

# Liquid Phase Optimisation in a Horizontal Flow Biofilm Reactor (HFBR) Technology for the Removal of Methane at Low Temperatures

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## Abstract

Methane (CH<sub>4</sub>) is a potent greenhouse gas often emitted in low concentrations from waste sector activities. Biological oxidation techniques have the potential to offer effective methods for the remediation of such emissions. In this study, methods of improving the CH<sub>4</sub> oxidation performance of a horizontal flow biofilm reactor (HFBR) technology, operated at low temperatures, were investigated.

Three pilot scale HFBRs were operated over three phases (Phases 1, 2 & 3) lasting 310 days in total. The reactors were loaded with 13.2 g CH<sub>4</sub>/m<sup>3</sup>/hr during each phase and operated at an average temperature of 10°C.

In Phase 1, nutrients were added to the biofilm via a liquid nutrient feed (LNF) comprising water and nutrient mineral salts. Average removals were 4.2, 3.1 and 2.3 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1, 2 and 3 respectively.

**Commented [NU1]:** In response to Reviewer #1 comment; "Authors must start the abstract with the motivational statement related to the background of study"

**Commented [NU2]:** In response to Reviewer #2 comment; "Standardize the format of units for performance variables (methane load, methane removal, etc.) through the document included the abstract" units have been standardised as g XX/m<sup>3</sup>/hr

In Phase 2 silicone oil was added to the LNF of all three HFBRs. Average removals increased, when compared to Phase 1, by 31%, 79% and 78% for HFBRs 1, 2 and 3 respectively.

In Phase 3 a non ionic surfactant (Brij 35) was added to the LNF and silicone oil liquid phase of HFBRs 1 and 2. The operating conditions of HFBR 3 were not changed and it was used as a control. A concentration of 1.0 g Brij 35/L proved most effective in improving reactor performance; with removal rates increasing by 105% and 171% for HFBRs 1 and 2 respectively when compared to Phase 1.

These results indicate the potential of liquid phase optimisation for improving mass transfer rates and removal performances in biological reactors treating CH<sub>4</sub>.

**Commented [NU3]:** In response to Reviewer #2 comment, "The abstract length is 329 words exceeding the maximal length suggested by the journal", the abstract has been restructured to reduce word count to 260 words.

## Keywords

Biological oxidation, biofilm reactor, methane, non ionic surfactant, secondary liquid phase, silicone oil

## 1. Introduction

Methane is a prominent greenhouse gas with a global warming potential 25 times that of carbon dioxide (CO<sub>2</sub>) and comprises almost a quarter of worldwide greenhouse emissions (Haubrichs and Widmann, 2006; Rocha Rios et. al., 2009). 55% of anthropogenic methane (CH<sub>4</sub>) emissions are below the lower explosive limit (LEL) for CH<sub>4</sub> and cannot be thermally oxidised (Avalos Ramirez et. al., 2012a). In such cases, biological waste treatment technologies can be an effective mitigation measure against these emissions (Chiemchaisri et. al., 2013; Kraakman and Witherspoon, 2013). Biofilm reactors are a practical alternative for the control and mitigation of these emissions (Kraakman and

**Commented [NU4]:** In response to reviewer #2 comment, "Carefully review and correct the format to cite references through the manuscript", all citations and references have been checked and verified as recommended.

Witherspoon, 2013; Veillette et. al., 2012; Kennelly et. al., 2012). Biofilm reactors are low cost, energy efficient and are simple to construct and operate (Veillette et. al., 2012). Biofilm reactors treating CH<sub>4</sub> have been previously shown to be capable of achieving high removals of up to 100 g CH<sub>4</sub>/m<sup>3</sup>/hr (Rocha Rios et. al., 2009; Nikiema and Heitz, 2009) and have successfully been deployed at site scale (Melse and Van der Werf, 2005). There are, however, a number of challenges when designing CH<sub>4</sub> biofilm reactor, foremost of which is the low solubility of CH<sub>4</sub> in water. This presents a barrier to mass transfer and necessitates long hydraulic retention times, especially at low temperatures (Rocha Rios et. al., 2009). Biofilm reactors treating CH<sub>4</sub> have typically required retention times 100 times greater than biofilm reactors treating odorous compounds such as hydrogen sulphide or ammonia (Streese and Stegmann, 2003), with required empty bed retention times (EBRT) of over 1 hour previously reported (Gerbert and Grongroft, 2006; Melse and Van der Werf, 2005; du Plessis et. al., 2003).

Recent studies have shown, however, that the limiting effect of low solubility can be alleviated in a number of different ways. Optimising the nutrients in the liquid phase to maximise methanotrophic activity in the biofilm can significantly improve performance (Clifford et. al., 2012; Nikiema et. al., 2009). The use of a secondary organic liquid phase with a higher affinity for methane than water such as polydimethylsiloxane (silicon oil) or hexadecane have been shown to result in greater rates of mass transfer in both a packed bed biotrickling filter and in a stirred tank reactor and lead to improved oxidation rates (Gulfam et. al., 2011; Bordel et. al., 2010; Munoz et. al., 2007). Addition of silicone oil leads to improved methane solubility as the partition coefficient of methane in silicone oil is approximately 10 times lower than in water; thus at equilibrium, the ratio of concentrations of methane dissolved the oil and water phases will be 10:1 (Rocha Rios et. al., 2009).

In other studies, non-ionic surfactants such as Brij 35 and Tween 20 have been used to improve reactor performance (Avalos Ramirez et. al., 2012a). Non ionic surfactant molecules contain both hydrophilic and hydrophobic elements and when added to the aqueous phase of a biofilm reactor, can increase the solubility of low water soluble compounds such as methane (Avalos Ramirez et. al., 2012a; King, 2001). Non ionic surfactants have successfully improved performances of packed bed biofilters (Avalos Ramirez et. al., 2012a; Jurado et. al., 2007) and are largely biodegradable and non toxic in low concentrations (< 0.5 % w/w), (Avalos Ramirez et. al., 2012b). Brij 35 can also be used as an oil water emulsifier (its hydrophobic-lipophilic balance (HLB) number is 16.9 - within the range for solubilising oils into water).

To date limited work has focused on the combined use of transfer vectors such as silicone oil and non-ionic surfactants (e.g. Brij 35) to aid mass transfer of CH<sub>4</sub> into the liquid phase. Rocha Rios and Revah (2013) found that the effectiveness of silicone oil as a transfer vector is dependent on the degree of oil dispersion in the liquid phase. While a number of studies use mechanical turbulence to create dispersion (Arriga et. al., 2006; Rocha Rios et. al., 2009; Quijano et. al., 2010a) previous studies have not examined the possibility of combining transfer vectors to both enhance mass transfer and improve oil dispersion in the water phase.

Furthermore most studies are carried out at temperatures of 20°C or more. In many scenarios (due to the facility in question or the climate) temperatures can be significantly lower.

The horizontal flow biofilm reactor (HFBR) has been previously applied successfully to both wastewater and waste gas treatment (Kennelly et. al, 2012; Clifford et. al., 2010). The unique flow regime in the HFBR ensures good contact with the biofilm in the reactor and alleviates problems that can be associated with conventional biofilm reactors such as

clogging, channelling, compaction and pressure drop. In this study, the effect of adding silicone oil, both with and without Brij 35, to the liquid phase of HFBRs treating methane gas, was investigated.

**Commented [NU5]:** In response to Reviewer #2 comment, "Page 7 lines 14-32 (end of introduction) includes information that must be included in materials and methods as analytical procedures", this paragraph has been removed from here and included in the materials and methods section as recommended.

## 2. Materials and methods

**Commented [NU6]:** In response to Reviewer #2 comment, "The title of this section should be changed from "Methods and materials" to "Materials and methods", this change has been made as recommended.

### 2.1. Horizontal Flow Biofilm Reactor (HFBR)

Three HFBR units (HFBR 1, HFBR 2 and HFBR 3) were commissioned during these trials. Each HFBR comprised 55 horizontal plastic sheets with integrated frustums stacked vertically - one above the other. The sheet stack was placed in a sealed enclosure that could be opened for visual assessment and biofilm sampling. The working volume of each reactor was 18 L and the top plan surface area (TPSA) of the plastic media was 0.04 m<sup>2</sup>, giving a total media plan area of 2.4 m<sup>2</sup>. 6 intermediate sample ports were located along the vertical profile of each reactor allowing for intermediate samples of air and water to be taken.

The HFBR units were housed in a temperature-controlled laboratory, maintained at 10°C. The influent gas mixture stream comprised a mixture of atmospheric air with a CH<sub>4</sub> gas supply. Mass flow controllers (Bronkhorst High Tech BV, Ruurlo, Netherlands), flowmeters (Key Instruments, Trevoise, USA) and pressure regulators (GCE DruVa, Cheshire, United Kingdom) were used to control gas flow rates and gas mix proportions as required (Figure 1).

**Commented [NU7]:** In response to Reviewer #2 comment, "In page 8 lines 10-15 more information on suppliers must be provided", this information has now been included.

