maximum of 57%. The dominant plant species were common reed and cattail, and these contributed around 23% and 26%, respectively, when the coverage reached a stable state. The area covered by lotus contributed to about 8% of the total vegetation coverage.

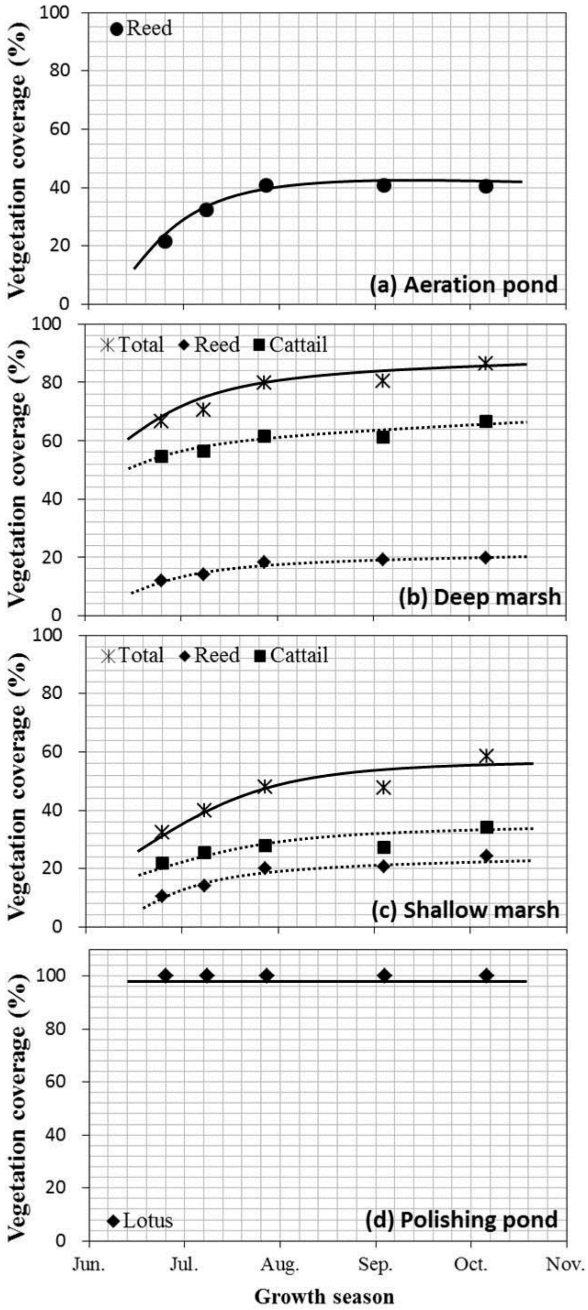

Fig. 4. Development of vegetation coverage in each treatment cell in this wetland

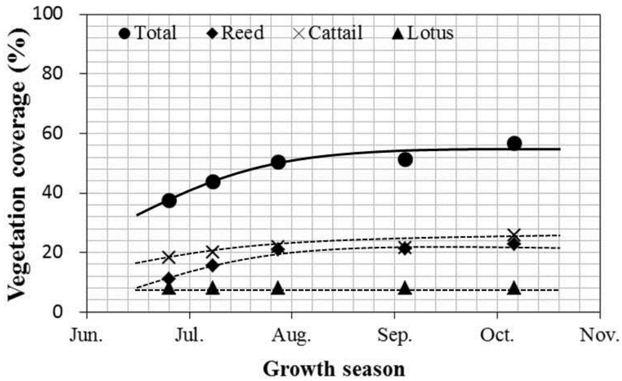

Fig. 5. Overall change in vegetation coverage in the wetland with respect to the growth season

3.2 Surface area provided by submerged plant parts

As shown in Fig. 6, the increase in the surface area provided by the submerged plant parts showed a trend

