The expanded bed biofilter: combined nitrification, solids destruction, and removal of bacteria

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Abstract Developed for tertiary nitrification, this biofilter also removed carbonaceous BOD (cBOD) and (SS). Because the biofilter is expanded, it cannot clog, and therefore does not require backflushing; yet, it removed a significant proportion of the influent SS. This unanticipated capability was due to the activities of heterotrophic bacteria, protozoa, and metazoa (nematode and oligochaete worms). The expanded bed is an intensified process, which is based on natural immobilization of microbes to small support particles. Using glassy coke as the support material, an attached layer of microbes develops, forming particulate biofilms having a superficial surface area of 1 800 $m^2\,m^{-3}_{expanded\,bed}$. Autotrophic nitritifiers (*Nitrosomonas* spp.) were detected in the biofilm using rRNA-based molecular methods and were likely responsible, at least in part, for reducing the ammonia concentration by up to 99% (to 0.1 $mg\,L^{-1}$), while the other organisms reduced cBOD and SS by up to 56% and 62%, respectively. Furthermore, the influent concentrations of *Escherichia coli*, coliform and heterotrophic bacteria were reduced by over 80%. It thereby provides a single process solution for combined tertiary nitrification and solids removal. Operating the process to consistently achieve $<0.5\,mg\,NH_3N\,L^{-1}$ and at the same time removing a significant fraction of cBOD and SS, it can replace processes such as SAFs or NTFs followed by a sandfilter.

Keywords Bacteria removal; biofilter; expanded bed; nitrification; solids removal; sustainability

Introduction

The main problems encountered during the removal of nitrogen from secondary wastewater are that it is in relatively low concentration, there is a very large volume to treat, and some autotrophic bacteria involved (nitrifiers or denitrifiers) have very slow ($t_d = 1–3$ d) or extremely slow ($t_d = 11$ d, (Jetten *et al.*, 1998)) doubling times. Owing to these growth rate limitations, the process microbes will be washed out easily from the treatment system unless they are retained, e.g. by immobilization or membranes.

In biological wastewater treatment processes, organic matter (Carbonaceous BOD (cBOD) and SS) is removed mainly by microbial metabolism and growth. Although microbial growth can be estimated by comparing the inlet and outlet biomass concentrations, such simplistic measurements do not take account of endogenous biological processes. These processes include maintenance energy requirements, predation by eukaryotic microorganisms, cell death or dormancy, cell lysis and cryptic growth using lysis products (van Loosdrecht and Henze, 1999). For example, Ghyoot and Verstraete (2000) have stated that mineralization by protozoa and metazoa releases ammonia and phosphate, and that in a 2-stage MBR, there was a 20–30% reduction in sludge volume through grazing; although over-grazing of the slow-growing nitrifyers was a problem.

In bioprocesses with long sludge ages, a complex community of organisms can develop that is capable of significant cBOD and SS removal, which is a form of

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endogenous (internal) nutrient cycling. For example, Lee *et al.* (2004) consider decomposers (bacteria and fungi), which utilize the dissolved organic matter in the wastewater, and consumers (heterotrophic flagellates, ciliates, and small metazoa), where, e.g. ciliates feed upon dispersed bacteria and other organisms. In a recent study of filter-feeding, Eisenmann *et al.* (2001) demonstrated that ciliates are able to remove up to 1 200 bacteria-sized particles (1 μm) each per hour. Luxmy *et al.* (2000) have demonstrated that bacterial flocs $<10\,\mu m$ are ideal for ciliates to consume, while the same group demonstrated that metazoa grazing on membrane-attached biofilm in an MBR were able to reduce the transmembrane pressure (Luxmy *et al.*, 2001). In a similar vein, Rensink and Rulkens (1997) demonstrated that tubificid worms deliberately added to trickling filters were able to substantially reduce the effluent SS and COD. Therefore, the presence of a diverse and large population of protozoa and metazoa is important for SS and BOD removal.

