



## Bacteria distribution and dynamics in constructed wetlands based on modelling results



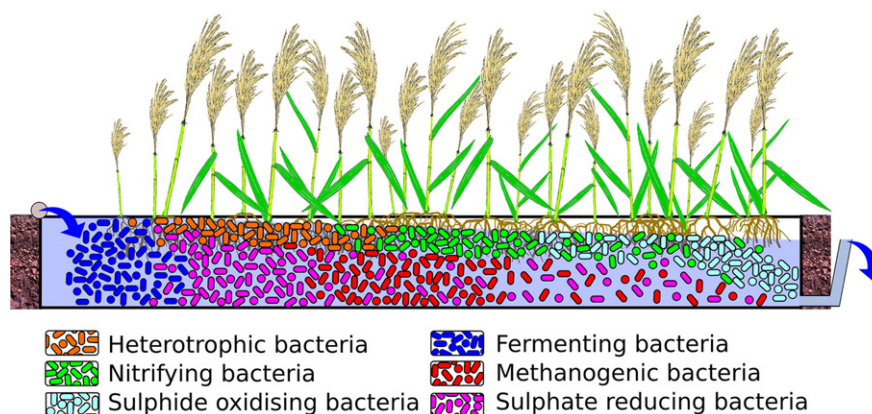
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### HIGHLIGHTS

- This work presents the first long term simulation of bacteria dynamics in wetlands.
- Bacteria biomass and distribution were studied for a 3 year period.
- Bacteria stability was reached between 400 and 700 days.
- Spatial distribution was consistent with previous experimental observations.
- This work presents a full view of how wetlands behave in the long term.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Bacteria communities growing in constructed wetlands play a major role on the removal of pollutants from wastewater and the presence of a stable community is a critical factor affecting their performance. With this work we aimed at finding how long it takes for bacterial communities to stabilise in constructed wetlands and at answering specific questions regarding their abundance, spatial distribution and their relative importance on the treatment processes. To this end the numerical model BIO\_PORE was used to simulate the dynamics of 6 functional bacteria groups (heterotrophic, autotrophic nitrifying, fermenting, acetotrophic methanogenic, acetotrophic sulphate reducing and sulphide oxidising bacteria) within a wetland for a period of 3 years. Three indicators of bacterial stabilisation were used: 1) total biomass; b) effluent pollutant concentrations and c) Shannon's diversity index. Results indicate that aerobic bacteria dominated the wetland until the 80th day of operation. Anaerobic bacteria dominated the wetland from that moment and until the end of the studied period. Bacteria stability was reached between 400 and 700 days after starting operation. Once the wetland reached stability, sulphate reducing bacteria accounted for the highest biomass of all bacterial groups (46%). The distribution of bacterial communities obtained after bacterial stability is consistent with available experimental results, and was clearly controlled by dissolved oxygen (SO) concentrations and H<sub>2</sub>S toxicity. After stability, the progressive accumulation of inert solids pushed the location of the active bacteria zone towards the outlet section.

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## 1. Introduction

Subsurface flow constructed wetlands (SSF CWs) are nowadays one of the most common types of eco-technologies for wastewater treatment used throughout the world. In SSF CWs three relevant elements contribute to wastewater treatment: granular medium, plants and bacterial communities. However, from these three, it is widely accepted that the activity of diverse bacterial communities growing in the form of biofilms is the most important element in pollutant transformation and removal (Ahn et al., 2007; Faulwetter et al., 2009; García et al., 2010; Iasur-Kruh et al., 2010; Krasnits et al., 2009; Ramond et al., 2012; Truu et al., 2009).

The idea that in SSF CWs coexist diverse bacterial communities with completely different metabolic requirements and functional roles (i.e. from strictly aerobes to anaerobes, from autotrophs to heterotrophs) has been progressively built through intense fundamental research on this technology. The role of bacterial communities was put into evidence when the activity of bacterial groups was inferred by measuring concentration changes of different electron acceptors (i.e. oxygen, nitrate and sulphate) and donors (i.e. ammonia and dihydrogensulphide) in pilot wetland systems (Aguirre et al., 2005; García et al., 2004b; Huang et al., 2005). The detection of gaseous or volatile intermediate and end-products such as methane, nitrous oxide and dimethylsulphide was also an indication of the metabolic activity of different bacterial groups (García et al., 2005; Huang et al., 2005; Mander et al., 2003; Teiter and Mander, 2005). More recently, characterisation of bacterial communities in SSF CWs has proliferated with the advent of molecular microbiology methods such as fluorescence in-situ hybridisation and PCR-based techniques, and other advanced methods such as community-level physiological profiling (Criado and Becares, 2005; Krasnits et al., 2009; Ramond et al., 2012; Weber and Legge, 2011). Results obtained with such studies revealed that SSF CWs account for a diversity of bacterial strains comparable to that found in natural ecosystems (Calheiros et al., 2010; Criado and Becares, 2005; Hadwin et al., 2006; Hallberg and Johnson, 2005; Hench et al., 2004; Sims et al., 2012; Sleytr et al., 2009). However, most of these studies consist in point-in-time observations and only a few investigated temporal dynamics of bacterial communities in SSF CW (Ramond et al., 2012; Truu et al., 2009; Weber and Legge, 2011). This is probably due to the fact that such experimental studies involve complex methodologies which are generally very time and resource-consuming. Therefore, complementary techniques could help to give a wider image of the temporal evolution of microbial communities in SSF CWs. Mathematical models are one such complementary technique, with which bacteria dynamics and interrelations can be studied under different scenarios and at much lower costs.

