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

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## ABSTRACT

In this study, methods of improving the methane oxidation performance of a biofilm technology, the horizontal flow biofilm reactor (HFBR), operated at low temperatures were investigated. Three pilot scale HFBRs were commissioned to treat an air mixture containing methane (CH<sub>4</sub>) gas and were operated over 3 phases (Phases 1, 2 & 3) lasting 310 days in total. The reactors, loaded with 13.2 g CH<sub>4</sub>/m<sup>3</sup> reactor volume/hr during each phase, were operated at an average temperature of 10°C throughout.

In Phase 1, nutrients were added to the biofilm via a liquid nutrient feed (LNF) comprising water and nutrient mineral salts. Removals averaged 4.1, 3.1 and 2.7 g CH<sub>4</sub>/m<sup>3</sup> /hr for HFBRs 1, 2 and 3 respectively.

In Phase 2 silicone oil was added to the LNF of all three HFBRs to enhance mass transfer of methane to the liquid phase and thus improve treatment performance. Following this removal rates for Phase 2 averaged 5.6, 5.5 and 4.0 g CH<sub>4</sub>/m<sup>3</sup>/hr 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. Various concentrations were trialled, with 1.0 g Brij 35/L proving most successful. Removal rates increased to 8.6 g CH<sub>4</sub>/m<sup>3</sup>/hr and 8.4 g CH<sub>4</sub>/m<sup>3</sup>/hr for HFBRs 1 and 2 respectively under these conditions, representing increases of 54% and 53% for HFBRs 1 and 2 respectively.

These results indicate the potential of liquid phase optimisation as an efficient solution to improving the performance of biological reactors treating CH<sub>4</sub> emissions and overcome traditional constraints posed by mass transfer limitations.

## INTRODUCTION

Methane (CH<sub>4</sub>) 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 (Rocha Rios et. al., 2009a). 55% of anthropogenic methane emissions are below the lower explosive limit (LEL) and cannot be thermally oxidised (Avalos-Ramirez et. al., 2012a). Treatment in biofilm reactors presents a viable alternative for the control of these emissions (Clifford et. al., 2012; Kennelly et. al., 2012; Streese and Stegmann, 2003). One of the main challenges in designing CH<sub>4</sub> oxidising biofilm reactors is the low solubility of CH<sub>4</sub> in water. This presents a barrier to mass transfer and necessitates long hydraulic retention times; particularly at low temperatures and thus increases reactor size (Streese and Stegmann, 2003). Recent research has focused on methods of chemically optimising the liquid phase in biofilm reactors to maximise mass transfer. One such method is the addition of a secondary, non aqueous, organic liquid phase such as silicon oil or hexadecane. Silicone oil has previously been successfully applied as a mass transfer vector to a stirred tank reactor and a biotrickling filter (Rocha-Rios et. al., 2009a).

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

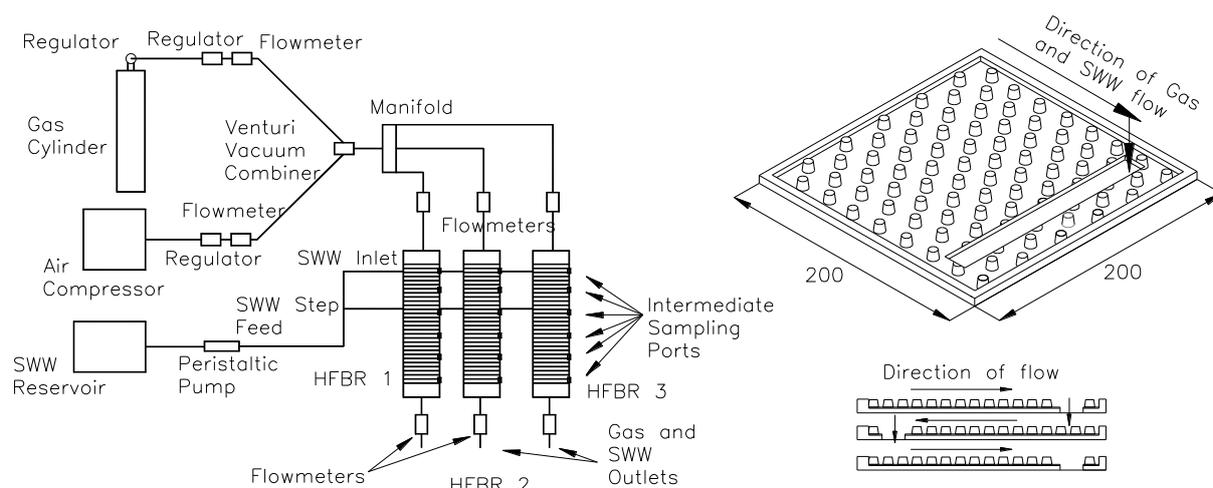
surfactants have successfully improved performances of packed bed biofilters (Avalos-Ramirez et. al., 2012a; Jurado et. al., 2007) and are largely biodegradable and non toxic in low concentrations (Avalos-Ramirez et. al., 2012b). Brij 35 can also be used as an oil water emulsifier (HLB: 16.9). To date, 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. Brij 35 can enhance mass transfer directly by increasing solubility of the methane in the liquid phase and also indirectly by acting as an emulsifier for the silicone oil and water phases. 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.

