

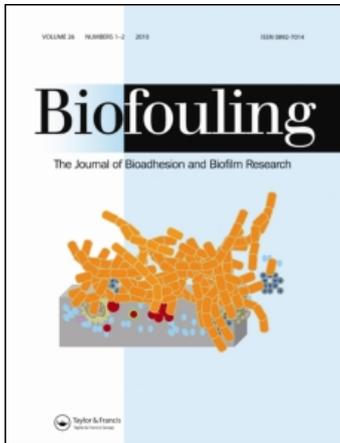
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## Biofouling

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## The use of aeration as a simple and environmentally sound means to prevent biofouling

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Biofouling is a major problem faced by marine industries. Physical and chemical treatments are available to control fouling, but most are costly, time consuming or negatively affect the environment. The use of aeration (ie continuous streams of air bubbles) to prevent fouling was examined. Experiments were conducted at three sites with different benthic communities. Experimental panels (10 cm × 10 cm; PVC and concrete) were deployed with or without aeration. Aeration flowed continuously from spigots 0.5 m below the panels at a rate of ~3.3 to 5.0 l min<sup>-1</sup>. After 1 and 4 weeks, aerated PVC panels from all sites had significantly less fouling than non-aerated controls. Aeration reduced fouling on both the PVC and concrete surfaces. Fouling was reduced on panels directly in bubble streams while panels 30 cm and 5 m away had significantly more fouling. Thus, under the conditions used in this study, aeration appears to be an effective and simple way to prevent fouling.

**Keywords:** aeration; antifouling; biofouling; bubbles; settlement

### Introduction

Biofouling, the accumulation of plants and animals on hard surfaces in the marine environment, is a major problem for marine industries with numerous economic and ecological impacts. Biofouling affects recreational boaters and commercial shipping by adding drag that increases fuel consumption (Townsin 2003). At aquaculture sites, biofouling increases labor costs, reduces the value of product and harms cultured species (Daigle and Herbingier 2008; de Nys and Ison 2008; Watson et al. 2009). Biofouling also plays a critical role in the transport and introduction of invasive species as exotic fouling species on ships' hulls and on aquaculture organisms are readily transported to new areas (Gollasch 2002; Ashton et al. 2006; Drake and Lodge 2007; Locke et al. 2007; Bullard and Carman 2009). Given these problems, considerable effort has been spent developing techniques to prevent and control biofouling (eg Armstrong et al. 2000; Yebra et al. 2004; Chambers et al. 2006; Coutts 2006). Although control methods are often effective, most are costly, time consuming or negatively affect the environment (Evans et al. 2000; Voulvoulis 2006).

To address these issues, the use of continuous streams of air bubbles (hereafter aeration) to prevent biofouling was investigated. The concept of using aeration as an antifouling (AF) technique was first

proposed by Smith (1946a) who had previously found that water currents, such as those generated by a ship in motion, could prevent fouling. Expanding on these ideas, he conducted experiments to determine if aeration could similarly prevent fouling (Smith 1946b). In a series of experiments conducted in Florida, he found that continuous aeration greatly reduced barnacle fouling (Smith 1946b). Subsequently, the UK Admiralty conducted a large-scale applied experiment to see if aeration could prevent fouling on ships' hulls (UK Admiralty 1951, unpublished). Although it was found that aeration was very effective in preventing fouling on some hull surfaces, it was concluded that AF paints were more effective (UK Admiralty 1951, unpublished). While these paints remain effective, most are toxic and many of their components are currently being phased out of production.

Since these early experiments, the idea of using aeration for AF purposes has largely been forgotten. Recently, however, the idea has begun to be reevaluated. A study by Scardino et al. (2009) documented the ability of aeration to reduce fouling over long time scales (months to years) in Australia. These experiments used panels deployed at an angle similar to the slope of a ship's hull (22.5° to the vertical) and found that aeration, and aeration with ozone, could significantly reduce fouling. The authors also conducted a large-scale applied experiment where they found

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reduced fouling on the hull of a small ship after aeration for approximately 4 months (Scardino et al. 2009).

