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K e y w o r d s
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A B S T R A C T

In this study, a mathematical biofilm reactor model based on the structure of the Constructed Wetland Model No.1 (CWM1) coupled to AQUASIM's biofilm reactor compartment has been used to reproduce the sequence of transformation and degradation of organic matter, nitrogen and sulphur observed in a set of constructed wetland mesocosms and to elucidate the development over time of microbial species as well as the biofilm thickness of a multispecies bacterial biofilm in a subsurface constructed wetland. Exper-imental data from 16 wetland mesocosms operated under greenhouse conditions, planted with three different plant species (Typha latifolia, Carex rostrata, Schoenoplectus acutus) and an unplanted control were used in the calibration of this mechanistic model. Within the mesocosms, a thin (predominantly anaerobic) biofilm was simulated with an initial thickness of 49 mm (average) and in which no con-centration gradients developed. The biofilm density and area, and the distribution of the microbial species within the biofilm were evaluated to be the most sensitive biofilm properties; while the substrate diffusion limitations were not significantly sensitive to influence the bulk volume concentrations. The simulated biofilm density ranging between 105,000 and 153,000 gCOD/m^3 in the mesocosms was observed to vary with temperature, the presence as well as the species of macrophyte. The biofilm modeling was found to be a better tool than the suspended bacterial modeling approach to show the influence of the rhizosphere configuration on the performance of the constructed wetlands.

Introduction

Subsurface flow constructed wetlands (SSF-CWs) are finding extensive application for domestic and municipal wastewater treatment (Haberl, 1999; Neralla et al., 2000; Vymazal, 2010; Mburu et al., 2013b; Ranieri et al., 2013) because of their simple and robust configuration together with low energy requirements and operating cost. SSF-CWs are generally constructed with a porous material (e.g. soil, sand, or gravel) as a substrate for growth of rooted wetland plants in addition to various microbes. The mi-croorganisms and their extracellular products adhere to the solid support provided by the porous media and plant roots, forming a biofilm layer in which the contaminant compounds disperse and are degraded by the microorganisms (Wichern et al., 2008; Kadlec and Wallace, 2009).

Thus, the organic content in the wastewater is reduced by biological degradation rather than by simple screening (Krasnits et al., 2009). Aerobic respiration, denitrification, sulphate reduction and methanogenesis are the principal biochemical re-actions involved in the oxidation and net removal of organic matter in subsurface flow constructed wetland systems (Baptista et al., 2003; Caselles-Osorio et al., 2007; Langergraber et al., 2009).

Despite there is a recognition that the improvement of water quality in treatment wetland applications is primarily due to mi-crobial activity (Faulwetter et al., 2009; Kadlec and Wallace, 2009), the mechanistic understanding of the dynamics of microbial bio-film biomass, activity, and community composition in constructed wetlands is still evolving, albeit significantly in the past couple of years (Faulwetter et al., 2009; Truu et al., 2009; Samsó and Garcia, 2013). These aspects have been completely overlooked in tradi-tional wetland models using reaction rate constants (Rousseau et al., 2004). Only recently constructed wetland mechanistic modeling started to incorporate these aspects (Langergraber, 2001; Langergraber and Šimůnek, 2005; Rousseau, 2005). However,

barely a few of the mechanistic models consider multispecies bacterial biofilms (McBride and Tanner, 1999; Langergraber and Šimůnek, 2005; Mayo and Bigambo, 2005; Langergraber and Šimůnek, 2012; Samsó and Garcia, 2013), but rather are formulated with "suspended cells" (i.e. bacterial community without substratum) under batch or continuous flow modes (Wynn and Liehr, 2001; Mayo and Bigambo, 2005; Rousseau, 2005; Llorens et al., 2011b; Mburu et al., 2012). Further, there are variations in the modeling of microbial reactions including type and number of bacterial populations considered and kinetics of growth and processes affecting the bacterial-biofilm growth (Kumar and Zhao, 2011) due to the complex nature of constructed wetland systems. In this context, the biokinetic Constructed Wetland Model number 1 (CWM1) (Langergraber et al., 2009) is seen as the most advanced theoretical biokinetic model developed for SSF-CWs.

A biofilm modeling approach has been used here to reproduce the sequence of transformation and degradation of organic matter, nitrogen and sulphur observed in a set of constructed wetland mesocosms and to elucidate the biofilm growth dynamics in a multispecies bacterial biofilm. The growth of six microbial groups (heterotrophic, autotrophic nitrifying, fermenting, acetotrophic methanogenic, acetotrophic sulphate reducing and the sulphide oxidising bacteria) and the subsequent consumption of electron donors and acceptors in 16 batch operated subsurface flow wetland mesocosms operated under controlled greenhouse conditions with three different plant species (*Typha latifolia*, *Carex rostrata*, *Schoenoplectus acutus*) and an unplanted control is simulated. The processes occurring in the biofilms attached to the gravel and plant roots in the mesocosms are simulated in the one-dimensional (1-D) mathematical biofilm model of the simulator AQUASIM (Reichert, 1998), a programme for identification and simulation of aquatic systems. For the rate equations and kinetics of the microbiological processes, the Constructed Wetland Model No.1 (CWM1) biokinetic model as described in Langergraber et al. (2009) is used. The results are compared and contrasted with those in the work of Mburu et al. (2012) in which a non-biofilm (i.e. suspended) multipopulation bacterial growth and uptake approach together with plant related processes (growth, physical degradation, decay, and oxygen leaching), physical re-aeration, as well as adsorption and desorption processes for COD and ammonium were applied in the simulation of the 16 mesocosms to describe the transformation and degradation processes of organic matter, nitrogen and sulphur (Mburu et al., 2012). Thus although biofilm modeling may represent a theoretical improvement over the "suspended bacteria" approach in constructed wetland modeling, a direct comparison of the two modeling approaches is important to determine if the biofilm modeling approach yields new qualitative information, specifically on the possible influence of the boundary conditions of temperature and macrophyte species on the prediction of substrate removal in constructed wetlands.

