Process Safety and Environmental Protection, Volume 91, Issue 4, July 2013, Pages 321–324

Media Surface Properties and the Development of Nitrifying Biofilms in Mixed Cultures for Wastewater Treatment

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ABSTRACT

Plastic was tested to select biofilm support media that would enhance nitrification in the presence of heterotrophs. Eight different types (acrylonitrile butadiene styrene, nylon, polycarbonate, polyethylene, polypropylene, polytetraflouroethylene (PTFE), polyvinyl chloride and tufnol) were immersed in an aerobic fed-batch reactor receiving domestic settled wastewater. Nitrification rates did not correlate with biomass concentrations, nor surface roughness of the plastics as measured by atomic force microscopy (AFM). The maximum nitrification rate of 1.5 g/m^2d^1 was obtained from biofilms growing on PTFE which had the lowest surface adhesion force (8 nN). Nitrification rates for the biofilms were inversely correlated with the attraction forces as measured by AFM.

Research highlights:

- Eight plastics supported nitrifying biofilms in wastewater
- Nitrification did not correlate with surface roughness nor biomass concentration
- Nitrification was inversely proportional to adhesion force with heterotrophs present

Keywords: Atomic force microscopy, biofilms, nitrification, plastic media, wastewater treatment

1. Introduction

Biofilm processes are frequently used for aerobic biological wastewater treatment. The conventional trickling filter has been a mainstay and recent developments include rotating biological contactors (RBCs) and biological aerated filters (BAF) (Boller et al., 2004; Mendoza-Espinosa and Stephenson, 1999). These processes are often designed to remove ammonia as well as organic carbon, as measured by biochemical oxygen demand (BOD). As heterotrophic bacteria responsible for BOD removal have faster growth rates and higher yield coefficients compared to autotrophic nitrifying bacteria, in order for combined BOD removal and nitrification to occur, the reactor is designed to operate at a lower BOD loading (Grady et al., 1999). Common to all the processes is an inert support media on which a biofilm grows. Selection of appropriate materials has been the aim of much research in the past but has almost entirely focussed on mesoscale, i.e. reactor-scale, investigations such as using laboratory scale reactors or pilot plants e.g. Moore et al., (1998). Most attempts to manipulate bacterial population to improve treatment processes are undertaken with suspended growth systems (Stephenson and Stephenson, 1992); for example, the use of immobilisation in gels to enhance performance of activated sludge (Bouchez et al., 2009). However, for biofilm systems, research has concentrated on the selection of the media, and has usually been for a single species under laboratory conditions, e.g. Robledo-Ortiz et al. (2010). The experiments described in this paper were designed to select biofilm support media to treat wastewaters that would enhance growth of nitrifiers in the presence of heterotrophs. Atomic force microscopy (AFM) was used to determine the important media properties for the enhancement of nitrification.

2. Materials and Methods

A 175 l tank was set up as a fed-batch aerobic reactor treating settled primary sewage in order to grow biofilm on 8 different plastic media. In one half of the tank 9 aquarium diffuser stones (Aquatics-online Ltd., Bridgend, UK) were used to aerate the tank at a rate of 2 – 4 l/min; the plastic media samples were submerged in the other half to reduce the scouring effect on biofilm that would attach to the plastic. Each sample was end on to the flow in order to aid the even distribution of liquid over the surface of the plastic. A centrifugal pump (Platon Flowbits, Basingstoke, UK) circulated the tank contents to ensure good mixing. The tank was initially seeded with 20 l of Return Activated Sludge (RAS) taken from an on-site pilot plant; 80 l of tap water and 75 l of settled sewage from Cranfield University's sewage treatment works were then added to make up the volume. A hydraulic retention time (HRT) of 48 h was maintained by draining half the volume of the tank once a day and refilling with 87.5 l of fresh settled sewage with BOD concentrations around 90-190 mg/l and ammonia 5 to 37 mg/l (Chang et al., 2001).

