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Organic Carbon Removal from Wastewater by a PHA Storing Biofilm using Direct Atmospheric Air Contact as Oxygen Supply

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Abstract:

The principal reason for the high energy costs for biological wastewater treatment is the poor transfer efficiency of oxygen to the bulk water phase. The current paper describes a biofilm reactor in which oxygen transfer to the bulk solution is avoided by alternating anaerobic submersed (2h) and drained (1 h) operation of the biofilm. During the submersed phase the biofilm enriched for glycogen accumulating organism (GAO) stored the organic carbon (acetate) as poly-hydroxy-alkanoate (PHA). After draining the reactor, this carbon stored as PHA was biologically oxidised, using oxygen directly from the atmosphere. The 12 Cmmol/L (384 mg/L BOD) of acetate was completely removed during long term automated operation of the reactor for 9 months with a cycle length of 3.3 hours. As the process specifically removes dissolved organic carbon but not N or P it could possibly be coupled with novel processes such as Anammox or nutrient recovery.

KEYWORDS: Aeration reduction, Organic removal, Biofilm, PHA/PHB, Wastewater, Glycogen accumulating bacteria.

1. Introduction

Next to the removal of organic carbon (biological oxygen demand, BOD), biological wastewater treatment aims at removing nutrients: nitrogen (N), phosphate (PO_4^{3-}). Most recent research has focussed on the effective removal of nutrients as they are responsible for eutrophication. However, the biological aerobic removal of the BOD from wastewater is the main energy cost to the treatment plant operator (Young & Koopman, 1991). Significant energy is required for the supply of poorly soluble oxygen from the atmosphere into the bulk solution. For example the removal of 10 Cmmol/L dissolved organic pollution (320 mg/L BOD) requires 0.245 L of dissolved oxygen (DO) available to 1 L of bacterial suspension. Assuming a practical oxygen transfer efficiency of 10 % and an air oxygen content of 21 %, a treatment plant would provide about 50 times this volume of air (about 11.7 L of air) per L of wastewater. As this air has to be provided under sufficient pressure to lift a water column of typically 5 m height, it is understandable that despite advances in air supply technologies, the energy cost for air supply is high (570 $J/L_{wastewater}$).

The oxygen supply to the biomass (activated sludge) also initiates the oxidation of ammonia to nitrite or nitrate, which represents an additional oxygen demand (Marcos, 2007). A wastewater with a typical C:N ratio content of 6 g-C/g-N (Sheng-Peng et al., 2010) approximately requires an additional 30 % of oxygen for the oxidation of NH_4^+ to NO_2^-. While this component of air supply may seem unavoidable in the case of traditional N removal by nitrification and denitrification, there are current trends suggesting alternatives for N removal. These include the anaerobic ammonia oxidation (Anammox (Kuenen, 2008)) and other forms of nitrogen recovery, for example

completely autotrophic nitrification over nitrite (CANON) (Schmidt et al., 2003; Third et al., 2001).

In principle, bacteria tend to store organic compounds (poly-hydroxy-alkanoates, PHA) if there are limiting growth factors, which prevent organisms to use the BOD as a source for biomass growth and energy (Lenz & Marchessault, 2005). In wastewater, where inorganic nutrients are typically available, the key mechanism that provokes bacteria to store BOD, as PHA, is the short term depletion of oxygen (Mino et al., 1998; Smolders et al., 1995). In the literature, conditions of alternating oxygen supply have been demonstrated to encourage activated sludge biomass to gradually build up increasing reservoirs of reducing power in the form of PHA (Satoh et al., 1999; Third et al., 2003). In biofilms this reducing power could be used to subsequently drive denitrification in the form of storage driven denitrification (Hughes et al., 2006; Krasnits et al., 2013).

In the current paper we describe the use of the above principle of anaerobic storage of soluble carbon for removing BOD compounds without the costly transfer of oxygen into the bulk wastewater. The approach is to selectively develop and maintain a biofilm rich in PHA accumulating bacteria and to provide it with oxygen by draining the reactor and thus enabling direct contact of the bacterial biomass (here biofilm) with the atmosphere.

2. Materials and Methods

2.1 Reactor dimensions and set-up

A cylindrical 2 L PVC reactor (12cm Ø and 29cm height) with openings at the top and bottom (Figure 1) was filled with packing material (AMB^{TM} Biomedia Bioballs with a specific surface area of 850 m^2/m^3) and approximate active surface of 500 m^2/m^3, such that the material filled the entire volume of the reactor. The volume taken by the packing material was 300 mL such that the working volume of the reactor without biomass was 1.7 L.

2.2 Reactor operation

Prior to operation, the described reactor was inoculated with activated sludge and biomass from a previously used biofilm reactor for storage driven denitrification (Hughes et al., 2006). After seeding, the reactor was operated automatically by specifically timed phases (Table 1). The reactor was filled with synthetic wastewater (within 5 min through a peristaltic pump) then maintained under anaerobic conditions for about 2 hours. The anaerobic phase was followed by gravity drainage of 10 min. This allowed air penetration within the reactor of equal volume to the liquid drained. Thereafter further air intake was prevented, using a solenoid, and the volume of air was recirculated for 1 hours. The oxygen in the head space was measured by a dissolved oxygen probe (Mettler Toledo, InPro 6800).

