

Performance of permeable media rotating reactors used for pretreatment of wastewaters

F. Hassard, E. Cartmell, J. Biddle and T. Stephenson

ABSTRACT

The impact of organic loading rate (OLR) on carbonaceous materials and ammonia removal was assessed in bench scale rotating media biofilm reactors treating real wastewater. Media composition influences biofilm structure and therefore performance. Here, plastic mesh, reticulated coarse foam and fine foam media were operated concurrently at OLRs of 15, 35 and 60 g sCOD m⁻² d⁻¹ in three bench scale shaft mounted advanced reactor technology (SMART) reactors. The sCOD removal rate increased with loading from 6 to 25 g sCOD m⁻² d⁻¹ ($P < 0.001$). At 35 g BOD₅ m⁻² d⁻¹, more than double the arbitrary OLR limit of normal nitrifying conditions (15 g BOD₅ m⁻² d⁻¹); the removal efficiency of NH₄-N was 82 ± 5, 27 ± 19 and 39 ± 8% for the mesh, coarse foam and fine foam media, respectively. Increasing the OLR to 35 gm⁻² d⁻¹ decreased NH₄-N removal efficiency to 38 ± 6, 21 ± 4 and 21 ± 6%, respectively. The mesh media achieved the highest stable NH₄⁺-N removal rate of 6.5 ± 1.6 gm⁻² d⁻¹ at a sCOD loading of 35 g sCOD m⁻² d⁻¹. Viable bacterial numbers decreased with increasing OLR from 2 × 10¹⁰–4 × 10⁹ cells per ml of biofilm from the low to high loading, suggesting an accumulation of inert non-viable biomass with higher OLR. Increasing the OLR in permeable media is of practical benefit for high rate carbonaceous materials and ammonia removal in the pretreatment of wastewater.

Key words | ammonium removal, media, organic loading, rotating biological contactor, viability, wastewater treatment

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INTRODUCTION

The main priorities for wastewater treatment are effluent quality, cost, energy efficiency and nutrient removal/recovery (STOWA 2010). In traditional biological treatment, the achievable effluent standard is largely dependent on the energy applied through aeration and extended reactor retention time. This increases the cost to the operator/consumer and the environmental impact of treatment (Vanrolleghem *et al.* 1996). These challenges are important when commissioning or upgrading wastewater treatment plants. To achieve discharge limits with financial constraints it is imperative to optimize process operation. A biofilm technology known as shaft mounted advanced reactor technology (SMART) has shown promise in addressing these challenges (Hoyland *et al.* 2010). These units are similar to rotating biological contactors (RBCs) but the media composition is permeable with heterogeneous architecture and a high porosity, which is thought to overcome the limitations of RBC-like reactors (Chen *et al.* 2006). The SMART units

usually operate in conjunction with an activated sludge process collectively known as hybrid activated sludge. The SMART unit operates as a roughing bulk carbon oxidation step with nutrient removal followed by clarification in the activated sludge stage (Hoyland *et al.* 2010). In this study, the impact of novel media as a roughing stage was compared for the first time against other high specific surface area, high porosity, commercially available alternatives.

The organic loading rate (OLR) is the principal parameter when deploying a rotating biofilm reactor (RBR) (Cortez *et al.* 2008) and impacts on biofilm treatment performance (Ayoub & Saikaly 2004). A traditional RBR process should have an overall maximum surface loading of 15 g BOD₅ m² d⁻¹ to achieve simultaneous biochemical oxygen demand (BOD₅) removal and nitrification (Rittmann & McCarty 2001). However, SMART units are designed as a 'roughing step' to rapidly remove BOD₅, to reduce load for the secondary treatment processes and improve the nitrogen

removal capacity of the system. In RBRs the microbial population is attached on a solid medium and mean cell residence time is independent of hydraulic residence time (HRT), which allows greater flow rates, organic loadings and process stability than is possible in suspended culture systems (Cortez *et al.* 2008). The addition of a roughing step to a wastewater treatment plant can improve the stability of a process that has strong or variable loading, increase capacity or improve the achievable effluent standard (Hoyland *et al.* 2010).