Experiments were conducted on 8 different plastic sheets: acrylonitrile butadiene styrene (ABS), nylon (Ny), polycarbonate (PC), polyethylene (PE), polypropylene (PP), polytetraflouroethylene (PTFE), polyvinyl chloride (PVC) and tufnol (Tu), all supplied by Model Products Ltd (Wootton, UK). Each sheet was cut into 5 strips 40 cm long and 6 cm wide. These were fixed to a frame made of PVC pipe and suspended horizontally in the tank. The strips could be removed from the tank and sacrificed for nitrification tests. The nitrification rates of the biofilms grown on the plastics were tested at 8 and 10 weeks by withdrawing plastic strips from the tank. These were then immersed in a measuring cylinder with 2 l of a synthetic feed adapted from Hanaki et al. (1990) containing 80mg/L as N ammonium bicarbonate as the sole source of ammoniacal

nitrogen, 76 mg/l K_2HPO_4, 30 mg/l KH_2PO_4, 33.75 mg/l $MgSO_4.7H_2O$, 41.25 mg/l $CaCl_2.2H_2O$, 0.375 mg/l $FeCl_3.6H_2O$, 0.0338 mg/l $MnSO_4.H_2O$, 0.0021 mg/l $CuSO_4$, 0.0012 mg/l $Na_2MoO_4.2H_2O$, 0.035 mg/l $ZnSO_4.7H_2O$. The strips were aerated via an aquarium diffuser stone for 24 h and the ammonia decay monitored. Dissolved oxygen was maintained at >7.5 mg/l and temperature ranged from 18.0 to 23.5 ^oC. When needed, the pH was maintained between pH 7 and 8 by addition of 0.1M hydrochloric acid (Analar Grade, Merck, Poole, UK). Biomass was determined at the end of each test by combining suspended solids that had scoured off during the test with the biofilm removed from the plastic. All 2 l of the suspension was filtered through a pre-weighed glass microfibre paper (MF 200, Fisherbrand, UK) prior to determination of volatile solids by a standard method (Clesceri et al., 1998).

In parallel, each plastic was analysed for its surface properties by atomic force microscopy (AFM). A Nanoscope IIIA Atomic Force Microscope (Digital Instruments, Santa Barbara, CA, USA) was used to image and obtain surface roughness values and to measure force on the surface. The nanoprobe cantilevers (Lot Oriel Group, UK) were made of silicon nitride (Si_3N_4) with a spring constant of k = 1 N/m. Digital software (Nanoscope version 4.42r4) was used to analyse the topographic images of the surface, as well as the force-distance. The scanning rate in z-direction was maintained at 45 Hz. The AFM tapping mode in air was used to image the surface characteristics of the plastics. From the topographic images, the roughness of the plastics was expressed in three ways: as the surface area (SA) taking into account the topography for a given x-y distance; as the average roughness value for the area sampled (R_a), i.e. the overall average distance between the peaks and valleys; and as the maximum range of the profile (R_{max}), i.e. the height difference between the deepest valley and the highest peak. Surface adhesion and

repulsion forces were also determined for plastics using AFM. Force curves were generated for all plastics with the exception of ABS and surface adhesion forces were determined according to Fang et al. (2000).