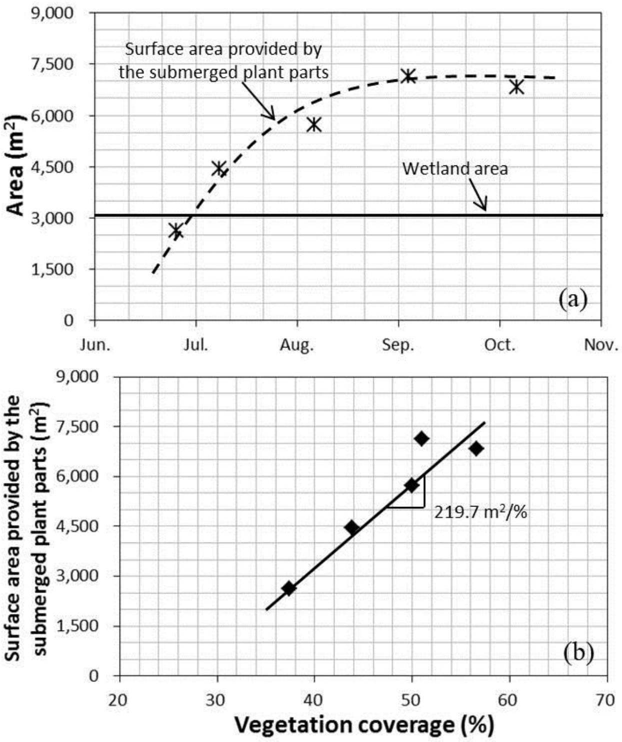

Fig. 6. Change in the surface area provided by submerged plant parts with respect to the (a) growth season and (b) vegetation coverage

