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Microbial biomass and heterotrophic production of surface flow mesocosm wetlands treating woodwaste leachate: Responses to hydraulic and organic loading and relations with mass reduction

Wendong Tao[a,*], *Ken J. Hall*[b], *Sheldon J.B. Duff*[c]

[a] *Environmental Engineering Program, University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4Z9*
[b] *Department of Civil Engineering, University of British Columbia, 6250 Applied Science Lane, Vancouver, BC, Canada V6T 1Z4*
[c] *Department of Chemical and Biological Engineering, University of British Columbia, 2216 Main Mall, Vancouver, BC, Canada V6T 1Z4*

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ABSTRACT

Microbial degradation is the major mechanism for removal of organic carbon from woodwaste leachate in surface flow constructed wetlands. To explore the relations of microbial biomass and heterotrophic production with hydraulic and organic loading rates as well as mass reduction rates, this study examined microbial ATP concentration and leucine incorporation rate in water, epiphytic biofilm and sediment of four surface flow mesocosm wetlands fed at different hydraulic loading rates during two periods with 10× and 3× diluted woodwaste leachate. The hydraulic loading rates from 13 to 49 mm d^{-1} had no effects on microbial biomass and heterotrophic production. The organic loading rates between 32 and 396 g COD d^{-1} m^{-3} had significant, negative correlations with heterotrophic production rate and microbial biomass of sediment, and positive correlations with heterotrophic production rates of water and epiphytic biofilm. Higher organic loading yields greater substrate availability for planktonic and epiphytic bacteria, and may induce inhibitory effect on sedimentary bacteria. Microbial attachment played an important role in the establishment of epiphytic and sedimentary communities. The reduction rates for chemical oxygen demand (COD), tannin and lignin (T&L), and volatile fatty acids (VFAs) were significantly correlated to the heterotrophic production rates of water, epiphytic biofilm and sediment together.

1. Introduction

Surface flow constructed wetlands have been tested at pilot- and mesocosm-scales for treatment of woodwaste leachate (Hunter et al., 1993; Frankowski, 2000; Masbough et al., 2005; Tao et al., 2006a,b). Microbial degradation is the major mechanism of surface flow constructed wetlands for removal of chemical oxygen demand (COD), tannin and lignin (T&L) and volatile fatty acids (VFAs) from woodwaste leachate (Frankowski, 2000; Tao et al., 2007). Heterotrophic bacterial activity in surface flow constructed wetlands has been found to be influenced by substrate availability (Kozub and

* *Corresponding author*. Tel.: +1 250 9605277; fax: +1 250 9605845.
E-mail address: wdtao@yahoo.ca (W. Tao).
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Liehr, 1999; Toet et al., 2003; Tao and Hall, 2004; Tao et al., 2006a). In addition to substrate availability, other factors such as toxicity and supply of electron acceptors may affect biomass and heterotrophic production of bacteria. In constructed wetlands, these factors are related with such design parameters as hydraulic and organic loading rates. The previous studies on microbial processes (Tao and Hall, 2004; Tao et al., 2006a) investigated spatial variations, effects of vegetation and nutrient amendment, and initial development of heterotrophic production and substrate utilization in pilot-scale constructed wetlands fed with specified dilutions of woodwaste leachate. However, no relations between influent loading and heterotrophic production or microbial biomass have been systematically investigated in surface flow constructed wetlands.

Field applications and pilot-scale wetlands may undergo daily and seasonal hydraulic variations due to rainfall events, mechanical failure, or leakage. Mesocosm wetlands are more controllable to keep constant water depth, avoid influence of rainfall, and minimize mechanical failure. Mesocosm wetlands are hence better facilities to examine the effects of individual design parameters on heterotrophic production and microbial biomass. Four surface flow mesocosm wetlands were operated in the present study at different hydraulic loading rates during two periods with different strengths of influent, yielding eight organic loading rates. Significant effects of hydraulic retention time and organic loading rate on treatment performance of the mesocosm wetlands have been reported (Tao et al., 2006b). Tao et al. (2006b) also demonstrated a maturation time of <6 weeks for the microbial communities, significant decreases of water temperature, dissolved oxygen and redox potential with water depth, and insignificant vertical variation of microbial biomass in the mesocosm wetlands. To promote kinetic-based design of constructed wetlands, the internal treatment mechanisms have to be revealed. The main objective of this paper is to evaluate the effects of hydraulic and organic loading rates on microbial biomass and heterotrophic production in surface flow wetlands treating woodwaste leachate.