3. Results and Discussion

Plastics ranked in order of roughness (roughest to smoothest) based on AFM measurement of R_a were PE>PTFE>Ny>PVC>Tu>ABS>PP>PC (Table 1). Average roughness (R_a) values ranged from 6 to 603 nm (PC and PE respectively). The R_{max} and SA measurements were also greatest for PE at 6.58 nm and 6322 nm^2 respectively and least for PC at 0.28 nm and 5369 nm^2 respectively. Plastics could generally be ranked in a similar order based on R_{max} and SA values, with the exception of PTFE and ABS. Irregular, rough polymeric surfaces generally promote bacterial adhesion and biofilm deposition (Katsikogianni and Missirlis, 2004). Biomass was monitored in the early weeks until growth had stabilised from week 8 onwards. Variations between weeks 8 and 10 will in part be caused by changes in influent BOD and ammonia concentrations as real settled sewage was used. Whilst mean biomass accumulation over the experimental period was greatest in ABS and Nylon at 52.5 and 47.5 g/m^2 respectively and least in PVC at 19 g/m^2 (Table 1), there was no correlation with surface roughness. Additionally, no significant correlation was observed between surface roughness or topography of plastics and nitrification rate. Indeed, the nitrification rate was at the lower end of the range for the plastic which had the highest surface roughness, PE, at 0.04 – 0.15 $g/m^2/d$. Conversely, PC, which had the lowest observed surface roughness, had a relatively high nitrification rate at 0.24 – 0.39 $g/m^2/d$ (Table 1). In one example Quirynen and Bollen (1995) noted surface roughness as the more influential property in determining formation of dental plaque but also identified that surface energy was important.

Growth on Nylon and PVC appeared evenly distributed, the former thinly the latter thicker, whilst growth on other plastics tended to appear less uniform. Based upon a biofilm range of 10

– 60 g/l (Morgenroth, 2008), the mean biofilm thicknesses ranged from 288 μm/m^2 for PVC to 5.64 mm/m^2 for ABS. Sousa et al. (1997) also compared polymeric support materials for the adhesion of autotrophic nitrifying bacteria and characterised surface properties of the plastics by hydrophobicity and surface charge. Of the five plastics tested (PE, PP, PVC, high density polystyrene and polymethyl-methacrylate), it was observed that PP, the most hydrophobic material, provided the best surface for biofilm development. In contrast, the current study showed that PP exhibited relatively low nitrification compared with over half of the other plastics tested. However, both Sousa et al. (1997) and the current study ranked comparable plastics PP>PE>PVC based on nitrification rate. Liu (1995) ranked 3 polymers in the order PS (polystyrene) >PP>PE in terms of maximum accumulation of nitrifiers. Again, this corroborates data from the current study in which nitrification rates were greater for PP than PE. Kim et al. (1997) demonstrated that a support medium with favourable (negative) surface free energy values promoted formation of a nitrifying biofilm. However the work was carried out using laboratory cultures of nitrifying bacteria and thus the interaction with heterotrophic biofilm was not investigated.

The current preliminary study provides important evidence of a relationship between the surface characteristics of plastics and the nitrification rate of associated biofilm. Mean nitrification rates ranged from <0.01 to 1.52 $gm^{-2}d^{-1}$ with the highest rates observed for PTFE and lowest for PE (Table 1). There was no evidence of a correlation between nitrification rate and dry biomass, which is perhaps unsurprising as the biofilms have grown in aerated settled sewage with a significant organic carbon content, so will be dominated by heterotrophs removing BOD. However, there was a significant negative correlation between nitrification rate and the adhesion force of the plastics, observed both at week 8 (R^2 0.91; P<0.001) and at week 10 (R^2 0.61;

P<0.05) (Fig. 1). Adhesion forces ranged from 8nN for PTFE to 40 nN for nylon (Table 1). The correlation was stronger at week 8 compared to week 10, probably due to different stages of biofilm development. The results indicated that nitrifiers appear to be able to attach more strongly to inert surface media compared to one with a higher adhesion force and that this could help encourage their growth in the presence of heterotrophs. Recent research on pure cultures of nitrifiers and heterotrophs corroborates this conclusion (Khan et al. 2011). This also indicates that controlled backwashing could maintain the biofilm in an optimal state as used in processes such as biological aerated filters (BAFs) (Mendoza-Espinosa and Stephenson, 1999).

