OXIDATION OF METHANE IN BIOTRICKLING FILTERS INOCULATED WITH METHANOTROPHIC BACTERIA

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Abstract

The oxidation of methane (CH_4) using biofilters has been proposed as an alternative to mitigate anthropogenic greenhouse gas emissions with low concentration of CH_4 that cannot be used as a source of energy. However conventional biofilters utilize organic packing materials that have a short lifetime, clogging problems and are commonly inoculated with non-specific microorganisms leading to unpredictable CH_4 elimination capacities (EC) and removal efficiencies (RE). The main objective of this work was to characterize the oxidation of CH_4 in two biotrickling filters (BTFs) packed with polyethylene rings and inoculated with two methanotrophic bacteria *Methylomicrobium album* and *Methylocystis* sp. in order to determine the CH_4 elimination capacity (EC) and CO_2 production (pCO_2) when using a specific inoculum. The repeatability of the results in both BTF was determined when operated at the same inlet load of CH_4. A dynamic mathematical model that describes the CH_4 abatement in the BTFs was developed and validated using mass transfer and kinetic parameters estimated independently. Results showed that EC and pCO_2 of the BTFs are not identical but very similar at all the conditions tested. The use of specific inoculum has shown a faster start-up and higher EC per unit area (0.019 $g_{CH4}\cdot m^{-2}\cdot h^{-1}$) in comparison to most of previous studies at the same CH_4 load rate (23.2 $g_{CH4}\cdot m^{-3}\cdot h^{-1}$). Global mass balance shown that the maximum reduction of CO_2 equivalents was 98.5 $g_{CO2eq}\cdot m^{-3}\cdot h^{-1}$. Model developed described satisfactorily CH_4 abatement in BTFs in a wide range of conditions.

Key words: biofiltration, biotrickling filters, greenhouse gases, global warming, methane oxidation, methanotrophs.

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INTRODUCTION

Methane (CH_4) is considered the second largest contributor to the greenhouse effect with a global warming potential (GWP) of about 23 times greater than carbon dioxide (CO_2). For this reason there is a growing interest in reducing anthropogenic emissions of this gas when its use as source of energy is not feasible due to its low concentration. There are many anthropogenic sources of gaseous emissions with such characteristic that are emitted to the atmosphere, such as those emitted from abandoned landfills, livestock facilities, animal husbandry and some sections of wastewater treatment plants. In all these cases, the microbial oxidation of CH_4 could be a low cost solution compared with physical/chemical technologies (Lopez et al. 2013). This biotechnology takes advantage of the ability of methane-oxidizing bacteria (MOB), also called methanotrophs, which utilize CH_4 as a source of carbon and energy (Sohngen, 1906). In MOBs the incorporation of CH_4 into the metabolism is mediated by the enzyme methane monooxygenase (MMO) that oxidase CH_4 to methanol. In a second reaction, methanol is converted to formaldehyde by a methanol dehydrogenase. Then, the carbon from CH_4 follows its catabolism by the RuMP or serine pathway depending on the type of microorganism, giving rise to the classification of methanotrophs type I and II respectively, being assimilated to biomass or released as carbon dioxide (CO_2) (Hanson and Hanson 1996).

The bio-oxidation of CH_4 has been applied in landfills using covers of compost bio-augmented with MOBs, achieving good CH_4 reductions but without control of the operational conditions (Scheutz et al. 2009; Sadasivam and Reddy 2014). Different configurations of closed bioreactors, like traditional biofilters and biotrickling filters (BTFs), have been tested looking for an improved configuration that allows a better control of the factors that determine the rate of CH_4 bio-oxidation (Nikiema et al. 2009; Rocha-Ríos et al. 2009; Pfluger et al. 2011; Veillette et al. 2011). Traditional biofilters generally utilize organic materials, like soil or compost, as support of the microbial communities established in biofilms over the surfaces of the particles that at the same time can be source of nutrients for the microorganisms. In the bio-oxidation of CH_4 these organic materials have shown to have a short lifetime (<6 months) and problems associated with channeling, clogging and pressure drop in long-term operations (Veillette et al. 2012). In BTFs a biofilm develops on an inorganic material and nutrients are provided by a recirculating solution. Inorganic packing materials have several advantages like good mechanical resistance, low-pressure drop and a more stable behavior in long-term operation. BTFs have been used to study the effect of nutrients concentrations, pH and

temperature on the bio-oxidation of CH_4 because it allows a better control of the operation conditions. Until now there have been reported the use of non-specific microbial communities like active sludge or natural inoculation for the bio-oxidation of CH_4 in biofiltration systems. Table 1 shows a summary of the values reported by different authors using inorganic and organic materials as a support for non-specific microbial communities. There are no reports of bio-oxidation of CH_4 using BTFs inoculated with pure cultures of methanotrophic bacteria.

Table 1 Bio-oxidation of CH_4 in different reactors with different inoculum and packing materials

The use of non-specific microbial communities can lead to long startup periods and different communities can evolve obtaining different performances. Although biofiltration studies generally do not have duplicate systems to evaluate the repeatability in biofilters (Jimenez et al. 2016) the complexity of the mechanisms involved leads to accept that repeatability is not ensured. In this sense the use of specific methanotrophic bacteria as inoculum of a BTF can be an effective way to get more reproducible CH_4 results that can be predicted through a dynamic model that considers the kinetic parameters of these microorganisms and mass transport processes of the system. There are few reports on the modeling and simulation of the biofiltration of CH_4 probably due to the scarce experimental data to build and validate reliable models. Mrazovac et al. 2012 focused on the preliminary stage of the biological degradation of CH_4; the diffusion of CH_4 in water by irreversible absorption and desorption. Ordaz et al. (2014) characterized the impact of a non-aqueous phase on the kinetics of CH_4 bio-oxidation using respirometry techniques. Only one empirical model has been reported and was developed for a compost-based biofilter (Plessis 2003). Nikiema et al. (2009b) proposed a model for the biofiltration of CH_4 taking into account the variables concentration, velocity and temperature. This model simulates the biofiltration of CH_4 at steady conditions in a range of concentrations between 1500 and 9500 ppm and considering a constant biomass concentration. Other studies determining kinetic parameters of methanotrophs have set different kinetic parameters for different concentrations ranges (Delhoménie et al. 2008; Ménard et al. 2014; Rodrigues et al. 2009; Boiesen et al. 1993; Ordaz et al. 2014).

The main objective of this work was to characterize the oxidation of CH_4 in biotrickling filters (BTFs) inoculated with *Methylomicrobium album* and *Methylocystis* sp., type I and type II methanotrophic bacteria respectively, assessing the CH_4 bio-oxidation repeatability through a statistical comparison of two identical BTFs. A

comprehensive dynamic model of the bio-oxidation of CH_4 using BTFs was also developed and validated using the experimental set of data.

