Kinetics of heterotrophic biomass and storage mechanism in wetland cores measured by respirometry
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ABSTRACT

Although oxygen uptake rate has been widely used in activated sludge for measuring kinetic and stoichiometric parameters or for wastewater characterization, its application in constructed wetlands (CWs) cores has been recently proposed. The aim of this research is to estimate the kinetic and stoichiometric parameters of the heterotrophic biomass in CW cores. Respirometric tests were carried out with pure carbonaceous substrate and real wastewater. Endogenous respiration was about 2 gO₂ m⁻³ h⁻¹ (per unit of bed volume), while the kinetic parameters obtained for COD oxidation were very high (maximum rate per unit of bed volume of 10.7–26.8 gCOD m⁻³ h⁻¹) which indicates high biodegradation potential in fully aerobic environment. Regarding to stoichiometric parameter, the maximum growth yield, Yₓₕ, was 0.56–0.59 mgCOD/mgCOD, while the storage yield, Yₓₜₒ, was 0.75–0.77 mgCOD/mgCOD. The storage mechanism was observed in CW cores during COD oxidation, which leads to the transformation of the external soluble substrate in internal storage products, probably as response to intermittent loads applied in CW systems, transient concentrations of readily biodegradable substrate and alternance of feast/famine periods.

Key words | constructed wetland, heterotrophic biomass, kinetic parameters, respirometry, specific growth yield, storage products

INTRODUCTION

Numerical models of differing complexity have been published in recent years to simulate hydraulic behaviour, biochemical transformation and degradation processes for organic matter and/or nitrogen in constructed wetlands (CWs), for example, using mechanistic models describing reactive transport in saturated or variably saturated conditions as well reviewed by Langergraber (2008). Although some of these models have been used in several applications and a good match of the measured data has been obtained, the parameters used in the models are often assumed from literature and not always obtained from the specific CW plant. Kinetic and stoichiometric parameters involved in biological processes such as CWs can not be taken as universal, since they may be influenced by many factors e.g., the influent wastewater composition, properties of gravel/sand bed, operational strategies, etc. Procedures for the direct and experimental measurement of the kinetic parameters of microbial biomass in CWs are still rare or absent today (Langergraber & Šimůnek 2005). Some authors have highlighted the need for further research to develop experimental methods for estimating model parameters, both kinetic and stoichiometric, with the aim of improving the accuracy of numerical models to be used as a reliable design tool for CWs (Langergraber 2008).

The application of respirometric techniques to investigate carbonaceous substrate oxidation or nitrification using columns that simulate cores of vertical subsurface flow CW was first proposed recently by Andreottola et al. (2007). The dynamic of the oxygen uptake rate (OUR) is usually known as respireogram, and batch OUR tests have been widely used in activated sludge processes to measure kinetic and stoichiometric parameters or to characterise wastewater biodegradability. Furthermore, respirometry is often considered as a traditional method in the calibration of activated sludge models (inter alia Vanrolleghem et al. 1999). Although the use of OUR dynamics is still new and not yet fully investigated and understood in the field of CWs, this approach seems promising to obtain kinetic and stoichiometric parameters involved in the oxidation of organic matter, such as the maximum oxidation rate of...
readily biodegradable COD, endogenous respiration and maximum growth yield.

This research aimed to further improve our understanding of the kinetic and stoichiometric parameters of heterotrophic biomass processes occurring in CW cores, interpreting the respiromgrams obtained under aerobic and controlled conditions. Respirometric batch tests were carried out in the presence of (1) pure soluble and rapidly biodegradable carbonaceous substrate (acetate) and (2) raw municipal wastewater. The respirometric tests carried out with pure substrate permitted the calculation of the maximum growth yield of heterotrophic biomass, while the respirometric tests carried out with raw wastewater allowed us to quantify the biodegradable COD of wastewater when applied in a CW system. Particular emphasis was given to the description of the storage mechanisms, which lead to the formation of internal storage products under “feast” conditions and their degradation under ‘famine’ conditions, very similar to the phenomena already observed in activated sludge systems (inter alia Majone et al. 1999; Sin et al. 2005).

