Comparison of measured and simulated distribution of microbial biomass in subsurface vertical flow constructed wetlands

G. Langergraber, A. Tietz and R. Haberl

Institute for Sanitary Engineering and Water Pollution Control, University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Muthgasse 18, A-1190 Vienna, Austria
(E-mail: *guenter.langergraber@boku.ac.at*)

Abstract The multi-component reactive transport module CW2D has been developed to model transport and reactions of the main constituents of municipal wastewater in subsurface flow constructed wetlands and is able to describe the biochemical elimination and transformation processes for organic matter, nitrogen and phosphorus. It has been shown that simulation results match the measured data when the flow model can be calibrated well. However, there is a need to develop experimental techniques for the measurement of CW2D model parameters to increase the quality of the simulation results. Over the last years methods to characterise the microbial biocoenosis in vertical subsurface flow constructed wetlands have been developed. The paper shows measured data for microbial biomass and their comparison with simulation results using different heterotrophic lysis rate constants.

Keywords CW2D; microbial biomass; numerical simulation; vertical subsurface flow constructed wetlands; wastewater treatment

Water Science & Technology **Vol 56 No 3 pp 233–240** © IWA Publishing 2007

Introduction

Constructed wetlands (CWs) are still often looked at as a "black box" in which water is purified. This is although there is much experience available in constructing and operating these systems. Numerical models can provide insight into the "black box" and are therefore a means to increase the understanding of the complex processes in constructed wetland systems. Once reliable numerical models exist they can be used for evaluating and improving existing design criteria which are mainly based on rules of thumb using specific surface area requirements or simple first-order decay models (e.g. Kadlec *et al.*, 2000).

Many models available have focused on the description of seasonal trends (e.g. Wynn and Liehr, 2001) or using simplifications such as first-order reaction rates to describe the degradation of a substance along the flow path (Werner and Kadlec, 2000). Rousseau (2005) developed a mechanistic reaction model for subsurface horizontal flow constructed wetlands using tanks-in-series for modelling water flow. Rousseau's model can therefore only be used for horizontal flow systems where saturated conditions occur.

The numerical model used in this study, HYDRUS-2D/CW2D (Langergraber and Šimůnek, 2006), uses mechanistic models for both water flow and reactions. This enables modelling of both unsaturated and saturated water flow and therefore the model can be applied for subsurface vertical and horizontal flow constructed wetlands. CW2D is an extension of the variably saturated water flow and solute transport program HYDRUS-2D (Šimůnek *et al.*, 1999) and was developed to model transport and reactions of the main constituents of municipal wastewater in subsurface flow constructed wetlands. CW2D is able to describe the biochemical elimination and transformation processes for organic

doi: 10.2166/wst.2007.496

matter, nitrogen and phosphorus based on the mathematical formulation introduced by Henze *et al.* (2000) for the IWA Activated Sludge Models (ASMs).

The experience showed that the results of multi-component reactive transport simulations with CW2D match well with measured effluent concentrations when the water flow model can be calibrated. However, there is still a need to develop methods to measure CW2D model parameters (Langergraber, 2003; Langergraber and Šimůnek, 2005). This is required for the calibration of the reactive transport part of the model and a precondition before the model can be used for evaluating design criteria. A number of measurement techniques have been developed to characterise the parameters of ASMs (Vanrolleghem *et al.*, 1999; Henze *et al.*, 2000). However, no experimental techniques are currently available to measure CW2D model parameters.

Over the last years methods to characterise the microbial biocoenosis in vertical subsurface flow constructed wetlands have been developed (Tietz *et al.*, 2007). This paper shows how the measured data have been converted into parameters that can be used to compare the measured values with simulation results of CW2D. Simulation results with different rate constants for heterotrophic lysis are shown and compared with the measurements.