In this study, the effect of adding silicone oil both with and without Brij 35, to the liquid phase of a Horizontal Flow Biofilm Reactor (HFBR), previously applied successfully to methane oxidation (Clifford et. al., 2012) was investigated at lower temperatures.

## METHODS

### Horizontal Flow Biofilm Reactor (HFBR)

The HFBR is a novel biofilm reactor designed to eliminate problems traditionally associated with packed bed biofilm reactors such as clogging and pressure drop. The unique flow regime also maximises contact time between the target contaminants and the biofilm due to the alternating liquid and gas flow paths through the unit. The construction of the HFBR and configuration of the gas and water flow system is shown in Figure 1 and has been previously described in detail (Kennelly et. al., 2012).



**Figure 1** – Schematic of the HFBR system

The influent gas stream comprised a mixture of atmospheric air mixed with a CH<sub>4</sub> gas supply. Gas flow and loading parameters are summarised in Table 1.

**Table 1** – Current flow and loading parameters for CH<sub>4</sub> HFBR reactors

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/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

### Liquid Nutrient Feed (LNF)

The LNF used in this study is presented in Table 2. Previous work has differed on the optimal nitrogen source, in the liquid feed of for methane oxidation reactors (Clifford et. al., 2012; Nikiema et. al., 2009; Bodelier et. al., 2000); thus, initially HFBRs 1, 2 and 3 used nitrate-nitrogen as the main nitrogen source in the influent liquid phase.

**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	0.03*	KI	0.000166		
NaNO <sub>3</sub>	1.51	H <sub>3</sub> BO <sub>3</sub>	0.000124		

\* Added to HFBR 3 only after 25 days

### Biofilm growth and inoculation

On commissioning the HFBR, an enrichment strategy was employed to cultivate a mixed microbial community capable of CH<sub>4</sub> oxidation at low temperatures. The enrichment was carried out over 4 months by feeding CH<sub>4</sub> to a mixture of landfill soil, compost, landfill leachate and compost leachate, at 10°C in batch cultures. GC analysis was used to monitor CH<sub>4</sub> oxidation in the enrichment cultures, which were sub-cultured to new medium every 2-3 weeks. The biomass was suspended in an adapted Whittenbury medium (Whittenbury et al., 1970). An aliquot of 2 L of the enriched biomass was then added to each of the HFBRs and was re-circulated through the reactors for 7 days using peristaltic pumps.

### Sampling and Analytical Methods

Gas samples were taken with a syringe and analyzed for CH<sub>4</sub> and CO<sub>2</sub> using an Agilent 7280 GC. The system was operated, maintained and calibrated as per manufacturer's guidelines.

