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APPLICATION OF BIOFILM MODEL IN FREE WATER SYSTEM CONSTRUCTED WETLANDS

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This study provides an integrated modeling approach of free water system constructed wetlands by considering dispersed flow conditions of the wetlands, and contribution of suspended biomass in bulk liquid phase and biofilm biomass attached on various surfaces in removing the organic matters. Model parameters such as kinetic and dispersion characteristics, biofilm thickness, and density were obtained experimentally. A significant role of attached biomass was established. The model was validated with the data obtained from a pilot-scale plant and compared with other existing plug-flow models and found more applicable in wetland design.

Key Words: *constructed wetlands, wastewater treatment, mathematical modeling, biofilm and suspended biomass, dispersion number, effective specific surface area, rate constants*

1. INTRODUCTION

Constructed or treatment wetlands have been extensively operated in both developing as well as industrialized countries as a cost-effective and ecologically acceptable alternative to many conventional wastewater treatment systems. They can be used for treatment of primary and secondary wastewater as well as wastewater from variety of other sources including industrial and agricultural practices[1].

Constructed wetlands (CW) can be classified into two categories: free water system (FWS) and subsurface flow system (SFS). FWS CWs are densely vegetated by a variety of plant species and typically have shallow water depth less than 0.30 m to encourage plant growth in the free water interface and consist of parallel basins or channels with relatively impermeable bottom and soil to support the emergent vegetation[1]. On the other hand, SFS CWs use a bed of soil or gravel as a substrate for growth of rooted wetland plants and water flows horizontally through the bed substrate leaving no visible surface water flow. Typically, the soil substratum in SFS wetlands is 0.3 to 0.6 m deep and made up of various sizes of gravel, crushed rock, and soil[2]. FWS CWs are favored in tropical countries and in North America, whereas SFS CWs have been widely used in European countries. Worldwide there are approximately 1000 FWS CWs in operation for various treatment purposes[2]. Constructed wetlands have been extensively used to adequately treat many different types of wastewater, including: municipal, acid mine drainage, industrial, and agricultural wastewaters[1),2),3)]. The possibility of using CWs for agricultural non-point and single discharge wastewater pollution control has been investigated in great detail[1),3),4),5),6),7),8)].

In FWS CW long retention time and extensive surface area in contact with the flowing water provide effective removal of particulate and organic matter. The sediments, plant biomass, and plant litter surfaces are also important for most of the microbial activity, including oxidation of organic matter and transformation of nutrients[9]. Sedimentation, filtration, microbial anaerobic and aerobic degradation, nitrification-denitrification, soil sorption, plant uptake are examples of the mechanisms responsible for the removal of suspended solids, BOD, nitrogen, phosphorus in the CWs, whereas pathogen removal is achieved mainly due to natural die-off, UV radiation, excretion of antibiotics from the roots of macrophytes as well as sedimentation and filtration[10),11)]. Plant harvesting is thought to be one of the possible sinks for nitrogen in CW. Reddy and DeBusk[12)] reported that treatment

wetland cattails can uptake as much as 600 to 2630 kg nitrogen per hectare annually. Hence, routine harvesting of plant material may optimize nutrient removal potential[8),13)].

Biologically CW systems are far more diverse than mechanical systems. Integrated combination of physical, chemical, and microbial interaction is the key of wastewater treatment in wetlands. Plants are the most obvious components of any wetland system. Cattails (*Typha spp.*), bulrushes (*Scirupus spp.*), and reeds (*Phragmites communis*) are the most commonly used plant species. Apart from evapotranspiration and nutrient uptake, the most important function of the vegetation is to provide an extensive surface on roots, stems, and litter for attached biofilm formation.

Despite their extensive use in wastewater and other potential pollution treatment all over the world, the design models of FWS CWs available so far are primarily based on first-order kinetics of BOD removal and plug-flow hydrodynamics[14),15)]. These models assume that the dispersion characteristic of the wetlands is negligible and all fluid particles spend equal time in the system.

The first-order plug-flow models remain predominant in all types of natural treatment systems and wetland systems are not an exception. However, according to Kadlec, and Kadlec and Knight[1),16)], the hydrodynamics of a FWS CW is an intermediate between plug flow and well mixed conditions, where the void areas are likely to be well mixed and dense areas plug flow, even for narrow wetlands. As a result of non-ideal flow FWS CWs simultaneously behave as plug-flow reactor with dispersion, tank in series, and parallel stir tank reactors. With an example of waste stabilization ponds, Thirumurthi[17)] suggested that the completely mixed model, which considers equilibrium condition with no allowance for seepage and evaporation losses, to define the wetland flow is also misleading. Many tracer studies conducted on wetlands also do not indicate plug- or completely mixed flow patterns.

Similarly, the conventional plug-flow models pay a very little attention on the role of biofilm attached on the vast surfaces provided by the plant-media-litter matrix of the wetlands. Effectiveness of attached biofilm in removing organic matter better than the suspended biomass in shallow stream and river has been proved by many researchers[18),19)].

This study presents a design model for FWS CW incorporating the significant role of biofilm attached on the plant stems-roots-media- litter matrix and dispersion characteristics of the system on the degradation of organic matter.