Materials and methods

The multi-component reactive transport module CW2D

The multi-component reactive transport module CW2D (Langergraber, 2001) was developed to describe the biochemical transformation and degradation processes in subsurface flow constructed wetlands. CW2D is incorporated into the HYDRUS-2D variably saturated water flow and solute transport program (Šimůnek *et al.*, 1999) and considers 12 components and nine processes. The components include dissolved oxygen, organic matter (three fractions of COD with different degradability: CR, CS, CI = readily, slowly biodegradable and inert COD, respectively), ammonium, nitrite, nitrate and nitrogen gas, inorganic phosphorus and heterotrophic and autotrophic microorganisms. Organic nitrogen and organic phosphorus are modelled as nutrient contents of the organic matter. The processes considered in the model are hydrolysis, mineralisation of organic matter, nitrification (modelled as a two-step process), denitrification and lysis processes for microorganisms. The mathematical structure of CW2D is based on the mathematical structure of the ASMs (Henze *et al.*, 2000). ASMs are based on mass balances for COD, nitrogen and phosphorus. For a detailed discussion of the CW2D module see Langergraber and Šimůnek (2005).

The indoor pilot-scale constructed wetlands (PSCWs)

The experiments have been carried out at indoor vertical flow pilot-scale constructed wetlands (PSCWs) in the technical laboratory hall of the Institute. Each PSCW had a surface area of 1 m^2 and was loaded intermittently four times per day. The 50 cm main layer of the eight PSCWs sampled for the investigations consisted of a sandy substrate with a grain size of 0.06–4 mm. The organic load applied was 20 g COD/m^2/d (i.e. a specific surface area requirement of 4 m^2 per person), the hydraulic load about 60 mm/d. The PSCWs have been planted with *Miscanthus gigantea* (6) and unplanted (2), respectively.

Samples from the main layer were collected from different depths (0–1, 1–5, 5–10, 10–20, 20–30, 30–40 and 40–50 cm) and were analysed immediately for bacterial production and stored at 4 °C for analysis of microbial biomass and TOC within 10 days (Tietz *et al.*, 2007). Water samples were taken on a monthly basis and analysed in the laboratory of the Institute.

Methods for the determination of the microbial and bacterial biomass

As CW2D is based on mass balances for COD biomass concentrations are also expressed in terms of COD. A number of methods exist to determine the COD of the microbial biomass in activated sludge systems (e.g. Bullock *et al.*, 1996; von Münch and Pollard, 1997; Contreras *et al.*, 2002). However, no method exists for determining the COD of soil biomass. Therefore for the determination of microbial COD it was decided to measure the C and N content of the biomass and calculate the biomass COD using conversion factors based on stoichiometry.

A number of methods were applied to measure parameters to characterise microbial and bacterial biomass in a previous study. These methods include conversion of bacterial abundance determined by microscopic direct counts into biomass by measurement of the cell volume, fumigation-extraction for biomass-C and -N, ATP measurements for biomass-C, and substrate-induced respiration (SIR) for biomass-C (Tietz *et al.*, 2007).

Calculation of the theoretical biomass COD

As described above, the biomass COD is calculated from the measured C and N content of the biomass. The conversion factor for microbial C and N into the COD of the biomass is derived from the reactions of the aerobic degradation of biomass.

Several chemical formulae are used to describe the composition of biomass in wastewater systems (e.g. von Münch and Pollard, 1997; Contreras *et al.*, 2002; Sötemann *et al.*, 2005). For the calculation of biomass COD, a simple biomass composition ($C_5H_7O_2N$) that is widely used for characterising biomass in activated sludge systems was chosen. It is assumed that the mineralisation of biomass occurs in two steps: at first biomass is hydrolysed and in a second step oxidised.