Expanded bed (EB) technology relies on the suspension (fluidization) of small particles in upward flowing wastewater. Microbes attach to the particles and grow as a mixed biofilm, typically dominated by bacteria and protozoa. Because the particles provide a large surface area and are retained in the bioreactor, a high concentration of immobilized biomass with a long sludge age develops. Bed expansion not only prevents the filter from clogging, but also allows advantage to be taken of the excellent mixing characteristics of fluidization. The net result is a biofilter containing a high concentration of highly active biomass and, hence, a high-rate, but compact process.

Glassy coke has been found to be an excellent biomass support material (Dempsey, 2003b). Being carbon-based and porous, it is less dense than silica sand. This means that coke requires a lower upward velocity of wastewater to expand the bed and, therefore, the surface where microbes attach experiences a lower shear force. Bacteria first colonize the pores and then overgrow the surface to form a continuous biofilm that encloses a particle, to which protozoa attach. These biofilm-coated particles are referred to as particulate biofilms (Nicolella *et al.*, 2000).

We have used EB technology to develop an intensified nitrification process that is suitable as a tertiary treatment. Although this is only the first step of a complete nitrogen removal process, the nitrified effluent could be denitrified by mixing with wastewater that provides readily-degradable cBOD, e.g. settled sewage. The key advantages of EB technology are that it is simple, energy-efficient, and compact (Dempsey *et al.*, 2005). It therefore meets several criteria of sustainability that are important for all future wastewater treatment processes.

Materials and methods

A pilot-scale EB was constructed from transparent PVC, and the design is shown in Figures 1 and 2. EB section had a diameter of 500 mm and a depth of 3 000 mm. Subtracting the depth of the moving bed distributor (Dempsey, 2003a), it had an EB volume of $0.5\,m^3$. The unit was operated throughout on activated sludge final effluent (ASFE) at the Davyhulme wastewater treatment works. Glassy coke (Dempsey, 2003b) was used as the biomass support material. Approximately 30% of the bioreactor volume was filled with 1 mm coke particles and expanded by 50% by the upflowing wastewater (upward velocity $= 36\,m\,h^{-1}$). As the biofilm developed, the degree of bed expansion increased to 100%, and the biofilm thickness was controlled by recycling the bioparticles from the top of the bed and injecting them into the moving bed distributor (Dempsey, 2003a).

The ASFE flow rate was monitored using a Micronics ultrasonic flowmeter and proportioned to the works flow using an ABB inverter with PID control to alter the speed of a submersible Best pump (all supplied by Pumps and Energy, Stockport, UK).

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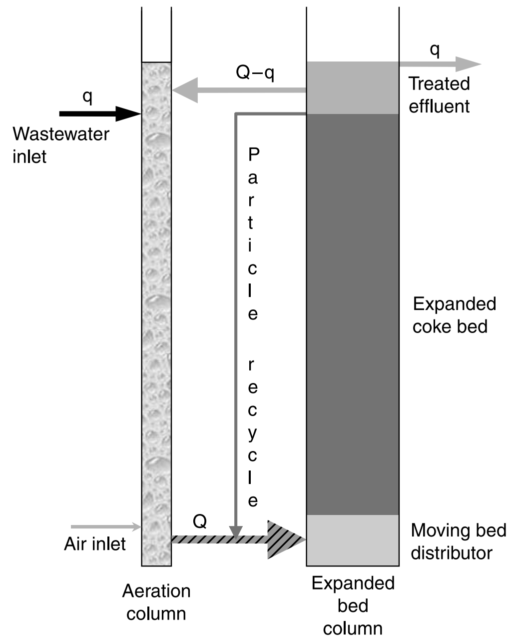