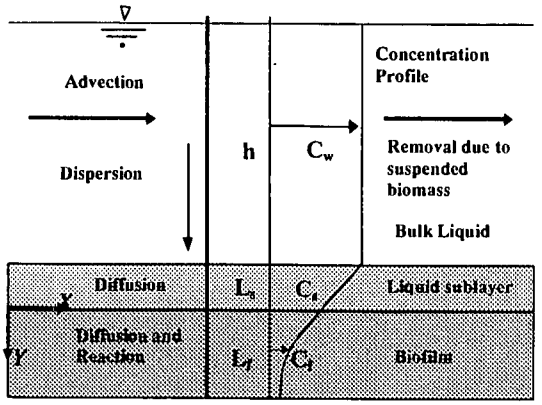

Fig. 1 Biofilm model

The scope of the study discussed here is limited to chemical oxygen demand (COD) removal in FWS CWs.

2. MODEL DEVELOPMENT

In many natural aquatic systems, especially those having a high specific surface area and low nutrient concentrations, it is presumed that biofilm consists of 90 to 99.9 % of the bacteria. The density of biofilm varies with film thickness, but most of the biofilm models assume a constant density[20),21),22)]. A steady-state biofilm is defined as the one in which, for a given bulk-liquid substrate concentration, the rates of growth and decay are equal. The major process governing the steady-state biofilm are: diffusion of the substrate from the bulk liquid to the biofilm surface; substrate utilization and diffusion within the biofilm; growth of biomass; loss of biomass due to decay; transport of substrate down the reactor etc. It is important to note that any of the above mentioned processes may be rate limiting for the overall reaction of the biofilm.

Since biofilm is a dynamic biological entity, all of its characteristics are not amenable to mathematical analysis. Hence, in order to develop the model accounting both suspended and biofilm biomass activities, a few assumptions were made in this study. The biofilm was assumed to be ideal, i.e. having constant thickness and density. The assumptions are graphically illustrated in **Fig.1.**

Lau[22)] suggested that the effect of heterogeneity of biofilm should be incorporated into the diffusion coefficient and rate constants. The substrate concentration in the bulk liquid was assumed to be constant. In the bulk liquid flow, diffusion of substrate was considered negligible as compared to advection, while in the biofilm diffusion was predominant compared to advection. A mass

balance for the substrate in an imaginary control volume, within the bulk liquid flow of FWS CW, with a length of x and volume of V can be written mathematically as:

$$\Delta V\frac{\partial C_w}{\partial t}+\Delta V r=\left(qC_w-AD\frac{\partial C_w}{\partial x}\right)_x-\left(qC_w-AD\frac{\partial C_w}{\partial x}\right)_{x+\Delta x} \quad (1)$$

Considering steady-state condition and no lateral flow, Eq. (1) in terms of fractional distance z becomes

$$d\frac{d^2C_w}{dz^2}=\frac{dC_w}{dz}+t r_s+t a_s J \quad (2)$$

where,

C_w - substrate concentration in the bulk liquid (g/m^3); $r = r_s + r_a$, losses due to reaction ($g/m^3.d$); r_s - substrate utilization rate by suspended media ($g/m^3.d$); r_a - substrate utilization rate by attached media ($g/m^3.d$); q - bulk liquid flow rate (m^3/d); A - cross-sectional area of the wetland control volume perpendicular to the direction of flow (m^2), V - wetland volume (m^3), a_s - specific surface area (m^2/m^3); J - flux of substrate into the biofilm ($g/m^2.d$); $z = x/L$, fractional length; x - distance measured from influent along wetland length (m); L = wetland length (m); $t = L/U$, mean hydraulic retention time (day); U - uniform flow velocity (m/d), $d = D/UL$, dispersion number; D-longitudinal dispersion coefficient (m^2/d).

Neglecting the accumulation of substrate on the biofilm surface, the substrate mass balance within the biofilm based on first order kinetics can be given as[22]

$$D_f\frac{d^2C_f}{dy^2}=k_{fa}C_f \quad (3)$$

And the flux (J) at the biofilm surface ($y = 0$) is

$$J|_{y=0}=\left(\frac{\tanh(\phi)}{\phi}\right)k_{fa}L_f C_s \quad (4)$$

Assuming first order kinetics for substrate removal rate by suspended biomass[17] we finally get,

$$d\frac{d^2C_w}{dz^2}=\frac{dC_w}{dz}+t k_{fs}C_w+t a_s\left(\frac{\tanh(\phi)}{\phi}\right)k_{fa}L_f C_s \quad (5)$$

where,

k_{fs} - first order rate constant for suspended biomass (d^{-1}); k_{fa} - first order rate constant in the biofilm (d^{-1}); D_f - substrate diffusion coefficient in the biofilm(m^2 / d); C_f - substrate concentration within the biofilm (g/m^3); C_s - substrate concentration on the biofilm surface (g/m^3); L_f - biofilm thickness (m); ϕ - characteristic biofilm parameter,

$$\phi=\sqrt{\frac{k_{fa}\cdot L_f^{\,2}}{D_f}} \quad (6)$$

Assuming linear variation of substrate concentration across the liquid sublayer, the substrate flux (J_c) is given by Fick's Law:

$$J_c=\frac{D_w}{L_s}(C_w-C_s) \quad (7)$$

where L_s is thickness of liquid sublayer (m) and D_w is sbstrate diffusion coefficient through liquid-sublayer (m^2/d).