Additional work on the AF properties of aeration is timely and important for a variety of reasons: First, because of their toxicity, many traditional AF paints are no longer in use (Evans et al. 2000; Yebra et al. 2004). Secondly, due to innovations in aeration technology a wide variety of inexpensive and highly efficient aeration production devices are now commercially available. Thirdly, Scardino et al. (2009) did not assess the susceptibility of individual fouling species to aeration; different benthic communities may be more or less susceptible to the effects of aeration. Fourthly, the initial studies created aeration by bubbling air through copper piping (Smith 1946b; UK Admiralty 1951, unpublished). Copper is highly toxic to marine organisms (Rygg 1985; Stark 1998; Bellas et al. 2001), and it is likely that some of the AF effects seen in these early studies were due to the toxic effects of the copper piping rather than from the effects of aeration. Finally, due to numerous invasions by non-native species, many benthic communities have substantially changed since the mid-twentieth century (Olenin and Leppäkoski 1999; Lambert and Lambert 2003; Streftaris et al. 2005; Needles 2007). It is unclear whether aeration can prevent fouling by ubiquitous, highly competitive invasive species. If successful and broadly applicable (eg Scardino et al. 2009), aeration has the potential to provide an effective, inexpensive and environmentally friendly way to control biofouling.

## Methods

Experiments were conducted during the summers of 2008 and 2009. During both years, the primary research site was located in eastern Long Island Sound on floating docks adjacent to the University of Connecticut's Avery Point Campus, Groton, Connecticut (41° 18'59 N, 72° 03'38 W) (CT site). Water depth at this site is ~2–3 m; current velocities range from 0 to 10 cm s<sup>-1</sup>. A diverse benthic fouling community grows on the underside of the docks and pilings in this area (see Osman and Whitlatch 1995a,b). Dominant sessile fauna include ascidians, bryozoans and polychaetes, with ascidians typically occupying > 80% of primary space during warmer months (Osman and Whitlatch 2004). The 2009 experiments were conducted at the CT site and on similar floating docks at Martha's Vineyard, Massachusetts (41° 26'29 N, 70° 36'02 W) (MA site) and near Yorktown, Virginia (37° 15'34 N, 76° 28'03 W) (VA site). The benthic community at the MA site is very similar to the CT site, but with an even greater abundance of ascidians. The community at the VA site is very different; ascidians

are rare, while barnacles, sponges and hydroids are common.

For all experiments, PVC or concrete panels (10 cm × 10 cm) were attached to PVC racks and deployed facing the seafloor either in the presence of a steady stream of air bubbles (aerated panels) or without bubbles (control panels) (Figure 1). Aeration at each site was generated using a dock-mounted, 1/3 hp regenerative air pump. Experimental racks were composed of PVC piping formed into a rectangular frame, 0.5 m high by ~0.75 m long (Figure 1). Experimental panels were attached to the upper level of the frame while air spigots (adjustable plastic airline valves) were present on the lower level. The vertical distance from the spigots to the panels was 50 cm for all experiments. Each panel was positioned directly over a spigot so the aeration for each panel was provided by a point source directly below it. Bubbles flowed continuously from the spigots at a rate of 3.3–5.0 l min<sup>-1</sup> (measured by the time needed to fill a 500 ml bottle with air). Once released from the spigots, bubbles diverged into a cone-shaped pattern that struck the entire surface of test panels. Spigot openings remained free of fouling during experiments and did not require cleaning. Each rack held three test panels, each ~5 cm apart. Three aeration racks and three control racks were deployed at each site with aeration and control racks interspersed in an alternating manner. Racks were ~5 m apart in CT and ~1 m apart in MA and VA. The difference in spacing was due to differences in the amount of space available at each location.

The first set of experiments assessed whether aeration could reduce fouling over short, 1-week time

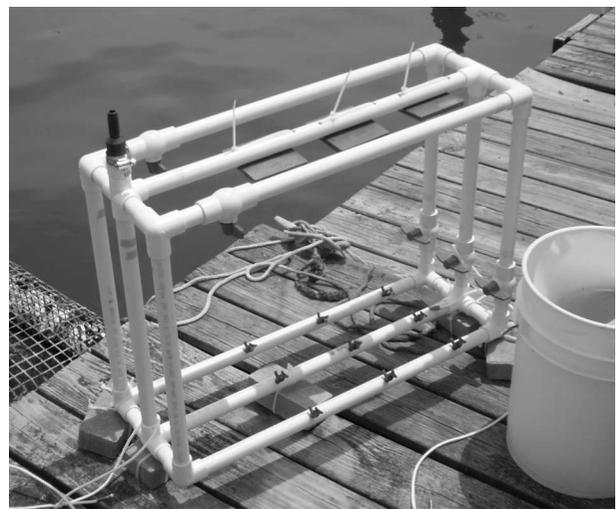


Figure 1. Aeration bubble rack ready for deployment. Three 10 cm × 10 cm PVC panels were attached to the upper level of the rack. Adjustable air spigots were present on each PVC pipe on the lower level of rack.