Figure 1 Schematic diagram of expanded bed process plant

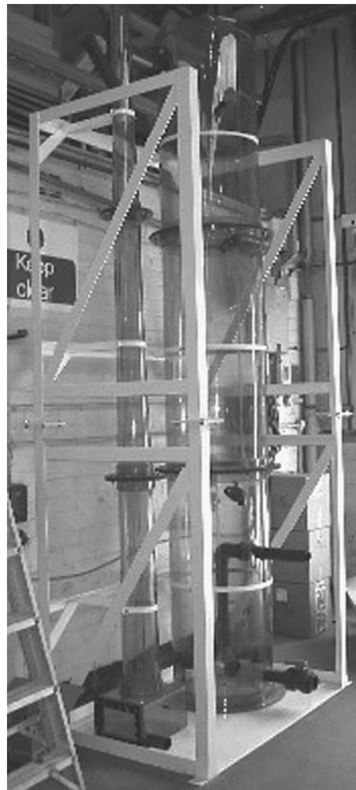

Figure 2 Photograph of expanded bed plant

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Bed expansion and particle fluidization was achieved using a Weir isoglide pump controlled by a Teco inverter (both from Pumps and Energy, Stockport, UK). Nitrogen was removed from compressed air using an OG-15 pressure-swing oxygen concentrator (OGSI, Amherst, NY, USA), so that sufficient dissolved oxygen could be supplied to the high concentration of active biomass.

Samples (20 cm^3) of influent and effluent wastewater were filter-sterilized for chemical analyses using Acrodisc syringe filters (0.2 μm Supor membrane, Pall, UK). The concentrations of ammonia (hypochlorite, salicylate, and nitroprusside, (ISO-7150-1 1984)), nitrite (azo dye, (ISO-6777, 1984)), and nitrate (UV absorption, (ISO-7890-3, 1988)) were measured spectrophotometrically.

A second pair of samples (1 000 cm^3) was used for measurement of viable bacteria, suspended solids, and cBOD. A small sub-sample was used to determine viable bacterial counts using a Spiral Plater (Don Whitley Model D) and Tryptone Soya Agar (TSA, LabM, Bury, UK) for enumerating heterotrophs; and Chromogenic *E. coli*/coliform Agar (CM0956, Oxoid) for enumerating coliforms and *Escherichia coli*. Sub-samples were used neat or diluted 10-fold before inoculating 50 μl aliquots onto 9 cm plates of growth medium, when the spiral-plater gave a 1000-fold dilution factor. Plates were incubated at 37 °C, and colonies counted after 24 h. The concentration of SS was determined using Whatman GF/C (47 mm diameter) filters dried to constant mass at 105 °C (BS-EN-872-BS-6068-2.54, 2005). Filtered wastewater was collected for determination of the soluble cBOD using a polarographic dissolved oxygen (DO) probe (ISO-5814, 1990), and allylthiourea (Sigma) to inhibit nitrification (BS-6068-2.63, 1998). In addition, pH, DO, and temperature were logged automatically using online probes linked to a Microlink USB data logger (Biodata, Manchester, UK) and measured on site using portable instruments (Jenway).

Samples of wastewater were taken from the inlet and outlet of the reactor, as well as bioparticles from within the reactor, for identification of the AOB (ammonia oxidising bacteria) populations. Inlet and outlet wastewater samples were filtered through 0.2 μm polycarbonate (Nucleopore) membrane filters to harvest the cells. Samples of bioparticles were taken 0, 70, and 186 cm from the top of a 295 cm deep EB. All samples were stored in ethanol at −80 °C. DNA was extracted from the filters and bioparticle samples using a FastDNA soil kit (Q-biogene) according to manufacturer's instructions. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis was performed by a previously published method described by Rowan *et al.* (2003). PCR was used to amplify 16S rRNA gene fragments using primers selective for the betaproteobacterial AOB (nested PCR AOB specific CTO189f/654r (Kowalchuk *et al.*, 1997) followed by Primer 2/3 (Muyzer *et al.*, 1993)). The PCR-amplified fragments were analysed by

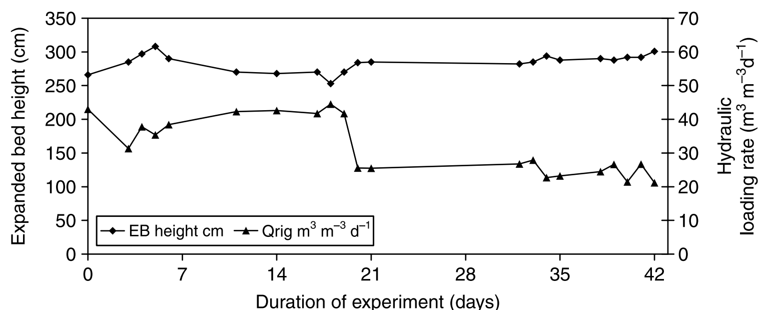

Figure 3 Variation in expanded bed height and bed loading rate with activated sludge final effluent during high to low loading rate trial

