The impact of hydraulic retention time on the performance of two configurations of anaerobic pond for municipal sewage treatment

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Abstract

Anaerobic ponds have the potential to contribute to low carbon wastewater treatment, however are currently restricted by long hydraulic residence time (HRT) which leads to large land requirements. A two stage anaerobic pond (SAP) design was trialled against a single stage control (CAP) over four HRTs down to 0.5 days, to determine the lowest HRT at which the ponds could operate effectively. No statistical differences were observed in particulate removal between the ponds over all four HRTs, suggesting solids loading is not a critical factor in AP design. Significantly higher biogas production rates were observed in the SAP than the CAP at 1.5 d and 1.0 d HRT, and microbial community

profiling suggest the two stage design may be facilitating spatial separation of the anaerobic digestion process along reactor length. Hydrogenotrophic methanogensis dominated over aceticlastic, with acetate oxidization a likely degradation pathway. Experimental tracer studies were compared to CFD simulations, with the SAP showing greater hydraulic efficiency, and differences more pronounced at shorter HRTs. Greater flow recirculation between baffles was observed in CFD velocity profiles, demonstrating baffles can dissipate preferential flow patterns and increase effective pond volume, especially at high flow rates. The study demonstrates the potential of APs to be operated at shorter HRTs in psychrophilic conditions, presenting an opportunity for use as pre-treatments (in place of septic tanks) and primary treatment for full wastewater flows. Two stage designs should be investigated to separate the stages of the anaerobic digestion process through creating preferential conditions along the pond length.

Keywords: wastewater; stabilization lagoons; hydrodynamics; biogas; psychrophilic treatment

1 Introduction

The traditional approach to designing anaerobic ponds (APs) is currently being challenged, as the opportunities for shorter hydraulic retention times (HRTs) (Peña et al., 2003), the use of baffling (Peña et al., 2003; Vega et al., 2003; Shilton and Harrison, 2003), and the covering of APs for biogas collection (DeGarie et al., 2000; Parissopoulos et al., 2003; Noyola et al., 2006; Shilton et al., 2008) are being realised. Temperature-dependent design organic loading rates were developed through empirical observation, ranging from 100 gBOD m^{-3} d^{-1} for ambient temperatures < 10 ^{o}C, to 350 gBOD m^{-3} d^{-1}

for temperatures >25 ^{o}C. The design loading rates were deliberately conservative, with the lower limit specified to ensure anaerobic conditions and the upper limit capped to minimise odour nuisance and the need for desludging (Mara and Pearson, 1998). In practice, even these conservative guidelines are rarely met, with odour nuisance cited as the most common reason for AP underloading (Pearson et al., 1996; Picot et al., 2005a; Archer and Mara, 2003; Alexiou and Mara, 2003). Covering of APs not only eliminates odour but reduces greenhouse gas emissions (Noyola et al., 2006), and the captured biogas can be used for energy generation thus providing an opportunity to reconsider appropriate loading rates based on the positive attributes of the technology rather than negating the negative ones (Hodgson and Paspaliaris, 1996; Park and Craggs, 2007). For instance, as the potential of APs for energy positive primary treatment has been recognised (McAdam et al., 2011), design focus is changing from primary sedimentation to more complete organic breakdown, with particular emphasis on identifying appropriate design geometry to maximise performance and reduce process scale (Vega et al., 2003; Agunwamba, 2006).

Currently, the costs associated with the extensive land requirements are the largest single barrier to uptake of APs (Xian-Wen, 1995; Agunwamba, 2001, Dias et al., 2018), with hydraulic retention times (HRTs) ranging from 1 and 4 days but most commonly between 2 and 3 days (Mara and Pearson, 1998). Reduction of land requirement, through shorter HRTs, improves the economic viability of APs whilst also offering process improvements. Higher organic loading rates provide more substrate for microbial growth, whilst the increased flow rates lead to greater mixing, reducing

hydraulic dead space in the pond and facilitating biomass/substrate contact (Peña et al., 2003). However, shorter HRTs increase the potential for biomass washout, which must be avoided in order to allow sufficient solids retention time (SRT) within the process for degradation. For instance, Craggs et al. (2008) suggested that the methane yield (and hence solids degradation) in low temperature APs could equal those of mesophilic ADs, provided solids retention time were doubled to compensate for the lower kinetic rate. Therefore, separation of SRT from HRT is vital, to ensure sufficient retention and degradation time for particulate carbon, whilst contact between the retained biomass and the liquid layer must also be facilitated to target soluble carbon fractions that are an essential step in methanogenesis (Lettinga et al., 2001; Lew et al., 2009).

The separation of HRT and SRT can be facilitated through the use of baffling. Incorporation of baffles into passive treatment systems has been found to improve hydrodynamic performance and increase mixing (Peña et al., 2003; Langenhoff and Stuckey, 2000). Horizontal baffles, which produce a lane system creating 'side to side' flow, reduce hydraulic short circuiting and therefore promote sedimentation and particulate retention (Muttamara and Puetpaiboon, 1997). In contrast, vertical baffles create 'up-and-under' flow, which provides greater biomass contact and has been demonstrated to separate the stages of anaerobic digestion along the reactor length. Consequently, in anaerobic baffled reactors (ABRs), acidogenesis has been observed in the compartments closest to the inlet and methanogenesis further down the reactor (Wang et al., 2020), increasing acidogenic and methanogenic activity by up to a factor of four (Barber and Stuckey, 1999). The incorporation of baffles into anaerobic reactors

has led to the development of high-rate anaerobic ponds with 0.5 day HRTs (Peña et al., 2003), and ABRs with typical HRT <1 day, and as low as 1 hour (Barber and Stuckey, 1999).

Recently, further understanding of high-rate upflow anaerobic sludge blanket reactors (UASBs) has identified benefit can be delivered through inclusion of an anaerobic pre-treatment stage, in order to decrease solids loading onto the UASB and provide a more acidified substrate (Elmitwalli et al., 1999; Van Haandel et al., 2006). This has led to the development of two-stage high-rate anaerobic reactors, where downstream UASBs have been preceeded by septic tanks (Luostarinen and Rintala, 2005), anaerobic filters (Sawajneh et al., 2010), and lower-rate UASBs (Sayed and Fergala, 1995; Halalsheh et al., 2005). Whilst it has been identified that, especially at low temperatures, two-stage anaerobic designs are essential for both maximising solids retention and degradation in the first stage, and providing preferential substrate to the second stage (Lettinga et al., 2001; Van Haandel et al., 2006), two-stage designs have not been applied to low-rate technologies to date. The context relates to remote/rural; communities that are commonly served by septic tanks and where a short HRT anaerobic pond could offer real advantages in terms of treatment and desuldging frequency. To illustrate, Scottish Water currently operate over 1,250 spetic tanks with 100 of these treating population equivalents of over 1,000 PE. The septic tanks operate at HRTs of around 0.5-1.0 days and require desludging every 6 months to 2 years which incurs significant costs and disruption to the local community. Two stage anaerobic ponds have been posited as a

future alternative but must be able to operated at short HRTs to be considered economically viable (Mason, 2018).

