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Modelling faecal *streptococci* mortality in constructed wetlands implanted with *Eichhornia crassipes*

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Abstract Faecal *streptococci* mortality was investigated in a water hyacinth (*Eichhornia crassipes*) constructed wetland pond. The wetland was 7.5 m long, 1.5 m wide and 1.0 m deep, and was implanted with *E. crassipes*. In order to assess the performance of the system towards bacterial mortality, a mathematical model, based on plug flow philosophy was developed. The model incorporated the role of factors, namely solar intensity, pH, dissolved oxygen, temperature, sedimentation, and root attached growth. Model analysis strongly suggests that bacterial mortality rate constant was largely influenced by two factors, namely solar intensity and root biofilm attachment, with both contributing approximately 70.5% of removal. The contribution of other factors like temperature, dissolved oxygen, pH and sedimentation on bacterial mortality rate were less significant. For example, dissolved oxygen, pH and sedimentation contributed 5%, 8% and 0.82%, respectively. Thus, the sedimentation factor was omitted from the model because of its insignificant contribution. The same was done for temperature, due to low ambient temperature range (3.1°C) in the study area. The overall model bacterial removal efficiency was 83%.

Keywords Bacterial mortality · *Eichhornia crassipes* · Faecal *streptococci* · Wetlands

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Introduction

Waste stabilization ponds (WSPs), particularly those modified by implanting aquatic flora, are becoming popular in wastewater treatment, especially in tropical and subtropical regions (Metcalf and Eddy 1995; Senzia et al. 2003; Mayo and Bigambo 2005). This is due to the fact that they have depicted satisfactory efficiency in treating secondary and tertiary effluents (Vymazal 1995; Kayombo et al. 2000). These modifications have actually made WSPs partially function as wetlands (Vymazal et al. 1998; Billore et al. 1998; Mayo and Bigambo 2005). The abundance of solar intensity and ambient temperature in those areas are in most cases reliable.

The capacity of constructed wetland ponds to efficiently treat organic loading is also well documented (Cooper et al. 1996; Vymazal et al. 1998). Because of their ability to provide quality effluents, *E. crassipes* plants have received enormous attention in wastewater treatment systems, and wetlands in particular (Trivedy and Gudekar 1978; Pinto et al.

1987; Kalibbala 2001). However, due to lack of sufficient design criteria, it is still doubtful whether *E. crassipes* wetlands, can consistently meet the present pathogenic effluent standards, since some studies have shown that plants accumulate pathogens around their root zones (Reed et al. 1988; Gersberg et al. 1989).

As early as 1900, pathogens (e.g. coliforms and faecal *streptococci*) were, and are still used as classical indicators for analyzing bacteriological presence and quality in water and wastewater (El-Zanfaly and Shaban 1991; Lahti 1993; Eckner 1998; Ford 1999). These classical indicator bacteria have proved to be useful in studies describing the environmental distribution of wastewater discharges to natural water bodies, and wetland plant attachment enumeration (Edwards et al. 1998; Tryland and Fiksdal 1998).

Although it is not recommended to rely solely on faecal *streptococci* for bacteriological analysis—due to their limited survival in some environments (Morigino et al. 1990; APHA et al. 1992)—the ratio between faecal coliforms and faecal *streptococci* (*FC*/*FS* ratio) is widely used to define the possible source of wetland influent pollution (APHA et al. 1992; Mahasneh 1992). This makes them the most viable species in performing bacterial and food poisoning monitoring (Sinton et al. 1993).

In addition, various tenable hypotheses, explanations and studies have tried to vindicate the causes of bacterial mortality (Polprasert et al. 1983; Mayo and Gondwe 1989; Mayo 1995; Kalibbala 2001). Studies have also revealed that bacterial mortality in WSPs—including those implanted with aquatic plants—depends on environmental and climatological factors (Mara 1974; Vymazal 1995; Vymazal et al. 1998). For that matter, any attempts to develop a comprehensive model should consider them.

These factors include, temperature (Mara 1974), effect of pH (Parhad and Rao 1974), depletion of nutrients i.e., starvation (Gann et al. 1968), biocide excretion (by flora like algae, water hyacinth, etc.) (Gersberg et al. 1989), microbial antagonism (Polprasert et al. 1983), sedimentation (Gannon et al. 1983; Auer and Niehaus 1993), dissolved oxygen (DO) (Curtis et al. 1992), solar radiation (Gameson and Saxon 1967; Calkins et al. 1976; Sierack 1980), adsorption under aerobic conditions (Ohgaki et al. 1986), and biofilm attachment (Swanson and Williamson 1980; Mayo 2004).

A few studies that have attempted to model bacteriological mortality have concentrated on WSPs and subsurface flow gravel beds (Mayo 1995; Mayo 2004). On the other hand, many mathematical models and illustrations have been developed to describe the kinetics of their organic matter degradation (DiToro et al. 1971; Reed et al. 1988; Mayo 1995; Mayo and Mutamba 2004; Mayo and Bigambo 2005). Others have used dispersed flow model concepts (Polprasert and Bhattarai 1985), and kinetics (Polprasert and Khatiwada 1998).

