Chemical Engineering Journal 152 (2009) 289–292

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Short communication

Methane degradation in two-phase partition bioreactors

José Rocha-Rios [a], Sergio Bordel [b], Sergio Hernández [a], Sergio Revah [b,*]

[a] Departamento de Ingeniería de Procesos e Hidráulica (IPH), Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco No. 186, 09340 México, D.F., Mexico
[b] Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, c/o IPH, UAM-Iztapalapa, Av. San Rafael Atlixco No. 186, 09340 México, D.F., Mexico

ARTICLE INFO

Article history:
Received 3 February 2009
Received in revised form 3 April 2009
Accepted 7 April 2009

Keywords:
Two-phase partition bioreactors
Silicone oil
Methane biodegradation
Biotrickling filter
Stirred tank bioreactor

ABSTRACT

Biological degradation of methane by an adapted consortium in steady state was compared in two reactor configurations (stirred tank, STR and trickling bed, TBR) with and without 10% (v/v) silicone oil. Silicone oil addition increased the methane average volumetric elimination capacity by 41% in STR up to 106 g m^{-3} h^{-1} and by 131% in TBR up to 51 g m^{-3} h^{-1}. Specific elimination capacities showed higher degradation (69% in STR and 98% in TBR) suggesting increased bioavailability. The elimination capacities obtained in both reactors with oil addition exceed most of experimental reports for methane biofiltration.

1. Introduction

Methane is an important greenhouse gas; it has 21 times more greenhouse impact on the atmosphere than CO_2 at the same concentration. It represents approximately 23% of the total worldwide greenhouse emissions and atmospheric methane concentration has been reported to increase twice as fast than CO_2 [1]. Therefore, the control of methane release is a relevant issue of the global warming problem.

An important source of methane emissions is the biogas generated from landfills [2]. Utilization of methane from biogas emissions for energy production is only possible when methane concentration in the biogas and the overall biogas quantities are important, i.e. more than 30% (which occurs during the first 25 years of a landfill) and 50 m^3 h^{-1}, respectively [3]. For biogas streams over 10–15 m^3 h^{-1} and methane concentrations greater than 20% [3], it is possible to remove methane by burning it in flares. However, biogas combustion requires concentrating methane if the flow and/or concentration are below those values, which is not economically viable, as in the biogas emissions from small or old landfills, anaerobic digestors, sewer emissions, etc.

Biological oxidation is a good alternative to reduce atmospheric emissions of methane if its gas concentration is below its explosion limit in air (5%) [2,4]. These methods are based on a group of aerobic bacteria called methanotrophs which can use methane as carbon and energy source [5]. Biofilters have been already used for methane removal, Nikiema et al. [2] published a complete review on the topic. The main disadvantage of biofiltration for methane abatement is the required high gas residence time, which ranges between several minutes to hours [2,4], while the typical residence times for the removal of VOCs are between 30 and 120 s [6]. The long residence times are due to the low water solubility of methane which has a dimensionless Henry constant at 30 °C of 33.5 [7].

Two-phase partition bioreactors have been proposed as an alternative to improve the removal of low solubility compounds [8,9]. This technology is based on the addition of an organic phase with more affinity for the target compound than water. Two-phase bioreactors have shown to improve toluene [10] and hexane removals [11], oxygen transfer rate [12] and to improve performance under transient conditions. The most used organic phases are silicone oils, long chain hydrocarbons (hexadecane, tetradecane, etc.) and fluorocarbons such as $C_{10}F_8$ [9]. The non-biodegradable silicone oil has consistently been shown to favor the elimination capacity of hydrophobic compounds [9,11] without toxic effects. Nevertheless, silicone oil has certain drawbacks as transfer vector, as it is relatively expensive and its recovery may increase process costs. Alternative organic phases, including solid polymers, have been proposed for the degradation of poorly soluble compounds [13].

The aim of this work is to evaluate, under steady state conditions, methane abatement by a mixed bacterial culture in stirred tank and trickling bed bioreactors with two liquid phases as compared to an aqueous system. Volumetric and specific methane uptake rates will be evaluated to determine the direct influence of oil addition.

* Corresponding author. Tel.: +52 5558046408; fax: +52 5558046408.
E-mail address: srevah@xanum.uam.mx (S. Revah).