A pH and ORT probe were used to measure and record the values continually into a spreadsheet, using a LabJack U12 data acquisition card and the process control software LabVIEW^{TM} (version 7.1 National Instrument).

2.3 Synthetic wastewater composition

A synthetic wastewater was used and consisted of ($mg.L^{-1}$): CH_3COONa 660, NH_4Cl 160, $NaHCO_3$ 125, KH_2PO_4 44, $MgSO_4.7H_2O$ 25, yeast extract 50, and 1.25 $mL.L^{-1}$ of trace element solution, which contained ($g.L^{-1}$): ethylene-diamine-tetra-acetic acid

(EDTA) 15, $ZnSO_4.7H_2O$ 0.43, $CoCl_2.6H_2O$ 0.24, $MnCl_2.4H_2O$ 0.99, $CuSO_4.5H_2O$ 0.25, $NaMoO_4.2H_2O$ 0.22, $NiCl_2.6H_2O$ 0.19, $NaSeO_4.10H2O$ 0.21, H_3BO_4 0.014 and $NaWO_4.2H_2O$ 0.050.

2.4 Analytical

2.4.1 Acetate analysis

An Agilent 7820A gas chromatograph (GC) with auto-sampler was used to quantify acetate concentrations. Samples were acidified with formic acid (1 % (v/v)) before 0.4 uL samples were injected onto an Alltech ECONOCAP^{TM} EC^{TM} 1000 column (15 m x 530 um (i.d.) 0.25 μm). The carrier gas (N_2) was set at a flow rate of 3 mL/min and at the inlet the sample was split 10:1. The oven temperature was programmed as follows: initial temperature 70 ^oC; temperature ramp 5 ^oC/min to 100 ^oC; held for 2 min; temperature ramp 70 ^oC/min to 230 ^oC; held for 2 min. Injector and detector were set at 200 and 250 ^oC respectively. The peak area of the Flame Ionisation Detector (FID) output signal was computed via integration using the EzChrom Elite Compact software (© 2005, V.3.3.2SP2). The detection limit determined was 0.5 μmol/L of acetate.

Chemical oxygen demand (COD) was determined by the closed reflux, colorimetric method according to the standard method (Rice et al., 2012). The COD readings were obtained against known concentration of acetate in wastewater (1 to 10 mmol/L).

2.4.2 PHB analysis

The poly-hydroxybutyrate was extracted from the biomass using a method adapted from Smolders et al. (1995). Dichloromethane was used instead of dichloroethane. The samples were esterified in 1:4 concentrated HCl:propanol solution for 2 h at 150 ^oC in a Hach COD reactor. The culture tubes were sealed with Teflon lids to prevent loss of

volatile solvents. Aliquots of 3 mL of DI water were used to clearly separate the organic and the aqueous layers. The organic layer was transferred to a GC vial for analysis. Similarly standards of 0, 3.3, 6.6, 9.9, 13.2 mM beta-hydrybutyrate were prepared using a stock solution of 200 mmol/L HB (Sigma-Haldrich).

After the above steps of PHB hydrolysis and esterification of the hydrolysed product hydroxybutyric acid, the resulting ester (propyl-hydroxy-butanoate) was analysed using the same GC and column as above with the following conditions. The sample was split at the inlet 5:1. The oven temperature program was: initial temperature 80 ^{o}C; temperature ramp 70 ^{o}C to 152 ^{o}C ; temperature ramp 4 ^{o}C/min to 160 ^{o}C; temperature ramp 70 ^{o}C/min to 230 ^{o}C hold for 2 min.

The chromatogram of the PHB produced two additional peaks at higher retention times. These peaks were assumed to be hydroxy-valerate (PHV) in two different isomeric forms. The amount of the two additional peaks was estimated from the HB standard curve using benzoic acid as an internal standard.

2.4.3 Glycogen analysis

Biomass was collected and freeze dried (Hetosicc-CD 4) between 10 and 20 hours. Dried biomass was accurately weighed in a digestion tube. The biomass was then digested in a solution of 0.9 mol/L HCl for 4h at 100 ^{o}C in a water bath. The insoluble biomass was removed and the pH of a 3 mL supernant of the digested solution was adjusted to 7.2 (±0.2) using 0.35 mL of 10 mol/L NaOH and 0.5 mL of 0.9 mol/L KH_2PO_4. The sample was tested by an enzymatic glucose analyser (AccuCheck) against a linear glucose standard curve (0 to 10 mmol/L glucose) (Flavigny, 2014).