Combination of all above equations and assumption of steady-state condition result in

$$d\frac{d^2C_w}{dz^2}=\frac{dC_w}{dz}+t\left(k_{fs}+a_s\frac{\alpha\cdot\beta}{\alpha+\beta}\right)C_w \quad (8)$$

where $\beta=\dfrac{\tanh(\phi)}{\phi}\cdot k_{fa}\cdot L_f$; $\alpha=\dfrac{D_w}{L_s}$

If D_w, L_s, and L_f are assumed to be constants, the terms α and β will not vary along the wetland length for a particular set of climatic conditions. Based on the assumption that organic matter concentration near the wetland outlet do not change considerably or its gradient approaches zero at the outlet, the boundary conditions for Eq. (8) are $C_w = C_i$ at $z = 0$ and $dC_w / dz = 0$ at $z = 1$, in which C_i is influent substrate concentration (g / m^3).

For the above boundary conditions, Eq. (8) can be integrated to obtain,

$$\frac{C_e}{C_i}=\frac{2a_1 e^{\frac{1}{2d}}}{(1+a_1)e^{\frac{a_1}{2d}}-(1-a_1)e^{-\frac{a_1}{2d}}} \quad (9)$$

where, C_i and C_e are influent and effluent substrate concentrations (g/m^3), respectively, e is exponential and

$$a_1=\sqrt{1+4Ktd} \quad (10)$$

where, K = overall rate constant (d^{-1})

$$K=k_{fs}+a_s\frac{\alpha\cdot\beta}{\alpha+\beta} \quad (11)$$

Equation (9) is the model for substrate biodegradation in constructed wetlands that incorporates the reactions of both suspended and as well as biofilm biomass. By making k_{fs} in Eq. (11) equal to zero, Eq. (9) would determine the removal efficiency due to the biofilm biomass only.

The following methods were used to determine various parameters of the model. The parameters, which could not be obtained experimentally, were taken from literature. The details of modeling procedures and methodologies for the determination of the model parameters are given by Bhurtel[23].

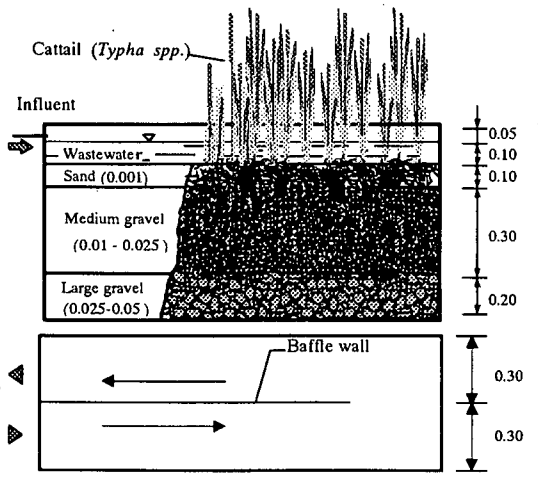

Fig. 2 Schematic diagram of FWS CW (all units in m)

3. METHODOLOGY

(1) Experimental set-up

This study was carried out using 3 laboratory scale FWS CW units with the theoretical HRT of 1, 3, and 5 days and a pilot-scale unit located at the Environmental Research Station, Asian Institute of Technology (AIT), Thailand. All the wetlands were open space units. The primary-treated AIT campus wastewater was fed into these units for the determination of treatment performance, kinetic constants, and flow characteristics.

The lab-scale units, made of acrylic plates, had a working dimension of 0.60 × 1.65 × 0.75 m (width × length × depth) and with a length to width ratio (L/W) of about 10:1. The baffle was installed to minimize short-circuit as shown in **Fig. 2**.

The pilot-scale unit operating at HRT of 5 days was 0.75 m deep, 1m wide, and 10 m long with a slope of 1%. The support media, water depth and cultured plants were similar as in the lab-scale units. Cattail plants (*Typha spp.*) were cultured in the wetland beds at an initial density of 40 - 45 plants/m^2. There were 3 layers of support medium: large gravel (2.5 - 5 cm in diameter) base of 20 cm depth, the middle section of 30 cm depth with comparatively small gravel (1.0 - 2.5 cm in diameter); and 10 cm deep sand on top. Ten cm of water depth was maintained on the surface of these units to operate the CW in FWS mode.

(2) Tracer study

Tracer study was performed to determine the dispersion characteristics and actual HRT of the lab-scale unit operating at 5 days HRT.

Traditionally, dye studies and NaCl were used for this purpose[24),25)]. However, introducing NaCl for dispersion studies seems to be not effective because of high interference of Cl and Na ions even in tap water. A laboratory study with column packed with sand, conducted by Wrenn et al.[26)], revealed that Rhodamine WT strongly adsorbed on the sand surface, whereas lithium (Li) did not, and the recovery of Li was much higher than that of Rhodamine WT (100 % against 50 %). Similarly, Kadlec et al.[27)] also used Li as tracer in FWS constructed wetlands and the results obtained were of high reliability. Therefore, in this experiment Li was selected as the tracer.

Before introducing LiCl solution the unit was flushed properly and fed with tap water. Monitoring of tap water for Li ion background concentration was performed at an hourly interval for two days and no Li ion concentration was detected. Next, a 1 L solution of LiCl (grade analytical, Merck, Germany) with distilled water was prepared and introduced at the inlet section. Samples were taken from the outlet at an interval of 8 hours for an approximate duration of 3 times the HRT. Li ion concentration was analyzed by atomic absorption spectrophotometry (Zeeman Polarized, Flame type, detection limit = 0.01 mg/L). From the concentration response data, dispersion numbers was calculated according to the closed-vessel equation of Levenspeil[28)].