1385-8947/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2009.04.028

2. Materials and methods

2.1. Microorganisms and culture conditions

A methanotrophic consortium obtained from a sample of activated sludge from the waste water treatment plant of UAM-Iztapalapa (México City). One liter of activated sludge was filtered through a filter paper (8 μm particle retention; Whatman, UK), the filtrate was centrifuged and the cells were resuspended in 0.5 L of saline mineral medium described by Aaronson [14]. A gas stream with 1% (v/v) of methane was bubbled through the culture during 4 weeks with weekly medium change before using it to inoculate the reactor.

The mineral medium used for the culture contained ($g\,L^{-1}$): $NaNO_3$, 2; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 10^{-3}; Na_2HPO_4, 0.2; $NaH_2PO_4{\cdot}H_2O$, 0.09; $CoSO_4{\cdot}5H_2O$, 5×10^{-6}; H_3BO_3, 10^{-5}; $MnSO_4{\cdot}5H_2O$, 10^{-5}; $ZnSO_4{\cdot}7H_2O$, 7×10^{-5}; MoO_3, 10^{-5}; KCl, 0.04; $CaCl_2$, 0.015. The final pH of medium was 7.

2.2. Chemicals

Methane gas at 99.9% was from Praxair (Mexico). Silicone oil (polydimethylsiloxane, 200 fluid) was from Sigma–Aldrich (USA). An organic fraction of 10% (v/v) was chosen based on previous reports [9,11]. Silicone oil was shown not to be biodegraded nor toxic for the microorganisms [11].

2.3. Partition coefficient of methane in silicone oil

Glass serological bottles (0.125 L) sealed with mininert valves (Supelco, USA) were added with 10 mL of silicone oil and 1, 2, 3, 4 and 5 mL of methane. Bottles by triplicate were placed in a rotary shaker at 30 °C and once the partition equilibrium was attained (when headspace concentration was constant in two consecutive injections with two hours difference), 0.1 mL of headspace were used for methane quantification by chromatography. The methane partition coefficient was determined by mass balance.

2.4. Stirred tank reactor (STR)

A 3.5 L fermentor (Bioflo III New Brunswick, USA) with an operation volume of 2 L and an agitation system with two Rushton turbines operated at 800 rpm was used for methane degradation in suspension. The gas flow through the system was $0.42\,L\,min^{-1}$ (corresponding to 0.21 vvm or empty bed residence time, EBRT, of 4.8 min) and the average methane load was $200\,g\,m^{-3}\,h^{-1}$ (average methane concentration of $15.9\,g\,m^{-3}$). Mineral medium was continuously added with a dilution rate of $0.1\,d^{-1}$ ($0.2\,L\,day^{-1}$).

2.5. Trickling bed reactor (TBR)

This system consisted of a glass cylindrical column of 1 m with an inner diameter of 0.08 m packed with 1 L of polyurethane open pore foam (EDT, Germany) with a porosity of 0.97, a specific area of $600\,m^2\,m^{-3}$ and a density of $35\,kg\,m^{-3}$. A magnetic stirring system at the bottom of the column allowed continuous mixing of the liquid (volume of 0.3 L) prior to recirculation. The inlet gas flow in system was $0.21\,L\,min^{-1}$ equivalent to an EBRT of 4.8 min and the average methane load was $140\,g\,m^{-3}\,h^{-1}$ (average methane concentration of $11.1\,g\,m^{-3}$). As in the STR, mineral medium was continuously added with a $0.1\,d^{-1}$ dilution rate. Steady state was considered for both STR and TBR when constant EC and biomass values were obtained for 10 days.

Reactor performance is described for both systems by the volumetric methane load $L = C_{Gin}QV_R^{-1}$; the volumetric elimination capacity $EC = (C_{G\,in} - C_{G\,out})QV_R^{-1}$; the volumetric CO_2 production rate $R_{CO_2} = (C_{CO_2\,in} - C_{CO_2\,out})QV_R^{-1}$; the percent removal efficiency $\%RE = 100 \times \{(C_{G\,in} - C_{G\,out})/C_{G\,in}\}$; and the gaseous elimination capacity $EC_g = EC/\alpha$. Where $C_{G\,in}$, $C_{CO_2\,in}$, $C_{G\,out}$ and $C_{CO_2\,out}$ are the inlet and outlet methane and CO_2 concentrations ($g\,m^{-3}$); Q is the gas volumetric flow ($m^3\,h^{-1}$), V_R is the operation reactor volume (m^3) and α is the gas hold-up (STR) or the porosity (TBR).

