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Biofilm control for plate heat exchangers using surface seawater from the open ocean for the OTEC power plant

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Abstract

At the proposed site for the Ocean Thermal Energy Conversion power plant on the east coast of India, biofilm formation and its control using intermittent chlorination in plate heat exchangers was studied prior to commissioning of the plant. Settlement of fouling larvae was not observed on the plates, which may be attributed to the low density of larvae of fouling organisms in the open ocean (65 km from the coast). Significant reduction in biofilm thickness was observed after chlorination at a dosage of 1.2 ppm residual. Microalgae were absent on untreated and chlorinated heat exchanger plates. Significant differences were also observed with respect to bacterial density and diversity between chlorinated and control surfaces. Total counts of viable bacteria in untreated controls increased with time. Relative to controls, a significant reduction in bacterial density in biofilms was observed upon chlorination. Sulphate reducing bacteria were absent in chlorinated biofilms, whereas their numbers increased with time in controls. Counts of heterotrophic bacteria in biofilms also showed an increase with time in controls and were significantly lower on chlorinated surfaces. Counts of slime formers, e.g. *Pseudomonas* sp. and *Aeromonas* sp., were low compared to total viable bacterial counts.

Keywords: Biofouling; Biofilms; Plate heat exchanger; Intermittent chlorination

1. Introduction

Biofilms are localized concentrations of microorganisms attached to a substratum comprising of a single species, or a multi-species community, that are distributed heterogeneously (White et al., 1999). Operationally, the problem due to biofouling manifests when "biofilm development exceeds a given threshold of interference" (Flemming and Griebe, 2000). Keeping biofilm development below the threshold is of importance to industrial operations. In cooling water circuits, the presence of biofilms can restrict flow in pipelines and heat exchangers (Bott, 1999), reduce heat transfer across the metal and alter surface roughness, which in turn can increase fluid frictional resistance and result in decreased flow (Aftring and Taylor, 1979; Geesey and Bryers, 2000). Control of biofilms and macrofouling in industrial systems is an important component of a successful water treatment program (Ludensky, 2003). Natural biofilms are heterogeneous with respect to the distribution of the organisms and in their metabolic activity.

Biofilms are dynamic systems where deposition, growth, mortality and detachment of bacteria intricately coexist and are difficult to separate (Characklis and Marshall, 1990). Biofilms are not a single homogenous entity (Allison and Gilbert, 1992), comprising a complex functional consortium made up from microorganisms, organic and inorganic solids. Attachment in biofilms is advantageous for microorganisms in increasing the availability of nutrients, concentrated at the surface in flowing systems. This, in turn, increases their infusibility into biofilms at low Reynolds numbers (White et al., 1999). Development of biofilm depends on various factors such as the availability of nutrients and oxygen in the water that influence cell metabolism and physical conditions of the flowing system such as flow velocity, temperature, and nature of the solid surface (Bott, 1999). As the biofilm ages, microniches are generated by the metabolic activity of multispecies, such as the generation of anaerobic sites in an aerobic environment and the generation of a glycocalyx

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to block exposure to biocides. In flowing systems the availability of nutrients at surfaces, infusibility of these nutrients, development of a glycocalyx matrix to restrict predation and exposure to toxicants are advantageous. Moreover the extracellular material produced by biofilms can facilitate incorporation of particulate matter in the biofilm.

In the choice of biocides for biofilm control, several factors have to be taken into account including:

(a) The control of microorganisms in the bulk fluid.
(b) Fluctuations in composition and condition of the water.
(c) The toxicity of the biocide(s).
(d) Cost effectiveness and safety implications.
(e) The ability to determine their concentration in treated water.
(f) The persistence in the water of the biocide at residual concentration to prevent possible recontamination (Walker and Percival, 2000).

Notable amongst the unique properties of biofilms is their high level of resistance towards antibiotics, biocides and disinfectants (Allison et al., 2000). Costerton et al. (1995) reported that biofilm cells are 500 times more resistant to antimicrobial agents than their planktonic counterparts. Hence control measures adopted should concentrate on the initial stages of biofilm development. Biocides should be evaluated against the organisms, which they are expected to kill, i.e. the dominant organisms in the system to be treated (Cloete et al., 1992). Studies by Cheng et al. (1993) revealed that the efficacy of the biocide monochloramine against *Pseudomonas* biofilms varied on different metal substrata. Similarly, depending on the morphology of the biofilm and the density of the planktonic forms, the biocidal concentration required for control differs from case to case (Tashiro et al., 1991).