Heterotrophic bacteria may inhabit the water column, plant surface (epiphytic biofilm) and sediment in surface flow constructed wetlands. Flood et al. (1999) reported that bacterial abundance and production of the epiphytic community differed in two surface flow constructed wetlands for treatment of secondary municipal effluent at different influent phosphorus concentrations. However, different relative contributions of water, epiphytic biofilm and sediment to total bacterial activity have been reported for constructed wetlands (Toerien and Toerien, 1985; Toet et al., 2003; Tao and Hall, 2004; Tao et al., 2006a). The present study examined microbial biomass and heterotrophic production, respectively, by measuring cellular ATP and ^{3}H-leucine incorporation into bacterial protein in water, epiphytic biofilm and sediment of four surface flow mesocosm wetlands treating woodwaste leachate. Simultaneous determination of microbial biomass and heterotrophic production in all of the three components of the mesocosm wetlands would assist in understanding the biological treatment processes and generate a more reliable database to connect treatment performance with heterotrophic production. The second objective of the present study is to examine the connections of heterotrophic production and microbial biomass with mass reduction rates for treatment of woodwaste leachate in the mesocosm wetlands. Such integrated investigation into connections of heterotrophic production with leachate loading and to mass reduction is expected to facilitate mechanistic modeling of surface flow constructed wetlands.

2. Materials and methods

2.1. Operating conditions of mesocosm wetlands

Four surface flow mesocosm wetlands were operated on the south University of British Columbia campus, Vancouver, British Columbia, Canada. Each had internal dimensions of 0.36 m wide, 1.98 m long and 0.58 m deep. There was a 0.22-m layer of sandy loam on the bottom, which supported broadleaf cattails (*Typha latifolia*). The mesocosm wetlands were continuously fed at different flow rates with 10% woodwaste leachate in tap water from June 1 to August 25 and 30% leachate from August 25 to November 3, 2003. Effluent overflowed through an elbow, and water depth was kept at 0.25 m.

Woodwaste leachate in this study was generated by rainfall on an uncovered pile of woodwaste from several cedar processing mills in Mission, British Columbia, Canada. The leachate was dark and acidic, and had a high oxygen demand. The leachate had a pH of 4.3, COD 6068 mg L^{-1}, T&L 1839 mg L^{-1} and VFAs 994 mg L^{-1} on average (Tao et al., 2006b).

Hydraulic loading rates to the mesocosm wetlands were, respectively, at 49, 30, 18, and 13 mm d^{-1}. A wide range of organic loading rates were applied to the mesocosm wetlands during the two periods, i.e., 32.2–396.4 g COD d^{-1} m^{-3}, 8.9–131.7 g T&L d^{-1} m^{-3} and 2.2–44.4 g VFAs d^{-1} m^{-3} on average of individual mesocosm wetlands. The influent had low levels of inorganic nutrients relative to COD, 0.06–0.17 mg NH_4^+–N L^{-1}, 0.07–0.24 mg NO_x^-–N L^{-1}, and 0.07–0.19 mg PO_4^{3-}–P L^{-1} (Tao et al., 2006b). Detailed operating parameters have been reported by Tao et al. (2006b). The mean water temperature ± standard deviation was 21.2 ± 1.2 °C during the first sampling period (July 21 to August 18) and 13.5 ± 3.1 °C during the second period (September 29 to October 27). The bulk water in all of the mesocosm wetlands was anaerobic, with dissolved oxygen concentrations <0.6 mg L^{-1}. The top 0.02-m sediment had similar densities, 0.72–0.75 kg (dry weight) L^{-1}, and organic contents, 5.2–6.0% across the mesocosm wetlands. There were 24–30 bigger, green cattails per mesocosm during the first sampling period operated with 10% leachate and 46–48 smaller, dying cattails during the second period with 30% leachate. Specific plant surface area of the mesocosm wetlands was estimated at 9.0–10.6 m^2 plant surface m^{-3} water during the first period and 10.6–12.7 m^2 m^{-3} during the second period.