2.6. Analytical procedures

Methane concentrations in the gas phase were measured using a gas chromatograph (Agilent Technologies 6890N, USA) equipped with an AT-WAX 25 m × 0.25 mm × 0.2 μm column (Alltech, USA) and a FID detector. CO_2 concentrations were measured using the same chromatograph with a Porapack Q 80/100 6′ × 1/8″ column and a TCD detector. The volumetric CO_2 production rate (R_{CO_2}) considered the concentration difference between inlet and outlet. The helium (carrier), H_2 and air flows were 7, 25 and 250 mL min^{-1} respectively; and temperatures of injector, oven and both detectors were maintained at 200, 70 and 250 °C, respectively. Measurements in each system were carried out daily by triplicate.

Biomass in liquid of both reactors was calculated from the protein content using Lowry's method (BioRad kit, USA) and a protein content of 50% which was experimentally obtained through independent dry weight measurements. Triplicate samples were centrifuged at 13000 rpm for 10 min to separate biomass from the culture medium prior to protein determination. At the end of TBR operation, the biomass attached to the package was dissolved in NaOH solution (0.5 M at 70 °C) during two hours using an ultrasonic cleaner. The obtained solution was homogenized and the protein content was determined with Lowry's method.

3. Results and discussion

The partition coefficient ($K_O = C_{G\,eq}/C_{O\,eq}$) of methane in silicone oil at 30 °C was found to be in 3.2 ± 0.3, which is approximately 10 times lower than the value in water of 33.5. This partition coefficient and the fact that the oil was neither toxic nor degraded by the methanotrophic consortium confirmed its suitability as a transference vector for methane. Table 1 shows the performance results for the steady state operation in both experiments.

The increase in volumetric elimination capacities with silicone oil addition (10%, v/v) were 41% and 131% for STR and TBR respectively with corresponding improvements both in the %RE and CO_2 production rates, despite the fact that the average loads were slightly lower for the experiments with oil addition. The average surface elimination capacities (expressed by reactor cross-section) were calculated for the STR and TBR with oil addition and found to be 344 and $214\,g\,m^{-2}\,d^{-1}$, respectively, which were superior to most of methane biofiltration experiments reviewed by Nikiema et al. [2].

Moreover, to compare both systems, the volumetric elimination capacities were expressed per unit of gas volume contained in the reactors (considering a measured gas hold up value of 0.15

Table 1
Performance variables in both systems with and without silicone oil addition during stationary operation.

	STR		TBR	
	Control	Added	Control	Added
L ($g\,m^{-3}\,h^{-1}$)	209 ± 26	187 ± 11	157 ± 15	131 ± 9
EC ($g\ m^{-3}_{reactor}\,h^{-1}$)	75 ± 6	106 ± 7	22 ± 3	51 ± 7
R_{CO_2} ($g\,m^{-3}\,h^{-1}$)	80 ± 8	109 ± 12	36 ± 2	69 ± 5
%RE	34 ± 5	57 ± 4	15 ± 3	40 ± 4
EC_g ($g\ m^{-3}_{gas}\,h^{-1}$)	500 ± 40	707 ± 47	24 ± 3	57 ± 8

in STR and a bed porosity of 0.9 in TBR) similarly to Arriaga et al. [11]. Gaseous elimination capacities (summarized in Table 1), which are equivalent to the global transfer rates under steady state, were much higher for the STR than for the TBR (21 and 12 times higher for the control and with oil addition, respectively). This important difference can be explained considering the agitation energy applied in the STR that promotes both the transfer coefficients and the gas–aqueous interfacial area. Furthermore, in STR the agitation reduces the coalescence of the oil particles and the consequent decrease of the aqueous-organic interfacial area. In TBR, despite the longer retention time, mass transfer may be reduced by clogging or channeling of gas flow in the support due to heterogeneous biofilm formation [11]. To evaluate the specific rates, biomass was measured in each reactor and correlated to the volumetric rate values. Fig. 1 shows biomass values and the specific methane elimination capacity and CO_2 production rates for steady state operation in each experiment.

Results in Fig. 1 indicate that the specific methane elimination capacity increased around 70% for the STR and 100% for the TBR when oil was present. The specific methane elimination capacities obtained were superior to most of values reported in literature which are in the order of 10^{-6} to 10^{-2} g $g_{biomass}^{-1}$ h^{-1} [15–20]. Concurrent specific CO_2 production rates increased with the higher specific methane uptake. The CO_2 yields were 1.04 ± 0.02 g_{CO_2} $g_{CH_4}^{-1}$ (0.378 g_C g_C^{-1}) and 1.35 ± 0.1 g_{CO_2} $g_{CH_4}^{-1}$ (0.491 g_C g_C^{-1}) for the STR and TBR respectively, these values are similar to others reported for metanotrophic bacteria [16,21].