MATERIALS AND METHODS

Biotrickling filters set up

Two identical biotrickling filters (BTFs) were set up using transparent tubes of polyvinyl chloride (PVC) of 0.153 m internal diameter (ID) and 1.20 m of height with gas sampling ports located every 30 cm from inlet to outlet. Polyethylene rings (OD =15 mm, ID = 13 mm, H=10 mm, density 1.02 $kg \cdot L^{-1}$, external specific area 316 m^{-1} and 77% void fraction) were used as a support for the biofilm. The total packing volume (*V*) was 20 L. Both BTFs were inoculated with active cultures of methanotrophic bacteria type I and II, *Methylomicrobium album* (ATCC 33003) and *Methylocystis* sp. (ATCC 49242), grown using CH_4 as sole carbon and energy source in a nitrate mineral salts liquid medium (NMS, ATCC 1306). The composition of *NMS medium* was: 1.0 $g \cdot L^{-1}$ $MgSO_4x7H_2O$, 0.2 $g \cdot L^{-1}$ $CaCl_2x\ 6H_2O$, 1.0 $g \cdot L^{-1}$ KNO_3, 0.272 $g \cdot L^{-1}$ KH_2PO_4, 0.717 $g \cdot L^{-1}$ $Na_2HPO_4x12H_2O$. 2.0 ml of a chelated iron solution and 0.5 ml of a trace elements solution was also added to 1 L of the NMS solution. *Chelated Iron Solution*: Ferric (III): 1.0 $g \cdot L^{-1}$ ammonium citrate, 2.0 $g \cdot L^{-1}$ EDTA sodium salt, 0.3 ml of HCl (concentrated), 100 ml of distilled deionized water. *Trace Element Solution*: 0.5 $g \cdot L^{-1}$ EDTA, 0.2 $g \cdot L^{-1}$ $FeSO_4x7H_2O$, 0.01 $g \cdot L^{-1}$ $ZnSO_4x\ 7H_2O$, 0.003 $g \cdot L^{-1}$ $MnCl_2x4H_2O$, 0.03 $g \cdot L^{-1}$ H_3BO_3, 0.02 $g \cdot L^{-1}$ $CoCl_2x6H_2O$, 0.001 $g \cdot L^{-1}$ $CaCl_2x2H_2O$, 0.002 $g \cdot L^{-1}$ $NiClx6H_2O$, 0.003 $g \cdot L^{-1}$ $Na_2MoO_4x2H_2O$.

The BTFs were continuously fed with a mixture of pre-humidified air and pure CH_4 (99.8% v/v). Different CH_4 concentrations were obtained by mixing air and CH_4 using two mass flow controllers (AFC-37, Aalborg, USA). Inlet concentrations ($[CH_4]_{in}$) between 0.5% - 3.9% (v/v) of CH_4 were fed initially at different gas flow rates to determine the best condition for kinetic tests. The gas flow rate (*F*) used were between 0.2 - 1.0 $L \cdot min^{-1}$. CH_4 and CO_2 were measured on line using an IR detector and O_2 using an electrochemical sensor with a multigas analyzer (Xam 5600, Dräger, Germany). Fresh NMS medium (0.5 L) was supplied every day by spraying it to the top of the columns at rate of 0.5 $L \cdot min^{-1}$. Figure 1 shows a diagram of the experimental setup used for the oxidation of CH_4 in BTFs.

Fig. 1 Diagram of the experimental system for the oxidation of CH_4 in biotrickling filters.

The BTFs operation was characterized by measuring the CH_4 removal efficiency (*RE*) in %, and the CH_4 elimination capacity (*EC*) in $gCH_4 \cdot m^{-3} \cdot h^{-1}$ at different CH_4 loads (*L*) in $gCH_4 \cdot m^{-3} \cdot h^{-1}$ after reaching steady state. Production of CO_2 (pCO_2) in $gC0_2 \cdot m^{-3} \cdot h^{-1}$ and consumption of O_2 (cO_2) in $gO_2 \cdot m^{-3} \cdot h^{-1}$ were also measured. A steady state was considered to be reached when *RE* have a variation less than 5% in consecutive days. These parameters were determined according to the following equations:

$$RE = \frac{[CH_4]_{in} - [CH_4]_{out}}{[CH_4]_{in}} \cdot 100 \quad (1)$$

$$EC = \left([CH_4]_{in} - [CH_4]_{out}\right) \cdot \frac{F}{V} \quad (2)$$

$$L = [CH_4]_{in} \cdot \frac{F}{V} \quad (3)$$

$$pCO_2 = ([CO_2]_{out} - [CO_2]_{in}) \cdot \frac{F}{V} \quad (4)$$

$$cO_2 = ([O_2]_{out} - [O_2]_{in}) \cdot \frac{F}{V} \quad (5)$$

Repeatability assessment

The repeatability of CH_4 bio-oxidation in the BTFs was evaluated with two indicators: EC and pCO_2; using the paired samples Student's t-test according to the methodology proposed by Jimenez et al. (2016). A two-tailed hypothesis testing was used considering that the mean of the differences is equal to zero, i.e. no significant differences exist between BTFs, at a 95% confidence level. Repeatability between BTFs is established qualitatively when the calculated *t* value is under a specific tabulated $T_{critical}$ value, based on the degrees of freedom of the data set (n−1). Similarly, no significant difference exists between biofilters for a p-value > 0.05.

Model of the bio-oxidation of CH_4 in a biotrickling filter

The model developed here considers the most relevant phenomena occurring during the biofiltration process for the bio-oxidation of CH_4 in a biotrickling filter like advection, absorption and diffusion. The assumptions underlying this model are based on a consolidated model reported previously (Dorado et al. 2012):

(1) Gas phase circulation regime is modelled as plug flow pattern. Thus, axial dispersion is not considered.

(2) Gas-biofilm interface equilibrium is described by Henry's law.

(3) Planar geometry and perpendicular diffusion in biofilm are used to derive model equations considering that the solid support size is significantly higher than the biofilm thickness. Diffusion in the biofilm is described by Fick's law.

(4) Biofilm is formed on the external surface of the packing material. Thus, biomass does not grow in the pores of the packing material and reactions only take place in the biofilm phase.

(5) Physical properties of the species in the biofilm are assumed to be the same as in water since this is the main component.

(6) There is no accumulation of biomass in the filter bed in each period and biomass properties (thickness, specific surface area and kinetic coefficients) are uniform along the bed.

(7) Adsorption of pollutant onto the support is neglected due to the low pollutant concentration and the low adsorption capacity of the packing material. Moreover, under steady-state conditions, the adsorption process is in equilibrium.

Dynamic mass balances in the gas phase and within the biofilm serve to describe changes in the biodegradation capacity of the biofilter during operation, overcoming the limitations of previous biofiltration models. The resulting equations are summarized as following:

$$\frac{\partial[CH_4]}{\partial t} = -v_z \cdot \frac{\partial[CH_4]}{\partial z} - \frac{a}{\varepsilon} k_g([CH_4] - \frac{[CH_4]}{H}) \quad (6)$$

$$\frac{\partial [CH_4]_b}{\partial t} = D_B \cdot \frac{\partial^2 [CH_4]_b}{\partial x^2} - \frac{1}{Y_{X/S}} \cdot \mu_{max} \cdot \frac{[CH_4]}{K_S + \frac{[CH_4]}{H}} \cdot X \quad (7)$$

Where $\boldsymbol{v_z}$ is the gas velocity in $m \cdot h^{-1}$; $\boldsymbol{z}$ is the height position from the inlet in m; $\boldsymbol{a}$ is the specific surface area in m^{-1}; ε is the porosity; $\boldsymbol{k_g}$ is the mass transfer coefficient in m h^{-1}; $\boldsymbol{H}$ is the adimensional partition coefficient; $\boldsymbol{[CH_4]_b}$ is the concentration in the biofilm in $g \cdot m^{-3}$; $\boldsymbol{D_B}$ is the diffusion coefficient for CH_4 in the biofilm in $m \cdot h^{-1}$; $\boldsymbol{Y_{X/S}}$ is the yield coefficient biomass/methane; $\boldsymbol{\mu_{max}}$ is the maximum specific growth rate in h^{-1}; $\boldsymbol{K_S}$ is the half saturation constant in $g \cdot m^{-3}$; and $\boldsymbol{X}$ is the biomass concentration in $g \cdot m^{-3}$.

With the following initial and boundary conditions:

At t = 0 $\quad [CH_4] = 0 \; and \; [CH_4]_b = 0;$

z = 0 $\quad [CH_4] = [CH_4]_{in}$

x = 0 $\quad [CH_4]_b = \frac{[CH_4]}{H}$

x = δ $\quad \frac{\partial [CH_4]_b}{\partial x} = 0$

The resulting set of ordinary differential equations was solved using MATLAB. A variable order method was used for solving stiff differential equations based on the numerical differentiation formulas (NDFs). The parameter estimation was performed using a MATLAB algorithm based on a multidimensional unconstrained nonlinear minimization (Nelder–Mead) algorithm.

