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Estimation of flow and transport parameters for woodchip-based bioreactors: I. laboratory-scale bioreactor

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In subsurface bioreactors used for tile drainage systems, carbon sources are used to facilitate denitrification. The objectives of this study were to investigate the validity of first- and zero-order reactions for a laboratory-scale bioreactor, and to estimate flow and transport parameters for a laboratory-scale bioreactor. The laboratory-scale bioreactor used in this study consisted of a polyvinyl chloride (PVC) pipe (0.25 m in diameter and 6.1 m in length) filled with woodchips, with a drainage control structure attached to each end. Creek water and deionised water with NO_3-N concentrations ranging from 8 to 33.7 mg l^{-1} was passed through the bioreactor at several flow rates. For the tests with creek water, complete (100%) nitrate reduction and approximately 10–40% nitrate reduction were observed at high retention times and at low retention times, respectively. The model CXTFIT2, developed by Toride *et al.* (1999, Research report No. 137, U.S. Salinity Lab., ARS, USDA, Riverside, California), and an analytical solution introduced by van Genuchten and Alves (1982, Technical Bulletin No. 1661, U.S. Salinity Lab., USDA, Riverside, California) were used to investigate the assumptions that the reactions obeyed first- and zero-order kinetics. The results revealed that the assumption of first-order reaction kinetics resulted in closer agreement between the model results and the data. However, the estimated reaction terms varied over a factor of 5. These variations were not improved with the single parameter estimation. From these laboratory-scale bioreactor studies, it was concluded that the assumptions of first-order decay for nitrate transport are justified for each single run, and that woodchip-based bioreactors may be suitable for significant nutrient reduction from agricultural fields, artificially drained by tile drain systems.

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Nomenclature

S	rate-limiting substrate concentration, mg l^{-1}
k_m	maximum specific rate of substrate utilisation, h^{-1}
X	concentration of microorganisms, mg l^{-1}
K_s	half saturation concentration, mg l^{-1}
x	distance in the longitudinal direction, cm
t	elapsed time, h
C	nitrate concentration, mg l^{-1}
D	hydrodynamic dispersion coefficient, $cm^2\ s^{-1}$
q	Darcy velocity, cm s^{-1}
n_e	effective porosity
R	reaction term, k_1C, for a first-order reaction; k_0, for a zero-order reaction
k_1	first-order decay coefficient, h^{-1}
k_0	zero-order decay coefficient, mg $l^{-1}\ h^{-1}$
K_e	effective hydraulic conductivity, cm s^{-1}
ΔH	head difference over a column of porous media, cm
L	column length, cm
$\bar{v}$	pore water velocity, cm s^{-1}
t_2	travel time, h
α	dispersivity, cm

1. Introduction

Nitrogen fertiliser is generally applied to crops to increase yield. Applied nitrogen is transformed in the soil through many processes, including nitrification and denitrification. Nitrification, typically by chemoautotrophic bacteria, is the oxidisation of ammonium to nitrate; denitrification, typically by facultative bacteria, is the transformation of nitrate into nitrous oxide or elemental N (Shah and Coulman, 1978). Denitrification occurs under certain specific conditions: an adequate supply of nitrates, an energy (carbon) source, and a marginally anaerobic environment (Tiedje *et al.*, 1989). During this reaction, nitrate serves as a terminal electron acceptor. However, the lack of an adequate energy source is generally a limitation on the reduction of nitrate in groundwater (Starr and Gillham, 1993).

Since nitrate is highly soluble and mobile, excess nitrate that cannot be assimilated by plant life may be directly released to groundwater and surface water, especially where soils are artificially drained by subsurface (tile) systems. Released nitrate can lead to environmental problems (e.g., eutrophication of rivers and lakes), contamination of raw water supplies (e.g., methemoglobinemia in infants – Comly, 1987), and deterioration of water quality (Goolsby and Battaglin, 2000). Nitrates delivered from the Mississippi River Basin to the Gulf of Mexico have contributed to hypoxia (defined as dissolved oxygen concentrations less than 2 mg l^{-1}) in the Gulf of Mexico. Nitrogen fertilisers and mineralised soil nitrogen were determined to be the main sources of nitrate flowing from the Mississippi River Basin to the Gulf of Mexico (Goolsby *et al.*, 1999, Rabalais *et al.*, 2002).

Several substances such as sucrose (Sison *et al.*, 1995), methanol (Wang *et al.*, 1995; McCleaf and Schroeder, 1995; Reising and Schroeder, 1996), and glucose (Shah and Coulman, 1978) have been used as carbon sources to stimulate denitrification. Solid carbon sources such as sawdust (Robertson and Cherry, 1995; Schipper and Vojvodic-Vukovic, 1998; Bedessem *et al.*, 2005) and shredded newspaper (Volokita *et al.*, 1996) have been also used. Wang *et al.* (1995) reported that the optimal pH for nitrate degradation at 30 °C was between 7.4 and 7.6, and the optimal temperature for the process was approximately 38 °C. Shah and Coulman (1978) reported that the denitrification process can be described by a first-order reaction, and that three parts of glucose were required to completely reduce one part of nitrate. Schipper and Vojvodic-Vukovic (1998) reported that the total concentration of carbon did not decrease significantly over time, in spite of a decline of the availability of the remaining carbon. Blowes *et al.* (2000) used permeable reactive barriers containing solid-phase organic carbon in the form of municipal compost, and reported that the barriers removed inorganic contaminants and dissolved nutrients. Robertson *et al.* (2007) reported that high nitrate reduction (6–99 mg [N] l^{-1} *vs.* <0.1 mg [N] l^{-1}) was observed in a permeable reactive layer using wood particles.

Research has been conducted on the use of bioremediation to reduce nitrate from tile drains for the past two decades. Janes *et al.* (2008) reported that 55% of nitrate reduction rate was observed in their denitrification wall provided woodchips as a carbon source. Blowes *et al.* (1994) used organic carbon (tree bark, woodchips, and leaf compost) for their pilot systems, and concluded that the conditions of nitrate removal were more rapidly established in the reactor with the mixed organic substances (sand, grow-bark, woodchips, and leaf compost) than a single source of organic carbon, grow-bark. Greenan *et al.* (2006) used cornstalks, cardboard fibres, woodchips with oil, and woodchips alone for their laboratory study. They found that cornstalks as a carbon source were most effective among those substances, and that the reduction rates of nitrate for woodchips were steady over the experiments. Furthermore, they reported that a significant increase in reduction rate was observed by adding soybean oil to woodchips over woodchips alone. Wildman (2002) evaluated the rate of nitrate removal of field-scale bioreactors with woodchips and a mixture of woodchips and gravel. It was reported that nitrate was reduced at a low rate ranging from 3 to 11% for the first few months after the installation of the bioreactors, and that after this period, the reduction rate increased to 95%. These studies have been devoted to determining the feasibility of carbon sources for bioreactors or the nitrate reduction rates of bioreactors.

