Elsevier Editorial System(tm) for Journal of Environmental Management

Manuscript Draft

Manuscript Number: JEMA-D-18-00511R1

Title: A systematic comparison of two empirical gas-liquid mass transfer determination methodologies to characterize methane biodegradation in stirred tank bioreactors

Article Type: VSI:ISEAC 5 - Asia

Keywords: Biological gas treatment; Greenhouse gas; CH4; Suspended growth bioreactor; Volumetric mass transfer coefficient.

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Special Issue Managing Guest Editor
Journal of Environmental Management

Ref: Ms. No. JEMA-D-18-00511: Former title: **"New insights on CH_4 mass transfer characterization in stirred tank bioreactors devoted to CH_4 bioconversion",** current title: **"A systematic comparison of two empirical gas-liquid mass transfer determination methodologies to characterize methane biodegradation in stirred tank bioreactors"**, submitted for publication in the special issue ISEAC5-Asia in **Journal of Environmental Management**.

Dear editor,

The authors would like to thank you for the attention given to our manuscript during this peer-review process. We have carefully addressed all reviewers and editor comments, and therefore, the quality of the paper has been significantly improved. More specifically:

REVIEWER 1

Muñoz et al. compared the potential of two experimental methodologies for the estimation of $klaCH_4$ at multiple stirring rates (400, 600 and 800 rpm). These two methodologies are somewhat useful. Although authors have used a simple methodology to accurately describe CH_4 mass transport in continuous bioreactors. I recommend it for the publication only after major changes for the following reasons:

The authors acknowledge the positive feedback from Reviewer 1

1. Research highlights need to be re-refined.

The research highlights were reworded in order to better reflect the main outcomes of the present study in accordance to Reviewer 1 request (see highlight section).

k_La_{O2} determination based on SO_3^{-2} oxidation accurately described CH_4 mass transfer
Microbial CH_4 uptake accelerated CH_4 mass transport similarly to SO_3^{-2} oxidation
The gassing-out methodology underestimated CH_4 mass transport
Maximum CH_4 elimination capacities of 62 ± 5 g CH_4 m^{-3} h^{-1} were achieved at 800 rpm

2. Abstract and conclusions are written very poorly, I would suggest authors' to modify carefully. Provide more systematic explanation and important results. A complete re-write is necessary.

The abstract and conclusion sections were significantly modified in order to highlight the main results and provide a more systematic explanation of the findings obtained as requested by Reviewer 1. In addition, the length of both sections was reduced to ≥150 words as requested by the guest editor (current pages 2 and 14-15).

Abstract (150 words)

"This study aimed at systematically comparing the potential of two empirical methods for the estimation of the volumetric CH_4 mass transfer coefficient (k_la_{CH4}), namely gassing-out and oxygen transfer rate (OTR), to describe CH_4 biodegradation in a fermenter operated with a methanotrophic consortium at 400, 600 and 800 rpm. The kla_{CH4} estimated from the OTR methodology accurately predicted the CH_4 elimination capacity (EC) under CH_4 mass transfer limiting conditions regardless of the stirring rate (~ 9 % of average error between empirical and estimated ECs). Thus, empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} vs predicted CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} were recorded at 400, 600 and 800 rpm, respectively. The rapid Co^{2+}-catalyzed reaction of O_2 with SO_3^{-2} in the vicinity of the gas-liquid interphase during OTR determinations, mimicking microbial CH_4 uptake in the biotic experiments, was central to accurately describe the k_la_{CH4}."

Conclusions (119)

"This work empirically validated a simple methodology to accurately describe CH_4 mass transport and biodegradation in continuous stirred tank bioreactors based on OTR estimations. The rapid O_2 uptake at the gas-liquid interphase mediated by sulfite and Co^{2+}, mimicking microbial CH_4 uptake during continuous CH_4 treatment, supported the superior performance of the OTR methodology for the description of k_la_{CH4} compared to the classical gassing-out methodology. Thus, the empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} recorded at 400, 600 and 800 rpm, respectively, were comparable to the CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} predicted by the OTR methodology. This finding will help in the design of suspended growth bioreactors devoted to CH_4 bioconversion."

4. Authors should compare two methods. Figure 2 is simply comparison at three rates (400, 600 and 800 rpm), how about 1000 rpm or 1200 rpm? What would happen at high rates.

The authors agree with Reviewer 1 on the additional value of the information provided by tests conducted at 1000 and 1200 rpm. Unfortunately, these experiments were not conducted based on the fact that these agitation rates would be hardly applicable in full scale fermenters. In our humble opinion, the main findings of this study were validated with the experiments carried out at 400, 600 and 800 rpm. An explanatory remark was included in current page 5.

"*These aeration rates were selected as potential operational conditions implemented in large-scale fermenters devoted to the bioconversion of CH_4.*"

3. The authors' interpretation of the methodologies is weak. Many sentences are merely left to the speculation of the scientific community. Provide more sound discussion on the mechanisms.

The interpretation and discussion of the results obtained was significantly improved as requested by Reviewer 1, 3 and 4. More sound discussions of the mechanisms underlying O_2 mass transfer during the gassing-out and sulphite oxidation experiment, and CH_4 mass transfer during the biotic experiments, was also provided. More specifically:

Current Page 9

"The type of O_2 sensor (polarographic vs optical) used in the gassing-out methodology did not significantly ($p \leq 0.05$) influence the measurement of k_La_{O2} (average error < 2.75 %), which confirmed that the significant ($p \leq 0.05$) differences"

Current page 10

"At this point it should be highlighted that moderate salinity media, despite slightly reducing the solubility of gases, typically result in enhanced gas-liquid k_La induced by the higher gas-liquid interfacial areas (Rodero et al., 2018)."

"Figure 2, which depicts the O_2 profiles in the liquid phase during the gassing-out and OTR experiments, clearly shows the higher mass transfer gradients (estimated locally from the slope of the decrease in dissolved O_2 concentration at the gas-liquid interphase) despite the overall difference in O_2 concentration between the interphase and the bulk liquid (200 mg L^{-1} – 0 mg L^{-1}) remained similar. These higher gradients mediated by the rapid oxidation of O_2 in the presence of SO_3^{-2} and Co^{2+} entailed a superior O_2 mass transfer, which mathematically resulted into higher $klaO_2$ (obtained from equations 1 and 3). Finally, it should be highlighted that the k_La_{CH4} here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017)."

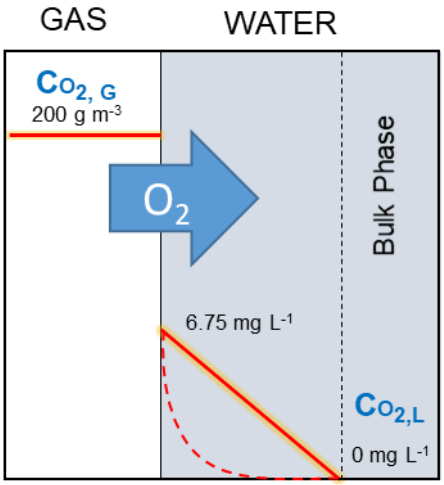


Figure 2. *O_2 concentration profiles in the liquid phase during the gassing-out (continuous line) and OTR (dashed line) methods.*

Current page 11

"At this point it should be also stressed that high k_La_{CH4} values are required in CH_4 treating bioreactors in order to compensate for the low concentration differences imposed by the high Henry law constant of CH_4 (~ 30 at 25 ºC) (Lopez et al., 2013). Thus, the higher the k_La_{CH4} values, the lower the bioreactor volumes and consequently, the lower the investment costs."

"This implies that the removal of CH_4 recorded at 400, 600 and 800 rpm can be attributed to the biocatalytic action of the microbial consortium present in the fermenter, which converts CH_4 to CO_2, H_2O and new biomass (Lopez et al., 2014)."

Current page 12

$$CH_4 - EC = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \quad \text{(Eq. 7)}$$

"Where H_{CH4} represents the dimensionless Henry law constant of CH_4 (28.7 at 30 ºC) and C_{LCH4} the aqueous concentration of CH_4 in the bulk phase, which is negligible under the mass transfer limiting scenario here encountered. In fact, no significant differences ($p \leq 0.05$) between the empirical and estimated CH_4-ECs were found at 400 and 800 rpm."

"In this context, Cu positively regulates the activity of the enzymes pMMO and sMMO (particulate and soluble CH_4 monooxygenase, respectively) and controls the expression of their genes. Most methanotrophs grow optimally at Cu concentrations lower than 270 µg L^{-1}, though previous enzymatic assays have demonstrated that sMMO in type II methanotrophs is properly synthesized at low Cu concentrations (below 50 µg L^{-1}) (Graham et al., 1993; Bender and Conrad, 1995; Semrau et al., 2010)."

"Thus, the rapid CH_4 biodegradation in the vicinity of the gas-liquid interphase mediated by methanotrophic bacteria likely supported an enhanced CH_4 mass transfer (induced by the higher local gradient close to the gas-liquid interphase)"

Current page 13-14

"3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of k_La_{CH4}, whose characterization has been conducted based on either classical k_La_{O2} determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the k_La_{CH4} estimated at the

three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016).”

Detailed Comments

Page 2
Line 29: what do authors means by "accurately predicted the CH4 elimination capacity"?

The original sentence was modified as recommended by Reviewer 1 in order to quantify the error between the empirical and estimated methane elimination capacities in the fermenter (current page 2).

“The kla_{CH4} estimated from the OTR methodology accurately predicted the CH_4 elimination capacity (EC) under CH_4 mass transfer limiting conditions regardless of the stirring rate (~ 9 % of average error between empirical and estimated ECs)”

Page 5
Line 29: "The klaO2 was recorded at an aeration rate of 0.42 L min-1 under three stirring rates (400, 600 and 800 rpm) ? Why chose three stirring rates? Should carefully explain in detail.

These aeration rates were selected as potential operational conditions implemented in large-scale fermenters devoted to the bioconversion of CH_4. An explanatory remark was included in page 5 to avoid further misunderstandings:

“These aeration rates were selected as potential operational conditions implemented in large-scale fermenters devoted to the bioconversion of CH_4.”

Page 6
Line 1: "O2 concentration at the beginning of the test (DO = 0 mg L-1 at t =0 s)", DO = 0 ?

Please, kindly note that the cultivation broth in the fermenter was always initially degassed with Argon prior to each gassing-out experiment (former page 5 lines 32-35), which supported the negligible dissolved oxygen concentrations reported at t = 0 s (DO = 0 mg L^{-1}). An explanatory remark was included in this revised manuscript in order to avoid further misunderstandings (current page 6):

"……, $C_{O_2,L}$ the dissolved O_2 concentration at time t, and $(C_{O_2,L})_0$ the dissolved O_2 concentration at the beginning of the test (DO = 0 mg L^{-1} at t =0 s, since the cultivation broth was initially degassed with argon)."

Page 7
Line 43: Analytical methods is too simple and should be explained and supplemented. Do not refer to literatures in this section. Provide brief explanation on how the authors did the work in real situation in the lab.

The authors agree with Reviewer 2 on the fact that this section can be systematically improved by providing the description of the dissolved oxygen sensors, and the detailed methodologies for the determination of biomass and sulphite concentrations (current page 8-9):

"A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again."

Page 10
Line 43: How unique and useful is this "first time" of authors compared to other study? It should be summarized and explained in the text with proper comparisons. A new Table has to be prepared.

To the best of our knowledge, this was the first work validating a simple methodology to accurately describe CH_4 mass transport in continuous bioreactors based on OTR estimations. However, since other methodologies have been used to characterize the gas-liquid methane mass transport in bioreactors (see Table S1 in the *supplementary material* section), the term "for the first time" was deleted in this revised version of the manuscript. An explanatory remark in the introduction section (page 4) and a new table (Table S1 in *supplementary materials*) were included in this revised version of the manuscript in order to provide a fairer and more detailed overview of the previous studies on the topic.

"Table S1 presents the main studies devoted to the characterization of the volumetric CH_4 mass transfer coefficients in bioreactors"

1. Compilation of previous studies on the determination of kla_{CH4}

***Table S1**. Previous studies on the characterization of the gas-liquid CH_4 mass transport in bioreactors*			
Bioreactor Configuration	***Methodology***	***kla_{CH4} (h^{-1})***	***Reference***
Immobilized soil bioreactor & Fermenter	*kla_{O2} based on the dynamic method combined with gas phase CH_4 analysis Model validation*	*16.3 ±0.67*	*Yu et al. (2010)*
Airlift bioreactor with internal recirculation	*kla_{O2} based on OTR No validation of CH_4-EC was achieved*	*28.6 -200**	*Rocha-Rios et al. (2011)*
Two-phase partitioning fermenter	*kla_{O2} based on static method No validation of CH_4-EC was achieved*	*6.2-35.4**	*Rocha-Rios et al. (2010)*
250 mL bottle	*kla_{CH4} based on CH_4 absorption experiments No methodology validation*	*22.7-41.9*	*Lee et al. (2015)*

**- Estimated from kla_{O2} using equation 4.*

Lee, S.Y., Mo, K.S., Choi, J.H., Hur, N.H., Kim, Y.K., Oh, B.K., Lee, J., 2015. Enhancement of CH_4-water mass transfer using methyl-modified mesoporous silica nanoparticles. Korean Journal of Chemical Engineering. 32(9), 1744-1748.

Rocha-Rios, J., Muñoz, R., Revah, S., 2010. Effect of silicone oil fraction and stirring rate on methane degradation in a stirred tank reactor. Journal of Chemical Technology and Biotechnology. 85, 314-319.

Rocha-Rios, J.M., Quijano, G., Thalasso, F., Revah, S., Muñoz, R., 2011. Methane biodegradation in a two-phase partitiong airlift reactor with gas recirculation. Journal of Chemical Technology and Biotechnology. 86(3), 353-360.

Yu, Y., Ramsay, J.A., Ramsay, B.A., 2006. On-Line estimation of dissolved methane concentration during methanotrophic fermentations. Biotechnology and Bioengineering. 95, 788-793.

REVIEWER 2

This study contributes to the scientific literature of the methanotrophic fermentation; also the findings of this study can be useful for engineers and scientist that use mathematical modelling in order to design a methanotrophic bioreactor or to estimate the performance of a methanotrophic process. However, the authors need to elaborate more on several key parameters that have investigated.

The authors acknowledge the positive feedback from Reviewer 2

1) The title can be more specific and can describe in a higher accuracy the experiments and the comparison.

The title of the paper was modified in accordance to Reviewer 2 request to "*A systematic comparison of two empirical gas-liquid mass transfer determination methodologies to characterize methane biodegradation in stirred tank bioreactors*" (current page 1)

2) At the abstract the aim of this study need to be clearly stated.

The aim of the study was clearly stated in the first sentence of the revised abstract (current page 2)

"*This study aimed at systematically comparing the potential of two empirical methods for the estimation of the volumetric mass transfer coefficient of CH_4 (k_la_{CH4}), namely gassing-out vs oxygen transfer rate (OTR), to describe CH_4 biodegradation in a fermenter operated with a methanotrophic consortium at 400, 600 and 800 rpm.*"

3) Page 2 Line 12 : Which biorefineries? Please be specific – which industries? [UTF-8?]"â€¦has raised increasing attention in biorefineries"

Please note that the abstract in this revised manuscript was reduced from 230 words to 150 words by request of the guest editor. Thus, the introductory sentences of the original abstract (including former line 12 in page 2) were deleted and this comment does not longer apply in the revised abstract.

4) At the introduction more information are needed regarding the "klaCH4, namely gassing-out vs Oxygen Transfer Rate (OTR)" Why is it important ?