Biofilter model parameters estimation

The measuring of CH_4 concentration in the gaseous phase of flasks containing an active culture of methanotrophs were used to characterize separately the kinetic of CH_4 bio-oxidation by methanotrophs type I and II for initial concentrations between 1.0 and 6.8 g m^{-3} of CH_4. Maximum specific growth rate (μ_{max}) and half saturation constant (K_S) were determined by using a dynamic model of the batch culture of the microorganisms using methane as a sole source of carbon and energy. In this model the specific growth rate (μ) is replaced by Monod expression (equations 8

and 9). A non-linearization process minimizing the norm of the differences between experimental CH_4 concentration and the model predictions was used for determining the parameters.

$$\frac{d[CH_4]}{dt} = -\frac{1}{Y_{X/S}} \cdot \mu_{max} \cdot \frac{[CH_4]}{K_S + \frac{[CH_4]}{H}} \cdot X \qquad (8)$$

$$\frac{dX}{dt} = \frac{\mu_{max}}{H} \cdot \frac{[CH_4]}{K_S + \frac{[CH_4]}{H}} \cdot X \qquad (9)$$

RESULTS AND DISCUSSION

Elimination capacity

Figure 2 shows the CH_4 elimination capacity (EC) of the BTFs operated both in parallel at the same CH_4 inlet load. The maximum CH_4 elimination capacity (EC_{max}) reached was in average 6.2 $gCH_4 \cdot m^{-3} \cdot h^{-1}$ at an inlet load of 23.2 $g_{CH4} \cdot m^{-3} \cdot h^{-1}$ given by an inlet CH_4 concentration of 3.9% (v/v). Compared with other studies using similar reactor volumes (Table 1) the EC_{max} was low, probably due to the low specific area of the polyethylene rings used as packing material for biofilm formation in the BTFs. However, the maximum specific elimination capacity (EC_{sp}) was 0.019 $g_{CH4} \cdot m^{-2} \cdot h^{-1}$ being greater than the values reported by Nikiema et al. (2009a) at the same inlet load of CH_4 using packing materials with similar and higher specific area, indicating that the high biological CH_4 oxidation activity observed in this work could be related to the use of massive specific methanotrophic inoculum. Likewise, Rocha-Ríos et al. (2009) reported a EC_{sp} of 0.037 $g_{CH4} \cdot m^{-2} \cdot h^{-1}$ in a BTF using polyurethane foam as support with specific area of 600 m^{-1} and inoculated with a methanotrophic consortium. Figure 3 shows photographs taken with scanning electron microscopy (SEM, Jeol/Jem 1200 EX II, camera Gatan ES500W Model 782, USA) of the biofilm formed on the surface of the rings used as support in the lower section of the BTFs. It is possible to observe that the methanotrophic bacteria were properly immobilized on packing material forming a robust biofilm with a similar degree of colonization in both BTFs.

Fig. 2 Elimination capacity (EC) of CH_4 in BTF1 (○) and BTF2 (●) as function of the inlet load of CH_4 (L).

Fig. 3 SEM pictures (5000x) of the biofilm formed in the external side of rings extracted from the lower section of BTF1 (a) and BTF2 (b).

Figure 4 shows that the average production of CO_2 and the consumption of O_2 in BTFs were almost equivalent to the stoichiometric amount of CH_4 oxidized. The proposed stoichiometry for the complete oxidation of CH_4 indicates that 1 mol of CH_4 requires 2 mol of oxygen (O_2) to generate 1 mol of CO_2 (Havran et al. 2011). The difference between the production of CO_2 in the BTFs and the theoretical value obtained for the complete oxidation of CH_4 can be explained by its use as carbon source for microbial growth. The low difference indicates that a high degree of mineralization was achieved in BTFs at inlet loads of CH_4 lower than 10 $g_{CH4} \cdot m^{-3} \cdot h^{-1}$.

Fig. 4 O_2 consumed (♦) and CO_2 produced (▲) as a function of CH_4 elimination capacity.

A carbon mass balance was made considering the carbon from CH_4 (C_{CH4}) and CO_2 (C_{CO2}) in $gC \cdot m^{-3} \cdot h^{-1}$ at the inlet and the outlet of the BTFs to estimate the amount of carbon accumulated (C_{ac}) as biomass into the BTFs, Equations 8, 9 and 10. Figure 5 shows the C_{ac} (in $gC \cdot m^{-3} \cdot h^{-1}$) as function of the CH_4 load. An estimation of the reduction of the global warming potential (GWP) in the gaseous stream was made considering that the GWP of CH_4 is 23 related to the CO_2 (Equation 11).

$$C_{in} = (C_{CH4})_{in} + (C_{CO2})_{in} \quad (8)$$

$$C_{out} = (C_{CH4})_{out} + (C_{CO2})_{out} \quad (9)$$

$$C_{ac} = (C_{in} - C_{out}) \quad (10)$$

$$\text{Red } GWP = (GWP_{CH4}) \cdot EC_{CH4} - pCO_2 \quad (11)$$

For inlet loads of CH_4 below 10 $gCH_4m^{-3}h^{-1}$ the amount of accumulated carbon in the BTFs (C_{ac}) was around $0.1gCm^{-3}h^{-1}$ but when the load of CH_4 was increased over 10 $gCH_4m^{-3}h^{-1}$ it was observed a proportional increase of accumulated carbon in the BTFs. This could be due to the higher availability of CH_4 stimulate the growth of

biomass. In addition methanotrophic bacteria are known for their ability to produce exopolysaccharides (EPS) at high methane flux rates (Huq et al. 1978). According to Equation 11 the maximum reduction of CO_2 equivalents (Red GWP) was 98.5 $gCO_2m^{-3}h^{-1}$ at load of 23.2 $gCH_4 \cdot m^{-3} \cdot h^{-1}$.

Fig. 5 Accumulated carbon (C_{ac}) in the BTF1 (□) and BTF2 (■)

Figure 6 shows the concentrations of CH_4 along the BTF1 at different empty bed residence times (EBRT), and the effect of different inlet concentration of CH_4. The higher variation in the CH_4 concentration along the column was observed in the first section of the BTF1. This effect was accentuated at inlet CH_4 concentrations over 1.0% v/v. The higher variation on CH_4 concentration along the BTF1 was observed at the lower gas velocity tested (1.1 h of EBRT). A similar behavior was observed in the BTF2. This behavior is consistent with the decrease of CH_4 and O_2 concentration from the gas phase to the biofilm as the gas moves through the column of the BTFs and CH_4 is oxidized, decreasing CH_4 and O_2 concentration. Due the high free volume given by polyethylene rings, clogging episodes or even increases of pressure drop after one year of continuous operation were not detected.

Fig. 6 Profiles of CH_4 concentration along the height (H) of the BTF1 at different inlet CH_4 concentration and different empty bed residence time: ♦ 67, ▲ 50, ■ 40, x 30 minutes.

Operation repeatability

Statistical analysis (Student's t test) of the data considering as hypothesis that the BTFs have identical EC and pCO_2 (difference between the means is equal to zero) and 80 degrees of freedom indicated that significant differences between the BTFs were established since the calculated *t* value was higher than the specific tabulated $T_{critical}$ value for both indicators. However, if is used a more flexible comparison criteria (like a reasonable difference between the means), the values measured for EC and pCO_2 are quite similar between the BTFs to consider that the two systems of bio-oxidation of CH_4 have similar behavior. Moreover when the statistical analysis was made by periods of operation, the results indicated an identical behavior ($t < T_{critical}$) to the first 45 days of operation, after which their performance began to distancing probably due to the sum of small differences in the operation like channeling of the gas flow, temperature or pH. In Table 2 are summarized the results for statistical analyses.

