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Mesh rotating reactors for biofilm pre-treatment of wastewaters – Influence of media type on microbial activity, viability and performance

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ABSTRACT

The impact of using different plastic mesh in rotating biofilm reactors (RBRs) on the treatment performance, biofilm activity and viability under varying organic loading rates (OLRs) was investigated. Laboratory-scale RBRs treating real wastewater were operated under OLR loading conditions typical of pre-treatment processes. A fully-crossed, three-factorial design series of experiments was undertaken with low and high surface area mesh made from polyvinyl chloride (PVC) and polypropylene (PP) operated at low, medium, high and very high OLR. The maximum volumetric removal rate of 2.4 kg sCOD m^3 d^{-1} occurred at the high OLR, for low surface area mesh, irrespective of plastic used. The highest OLR at which nitrification could be attained was 35 g sCOD m^{-2} d^{-1}. The biofilm growth decreased under medium compared to low OLR on all mesh. This coincided with a ~2 fold decrease in the microbial viability. Higher surface area mesh was important for high nitrification rates at medium OLR ($p < 0.05$). In contrast the low surface area PVC and PP mesh was best at very high OLR (160 g sCOD m^{-2} d^{-1} or ~320 g BOD_5 m^{-2} d^{-1}) for bulk organics removal ($p < 0.05$). Therefore, lower surface area mesh is recommended for wastewater pre-treatments at high OLR, whilst high surface area mesh was best for elevated nitrification rates at medium OLR. The microbial activity and viability had a strong positive correlation with OLR ($R^2 = 0.92$, $p < 0.001$ and 0.81, $p < 0.001$ respectively). The microbial activity and viability also positively correlated ($R^2 = 0.4$, $p < 0.05$ and 0.29, $p < 0.01$ respectively) to the sCOD removal performance but not the ammonia removal in mesh RBRs. This confirms the importance of maintaining biofilm activity and viability for bulk organics removal in biofilm processes in wastewater treatment.

1. Introduction

Achieving more stringent effluent standards in conventional biological treatment is usually contingent on factors such as extended reactor retention times and aeration rates, both of which increase the cost of treatment. To achieve discharge limits within financial constraints with low energy usage, it is imperative to optimise process operation and develop new technology (STOWA, 2010). A rotating biofilm reactor (RBR) known as shaft mounted advanced reactor technology (SMART) has shown promise for high organic load treatment (Hoyland et al., 2010). These units are similar to rotating biological contactors (RBCs) but with an open architecture mesh comprised of fibres arranged in a high porosity mesh, which overcomes the limitations of RBC-like reactors under high load conditions (Chen et al., 2006; Hassard et al., 2014, 2015). In order to minimise the cost of treatment and maximise value of existing assets, the SMART unit operates as a roughing biofilm, for

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oxidation of bulk organics prior to existing secondary process such as activated sludge (HYBACS) or trickling filters (HYFILT) (Hassard et al., 2014; Hoyland et al., 2010).

The organic loading rate (OLR) is important when deploying a biofilm reactor as this impacts on biofilm treatment performance and effluent quality (Wijeyekoon et al., 2004; Chen et al., 2006). Previous research suggested that rotating biofilm processes achieved simultaneous biochemical oxygen demand (BOD_5) removal and nitrification at an arbitrary surface loading of ~6 g BOD_5 m^2 d^{-1} although some high rate systems have exceeded this limit (Hassard et al., 2014). Above 6 g BOD_5 m^{-2} d^{-1} biofilm/solids accumulation occurs, preventing stable nitrification. Increasingly, biofilm accumulation is seen as an advantage by facilitating a greater range of treatment regimens in a simultaneous volume, as biofilm reactors have an undefined sludge retention time and growth kinetics whilst maintaining distinct gradients in substrate and electron acceptors (Bryers, 2000). High biomass concentrations in biofilm systems constitute an opportunity to construct simple, cheap and compact reactors (Mendoza-Espinosa and Stephenson, 1999; Hassard et al., 2015). In biofilm reactors, the microbial population is attached on a solid media, which allows greater flow rates, OLR and process stability than is possible in most suspended culture systems (Stephenson et al., 2013).