The gas mixture, containing approximately 1.6% v/v CH<sub>4</sub>, was introduced at the top of the reactor (Sheet 1) and flowed horizontally across each sheet before moving to the sheet below. Similarly a liquid phase, introduced onto Sheets 1 and 30 of the reactor, flowed over each sheet before dropping to the sheet below (i.e. the unit does not operate as a

submerged reactor). The gas and liquid exited the reactor below Sheet 55 (the bottom sheet in the reactor). Operating parameters are summarised in Table 1

Nutrients were added to each of the reactors in the form of a Liquid Nutrient Feed (LNF) mixture, similar to that used by Nikiema et al., (2009) (Table 2). The LNF was delivered intermittently (10 mins/hr) via a peristaltic pump. The LNF was delivered in a step feed manner, i.e. 75% of the LNF (3 L/day) was dosed onto Sheet 1 and 25% of the LNF (1 L/day) onto Sheet 30.

**Table 1**

Flow and loading parameters during the study

Parameter	Phase 1	Phase 2	Phase 3
Air Mixture Flow Rate (m <sup>3</sup> /m <sup>3</sup> /hr)	1.3	1.0	1.1
Gas Loading Rate (g CH <sub>4</sub> /m <sup>3</sup> /hr)	13.2	13.2	13.2
Average Influent Concentration (ppm <sub>v</sub> )	14000	18000	17000
Empty Bed Retention Time (EBRT) (mins)	45	55	52

**Table 2**

Composition of SWW

Component	(g/L)	Component	(g/L)	Component	(g/L)
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.037	K <sub>2</sub> SO <sub>4</sub>	0.17	Na <sub>2</sub> HPO <sub>4</sub>	0.86
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.00112	CaCl <sub>2</sub> .2H <sub>2</sub> O	0.007	KH <sub>2</sub> PO <sub>4</sub>	0.53
Urea	0.03 *	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.000576	NaMoO <sub>4</sub> .2H <sub>2</sub> O	0.000096
MnSO <sub>4</sub> .H <sub>2</sub> O	0.000466	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.000250	CoCl <sub>2</sub> .6H <sub>2</sub> O	0.000096
NH <sub>4</sub> Cl	0.06 *	KI	0.000166		
NaNO <sub>3</sub>	1.51	H <sub>3</sub> BO <sub>3</sub>	0.000124		

\* Added to HFBR 3 only

**Commented [NUS]:** In response to Reviewer #2 comment, "Standardize the format of table title and figure captions. Phase 1 for example sometimes is written with capital letter and other times as lowercase", each caption has been checked for consistency. Where the word "phase" refers to the title of the phase of study, e.g. "for Phase 1" or "during Phase 3" then it is capitalised. Where it refers to a description of the phase of study, e.g. "for the next phase" or "typical of all phases", it remains lower case. Please note that the states of matter within the reactor are also referred to as phases, e.g. the liquid phase or gas phase. As this is also a description, the word phase is lower case for these instances also.

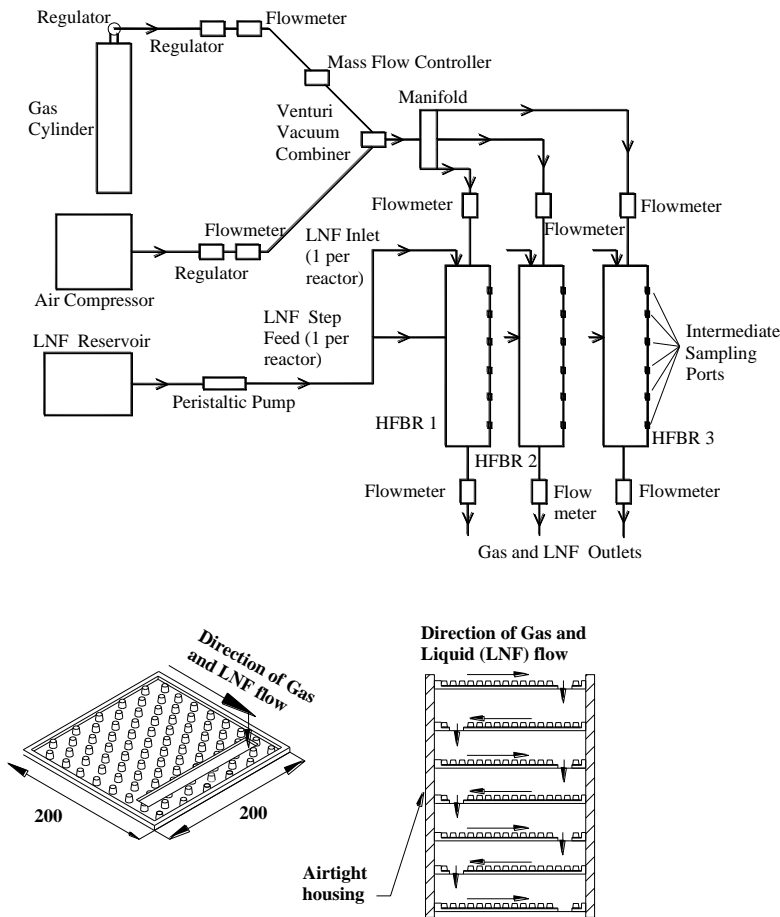


Figure 1. Schematic of the HFBR system

## 2.2. Biofilm growth and inoculation

An enrichment strategy was employed to cultivate a methanotroph-rich biomass capable of methane oxidation which could be used to seed the HFBRs. A biomass mix comprising landfill cover soil, landfill leachate, composted OFMSW and compost leachate in a 1:1:1:1 ratio was used for the enrichment culture. Briefly, 2 ml of the biomass mix were

**Commented [NU9]:** In response to Reviewer #1 comment, "Please write more details such as more details about ratio and method of mixture of landfill soil, compost, landfill leachate and compost leachate (2.2 Biofilm growth and inoculation)", this section has been re-written to give more detail on the enrichment strategy carried out.

placed in each of several 40-ml crimp-top, glass vials with 8 ml Adapted Whittenbury Medium (AWM; Whittenbury et. al., 1970). The vials were sealed and the headspace was adjusted to a methane concentration of 10% at atmospheric pressure. Vials were incubated in the dark at 10°C shaking at 80 rpm. The headspace methane concentration was monitored by gas chromatography (GC; Varian CP-3800 Gas Chromatograph) analysis of twice-weekly headspace samples. Once the headspace methane concentration was <0.5%, the headspace was flushed with air and a 10% methane headspace was re-instated. Over the course of 4 months, the cultures were subcultured (c. 10% inoculum) to fresh medium and eventually were scaled to 2-l enrichment cultures to cultivate sufficient biomass for HFBR inoculation. The enriched culture was added to the HFBRs at the beginning of the trial and then re-circulated around the systems for several days to encourage biofilm formation.

### 2.3. *Experimental plan and data analysis*

The study was divided into 3 phases (Phases 1, 2 and 3 lasting 150, 90 and 70 days respectively). The 3 phases of study ran consecutively with the end of one phase marking with the beginning of the next phase.

During Phase 1 the LNF (Table 2) was the sole component of the liquid phase. All reactors were operated identically however, HFBRs 1 and 2 used nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) as the main nitrogen source in the LNF whereas HFBR 3 used both ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and  $\text{NO}_3\text{-N}$ .

In Phase 2, silicone oil (10% v/v, Mistral, Northern Ireland) was added each of the three reactors as a second liquid phase. The silicone oil was added to the LNF and continuously

**Commented [NU10]:** In response to Reviewer #1, "Please add a new subtitle namely Experimental plan and data analysis" subsection entitled Sampling and Analytical methods has been renamed thus.

agitated with a magnetic stirrer in a feed container, before being pumped into each of the reactors with the LNF.

During Phase 3, Brij 35 (Acros Organics, NJ, USA) was added in concentrations of 0.5 g/L (Phase 3a), 1.0 g/L (Phase 3b), 2.0 g/L (Phase 3c), 1.0 g/L (Phase 3d) and 0.75 g/L (Phase 3e) to HFBRs 1 and 2. The Brij-35 was added to the LNF and silicone oil in the LNF reservoir tank. Brij 35 was not added to HFBR 3, which was used as a control during Phase 3.

The study was carried out at 10°C, which can be typical of onsite temperature in Ireland, Northern Europe and other temperate climates.

Throughout the study, influent, effluent and profile samples of the gas mixtures were taken from each reactor and analysed. The removal efficiency (RE) of each reactor was measured by comparing influent and effluent samples of the gas air mixture. Intermediate samples were taken from the 6 sampling ports located along the vertical profile of each reactor, to allow detailed profiling of CH<sub>4</sub> oxidation and CO<sub>2</sub> production to be carried out. Gas samples were taken with a syringe and analysed for CH<sub>4</sub> and CO<sub>2</sub> concentrations using an Agilent 7890A GC. In order to give further insight into microbial activity in the biofilm, LNF samples from influent, effluent and intermediate sampling ports were taken regularly and analysed in accordance with standard methods (APHA-AWWA-WEF, 2005). Total Nitrogen (TN) and Total Phosphorous (TP) were analysed using a Biotector TOC TN TP Analyser (Biotector, Cork, Ireland). NH<sub>4</sub>-N, nitrite-nitrogen (NO<sub>2</sub>-N), NO<sub>3</sub>-N and phosphate-phosphorous (PO<sub>4</sub>-P) concentrations were determined using a Thermo Clinical Labsystems, Konelab 20 Nutrient Analyser (Fisher Scientific, Waltham, Massachusetts, USA). All analytical equipment was checked, maintained and calibrated as per manufacturer's guidelines.

Experimental results were statistically compared to check for significant improvements (between experiments) or differences (between replications). One-way analysis of variance (ANOVA) carried out using GraphPad InStat software (Version 3.10). The analysis was carried out at a confidence interval of 95% (a significance level of 0.05).