Table 2 Results for statistical analyses for BTFs

Estimation of the kinetic parameters of the model

Figures 7 show the experimental data and the model simulation for the bio-oxidation of CH_4 by *Methylomicrobium album* and *Methylocystis* sp. respectively, at different initial concentration of CH_4.

Fig. 7 Experimental data (dots) and model estimation (lines) for the bio-oxidation of CH_4 by (a) *Methylomicrobium album* and (b) *Methylocystis* sp. at different initial concentration of CH_4.

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The estimated kinetic parameters are presented in Table 3 for *Methylomicrobium album* and *Methylocystis* sp., respectively.

Table 3 Kinetics parameters for *Methylomicrobium album* and *Methylocystis* sp.

Simulation of the bio-oxidation of CH_4 in BTFs

Figure 8 shows the effect of flow rate (i.e. contact time) on the RE measured experimentally and predicted for the model developed. In this figure the RE is normalized with respect to RE achieved at the most favorable condition of contact time (100 min) to evaluate the influence of the flow rate on the loss of efficiency at 3 different concentrations (0.5, 1 and 2% of CH_4). RE at contact times of 100 min were respectively 28, 39 and 70% in ascending order of concentration in the case of BTF1 and 31, 40 and 78% in the case of BTF2. Data analysis shows that, independently of methane inlet concentration, the effect of contact time is equivalent in both BTFs: from 0.2 to 0.6 l min^{-1} the loss of efficiency is maximum (50%), considerably inferior (25%) between 0.6 and 1.0 l min^{-1}, and being practically constant from then on (5%). Thus, the critical effect of mass limitation due the low solubility of CH_4 is highly sensitive between 30 and 100 min.

The degree of agreement between experimental RE and model predictions is significantly high according to Figure 8, demonstrating that the model proposed based on mass balances, transport phenomena and biological characterization

can predict the observed behavior by means of a low set of parameters (Table 4). Mainly, it is noteworthy that the model proposed is able to describe satisfactorily 36 different situations were flow rate (from 0.2 to 2.0 l min^{-1}); inlet concentration (0.5, 1.0 and 2.0%) and bio-system (BTF1 and BTF2) were varied in each case. In this table is also possible to compare the parameters values with previous works reported in the field of methane biodegradation. Although in the present work the range of concentrations is wider than those previously studied, a unique set of parameters was able to describe all scenarios monitored, not differing significantly than those reported for other studies.

Table 4 Physical and kinetics parameters values for the bio-oxidation of CH_4.

Fig. 8 Removal efficiency (RE) of CH_4 in the BTFs as function of the gas flow rate at inlet CH_4 concentrations of 0.5% v/v (x), 1.0% v/v (○) and 2.0% v/v (*) for model predictions (continuous lines) and experimental monitoring (discontinuous signs) in the case of BTF1 (a) and BTF2 (b).

CONCLUSIONS

The high degree of feasibility and reproducibility of CH_4 bio-oxidation has been demonstrated in a long-term operation of 1 year for two identical biotrickling filters inoculated with methanotrophic bacteria type I and II, *Methylomicrobium album* and *Methylocystis* sp. The maximum CH_4 elimination capacity reached was in average 6.2 $gCH_4 \cdot m^{-3} \cdot h^{-1}$. The use of specific inoculum has shown a faster start-up and higher EC per unit area (0.019 $g_{CH4} \cdot m^{-2} \cdot h^{-1}$) in comparison to most of previous studies. Plant monitoring let to develop a more comprehensive mathematical model to describe CH_4 biofiltration by means of kinetic and mass transport characterization that predicts the wide range of conditions tested with high agreement with the experimental observations.

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Table captions

Table 1 Bio-oxidation of CH_4 in different reactors with different inoculum and packing materials

Table 2 Statistical analysis of experimental results in Biotrickling filters

Table 3 Kinetics parameters for the bio-oxidation of CH_4 by *Methylomicrobium album* and *Methylocystis* sp.

Table 4 Physical and kinetics parameters values for the bio-oxidation of CH_4.

Figures captions

Fig. 1 Diagram of experimental system for the oxidation of CH_4 in biotrickling filters.

Fig. 2 Elimination capacity (EC) of CH_4 in BTF1 (○) and BTF2 (●) as function of the inlet load of CH_4 (L).

Fig. 3 SEM pictures (5000x) of the biofilm formed in the external side of rings extracted from the lower section of BTF1 (a) and BTF2 (b).

Fig. 4 Moles of O_2 consumed (□) and CO_2 produced (▲) as function of CH_4 elimination capacity.

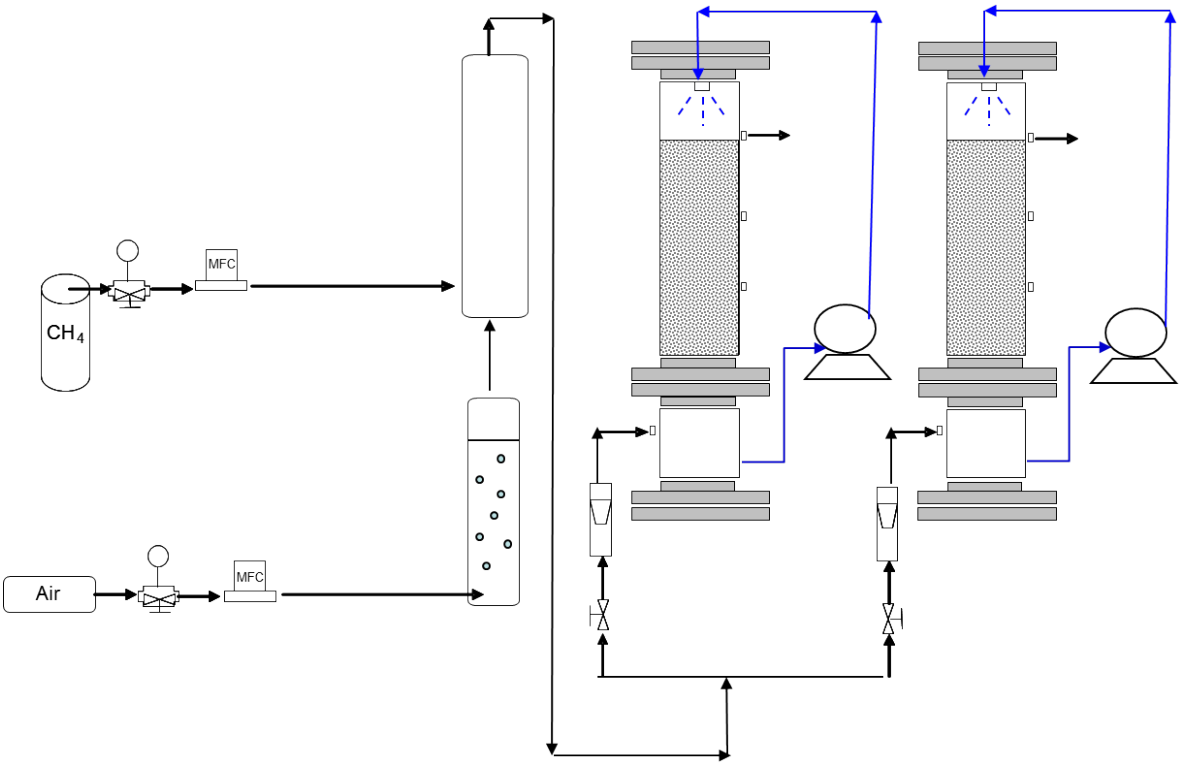
Fig. 5 Accumulated carbon (C_{ac}) in the BTF1 (□) and BTF2 (■)

Fig. 6 Profiles of CH_4 concentration along the height (H) of the BTF1 at different inlet CH_4 concentration and different empty bed residence time: ♦ 67, ▲ 50, ■ 40, x 30 minutes.