One of the main functions of constructed wetlands (both natural and constructed) is to remove pathogenic organisms, like faecal *streptococci*. However, the above-mentioned factors affecting their mortality have not been exhaustively modeled, especially using the root biofilm attachment contribution, particularly for constructed wetlands implanted with free floating aquatic plants, e.g. *E. crassipes*. Not much endeavour has been done so far to develop a model in order to address this issue.

Therefore, the primary objective of this study is to develop a simple, practical model that depicts the kinetics of *streptococci* mortality in *E. crassipes* wetlands, including the effect of root biofilm attachment. The specific objectives are to determine the main factors influencing bacterial mortality rates in such schemes, and to develop and determine a model parameter governing equation.

Materials and methods

Description of the study site

The water hyacinth (*E. crassipes*) constructed wetland is located at the University of Dar es Salaam premises, Tanzania, at Longitude 39°13′E and Latitude 6°48′S. It is close to mean sea level with a mean monthly temperature range of 28°C–31.1°C (Mayo 1989; Kalibbala 2001). The layout of the wetland system is as stipulated in Fig. 1. It was constructed downstream of the university's primary facultative ponds.

The wetland unit is 7.5 m long by 1.5 m wide by 1.0 m deep, with a freeboard of 0.15 m and bed slope of 1%. It was designed and dimensioned to ensure plug flow behaviour, with a retention time of 10 days, under surface flow hydraulics (Middlebrooks 1995;

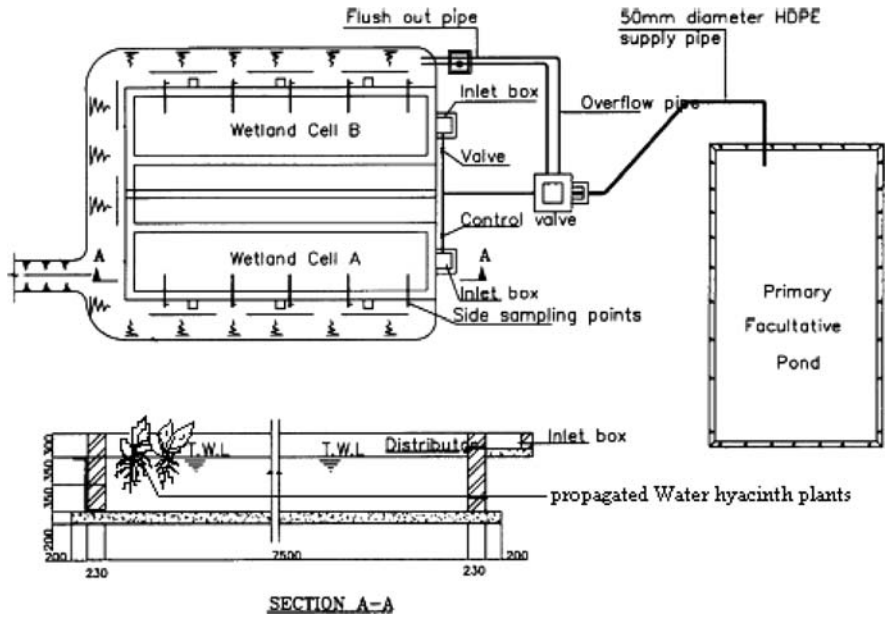

Fig. 1 Layout and cross section of the experimental constructed wetland implanted with *E. crassipes* plants

Cooper et al. 1996). Wastewater was fed into the wetland from the primary facultative pond, treating wastewater of largely domestic characteristics, with a small portion of chemicals from laboratories, health center and workshops. The influent was fed into the scheme at a flow rate of 1 m^3 day^{-1}.

From literature, *E. crassipes* plants are known to germinate, acclimatize to their environment and double the biomass after every 12–20 days (Gopal 1987). Thus, after planting in wetland cell B (see Figure 1), about 16 days were given for acclimatization, i.e., before any sampling, data collection and analysis was done. In addition, during the study period, the wetland surface area covered by *E. crassipes* plants per unit time, was determined with the aid of the quadrant's technique.

Sampling and sample measurements

In order to obtain reliable results from the bacteriological analysis, the standard procedures laid down for sample collection, storage and examination were strictly followed. Grab samples were collected from the influent and effluent of the unit between 9:00 and 10:00 am for every day of sampling, and examination of samples was done immediately. All sampling and analyses were conducted as stipulated in the Standard methods for the examination of water and wastewater (APHA et al. 1992).

Temperature and pH were simultaneously measured in the field using a pH meter (Metrohm, model 704, Britain). Solar intensity data was recorded and collected from a nearby computerized digital solar intensity recorder (Stevens WMS Inc., USA), at the Department of Physics, University of Dar es Salaam. Dissolved oxygen was measured in situ using a DO meter (TOA Electronics Limited, Japan).

The enumeration of faecal *streptococci* closely followed the procedure stipulated by APHA et al. (1992), using KF streptococcal broth base as the culturing medium, coupled with the filter membrane technique. The broth base was prepared according to the manufacturer's specifications (Techno Pharm-Chem, India). The mixture of broth and wastewater samples on the petri dishes was carefully and uniformly spread, to avoid trapping air bubbles (APHA et al. 1992). The cultured petri dishes were then inverted and incubated at 35°C for 48 h. Thereafter, the colonies formed by faecal *streptococci* were counted and recorded as colony–units per 100 ml of wastewater sample.