Additional information was provided in the introduction section of this revised manuscript regarding the relevance of kla_{CH4} in accordance to Reviewer 2 request (current page 4)

"Unfortunately, the validation of empirical methodologies for the determination of the volumetric CH_4 mass transfer coefficient (k_la_{CH4}) has been poorly addressed in literature (García-Ochoa et al., 2009; Rocha-Ríos et al., 2010). Indeed, the lack of cheap and reliable dissolved CH_4 sensors along with the low solubility and limited industrial interest of CH_4 to date have restricted the development of accurate methodologies for the characterization of k_la_{CH4}. Table S1 presents the main studies devoted to the characterization of the volumetric CH_4 mass transfer coefficients in bioreactors."

"This study aims at comparing and validating, for the first time, two experimental methodologies for the estimation of k_la_{CH4} (gassing-out vs oxygen transfer rate methods) in

order to accurately describe CH_4 abatement in a stirred tank reactor operated with a methanotrophic consortium at multiple stirring rates. This information is expected to provide the basis for a correct bioreactor design for CH_4 bioconversion into added-value products."

5) What are the literature estimated values for question 4? How was it calculated?

A new table (Table S1) compiling the most recent attempts to characterize the gas-liquid mass transfer in CH_4 treating bioreactors, along with the methodologies used to estimate kla_{CH4}, was added in the *supplementary materials* section accordingly.

1. Compilation of previous studies on the determination of kla_{CH4}

***Table S1**. Previous studies on the characterization of the gas-liquid CH_4 mass transport in bioreactors*

Bioreactor Configuration	***Methodology***	***kla_{CH4} (h^{-1})***	***Reference***
Immobilized soil bioreactor & Fermenter	*kla_{O2} based on the dynamic method combined with gas phase CH_4 analysis Model validation*	*16.3 ±0.67*	*Yu et al. (2010)*
Airlift bioreactor with internal recirculation	*kla_{O2} based on OTR No validation of CH_4-EC was achieved*	*28.6 -200**	*Rocha-Rios et al. (2011)*
Two-phase partitioning fermenter	*kla_{O2} based on static method No validation of CH_4-EC was achieved*	*6.2-35.4**	*Rocha-Rios et al. (2010)*
250 mL bottle	*kla_{CH4} based on CH_4 absorption experiments No methodology validation*	*22.7-41.9*	*Lee et al. (2015)*

**- Estimated from kla_{O2} using equation 4.*

Lee, S.Y., Mo, K.S., Choi, J.H., Hur, N.H., Kim, Y.K., Oh, B.K., Lee, J., 2015. Enhancement of CH_4-water mass transfer using methyl-modified mesoporous silica nanoparticles. Korean Journal of Chemical Engineering. 32(9), 1744-1748.

Rocha-Rios, J., Muñoz, R., Revah, S., 2010. Effect of silicone oil fraction and stirring rate on methane degradation in a stirred tank reactor. Journal of Chemical Technology and Biotechnology. 85, 314-319.

Rocha-Rios, J.M., Quijano, G., Thalasso, F., Revah, S., Muñoz, R., 2011. Methane biodegradation in a two-phase partitiong airlift reactor with gas recirculation. Journal of Chemical Technology and Biotechnology. 86(3), 353-360.

Yu, Y., Ramsay, J.A., Ramsay, B.A., 2006. On-Line estimation of dissolved methane concentration during methanotrophic fermentations. Biotechnology and Bioengineering. 95, 788-793.

In addition, a brief statement was included in the revised *Results and Discussion* section in order to compare the kla_{CH4} herein obtained with those previously reported in literature (current page 10-11)

"Finally, it should be highlighted that the k_la_{CH4} here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017)."

6) What is the impact of Kla CH4 in bioreactors? Several examples from the literature need to be stated because CH4 is poorly water soluble.

An explanatory remark describing the impact of k_la_{CH4} on bioreactor design was included in the *Results and Discussion* section of this revised manuscript in accordance to Reviewer 2 request (current page 11).

"At this point it should be also stressed that high k_la_{CH4} values are required in CH_4 treating bioreactors in order to compensate for the low concentration gradients imposed by the high Henry law constant of CH_4 (~ 30 at 25 ºC) (Lopez et al., 2013). Thus, the higher the k_la_{CH4} values, the lower the bioreactor volumes and consequently, the lower the investment costs."

7) Page 4 line 12 a reference is needed. [UTF-8?]"â€¦using a biorefinery approach"

A recent reference was included in former page 4 line 12 in accordance to Reviewer 2 request (current page 4).

"Indeed, CH_4 can be bioconverted into biopolymers, ectoine, protein, exopolysaccharides, etc., using a biorefinery approach (Cantera et al., 2017)."

Cantera, S., Frutos, O., López, J.C., Lebrero, R., Muñoz, R., 2017. Technologies for the bio-conversion of GHGs into high added value products: current state and future prospects. In: R. Álvarez (Ed.), Carbon Footprint and the Industrial Life Cycle, Springer, Cham, pp. 359-388.

Material and methods:

8) Apart from Sphingobacterium sp. CZ-UAM what are the other strains of the methanotrophic consortium. If the authors do not know the microbial composition they can report the inoculum source for of the methanotrophic consortium.

Unfortunately, a detailed microbial characterization of the methanotrophic consortium was not carried out. The source of the inoculum was included in this revised version of the manuscript in accordance to Reviewer 2 request (current page 5).

"The consortium was enriched from the UAM-Iztapalapa wastewater treatment plant (México City)."

9) The iodometric back-titration need to be briefly descripted.

A detailed description of the iodometric back-titration methodology was included in the revised *analytical methods* section in accordance to Reviewer 2 request (current page 8)

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

10) More information need to be provided regarding the KlaCH4 = KlaO2/1.16

This correlation was derived and empirically validated by Yu et al. (2010). This information was included in the revised version of the manuscript accordingly (current page 7).

"Finally, the volumetric mass transfer coefficient of CH_4 was estimated from k_La_{O2} according to Yu et al. (2010) using the correlation empirically validated for stirred tank reactors based on dissolved CH_4 measurements and a dynamic methodology to characterize k_La_{O2} (equation 4):"

Results:

11) What are the main conclusions from the first test? The statement page 9 line 4 [UTF-8?]"â€¦remains unclear in literature, despite its significant [UTF-8?]impactâ€¦" do not help the reader.

The statement in former page 8 line 55-page 9 line 7 was deleted in this revised manuscript in order to avoid further misunderstandings.

12) Why MSM supplementation with Cu at 50 µg L-1 was required to overcome process limitation? The authors need to explain the affinity of several methanogens for Cu.

A brief discussion to describe the role of Cu on CH_4 biodegradation and the typical levels of Cu to support the synthesis of the enzymes pMMO and sMMO was included in this revised version of the manuscript in accordance to Reviewer 2 request (current page 12).

"In this context, Cu positively regulates the activity of the enzymes pMMO and sMMO (particulate and soluble CH_4 monooxygenase, respectively) and controls the expression of their genes. Most methanotrophs grow optimally at Cu concentrations lower than 270 µg L^{-1}, though previous enzymatic assays have demonstrated that sMMO in type II methanotrophs is properly synthesized at low Cu concentrations (below 50 µg L^{-1}) (Graham et al., 1993; Bender and Conrad, 1995; Semrau et al., 2010)."

Bender, M., Conrad, R., 1995. Effect of CH_4 concentrations and soil conditions on the induction of CH_4 oxidation activity. Soil Biology and Biochemistry. 27(12), 1517-1527.

Graham, D.W., Chaudhary, J.A., Hanson, R.S., Arnold, R.G., 1993. Factors affecting competition between type I and type II methanotrophs in two-organism, continuous-flow reactors. Microbial ecology. 25(1), 1-17.

Semrau, J.D., DiSpirito, A.A., Yoon, S., 2010. Methanotrophs and copper. FEMS microbiology reviews. 34(4), 496-531.

13) The authors can report more studies apart from [21]. Page 10 line 21. Also a table that can contain KlaCH4 values from other studies, measuring methods, type of bioreactor can be included.

A table containing kla_{CH4} values from recent studies, along with the methodologies used and the type of bioreactor, was included in the *supplementary material* section as requested by Reviewer 2.

1. Compilation of previous studies on the determination of kla_{CH4}

***Table S1**. Previous studies on the characterization of the gas-liquid CH_4 mass transport in bioreactors*

Bioreactor Configuration	***Methodology***	***kla_{CH4} (h^{-1})***	***Reference***
Immobilized soil bioreactor & Fermenter	*kla_{O2} based on the dynamic method combined with gas phase CH_4 analysis Model validation*	*16.3 ±0.67*	*Yu et al. (2010)*
Airlift bioreactor with internal recirculation	*kla_{O2} based on OTR No validation of CH_4-EC was achieved*	*28.6 -200**	*Rocha-Rios et al. (2011)*
Two-phase partitioning fermenter	*kla_{O2} based on static method No validation of CH_4-EC was achieved*	*6.2-35.4**	*Rocha-Rios et al. (2010)*
250 mL bottle	*kla_{CH4} based on CH_4 absorption experiments No methodology validation*	*22.7-41.9*	*Lee et al. (2015)*

**- Estimated from kla_{O2} using equation 4.*

Lee, S.Y., Mo, K.S., Choi, J.H., Hur, N.H., Kim, Y.K., Oh, B.K., Lee, J., 2015. Enhancement of CH_4-water mass transfer using methyl-modified mesoporous silica nanoparticles. Korean Journal of Chemical Engineering. 32(9), 1744-1748.

Rocha-Rios, J., Muñoz, R., Revah, S., 2010. Effect of silicone oil fraction and stirring rate on methane degradation in a stirred tank reactor. Journal of Chemical Technology and Biotechnology. 85, 314-319.

Rocha-Rios, J.M., Quijano, G., Thalasso, F., Revah, S., Muñoz, R., 2011. Methane biodegradation in a two-phase partitiong airlift reactor with gas recirculation. Journal of Chemical Technology and Biotechnology. 86(3), 353-360.

Yu, Y., Ramsay, J.A., Ramsay, B.A., 2006. On-Line estimation of dissolved methane concentration during methanotrophic fermentations. Biotechnology and Bioengineering. 95, 788-793.

In addition, a brief statement was included in the revised *Results and Discussion* section in order to compare the kla_{CH4} herein obtained with those previously reported in literature (current page 10-11)

"Finally, it should be highlighted that the kla_{CH4} here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017)."

14) At the conclusion the estimated values or the absolute error between the calculated and the predicted values need to be recorded.

The CH_4 elimination capacities experimentally recorded in the continuous bioreactor and those estimated from the OTR methodology are now described in the conclusion section of this revised manuscript in accordance to Reviewer 2 request (current page 15)

"Thus, the empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} recorded at 400, 600 and 800 rpm, respectively, were comparable to the CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} predicted by the OTR methodology."

15) How can the results be used in practice, in which circumstance, which industry and how. Provide a good guideline to the reader.

The discussion section was modified in accordance to Reviewer 2 suggestion (current page 13 and 14). In addition, a practical application of the kla_{CH4} determined is shown in the *supplementary materials* section.

"3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although

recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of k_La_{CH4}, whose characterization has been conducted based on either classical k_La_{O2} determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the k_La_{CH4} estimated at the three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016)."

4. Application of the kla_{CH4} for process design

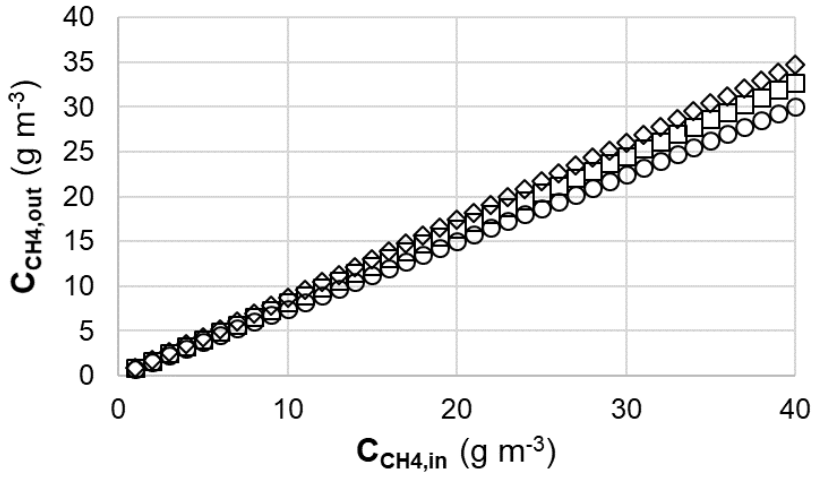


***Figure S3**. Model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the kla_{CH4} estimated at 400 (◊), 600 (□) and 800 rpm (○).*

The CH_4 outlet concentration, $C_{CH4,out}$, was derived from the definition of the CH_4 elimination capacity as follows (equation S1 and S2)

$$CH_4 - EC = \frac{Q}{V} \times \left(C_{CH4,in} - C_{CH4,out}\right) = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \quad (Eq.\ S1)$$

$$C_{CH4,out} = \frac{\left(\frac{Q}{V} \times C_{CH4,in}\right)}{\left(\frac{kla_{CH4}}{H_{CH4}} + \frac{Q}{V}\right)} \quad (Eq.\ S2)$$

REVIEWER 3

This manuscript presents data on the CH4 mass transfer characterization in stirred tank bioreactors devoted to CH4 bioconversion. While the manuscript provides useful practical information, it needs to be significantly improved so that the ideas and data presented in the manuscript and data interpretation and discussion are clear. I suggested MAJOR REVISION.

The authors acknowledge the positive feedback from Reviewer 3

1. The novelty/originality shall be further justified by highlighting that the manuscript contains sufficient contributions to the new body of knowledge.

The novelty of this work was highlighted in the introduction section of this revised version of the manuscript as requested by Reviewer 3 and the guest editor (current page 4)

"Unfortunately, the validation of empirical methodologies for the determination of the volumetric CH_4 mass transfer coefficient (kla_{CH4}) has been poorly addressed in literature (García-Ochoa et al., 2009; Rocha-Ríos et al., 2010). Indeed, the lack of cheap and reliable dissolved CH_4 sensors along with the low solubility and limited industrial interest of CH_4 to date have restricted the development of accurate methodologies for the characterization of kla_{CH4}. Table S1 presents the main studies devoted to the characterization of the volumetric CH_4 mass transfer coefficients in bioreactors.

*This study aims at comparing and validating, for the first time, two experimental methodologies for the estimation of kla_{CH4} (gassing-out vs oxygen transfer rate methods) in order to accurately describe CH_4 abatement in a stirred tank reactor operated with a methanotrophic consortium at multiple stirring rates. This information is expected to provide the basis for a correct bioreactor design for CH_4 bio*conversion into added-value products.*"*

In other words, literature survey is not sufficient. Therefore, I suggest that you should read the following references:

a) Photosynthetic CO2 uptake by microalgae for biogas upgrading and simultaneously biogas slurry decontamination by using of microalgae photobioreactor under various light wavelengths, light intensities, and photoperiods. Applied Energy
b) Review Microalgal cultivation with biogas slurry for biofuel production. Bioresource Technology
c) The effects of various LED lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae Chlorella sp. Energy

d) Biorefinery as a promising approach to promote microalgae industry: an innovative framework. Renewable and Sustainable Energy Reviews

The literature survey was improved in this revised version of the manuscript as recommended by Reviewer 3. The recommended references were included in a new 1.5 page section entitled "**3.3 Practical applications and future research perspectives"** (current page 14)

"In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016)."

2. More discussion and literature reference should be provided in the results and discussions. A lot of discussion is missing.

