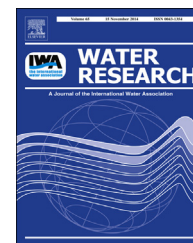


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Assessment of methane biodegradation kinetics in two-phase partitioning bioreactors by pulse respirometry

Alberto Ordaz^{a,b}, Juan C. López^a, Ivonne Figueroa-González^a,
Raúl Muñoz^a, Guillermo Quijano^{a,*}

^a Department of Chemical Engineering and Environmental Technology, University of Valladolid, Spain

^b Universidad Politécnica de Quintana Roo, Av. Tulum, Manzana 1 Lote 40, SM2, Cancún, Quintana Roo, CP 77500, Mexico

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ABSTRACT

Biological methane biodegradation is a promising treatment alternative when the methane produced in waste management facilities cannot be used for energy generation. Two-phase partitioning bioreactors (TPPBs), provided with a non-aqueous phase (NAP) with high affinity for the target pollutant, are particularly suitable for the treatment of poorly water-soluble compounds such as methane. Nevertheless, little is known about the influence of the presence of the NAP on the resulting biodegradation kinetics in TPPBs. In this study, an experimental framework based on the *in situ* pulse respirometry technique was developed to assess the impact of NAP addition on the methane biodegradation kinetics using *Methylosinus sporium* as a model methane-degrading microorganism. A comprehensive mass transfer characterization was performed in order to avoid mass transfer limiting scenarios and ensure a correct kinetic parameter characterization. The presence of the NAP mediated significant changes in the apparent kinetic parameters of *M. sporium* during methane biodegradation, with variations of 60, 120, and 150% in the maximum oxygen uptake rate, half-saturation constant and maximum specific growth rate, respectively, compared with the intrinsic kinetic parameters retrieved from a control without NAP. These significant changes in the kinetic parameters mediated by the NAP must be considered for the design, operation and modeling of TPPBs devoted to air pollution control.

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1. Introduction

Methane is a greenhouse gas with a global warming potential 23 times higher than that of CO₂ and an atmospheric

concentration increasing at 0.2–1% year⁻¹, representing ~14% of the total greenhouse gas emissions worldwide (IPCC, 2007; Lopez et al., 2013). Methane is produced in key waste management activities such as wastewater treatment, landfilling and composting (Benbelkacem et al., 2010; Daelman et al.,

* Corresponding author. Tel.: +34 983186424; fax: +34 983423013.

E-mail address: gquijano@iq.uva.es (G. Quijano).

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Nomenclature	
C_{L,O_2}^*	O ₂ saturation concentration in aqueous phase (gO ₂ m ⁻³)
C_{L,O_2}	O ₂ concentration in aqueous phase at time t (gO ₂ m ⁻³)
C_{L,O_2}^i	O ₂ concentration in aqueous phase at t = 0 (gO ₂ m ⁻³)
C_{L,O_2}^f	O ₂ concentration in aqueous phase at the end of the respirometric test (gO ₂ m ⁻³)
C_{L,CH_4}^i	CH ₄ concentration in aqueous phase at t = 0 (gCH ₄ m ⁻³)
C_{L,CH_4}^f	CH ₄ concentration in aqueous phase at the end of the respirometric test (gCH ₄ m ⁻³)
K_S	Half-saturation constant (gCH ₄ m ⁻³)
OUR	Exogenous O ₂ uptake rate (gO ₂ m ⁻³ h ⁻¹)
OUR _{max}	Maximum exogenous O ₂ uptake rate (gO ₂ m ⁻³ h ⁻¹)
$Y_{O_2/S}$	Substrate oxidation yield (gO ₂ gO ₂ ⁻¹)
$Y_{x/S}$	Biomass growth yield (gO ₂ gO ₂ ⁻¹)
R_{max}	Maximum CH ₄ uptake rate (gCH ₄ m ⁻³ h ⁻¹)
μ_{max}	Maximum specific growth rate (h ⁻¹)
K_{LaO_2}	Volumetric mass transfer coefficient for O ₂ (h ⁻¹)
K_{LaCH_4}	Volumetric mass transfer coefficient for CH ₄ (h ⁻¹)
OTR	Maximum O ₂ transfer rate (gO ₂ m ⁻³ h ⁻¹)
MTR	Maximum CH ₄ transfer rate (gCH ₄ m ⁻³ h ⁻¹)
MOD	Methane oxygen demand (gO ₂ gCH ₄ ⁻¹)
V_{mO_2}	Molar volume for O ₂ (mL mol ⁻¹)
V_{mCH_4}	Molar volume for CH ₄ (mL mol ⁻¹)