Considering the partition coefficients mentioned previously and gas methane concentration in STR added with silicone of 6.6 g m^{-3} (average effluent concentration and perfect mixing), the concentration in the aqueous phase would be 0.197 and 2.06 g m^{-3} in the oil phase with 3.5×10^{-4} g of methane dissolved in 1.8 L of water and 4.1×10^{-4} g dissolved in 0.2 L of silicone oil. Therefore, in 10% (v/v) of silicone oil there is about 10 times the concentration and 1.2 times more methane available for cells than in 90% of water. The steady state mass balance in STR indicates 1.4 times more methane available for cells in the oil added reactor and 2.3 times for TBR.

The enhancement in performance of two-phase partition bioreactors can be attributed to improved methane availability originated by (a) better transfer of the pollutant and oxygen from the gaseous phase to cells and, (b) possible direct substrate uptake from the organic phase. With respect to the mass transfer increase, no consistent relation has been found between the volumetric mass transfer (k_La) and organic phase addition as reported by Clarke and Correia [22]. For silicone oil, Morao et al. [23] reported that there is a "critical fraction" from which an improvement in k_La may be obtained. The net effect of organic phase on mass transfer is the result first of the film coefficient (k_L) which is affected on one hand as there is a new resistance to the methane transfer from the gas phase to cells and by an increase in the liquid viscosity, and on the other hand, this coefficient is benefited by an increase in the driving force caused by the more favorable substrate partition coefficient in the liquid phase. Secondly, oil addition reduces gas-liquid surface tension favoring smaller air bubbles and consequently a higher specific mass transfer area (a). As a result of these effects, the maximum increase reported for k_La has been lower than 20% which is significantly inferior to the increase in methane EC in our study of 41% in the STR and 131% in the TBR. These considerations suggest that direct methane uptake from the cells adhered to the oil drops may be improving the overall EC as the higher methane and oxygen concentration in the oil drops, tentatively up to ten times based on the partition coefficient, foster higher specific growth and methane uptake rates.

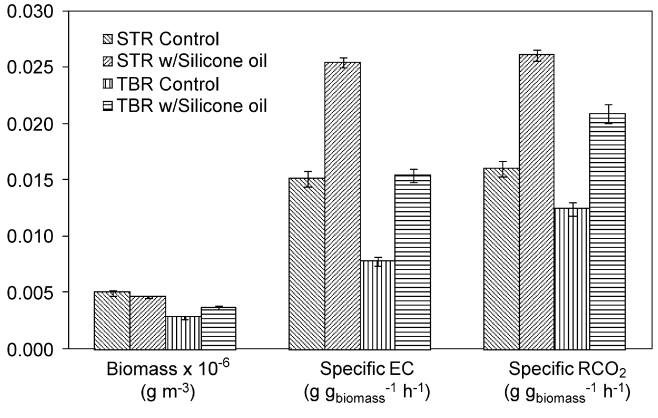

Fig. 1. Biomass, specific elimination capacity and specific CO_2 production rate in the STR and TBR during steady operation with and without silicone oil addition.

4. Conclusions

Ten percent (v/v) silicone oil addition augmented significantly the biodegradation performance in a stirred tank and trickling bed reactors, making two-phase partition bioreactors a promising alternative to improve methane removal in bioreactors with shortcomings in mass transfer to cells. A mass balance and the fact that specific elimination capacities (per gram of biomass) in both systems were increased, suggest that a direct uptake of methane and oxygen with higher rates cells adhered to oil drops adds to the dissolved methane uptake from the aqueous phase. Elimination capacities obtained in this paper are superior to most of methane biofiltration experiments reported in literature and current research is being pursued in alternative organic phase selection and mass transfer coefficients determination.