Fig. 7 Experimental data (dots) and model estimation (lines) for the bio-oxidation of CH_4 by (a) *Methylomicrobium album* and (b) *Methylocystis* sp. at different initial concentration of CH_4.

Fig. 8 Removal efficiency (RE) of CH_4 in the BTFs as function of the gas flow rate at inlet CH_4 concentrations of 0.5% v/v (x), 1.0% v/v (○) and 2.0% v/v (*) for model predictions (continuous lines) and experimental monitoring (discontinuous signs) in the case of BTF1 (a) and BTF2 (b).

Figure 1 was created using Microsoft Office.



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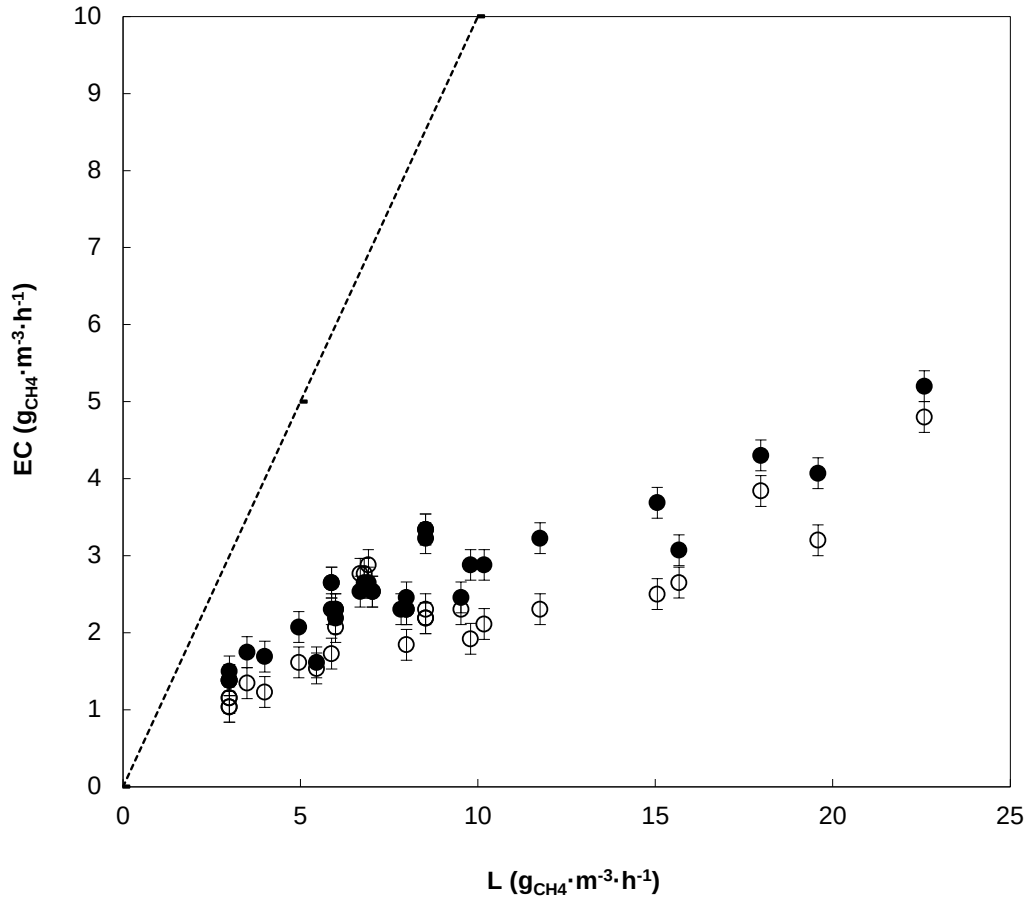
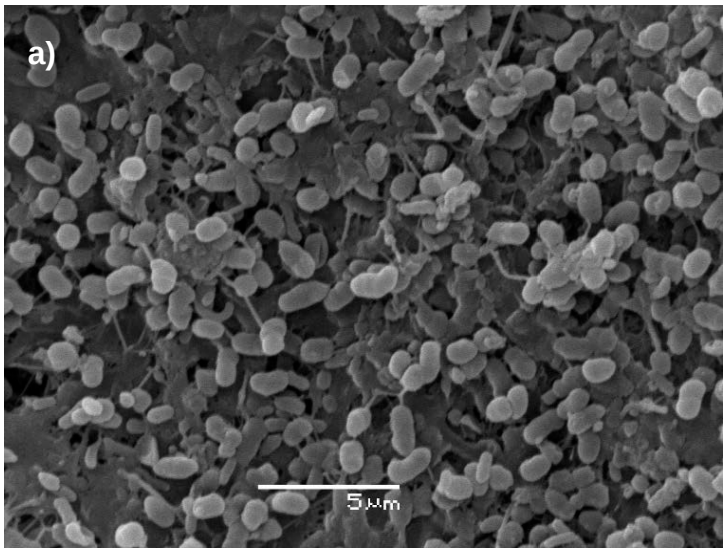
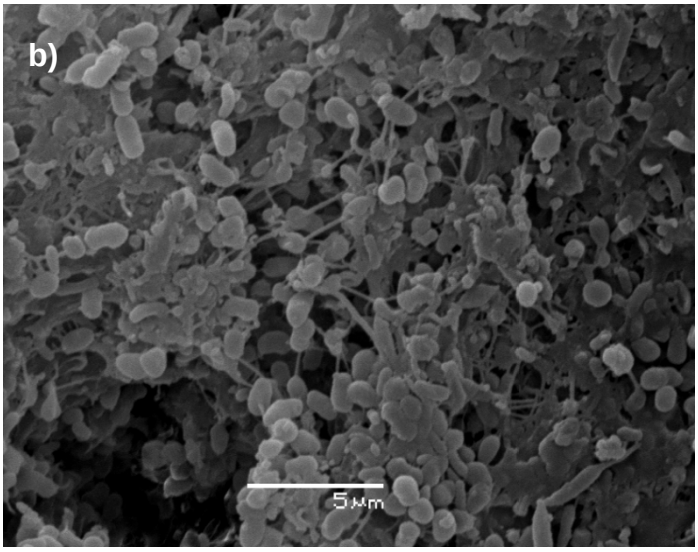


Figure 3. SEM pictures were taken using DigitalMicrograph (DM).





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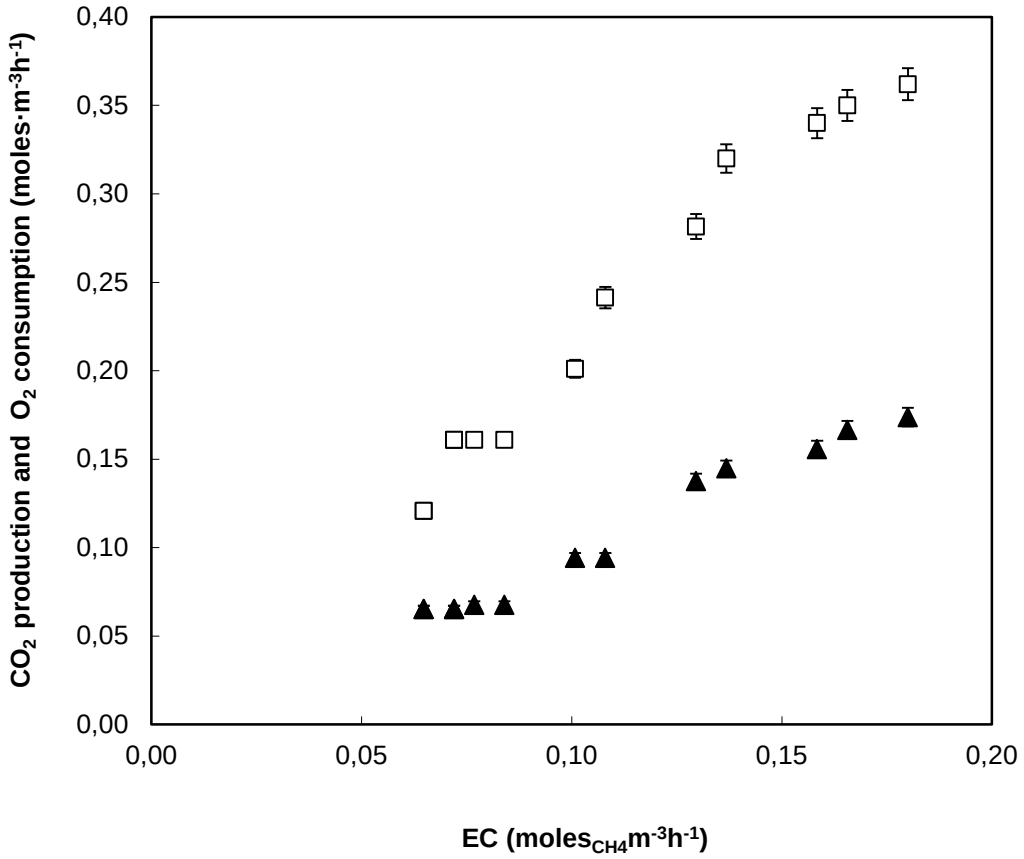