2.4.4 DNA analysis

DNA from the reactor and from activated sludge were extracted using Power Soil DNA analysis extraction kit (MO-Bio). The DNA was stored at – 20 ^{o}C until further analysis. Variable regions of the bacterial 16S rRNA gene were amplified by barcoded pyrosequencing as previously described in Coghlan et al. (2012). Briefly, universal bacterial fusion primers (Hamady et al., 2008) were used to generate PCR amplicons in triplicate and pooled. The forward primer F515 (5´ GTGCCAGCMGCCGCGGTAA 3´) and the reverse primer R806 (5´ GGACTACHVGGGTWTCTAAT 3´) targeted the V4 hypervariable region of the 16S rRNA. PCR was carried out in a 25 µL total volume including 4 µL of template DNA, containing: 2.5 mM $MgCl_2$ (Fisher Biotec, Aus), 1× Taq polymerase buffer (Fisher Biotec, Australia), 0.4 µM dNTPs (Astral Scientific, Australia), 0.4 mg BSA (Fisher Biotec, Australia), 0.4 µM of each primer, and 0.25 µL of AmpliTaq Gold DNA polymerase (ABI). The PCR conditions included: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 s, 54°C 30 s, 72°C for 30 s, and a final extension at 72°C for 10 minutes (Corbett Research, NSW, Aus). Amplicons were purified (AMpure beads, Invitrogen) and DNA concentration estimated by ethidium gel staining to approximate equimolar concentrations for emulsion PCR. Bead: template rations for the emulsion were determined by qPCR (Bunce et al., 2012). The Roche GS Junior run set up included an emulsion PCR step, bead recovery, and the sequencing run. All of these procedures were carried out according to the Roche GS Junior protocols (http://www.454.com). In order to screen for high quality sequences, the sequencing output files were processed as described in Coghlan et al. (2012). This yielded 269 and 165 high quality sequences for the reactor's biomass and the activated sludge respectively. The resultant BLAST files were imported into the program-MEtaGenome ANalyzer (MEGAN version 4.62.1)

(Huson et al., 2007) for taxonomy using the following lowest common ancestor parameters: min score of 65, top percent of 5, and min support of 1.

3. Results and Discussion

3.1. Development of a biofilm that can remove soluble acetate anaerobically

For the purpose of developing a biofilm reactor that specifically selects for bacteria that maximise the BOD (i.e. acetate) storage from the inflow, rather than oxidising the acetate, as observed in activated sludge and in trickling reactors, strict selective conditions were applied after seeding the reactor. The selective conditions entailed the provision of acetate in the absence of oxygen followed by providing oxygen in the absence of soluble acetate. To accomplish this, the reactor was operated in a sequencing batch mode for 2 months as follows:

- anaerobic flooding of the biofilm with synthetic wastewater using acetate (16 mmolC/L 512 mg/L BOD) as the carbon source for 2 hours
- draining of the synthetic wastewater after completed acetate storage as PHA
- passive aeration of the biofilm by merely keeping it drained in the presence of atmospheric air for 1 hour.

The exposure to air was for the purpose of providing oxidative power to the biomass to oxidise the stored soluble carbon (ie: PHA) and by-passing the costly transfer of oxygen to the bulk liquid. This oxygen exposure to the biomass was expected to form glycogen from PHA thus enabling carbon uptake (ie: acetate) in the subsequent anaerobic phase (Liu et al., 1996). The continued operation under this scheme of

anaerobic storage and biomass exposure to air was expected to selectively enrich for bacterial species capable of effective anaerobic storage of acetate (i.e. GAO).

The reactor was operated continuously and its anaerobic acetate storage monitored (Figure 2). After only partial storage of acetate at the beginning (3 Cmmol/L or 384 mg/L, over 2 hours), the rate and storage capacity of acetate improved after 9 weeks of operation, reaching a rate of acetate storage of 10 Cmmol/L/h acetate (320 mg/L/h BOD) and a storage capacity of 40 Cmmol/L (1280 mg/L BOD). Clearly, the removal rate as well as the mass of acetate taken up now exceeded the capacity of typical activated sludge process achieving < 1 Cmmol/L/h and 128 mg/L/h BOD respectively (Tandukar et al., 2007).

To eliminate the possibility of acetate being converted to another soluble organic compound, COD analysis was carried out in parallel to acetate analysis of the effluent. No evidence of organic species other than acetate was found (data not shown).

3.2. Specialised biofilm with GAO and its metabolism

In general the intermittent supply of oxygen is known to lead to BOD storage as PHA by phosphate accumulating organisms (PAO) (Hesselmann et al., 1999). PAO bacteria accumulate phosphate as an energy store under aerobic conditions. This is then hydrolysed and released under anaerobic conditions providing sufficient energy for anaerobic BOD uptake and its polymerisation as PHA. However, in the present reactor the aerobic phosphate accumulation cannot occur because in the aerobic phase the phosphate containing bulk liquid has been drained. As a consequence an alternative anaerobic energy source to polyphosphate must be used in the biofilm described here.

The known alternative to PAO metabolism is the metabolism of the glycogen accumulating organisms (GAO). These synthesize glycogen from PHA under aerobic conditions, which serves via fermentation as the energy source for BOD uptake and PHA storage under anaerobic conditions (Liu et al., 1996). So clearly our biofilm operation would be likely to select for GAO rather than the traditional PHA storing PAO. Furthermore, at the low P/C (Pmol/Cmol) ratio of ≤ 0.02 used in our experiments PAO would be outcompeted by GAO (Liu et al., 2000; López-Vázquez et al., 2007).

After 9 months of operation a biomass sample from the biofilm and from activated sludge from Woodman point wastewater treatment (Perth, Australia) were used for DNA extraction and sequencing. The aim was to compare obvious differences in biomass composition (Figure 3).