Research on permeability of woodchip-based bioreactors has been conducted. Robertson *et al.* (2005) proposed a more efficient design for permeable reactive subsurface barriers (PRBs) through a modelling approach. van Driel *et al.* (2006) estimated effective porosity and hydraulic conductivity of wood-based bioreactors, and reported that the values of those

parameters for a lateral flow reactor were approximately 0.7 and $1.2 \pm 10\ \mathrm{cm\ s^{-1}}$, respectively. However, transport parameters such as hydrodynamic dispersion coefficients (or dispersivity) and reactive coefficients (first- or zero-order decay coefficients for nitrate reduction) are needed to characterise nitrate transport, since nitrate transport through bioreactors is a reactive process.

Monod kinetics can be used to represent substrate utilisation rates in porous media (Monod, 1949; Lawrence and McCarty, 1970; Rifai and Bedient, 1995; Hughes, 1999):

$$\frac{dS}{dt} = -\frac{k_m X S}{K_s + S} \tag{1}$$

Where S is a rate-limiting substrate concentration, k_m is maximum specific rate of substrate utilisation, X is a concentration of microorganisms, and K_s is a half saturation concentration.

Depending on substrate concentration, the substrate utilisation can be simplified as follows:

For low substrate concentration ($S << K_s$), pseudo first-order conditions prevail

$$-\frac{dS}{dt} = \frac{k_m}{K_s} X S \tag{2}$$

For high substrate concentration ($S >> K_s$), pseudo zero-order conditions prevail

$$-\frac{dS}{dt} = k_m X \tag{3}$$

Monod kinetics have been used to describe denitrification (Shah and Coulman, 1978; Dincer and Kargi, 2000). For denitrification, a first-order reaction (Parker *et al.*, 1976; Shah and Coulman, 1978; Novotny and Olem, 1994) and a zero-order reaction (Roš, 1995; Robertson and Cherry, 1995; Volokita *et al.*, 1996; Bedessem *et al.*, 2005; Greenan *et al.*, 2006) has been assumed.

The objectives of this study were to investigate the validity of first- and zero-order reactions for a laboratory-scale bioreactor, and to estimate flow and transport parameters (i.e. hydraulic conductivity, effective porosity, dispersivity, or first- and zero-order decay coefficients) for a laboratory-scale bioreactor. These parameters could then be used to improve the operation and the design of field-scale bioreactors.

2. Methods and materials

2.1. Experimental data

To achieve better control and more cost-efficient observation, a laboratory-scale bioreactor (Fig. 1) was designed to simulate nitrate transport through field-scale bioreactors at a scale that reduces the scale-differences in dispersivity values reported by Gelhar *et al.* (1992). The reactor was set up to estimate the flow and transport parameters that are commonly used in reactive transport models in a laboratory of the Department of Agricultural and Biological Engineering, University of Illinois, USA. It consisted of a polyvinyl chloride (PVC) pipe (0.25 m in diameter and 6.1 m in length) filled with woodchips, with drainage control structures attached to the inlet and outlet. The water flow rate through the bioreactor was regulated by adjusting the water levels in the drainage control structures, and was determined by measuring the volume of water that flowed through the outlet in a specified time. The overflow pipe in Fig. 1 was used to maintain a constant upstream head, and was not used in the calculation of flow rate.

For this study, woodchips used as a carbon source were of mixed species with a bulk density of $0.2\ \mathrm{g\ ml^{-1}}$ and an overall size range of 0–5.1 cm in length, and a mass-mean screened size of 1.3 cm. The size distribution by weight was: 2.5–5.1 cm: 4.2%, 1.3–2.5 cm: 32.5%, 0.6–1.3 cm: 33.8%, and <0.6 cm: 29.5%. The woodchips were packed by standing the 0.25-m PVC pipe on end while filling and running water downward through them, which further packed them. Ten sampling ports were installed along the length of the bioreactor for collecting water samples that were analyzed for nitrate concentration. Two sampling ports were located in the inlet and outlet to determine system-wide variations in nitrate concentrations, six sampling ports were installed at 0.87 m intervals along the PVC pipe to obtain sectional profiles of nitrate concentrations, and two sampling ports were placed at the end of the PVC pipe, one on top and one in the centre, to check the possibility of by-pass flow, due to slumping, along the top inside edge of the pipe. All of the ports, with the exception of the port on top of the pipe at the outlet (i.e. 6.1 m from the inlet of the bioreactor), were installed to collect water samples at the middle of the woodchip column. That additional port collected water from the edge of the pipe. The data from the

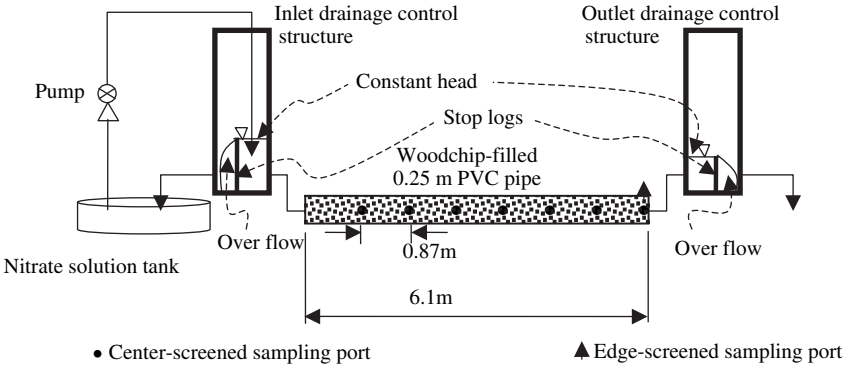

Fig. 1 – Schematic of the laboratory-scale bioreactor.

