

Changes in bacteria composition and efficiency of constructed wetlands under sustained overloads: a modeling experiment

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Abstract

The average organic and hydraulic loads that Constructed Wetlands (CWs) receive are key parameters for their adequate long-term functioning. However, over their lifespan they will inevitably be subject to either episodic or sustained overloadings. Despite the consequences of sustained overloading are well known (e.g., clogging), the threshold of overloads that these systems can tolerate is difficult to determine. Moreover, the mechanisms that might sustain treatment efficiency during overloads are not well understood. The aim of this work is to evaluate the effect of sudden but sustained organic and hydraulic overloads on the general functioning of CWs. To that end, the mathematical model BIO_PORE was used to simulate five different scenarios, based on the features and operation conditions of a pilot CW system: a control simulation representing the average loads; 2 simulations representing +10% and +30% sustained organic overloads; one simulation representing a sustained +30% hydraulic overload; and one simulation with sustained organic and hydraulic overloads of +15% each. Different model outputs (e.g., total bacterial biomass and its spatial distribution, effluent concentrations) were compared among different simulations to evaluate the effects of such operation changes. Results reveal that overloads determine a temporary decrease in removal efficiency before microbial biomass adapts to the new conditions and COD removal efficiency is recovered. Increasing organic overloads cause stronger temporary decreases in COD removal efficiency compared to increasing hydraulic loads. The pace at which clogging develops increases by 10% for each 10% increase on the organic load.

Keywords:

organic load, hydraulic load, buffering capacity, BIO_PORE, CSO

Highlights

- Little experimental evidence on the assumed buffering capacity of CWs has so far been provided
- Five simulations representing overloading scenarios on a HF CW were run
- Hydraulic overloadings have bigger effects than organic ones

Acronyms

- HF CWs: Horizontal flow constructed wetlands
- HLR: Hydraulic loading rate
- HRT: Hydraulic retention time
- OLR: Organic loading rate

1. Introduction

Experience shows that when adequately designed and operated, Horizontal Flow Constructed wetlands (HF CWs) are an efficient and reliable wastewater treatment technology for suspended solids and organic matter removal. The benefits of these systems are maximized when applied to small communities and in places where seasonal population changes may occur [Puigagut et al., 2007; Masi et al., 2007]. CWs are renowned for tolerating relatively high variability of loading rates and influent wastewater quality [Weerakoon et al., 2013]. However a recently published study seems to put limits to this statement [Galvão and Matos, 2012]. Even though complex multi-stage CW systems have been proved to be able to cope with load fluctuations [Ávila et al., 2016], the buffering capacity of single-stage CWs has been generally assumed while little consistent experimental evidence has been provided to show the extent of such capacity.

Several experimental studies exist on the effects of organic and hydraulic loadings, but those studies analyze the response of the system to isolated steady state conditions [e.g., Ojeda et al., 2008], rather than their response when those conditions suddenly change [Galvão and Matos, 2012]. Moreover, inter-

dependence of HLR and OLR makes it difficult to study the effects of these two operating parameters separately [Galvão and Matos, 2012]. Examples of those facts are the works of Caselles-Osorio and García [2006] and Weerakoon et al. [2013]. In the first work two identical pilot CWs were used, one fed with glucose and the other fed with starch. Their experiment was divided in 4 phases with 2 months duration. During each of them, several experimental parameters were varied: presence/absence of sulphate and $\text{NH}_3\text{-N}$ in the inflow water, type of water (tap/distilled water), HRT, and inflow COD concentration. On the other hand, Weerakoon et al. [2013] evaluated the performance of 3 lab scale wetlands (two planted and an unplanted control) in a tropical region under different HLR and fed with synthetic wastewater mixed with septage sludge. For all CWs, HLRs were progressively increased from an initial value of 2.5 cm day^{-1} to 30 cm day^{-1} , each value being kept constant for a 12 days. Results from both studies seem to point to the fact that lower HLRs improve pollutants removal efficiencies. However, in Weerakoon et al. [2013] the HLR was linked to the OLR and thus the effects of each could not be analyzed separately. Although in Caselles-Osorio and García [2006] the HLR was changed while maintaining a constant OLR, several other operation parameters were modified for each of the 4 phases of the study. Moreover, neither of these studies addresses the adaptation of the systems between changing operation conditions, nor they considered the influence of long-term evolution of the microbial communities over their results.

Acknowledging the difficulty of extracting reliable results on the effects of organic and hydraulic overloadings from the available experimental studies, Galvão and Matos [2012] studied the effects of punctual and sudden organic overloadings on 9 lab scale HF CWs, divided in 3 different groups (A, B, and C). All beds were initially batch fed with synthetic wastewater at constant organic loadings, and the total mass loading was then increased for 2 weeks while the HLR was unchanged. Although the mass removal rate increased (indicating an adaptation of the microbial communities) they observed a general increase in the effluent COD concentrations and a decrease in the removal efficiencies. When the OLR was set back to the initial conditions, microbial activity and removal efficiencies also went back to match the initial ones. From these results Galvão and Matos [2012] concluded that, for the organic loads applied in their study, the buffering capacity of CWs in the short term was not sufficient to absorb the rapidly increasing mass loadings. They also stated that the buffering capacity of CWs is not yet well understood and that further work is required to understand their capacity to adapt to sudden changes of their operating conditions.

The data from the Galvão and Matos [2012] experiments were employed by Rizzo et al. [2014] to calibrate a HYDRUS-CWM1 model. The calibrated model was later used [Rizzo and Langergraber, 2016] to investigate the response of the pilot systems to sudden changes of influent organic load. In these numerical experiments, daily sudden loads and two weeks of high and low load phases were simulated following the European Standard 12556-3 [2005]. The simulated results suggested a

good buffer capacity of the CW to daily sudden loads. On the other hand, an increase in COD effluent concentration was also predicted during the two weeks of high load due to slow response of anaerobic bacteria. Moreover, the increase in influent organic load led to a longitudinal shift of fermenting bacteria towards the outlet, suggesting that an undersized HF CW could efficiently treat nominal loads but would fail in case of an influent sudden load. These results confirm that duration timescale of overload application significantly affects treatment efficiency.

In order to elucidate the long-term response of CWs to increase in inflow loads, the objective of this work was to evaluate the effect of sudden but sustained organic and/or hydraulic overloads on pollutants removal efficiencies, bacterial communities, and accumulated solids. We also investigated which of the two, either the organic or the hydraulic overloads, has the largest impact on the overall functioning of CWs. To that end, in this work we use a mathematical model called BIO_PORE [Samsó and García, 2013a] which was specifically developed to simulate subsurface-flow Constructed Wetlands general functioning. This model has already been calibrated and used in several works, which have provided new insights on the internal dynamics of CWs and the dynamics of solids accumulation and bacterial growth [Samsó and García, 2013b, 2014].

In this work, five numerical simulations were carried out. First off, the model was run under steady state conditions until bacterial communities and effluent pollutant concentrations reached a noticeable equilibrium. At that point five separate simulations were performed that correspond to four different overloading scenarios and one scenario with no change in inflow load. The simulations involved sudden organic overloads, hydraulic overloads, and both organic and hydraulic overloads at the same time. Each simulation was continued after the sudden inflow load variation until a new equilibrium state was reached. This work puts to tests well accepted beliefs on CWs capacities and provides further insights on their internal functioning and long-term response to operation changes. Moreover, despite in this work we have considered a wetland treating domestic wastewater, the results can be extrapolated to other types of wastewaters and wetland configurations. In particular, the findings of the present study are useful to understand the potential limits of CWs whose performance depends mostly on their short-term buffering capacity.