scales. For these experiments, aeration and control PVC panels were deployed for 1 week and then all attached settlers were identified and counted. To aid counting, panels were marked with a grid in permanent ink (1 cm × 1 cm). Three panels were deployed on each rack, for a total of nine aeration and nine control panels per assay. For statistical analyses, however, each rack was treated as an independent replicate. Thus, differences in settlement on aerated *vs* control panels were examined using *t*-tests on the mean number of settlers per rack ( $n = 3$ ). Three separate week-long assays were conducted in CT in 2008. Additional similar assays were conducted during the summer of 2009, but in this case assays were conducted at three different sites to test the effectiveness of aeration in areas with different fouling communities. The experimental design was the same as above, but one assay was conducted in CT, one in MA and one in VA. At the end of the assay at the VA site, it was found that large colonies of the hydroid *Obelia geniculata* had overgrown portions of the panels. Because the colonies were interwoven and could not be counted individually, the percentage cover occupied by hydroids was assessed. This was done by counting the number of grid squares containing hydroids.

Data from the six 1-week assays described above were used to determine the effectiveness of aeration at preventing fouling by different taxa of organisms. The total number of settlers for each species was assessed for control and aerated panels. For each species, the number of settlers on aerated panels was divided by the number of settlers on controls. Subtracting the resulting values from one yielded the percentage of fouling reduction caused by aeration for each species. The mean reduction for each taxa (eg all ascidians) was then determined to assess the overall effectiveness of aeration for different taxa.

A second experiment assessed whether aeration could reduce fouling over longer, 4-week time scales. For these assays, aeration and control PVC panels were deployed in CT for 4 weeks and the total mass of fouling on each panel determined. Two assays were conducted, one in 2008, one in 2009. Three panels were deployed on each rack (for a total of nine aeration and nine control panels). For statistical analysis, each rack was treated as an independent replicate. Thus, differences in fouling on aerated *versus* control panels were examined using *t*-tests on the mean mass of fouling per rack ( $n = 3$ ).

A third experiment, conducted in CT in 2008, examined the ability of aeration to prevent fouling on different types of hard surfaces. Three aeration and three control racks were deployed, each with one PVC and one concrete panel 30 cm apart. After 1 week, panels were recovered and all settlers identified and counted.

Differences in settlement were examined using a one-way ANOVA on the mean number of settlers per treatment ( $n = 3$  each). Although one PVC and one concrete panel were attached to each rack, for analysis all panels were considered independent because panels were at least 30 cm apart and because each aerated panel was isolated within its own bubble stream and aerated independently.

A final experiment assessed the horizontal distance over which aeration could reduce fouling. PVC panels were deployed in CT in 2008 for 1 week. Three panels were placed directly inside aeration, three were placed 30 cm away from aeration and three 5 m away. After 1 week, panels were recovered and all settlers identified and counted. Differences in settlement were examined using a one-way ANOVA on the mean number of settlers per treatment ( $n = 3$  each).

## Results

Significantly fewer settlers attached to aerated *vs* control PVC panels after 1 week (Figure 2; Table 1). Fouling was very low on all aerated panels despite seasonal and geographic differences in settlement intensity and species composition. During the six separate 1-week assays (three in CT in 2008 and one each in CT, MA, VA in 2009), a total of 31,244 settlers attached to control panels compared with only 198 on aerated panels (0.6% of the controls). The comparatively heavy fouling found on aerated panels in VA was almost entirely caused by barnacles (with barnacles accounting for 135 of 136 settlers).

On 1-week timescales, aeration varied in AF effectiveness for different taxa of organisms (Table 2). Aeration reduced fouling by ascidians, bryozoans, polychaetes and sponges by >99%. Aeration reduced fouling by barnacles and hydroids by 68% and 57% respectively.