2. Methodology

2.1. The experimental constructed wetlands

The column experimental constructed wetlands were operated under controlled greenhouse conditions at Montana State University in Bozeman (Montana, USA). Details of column design, construction and planting, as well as sampling and measurement are described in Allen et al. (2002) and Stein et al. (2006). Briefly, 16 subsurface constructed wetland mesocosms were constructed from polyvinyl chloride (PVC) pipes (60 cm in height × 20 cm in diameter) and filled to a depth of 50 cm with washed pea-gravel (0.3–1.3 cm in diameter). Four columns each were planted with *C. rostrata* (Northwest Territory sedge), *Schoenoplectus acutus* (hardstem bulrush) and *T. latifolia* (broadleaf cattail), while four were left unplanted as controls. A series of 3-6-9-20-d incubations with synthetic wastewater was conducted over 20 months at temperatures ranging from 12 to 24 °C at 4 °C steps. A synthetic wastewater simulating secondary domestic effluent was used with mean influent concentrations of 470 mg/l COD, 44 mg/l N (27 Org-N, 17 NH_4^+–N), 8 mg/l PO_4^{3-}–P, and 14 mg/l SO_4^{2-}–S. Columns were gravity drained 3 days prior to each incubation and then again at the start of each incubation. Upon each emptying, columns were refilled from above with new wastewater. Sampling from all 16 columns occurred at days 0, 1, 3, 6, 9, 14 and 20 of each incubation and those sub-samples were analyzed afterwards for the constituents.

2.2. Model description and implementation

The constructed wetland mesocosm is mathematically described as a reactor with completely mixed bulk water volume and with a biofilm growing on a substratum (gravel media and plant roots) surface inside the reactor. The mesocosm was implemented into the biofilm compartment of the AQUASIM 2.1d (win/mfc) software (Reichert, 1998). The biofilm reactor compartment in AQUASIM enables the simulation of biofilm systems with several microbial species and substrates. It describes the spatial distribution and development in time of dissolved and particulate components in the biofilm, as well as the development in time of the biofilm thickness. The AQUASIM user manual (Reichert, 1998) details the mathematical formulation executed by the software to determine the biofilm thickness. The biofilm is divided into a liquid phase consisting of water (80%) in which the dissolved substances are transported by diffusion and a solid matrix (20%) consisting of particulate components such as active and inactive bacteria and their extracellular polymeric substances (EPS). The compartment was configured with the following aspects: (a) "Unconfined" reactor (i.e. volume of bulk liquid was assumed constant and did not change with biofilm thickness, whereas the biofilm can grow freely as may be the case in a trickling filter (Wanner and Morgenroth, 2004)), (b) a rigid structure (there is no diffusive mass transport of solids, i.e. the biofilm matrix can change its volume due to microbial growth and decay only), (c) no suspended solids in the pore volume (no particle transport through the biofilm), and (d) no surface or volume attachment or detachment (there is no exchange of particulate components between the biofilm solid matrix and the bulk liquid). Diffusivities were taken from Boltz et al. (2011), considering that the diffusivity of a solute inside the biofilm is generally lower than that in water because of the tortuosity of the pores and minimal biofilm permeability.

The transformation and degradation processes were defined based on the biokinetic model CWM1. The values of the kinetic and stoichiometric parameters required by the model are available in Langergraber et al. (2009), with the exception of those that were modified in Mburu et al. (2012). Dynamic processes were used for the growth and decay rate of all bacterial groups as included in the CWM1 model. Because of the serial execution of the equation of the heterotrophic bacteria processes as described in CWM1, growth failed when switching between readily biodegradable COD and the fermentation product acetate, both under aerobic and anoxic conditions. The implementation of these process rates as described in Langergraber et al. (2009) was thus not possible. Instead, the implementation was carried out as described in the work of Llorens et al. (2011a, b), where the solution was to divide the heterotrophic bacteria group into two subgroups according to the substrate they consume.