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Table 1 Performance of pilot-scale expanded bed for removal of ammonia, suspended solids, and carbonaceous BOD during 42 days operation with high to low loading rate

	EB height cm	Loading rate $m^3 m^{-3} d^{-1}$	DO %	NH_3-N in mg L^{-1}	NH_3-N out mg L^{-1}	NH_3-N oxidation %	SS in mg L^{-1}	SS out mg L^{-1}	SS removal %	$cBOD_7$ in mg L^{-1}	$cBOD_7$ out mg L^{-1}	cBOD removal %
High loading rate												
Mean	278	40	6	18.5	5.2	75%	15.1	6.8	46%	32	18	41%
Min.	253	31	4	6.3	0.1	23%	7.7	4.7	13%	16	9	18%
Max.	308	45	11	23.4	14.8	99%	37.7	13	88%	41	28	78%
n	10	10	10	10	10	10	8	8	8	6	6	6
SD	17	4.1	2.7	5.3	5.1	24%	9.8	2.6	21%	8.9	7.0	22%
Low loading rate												
Mean	289	25	11	21.3	0.4	98%	17.0	5.4	62%	20	9	56%
Min.	282	21	6	8.7	0.1	95%	7.9	3.6	32%	13	5	38%
Max.	301	28	23	31.2	1.5	100%	29.8	7.9	82%	29	14	69%
n	11	11	11	11	11	11	10	10	10	5	5	5
SD	5	2.3	4.8	5.9	0.4	1%	7.6	1.2	19%	7.1	3.4	12%

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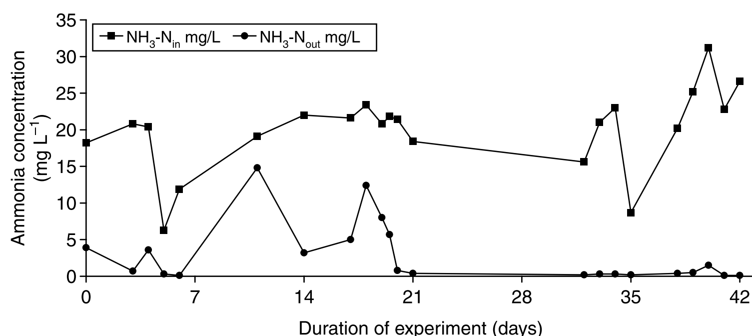

Figure 4 Variation in inlet and outlet ammonia concentrations during high to low loading rate trial

DGGE and the predominant DGGE bands (9 bands; Figure 7) were excised. DNA from the DGGE bands was reamplified using primers 2/3 (Muyzer *et al.*, 1993), and the PCR product was sequenced. The resulting sequences (approx. 150 base pairs) were compared to the GenBank database using Fasta3, and information on their closest relatives was obtained.

Results and discussion

By reducing the average loading rate from 40 to 25 $m^3\,m^{-3}\,d^{-1}$ (Figure 3 and Table 1), it was possible to achieve a consistent effluent ammonia concentration of <0.5 mg/L (Figure 4), which is necessary if discharge consents of 2 or 3 mg/L are to be complied with. Despite the decreased loading rate, the EB volume remained virtually constant (Figure 3), indicating that biofilm growth still occurred and was controlled by the bioparticle recycle and re-injection system. This system is able to control the bed height completely automatically using a device with no moving parts and the minimum consumption of energy, as the driving force comes from the recirculation pump that is used for bed expansion and particle fluidization (Dempsey, 2003a).