Common to most biofilm processes is an inert solid support medium on which the microbial community grows (Stephenson *et al.* 2013). The physical composition and architecture of the medium has an impact on the biofilm and the removal rate of both BOD₅ and ammonia (Tawfik & Klapwijk 2010). Biofilm processes have unique features that affect their biofilm structure, microbial composition and therefore substrate utilization (Wuertz *et al.* 2004). The biofilm biomass, usually expressed through the concentration of volatile solids, does not measure biological information about the health, viability or activity of the biofilm (Ziglio *et al.* 2002). The use of viability as an indicator is useful for determining the impact of operating conditions on biofilm bacterial performance. The objectives were to establish the impact of OLR on the substrate removal rate and to assess how the media type affects this dependency. To elucidate this relationship, the viability of the microbial population was used to evaluate the bacterial viability within the biofilm at different surface OLRs.

MATERIALS AND METHODS

Pilot studies at varying OLRs

Three bench scale SMART units were situated at Cranfield University sewage treatment works; each consisted of a plastic vessel and a single rotating shaft with permeable plastic frames for housing the media. The media consisted of circular plates of a PVC-derived mesh, polyester reticulated foam and polyurethane reticulated foam with specific surface area of ± 450 , ± 800 and $\pm 1,000 \text{ m}^{-2} \text{ m}^{-3}$ – henceforth called mesh, coarse foam and fine foam, respectively. 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%). The SMART units were operated at a constant tip speed of 0.08 m/s (8 rpm); at this speed, the rpm itself is unlikely to limit the substrate removal rate (Di Palma & Verdone 2009). The SMART units were fed with

Table 1 | Operating conditions of the SMART units

Load	HRT (d)	Flow rate (Ld ⁻¹)	sCOD loading rate (g m ⁻² d ⁻¹)	NH ₄ -N loading rate (g m ⁻² d ⁻¹)	COD/N (g COD g ⁻¹ N)
Low	0.115	26	15 ± 0.37	4 ± 0.14	3.25 ± 0.32
Med.	0.057	53	35 ± 2.51	10 ± 1.19	3.34 ± 0.17
High	0.028	106	60 ± 3.30	14 ± 0.27	4.35 ± 0.09

real settled sewage and were operated for a minimum of 3 weeks prior to monitoring to ensure pseudo-steady state conditions. Different OLRs were applied to each reactor: 15, 35 and 60 g sCOD m⁻² d⁻¹, which corresponded to 1.1, 2.2 and 4.4 L of settled sewage per hour (Table 1). To achieve stable nitrification, a surface loading of <15 g BOD₅ m⁻² d⁻¹ is recommended for biofilm processes (Rittmann & McCarty 2001). The equivalent BOD₅ loading rates were ~35, 81 and 140 g BOD₅ m⁻² d⁻¹ based on an average sCOD: BOD₅ ratio of 1:2.3 from influent wastewater from the study period ($n = 78$) for low, medium and high OLRs, respectively. The impact of OLR on trial media for SMART units was assessed at ~2X (low), 5X (medium) and 10X (high) this value, as these units have high voidage and are used in a roughing configuration (Table 2). The removal rates of sCOD and NH₄⁺-N are compared to other process characteristics. The total operating time for the study was 9 months with, approximately 3 months at each loading. The SMART reactors were temperature controlled to 15 °C using a 50 W thermostatic aquarium heater (Superfish, UK).