Using the general formula for biomass hydrolysis given by Sötemann *et al.* (2005):

$$C_XH_YO_ZN_A \Rightarrow \frac{Y+4X-2Z-3A}{24}C_6H_{12}O_6 + ANH_3 + \frac{Y-4X+2Z-3A}{4}H_2O + \frac{2Z+3A-Y}{4}CO_2 \quad (1)$$

hydrolysis of $C_5H_7O_2N$ results in

$$6 \times C_5H_7O_2N \Rightarrow 5 \times C_6H_{12}O_6 + 6 \times NH_3 - 18 \times H_2O \quad (2)$$

and the oxidation of the hydrolysis product $C_6H_{12}O_6$ in

$$C_6H_{12}O_6 + 6 \times O_2 \Rightarrow 6 \times H_2O + 6 \times CO_2 \quad (3)$$

Summarising the two reactions results in

$$C_5H_7O_2N + 5 \times O_2 \Rightarrow 2 \times H_2O + 5 \times CO_2 + NH_3 \quad (4)$$

as also given by, for example, von Münch and Pollard (1997).

Therefore for oxidising 113 g $C_5H_7O_2N$ 160 g O_2 are required resulting in an oxygen demand, i.e. COD, of 1.416 g O_2 per g $C_5H_7O_2N$. 113 g $C_5H_7O_2N$ biomass consists of 60 g C and 14 g N. Relating the oxygen demand to the biomass C and N results in 2.667 g O_2 per g biomass-C and 11.429 g O_2 per g biomass-N, respectively.

Using the formula $C_8H_{14}O_4N$ for biomass composition (Contreras *et al.*, 2002) results in a similar COD of 1.489 g O_2 per g $C_8H_{14}O_4N$, and related to the biomass C and N the results are 2.917 g O_2 per g biomass-C and 20.0 g O_2 per g biomass-N, respectively.

Another possibility for calculating biomass COD would be to determine biomass COD from VSS measurements using given ratios for $C_5H_7O_2N$ and $C_8H_{14}O_4N$ of 1.42 and

1.49 mg COD/mg VSS, respectively. However, COD/VSS ratios have been only reported to be valid for activated sludge systems and pure cultures (Contreras *et al.*, 2002).

Results and discussion

Measured microbial and bacterial biomass

Mean values and standard deviations for measured C and N content of the biomass in different depths of the main layer as reported by Tietz *et al.* (2007) are shown in Table 1. For all parameters characterising the biomass a similar decrease via depth in the vertical flow bed could be observed. There was no statistically significant difference between planted and unplanted PSCWs. Most of the biomass could be found in the top 10 cm of the main layer; the biomass concentrations measured in depths from 10 to 50 cm are very small.

Calculated biomass COD

Table 2 shows the calculated biomass COD figures using the measured data from Table 1 and the biomass composition $C_5H_7O_2N$. In the first centimetre of the main layer the mean values range from 3,400 to 5,100 mg COD/g DW, from 1 to 5 cm 1,100 to 2,600 mg COD/g DW, and from 5 to 10 cm 640 to 1,400 mg COD/g DW, respectively. Biomass COD calculated from C fumigation measurements resulted in the lowest values. No significant difference could be found when comparing the calculations using biomass measurements that are based on different methods (Figure 1).

Model set-up

To reduce the simulation time only a part of the PSCW cross section was considered for the numerical simulations. The width of the transport domain was 20 cm and its depth 50 cm (the depth of the main layer), while the transport domain itself was discretised into three columns and 25 rows. This results in a two-dimensional finite element mesh

Table 1 Measured biomass C and N content in different depths of the main layer (Tietz *et al.*, 2007)

Depths	SIR		ATP		C fumigation		N fumigation	
cm	Mean mg C/g DW	Std. Dev.	Mean mg C/g DW	Std. Dev.	Mean mg C/g DW	Std. Dev.	Mean mg N/g DW	Std. Dev.
0–1	1,831	315	1,912	755	1,287	85	359	85
1–5	979	295	596	334	401	26	199	26
5–10	522	104	358	218	241	27	73	27
10–20	57	*	8	7	5	*	6	*
20–30	49	*	3	1	2	*	6	*
30–40	31	*	4	1	3	*	2	*
40–50	106	*	4	2	3	*	7	*

*Not reported

Table 2 Calculated biomass COD in different depths of the main layer (mg COD/g DW)