two outlet ports were used to test the significance of differences of concentrations across the flow cross-section. The Wilcoxon Paired-sample test (Wilcoxon, 1945) was used for this investigation with the R 2.8.1 statistical package (R Development Core Team, 2008). The test is an alternative non parametric test of paired *t*-tests, and requires no *a priori* assumptions about data distributions. The initial intent was to use tile drainage water for this research so that microbial cultures similar to those in the field-scale bioreactors could be formed. However, because of dry weather, little tile flow water was available during the course of the experiments, and creek water and deionised water were used in this study. Blowes *et al.* (1994) reported that the chemical constituents of tile outflow and the stream water employed in their study were similar. Creek water was collected at a place on the Embarrass River (520 m east of First Street and 305 m south of Windsor Road, Champaign, IL) and mostly one day before each run from 2 pm to 5 pm (31/10/2006, 07/11/2006, 13/11/2006, and 16/11/2006 are for 01/11/2006, 08/11/2006, 14/11/2006, and 17/11/2006, respectively) except for the run on 06/11/2006 (collected on 03/11/2006). Nitrate concentration of the collected creek water varied from below the detection limit, which was 0.07 mg [N] l^{-1}, to 1 mg [N] l^{-1} which was lower than that of tile outflow. This lower nitrate concentration may be explained by considering that the creek receives urban runoff which then passes through a stilling pond just upstream of the abstraction point. For this study, potassium nitrate (KNO_3) was added to the creek water and deionised water to replicate the range of the nitrate concentrations measured in tile drain outflow. The targeted upper concentration limit for nitrate was 39 mg [N] l^{-1} based on field measurements reported by Mitchell *et al.* (2000). Approximately 950 l of creek water and deionised water were passed through the bioreactor for each run. The 950 l of creek or deionised water represents approximately 4.4 times the pore volume of the bioreactor. This amount of creek water could be pumped into the bioreactor for approximately 48 h for the runs on 08/11/2006 and on 14/11/2006. Before each run, the bioreactor was flushed with approximate 380 l of creek or deionised water to initialise the nitrate concentrations of the bioreactor. At each sampling event, 100 ml of water were collected from each sampling port. The samples were preserved with concentrated sulphuric acid (0.2 ml), and stored at 4 °C until they were analysed by EPA Method 353.1 (EPA, 2001). The woodchip column bioreactor was installed in an indoor facility whose temperature was maintained at approximately 20–25 °C over the experiments. During the period between creek water and deionised water experiments, the column was constantly saturated in an attempt to maintain anaerobic conditions.

The experimental data for creek water are summarised in Table 1. The pH and temperature of the creek water ranged from 7.5 to 7.9 and from 13.0 to 18.8 °C, respectively. This was within the optimal pH range of 7.0–8.2 (Painter, 1970) for denitrification. The measured temperatures were also within the range of 5–30 °C, the range over which Timmermans and van Haute (1983) reported observing the process of denitrification. The measured ranges of these parameters in the creek water employed in this study indicate that the process of denitrification may not have been significantly inhibited. The head differences and sampling intervals in this study were determined by considering the inlet tank size. By using stop logs to set overflow levels in both the inlet and the outlet drainage control structures, constant head differences of 15–68 mm were generated. The water samples were collected at 1–4 h intervals as specified in Table 1.

The experimental data for deionised water are also presented in Table 1. Deionised water was used as a feedstock in another experiment to investigate, by difference, any potential effects of sediment in the creek water, and to investigate changes in flow and transport parameters after a significant period of inactivity. The runs on 12/06/2007 and on 21/06/2007 were set up with similar initial and boundary conditions, while the flow rate through the system for the run on 13/06/2007 was set lower to examine the difference in biofilm formation between high flow rate and low flow rate (i.e. high retention time and low retention time). The pH and temperature for all three runs were close to the optimal values for denitrification.

2.2. Governing equation

In studies of porous media, it is most often assumed that the nitrate is always dissolved and mobile (i.e. the retardation factor $R_d = 1$) (Novotny and Olem, 1994). Under this assumption, the one-dimensional transport can be described by the following governing equation:

$$\frac{\partial C}{\partial t} = -\frac{\partial}{\partial x}\left(\frac{q}{n_e}C\right) + \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) - R \tag{4}$$

where x is the distance in the longitudinal direction, t is elapsed time, C is the nitrate concentration, D is the hydrodynamic dispersion coefficient (sum of molecular diffusivity and mechanical dispersion coefficient), q is the Darcy velocity, and n_e is the effective porosity. R, the reaction term, for a first-order reaction, is k_1C, where k_1 is the first-order decay coefficient. For a zero-order reaction, R would be k_0, where k_0 is the zero-order decay coefficient.

2.3. Parameter estimation

Hydraulic conductivity is a fundamental parameter in groundwater modelling. The (effective) hydraulic conductivity can be obtained from Darcy's equation:

$$q = K_e\frac{\Delta H}{L} \tag{5}$$

where q is the Darcy velocity, K_e is the effective hydraulic conductivity, ΔH is a head difference over a column of porous media, and L is the column length.

The model CXTFIT2 (Toride *et al.*, 1999) was used for the first-order reaction assumption for the denitrification through the bioreactor. Solutions for infinite boundary conditions are generally used to describe breakthrough curves at the outlet of finite boundary cases (Bear, 1979) with an assumption that the outlet boundary does not affect upstream solute concentrations (Parker and van Genuchten, 1984). Third-type inlet conditions and infinite outlet conditions were used for both first- and zero-order reaction assumptions.

Since the model CXTFIT2 does not provide an inverse problem for zero-order reaction, an analytical solution

Table 1 – Creek water experimental data. (a) Creek water experiment and (b) deionised water experiment

Date of run	pH	Temperature, °C	NO_3-N of water in the tank before KNO_3 added, mg $[NO_3\text{-}N]\ l^{-1}$	Input NO_3-N after KNO_3 added,[a] mg $[NO_3\text{-}N]\ l^{-1}$	Initial NO_3-N in the bioreactor[b] mg $[NO_3\text{-}N]\ l^{-1}$	Head difference, cm	Sampling interval,[c] h
01/11/2006(a)	7.8	16.0	0.3	11.2	0.2	4.4	1
06/11/2006(a)	7.6	17.5	0.8	10.4	0.7	6.8	1
08/11/2006(a)	7.9	18.0	0.5	8.0	0.4	1.5	3–4
14/11/2006(a)	7.7	18.8	<D.L[d]	25.7	<D.L[d]	1.5	3–4
17/11/2006(a)	7.5	13.0	1.7	31.5	1.7	6.3	1
12/06/2007(b)	6.8	25.8	<D.L[d]	23.0	<D.L[d]	6.5	1
13/06/2007(b)	6.8	25.6	<D.L[d]	33.7	0.1	1.5	3–4
21/06/2007(b)	7.0	25.6	<D.L[d]	21.1	<D.L[d]	6.5	1

a $C(x=0,\ t\geq 0)=C_o$.
b $C(x>0,\ t=0)=C_i$, These concentrations were after flushing and before pumping into the bioreactor.
c Samples were taken at 20–30 min intervals for the first hour of each run.
d Below detection limit (0.07 mg $[NO_3\text{-}N]\ l^{-1}$).