2.2. Preparation of microbial samples

After 7 weeks of initial operation and more than 4 weeks after switching influent strength, the mesocosm wetlands were sampled weekly to determine microbial dynamics in water, epiphytic biofilm and sediment. Water was collected at

70 mm deep in the middle of the mesocosm wetlands, using a 60-mL syringe with a long needle. Water samples were transferred to 125-mL autoclaved polypropylene bottles through a 0.2-mm nylon sieve to remove macroorganisms and detritus. Epiphytic samples were collected on 600-grit silicon carbide waterproof sandpaper discs (95 mm^2 each) adhered to PVC duct tape, wrapped around Perspex plates (125 mm × 80 mm) and immersed 70 mm deep in the middle of the mesocosm wetlands. Sandpaper discs were retrieved after 6 weeks of microbial colonization. Sediment was sampled with 20-mL plastic syringe barrels (lock end removed). Three sediment cores were taken near the inlet, middle and outlet of each mesocosm. The cores were sealed with rubber stoppers at both ends, and kept in an upright position. Before microbiological examination, the overlying water was poured out. The top 20-mm soft sediment was pushed out of the three cores collected near inlet, middle and outlet of each mesocosm, cut into a flask, mixed, and passed through a 1-mm nylon sieve. Two millilitres of the sediment slurry was diluted with 40 mL filter-sterilized mesocosm water to make sediment suspension.

2.3. Determination of heterotrophic production

Heterotrophic bacterial production was tracked by determining the rate of ^{3}H-leucine incorporation into protein, using a modification of the method described by Ward and Johnson (1996). Mesocosm water (9-mL) in 20-mL syringes, epiphytic biofilm (1 disc plus 9-mL sterile mesocosm water) in 20-mL syringes, or sediment (1 mL suspension plus 8 mL sterile mesocosm water) in 20-mL scintillation vials was added 0.9 mL of L-[4,5-^{3}H]leucine (Amersham Biosciences UK Ltd., Buckinghamshire, UK; 2.9 mCi radioactivity per litre of working solution) and 0.1 mL of 1.31 mg L^{-1} L-leucine. For each sample, one control and two live subsamples were prepared. One millilitre of 25% (w/w) glutaraldehyde solution was used to fix the controls and terminate incubation of the live subsamples.

During the operating period with 10% leachate, the samples were incubated at in situ temperatures for 0.5 h. During the period with 30% leachate, water and epiphytic biofilm were incubated for 0.75–1 h and sediment for 1–1.5 h. Time course experiments (0.25–2 h) were conducted initially with water, epiphytic biofilm and sediment to estimate incubation times. Isotope dilution experiments were conducted initially to estimate leucine saturation levels for bacteria in water, sediment and epiphytic biofilm. The final concentration of 13.1 μg L^{-1} non-labeled leucine was supposed to saturate bacterial uptake and reduce the potential of isotope dilution.

At the end of incubation, the fixed epiphytic samples were sonicated for 3 min in an Aquasonic Model ultrasonic cleaner (50/60 Hz, average sonic power of 45 W) to disperse bacteria. Bacterial protein was extracted with 2.5 mL of 20% cold trichloroacetic acid for 0.25 h at 4 °C. The extracted protein was collected on polycarbonate filters (pore size of 0.2 μm) through vacuum filtration, followed by ice-cold 5% trichloroacetic acid washing and 80% ethanol washing. Each polycarbonate filter was placed in 5 mL Ecolite scintillation cocktail (ICN, Costa Mesa, CA). Radioactivity of the ^{3}H-leucine incorporated in bacterial protein was determined with a Beckman LS6500 multi-purpose scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Heterotrophic production rate was calculated with radioactivity measurements as described by Ward and Johnson (1996).

In order to compare the relative contributions of water, epiphytic biofilm and sediment toward the total heterotrophic production of a mesocosm wetland, the measured areal production rate of epiphytic biofilm and the volumetric production rate of sediment were converted to water equivalent rate as follows:

$$U = U_e \times a, \text{ for epiphytic biofilm} \quad (1)$$

$$U = U_s \times \frac{0.02}{0.25}, \text{ for sediment} \quad (2)$$

where U is the heterotrophic production rate of epiphytic biofilm or sediment, mg C h^{-1} m^{-3} (water equivalent); U_e the measured heterotrophic production rate of epiphytic biofilm, mg C h^{-1} m^{-2} (biofilm); U_s the measured heterotrophic production rate of sediment, mg C h^{-1} m^{-3} (sediment); a the specific surface area, m^2 (plant surface) m^{-3} (water); 0.02 the depth of microbiologically active surface sediment, m; and 0.25 is the water depth of the mesocosm wetlands, m.