2. Methods

2.1. The BIO_PORE model

BIO_PORE is a 2D mechanistic model built in COMSOL Multiphysics™ software that includes a wide range of physical and biological processes to reproduce the general functioning of CWs [Samsó and García, 2013a]. To that end, it includes fluid flow and transport equations together with the biokinetic model Constructed Wetland Model number 1 (CWM1) [Langergraber et al., 2009]. CWM1 is based on ASM and ADM formulations [Batstone et al., 2002; Henze et al., 2000], and is seen as the most advanced biokinetic model developed for CWs. The wastewater constituents and the bacteria

147 groups considered in CWM1 are listed in Table 1. Calibrated
 148 values of model biokinetic parameters can be found in Samsó
 149 and García [2013a].

150 2.2. Simulation strategy

151 2.2.1. System characteristics

152 This work analyzes the same domain configuration used by
 153 Samsó and García [2013b], who considered a pilot wetland lo-
 154 cated at les Franqueses del Vallès, Barcelona, Spain [García
 155 et al., 2004]. The pilot HF CW has a horizontal area of 54.6 m²
 156 (10.3 x 5.3 m), and it is planted with *Phragmites australis*. It
 157 contains a layer of fine gravel ($D_{60} = 3.5$ mm, initial porosity
 158 $n = 0.4$, hydraulic conductivity $K = 50$ m d⁻¹) whose depth
 159 varies between 0.6 m at the inlet and 0.7 m at the outlet.

160 Samsó and García [2013b] performed a numerical simulation
 161 of the behavior of this HF CW for a period of three years under a
 162 constant HLR of 36.6 mm d⁻¹ of pre-treated urban wastewater.
 163 Results of the simulations at the end of this period showed that
 164 effluent concentrations and bacterial biomass within the CW
 165 substantially attained steady-state conditions. This final state
 166 after three years is employed as the initial condition at $t = 0$ for
 167 the simulations presented in this work.

168 2.2.2. Numerical simulations

169 Five simulations were performed in total. A first simulation²⁰¹
 170 (S_0) was run using the same flow rates and inflow pollutant con-²⁰²
 171 centrations used by Samsó and García [2013b] for an additional²⁰³
 172 year of simulation. These flow rates and pollutant concentra-²⁰⁴
 173 tions correspond to average values measured in the pilot plant
 174 and are summarized in Table 1. The Table shows that inflow²⁰⁵
 175 COD is mainly composed of particulate components (60%, X_S
 176 and X_I) compared to soluble ones (40%, S_F , S_A , and S_I). The²⁰⁶
 177 fraction of inert components (S_I , X_I) represents 15% of total²⁰⁷
 178 COD. Simulation S_0 represents normal operation conditions,²⁰⁸
 179 in the CW receives the organic and hydraulic loads for which it²⁰⁹
 180 was designed and no overload occurs.²¹⁰

181 The response of the system to sustained overloads was ana-²¹¹
 182 lyzed by performing four additional simulations in which the²¹²
 183 inflow is suddenly modified at $t = 0$ and it is then kept constant²¹³
 184 for one year. The organic and hydraulic overloading considered²¹⁴
 185 in these four simulations are shown in Table 2. The increase²¹⁵
 186 in organic wastewater loads can be driven by a change in in-²¹⁶
 187 flow pollutant concentration (S^C), hydraulic load (S^q), or both²¹⁷
 188 ($S^{C,q}$). In this study, simulation $S_{+10\%}^C$ and $S_{+30\%}^C$ considered the²¹⁸
 189 effect of an increase in inflow COD concentration of +10% and²¹⁹
 190 +30%, respectively, without changes in inflow COD fractioning²²⁰
 191 and NH₃-N concentration. The effect of variations in the HRT²²¹
 192 was investigated by introducing a +30% increase in the flow²²²
 193 rate ($S_{+30\%}^q$) while keeping all influent concentrations unaltered.²²³
 194 Finally, inflow COD concentration and flow rate were jointly²²⁴
 195 increased by +15% each in simulation $S_{+15\%,+15\%}^{C,q}$. It should be²²⁵
 196 noted that the OLR is the same for simulations $S_{+30\%}^C$, $S_{+30\%}^q$ ²²⁶
 197 and $S_{+15\%,+15\%}^{C,q}$, and their comparison hence allowed to identify²²⁷
 198 the effect of sustained overloads of equal intensity but caused²²⁸
 199 by different combinations of contaminant concentrations and²²⁹
 200 HLRs. The response variables that were analyzed to quantify²³⁰

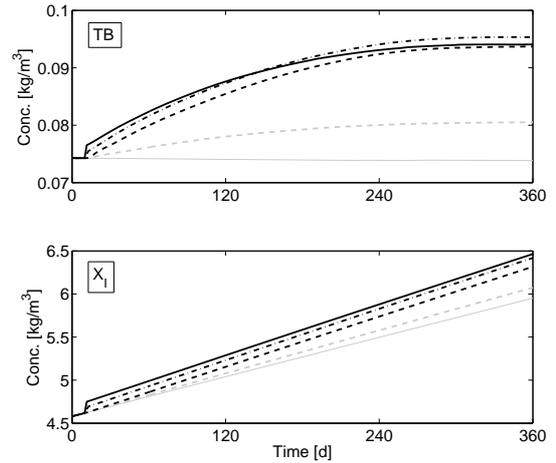


Figure 1: Total biomass (TB - upper panel) and accumulated solid (X_I - lower panel) at the end of the simulation (i.e., after four years of HF-CW functioning) for different influent overload conditions: +0% ($S_{+0\%}^C$ - gray continuous line), +10% and +30% of concentrations ($S_{+10\%}^C$ - gray dashed line - and $S_{+30\%}^C$ - black dashed line, respectively); +15% of hydraulic load and +15% of concentration ($S_{+15\%,+15\%}^{C,q}$ - black dash-dotted line); +30% of hydraulic load ($S_{+30\%}^q$ - black continuous line).

the influence of the different overloading scenarios are: spatial distribution in the CW of the total bacterial biomass and of the main functional groups, COD distribution and fractionation in the CW, effluent concentrations, and removal efficiencies.

3. Results

3.1. Bacterial distribution in the CW

The total amount of bacterial biomass increases with higher inflow mass loads for all simulations. Fig. 1a shows that the simulated total biomass in the CW increases steadily over time, and that an equilibrium value is attained after one year since the beginning of the overload. The increase in total biomass is very similar for all simulations with a +30% increase of OLR ($S_{+30\%}^C, S_{+30\%}^q, S_{+30\%}^{C,q}$). This result indicates that the amount of bacterial biomass at the equilibrium is controlled by the amount of inflow rate of substrate.