(van Genuchten and Alves, 1982) was used for the zero-order reaction assumption. For the implementation of a computer model, an approximate solution was used, given as:

$$C(x,t)=C_i+(C_0-C_i)A(x,t)+B(x,t) \tag{6}$$

$$\begin{aligned}A(x,t)=&\frac{1}{2}erfc\left[\frac{x-\bar{v}t}{2\sqrt{Dt}}\right]+\sqrt{\frac{\bar{v}^2t}{\pi D}}\exp\left[-\frac{(x-\bar{v}t)^2}{4Dt}\right]\\&-\frac{1}{2}\left(1+\frac{\bar{v}}{D}+\frac{\bar{v}^2t}{D}\right)\exp\left(\frac{\bar{v}x}{D}\right)erfc\left[\frac{x+\bar{v}t}{2\sqrt{Dt}}\right]\\&+\sqrt{\frac{4\bar{v}^2t}{\pi D}}\left[1+\frac{\bar{v}}{4D}(2L-x+\bar{v}t)\right]\exp\left[\frac{\bar{v}L}{D}-\frac{(2L-x+\bar{v}t)^2}{4Dt}\right]\\&-\frac{\bar{v}}{D}\left[2L-x+\frac{3\bar{v}t}{2}+\frac{\bar{v}(2L-x+\bar{v}t)^2}{4D}\right]\\&\exp\left(\frac{\bar{v}L}{D}\right)erfc\left[\frac{2L-x+\bar{v}t}{2\sqrt{Dt}}\right]\end{aligned} \tag{7}$$

$$\begin{aligned}B(x,t)=k_0\Bigg\{&t+\frac{1}{2\bar{v}}(x-\bar{v}t+\tfrac{D}{\bar{v}})erfc\left[\frac{x-\bar{v}t}{2\sqrt{Dt}}\right]+\sqrt{\frac{t}{4\pi D}}(x+\bar{v}t+\frac{2D}{\bar{v}})\\&\exp\left[-\frac{(x-\bar{v}t)^2}{4Dt}\right]+\left[\frac{t}{2}-\frac{D}{2\bar{v}^2}+\frac{(x+zt)^2}{4D}\right]\\&\exp\left(\frac{\bar{v}}{D}\right)erfc\left[\frac{x+\bar{v}t}{2\sqrt{Dt}}\right]-\frac{D}{2\bar{v}^2}\exp\left[\frac{\bar{v}(x-L)}{D}\right]\\&erfc\left[\frac{2L-x-\bar{v}t}{2\sqrt{Dt}}\right]+\frac{D}{2\bar{v}^2}\left[1-\frac{\bar{v}(2L-x)}{2D}+\frac{\bar{v}^2}{2D^2}(2L-x+\bar{v}t)\right.\\&\left.(2L-x+3\bar{v}t)+\frac{\bar{v}^3}{6D^3}(2L-x+\bar{v}t)^3\right]\exp\left(\frac{\bar{v}L}{D}\right)\\&erfc\left[\frac{2L-x+\bar{v}t}{2\sqrt{Dt}}\right]-\frac{1}{\bar{v}}\left[-1+\frac{\bar{v}}{2D}(2L-x+7\bar{v}t)\right.\\&\left.+\frac{\bar{v}^2}{6D^2}(2L-x+\bar{v}t)^2\right]\sqrt{\frac{Dt}{\pi}}\exp\left[\frac{\bar{v}L}{D}-\frac{(2L-x+\bar{v}t)^2}{4Dt}\right]\Bigg\}\end{aligned} \tag{8}$$

where $C(x,0)=C_i$, $(-D\frac{\partial C}{\partial x}+\bar{v}C)|_{x=0}=C_0$, and $\bar{v}=\frac{q}{n_e}$.

A nonlinear least-squares technique for fitting the analytical solution to the observed breakthrough curves was used to estimate the hydrodynamic dispersion coefficients (or dispersivity) and the zero-order decay coefficient of the laboratory-scale bioreactor. The parameters were determined by minimising the sum of squared residuals.

The validity of both first- and zero-order reaction assumptions was investigated by comparing with the observed breakthrough curves. $\bar{v}$, D, and k_1 or k_0 were estimated by the model CXTFIT2 or the analytical solution (Eqs. (6)–(8)) (three parameters estimation). Also, k_1 or k_0 was estimated by the model or the analytical solution with setting a fixed α as an average dispersivity α for better assumptions between first- and zero-order reaction assumptions. This is denoted as single parameter estimation. This additional estimation was conducted to investigate if the single parameter estimation provided more consistent reaction terms (k_1 or k_0). Effective porosity n_e can be calculated with travel time t_2 (i.e. retention time), the length of the bioreactor L, and Darcy's velocity q, with the following expression:

$$n_e=\frac{qt_2}{L} \tag{9}$$

Travel time of solute can be estimated as a point of inflection of a breakthrough curve (Freeze and Cherry, 1979). The travel time of solute can also be obtained by dividing the length of the bioreactor L by $\bar{v}$. Both estimated travel times were compared. D can most often be assumed as $\alpha\times\bar{v}$ for high flow rates. Therefore, the reaction terms will be the only unknown in Eq. (4), assuming a value of α. For the three parameters estimation, Sim_first3 and Sim_zero3 were denoted for the first- and zero-order reaction assumptions, respectively, and for the single parameter estimation, Sim_first1 and Sim_zero1 for the first- and zero-order reaction assumptions, respectively.

3. Results and discussion

3.1. Concentration profiles

The by-pass effect was investigated separately for the creek water and deionised water experiments, because they were performed approximately seven months apart. Fig. 2 shows the comparison of the nitrate concentrations from the center, with those from the edge of the woodchip column. The slope of the fitted line for the creek water experiment was close to 1 and the coefficient of determination (R^2) of the fitted line was

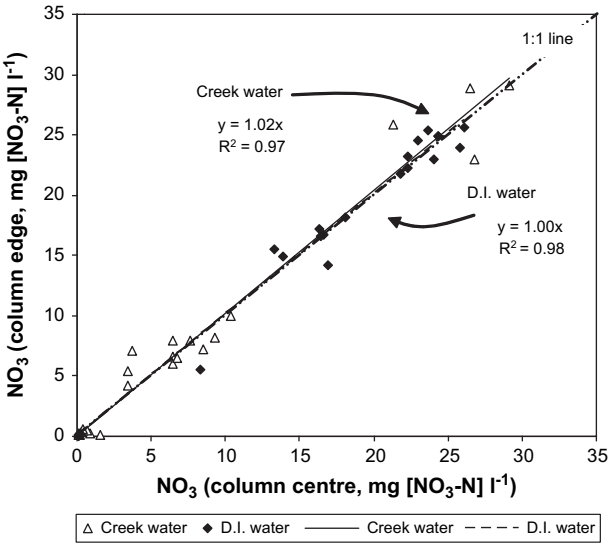

Fig. 2 – Comparison of the nitrate concentrations from the centre and the edge of the woodchip column (D.I water: Deionised water).