In the biofilm reactor the second largest population was *Candidatus competibacter* (10.7 %) which is a known GAO (Filipe et al., 2001; Lopez-Vazquez et al., 2009). In theory, this could be expected because of the selective operation of the system offering anaerobic condition with acetate followed by an aerobic environment without acetate (and without phosphate which could otherwise lead to PAO). However, the presence of the genus *Haliangium* is unusual, as these belong to the myxobacteria which are known as predators to other bacteria (Ivanova et al. (2010). Similar to GAO, which oxidise stored PHA in the aerobic phase, myxobacteria also have their food source in the aerobic phase, while other typical heterotrophs have no organic feed supply after draining. This aerobic feeding of predators in the biofilm provides one possible explanation for the low sludge production observed. Representatives of PAO (*C. accumulibacter*) were not detected. This is because PAO require oxygen and phosphate together (Cech & Hartman, 1993; Smolders et al., 1995) whereas in the reactor, after the liquid is drained for aeration, the phosphate has also been drained.

A proper carbon balance including PHA, glycogen and acetate would be able to evaluate whether the biofilm behaviour is in accordance with GAO metabolism. The large volume of the biofilm biomass in the reactor prevents representative sampling which is needed for carbon balance purposes. Therefore, after thorough mixing of the reactor's biomass, a subsample was used for the carbon balance experiment. An extended aerobic period and an increased dose of acetate was provided to generate changes in the overall storage products (PHA and glycogen) that were sufficiently large to show significant differences against the background storage products.

To test whether the carbon removal behaviour under anaerobic conditions was consistent with the glycogen metabolism of typical GAO a simple carbon balance was established. The biofilm subsample (5.6 g dry biomass) was first aerated for 6 hours. It was then suspended anaerobically in a solution of synthetic wastewater (100 mL) with excess acetate (20 Cmmol/L) overnight, to record glycogen and acetate conversion to PHA (Figure 4). Overall the carbon balance was maintained throughout this anaerobic phase. During the anaerobic phase, the glycogen oxidation (ie: decrease) provides the ATP source for acetate uptake and the resulting production of PHA (Figure 4) as expected from the literature (Bengtsson, 2009; Filipe et al., 2001; Smolders et al., 1995). In the anaerobic period 1.0 Cmol of PHAs was produced per Cmol of the combined reactants, acetate and glycogen. This is in line with the reported anaerobic PHA yield ranging from 0.87 to 0.99 Cmol of PHB produced per Cmol consumed (VFA + glycogen) (Dai et al., 2007; Pisco et al., 2009).

Also under aerobic conditions the carbon balance was reasonably conserved indicating a glycogen production of 1.1 $Cmmol_{glyc}/Cmmol_{PHA}$ which is slightly higher than the result of 0.8 obtained by Filipe et al. (2001) and similar to the value of 1.0 by

(Liu et al., 1994) . The expected carbon loss as CO_2 originating from carbon respiration could not be accounted for in the carbon balance.

Overall the results show that the biofilm behaviour is in line with the GAO metabolism demonstrated in the literature (Bengtsson, 2009; Filipe et al., 2001). Anaerobically, the carbon taken up via acetate was accounted for by the combination of PHA gain and glycogen loss. Aerobically, carbon usage for glycogen production was similar to carbon release from PHA consumption.

3.3. Direct passive oxygen supply to the drained biofilm

Once the soluble acetate was removed from the wastewater, the liquid was drained by opening the bottom and the top of reactor (valve operated system) to allow air to fill the void volume. This allowed the supply of oxygen to the microbial cells while by-passing costly oxygen transfer to the bulk solution. Then the reactor was closed to provide a reproducible amount of oxygen for all trials.

Considering the volume of the liquid contained in the reactor was approximately 1 L then there is 210 mL or 8.5 mmol of O_2 available for the oxidation of the stored carbon (ie: 8.5 Cmmol of acetate). Mass balance showed that, of the acetate removed from solution (12 Cmmol/L), only about half was oxidised by oxygen (Figure 5). Therefore carbon accumulated within the system, either in the form of biomass or alternatively as storage material.

3.4. Minimum oxygen requirements

To test whether providing one pore volume of air was sufficient for the long term operation and acetate removal, the reactor was run continuously for 24 cycles. Over 80 hours it was demonstrated that 14 Cmmol/L acetate (448 mg/L BOD) present in the

synthetic wastewater were removed in cycles of 3.5h (Figure 6). Therefore the removal rate of acetate was 4 Cmmol/L/h (123 mg/L/h BOD), which is a carbon removal rate that is about 3 times higher than typically observed in activated sludge plants (Tandukar et al., 2007). No significant biomass output was recorded over this time. From the reproducible oxygen uptake curves (data not shown) it could be predicted that approximately 50 % of the acetate added was respired (Table 2).

Above, the acetate was continuously removed with a single reactor void volume of air. To test for the maximum carbon to oxygen ratio needed to enable sustained operation, the acetate concentration was incremented to 22 Cmmol/L and 30 Cmmol/L (Figure 6). The time provided for acetate uptake was increased from 2 hours to 4 hours and to 7.5 hours for the highest concentration. The oxygen supply was maintained to a single void pore volume, the C:O_2 ratio is therefore increased from1.3 to 2.1 and 2.9 for the highest concentration.