2012; Yamamoto et al., 2011). Recent reports warned about the fact that the methane produced in the above mentioned industrial activities is in many cases not further used for energy generation (Farhad et al., 2010; Foresti et al., 2006; McCarty et al., 2011; Rocha-Rios et al., 2009). So far, flaring is the simplest and most extended way to avoid direct methane release to the atmosphere (Noyola et al., 2006). Nevertheless, flaring is not an environmentally friendly practice since complete combustion in conventional open flares is rarely achieved, combustion efficiencies of 10–15% being reported at wind speeds higher than 20 km h⁻¹ (Leahey et al., 2001). Additional gas pollutants such as NO_x, CO, dioxins and furans can also be generated from incomplete methane burning (Caine, 2000).

Methane oxidation by means of biological processes has been identified as a sustainable and environmentally friendly alternative to flaring (Hatamoto et al., 2010; Nikiema et al., 2007; Rocha-Rios et al., 2009). In recent years, biological methane removal has been regarded as a promising treatment alternative when methane is not used for energy production in waste management processes (Lopez et al., 2013). However, the low water solubility of methane (dimensionless Henry's law constant, H, of 30 at 25 °C) is one of the main limitations for its efficient biodegradation by the microbial communities present in the aqueous phase (Avalos-Ramirez

et al., 2012). Consequently, biological systems treating methane require long gas residence times and therefore, large bioreactor volumes (Kennelly et al., 2012). In this regard, several studies suggested that two-phase partitioning bioreactors (TPPBs) can overcome this drawback, which is common for gas pollutants exhibiting a dimensionless Henry's law constant higher than 1 (Muñoz et al., 2012; Rocha-Rios et al., 2011; Volckaert et al., 2014). TPPBs are multiphase systems based on the addition of a non-aqueous phase (NAP) with high affinity for the target pollutant, resulting in higher driving forces for mass transfer and, therefore, higher pollutant removal rates (Clarke et al., 2006; Nielsen et al., 2006). Nevertheless, little is known about the impact of NAP addition on the resulting biodegradation kinetics in TPPBs. Recent studies revealed that NAP addition can dramatically modify the way the microorganisms take up both the gas pollutant and oxygen due to potential microbial growth in the NAP (Hernandez et al., 2012; Muñoz et al., 2013). Furthermore, there is a lack of systematic studies on methane biodegradation kinetics in TPPBs likely due to the analytical difficulties associated with the low water solubility of methane. In this context, the assessment of the impact of NAP addition on microbial kinetics would certainly improve the understanding of the mechanisms underlying the enhanced methane removal in TPPBs, allowing for optimized bioreactor design and operation.

In this study, an experimental framework based on the pulse respirometry technique was developed to assess the impact of NAP addition on methane biodegradation kinetics using *Methylosinus sporium* as a model methane-degrading microorganism. Useful rules of thumb were also proposed to guarantee the absence of mass transfer limiting conditions during the kinetic parameter determination.

2. Material and methods

2.1. Microorganism and culture medium

M. sporium (DSMZ 17706), a methane oxidizing bacterium (Hoefman et al., 2012) was used as a model microorganism in the present study. This microorganism was selected to avoid long acclimation periods during the respirometric experiments. Additionally, inhibition or competition phenomena were avoided by using a pure strain. The mineral salt medium (MSM) used for microbial growth was composed of (g L⁻¹): Na₂HPO₄–12H₂O, 6.15; KH₂PO₄, 1.52; MgSO₄·7H₂O, 0.2; CaCl₂·2H₂O, 0.05; NaNO₃, 2.6 and 10 mL L⁻¹ of a trace element solution composed of (g L⁻¹): EDTA, 0.5; FeSO₄–7H₂O, 0.2; ZnSO₄–7H₂O, 0.01; MnCl₂–4H₂O, 0.003; H₃BO₃, 0.03; CoCl₂, 0.011; CuCl₂–2H₂O, 4.43; NiCl₂–6H₂O, 0.002; Na₂MoO₄–2H₂O, 0.003. The final pH of the medium was 7. The microorganism was cultured in 120-mL glass serum bottles containing 25 mL of MSM and initial methane concentration in the headspace of 20% v/v. The bottles were closed with butyl septa, sealed with aluminum caps and incubated at 25 °C and 150 rpm in an orbital shaker for two weeks. These cultures served as inocula for the respirometric assays.

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