Wastewater analysis and calculations

Influent samples were collected at 09:00, with effluent samples collected at 1, 2 and 4 h post-influent sampling depending on the OLR studied. Wastewater was analysed using proprietary cell test kits (VWR, UK) for total chemical oxygen demand (tCOD) and total nitrogen (TN). The wastewater was filtered through a 1.2 µm glass fibre filter (Whatman, UK). The sCOD ammonia-nitrogen (NH₄⁺-N), nitrite-nitrogen (NO₂-N) and nitrate-nitrogen (NO₃-N) were measured using a NOVA60 photometer (VWR, UK). Total suspended solids (TSS) and volatile suspended solids were measured according to *Standard Methods* (APHA 1998). The dissolved oxygen (DO) of the effluent was measured using an HQ30d DO probe (Hach, Germany) and the pH of the influent and effluent was measured using a Jenway 320 pH meter (Bibby, UK). The COD was used to assess the OLR applied as it is the more fundamental

Table 2 | Wastewater characteristics of influent feed and effluent of SMART units and performance running settled sewage at different OLR $\pm 95\%$ confidence interval

OLR parameter (mgL ⁻¹)	Low			Medium			High		
	Mesh	Coarse foam	Fine foam	Mesh	Coarse foam	Fine foam	Mesh	Coarse foam	Fine foam
tCOD _i	450 \pm 82	440 \pm 60	459 \pm 67	297 \pm 33	268 \pm 39	300 \pm 41	345 \pm 43	347 \pm 53	385 \pm 60
tCOD _e	126 \pm 27	98 \pm 60	134 \pm 36	135 \pm 24	163 \pm 26	189 \pm 32	221 \pm 36	183 \pm 60	250 \pm 48
sCOD _i	85 \pm 13	80 \pm 10.0	85 \pm 9	117 \pm 15	109 \pm 35	129 \pm 21	106 \pm 15	99 \pm 17	93 \pm 13
sCOD _e	44 \pm 8	40 \pm 6	53.4 \pm 11	73 \pm 12	86 \pm 13	101 \pm 17	63 \pm 8	67 \pm 11	67 \pm 12
TN _i	45 \pm 4	44 \pm 4	42 \pm 4	52 \pm 9	50 \pm 8	53 \pm 8	42 \pm 3	42 \pm 2	42 \pm 2
NH ₄ ⁺ -N _i	28 \pm 4	26 \pm 5.5	26 \pm 3.5	36 \pm 10	35 \pm 11	29 \pm 11	24 \pm 3	24 \pm 2	23.1 \pm 2
NH ₄ ⁺ -N _e	5.3 \pm 1.5	22 \pm 7	23 \pm 7	14 \pm 6	30 \pm 9	39.2 \pm 12	23 \pm 3	23 \pm 2	21.0 \pm 2
NO ₃ ⁻ -N _e	18.5 \pm 2	1.5 \pm 0.25	2.0 \pm 0.25	6.5 \pm 1.25	1.0 \pm 0.25	1.5 \pm 0.5	1 \pm 0.25	1 \pm 0.25	1 \pm 0.5
DO _e	8.4 \pm 1.3	8.6 \pm 0.4	7.0 \pm 1.0	8.3 \pm 0.3	7.4 \pm 0.2	6.4 \pm 0.6	4.5 \pm 0.3	4.9 \pm 0.5	3.4 \pm 0.4
TSS _i	213 \pm 56	222 \pm 38	154 \pm 23	150 \pm 33	138 \pm 23	124 \pm 23	177 \pm 29	161 \pm 21	187 \pm 35
TSS _e	71 \pm 13	56 \pm 16	77 \pm 44	25 \pm 15	34 \pm 11	53	93 \pm 17	82 \pm 15	103 \pm 23

parameter compared to the BOD₅ test (Roeleveld & van Loosdrecht 2002). The media nominal surface area (A_{nominal}) and OLR were calculated according to Equations (1) and (2). Nominal rates were selected, as specific surface area is less important under high biofilm growth conditions such as the OLRs studied. The removal efficiency and substrate removal rate were calculated normally

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

$$\text{OLR} = S_i \times Q_i / A_{\text{nominal}} \quad (2)$$

where r is the radius of the plate, h is the plate thickness, S_i = influent substrate concentration (sCOD or NH₄⁺-N) and Q_i is influent flowrate.