The relative abundance of different bacterial groups is summarized in Fig. 2 for the final day of each simulation. This relative abundances refers to the total mass of bacteria in the CW. In all simulations the most abundant groups are sulphate reducers (X_{ASRB}) and fermenters (X_{FB}), followed by methanogens (X_{AMB}). These three groups account for more than 90% of the total biomass, while heterotrophic bacteria (X_H), autotrophic nitrifiers (X_A), and sulfur oxidizers (X_{SOB}) are predicted to be scarce because of the aerobic conditions and the absence of nitrate in the inflow (see Table 1). Hence, the discussion about biomass distribution and microbial reactions will hereinafter focus on the prevailing bacterial groups X_{ASRB} , X_{FB} , and X_{AMB} because of their leading role in the system.

The presence of bacteria and their distribution in the gravel bed is strongly affected by the inflow characteristics. The com-

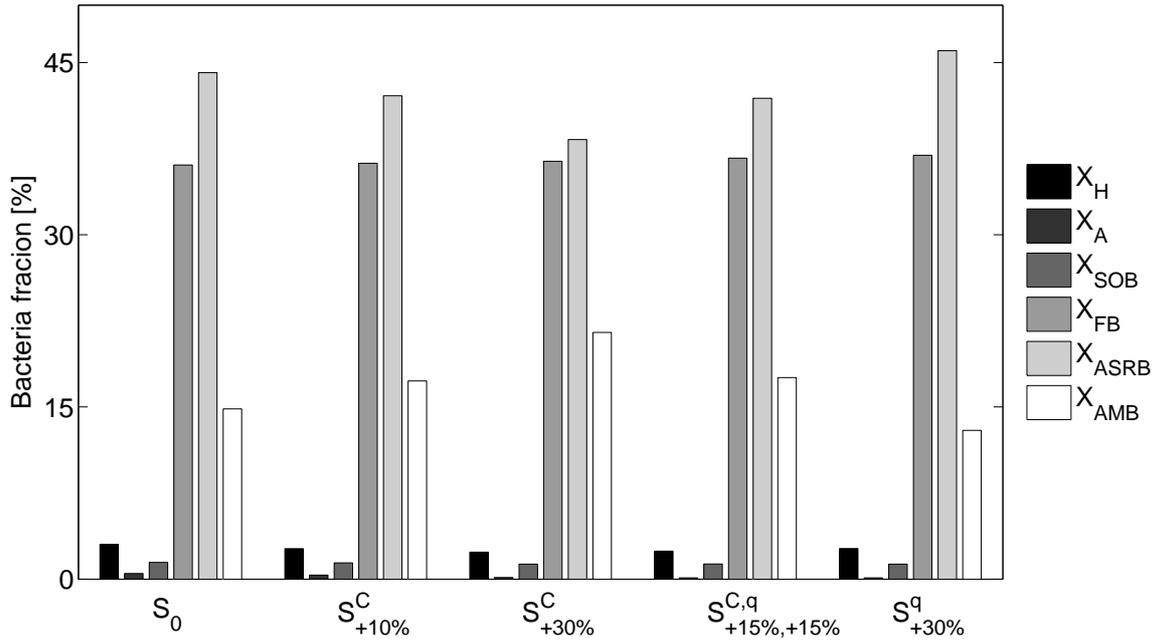


Figure 2: Relative proportion of the total biomass of heterotrophic (X_H), autotrophic nitrifying (X_A), sulphide oxidising (X_{SOB}), fermenting (X_{FB}), acetotrophic sulphate reducing (X_{ASRB}), and acetotrophic methanogenic (X_{AMB}) bacteria within the CW at the end of the simulation (i.e., after four years of HF-CW functioning) for different influent overload conditions: +0% ($S^C_{+0\%}$), +10% and +30% of concentrations ($S^C_{+10\%}$ and $S^C_{+30\%}$, respectively); +15% of hydraulic load and +15% of concentration ($S^{C,q}_{+15\%,+15\%}$); +30% of hydraulic load ($S^q_{+30\%}$).

231 parison between S_0 , $S^C_{+10\%}$, and $S^C_{+30\%}$ in Fig. 2 shows how 259
 232 changes in inflow COD concentration alters the microbial com- 260
 233 munity composition. Organic loads with higher COD concen- 261
 234 tration result in a slightly lower relative amount of sulphate 262
 235 reducers X_{ASRB} and in an increase of methanogenic bacteria 263
 236 X_{AMB} . On the other hand, Fig. 2 also reveals that increas- 264
 237 ing hydraulic load influences the composition of the microbial 265
 238 community differently than increasing inflow COD concentra- 266
 239 tion. The comparison between results of $S^q_{+30\%}$, $S^C_{+30\%}$, and 267
 240 $S^{C,q}_{+15\%,+15\%}$ indicates that shorter HRTs favor the presence 268
 241 of sulphate reducers over methanogens. All these results about 269
 242 steady-state contribution of bacterial groups agree very well 270
 243 with those reported by Ojeda et al. [2008]. 271

244 The spatial distribution of the most abundant bacterial groups 272
 245 is presented in Fig. 3, which shows the development of a mi- 273
 246 crobrial zonation within the CW along the flow direction. The 274
 247 downstream sequence of fermenting bacteria (X_{FB}), sulphate 275
 248 reducers (X_{ASRB}), and methanogens (X_{AMB}) indicates the estab- 276
 249 lishment of a longitudinal redox gradient in the CW that leads to 277
 250 a well defined spatial arrangement of microbial species. Simu- 278
 251 lated overloads do not modify the overall biomass zonation but 279
 252 lead to slight changes in the amount and location of the bacterial 280
 253 groups. When inflow COD concentration increases, the area 281
 254 occupied by each bacterial group tends to slightly extend in the 282
 255 downstream direction, as shown by the comparison between S_0 , 283
 256 $S^C_{+10\%}$, and $S^C_{+30\%}$ in Fig. 3. Instead, if the +30% load increment 284
 257 is caused by an increase in hydraulic load ($S^q_{+30\%}$) rather than in 285
 258 COD concentration ($S^C_{+30\%}$), the simulated results predict little 286

variations in the area occupied by methanogens while sulfate 287
 288 reducers occupy a remarkably wider portion of the CW. The 289
 290 case of an increase in both influent COD concentration and hy- 291
 292 draulic load ($S^{C,q}_{+15\%,+15\%}$) leads to a bacterial distribution within 292
 293 the CW which is intermediate between $S^C_{+30\%}$ and $S^q_{+30\%}$. 293

The absence of bacteria in the first part of the CW (see Fig. 294
 295 3) is caused by the progressive accumulation of particulate in- 296
 297 ert solids (X_I) and biodegradable solids (X_S) that reduce the 297
 298 amount of pore spaces available for biofilm growth [Samsó and 298
 299 García, 2014]. Specifically, simulated results (not shown) indi- 299
 300 cate that the concentration of inert particles (X_I) is larger by 300
 301 two orders of magnitude than the one of biodegradable particles 301
 302 (X_S), whose accumulation is hence responsible for the clogging 302
 303 of the CW. Fig. 1b shows the temporal evolution of the average 303
 304 concentration of accumulated inerts X_I for the different simu- 304
 305 lated overloads. The results show relatively minor differences 305
 306 in the rate of particle accumulation for different overloads over 306
 307 the one year of simulation, thus explaining the similar sizes of 307
 308 clogged areas near the CW inlet in Fig. 3. A more accurate ex- 308
 309 amination of Fig. 1b reveals that the rate of clogging increases 309
 310 of 10% for every 10% increase in inflow mass load, which de- 310
 311 rives from the fact that all influent solid particles are removed 311
 312 by filtration in the CW. This finding implies that clogging in- 312
 313 duced by the increase in wastewater load would proportionally 313
 314 shorten the lifespan of the considered CW. 314