(3) Biofilm thickness and density

Because of the irregular shape and formation in the wetlands, a direct measurement of the biofilm thickness and density was not feasible. Therefore, the thickness of biofilm growing on the stems and bottom surface area of the wetland beds was measured by installing eighteen acrylic sampling slides (each with a dimension of 7 × 7 cm) at different locations randomly along the bed length of the lab-scale units. After two to three months when biofilm growth was observed to have established fully on those plates, the plates were removed from the wetland beds and the attached biofilm was thoroughly scraped into a 100-mL volumetric flask. Distilled water was filled to the flask mark using a 50-mL burette and the biofilm volume was measured by water displacement method. The content of the flask was used to determine total solid (TS) concentrations. The average biofilm thickness was determined by dividing the wet volume of the biofilm by the surface area of the plate. The density of biofilm was expressed in terms of grams of TS per L[29)].

(4) Batch kinetic experiments

Batch kinetic experiments were performed to determine the first-order rate constants of COD removal by the suspended and biofilm biomass

individually. For the biofilm biomass, three runs of experiments were conducted. Biomass from the above mentioned plates (section 3.3) located on the surface of the sand media was transferred into a 2.5-L cylindrical acrylic batch reactor filled with 2-L of synthetic wastewater with a COD concentration ranging from 100 to 120 mg/L. The synthetic wastewater used in the batch kinetic experiment of biofilm biomass was composed of glucose with the usual composition of nutrients such as KH_2PO_4, K_2HPO_4, NH_4Cl, and $CaCl_2$[30]. The given range of COD was selected to simulate the actual COD concentration of campus wastewater fed to the wetland units. The mixed liquor contents were subjected to thorough mixing initially for 15 minutes in a jar test apparatus and aerated by diffused air under ambient conditions to maintain a dissolved oxygen (DO) level of about 4 mg/L. The volatile suspended solid (VSS) concentration of the mixture was maintained at about 430 mg/L. Ten-mL samples were taken for filtered COD analysis at an interval of every 30 minutes for the first 90 minutes and then every hour until negligible amount of reduction in COD concentration was observed. COD and other parameters were analyzed in accordance with the method recommended by Standard Methods[31].

Similarly, for determining the suspended biomass rate coefficient, a grab sample of 500 mL of mixed liquor was collected from the liquid layer from the mid-section of the wetland unit using siphon-technique. It was then placed into a batch reactor filled with 1.5 liters of 45 μm glass-fiber filtered influent, so as to achieve a filtered COD of about 50 mg/L. To satisfy the nutrient requirements for proper bacterial growth KH_2PO_4, K_2HPO_4, NH_4Cl and $CaCl_2$ were added to the solution. The contents were thoroughly mixed and supplied with diffused air. Ten-mL samples were taken, filtered, and analyzed for filtered COD and VSS concentration on a daily basis. Similar methodology has been used by many researchers[26),29),32)] for conducting batch kinetic experiments.

First-order kinetics was used in batch kinetic experiments. The first-order rate equation for a chemical process is given by[28)]

$$r = - dC / dt = k C \qquad (12)$$

where, k - first order rate constant (d^{-1}).

Therefore, a plot between $-ln\ (C_t / C_0)$ and reaction time (t) would yield a slope equal to the first-order reaction rate, k.

Table 1 Performance summary of constructed wetland units

Unit	HRT (theore-tical)	Evapo-trans-piration	OLR	Avg. Infl. COD	Avg. Effl. COD	Avg Rem. Effi.
	days	m^3/ (ha × d)	kg COD/ (ha ×d)	g/d	g/d	%
L-1	1	582	269	25.28	16.13	42
L-2	3	320	114	11.34	3.92	60
L-3	5	334	63	6.0	1.05	80
Pilot	5	291	70	68.19	11.54	82

OLR – organic loading rate, L–Lab-scale

Table 2 Results of the tracer study

Unit	Theoretical HRT (days)	Actual HRT days (Tracer study)	Dispersion Number
Lab - 3	5	6.65	0.15

4. RESULTS AND DISCUSSION

Table 1 summarizes the performance of lab- and pilot-scale CW units. The wetland units were fed with a low strength wastewater and daily variation in COD, SS, and VSS concentrations were considerable.

The primary-treated campus wastewater had COD concentrations ranging from 43 to 184 mg/L, SS concentrations of 24 to 88 mg/L, VSS concentrations of 8 to 58 mg/L and pH ranging from 7.7 to 8.3. A HRT of 5 days gave the maximum COD removal efficiency of about 80% in both lab- and pilot-scale units, based on COD load in g/d. The removal efficiencies were calculated based on mass basis to eliminate the effect of evapotranspiration. During the experimental period of 4½ months (1st Feb. - 15th June 1997), the water temperature was in the range of 24 to 34 °C. Plant density increased to 85-110 rhizomes/m^2 and the plants were harvested twice during the course of experiments.

(1) Tracer study

Lab-scale FWS CW unit operating at theoretical HRT of 5 days was used for conducting dispersion studies. As stated in section 3.2, tap water was used in order to minimize the interference of Li ion background concentration. **Table 2** shows the actual HRT and dispersion numbers calculated from dispersion data of the unit.