**Commented [NU11]:** In response to Reviewer #1 comment, "Please explain the statistical analysis of this study in details", details of statistical significance testing carried out between replicates and between Phases is included.

### 3. Results and discussion

#### 3.1. CH<sub>4</sub> Removal – Phase 1

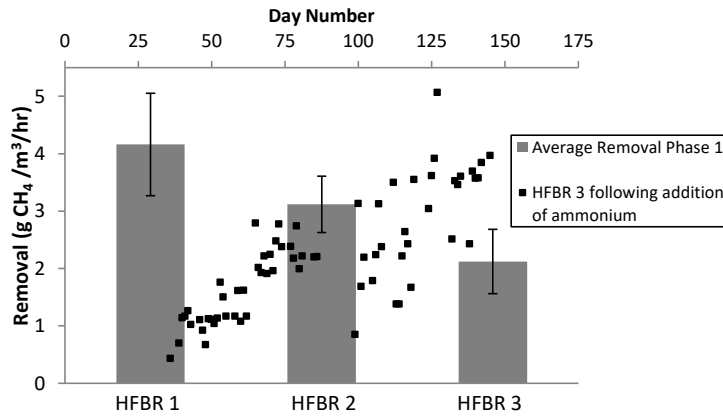
##### 150 days – 10°C: LNF only in the liquid phase

After inoculation with the enriched biomass, the 3 HFBRs were continuously operated and monitored under the Phase 1 conditions outlined in Table 1. After a 25 day acclimation period, reasonably consistent removals were observed. Results for Phase 1 are outlined in Table 3 and in Figure 2.

**Table 3**

CH<sub>4</sub> Removal Results for Phase 1

Reactor	Average Removal (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Standard Deviation (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Maximum (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Number of Samples (n)
HFBR 1	4.2	1.78	8.3	75
HFBR 2	3.1	0.98	5.2	71
HFBR 3	2.3	1.12	5.1	69



**Figure 2.** Average CH<sub>4</sub> removals for each HFBR during Phase 1. Standard deviation is shown using error bars. The effect of adding NH<sub>4</sub>Cl to the liquid nutrient feed is illustrated using a scatter plot.

Average removals for the reactors were 4.2, 3.1 and 2.3 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1, 2 and 3 respectively. These results compare reasonably well with results from previous trials by Kennelly et. al. (2012), who reported average removals of 5.19 g CH<sub>4</sub>/m<sup>3</sup>/hr for a HFBR unit operated under similar conditions and using the LNF outlined in Table 2.

HFBR 3, although identical in operation and influent loading, initially underperformed in comparison to HFBRs 1 and 2. In previous HFBR trials the best performing units were those which used feed containing ammonium salts in the LNF and had organic carbon removed (Clifford et. al., 2012; Kennelly et. al., 2012). Thus NH<sub>4</sub>Cl was added to the LNF of HFBR 3. Following the addition of NH<sub>4</sub>Cl (on Day 30) average removals steadily increased, from 1.0 g CH<sub>4</sub>/m<sup>3</sup>/hr to 3.0 g CH<sub>4</sub>/m<sup>3</sup>/hr for the latter 2 months of the trial - a performance more in line with HFBR 2. This is illustrated in Figure 2 with the scatter plot representing performance in HFBR 3 following addition of NH<sub>4</sub>Cl to the LNF. It can be observed from Figure 2 that a time lag of about 20 days was observed between the addition of NH<sub>4</sub>-N and subsequent improvement in reactor performance. This was most

**Commented [NU12]:** In response to Reviewer #2 comment, "Standardize the format of units for performance variables. Hours are represented in some cases as hr in other as Hours. In Table 6 units of specific methane elimination capacities are reported as g CH<sub>4</sub> oxidised/gVSS which lack of time units", all units have been checked and edited where appropriate, including units used in figures and tables.

likely due to the specialised microbial consortia requiring time to adapt to the feed modification i.e. methanotrophic species which are less tolerant to  $\text{NH}_4^+$  would be replaced by species which are more tolerant to  $\text{NH}_4^+$  (Veillette et. al., 2012). This observed improvement in performance following the addition of  $\text{NH}_4$  salts indicates low levels of  $\text{NH}_4\text{-N}$  may improve reactor performance. It should be noted that previous studies by Nikiema et. al. (2009), who noted that in the presence of high quantities of ammonium in the liquid phase (12 – 28%), some methanotrophic activity, is diverted towards the nitrification. However in this case, the proportions of  $\text{NH}_4\text{Cl}$  are significantly lower (0.0025% or 25 mg N/L). Hernandez and Omil, (2013) observed a severe decrease in removal efficiency when  $\text{NH}_4\text{-N}$  concentrations reached 1000 mg  $\text{NH}_4\text{-N/L}$  but noticed a significant increase in removal efficiency by increasing the ammonium nitrogen concentration (as  $\text{NH}_4\text{Cl}$ ) from 260 mg  $\text{NH}_4\text{-N/L}$  to 520 mg  $\text{NH}_4\text{-N/L}$ . This suggests that ammonia only inhibits methanotrophic activity beyond a threshold concentration. A similar view was presented by Veillette et. al. (2011), who suggested that methanotrophic activity in a packed bed biofilter, is only inhibited by  $\text{NH}_4^+$  beyond a tolerance limit of methanotrophic microbes in the biofilm of between 0.1 and 0.2 g N/L.

Previous studies differ as to the most appropriate species and concentrations of nitrogen for use in  $\text{CH}_4$  biofilm reactors (Bodelier et. al., 2000; Nikiema et. al., 2009; Clifford et. al., 2012; Hernandez and Omil, 2013). The difference between these studies may be due to environmental factors such as temperature and reactor design though further studies are necessary to examine and adequately describe nitrogen dynamics in a  $\text{CH}_4$  oxidising bioreactors.

One-way ANOVA carried out between replications for steady state removals during Phase 1 revealed that variations in the performances of the triplicate reactors were significant. This was previously observed in similar trials with the HFBR (Clifford et. al.,

2012; Kennelly et. al., 2012). As the reactors were operated and sampled identically (except for the 25 mg/L of NH<sub>4</sub>Cl that was added to the LNF to HFBR 3 after Day 30), the variations in each reactor performance may be due to in-situ (i.e. within each individual reactor) differences in microbial immobilisation rates following inoculation, dissimilarities in microbial growth rates and in-situ variations environmental conditions.

**Commented [NU13]:** In response to Reviewer #1 comment, "Please explain the statical analysis of this study in details", details of statistical significance testing carried out between replicates and between Phases is included.

### 3.2. CH<sub>4</sub> Removal – Phase 2

#### 90 days – 10°C: Liquid Phase comprises LNF and Silicone Oil

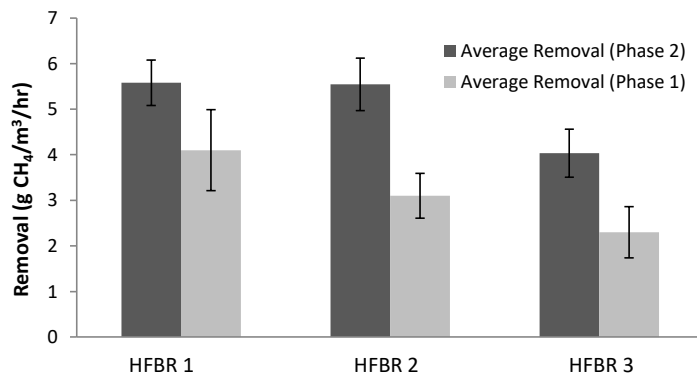
Rocha Rios et. al. (2009) observed that methane biodegradation in a packed bed biofilter is limited by mass transfer and not by biological reaction. Therefore, during Phase 2 silicone oil was added to the LNF to investigate its potential to enhance mass transfer rates and potentially increase reactor performance. Silicone oil was selected as a suitable transfer vector as methane is more readily dissolved into silicone oil when compared to water (Rocha Rios et. al., 2010). Moreover, it has previously been demonstrated that silicone oil is not biodegraded by the methanotrophic consortia in a biofilm reactor (Munoz et. al., 2007; Rocha Rios et. al., 2009) and so does not result in the introduction of excessive concentrations of biodegradable carbon into the LNF. The results for Phase 2 are summarised in Table 4 and Figure 3.

**Table 4**

CH<sub>4</sub> Removal Results for Phase 2

Reactor	Average	Standard	Maximum	Performance	Number of
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	Removal (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Deviation (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Removal (g CH <sub>4</sub> /m <sup>3</sup> /hr)	Improvement (compared to Phase 1)	Samples (n)
HFBR 1	5.5	0.99	7.1	31 %	42
HFBR 2	5.5	1.15	7.9	79 %	43
HFBR 3	4.0	1.05	6.1	78 %	42



**Figure 3.** CH<sub>4</sub> Removals for Phase 1 and Phase 2. Standard deviation shown using error bars

Significant improvements in reactor performance were observed in each of the three reactors. Average removal rates increased to 5.6, 5.5 and 4.0 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1, 2 and 3 respectively, representing improvements, when compared to Phase 1, of 31% for HFBR 1, 79% for HFBR 2 and 78% for HFBR 3.

As can be seen from Figure 3, similar removals were recorded in HFBRs 1 and 2 and removal rates in HFBR 3 were lower. This is supported by one way statistical analysis (ANOVA testing), which shows removals in HFBRs 1 and 2 were similar and that the difference in removal rates between them and HFBR 3 were significant. As the reactors were operated and sampled in triplicate during Phase 2, in-situ variations in microbial

ecology and environmental conditions were most likely the reason for the disparity between HFBR 3 and the other reactors.