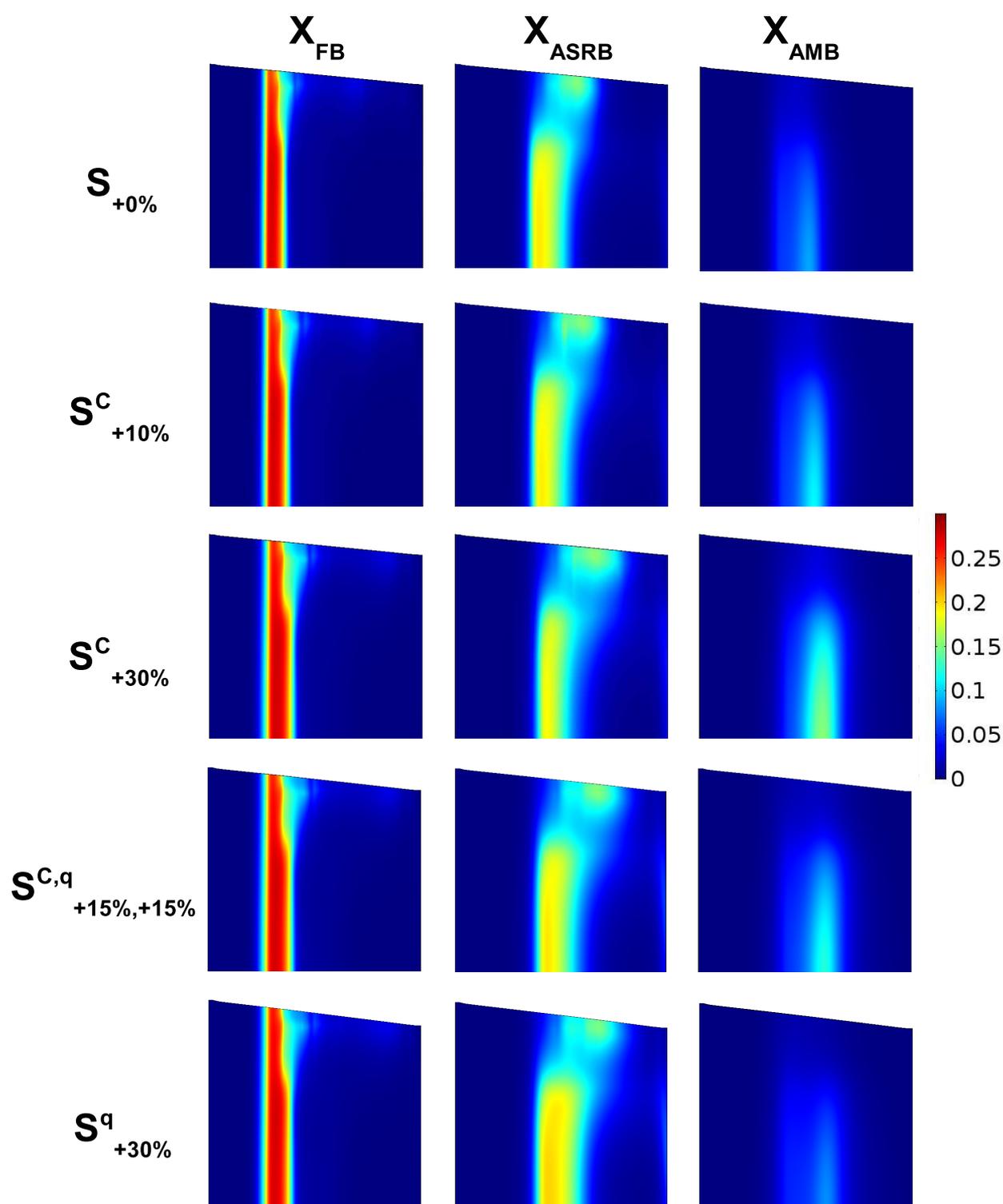


Figure 3: Fermenting (X_{FB}), acetotrophic sulphate reducing (X_{ASRB}), and acetotrophic methanogenic (X_{AMB}) bacteria spatial distribution at the end of simulation (after four years of HF-CW functioning) for different influent overload conditions: +0 % ($S_{+0\%}^C$), +10% and +30% of concentrations ($S_{+10\%}^C$ and $S_{+30\%}^C$, respectively); +15% of hydraulic load and +15% of concentration ($S_{+15\%,+15\%}^{C,q}$); +30% of hydraulic load ($S_{+30\%}^q$). Color scale is expressed in kg m^{-3} and the longitudinal scale is deformed to facilitate the visualization.

3.2. COD distribution in the CW

The zonation of bacterial biomass in the CW is mirrored by the spatial distribution of the different COD components, which is displayed in Fig. 4 that reports concentration patterns for total COD and for its reactive components (X_S, S_F, S_A) at the end of the one year transient. In all simulations total COD is concentrated near the inlet, in accordance with the expected HF CW functioning reported in literature [Kadlec and Wallace, 2009]. The first part of the CW shows no variation in COD concentration because of the previously discussed absence of bacteria and hence of reactions. It should be noted that this behavior stems from the assumption in BIO.PORE that the wastewater can flow through this clogged area, while wastewater at the inlet should flow over this first part of CW gravel bed and infiltrate only when the non-clogged part is reached. However, this simplification does not have any impact on results on water flow and reaction rates for the rest of CW. Downstream of the clogged part, fermenting bacteria (X_{FB} , Fig. 3) first hydrolyze solid biodegradable COD (X_S) into the soluble form S_F and then convert it to acetate (S_A) through fermentation (Fig. 4). Acetate is then sequentially degraded by sulphate reducers (X_{ASRB}) and methanogens (X_{AMB}).

When an overload induced by higher COD concentration occurs (S_0^C to $S_{+30\%}^C$, Fig. 4), the total COD concentration in the CW increases as well. This increase is particularly evident for acetate (S_A), with the portion of CW with high acetate concentrations extending further downstream with increasing inflow COD concentration. While the higher COD availability enhances growth for all bacterial groups (Fig. 3), the relative predominance of acetate explains the higher presence of methanogens (X_{AMB} , Figs. 2 and 3) compared to other microbial groups. In BIO.PORE, acetate is the substrate used by methanogenic bacteria (X_{AMB}) and also by sulphate reducers (X_{ASRB}), but the latter also require SO_4^{2-} for their metabolism. Since inflow concentration of sulphate always remained constant, the growth rate of sulphate reducers did not increase as much as the one of methanogens.

On the other hand, the proportion of the different COD fractions within the CW is less sensitive to overloads caused by increases in HLR compared to increases in COD inflow concentration. For a +30% hydraulic overload, the increase in total COD and in particular in acetate is less evident than for organic overloads, as shown by the comparison between $S_{+30\%}^C$, $S_{+15\%,+15\%}^{q,C}$ and $S_{+30\%}^q$ in Fig. 4). These small changes in COD fractioning within the CW explains the relative small variation in the composition of the bacterial community (Fig. 2 - compare S_0 and $S_{+30\%}^q$).