As seen from the tracer output curve depicted in **Fig. 3**, the response at the wetland outflow was intermediate between well mixed and plug flow, showing large dispersion and asymmetry. Actual HRT was found to be 6.65 days, 33% more than the

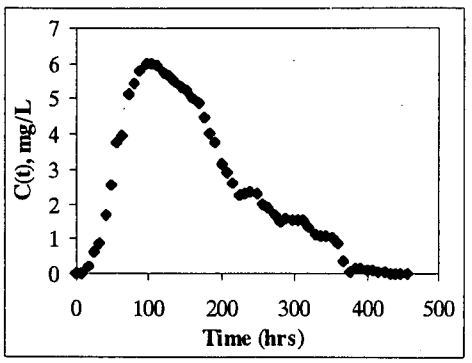

Fig. 3 Concentration of tracer output curve

theoretical value. The tracer mass balance of the unit was around 90%.

Large dispersion, as indicated by dispersion number of 0.15, can be expected considering the possibility of formation of internal islands in the corners of a rectangular tank. These internal islands are inaccessible to the main flow and may cause a great deviation from plug flow conditions[33]. This might be due to solid loading or biofilm growth inside the sand and gravel beds leading to clogging and/or preferential flows.

Nonetheless, the dispersion number of 0.15 obtained from this experiment is consistent with the values reported by various researchers. Mattaraj[24] found a dispersion number of 0.15 for a HRT of 5 days for the constructed wetlands functioning under similar climatic and operating conditions as in this study. For surface flow wetlands planted with cattails, Kadlec and Knight[1] suggested a dispersion number of 0.26 ± 0.10.

(2) Biofilm thickness and density

The thickness of the biofilm growing on the stems and top surface area of the sand media was approximately determined by scraping the biofilm attached on the acrylic plates located at influent and effluent sections of the all three lab-scale units; and measuring the volume by water displacement. The results are depicted in **Table 3**. As seen in the table, average thickness and density of biofilm obtained were 1036 μm and 16.18 gTS/L, respectively. Depending on the location of plates these parameters varied considerably.

Thicker and denser layer of biofilm was formed on the plates located at the influent section as compared to the effluent section. It may be because the biofilm microorganisms responsible for carbon oxidation grow predominantly in the earlier part of the pond, while those responsible for nitrification attach mainly in the latter portion of the pond. Since carbon oxidation normally yields more energy for cell growth than that of nitrification, the quantity of

Table 3 Biofilm density and thickness

HRT	PL	TS	TVS	TVS/TS	Thick-ness	Density
days	-	g/m^2	g/m^2	%	μm	gTS/L
5	IS	28.53	16.22	57	1490	19.15
5	IS	29.00	17.59	61	1429	20.30
5	ES	3.80	3.47	91	347	10.94
5	ES	11.35	7.51	66	837	13.56
3	IS	28.94	17.45	60	1592	18.18
3	IS	28.12	16.80	60	1531	18.37
3	ES	11.53	9.10	79	1020	11.30
3	ES	8.84	6.71	76	714	12.37
1	IS	24.77	8.41	34	1143	21.68
1	IS	23.12	13.08	57	1102	20.98
1	ES	7.84	4.41	56	592	13.24
1	ES	8.90	5.59	63	633	14.07
Average					1036	16.18

PL: Plate Location, IS: Influent Section, ES: Effluent Section

biofilm growth in the earlier part should be more than that in the latter part along the pond length.

In order to determine the biomass present in interstitial spaces of soil media, samples were taken from the influent, effluent and middle sections of the wetland beds and analyzed for TS and TVS. The result showed the maximum TVS/TS content of only 6.7% for HRT of 5 days, revealing that there was no significant amount of biomass in the interstitial spaces of the media. Hence, it can be concluded that the litter, stems, and top of sand media play dominant role of providing surfaces for biofilm formation.

(3) Batch kinetics experiments

a) First-order rate constant of suspended biomass

Samples from the bulk liquid portion of the laboratory and pilot scale wetlands were extracted by siphoning to estimate the quantity of available suspended biomass inside the units. The samples were collected weekly for a period of 4 months and analyzed for VSS. The VSS concentration was found to be very low, ranging from 4 - 26 mg/L, with an average value of 11.78 mg/L. The primary-treated campus wastewater was found to have an average VSS of 27 mg/L. During the batch kinetic experiments, it was observed that even after 4 days of aeration, there was neither significant reduction in initial COD nor increase in VSS concentrations throughout the experiment, indicating insignificant activity of the suspended biomass. It was suspected that a shallow water depth (10 cm) and primary treated wastewater with very low VSS content could not provide favorable conditions for suspended bacterial activity.

Table 4 First-order rate constant of biofilm biomass at various temperatures

Batch No	k*	Calculated k	Temp (T)	Avg. VSS	Avg. Density	k_{fa}**	k_{fa}***
	min^{-1}	d^{-1}	°C	mg/L	gTS/L	d^{-1} at T	d^{-1} at 20 °C
1	0.0093	13.392	29.5	428	16.18	506	318
2	0.0070	10.080	28.5	428	16.18	381	251
3	0.0059	8.496	27.3	428	16.18	321	225
Average value							265

* Slope of the curve

** Corrected for biofilm density (multiplied by density and divided by VSS)

*** Corrected for temperature

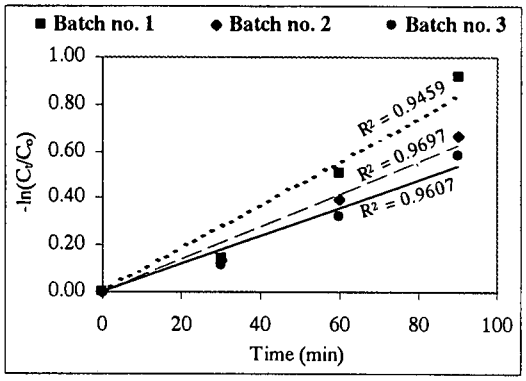

Fig.4 First-order rate constants of COD degradation by biofilm bacteria

Therefore, in this study the first-order rate constant of the suspended biomass was taken as negligible or $k_{fs} = 0$.