The biofilm is modeled as a film growing on the spherical surfaces of the gravel media inside the mesocosm and assumed to be an ideal biofilm of uniform thickness (L_F) and density (*rho*), with the diffusion and kinetic coefficients assumed to be constant

throughout the wetland unit. The equations to calculate the biofilm growth area on the washed pea gravel followed the approach of Wichern et al. (2008). In an idealized way, the washed gravel is approximated by spheres which all have the same diameter and which can touch each other at up to eight points. Where the spheres touch, biomass growth on the surface of the spheres is not possible. Thus, it is possible to determine the loss of biofilm surface area (A_{loss}) between two pieces of gravel (considered as spheres) in relation to the radius (r) of the single sphere and the thickness of the biofilm (L_F) with the equation:

$$A_{loss} = B\pi L_F(2r + 2L_F) \quad \left[m^2\right] \tag{1}$$

where B represents the number of contact points per sphere. The number of pieces of gravel (N) in the mesocosms volume (V) is estimated depending on the porosity ε as:

$$N = \frac{(1-\varepsilon)V}{\frac{\pi}{6}(2r)^3} \quad [-] \tag{2}$$

The remaining surface area of the biofilm $A_{remaining}$ in relation to the number of contact points, the diameter of the spheres, and the biofilm thickness amounts to:

$$A_{remaining} = N\pi(2r)^2 - NA_{loss}\left[m^2\right] \tag{3}$$

Reduction in concentration of any substrate is modeled as a mass transfer or boundary layer mass transfer resistance, R_L ($=L_L/D$), which depends on the diffusivity (D) of a substrate inside the biofilm and an accurate estimate of the mass transfer liquid layer of thickness L_L. Very low diffusion coefficients will lead to high boundary layer resistance and the model will not yield a converging solution. The diffusion coefficients of the components in the biofilm were taken as their "effective" values approximated as 0.8 times their diffusion coefficient through pure water (Boltz et al., 2011). Temperature dependency of diffusion coefficients was accounted for according to:

$$D(T) = D(20^{\circ}C)\cdot\frac{273+T}{273+20^{\circ}C}\cdot\frac{\mu(20^{\circ}C)}{\mu(T)}\left[m^2d^{-1}\right] \tag{4}$$

where D is the diffusion coefficient, T the temperature in °C, and μ the dynamic viscosity of water in N m^{-2}s (Boltz et al., 2011).

Four plant processes (growth, physical degradation, decay, and oxygen leaching), physical re-aeration, as well as adsorption and desorption processes for COD and ammonium nitrogen were also included as dynamic processes following the work of Mburu et al. (2012).

To run the model, 16 inputs characterizing the influent (Oxygen, COD fractions, N compounds, and S compounds), and one input for water temperature are required. Other inputs concerning initial data i.e. the biofilm (density, thickness, area and volume fractions), the boundary liquid layer thickness, the reactor volume and the diffusion coefficients of 13 substrate is necessary. Fractionation of the influent wastewater COD was based on standard ratios given in the ASM models (Henze et al., 2000) and implemented following the work of Mburu et al. (2012).

2.3. Sensitivity analysis

A sensitivity analysis was carried out to recognize the most important parameters influencing the prediction of carbon, nitrogen and sulphur concentrations and the development of the biofilm. With the "sensitivity analysis" function in AQUASIM, it is possible to investigate whether the time series of the calculated values are affected noticeably by a change in the value of a model parameter. The sensitivity analysis feature enables calculation of linear sensitivity functions of arbitrary variables with respect to each of the parameters included in the analysis (Reichert, 1995). The sensitivity analysis results described in this study are those of the absolute-relative sensitivity function of AQUASIM (Eq. (5)) that computes the absolute change in a model output variable, y, for a 100% change in any parameter of interest, p:

$$\delta_{y,p}^{a,r} = p\frac{\partial y}{\partial p} \tag{5}$$

This makes quantitative comparisons of the different parameters on a common variable possible.

The uncertainty is determined by using the error propagation formula (Eq. (6)), which is based on the linearized propagation of standard deviations of the parameters of interest, neglecting their correlation:

$$\sigma_y = \sqrt{\sum_{i=1}^{m}\left(\frac{\partial y}{\partial p}\right)^2\sigma_{pi}^2} \tag{6}$$

Where p_i are the uncertain model parameters, σ_{p_i} their standard deviations, $y(p_i.....p_m)$ the solution of the model equations for a given variable at a given location and time, and σ_y is the approximate standard deviation of the model result. Identifiability of the model parameters was evaluated by use of the parameter correlation matrix in AQUASIM.

2.4. Model calibration and simulations

Data based on bulk measurements of COD, NH_4^+–N and SO_4^{2-}–S at 12 °C, 16 °C, 20 °C and 24 °C from the unplanted (control) mesocosms were used for optimization of the microbial biokinetic parameters of CWM1 and the calibration of the biofilm parameters. The physical re-aeration coefficient, initial amount of sorbed ammonia and the COD adsorption parameters were adopted as determined in the work of Mburu et al. (2012). To optimize the parameter sets, the result of the sensitivity analysis was used to guide the selection and calibration of the kinetic coefficients and the biofilm parameters with the "parameter estimation" function of AQUASIM. The function attempts to determine unknown values of model parameters by iteratively best-fit matching time-series of calculated and measured values. The simulations were conducted with data from planted mesocosm, at 12 °C, 16 °C, 20 °C and 24 °C.

3. Results

3.1. Sensitivity and identifiability analysis

Prior to calibration of the biofilm parameters, the parametric sensitivity of the dynamic model was conducted in AQUASIM (Table 1). The importance of the constructed wetland biofilm

Table 1
Sensitivity of the biofilm parameters on the bulk liquid concentrations in the constructed wetland mesocosms.