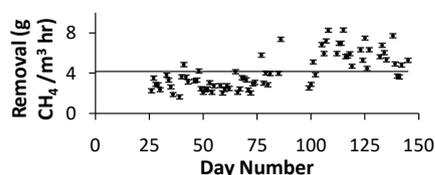
## RESULTS

### CH<sub>4</sub> Removal – Phase 1

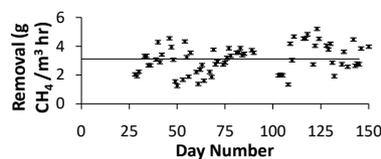
150 days – 10°C: LNF only in the liquid phase. After inoculation with the enriched biomass, the 3 HFBRs were continuously operated and monitored under the Phase 1 conditions outlined in Table 1. After a 25 day acclimation period, reasonably consistent removals were noted and a pseudo steady state was observed. Removal results for Phase 1 are outlined in Figures 2 (a), (b) & (c) and in Table 3.

**Table 3 - CH<sub>4</sub> Removal Results for Phase 1**

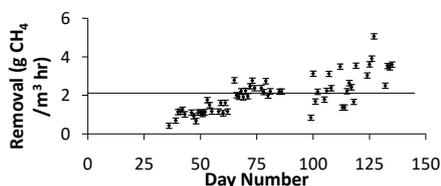
Reactor	Average Removal (g/m <sup>3</sup> .hr)	Standard Deviation (g/m <sup>3</sup> .hr)	Maximum (g/m <sup>3</sup> .hr)
HFBR 1	4.1	1.78	8.3
HFBR 2	3.1	0.98	5.2
HFBR 3	2.3	1.12	5.1



(a)



(b)



(c)

**Figure 2** –CH<sub>4</sub> Removal for Phase 1; (a) HFBR 1; (b) HFBR 2; (c) HFBR (3). Average removal shown using a straight line

HFBR 3, although identical in operation and influent loading, initially underperformed in comparison to HFBRs 1 and 2. In previous HFBR trials (Clifford et. al., 2012) the best performing units were those which used feed containing ammonium salts in the LNF, and for this reason NH<sub>4</sub>Cl was added to the LNF for HFBR 3. As can be seen from Figure 2 (c), the performance following addition of NH<sub>4</sub>Cl, on Day 30, steadily improved. The average removal increased from 1.0 g CH<sub>4</sub>/m<sup>3</sup> /hr during the first two months of the trial, during which time the NH<sub>4</sub>Cl was added, 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. While some previous research has found ammonium-nitrogen (NH<sub>4</sub>-N) can limit methane oxidation (Nikiema et. al., 2009), the studies involving methane oxidation in the HFBR to date have found that the presence of NH<sub>4</sub>-N in the LNF (in concentrations of about 20 mg NH<sub>4</sub>-N/l) have improved reactor performance.

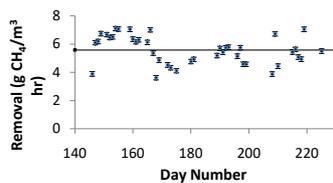
### CH<sub>4</sub> Removal – Phase 2

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

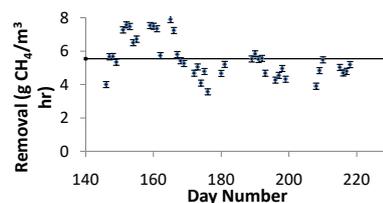
Recent work has shown silicon oil to be a suitable transfer vector as it not toxic to methanotrophic biomass nor is it biodegraded by the methanotrophs. (Rocha Rios et al, 2009a); silicone oil has a partition coefficient 10 times lower than that of water. Silicone oil (10% v/v) was therefore added to the LNF during Phase 2 to enhance mass transfer rates. The silicone oil and LNF mixture was constantly agitated using magnetic stirrers. Results for Phase 2 are outlined in Table 4 and Figures 3 (a), (b) & (c) respectively.

**Table 4** - CH<sub>4</sub> Removal Results for Phase 2

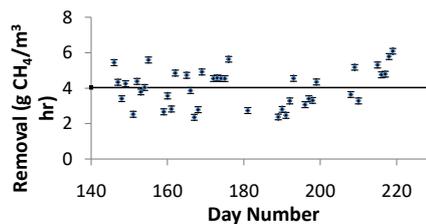
Reactor	Average Removal (g/m <sup>3</sup> . h)	Standard Deviation (g/m <sup>3</sup> . h)	Maximum Removal (g/m <sup>3</sup> . h)	Performance Improvement (compared to Phase 1)
HFBR 1	5.5	0.99	7.1	31 %
HFBR 2	5.5	1.15	7.9	79 %
HFBR 3	4.0	1.05	6.1	78 %