2.4. Measurement of microbial ATP

Extraction and assay of microbial ATP followed the procedures described by Karl (1993). Water sample (5 mL) was concentrated by vacuum filtration through a nitrocellulose membrane filter (0.45 μm pore size). Planktonic ATP was extracted in 5-mL boiling Tris buffer (20 mM; pH 7.4) for 5 min. Epiphytic ATP and sedimentary ATP were extracted in 10-mL of 49 g L^{-1} cold phosphoric acid for 0.25 h at 4 °C with three discs of epiphytic biofilm and 0.1 mL of sediment slurry, respectively. The extracts were separated from discs and sediment particles by centrifugation at 2800 × g for 7 min. Field samples were extracted in duplicate. One extraction blank was processed with each set of water, epiphytic biofilm or sediment samples. Two additional subsamples were spiked with an ATP standard and extracted to assess recovery.

The extracts were analyzed for ATP with the luciferin-luciferase method using an Aminco Model J4-7441B Chem-Glow photometer equipped with a strip chart recorder. The enzyme preparation was made from lyophilized firefly lantern extract (Sigma FLE50). ATP standards (2, 5, 10, 20, 40 and 70 μg L^{-1}) were made from disodium salt recovered from equine muscle (Sigma Chemical, St. Louis, MO) with 20-mM Tris buffer for water samples, and potassium phosphate buffer (8.1 g L^{-1} K_2HPO_4) for sediment and epiphytic samples.

The ATP measurements were converted to carbon biomass using a factor of 1 g ATP in 250 g carbon (Karl, 1993). Bacterial generation time was calculated as biomass divided by heterotrophic production rate. In the same way as heterotrophic production rate, the areal biomass concentration of epiphytic biofilm and volumetric biomass concentration of sediment were converted to water equivalent concentrations.

2.5. Statistical analysis

The differences between the mesocosm wetlands and between the two sampling periods were assessed by analy-

sis of variance (ANOVA). Spearman's rank correlation analysis was used to test the significance of a monotonic relationship between two variables, giving P value and coefficient r_s. Regression was employed to test the linear relationship between variables, giving P value and coefficient of determination R^2. Correlation and difference were considered significant at a $P \leq 0.05$.

3. Results and discussion

3.1. *Effect of hydraulic loading rate and interactions between microbial communities*

Fig. 1 shows the variations of heterotrophic production rate with hydraulic loading rate during the two periods operated with different strengths of influent. Hydraulic loading rate did not have a significant effect on the heterotrophic production rates of water, epiphytic biofilm and sediment ($P = 0.36$–0.52). Table 1 shows microbial biomass concentrations in water, epiphytic biofilm and sediment of the mesocosm wetlands during the two periods operated with different strengths of influent. Hydraulic loading rate had no significant influence on microbial biomass across the four mesocosm wetlands ($P = 0.10$–0.81).

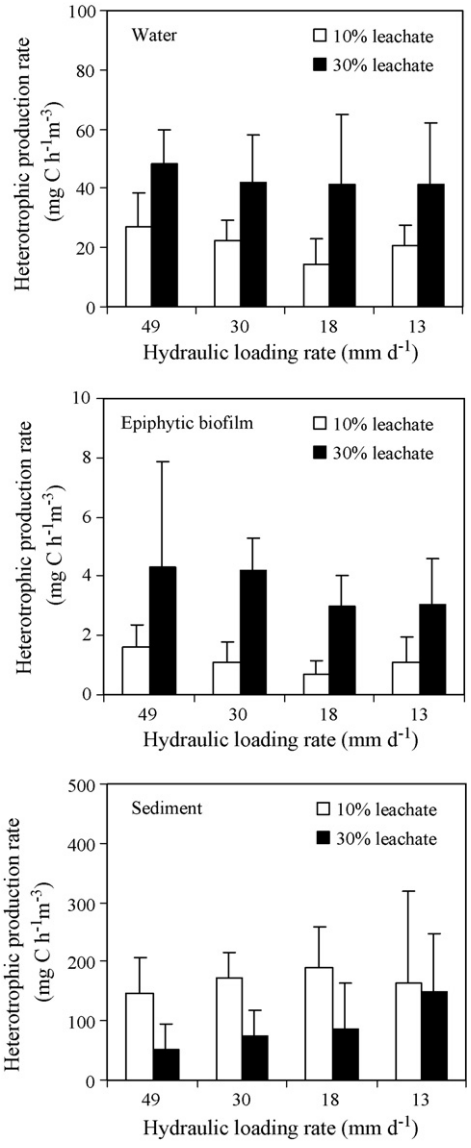

Fig. 1 – Variation of heterotrophic production rate across mesocosm wetlands fed at different hydraulic loading rates during two periods operated with 10 and 30% leachate in tap water. Error bar = S.D. $n = 5$.