The mortality rate constants of faecal *streptococci* were calculated based on the plug flow model as depicted in Eq. 1 (Cooper et al. 1996).

$$N_e/N_i = \exp(-k \times t) \quad (1)$$

Where N_e is the effluent bacterial (faecal *streptococci*) density per 100 ml, N_i is the influent bacterial density

per 100 ml, k is the bacterial mortality rate (day^{-1}), and t is the retention time of the wetland system (*days*).

Results and discussion

Model development

Based on first order kinetics and plug flow conditions as assumptions, various researchers have vindicated that plug flow models are more favorable in terms of simplicity, compared to completely mixed flow models (Klock 1971; Sarikaya and Saatci 1987; Mayo 1989; Mogensen and Lundall 1991). Thirumurthi (1969) reported that ponds are seldom completely mixed, but operate near plug flow conditions.

Alvaer (1995) found out that ponds planted with aquatic flora had the same dispersion number compared to those without plants. Using tracer studies, their dispersion numbers were 0.017 and 0.016, respectively. From this, he concluded that floating flora had trivial effect on the hydraulics of ponds, whether designed for plug or completely mixed flow. However, other researchers have used completely mixed flow models (Mara 1974; Nascimento 1987; Polprasert and Khatiwada 1998). In the above regard, and aforementioned reasons, this study adopted the plug flow model.

A model was developed incorporating major mechanisms influencing faecal *streptococci* mortality in the *E. crassipes* wetland. It assumed that the removal of bacteria in the wastewater layer was largely influenced by solar radiation and other environmental factors such as temperature, pH and DO. Also, the contributions of physical factors like root biofilm attachment, and sedimentation were added, their effects were then analyzed. The proposed model is as depicted in Eq. 2.

It is worth mentioning that the effects of bacterial antagonism and biocide production on mortality were assumed to be insignificant. Organic loading rates and retention time were also neglected based on the findings of Mayo and Gondwe (1989) and Mayo (1995). Studies have shown the importance of biofilm in wastewater purification (Reynolds et al. 1975; Polprasert and Agarwalla 1994; Polprasert and Khatiwada 1998). This study aimed at adding and investigating its effect on faecal *streptococci* mortality rates. For purposes of simplicity, the symbols used in all mathematical equations, plus their respective meanings, are presented in Table 1.

Thus, the total mortality rate k (day^{-1}) is given below:

$$k = k_W + k_{fb} + k_{SD} \tag{2}$$

Where,

$$k_W = \frac{k_S(\lambda_t S_O^a)}{KH}(1 - e^{-KH})(1 - l_o) + (k_{pH})(pH) + (k_{DO})(DO) + (k_{20})\theta^{(T-20)} \tag{3}$$

$$k_{fb} = (a_S)_t \frac{\gamma\beta}{\gamma + \beta} \tag{4}$$

$$k_{SD} = \frac{4}{\pi}\eta\alpha\frac{u(1-\theta)}{d_C} \tag{5}$$

Where, all the terms used in the equations are as defined in Table 1. The wastewater mortality term, k_W (day^{-1}) incorporates solar intensity, pH, DO, and temperature. k_{fb} (day^{-1}) accounts for the biofilm mortality rate emanating from faecal *streptococci* root attachment. k_{SD} (day^{-1}) is the mortality rate coefficient due to sedimentation by a cylindrical collector (Bales et al. 1991; Logan et al. 1993). For purposes of simplicity, the roots of *E. crassipes* were assumed to be cylindrically shaped.

Thus, combining Eqs. 3, 4 and 5, the overall proposed model for the prediction of faecal *streptococci* mortality becomes:

$$k = \frac{k_S(\lambda_t S_O^a)}{KH}(1 - e^{-KH})(1 - l_o) + (k_{pH})(pH) + (k_{DO})(DO) + (k_{20})\theta^{(T-20)} + \frac{4}{\pi}\eta\alpha\frac{u(1-\theta)}{d_C} + (a_S)_t\frac{\gamma\beta}{\gamma + \beta} \tag{6}$$

As *E. crassipes* increasingly covered the surface, the exposed wastewater area of the pond to solar intensity decreased. Thus, a solar correction factor λ_t, ranging between $0.0 \leq \lambda_t \leq 1.0$ was introduced as shown in Eq. 6. This unitless factor took into account the ratio of the surface area exposed to solar intensity (i.e. not covered by plants), to the total surface area of the *E. crassipes* wetland system. Logically, λ_t decreased as the plants continued to spread and cover the entire system.