At a feed concentration of 22 Cmmol/L > 90% of acetate was continually removed over 18 subsequent cycles with providing still only 1 pore volume of air (Figure 6). When elevating the acetate concentration to 30 Cmmol/L acetate could no longer be stored sustainably, as indicated by 50 % residual acetate being present in the effluent after 5 cycles (Figure 6).

Using stoichiometric considerations, the fact that up to 22 Cmmol/L of acetate (26.4 Cmmol of acetate per reactor) could be removed continuously with 1 pore volume of air continuously suggests that sufficient stored acetate is oxidised. In fact, calculated from the oxygen content, approximately 27 % of the added acetate was oxidised (Table 2) with the remainder retained within the biomass. On the contrary, when 30 Cmmol/L were added the cycles were not sustained (Figure 6). In this case, the 1 pore volume

oxidised 20 % of the added acetate. This suggests that there is a minimum of PHA oxidation required to sustain BOD uptake.

Overall, if 27% of the added acetate oxidised, 22 Cmmol/L removal was sustained. However, if 20% or less of the added acetate, 30 Cmmol/L removal was not sustained. It seems that if more than a quarter of the added BOD is oxidised then the BOD storage can be sustained (Table 2).

3.5. Operational considerations:

In a number of ways the described biofilm reactor is similar to trickle filters used for wastewater treatment. However, significant differences can be pointed out both in terms of microbial composition and operational attributes.

Because of the strict cycling of anaerobic acetate storage to PHA followed by aerobic PHA oxidation, only those heterotrophic bacteria than can effectively store BOD as PHA, namely GAO can be sustained in the biofilm. The current sequencing batch operation of the biofilm reactor would hence select for the development of a distinctly different biomass to that in trickle reactors.

With an acetate removal rate of 4 Cmmol/L/h (123 $g/m^3/h$) the described biofilm process demonstrated a 10 to 20 times faster volumetric carbon removal rate than that obtained for traditional trickle reactors (Table 3). Possible reasons for the rather high rates of BOD removal could be:

- the high surface area of carrier material used in the bioreactor (850 m^2/m^3)
- the high biomass content of the biofilm (50 g dry biomass per L of reactor volume)

Assuming a 5 m high reactor and 3.5 h treatment time, the energy required is 4 W/m^3. Considering that trickling reactors (high rate with plastic media) are recirculating 4 to 7 times, their energy usage is typically 6 to 10 W/m^3 (Metcalf et al., 1972), our biofilm requires 1.5 to 2.5 times less energy expense compared to trickle reactors.

The biofilm reactor described removes BOD rapidly and energy-efficiently but does not remove phosphorus and nitrogen. The nutrient removal can be achieved by the novel low-energy nitrogen removal processes that include the Anammox bacteria, such as the completely autotrophic nitrogen removal over nitrite (CANON) which uses limited aeration for partial oxidation of ammonia to nitrite followed by the Anammox process leading to N_2 formation (Third et al., 2001). However, this process cannot be effectively applied with existing wastewater streams because of the BOD (Kartal et al., 2010). The currently described process would be a fast and cost effective way to remove soluble BOD from wastewater prior to nitrogen removal treatments, which requires low BOD wastewater. The additional removal of nitrogen by a process linked to the described biofilm reactor has been designed and will be described later.

4. Conclusion:

- A simple sequence of anaerobic conditions (filling) and aerobic condition (drainage) selectively enriched for glycogen accumulating organisms (GAO) from activated sludge. Such method is easily applicable for existing biofilm reactors.
- The biofilm was able of sustained acetate removal from synthetic wastewater without transferring air into the bulk wastewater and hence by-passing the majority of energy expense for oxygen transfer.

- Atmospheric air provides oxidative power via passive aeration to the biofilm for PHA oxidation, hence recovering the biofilm's ability to store acetate in subsequent cycles. A repeated liquid recycle as needed for trickling reactors was not needed.

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Captions:

Figure 1: Schematic diagram of the operated reactor.

Table 1: Time schedule of operation of the BOD to PHA storage biofilm reactor in sequencing batch mode.

Figure 2: Acetate storage after 2 (●), 8 (■) and 9 (▲) weeks of operations. The acetate supplied was lower, from 12Cmmol/L to 7.5 Cmmol/L, after the week 2 to prevent the development of non-storing bacteria during the aerobic phase when acetate is present in the water.

Figure 3: Comparison of the microbial population of biofilm described (white) with that of activated sludge (grey). Size of the node labels is proportional to the number of sequence reads at each taxonomic level. The pie slices are proportional to differences in sequence reads at the taxonomic level.