Acknowledgements

To Conacyt for financial support for the project, Sergio Bordel's postdoctoral position and José Rocha's doctoral scholarship. To Raúl Muñoz for fruitful discussions

References

[1] U.S. Environmental Protection Agency (USEPA), Global mitigation of non-CO_2 greenhouse gases, 2006, http://www.epa.gov/climatechange/economics/downloads/GlobalMitigationFullReport.pdf.
[2] J. Nikiema, R. Brzezinski, M. Heitz, Elimination of methane generated from landfills by biofiltration: a review, Rev. Environ. Sci. Biotechnol. 6 (2007) 261–284.
[3] R. Haubrichs, R. Widmann, Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas, Waste Manage. Assoc. 26 (2006) 408–416.
[4] C. DuPlessis, J. Strauss, E. Sebapalo, K. Riedel, Empirical model for methane oxidation using a composted pine bark biofilter, Fuel 82 (2003) 1359–1365.
[5] R. Hanson, T. Hanson, Methanotrophic Bacteria Microbiol. Rev. 60 (1996) 439–471.
[6] Z. Shareefdeen, A. Singh, Biotechnology for Odor and Air Pollution Control, 1st ed., Springer-Verlag, Berlin, Heidelberg, 2005, p. 406.
[7] D. Zenhao, N. Moller, J. Greenberg, J. Weare, The prediction of methane solubility in natural waters to high ionic strength from 0 to 250 °C and from 0 to 1600 bar, Geochim. Cosmochim. Acta 56 (1992) 1451–1460.
[8] A. Daugulis, Two-phase partitioning bioreactors: a new technology platform for destroying xenobiotics, Trends Biotechnol. 19 (2001) 457–462.
[9] R. Muñoz, S. Villaverde, B. Guieysse, S. Revah, Two-phase partitioning bioreactors for treatment of volatile organic compounds, Biotech. Adv. 25 (2007) 410–422.
[10] A. Daugulis, N. Boudreau, Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*, Biotechnol. Lett. 25 (2003) 1421–1424.
[11] S. Arriaga, R. Muñoz, S. Hernandez, B. Guieysse, S. Revah, Gaseous hexane biodegradation by *Fusarium solani* in two liquid phase packed-bed and stirred tank bioreactors, Environ. Sci. Technol. 40 (2006) 2390–2395.
[12] M. Cesário, H. de Wit, J. Tramper, H. Beeftink, Dispersed organic solvent to enhance the overall gas/water mass transfer coefficient of apolar compounds in the biological waste-gas treatment. Modeling and evaluation, Biotechnol. Prog. 13 (1997) 399–407.

[13] A. Daugulis, N. Boudreau, Solid-liquid two-phase partitioning bioreactors for the treatment of gas-phase volatile organic carbons (VOCs) by a microbial consortium, Biotechnol. Lett. 30 (2008) 1583–1587.
[14] S. Aaronson, Experimental Microbial Ecology, 1st ed., Academic Press, New York, 1970, pp. 109–111.
[15] I. Watanabe, T. Hashimoto, A. Shimoyama, Methane-oxidizing activities and methanotrophic populations associated with wetland rice plants, Biol. Fertil. Soils 24 (1997) 261–265.
[16] G. Börjesson, I. Sundh, A. Tunlid, A. Frostergard, B. Svensson, Microbial oxidation of CH_4 at high partial pressures in an organic landfill cover soil under different moisture regimes, FEMS Microbiol. Ecol. 26 (1998) 207–217.
[17] J. Xin, J. Cui, J. Chen, S. Li, C. Xia, L. Zhu, Continuous biocatalytic synthesis of epoxypropane using a biofilm reactor, Proc. Biochem. 38 (2003) 1739–1746.
[18] D. Reay, D. Nedwell, Methane oxidation in temperate soils: effects of inorganic N, Soil Biol. Biochem. 36 (2004) 2059–2065.
[19] S. Lontoh, J. Semrau, Methane and trichloroethylene degradation by *Methylosinus trichosporium* OB3b expressing particulate methane monooxygenase, Appl. Environ. Microbiol. 64 (1998) 1106–1114.
[20] K. Chu, L. Alvarez-Cohen, Effect of nitrogen source on growth and trichloroethylene degradation by methane-oxidizing bacteria, Appl. Environ. Microbiol. 64 (1998) 3451–3457.
[21] V. Acha, J. Alba, F. Thalasso, The absolute requirement for carbon dioxide for aerobic methane oxidation by a methanotrophic-heterotrophic soil community of bacteria, Biotechnol. Lett. 675 (2002) 675–679.
[22] K. Clarke, L. Correia, Oxygen transfer in hydrocarbon—aqueous dispersions and its applicability to alkane bioprocesses: a review, Biochem. Eng. J. 39 (2008) 405–429.
[23] A. Morao, C. Maia, M. Fonseca, J. Vasconcelos, S. Alves, Effect of antifoam addition on gas–liquid mass transfer in stirred fermenters, Bioproc. Eng. 20 (1999) 165–172.