Microbial viability

Wastewater biofilm was sampled from the media surface using a 10 mL sterile plastic bottle at the same time as effluent samples. A dilute, dispersed cellular fraction was obtained according to Ziglio *et al.* (2002). A 5 mL sub-sample was mechanically disaggregated using a homogeniser (Powergen 125, Fisherbrand, UK) for 10 min at speed setting 2 (12,250 1/min). Samples were diluted using 0.22 μm filter sterilised NaCl solution (0.085%) (Boulos *et al.* 1999). The samples were then disaggregated for a further 5 min to obtain the maximum number of viable bacteria (Ziglio *et al.* 2002). Differential centrifugation 180 \times g ($r_{\text{max}} = 3.5$, microcentaur, MSEUK, UK) was used to separate the bacteria (in the supernatant) from the solids and extracellular debris (pellet) (Lunau *et al.*

2005). The bacteria were diluted to give approximate bacterial numbers per field of view. Bacterial samples were stained with LIVE/DEAD[®] BacLight[™] test (Invitrogen, Glasgow, UK) according to the manufacturer's guidelines with modifications according to Boulos *et al.* (1999). The bacterial sample was then vacuum filtered onto a black polycarbonate membrane filter (0.22 μm pore size, 25 mm diameter; Nucleopore, Whatman, UK). The filter was washed with sterilised NaCl solution (0.085%) and mounted on a microscope slide with a drop of LIVE/DEAD[®] BacLight[™] mounting oil, and a coverslip was placed over the filter and fixed with clear nail polish (Rimmel London, UK). Cells were viewed under oil immersion on an LSM 510 META confocal laser scanning microscope (Carl Zeiss, Inc., Germany) with Axiovision software. Images for cell counts were acquired using a Zeiss LSM camera. Image processing with the imageJ program (Abramoff *et al.* 2004) was used to prepare images for counting. The number of viable (green) and dead (red) bacteria was then calculated, taking dilutions into account.

Experimental design

The performance aspect of this study was based on a pilot ($n = 10$); the averages and standard deviations were used to calculate the sample size using G*power 3. There were 12 and 11 samples per group for sCOD and NH₄⁺-N (95% confidence interval, 50% power), respectively. A balanced statistical design was used and the assumptions of parametric statistics were met. The grouped data of loading and media data were normally distributed Kolmogorov-Smirnov, sCOD $p > 0.01$; NH₄⁺-N $p > 0.01$). To test if

whether there was a difference in the removal rate of sCOD/ $\text{NH}_4^+\text{-N}$ with both organic loading and media type, separate one way analysis of variance (ANOVA) was undertaken (SPSS, IBM, USA). The viability test was performed on the last 3 days of performance data. Each sample was analysed in triplicate and incorporated recommendations by Lisle & Hamilton (2004) to achieve representative counts.