From Eq. 6 above, the surface layer effect coefficient l_o generally varies from 0 to 0.003 (Qin et al. 1991). Therefore, the term $(l - l_o)$, in the solar

Table 1 Explanation and nomenclature of all the symbols used in Fig. 2 and mathematical equations

Nomenclature:

N_i = Influent number of faecal *streptococci* (/100 ml)	N_e = Effluent number of faecal *streptococci* (/100 ml)
y = Bacterial travel distance by diffusion biofilm (m)	π = 22/7 Mathematical constant pi,
t = Hydraulic retention time (days)	S_o = Solar intensity (cal/cm^2/d)
ϕ = Characteristic biofilm parameter	L = Length of the wetland cells (m)
DO = Dissolved Oxygen (mg/l)	W = Width of the wetland cells (m)
T = Water temperature (°C)	H = Depth of the wetland cells (m) i.e. 1.0 m
x = Distance from influent point along L (m),	L_s = Wastewater sublayer thickness (m)
T' = Absolute temperature of pond water (K)	K = Light Attenuation coefficient (1/m)
μ = Viscosity (K) of liquid (N.s/m^2)	μ_{20} = Viscosity of liquid at 20°C (N · s/m^2)
L_f = Biofilm thickness (m)	D_S = Diffusion coefficient of sublayer (m^2/d)
k = Overall mortality rate (d^{-1})	D_f = Diffusion coefficient of biofilm (m^2/d)
k_{DO} = DO mortality rate constant (d^{-1})	k_{fb} = Biofilm mortality rate constant (d^{-1})
k_{pH} = pH mortality rate constant (d^{-1})	k_S = Solar Intensity mortality rate constant (d^{-1})
k_{20} = First order bacterial mortality rate at 20°C (d^{-1})	k_W = Overall mortality rate in wastewater by physical parameters (d^{-1})
k_{SD} = Mortality rate due to Sedimentation (d^{-1})	$(a_S)_t$ = Specific surface area of biofilm per unit control volume at time t (m^2/m^3)
k'_{fb} = First order rate constant for biofilm bacteria (d^{-1})	$(R_S)_t$ = Effective root surface area per unit pond areal surface area at time t (m^3/m^3)
d_P = Diameter of sediment particles (m)	η = Single collector removal efficiency
$\gamma = D_w/L_S$	α = Sticking coefficient
$\beta = \{tanh(\phi)*k'_{fb}*L_f/\phi\}$	u = Flow velocity (m/day)
K = Light Attenuation coefficient (m^{-1})	θ = Porosity of medium (%)
l_o = Surface layer effect coefficient	d_C = Diameter of collector (m)
a = Solar intensity term coefficient	g = Acceleration due to gravity (m/s^2)
ρ_p = Density of particles (kg/m^3)	ρ = Fluid (water) density (kg/m^3)

λ_t = Solar intensity correction factor i.e. the relative area A' of the pond exposed to solar energy at any time t

A' = (Surface area exposed to solar energy & uncovered by plants)/(Total pond surface area)

$0.0 \leq \lambda_t \leq 1.0$, as growth of the water hyacinth increased, the exposed area of the pond to solar energy decreased, therefore λ_t decreased. Therefore, λ_t decreases with time

intensity contribution can be neglected. In addition, for $KH > 4.6$, the term e^{-KH} in Eq. 6 above would be less than 0.01, implying that the solar radiation fraction of k_W would have an error of <1% if e^{-KH} was neglected.

Furthermore, the light attenuation coefficient in WSPs is 7.8–16 m^{-1} (Calkins et al. 1976; Sarikaya and Saatci 1987) and that of wetlands is 7.8–24.0 m^{-1} (Moeller and Calkins 1980, Sarikaya et al. 1987). The typical depth range of facultative and maturation ponds is 1.0 to 1.5 m—for this study, the wetland pond depth was 1.0 m—even under the worst conditions, $KH > 5.4$ (Metcalf and Eddy 1995). Therefore, the term e^{-KH} is negligible. From the above discussion, Eq. 6 can be reduced to Eq. 7 below.

$$k = \frac{k_S(\lambda_t S_O^a)}{KH} + (k_{pH})(pH) + (k_{DO})(DO) + (k_{20})\theta^{(T-20)} + \frac{4}{\pi}\eta\alpha\frac{u(1-\theta)}{d_C} + (a_S)_t\frac{\gamma\beta}{\gamma+\beta} \quad (7)$$

Mara (1974) reported that temperature was one of the main factors contributing to bacterial mortality in wastewater, especially for regions which exhibit relatively large temperature ranges. However, other studies have reported that temperature played a trivial role. In their studies of bacterial mortality, under almost similar temperature ranges, Mitchell and

Chamberlain (1978), and Sierack (1980) found no differences in mortality rates. Moeller and Calkins (1980) also observed no differences in mortality rates, among five sets of temperature ranges.