3.3. Effluent concentration

Variations in COD effluent concentrations over the considered period are shown in Fig. 5 (top panel). All simulations display a transient, with a sharp increase in COD concentration shortly after the beginning of the overload. COD concentrations peak after approximately after 45-60 days of overload and then decrease to values (22.3-24.3 mg l⁻¹) that are close to the original one after almost one year. The existence of this

transient shows that the CW requires a considerable amount of time before it can effectively treat the increased load. The maximum peak concentrations occur for the +30% mass overloads and is highest for simulation $S_{+30\%}^C$, followed by $S_{+15\%,+15\%}^{q,C}$ and $S_{+30\%}^q$. Increases in concentration are hence more critical than increases in flow rate.

The fractionation of the effluent is shown in Fig. 6 for the final steady configuration. Total effluent COD is essentially composed of inert soluble COD (S_I) and acetate (S_A), plus negligible amounts of fermentable COD (S_F) which has mostly been converted into acetate (see Fig. 4). Particulate components (X_S, X_I) are absent after having completely been filtered out by the CW. Overload conditions increase the proportion of acetate (S_A). This increase is slightly higher when the overload is caused by an increase in HLR (compare $S_{+30\%}^C$, $S_{+15\%,+15\%}^{q,C}$ and $S_{+30\%}^q$ in Fig. 6).

Effluent concentrations of Total Nitrogen (TN) are displayed in Fig. 5 (center). After a sharp increase that is similar to the increase in COD concentration, TN effluent concentration does not show any decrease and instead stabilize to a constant value. This equilibrium value ranges 53.7 and 55.9 mg l⁻¹ in the case of a +10% and +30% overload, respectively. The HF CW is unable to manage the increased amount of TN – mostly composed of NH_4^+ – because the competition for dissolved oxygen results in a marginal presence of autotrophic nitrifiers (X_A), which are outcompeted by heterotrophic bacteria (X_H – Fig. 2). NH_4^+ hence cannot be oxidized to nitrate for further denitrification, and it is transferred as TN to the CW outlet with almost no removal.

Fig. 5 (bottom panel) show sulphate concentrations at the CW outlet. Overload conditions enhance SO_4^{2-} removal for all simulations. The decrease in SO_4^{2-} effluent concentration reflects the development of acetotrophic sulphate reducing bacteria (X_{ASRB} , see Fig. 3) driven by increased availability of organic carbon in the CW. Sulphate removal hence improves for more intense overloads, as demonstrated by the comparison between $S_{+10\%}^C$ and $S_{+30\%}^C$, $S_{+15\%,+15\%}^{q,C}$, and $S_{+30\%}^q$ in Fig. 5.

Removal efficiency of COD and TN are presented in Fig. 7. COD removal efficiency is equal to 91% before the beginning of the overload, and then varies in the range between 85% and 93% depending on the considered overload simulation. Efficiencies at the end of the simulation are equal or even higher than at the beginning. However, the time required to recover from the transient efficiency drop is high due to the slow growth rates of anaerobic bacterial groups. This behavior results in a long transition phase (almost six months) in which COD removal efficiency is lower and potentially insufficient to ensure the design effluent concentrations. Organics overloads induce strongest fluctuations in COD removal efficiencies, but they also exhibit shorter recovery times compared to hydraulic overloads (compare $S_{+30\%}^C$ and $S_{+30\%}^q$ in Fig. 7, upper panel). TN removal efficiencies (Fig. 7 - lower panel) always decrease in presence of overloads because of the previously discussed limits of the HF CW in efficiently removing NH_4^+ and of the resulting higher TN effluent concentrations (Fig. 5 - central panel). Efficiency in TN removal drops from 21% before the overload

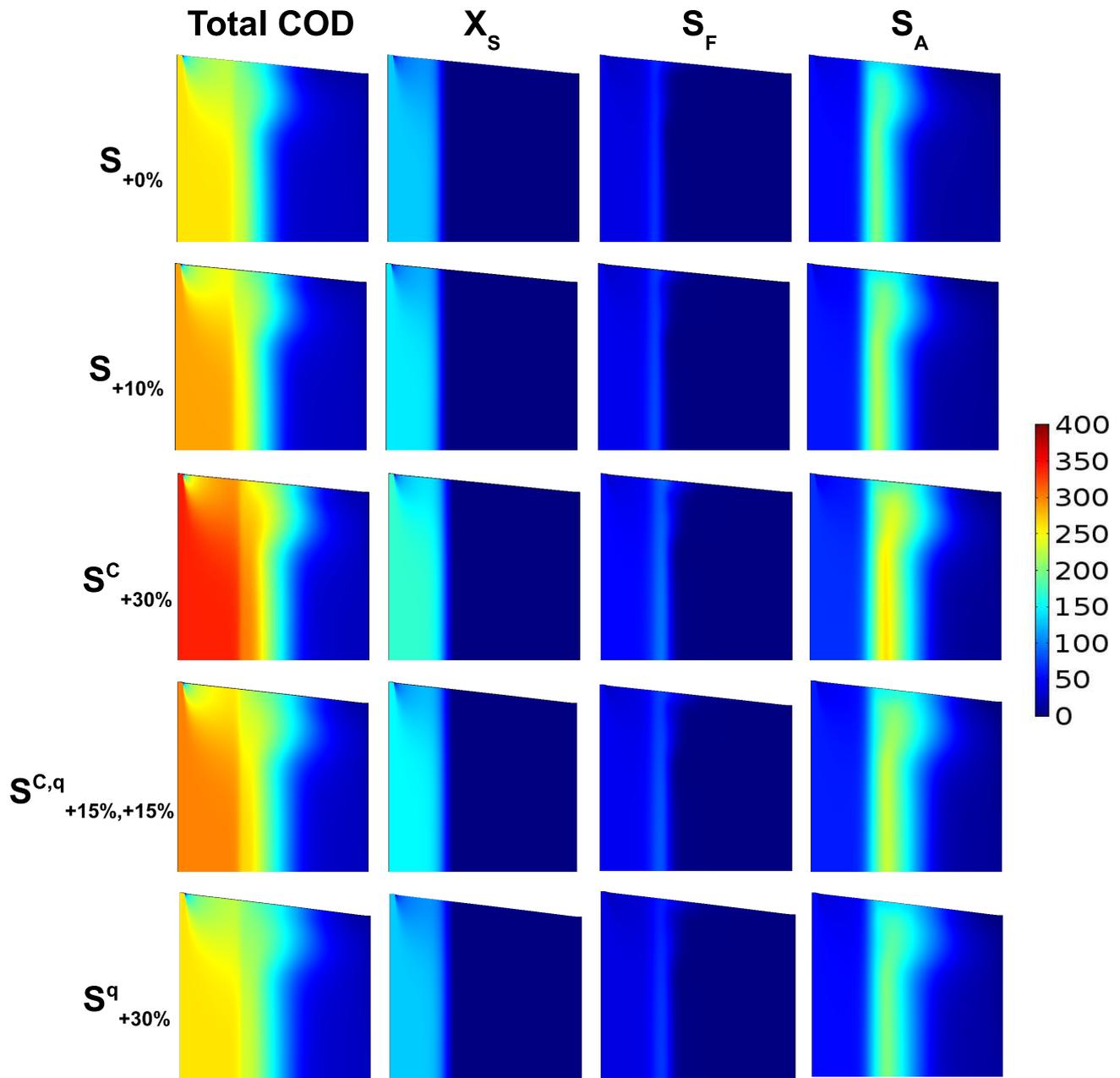


Figure 4: Total, slowly biodegradable particulate (X_S), fermentable readily biodegradable (S_F), fermentation products as acetate (S_A) COD spatial distribution at the 360th day of simulation (end of the fourth year of HF-CW functioning) for different influent overload conditions: +0 % ($S^C_{+0\%}$), +10% and +30% of concentrations ($S^C_{+10\%}$ and $S^C_{+30\%}$, respectively); +15% of hydraulic load and +15% of concentration ($S^{C,q}_{+15\%,+15\%}$); +30% of hydraulic load ($S^q_{+30\%}$). Colour scale is expressed in mg l^{-1} and the longitudinal scale is deformed to facilitate the visualization. Note as well that *Total COD* corresponds to the sum of S_F , S_A , S_I , X_S and X_I .