Similarly, by performing a comparison of variance between steady state removals during Phase 1 and Phase 2, the impact of adding the silicone oil to the LNF during Phase 2 was found to be statistically significant.

The principal mechanism for this enhancement is most likely increased mass transfer of methane into the LNF. The partition coefficient between a gas and liquid phase is closely related to the Henry's law constant and can be defined as:

$$K_{g,l} = \frac{C_g}{C_l} \quad (\text{Equation 1})$$

Where  $C_g$  and  $C_l$  are the concentrations of the gas in the gas phase and the liquid phase at equilibrium, respectively (Lomond and Tong, 2011).

Rocha Rios et. al. (2009) previously determined that partition coefficients of methane in water and silicone oil at 30°C (303.15 K) were 33.5 and 3.2 respectively. Using the following Van't Hoff extrapolation;

$$K_{g,l}^{T_1} = K_{g,l}^{T_2} \times \exp\left(-X\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right) \quad (\text{Equation 2})$$

where;

$$X = \frac{-d \ln K_{g,l}}{d\frac{1}{T_1}} = \frac{\Delta_{soln}H}{R} \quad (\text{Equation 3})$$

and  $K_{g,l}^{T_1}$  is the partition coefficient at temperature  $T_1$  (K),  $K_{g,l}^{T_2}$  is the partition coefficient at temperature  $T_2$  (K),  $\Delta_{soln}H$  is enthalpy of solution and  $R$  is a gas constant.

**Commented [NU14]:** In response to Reviewer #1 comment, "Please explain the statistical analysis of this study in details", details of statistical significance testing carried out between replicates and between Phases is included.

The partition coefficients for methane in water and oil at 10°C (283.15 K) can be calculated as  $22.5 \pm 0.2$  and  $2.15 \pm 0.2$ .

Therefore, despite the lower operating temperatures and consequently lower partition coefficients used during this study, the effect of silicone oil addition was in line with that observed by Rocha Rios et. al. (2009). This is reflected in the fact that performance improvements following the addition of the silicon oil during Phase 2 of this trial (31%, 78% and 79%) were similar to those observed by Rocha Rios et. al (2009), (41% and 131%). The remaining disparity between the two studies may be partly due to differences in the degree of dispersion of the oil within the respective reactor systems.

Given that the average concentration of CH<sub>4</sub> during this phase of study was 12.4 g CH<sub>4</sub>/m<sup>3</sup> gas air mixture (i.e. 1.8 % v/v). The increase in CH<sub>4</sub> solubility can be calculated as follows;

$$C_{CH_4} = X \cdot C_{CH_{4,w}} + Y \cdot C_{CH_{4,o}} \quad (\text{Equation 4})$$

Where  $C_{CH_{4,w}}$  and  $C_{CH_{4,o}}$  are the concentrations of CH<sub>4</sub> in the water and silicone oil phases respectively and X and Y are the volumetric fractions of water and silicone oil respectively (Rocha Rios et. al., 2010). In this case the volumetric fractions are 0.9 for water and 0.1 for silicone oil. Given the partition coefficients for water and oil at 10°C are 22.5 and  $2.15 \pm 0.2$ , the values of  $C_{CH_{4,w}}$  and  $C_{CH_{4,o}}$  in Phase 2 can be calculated as 0.55 g CH<sub>4</sub>/m<sup>3</sup> and 5.79 g CH<sub>4</sub>/m<sup>3</sup> respectively. Thus given an average liquid phase flow rate of 4 L/day,  $2.21 \times 10^{-3}$  g CH<sub>4</sub> is dissolved in the water phase and  $2.31 \times 10^{-2}$  g CH<sub>4</sub> is dissolved in the silicone oil phase. Hence the concentration of CH<sub>4</sub> in the silicone oil is about 10 times that of the concentration in water.

As mass transfer is the limiting step in biological methane oxidation, particularly at such low temperatures, this increased mass transfer significantly enhanced reactor performance during Phase 2.

There was a visually observed improvement in biofilm growth and consistency compared to Phase 1. Biofilm on the sheets was slightly thicker and more evenly distributed throughout each of the sheets in the reactors. This, along with a consistent distribution of silicone oil on each sheet in each reactor, suggests a reasonable level of oil dispersion within the system.

In previous experiments examining two-phase biofilm reactors, a suitable degree of silicone oil dispersion could be generated within the reactor mechanically (Rocha Rios et. al., 2009; Bordel et. al., 2010; Gulfam et. al., 2011) The flow regime employed by the HFBR precludes the generation of mechanical dispersion in this manner, with magnetic agitation at the LNF reservoir rather than within the reactor. As the effectiveness of silicone oil on mass transfer is dependent on the degree of oil dispersion (Rocha Rios and Revah, 2013), increasing the degree of dispersion within the HFBR could lead to additional performance improvements.

### 3.3. *CH<sub>4</sub> Phase 3*

(70 days – 10°C): Liquid phase of HFBR 1 and 2 comprises LNF, silicone oil and Brij 35.

Non-ionic surfactants such as Brij 35 have previously been shown to act as transfer vectors in a methane oxidising biofilm (Avalos Ramirez et. al., 2012a). Brij 35 has a hydrophilic-lipophilic balance (HLB) number of 16.9 - within the range for solubilising oils into water. Thus the efficacy of including a suitable concentration of a non-ionic

surfactant could also lead to a greater degree of silicone oil dispersion within the HFBR. Furthermore, low concentrations of Brij 35 (< 0.5% w/w or 5 g/L) have previously been shown to be both biodegradable and non-toxic to methanotrophic biomass (Avalos Ramirez et. al., 2012b).

Phase 3 was divided into 5 sub-phases, namely Phase 3a, Phase 3b, Phase 3a, Phase 3c, Phase 3d and Phase 3e lasting for 16, 17, 11, 14 and 12 days respectively.

Average removal rates, in Phase 3a, increased by 28% (to 7.0 g CH<sub>4</sub>/m<sup>3</sup>/hr) and 23% (to 6.9 g CH<sub>4</sub>/m<sup>3</sup>/hr) for HFBRs 1 and 2 respectively when compared to Phase 2. In Phase 3b average removals further increased by 19% to 8.6 g CH<sub>4</sub>/m<sup>3</sup>/hr and 23% to 8.4 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1 and 2 respectively.

During Phase 3c, where concentrations of Brij-35 was increased to 2 g/L in the liquid phase, average removals decreased by 41% (to 4.9 g CH<sub>4</sub>/m<sup>3</sup>/hr) and 33% (to 5.6 g CH<sub>4</sub>/m<sup>3</sup>/hr) for HFBRs 1 and 2 respectively.

Following this decrease in performance the concentration of non-ionic surfactant was reduced to 1.0 g CH<sub>4</sub>/m<sup>3</sup>/hr (Phase 3d) for 14 days. Average removal rates recovered to 5.6 and 6.0 g CH<sub>4</sub>/m<sup>3</sup>/hr, representing an improvement of 15% and 7% for HFBRs 1 and 2 respectively when compared to Phase 3c. The concentration was further reduced to 0.75 g/L during Phase 3e and average removal rates further increased by 39% and 27% to 7.8 g CH<sub>4</sub>/m<sup>3</sup>/hr and 7.6 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1 and 2 respectively. Throughout Phase 3, the performance of HFBR 3 – which did not have Brij 35 – added remained steady.

The performance of each reactor during Phase 3 is illustrated in Figure 4 and in Table 5.

**Table 5**

CH<sub>4</sub> Removal Results for Phase 1 – Standard deviation shown in parentheses.

All removals in g CH<sub>4</sub>/m<sup>3</sup>/hr.

	Phase 3 a		Phase 3 b		Phase 3 c		Phase 3 d		Phase 3 e	
	Average Removal	Maximum Removal	Average Removal	Maximum Removal	Average Removal	Maximum Removal	Average Removal	Maximum Removal	Average Removal	Maximum Removal
HFBR 1	7.0 (0.36)	7.8	8.6 (0.81)	10.1	4.9 (1.09)	7.5	5.8 (0.64)	7.0	7.8 (0.81)	8.7
HFBR 2	6.9 (0.75)	8.1	8.5 (1.14)	10.4	5.6 (1.27)	8.3	6.0 (0.56)	6.9	7.6 (0.54)	8.21
HFBR 3	4.4 (0.56)	5.2	4.9 (0.84)	6.5	4.3 (0.62)	5.6	4.4 (0.68)	5.5	4.5 (0.30)	5.1

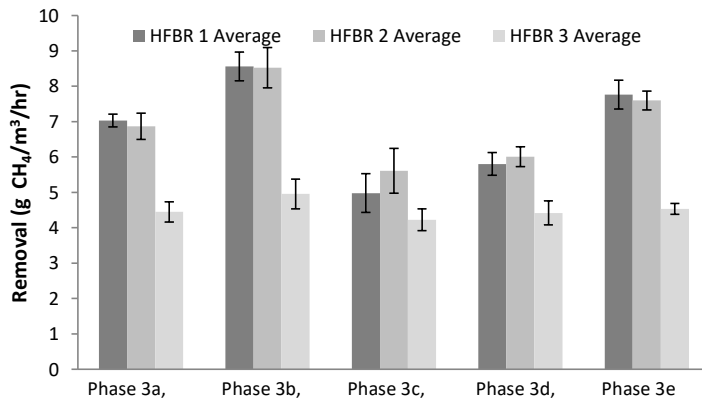


Figure 4. Average CH<sub>4</sub> Removal for Phase 3

As can be seen in Figure 4, the performances of HFBRs 1 and 2 initially improved significantly following the addition of Brij 35. Comparison of variance (ANOVA) between steady state removals during Phase 2 and Phase 3 revealed the increase in the removal performances of HFBRs 1 and 2 were significant, whereas the average removal

for HFBR 3, which did not have Brij 35 added, remained similar during both Phase 2 and Phase 3.