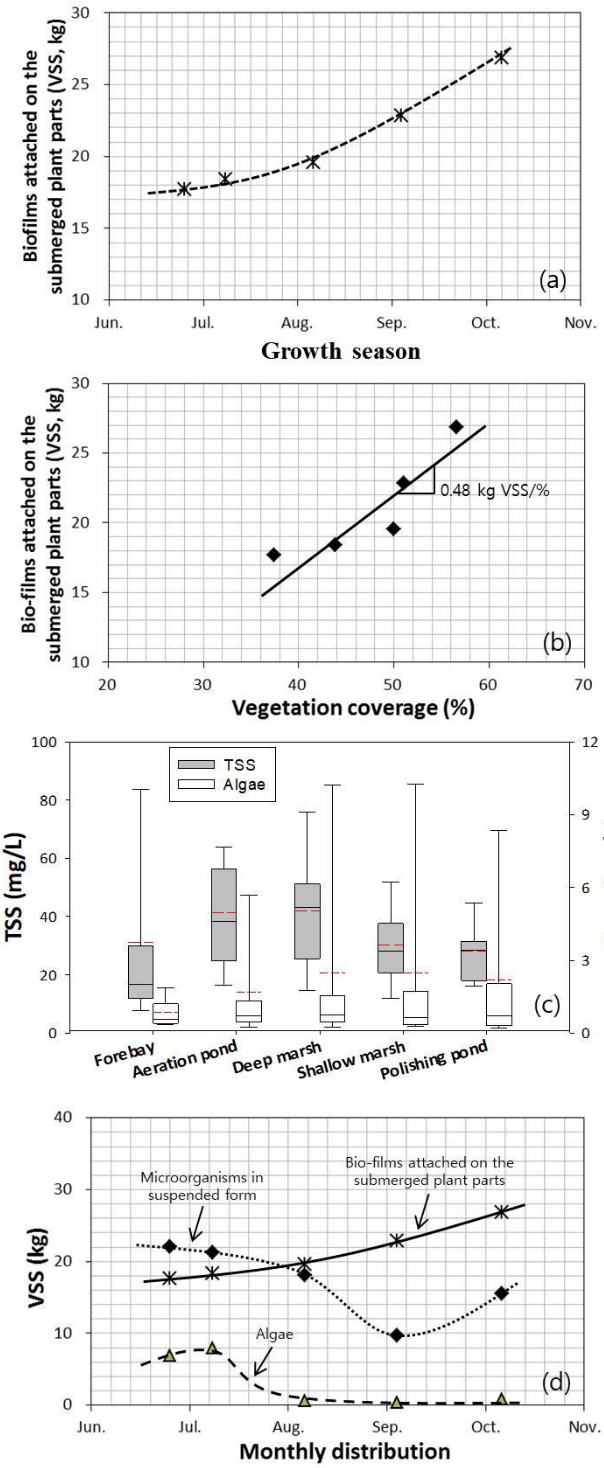

Fig. 7. Production of attached biofilms with respect to the (a) growth season and (b) percentage of vegetation cover in the wetland, and (c and d) comparison of microorganisms to which biodegradation is attributed

similar to that of vegetation coverage, gradually increasing during the growth seasons and reaching about 2.3 times that of the wetland area at the stable state. In general, a 1% (31 m^2) increase in vegetation coverage produced about 220 m^2 of surface area of submerged plant parts.

The rate of pollutant removal in an FWS wetland is partly determined by the surface area available for growth of attached biofilm bacteria (Khatiwada and Polprasert 1999, Yi *et al.* 2009). A higher submerged surface area indicates a higher potential to house more microbes and epiphytes, likely enhancing water treatment. The submerged surface area of the vegetation in a wetland is a function of the plant type, plant density, and water depth. The amount of surface area contributed by submerged stems and leaves in FWS wetlands was from 1.0 to 7.6 times that of the bottom area of the wetland (USEPA 1999). The relatively low amount of submerged surface area in this study was a result of a lower amount of vegetation coverage, even during the stable state.

3.3 Biofilms attached to the submerged plant parts

Two basic bacterial groups are mainly responsible for pollutant degradation in FWS wetlands: bacteria suspended in the liquid portion and biofilm bacteria attached to the surfaces of submerged plant parts, sediment, and litter (Polprasert *et al.* 1998, Yi *et al.* 2009). The contribution of the suspended growth is believed to be dependent on water depth, porosity, and wastewater characteristics while the contribution of attached biofilms depends on the amount of surface area available for biofilm attachment (Khatiwada and Polprasert 1999).

The quantitative change in the biofilms attached to the submerged plant parts with respect to the changes in season and vegetation coverage are shown in Figs. 7a and 7b. The amount of attached biofilms continuously increased as time went on since an increase in the vegetation coverage supplied additional surface area for biofilm growth. On average, during the growth seasons, every 1% increase in vegetation coverage yielded 0.48 kg (VSS) of attached biofilms. However, it is worth noting that unlike the increase in the submerged surface area, the increase in the attached biofilms in the summer was relatively slow (refer to the concave shape in Fig. 7a). This was probably a result of the fall-off of attached biofilms caused by water scouring since rainfall during the summer was frequent and heavy and resulted in a rapid water flow into the wetland.

The seasonal distribution of the attached bacteria, suspended bacteria, and algae are shown in Fig. 7d. As seen in the figure, the abundance and the proportion of the attached biofilms are strongly correlated with a change in the vegetation coverage, and at the same time, the mass of the suspended bacteria and of the algae decreased. It is clear that the attached biofilms were important and were dominant, especially at a higher vegetation coverage. The biofilms accounted for up to 64% of the total bacteria present when the vegetation coverage reached its peak. This observation suggests that the attached biofilms play a major role in the degradation of pollutants.

3.4 Effects of vegetation coverage on wetland effluent

The relationships between the vegetation coverage and the quality of the wetland effluent are illustrated in Fig. 8. The data appeared to be scattered, especially at the stage with a higher vegetation coverage. These changes were attributed to the effect of seasonal changes in water temperature since temperature highly affects the reaction rates of biological processes (Beauchamp *et al.* 1989, Jørgensen 1994), which is often expressed by the modified Arrhenius relationship (Kadlec and Wallace 2009):

$$k_T = k_{20} \times \theta^{(T-20)} \tag{5}$$

where k_T is the reaction rate at a specified temperature T°C, k_{20} is the reaction rate at water temperature = 20°C, T (°C) is the water temperature, and θ is the temperature-activity coefficient.