The interpretation and discussion of the results obtained was significantly improved as requested by Reviewer 3, 2 and 1. More sound discussions of the mechanisms underlying O_2 mass transfer during the gassing-out and sulphite oxidation experiment, and CH_4 mass transfer during the biotic experiment, was also provided. More specifically:

Current Page 9

"The type of O_2 sensor (polarographic vs optical) used in the gassing-out methodology did not significantly ($p \leq 0.05$) influence the measurement of k_La_{O2} (average error < 2.75 %), which confirmed that the significant ($p \leq 0.05$) differences"

Current page 10

"At this point it should be highlighted that moderate salinity media, despite slightly reducing the solubility of gases, typically result in enhanced gas-liquid k_La induced by the higher gas-liquid interfacial areas (Rodero et al., 2018)."

"Figure 2, which depicts the O_2 profiles in the liquid phase during the gassing-out and OTR experiments, clearly shows the higher mass transfer gradients (estimated locally from the slope of the decrease in dissolved O_2 concentration at the gas-liquid interphase) despite the overall difference in O_2 concentration between the interphase and the bulk liquid (200 mg L^{-1} – 0 mg L^{-1}) remained similar. These higher gradients mediated by the rapid oxidation of O_2 in the presence of SO_3^{-2} and Co^{2+} entailed a superior O_2 mass transfer, which mathematically resulted into higher k_La_{O2} (obtained from equations 1 and 3). Finally, it should be highlighted that the k_La_{CH4} here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017)."

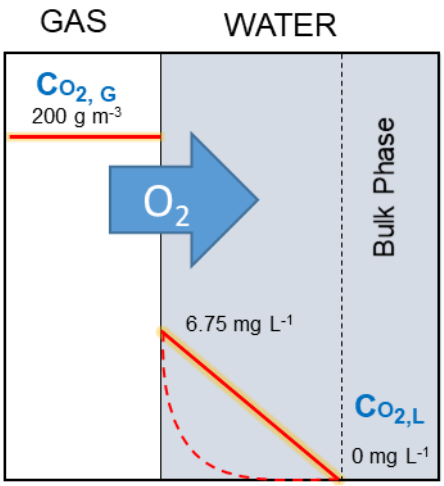


Figure 2. *O_2 concentration profiles in the liquid phase during the gassing-out (continuous line) and OTR (dashed line) methods.*

Current page 11

"At this point it should be also stressed that high kla_{CH4} values are required in CH_4 treating bioreactors in order to compensate for the low concentration differences imposed by the high Henry law constant of CH_4 (~ 30 at 25 ºC) (Lopez et al., 2013). Thus, the higher the kla_{CH4} values, the lower the bioreactor volumes and consequently, the lower the investment costs."

"This implies that the removal of CH_4 recorded at 400, 600 and 800 rpm can be attributed to the biocatalytic action of the microbial consortium present in the fermenter, which converts CH_4 to CO_2, H_2O and new biomass (Lopez et al., 2014)."

Current page 12

$$CH_4 - EC = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \qquad \textit{(Eq. 7)}$$

"Where H_{CH4} represents the dimensionless Henry law constant of CH_4 (28.7 at 30 ºC) and C_{LCH4} the aqueous concentration of CH_4 in the bulk phase, which is negligible under the mass transfer limiting scenario here encountered. In fact, no significant differences ($p \leq 0.05$) between the empirical and estimated CH_4-ECs were found at 400 and 800 rpm."

"In this context, Cu positively regulates the activity of the enzymes pMMO and sMMO (particulate and soluble CH_4 monooxygenase, respectively) and controls the expression of their genes. Most methanotrophs grow optimally at Cu concentrations lower than 270 µg L^{-1}, though previous enzymatic assays have demonstrated that sMMO in type II methanotrophs is properly synthesized at low Cu concentrations (below 50 µg L^{-1}) (Graham et al., 1993; Bender and Conrad, 1995; Semrau et al., 2010)."

"Thus, the rapid CH_4 biodegradation in the vicinity of the gas-liquid interphase mediated by methanotrophic bacteria likely supported an enhanced CH_4 mass transfer (induced by the higher local gradient close to the gas-liquid interphase)"

Current page 13-14

"3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of k_La_{CH4}, whose characterization has been conducted based on either classical k_La_{O2} determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the k_La_{CH4} estimated at the three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016)."

REVIEWER 4

The research focuses on the methane mass transfer in conventional fermenters. The authors compared two experimental methodologies for the estimation of the volumetric mass mass transfer coefficient for methane, concluding that the Oxygen Transfer Rate method described the methane mass transfer coefficient. The topic of the manuscript is interesting and related to the field of the journal. The objectives, results and conclusions are clearly presented.

The authors acknowledge the positive feedback from Reviewer 4

However, in my opinion some information in the methodology part are missing and the results of the control tests need to be shown.

The methodology part was significantly improved by adding a more detailed description in the *analytical methods* section and a new statistical treatment section (current page 8-9)

"A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again."

2.5 Statistical Treatment

All experiments were performed in duplicate and each assay was considered as a replicate. The results are given as the average value ± standard deviation from the duplicate assays. In addition, the results were analysed using a one-way ANOVA with significance at $p \leq 0.05$. The Excel statistical package (Microsoft Corporation, USA) was used for data treatment."

The gassing-out control test in the presence of sulphite during the kla_{O2} determination, along with the control abiotic test in the continuous bioreactor, are now provided in the *supplementary materials* section as Figure S1 and Figure S2.

2. Influence of stirring rate on the volumetric mass transfer coefficient of O_2

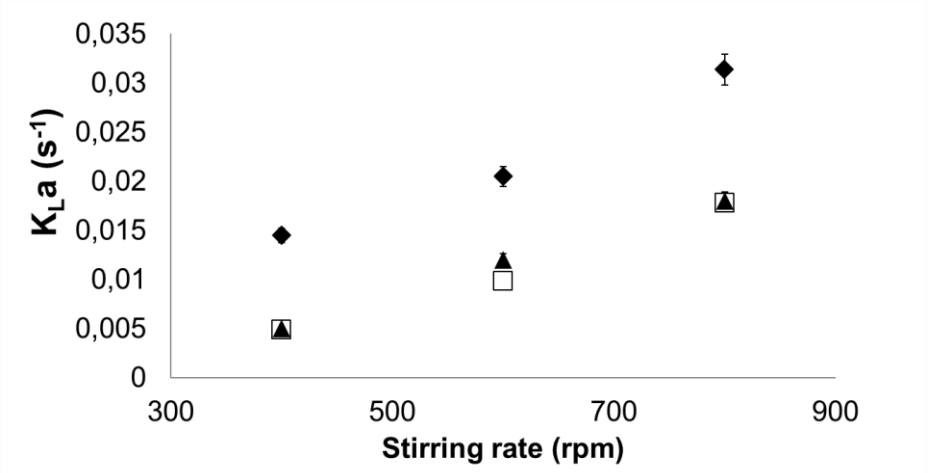


Figure S1. Influence of the stirring rate on the *kla* of O_2 determined by the OTR method (♦), gassing-out (□) and control gassing-out (▲).

3. Abiotic Test

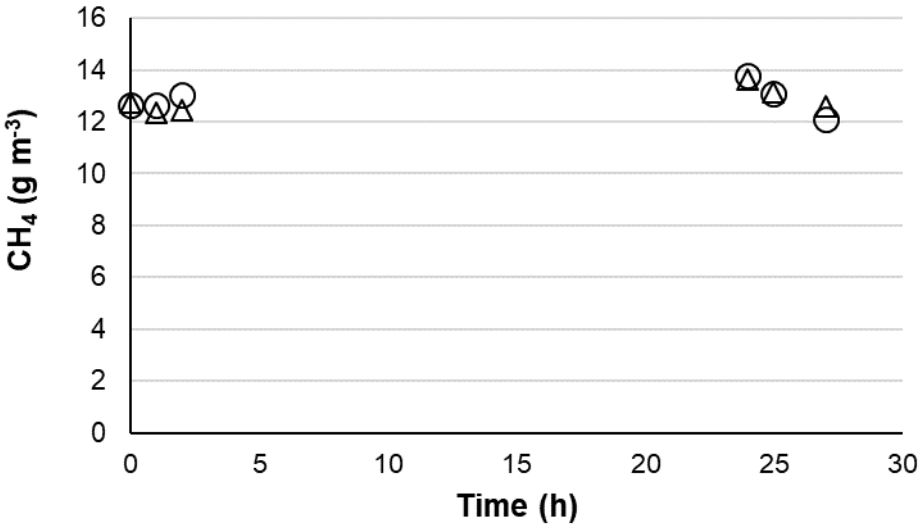
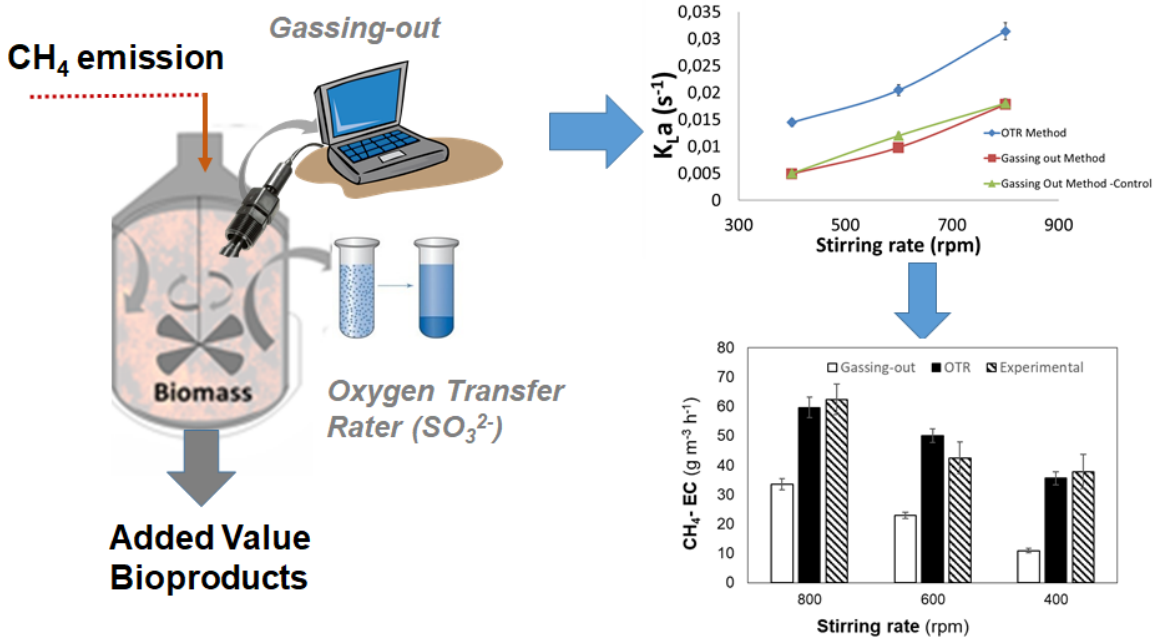


Figure S2. Time course of the inlet (○) and outlet (Δ) CH_4 concentration in the continuous bioreactor during the abiotic test.

1) Graphical abstract: images not clear. Moreover, the organization flow of the graphical abstract is not coherent with the text. From the way it is drawn, the gassing-out method seems the best method, which is not. The graph "kLa vs stirring rate" is not clear, missing units and legend.

The quality of the images, the organization flow of the information and the quality of figures provided in the revised graphical abstract were significantly improved in accordance to the suggestions from Reviewer 4 and the guest editor.



2) Third Highlight need to be rephrased: "Microbial methane uptake accelerated methane transport", where is this explained in the text?

The highlights were rephrased as requested by Reviewer 1 and Reviewer 4 as follows (see *Highlights* section):

k_La_{O2} determination based on SO_3^{-2} oxidation accurately described CH_4 mass transfer
Microbial CH_4 uptake accelerated CH_4 mass transport similarly to SO_3^{-2} oxidation
The gassing-out methodology underestimated CH_4 mass transport
Maximum CH_4 elimination capacities of 62 ± 5 g CH_4 m^{-3} h^{-1} were achieved at 800 rpm

Please note that the original Highlight 3 was supported by the discussion provided in former page 9 lines 43-56: *"Overall, the results here obtained confirmed that the classical gassing-out methodology for the estimation of k_La_{CH4} clearly underestimated CH_4 mass transfer under continuous biotic operation of the fermenter. Thus, the instantaneous CH_4 biodegradation at the gas-liquid interphase mediated by methanotrophic bacteria likely supported an enhanced CH_4 mass transfer, similarly to the acceleration in the O_2 mass transfer recorded in the OTR experiments. "*

3) kLa should be defined also in the abstract.

The acronym *kla* was defined in the revised abstract section in accordance to Reviewer 4 suggestion (current page 2).

"This study aimed at systematically comparing the potential of two empirical methods for the estimation of the volumetric mass transfer coefficient of CH_4 $(k_La_{CH4})_{...}$"

4) Lacking reference of the two methodologies (Page 5 Lines 24-26).

The corresponding references to the gassing-out and OTR methodologies were included in this revised version of the manuscript in accordance to Reviewer 4 request (current page 5)

"A 3.5 L ez-control fermenter (Applikon, The Netherlands) equipped with two rushton turbines and containing 2.5 L of MSM was used for the determination of the volumetric mass transfer coefficient of O_2 (k_La_{O2}) using the gassing-out and the oxygen transfer rate (OTR) methods (Quijano et al., 2009, Estrada et al., 2014b)."

The new reference (Estrada et al., 2014) was included in the revised reference section

Estrada, J.M., Dudek, A., Muñoz, R., Quijano, G., 2014b. Fundamental study on gas–liquid mass transfer in a biotrickling filter packed with polyurethane foam. Journal of Chemical Technology and Biotechnology. 89(9), 1419-1424.

5) Provide brief description of all the analytics used. Do not directly refer to literatures.

A more detailed description of the analyses conducted was included in the *analytical methods* section along with a new statistical treatment section (current page 8-9)

"A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again."

2.5 Statistical Treatment

All experiments were performed in duplicate and each assay was considered as a replicate. The results are given as the average value ± standard deviation from the duplicate assays. In addition, the results were analysed using a one-way ANOVA with significance at $p \leq 0.05$. The Excel statistical package (Microsoft Corporation, USA) was used for data treatment."

6) In page 6 Eq. 3, it is not defined how the OTR was determined. Please add one more equation or the correct reference. Moreover, the CO2,G is not defined in the text.

The parameters OTR and $C_{O2,G}$ are now defined in this revised manuscript and the corresponding reference was also included in accordance to Reviewer 4 suggestion (current page 7)

"Where OTR is calculated from the slope of the absorbed O_2 concentration (estimated from the SO_3^{-2} concentration multiplied by the O_2/SO_3^{2-} stoichiometric coefficient derived from equation 3) versus time plot, H_{O2} stands for the dimensionless Henry law constant of O_2 (30.9 at 30 ºC) and $C_{O2,G}$ represents the gas O_2 concentration in the exhaust gas (Zhao et al. 1999)"

The new reference (Zhao et al., 1999) was included in the revised reference section

Zhao, S., Kuttuva, S.G., Ju, L.K., 1999. Oxygen transfer characteristics of multiple-phase dispersions simulating water-in-oil xanthan fermentations. Bioprocess Engineering. 20, 313-323.

7) Please make sure the references are correct. For instance, Page 6 Line 18, ref. 15 is not describing the sulfite method, I think reference 17 is describing the method.

The citations used along the main text were double checked in accordance to Reviewer 4 request. In addition, former reference 15 was replaced by former reference 17 (current page 6)

"The OTR (g L^{-1} h^{-1}) from the gas phase to the MSM was estimated (in duplicate) by periodically monitoring the time course of sulfite oxidation in the fermenter according to Quijano et al. (2009)."

8) A lot of reference formatting errors. Use manual editing.

The authors apologize for the above mentioned formatting errors in the *references* section of the original manuscript. The format of the references was double checked manually in this revised submission in order to avoid further typos. The previous errors were highlighted in red colour (see *references* section).

9) Page 9 lines 24-26. The equation is not well explained.