The most probable reasons for this are the increase in CH<sub>4</sub> availability in the liquid phase with the non-ionic surfactant (Brij 35) acting as a direct transfer vector, in addition to the emulsifying effect of the Brij 35 on the oil-water phase. Thus the addition of Brij may provide an alternative to mechanical agitation used in previous studies to improve oil dispersion (Rocha Rios et. al., 2009; Bordel et. al., 2010; Gulfam et. al., 2011).

There was a notable improvement in the consistency of results (as measured by the standard deviation of the average removals) during Phase 3 (and indeed Phase 2) when compared to Phase 1 (during pseudo steady state). This indicates that the improved (and possibly more consistent) mass transfer resulted in a more stable reactor performance.

It can be observed from Figure 4 that a disimprovement occurred during Phase 3c when the concentration of Brij was increased from 1.0 g/L to 2.0 g/L (0.1% up to 0.2% w/w).

The most probable reason for this was excessive stripping of useful biomass brought about by the detergent effect of the non-ionic surfactant. It is also possible that the bacterial population utilised the Brij 35 as carbon source, leading to a decrease CH<sub>4</sub> oxidation and corresponding increase in CO<sub>2</sub> production (discussed in more detail in Chapter 3.5). Excessive concentrations of biodegradable carbon have previously been

shown to be detrimental to the CH<sub>4</sub> oxidation performance of a HFBR (Clifford et. al., 2012). The reactors quickly recovered when Brij 35 concentrations were returned to previous levels (Phases 3d and 3e). This observation contrasts with studies by Avalos Ramirez et. al. (2012a), who observed that although biomass production was reduced as the concentration of non-ionic surfactant increased from 0.1 to 1.0% w/w due to biomass stripping, overall oxidation performance was not diminished in this range. This contrast may be due to the design of the HFBR when compared to a packed bed reactor such as

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**Commented [NU16]:** Reference

**Commented [NU17]:** In response to Reviewer #2 comment "The reviewer considers however that above of a threshold concentration of Brij 35 (1%), the bacterial population used the surfactant as carbon source decreasing methane consumption but increasing CO<sub>2</sub> production. A discussion of this possibility should be included", the authors agree and have included the highlighted paragraph. We also refer the reviewer to Chapter 3.5 (page 29, lines 39 – 49, However as can be seen from Figure 10...) where this possibility is discussed with the support of CO<sub>2</sub> data.

was used in the study by Avalos Ramirez (2012a). The HFBR is designed to maximise hydraulic retention time and promote good axial dispersion and mixing in the liquid phase (Rodgers and Clifford, 2009). This may accentuate the detergent effect of the Brij-35 on the biomass as the liquid phase moves through the reactor. Avalos Ramirez et. al., (2012a) also observed that the method of delivery of a non-ionic surfactant to a biotrickling filter had a significant effect on performance. Therefore further optimisation of the method of delivery of the Brij 35 to the HFBR could reduce susceptibility to higher concentrations and maximise its effect on mass transfer and reducing surface tensions in the liquid phase.

#### *3.4. Profile Removal Analysis*

Profile analysis indicated removals were reasonably consistent down through the reactor during each phase, with no particular zone producing consistently higher or lower removal rates. Profile results for each of the three reactors during Phase 1 are illustrated in Figure 5. Phase 2 results are presented in Figure 6 and Phase 3 results are presented in Figure 7. During Phase 1 of this study, influent organic carbon concentration remained low (< 20 mg TOC/L) and the performance of uppermost sheets was consistent with the rest of the reactor in each case. A similar case was observed during Phase 2, where organic carbon concentrations were slightly higher throughout the reactor, in the range 20 – 70 mg TOC/L. This additional carbon, due to the addition of silicone oil, and has been shown previously to be non-biodegradable (Rocha Rios et. al., 2009; Munoz et. al., 2007; Arriaga et. al., 2006).

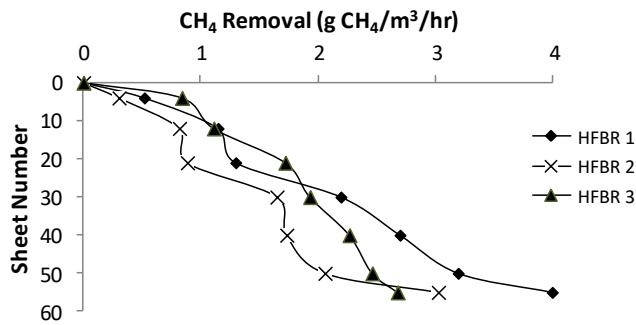


Figure 5. CH<sub>4</sub> profile removal results for Phase 1

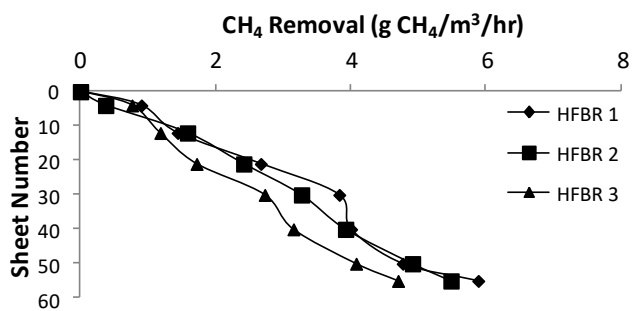
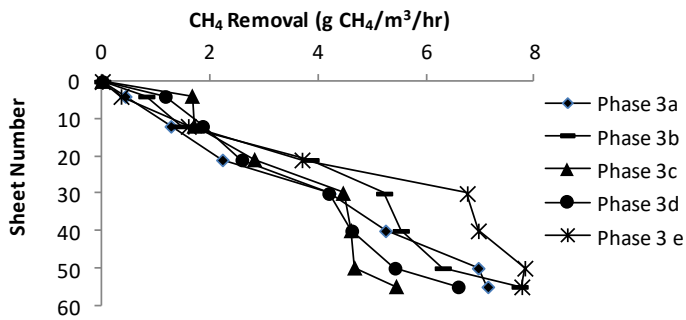


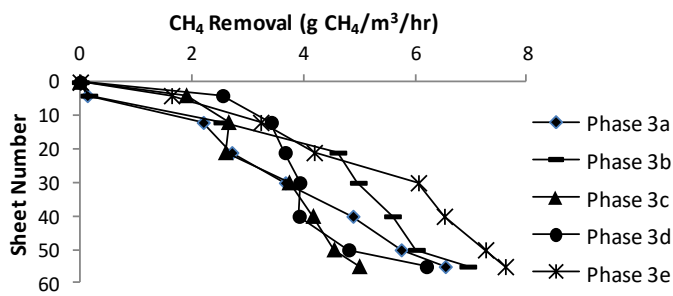
Figure 6. CH<sub>4</sub> profile removal results for Phase 2

Organic carbon concentrations during Phase 3 were higher in HFBRs 1 and 2 (250 – 350 mg TOC/L) than in HFBR 3 (30 – 80 mg TOC/L). This additional organic was due to the presence of Brij 35 and was biodegradable. This is further reflected by the increased CO<sub>2</sub> production observed during Phase 3 for HFBRs 1 and 2 (Figure 10). A similar increase in CO<sub>2</sub> production was observed by Avalos Ramirez et. al. (2012a) when increasing the concentrations of a non-ionic surfactant in a packed bed bioreactor from 0.1 to 1.0% w/w. In previous trials examining the effect of the presence of organic carbon, lower removal rates were recorded in the uppermost sheets where most organic carbon

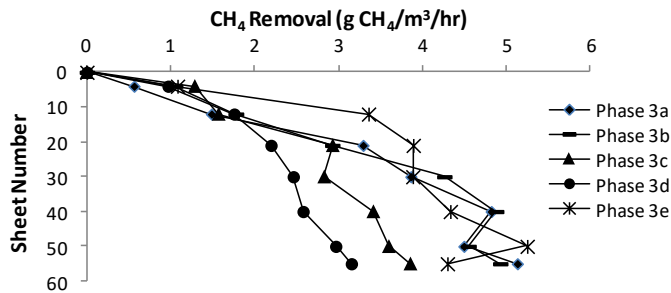
was removed (Kennelly et. al., 2012). However in this study removals in the uppermost sheets were consistent with other regions in the reactor. Therefore the addition of biodegradable carbon as Brij 35 did not appear to cause any significant inhibition of methanotrophic activity in HFBRs 1 and 2 during Phase 3, with any negative effect potentially being offset by the increased solubility of the CH<sub>4</sub> following addition of Brij 35.