Significantly less fouling accumulated on aerated *vs* control PVC panels after exposure for 4 weeks (Figures 3 and 4). During both 2008 and 2009, aerated panels

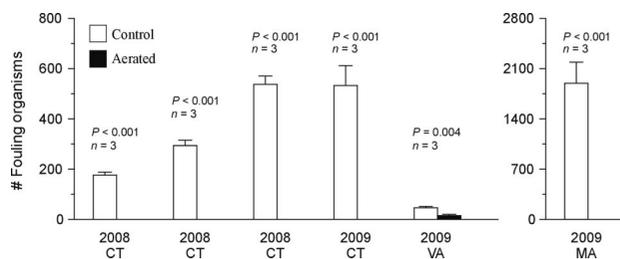


Figure 2. Mean number ( $\pm 1$  SE) of fouling organisms on 100 cm<sup>2</sup> PVC panels after exposure for 1 week. From left to right, assays were conducted: in 2008 in CT 1–8 July, 8–15 July, 19–26 August; in 2009 in CT 26 August–2 September, VA 21–28 July, MA 11–18 August.

Table 1. Abundance of fouling organisms on PVC panels after exposure for 1 week.

	# Control	# Aerated
Barnacles	456	147
Ascidians		
<i>Aplidium constellatum</i>	45	1
<i>Asciella aspersa</i>	390	0
<i>Botryllus schlosseri</i>	4040	30
<i>Botrylloides violaceus</i>	2105	1
<i>Didemnum vexillum</i>	303	5
<i>Diplosoma listerianum</i>	2561	5
<i>Molgula manhattensis</i>	157	0
Bryozoans		
<i>Bowerbankia gracilis</i>	41	0
<i>Bugula spp.</i>	18567	4
<i>Bugula neritina</i>	427	0
<i>Cryptosula pallasiana</i>	761	0
<i>Electra crustulenta</i>	37	0
<i>Electra pilosa</i>	24	0
<i>Electra sp.</i>	21	1
<i>Schizoporella errata</i>	141	0
Hydroids		
<i>Tubularia croceaca</i>	2	1
<i>Obelia sp.</i>	5	2
Polychaetes		
<i>Hydroides dianthus</i>	364	0
<i>Spirorbis spp.</i>	629	0
Sponge		
<i>Halichondria sp.</i>	168	1
Hydroid (% Cover, VA panels)		
<i>Obelia geniculata</i>	51.3%	9.1%

Values indicate the total number of individuals. Values for *O. geniculata* indicate the mean percentage cover of the hydroid (only present on VA 2009 panels). Data were pooled from the six 1-week assays as described in Figure 2.

Table 2. Effectiveness of aeration on different taxa of marine organisms.

	% Reduction
Ascidians	99.3
Barnacles	67.7
Bryozoans	99.4
Hydroids	57.4
Polychaetes	100.0
Sponge	99.4

Values indicate the mean percentage reduction of fouling for each taxon from the six 1-week assays as described in Figure 2.

had only 4% of the mass of fouling as control panels. Though not rigorously assessed, fouling material extended ~1.5 cm from the surface of control panels in both assays, while fouling on aerated panels was nearly flush with the panel surfaces (Figure 4).

Settlement was significantly lower on aerated PVC and concrete panels than on controls (Figure 5). Levels of settlement on control PVC and concrete panels were not significantly different from each other. A total of 1989 settlers attached to control PVC

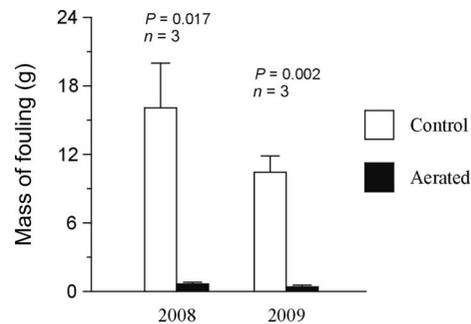


Figure 3. Mean mass ( $\pm 1$  SE) of fouling on 100 cm<sup>2</sup> PVC panels after exposure for 4 weeks. Both assays conducted in CT, 15 July – 12 August 2008, 17 July – 17 August 2009.



Figure 4. PVC panels after exposure for 4 weeks (15 July–12 August 2008). Non-aerated control panels are in the back row, aerated panels are in the front row.

panels, 2484 to control concrete panels and 1 each to aerated PVC and concrete panels (<0.1% of controls in both cases).