0.97, with the *p*-value of the Wilcoxon Paired-sample Test being 0.676. For the deionised water experiments, the slope and the coefficient of determination (R^2) of this graph were approximately 1 and 0.98, respectively, and the *p*-value for the Wilcoxon Paired-sample Test was 0.458. These results indicate that there was no significant difference in concentration between the edge and the center of the PVC pipe, and therefore, no significant by-pass flow.

The two nitrate concentration profiles for the creek water runs which were not used for the estimation of the flow parameters of the bioreactor are shown in Fig. 3. Complete (100%) nitrate reduction was observed at low flow rates for influent concentrations of 10.4 mg $[NO_3\text{-}N]\,l^{-1}$ and 25.7 mg $[NO_3\text{-}N]\,l^{-1}$ (Fig. 3a and b, respectively), while 10–40% nitrate reductions were measured at relatively high flow rates (Fig. 4). This result is consistent with earlier findings suggesting that more than eight hours of retention time was required for complete nitrate reduction (Doheny, 2002). The removal of nitrate through the bioreactor was similar to that (30–40%) of wood-based reactors by van Driel *et al.* (2006). Blowes *et al.* (1994) reported that 2–6 mg $[NO_3\text{-}N]\,l^{-1}$ reduced to less than 0.02 mg $[NO_3\text{-}N]\,l^{-1}$ through organic carbon served barrels. However, this result was at much longer retention times (up to 2 weeks). For the first deionised water run (Fig. 4d), the retention time was 2.7 h, and there was no appreciable reduction in nitrate concentration. This is most likely due to the absence of microbes in the deionised water to inoculate the bioreactor. The retention time for the second deionised water run was 12.1 h. However, once the biofilm was formed during the second run, a retention time of 2.9 h in the third deionised water run resulted in about a 20% decrease in nitrate concentration (Fig. 4f).

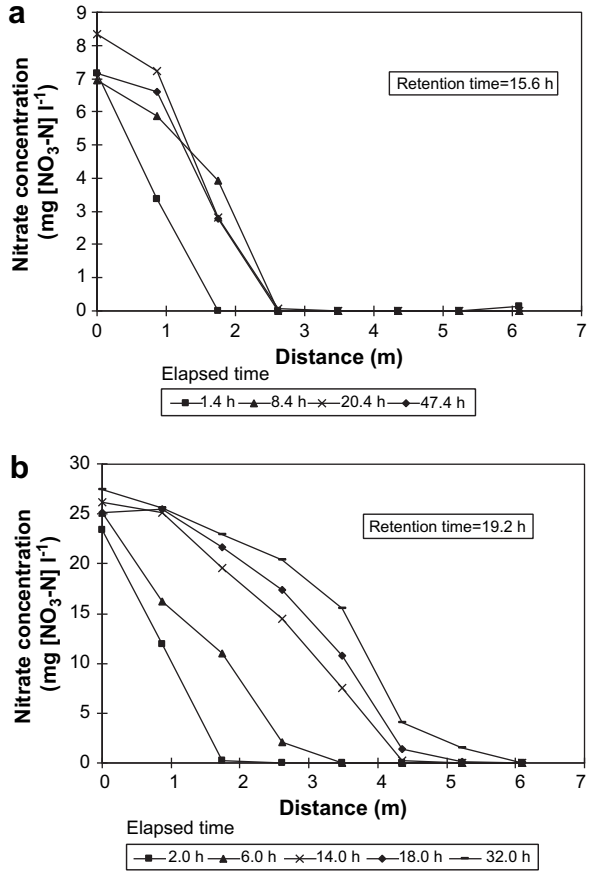

Fig. 3 – Nitrate concentration profiles of runs on (a) 08/11/2006 and (b) on 14/11/2006.

3.2. *Flow parameters*

Flow rates at the outlet are presented in Table 2. These flow rates were taken at irregular intervals, and not all measured flow rates are presented. As shown in Table 2, the flow rate increased over time for the runs with inlet/outlet head differences of 6.3, 6.5, or 6.8 cm of head difference over the run, and decreased for the runs with 1.5 cm of head difference. This tendency may be indicative that stable biofilms were formed at low flow rates (i.e. 1.5 cm of head difference), and that biofilms may be washed off at high flow rates (i.e. 6.3, 6.5 or 6.8 cm of head difference). At high flow rates, it is possible that woodchip fines will be washed off, leading to higher porosity and conductivity. However, we observed no appreciable change in overall head difference at the same throughput rate between the beginning and the end of the whole set of experimental runs.

Table 2 summarises the hydraulic parameters of the laboratory-scale bioreactor. The flow rate, Darcy's velocity, and effective hydraulic conductivity were determined by averaging observed data. The effective hydraulic conductivity ranged from 2.7 to 4.9 cm s^{-1}. These results were similar to the effective hydraulic conductivity estimated by van Driel *et al.* (2006). At comparative flow rates, the effective hydraulic conductivity of the deionised water experiments exceeded those of the creek water experiments. The effective hydraulic conductivity of the bioreactor decreased with successive runs for both the creek water and the deionised water experiments. These results may indicate that there was significant microbial growth in the bioreactor as the experiments proceeded. Several researchers have suggested that biofilm formation leads to the reduction of