Accordingly, the current study reports on the operation of a pilot scale two stage anaerobic pond (SAP) over four HRTs, decreasing from 2.3 days to 0.5 days, to assess the potential for two-stage passive anaerobic treatment at higher loading rates than traditionally applied. The specific objectives of the study were:

1. Compare the performance of a staged AP to a single control AP over four HRT to determine differences in key indicators: hydrodynamic efficiency and flow characteristics; removal efficiency, specifically of carbon fractions; sludge accumulation and where it is retained; biogas production quantity and quality
2. Identify the effect of decreasing HRT on the APs for the above indicators, to determine optimal loading rates for APs at low temperature and its impact on AP operation for effluent quality, sludge management and energy generation

2 Materials and Methods

2.1 *Experimental reactor design*

The anaerobic reactors, designed as pilot-scale ponds, were constructed of 12 mm uPVC sheeting and sealed with PVC hot welding. The internal dimensions were 1.5 m x 0.5 m x 0.25 m, giving hydraulic volumes of 188 L. A 3:1 Length:Width ratio was used in accordance with recommended AP design (Mara and Pearson, 1998). The SAP was created by connecting two single stage ponds in series, with a horizontally baffled

anaerobic pond (HBAP) located upstream of a vertically baffled anaerobic pond (VBAP, designed with ABR principles but under AP loading conditions) with configurations previously reported (Cruddas et al., 2018). A control pond (CAP) was constructed with the same specifications as the HBAP. The reactors were initially seeded with 7 % by volume anaerobic sludge (volatile solids, VS = 36 g L^{-1}) from a previous study (Cruddas et al., 2014), filled with crude wastewater from the Cranfield University sewage treatment works and left in batch for one day. The reactors were operated for three months at each of four HRTs, with a 2.3 d HRT applied at start up, then subsequent HRTs of 1.5, 1.0, and 0.5 d.

2.2 *Analytical methods*

Influent and effluent were analysed three times a week in duplicate, whilst liquid samples were also collected and analysed once a month from side ports in each of the chambers created by the baffles (Figure 1). Ambient and liquid temperatures were recorded at the time of sampling using a digital probe thermometer, with a sensitivity of ±0.05 ^{o}C. Samples were analysed for BOD_5, COD, TSS and VSS according to standard methods (APHA, 1998). Samples for sCOD were filtered through a 1.2 µm glass fibre filter (Whatman, Maidstone, UK). Particulate COD fraction (pCOD) was calculated by subtracting sCOD from tCOD. Volatile fatty acids (VFA) and biogas volumes and composition were measured with previously described methods (Cruddas et al, 2018). Sludge depth was measured at the end of each loading rate on a grid of 0.1 m x 0.1 m using a perspex tube graduated at 1 mm intervals. ANOVA tests were performed on all data sets to determine statistical significance to 95 % confidence. Tracer studies and

specific methanogenic activity (SMA) tests were carried out according to previously described methods (Cruddas et al, 2018)

2.3 *Microbial community analysis*

Sludge samples from different chambers of the reactors were taken at the end of the trial. Biomass (10 g) was collected in sterile tubes and stored at -20°C for their processing. A fully automated nucleic acid extractor employing magnetic bead technology (Maxwell 16 DNA Purification Kits; Promega) was used to extract and purify genomic DNA from the samples following the manufacturer´s instructions. The DNA was stored at -20°C.

Quantitative real-time polymerase chain reaction (qPCR) was used to quantify 16S rRNA gene of three methanogenic orders, *Methanomicrobiales*, *Methanobacteriales* and *Methanosarcinales*, and two families of the *Methanosarcinales* order, *Methanosaetaceae* and *Methanosarcinaceae*. All qPCR reactions were performed using 20 µl reaction capillary tubes with the LightCycler 480 Probe Master (Roche Diagnostics). The Archaeal 16S rRNA gene from each sample was amplified using the primers Arch109F (5'-ACKGCTCAGTAACACGT-3') (6-FAM fluorescent labelled) (Luna *et al*., 2009) and Arch958R (5'-AGGAATTGGCGGGGGAGCAC-3') (Ferris *et al.*, 1996). Full details of the experimental procedure are described in Supporting Information S1.

2.4 *CFD modelling*

Three dimensional single phase CFD simulations were performed using the commercial software FLUENT v14.0.0 (ANSYS) using geometries and methods previously reported (Cruddas et al, 2018).

3 Results

3.1 *Hydrodynamic comparison using experimental tracer studies and computational fluid dynamics*

In both the experimental tracer studies and CFD simulations, lower dead space volumes were found in the SAP compared to the CAP at all HRTs. The differences between AP configurations became more pronounced with each step decrease in HRT (Table 1), indicating greater utilisation of reactor volume in the SAP, especially at shorter HRT. Overall, the CFD trends suggest the general flow characteristics of the SAP tending towards plug flow to a greater extent than the CAP. This is evidenced by lower dispersion numbers and higher N (tanks in series) values, with the differences between the reactors increasing with decreasing HRT. Comparison between experimental and CFD profiles revealed differences with respect to lower dead space volumes and lower S (short-circuiting) quotients for the CAP at short HRTs. The differences are attributed to the interaction between the tracer and the retained sludge in the experimental measurements.

The enhanced hydrodynamic profiles observed in the SAP are attributed to the baffles generating a greater degree of recirculation. As such, flow is forced back into the chamber by the small aperture created by the baffles, consequently utilising more of the chamber and thus reducing short circuiting (Figure 2). Recirculation is most pronounced in the front chamber of the reactors, where velocities are highest due to the inlet jetting effect. Velocities decrease through subsequent chambers, reducing recirculation and creating preferential flow patterns, most evident in the CAP. In the first stage of the SAP, velocities in the second chamber are high enough to cause noticeable recirculation (Figure 2), improving the mixing profile and reducing dead space whilst also creating an overall plug flow effect through the reactor.

3.2 *Removal efficiencies over four HRTs from the staged and control anaerobic ponds*

No significant difference between reactors was observed in relation to TSS or pCOD removal over all HRTs to a 95 % confidence level. In comparison, TSS removal rates were found to increase in both ponds with decreasing HRT from 2.3 to 1.0 days. However, at a HRT of 0.5 days, the TSS removal increased in the SAP but reduced in the CAP. This suggests both reactors were operating beneath their maximum solids loading limits until 1.0 d HRT, and the SAP could prevent solids washout even at the highest loading applied. In contrast, mean sCOD removals were statistically different, and were lowest at 1.5 d HRT in both reactors at -40 % and -44 % for the CAP and SAP, respectively, with the highest removal observed at 0.5 d HRT, CAP -5 % and SAP 2 % (Figure 3).

However, these removal efficiencies correlate with the temperature profile in the reactors, with the highest mean effluent temperatures recorded at 1.5 d HRT (CAP 17.1 ^{o}C; SAP 17.0 ^{o}C) and the coldest temperatures observed during the 0.5 d HRT period (CAP 9.3 ^{o}C; SAP 9.1 ^{o}C). Removal efficiencies of VFA were similar to the sCOD trend, with the largest addition of VFA to the effluent occurring at 1.5 d HRT whilst removal efficiency increased in the shortest HRT period (Figure 3). The negative removal, or generation of sCOD and VFA, suggests particulate COD breakdown to soluble components which are then not degraded further before leaving the reactors.