The mechanism of resistance in biofilms seems to depend on multicellular strategies from the now familiar plasmids, transposons and mutations that confer innate resistance to individual bacterial cells (Stewart and Costerton, 2001). On the other hand, it is now believed that attachment of microorganisms to surfaces causes the expression of biofilm-specific phenotypes, possibly regulated through quorum sensing mechanisms, which may contribute to biofilm resistance (Allison et al., 2000). In order to control biofilm development in heat exchangers with effective biocide addition, effects of the type of system, flow rates, density and species composition have to be investigated.

Plate heat exchangers for the Indian 1-MW Ocean Thermal Energy Conversion (OTEC) power plant are selected because of their compactness and heat transfer efficiency of 1°C. Heat exchanger cleanliness is a vital factor in plate type because the gap between successive plates is only 3.7 mm, with very little tolerance for any fouling. The design value for seawater velocities between the heat exchanger plates is in the range of 0.6–0.8 m s^{-1}, which is ideal for slime formation. A rigorous treatment regime has to be put in place to ensure maximum heat transfer efficiency and uninterrupted plant operation. Further, online mechanical cleaning methods are not available for these heat exchangers necessitating the use of chemical methods to combat fouling. Liquid sodium hypochlorite was chosen as biocide for the OTEC plant based on its easy availability, cost effectiveness for open cycle systems, easy handling, transport, midsea transfer and replenishment to the OTEC barge at the offshore site.

Near-shore fouling control experiments, conducted earlier at a seacoast test facility near Tuticorin on the East Coast of India, using flat coupons in a model flow through system—a modified Pederson device (Pedersen, 1982), revealed that settlement of larvae of macrofouling organisms was prevented at continuous chlorine residuals of 0.8 ppm upwards. However, slime control was achieved at 1.2-ppm residuals. The effectiveness of this dosage applied to plate heat exchangers was of concern. Hence the present study was carried out to assess the extent of fouling buildup, and effectiveness of the chlorination regime, using plate heat exchangers at the proposed OTEC plant site prior to commissioning. Further there is scant information on the nature and extent of fouling and control measures to be adopted for plate heat exchangers used for seawater applications.

2. Materials and methods

The Indian OTEC Plant is to be commissioned 35 nautical miles (8°15′N and 78°35′E) off the Tuticorin coast in the Gulf of Mannar on the East coast of India. The platform comprises a non-self-propelled barge housing the plant components and moored using the cold water pipeline.

2.1. Experimental site and flow system

Experiments were carried out during the 43rd cruise of the research vessel "*Akademik Alexander Sidorenko*" using a seawater flow-through system. The experimental system was composed of stainless steel plate heat exchangers (Alfa Laval A45-FG) containing ten plates. These plates were separated using CFC gaskets and the plates are equidistantly spaced (3.7 mm). The heat exchanger was connected to two seawater pumps using 5.08-cm diameter carbon steel piping. One pump fed the odd numbered plates whereas and the other fed seawater to the even numbered plates. The seawater line feeding the even numbered plates was chlorinated before it entered the plate heat exchangers using a variable stroke chemical dosing pump connected to a programmable timer device. This arrangement ensured that every alternate plate was receiving chlorinated water. Thus one set of plates of the plate heat exchanger was left untreated and served as a control, and other set was chlorinated using sodium hypochlorite (NaOCl). A chlorine residual of 1.2 mg l^{-1} applied for 2 h with 2-h intervals between applications, determined from experiments conducted near the shore, was tested. Subsequently, the effectiveness of this

dose was tested at the offshore plant site using model heat exchangers. Residual chlorine was measured by the iodometric method (White, 1986). The chlorine demand of the seawater was ascertained and the net chlorine residual was maintained and checked once every 4 h. Seawater velocities between the plate surfaces were maintained at 0.6 m s^{-1}. Excess flow was throttled and routed out using an intermediate bypass valve. The flow through system was operated continuously and the vessel was maintained at a speed of 0.2 knots, set on a circular trajectory at the oceanic site (8°15′N and 78°35′E) during the experimental period.

2.2. Biofilm characterization

The heat exchanger was opened and biofilm samples collected at daily intervals from day 1 to day 4 of continuous operation. Biofilm thickness on the heat exchanger plates was measured with a modification of the method of Bakke and Olsson (1986). Acridine orange staining (Hobbie et al., 1977) and epifluorescence microscopy (Carl Zeiss Axioscope 2 plus) were used since the surface was opaque. The region of the plate where seawater entered the pass was stained with acridine orange. Excess stain was drained off and the plates air-dried. The plates were then viewed under 100 × Neofluor objectives with oil immersion. Biofilm thickness was calculated by determining the distance travelled by a 100 × objective lens when focused on the metal surface and on the biofilm surface.