Similarly, our study checked whether temperature had a significant effect on mortality within the observed temperature range, by plotting mortality rates and temperature data. The resultant regression coefficient (R^2) was 0.07. This poor correlation justified similar observations made in other studies (Auer and Niehaus 1993; Mayo 1995). The unfruitful contribution of the temperature term is most probably attributed to the limited temperature range $28.0^{\circ}\text{C} \leq T \leq 31.1^{\circ}\text{C}$ in the geographical area where this study was conducted. The temperature term was omitted, thus, Eq. 7 was modified to Eq. 8 below.

$$k = \frac{k_S(\lambda_t S_O^a)}{KH} + (k_{pH})(pH) + (k_{DO})(DO) + \frac{4}{\pi}\eta\alpha\frac{u(1-\theta)}{d_C} + (a_S)_t\frac{\gamma\beta}{\gamma+\beta} \quad (8)$$

The value of η was further computed from the expression of Stoke's law as given by O'Melia (1985).

$$\eta = \frac{(\rho_P - \rho)g d_p^2}{18\mu u} \quad (9)$$

Further to the above, the viscosity of water μ was considered a variable, obeying the equation below (Weast 1981). For $0^{\circ}\text{C} \leq T \leq 20^{\circ}\text{C}$ then,

$$\log\mu = \frac{1.301}{998.333 + 8.1855(T-20) + 0.00585(T-20)^2} \quad (10)$$

and for $20^{\circ}\text{C} \leq T \leq 100^{\circ}\text{C}$ then,

$$\log\left(\frac{\mu}{\mu_{20}}\right) = \frac{1.3272(20-T) - 0.001053(T-20)^2}{(T+105)} \quad (11)$$

Where all the parameter symbols are as defined in Table 1, $\mu_{20} = 1.002 \times 10^{-3}$ N.s/m^2 (=1.002×10^{-2}poise). In addition, all values of the parameters in Eqs. 8 and 9 pertaining to the sedimentation term were either sourced from literature ($\rho_P, \rho, g, d_P, \theta$ and α) or were measured as field data (u and d_C), as depicted in Table 2. Assuming horizontal flow, the velocity of wastewater u was calculated from the influent flow rate, Q and effective cross sectional area of the wetland system, A, and was computed from $u = Q/A$.

The mean root hair diameter, d_C of *E. crassipes* in this study was measured and found to be 0.068 ± 0.031 mm. This value compared well with that of Polprasert and Khatiwada (1997), who reported a mean root diameter of 0.075 ± 0.025 mm. Other researchers reported values in the same range, $0.06 \leq d_C \leq 0.30$ mm (Gopal 1987; Reddy 1988; Alvaer 1995). The sticking coefficient α was 0.008, which was also within the range reported in literature (Polprasert and Khatiwada 1997).

Table 2 Parameters incorporated and used in the model

Parameter	Notation	Range	Units	Source
-Diameter of particles	d_P	0.5–40	μm	Metcalf and Eddy (1995)
-Density of water at standard temperature and pressure	ρ	995.7	kgm^{-3}	Weast (1981)
-Density of particles	ρ_P	1050–1500	kgm^{-3}	Metcalf and Eddy (1995)
-Acceleration due to gravity	g	9.81	ms^{-2}	Weast (1981)
-Sticking coefficient	α	0.005–0.03	–	Polprasert and Khatiwada (1998)
-Pond porosity	θ	96.5	%	Kim and Kim (1999)
-Wetland depth	H	1.0	m	From the field
-Diameter of root collectors	d_C	0.068 ± 0.031	cm	From the field
-Flow velocity	u	0.702	mday^{-1}	From the field
-Light attenuation coefficient	K	7.8–25	m^{-1}	Moeller and Calkins (1980), Sarikaya and Saatchi (1987), Polprasert and Khatiwada (1998)
-Light mortality constant	k_S	0.00824–0.011	cm^2 cal^{-1}	Auer and Niehaus (1993); Mayo (1995)

The effect of pH on faecal *streptoccocci* mortality was higher at the beginning of the study when only a portion of the wetland was covered by *E. crassipes*. During that time, algae coexisted with the plants and effluent pH was relatively higher than influent pH, leading to some mortality. Subsequently, algae were phased out in the latter stage because of competition.

Also, the successive growth of *E. crassipes* reduced wind mixing effect, minimized thermal mixing (Kalibbala 2001), caused shading effect against algae growth (Gopal 1987; Vymazal 1995), caused shifts in carbon equilibrium states (Kim and Kim 1999), trapped algae and particulate matter in its roots (Dinges 1982; Kim and Kim 1999). Kim and Kim (1999) observed similar pH trends in their study.

In their study on the effect of pH on the mortality rates of *Escherichia coli*, Parhad and Rao (1974) observed that for pH values around and above 9.0, mortality rates were significant. The pH of the constructed wetland ranged between 6.91 and 9.20. The relatively low contribution of pH towards mortality, in this study, is thus not surprising. Nevertheless, the pH term was included in the model, as shown in Eq. 8.

From Eq. 8, the DO component in *E. crassipes* ponds is influenced by its concentration in the influent and transportation rates in the plants' dense mesh of roots, which is estimated to vary between 0.11 g O_2/kg/h and 3.73 g O_2/kg/h (Moorhead and Reddy 1988). Information in literature depicts conflicting opinions on the effect of DO on bacterial mortality rates. Pearson et al. (1987) and Kalibbala (2001) did not observe the effect of DO on bacterial mortality.

However, Curtis et al. (1992) suggested that in the presence of humic substances, oxygen radicals such as singlet oxygen, hydrogen peroxide and super-oxide were toxic to bacteria. This toxicity was reported to increase proportionally with an increase in DO concentration. From this study, preliminary analyses of field data by using correlation matrices, plus a plot of observed mortality versus DO indicated that the mortality rate of faecal *streptococci* increased slightly with DO concentrations. Therefore, the DO term was justifiably included in the model.