3.3 *Sludge accumulation*

Solids accumulation rate within the reactors was found to be more dependent on temperature than loading. In both reactors, per capita normalised sludge accumulation rates were comparable at three of the HRTs studied. In the CAP, accumulation rates over the 2.3, 1.0 and 0.5 d HRT periods were 0.04 m^3 PE^{-1} y^{-1} (mean effluent temperature, T_{eff} = 10.5 ^{o}C), 0.04 m^3 PE^{-1} y^{-1} (T_{eff} = 13.9 ^{o}C) and 0.06 m^3 PE^{-1} y^{-1} (T_{eff} = 9.3 ^{o}C), respectively. In comparison, accumulation rates in the SAP were 0.06 m^3 PE^{-1} y^{-1} (T_{eff} = 10.5 ^{o}C), 0.04 m^3 PE^{-1} y^{-1} (T_{eff} = 13.7 ^{o}C) and 0.06 m^3 PE^{-1} y^{-1} (T_{eff} = 9.1 ^{o}C) calculated for the same periods, respectively. However, during the warmest HRT period, 1.5 d, a reduction in total sludge volume was recorded in both reactors, with an accumulation rate of -0.02 m^3 PE^{-1} y^{-1} for both reactors (CAP T_{eff} = 17.1 ^{o}C, SAP T_{eff} = 17.0 ^{o}C). Whilst the normalised accumulation rates were comparable across the decreasing HRT periods at low temperature, the higher loadings applied relate to higher absolute sludge volumes within the reactors. To illustrate, in the SAP the accumulation rate of 0.06 m^3 PE^{-1} y^{-1}

during the 2.3 d HRT period related to an accumulated sludge volume of 16.11 L, or 3 % of total pond volume, whilst the 0.04 m^3 PE^{-1} y^{-1} accumulation rate over the 0.5 d HRT period related to an accumulated sludge volume of 73.11 L, or 14 % of total reactor volume. Solids were mostly deposited in the front chamber of each reactor (Figure 4), with 63, 49, 30 and 73 % of total CAP sludge volume found in this chamber after the 2.3, 1.5, 1.0 and 0.5 d HRT periods, respectively, whilst this chamber comprised only 33 % of total reactor volume. In the SAP, sludge accumulation in the front chamber contained 39, 28, 37, and 43 % of total sludge volume, despite this chamber comprising only 17 % of total reactor volume.

3.4 *Biogas methane production and specific methanogenic activity of sludge*

Rapid start up of methane biogas production was observed in both reactors, with mean flow normalised production of 3.86 LCH_4 m^{-3} wastewater treated (WWT) in the CAP and 5.40 LCH_4 m^{-3}WWT in the SAP during the first operational period, at 2.3 d HRT. The highest mean biogas production occurred during the second period, 1.5 d HRT, with 5.40 LCH_4 m^{-3}WWT in the CAP and 8.82 LCH_4 m^{-3}WWT in the SAP, which coincided with the highest mean effluent temperatures (Figure 5). The volumetric biogas production rates at this HRT were 3.6 L $CH_4/m^3/d$ for the CAP and 5.9 L $CH_4/m^3/d$ for the SAP.

With decreases in both temperature and HRT, large reductions in biogas production were observed for the final two operational periods, with mean production rates of 0.05 LCH_4 m^{-3}WWT and 0.11 LCH_4 m^{-3}WWT in the CAP and 0.74 LCH_4 m^{-3}WWT and 0.08 LCH_4 m^{-3}WWT in the SAP for the 1.0 and 0.5 d HRT periods, respectively. No statistical

difference was observed in biogas production between the two reactors at 2.3 d HRT, nor at 0.5 HRT due to low production rates in both reactors. However, at 1.5 and 1.0 d HRT, biogas production in the SAP was significantly higher than the CAP to a 95 % confidence level.

The highest measured production rate was in the chamber closest to the inlet for both reactors at all four loading rates (Figure 6), with 95 and 84 % of total biogas CH_4 recorded in this chamber for the CAP and SAP, respectively. In the CAP, production rates decreased in subsequent chambers at 2.5 and 1.5 d HRT, although at 1.0 d an increase was evident in the final chamber, suggesting production at the outlet may have been increasing respective to the centre of the reactor. Due to the low temperature during the 0.5 d HRT, no biogas was recorded in either chamber 2 or 3 for this final loading rate. In the SAP, biogas production decreased throughout the first stage reactor, but increased in first chamber of the second phase at 2.3, 1.5 and 1.0 d HRTs. To illustrate, mean biogas methane production rates at the outlet of the first stage were 0.08, 0.03 and 0.06 LCH_4 m^{-3} WWT *cf.* 0.26, 1.05 and 0.17 LCH_4 m^{-3}WWT at the inlet of the second stage at 2.3, 1.5, and 1.0 d HRT, respectively. This may be induced by both the jetting effect of the connection pipe between the two stages creating high mixing at the inlet of the second stage, and through a change in microbial community found in the reactors. Specific methanogenic activity tests conducted on sludge at the end of the study period show activity rates were lower at the inlet of both reactors than the subsequent chambers (Figure 6). Hydrogenotrophic methanogenic activity was found to be over two orders of magnitude greater than aceticlastic activity, with mean hydrogen specific SMA

of 1,001 $mgCH_4$ $gVSS^{-1}$ d^{-1} and 1,489 $mgCH_4$ $gVSS^{-1}$ d^{-1} recorded in the CAP and SAP, respectively, *cf*. 0.27 $mgCH_4$ $gVSS^{-1}$ d^{-1} and 1.36 $mgCH_4$ $gVSS^{-1}$ d^{-1} for acetate specific SMA. Interestingly, acetate specific SMA was over two orders of magnitude higher in the second phase of the SAP than the first, with mean acetate specific SMA of 0.01 $mgCH_4$ $gVSS^{-1}$ d^{-1} in the first stage *cf.* 2.71 $mgCH_4$ $gVSS^{-1}$ d^{-1} in the second stage.

3.5 *Microbial community profiling of methanogenic orders and families in the sludge*

Microbial community profiling of methanogenic archea in sludge taken at the end of the study found the hydrogenotrophic order *Methanomicrobiales* dominant, producing a mean of $1.87x10^7$ copies from the qPCR process *cf.* $1.26x10^6$ copies of the aceticlastic *Methanosarcinales* order in the CAP, and $2.51x10^7$ copies *cf.* $4.53x10^6$ copies in the SAP (Figure 7). In addition, another hydrogenotrophic order, *Methanobacteriales*, was also present with mean $3.25x10^5$ copies in the CAP and $2.44x10^5$ copies in the SAP, increasing the dominance of hydrogen utilisers. The relative presence of these orders supports the SMA findings of hydrogen pathways dominating the anaerobic digestion process in both reactors. In the SAP, an increase in the *Methanosarcinales* order from mean $1.27x10^6$ copies in the first stage to $6.48x10^6$ copies in the second stage, also reflects the increase in acetate specific SMA found the in the second stage at the end of the study. Within the *Methanosarcinales* order, the *Methanosaetaceae* family was found to dominate the *Methanosarcinaceae* family in both reactors, with mean copy numbers $2.61x10^6$ *Methanosaetaceae cf.* $3.86x10^4$ *Methanosarcinaceae* in the CAP and $6.53x10^6$ *Methanosaetaceae cf.* $9.37x10^4$ *Methanosarcinaceae* in SAP (Figure 7). Interestingly, in the SAP the Methanosarcinales families were found in closest relative abundance in the

first chamber, with $1.15x10^6$ copies of *Methanosaetaceae cf.* $4.85x10^5$ *Methanosarcinaceae*, with Methanosaetaceae dominating further along the reactor, particularly in the second stage, with mean $9.28x10^6$ copies of *Methanosaetaceae cf.* $3.26x10^4$ *Methanosarcinaceae*. The dominance of Methanosarcinaceae within the Methansarcinales order has been found to be consistent with low acetate concentrations and indicative of increased acetate oxidation, leading to hydrogenotrophic methanogenesis, rather than aceticlastic pathways (Karakashev et al., 2006).