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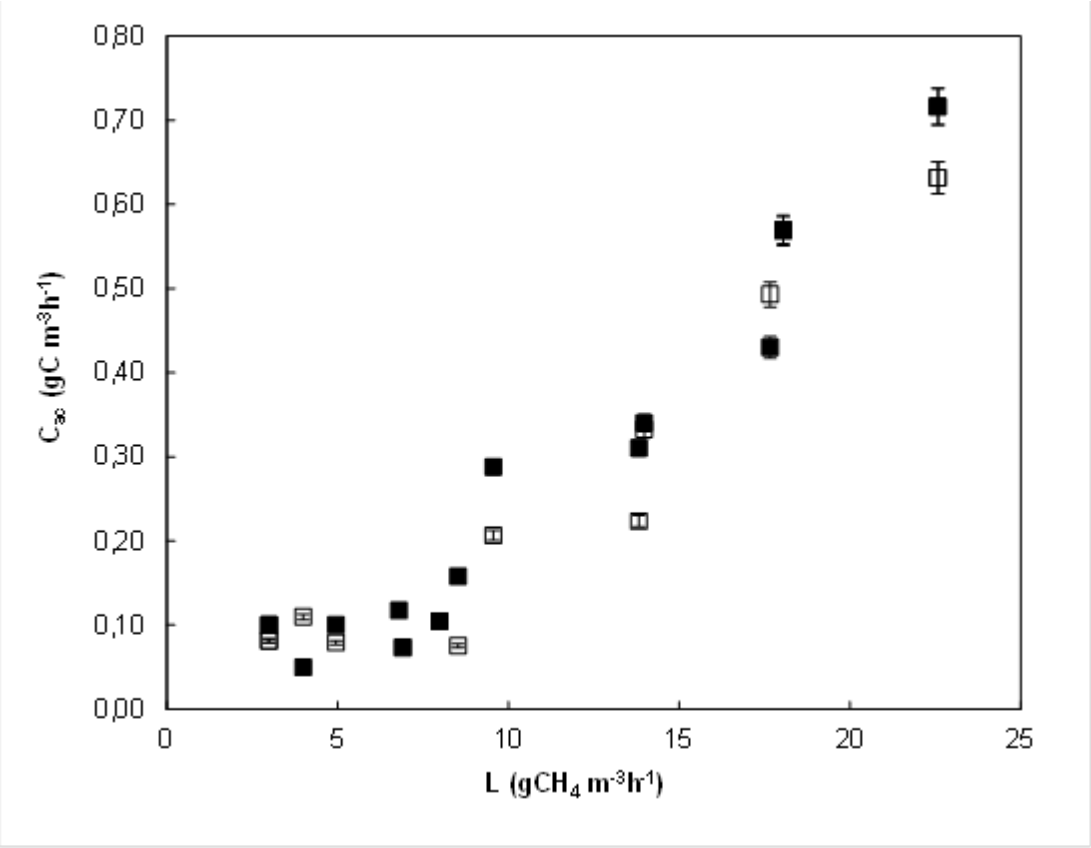

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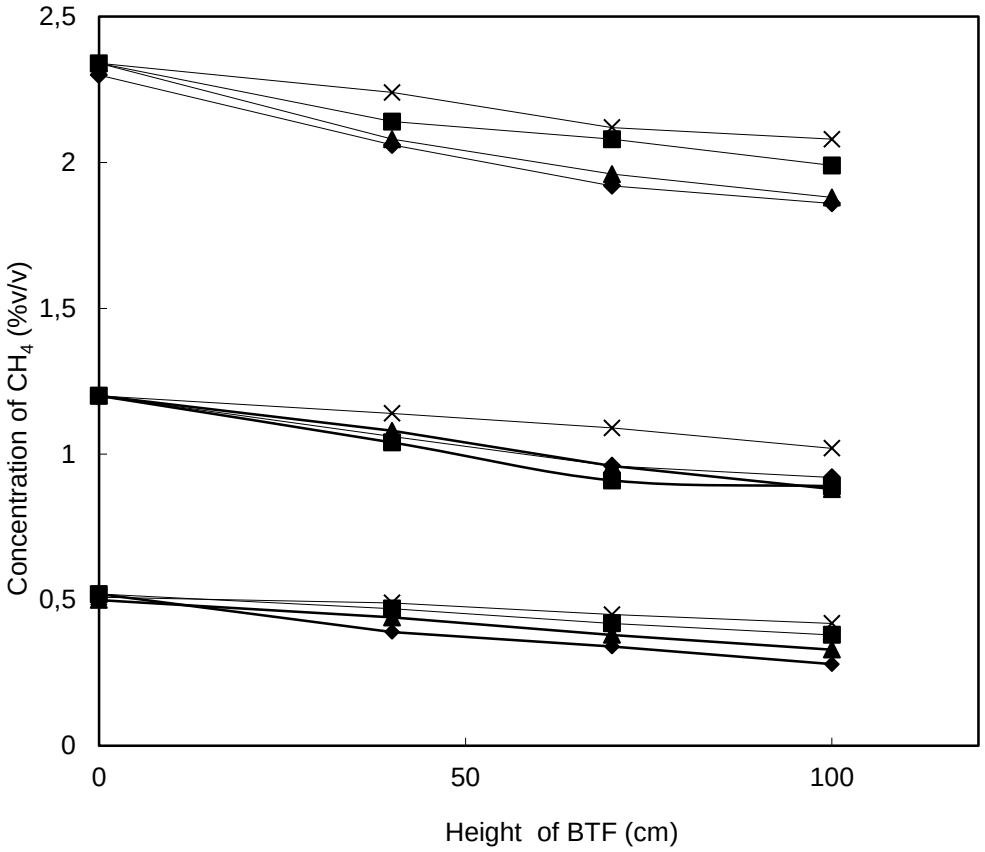
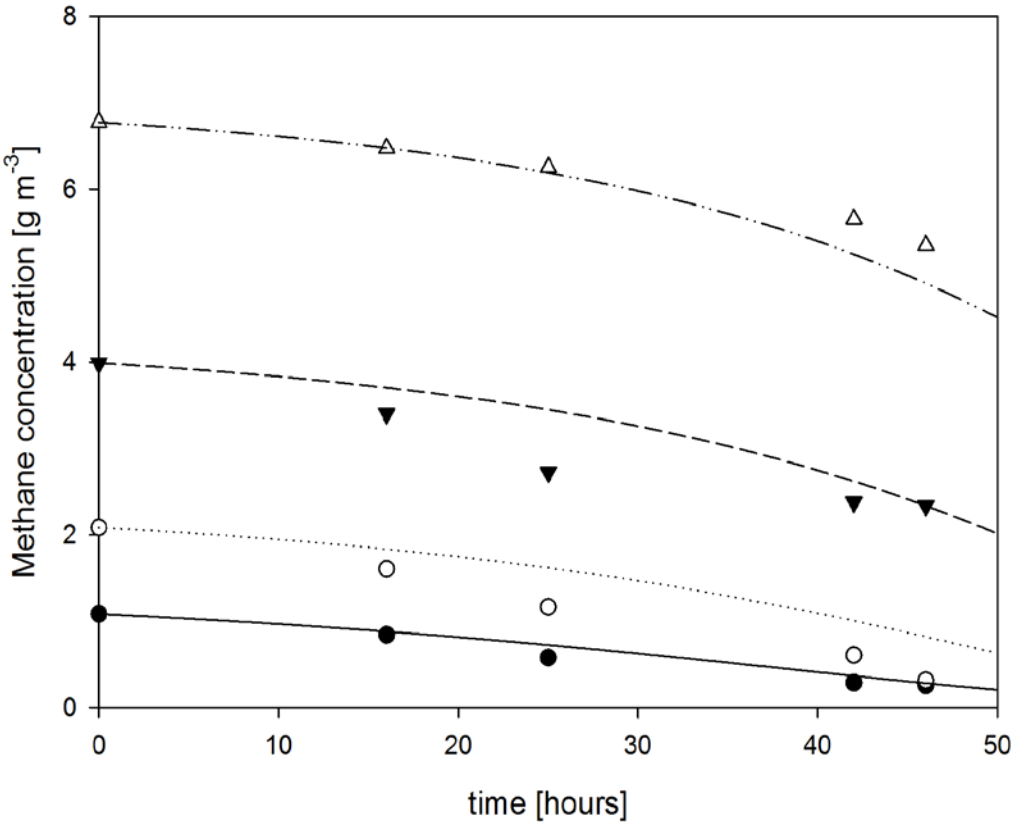