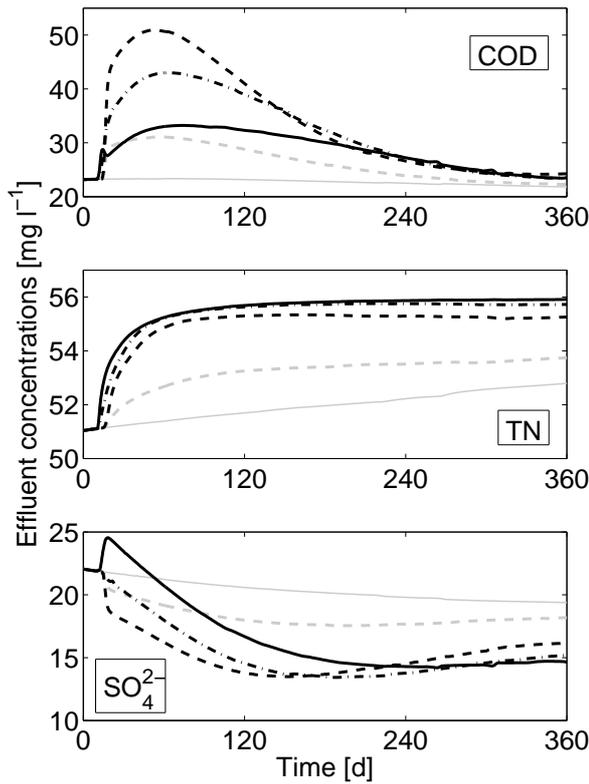


Figure 5: Effluent concentration of COD (upper panel), total nitrogen ($S_{NH} + S_{NO}$)(TN - middle panel), and sulphates (SO_4^{2-}) during the 360th day of simulation (i.e., during fourth year of HF-CW functioning) for different influent overload conditions: +0% ($S_{+0\%}^C$ - gray continuous line), +10% and +30% of concentrations ($S_{+10\%}^C$ - gray dashed line - and $S_{+30\%}^C$ - black dashed line, respectively); +15% of hydraulic load and + 15% of concentration ($S_{+15\%,+15\%}^{C,q}$ - black dash-dotted line); +30% of hydraulic load ($S_{+30\%}^q$ - black continuous line).

395 to values ranging between 13% ($S_{+30\%}^q$) and 17% ($S_{+10\%}^C$). In-
 396 crease in HLR are more critical in terms of TN removal effi-
 397 ciency compared to increases in inflow concentration.

398 4. Discussion

399 Simulation results from the present numerical analysis show
 400 that sustained organic and hydraulic overloads produce a tran-
 401 sient in the functioning of the CW, with temporarily higher ef-
 402 fluent concentrations and lower removal efficiencies. This tran-
 403 sient occurs because the amount of bacterial biomass is not
 404 suited to the modified biochemical conditions induced by the
 405 overloads, and almost one year is required for bacteria to adapt
 406 to the new conditions considered in this study. During this tran-
 407 sient, overloads due to increases in inflow concentration deter-
 408 mine high nutrient concentration within the CW that the initial
 409 amount of bacteria cannot fully metabolize. Overloads due to
 410 increases in HLR instead reduce contact times between nutri-
 411 ents and microbial biomass and hence decrease removal effi-
 412 ciencies. In both cases, total bacterial biomass in the CW in-
 413 creases, with slight modifications in its composition, and CW
 414 performance in COD removal increases back to values simi-
 415 lar or higher than the original ones. The same behavior occurs
 416 for SO_4^{2-} removal, while efficiencies in total nitrogen decrease
 417 because the competition for dissolved oxygen among bacterial
 418 groups limits the growth of nitrifying bacteria.

419 The simulated scenarios considered a set of overload con-
 420 figurations in which COD concentrations and HLR have been
 421 independently varied after three years of constant load. For do-
 422 mestic wastewater, changes in influent COD concentration may
 423 be caused by the introduction of water saving techniques and
 424 practices [Marleni et al., 2015] as well as by variations in pri-
 425 mary treatment, while variations in HLR may derive from the
 426 increase in population equivalents (e.g., variations in the num-
 427 ber of residents). The present results provide insights on the
 428 effect of sustained overloads that can be helpful even for situa-
 429 tions in which both concentrations and HLR are changed. How-
 430 ever, it is important to stress that the present study has always
 431 considered the same composition of inflow COD, and the CW
 432 response to overloads is likely to be affected by COD fractiona-
 433 tion. Differences in COD fractionation would impact the rate
 434 of solid accumulation and clogging (Fig. 1) and the relative
 435 composition of the bacterial community (Fig. 2). Nonetheless,
 436 we expect that the qualitative picture here discussed will still be
 437 valid, even though some quantitative changes are expected.

438 The findings of the present work are coherent with previous
 439 numerical studies [Ojeda et al., 2008] on the steady state con-
 440 ditions developed in CWs after changes in inflow wastewater
 441 loads, with effluent COD concentrations increasing in response
 442 to increases in either inflow COD concentrations or HLR. The
 443 present results are also in line with those obtained from the lab-
 444 oratory experiments performed by Galvão and Matos [2012],
 445 who also observed a decrease in COD removal efficiency af-
 446 ter the application of an overload. However, the study was fo-
 447 cused on the effects of overloads with a short duration (i.e., two
 448 weeks), a period that did not allow for bacterial species to reach
 449 a new steady condition and for the CW to recover its original