(a)



(b)



(c)

**Figure 7.** Phase 3 CH<sub>4</sub> profile removal results for (a) HFBR 1, (b) HFBR 2 and (c) HFBR 3

Profiles of the specific methane elimination capacities (i.e. g CH<sub>4</sub> oxidised/g VSS/hr, where VSS refers to volatile suspended solids) for each reactor at the end of Phase 3 are shown in Table 6. The values are in the range of 10<sup>-4</sup> – 10<sup>-2</sup> g CH<sub>4</sub> oxidised/g VSS/hr. Although the average values for specific methane elimination are lower than those presented by Rocha Rios et. al. (2009), they are in line with values from other studies in the literature, which were found to range between 10<sup>-6</sup> – 10<sup>-2</sup> g CH<sub>4</sub> oxidised/g VSS/hr (Xin, et. al., 2003; Watanabe et. al., 1997). The most likely reason for the values presented in Table 6 being lower than those presented by Rocha Rios et. al. (2009) is the lower temperatures and lower loading rates used during this study. It was also observed that following the addition of Brij 35, the biofilm appeared to become thicker and more consistent. While this may be mostly a consequence of improved mass transfer and CH<sub>4</sub> oxidation (a similar observation of increased biofilm thickness and consistency was made at the end of Phase 2), it is possible also that the presence of biodegradable carbon as Brij 35 in the LNF resulted in the growth of some carbonaceous heterotrophs, leading to increased biomass yield (Clifford et. al., 2012) and reduced specific methane elimination capacities.

**Table 6**Specific CH<sub>4</sub> Elimination Capacities

	g CH <sub>4</sub> oxidised/gVSS/hr		
	HFBR 1	HFBR 2	HFBR 3
Sheets 1-4	9.90 x 10 <sup>-3</sup>	1.77 x 10 <sup>-2</sup>	3.78 x 10 <sup>-3</sup>
Sheets 5-12	9.74 x 10 <sup>-4</sup>	1.35 x 10 <sup>-3</sup>	9.67 x 10 <sup>-4</sup>
Sheets 13-21	2.46 x 10 <sup>-3</sup>	1.08 x 10 <sup>-3</sup>	1.36 x 10 <sup>-3</sup>
Sheets 22-30	1.88 x 10 <sup>-3</sup>	1.58 x 10 <sup>-3</sup>	5.69 x 10 <sup>-4</sup>
Sheets 31-40	5.98 x 10 <sup>-4</sup>	4.77 x 10 <sup>-4</sup>	1.36 x 10 <sup>-3</sup>
Sheets 41-50	1.11 x 10 <sup>-3</sup>	6.95 x 10 <sup>-4</sup>	2.02 x 10 <sup>-4</sup>
Sheets 51-55	1.24 x 10 <sup>-3</sup>	2.41 x 10 <sup>-3</sup>	5.11 x 10 <sup>-4</sup>

As CH<sub>4</sub> moves down through the reactor, diffusion profiles through biofilm on each sheet will vary. Diffusion of methane through a methanotrophic biofilm can be given as follows (Nikiema et.al. 2009b):

$$D_{ef} \frac{\partial^2 S_{CH_4}}{\partial x^2} \quad (\text{Equation 5})$$

Where:

$D_{ef}$  = Effective diffusion coefficient of CH<sub>4</sub> in the biofilm (m<sup>2</sup>/s)

$S_{CH_4}$  = Concentration of CH<sub>4</sub> within the biofilm (g CH<sub>4</sub>/m<sup>3</sup>)

$x$  = depth coordinate in the biofilm (m).

The boundary conditions of this equation can be given follows:

$$\text{At } x = 0 \text{ and for } 0 \leq z \leq H: S_{CH_4}(0, z) = \frac{C_{CH_4}(z)}{H_{CH_4}(T)} \text{ where:} \quad (\text{Equation 6})$$

$C_{CH_4}$  = Concentration of CH<sub>4</sub> in the gas phase (g CH<sub>4</sub>/m<sup>3</sup>)

$H_{CH_4}$  = Henry's coefficient of CH<sub>4</sub>

$$\text{At } x = d \text{ and for } 0 \leq z \leq H: \frac{\delta S_{CH_4}(\delta, z)}{\delta x} = 0 \quad (\text{Equation 7})$$

i.e. no biodegradation takes place before mass transfer from the gas phase and biodegradation reaches zero at a given depth through the biofilm (i.e. at a critical biofilm depth, below which no further oxidation of the substrate occurs). This critical biofilm depth depends on the coefficient of diffusion of the gas into the biofilm and the thickness of the biofilm (as well as the available concentration of substrate).

This critical depth was modelled by Stewart (2003) who, having regard to biofilm phenomena such as nutrient gradients, diverse microbial ecologies and solute reactivity described the following equation to estimate the penetration depth of a reacting solute in a “flat-slab” shaped biofilm community:

$$a = \sqrt{\frac{2D_{ef}C_{CH_4}}{k_0}} \quad \text{(Equation 8)}$$

Where:

$D_{ef}$  = Effective diffusion coefficient of  $CH_4$  in the biofilm ( $m^2/s$ )

$C_{CH_4}$  = Concentration of  $CH_4$  at the gas-biofilm interface ( $g\ CH_4/m^3$ )

$k_0$  = Volumetric reaction rate =  $\mu \frac{X}{Y_{XS}}$

Where:

$\mu$  = specific growth rate of microorganisms within the biofilm ( $s^{-1}$ )

$X$  = Cell density in the biofilm ( $g/m^3$ )

$Y_{XS}$  = Biomass yield coefficient ( $g\ biomass/g\ CH_4$ )

The dependence of penetration depth of substrate on concentration of  $CH_4$  at the gas-biofilm interface leads to a close correlation between the  $CH_4$  concentration profile (as a function of height), with the predicted critical depth in each of the zones. The estimates stoichiometric and kinetic values used to estimate the critical depth at each point in the HFBR is presented in Table 7.

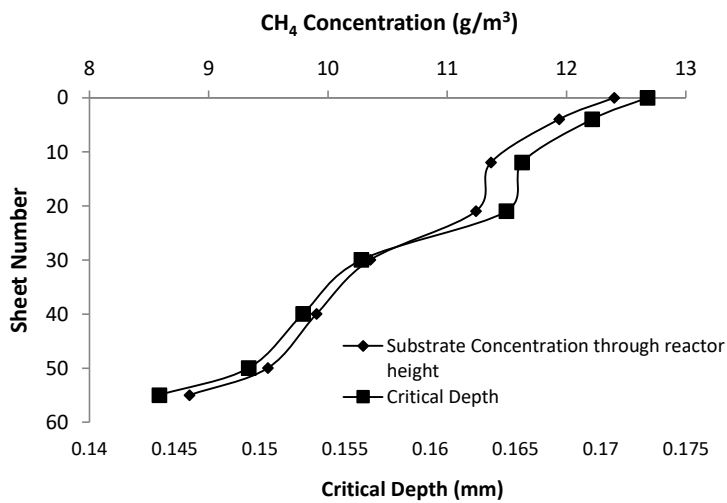
**Table 7**

Estimated stoichiometric and kinetic values for methane oxidising biofilm operated at 10°C and without additional transfer vectors.

Parameter	Value	Reference
$D_{ef}$	1.427 $m^2/s$	Wetherspoon and Seref, 1965
$C_{CH_4}$	12.4 $g/m^3$	
$\mu_{max}$	$4.98 \times 10^{-6} s^{-1}$	Nikiema et. al., 2009
$\mu$	$4.86 \times 10^{-6} s^{-1}$	Delhmoenie et. al., 2008
X	100,000 $g/m^3$	Nikiema et. al., 2009
$Y_{XS}$	0.41 $g/g CH_4$	Delhomenie et. al., 2008; Arcangeli and Arvin, 1999

**Commented [EC18]:** Seems high – is it the wet or dry density?  
Do we have our own values based on VSS?

This correlation is illustrated in Figure 8 below, which estimates the  $CH_4$  concentration profile down through the reactor height for HFBR 1 during Phase 1 and the expected critical biofilm depth for  $CH_4$  in each zone.



**Figure 8 -**  $CH_4$  concentration profile down through the reactor height for HFBR 1 during Phase 1 and the expected critical depth for  $CH_4$  in each zone.

Each HFBR contained 55 sheets and the EBRT during Phase 1 was 45 minutes. This equates to a contact time of approximately 49 seconds for each sheet. The diffusive

penetration time for a solute added to a biofilm was described by Stewart (2003) using the following relationship;

$$t = 1.03 \frac{L^2}{D_{ef}} \quad (\text{Equation 9})$$

Where:

$t$  = diffusive penetration time (s)

$L$  = biofilm thickness (m)

$D_{ef}$  = Effective diffusion coefficient of  $\text{CH}_4$  in the biofilm ( $\text{m}^2/\text{s}$ )

Thus, it can be approximated that the time taken to reach the upper critical depth illustrated in Figure 8 is 21.5 s. This suggests that the system was not limited by diffusion, given the retention time on each sheet was estimated at about 49 seconds.

In two phase partitioning biofilm reactors (i.e. biofilm reactors which use a secondary non-aqueous phase such as silicone oil), interactions between the gas phase, liquid phase and microorganisms are more complex, but the flow of substrate through the biofilm is still determined as the product of the mass transfer coefficient and the concentration gradient between the interface and the bulk substrate concentration (Munoz et. al., 2007). Therefore the above analysis provides an insight into the general trends expected for diffusion of  $\text{CH}_4$  into the biofilm following the addition of transfer vectors. Given that mass transfer and, therefore, rate of diffusion of  $\text{CH}_4$  into the biofilm increased during Phases 2 and 3, in addition to the slightly longer EBRTs that were applied, it is unlikely that diffusion was limiting during these Phases.