RESULTS AND DISCUSSION

Effect of OLR on sCOD removal

The removal rate of sCOD was ranked from greatest to least mesh > coarse foam > fine foam for all studied loadings except for the medium OLR, where the fine foam had a higher removal rate of $8 \text{ gm}^{-2} \text{ d}^{-1}$ compared to $6.5 \text{ gm}^{-2} \text{ d}^{-1}$ for the coarse foam (Figure 1(a)). The mesh and the coarse foam media had significantly higher removal rates at this loading ($p < 0.05$). The mesh media achieved superior sCOD removal of 6, 14 and $26 \text{ gm}^{-2} \text{ d}^{-1}$ at low, medium and high loads, respectively (Figure 1). The foam media sCOD removal rate did not increase in proportion to OLR, unlike the mesh media. Biofilm bridging probably reduced the active biofilm surface area and reduced the transfer of oxygen and substrates (Gujer & Boller 1990). At a higher OLR, the removal rate of the permeable media increased but the removal efficiency decreased, which has been noted previously (Hiras *et al.* 2004). Sayess *et al.* (2013) achieved consistently high COD removal efficiency of >86% at loadings of 5 or $10 \text{ gm}^{-2} \text{ d}^{-1}$ in RBC systems. Chen *et al.* (2006) obtained an increase in removal rate from 30 to $38 \text{ g tCOD m}^{-2} \text{ d}^{-1}$ with increasing OLR. Di Palma & Verdone (2009) found that $23 \text{ g TOC m}^{-2} \text{ d}^{-1}$ was the OLR threshold, after which the removal rate of organic carbon decreased due to reduced oxygen transfer. However, increasing rotational speed reduced this effect. Most of these studies utilised synthetic sewage, which is more readily biodegradable than real sewage, so results should be compared with caution. At a volumetric organic loading rate (vOLR) of $4.0 \text{ kg sCOD m}^{-3} \text{ d}^{-1}$ the mesh media achieved a reactor volumetric removal rate of $1.4 \text{ kg sCOD m}^{-3} \text{ d}^{-1}$, which is similar to the upper limit for high-rate suspended growth systems that have vOLR from $1.5\text{--}3 \text{ kg BOD}_5 \text{ m}^{-3} \text{ d}^{-1}$ (WEF 1998). Roughing trickling filters are operated with a vOLR of $>1.5 \text{ kg m}^{-3} \text{ d}^{-1}$ with a reported removal rate of $<1 \text{ kg m}^{-3} \text{ d}^{-1}$ (WEF 2000). The SMART unit achieves a higher removal rate with a lower footprint, without excessive odour or flies.