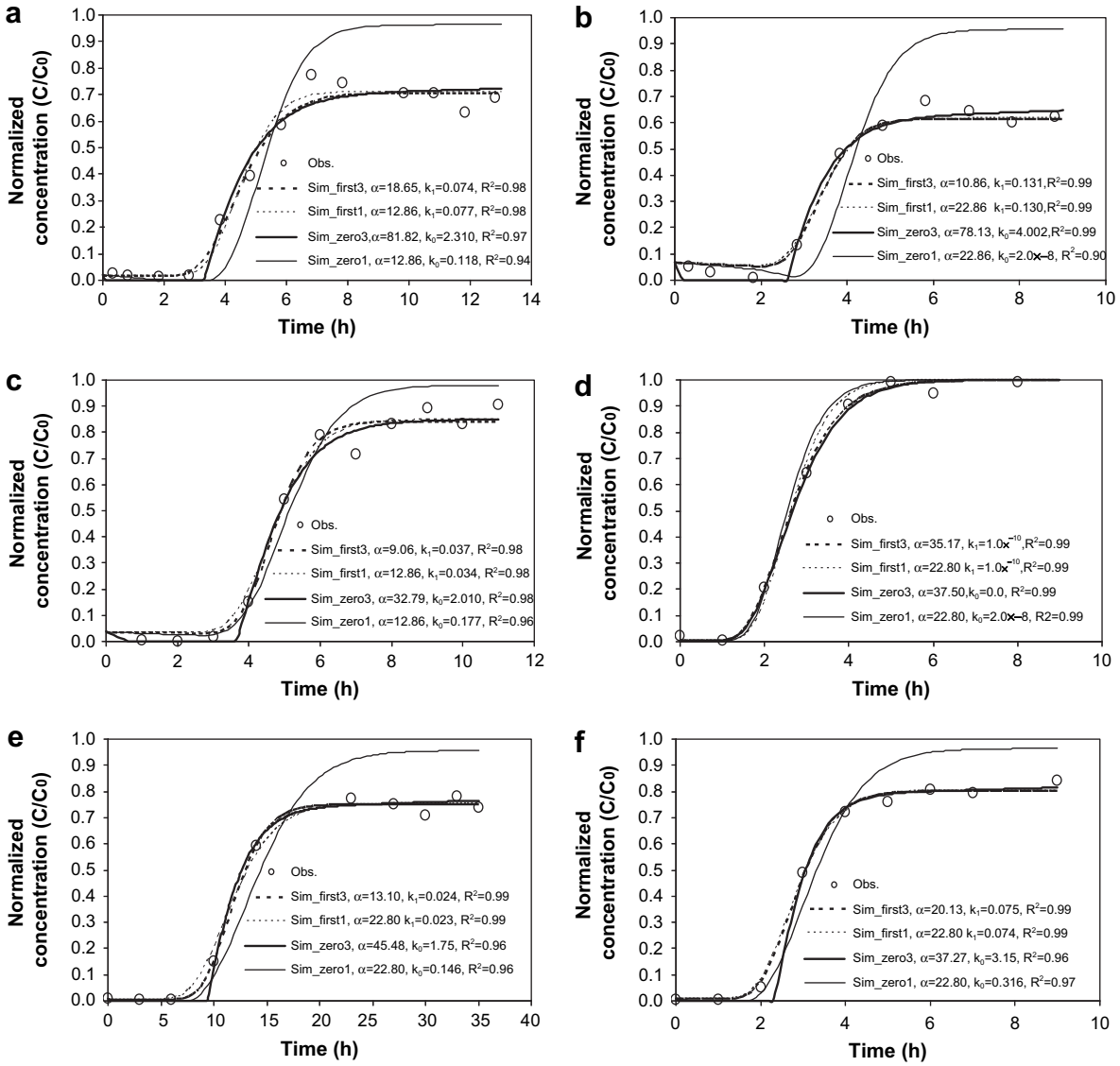

Fig. 4 – Comparison of the observed and fitted normalised concentrations for creek water experiment (a) 01/11/2006, (b) 06/11/2006, and (c) 17/11/2006) and deionised water experiment ((d) 12/06/2007, (e) 13/06/2007, and (f) 21/06/2007) (Obs.: observed data, Sim_first3: simulated data from three parameters estimation (pore water velocity $\bar{v}$, hydrodynamic dispersion coefficient D, and first-order decay coefficient k_1), Sim_first1: simulated data from single parameter estimation (k_1), Sim_zero3: simulated data from three parameters estimation ($\bar{v}$, D, and zero-order decay coefficient k_0), and Sim_zero1: simulated data from single parameter estimation (k_0), α: dispersivity, and R^2: coefficient of determination.

hydraulic conductivity in bioreactors (e.g., Taylor *et al.*, 1990; Dennis and Turner, 1998; Daniels and Cherukuri, 2005). Since the deionised water and the creek water experiments showed the same trends, sediments in the creek water may be not a factor in the decrease of hydraulic conductivity. The variation in conductivity for the deionised water experiment was smaller than that for the creek water experiment. A possible explanation is that the creek water experiment was carried out over a longer time period than the deionised water experiment.

3.3. *Transport parameters*

The three creek water runs (01/11/2006, 06/11/2006, and 171/11/2006) with observed breakthrough curves at the outlet, and the three deionised water runs were used to estimate transport parameters for the bioreactor. The model CXTFIT2 and an analytical solution (van Genuchten and Alves, 1982) were used to estimate $\bar{v}$, D, and k_1 or k_0 for first- and zero-order reaction assumptions, respectively. The parameters were estimated by the models, although the measured flow rates varied throughout each run (Table 2). Since the variation in flow rate only exceeded 10% for the relatively long runs (36 or 48 h), the steady state representation of flow was deemed appropriate.

Table 3 summarises the estimated values for $\bar{v}$, D, and k_1 or k_0 with three parameters estimation. For the first-order reaction assumption (Sim_first3), the estimated retention times by dividing the length of the bioreactor L with $\bar{v}$ were nearly

Table 2 – Measured flow rates of the laboratory-scale bioreactor. (a) Creek water experiment and (b) deionised water experiment

Date of run	Head difference, cm	Number of hours after start, h	Flow rate, ml s^{-1}	Darcy's velocity, cm s^{-1}	Effective hydraulic conductivity,[a] cm s^{-1}
01/11/2006[(a)]	4.4	0.5	14.3	0.03	3.90
		6.8	14.4	0.03	3.95
		12.8	12.7	0.02	3.46
06/11/2006[(a)]	6.8	0.5	20.1	0.04	3.56
		3.3	20.9	0.04	3.71
		8.3	20.8	0.04	3.68
08/11/2006[(a)]	1.5	3.4	4.4	0.01	3.57
		20.4	4.2	0.01	3.40
		44.4	3.8	0.01	3.06
14/11/2006[(a)]	1.5	2.0	4.3	0.01	3.47
		13.0	3.5	0.01	2.80
		48.0	3.1	0.01	2.52
17/11/2006[(a)]	6.3	1.0	13.1	0.03	2.50
		6.0	14.6	0.03	2.78
		11.0	15.4	0.03	2.94
12/06/2007[(b)]	6.5	0.5	24.8	0.05	4.59
		4.1	27.1	0.05	5.01
		6.1	28.0	0.06	5.19
13/06/2007[(b)]	1.5	0.5	6.8	0.01	5.48
		23.0	5.6	0.01	4.52
		36.0	5.3	0.01	4.28
21/06/2007[(b)]	6.5	0.1	24.3	0.05	4.50
		3.0	24.2	0.05	4.48
		9.0	26.9	0.05	4.97

a Averaged values over each run were used for the model CXTFIT2.

identical to the points of inflection of the corresponding observed breakthrough curves (Fig. 4). However, for the zero-order reaction assumption (Sim_zero3), these retention times did not well match the points of inflection of the observed breakthrough curves. The estimated retention times were lower for the zero-order reaction assumption than for the first-order reaction assumption. This can be explained by considering that the estimated pore water velocities were faster for the zero-order reaction assumption than for the first-order reaction assumption.