	Rho	D	LL	LF_{ini}	A	eps_X
COD	+++	−	++	++	+++	++
NH_4^+–N	+++	−	+	++	++	++
SO_4^{2-}–S	+++	−	+	++	++	++

(−) insignificant effect; (+) moderate effect; (++) significant effect; (+++) strong effect.
(rho: biofilm density, D: substrate diffusivity, LL: liquid layer thickness, LF_{ini}: initial biofilm thickness, A: area of biofilm, eps_X: biomass volume fraction).

structure was reflected in the dependence of the state variables on the biofilm density and area. Table 1 shows the sensitivities of the main bulk liquid concentrations (i.e. COD, NH_4^+—N and SO_4^{2-}—S) on biofilm characteristics. Here, "strong effect", "significant", "moderate" and "insignificant" indicate SF $\geq$ 1, 1 > SF $\geq$ 0.1, 0.1 > SF $\geq$ 0.01 and SF < 0.01, respectively, where SF is the absolute-relative sensitivity function (unit of g-COD/m^3, g-N/m^3 or g-S/m^3).

The correlation matrix for the biofilm parameters (Table 2) shows the biofilm density and the biofilm area to be strongly correlated and hence not simultaneously theoretically identifiable from the measured data (Petersen et al., 2001). This suggests that other factors (e.g. diffusivity or mass transport limitation within the biofilm) may have influenced parameter identifiability, especially for parameters that are otherwise uncorrelated or with a low linear dependency (Brockmann et al., 2008).

3.2. Biofilm properties

3.2.1. Simulated volume fractions and the activity of microbial species

The simulated volume fractions of the microbial species within the biofilm at different incubation temperatures are presented in Table 3. The fractions represent the interactions within the biofilm depth among the bacteria species involved and their competition for existence within the biofilm as a function of the substrate flux. The simulated volume fractions were not varying significantly with temperature, while the sulphide oxidising bacteria were not growing in the biofilm, probably due to oxygen and nitrate limitation during the biofilm development.

3.2.2. Physical and geometric parameters

The characterization of the physical and geometrical parameters of the biofilm in the control and the planted mesocosms is presented in Tables 4 and 5, respectively. The calibrated values for initial biofilm thickness (LF_{ini}), biofilm density (*rho*), liquid boundary layer (*LL*) and the estimated biofilm area (*A*) were obtained by fitting the model to the experimental measurements of the bulk liquid concentrations of the wetland mesocosms set-ups observed at the different incubation temperatures.

The initial biofilm thickness (average 49 μm) was model predicted to shrink with time during the batch simulations without reaching a steady state under the experimental conditions. It is clear that the progressive decrease in biofilm thickness follows a trend of diminishing substrate availability under the batch loading conditions. The thickness of the biofilm is governed by the flux of substrate to the biomass, as well as the growth and decay of the microorganisms in the model. The attachment and detachment of cells at the biofilm surface and inside the biofilm was not considered in this work.

3.3. Bulk volume simulations

Model predictions showed a good qualitative agreement with the measured bulk volume concentrations of COD, NH_4^+—N and SO_4^{2-}—S (Figs. 1–3). However, discrepancies exist between measured and simulated NH_4^+—N and SO_4^{2-}—S concentrations. The simulations of NH_4^+—N and SO_4^{2-}—S conversion/consumption show an offset (both over-estimation and under-estimation) in the planted mesocosms. These discrepancies between the measured and simulated values might be due to the fact that the mean density of the biofilm in the model is estimated rather than experimentally determined. Ideally, the mean biofilm density is known and is considered as an input parameter. Alternatively, omitting the influence of plant roots on the biofilm development represents an oversimplification (of the mathematical model) in attempting to determine biofilm model parameters based on experimental data. This is possibly the case, as the obtained planted-unplanted biofilm density ratio was found to range between 7 and 13 as described in Munch et al. (2005). Further, the non-development of the sulphide oxidising bacteria in the biofilm (Table 3) caused the poor fit for the SO_4^{2-}—S profiles.

Table 2
Correlation matrix during biofilm parameter estimation in AQUASIM.

	A	LF_{ini}	*LL*	rho
A	1			
LF_{ini}	−7.0E-06	1		
LL	−7.9E-06	−4.5E-07	1	
rho	1	1.7E-01	0.46	1

Table 3
Simulated volume fractions of bacterial functional groups of CWM1 in the control mesocosms.

Temp °C	Bacterial functional group					
	*X*_FB	*X*_ASRB	*X*_AMB	*X*_A	*X*_H	*X*_SOB
12	0.026	0.047	0.057	0.002	0.047	0.000
16	0.029	0.047	0.061	0.005	0.038	0.000
20	0.029	0.047	0.061	0.003	0.039	0.000
24	0.029	0.048	0.062	0.005	0.037	0.000

*X*_FB: Fermenting bacteria; *X*_ASRB: Acetotrophic sulphate reducing bacteria; *X*_AMB: Acetotrophic methanogenic bacteria; *X*_A: Autotrophic nitrifying bacteria; *X*_H: Heterotrophic bacteria; *X*_SOB: Sulphide oxidising bacteria.

Table 4
Calibrated values of the initial biofilm thickness (LF_{ini}), *density* (rho), liquid layer (*LL*) and area (*A*) in the control mesocosms.