In this case, the overall rate constant (K) becomes equal to the rate constant of biofilm biomass, and Eq.(11) becomes,

$$K = a_s \frac{\alpha \cdot \beta}{\alpha + \beta} \qquad (13)$$

b) First-order rate constant of biofilm biomass

The results of the batch experiments with biofilm are illustrated in **Fig. 4** and the values of first order rate constant, k_{fa}, are given in **Table 4**.

The values of k_{fa} were obtained by correcting the calculated k values for the biofilm density and temperature, and found to be in the range of 225 to 319 d^{-1} at 20°C. The variation was due to different ambient temperatures. The average k_{fa} value of 265 d^{-1} appeared to be slightly higher. Since contribution of suspended biomass in removing the organic matter was found negligible, the biofilm bacteria was the only responsible factor for COD removal, which justifies a higher value of the first-order rate constant for the biofilm bacteria.

(4) Effective specific surface area of biofilm formation

A precise measurement of biofilm attached on stems of cattail plants, in the interstitial spaces of various media, wetland walls, and top of the sand media was not possible. Furthermore, not all the specific surface area is actively available for biofilm formation. The required surface area to bring about the observed removal was back-calculated by solving Eq.(9) using trial and error approach with the performance data of the lab-scale constructed wetland units. The mean value thus obtained was considered as the effective biofilm specific surface area, a_s. It was observed that the actual HRTs of the units were increased due to evapotranspiration. Actual HRT was calculated with an average flow rate as depicted in **Table 5**. The effect of evapotranspiration was more pronounced in the unit operating at a theoretical HRT of 5 days as evident from **Table 5**.

Table 5 Theoretical and actual HRT, average flow rates of lab-scale experimental FWS CW.

Lab unit	Avg. inflow	Avg. outflow	Theoretical HRT	Actual HRT (evapt)
#	mL/min	mL/min	day	day
1	220	180	1	1.30
2	93	71	3	3.20
3	51	28	5	6.50

Note: Volume of all units is 0.37125 m^3.

The actual HRT of this unit was 6.5 days. Hence, to account for the effect of HRT, the corresponding effluent COD values for a given influent were obtained 7 days later than the influent. The dispersion number obtained from the tracer study was 0.15. The effective specific surface area calculated from Eq.(9), were found to be in the range of 0.83 m^2/m^3 to 7.33 m^2/m^3 with a mean value of 4.40 m^2/m^3. Similarly, for the other two lab-scale units, the mean values of a_s obtained were 5.43 and 6.39 m^2/m^3, respectively. A more detailed explanation of a_s determination is given by Polprasert et al.[34].

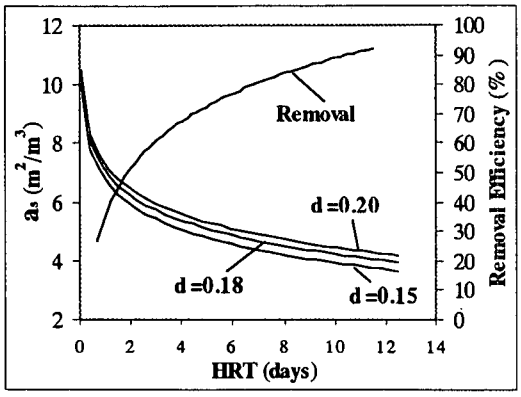

Fig. 5 Relationship between a_s, COD removal and HRT with respect to dispersion numbers

Using Eq.(9), a relationship between a_s, COD removal, d, and HRT was developed and is depicted in **Fig. 5**. From the figure, it is apparent that with the increase in HRT the removal efficiency of wetlands increases, whereas the requirement of effective specific surface area decreases. Besides, to bring about a given percentage removal, the effective specific surface area requirement increases with the increase in dispersion number. Higher dispersion number requires more effective specific surface area for achieving desired removal efficiency.

(5) Diffusion coefficients (D_w and D_f)

The diffusion coefficient through the stagnant liquid sub-layer (D_w) is defined as equal to the diffusion coefficient through water, and it uniquely characterizes the degree of back mixing during flow[28]. Perry and Green[35] have proposed a D_w value of 6.9×10^{-6} cm^2/s (59.6×10^{-6} m^2/d) using glucose and water as solute and solvent at 25 °C. At 20 °C the value of D_w (molecular diffusivity of glucose in water) is 50.90×10^{-6} m^2/d.

The diffusivity of a substrate in biofilm (D_f) is usually considered constant or independent of concentration. It is defined as the ratio of the flux of substrate (perpendicular to the film surface) to the concentration gradient. La Motta[36], proposed D_f values of glucose to be 1.25×10^{-6} cm^2/s to 4.86×10^{-6} cm^2/s at 22 °C with an average value being 2.83×10^{-6} cm^2/s or 44% of D_w at the same temperature. At 20 °C the value of D_f is 22.10×10^{-6} m^2/d.