MATERIALS AND METHODS

CW cores

The cores (height = 0.60 m; diameter = 0.125 m, volume = 7.4 L) were prepared with filling media with the following characteristics from the bottom of the column: 0.2 m gravel 15–30 mm (porosity p = 31%); 0.1 m gravel 7–15 mm (p = 50%); 0.2 m sand 1–6 mm (p = 28%); 0.1 m sand 1–3 mm (p = 31%). The columns were differently acclimatised for several months using raw municipal wastewater and applying average COD loads of 40 gCOD m⁻² day⁻¹ (equivalent to 2.8 m²/PE). During the acclimatisation period, oxygen transfer was provided only spontaneously during filling and discharge of the cores. All CW cores performed COD removal and nitrification.

Respirometry of CW cores

A new respirometric set-up suitable for application with CW cores and recently developed by Andreottola et al. (2007) was used (Figure 1). The respirometer consists of the CW core connected to pipes and to a peristaltic pump with known flow rate (Q = 18 L/h in our tests) to force a continuous recirculation of flow from the bottom to the top of the core. Aeration is supplied continuously at the top of the core at aeration rate ranging from 2 to 7 NL/min. The liquid volume ($V_L$) in the respirometer was 3.2 L, including the porosity of the media (2.20 L) and the water in the pipes, dissolved oxygen (DO) chamber and a small water column at the top of the core (0.98 L). Two DO probes are placed at the top and at the bottom of the core.

OUR, expressed as mgO₂ L⁻¹ h⁻¹, was calculated by the difference in DO concentrations between the top and the bottom of the core and taking into account the hydraulic retention time in the core (which was 0.18 h in our tests), according to the following expression:

$$\text{OUR (mgO}_2\text{ L}^{-1}\text{h}^{-1}) = \frac{\text{DO}_\text{top} - \text{DO}_\text{bottom}}{\text{HRT}}$$

where $\text{DO}_\text{top}$ = dissolved oxygen measured at the top of the core, expressed as mgO₂ L⁻¹; $\text{DO}_\text{bottom}$ = dissolved oxygen measured at the bottom of the column, expressed as mgO₂ L⁻¹; HRT = hydraulic retention time in the core calculated as the ratio $V_L/Q$ and expressed in hours.

Usually the CW cores were first aerated overnight until endogenous respiration was achieved. Sodium acetate was used as a readily biodegradable carbonaceous substrate and a spike addition correspondent to 187 mgCOD/L was applied in the core. Alternatively, raw municipal wastewater (from Trento Nord WWTP, Italy) with COD concentrations of 324–465 mgCOD/L was used. During the respirometric test with wastewater, the CW core was aerated until endogenous conditions were achieved and then 1.5 L of wastewater was gently added to the water column at the top of the core, replacing 1.5 L of liquid which was extracted from the bottom of the core, being careful to avoid air entrapment. Ammonia in excess was present in all the tests. Allylthiourea was always added to the CW cores to avoid nitrification and to measure only the oxygen consumption of heterotrophic bacteria.

![Figure 1](http://iwaponline.com/wst/article-pdf/64/2/409/444277/409.pdf)
Respirometry of activated sludge

Closed-respirometers consisted of temperature controlled batch reactors where 1.2 L of activated sludge (taken from the oxidation tank of Trento Nord WWTP, Italy) was aerated intermittently between two DO set-points. Aeration and mixing were provided by compressed air and magnetic stirrer. DO was monitored by oximeters (OXI 340, WTW GmbH, Weilheim, Germany) connected to a data acquisition system. OUR was calculated as the slope of DO concentration during a phase without aeration and between the two set-points. In order to inhibit nitrification, allylthiourea was added at the beginning of the respirometric tests.

Chemical analyses

COD was analysed according to Standard Methods (APHA 1995).

RESULTS

Calculation of the respirogram of the CW cores

A respirometric test starts providing aeration overnight in the CW core in order to achieve endogenous respiration. DO concentrations at the top and the bottom of the column are indicated in the example in Figure 2(a) and depend on:

- room temperature (ranging from 22.3 to 26.1 °C), which causes daily DO variations;
- the substrate spike addition (acetate) which causes an immediate significant decrease of DO concentration at the bottom of the column and a slight decrease at the top.