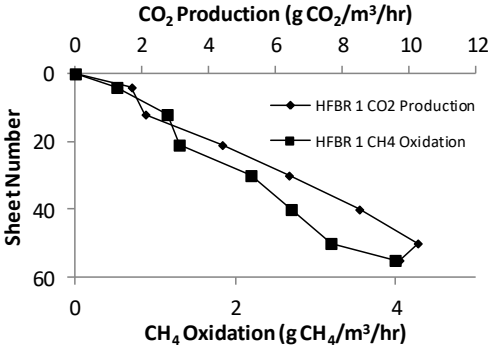
### 3.5. $\text{CO}_2$ Production

$\text{CO}_2$  production throughout HFBRs 1, 2 and 3 was monitored over each phase to give additional insight into methanotrophic activity in the biofilm. During each of the 3 phases

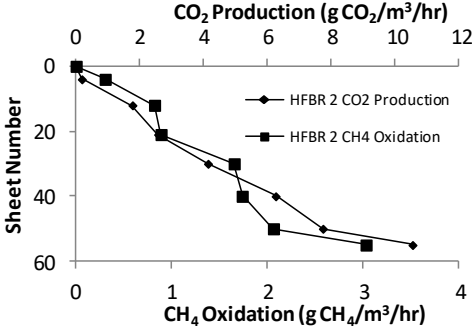
**Commented [EC19]:** Use same symbols for biofilm thickness as you did above.

**Commented [NU20]:** In response to Reviewer #2 comment, "It would be interesting if authors could relate the axial profile through the reactor with axial profiles inside the biofilm in each sheet", the highlighted section is included. Diffusion through the biofilm and biodegrading profiles through the biofilm are considered and correlation between the removal profile as a function of height and removal of substrate with depth through the biofilm are considered.

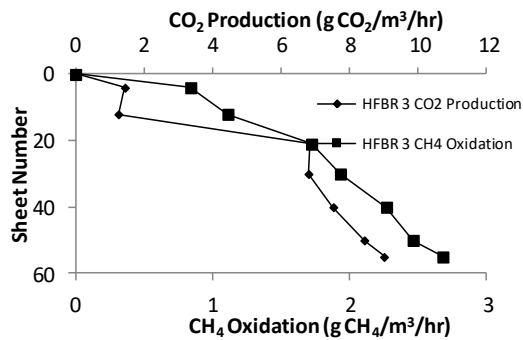
the CO<sub>2</sub> production followed a similar profile to CH<sub>4</sub> oxidation (Figure 9 shows the pattern during Phase 1, similar patterns were observed for all reactors during Phase 2 and Phase 3).



(a)



(b)



(c)

**Figure 9.** CO<sub>2</sub> production profile for Phase 1; (a) HFBR 1; (b) HFBR 2; (c) HFBR 3. The profile is typical of all phases of the trial

The CO<sub>2</sub> yield (g CO<sub>2</sub> produced per g CH<sub>4</sub> oxidised - YCO<sub>2</sub>), during Phase 1 of these trials is somewhat less than that observed in previous, similar studies examining CH<sub>4</sub> oxidation in a HFBR (Clifford et. al., 2012). This was most probably due to the different inoculation process used and nutrient feeding regime used in this study. A procedure to estimate the carbon diverted into biomass growth has previously been detailed (Clifford et al., 2012). Using this procedure it was estimate that  $9 \times 10^{-3}$ ,  $5 \times 10^{-3}$  and  $4 \times 10^{-3}$  mol C/day were diverted into biomass growth in HFBRs 1, 2 and 3.

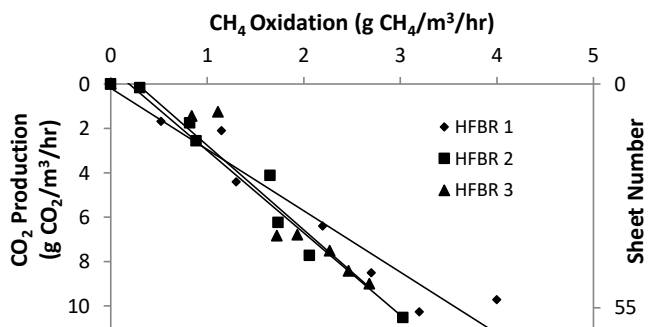
During Phase 2, effluent CO<sub>2</sub> emissions increased slightly to 0.190, 0.169 and 0.133 mol/day for HFBRs 1, 2 and 3 respectively, representing increases of 74%, 59% and 46%. These increases are roughly in line with the CH<sub>4</sub> performance improvements of 31%, 79% and 78% for HFBRs 1, 2 and 3 respectively. An increase in CO<sub>2</sub> production correlated well with increased CH<sub>4</sub> oxidation (Figure 9) and thus the addition of the organic oil phase likely had a limited impact on CO<sub>2</sub> production. In all phases linear relationship observed between CH<sub>4</sub> oxidation and CO<sub>2</sub> production (Figures 10a, 10b & 10c). Moreover, the carbon mass balance appeared to be largely unaffected, with  $4 \times 10^{-4}$ ,  $6 \times$

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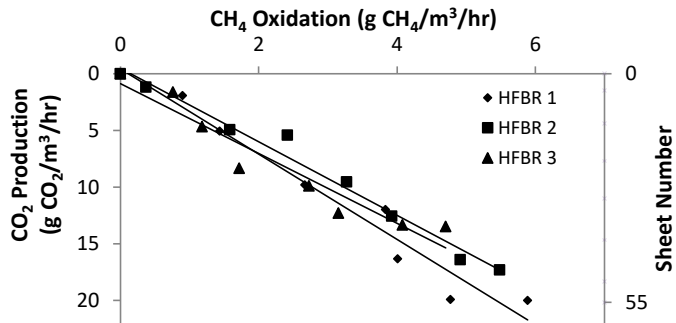
$10^{-3}$  and  $6 \times 10^{-4}$  mol C/day used for biomass growth in HFBRs 1, 2 and 3 respectively. The close correlation between  $\text{CH}_4$  oxidation and  $\text{CO}_2$  production with reactor depth was maintained during Phase 3 (Figure 10c) indicating the  $\text{CO}_2$  emitted in the effluent was due mainly to  $\text{CH}_4$  oxidation, which is in line with previous studies (Avalos Ramirez et. al., 2012b).

However, as can be seen from Figure 11,  $\text{YCO}_2$  increased in HFBRs 1 and 2 during Phase 3c and 3d. This indicates additional organic carbon oxidation and subsequent  $\text{CO}_2$  production, probably due to the presence of the Brij 35 in the LNF. This has been observed previous studies (Avalos Ramirez et. al., 2012b), which also recorded an increase in  $\text{YCO}_2$  following addition of non-ionic surfactant.

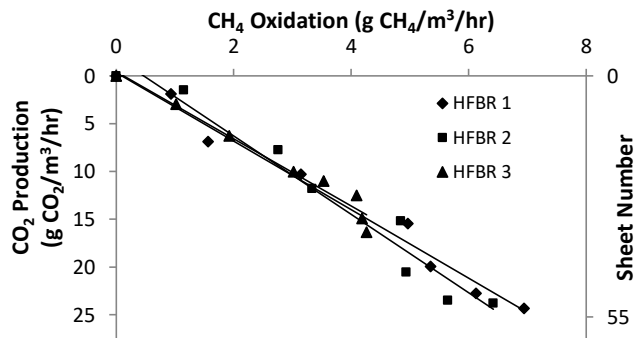
The presence of biodegradable organic carbon has previously been shown to inhibit performance of a  $\text{CH}_4$  oxidising HFBR (Clifford et. al., 2012) and therefore may have been a factor in the reduced oxidation performance that was observed at higher concentrations of Brij 35 (Phase 3c).



(a)

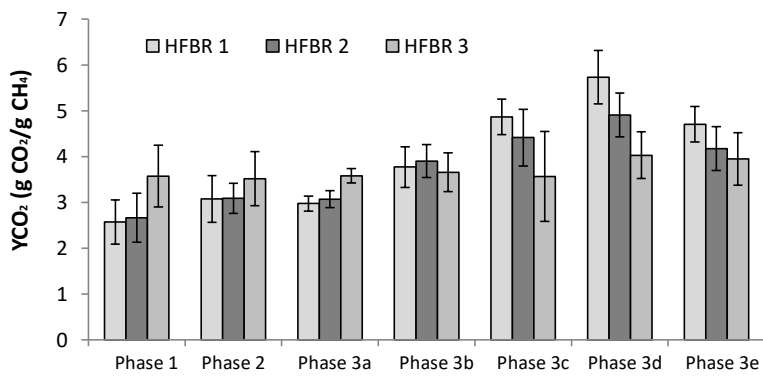


(b)



(c)

**Figure 10.** Relationship between CO<sub>2</sub> production and CH<sub>4</sub> oxidation at different depths of each reactor for; (a) Phase 1; (b) Phase 2 and (c) Phase 3 (all sub-phases). R<sup>2</sup> for each linear equation varied between 0.92 and 0.98 for all phases. Slopes are within 1 standard deviation of overall effluent YCO<sub>2</sub> for each phase.