In theory, the proper temperature, coupled with a higher vegetation coverage, may provide the best condition for biochemical processes. However, this situation may not always occur in field treatment systems. In this case, seasonal temperature changes combined with the development of vegetation coverage resulted in four different situations: lower temperature + lower vegetation coverage (in May), higher temperature + lower vegetation coverage (in June), higher temperature + higher vegetation coverage (in August), and lower temperature + higher vegetation coverage (in October). These various environmental conditions led to variation in the reduction of pollutants at a specific vegetation coverage. Nevertheless, the data obtained at a temperature of over 20°C (Maximum: 33.7°C; Mean ± STDEV: 25.1 ± 3.9°C) still suggests that there is a significant correlation between the vegetation coverage and the quality of the wetland effluent.

As shown in Figs. 8a and b, the COD and TKN content in the effluent decreased as the vegetation coverage increased (r = -0.41, p<0.05; and r = -0.37, p<0.05, respectively). This is a result of the higher coverage of the vegetation, which brought about a greater subsurface area

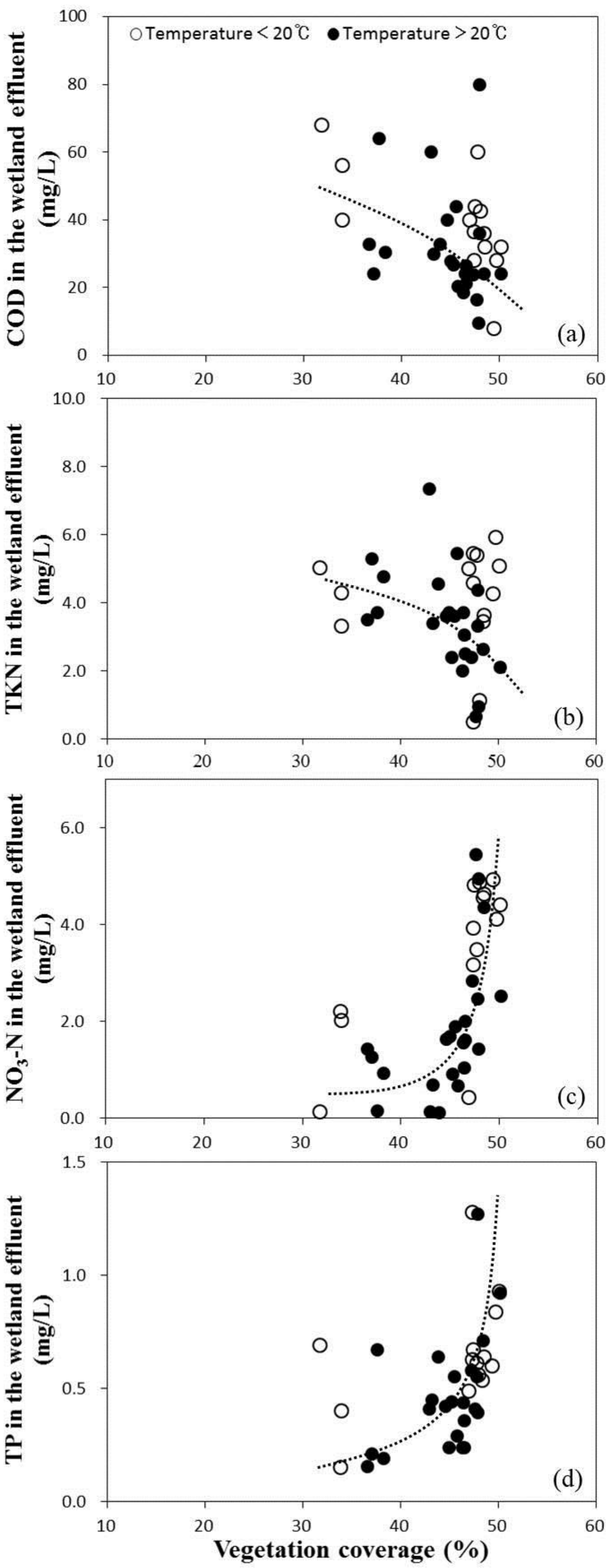

Fig. 8. Relationship between the percentage of vegetation cover and pollutant concentrations in the wetland effluent

for the growth of attached biofilms and thus provided more opportunity for contact between the pollutants and the active microorganisms responsible for the degradation of organic matter and transformation of TKN, which in turn enhanced the removal of organic matter and TKN.