Depths	SIR		ATP		C fumigation		N fumigation	
cm	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
0–1	4,884	839	5,100	2,012	3,431	226	4,103	970
1–5	2,611	787	1,590	891	1,070	70	2,269	299
5–10	1,392	276	955	581	642	72	834	307
10–20	151	–	22	19	15	–	69	–
20–30	130	–	8	3	5	–	69	–
30–40	82	–	10	3	7	–	23	–
40–50	283	–	10	6	7	–	80	–

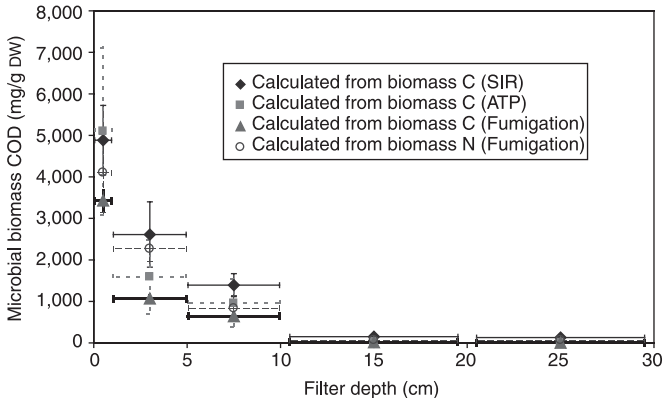

Figure 1 Calculated microbial biomass COD in different depths of the main layer

consisting of 75 nodes and 96 triangular finite elements. An atmospheric boundary condition was assigned to the top of the system representing the influent distribution system, and a constant pressure head boundary condition (constant head of -2 cm) to the bottom of the main layer. Parameters for the flow model, i.e. the parameters to describe the unsaturated hydraulic properties of the main layer, have been taken from Mollner (2005).

Simulation results

Heterotrophic organisms are the majority of the microbial community and therefore represent the main component of the total microbial biomass. Simulations have been carried out for different rate constants b_H for lysis of heterotrophic microorganisms. Lysis represents the sum of all decay and sink processes, and produces organic matter (CS, CR and CI) as well as nutrients incorporated in the biomass (ammonium and inorganic phosphorus). The values for other rate constants of CW2D (as defined in Langergraber (2001)) have been kept constant: the maximum heterotrophic aerobic growth rate on CR was 2.4/d, the maximum denitrification rate was 1.9/d, the maximum autotrophic aerobic growth rate on NH_4^+ was 0.36/d, the maximum autotrophic aerobic growth rate on NO_2^- was 0.40/d, and the autotrophic rate constant for lysis was 0.036/d.

The influent concentrations used for the simulations are shown in Table 3 and that also shows the effluent concentrations for simulations with different rate constant for lysis of heterotrophic microorganisms. The effluent concentrations are median values of the last five days of a simulation time of 200 days. Steady-state conditions could be reached after this period. With increasing b_H values the COD effluent is increasing due to more decay processes of biomass resulting in CS and CI, respectively. Effluent concentrations of ammonia and nitrite nitrogen are not affected by different b_H values whereas

Table 3 CW2D influent concentrations and simulated effluent concentrations

Parameter	b_H	CR	CS	CI	COD	NH_4-N	NO_2-N	NO_3-N
Unit	(d)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Influent	–	160	120	20	300	60	0.1	0.1
Effluent	0.10	0.2	2.9	28.9	32.0	0.060	0.019	50.6
	0.20	0.4	5.8	33.3	39.5	0.060	0.019	48.1
	0.25	0.5	7.5	34.4	42.4	0.061	0.019	48.6
	0.30	0.6	9.9	34.5	44.9	0.060	0.019	50.5
	0.35	0.7	12.5	33.8	47.0	0.061	0.019	53.0

CR, CS, CI: readily, slowly biodegradable and inert organic matter, respectively (Langergraber, 2001)

nitrate nitrogen concentrations change due to different availability of readily biodegradable organic matter that is required for denitrification.