**Figure 11.** YCO<sub>2</sub> for HFBRs 1, 2 and 3

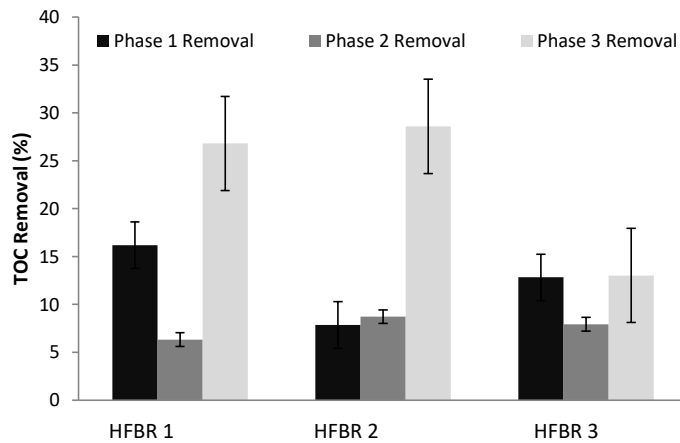
### *3.6. Liquid Phase Analysis*

#### *3.6.1. Total Organic Carbon*

The absence of external biodegradable carbon in the nutrient feed leads to improved methanotrophic activity in a methane oxidising HFBR (Clifford et. al., 2012; Kennelly et. al., 2012). Therefore during Phase 1 of these trials, influent organic carbon was minimal (< 18 mg TOC/L) and removal of liquid phase organic carbon was consistently below 15%.

Organic carbon was slightly higher in each of the reactors during Phase 2. This additional carbon was due to the addition of silicone oil (non-biodegradable), and therefore of liquid phase organic carbon remained consistently below 10%.

As can be seen from Figure 12, influent TOC removal increased in HFBRs 1 and 2 during Phase 3. This additional organic carbon removal was due to the addition of Brij 35, which was biodegradable. Liquid phase TOC removal increased to 27% for HFBR 1 and 29% for HFBR 2. No increases in liquid phase TOC removal was observed for HFBR 3 (which had no Brij 35 added during Phase 3).



**Figure 12.** Total Organic Carbon (TOC). Average removals are shown as columns.

### 3.6.2. Nitrogen

Previous studies (Nikiema et. al., 2009) established that the optimum nitrogen concentration for a packed bed biofilter treating similar loads of CH<sub>4</sub> to this study is 0.75 g N/L as nitrate (NO<sub>3</sub>-N). The results of that study also found that concentrations of 0.25 g N/L were generally as effective as concentrations of 0.75 up to a loading rate of 20 g CH<sub>4</sub>/m<sup>3</sup>/hr, therefore 0.25 g N/L as nitrate were included in the LNF for this study.

However during these trials, NO<sub>3</sub>-N uptake was variable (4 – 34 %) and no clear pattern of uptake could be established from the LNF profile analysis (Figure 13). This contrasts with the uptake pattern of NH<sub>4</sub> added to HFBR 3 during Phase 1, which steadily reduced in concentration as it passed through HFBR 3 (Figure 14). The performance of HFBR 3 improved following the addition of 25 mg NH<sub>4</sub>-N/L suggesting that ammonia may be the preferred nitrogen species in a CH<sub>4</sub> oxidising HFBR, in line with previous studies (Clifford et. al., 2012; Kennelly et. al., 2012).

However given the complex nature of nitrogen dynamics in such a system, further studies are necessary to describe how nitrogen addition can be optimised and reconciled with other operating and environmental parameters such as CH<sub>4</sub> loading rate, optimised LNF flow regime, temperature, pH, inoculation techniques etc.

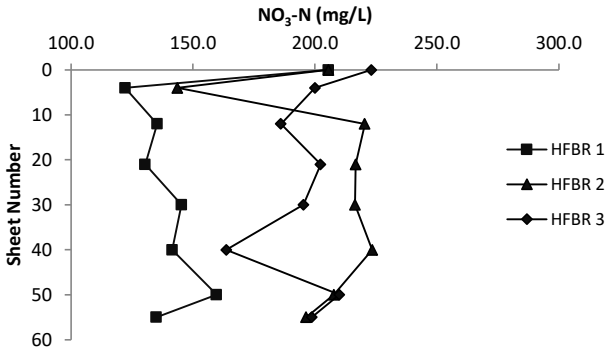


Figure 13. Nitrate Nitrogen (NO<sub>3</sub>-N) average removals during Phase 1. The profile is typical of all phases of the trial

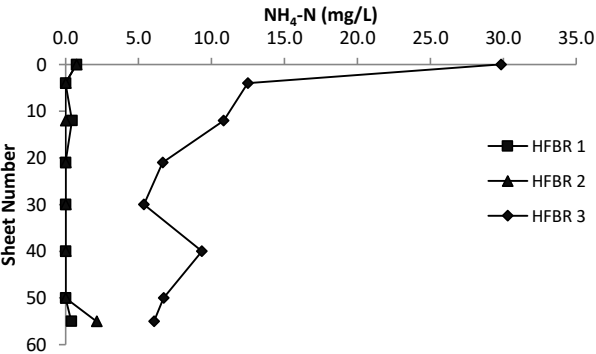


Figure 14. Ammonium Nitrogen (NH<sub>4</sub>-N) average removals during Phase 1. The profile is typical of all Phases of the trial

### 3.7. Cost Effectiveness of Chemical Transfer Vectors

The results of this study demonstrate that significant performance improvements can be achieved following the addition of chemical transfer vectors (e.g. silicone oil and Brij 35). However, it is important to consider the cost implications of using transfer vectors for CH<sub>4</sub> oxidation at site scale. The use of silicone oil can add to both material costs, operational costs and to whole life energy costs due to increased power consumption and pumping costs (Rocha Rios et. al., 2013; Clarke and Correia, 2008).

Similarly, adding Brij 35 can add to whole life material costs. Furthermore, in traditional reactor designs, the addition of Brij 35 can lead to clogging due to increased biomass detachment, adding to maintenance costs and pumping requirements (Ramirez et. al., 2009a). Such considerations have raised concerns over the cost effectiveness of chemical transfer vectors at site scale (Rocha-Rios et. al., 2013; Quijano et. al., 2010b).

A larger pilot scale study would be required to complete an accurate cost-benefit analysis. For example the addition of transfer vectors may result in additional running costs but could also lead to significant reductions in capital costs due to increased removal rates. With further optimisation it may be possible to circumvent many of the additional costs incurred by using chemical transfer vectors. For example, an effective liquid phase recirculation strategy could significantly reduce material costs, although further research would be necessary to determine the degradation rates of nutrients and Brij 35 in the liquid phase. In addition, the HFBR has previously been shown to be a low cost and energy efficient reactor technology, both at laboratory and site scale (Clifford et. al., 2010; Rodgers and Clifford, 2009).

## 4. Conclusions

**Commented [NU22]:** In response to Reviewer #2 comment, "A cost-benefit analysis should be done to determine if the improvement in methane removal with addition 1% of Brij 35 is significant compared to methane removals obtained only with addition of silicone oil (10%)", The authors have included a general discussion on costs vs benefits for chemical transfer vectors in a biofilm reactor in response to this comment.

**Commented [NU23]:** In response to Reviewer comment #2, "The information is repetitive and it represents a summary of paper more than conclusions. The authors should rewrite this section" the conclusions section has been shortened and made more concise

In this study, methods of improving the performance of a CH<sub>4</sub> oxidising Horizontal Flow Biofilm Reactor (HFBR) operating at low temperatures were investigated. The study shows how liquid phase improvement using secondary organic liquid phases and non-ionic surfactants can significantly reduce mass transfer limitations in a CH<sub>4</sub> biofilm reactor. Furthermore, despite the low operating temperatures employed in this study, the HFBR technology has excellent potential to treat emissions of low concentrations of CH<sub>4</sub>. The key conclusions are as follows:

- Given the low operating temperature (10°C) during this trial, the results compare favourably with previous studies, even without the addition of chemical transfer vectors. Maximum average removals of 8.6 g CH<sub>4</sub>/m<sup>3</sup>/hr were observed. The maximum observed removal was 10.5 g CH<sub>4</sub>/m<sup>3</sup>/hr.
- The results suggest that the addition of ammonium salts in low concentrations can have a positive influence on the performance a methanotrophic biofilm. It should be noted that reports in literature vary as to the optimum species and concentrations of nitrogen that should be added to the liquid phase of a CH<sub>4</sub> oxidising biofilm reactor. This is an area that may require further research.
- The addition of silicone oil to the liquid nutrient feed (LNF) led to performance improvements of 31%, 79% and 78% for HFBRs 1, HFBR 2 and HFBR 3 respectively. The most probable reason for this improvement was increased mass transfer of CH<sub>4</sub> into the liquid phase following the addition of the silicone oil.
- Subsequent addition of a non-ionic surfactant (Brij 35) to HFBRs 1 and 2 led to further significant improvements in reactor performance of up to 110% and 174% respectively when compared to the addition of silicone oil alone. This was most likely due to the increase in CH<sub>4</sub> availability in the liquid phase. The non-ionic surfactant

(Brij 35) acted as a direct transfer vector, in addition to its emulsifying effect on the oil-water phase.

- Profile analysis indicated consistent CH<sub>4</sub> oxidation throughout each zone of each HFBR throughout the study. Although microbial communities will vary with depth due to changes in substrate and nutrient availabilities, the consistent removal profile is in keeping with the structured, modular nature of the HFBR that limits bypass and maximises contact time.

Further work could focus on the costs and cost benefits of using transfer vectors at a larger scale. Detailed microbial analysis currently underway will help characterise the microbial community's dominant in the HFBR during these trials and may illustrate provide additional information on processes within in the HFBR system.

## 5. Acknowledgements

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