Furthermore, algal solids in FWS wetlands had been reported to be a source of organic matter and nutrients (USEPA 1999), and a higher vegetation coverage indicated a greater vegetated area and smaller open area, which reduced the production of algae because the vegetated area decreases algal photosynthesis by preventing light from penetrating into the water column.

Denitrification is considered to be the main pathway to remove nitrogen in constructed wetlands, while it is often restricted by lack of organic carbon (Saeed and Sun 2012). Denitrification occurs most readily in wetland sediments and in the water column below fully vegetated growth due to low concentrations of DO and a high availability of organic carbon (USEPA 1999). Fig. 8c shows how the nitrate concentration in the effluent gradually increases as the vegetation coverage increases ($r = 0.54$, $p < 0.05$). Nitrate accumulated as a result of enhanced nitrification and incomplete denitrification. In other words, the nitrate produced via nitrification was not immediately removed by further denitrification. Thus, a higher vegetation coverage did not result in an effective reduction of nitrate, even though it provided an increased submerged surface area for the potential attachment of denitrifying bacteria and drew down the concentration of DO in the water column. The factor controlling the efficiency of the denitrification was possibly the availability of a carbon source because the amount of algae in the water column significantly decreased as the vegetation coverage increased.

With respect to phosphorus (Fig. 8d), the concentration was positively correlated with the development of vegetation coverage ($r = 0.42$, $p < 0.05$). The increase of TP in the effluent probably resulted from the release of phosphorus from senescent biofilms and sediments due to the decrease in the DO content. The DO concentrations in the water column were observed to decrease as vegetation coverage increased, especially in the deep and shallow marshes (Fig. 9). In contrast, the change of DO in the aeration pond is not evident, as the effect of the vegetation growth is enshrouded by the artificial aeration. The increase in the vegetation coverage resulted in a decrease in the open water area and also casted greater shade, reducing the availability of light for algae to grow in the water column, and therefore caused a reduction in the oxygen supply in the water column that normally occurs as a result of algal photosynthesis (Thullen *et al.* 2002). Previous studies reported that the redox potential influences the stability of the phosphate minerals within the soils and overlying waters, and that phosphorus associated with Fe may be released from the soil to the water under anaerobic conditions (Wildung *et al.* 1977, Hosomi *et al.* 1982, Furumai and Ohgaki 1982).

The results discussed above indicate that vegetated area was a necessary component to stimulate biochemical processes in an FWS wetland but that a higher vegetation coverage did not always improve treatment. Even for ammonia, removal may not be effective if the vegetation coverage is too high. A study by Thullen *et al.* (2002) stated that a 70% vegetated wetland removed 58% of NH_4-N while a fully vegetated wetland only removed 6% due to the lack of open water areas. This is because, as mentioned previously, the open water areas are also essential