The equation embedded in the text in former page 9 lines 24-26 (from now on equation 7) was more clearly described in the revised version of the manuscript in accordance to Reviewer 4 request (current page 11).

"These values were similar to the CH_4-ECs estimated using the OTR kla_{CH4} at a maximum concentration gradient (equation 7): 35.6 ± 2.2, 50.1 ± 2.3 and 59.6 ± 3.4 g CH_4 m^{-3} h^{-1} at 400, 600 and 800 rpm, respectively (Figure 2).

$$CH_4 - EC = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \qquad (Eq.\ 7)$$

Where H_{CH4} represents the dimensionless Henry law constant of CH_4 (28.7 at 30 ºC) and C_{LCH4} the aqueous concentration of CH_4, which is negligible under the mass transfer limiting scenario here encountered."

10) Page 9 Lines 11-16, no results are presented in the manuscript to show that abiotic methane removal was negligible. In my opinion, this is an important results considering that you are assuming that O2 uptake at the gas-liquid inter phase is mimicking the microbial methane uptake (Page 11 lines 6-12).

The results from the abiotic test conducted in the continuous bioreactor are included as Figure S2 in the *supplementary materials* section. In addition, a brief discussion of the implications of the absence of abiotic CH_4 removal in the bioreactor was included in current page 11.

3. Abiotic Test

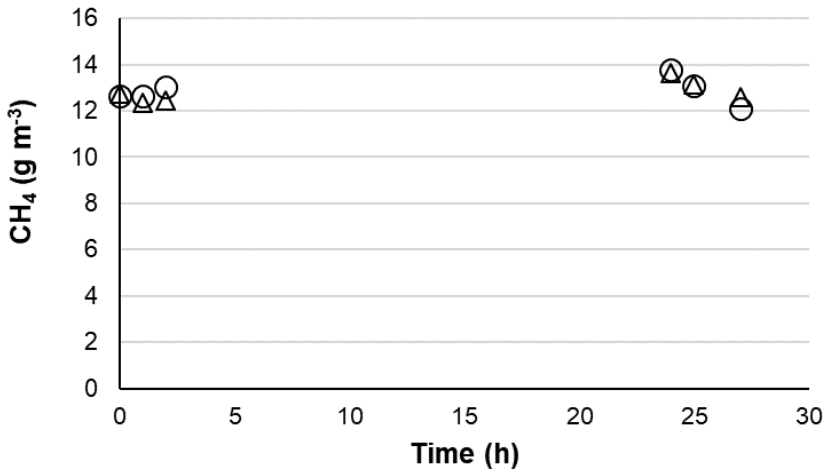


Figure S2. Time course of the inlet (○) and outlet (Δ) CH_4 concentration in the continuous bioreactor during the abiotic test.

"The abiotic test carried out in the fermenter under continuous gas phase supply revealed a negligible contribution of adsorption or photolysis to CH_4 removal in this particular experimental set-up (Figure S2). This implies that the removal of CH_4 recorded at 400, 600 and 800 rpm can be attributed to the biocatalytic action of the microbial consortium present in the fermenter, which converts CH_4 to CO_2, H_2O and new biomass (Lopez et al., 2014)."

Minor points of concern:

11) Page 10 lines 26-29, rephrase " has emerged in the past 5 years based on.... based on the bioconversion.

Former lines 26-29 in page 10 were reworded to **"***A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012)"* in accordance to Reviewer 4 request (current page 13).

12) Page 10 Line 43. full stop missing at the end of the phrase.

The sentence in former page 10 line 43 was reworded to "*Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries.*" and a full stop was included in accordance to Reviewer 4 request.

13) Many parts of the results and discussion section contain only results. It will be highly beneficial for the reader, if the authors also provide more insights and discuss based on their expertise so far. Some of the results are just stated without proper explanation/scientific discussion. This section has to be revised carefully.

The interpretation and discussion of the results obtained was significantly improved as requested by Reviewer 1, 3 and 4. More sound discussions of the mechanisms underlying O_2 mass transfer during the gassing-out and sulphite oxidation experiment, and CH_4 mass transfer during the biotic experiments, was also provided. More specifically:

Current Page 9

"The type of O_2 sensor (polarographic vs optical) used in the gassing-out methodology did not significantly ($p \leq 0.05$) influence the measurement of k_La_{O2} (average error < 2.75 %), which confirmed that the significant ($p \leq 0.05$) differences"

Current page 10

"At this point it should be highlighted that moderate salinity media, despite slightly reducing the solubility of gases, typically result in enhanced gas-liquid k_La induced by the higher gas-liquid interfacial areas (Rodero et al., 2018)."

"Figure 2, which depicts the O_2 profiles in the liquid phase during the gassing-out and OTR experiments, clearly shows the higher mass transfer gradients (estimated locally from the slope of the decrease in dissolved O_2 concentration at the gas-liquid interphase) despite the overall difference in O_2 concentration between the interphase and the bulk liquid (200 mg L^{-1} – 0 mg L^{-1}) remained similar. These higher gradients mediated by the rapid oxidation of O_2 in the presence of SO_3^{-2} and Co^{2+} entailed a superior O_2 mass transfer, which mathematically resulted into higher kla_{O2} (obtained from equations 1 and 3). Finally, it should be highlighted that the kla_{CH4} here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017)."

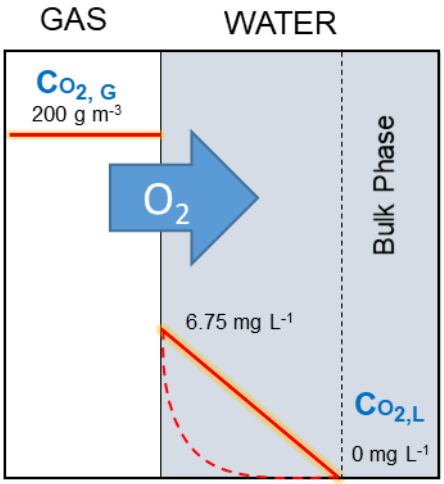


Figure 2. *O_2 concentration profiles in the liquid phase during the gassing-out (continuous line) and OTR (dashed line) methods.*

Current page 11

"At this point it should be also stressed that high kla_{CH4} values are required in CH_4 treating bioreactors in order to compensate for the low concentration differences imposed by the high Henry law constant of CH_4 (~ 30 at 25 ºC) (Lopez et al., 2013). Thus, the higher the kla_{CH4} values, the lower the bioreactor volumes and consequently, the lower the investment costs."

"This implies that the removal of CH_4 recorded at 400, 600 and 800 rpm can be attributed to the biocatalytic action of the microbial consortium present in the fermenter, which converts CH_4 to CO_2, H_2O and new biomass (Lopez et al., 2014)."

Current page 12

$$CH_4 - EC = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \qquad \text{(Eq. 7)}$$

"Where H_{CH4} represents the dimensionless Henry law constant of CH_4 (28.7 at 30 ºC) and C_{LCH4} the aqueous concentration of CH_4 in the bulk phase, which is negligible under the mass transfer limiting scenario here encountered. In fact, no significant differences ($p \leq 0.05$) between the empirical and estimated CH_4-ECs were found at 400 and 800 rpm."

"In this context, Cu positively regulates the activity of the enzymes pMMO and sMMO (particulate and soluble CH_4 monooxygenase, respectively) and controls the expression of their genes. Most methanotrophs grow optimally at Cu concentrations lower than 270 µg L^{-1}, though previous enzymatic assays have demonstrated that sMMO in type II methanotrophs is properly synthesized at low Cu concentrations (below 50 µg L^{-1}) (Graham et al., 1993; Bender and Conrad, 1995; Semrau et al., 2010)."

"Thus, the rapid CH_4 biodegradation in the vicinity of the gas-liquid interphase mediated by methanotrophic bacteria likely supported an enhanced CH_4 mass transfer (induced by the higher local gradient close to the gas-liquid interphase)"

Current page 13-14

"3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of k_La_{CH4}, whose characterization has been conducted based on either classical k_La_{O2} determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the k_La_{CH4} estimated at the three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016)."

REVIEWER 5

Authors present interesting experimental results highlighting the removal of methane under aerated condition.

The authors acknowledge the positive feedback from Reviewer 5

Comments

1) At least for the experimental conditions investigated in the work, they should try to simulate the observed CH4 concentration once it enters the reactor and leaves the reactor.

A simple model simulation of the CH_4 concentration leaving the reactor was conducted based on the kla_{CH4} estimated as requested by Reviewer 5. This information was highlighted in this revised version of the manuscript (current page 14) and comprehensively described in the *supplementary materials* section.

"For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the kla_{CH4} estimated at the three stirring rates tested."

4. Application of the kla_{CH4} for process design

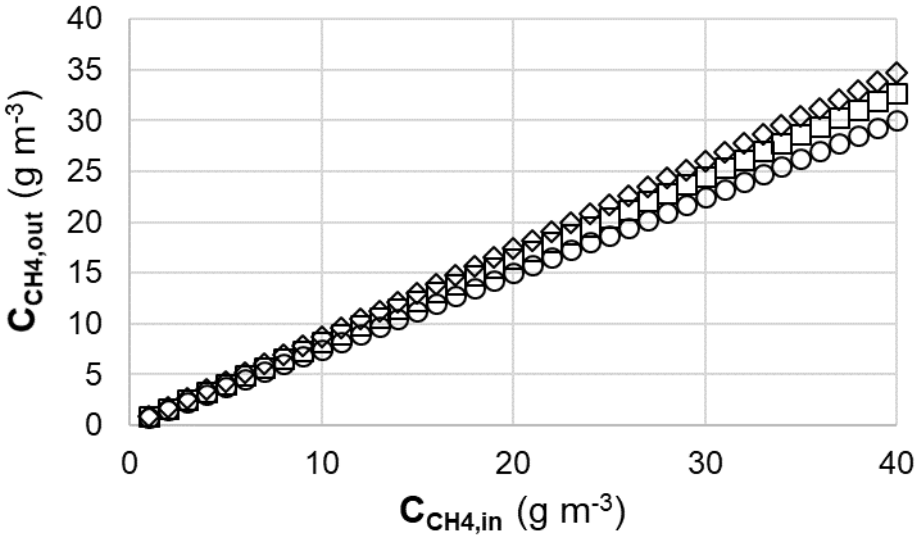


***Figure S3**. Model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the kla_{CH4} estimated at 400 (◊), 600 (□) and 800 rpm (○).*

The CH_4 outlet concentration, $C_{CH4,out}$, was derived from the definition of the CH_4 elimination capacity as follows (equation S1 and S2)

$$CH_4 - EC = \frac{Q}{V} \times \left(C_{CH4,in} - C_{CH4,out}\right) = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \quad (Eq.\ S1)$$

$$C_{CH4,out} = \frac{\left(\frac{Q}{V} \times C_{CH4,in}\right)}{\left(\frac{kla_{CH4}}{H_{CH4}} + \frac{Q}{V}\right)} \qquad \textit{(Eq. S2)}$$

2) Governing differential equations, used if any, must be stated along with factors affecting the kinetic parameters. This will help in further improvement of the quality of work.

The authors also acknowledge the relevance of the determination of the microbial kinetic parameters, but unfortunately, the study was focused on investigating process performance under mass transfer limiting conditions and steady state (where microbial kinetics are not governing the process). Similarly, since the process was studied under steady state conditions, no differential equation was used in the description of the process.

3) On page 5, line 56, in place of Mexico city, temperature should be a better choice.

The temperature used in the experiments carried out in the fermenter was included in this revised version of the manuscript in accordance to Reviewer 5 request (current page 6).

"Where $C^*_{O_2L}$ stands for the dissolved O_2 concentration at saturation (6.75 mg L^{-1} at Mexico city at 30 ºC),…"

4) on page 7: line 50, basis for these temperatures should be provided.

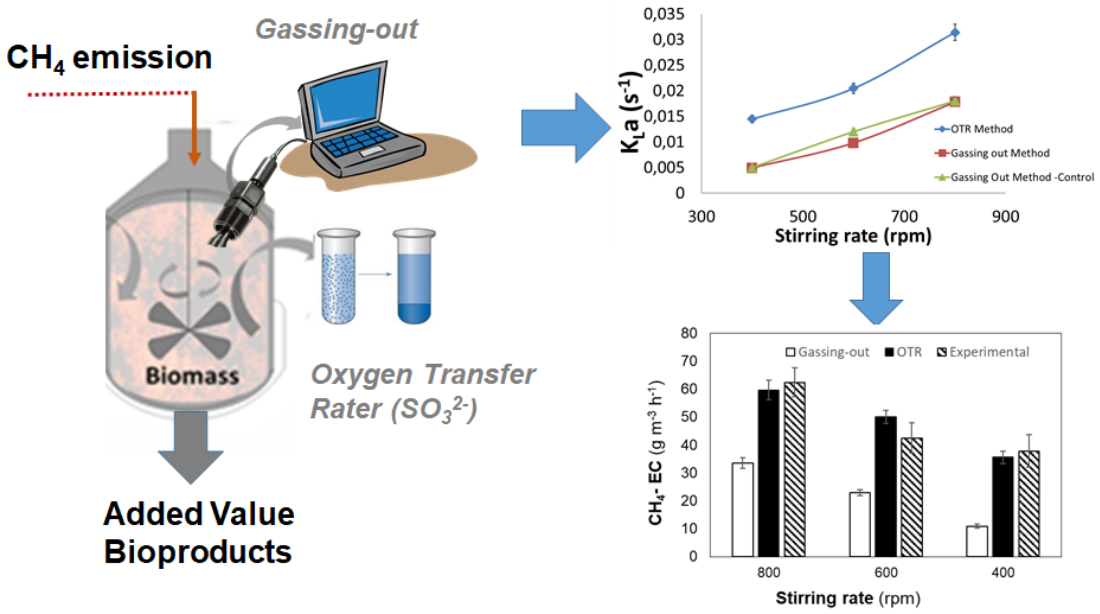
The temperatures set in the GC-TCD method in the injector, oven and detector were recommended by the manufacturer of the column for the separation of permanent gases. This information was provided in the revised manuscript in order to avoid any further misunderstanding (current page 8)

"The injector, oven and detector temperatures were maintained at 50, 40, 115 ºC, respectively, as recommended by the column manufacturer for the separation of permanent gases. "

GUEST EDITOR PROBLEMS

1) Check the quality of the image provided in the graphical abstract. The graph should be readable and understandable.

The quality of the graphical abstract was significantly improved (Arial font also used) in this revised submission as requested by the guest editor (and also by Reviewer 4).



2) Use times new roman, 12 font size in the document.

Times New Roman 12 is used along this revised manuscript in accordance to the request from the guest editor.

3) Use Arial font type for the graphs.

Arial font was used in the text and numbers contained in figures 1, 2 and 3 (former figure 2) as requested by the guest editor.