The effect of biofilm attached growth is described by the last term in Eq. (8). The values of the parameters for this term—especially biofilm coefficients—were sourced both from literature (Polprasert and Agarwalla 1994) and field data. Coefficients γ and β depend on liquid sublayer thickness plus sublayer diffusion, and thickness–diffusion effects of the biofilm, respectively. $(a_S)_t$ is the specific biofilm surface area per unit control volume at time t (m^2/m^3). The characteristic biofilm coefficients γ, β and ϕ were computed from Eqs. 12, 13 and 14, respectively. All the parameter symbols in the equations below are defined in Table 1.

$$\gamma = \frac{D_S}{L_S} \tag{12}$$

$$\beta = \tanh(\phi) \times k'_{fb} \times (L_f/\phi) \tag{13}$$

$$\phi = \sqrt{\frac{(k'_{fb} \times L_f^2)}{D_f}} \tag{14}$$

For this study, L_f was 2.5×10^{-4} m which was outside the range of 1.462×10^{-3} m $\leq L_f \leq 1.615 \times 10^{-3}$ m reported by Polprasert and Agarwalla (1994) probably because we conducted it almost immediately after the system started operating. No ample time was given for sufficient biofilm thickness to develop. Williamson and McCarty (1976b) reported that the sublayer thickness consisted of two specific layers namely, L_1 and L_2. The outer liquid sublayer L_1 can be totally omitted with adequate mixing in the pond, the inner layer L_2 being constant, with a thickness of 56 mm.

Also, Rittmann and McCarty (1980) conducted column reactor experiments and found that liquid sublayer thickness, L_S was in the range 1.19×10^{-4} m $\leq L_S \leq 2.26 \times 10^{-4}$ m for superficial flow velocities between 3220 and 43000 mm/day. The flow velocity of the study was 702 mm/day, and in the absence of adequate flow data, the entire range was used during parameter optimization.

Perry and Chilton (1973) and Lamotta (1976) reported a sublayer diffusion coefficient D_S of 5.26×10^{-5} m^2/day. In addition, the ratio of the diffusion coefficient of biofilm D_f to diffusion coefficient of sublayer D_S was reported to be 0.1 $\sim$0.3 for aerobic conditions in biofilms (Pavlostathis and Gilardo 1991), and 0.8 for anaerobic conditions in biofilms (Williamson and McCarty 1976a, 1976b).

Some researchers have adopted D_f to be a percentage of D_S (Rittmann and McCarty 1980; Pavlostathis and Gilardo 1991). Since the entire range between aerobic and anaerobic conditions is $0.1 \leq D_f/D_S \leq 0.8$ and *E. crassipes* ponds are usually facultative in nature, a D_f/D_S ratio of 0.4

was utilized, which is midway between the range. Therefore the diffusion coefficient of biofilm $D_f = 2.30 \times 10^{-5}$. Coefficient γ was optimized and observed to vary in the range 0.233 m/d $\leq \gamma \leq 0.442$ m/d.

For simplicity, $(a_S)_t$ and $(R_S)_t$ were assumed to be proportional to A' and/or λ_t (Eqs. (15) and (16)). Thus, $(R_S)_t$ and $(a_S)_t$ are functions of the pond area covered by *E. crassipes* with time, since the covered pond surface area varied with time. Furthermore, $(a_S)_t$ of a pond without baffles is given by Eq. 17, while that of biofilm attached growth system like water hyacinth is depicted by Eq. 18. From literature, a_S is known to vary in the range 5.76 $m^2/kg \leq a_S \leq 20.83$ m^2/kg for *E. crassipes* (Polprasert and Khatiwada 1997; Kim and Kim 1999). As reiterated earlier, all the symbols are as defined in Table 1.

$$(R_S)_t = R_S(1 - \lambda_t) \tag{15}$$

$$(a_S)_t = a_S(1 - \lambda_t) \tag{16}$$

$$(a_S)_t = (1/H + 2/W + 2/L) \tag{17}$$

$$(a_S)_t = (R_S)_t/H \tag{18}$$

All the data of parameters sourced from the field, plus those from literature (as discussed prior), were optimized by using a FORTRAN program, coupled with the fourth order Runge–Kutta method, which is widely utilized because of its low truncation error and fast convergence, for a given set of initial conditions. As a result, closer investigations gave the following optimized parameters for sedimentation, namely, $d_P = 4 \times 10^{-6}$ m, $0.008 \leq \alpha \leq 0.012$, $d_C = 7.0 \times 10^{-4}$ m and $\rho_P = 1100$ kgm^3. The value of d_P is way smaller than 4×10^{-5} m cited by Metcalf and Eddy (1995). Also, the contribution of sedimentation improved the model efficiency by about 0.82%.