The effective porosity of the bioreactor, n_e, was estimated with Eq. (9). As summarised in Table 3, the effective porosity of the bioreactor, n_e, ranged from 0.75 to 0.86 for the first-order reaction assumption (Sim_first3). This high range may

Table 3 – Estimated flow and transport parameters of the laboratory-scale bioreactor with three parameters estimation. (a) Creek water experiment and (b) deionised water experiment

Date	First-order reaction assumption						Zero-order reaction assumption					
	n_e[a]	t_2,[b] h	$\bar{v}$,[c] cm s^{-1}	D,[e] cm^2 s^{-1}	α,[f] cm	k_1,[d] h^{-1}	n_e[a]	t_2,[b] h	$\bar{v}$,[c] cm s^{-1}	D,[e] cm^2 s^{-1}	α,[f] cm	k_0,[g] mg l^{-1} h^{-1}
01/11/2006[(a)]	0.75	4.6	0.04	0.69	18.65	0.074	0.48	1.54	0.11	9.00	81.82	2.31
06/11/2006[(a)]	0.84	3.5	0.05	0.53	10.86	0.131	0.08	1.06	0.16	12.50	78.13	4.00
17/11/2006[(a)]	0.79	4.8	0.04	0.32	9.06	0.037	0.81	2.78	0.06	2.00	32.79	2.01
12/06/2007[(b)]	0.82	2.6	0.06	2.27	35.17	1.0×10^{-10}[h]	0.88	2.82	0.06	2.25	37.50	1.0×10^{-10}[h]
13/06/2007[(b)]	0.86	12.0	0.01	0.18	13.10	0.024	0.39	5.46	0.03	1.41	45.48	1.75
21/06/2007[(b)]	0.84	2.9	0.06	1.19	20.13	0.075	0.45	1.54	0.11	4.10	37.27	3.15

a Effective porosity.
b Retention time.
c Pore water velocity.
d First-order decay coefficient.
e Hydrodynamic dispersion coefficient.
f Dispersivity.
g Zero-order decay coefficient.
h Negligible.

indicate the presence of secondary porosity in woodchips. This range was greater than the range (0.48–0.65) measured by Wildman (2002) in his study to estimate the effect of soil overburden on the porosity of woodchips. However, this range was close to the value of 0.7 obtained by van Driel *et al.* (2006). The effective porosity inferred from the deionised water experiment was higher than that from the creek water experiment. This difference may be due to the decomposition of small particles or the decrease of biofilm in the bioreactor in the period between the experiments. However, for the zero-order reaction assumption (Sim_zero3), n_e varied with the much wider range from 0.08 to 0.88. 0.08 of effective porosity may not practically be observed in woodchip particles.

In this study, molecular diffusion may be negligible because the flow rates and mechanical dispersion were relatively high. Therefore, dispersivity can be represented as the quotient of hydrodynamic dispersion coefficient to pore water velocity. The dispersivity of the laboratory-scale bioreactor ranged from approximately 9 to 30 cm and 30 to 80 cm for Sim_first3 and Sim_zero3, respectively. These ranges are within dispersivities of sandy aquifers. For examples, 2–8 cm of dispersivity for fine to medium sand (Schreiber and Bahr, 2002), 96 cm for medium to coarse sand (Garabedian *et al.*, 1988) were reported. However, the estimated dispersivities of the bioreactor cannot be compared because of the scale-differences in dispersivity values reported by Gelhar *et al.* (1992). In fact, Pickens and Grisak (1981) reported the scale-differences in dispersivity values varied from 0.035 cm (laboratory column tracer tests) to 50 cm (full-aquifer dispersivity from the two-well test). No appreciable trend was observed in the estimated dispersivities of the bioreactor. This result is inconsistent with the result of Taylor and Jaffé (1990) who reported that the dispersivity in their study increased as a result of significant biofilm growth. This inconsistency could be resolved by independent determination of the hydrodynamic dispersion and the first-order decay coefficients by the method of moments for step input cases as proposed by Yu *et al.* (1999). The best-fit first-order decay coefficients varied from approximately 0–0.13 h^{-1} and 0–4 mg l^{-1} h^{-1} (Table 3).

Fig. 4 presents the comparison of the observed and fitted breakthrough curves. In the early breakthrough ($t < 2$ h) of nitrate in the creek water experiment (Fig. 4a–c), the models except for Sim_zero3 predicted a slight decrease as the observed concentrations decreased. This decrease was largest at the highest estimated k_1 (the run on 06/11/2006) in creek water experiment. Since the decrease occurred within one residence time (e.g., 3.5 h for the run on 06/11/2006), during this time, pore water (e.g., 0.7 mg [N] l^{-1} for the run on 06/11/2006) seemed to contribute more to the breakthrough than pumping water (e.g., 10.4 mg [N] l^{-1} for the run on 06/11/2006). The nitrate concentration could decrease with reaction (the estimated first-order decay coefficient, k_1 was 0.131 h^{-1}) through the bioreactor before pumping water started to contribute to the breakthrough. Pumping water seemed to mainly contribute to the breakthrough only when the breakthrough started to increase. However, in the deionised water experiment, this phenomenon was not observed (Fig. 4d–f). This phenomenon could be removed by flushing the bioreactor with deionised water so that initial nitrate concentration in the bioreactor was close to zero. In fact, the initial concentrations of the bioreactor became similar to concentrations of the flushed creek water in these circumstances. For example, 0.8 mg [NO_3-N] l^{-1} of creek water was used to flush the bioreactor for the run on 06/11/2006, and 1.7 mg [NO_3-N] l^{-1} for the run on 17/11/2006 (Table 1). The initial NO_3-N concentrations were 0.7 and 1.1 mg [NO_3-N] l^{-1} for the runs on 06/11/2006 and 17/11/2006, respectively. Sudden drops in the fitted breakthrough curves were observed for Sim_zero3 in Fig. 4b and c. This result may indicate that the reaction terms were overestimated for the zero-order reaction assumption at the early breakthrough.

The average of the estimated dispersivities of the bioreactor for the first-order reaction assumption (Sim_first3) was used to investigate if the single parameter estimation provided more consistent reaction terms. However, simulations of the creek and deionised water experiments were split to take the average of the dispersivities, since the range of the dispersivities were narrower in each experiment and since they were performed approximately seven months apart. Values of 12.86 and 20.80 cm for the averaged dispersivities were used for Sim_first1 and Sim_zero1, respectively. Pore water velocities were calculated by dividing q by n_e for the first-order reaction assumption in Table 3, because the estimated effective porosities better represent the retention times than the points of inflection of the observed breakthrough curves. Table 4 summarises the single parameter estimation, for which the first-order decay coefficients were found to be similar to those for the three parameters estimation. The estimated zero-order decay coefficients were lower for the three parameters estimation than for the single parameter estimation. However, for the three parameter estimation and the single parameter estimation, these reaction coefficients ranged similarly over factors of 5 and 2 for k_1 and k_0, respectively, excluding the run on 13/06/2007.