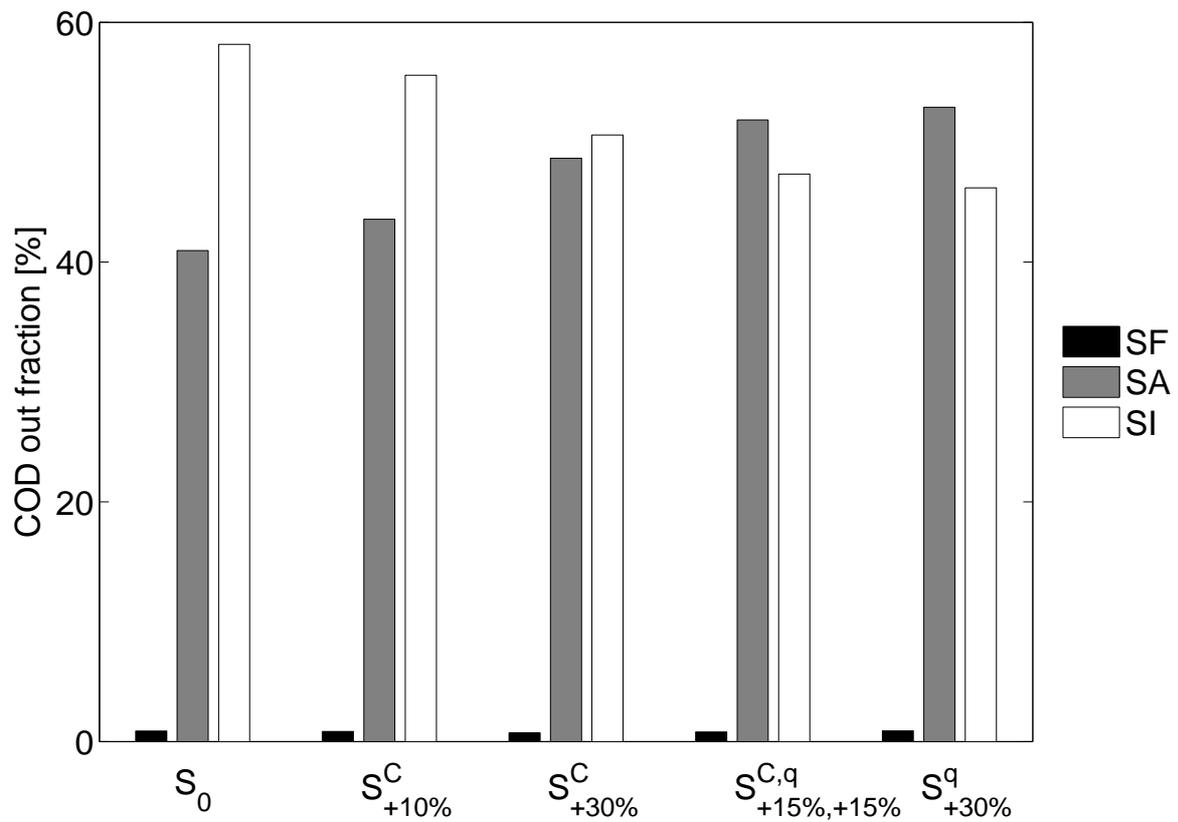


Figure 6: Relative fraction of fermentable readily biodegradable (S_F), acetate fermentation products (S_A), and inert soluble (S_I) effluent COD concentration during the 360th day of simulation (i.e., during fourth year of HF-CW functioning) for different influent overload conditions: +0% ($S^C_{+0\%}$), +10% and +30% of concentrations ($S^C_{+10\%}$ and $S^C_{+30\%}$, respectively); +15% of hydraulic load and +15% of concentration ($S^{C,q}_{+15\%,+15\%}$); +30% of hydraulic load ($S^q_{+30\%}$).

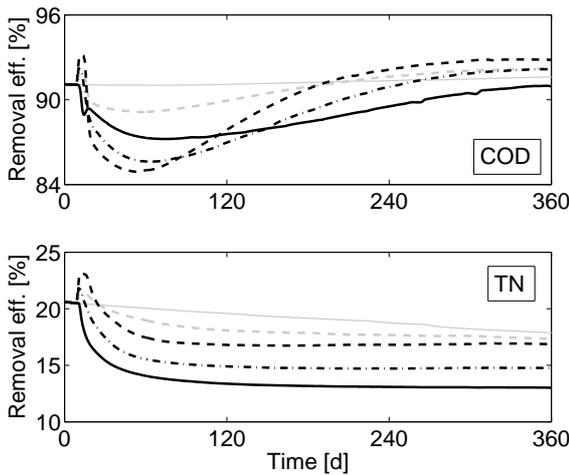


Figure 7: Removal efficiency of COD (upper panel) and total nitrogen (TN - lower panel) during the 360th day of simulation (i.e., during fourth year of HF-CW functioning) for different influent overload conditions: +0% ($S_{+0\%}^C$ - gray continuous line), +10% and +30% of concentrations ($S_{+10\%}^C$ - gray dashed line - and $S_{+30\%}^C$ - black dashed line, respectively); +15% of hydraulic load and +15% of concentration ($S_{+15\%,+15\%}^{C,q}$ - black dash-dotted line); +30% of hydraulic load ($S_{+30\%}^q$ - black continuous line).

480 to recover the original efficiency. An additional problem is that
 481 the ability of the CW to cope with organic overloads is cou-
 482 pled with a reduction of the CW lifespan due to the increased
 483 rate of clogging [Samsó and García, 2014]. Although the CW
 484 successfully remove the increased inflow of suspended particle,
 485 this effectiveness entails that the clogging rate increases pro-
 486 portionally to the increase in organic load. The impact of the
 487 increased clogging rate on the CW lifespan should thus be care-
 488 fully considered if the load conditions are expected to change
 489 during the CW.

450 removal efficiency. Our results indicate that sustained overloads
 451 are less critical than relatively frequent variations in organic
 452 load. The findings of the present study are useful to describe
 453 the evolution of CW performance during the transient between
 454 a sudden change in inflow load and the establishment of new
 455 equilibrium conditions.

456 5. Conclusions

457 The numerical simulations described in the present work has
 458 shown the response of a HF CW to sustained hydraulic and or-
 459 ganic overloads. The simulation results provide insights on the
 460 buffer capacity of CWs that have to deal with such variations
 461 in inflow wastewater load. The common claim about CW effi-
 462 ciency in treating organic loads is generally confirmed, as the
 463 simulation results show that the considered overloads cause a
 464 moderate drop in COD removal efficiency, which is never lower
 465 than 85% (compared to an original 91% value before the over-
 466 load application) for a +30% overload condition. Hence, even
 467 though the adaptation to the new load conditions is slow, the
 468 CW always exhibits a rather high COD treatment performance.
 469 After the microbial biomass in the CW has fully adapted to
 470 the new conditions, the system eventually shows a slight im-
 471 provement in removal efficiency compared to pre-overload con-
 472 ditions.

473 Despite the capacity of the HF CW to effectively manage in-
 474 creases in wastewater inflow, the present findings indicate that
 475 some caution should be exercised to verify that higher COD ef-
 476 fluent concentration during the transient does not negatively af-
 477 fect the receiving water body. The occurrence of overloads with
 478 relatively short duration that prevent adaption of the microbial
 479 groups is particularly critical because the CW would be unable

Table 1: Constant inflow concentrations used for simulations. These values were obtained from averages of an experimental study conducted in the pilot wetland [García et al., 2004]

Component	Description	Inflow concentration	Unit
S_O	Dissolved oxygen	0	$mgCOD \cdot L^{-1}$
S_F	Soluble fermentable COD	39	$mgCOD \cdot L^{-1}$
S_A	Fermentation products as acetate as COD	52	$mgCOD \cdot L^{-1}$
S_I	Inert soluble COD	13	$mgCOD \cdot L^{-1}$
X_{Sm}	Aqueous slowly biodegradable particulate COD	130	$mgCOD \cdot L^{-1}$
X_{Sf}	Solid slowly biodegradable particulate COD	0	$mgCOD \cdot L^{-1}$
X_{Im}	Aqueous inert particulate COD	26	$mgCOD \cdot L^{-1}$
X_{If}	Solid inert particulate COD	0	$mgCOD \cdot L^{-1}$
S_{NO}^a	Nitrite and nitrate nitrogen	0	$mgN \cdot L^{-1}$
S_{NH}	Ammonium and ammonia nitrogen	57	$mgN \cdot L^{-1}$
S_{SO4}	Sulphate sulphur	72	$mgS \cdot L^{-1}$
S_{H2S}	Hydrogen sulphide sulphur	0	$mgS \cdot L^{-1}$

^aNote that S_{NO} is assumed to include all nitrite and nitrate nitrogen, since nitrite is not included as a separate model component.