There were significantly fewer settlers on PVC panels deployed directly in aeration than on panels 30 cm or 5 m away from aeration (Figure 6). The levels of settlement on 30 cm and 5 m panels were of similar intensity and were not significantly different from each other (with a mean of 281 and 275 settlers panel<sup>-1</sup> respectively; Figure 6). During this assay, a strong storm damaged the main airline and prevented panels from being bubbled for ~2 days; this accounts for the presence of more settlers on aerated panels in this assay compared to other similar 1-week assays.

## Discussion

Continuous aeration significantly reduced biofouling on the PVC and concrete panels. After 1 week, aerated panels typically had ~0.1% of the number of settlers

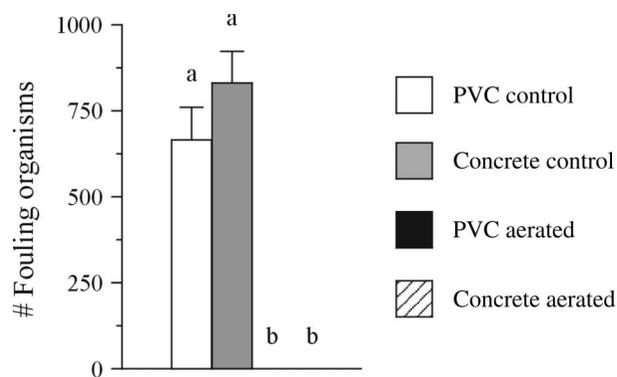


Figure 5. Mean number ( $\pm 1$  SE) of fouling organisms on 100 cm<sup>2</sup> PVC and concrete panels after exposure for 1 week (12–19 August 2008).

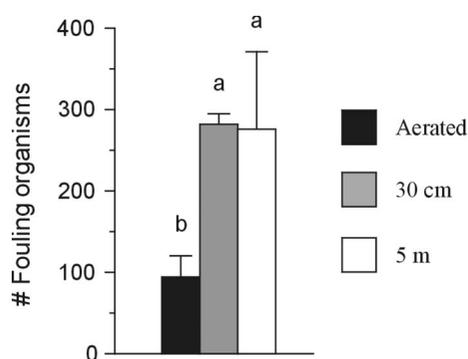


Figure 6. Mean number ( $\pm 1$  SE) of fouling organism on 100 cm<sup>2</sup> PVC panels after 1 week (24 June–1 July 2008). Aerated panels were directly in bubble streams, other treatment panels were 30 cm and 5 m away from aeration. Storm damage prevented bubbling for  $\sim 2$  days during this assay.

as non-aerated control panels (Figure 2). After 4 weeks aerated panels had only  $\sim 4.0\%$  of the mass of fouling as the controls (Figures 3 and 4). Aeration worked equally well at preventing fouling on PVC and concrete surfaces (Figure 5), but panels had to be directly exposed to aeration to gain fouling protection (Figure 6). These results suggest that aeration could be an effective way to control biofouling in marine systems and support previous studies that have also shown the AF potential of aeration (Smith 1946b; Scardino et al. 2009).

In 1-week and 4-week assays, aerated surfaces accumulated very few fouling organisms compared to non-aerated controls. After 1 week, aerated PVC and concrete panels looked relatively 'clean', while control panels were covered with attached settlers. Similarly, after 4 weeks, control PVC panels were 100% covered with fouling material, while aerated panels had very few attached organisms (Figure 4). Not only was a

significant mass of fouling attached to controls, but this fouling also hung from the panels into the water column for a distance of  $\sim 1.5$  cm. In contrast, most of the fouling found on aerated panels was composed of small amounts of mud and sediment that were flush with the surface of the panels. This is potentially important, as one of the main applications for aeration could be its use in preventing hull fouling. If aeration can similarly protect ships' hulls (eg Scardino et al. 2009), it would significantly reduce fouling-related drag and decrease fuel expenditures (eg Townsin 2003).