Careful media selection has been suggested as a method to control biofilm thickness (Morgenroth and Wilderer, 2000), select for and against different strains (Khan et al., 2013; Stephenson et al., 2013) ensure biofilm stability microbial viability or activity of microbial populations (van der Mei et al., 2008; Lackner et al., 2009; Jurecska et al., 2013; Hassard et al., 2014). The aim of these studies was to improve performance, through harnessing different media/bacterial interactions. Research has suggested that the media physical composition and architecture affects the reactor microbial activity and the removal rate of BOD_5 and ammonia (Hassard et al., 2014). Traditional RBC media are ineffective under high OLR as biofilms excessively grow on the media, which can inhibit mass transfer to the biofilm (Kim et al., 2010; Hassard et al., 2015), leading to inactivation of some consortia or mechanical failure of media, shaft or bearings (Mba et al., 1999). Flexible fibres with a heterogeneous architecture propagate differential shear and boundary layer conditions and could maintain appropriate thickness to prevent clogging, whilst tortuosity provides heterogeneous microniches in hydrodynamics, substrate and electron acceptor conditions. This could result in unique microbial communities and removal regimes in biofilms compared to conventional suspended growth alternatives (Singer et al., 2010). In addition, media should have chemical stability in wastewater, be resistant to microbial degradation, extremes of temperature, pH, salinity and ultra-violet radiation whilst having appropriate tensile strength, reliability and life cycle costs (Jurecska et al., 2013). Hassard et al. (2014) tested a novel polyvinylchloride fibre mesh media in isolation against high porosity reticulated foam. This mesh media offered elevated removal of substrates and microbial resistance to high OLR treatments, despite similar porosities, demonstrating the role of media architecture in determining performance. Jechalke et al. (2010) found that coconut fibres were more effective than polypropylene mesh for removal of a variety of micropollutants suggesting that material selection is important. Jurecska et al. (2013) compared polypropylene to polyester fibres and showed polypropylene was superior for biofilm attachment, performance and microbial activity despite similar macroscale media architecture.

The impact of mesh material on the initial biofilm deposition and long-term biofilm establishment, viability and activity has not been studied previously in RBRs (Busscher et al., 1995; Hassard et al., 2014). This is pertinent considering RBRs often operate as a roughing stage that are subjected to extremes of high OLR, low dissolved oxygen and redox potential. Therefore, the objective of this research was to examine the effect of macroscale properties on the performance of mesh media under high OLRs. To test the impact of OLR on substrate removal (sCOD, NH_4-N), experiments were performed with low and high surface area mesh made from polyvinyl chloride (PVC) and polypropylene (PP) operated at four different OLRs. Previous research has shown that the viability of bacteria can be affected by the media and operating conditions (Lackner et al., 2009; van der Mei et al., 2008). The activity and viability of the microbial population was therefore determined to test whether either media composition or operating conditions were more important to biofilm function or performance at high OLRs.

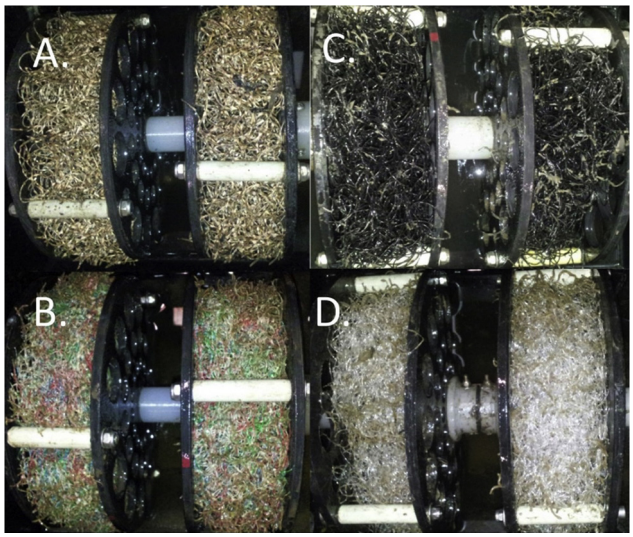

Fig. 1 – Mesh media. (A) Polyvinylchloride low surface area (PVC-L). (B) Polyvinylchloride high surface area (PVC-H). (C) Polypropylene low surface area (PP-L). (D) Polypropylene high surface area. (PP-H).

2. Materials and methods

2.1. Bench-scale reactors

Four bench scale RBRs, each being a polycarbonate vessel with plastic frames for housing the media, treated settled wastewater from Cranfield University wastewater treatment plant (WWTP). The media consisted of two circular mesh plates (Bluewater Bio, UK) in each reactor: two reactors containing polyvinyl chloride-like (PVC) material and two of polypropylene (PP) material, mounted on a single rotating shaft (Fig. 1). Two reactors contained PVC of low (150 m^2 m^{-3}) and high (360 m^2 m^{-3}) specific surface areas (PVC-L and PVC-H respectively) and the other two contained PP of low (160 m^2 m^{-3}) and high (277 m^2 m^{-3}) specific surface areas (PP-L and PP-H respectively) (Table 1). The total media volume per reactor was 0.003 m^3 (d = 0.2, surface area = 0.19 m^2, disk n = 2, wetted volume = 3 L, submergence = 40%) and the RBRs were operated at a constant tip speed of 0.08 m/s (8 rpm). The media nominal surface area ($A_{nominal}$) was calculated according to Eq. (1):

$$A_{nominal} = 2\pi r^2 + 2\pi rh \quad (1)$$

where r is the radius of the plate and h is the plate thickness. Each mesh media was analysed for surface physical properties by atomic force microscopy (AFM) and surface roughness values were obtained as outlined in Stephenson et al. (2013). The reactors were kept at approximately 15 °C using a 50 W thermostatic aquarium micro heater (Superfish, UK).