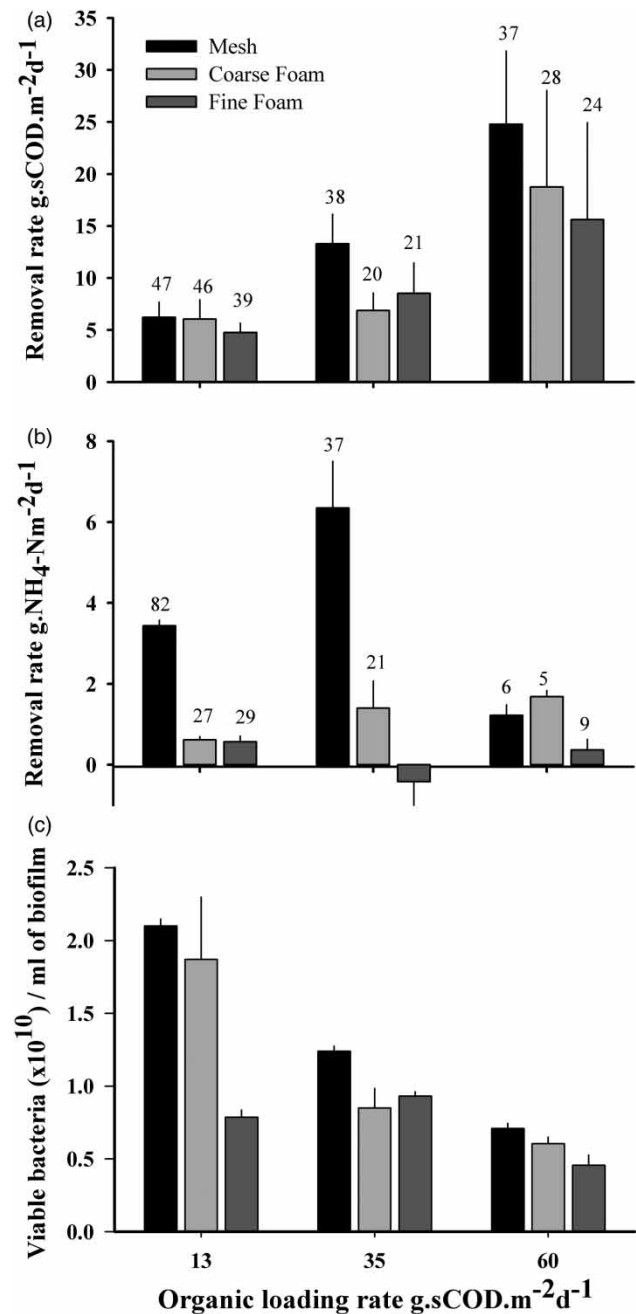


Figure 1 | (a) Surface removal rate of sCOD ($\text{g sCOD m}^{-2} \text{ d}^{-1}$) related to OLR. Error bars indicate $\pm 95\%$ confidence intervals (based on SEM and $n = 12$). Numbers above bars are percentage removal: (b) Removal rate of $\text{NH}_4^+\text{-N}$ ($\text{g NH}_4^+\text{-N m}^{-2} \text{ d}^{-1}$) related to OLR. Error bars indicate $\pm 95\%$ confidence intervals (based on SEM and $n = 11$). (c) Viable 'membrane' 'intact' and bacterial counts per ml of biofilm with OLR. Error bars indicate ± 1 standard deviation from the mean.

Effect of OLR on nitrogen oxidation

The mesh media achieved the highest ammonia removal rate of 3.8 and $6.5 \text{ gm}^{-2} \text{ d}^{-1}$ at the low and medium OLRs, which was greater than the foam media ($p < 0.05$). The mesh media

achieved greater sCOD and $\text{NH}_4^+\text{-N}$ removal and stable nitrification at OLR of up to $35 \text{ g sCOD m}^{-2} \text{ d}^{-1}$ (Figure 1(b)). Chen et al. (2006) achieved a first stage removal of $1.9 \text{ g.NH}_4^+\text{-N}$ removal, at a COD loading of $51 \text{ g tCOD m}^{-2} \text{ d}^{-1}$ at 2 hours HRT. There was little or no TN removal as no solids separation was incorporated in the SMART units. The mesh media exhibited three times the $\text{NH}_4^+\text{-N}$ removal at double the soluble COD concentration than previously reported for traditional solid disc RBCs fed with real municipal wastewater. An incremental increase in OLR reduced the $\text{NH}_4^+\text{-N}$ removal rate by a factor of 4.3; however, an increase in COD/N ratio could have exacerbated this (Table 1). At the high OLR, nitrifiers incur greater mass transport resistance and physical competition from heterotrophs (Okabe et al. 1996); these performance data for novel media suggest that a washout, selection or inhibition mechanism occurred (Gujer & Boller 1990). Ayoub & Saikaly (2004) showed that the negative effects of OLR on $\text{NH}_4^+\text{-N}$ removal efficiency can be reduced using step feeding and an internal recycle. To provide nitrification, a trickling filter OLR should be $<0.5 \text{ kg BOD}_5 \text{ m}^{-3} \text{ d}^{-1}$ (WEF 2000). The maximum $\text{NH}_4^+\text{-N}$ reactor agreed volumetric removal rate (VRR) of the mesh media was $0.4 \text{ kg sCOD m}^{-3} \text{ d}^{-1}$ at a vOLR of $2.1 \text{ kg sCOD m}^{-3} \text{ d}^{-1}$.

Bacterial viability with OLR

The Baclight test is one of the most conservative indirect measures of viability and has been applied previously to wastewater matrices such as activated sludge and RBC biofilms (Ziglio et al. 2002). In this study, the number of viable cells ranked from mesh > coarse foam > fine foam for all studied loadings except for $35 \text{ g sCOD m}^{-2} \text{ d}^{-1}$ (Figure 1(c)). This reflected the sCOD removal data, as 8 and 6 $\text{g sCOD m}^{-2} \text{ d}^{-1}$ were removed from the fine foam and coarse foam, respectively (Figure 1(a)). The viable cell number ranged from 2×10^{10} – 4×10^9 cells per ml at the lowest loading for the mesh media and highest loading for the fine foam media respectively (Figure 1(c)) (Table 2). The viable bacterial cell number declined by a factor of 2.9, 2.7 and 1.6 from low to high loading for the mesh media. However, the sCOD removal rate increased by a factor of 5, 3.8 and 3.6 for mesh, coarse foam and fine foam, respectively. These data showed a strong negative correlation between removal rate and viable cell number where 92 and 73% of the variation was explained by the regression line for mesh and coarse foam, respectively ($P < 0.05$), but not for fine foam ($P > 0.05$), due to low removal rates demonstrated under all tests. If an identical quantity of biofilm was present on the media then the specific sCOD removal rate of each bacteria in the biofilm would increase by a factor of 14.5, 10.8 and 5.76

for mesh, coarse foam and fine foam, respectively. The data are consistent with the biofilm theory of reduced bacterial density with increasing OLR (Okabe et al. 1996).