4. Conclusions

The results may reflect species-related differences in adsorption capacity i.e. that nitrifiers are better adapted to adhere to low-energy surfaces, or possibly the inability of low-energy surfaces to support greater biomass associated with the rapid growth of heterotrophic biofilm. Experimental work on activated sludge flocs has shown that nitrifiers bind together very strongly, with extracellular polymers probably responsible (Larsen et al., 2008), which would aid their attachment to low energy surfaces. These findings have important implications for media selection in wastewater treatment systems where reliable nitrification is required.

Acknowledgements

This work was supported by a UK Engineering and Physical Science Research Council (EPSRC) Platform Grant (Grant No. GR/S 64523/01). The authors wish to acknowledge the inspiration of Derek Rodman now of Clearfleu Ltd.

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Table 1. Characteristics of plastics and biofilms

Parameter		Material							
		PC	ABS	PTFE	PP	Tu	Ny	PE	PVC
R_a (nm)		6	34	162	25	62	107	603	75
R_{max} (nm)		0.28	0.75	2.02	1.85	1.96	2.75	6.58	2.10
SA (nm^2)		5639	5891	5839	5719	5735	5799	6322	5737
Surface adhesion force (nN)		11.7	nd	8.0	10.5	12.5	40.0	21.0	23.5
Dry biomass (g/m^2)	Week 8	46.8	56.4	44.7	33.1	40.7	56.2	36.5	17.3
	Week 10	35.2	51.9	24.7	42.2	42.2	50.5	40.6	22.0
Nitrification rate (g/m^2d)	Week 8	0.24	0.02	1.52	0.19	0.26	0.00	0.04	0.01
	Week 10	0.39	0.36	0.32	0.17	0.49	0.07	0.15	0.06

nd = no data

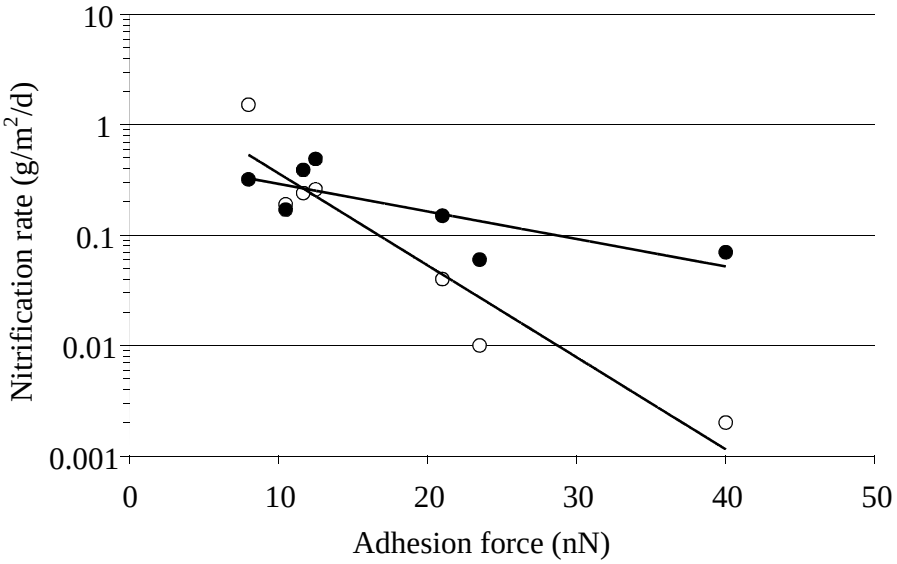

Figure 1. Biofilm nitrification rate for synthetic feed (based on ammonia decay measurements) against surface adhesion force of plastic supports. Open circles represent data from biofilms at week 8 (R^2 0.91; $P<0.001$); filled circles represent data from week 10 (R^2 0.61; $P<0.05$).

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T. Stephenson, E. Reid, L.M. Avery, B. Jefferson, Media surface properties and the development of nitrifying biofilms in mixed cultures for wastewater treatment, Process Safety and Environmental Protection, Volume 91, Issue 4, July 2013, Pages 321–324.
http://dx.doi.org/10.1016/j.psep.2012.07.002

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