Table 4 shows the simulated microbial biomass COD in different depths of the main layer for different heterotrophic lysis rates. With increasing b_H the microbial biomass COD decreases. Using heterotrophic lysis rates between 0.25 and 0.35/d results in microbial biomass COD between 5,600 and 3,400 mg COD/g DW for the first cm of the main layer. These values are in the range of the measured values (Table 2). Table 5 shows the simulated composition of the microbial biomass COD for $b_H = 0.30$/d. As expected the main contribution to the microbial biomass COD comes from heterotrophic organisms (more than 90% in the top layers).

Figure 2 compares calculated and simulated microbial biomass COD in different depths of the main layer for a heterotrophic lysis rate of $b_H = 0.30$/d. For comparison

Table 4 Simulated microbial biomass COD (mg COD/g DW) in different depths of the main layer for different heterotrophic lysis rates b_H

Heterotrophic lysis rate b_H (d)		0.10	0.20	0.25	0.30	0.35
Depth	0–1 cm	8,191	6,466	5,557	4,634	3,384
	1–5 cm	6,454	5,017	4,402	3,867	3,223
	5–10 cm	3,811	2,069	1,245	480	227
	10–20 cm	910	374	198	191	192
	20–30 cm	219	171	149	133	103
	30–40 cm	84	64	61	63	61
	40–50 cm	44	38	38	37	34

Table 5 Simulated composition of the microbial biomass COD (mg COD/g DW) in different depths of the main layer for $b_H = 0.30$/d

Depth	XH	XANs	XANb	Microbial biomass
0–1 cm	4,291	193	151	4,634
1–5 cm	3,549	168	150	3,867
5–10 cm	360	64	56	480
10–20 cm	108	43	41	191
20–30 cm	102	15	15	133
30–40 cm	57	3	3	63
40–50 cm	33	2	2	37

XH, XANs, XANb: heterotrophic organisms, *Nitrosomonas* sp. and *Nitrobacter* sp., respectively (Langergraber, 2001)

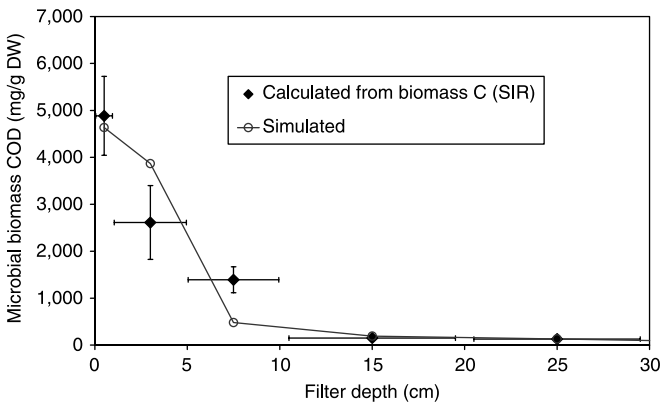

Figure 2 Calculated and simulated ($b_H = 0.30$ 1/d) microbial biomass COD in different depths of the main layer

only the calculated values from substrate induced respiration (SIR) are shown. The simulations overpredict biomass COD in 1–5 cm depth and underpredict biomass COD in 5–10 cm depth. This indicates that it might be necessary to include the influence of biomass growth (and volume) on the hydraulic flow properties of the sandy substrate. Biomass growth leads to reduced pore volume and therefore flow velocities are reduced resulting in longer contact times between the biomass and the wastewater consituents and therefore in higher degradation of organic matter in the upper 1 cm. Finally, less organic matter is available in deeper zones of the main layer resulting in reduced biomass growth in the 1–5 cm depth.

Summary and conclusions

This paper shows how measured data for microbial biomass in vertical subsurface flow constructed wetlands have been used to calculate biomass COD values needed for comparison with numerical simulations using the multi-component reactive transport module CW2D. Simulated microbial biomass COD in the first centimetre of the main layer are between 5,600 and 3,400 mg COD/g DW (the range of the measured values) when using heterotrophic lysis rates between 0.25 and 0.35/d. When comparing measured and simulated biomass COD in different depths of the main layer simulations seem to overpredict biomass COD in the 1–5 cm depth and underpredict biomass COD in the 5–10 cm depth. This could be an indication that the influence of biomass growth on the hydraulic properties has to be modelled as well. However, up to now this is not possible with the HYDRUS-2D/CW2D software package.