(6) Liquid Sub-Layer Thickness (L_s)

Williamson and McCarty[37),38] found the liquid sub-layer consisting of two layers, L_1 and L_2; the outer sub-layer (L_1) had varying thickness and could be reduced to zero thickness with adequate mixing. However, the inner sub-layer (L_2), adjacent to the biofilm, was found to have a constant thickness of 56 μm. Rittman and McCarty[39], conducted experiments on a column reactor and found the value of L_s to be in the range of 119 to 226 μm. In the absence of experimental data, L_s was assumed to be 200 μm for the purpose of this study.

Table 6 Model parameters and their values

Parameters	Value at 20 °C	Unit	Source
a_s	4.40	m^2/m^3	this study
d	0.15	-	this study
X_f	16.18	gTS/L	this study
k_{fa}	265	d^{-1}	this study
k_{fs}	0	d^{-1}	this study
L_f	1036×10^{-6}	m	this study
L_s	200×10^{-6}	m	Ritmann & McCarty[39]
$D_w^{\#}$	50.90×10^{-6}	m^2/d	Perry & Green [35]
$D_f^{\#}$	22.10×10^{-6}	m^2/d	LaMotta[36]

$^{\#}$ D_f and D_w values were corrected for temperature using the relationship given by Perry and Green[35].

Table 6 summarizes the parameters required to evaluate the model. The value of a_s varies according to HRT and dispersion number. In this regard, the chart given in Fig. 5 might be useful to obtain a_s at various HRT and desired removal efficiencies. For the validation purposes, the values of a_s and d were chosen as 4.4 m^2/m^3 and 0.15, respectively as obtained from the lab-scale unit with a theoretical HRT of 5 days.

5. MODEL VALIDATION

The model was validated with the data obtained from the pilot scale CW. Due to evapotranspiration, actual HRT calculated with an average flow rate was found to be 7.34 days. Therefore, it was concluded that the influent COD concentration measured on a day would correspondent to the effluent COD measured after an interval of 7 days. The observed and model predicted effluent COD mass loads (g/d) are illustrated in **Fig. 6**.

The model formulated in this study was compared with two most widely used design models of CWs: first-order plug-flow model and its modification[2]. The first-order plug-flow model is:

$$\frac{C_e}{C_i} = \exp[-K_T\, t] \qquad (14)$$

where, C_e and C_i are effluent and influent BOD loads (g/d), respectively. The temperature dependent first-order rate constant K_T is

$$K_T = K_{20}\,(1.06)^{(T-20)} \qquad (15)$$

where, T is the actual temperature (°C) and

$K_{20} = 0.678$ d^{-1}

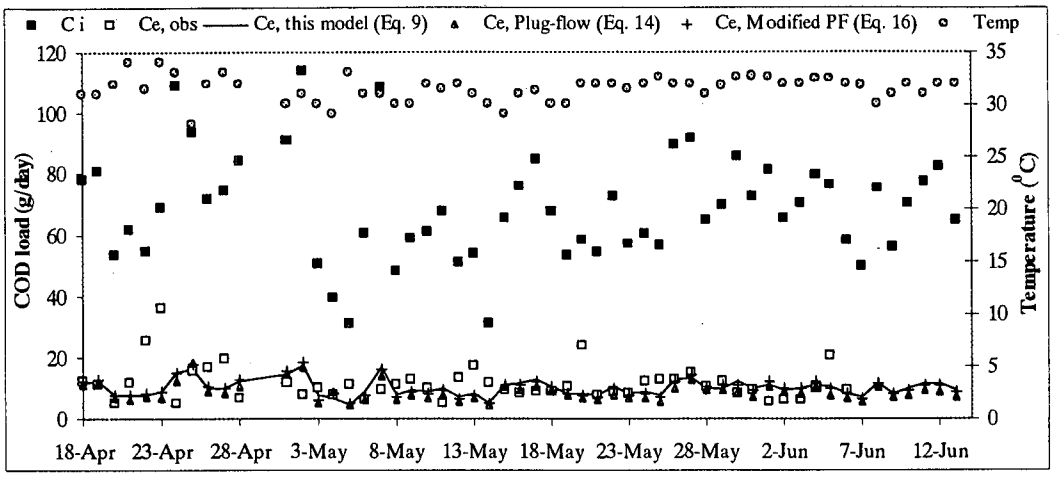

Fig. 6 Comparison of this model with plug-flow and modified plug-flow models and actually measured effluent COD load

The plug-flow model was modified by Reed et al.[2] to encompass other characteristics of wetlands. The modified plug-flow (modified PF) model[2] considers fractions of organic matter unremoved by settling at the head of the system (A):

$$\frac{C_e}{C_i} = A \exp\left[-0.7\, K_T\, (A_v)^{1.75}\, t\right] \qquad (16)$$

The rate constant at 20 °C for this formula is

$K_{20} = 0.0057\ d^{-1}$.

and 0.7 - empirical constant; A = 0.52; A_v - specific surface area made available by wetland vegetation for microbial activities (m^2/m^3). Reed et al.[2] recommended an A_v of 15.7 m^2/m^3. The value, however, gives much lower prediction of COD removal than actually observed during the experimental period. Therefore, the A_v value was also back-calculated by solving Eq. (16) using trial and error approach with the performance data of the lab-scale constructed wetland unit as done in this model to obtain the effective specific surface area (a_s). The mean value of A_v thus obtained was of 14.2 m^2/m^3; t is the hydraulic detention time (d) and equals to

$$t = \frac{L \cdot W \cdot n \cdot d}{Q} \qquad (17)$$

where, L, W are length, width of the system and d is average water depth in the system (m); n - fraction of cross-sectional area not occupied by plants and equals to 0.75. Average flow (m^3 / d) is

$$Q = (Q_{influent} + Q_{effluent}) / 2\ , \qquad (18)$$

The validation results obtained from first-order plug-flow and modified PF models are shown in **Fig.6**. It is obvious from **Fig. 6** that prediction of effluent COD mass load made by the model proposed in this study fits better with the observed COD load of the pilot-scale wetland as compared to the values predicted by the plug-flow models. Few irregular peaks encountered in the observed COD effluent curve might be attributed to various phenomena such as occasional change in microbial communities, biofilm sloughing, scouring due to heavy rainfall events, wind effect and other ecological factors not included in the model.