4 Discussion

Comparison of the proposed staged AP (SAP) to a conventional design (CAP) revealed the potential of SAPs to enhance both biogas production and overall hydrodynamic efficiency. The latter was seen in terms of less short circuiting with associated less dead space. The enhancements were observed across all HRTs with velocity profiles demonstrating the increased recirculation between baffles (Peña et al., 2003) leading to greater utilisation of the reactor volume. Solids accumulation reduced the clarity of the impacts congruent with previous studies on unbaffled ponds (Peña et al., 2000; Alvarado et al., 2012). Further, vertically baffled systems similar to the VBAP, such as ABRs, can be particularly susceptible to channelling (Grobicki and Stuckey, 1992), as the flow is forced through the sludge layer at every 'hanging' baffle, thus optimisation of the baffling arrangement will be critical and as such is one of the key areas for further investigation. The differences in hydrodynamics did not manifest in terms of bulk removal which remained statistically similar for both reactors. This extended to soluble

COD removal where improvements in removal were not observed to a statistically significant level. Improvements in gas production, however, were observed in the SAP, with increased SMA and an aceticlastic methanogenic community measured in the second stage suggesting the spatial distribution of anaerobic digestion was starting to occur (Barber and Stuckey, 1999; Paing et al., 2000). Furthermore, many of the advantages seen in the SAP over the CAP were more pronounced at the shorter HRTs indicating that the proposed design can provide a route to using APs with smaller footprints more attractive to potential adopters.

Results from the reductions in HRT suggest APs can tolerate higher loadings than currently applied. Decreasing HRT in unbaffled ponds is known to increase short circuiting, however the results of the CFD simulations suggest that these impacts can be lessened in a baffled system, and advantages can also be gained in reducing hydraulic dead space through the recirculation effect between baffles. As temperature profiles changed over the course of the study, the influence of temperature must be considered when comparing the HRTs applied. The removal of solids has been reported to be independent of operating temperature (Picot et al., 2003; Papadopoulos et al., 2003), and in this study a clearer relationship was found with loading rate. Consistent effluent TSS profiles down to 1.0 d HRT in both reactors reinforced the ability of the APs to handle shock loadings, whilst also confirming that solids loading rates are unlikely to be a restricting factor in AP design (Cruddas et al., 2014). However, biological activity was clearly strongly associated with temperature (Toprak, 1995; Picot et al., 2003; Parissopoulos et al., 2003). Soluble carbon removal efficiency was observed to be more

strongly linked with temperature than loading rate during the study, and may be attributed to a reduction in soluble carbon generated in the digestion process (Cruddas et al., 2014) rather than improvement of soluble degradation due to biological establishment (Paing et al., 2000; Picot et al., 2003). Sludge accumulation rates were also temperature dependent, with volume reduction occurring above 17 ^{o}C as suggested by Papadopoulos et al. (2003). The sludge reduction at warmer temperatures was linked to the highest biogas production rates, and supports previous evidence that APs can store particulate carbon in winter periods to be subsequently degraded in summer (Safley Jr. and Westerman, 1989; Papadopoulos et al., 2003; Picot et al., 2003). Therefore, in order to accurately estimate the effect of HRT on sludge accumulation and biogas production, studies must be conducted over an annual cycle. Furthermore, sludge accumulation rates have been found to lower, and biogas production rates increase, with extended AP operation (Paing et al., 2000; Picot et al., 2005b), and the minimum temperature at which methanogenesis occurs has been found to decrease with AP age as biomass acclimatises (Heubeck and Craggs, 2010). Therefore, it can be posited that these characteristics would improve from the current study over time.

Shorter HRTs can mitigate the largest single problem with AP uptake in reducing the land requirement, and therefore the cost (Agunwamba, 2001; Alexiou and Mara, 2003), and the results from this study suggest that shorter HRTs than currently recommended are feasible. The severe reduction in gas production at 0.5 d HRT is likely to be a cause of the temperature but also the loading, and whilst the sludge accumulation rate per capita was comparable to longer HRTs, the volume of sludge produced at this HRT would likely

reduce the advantages APs can bring in reduced sludge handling (Cruddas et al., 2014). To illustrate, whilst sludge accumulation rates in the SAP were 0.06 m^3 PE^{-1} y^{-1} at both 2.3 and 0.5 d HRT, desludging at 50 % volume would lead to a desludge frequency of 3.8 years at 2.3 d HRT, but 0.4 years at 0.5 d HRT. Therefore, extended trials of APs at 1.0 and 1.5 d HRTs are recommended, which would reduce AP volume by two to three times the current recommendations.

5 Conclusions

The work presented has demonstrated efficacy of anaerobic ponds, even at low temperatures, to operate at shorter HRTs than commonly considered. Further, an HRT of 1 day is seen as a conservative estimate to base future development around. The advantages in hydraulic performance of using a two-stage design was observed predominately at these shorter HRTs and indicates that appropriate baffling arrangement will be critical in future designs. Importantly, management of the degradation of both solids and soluble organics appears to be a temperature issue rather than a hydraulic one. Accordingly, future development needs to consider the use of heat sources, including the utilisation of any produced gas in maintaining higher temperatures. Whilst heating of full wastewater flows would not be practical, targeted heating of the sludge layer could be considered, to extend periods where the temperatures can be around 17^{o}C, where a net overall reduction in solids was observed. The presented results show promise but were based on relatively short-term experiments which will underestimate performance compared to a fully acclimatised

system. As such the findings support the idea of development of an alternative to septic tanks that can offer improved treatment and reduced desludging frequencies without requiring excessive land use.

Acknowledgements

This work was supported by the Engineering & Physical Sciences Research Council through an industrial CASE studentship. Gavin Collins and Estefania Porca were supported by a European Research Council Starting Grant (3C-BIOTECH 261330)

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Table 1 Hydrodynamic data calculated for a control anaerobic pond (CAP) and staged anaerobic pond (SAP), over four hydraulic loading rates. Data is shown for experimental data collected from tracer studies and for computational fluid dynamics (CFD) simulations