2.2. Experimental protocol

All 4 RBRs were operated over a total period of 240 d at 4 different OLRs. The OLR was increased at approximately 60 d intervals: at the end of this period, the biofilm on the media was sacrificed. The reactors were subsequently restarted at a new OLR by changing the influent settled wastewater flowrate.

Table 1 – Properties of media used in rotating biofilm reactors (RBRs).

Mesh media name	PVC-L	PVC-H	PP-L	PP-H
Base material	PVC	PVC	PP	PP
Filament diameter[a] (μm)	544 ± 84	300 ± 46	578 ± 36	394 ± 22
Filament linear mass density[b] ($g{\cdot}10^4\ m^{-1}$)	4400	2222	1100	504
Porosity[c] (%)	96	93	96	95
Surface area per unit weight[b] ($m^2\ g^{-1}$)	0.26	0.41	0.84	0.98
Specific surface area of mesh[a] ($m^2\ m^{-3}$)	~150	~360	~160	~277
Average roughness (R_a surface)[a] (nm)	8.72 ± 4.2	11.75 ± 3.08	26.75 ± 6.8	23.2 ± 4.2

[a] Experimental results.
[b] Manufacturer data.
[c] Derived results.

The reactors were allowed to stabilise for a minimum of 21 d prior to sampling in order to monitor performance and biofilm conditions.

The OLRs based on soluble COD (sOLR) or ammonia (NH_4^+-N) were calculated based on the media surface area according to Eq. (2) in order to obtain a normalised comparison of the relative performance of the different media.

$$\text{sOLR} = \frac{S_i \times Q_i}{A_{\text{nominal}}} \quad (2)$$

where S_i is the influent substrate concentration (sCOD or NH_4^+-N) and Q_i is the influent flowrate. The OLRs based on reactor volume (vOLR) were calculated according to Eq. (3).

$$\text{vOLR} = \frac{S_i \times Q_i}{V_{\text{nominal}}} \quad (3)$$

The reactors were commissioned at a low flowrate of $1.1\ L\ h^{-1}$ and increased over time to 2.2, 4.4 and $8.8\ L\ h^{-1}$. These flowrates corresponded to nominal hydraulic retention times of 200, 100, 50 and 25 min respectively, thereby resulting in nominal media surface OLRs of 16, 35, 60, 35 and $160\ g\ sCOD\ m^{-2}\ d^{-1}$ described as low, medium, high and very high OLRs respectively (Table 2). The corresponding BOD_5 loading rates were ~34, 80, 138, $368\ g\ BOD_5\ m^{-2}\ d^{-1}$ respectively based on the sCOD:BOD_5 ratio of 2.1:1 averaged from 25 analyses of influent settled wastewater over the duration of the experiments. To achieve stable nitrification, a surface loading of <6 g $BOD_5\ m^{-2}\ d^{-1}$ is recommended for rotating biofilm processes (Rittmann and McCarty, 2001). The impact of OLR on the RBR media was therefore assessed at loadings several times greater than this stated threshold (Table 2), as would be expected when the process is applied as a pre-treatment stage.

2.3. *Biofilm analysis*

The biofilm was removed from the media at the end of each sampling run to ensure complete biofilm removal as described by Regmi et al. (2011). Three fibres per sample were checked with SYTO-9 staining under confocal laser scanning microscopy (CLSM, Carl Zeiss, Inc., Germany) to ensure complete biofilm removal had occurred. The removed biofilm was measured for total solids (TS) and volatile solids (VS) by standard methods (APHA, 2012). Macro observations of the biomass were made using a light microscope (M80 Stereo, Leica, Germany).

A homogenous biofilm distribution on the mesh was assumed and the microbial activity and viability analyses were undertaken by removing a sample of biofilm from the mesh media at the start, mid and end points for each treatment. An average of the microbial viability and activity was then calculated. The analyses were undertaken on a composite, randomly selected sample which was analysed in triplicate to account for method error.