Not only was it possible to reduce the effluent ammonia concentration to a minimum of 0.1 $mg\,L^{-1}$ (Table 1), but the EB was also able to substantially reduce the cBOD and SS (Table 1). Prior to decreasing the loading rate, the average reduction in SS was 46%, but afterwards it was 62%. A similar effect was found for the reduction in cBOD, where the reductions were 41% before and 56% after the decrease. Decreasing the flow rate of ASFE (q) has the effect of increasing the recycle rate ($Q-q$) because the flow for bed expansion (Q) is fixed (Figure 1). In this case, the recycle ratio increased from 4.3 to 6.8, thereby allowing an increase in bed contact time of 1.6. This resulted in a 35–37% increase in the SS and cBOD removed. Thus, the nitrifying bacteria had more time to oxidize ammonia, heterotrophic bacteria more time to consume cBOD, while the protozoa and metazoa had more time to consume SS. Although not strictly comparable with activated sludge, at the lower hydraulic loading rate, the estimated sludge age for the biofilms was in excess of 30 days, and the food to microorganism (F:M) ratio was approximately 0.001. These estimates help to explain the destruction of suspended solids and cBOD (Lee *et al.*, 2004).

Furthermore, the influent concentrations of *Escherichia coli*, coliform and heterotrophic bacteria were reduced by 70, 66, and 73% (Table 2), respectively. Figure 5 illustrates the removal of *E. coli* (dark colonies) and coliform bacteria (dark halo) from the ASFE feed.

Because the biofilter was operated as an expanded bed, removal of SS was unexpected as solids with a sedimentation velocity $<1\,cm\,s^{-1}$ were expected to pass straight through.

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Table 2 Performance of pilot-scale expanded bed for removal of heterotrophic and coliform bacteria and *E. coli* during 42 days operation with high to low loading rate

	High loading rate								
	Heterotrophs			Coliforms			*Escherichia coli*		
	In (CFU cm^{-3})	Out (CFU cm^{-3})	Removal (%)	In (CFU cm^{-3})	Out (CFU cm^{-3})	Removal (%)	In (CFU cm^{-3})	Out (CFU cm^{-3})	Removal %
Mean	19 187	8 601	74%	1 663	1 108	84%	1 008	88	93%
Min.	3 980	520	4%	160	0	67%	40	0	83%
Max.	54 666	52 571	95%	4 880	7 120	100%	3 120	360	100%
n	8	8	8	8	8	7	8	8	7
SD	15 516	17 868	30%	1 607	2 444	12%	986	116	6%
	Low loading rate								
Mean	10 336	1 424	86%	440	88	82%	92	16	78%
Min.	5 380	620	77%	0	0	64%	0	0	50%
Max.	14 363	2 480	94%	840	300	97%	180	40	100%
n	5	5	5	5	5	3	5	5	4
SD	3 813	687	6%	409	129	16%	77	17	22%

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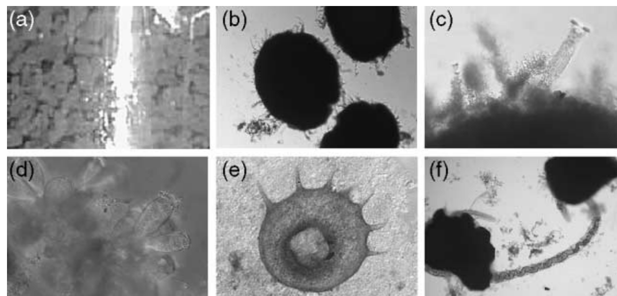

Figure 5 Particulate biofilms with associated protozoa and metazoa from expanded bed; (a) bioparticles in expanded bed; (b) bioparticles with surface-attached, stalked protozoa; (c) close-up of rotifer attached to bioparticle; (d) stalked protozoa on surface of particulate biofilm; (e) testate amoeba grazing on biofilm; (f) oligochaete worm grazing on bioparticles

However, microscopic examination of bioparticles revealed a large number of stalked protozoa attached to the bacterial biofilms (Figure 5). Protozoa filter-feed on suspended particles, which include bacteria that were either in the secondary effluent feed or grew in the process. Furthermore, nematode and oligochaete worms also were closely associated with the bioparticles (Figure 5). These organisms can graze on flocs carried over from the activated sludge process (Luxmy *et al.*, 2000), detached biofilm, and the bioparticles.