	Experimental data											
	2.3 d HRT			1.5 d HRT			1.0 d HRT			0.5 d HRT		
	CAP	SAP	Diff	CAP	SAP	Diff	CAP	SAP	Diff	CAP	SAP	Diff
HRT_a (d)	1.85	2.07	0.22	1.03	1.33	0.30	0.72	0.97	0.25	0.46	0.80	0.34
HRT_a/HRT_t (%)	80	90	10	69	89	20	72	97	25	92	160	68
Short circuiting quotient, S	0.40	0.47	0.07	0.25	0.36	0.11	0.16	0.34	0.18	0.46	0.79	0.33
Dead space volume (%)	20	10	-10	31	11	-20	28	3	-25	8	-60	-68
Variance, σ^2 ($days^2$)	0.90	0.76	-0.14	0.68	0.90	0.22	0.33	0.34	0.01	0.25	0.13	-0.12
Dispersion number, δ	0.16	0.10	-0.06	0.32	0.34	0.02	0.63	0.24	-0.39	0.19	0.12	-0.07
Tanks in series, N	5.96	7.06	1.10	4.82	2.76	-2.06	3.05	2.96	-0.09	3.93	1.89	-2.04
Tracer recovered (%)	100	100	0	48	55	7	35	52	17	40	88	48
Sludge volume (% of reactor)	13	14	1	11	12	1	18	19	1	46	38	-8
	CFD simulations											
	2.3 d HRT			1.5 d HRT			1.0 d HRT			0.5 d HRT		
	CAP	SAP	Diff	CAP	SAP	Diff	CAP	SAP	Diff	CAP	SAP	Diff
HRT_a (d)	1.33	1.48	0.15	0.89	0.98	0.09	0.63	0.69	0.06	0.34	0.36	0.02
HRT_a/HRT_t (%)	57	64	7	59	66	7	63	69	6	68	72	4
Short circuiting quotient, S	0.29	0.36	0.07	0.39	0.36	-0.03	0.41	0.38	-0.03	0.63	0.38	-0.25
Dead space volume (%)	43	36	-7	41	34	-7	37	31	-6	32	28	-4
Variance, σ^2 ($days^2$)	0.62	0.68	0.06	0.48	0.47	-0.01	0.37	0.36	-0.01	0.21	0.18	-0.03
Dispersion number, δ	0.13	0.12	-0.01	0.18	0.13	-0.05	0.23	0.17	-0.06	0.26	0.15	-0.11
Tanks in series, N	13.76	11.43	-2.33	9.77	10.03	0.26	7.31	7.46	0.15	5.67	7.75	2.08
Tracer recovered (%)	91	94	3	96	98	2	98	100	2	89	66	-23
Maximum velocity v_{max} ($m\ s^{-1}$)	$1.47x10^{-2}$	$1.04x10^{-1}$	$8.90x10^{-2}$	$2.39x10^{-2}$	$1.55x10^{-1}$	$1.3x10^{-1}$	$3.76x10^{-2}$	$2.27x10^{-1}$	$1.9x10^{-1}$	$7.51x10^{-2}$	$4.00x10^{-1}$	$3.2x10^{-1}$
Minimum velocity v_{min} ($m\ s^{-1}$)	$1.21x10^{-9}$	$7.22x10^{-8}$	$7.10x10^{-8}$	$7.22x10^{-8}$	$5.40x10^{-8}$	$-1.82x10^{-8}$	$1.74x10^{-9}$	$1.02x10^{-7}$	$1.00x10^{-7}$	$3.67x10^{-9}$	$1.71x10^{-5}$	$1.71x10^{-5}$

CAP –Control anaerobic pond; SAP – Staged anaerobic pond; Diff – Difference between CAP and SAP; HRT – Hydraulic retention time; HRT_a – actual (measured) HRT; HRT_t – theoretical HR

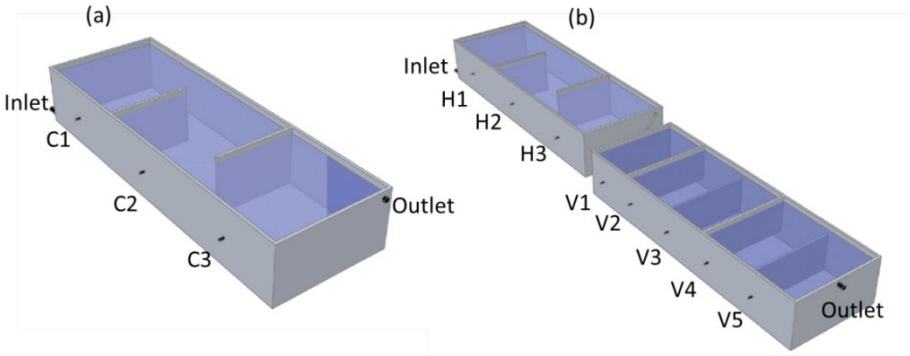

Figure 1 Layouts of the ponds used in the study, indicating sampling points at the inlets and outlets, and in each baffle chamber in the (a) control anaerobic pond and (b) staged anaerobic pond, with horizontally (H) baffled stage followed by vertically (V) baffled stage

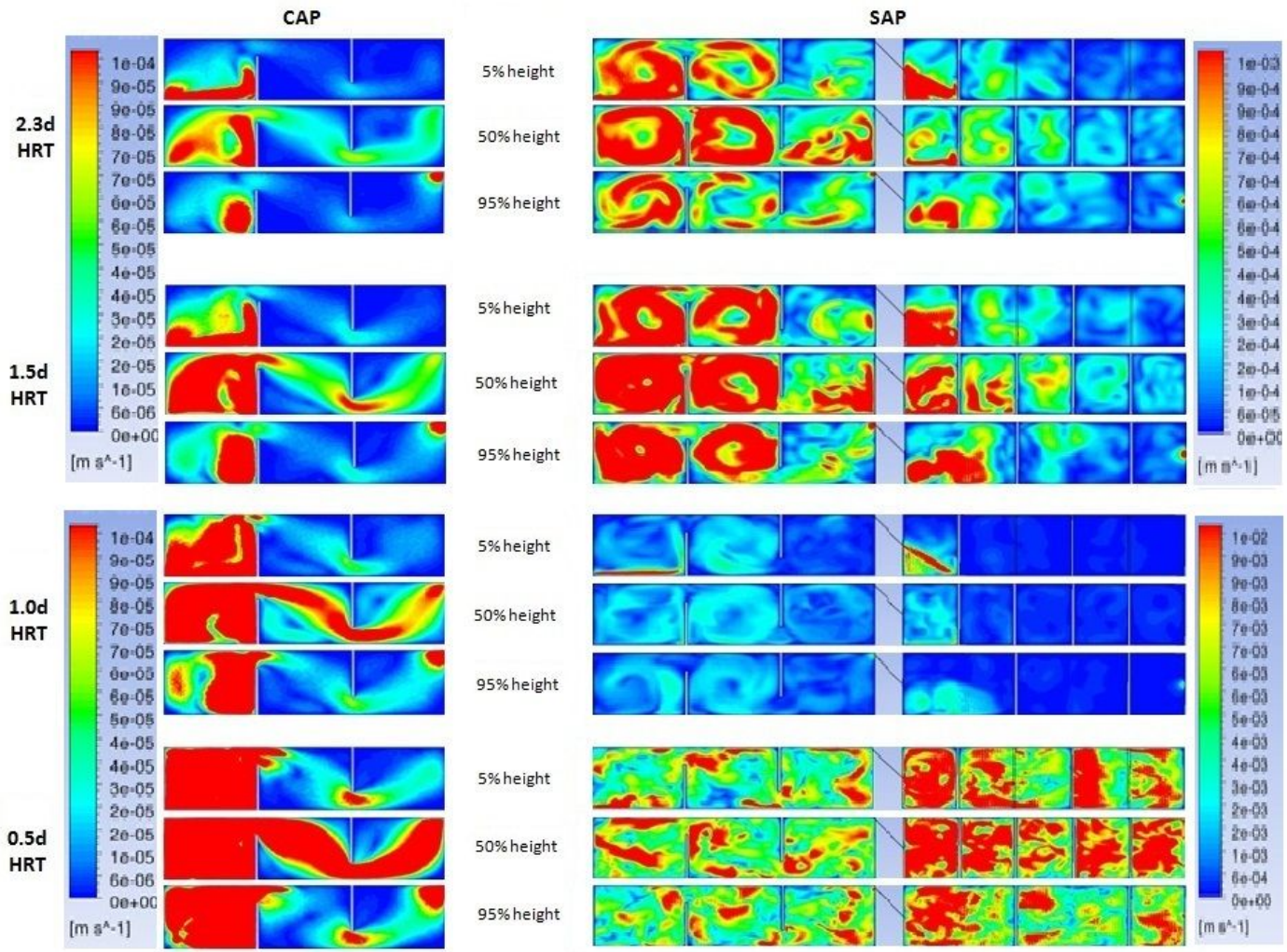

Figure 2 CFD generated velocity profiles for the control anaerobic pond (CAP), and the staged anaerobic pond (SAP)