This finding implied that the contribution of sedimentation to mortality, in the wetland pond was insignificant. A model sensitivity analysis done on $d_P(\pm 30\%)$, revealed that the model efficiency was improved by at most 6.6%. This further implied that sedimentation played a significant role only if raw wastewater was fed into the system, assuming that bacteria trapped by suspended particles are removed by settling to the bottom, or by plant roots. *E. crassipes* is known to trap sediment using its dense root mesh (Dinges 1982; Kim and Kim 1999).

It's not surprising that sedimentation was insignificant in this study because wastewater was abstracted from the primary facultative pond, where most of the sediment was removed. Also, a strainer at the intake structure, plus distribution chambers in the system did some additional particle screening to the influent. Consequently, the sedimentation term was omitted, thus, Eq. (8) becomes,

$$k = \frac{k_S(\lambda_t S_O^a)}{KH} + (k_{pH})(pH) + (k_{DO})(DO) + (a_S)_t \frac{\gamma\beta}{\gamma + \beta} \tag{19}$$

After optimization the parameters were $k_{pH} = 0.0015$, $k_{DO} = 0.0044$, $K = 6$ m^{-1}, $k_S/KH = 1.956 \times 10^{-3}$ cm^2cal^{-1}, $k_S = 0.01056$ cm^2 cal^{-1}, $a = 0.83$, $L_f = 2.5 \times 10^{-4}$ m, $k'_{fb} = 40$ d^{-1}, $R_S = 10.4$ m^2 m^{-2}, and biofilm parameter $\gamma\beta/(\gamma + \beta) = 0.0408$ d^{-1}. Since the wetland depth $H = 1.0$ m (with a freeboard of 0.1 m inclusive), the value of k_S above is within the recommended range (0 cm^2 $cal^{-1} \leq k_S \leq 0.011$ cm^2 cal^{-1}) reported in literature (Lantrip 1983). However, the light attenuation coefficient $K = 6$ m^{-1} observed in this study is slightly outside the range for WSPs (7.8–16 m^{-1}) (Calkins et al. 1976; Sarikaya and Saatci 1987) and wetlands (7.8–24.0 m^{-1}) (Sarikaya and Saatci 1987). Substituting all the optimized parameters in Eq. 19, the predicted mortality equation then becomes;

$$k_{pred} = 1.956 \times 10^{-3}(\lambda_t S_O^{0.83}) + 0.0015(pH) + 0.0044(DO) + 0.0414 \times R_S(1 - \lambda_t) \tag{20}$$

Figure 3 depicts the variation of influent and effluent (both observed and predicted) faecal *streptococci* densities for the *E. crassipes* wetland system. It was observed that the values of the observed effluent densities fitted quite well with the predicted ones. With all factors combined, and using the optimized parameters, the overall model efficiency was approximately 0.83. However, in some instances (days 5–12), the model displayed relatively lower predictions of the observed data.

This is attributed to the fact that some small plants namely, *Lemna aequinoctialis Welw* invaded the wetland, yet about 15% of the wetland was covered by *E. crassipes*. They became a nuisance by hindering penetration of solar intensity, which plays a vital

role in faecal *streptococci* mortality (Gameson and Saxon 1967; Calkins et al. 1976; Sierack 1980). The remedial measure was scrapping them off daily by raking, till they became totally extinct. Also, the fast growth of *E. crassipes* aided in out-competing them (Gopal 1987; Reddy 1988).

The faecal *streptococci* density data used in this study—especially that of the influent—are comparable with those reported elsewhere (Power 1991; Sinton et al. 1993). Water quality surveys which include faecal *streptococci* are becoming more common (Power 1991). Some factors have contributed to the increasing interest in these bacterial species. One of the factors is the fact that pollution control bodies worldwide have began considering the use of faecal *streptococci* as a means of assessing the relative contributions of animal and human faecal sources to observe the levels of microbiological contamination in natural water systems (APHA et al. 1992; Mahasneh 1992; Sinton et al. 1993).

Model application

In order to utilize the proposed mortality model—for a similar constructed wetland pond designed to treat wastewater—DO, pH, solar intensity (S_O), and the effective root surface area per unit pond areal surface area (R_S), ought to be measured. Fortunately, they can be measured or estimated by using simple, relatively cheap and available equipment and/or methods. For instance, R_S can be estimated by quantifying the relative surface area covered by *E. crassipes* on water, at a given point in time.

Thus, based on the desired effluent quality standards (WHO 1982; Metcalf and Eddy 1995), which in turn determine the retention time t (Cooper et al. 1996), the measured field data above can then be substituted into Eqs. 1 and 20 to obtain Eq. 22. Knowing the influent density of faecal *streptococci* N_i, the desired effluent bacterial density N_e, can be readily determined.

$$N_e/N_i = \exp\left(-k_{pred} \times t\right) \tag{21}$$

$$N_e = N_i \exp(-[1.956 \times 10^{-3}(\lambda_t S_O^{0.83}) + 0.0015(pH) + 0.0044(DO) + 0.0414 \times R_S(1-\lambda_t)] \times t) \tag{22}$$

It is worth mentioning that further studies are probably necessary to investigate the role of temperature on faecal *streptococci* mortality, coupled with the effect of *E. crassipes* under similar settings—especially in temperate climate—where high ambient temperature ranges are expected. Some studies have reiterated the role of temperature in temperate zones (Mara 1974; Metcalf and Eddy 1995). As discussed prior, the study found that the temperature factor played no role, probably due to low ambient temperature range (3.1°C) in the study area.