Table 2: Organic and hydraulic overloads considered in each simulation

Simulation	% Organic overload	% Hydraulic overload
S_0	0	0
$S_{+10\%}^C$	10	0
$S_{+30\%}^C$	30	0
$S_{+30\%}^q$	0	30
$S_{+15\%+15\%}^{C,q}$	15	15

490 **References**

- 491 J. Puigagut, J. Villaseñor, J. Salas, E. Bécares, J. García, Subsurface-flow
 492 constructed wetlands in Spain for the sanitation of small communities: A
 493 comparative study, *Ecological Engineering* 30 (4) (2007) 312–319, ISSN
 494 09258574.
- 495 F. Masi, N. Martinuzzi, R. Bresciani, L. Giovannelli, G. Conte, Tolerance to
 496 hydraulic and organic load fluctuations in constructed wetlands, *Water Sci.
 497 Technol.* 56 (3) (2007) 39–48, doi:10.2166/wst.2007.507.
- 498 G. M. P. R. Weerakoon, K. B. S. N. Jinadasa, G. B. B. Herath, M. I. M.
 499 Mowjood, J. J. a. van Bruggen, Impact of the hydraulic loading rate on pol-
 500 lutants removal in tropical horizontal subsurface flow constructed wetlands,
 501 *Ecol. Eng.* 61 (2013) 154–160, ISSN 09258574.
- 502 A. Galvão, J. Matos, Response of horizontal sub-surface flow con-
 503 structed wetlands to sudden organic load changes, *Ecological Engi-
 504 neering* 49 (2012) 123–129, doi:10.1016/j.ecoleng.2012.08.033, URL
 505 <http://dx.doi.org/10.1016/j.ecoleng.2012.08.033>.
- 506 C. Ávila, J. García, M. Garfí, Influence of hydraulic loading rate, simulated
 507 storm events and seasonality on the treatment performance of an experimen-
 508 tal three-stage hybrid constructed wetland system, *Ecological Engineering*
 509 87 (2016) 324–332, doi:10.1016/j.ecoleng.2015.11.042.
- 510 E. Ojeda, J. Caldentey, M. Saaltink, J. García, Evaluation of relative impor-
 511 tance of different microbial reactions on organic matter removal in hori-
 512 zontal subsurface-flow constructed wetlands using a 2D simulation model,
 513 *Ecological Engineering* 34 (1) (2008) 65–75, ISSN 09258574.
- 514 A. Caselles-Osorio, J. García, Performance of experimental horizontal subsur-
 515 face flow constructed wetlands fed with dissolved or particulate organic mat-
 516 ter., *Water research* 40 (19) (2006) 3603–11, ISSN 0043-1354.
- 517 A. Rizzo, G. Langergraber, A. Galvao, F. Boano, R. Revelli, L. Ridolfi, Mod-
 518 elling the response of laboratory horizontal flow constructed wetlands to un-
 519 steady organic loads with HYDRUS-CWM1, *Ecol. Eng.* 68 (2014) 209–213,
 520 ISSN 0925-8574, doi:10.1016/j.ecoleng.2014.03.073.
- 521 A. Rizzo, G. Langergraber, Novel insights on the response of horizontal
 522 flow constructed wetlands to sudden changes of influent organic load:
 523 A modeling study, *Ecological Engineering* 93 (2016) 242–249, doi:
 524 10.1016/j.ecoleng.2016.05.071.
- 525 E. S. 12556-3, Small wastewater treatment systems up to 50 PT. Part 3: Pack-
 526 aged and/or site assembled domestic wastewater treatment plants, *Tech.
 527 Rep.*, Österreichisches Normungsinstitut, Vienna, Austria, 2005.
- 528 R. Samsó, J. García, BIO.PORE, a mathematical model to simulate biofilm
 529 growth and water quality improvement in porous media: Application and
 530 calibration for constructed wetlands, *Ecol. Eng.* 54 (2013a) 116–127, doi:
 531 10.1016/j.ecoleng.2013.01.021.
- 532 R. Samsó, J. García, Bacteria distribution and dynamics in constructed wetlands
 533 based on modelling results, *Sci. Total Environ.* 461 (2013b) 430–440, ISSN
 534 0048-9697, doi:10.1016/j.scitotenv.2013.04.073.
- 535 R. Samsó, J. García, The Cartridge Theory: A description of the functioning of
 536 horizontal subsurface flow constructed wetlands for wastewater treatment,
 537 based on modelling results, *Sci. Total Environ.* 473 (2014) 651–658, ISSN
 538 0048-9697, doi:10.1016/j.scitotenv.2013.12.070.
- 539 G. Langergraber, D. P. L. Rousseau, J. García, J. Mena, CWM1: a general
 540 model to describe biokinetic processes in subsurface flow constructed wet-
 541 lands., *Water Science & Technology* 59 (9) (2009) 1687–1697, ISSN 0273-
 542 1223.
- 543 D. J. Batstone, J. Keller, I. Angelidaki, S. V. Kalyuzhny, S. G. Pavlostathis,
 544 A. Rozzi, W. T. M. Sanders, H. Siegrist, V. A. Vavilin, *Anaerobic digestion*
 545 *model No. 1 (ADM1)*, IWA Publishing, ISBN 1-900222-78-7, 2002.
- 546 M. Henze, W. Gujer, T. Mino, M. van Loosdrecht, *Activated sludge models*
 547 *ASM1, ASM2, ASM2d and ASM3*, IWA task group on mathematical mod-
 548 elling for design and operation of biological wastewater treatment, *Tech.
 549 Rep.*, IWA scientific and technical report, 2000.
- 550 J. García, J. Chiva, P. Aguirre, E. Álvarez, J. Sierra, R. Mujeriego, Hydraulic
 551 behaviour of horizontal subsurface flow constructed wetlands with different
 552 aspect ratio and granular medium size, *Ecol. Eng.* 23 (3) (2004) 177–187,
 553 ISSN 0925-8574, doi:10.1016/j.ecoleng.2004.09.002.
- 554 R. Kadlec, S. Wallace, *Treatment Wetlands*, 2nd edition, CRC press, Boca Ra-
 555 ton, FL, USA, ISBN 9781420012514, 2009.
- 556 N. Marleni, S. Gray, A. Sharma, S. Burn, N. Muttil, Impact of water man-
 557 agement practice scenarios on wastewater flow and contaminant concen-
 558 tration, *J. Environ. Manage.* 151 (2015) 461–471, ISSN 0301-4797, doi:
 559 10.1016/j.jenvman.2014.12.010.