(a)



(b)



(c)

**Figure 3** – CH<sub>4</sub> Removal for Phase 2; (a) HFBR 1; (b) HFBR 2; (c) HFBR (3). Average removal shown using a straight line

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, from those measured in Phase 1, of 31% 79% 2 and 78% for HFBRs 1, 2 and 3 respectively. These improvements are similar to those observed in recent studies investigating the effect of silicone oil in methane oxidising biofilm reactors. Rocha-Rios et al. (2009a) observed a 41% increase in performance in a stirred tank reactor and a 131% increased performance in a biotrickling filter when 10% v/v silicone oil was added.

### CH<sub>4</sub> Phase 3

(70 days – 10°C): LNF, silicone oil and brij 35 in the liquid phase of HFBR 1 and 2.

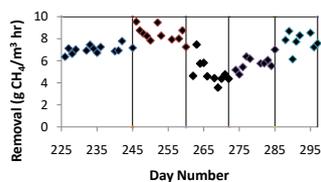
Phase 3 was divided into 5 stages, namely 3a, 3b, 3a, 3c, 3d and 3e lasting 16, 17, 11, 14 and 12 days respectively. In Phase 3 brij 35 was added to the LNF in HFBRs 1 and 2 at concentrations of 0.5 g/L, 1.0 g/L, 2.0 g/L, 1.0 g/L and 0.75 g/L in Stages 3a, 3b, 3a, 3c, 3d and 3e respectively.

During Stage 3a average removal rates 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. In Stage 3b average removal rates increased by a further 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. In Stage 3c average removal rates, when compared to Stage 3b 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 (Stage 3d) for 14 days. Removal rates recovered to 5.6 and 6.0 g CH<sub>4</sub>/m<sup>3</sup>/hr, representing a recovery of 15% and 7% for HFBRs 1 and 2 respectively. The concentration was further reduced to 0.75 g/L during Stage 3e and removals increased, when compared to Stage 3d, 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, and acted as a control – added remained steady.

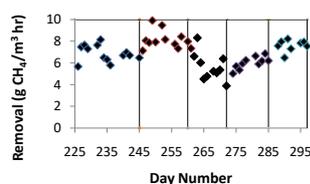
The performance of each reactor during Phase 3 is illustrated in Figure 4 and in Table 5.

**Table 5** - CH<sub>4</sub> Removal Results for Phase 1 – Standard deviation shown in ( ).

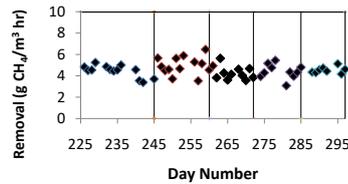
	Phase 3 a		Phase 3 b		Phase 3 c		Phase 3 d		Phase 3 e	
	(g CH <sub>4</sub> /m <sup>3</sup> .hr)									
	Average	Max	Average	Max	Average	Max	Average	Max	Average	Max
HFBR 1	7.0 (0.36)	7.8	8.6 (0.81)	10.1	4.9 (1.09)	7.5	5.8 (0.64)	7.0	7.8 (0.81)	8.7
HFBR 2	6.9 (0.75)	8.1	8.5 (1.14)	10.4	5.6 (1.27)	8.3	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



(a)



(b)



(c)

**Figure 4** – CH<sub>4</sub> Removal for Phase 3; (a) HFBR 1; (b) HFBR 2; (c) HFBR (3). Stage boundaries shown with a vertical line

As can be seen in Figures 4 (a) and (b) the performances of HFBRs 1 and 2 improved following the addition of brij 35. The most probable reasons for this are (i) the increase in CH<sub>4</sub> availability in liquid phase due to the increased solubility of CH<sub>4</sub> in a water phase containing brij 35 (i.e. non ionic surfactant acting as a direct transfer vector) and (ii) the emulsifying effect on the oil water phase (the hydrophilic-lipophilic balance number of brij 35 is 16.9). This could provide an alternative to the turbulence that Rocha Rios et. al., (2009) introduced to improve mixing and therefore, performance of a two phase partition bioreactor (i.e. water based and silicone oil based).