Acknowledgements

The work was carried out within the project "Characterisation of microbial biocoenosis to optimise removal efficiency and design of subsurface flow constructed wetlands for wastewater treatment" funded by the Austrian Science Fund (FWF, project No.: P16212-B06). The authors are grateful for the support.

References

Bullock, C.M., Bicho, P.A., Zhang, Y. and Saddler, J.N. (1996). A solid chemical oxygen demand (COD) method for determining biomass in waste waters. *Water Res.*, **30**(5), 1280–1284.

Contreras, E.M., Bertola1, N.C., Giannuzzi, L. and Zaritzky, N.E. (2002). A modified method to determine biomass concentration as COD in pure cultures and in activated sludge systems. *Water SA*, **28**(4), 463–467.

Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M.C.M. (2000). Activated sludge models ASM1, ASM2, ASM2D and ASM3. In: *IWA Scientific and Technical Report No. 9*. IWA Publishing, London.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P. and Haberl, R. (2000). Constructed wetlands for pollution control – processes, performance, design and operation. In: *IWA Scientific and Technical Report No. 8*. IWA Publishing, London.

Langergraber, G. (2001). Development of a simulation tool for subsurface flow constructed wetlands. *Wiener Mitteilungen 169*, Vienna, Austria, 207p, ISBN 3-85234-060-8.

Langergraber, G. (2003). Simulation of subsurface flow constructed wetlands – results and further research needs. *Wat. Sci. Technol.*, **48**(5), 157–166.

Langergraber, G. and Šimůnek, J. (2005). Modeling variably saturated water flow and multicomponent reactive transport in constructed wetlands. *Vadose Zone J.*, **4**(4), 924–938.

Langergraber, G. and Šimůnek, J. (2006). The multi-component reactive transport module CW2D for constructed wetlands for the HYDRUS Software Package. In: *Hydrus Software Series 2*, Department of Environmental Sciences, University of California Riverside, Riverside, CA, 72p.

Mollner, R. (2005). Numerical simulation of the experimental constructed wetlands Ernsthofen. Diploma thesis, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Rousseau, D. (2005). Performance of constructed treatment wetlands: model-based evaluation and impact of operation and maintenance. PhD thesis, Ghent University, Belgium.

Šimůnek, J., Senja, M. and van Genuchten, M.Th. (1999). *The HYDRUS-2D Software Package for Simulating the Two-Dimensional Movement of Water, Heat, and Multiple Solutes in Variably-Saturated Media*. Version 2.0.Manual, US Salinity Laboratory, USDA, Riverside, CA.

Sötemann, S.W., van Rensburg, P., Ristow, N.E., Wentzel, M.C., Loewenthal, R.E. and Ekama, G.A. (2005). Integrated chemical/physical and biological processes modelling Part 2 – anaerobic digestion of sewage sludges. *Water SA*, **31**(4), 545–568.

Tietz, A., Langergraber, G., Sleytr, K., Kirschner, A. and Haberl, R. (2007). Characterization of microbial biocoenosis in vertical subsurface flow constructed wetlands. *Science of the Total Environment*, DOI:10.1016/j.scitotenv.2006.11.034.

Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P. and Takács, I. (1999). Estimating (combinations of) activated sludge model No. 1 parameters and components by respirometry. *Wat. Sci. Technol.*, **39**(1), 195–214.

von Münch, E. and Pollard, P.C. (1997). Measuring bacterial biomass-COD in wastewater containing particulate matter. *Water Res.*, **31**(10), 2550–2556.

Werner, T.M. and Kadlec, R.H. (2000). Wetland residence time modelling. *Ecol. Eng.*, **15**, 77–90.

Wynn, M.T. and Liehr, S.K. (2001). Development of a constructed subsurface-flow wetland simulation model. *Ecol. Eng.*, **16**, 519–536.