Temp °C	Parameter			
	LF_{ini} (m)	rho (g/m^3)	*LL* (m)	*A* (m^2)
12	4.74E-05	134,025	6.24E-05	8.42
16	3.84E-05	132,655	6.29E-05	8.41
20	6.33E-05	105,190	5.93E-05	8.4
24	4.74E-05	108,512	3.40E-05	8.43

4. Discussion

CWM1 has been implemented in different simulation platforms (Llorens et al., 2011a; Langergraber and Šimůnek, 2012; Mburu et al., 2012; 2013a; Samsó and Garcia, 2013) and the resulting codes have been used to match experimentally measured effluent pollutant concentrations. Although these models provide insights

Table 5
Calibrated values of the initial biofilm thickness, density (rho), and area (*A*) in the planted mesocosms.

Set-up	Temp °C	Parameter	
		rho (g/m^3)	*A* (m^2)
Carex	12	152,519	8.55
	16	139,877	8.58
	20	143,015	8.57
	24	127,684	8.57
Schoenoplectus	12	150,290	8.57
	16	143,926	8.58
	20	144,135	8.58
	24	143,189	8.58
Typha	12	148,266	8.57
	16	145,365	8.55
	20	137,446	8.55
	24	130,818	8.57

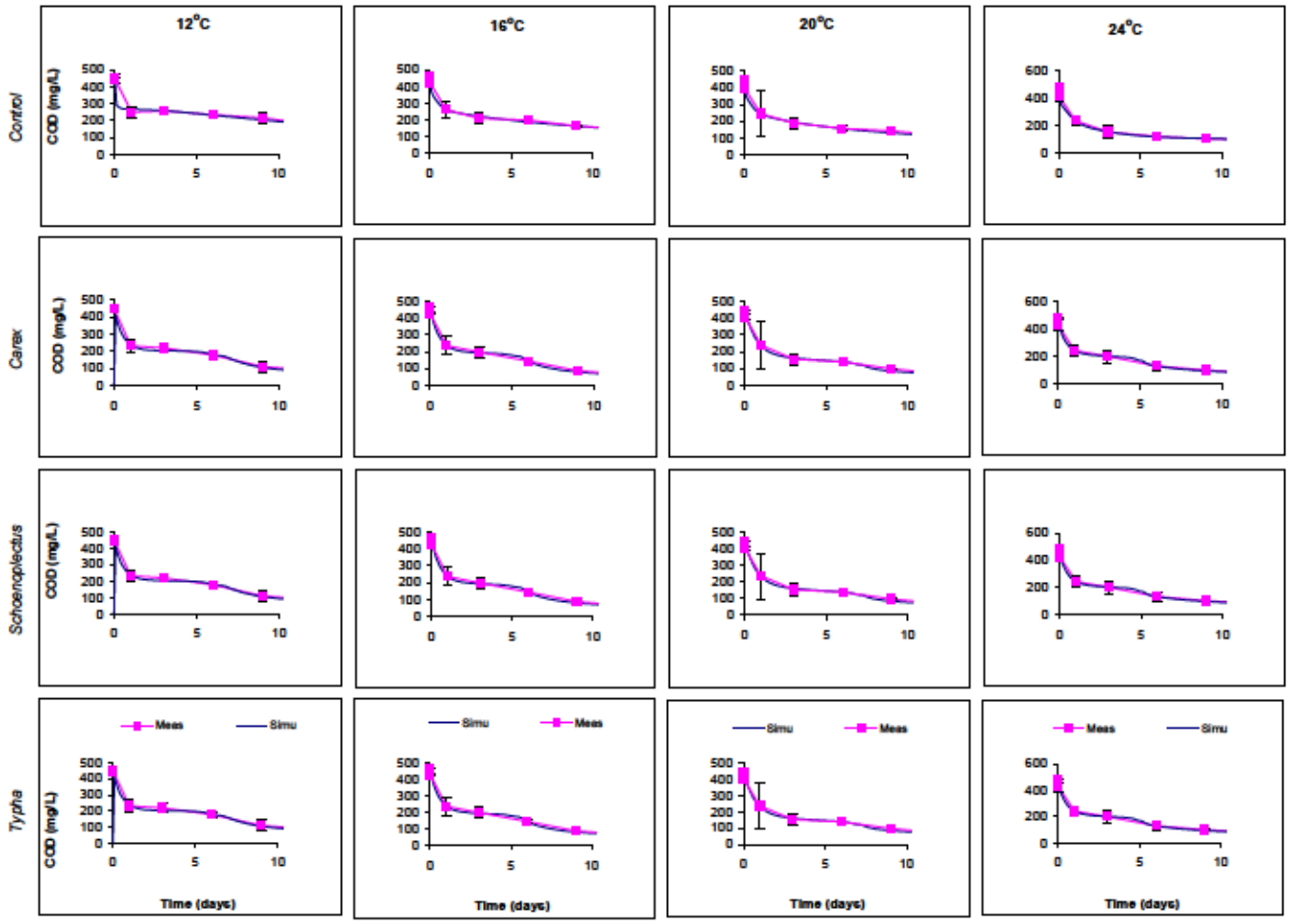

Fig. 1. Simulated compared with measured COD concentrations in the constructed wetland mesocosms. Symbols are means (±S.D.) of observed concentrations from four replicates for each treatment.

into the behavior of the SSF constructed wetland, they neglect certain potentially important phenomena influencing microbial reactions, such as diffusion limitation or the stratification of metabolic processes in the biofilm when several populations of bacteria are present, or the possible influence of macrophyte type on the development of biofilm biomass (Gagnon et al., 2007; Zhang et al., 2010). Knowledge on the growth dynamics of bacterial biofilms is essential for the design conceptualization of treatment processes in constructed wetlands. Compared with reactors with suspended bacteria, fixed biofilm bacteria reactors can be operated at high biomass concentrations in the reactor. This implies that biofilm units often require less land area than suspended bacteria units (Wik, 1999). Thus, the attached bacteria biofilm modeling approach should give better system insights to help to improve the performance of constructed wetlands by providing a scientific basis to find the optimal design and operating conditions of constructed wetlands systems (Rousseau, 2005).