Previous studies have showed how brij 35 increases the solubilisation of gases into the aqueous phase (King, 1992; King, 2001) and Avalos-Ramirez (2012b) demonstrated experimentally how this is one of the principal mechanisms by which brij 35 addition can enhance reactor performance. Non-ionic surfactants, such as brij 35, can also improve performance in a biofilm reactor by modifying the spatial structure of enzymes, increasing their substrate specificity, activity and stability, reducing the surface and interfacial tensions of liquids, and decreasing the thickness of the liquid layer covering the biofilm. Such factors can lead to an increase in mass transfer in hydrophobic compounds such as CH<sub>4</sub> Avalos-Ramirez (2012b).

Figures 4 (a) and 4 (b) show a sharp decrease in removal efficiency occurred during Phase 3c when the concentration of brij 35 was increased from 1.0 g/L up to 2.0 g/L (0.1% up to 0.2% w/w). It is unlikely that this is due to toxicity as non ionic surfactants have previously been shown to be non-hazardous to microorganisms at the concentrations employed in this study (Jurado et. al., 2007; Campbell, 2002). The concentrations employed in Stage 3c of this study were lower than those previously employed successfully by Avalos-Ramirez (2012a). Therefore, the most probable reason for this decrease is likely biomass detachment caused by the detergent effect of the non ionic surfactant. The reactors quickly recovered when Brij 35 concentrations were returned to previous levels (Stages 3d and 3e). Indeed, by end of Stage 3e a further improvement in biofilm growth and consistency was visually observed compared to Phase 2. Growth on each sheet was visually observed to be stable and consistent both across and underneath all sheets in HFBRs 1 and 2 by the end of Phase 3. This could have resulted in further improved reactor performance by increasing the biomass concentrations.

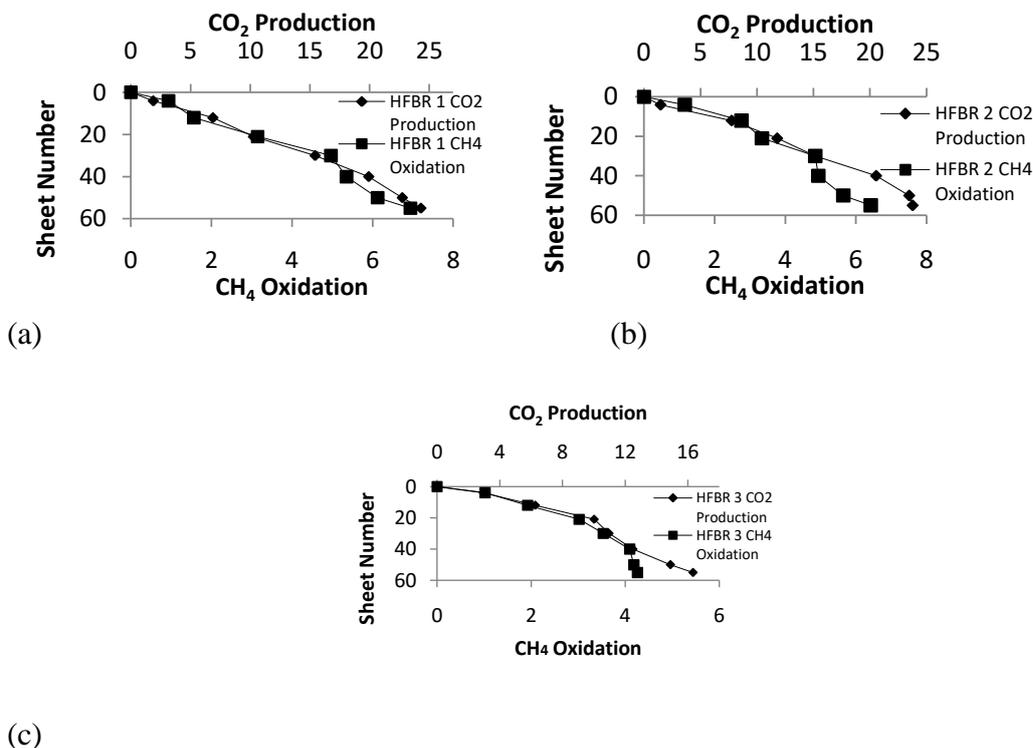
This observation contrasts with studies by Avalos-Ramirez et. al., (2012a) who observed that elimination capacity was not affected when the concentration of a similar non ionic surfactant (Tween 20) was incremented from 0.25% w/w to 1.0% w/w.