As shown in Fig. 4, for the three parameter estimation, the simulated breakthrough curves for Sim_first3 were better fitted to the observed breakthrough curves than for Sim_zero3. The single parameters estimation did not provided a better fit to the observed breakthrough than the three parameters estimation. For the single parameter estimation, the larger difference between the plateau of the simulated breakthrough curves for Sim_zero1 and the observed breakthrough curves was observed where the larger reduction amount of nitrate was observed. Since the slopes of the rising part of the breakthrough curve represent the effect of dispersion, and those for Sim_zero1 are similar to those of the observed breakthrough, the reaction terms can explain the difference of the plateau. These differences imply that the zero-order reaction assumption did not represent the reaction through the bioreactor well.

Overall, the first-order reaction assumption was better at explaining the nitrate reduction rate through the bioreactor than the zero-order reaction assumption. This may be explained by the reduced Monod kinetics, Eq. (2). Shah and Coulman (1978) determined the half saturation concentration, K_s as 88 mg l^{-1} in their experiments in which glucose was used as a carbon source. We surmised that, despite the lower molecular weight and higher degradability of glucose, more microbes would be able to colonise the woodchip lattice,

Table 4 – Estimated flow and transport parameters of the laboratory-scale bioreactor with one parameter estimation. (a) Creek water experiment and (b) deionised water experiment

Date	First-order reaction assumption						Zero-order reaction assumption					
	n_e[a]	t_2,[b] h	$\bar{v}$[c], cm s^{-1}	D,[e] cm^2 s^{-1}	α,[f] cm	k_1,[d] h^{-1}	n_e[a]	t_2,[b] h	$\bar{v}$[c], cm s^{-1}	D,[e] cm^2 s^{-1}	α,[f] cm	k0,[g] mg l^{-1} h^{-1}
01/11/2006[(a)]	0.75	4.6	0.04	0.47	12.86	0.077	0.75	4.6	0.04	0.47	12.86	0.118
06/11/2006[(a)]	0.84	3.5	0.05	0.62	12.86	0.130	0.84	3.5	0.05	0.62	12.86	0.176
17/11/2006[(a)]	0.79	4.8	0.04	0.46	12.86	0.034	0.79	4.8	0.04	0.46	12.86	0.177
12/06/2007[(b)]	0.75	4.6	0.04	1.47	22.80	1.0×10^{-10}[h]	0.75	4.6	0.04	1.47	22.80	2.0×10^{-8}[h]
13/06/2007[(b)]	0.84	3.5	0.05	0.32	22.80	0.023	0.84	3.5	0.05	0.32	22.80	0.146
21/06/2007[(b)]	0.79	4.8	0.04	1.34	22.80	0.074	0.79	4.8	0.04	1.34	22.80	0.316

a Effective porosity, estimated from three parameters estimation.
b Retention time, estimated from three parameters estimation.
c Pore water velocity, estimated from three parameters estimation.
d First-order decay coefficient.
e Hydrodynamic dispersion coefficient, dispersivity (f column) $\times\bar{v}$ (c column).
f Dispersivity, average of values estimated from three parameters estimation.
g Zero-order decay coefficient.
h Negligible.

leading to higher k_m, and that K_s in woodchip-served systems would be higher than in glucose served systems. Lobry *et al.* (1992) reported a linear increase of K_s with k_m from their mathematical approach for Monod kinetics and published data. It was also assumed that the K_s in woodchip-served systems is sufficiently higher than the mean of nitrate concentrations of tile outflow (13.5 mg $[NO_3\text{-}N]$ l^{-1}) reported by Mitchell *et al.* (2000) so that the first-order approximation, Eq. (2) could be valid. If the microbial concentrations are constant once significant biofilm are formed, then the term, $(k_m/K_s)X$ in Eq. (2) can be reduced to a constant (i.e. a first-order coefficient). Obviously, however, this constant is dependent on microbial concentration, and thus may change with time and position in the woodchip column.

4. Summary and conclusions

A laboratory-scale bioreactor was designed to simulate nitrate transport and transformation through field-scale bioreactors at a scale that reduces the scale-differences in dispersivity values reported by Gelhar *et al.* (1992), and was set up to estimate the flow and transport parameters. For the creek water runs, complete (100%) nitrate reduction and approximately 10–40% nitrate reduction were observed at high retention times and at low retention times, respectively. This result suggests that woodchip-based bioreactors may be suitable for nutrient reduction from artificially drained agricultural field by tile drain systems.

Flow and transport parameters were evaluated for a laboratory-scale bioreactor. The computer model CXTFIT2 for the first-order reaction assumption and an analytical solution (van Genuchten and Alves, 1982) for the zero-order reaction assumption were used to estimate$\bar{v}$, D, and k_1 and k_0. The estimated effective porosities were more consistent and more practically reasonable for Sim_first3 than Sim_zero3. The estimated retention times were nearly identical to the points of inflection of the observed breakthrough curves. The estimated dispersivity values varied from approximately 9–30 cm with no recognisable trend. The data observed were consistent with an assumption of no appreciable sorption of nitrate and a first-order decay coefficient ranging from approximately 0–0.13 h^{-1}.The results revealed that the first-order reaction assumption yielded a better fit to the data than the zero-order assumption. However, the estimated reaction terms ranged over a factor of 5, excluding the run on 13/06/2007. These variations were not improved by the single parameter estimation. It was concluded that the first-order reaction better explained nitrate reduction in the bioreactor. However, further examination of the laboratory-scale bioreactor experiment revealed that Eq. (2) may be needed to represent continuous nitrate transport through bioreactors, since the estimated reaction terms varied over each run.

A slight change in the permeability of bioreactors (i.e. woodchip column) was observed. However, over the long term, the permeability of bioreactors may vary within a fixed range, because the biofilm can be washed off at high flow rates, or, as they thicken, may slough off due to attachment weakness, even at moderate flow rates. The relationship between permeability and retention time may be instrumental in determining the flow rates at which the bioreactors are most efficacious. A further study on this relationship is recommended.

This study does not address the question of how long the woodchips, will last, and what happens as the supply of labile carbon is consumed. It is recommended that this question also be addressed in a future study. Such studies could include micro-scale studies of single woodchips that probe the biofilm and analyse labile carbon concentration within the woodchip.

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