4.1. Biofilm versus suspended bacterial growth modeling approach for simulating constructed wetland performance

The bacterial biofilm modeling approach is more complex than the suspended bacterial approach presented in Mburu et al. (2012), because the reactor mass balance is coupled with a diffusion reaction equation for the substrate in the biofilm. The biofilm model must account for the spatial aspects of biofilms, most notably the distribution of bacteria and substrates across the depth of a biofilm (Reichert, 1998).

Substrate gradients in the biofilm as a consequence of diffusion and reaction were not observed. The growth of bacterial biomass through substrate consumption in the biofilm is essentially under the same conditions or behavior as in the suspended bacterial model. Nevertheless, the evaluated bacterial volume fractions (Table 3), biofilm density and area (Table 4) show it was possible to evaluate more clearly how the individual functional groups developed in the biofilm of the constructed wetland. For example, the reason for the better performance of the planted mesocosm (with respect to substrate removal) compared to the unplanted mesocosm, is simulated as an increased colonizable surface area on which the biofilms can grow, together with the concomitant increase in biofilm density (due to incorporation of the plant). This is not obvious from the suspended bacteria model in Mburu et al. (2012), where these individual components of bacterial volume fractions, biofilm area and density are all lumped together as microbial concentration. Further, the biofilm density among the planted mesocosms is varying rather more significantly than the area of the biofilm, suggesting that the biofilm density (which is in qualitative agreement with the bacterial concentrations of the suspended biomass model) was the more significant factor influencing the performance of the wetlands. Hence, the dependence of

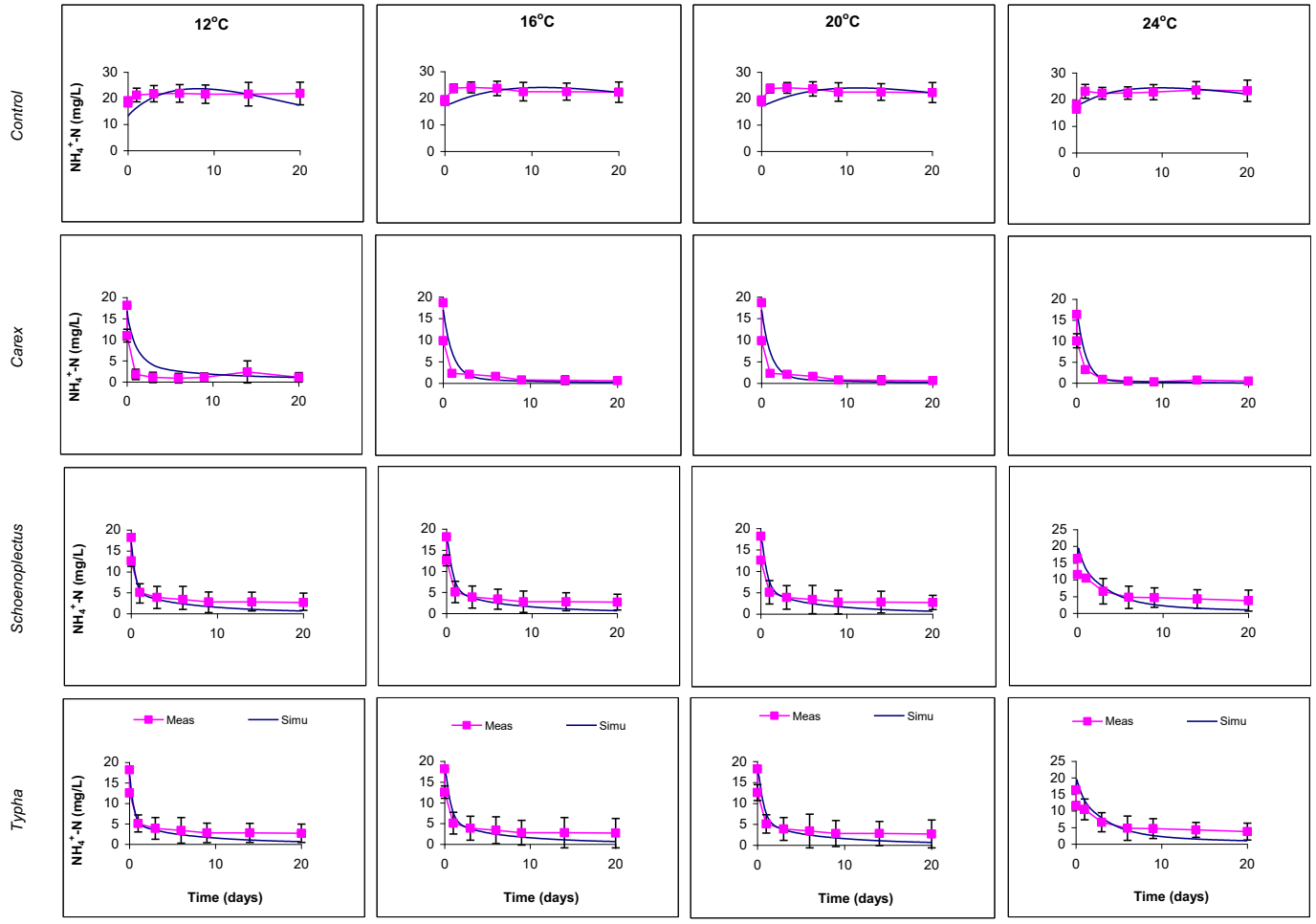

Fig. 2. Simulated compared with measured NH_4^+–N concentrations in the constructed wetland mesocosms. Symbols are means (±S.D.) of observed concentrations from four replicates for each treatment.