Table 7 Statistical evaluation of the models

Statistical Tests	This Model	Plug-flow (Eq. 14)	Modified PF (Eq. 16)
MEAN	10.01	7.97	9.83
STD DEV	2.63	2.84	2.90
ME	1.43	2.83	1.41
RE	0.40	0.44	0.41
MSE	46.63	53.76	48.20
RMSE	6.83	7.33	6.94

Mean of observed removal is 10.23 g/d

As shown in **Table 7**, the mean value of predicted COD load of 10.01 g/d of the present model is much closer to the observed effluent COD load of 10.23 g/d. Whereas the plug-flow and modified PF models produced mean values of only 7.97 and 9.83 g/d, respectively. Similarly, the mean error (ME), relative error (RE), the mean of the square error (MSE), and root mean of the square error (RMSE) of the current model are lesser than that of the other two models. It indicates better prediction ability of the model as compared to the other conventional approach. The modified PF model might be useful if more accurate estimation of A_v is made.

Apart from the above statistical analyses, the predicted and observed values given by the present model were subjected to paired T-test and Chi-squared test. The paired T-test with 5% significance level indicated insignificant difference between the values. Similarly, a very high Chi-squared value also approved the same.

Table 8 Sensitivity analysis of kinetic coefficients

Kinetic coefficient	Unit	Actual value	Degree of sensitivity
a_s	m^2/m^3	4.4	++
D_f	m^2/d	2.93×10^{-5}	+
D_w	m^2/d	6.75×10^{-5}	+
L_f	μm	1036	+
L_s	μm	200	+
k_{fa}	d^{-1}	453	++
d	-	0.15	+++
HRT	d	7.34	+++

Note: The actual values of the kinetic coefficients used in the analysis were obtained for a temperature of 31 °C.
+ less significant ++ moderately significant +++ significant

Sensitivity analysis was carried out to determine the influence of the kinetic parameters on the prediction of the model. The variation range given was from –40% to +100%. Dispersion number, HRT, and a_s were found to be the most sensitive parameters among all within the range. The results of the analysis are shown in **Table 8**.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study an integrated kinetic model of free water system constructed wetlands and methodologies to obtain the model parameters were presented. Batch kinetic studies were conducted to determine the rate constant of suspended as well as attached biofilm growth. As expected, the role of biofilm bacteria attached on various surfaces of wetland in the removal of organic matter was found to be significant, while the activity of suspended bacteria was negligible. The first-order rate constant of biofilm bacteria was found to be 265 d^{-1} at 20 °C. Tracer study was conducted to delineate the dispersion characteristic of wetland. The study unveiled the expected fact that wetland flow pattern was highly deviant from plug-flow conditions. A dispersion number of 0.15 was obtained for the lab-scale wetland with an HRT of 6.65 days. In principle, removal efficiency of the wetland unit increased with the increase in HRT. Effective specific surface area varied according to HRT and dispersion numbers. It was established that higher dispersion number needs higher value of effective specific surface area to obtain a given removal efficiency, whereas high HRT value requires lesser surface area. The model was validated with the data obtained from a pilot scale unit. The predicted effluent COD mass load values were found in close agreement with the observed values indicating the applicability of proposed kinetic model in FWS CW design. The model was further compared with two widely used CW design models. As compared to them, this model showed better prediction potential.

The study clearly demonstrated the significance of biofilm bacteria attached on the various surfaces of FWS CWs in the removal of organic matter. On the contrary, the activity of suspended bacteria was found to be negligible.

However, it should be noted that the model parameters were obtained in a tropical country with a mean temperature of 30 °C. Hence, the parameter values are applicable only in similar climatic and operational conditions. It is recommended that the proposed model should be modified to incorporate the effect of plant density, nutrient, and dissolved oxygen on the growth of attached biomass. In addition, the value of effective specific surface area of biofilm formation found in this study might have a universal significance. Hence, a further long-term study to validate it as well as the whole model in full-scale FSW CW with different vegetation types, bed media arrangement, climatic and operating conditions, and plant densities is desirable.

Considering the scarcity of conceptually sound approaches of natural treatment systems design, this model may represent a theoretical progress in constructed wetland research.

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自由水面型人工湿地における生物膜モデルの適用

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本研究では自由水面型人工湿地の総合的モデル化を行った．モデル化においては湿地における拡散流れを想定し，有機物除去に対する水相の浮遊生物群および表面に付着した生物膜の寄与を考慮した.動力学的特性値，拡散に関与する特性値，生物膜の厚さや密度などのパラメータは実験的に求めた．その結果，付着生物群が果たす役割の重要性が明らかになった．またパイロットプラントのデータを用いてモデルの検証を行い，既存の押し出し流れモデルと比較して人工湿地の設計に適用が可能であることが示された．