To determine microbial viability, biofilm was harvested as above and diluted using BOD_5 water ($1.25 \times 10^{-4}\ mg\ L^{-1}$ ferric chloride; $0.028\ mg\ L^{-1}$ calcium chloride; $0.025\ mg\ L^{-1}$ magnesium sulphate in a buffered aqueous solution), handled by pipetting (Finntip™ Wide Orifice Pipette Tips, Thermo Fisher, UK) and a 5 ml subsample was mechanically disaggregated on ice using a homogeniser (Powergen 125, Fisher-brand, UK) for 10 min at speed setting 2 (12, 250 L/min) after Hassard et al. (2014). Microbial viability was measured by staining biofilm samples with LIVE/DEAD® BacLight™ test (Invitrogen, Glasgow, UK). The fluorochromes SYTO® 9 and propidium iodide were added with biomass to a 96-well black flat bottom microtitre well (Porvair Sciences, UK) and incubated in the dark for 15 min with occasional linear shaking. Fluorescent and absorption spectroscopy was performed according to manufacturer's instructions for a plate reader for viability and activity measures respectively (Infinite M200, Tecan, UK).

Microbial activity was measured by using electron transport chain activity as a proxy to ascertain how the microbial biofilm was affected by OLR and media type, using a modified method of Nocker et al. (2011). This was measured using a water soluble tetrazolium salt (WST-8) (2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H/tetrazolium monosodium (Genscript, US) and electron mediator menadione (2-methyl-1,4-naphthoquinone) (Acros Organics, Fisher Scientific, UK). Diluted biofilm was added to a reaction mix of 2× Tryptic soy broth (TSB) and WST-8 detection reagent in a ratio of 4:5:1. The change in absorbance was measured after an

Table 2 – Operating conditions of the RBRs.

Load	HRT (min)	sCOD loading rate ($g\ m^{-2}\ d^{-1}$)	NH_4-N loading rate ($g\ m^{-2}\ d^{-1}$)	Temperature (°C)
Low	195.8	16 ± 3.2	5.7 ± 0.9	14.7
Medium	100	35 ± 3.7	6.7 ± 1.1	13.6
High	50.4	66 ± 6	20 ± 0.5	15.6
Very high	24.5	160 ± 14.1	30 ± 4.3	17.8

incubation time and the biofilm was removed by centrifugation at 10,000 × *g* for 1 min to minimise solids interference. The microbial activity was measured against appropriate controls at an absorbance of 460 nm in a 96-well clear flat bottom plate using a plate reader. The microbial activity (mol dye reduced per time) was calculated based on the molar absorption coefficient of WST-8 ($3.4 \times 10^4\ M^{-1}\ cm^{-1}$) taking into account dilutions. The specific microbial activity was measured based on reduction of WST-8 dye per gram of volatile solids in the biomass.

2.4. *Wastewater and statistical analysis*

Wastewater was analysed using proprietary cell test kits (VWR, UK) for total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total nitrogen (TN), ammonia-nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) using a NOVA60 photometer (VWR, UK). The BOD_5 was measured according to standard methods (APHA, 2012). The pH of the influent and effluent was measured using a Jenway 320 pH meter (Bibby, UK).

A two-way repeated measures ANOVA was run to determine the effect of different media with increasing OLR on sCOD removal rate and separately NH_4-N removal rate. Analysis of the studentised residuals suggested the data displayed normality (Shapiro–Wilk test, $p > 0.05$) and no outliers were observed, as assessed by lack of studentised residuals with a value greater than ±3 standard deviations. There was a significant interaction between media and OLR on sCOD removal rate ($p < 0.0005$) and NH_4-N removal rate ($p < 0.0005$) (Mauchly's test of sphericity). Therefore the effect of OLR on both sCOD and NH_4-N removal rate is different for each media/OLR treatment, therefore, simple main effects were run. The mean difference is significant at $p < 0.05$ level and multiple comparisons were adjusted with the Bonferroni correction and compared. The relationships between activity, viability, OLR and nitrogen loading rate with sCOD and NH_4-N removal rates were assessed by multiple linear regression analysis. Tests for normality and outliers was performed as above.

3. Results and discussion

3.1. *Treatment performance*

The pH was on average 7.7 ± 0.08, and the BOD:sCOD ratio was 2.1 ± 0.4 indicating that wastewater composition was similar during all experimental runs. The volumetric removal rate increased from 0.5 to 2.4 kg sCOD $m^{-3}\ d^{-1}$ from low through to high OLR, before decreasing to 0.9–2 kg sCOD $m^{-3}\ d^{-1}$ at very high OLR. The maximum removal rate of sCOD (g sCOD $m^{-2}\ d^{-1}$) ranked from greatest to least in the order: PVC-L > PP-H > PP-L > PVC-H (Fig. 2A). The removal rate of sCOD at the low and medium loadings were similar between media ($p > 0.05$). However under high OLR, the PVC-L