The results indicated that the most permeable media is preferred to achieve stable ammonia removal and simultaneous carbon oxidation at the lowest OLR (Figures 1(a) and 1(b)), even though the specific surface area for attachment and biomass thickness is lower. The biofilm substrate removal rate increases with biomass thickness up to a critical thickness, after which the rate of oxygen transfer is greater than the rate of oxygen respired (Stewart & Franklin 2008). Oxygen will become limiting and a further increase in biofilm thickness will not improve the removal rate (Arvin & Harremoës 1990). The mesh media with more open architecture allowed greater sCOD and ammonia removal while propagating conditions for a higher viable bacteria cell number (Figures 1(a)–1(c)). It is reasonable to suggest that open structure, non-uniform media would enhance the physical oxygen transfer to the biofilm compared to more closed pore foam alternatives. The inverse association between media specific surface area and porosity appears less important when considering OLR under high load scenarios as biofilm bridging reduces transfer and limits bacterial activity. There was growth of *Beggiatoa*-like species at loadings $>35 \text{ g sCOD m}^{-2} \text{ d}^{-1}$; this component of the biofilm was too large to feature in the viability analysis and contributed to the debris. Therefore, as the viability of the biofilm decreased, the amount of biomass could have increased, explaining the greater performance even though average cellular activity could be reduced. The results presented suggest that with increasing OLR, the concentration of viable cells decreases. Further study of the architectural properties of the media and the activity of the biofilm could further elucidate this trend with reference to performance. However, an association between operating conditions and biofilm physiology was noted. In full scale SMART reactors, a periodic air sparge controls the biofilm bridging of media; this method could be used to control biofilm growth rate analogous to sludge wasting in activated sludge systems.

CONCLUSIONS

- Increasing the OLR in all the permeable media resulted in greater removal rates, but lower percentage removal efficiency.
- The mesh media exhibited the highest ($6 \text{ g.NH}_4^+\text{-N m}^{-2} \text{ d}^{-1}$) ammonium removal rates, even at soluble sCOD loads of $35 \text{ gm}^{-2} \text{ d}^{-1}$ and low HRT.

- Media porosity was similar for the permeable media studied; the architecture and sizes of the apertures appeared to impact the maximum removal rate.
- The viability of the bacteria in the biofilm had a significant negative correlation with sCOD loading for the mesh and coarse foam media.
- Understanding the impact of process condition on bacterial viability could improve biofilm performance during wastewater treatment.