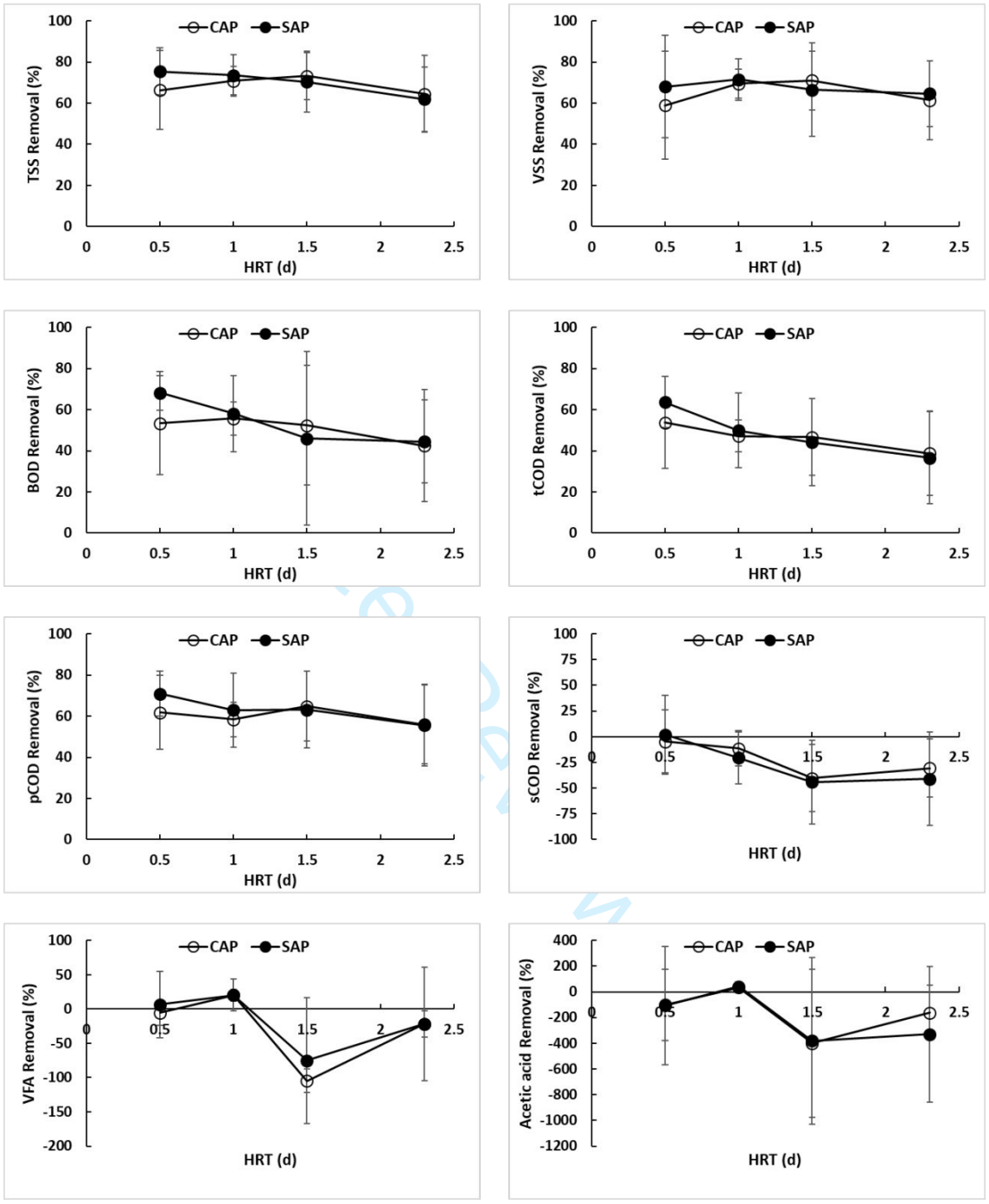

Figure 3 Removal efficiencies from the pilot scale trials on a horizontally baffled anaerobic pond as a control (CAP) and a staged anaerobic pond (SAP). Efficiencies shown for Total and Volatile Suspended Solids, total, particulate (>1.2µm) and soluble (<1.2µm) COD, volatile fatty acids, and acetic acid individually from the compound VFA measurement.

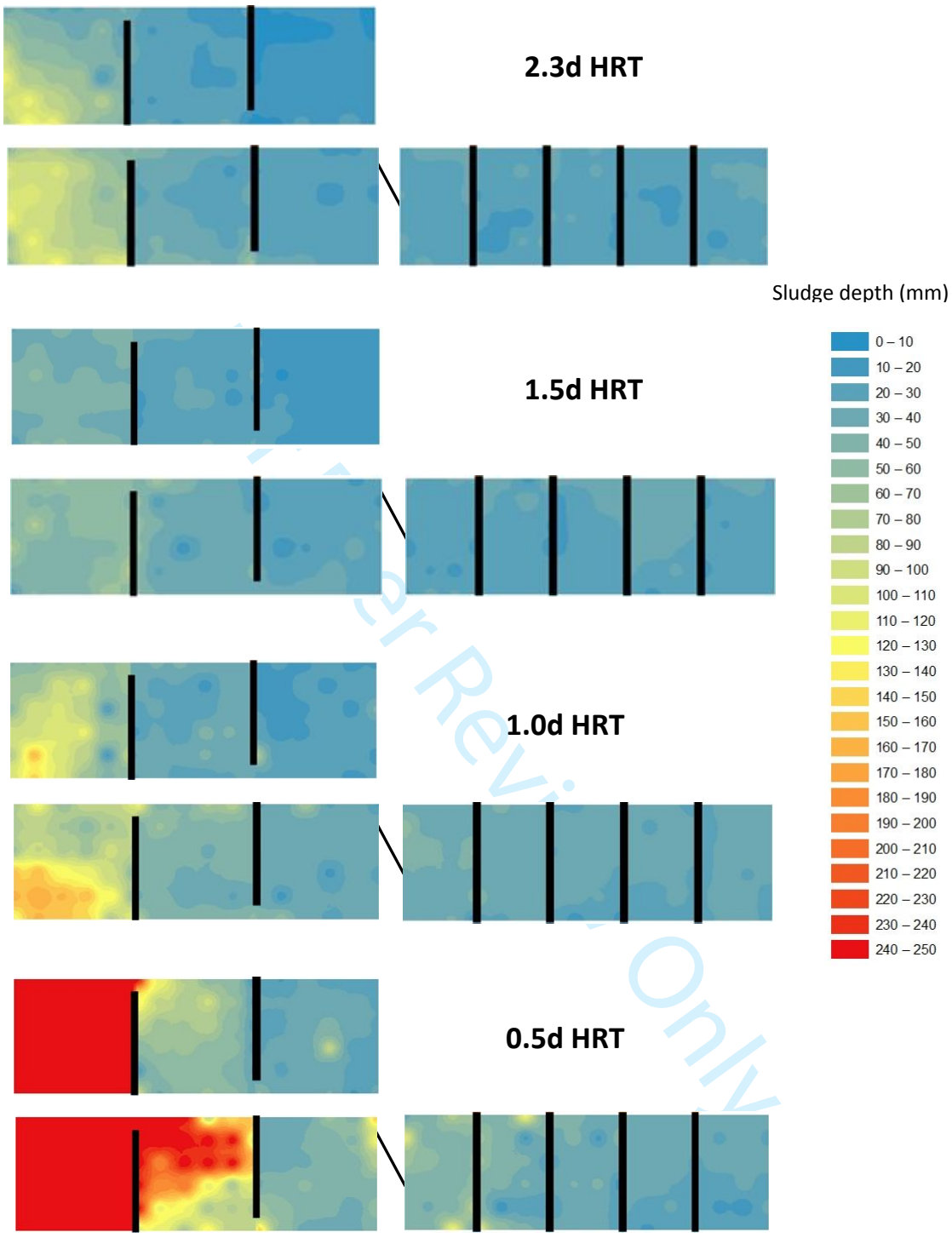

Figure 4 Sludge accumulation maps in the control anaerobic pond (CAP) and staged anaerobic pond (SAP) at the end of each of the four hydraulic retention times applied