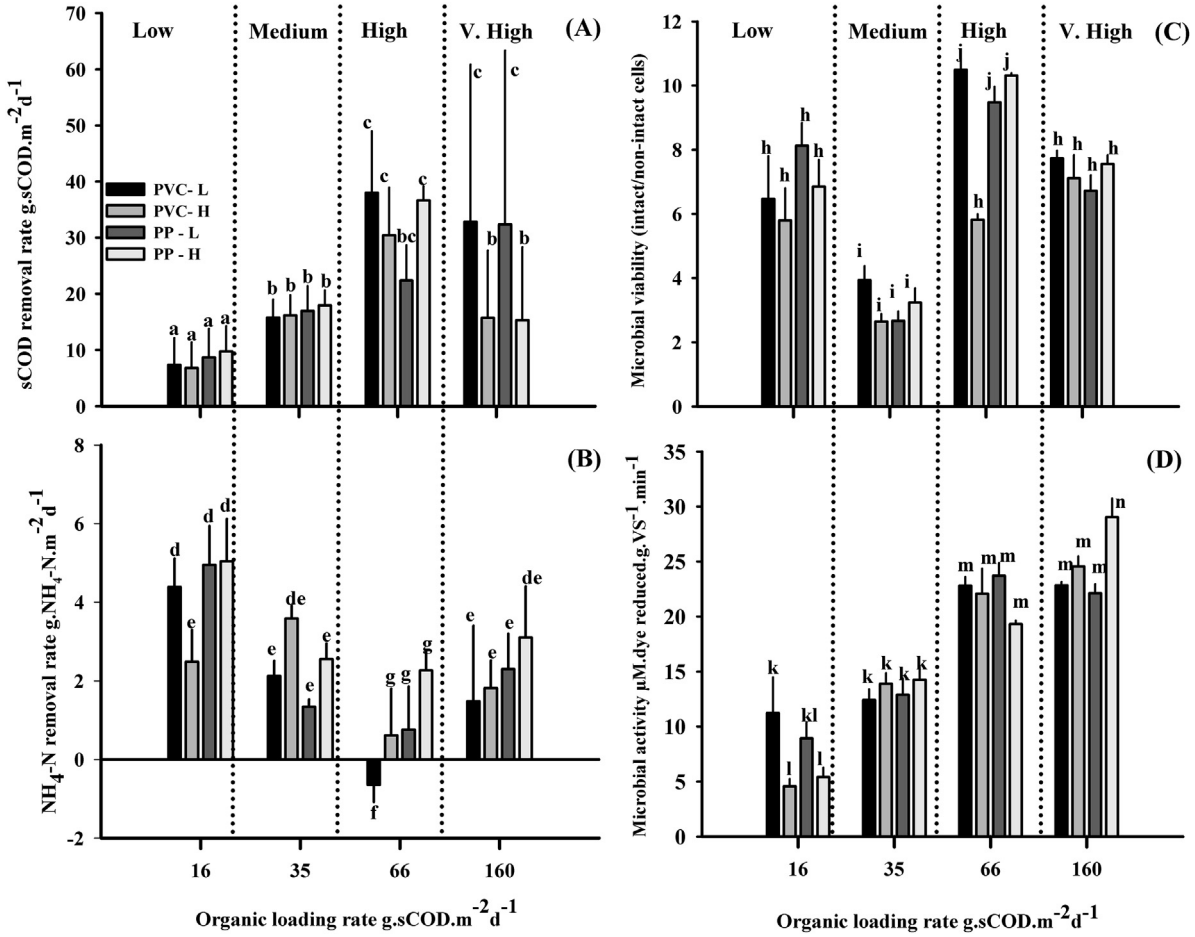

Fig. 2 – Performance mesh media for bulk organics and nitrification biomass 'viability' measures. (A) sCOD removal. (B) NH_4-N removal. (C) Microbial viability – measured by membrane integrity (ratio of intact over non-intact cells). (D) Microbial activity (moles dye reduced per minute) of biofilm from each media at different OLR error bars indicate ±standard deviation of repeat measurements. Like letters indicate no significant differences ($p > 0.05$) when comparing between equivalent treatments.