Based on the average heterotrophic production and microbial biomass measurements, the bacteria in mesocosm water had a generation time of 0.8–2.2 d. Hydraulic retention times of the mesocosm wetlands, 5–25 d (Tao et al., 2006b), were much longer than the generation times, thus minimizing washout of planktonic bacteria and providing sufficient time for establishment of a mature microbial community. Nevertheless, there was not a significant correlation between planktonic biomass and heterotrophic production rate of water ($r_s = 0.46$, $P > 0.05$). The concentration of planktonic biomass could be affected by heterotrophic production as well as colonization to solid surfaces, cell death, protozoan grazing, and viral attack (Pace, 1988; Hobbie and Ford, 1993).

Microorganisms colonize solid–liquid interfaces through attachment and growth (Caldwell, 1987). No significant correlation was found between epiphytic biomass and heterotrophic production rate of the epiphytic biofilm ($r_s = 0.04$, $P > 0.05$). The generation time of epiphytic bacteria was estimated at 3.1–6.3 d on average of individual mesocosm wetlands during the operating period with 10% leachate and 0.9–1.5 d during the period with 30% leachate. It took <1–6 weeks for maturation of the epiphytic community (Tao et al., 2006b). Therefore, both reproduction of epiphytic bacteria and attachment of planktonic bacteria could contribute to the development and dynamics of the epiphytic community in the mesocosm wetlands. The epiphytic generation time of the mesocosm wetlands is much longer than that (4.5–7.0 h) of wetlands receiving secondary sewage effluent (Flood et al., 1999). When compared to the sewage effluent, woodwaste leachate had a much higher oxygen demand and high concentrations of recalcitrant T&L that could result in conditions unfavorable to the epiphytic community.

Sedimentary bacteria had an average generation time of 78–169 d in the mesocosm wetlands. However, the sedimentary community reached maturity in terms of microbial ATP concentration in 42 days of initial operation (Tao et al., 2006b), suggesting that attachment of planktonic bacteria to sediment played an important role in establishment and dynamics of the sedimentary community in the mesocosm wetlands. Coincidently, there was no significant correlation between sedimentary biomass and heterotrophic production rate of the mesocosm sediment ($r_s = 0.71$, $P > 0.05$).

3.2. *Effect of organic loading rate*

There was a temperature difference of nearly 8 °C between the two operating periods of the mesocosm wetlands. However, temperature influence in surface flow constructed wetlands may not be as profound as it is for some of the more conventional processes (Wittgren and Maehlum, 1996). In a pilot-scale wetland receiving woodwaste leachate, heterotrophic activity

Table 1 – Microbial biomass distribution in mesocosm wetlands fed at different hydraulic loading rates during two periods operated with different strengths of influent

	Hydraulic loading rate (mm d^{-1})			
	49	30	18	13
Influent made of 10% leachate and 90% tap water				
Biomass in water (g C m^{-3})	1.26 ± 0.69	0.84 ± 0.42	0.76 ± 0.48	0.58 ± 0.40
Biomass in epiphytic biofilm (mg C m^{-2})	120 ± 23	83 ± 11	100 ± 41	108 ± 62
Biomass in sediment (g C m^{-3})	418 ± 143	390 ± 269	418 ± 150	418 ± 143
Total biomass as water equivalent (g C m^{-3})	36 ± 13	37 ± 23	40 ± 7	35 ± 13
Water:biofilm:sediment in percentage contribution	5:4:91	3:3:94	2:3:95	2:4:94
Influent made of 30% leachate and 70% tap water				
Biomass in water (g C m^{-3})	1.04 ± 0.28	0.78 ± 0.22	0.80 ± 0.20	0.88 ± 0.32
Biomass in epiphytic biofilm (mg C m^{-2})	98 ± 58	101 ± 56	74 ± 22	110 ± 70
Biomass in sediment (g C m^{-3})	151 ± 99	301 ± 218	245 ± 137	281 ± 115
Total biomass as water equivalent (g C m^{-3})	19 ± 13	24 ± 18	19 ± 11	25 ± 9
Water:biofilm:sediment in percentage contribution	12:7:81	7:12:81	7:11:82	4:5:91