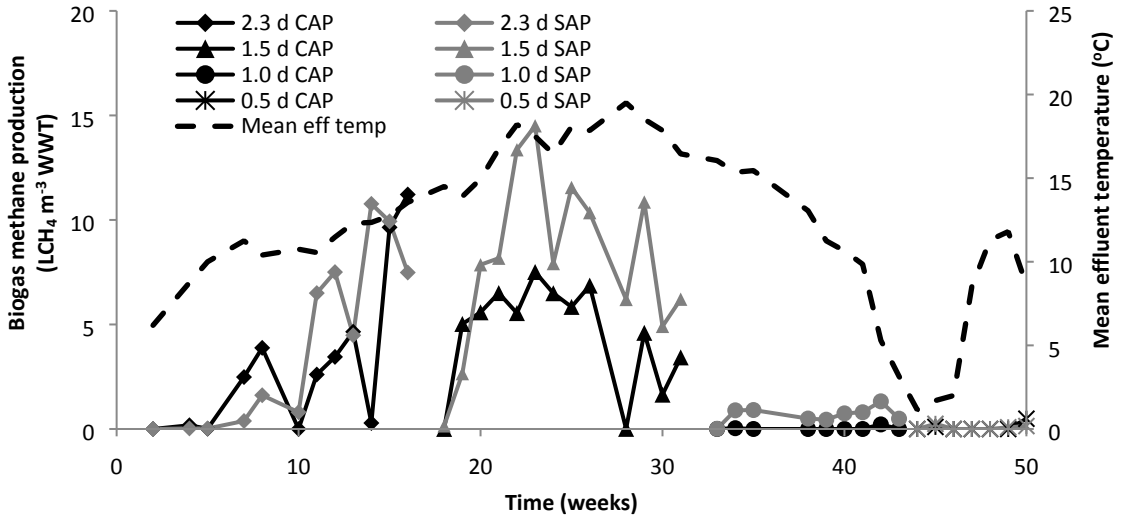

Figure 5 Mean flow-normalised biogas methane production in the control (CAP) and staged (SAP) anaerobic ponds.

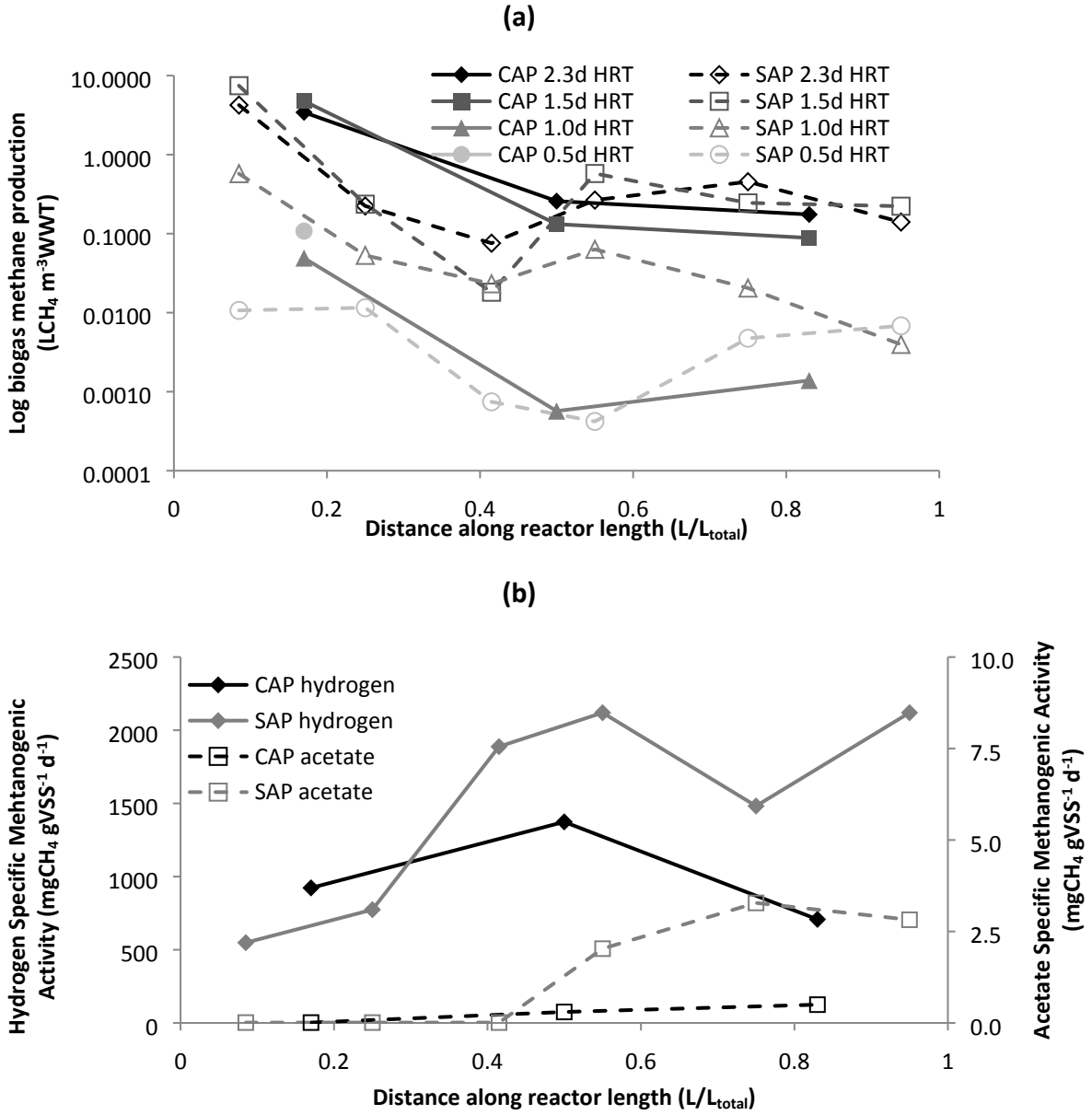

Figure 6 Mean flow-normalised biogas methane production (a), and specific methanogenic activity (b), from sludge samples along the length of the control anaerobic pond (CAP) and staged anaerobic pond (SAP) at the end of the study