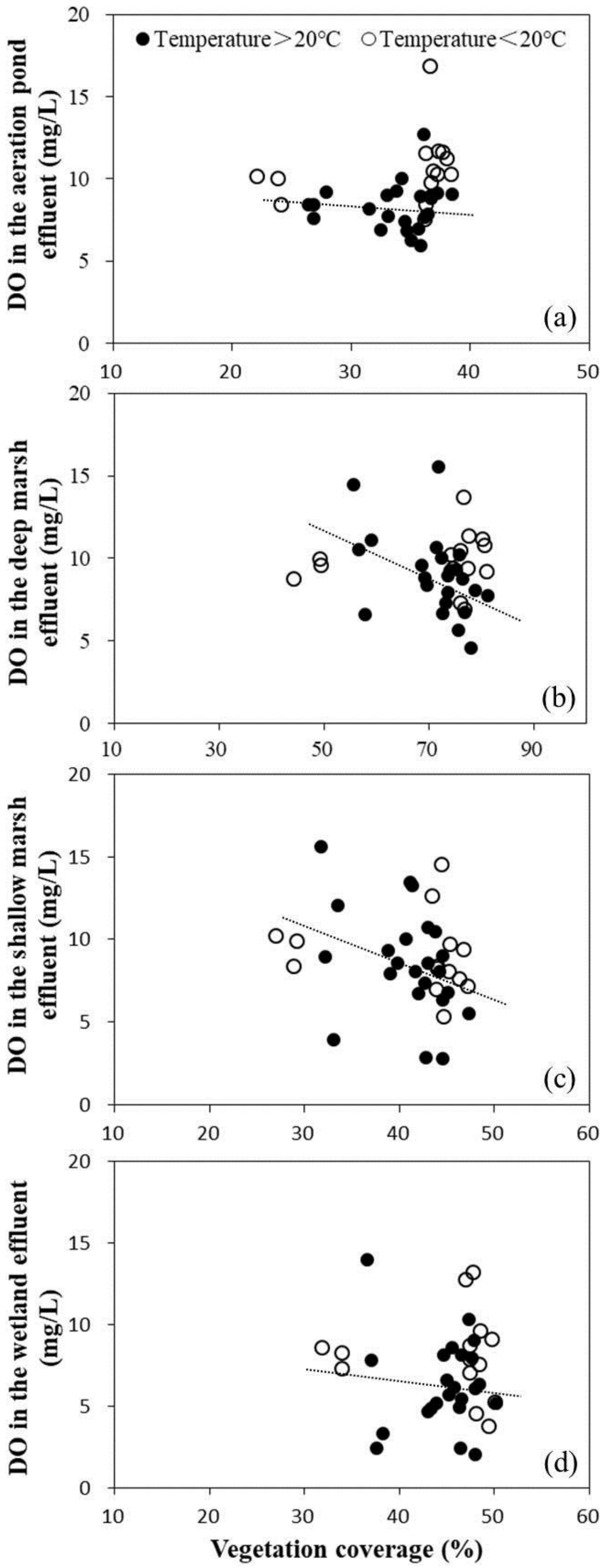

Fig. 9. Relationship between the vegetation coverage and DO concentrations in treatment cells and in the wetland effluent

components for wetland treatment since open areas provide many functions, such as supplying higher DO for nitrification by allowing sunlight penetration and mixing at the air/water interface, as well as creating diverse wildlife habitats and reducing mosquito production. Thus, proper vegetation coverage is required for wetland plant management. However, the ratio of open water areas and vegetated areas are quite different depending on the individual wetland settings and the specific water constituents of concern entering for wetland treatment. Hammer and Knight (1994) reported that 10-20% of total open water areas in a wetland system appeared to be adequate for nitrogen treatment while Pase and Brown (1994) argued that a 50% open water ratio was still not open enough to maintain the treatment benefits in a Californian wetland. According to the results in this study, the free water surface constructed wetlands were recommended to operate with a vegetation coverage below 50% to achieve a balanced removal of the pollutants.

5. Conclusions

This study investigated the contributions of emergent plants to the submerged surface area, subsequent production of attached biofilms, and treatment performance in an FWS wetland. The results drawn from this study concluded that biofilms attached to submerged plant parts were important and played a major role in the degradation of pollutants. The expansion of vegetation coverage inspired the breeding of attached biofilms and suppressed the production of algae, which resulted in enhanced removal of organic matter and TKN from the wetland. However, the reductions of nitrate and phosphorus were weakened, attributed to incomplete denitrification and lower DO concentration, respectively, caused by the higher vegetation coverage. As a result, in order to achieve a balanced pollutant removal, the vegetation coverage in an FWS wetland is recommended to be maintained just below 50%.

Acknowledgments

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