Although the EB removed over 80% of bacteria compared to the concentration in the inlet (Table 2 and Figure 6), the degree of *E. coli* removal fell from 93% to 78% when the loading rate was decreased. However, the degree of coliform removal remained about the same (84% vs. 82%), while the removal of heterotrophic bacteria increased from 74% to 86%. This was surprising because *E. coli* and coliforms are unlikely to grow during passage through the biofilter, while heterotrophs can grow in the biofilm. There is no simple explanation for this observation, but the study continues, in order to obtain additional data. Unfortunately, owing to the breakdown of ancillary equipment, it has not been possible to obtain data beyond 42 days operation at the reduced hydraulic loading rate (Figures 3 and 4).

A high degree of ammonia oxidation was observed in the mixed microbial biofilm process. Preliminary analysis of the AOB populations revealed that all the recovered AOB sequences belonged to the genus *Nitrosomonas* (Figure 7). This finding is consistent with other studies on wastewater treatment plants (WwTP), i.e. *Nitrosomonas* spp. tend to predominate in high ammonia engineered environments (WWTP), whereas low ammonia natural environments tend to harbour *Nitrosospira* spp. (Rowan *et al.*, 2003). There was no apparent difference in the bacteria identified in the upper, middle, or lower

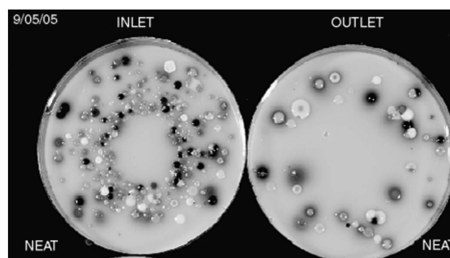

Figure 6 Comparison of inlet and outlet concentrations of *Escherichia coli* (dark colonies) and other coliform bacteria (dark halo around colonies), as revealed by Chromogenic *E. coli*/coliform Agar (CM0956, Oxoid)

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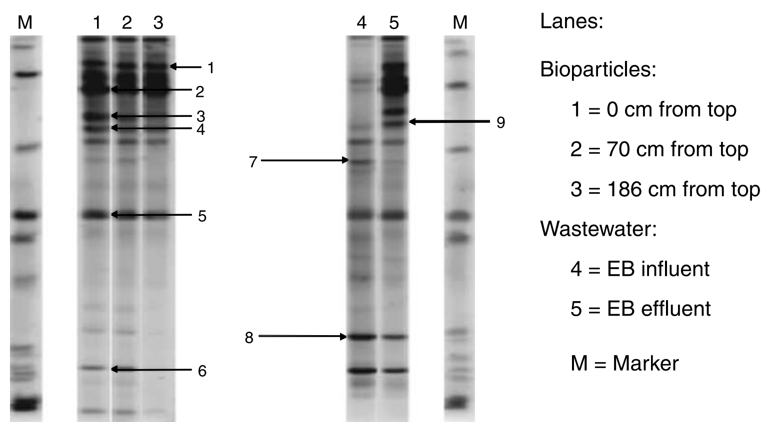

Figure 7 DGGE profiles of AOB communities (CTO 189f/654r, Kowalchuk *et al.*, 1997) from a EB reactor. The numbers in parentheses indicate the percentage similarity of 16S RNA sequences recovered from DGGE profiles with their nearest neighbour in the GenBank database

regions of the EB (Figure 7), despite the presence of gradients in pH, DO, and ammonia across the bed. This was most likely because particles are recycled continually from the top of the bed to the bottom (Figure 1), as a means of biofilm thickness control, and, therefore, constant particle redistribution occurs.

Conclusions

We have identified the operational conditions necessary for reducing the effluent ammonia concentration of our novel nitrification process to <0.5 (min. 0.1) $mg\,L^{-1}\,d^{-1}$, which is necessary for compliance with discharge consents of 2–3 $mg\,L^{-1}\,d^{-1}$. Furthermore, we have also clearly demonstrated that an EB biofilter is capable of significant SS and cBOD removal, despite the fact that particles with a sedimentation velocity $<1\,cm\,s^{-1}$ ought to pass straight through. The removal of $>60\%$ SS and $>50\%$ cBOD at the same time as reducing the effluent ammonia concentration to such a low level constitutes a serious contender for replacement of two-stage tertiary treatments to achieve the same aim. This novel EB biofilter therefore represents a potential replacement for SAFs or NTFs followed by a sandfilter.

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