Figure 1

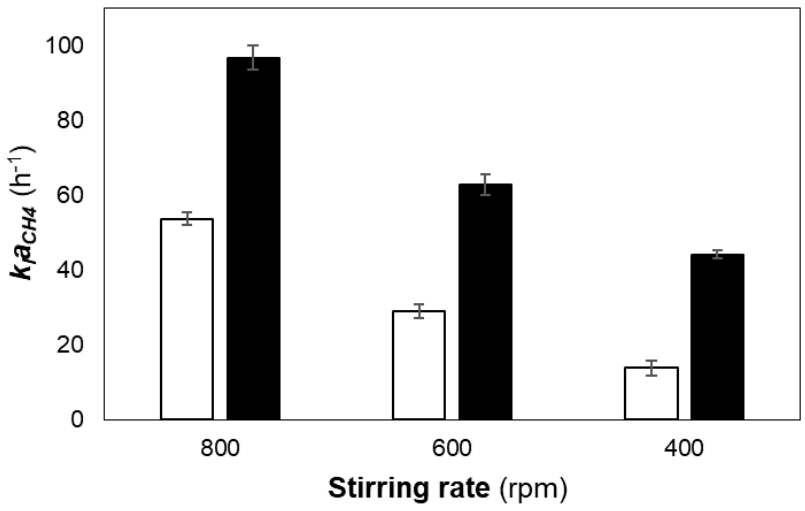


Figure 2

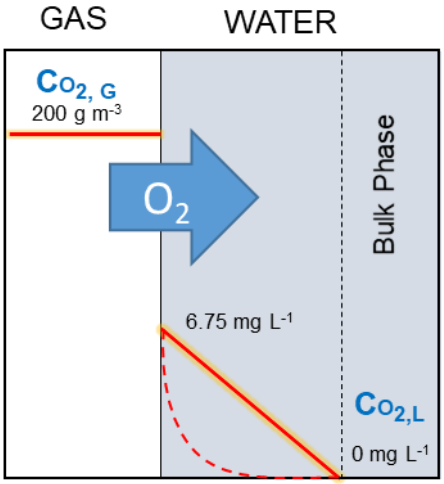
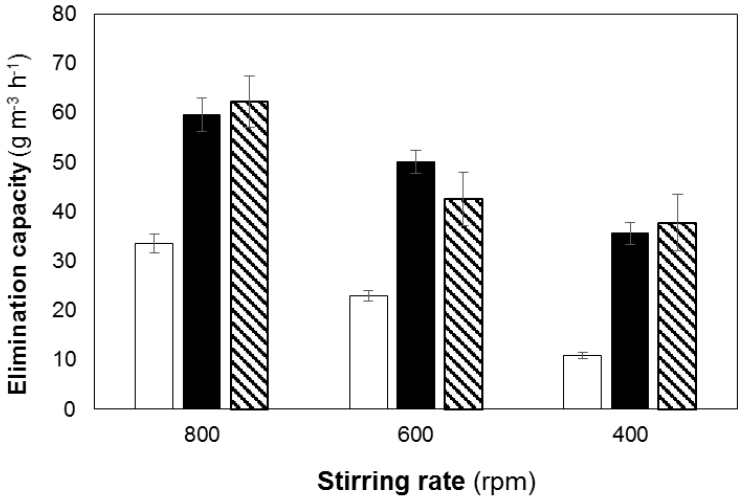


Figure 3



4) Write CH4 as methane in the title.

The title of the paper was modified in accordance to Reviewer 2 request to "*A systematic comparison of two empirical gas-liquid mass transfer determination methodologies to characterize methane biodegradation in stirred tank bioreactors*", which does not contain the acronym "CH_4" (current page 1)

5) Write the research gaps of this topic in 3-4 sentences.

The current research gaps in this topic are now clearly identified in the revised version of the introduction section (current page 4) as requested by the guest editor:

"Unfortunately, the validation of empirical methodologies for the determination of the volumetric CH_4 mass transfer coefficient (k_La_{CH4}) has been poorly addressed in literature (García-Ochoa et al., 2009; Rocha-Ríos et al., 2010). Indeed, the lack of cheap and reliable dissolved CH_4 sensors along with the low solubility and limited industrial interest of CH_4 to date have restricted the development of accurate methodologies for the characterization of

k_La_{CH4}. Table S1 presents the main studies devoted to the characterization of the volumetric CH_4 mass transfer coefficients in bioreactors."

6) Write the novelty of this work in 3-4 sentences.

The novelty of this work was highlighted in the introduction section of this revised version of the manuscript as requested by the guest editor and Reviewer 3 (current page 4)

"This study aims at comparing and validating, for the first time, two experimental methodologies for the estimation of k_La_{CH4} (gassing-out vs oxygen transfer rate methods) in order to accurately describe CH_4 abatement in a stirred tank reactor operated with a methanotrophic consortium at multiple stirring rates. This information is expected to provide the basis for a correct bioreactor design for CH_4 bioconversion into added-value products."

7) Provide the manufacture details of all equipment's, sensors, instruments used and their operational conditions clearly. Some are missing.

While the details of the fermenter and GC-TCD, along with their operational conditions, were initially provided in the original manuscript (former page 5 lines 19-29, former page 7 line 45-53), additional information of the sensors and analytical methodologies used in the study was included in current page 8-9:

"A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again."

8) Information on statistical analysis will be useful for the reader. Also, provide such information in the results and discussions section.

A new section on statistical data treatment (and its associated analyses) was included in both the *Material & Methods* and *Results & Discussion* sections of this revised version of the manuscript as requested by the guest editor (current page 9).

"2.5 Statistical Treatment

All experiments were performed in duplicate and each assay was considered as a replicate. The results are given as the average value ± standard deviation from the duplicate assays. In addition, the results were analysed using a one-way ANOVA with significance at P ≤0.05. The Excel statistical package (Microsoft Corporation, USA) was used for data treatment."

The revised *Results & Discussion* section also included the main findings of the statistical analysis as requested by the guest editor (current page 9 and 12)

"The type of oxygen sensor (polarographic vs optical) used in the gassing-out methodology did not significantly ($p \leq 0.05$) influence the measurement of k_la_{O2} (average error < 2.75 %), which confirmed that the significant ($p \leq 0.05$) differences observed in k_la_{CH4} between the OTR and gassing-out methodologies were not caused by the delay in the response of the polarographic sensor."

"In fact, no significant differences ($p \leq 0.05$) between the empirical and estimated CH_4-ECs were found at 400 and 800 rpm."

9) Write the abstract in less than 150 words and also the conclusions in less than 150 words.

The abstract and conclusion sections were significantly modified in order to highlight the main results and provide a more systematic explanation of the findings obtained as requested by Reviewer 1. In addition, the length of both sections was reduced to 150 words as requested by the guest editor (current pages 2 and 14-15).

Abstract (150 words)

"This study aimed at systematically comparing the potential of two empirical methods for the estimation of the volumetric CH_4 mass transfer coefficient (k_la_{CH4}), namely gassing-out and oxygen transfer rate (OTR), to describe CH_4 biodegradation in a fermenter operated with a methanotrophic consortium at 400, 600 and 800 rpm. The kla_{CH4} estimated from the OTR methodology accurately predicted the CH_4 elimination capacity (EC) under CH_4 mass transfer limiting conditions regardless of the stirring rate (~ 9 % of average error between empirical and estimated ECs). Thus, empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} vs predicted CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} were recorded at 400, 600 and 800 rpm, respectively. The rapid Co^{2+}-catalyzed reaction of O_2 with SO_3^{-2} in the vicinity of the gas-liquid interphase during OTR determinations, mimicking microbial CH_4 uptake in the biotic experiments, was central to accurately describe the k_la_{CH4}."

Conclusions (119)

"This work empirically validated a simple methodology to accurately describe CH_4 mass transport and biodegradation in continuous stirred tank bioreactors based on OTR estimations. The rapid O_2 uptake at the gas-liquid interphase mediated by sulfite and Co^{2+}, mimicking microbial CH_4 uptake during continuous CH_4 treatment, supported the superior performance of the OTR methodology for the description of k_la_{CH4} compared to the classical gassing-out methodology. Thus, the empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} recorded at 400, 600 and 800 rpm, respectively, were comparable to the CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} predicted by the OTR methodology.

This finding will help in the design of suspended growth bioreactors devoted to CH_4 bioconversion."

10) Do not use [1], [2] numbering style for references. We kindly suggest you to write the names of the authors in the text.

The style to refer to the citations in the main text was modified as requested by the guest editor.

11) Do not use UNWANTED upper case alphabets in the text without any reason. There are several such mistakes in the entire document.

The terms "Oxygen Transfer Rate", "Equation", "Helium" and "Rushton" now read "*oxygen transfer rate*", "*equation*", "*helium*", "*rushton*" along the main text as recommended by the guest editor.

12) Provide more details of the analytical techniques, the columns, the elution time, etc.

The *Analytical methods* section was significantly improved in this revised version of the manuscript by providing the description of the dissolved oxygen sensors, and the detailed methodologies for the determination of biomass and sulphite concentrations (current page 8-9):

"A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again."

13) What is fast kinetics? How fast is fast?

The presence of cobalt in the aqueous phase increases the kinetics of sulphite oxidation as previously reported by Zhao et al. (1999). This information is now included in the *Material & Method* section of the revised manuscript (current page 6)

"*The kinetics of O_2 absorption were recorded after the addition of the catalyst (5 mL of a 2.5×10^{-4} M $CoSO_4$ solution, which supports an instantaneous SO_3^{2-} oxidation) until complete SO_3^{-2} depletion according to equation 2.* "

In addition, the reference to the original method of determination of oxygen mass transfer rate was included in the revised paper (current page 10)

"*The extremely fast (almost instantaneous) kinetics of this reaction (faster than any gas-liquid diffusional mechanisms in the mass transfer process) in the presence of Co^{2+} likely accelerated O_2 uptake from the gas phase in the fermenter (Zhao et al. 1999),..*"

Zhao, S., Kuttuva, S.G., Ju, L.K., 1999. Oxygen transfer characteristics of multiple-phase dispersions simulating water-in-oil xanthan fermentations. Bioprocess Engineering 20, 313- 323

14) The results and discussions section should be divided according to the specific objectives of this study.

The *Results and Discussion* section in this revised manuscript was divided into three sub-sections in accordance to the specific objectives of the study (current page 9 and 10)

3.1 Volumetric mass transfer coefficient determination

3.2 CH_4 abatement under continuous operation: methodology validation

3.3 Practical applications and future research perspectives

15) Write a new section on practical applications and future research perspectives, before the conclusions section. In this new section, the authors should explain, how the results of this work can be applied in the succesful operation of bioreactors, what are the limitations, what future work can be done, etc. Write at least one page of information.

A new section discussing the practical applications of the results herein obtained and future research perspectives, including all items requested by the guest editor, was included in this revised version of the manuscript (current pages 13-14). In addition, a practical application of the kla_{CH4} determined is shown in the *supplementary materials* section.

"3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although

recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of k_La_{CH4}, whose characterization has been conducted based on either classical k_La_{O2} determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate k_La_{CH4}, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the k_La_{CH4} estimated at the three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016)."

3. Application of the kla_{CH4} for process design

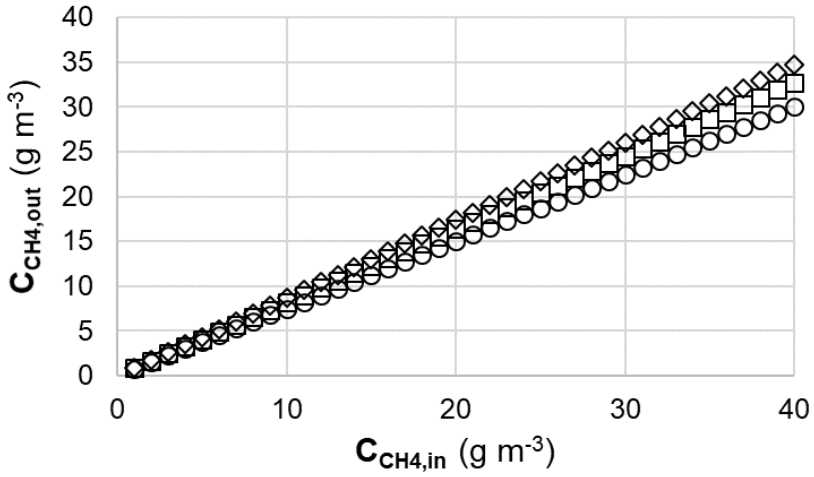


***Figure S3**. Model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the kla_{CH4} estimated at 400 (◊), 600 (□) and 800 rpm (○).*

The CH_4 outlet concentration, $C_{CH4,out}$, was derived from the definition of the CH_4 elimination capacity as follows (equation S1 and S2)

$$CH_4 - EC = \frac{Q}{V} \times \left(C_{CH4,in} - C_{CH4,out}\right) = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \quad (Eq.\ S1)$$

$$C_{CH4,out} = \frac{\left(\frac{Q}{V} \times C_{CH4,in}\right)}{\left(\frac{kla_{CH4}}{H_{CH4}} + \frac{Q}{V}\right)} \quad (Eq.\ S2)$$

16) References

- Should be carefully formatted. There are a lot of unwanted capital letters, small letters, etc. Please check LINE by LINE.
- There are SEVERAL punctuation errors.
- Journal names are not written in a consistent manner.
- Check a recent issue of JEMA or read the author guidelines carefully.
- Check how to refer to conference proceedings.
- Some author names are written without accents.
- Biological names should be written in italics.

The reference section was carefully double checked in order to avoid unwanted capital letters and punctuation errors, and to include biological names in italics and the journal names in a consistent manner (see revised *references* section with changes highlighted in red)

17) Once again, check all the formatting, spacing and sentence structure mistakes. As pointed out by the reviewers, there are several minor editing mistakes in the document.

The manuscript was double-checked prior submission to minimize the number of editing errors (typos, formatting, spacing and sentence structure mistakes) as requested by the guest editor.

18) The role of Co2+ and the mechanism should be discussed further.

The presence of cobalt in the aqueous phase increases the kinetics of sulphite oxidation as previously reported by Zhao et al. (1999). This information is now included in the *Material & Methoda* section of the revised manuscript (current page 6)

"*The kinetics of O_2 absorption were recorded after the addition of the catalyst (5 mL of a 2.5×10^{-4} M $CoSO_4$ solution, which supports an instantaneous SO_3^{2-} oxidation) until complete SO_3^{-2} depletion according to equation 2.* "

In addition, the reference to the original method of determination of oxygen mass transfer rate was included in the revised paper (current page 10)

"*The extremely fast (almost instantaneous) kinetics of this reaction (faster than any gas-liquid diffusional mechanisms in the mass transfer process) in the presence of Co^{2+} likely accelerated O_2 uptake from the gas phase in the fermenter (Zhao et al. 1999),..*"

Zhao, S., Kuttuva, S.G., Ju, L.K., 1999. Oxygen transfer characteristics of multiple-phase dispersions simulating water-in-oil xanthan fermentations. Bioprocess Engineering 20, 313- 323

19) What are the reasons for the under-estimation of the mass transfer values using the conventional method.

The reasons underlying the under-estimation of the mass transfer values using the conventional gassing-out methods were better explained in this revised manuscript in accordance to Reviewer 1 and the guest editor (current page 10)

"Therefore, the difference in the volumetric mass transfer coefficient of O_2, which directly impacted on the estimation of k_la_{CH4}, was attributed to a mass transfer acceleration mediated by the instantaneous chemical oxidation of O_2 by SO_3^{2-} (Eq. 2). The extremely fast (almost instantaneous) kinetics of this reaction (faster than any gas-liquid diffusional mechanisms in the mass transfer process) in the presence of Co^{2+} likely accelerated O_2 uptake from the gas phase in the fermenter (Zhao et al., 1999), which rendered the classical two-films model not valid since the O_2 concentration gradients in the liquid phase were no longer linear under this particular scenario (Van Krevelen et al., 1948). Figure 2, which depicts the O_2 profiles in the liquid phase during the gassing-out and OTR experiments, clearly shows the higher mass transfer gradients (estimated locally from the slope of the decrease in dissolved O_2 concentration at the gas-liquid interphase) despite the overall difference in O_2 concentration between the interphase and the bulk liquid (200 mg L^{-1} – 0 mg L^{-1}) remained similar. These higher gradients mediated by the rapid oxidation of O_2 in the presence of SO_3^{-2} and Co^{2+} entailed a superior O_2 mass transfer, which mathematically resulted into higher k_la_{O2} (obtained from equations 1 and 3)"

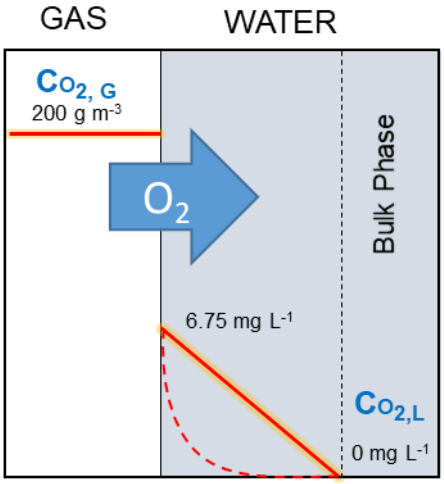


Figure 2. *O_2 concentration profiles in the liquid phase during the gassing-out (continuous line) and OTR (dashed line) methods.*

20) Be consistent in writing chemical names. Sometimes the authors are writing CH4, then sometimes, methane. Similarly, for a lot of other chemical names.