The BIO\_PORE model is a mathematical code built in the COMSOL Multiphysics™ platform that was specifically designed to simulate the behaviour of SSF CWs and incorporates the biokinetic reactions described in the Constructed Wetland Model Number 1 (CWM1) (Samsó and García, 2013). CWM1 was published with the aim of providing a widely accepted formulation for the main bacterial transformation and degradation processes of organic matter, nitrogen and sulphur in SSF CWs (Langergraber et al., 2009). It inherits the formulation of the Activated Sludge Model (ASM) series of the International Water Association (IWA) and includes 6 functional bacteria groups which, based on previous experimental and field evidences, are seen as the most relevant on wetlands functioning. CWM1 has been implemented in different simulation platforms (Langergraber and Šimunek, 2012; Llorens et al., 2011a,b; Mburu et al., 2012) and the resulting codes have been used to match experimentally measured effluent pollutant concentrations, while giving little attention to the results on bacteria abundance, distribution and interrelations. Moreover, to study the temporal dynamics of bacterial groups from modelling results, long-term simulations are required and BIO\_PORE is, to our knowledge, the only model able to run long-term simulations of continuously fed SSF CWs. Besides, one of the most useful features of BIO\_PORE is that it allows for

exploratory research, as each inhibitory/limiting term affecting the growth of the different bacteria groups can be studied individually and its relative impact on bacteria dynamics can be pinpointed. In our previous work, the equations of the BIO\_PORE model were presented, the model was calibrated and the main hypotheses, assumptions and limitations were discussed (Samsó and García, 2013).

The presence of a well-developed and stable microbial community is generally considered to be a critical factor for a good functioning of SSF CWs in terms of pollutants removal (Ramond et al., 2012; Torsvik and Ovreas, 2002; Weber and Legge, 2011; Wohl et al., 2004). Experimental studies in mesocosms suggested that bacteria community stabilisation in SSF CWs is reached between 75 and 100 days (Ramond et al., 2012; Truu et al., 2009; Weber and Legge, 2011). In the present study we demonstrate based on simulations in a pilot system with BIO\_PORE model that bacteria communities reach stability, but that it is a slower process than what has generally been reported. We also study the sequence of processes that lead to bacterial stability and analyse the abundance of the different bacteria groups at different times within a 3 years period. We also analyse the distribution of bacteria communities when stability is reached.

To our knowledge, this is the first time that bacterial communities' distribution and dynamics in SSF CWs are studied from modelling results. It is important to note, though, that by being this the first work of its kind on this topic we considered a simplified scenario (constant influent concentrations, flow-rates and water temperatures) which will gain in complexity once the basis are set.

## 2. Methods

### 2.1. The BIO\_PORE model (Samsó and García, 2013)

BIO\_PORE model was built using Comsol Multiphysics™ and implements fluid flow and transport equations coupled with the biokinetic expressions of CWM1 in a 2D domain representing a longitudinal section of a wetland (Fig. 1). As a distinctive feature from the original CWM1's formulation, in BIO\_PORE slowly biodegradable and inert particulate COD ( $X_S$  and  $X_I$ , respectively) are divided into aqueous ( $X_{S,m}$  and  $X_{I,m}$ ) and solid ( $X_{S,f}$  and  $X_{I,f}$ ) phases to be able to simulate attachment and detachment processes of particulate matter (refer to Samsó and García (2013) for further details).

From the 18 components used by BIO\_PORE, there are 8 dissolved components (all in the aqueous phase) corresponding to dissolved oxygen (SO), ammonia and nitrate nitrogen (SNH and SNO), sulphate and dihydrogensulphide sulphur (SSO4 and SH2S), soluble fermentable COD (SF), fermentation products as acetate (SA) and soluble inert COD (SI); the other 10 are particulate components:  $X_{S,m}$ ,  $X_{S,f}$ ,  $X_{I,m}$  and  $X_{I,f}$ , and 6 functional bacteria groups (solid phase): heterotrophic, nitrifying, fermenting, methanogenic, sulphate reducing and sulphide oxidising bacteria (XH, XA, XFB, XAMB, XASRB and XSOB, respectively). The most relevant interactions between the different bacteria groups and substrates are depicted in Fig. 2.

The most relevant features of BIO\_PORE are: 1) the inclusion of attachment and detachment processes for influent particulate components, which permits to simulate solids accumulation in the gravel media; 2) a complex hydraulic sub-model which calculates the exact location of the water table level at each time, preventing the growth of bacteria in dry areas of the wetland (above the water level); 3) a biofilm sub-model, which prevents unrealistic growth of biomass and takes into account the effect of inert solids accumulation on bacteria growth. For details on model equations, main hypothesis and assumptions, calibration and limitations, the reader is referred to Samsó and García (2013).

### 2.2. Simulation strategy

A simulation was conducted for a pilot wetland with size and features equivalent to those of one of the wetlands described in García et al.

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