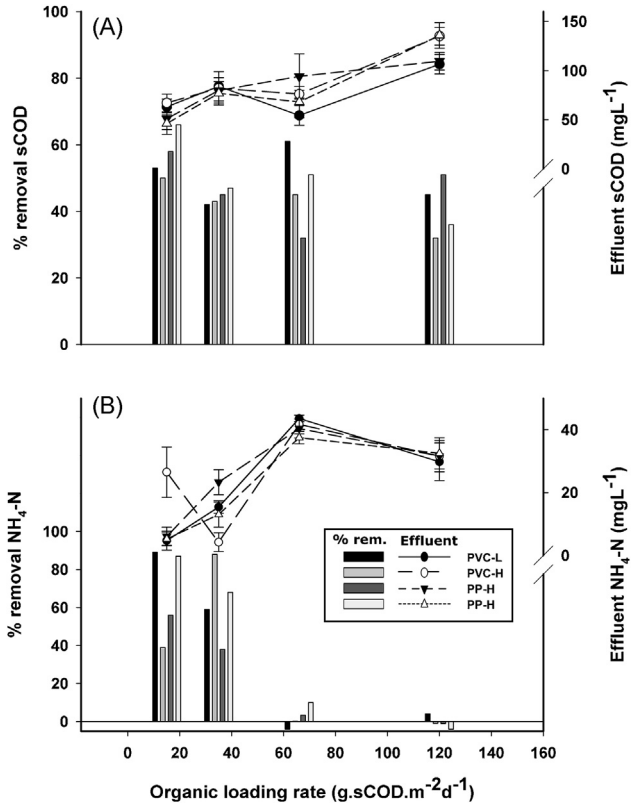

Fig. 3 – Impact of organic loading rate on average percentage removal and effluent quality. (A) sCOD and (B) NH_4-N for mesh media. Error bars represent ±1 standard deviation of repeat measurements.

significantly outperformed the PP-L with sCOD removal rates of $38 \pm 11.1\,g\,m^{-2}\,d^{-1}$ compared to $22.4 \pm 6.3\,g\,m^{-2}\,d^{-1}$ ($p = 0.038$). The removal efficiency decreased from low to very high OLR (Fig. 3A). This has been reported previously and can be attributed to mass transfer, kinetics and hydrolytic restrictions resulting in competition for resources or space (Hiras et al., 2004; Hassard et al., 2014).

The COD removal rate decreased by 15, 48 and 58% for PVC-L, PVC-H and PP-H respectively as OLR increased from high to very high. In contrast the PP-L media organic removal rate increased by 31%, although the consistency of this removal decreased (Fig. 2A). At very high OLR the sCOD removal rate was different between media ($p = 0.035$); however, pairwise comparisons revealed that differences between individual media alone did not account for all this difference. However, the low surface area mesh exhibited double the removal rate of $\sim 31\,g\,sCOD\,m^{-2}\,d^{-1}$ compared to $\sim 16\,g\,sCOD\,m^{-2}\,d^{-1}$ for the high surface area mesh ($p = 0.024$, one-way ANOVA on pooled removals) as media clogging reduced effective media/biofilm contact., Deliberate air scouring could allow for operation at higher OLRs, analogous to backwashing in submerged growth biofilm processes (Mendoza-Espinosa and Stephenson, 1999). Wijeyekoon et al. (2004) found that a wastewater biofilm had greater density and was increasingly resistant to shear under high OLR conditions resulting in clogging and reduced performance. Porous media clogging occurs under higher biofilm OLR and lower pore flow rate resulting in media bridging (Kim et al., 2010). The high surface area, low porosity mesh medias (PVC-L, PP-L) are therefore most appropriate for the pre-treatment application at the high and very high OLRs (Table 1 and Fig. 2).

The biofilm mass increased with OLR for most media studied except at the highest OLR (Table 3). The proportion of volatile solids ranged from 80.6 to 82% at the low OLR, which increased to >90% for higher OLRs. The PVC-L had significantly greater biofilm growth compared to other media tested ($p < 0.05$). The biofilm amount depends on media surface properties, packing density, shear conditions, attachment/detachment rates and grazing (Morgenroth and Wilderer, 2000). The biofilm did not accumulate in proportion to OLR as might be expected (Table 3). From low to medium OLR the biofilm mass increased by 78%, 22% and 39% on average for PVC-L, PVC-H and PP-H respectively, whilst the PP-L biofilm mass decreased by 60%. Grazing pressure could have reduced biomass concentrations at this OLR (Bryers, 2000). Microscopic observation noted visible aggregations of micro-fauna, likely to be nematodes suggesting grazing by higher organisms could have contributed to the decreased VS at this OLR. Low biofilm thickness and waste sludge production along with increased effective biofilm OLR are likely under this scenario (Chen et al., 2006). This biofilm data confirmed the significant impact of operating conditions as opposed to mesh properties at high OLR for bulk organics removal.