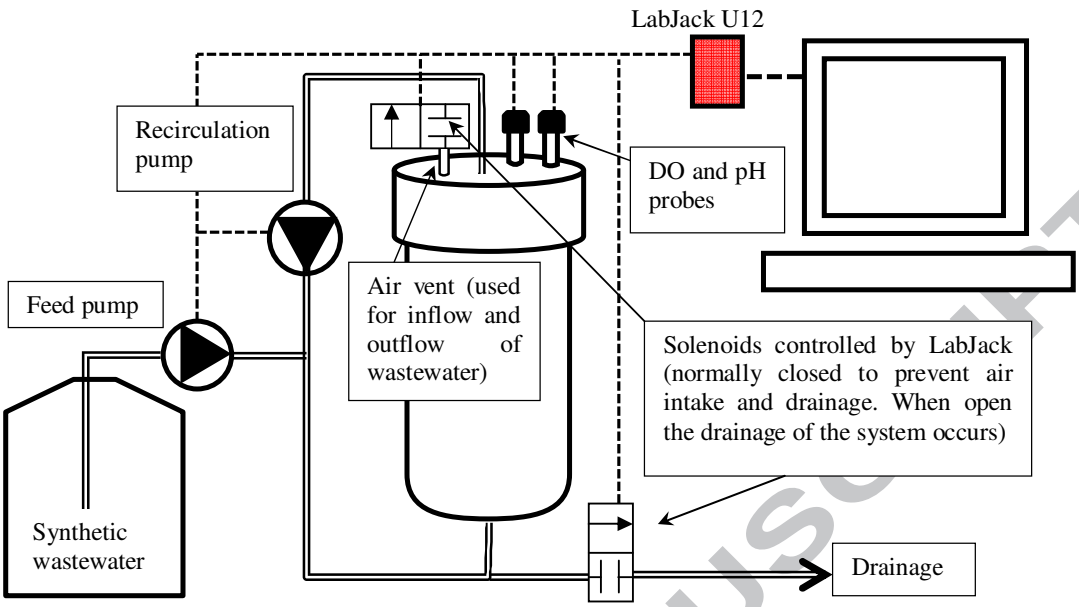
Figure 4: Acetate (●) removal, glycogen (▲), PHB (♦) and PHV (■) production by a subsample of the biofilm in suspension.

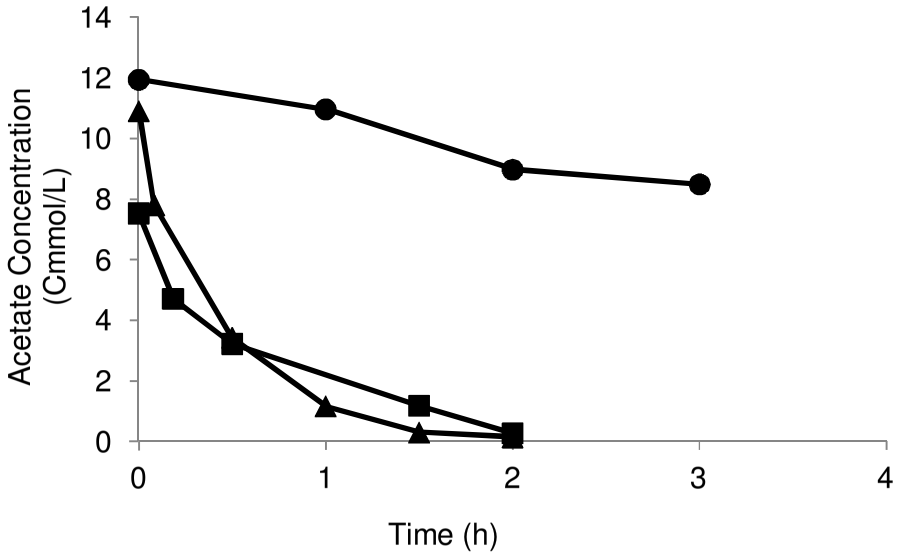
Figure 5: Typical behaviour of a single cycle of the storage biofilm reactor during anaerobic acetate storage (●) (0-2 h) and calculated aerobic acetate oxidation (○) (2-3h). Oxygen consumption (■) was used to calculate acetate oxidised.