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REFERENCES

- Abramoff, M. D., Magalhaes, P. J. & Ram, S. J. 2004 Image processing with Image J. *Journal of Biophotonics International* **11**, 36–42.
- APHA-AWWA-WEF 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edn. Washington, DC, USA.
- Arvin, E. & Harremoës, P. 1990 Concepts and models for biofilm reactor performance. *Water Science and Technology* **22**, 171–192.
- Ayoub, G. M. & Saikaly, P. 2004 The combined effect of step-feed and recycling on RBC performance. *Water Research* **38**, 3009–3016.
- Boulos, L., Prevost, M., Barbeau, B., Coallier, J. & Desjardins, R. 1999 LIVE/DEAD[®] Bac Light[™]: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of Microbiological Methods* **37**, 77–86.
- Chen, Z., Wen, Q., Wang, J. & Li, F. 2006 Simultaneous removal of carbon and nitrogen from municipal-type synthetic wastewater using net-like rotating biological contactor (NRBC). *Process Biochemistry* **41**, 2468–2472.
- Cortez, S., Teixeira, P., Oliveira, R. & Mota, M. 2008 Rotating biological contactors: a review on main factors affecting performance. *Reviews in Environmental Science and Biotechnology* **7**, 155–172.
- Di Palma, L. & Verdone, N. 2009 The effect of disk rotational speed on oxygen transfer in rotating biological contactors. *Bioresource Technology* **100**, 1467–1470.
- Gujer, W. & Boller, M. 1990 A mathematical-model for rotating biological contactors. *Water Science and Technology* **22** (1–2), 53–73.
- Hiras, D. N., Manariotis, I. D. & Grigoropoulos, S. G. 2004 Organic and nitrogen removal in a two-stage rotating biological contactor treating municipal wastewater. *Bioresource Technology* **95**, 91–98.
- Hoyland, G., Vale, P., Rogalla, F. & Jones, M. 2010 A new approach to nutrient removal using the HYBACS process. *WEF/IWA Biofilm Reactor Technology Conference 2010* **14**, 81–94.
- Lisle, J. & Hamilton, M. 2004 Comparison of fluorescence microscopy and solid-phase cytometry methods for counting bacteria in water. *Appl. and Environ. Microbiol.* **70**, 5343–5348.
- Lunau, M., Lemke, A., Walther, K., Martens-Habben, W. & Simon, M. 2005 An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environmental Microbiology* **7**, 961–968.
- Okabe, S., Hiratia, K., Ozawa, Y. & Watanabe, Y. 1996 Spatial microbial distribution of nitrifiers and heterotrophs in mixed-population biofilms. *Biotechnology and Bioengineering* **50**, 24–35.
- Rittmann, B. E. & McCarty, P. L. 2001 *Environmental Biotechnology: Principles and Applications*, 4th edn. McGraw-Hill Higher Education, New York.
- Roeleveld, P. J. & van Loosdrecht, M. C. M. 2002 Experiences with guidelines for wastewater characterisation in the Netherlands. *Water Science and Technology* **45** (6), 77–87.
- Sayess, R. R., Saikaly, P. E., El-Fadel, M., Li, D. & Semerjian, L. 2013 Reactor performance in terms of COD and nitrogen removal and bacterial community structure of a three-stage rotating bioelectrochemical contactor. *Water Research* **47**, 881–894.
- Stewart, P. S. & Franklin, M. J. 2008 Physiological heterogeneity in biofilms. *Nature Reviews Microbiology* **6**, 199–210.
- Stephenson, T., Reid, E., Avery, L. M. & Jefferson, B. 2013 Media surface properties and the development of nitrifying biofilms in mixed cultures for wastewater treatment. *Process Safety and Environmental Protection* **91**, 321–324.
- STOWA 2010 STOWA NEWS: The Dutch roadmap for the WWTP of 2030, STOWA report 2010–24, Amersfoort, The Netherlands.
- Tawfik, A. & Klapwijk, A. 2010 Polyurethane rotating disc system for post-treatment of anaerobically pre-treated sewage. *Journal of Environmental Management* **91**, 1183–1192.
- Vanrolleghem, P., Jeppsson, U., Carstensen, J., Carlsson, B. & Olsson, G. 1996 Integration of wastewater treatment plant design and operation – a systematic approach using cost functions. *Water Science and Technology* **34**, 159–171.
- WEF 1998 *Design of Wastewater Treatment Plants*, 4th edn. Manual of Practice no. 8, Water and Environment Federation, Alexandria, VA, USA.
- WEF 2000 *Aerobic Fixed-Growth Reactors; A Special Publication*. Water and Environment Federation, Alexandria, VA, USA.
- Wuertz, S., Okabe, S. & Hausner, M. 2004 Microbial communities and their interactions in biofilm systems: an overview. *Water Science and Technology* **49**, 327–336.
- Ziglio, G., Andreottola, G., Barbesti, S., Boschetti, G., Bruni, L., Foladori, P. & Villa, R. 2002 Assessment of activated sludge viability with flow cytometry. *Water Research* **36**, 460–468.

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