Using the expression described in the Material and Methods section, the OUR was calculated and indicated in Figure 2(b). The OUR profile calculated at room temperature is affected by the daily variations of temperature and therefore it is not easy to identify exactly the profile of endogenous respiration. In order to exclude the influence of temperature variations, the respirogram has to be corrected considering a reference temperature of 20 °C (however another reference temperature could be considered). The correction of temperature was calculated considering the following form of the Arrhenius equation:

\[ \text{OUR}_{20\,^\circ\text{C}} = \frac{\text{OUR}_t}{\alpha(T - 20)} \]  \[ (\alpha = 1.08) \]

but maintaining the same integral under the respirogram (by applying the trapezium rule and changing the time). The respirogram corrected to 20 °C is indicated in Figure 2(c), where the expected regular profile of the data can be immediately observed, which allows us a
better understanding of how the process and the endogenous respiration change over time.

**Respirometry of CW cores using acetate and storage mechanisms**

Respirometry of two different CW cores using acetate and storage mechanisms is indicated in Figure 3. The following phases can be observed:

- **Phase 1:** Initial endogenous respiration (OUR $= 4$–$5 \text{ mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$);
- **Phase 2:** Rapid increase of OUR immediately after the addition of S$_S$ (OUR peak of 14.5–32 mgO$_2$ L$^{-1}$ h$^{-1}$) and rapid decrease of OUR after S$_S$ depletion;
- **Phase 3:** Slow decrease of OUR due to the utilization of the stored compounds, until the endogenous respiration is reached;
- **Phase 4:** Endogenous respiration (OUR $= 4$–$5 \text{ mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$).

After S$_S$ addition, ‘feast’ conditions were formed in the CW core and the OUR increased rapidly up to a peak of 15 and 32 mgO$_2$ L$^{-1}$ h$^{-1}$ in the CW cores a and b respectively (phase 2 in Figure 3). Simultaneously, internal storage products were gradually formed (X$_{STO}$) until all the external S$_S$ was consumed. When S$_S$ was completely removed from the bulk liquid, OUR dropped from the maximum level to a level above the endogenous respiration. During the following phase (phase 3 in Figure 3) OUR decreased more slowly and in general in a non-linear way, to reach the values of endogenous respiration maintained prior to substrate addition. Phase 3 is associated with ‘famine’ conditions, in which the growth of heterotrophic biomass occurs using X$_{STO}$.

The internal storage products, mainly polysaccharides and lipids, are intermediate products in overall carbonaceous substrate removal, which are formed especially when the biomass is subjected to feast and famine conditions, as widely observed in activated sludge systems (inter alia Majone et al. 1999; Carucci et al. 2001; Dircks et al. 2000). The occurrence of the storage mechanism in CW cores is probably due to the intermittent loads applied to the CW cores, causing transient and highly dynamic load conditions, especially when long feast/famine periods are applied. Dynamic conditions can lead to a storage response, even in the absence of any external limitation for the growth. The storage of substrate available under feast conditions allows microorganisms capable of substrate storage to survive during the subsequent famine conditions when the external substrate is depleted (Karahan-Gül et al. 2005; van Loosdrecht et al. 1997). In biological systems with high SRT and low growth rate – CW systems can be considered as belonging to this category – the formation of storage polymers was frequently observed (Sin et al. 2005).

Important stoichiometric parameters in carbonaceous substrate oxidation and storage mechanisms are: the maximum growth yield Y$_H$ which represents the fraction of substrate converted into biomass and the storage yield Y$_{STO}$ which represents the fraction of substrate converted into storage products, then utilised for growth. These parameters can be easily determined by respirometry using acetate, also in the case of CW cores, as performed in this research. For the calculation of Y$_H$ the following expression was used:

$$Y_H(\text{mgCOD/mgCOD}) = 1 - \frac{\Delta \text{O}_2}{S_S} = 1 - \frac{\int_{t_0}^{t_f} \text{OUR}(t) - \text{OUR}_{\text{endogenous}}(t) \, dt}{S_S}$$
where the total amount of oxygen ($\Delta O_2$) needed for the oxidation of the external substrate was calculated as the integral between the respirogram and the endogenous respiration calculated from $t_0$ (time of addition of acetate) to $t_f$ (when endogenous respiration is reached again). $Y_H$ for CW cores was 0.56–0.59 (Table 1) which is lower than the typical value of 0.67 expected for conventional activated sludge.