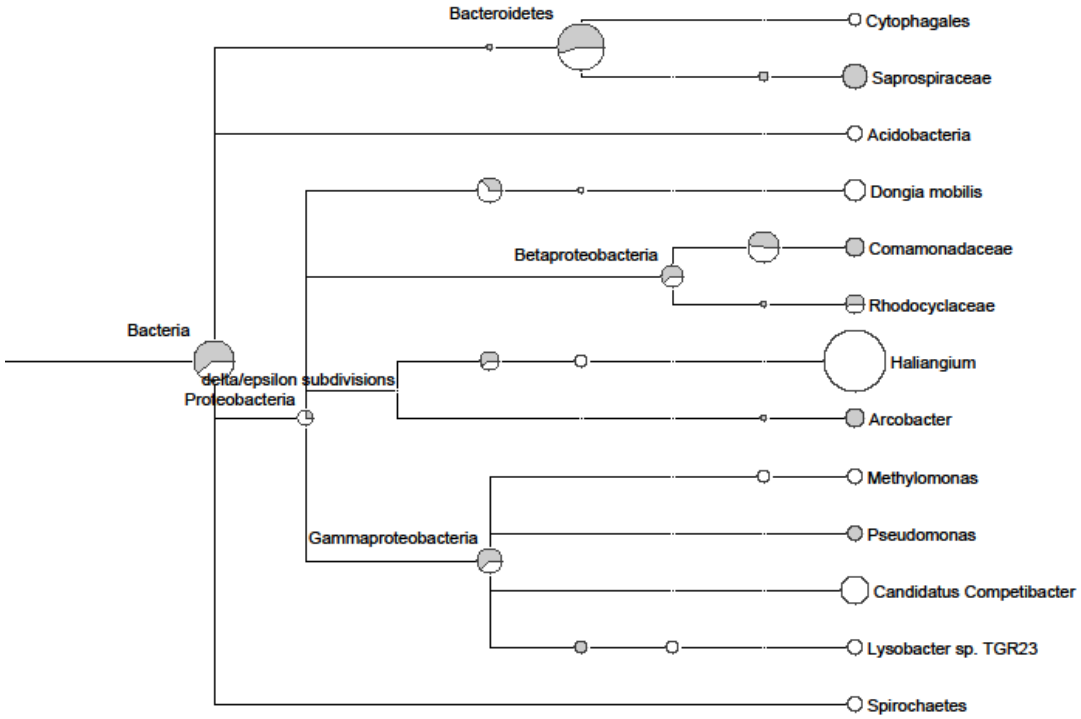
Figure 6: The effect of increasing the carbon to oxygen ratio, on the continuous removal of acetate. Continuous operation of the storage biofilm reactor under repeated cycles of synthetic wastewater with 1 pore volume of air provided, 24 cycles of 14 Cmmol/L, 18 cycles of 22Cmmol/L and 5 cycles of 30 Cmmol/L. Example cycles of 14 Cmmol/L (●) and carbon outflow (○), of 22 Cmmol/L (▲) and carbon outflow (Δ), and of 30 Cmmol/L (■) and carbon outflow (□).

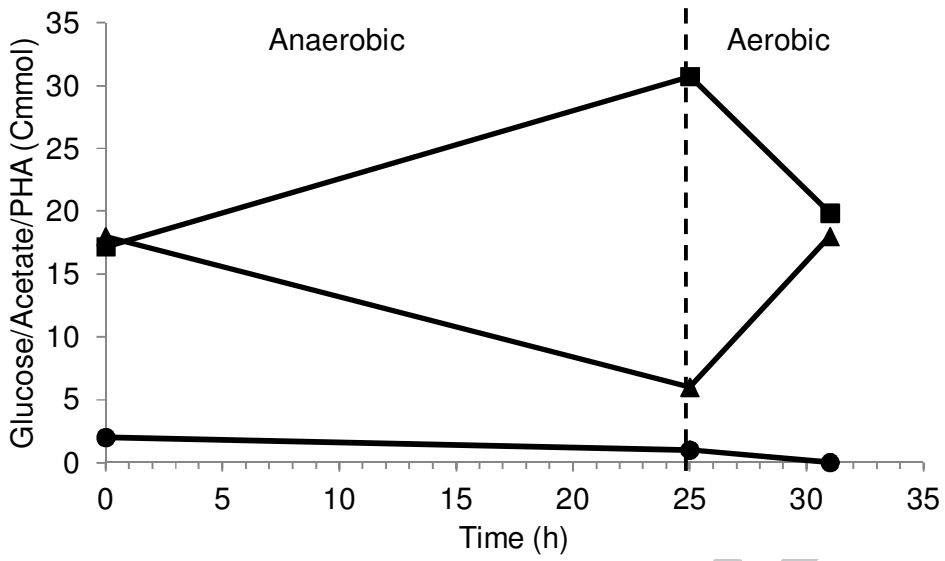
Table 2: Operation parameters of the reactor to test the effect of acetate to oxygen ratio to sustain continuous acetate removal. From the oxygen consumption, the acetate oxidised was calculated and the acetate storage determined.

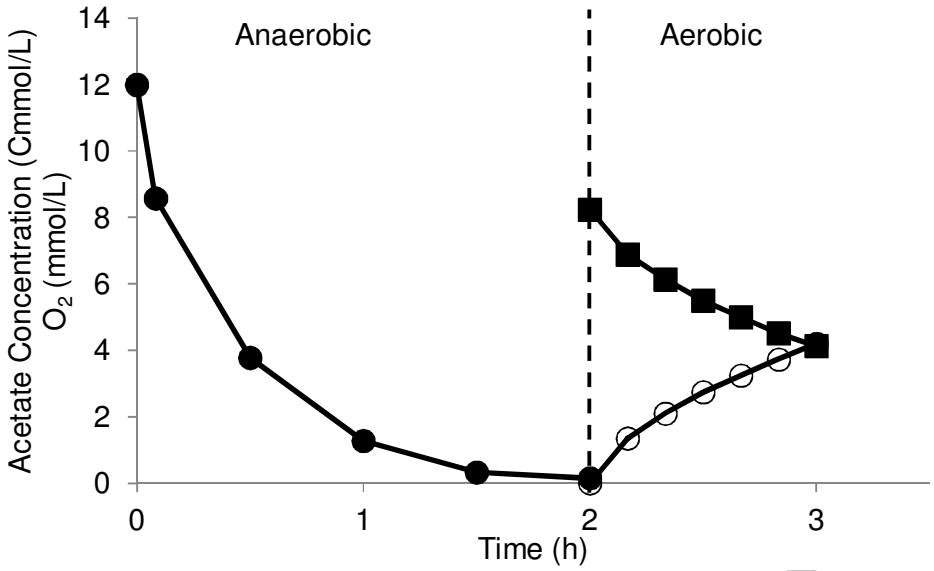
Table 3: Comparison of the hydraulic retention time (HRT) for trickle filters and proposed biofilm.