Mean ± S.D. (n = 5).

did not show a significant correlation with water temperature from 7 to 17 °C (Tao and Hall, 2004). The insignificant temperature effect was attributed to such possible reasons as presence of a mixed population of microorganisms that had different optimum temperatures. Heterotrophic bacteria in wetlands might be composed of mixed species established during a long residence time under variable substrate and ambient conditions (Westermann, 1993). Sirivedhin and Gray (2006) also found that denitrification rate in field-scale constructed wetlands was independent of temperature, although there was a positive relationship between denitrification potential and temperature in laboratory experiments. Similar studies in freshwater marshes and lake water (e.g., Moran and Hodson, 1992; Tulonen, 1993) found that there was no correlation between bacterial production and water temperature, or that bacterial production increased with increasing temperature up to a threshold of about 6–10 °C at which other factors became more limiting. Therefore, the eight sets of microbiological measurements on the mesocosm wetlands during the two operating periods at different temperatures were treated without bias while examining the effect of organic loading rate.

The loading rates of COD, T&L and VFAs were significantly correlated to heterotrophic production rate of the bacteria in water (r_s = 0.88–0.95, $P \leq 0.01$), epiphytic biofilm (r_s = 0.85–0.95, $P < 0.02$), sediment (r_s = −0.91 to −0.93, $P < 0.01$) and the total water equivalent (r_s = 0.90–0.98, $P < 0.01$). Fig. 2 demonstrates the monotonic relationships of COD load-

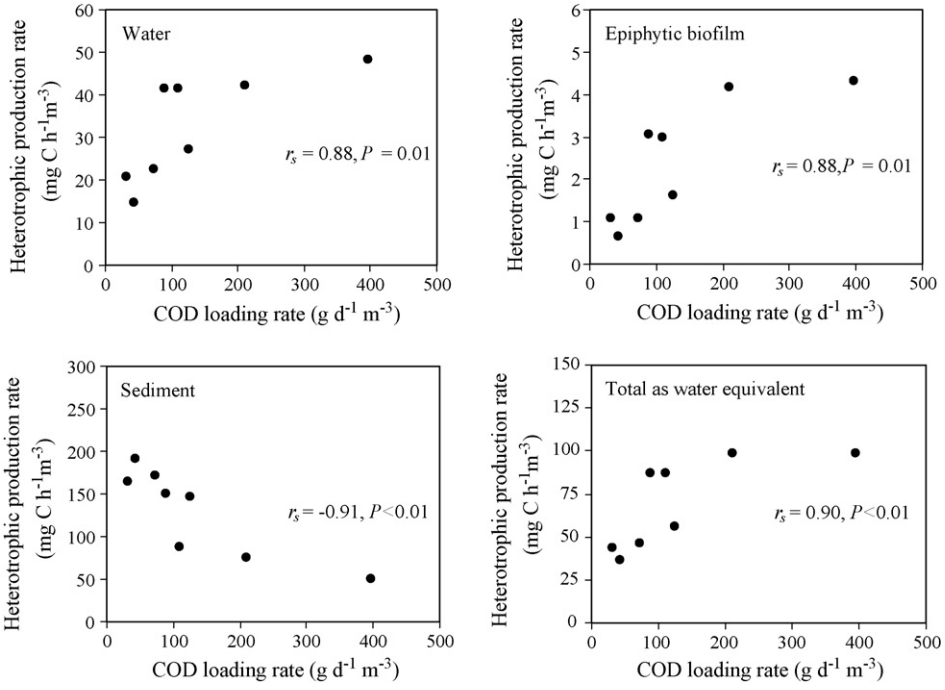

Fig. 2 – Spearman's rank correlation of heterotrophic production rate to chemical oxygen demand (COD) loading rate of the mesocosm wetlands. Data points represent the averages (n = 5) of individual mesocosm wetlands.