"Sulphite" was replaced by "SO_3^{2-}", "oxygen" was replaced by "O_2" and "methane" was replaced by "CH_4" in the main text of this revised manuscript in order to be consistent with the writing of the names of chemical species.

Kind advise:

A) Provide a separate word document on ''authors response to reviewers comments'' and explain how all the comments were incorporated. Highlight all the textual changes in YELLOW colour. Remove all the track changes and comments from the word file.

The authors included in this document a meticulous description of the changes carried out in the main text in response to the Reviewers' queries. In fact, an itemized response to all comments of each reviewer, along with a description of the changes performed in the revised manuscript, was included in this document. The changes performed in the main manuscript and *supplementary materials* files were however highlighted in **RED** colour (for a better readability).

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C) We strongly advise all the authors of this manuscript to carefully check the manuscript, at least 2 times, and correct all minor edits. Its very important for JEMA.

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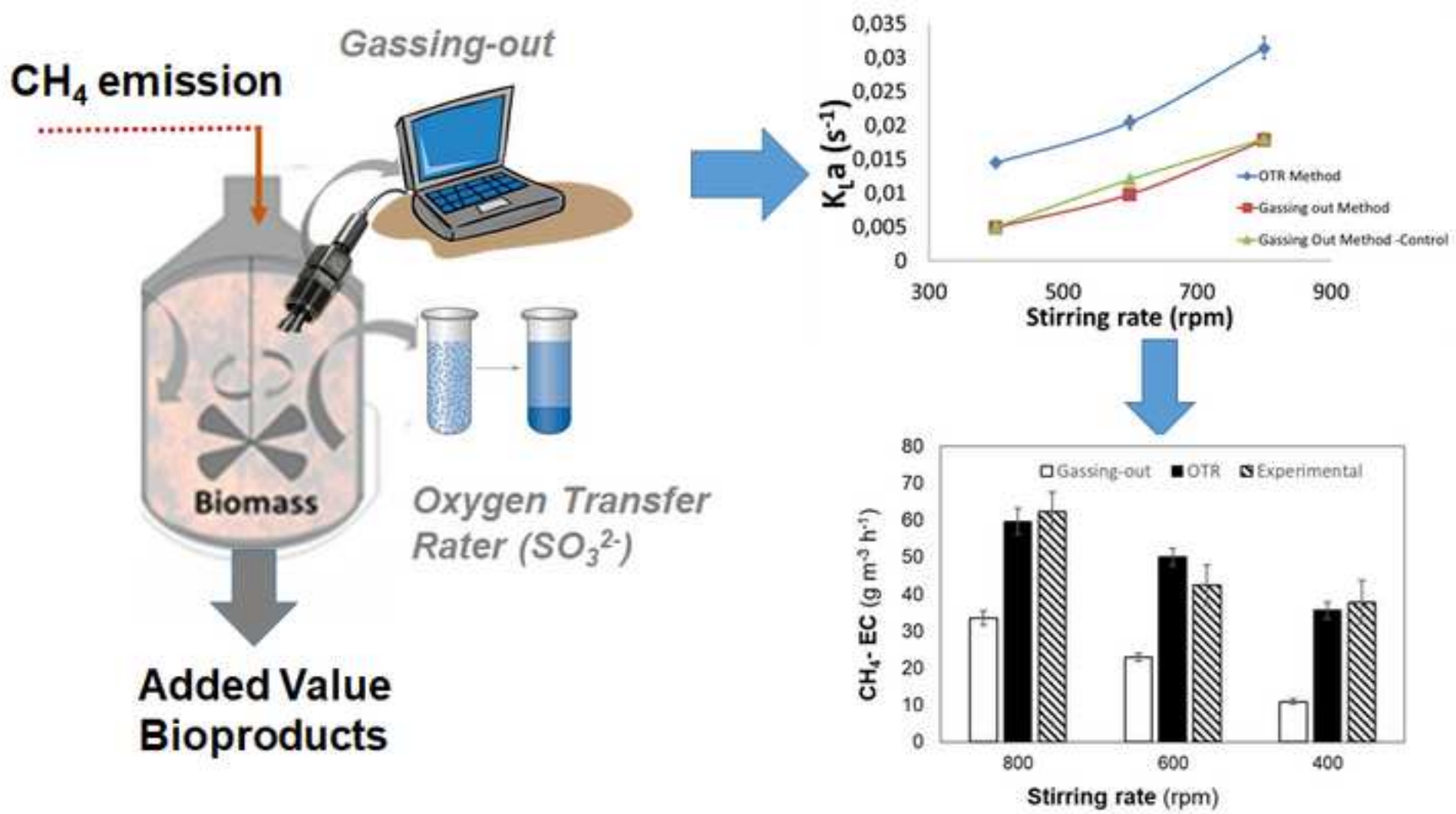
Raúl Muñoz **Sergio Revah Moissew**

Graphical Abstracts



***Highlights R1**

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- $k_l a_{O2}$ determination based on SO_3^{-2} oxidation accurately described CH_4 mass transfer
- Microbial CH_4 uptake accelerated CH_4 mass transport similarly to SO_3^{-2} oxidation
- The gassing-out methodology underestimated CH_4 mass transport
- Maximum CH_4 elimination capacities of 62 ± 5 g CH_4 m^{-3} h^{-1} were achieved at 800 rpm

A systematic comparison of two empirical gas-liquid mass transfer determination methodologies to characterize methane biodegradation in stirred tank bioreactors

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Abstract

This study aimed at systematically comparing the potential of two empirical methods for the estimation of the volumetric CH_4 mass transfer coefficient ($k_l a_{CH4}$), namely gassing-out and oxygen transfer rate (OTR), to describe CH_4 biodegradation in a fermenter operated with a methanotrophic consortium at 400, 600 and 800 rpm. The kla_{CH4} estimated from the OTR methodology accurately predicted the CH_4 elimination capacity (EC) under CH_4 mass transfer limiting conditions regardless of the stirring rate (~ 9 % of average error between empirical and estimated ECs). Thus, empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} *vs* predicted CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} were recorded at 400, 600 and 800 rpm, respectively. The rapid Co^{2+}-catalyzed reaction of O_2 with SO_3^{-2} in the vicinity of the gas-liquid interphase during OTR determinations, mimicking microbial CH_4 uptake in the biotic experiments, was central to accurately describe the $k_l a_{CH4}$.

Keywords: Biological gas treatment; Greenhouse gas; CH_4; Suspended growth bioreactor; Volumetric mass transfer coefficient.

1. Introduction

The assessment of current global greenhouse gas (GHG) emissions based on the 1-year pulse emission analysis has confirmed that CH_4 entails a contribution in terms of global warming potential (GWP) or global temperature change potential comparable to that of CO_2 for short time horizons (IPCC 2013). This fact can be attributed to the high GWP of CH_4 (90 and 72 on a 10 and 20 years horizon, respectively) (Global-Change 2012). However, despite the environmental relevance of CH_4 emissions, the development of cost-efficient and environmentally friendly treatment technologies (especially of those intended for the treatment of diluted emissions where no energy recovery is technically feasible) has been scarce. Today, physical/chemical CH_4 abatement technologies, such as activated carbon adsorption and incineration, are either inefficient or costly at the low concentrations typically found in emissions from waste treatment facilities (except young landfills), mines and animal farms, and possess a large CO_2 footprint as a result of their intensive energy use (Estrada et al., 2012; Melse and Van Der Werf 2009).

Biological methods represent an environmentally friendly alternative to physical/chemical methods for the abatement of CH_4. Biotechnologies, which rely on the biocatalytic action of specialized microorganisms, have been consistently proven as robust and efficient methods for the treatment of malodorous and industrial volatile organic compounds, exhibiting lower operating costs and environmental impacts than their physical/chemical counterparts (Estrada et al., 2012; Ferdowsi et al., 2017; Lopez et al., 2014). However, the cost-efficient application of conventional biotechnologies such as biofiltration or biotrickling filtration for the abatement of CH_4 is often limited by the poor mass transfer of this GHG from the gas emission to the methanotrophic community. This entails the need

for large gas residence times (0.1-10 h), and therefore large bioreactor volumes and investment costs, which typically jeopardizes the economics of CH_4 abatement (Lopez et al., 2013). In this context, the bioconversion of CH_4 into added-value products in stirred tank bioreactors represents a promising alternative to enhance the economic sustainability of CH_4 abatement (Garcia-Perez et al., 2018; Karthikeyan et al., 2015; Pieja et al., 2012) Indeed, CH_4 can be bioconverted into biopolymers, ectoine, protein, exopolysaccharides, etc., using a biorefinery approach (Cantera et al., 2017). The development of cost-competitive CH_4 biorefineries, inherently limited by CH_4 supply to the microbial community, requires the development of accurate methodologies for the characterization of CH_4 gas-liquid mass transfer in conventional fermenters (Cantera et al., 2017). Unfortunately, the validation of empirical methodologies for the determination of the volumetric CH_4 mass transfer coefficient ($k_l a_{CH4}$) has been poorly addressed in literature (García-Ochoa et al., 2009; Rocha-Ríos et al., 2010). Indeed, the lack of cheap and reliable dissolved CH_4 sensors along with the low solubility and limited industrial interest of CH_4 to date have restricted the development of accurate methodologies for the characterization of $k_l a_{CH4}$. Table S1 presents the main studies devoted to the characterization of the volumetric CH_4 mass transfer coefficients in bioreactors.

This study aims at comparing and validating, for the first time, two experimental methodologies for the estimation of $k_l a_{CH4}$ (gassing-out *vs* oxygen transfer rate methods) in order to accurately describe CH_4 abatement in a stirred tank reactor operated with a methanotrophic consortium at multiple stirring rates. This information is expected to provide the basis for a correct bioreactor design for CH_4 bioconversion into added-value products.

2. Material and methods

2.1. Microorganism and mineral salt medium

A mixed methanotrophic bacterial consortium containing *Sphingobacterium sp*. CZ-UAM (GenBankKJ411920) was used for CH_4 biodegradation purposes (Steffani-Vallejo et al., 2017). The consortium was enriched from the UAM-Iztapalapa wastewater treatment plant (México City). The composition of the mineral salt medium (MSM) used in the experiments was as follows (g l^{-1}): K_2HPO_4 0.7, KH_2PO_4 0.54, $MgSO_4 \cdot 7H_2O$ 1, $CaCl_2 \cdot 2H_2O$ 0.2, $FeSO_4 \cdot 7H_2O$ 0.004, NH_4CL 0.5, $ZnSO_4 \cdot 7H_2O$ 0.1, $MnCl_2 \cdot 4H_2O$ 0.03, H_3BO_3 0.3, $CoCl_2 \cdot 6H_2O$ 0.2, $CuCl_2 \cdot 2H_2O$ 0.01, $NiCl_2 \cdot 6H_2O$ 0.02 and $NaMoO_4 \cdot 2H_2O$ 0.06 (ATCC medium 1683). All chemicals were purchased from Sigma-Aldrich (USA). CH_4 (99.9%) was obtained from Praxair (Mexico).

2.2 Determination of the volumetric CH_4 mass transfer coefficient

A 3.5 L *ez*-control fermenter (Applikon, The Netherlands) equipped with two rushton turbines and containing 2.5 L of MSM was used for the determination of the volumetric mass transfer coefficient of O_2 (k_la_{O2}) using the gassing-out and the oxygen transfer rate (OTR) methods (Quijano et al., 2009, Estrada et al., 2014). The k_la_{O2} was recorded at an aeration rate of 0.42 L min^{-1} under three stirring rates (400, 600 and 800 rpm) at 30 ºC. These aeration rates were selected as potential operational conditions implemented in large-scale fermenters devoted to the bioconversion of CH_4.

In the gassing-out method, the MSM was initially degassed inside the fermenter for 15 min with argon at 0.42 L min^{-1} at the corresponding stirring rate (to complete dissolved O_2

depletion) and the dissolved O_2 concentration (DO) was then recorded every 5 seconds following the initiation of the aeration. The tests were carried out in duplicate using both polarographic and luminescent/optical dissolved O_2 sensors calibrated before each experimental series. The $k_l a_{O2}$ (h^{-1}) was determined using equation 1:

$$k_l a_{02} = -ln\left(\frac{C^*_{O_2L} - C_{O_2,L}}{C^*_{O_2L} - (C_{O_2,L})_0}\right)/t \qquad \text{(Eq. 1)}$$

Where $C^*_{O_2L}$ stands for the dissolved O_2 concentration at saturation (6.75 mg L^{-1} at Mexico city at 30 ºC), $C_{O_2,L}$ the dissolved O_2 concentration at time *t*, and $(C_{O_2,L})_0$ the dissolved O_2 concentration at the beginning of the test (DO = 0 mg L^{-1} at *t* =0 s, since the cultivation broth was initially degassed with argon). In addition, a similar test series as above described was conducted with MSM supplemented with 0.03 M Na_2SO_3.

The OTR (g L^{-1} h^{-1}) from the gas phase to the MSM was estimated (in duplicate) by periodically monitoring the time course of SO_3^{-2} oxidation in the fermenter according to Quijano et al. (2009). The bioreactor was initially filled with 2.5 L of MSM containing sodium sulfite at 0.03 M and aerated for 30 minutes prior to the test. The kinetics of O_2 absorption were recorded after the addition of the catalyst (5 mL of a 2.5×10^{-4} M $CoSO_4$ solution, which supports an instantaneous SO_3^{2-} oxidation) until complete SO_3^{-2} depletion according to equation 2.

$$SO_3^{2-} + 0.5\,O_2 \xrightarrow{Co^{2+}} SO_4^{2-} \qquad \text{(Eq. 2)}$$

Aqueous samples of 5 mL were drawn from the bulk phase of the fermenter and SO_3^{-2} concentration was determined by iodometric back-titration according to Zhao et al. (1999). The $k_l a_{O2}$ was determined using equation 3:

$$k_l a_{02} = \frac{OTR}{\frac{C_{O_2,G}}{H_{O2}} - C_{O_2,L}} \quad \text{(Eq. 3)}$$

Where OTR is calculated from the slope of the absorbed O2 concentration (estimated from the SO3-2 concentration multiplied by the O2/SO32- stoichiometric coefficient derived from equation 3) versus time plot, H_{O2} stands for the dimensionless Henry law constant of O_2 (30.9 at 30 ºC) and $C_{O2,G}$ represents the gas O_2 concentration in the exhaust gas (Zhao et al. 1999). Finally, the volumetric mass transfer coefficient of CH_4 was estimated from $k_l a_{O2}$ according to Yu et al. (2010) using the correlation empirically validated for stirred tank reactors based on dissolved CH_4 measurements and a dynamic methodology to characterize kla_{O2} (equation 4):

$$k_l a_{CH4} = \frac{k_l a_{O2}}{1{,}16} \quad \text{(Eq. 4)}$$

2.3 Determination of the influence of the stirring rate on CH_4 elimination capacity

The 3.5 L *ez*-control fermenter equipped with two rushton turbines was initially filled with 2.5 L of MSM and inoculated with the bacterial consortium. The bioreactor was continuously supplied with a CH_4-laden air emission (0.42 L min^{-1}) at a CH_4 concentration of 26.9 ± 1.7 g m^{-3} and Cu-supplemented MSM (50 µg L^{-1} of $CuSO_4$) at a dilution rate of 0.12 d^{-1}. The temperature and pH of the cultivation broth were automatically controlled at 30 ºC and 7, respectively. The system was initially operated for 60 days at 800 rpm to reach a steady state biomass concentration of 2.5 ± 0.4 g L^{-1} in order to prevent process limitation

by microbial activity. The influence of the stirring rate (800, 600 and 400 rpm) on the CH_4 elimination capacity (EC) was evaluated by monitoring the inlet and outlet CH_4 gas concentration ($C_{CH4,in}$ and $C_{CH4,out}$, respectively) for at least 7 consecutive days at each stirring rate. The EC was calculated according to equation 5:

$$EC = \frac{C_{CH4,in} - C_{CH4,out}}{C_{CH4,in}} \quad \text{(Eq. 5)}$$

A control test was initially conducted for 2 days in the absence of biomass in order identify any potential abiotic removal of CH_4.