Ammonia removal decreased significantly ($p < 0.05$) for all media as the OLR was increased from low through medium to very high (Fig. 2B), suggesting that nitrification was inhibited. Indeed, whilst ammonia removal was evident for at the low and medium OLRs, it was effectively zero at the high and very high OLRs (Fig. 3B). This was confirmed by a lack of NO_2-N and NO_3-N in the effluent (Table 3). This has been observed previously in suspended growth and biofilm reactor systems (Hiras et al., 2004). The suppression of nitrifying bacterial growth by competition from heterotrophs and inhibition through inefficient oxygen transfer is widely accepted as the dominant mechanism in biofilm reactors (Wijeyekoon et al., 2004; Di Palma and Verdone, 2009). Under medium OLRs, the media with high specific surface area is recommended to encourage nitrification, as evidenced by the higher effluent NO_3-N concentrations that averaged $30.9\,mg\,L^{-1}$ and $23.4\,mg\,L^{-1}$ for PVC-H and PP-H respectively compared to $18.5\,mg\,L^{-1}$ and $11.8\,mg\,L^{-1}$ for PVC-L and PP-L respectively. The same recommendation can be made for low OLRs. The COD:NH_4-N ratios were 2.8, 5.2, 3.3 and 5.3 during the experimental periods for low, medium, high and very high OLR respectively due to the natural variability in the real settled wastewater used as influent. Okabe et al. (1996) found that an increased C:N ratio from 0 to 1.5 led to stratification between functional groups in an RBC biofilm, whereby heterotrophs outcompeted the nitrifiers in the outer layers due to elevated growth rates. Therefore such conditions were met at all OLRs, expected as the RBRs were operating at as pre-treatment stages, resulting in the sub-optimal nitrification observed.

3.2. *Biofilm accumulation, viability and activity*

The average roughness of the media was 8.7, 11.7, 23.2 and 26.7 nm for PVC-L, PVC-H, PP-L and PP-H respectively (Table 1); therefore PP had the rougher surface. Recent evidence has suggested that increased media roughness can improve biofilm accumulation (Stephenson et al., 2013) and other microscale properties such as surface energy, charge density and wettability can influence biofilm adhesion and performance (Lackner et al., 2009; Khan et al., 2013). The PVC-L mesh media tested here had superior biofilm accumulation, as measured by VS, compared to the PVC-H and both of the PP media (paired *t* test,

Table 3 – Selected wastewater nitrogen constituents and biofilm parameters for each RBR media under incrementally increasing OLR.

Parameter ($mg\,L^{-1}$)	OLR							
	Low				Medium			
	PVC-L	PVC-H	PP-L	PP-H	PVC-L	PVC-H	PP-L	PP-H
TN_e	47.4 ± 4	45.7 ± 9	46.5 ± 5	47 ± 5	46.7 ± 7	40.0 ± 1	47 ± 8	47 ± 16
NO_2-N_e	3.4 ± 0.2	2.8 ± 1.4	2.6 ± 0.8	3.9 ± 1.9	2.9 ± 1	2.9 ± 0.8	2 ± 1.2	3.0 ± 0.4
NO_3-N_e	24.4 ± 4	10.3 ± 3	30.4 ± 5	27.7 ± 4	18.5 ± 2	30.9 ± 9	11.8 ± 4	23.4 ± 2
Biofilm VS ($g\,m^{-2}$)	36.9	30	65.8	40	157	38.4	26.6	65.8
Biofilm TS ($g\,m^{-2}$)	44.9	36.9	80.9	49.6	168.4	48.5	28.4	71.2

Parameter ($mg\,L^{-1}$)	OLR							
	High				Very high			
	PVC-L	PVC-H	PP-L	PP-H	PVC-L	PVC-H	PP-L	PP-H
TN_e	52.3 ± 1	50 ± 2	51 ± 17	47 ± 9	43.3 ± 4	42 ± 4	43 ± 4	40 ± 3
NO_2-N_e	1 ± 0.2	1 ± 0.3	1.2 ± 0.2	1.6 ± 0.5	1.3 ± 0.2	1.8 ± 0.2	1.9 ± 0.3	1.5 ± 0.2
NO_3-N_e	2.2 ± 1	2.4 ± 0.5	2.8 ± 1	5 ± 0.5	1.8 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.9 ± 0.2
Biofilm VS ($g\,m^{-2}$)	569.7	290.8	74.7	308.4	1499.2	501.2	325.9	629.6
Biofilm TS ($g\,m^{-2}$)	573.8	299.9	75.8	262.1	1514.8	528.4	351.9	661.9

$p < 0.05$); biofilm accumulation on this mesh increased incrementally with loading (Table 3). The PP-L mesh had similar biofilm accumulation at low OLR but lower biofilm accumulation at medium, high and very high OLR (paired t test, $p < 0.05$). However, there was no significant overall trend in biofilm accumulation rate with changes in mesh material type or porosity, nor microscale properties. As previously noted, this further confirms the significant impact of operating conditions as opposed to mesh properties at high OLR for bulk organics removal. In practical terms, the media filament linear mass density, which was least for the PP (Table 1), could influence selection for scale-up when considering the energetic costs of rotation (Mba et al., 1999).