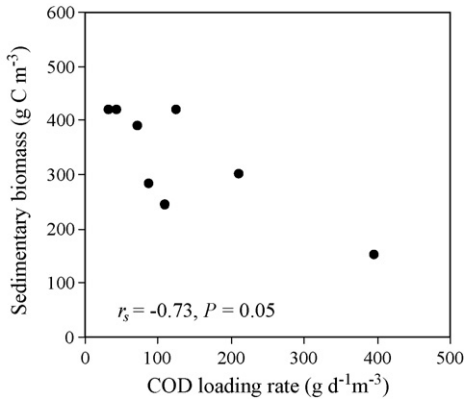

Fig. 3 – Spearman's rank correlation of sedimentary biomass concentration to chemical oxygen demand (COD) loading rate of the mesocosm wetlands. Data points represent the averages ($n = 5$) of individual mesocosm wetlands.

ing rate with heterotrophic production rates. Sedimentary biomass had negative correlations with the loading rates of COD, T&L and VFAs ($r_s = -0.73$ to -0.85, $P \leq 0.05$). Fig. 3 demonstrates the monotonic relationship of sedimentary biomass with COD loading rate.

The positive correlations of planktonic and epiphytic production rates with organic loading rates suggested substrate-limiting conditions in the mesocosm water. More bacterial substrates were supplied at higher organic loading rates, resulting in higher heterotrophic production rates. A recent study (Davies et al., 2005) also revealed non-toxicity on *Phragmites australis* peroxidase activity at higher organic loads of up to 105 g COD d^{-1} m^{-2} in a vertical flow constructed wetland. However, bacteria tend to colonize water–solid surfaces with increasing substrate limitation in water. The negative correlations of sedimentary production rate and biomass concentration with organic loading rates could be attributed to (1) increased microbial affinity to solid surfaces at lower organic loading rates that yielded more substrate-limiting conditions in mesocosm water and, (2) inhibitory effect of higher organic loading rates on the sedimentary bacteria. At the measured redox potential (Eh) of 50–131 mV in the surface sediment of these mesocosm wetlands (Tao et al., 2006b), sedimentary bacteria were likely facultative and strict anaerobes (Westermann, 1993), which are more sensitive to toxic substances (Metcalf and Eddy, 2003). The mesocosm water, however, had higher redox potentials (210–267 mV) and an aerobic water surface (Tao et al., 2006b).

The significantly lower sedimentary production rate ($P = 0.01$) and sedimentary biomass concentration ($P < 0.01$) during the operation with 30% raw leachate than with 10% leachate (Fig. 1 and Table 1) could be caused in part by the inhibitory effect of a stronger influent on the more sensitive sedimentary bacteria. The toxicity of wood leachate is usually attributed to low pH and phenolic compounds, such as tannins (Field et al., 1988; Taylor et al., 1996; Taylor and Carmichael, 2003), which were aggravated in the mesocosm wetlands fed with 30% leachate. Moreover, the higher production rate and biomass concentration of sediment during the first operating period from June 1 to August 25 could be partially attributed to root exudation when cattails were growing actively. The exudates from roots and rhizomes can increase microbial abundance (Rooney-Varga et al., 1997) and microbial activity (Edwards et al., 2006) in sediment.

The mesocosm wetlands had higher heterotrophic production rates in water and epiphytic biofilm ($P < 0.01$) during the operation with 30% leachate than the operation with 10% leachate (Fig. 1). In addition to the more bacterial substrates supplied by the influent of 30% leachate, the senescent cattails and litter that could release organic substrates by leaching and microbial decomposition (Kadlec and Knight, 1996; Alvarez and Becares, 2006) might also be responsible for the higher heterotrophic production rates of water and epiphytic biofilm during the second operating period from August 25 to November 3. Further investigations are needed to confirm the effects of influent strength on heterotrophic production.

3.3. *Relative contributions of wetland components*

Table 2 indicates that planktonic bacteria accounted for nearly one half of the total heterotrophic production rate of individual mesocosm wetlands. Epiphytic bacteria contributed more toward heterotrophic production during the operation with 30% raw leachate. In contrast, sedimentary bacteria contributed more during the operation with 10% raw leachate. Sedimentary bacteria accounted for the majority of the total biomass in the mesocosm wetlands (Table 1). Heterotrophic

Table 2 – Total heterotrophic production rate and the percentage contributions of bacteria in water, epiphytic biofilm and sediment of the mesocosm wetlands fed at different hydraulic loading rates