One reason for this contrast could be that because the HFBR is configured to maximise contact between the biofilm and the contaminated fluids it is treating, the liquid phase hydraulic retention time (HRT) will be significantly longer in a HFBR than in a traditional packed bed reactor, which could lead to the surfactant having an inhibitory effect on the HFBR biofilm at lower concentrations than those observed in a packed bed reactor.

Detailed microbial studies are currently underway to determine the nature of the methanotrophic community within the HFBRs during each phase of this study. This will allow the influence of the Brij 35 on the biofilm to be characterised in more detail.

### CO<sub>2</sub> Production

CO<sub>2</sub> production throughout HFBR units 1 – 3 was monitored in each phase to give additional insight into methanotrophic activity in the biofilm. During the 3 Phases CO<sub>2</sub> production was closely aligned to CH<sub>4</sub> oxidation (Figures 5), indicating, as expected, that a significant fraction of the CO<sub>2</sub> produced in the reactor was produced by CH<sub>4</sub> oxidation.



**Figure 5** – CO<sub>2</sub> production profile for Phase 3; (a) HFBR 1; (b) HFBR 2; (c) HFBR 3. CO<sub>2</sub> production and CH<sub>4</sub> oxidation figures are average for entire duration of Phase 3.

### CONCLUSIONS

3 pilot scale horizontal flow biofilm reactors (HFBRs 1, 2 and 3) were operated in a temperature controlled laboratory at 10°C, at a CH<sub>4</sub> loading rate of 13.2 g CH<sub>4</sub>/m<sup>3</sup> reactor/hr over 3 phases. Some of the main results are briefly highlighted and could result in significant improvements in biological methane oxidation, particularly at lower temperatures.

During Phase 1 a single liquid phase (LNF) was used. After a 21 day start up phase, removals consistent with previous HFBR trials were observed. HFBR 3 initially underperformed in comparison to HFBRs 1 and 2 during Phase 1. In previous studies examining CH<sub>4</sub> oxidation in a HFBR (Clifford et. al., 2012; Kennelly et. al., 2012); the addition of low concentrations of NH<sub>4</sub>Cl (≈ 20 mg/L) resulted in improved performances. Therefore, 20 mg/L of NH<sub>4</sub>Cl was added to the LNF to improve the performance of the HFBR 3. This (increasing 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) further indicates that ammonium-nitrogen may play an important role in biological reactor performance at lower temperatures.

During Phase 2 silicone oil was added to the reactors resulting in an immediate improvement in oxidation rates most likely due to improved mass transfer rates (generally the rate limiting step). An improvement in biofilm growth and consistency was also visually observed during Phase 2.

During Phase 3 oxidation rates were further increased following the addition of a non-ionic surfactant. Overall improvements in reactor performance between Phase 1 and Phase 3 were 73% and 130% for HFBR 1 and 2 respectively. The optimum concentration of the non-ionic compound (Brij 35) in the LNF, for this study, was found to be between 0.75 – 1.0 g Brij 35/L. It was observed at higher concentrations reactor performance declined rapidly and significantly. This is likely to be due to excessive biomass detachment from the plastic media. This has previously been observed in biodegradation studies (Whang et. al., 2008). Average removals were observed to be more consistent during Phases 2 and 3 than during Phase 1; the standard deviations of the average removals were significantly reduced when compared to Phase 1. This indicates that the improved (and possibly more consistent) mass transfer resulted in a more stable reactor performance.

CO<sub>2</sub> analysis revealed that CO<sub>2</sub> production followed a very similar profile pattern to CH<sub>4</sub> oxidation, with each suggesting that oxidation rates were relatively constant through the profile of the reactor.

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> oxidising biofilm reactor. Despite the low operating temperatures employed in this study, emissions of low concentrations of CH<sub>4</sub> can be effectively treated in biofilm reactors such as the HFBR.

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