To calculate $Y_{STO}$ (according to the definition in ASM No. 3, Gujer et al. (1999), in which it is considered that substrate storage is the preliminary step and the subsequent growth occurs solely on the stored products) from the respirometry of CW cores indicated in Figure 3 we adapted a simplified graphic method which does not require model simulation, as proposed by Karahan-Gül et al. (2002). As shown in Figure 3, a straight line was drawn connecting the first OUR point and the final OUR point of phase 2. The area between the respirogram and this straight line is oxygen used for storage ($\Delta O_{STO}$) and so $Y_{STO}$ can be easily calculated (Table 1):

$$Y_{STO} = \left(1 - \frac{\Delta O_{STO}}{S_S} \right)$$

The values of $Y_{STO}$ for CW cores using acetate were 0.75–0.77 mgCOD/mgCOD, lower than the value of 0.85 suggested in ASM No. 3 for activated sludge, but only slightly lower than the value of 0.78 obtained by Karahan-Gül et al. (2003) for activated sludge fed with acetate.

Although the storage mechanism has been extensively investigated and modelled in the field of activated sludge, this phenomenon is new in the field of CWs and models of CW processes have not yet been adapted to include the storage mechanism. In the field of activated sludge, some conceptual modifications have recently been introduced to describe storage mechanisms, such as the concept that biomass growth occurs during both feast and famine phases, using both $S_S$ and storage products (Karahan-Gül et al. 2005; Sin et al. 2005), but further research should be done to investigate whether these recent conceptual models are reliable in CW systems.

The main kinetic and stoichiometric parameters for CW cores are summarised in Table 1, where kinetics were calculated to 20°C and expressed per unit of volume and surface of CWs. The values of maximum OUR and maximum COD removal in Table 1 do not include the endogenous respiration. Considering the duration of the respirometry in Figure 3, a period longer than 24 h is needed to ensure the complete consumption of biodegradable substrate and to reach stable endogenous respiration. Therefore the organic load applied as a spike addition corresponds to a daily organic load of 81 gCOD m⁻³ day⁻¹ and 49 gCOD m⁻³ day⁻¹. The kinetics indicated in Table 1 are calculated per hour because the exogenous maximum rates last several hours. The values indicated in Table 1 may appear high when compared to the typical COD removal rates expected in real CWs. The reason is because during respirometric tests the conditions are prevalently aerobic and the biodegradation kinetics may be overestimated with respect to the kinetics expected in real CWs, where the oxygen transfer from the atmosphere is limited and not enough to ensure a fully aerobic environment. It is well known that anoxic/anaerobic processes are important in CWs, and anaerobic kinetics are considered slower than aerobic ones. However, the high kinetics found in CW cores suggest the high potential of CW systems in biodegradation, if oxygen were not the limiting factor.

Although the kinetics of CW cores a and b were different, and higher for core b, the parameters $Y_H$ and $Y_{STO}$ were similar. The reason is because the kinetics depend

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CW core a</th>
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<th>CW core b</th>
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<tbody>
<tr>
<td>Endogenous respiration (with acetate)</td>
<td>1.9 gO₂ m⁻³ h⁻¹</td>
<td>1.1 gO₂ m⁻³ h⁻¹</td>
<td>2.0 gO₂ m⁻³ h⁻¹</td>
<td>1.2 gO₂ m⁻² h⁻¹</td>
</tr>
<tr>
<td>Maximum OUR (with acetate)</td>
<td>4.4 gO₂ m⁻³ h⁻¹</td>
<td>2.7 gO₂ m⁻² h⁻¹</td>
<td>11.8 gO₂ m⁻³ h⁻¹</td>
<td>7.1 gO₂ m⁻² h⁻¹</td>
</tr>
<tr>
<td>Max COD removal rate (with acetate)</td>
<td>10.7 gCOD m⁻³ h⁻¹</td>
<td>6.5 gCOD m⁻² h⁻¹</td>
<td>26.8 gCOD m⁻³ h⁻¹</td>
<td>16.2 gCOD m⁻² h⁻¹</td>
</tr>
<tr>
<td>Maximum OUR (with wastewater)</td>
<td>5.0 gO₂ m⁻³ h⁻¹</td>
<td>3.0 gO₂ m⁻² h⁻¹</td>
<td>–</td>
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</tr>
<tr>
<td>$Y_H$</td>
<td>0.59 mgCOD/mgCOD</td>
<td>0.56 mgCOD/mgCOD</td>
<td></td>
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</tr>
<tr>
<td>$Y_{STO}$</td>
<td>0.75 mgCOD/mgCOD</td>
<td>0.77 mgCOD/mgCOD</td>
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on the amount of bacterial biomass in the core, while the stoichiometric parameters are independent of the biomass amount.