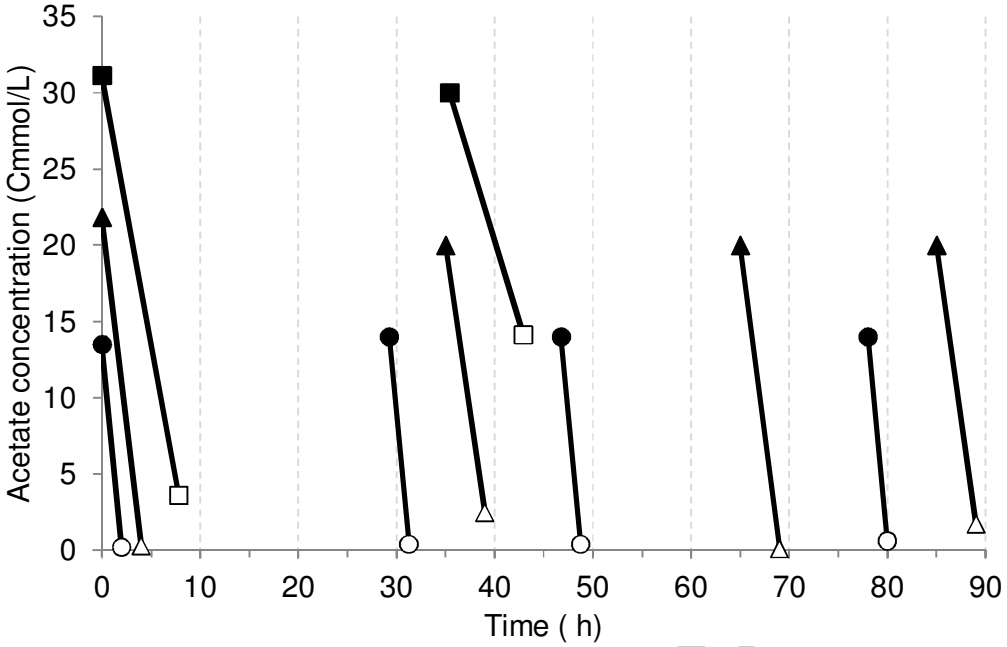








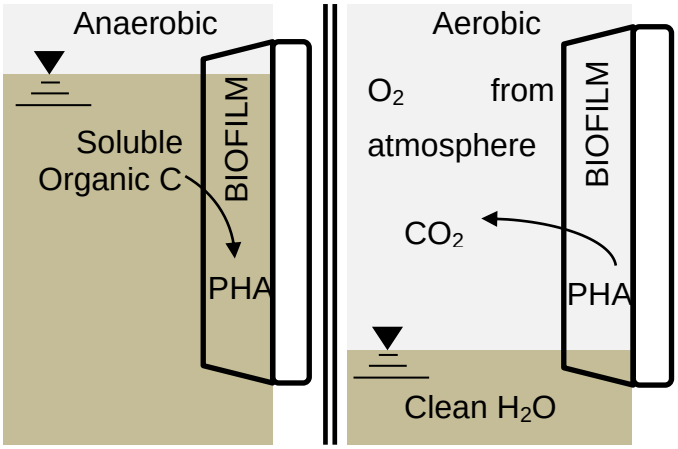




Operation time (min)	Phase	Purpose
0 - 5	Fill	Replacing air space by synthetic wastewater
5 - 120	Store	Uptake of soluble BOD as PHA under water circulation
120 - 130	Drain	Replacing treated wastewater by air
130 - 260	Vent	Provide air for oxidation of stored organics

Ac input rate CmM/h	Ac input per cycle CmM	O_2 input mM	Carbon to O_2 ratio C:O_2	Ac oxidised per cycle CmM	Percentage C oxidised %	Ac remaining in outflow	Sustained removal?
4	14	10.3	1.36	5.9	42	<1%	Yes
4	22	10.3	2.1	5.9	27	<1%	Yes
3.5	30	10.3	2.9	5.9	20	~50%	No

System	BOD removal rate $g/m^3/h$	BOD inflow g/m^3	HRT h	References
Trickle reactor for communal wastewater, rock media	11.7	599.5	51.2	Doan et al. (2008)
Trickle reactor for communal wastewater, rock media	4	250	62.5	Gray (2004)
Trickle reactor for communal wastewater, rock media	5	250	50	Forster (2003)
Sequencing operation of PHA storing biofilm	128	480	3.5	Present research



Highlights

- A biofilm, enriched in GAO from activated sludge, stored acetate effectively
- Soluble BOD was removed without air bubbling using atmospheric air transfer
- Atmospheric air supply was required to regenerate the biological storage capacity
- The simple operation of the biofilm required less energy than trickle filter