Figure 7 was created with MatLab.

a)



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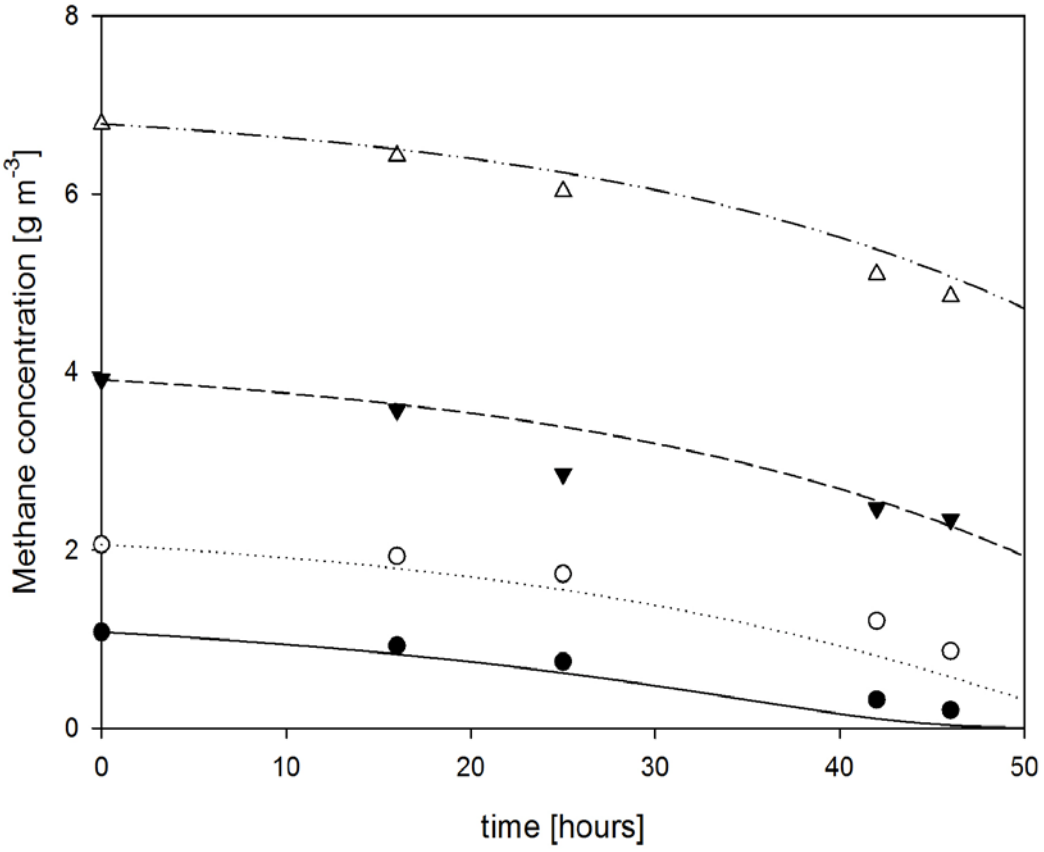
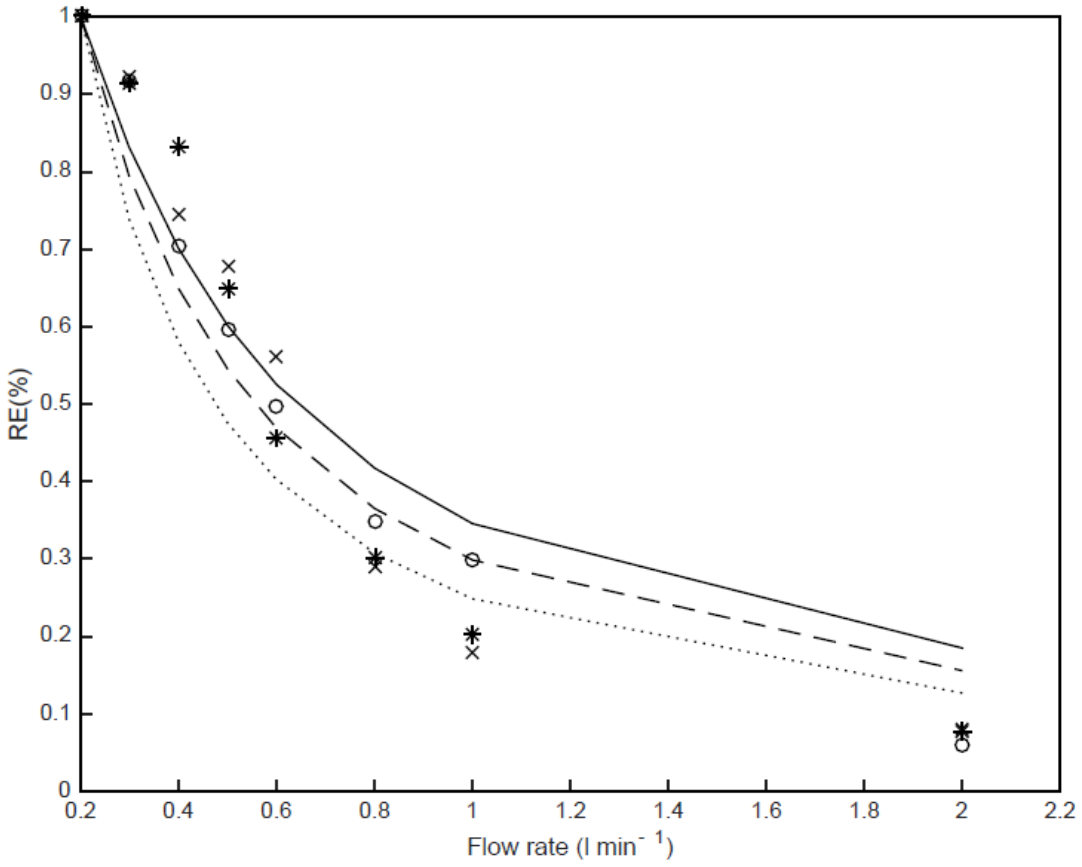