Therefore, for temperate climates, the proposed model might not suitably predict mortality, because high temperature fluctuations are expected to come into play. In addition, since the model was not sufficiently verified using data elsewhere (from other

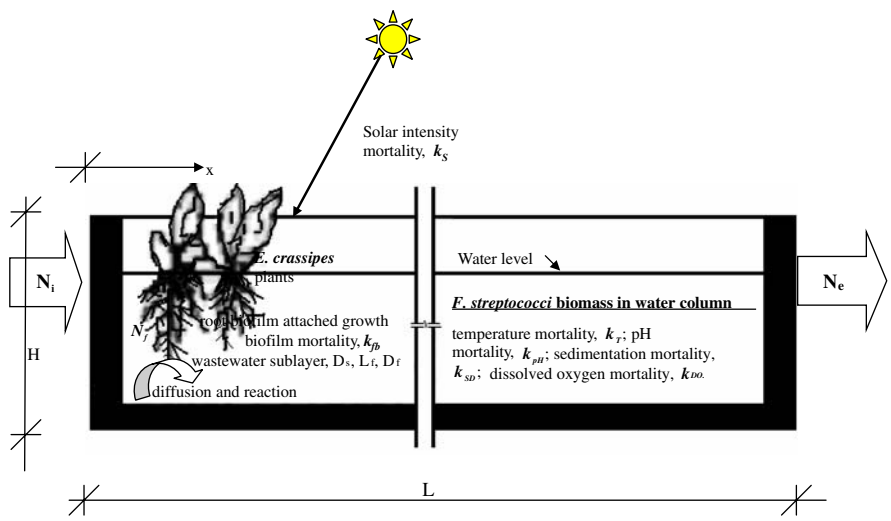

Fig. 2 Conceptual model showing the processes influencing faecal *streptococci* mortality in the *E. crassipes* constructed wetland. All symbols and abbreviations herein are defined in Table 1

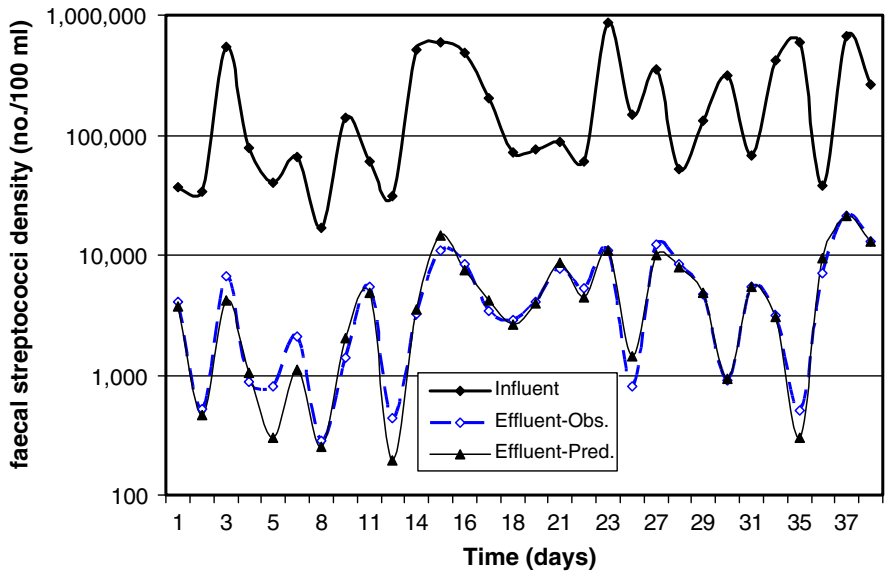

Fig. 3 Influent, observed effluent and model predicted effluent densities versus time for faecal *streptococci* bacteria. The vertical axis is in logarithmic scale

geographical locations due to lack of data in literature), it's probably necessary to do so in future when data becomes readily available. Consequently, this is a major shortcoming of the study.

It's known that data on the mortality and survival of faecal *streptococci* in the natural environment and groundwater are rare but crucial (McFeters et al. 1974; Sinton et al. 1993), the authors hope that this study provides a positive contribution, since this species is widely used as a pollution and food poisoning indicator (Sinton et al. 1993).

Conclusion

From this study, a simple, practical mathematical model (governing equation) for faecal *streptococci* mortality was developed. The role of factors namely, solar intensity, pH, DO, temperature, sedimentation, and root biofilm attachment, on faecal *streptococci* mortality was investigated. Model analysis strongly suggested that mortality rate k (day^{-1}) was mainly influenced by two factors i.e., solar intensity and root biofilm attachment, with both contributing a larger percentage of removal.

Other factors like temperature, DO, pH and sedimentation were less significant. For example, DO, pH and sedimentation contributed 5%, 8% and 0.82%, respectively. Therefore, the sedimentation term was omitted from the model because of its insignificant contribution. The same was done for temperature, due to the low ambient temperature range (3.1°C) in the study area.

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