The OLR and media type can influence viability of bacteria, and therefore biofilm growth and decay processes (Okabe et al., 1996; van der Mei et al., 2008). In this study the microbial viability ratio of live:dead ranged from 2.6 to 10.5 at medium and high OLR respectively (Fig. 2C) and was positively correlated to OLR ($R^2 = 0.81$, $p < 0.001$). The greatest microbial viability coincided with the highest removal rates of bulk organics under high OLR. Multiple regression analysis confirmed that viable microbial abundance correlated with the sCOD removal performance in biofilm reactors ($R^2 = 0.29$, $p < 0.001$). In this study the microbial viability decreased from low to medium OLR by 39, 54, 67 and 52% for PVC-H, PVC-L, PP-H, PP-L respectively (Fig. 2D) which could be due to higher organism grazing resulting in cellular fragmentation. A functional shift from a nitrifying, carbon limited biofilm to a heterotrophic oxygen limited biofilm one could result in temporary suppressed viability or growth rate as conditions became less conducive for the previous community (Fig. 2C and D). Further study could elucidate whether microscale deficiency in substrate or electron acceptor could account for greater microbial decay in wastewater biofilms (Okabe et al., 1996), which often precedes loss of treatment.

The data from the current study suggested that media surface properties may have little influence on the microbial viability during long term reactor operation. Further information is required to test if these media influence the viability or activity during initial adhesion (Busscher et al., 1995). The presence of a conditioning film could mask the impact of surface properties by acting as a barrier to chemical and spatial heterogeneity. In contrast Singh et al. (2011) suggested that the film provides a link between the media surface and bacteria. Importantly, after initial adhesion has occurred, additional bacterial accumulation will occur through the biofilm surface and suspended particle interaction, and not just media–biofilm interactions (Bryers, 2000, Hassard et al., 2015).

The microbial activity of the biofilm increased with OLR ($R^2 = 0.92$, $p < 0.001$). At low OLR, the low surface area media PVC-L and PP-L had 2.4× and 1.9× more microbial activity than PVC-H and PP-H respectively ($p = 0.002$, Fig. 2D). This could be due to less surface area for initial colonisation resulting in locally denser communities (Jurecska et al., 2013). However, as OLR increased from low to medium, this effect was masked as the microbial activity increased by 41% on average between media, although the increase was greatest for high surface area media (Fig. 2D). This suggested that the microbial activity in this study was dependent on OLR, not media properties ($p < 0.05$). As OLR increased from medium to high OLR, the microbial activity increased from a minimum of 12.4 to a maximum of 23.7 μM dye reduced $g\,VS^{-1}\,min^{-1}$ for PVC-H and PP-H respectively (Fig. 2D). However further OLR increases did not result in greater microbial activity for most media. The microbial activity positively correlated with the sCOD removal performance ($R^2 = 0.4$, $p < 0.05$) in mesh media reactors. The strength of this correlation was significantly greater than that of major process variables such as OLR and nitrogen loading rate (Table 4). However neither activity nor viability were important for NH_4-N removal rate. This confirms the importance of having an active, viable biofilm for wastewater treatment, particularly for bulk organics removal.

Okabe et al. (1996) showed that after long term operation 30% of the cells in the biofilm become physiologically inert, probably due to inactivation, decay or competition effects. In contrast, the current study showed that more active microbial communities were selected for at higher OLRs, which rapidly utilise available soluble substrates. However, this maximum activity remained unrealised due to mass transfer limitations in RBRs, which has been documented in other biofilm reactors (Di Palma and Verdone, 2009).

Table 4 – Multiple linear regression (MLR) output (β) coefficients for pooled removals, activity and viability for all mesh media.

	β coefficient	
Model (dependent variable →)	sCOD, $R^2 = 0.36^a$	NH_4-N, $R^2 = 0.49^a$
Independent variables		
OLR	-0.15^c	0.09^d
Nitrogen loading rate	0.16^b	-0.83^a
Activity	0.40^c	0.08^d
Viability	0.29^b	-0.05^d

Significance of MLR model, a ≤ 0.001, b ≤ 0.01, c ≤ 0.05, d > 0.05.

4. Conclusions

- Media with a specific surface area of ~150 $m^2\,m^{-3}$ performed the best for roughing application at high and very high OLR for RBRs. The nitrification rate was greatest at OLRs of 35 g sCOD $m^{-2}\,d^{-1}$.
- The PVC-L media demonstrated enhanced ability to support biofilm compared to other media studied. Particularly low biofilm accumulation was found for PP-L.
- The activity and viability positively correlated with sCOD removal performance ($R^2 = 0.4$ and 0.29).
- Lower surface area mesh is recommended for wastewater pre-treatments at high OLR, whilst high surface area mesh is recommended for elevated nitrification rates at medium OLR.

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