Figure 8 was created with MatLab.

a)



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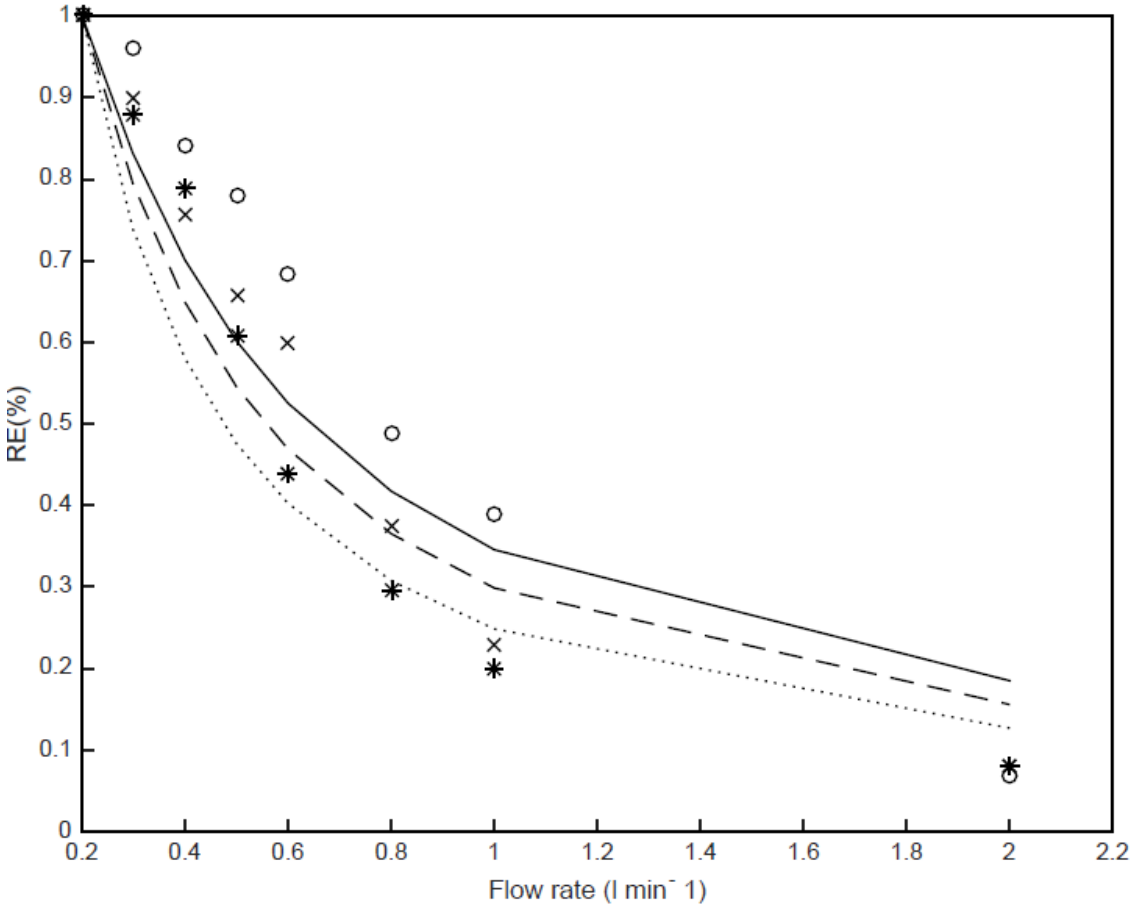
RE(%)
Flow rate (l min˙ 1)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2

Table 1 Bio-oxidation of CH_4 in different reactors with different inoculum and packing materials

Reactor	Inoculum	Packing	L_{CH4} ($g \cdot m^{-3} \cdot h^{-1}$)	EC_{max} ($g \cdot m^{-3} \cdot h^{-1}$)	Void fraction	Specific area (m^{-1})	EC_{sp} ($g \cdot m^{-2} \cdot h^{-1}$)	Reference
Biotrickling filter (Multiphase)	Methanotrophic consortium isolated from WWTP	Polyurethane foam With 10% (v/v) of silicon oil as nonaqueous phase	157 131	22 51	0.97 0.97	600 600	0.037 0.085	Rocha-Ríos et al. 2009
Biofilter	Leachate from methanotrophic biofilter	Expanded clay Rock-5mm Rock-2mm	23	5.0 10.5 17.3	0.55 0.40 0.37	470 1250 1360	0.010 0.008 0.013	Nikiema et al. 2010
Biofilter	Not specified	Gravel (4-8 mm)	25	14.5	0.40	8500	0.002	Girard et al. 2011
Biofilter	Indigenous microorganisms from the packing material	Compost	29	27.5	Not specified	Not specified	Not specified	Haubrichs and Widmann 2006
Biocover	Not specified	Manure compost/saw dust (9:1)	9	5	0.41	Not specified	Not specified	Perdikea et al. 2008
Biotrickling filter	Lixiviate from biofilter treating CH_4	Clay spheres Polypropylene spheres Stones	62	10 8 21	0.40 0.90 0.44	310 280 470	0.032 0.029 0.047	Avalos et al. 2012
Biotrickling filter with recirculation of gas	Methanotrophic consortium isolated from WWTP	Polyurethane foam in cubes of $1cm^3$	230	30	Not specified	1000	0.030	Estrada et al. 2014
Biotrickling filter	Methanotrophs type I (*Methylomicrobium album*) and type II (*Methylocystis* sp.)	Polyethylene rings (1cm id, 1.2 cm od, 1 cm height)	23	6.2	0.77	316	0.019	This work

Table 2 Statistical analysis of experimental results in Biotrickling filters

Parameter	**EC**	**pCO_2**	**EC**	**pCO_2**
(Units)	**($gCH_4m^{-3}h^{-1}$)**	**($gCO_2m^{-3}h^{-1}$)**	**($gCH_4m^{-3}h^{-1}$)**	**($gCO_2m^{-3}h^{-1}$)**
Degrees of freedom	80		45	
$T_{critical}$	1.97	2.14	1.74	1.86
Student *t* value	7.03	7.51	2.05	2.07

Table 3 Kinetic parameters for the bio-oxidation of CH_4 by *Methylomicrobium album* and *Methylocystis sp*.

Parameter	**Symbol**	***Methylomicrobium a.***	***Methylocystis* sp.**	**(Units)**	**Reference**
Maximum specific growth rate	μ_{max}	1.16	1.10	(d^{-1})	Fitted
Semi-saturation constant	K_S	0.29	0.43	($g\ m^{-3}$)	Fitted
Biomass-substrate yield	$Y_{X/S}$	0.28	0.28	($g\ g^{-1}$)	Experimental
Partition coefficient	H	29.4	29.4	-	Literature

Table 4 Physical and kinetics parameters values for the bio-oxidation of CH_4.

Parameter **(Units)**	**[CH_4]** **($g\ m^{-3}$)**	**μ_{max}** **(d^{-1})**	**K_S** **($g\ m^{-3}$)**	**$Y_{X/S}$** **($g\ g^{-1}$)**	**k_g** **($m\ h^{-1}$)**	**D_b** **($m\ h^{-1}$)**
Delhoménie et al. 2008	<10.4	0.43	5.37	0.36-0.8	-	-
Delhoménie et al. 2008	10.4-19.3	1.09	7.59	0.36-0.8	-	-
Menard et al. 2004	1.3-5.9	0.79	6.13	-	-	-
Santos-Rodrigues et al. 2009	0.03	0.77	-	0.68	-	-
Boiesen et al. 1993	-	0.43-1.30	0.05-0.19	0.27-0.89	-	-
Ordaz et al. 2014	1-20	2.23	0.11	0.69	-	-
This work	35-226	1.10-1.16	0.29-0.43	0.14-0.40	0.9	$1.87 \cdot 10^{-5}$