the substrate removal on the rhizosphere configuration, i.e. the extra microbial attachment surface area and the potential of enhanced biofilm density provided by different macrophyte species strongly determines the constructed wetland performance.

4.2. Influence of macrophyte species and temperature on the biofilm density

Biofilm density and thickness are the main design parameters used to evaluate the substrate consumption rate in biofilms (Vanhooren, 2002). In this study, the biofilm density was observed to vary with the presence and species of macrophyte as well as the temperature. Planted mesocosms were found to develop a higher density biofilm compared with the unplanted mesocosms. The presence of plants enhanced the microbial density and activity in experimental microcosm studies as has been reported by Gagnon et al. (2007) with results showing a bacterial density ratio of 10.3 between planted and unplanted microcosms. The ratio obtained from the simulations is, however, much lower, suggesting the simplifying assumptions made in the mathematical formulation of the biofilm with regard to microorganisms–plant interactions may cause some disagreements between the model output and field observations of the planted versus unplanted wetlands. For example, the estimation of the biofilm area (Tables 2 and 3) is based on the surface area provided by the gravel (equation (3)), ignoring the effect of plant root development. It is generally assumed that planted wetlands outperform unplanted controls, mainly because the plant rhizosphere stimulates microbial communities either through high carbon availability in the rhizosphere resulting of root exudates or extra attachment sites of the root surface correlated to plant species root morphology and development (Munch et al., 2005; Gagnon et al., 2007).

Biofilms are known to vary in their density (Lazarova and Manem, 1995). Biofilms with densities from 10 to 130 kg dry mass/m^3 wet volume have been reported in different aquatic systems including aquifers and wastewater treatment systems (Zysset et al., 1994; McBride and Tanner, 1999; Vanhooren, 2002; Melo, 2005). The simulated biofilm densities lie in this range, considering bacterial concentrations may be converted from COD units to DM units by using the conversion factor of 1.222 g COD (g biomass)$^{-1}$, as proposed in Rousseau (2005). There are many factors that could be responsible for the variation in biofilm density, such as culture morphology, i.e. changes in species, and amount of inactive material, and changes in biofilm porosity or lysis (Şeker et al., 1995; Wik, 1999). The simulated biofilm density variation with respect to temperature in this study (Table 5) is in agreement with the results of Honda and Matsumoto (1983), who observed the growth capacity of a microbial film in a model trickling filter to increase as temperature fell. This is due to the autolysis coefficient which becomes lower at low temperatures (Honda and Matsumoto 1983).