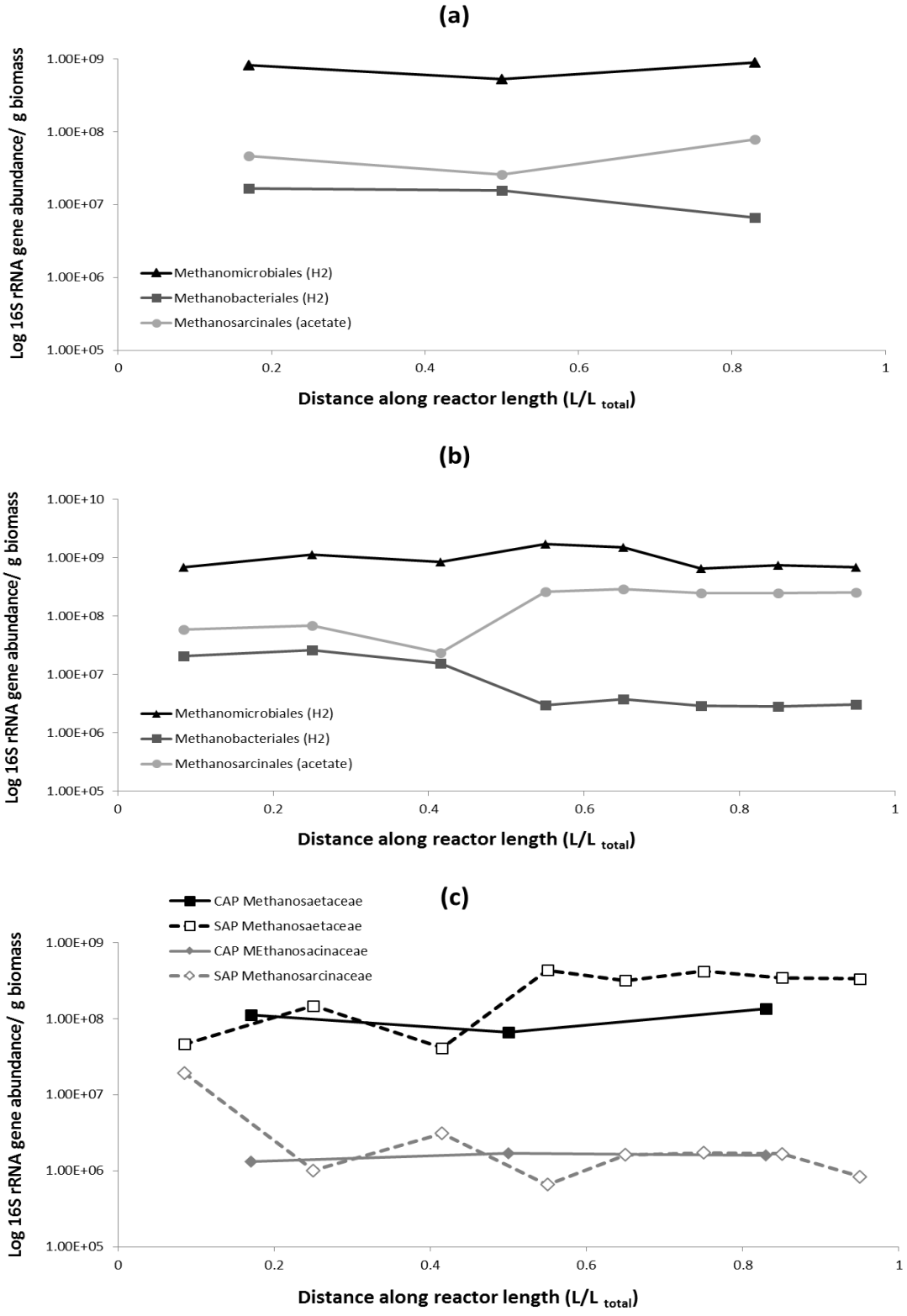

Figure 7 Microbial community qPCR data for three orders of methanogenic *archaea*, two hydrogenotrophic and one acetoclastic, in the (a) control anaerobic pond (CAP) and (b) staged anaerobic pond (SAP), and (c) two families of the *Methanosarcinales* order in the CAP and SAP.

The impact of hydraulic retention time on the performance of two configurations of anaerobic pond for municipal sewage treatment

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Supplementary Information S1 – Detailed Materials and Methods

Microbial community analysis

Sample collection

Samples from different zones of the ponds were taken at the end of the trial. Biomass (10 g), was collected in sterile tubes. All samples were stored at -20°C for their processing.

Genomic DNA extraction

A fully automated nucleic acid extractor employing magnetic bead technology (Maxwell 16 DNA Purification Kits; Promega) was used to extract and purify genomic DNA from the samples following the manufacturer´s instructions. The DNA was stored at -20°C.

Quantification of the 16S rRNA gene

Quantitative real-time polymerase chain reaction (qPCR) was used to quantify 16S rRNA gene of three methanogenic orders, *Methanomicrobiales*, *Methanobacteriales* and *Methanosarcinales*, and two families of the *Methanosarcinales* order, *Methanosaetaceae* and *Methanosarcinaceae*.

All qPCR reactions were performed using 20 µl reaction capillary tubes with the LightCycler 480 Probe Master (Roche Diagnostics). Each capillary tube was separately loaded with 5 µl of DNA dilution 1:10, followed by addition of: 10 µl of Taqman Mastermix (Roche Diagnostics); 1 µl of forward and reverse primers (10 µM); 1 µl of the TaqMan probe corresponding to each primer and probe set (10 µM); and PCR-grade sterile water to a final volume of 20 µl. A control without the corresponding DNA template (NTC) was included in every assay for each primer and probe set. All experiments were done in duplicate.

Two step amplification of the target DNA was performed applying the following conditions: an initial incubation at 94°C for 10 min, 45 cycles of denaturation at 94°C for 10 s and simultaneous annealing and extension at 60°C for 30 s, while the annealing and extension for the *Methanomicrobiales*-specific set was performed at 63°C. The transition rate was 20°C/s for all segments in the two-step cycling.

The standard curves were performed following Yu *et al.* (2006) method.

Microbial community fingerprinting: TRFLPs

The Archaeal 16S rRNA gene from each sample was amplified using the primers Arch109F (5'-ACKGCTCAGTAACACGT-3') (6-FAM fluorescent labelled) (Luna *et al*., 2009) and Arch958R (5'-AGGAATTGGCGGGGGAGCAC-3') (Ferris *et al.*, 1996). Each reaction mixture contained: 5 µl 10X reaction buffer, 2 µl MgCl2 (50 mM), 2 µl dNTPS (100 mM), 2 µl of each primer (10 µM), 0.2 µl of Taq DNA polymerase (Invitrogen), 2 µl of the DNA template and nuclease free water up to a final volume of 50 µl.

The DNA amplification was performed in a thermocycler with the following profile: initial denaturation at 95°C for 5 min; 33 cycles of denaturation (at 95°C for 30 sec), annealing (at 54°C for 45 sec) and elongation (at 72°C for 1 minute) and a final elongation at 72°C for 7 minutes. The success and yield of each amplification reaction were determined by electrophoresis in a 2% (w/v) agarose gel in 1X TAE buffer.

In order to decide the enzyme that would provide the best results for the samples, some *in silico* analysis were done using the NEBcutter software (http://tools.neb.com/NEBcutter2) trying different restriction enzymes. *Alu*I provided the best discriminating profiles and was used for further digestions. The reaction mixtures contained: 2 µl of 10X restriction enzyme buffer, 5 µl of the PCR rpoduct, 1 µl of *Alu*I (Invitrogen) and water nuclease free to a final volume of 20 µl. Labelled PCR products were digested at 37°C for 3 hours and the reactions were inactivated by incubation at 65°C for 20 min. After that, the samples were prepared following the company instructions and aliquots of 5 µl of each were sent to BioSciences (Dublin) for the analysis.

TRFLP profiles were aligned using T-Align program (Smith *et al.*, 2005) and multidimensional scaling (MDS) plots were constructed using Primer6 (Clarke & Warwick, 2001) software. MDS plots were based on relative abundance of the individual TRFs following square root transformation of data.

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