2.4. Analytical methods

CH_4 gas concentration was measured in a GOW MAC series 550 GC-TCD (USA) equipped with a CTR1 packed column (Alltech, USA) using helium as a carrier gas at 60 ml min^{-1}. The injector, oven and detector temperatures were maintained at 50, 40, 115 ºC, respectively, as recommended by the column manufacturer for the separation of permanent gases.

A polarographic sensor (Applikon, The Nethelands) and a luminescent/optical dissolved O_2 sensor (Intellical™ LDO101, Hach, USA) were used for the measurement of the dissolved O_2 concentration in the cultivation broth during the gassing-out experiments.

The determination of SO_3^{2-} concentration involved the careful sampling of 5 mL of cultivation broth to minimize their contact with ambient air. The sample was then transferred to a 50-ml Erlenmeyer containing 1 ml of sulfuric acid and 0.1 g of NH_2SO_3H crystals. After 1 ml of a starch solution (1%) was added as indicator, the sample was

titrated with standard KI-KIO_3 titrant until a faint permanent blue color developed. Similarly, a sample of distilled water was processed as the blank control. The SO_3^{2-} concentration was then calculated according to the following equation 6:

$$[SO_3^{2-}][M] = \frac{[Titrant\ Volume\ for\ Sample\ (ml) - Titrant\ volume\ for\ Blank\ (ml)]}{Volumen\ Sample\ (ml)} \quad (Eq.6)$$

Biomass concentration was determined by filtering 10 ml of cultivation broth in dry pre-weighted 0.2 µm acetate cellulose filters, which were then allowed to dry overnight and weighted again.

2.5 Statistical Treatment

All experiments were performed in duplicate and each assay was considered as a replicate. The results are given as the average value ± standard deviation from the duplicate assays. In addition, the results were analysed using a one-way ANOVA with significance at $p \leq 0.05$. The Excel statistical package (Microsoft Corporation, USA) was used for data treatment.

3. Results and Discussion

3.1 Volumetric mass transfer coefficient determination

The volumetric mass transfer coefficients for CH_4 increased when increasing the stirring rate regardless of the methodology used to estimate $k_l a_{O2}$ as a result of the increased turbulence and air bubble disruption caused by agitation. However, while $k_l a_{CH4}$ increased from 13.7±2.0 h^{-1} at 400 rpm to 52.6±1.7 h^{-1} at 800 rpm when the gassing-out method was used to characterize CH_4 mass transfer, the OTR methodology resulted in an increase from

44.1±1.1 h^{-1} at 400 rpm to 96.7±3.2 h^{-1} at 800 rpm (Figure 1). The type of O_2 sensor (polarographic *vs* optical) used in the gassing-out methodology did not significantly ($p \leq 0.05$) influence the measurement of $k_l a_{O2}$ (average error < 2.75 %), which confirmed that the significant ($p \leq 0.05$) differences observed in $k_l a_{CH4}$ between the OTR and gassing-out methodologies were not caused by the delay in the response of the polarographic sensor. In addition, the increase in MSM salinity as a result of the addition of 0.03 M Na_2SO_3 (=3.78 g L^{-1}) in the OTR methodology could only explain an average 9.5 % increase in $k_l a_{CH4}$ due to the reduction in bubble coalescence mediated by the slightly higher ionic strength of the liquid medium (Figure S1). Indeed, this methodology was optimized by Quijano et al. (2009) in 2009 to prevent a salinity effect of the reagent Na_2SO_3 on the determination of $k_l a_{O2}$. At this point it should be highlighted that moderate salinity media, despite slightly reducing the solubility of gases, typically result in enhanced gas-liquid $k_l a$ induced by the higher gas-liquid interfacial areas (Rodero et al., 2018). Therefore, the difference in the volumetric mass transfer coefficient of O_2, which directly impacted on the estimation of $k_l a_{CH4}$, was attributed to a mass transfer acceleration mediated by the instantaneous chemical oxidation of O_2 by SO_3^{2-} (Eq. 2). The extremely fast (almost instantaneous) kinetics of this reaction (faster than any gas-liquid diffusional mechanisms in the mass transfer process) in the presence of Co^{2+} likely accelerated O_2 uptake from the gas phase in the fermenter (Zhao et al., 1999), which rendered the classical two-films model not valid since the O_2 concentration gradients in the liquid phase were no longer linear under this particular scenario (Van Krevelen et al., 1948). Figure 2, which depicts the O_2 profiles in the liquid phase during the gassing-out and OTR experiments, clearly shows the higher mass transfer gradients (estimated locally from the slope of the decrease in dissolved O_2

concentration at the gas-liquid interphase) despite the overall difference in O_2 concentration between the interphase and the bulk liquid (200 mg L^{-1} – 0 mg L^{-1}) remained similar. These higher gradients mediated by the rapid oxidation of O_2 in the presence of SO_3^{-2} and Co^{2+} entailed a superior O_2 mass transfer, which mathematically resulted into higher kla_{O2} (obtained from equations 1 and 3). Finally, it should be highlighted that the $k_l a_{CH4}$ here recorded were similar to those reported in vertical tubular loop (70 h^{-1}) and airlift external loop reactors (85 h^{-1}), but higher than those observed in stirred tank, airlift bubble column and U-loop reactors (12-26 h^{-1}) (Stone et al., 2017). At this point it should be also stressed that high $k_l a_{CH4}$ values are required in CH_4 treating bioreactors in order to compensate for the low concentration differences imposed by the high Henry law constant of CH_4 (~ 30 at 25 ºC) (Lopez et al., 2013). Thus, the higher the $k_l a_{CH4}$ values, the lower the bioreactor volumes and consequently, the lower the investment costs.

<Figure 1>

3.2 CH_4 abatement under continuous operation: methodology validation

The abiotic test carried out in the fermenter under continuous gas phase supply revealed a negligible contribution of adsorption or photolysis to CH_4 removal in this particular experimental set-up (Figure S2). This implies that the removal of CH_4 recorded at 400, 600 and 800 rpm can be attributed to the biocatalytic action of the microbial consortium present in the fermenter, which converts CH_4 to CO_2, H_2O and new biomass (Lopez et al., 2014). On the other hand, the empirical CH_4 elimination capacities recorded in the biotic fermenter inoculated with the consortium at 400, 600 and 800 rpm accounted for 37.8 ± 5.8, 42.5 ± 5.4 and 62.3 ± 5.2 g CH_4 m^{-3} h^{-1}. These values were similar to the CH_4-ECs estimated using

the OTR $k_l a_{CH4}$ at a maximum concentration gradient (equation 7): 35.6 ± 2.2, 50.1 ± 2.3 and 59.6 ± 3.4 g CH_4 m^{-3} h^{-1} at 400, 600 and 800 rpm, respectively (Figure 3).

$$CH_4 - EC = kla_{CH4} \times \left(\frac{C_{CH4,in}}{H_{CH4}} - C_{L,CH4}\right) \quad \text{(Eq. 7)}$$

Where H_{CH4} represents the dimensionless Henry law constant of CH_4 (28.7 at 30 ºC) and C_{LCH4} the aqueous concentration of CH_4 in the bulk phase, which is negligible under the mass transfer limiting scenario here encountered. In fact, no significant differences ($p \leq 0.05$) between the empirical and estimated CH_4-ECs were found at 400 and 800 rpm. The assumption of process operation at a maximum CH_4 concentration difference [$C_{CH4,in}/H_{CH4}$-$C_{L,CH4}$] (which implies a negligible CH_4 concentration in the cultivation broth) was validated by the fact that the process was mass transfer limited during CH_4 biodegradation at a biomass concentration of 2.5 ± 0.4 g dry weight L^{-1} (as confirmed by the higher CH_4-ECs at higher stirring rates). Indeed, MSM supplementation with Cu at 50 µg L^{-1} was required to overcome process limitation by microbial activity, which increased CH_4-ECs from 24.4 ± 1.7 to 62.3 ± 5.2 g CH_4 m^{-3} h^{-1} at 800 rpm. In this context, Cu positively regulates the activity of the enzymes pMMO and sMMO (particulate and soluble CH_4 monooxygenase, respectively) and controls the expression of their genes. Most methanotrophs grow optimally at Cu concentrations lower than 270 µg L^{-1}, though previous enzymatic assays have demonstrated that sMMO in type II methanotrophs is properly synthesized at low Cu concentrations (below 50 µg L^{-1}) (Graham et al., 1993; Bender and Conrad, 1995; Semrau et al., 2010). Overall, the results here obtained confirmed that the classical gassing-out methodology for the estimation of $k_l a_{CH4}$ clearly underestimated CH_4

mass transfer under continuous biotic operation of the fermenter. Thus, the rapid CH_4 biodegradation in the vicinity of the gas-liquid interphase mediated by methanotrophic bacteria likely supported an enhanced CH_4 mass transfer (induced by the higher local gradient close to the gas-liquid interphase), similarly to the acceleration in the O_2 mass transfer recorded in the OTR experiments.

<Figure 3>

The CH_4-ECs here recorded (38-62 g m^{-3} h^{-1}) were significantly higher than those reported in biotrickling filter or two-phase airlift bioreactors operated with internal gas recirculation at similar inlet loading rates (CH_4-ECs ~ 20-25 g m^{-3} h^{-1}) (Quijano et al., 2009; Van Krevelen et al., 1948). Comparable CH_4-ECs of 35-54 g m^{-3} h^{-1} were recently achieved in a bubble column bioreactor operated with internal gas recirculation and *Methylocystis hirsuta* as a model CH_4 degrading microorganisms (Garcia-Perez et al., 2018).

3.3 Practical applications and future research perspectives

A renewed attention on suspended growth bioreactors for CH_4 abatement (i.e. stirred tank, airlift and bubble columns) has emerged in the past 5 years due to the popularization of biorefineries based on the feasible bioconversion of CH_4 into added-value bioproducts such as biopolymers, ectoine, exopolysaccharides, protein, etc. (Cantera et al., 2017; Karthikeyan et al., 2015; Khmelenina et al., 2015; Pieja et al., 2012). These novel biorefineries, which will be based both on dilute CH_4 emissions from abandoned landfills or coal mines and biogas from solid waste management, will create value out of GHG mitigation and envisage a new bioeconomy based on CH_4. In this context, suspended

growth systems represent nowadays the most popular bioreactor platform capable of supporting a cost-competitive methanotrophic biomass harvesting and bioproduct downstream, although recent attempts to produce added-value products from residual CH_4 in immobilized bioreactors have been reported (Cantera et al., 2016; Karthikeyan et al., 2017). Thus, the characterization of CH_4 mass transport from residual emissions or biogas streams into the microbial community is central to dimension the bioreactor and downstream processes. However, the absence of reliable commercial dissolved CH_4 sensors has hindered the development of accurate methodologies for the determination of $k_l a_{CH4}$, whose characterization has been conducted based on either classical $k_l a_{O2}$ determination methodologies without a further validation for CH_4 or complex experimental methodologies difficult to implement at large scale (Yu et al., 2010; Rocha-Rios et al., 2011). Therefore, the results herein obtained validated the SO_3^{2-} oxidation methodologies as the most accurate method to estimate $k_l a_{CH4}$, which is expected to support in the design of cost-effective CH_4 biorefineries. For instance, figure S3 depicts a simple model simulation of the CH_4 outlet concentration as a function of the CH_4 inlet concentration and the $k_l a_{CH4}$ estimated at the three stirring rates tested. This methodology entails the purchase of SO_3^{2-} and Co^{2+}, and will result in SO_4^{2-} as a final product in the cultivation medium after the experiment. Further research should focus on validating this methodology in high-performance off gas treatment bioreactors such as Taylor flow or two-phase partitioning bioreactors, which are foreseen as the only bioreactor configuration capable of supporting an effective CH_4 bioconversion at a reduced energy consumption (Cantera et al., 2017). In addition, this methodology could be also applied to the design of algal-bacterial photobioreactors devoted to biogas upgrading (CO_2 and H_2S removal), where both

chemically and biologically enhanced mass transport has been identified (Rodero et al., 2018, Yang et al., 2016a,b, Zhu, 2015, Zhu et al., 2016).

4. Conclusions

This work empirically validated a simple methodology to accurately describe CH_4 mass transport and biodegradation in continuous stirred tank bioreactors based on OTR estimations. The rapid O_2 uptake at the gas-liquid interphase mediated by SO_3^{-2} and Co^{2+}, mimicking microbial CH_4 uptake during continuous CH_4 treatment, supported the superior performance of the OTR methodology for the description of $k_l a_{CH4}$ compared to the classical gassing-out methodology. Thus, the empirical CH_4-ECs of 37.8±5.8, 42.5±5.4 and 62.3±5.2 g CH_4 m^{-3} h^{-1} recorded at 400, 600 and 800 rpm, respectively, were comparable to the CH_4-ECs of 35.6±2.2, 50.1±2.3 and 59.6±3.4 g CH_4 m^{-3} h^{-1} predicted by the OTR methodology. This finding will help in the design of suspended growth bioreactors devoted to CH_4 bioconversion.

Acknowledgements

The authors thank CONACyT for the mobility grant of Raul Muñoz to conduct a research stay at Universidad Autonoma Metropolitana-Cuajimalpa (Mexico). The support from the Spanish Ministry of Economy and Competitiveness and the European Union through the FEDER Funding Program (CTM2015-70442-R and Red NOVEDAR) are also gratefully acknowledged.

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Figure captions

Figure 1. Influence of the stirring rate on the volumetric mass transfer coefficient of CH_4 estimated based on the gassing-out (□) and OTR (■) methods for O_2.

Figure 2. O_2 concentration profiles in the liquid phase during the gassing-out (continuous line) and OTR (dashed line) methods.

Figure 3. Influence of the stirring rate on the CH_4-EC estimated from the gassing-out (□) and OTR (■) methods, and experimentally (▨).

Figure 1.