Respirometry of CW cores using municipal wastewater and comparison with activated sludge

Respirograms obtained in a CW core and in activated sludge taken from a conventional WWTP (3.5 kgTSS/m³) during the oxidation of the same raw municipal wastewater are compared in Figures 4(a) and (b), respectively. In both cases, after wastewater addition (at time $t_0$) the higher OUR values were due to the oxidation of readily biodegradable substrates, while a gradual decrease of OUR was observed successively, due to the consumption of slowly biodegradable compounds limited by hydrolysis. When the biodegradable substrates were completely oxidised the endogenous respiration (at time $t_f$) was achieved.

The calculation of the integral between the respirogram and endogenous respiration ($\Delta O_2$) allowed us to quantify the biodegradable COD ($COD_B$) in wastewater, according to the procedures proposed by Spanjers & Vanrolleghem (1998) and Vanrolleghem et al. (1999) for activated sludge, and adapted to the case of CW cores. In particular, the following expression was used for CW cores:

$$COD_B (mgCOD/L) = \frac{1}{1 - Y_H} \times \frac{V_{ww}}{V_L} \times \int_{t_0}^{t_f} [OUR(t) - OUR_{endogenous}(t)] dt$$

where $V_L$ is the liquid volume in the respirometer (3.2 L in this case) and $V_{ww}$ is the wastewater added (1.5 L in this case). Once $Y_H$ is known, as determined above (0.575 on average), the concentration of $COD_B$ in the wastewater was easily calculated.

Comparing the respirograms in Figure 4 some similarities and differences can be highlighted:

- A long time was required for the complete oxidation of biodegradable substrate in the CW core (29.6 h) compared to activated sludge (7.5 h);
- The maximum exogenous OUR obtained for the oxidation of readily biodegradable COD was higher in activated sludge (18.5 mgO₂ L⁻¹ h⁻¹) compared to the CW core (11.6 mgO₂ L⁻¹ h⁻¹);
- $Y_H$ was 0.575 in the CW core and assumed 0.67 in activated sludge;
- $COD_B$ in the wastewater was 182 mgCOD/L when applied in the CW core and 214 mgCOD/L when applied in the activated sludge, corresponding to 39.1 and 46% of total COD in the wastewater respectively.

Comparing the endogenous respiration in Figure 4, it can be observed that in the CW core the decay rate was close to zero, much slower than the value measured for activated sludge in Figure 4(b). This behaviour of CW cores suggests that the decay of biomass is slow or its variations are negligible within a relatively short period of 60 h.

**CONCLUSIONS**

This research demonstrated that respirometry may contribute to a better understanding of the mechanisms involved in the oxidation of carbonaceous substrates in CW systems by heterotrophic biomass. New information can help especially when models with mathematical structures similar to the Activated Sludge Models and including storage
phenomena are used (for example, similar to ASM; Gujer et al. 1999; Henze et al. 2000). From respirogams of CW cores, some kinetic and stoichiometric parameters ($Y_{H}$ and $Y_{STO}$) of heterotrophic biomass and wastewater biodegradability were evaluated. One important mechanism occurring in CW cores during the oxidation of carbonaceous substrates was the substrate storage mechanism by the heterotrophic biomass. This mechanism is probably a response to the intermittent/low feeding in CW systems, which creates transient concentrations of readily biodegradable substrate. In different CW cores the kinetics may vary significantly, probably due to the different amounts of heterotrophic bacterial biomass which can be present in the CW cores, while the stoichiometric parameters $Y_{H}$ and $Y_{STO}$ were similar, because stoichiometric parameters are independent from the biomass amount. However, to explain the difference in kinetics, further efforts to quantify the heterotrophic biomass are needed. Kinetics measured with respirometry can be overestimated when fully aerobic conditions are present in the respirometer, while in real CWs the oxygen transfer from the atmosphere is limited and not enough to ensure a fully aerobic environment. This new information may help in the optimisation of design procedures or to estimate maximum oxygen requirements in CWs.

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