	Hydraulic loading rate (mm d^{-1})			
	49	30	18	13
Influent made of 10% leachate and 90% tap water				
Total heterotrophic production rate (mg C h^{-1} m^{-3} water equivalent)	55.9	45.8	36.5	43.8
Water:epiphytic biofilm:sediment (percentage contribution)	49:30:21	49:21:30	40:18:42	47:23:30
Influent made of 30% leachate and 70% tap water				
Total heterotrophic production rate (mg C h^{-1} m^{-3} water equivalent)	98.6	98.2	86.6	86.5
Water:epiphytic biofilm:sediment (percentage contribution)	49:47:4	43:51:6	48:44:8	48:38:14

Mean ($n = 5$).

bacterial communities consist of heterogeneous populations in various physiological states (Wetzel and Likens, 2000). Presence of microorganisms does not equal activity (Hobbie and Ford, 1993). Measurement of microbial ATP does not differentiate metabolically active from inactive bacteria. Therefore, the relative contributions of bacteria in water, epiphytic biofilm and sediment to the entire wetlands were different in terms of microbial biomass and heterotrophic production rate.

Bacteria tend to colonize solid surfaces when the water is more substrate limiting (Caldwell, 1987; Mueller, 1996; Tao et al., 2006a). Subsequently, sedimentary bacteria in the mesocosm wetlands had more importance to the entire wetland system during the operation with 10% leachate relative to the operation with 30% leachate. In a pilot-scale constructed wetland treating the same woodwaste leachate, the bacteria in water, epiphytic biofilm and sediment contributed 53, 3 and 44%, respectively, toward the total heterotrophic production (Tao et al., 2006a). Compared to the pilot-scale wetland that had silt loam and only 1.2 $m^2\,m^{-3}$ specific plant surface area, the mesocosm wetlands with sandy loam and 9.0–12.7 $m^2\,m^{-3}$ specific plant surface area had lower contribution from sediment and much higher contribution from epiphytic biofilm to total heterotrophic production.

3.4. *Relationship between treatment performance and heterotrophic production*

Studies (Frankowski, 2000; Tao et al., 2007) have demonstrated that microbial degradation is the major mechanism for organic carbon removal from woodwaste leachate in surface flow wetlands. The mean reduction rates of individual mesocosm wetlands varied between 13.7 and 55.9 g COD $d^{-1}\,m^{-3}$, 5.0 and 10.6 g T&L $d^{-1}\,m^{-3}$ and 2.2 and 20.9 g VFAs $d^{-1}\,m^{-3}$, increasing with loading rates (Tao et al., 2006b). The reduction rates for COD, T&L and VFAs had no significant, linear relationships with microbial biomass concentrations in water, epiphytic biofilm and sediment ($R^2 = 0.49$–0.83, $P = 0.06$–0.15), but showed significant, linear relationships with the heterotrophic production rates of water, epiphytic biofilm and sediment together ($R^2 = 0.83$–0.88, $P = 0.02$–0.05). Nevertheless, heterotrophic production rate of individual wetland components was usually not correlated with mass reduction rate for COD, T&L or VFAs. When addressing treatment performance of surface flow constructed wetlands, it is suggested to examine microbial activity in all of the wetland components.

4. Conclusions

A hydraulic loading rate up to 49 mm d^{-1} was low enough to minimize washout of planktonic microorganisms from the surface flow mesocosm wetlands treating woodwaste leachate. The epiphytic community was established by attachment of planktonic bacteria and heterotrophic production of epiphytic bacteria. The establishment of the sedimentary community was likely contributed by attachment of planktonic bacteria.

Heterotrophic production rates of water, epiphytic biofilm and sediment responded differently to organic loading rates, 32–396 g COD $d^{-1}\,m^{-3}$ to the mesocosm wetlands. A lower organic loading rate increases microbial biomass concentration and heterotrophic bacterial production rate of sediment; a higher organic loading rate favors bacteria in water and epiphytic biofilm. Overall, the total heterotrophic production rate had significant, positive correlations with the organic loading rates.

The influent strength appeared to be another important factor affecting the heterotrophic production and microbial biomass in wetlands treating woodwaste leachate. The effects of influent strength that is associated with availability of bacterial substrates and inhibition to heterotrophic production need to be further explored in a wide range of influent strengths.

Mass reduction for COD, T&L and VFA was a combined indication of heterotrophic production of the microbial communities in water, epiphytic biofilm and sediment. The relative contributions of planktonic, epiphytic and sedimentary bacteria toward the total heterotrophic production of a mesocosm wetland varied with influent strength. However, microbial biomass provided limited information on the internal microbial process connecting organic loading and reduction rates of the mesocosm wetlands.

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