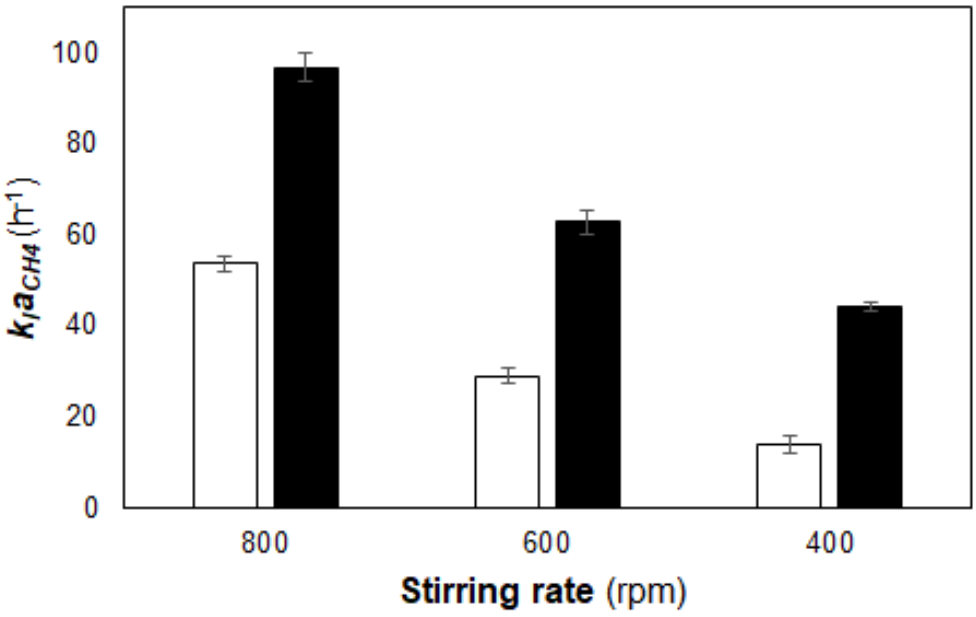
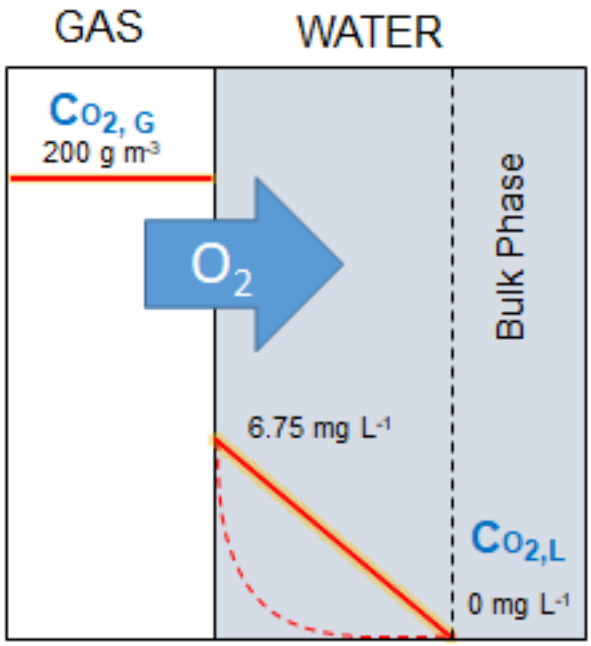


Figure 2.



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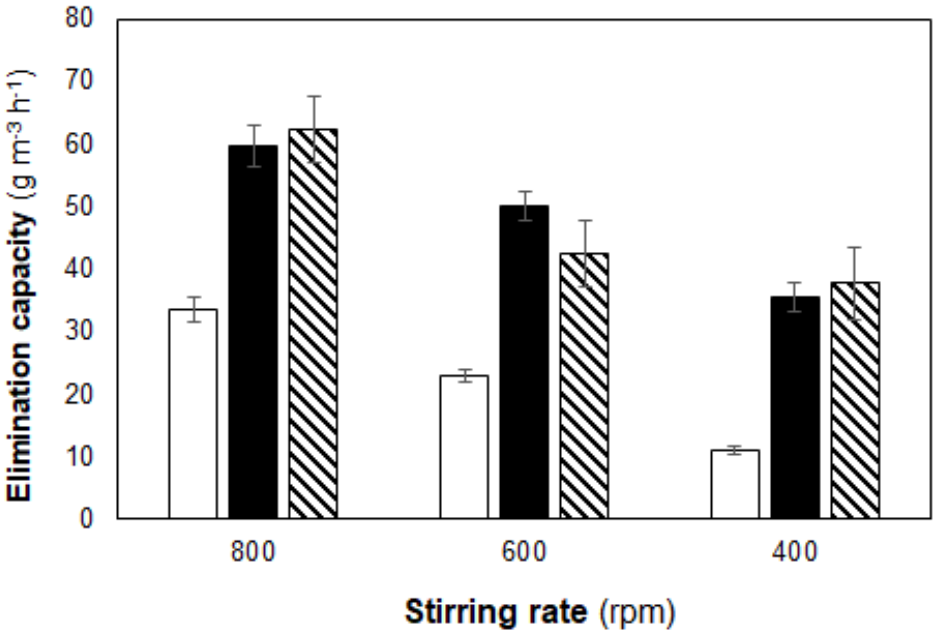
80
70
60
50
40
30
20
10
0
Elimination capacity (g m-3 h-1)
800
600
400
Stirring rate (rpm)

Figure 1

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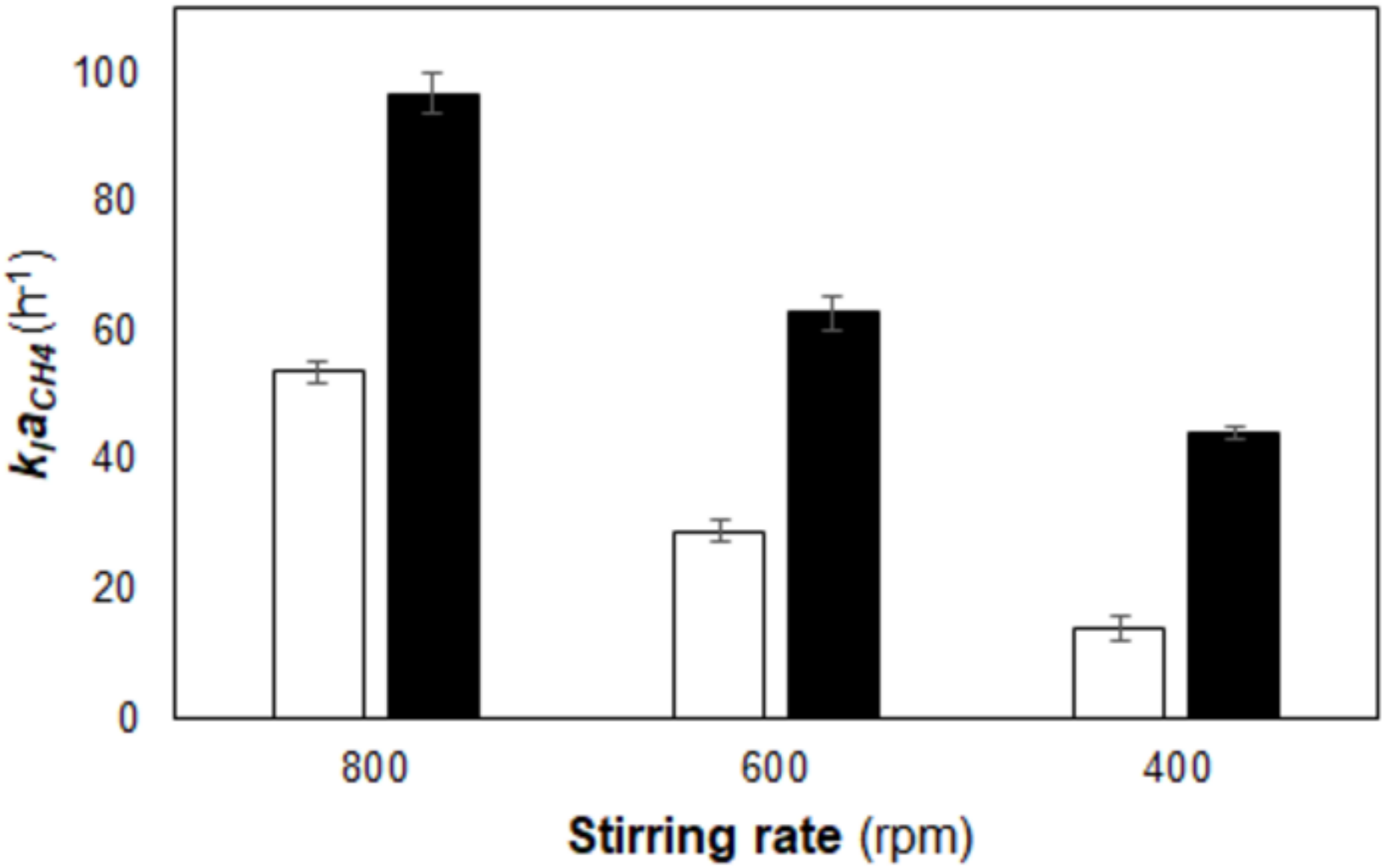

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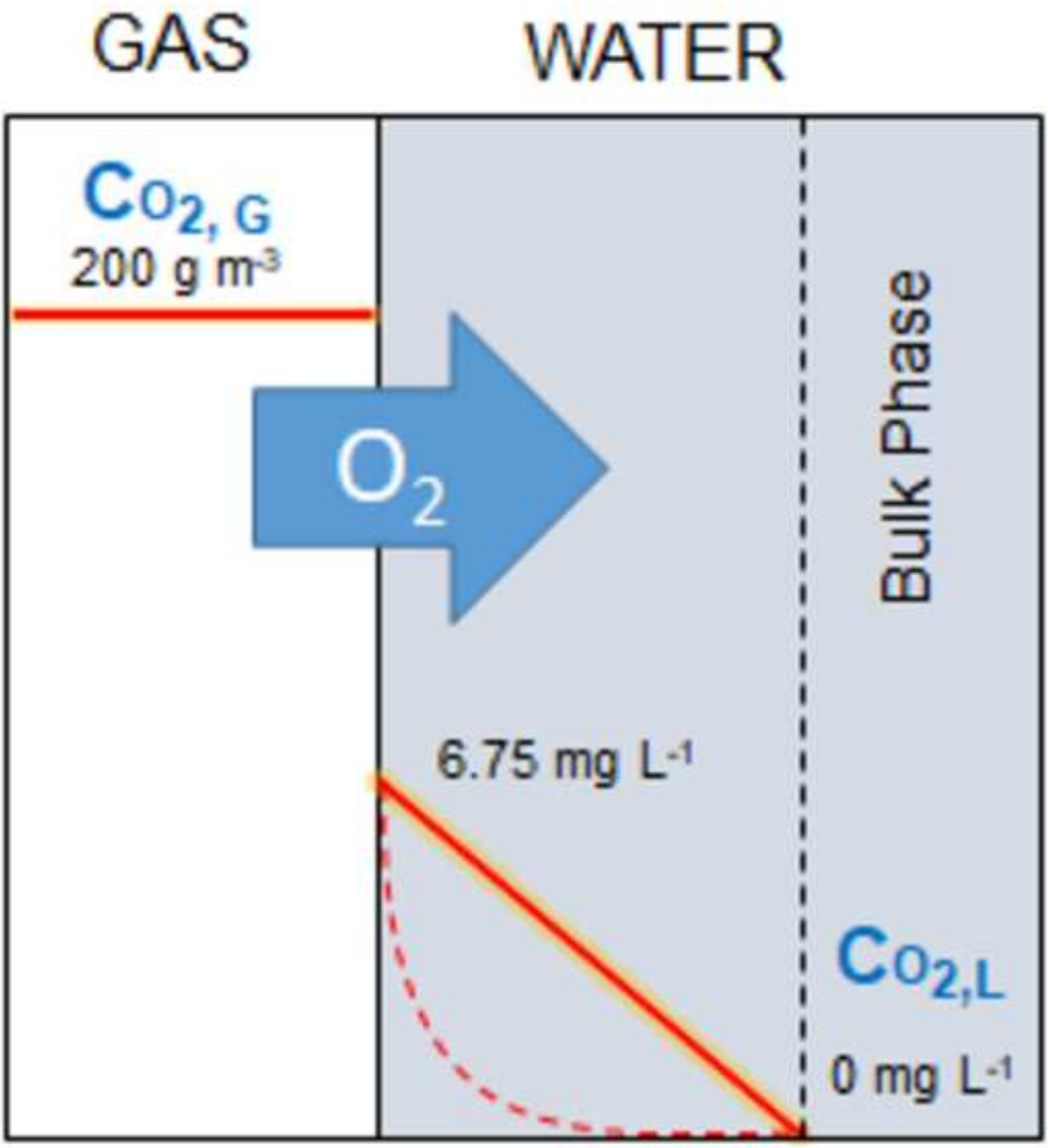

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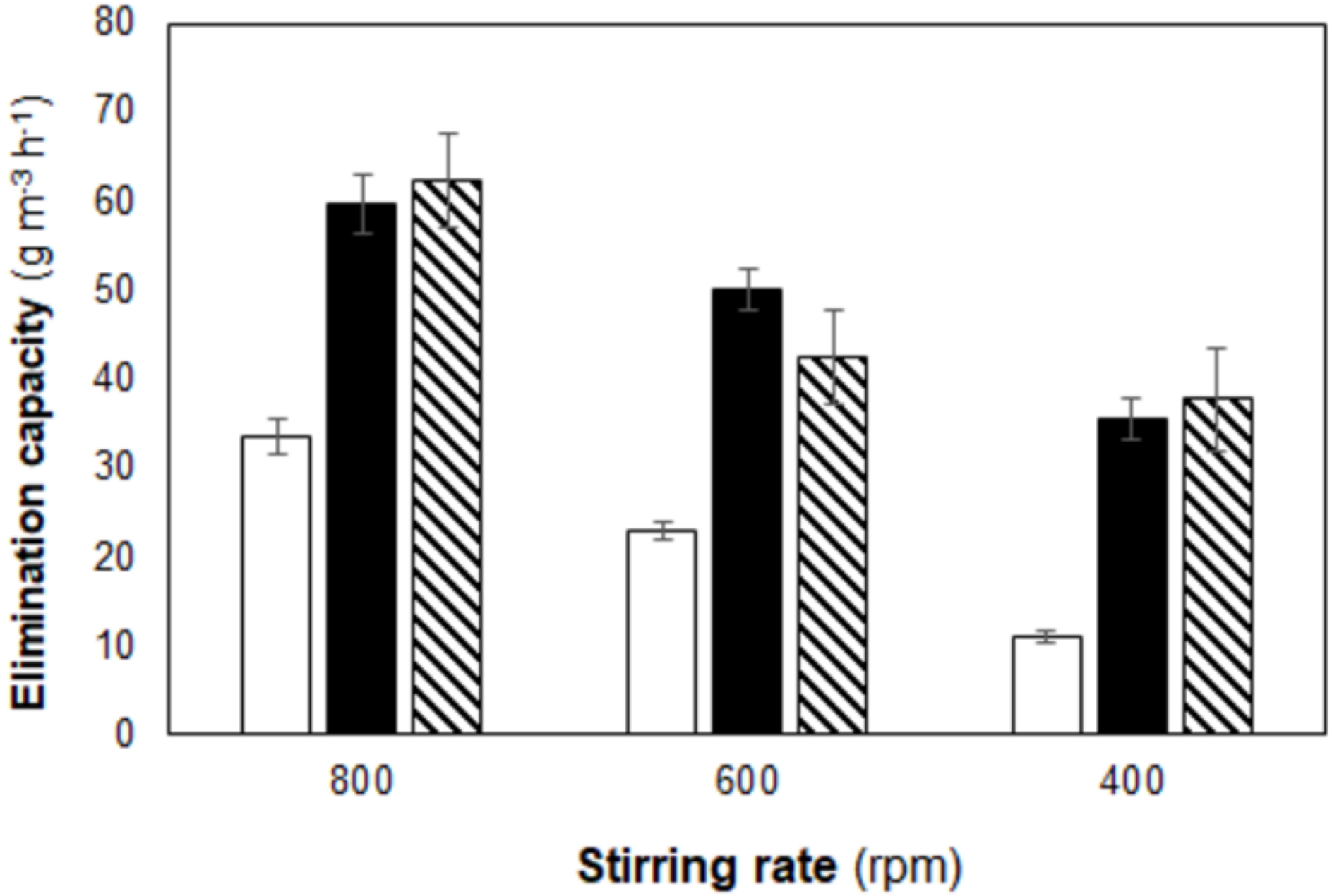

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