Aeration reduced fouling by all taxa examined (Tables 1 and 2). It was most effective against ascidians, bryozoans, polychaetes and sponges and reduced attachment of these organisms by  $> 99\%$ . Thus, aeration effectively prevented attachment by some of the most common and abundant fouling organisms. Additionally, because many of the species examined are highly invasive, such as *Botrylloides violaceus*, *Diplosoma listerianum* (Steneck and Carlton 2001) and *Didemnum vexillum* (Bullard et al. 2007), this suggests that commercial applications of aeration could help prevent the attachment and spread of invasive species. Aeration also reduced fouling by barnacles and hydroids, but to somewhat lesser degrees (by 68% and 57% respectively). Hydroids, in particular, seemed resistant to the AF properties of aeration. In the VA experiments, colonies of the hydroid *Obelia geniculata* attached to the surface of racks outside aeration and then grew into aerated areas. Scardino et al. (2009) also found high abundances of hydroids on aerated surfaces. Similarly, during a year-long preliminary assay conducted in CT (2007–2008), very high abundances of *Obelia* sp. were found on an aerated section of a concrete floating dock. Ascidians were virtually absent from the aerated dock section, while *Obelia* sp. was absent from adjacent, ascidian-dominated, non-aerated sections (personal observation). It is unclear why hydroids are less affected by aeration than other taxa. It could be due to unique characteristics of hydroid biology or growth patterns.

Aeration significantly reduced fouling at three different sites with different benthic communities. Thus, aeration appears to be broadly effective in many marine systems. Even so, there were some differences in fouling patterns among sites with different communities. While levels of fouling on aerated VA panels were significantly lower than on the controls, these panels were considerably more fouled than aerated panels from CT and MA (Figure 2). These differences were most likely due to differences in benthic community structure or seasonal settlement patterns among the sites. Almost all of the foulers attached to aerated VA were barnacles. At the time of the year sampled in this study, barnacles

were common in VA benthic communities (mean of ~34 per control panel), but relatively rare in CT and MA communities (mean of ~4 and 1 per control panel respectively). Had barnacles been more common in CT and MA, it is likely that they also would have successfully attached to aerated panels. These results were somewhat unexpected given that Smith (1946b) had previously found that aeration effectively controlled barnacle fouling in Florida. However, the fouling reduction Smith (1946b) observed could have been caused by the toxicity of copper piping rather than from aeration.

Aeration effectively prevented fouling on both PVC and concrete surfaces despite the fact that these materials have very different surface textures, ie concrete has a rough, irregular surface while PVC has a smooth, even surface. Because the surface characteristics of most hard substrata found in the marine environment (eg fiberglass, plastic, and bivalve shells) generally fall between these two extremes, the results presented here indicate that aeration will likely prevent fouling on most materials. Similarly, Scardino et al. (2009) found reduced fouling on acrylic and silicone and fluoropolymer fouling release coatings. Because different materials possess varied surface properties (eg rugosity, texture, surface chemistry, critical surface tension and wettability) (Brady and Singer 2000; Finlay et al. 2002; Ista et al. 2004) additional work is needed to empirically assess the AF effectiveness of aeration on different substances.

Surfaces had to be directly exposed to aeration to gain fouling protection. There was significantly less fouling on panels inside bubble streams than on panels 30 cm or 5 m away from aeration (Figure 6). The exact mechanism that causes aeration to prevent fouling is unclear. *In situ* observations revealed that when bubbles hit the surface of panels, they momentarily stuck then slowly rolled off. Thus, aeration may physically push larvae away from surfaces or dislodge or damage newly settled juveniles. If the main action of fouling prevention is for bubbles to push larvae away and prevent them from settling, this suggests that commercial applications will need to employ devices that continuously generate aeration. If aeration damages newly settled juveniles, commercial applications could be developed that release intermittent pulses of aeration that destroy new recruits. Regardless of the mechanism, fouling was only reduced on surfaces directly exposed to aeration.

In summary, these results are highly encouraging. The short-term experiments described in this study (ranging from 1 to 4 weeks) and previously conducted longer term experiments lasting several years (Scardino et al. 2009) have demonstrated the AF properties of aeration. The next step is to conduct additional

large-scale experiments to determine whether this process can be developed into a commercially viable AF treatment. It is highly likely that this process can be further developed (eg Scardino et al. 2009) because the equipment needed to produce aeration (pumps and chemically inert piping) is cheap and readily available. A major advantage of aeration is that it is environmentally friendly. Aeration produces no toxins and its only carbon footprint comes from the electricity needed to run the pumps. However, before commercial development is feasible, several significant questions remain to be answered. First, the minimum level of aeration needed to prevent most fouling must be determined. Secondly, the specific mechanism of fouling prevention should be identified.

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