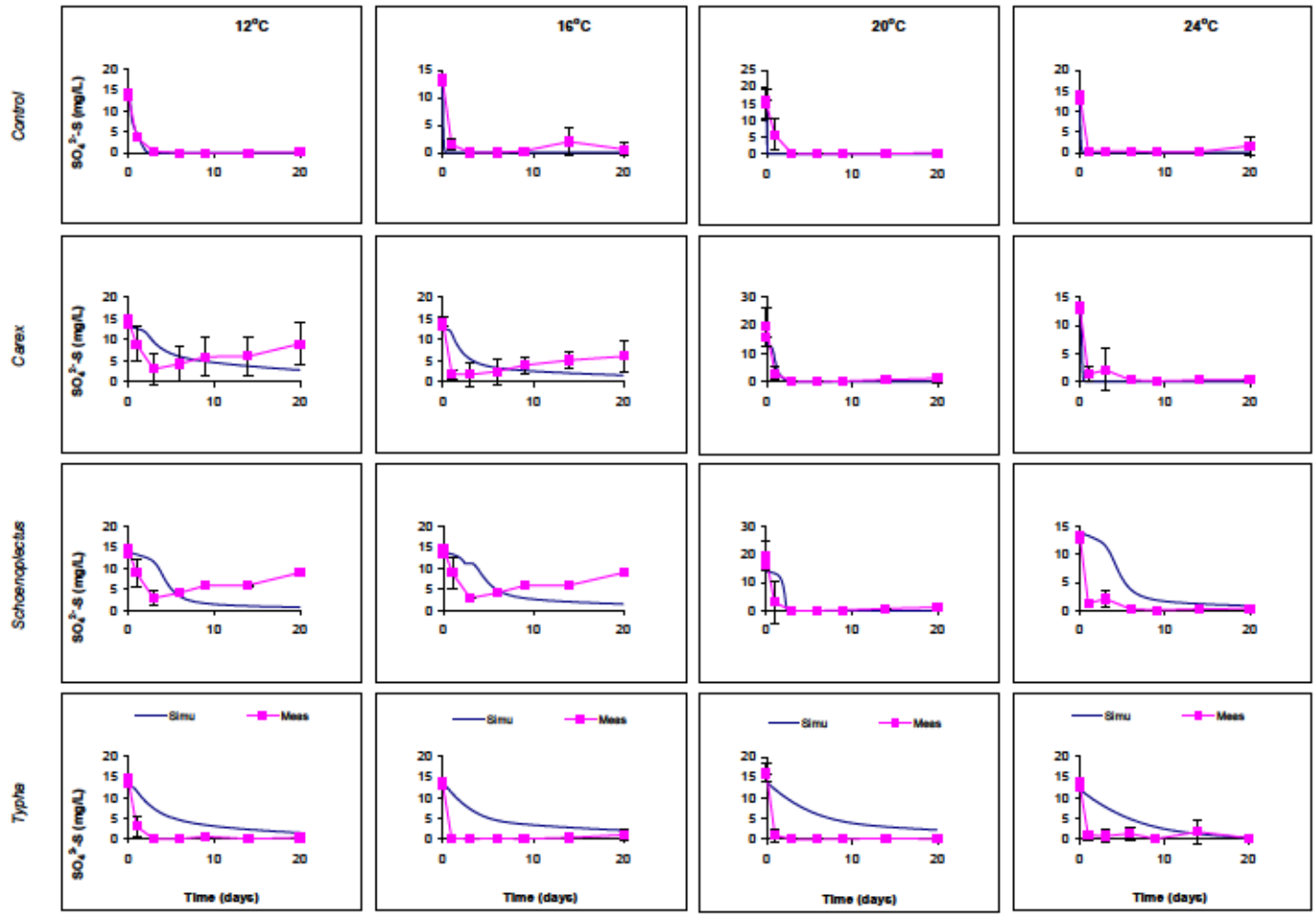

Fig. 3. Simulated compared with measured SO_4^{2-}–S concentrations in the constructed wetland mesocosms. Symbols are means (±S.D.) of observed concentrations from four replicates for each treatment.

4.3. Sensitivity of the biofilm parameters

The biofilm density, area and microbial volume fractions were the most sensitive biofilm characteristics for the majority of the variables in the bulk liquid zone (Table 1). This is an indication that the total biomass concentration, the flux area available for diffusion of substrate as well as the bacterial composition of the biofilm are important parameters in the dynamic simulation of the wetland mesocosms.

The sensitivity analysis revealed the diffusion coefficients of components into the biofilm have a low or insignificant sensitivity to the final simulation results at all temperatures. It appears that pore water diffusion was not limiting the transport and biodegradation of contaminants under the experimental conditions. This is also observed in the simulated pore water concentration profiles (not shown) found to be similar to the bulk water simulated concentration profiles presented in Figs. 1–3. This suggests the biofilm is fully penetrated (i.e. no substrate limitation and all reactions take place over the full depth of the biofilm), which may have allowed the omission of diffusion limitations in some previous modeling work for subsurface flow constructed wetland biofilms (García et al., 2010). The other factors influencing substrate transport, i.e. the biofilm thickness and the liquid boundary layer (through which transport from the bulk water to the biofilm surface occurs by molecular diffusion) had a moderate to significant sensitivity (Table 1). The sensitivity of the temperature (not shown) ranked high among the parameters with a "strong effect" on the bulk liquid concentrations only at the higher temperatures studied (i.e. at 20 °C and 24 °C).

There were no steep spatial gradients of the biomass profiles inside the biofilm within the simulation time (not shown), an expected result for a thin biofilm, whereas the simulation time scale (20 d) may also have been too short for the development of significant changes in bacterial species distribution (Vanhooren, 2002; Samsó and García, 2013). According to our current knowledge, a period of 3 year of continuous wetland operation is about sufficient for the bacterial communities to stabilize (Samsó and García, 2013). The apparent homogeneous distribution of the bacterial species involved can be of advantage for the processes, as microorganisms with differing redox potential requirements reside in close proximity, making the exchange of intermediate products between the species more efficient.

Anaerobic species favored by the oxygen limited conditions within the wetland mesocosm dominated the biofilm. The biomass volume fractions show the biofilm developed essentially as an anaerobic biofilm with a significant community of sulphate reducing and methanogenic bacteria (Table 3). Their population was enhanced with increase in temperature (Table 3), in agreement with the observation that in most cases the growth rates of both methanogens and sulphate reducing bacteria increase with increasing temperature (Baptista et al., 2003), while the methanogenic species were found to dominate the activity within the

biofilm across all temperatures during the simulation period, with activity defined here as the product of all substrate quotients in the Monod growth equation for a given population (Shanahan and Semmens, 2004).

5. Conclusions

Based on the CWM1 biokinetic model and the 1-D biofilm model of the AQUASIM software, the development over time of microbial species and substrates, as well as the biofilm thickness in a subsurface constructed wetland mesocosm have been simulated. The in silico analysis of constructed wetland biofilms indicates that aerobic, anoxic and anaerobic active biomass develops in the shallow biofilm. For operational analysis and constructed wetland technology development, a complex model such as the CWM1 model is recommended, with further extensions as required to address factors such as biofilm and rhizosphere development dynamics. The CWM1-Aquasim-Biofilm model is a useful tool to show the influence of the rhizosphere configuration on the performance of the constructed wetlands.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2014.01.005.

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