In the laboratory, the retrieved heat exchanger plates (chlorinated and untreated) were rinsed with filtered (0.2 μm, Millipore) and autoclaved (120°C for 15 min) seawater from the same site. The biofilm was scraped from the plates using a sterile nylon brush and dispersed in a fixed volume (500 ml) using the sterile filtered seawater. The biofilm on the corrugated regions of the plates were sampled. In general, the methods used for biofilm analysis followed those of workers such as Fletcher (1984), Bhosle et al. (1989), Sharma et al. (1990), Srivastava et al. (1990), Liu et al. (1993) and Rao et al. (1997). Biofilm solids were measured using GF/C 0.45 μm filter papers (Parsons et al., 1992). Combustible organic matter in biofilms was estimated using pre-ignited (400°C, 4 h) Whatman GF/C filter papers. Biofilm samples were also analyzed for total dissolved iron content by the phenanthroline method (APHA, 1998). Diatoms were counted on the plates as well as in biofilm samples using a haemocytometer.

Microbiological analysis was carried out using standard media packs prepared by HIMEDIA. Total viable counts (TVC) were estimated using Zobell Marine Agar, and counts of heterotrophic bacteria and sulfate-reducing bacteria (SRB) were made using standard microbiological methods as reported by Postgate (1984). SRB were assayed using Postgate medium, the inoculated plates being incubated in anaerobic jars and SRB colonies detected after incubation for 48 h. *Pseudomonas* counts were made on *Pseudomonas* agar (HIMEDIA pack) and *Aeromonas* counts on *Aeromonas* agar (HIMEDIA pack). Bacterial isolates were identified using standard biochemical tests according to Bergey's Manual of Systematic Bacteriology, Second Edition, 2001. Seawater quality parameters were assessed using the methods outlined by Grasshoff et al. (1999) and temperature measurements recorded using a CTD (Seabird-SBE 25). All analyses were carried out in triplicate, except for biofilm solids and combustible organic matter, and the results presented as mean values. The data were subjected to two-way analysis of variance.

3. Results

Table 1 describes the data on the water quality parameters at the site during the course of study. Settlement of larvae of fouling organisms was not observed on heat exchanger plates during the study period. Biofilm thickness on the heat exchanger plates increased from 5.0 μm after 24 h to 16.2 μm after 96 h (Fig. 1) in controls, whereas in chlorinated plates the increase in thickness was only from 3.2 μm after 24 h to 5.04 μm after 96 h. A significant difference ($p = 0.05$) in biofilm thickness was observed with respect to control and treated surface. No significant difference ($p = 0.098$) in total biofilm solids was observed with treated biofilms compared to controls. Epifluorescence microscopic observations on untreated heat exchanger plates revealed a sparse distribution of bacterial cells. Diatoms and other algal forms were absent. Accumulation of solids in untreated biofilms did not show any increase between 24 and 72 h, but an increase was observed at 96 h (Fig. 2). Similarly, no significant difference ($p = 0.640$) was observed in the total biomass fraction (estimated as total amount of combustible matter) between chlorinated biofilms and controls. Organic matter accumulation in biofilms showed an increasing trend with the age of the biofilm in controls, except for a marginal decrease observed after 72 h (Fig. 3). In

Table 1
Observed seawater parameters at OTEC site, Gulf of Mannar, during 4-day experimental period

Parameter	Unit	Mean ± SD	Range
pH	—	8.2 ± 0	(8.2)
Temperature	°C	27.4 ± 0.1	(27.4–27.1)
Dissolved oxygen	mg l^{-1}	4.8 ± 0	(4.1–4.8)
Salinity	ppt	35.5 ± 0	(35.1–35.5)
Total suspended solids	mg l^{-1}	2.1 ± 0	(2.1–2.1)
Combustible organic matter	mg l^{-1}	0.18 ± 0.03	(0.18–0.11)
Total viable bacteria	CFU ml^{-1}	800 ± 10	(680–800)
Pseudomonas sp.	CFU ml^{-1}	3 ± 1	(1.6–3)
Heterotrophic	CFU ml^{-1}	243 ± 15.2	(163.3–243)
Aeromonas sp.	CFU ml^{-1}	30 ± 10	(23.3–30)
SRB	CFU ml^{-1}	2.3 ± 0.5	(1.0–2.3)

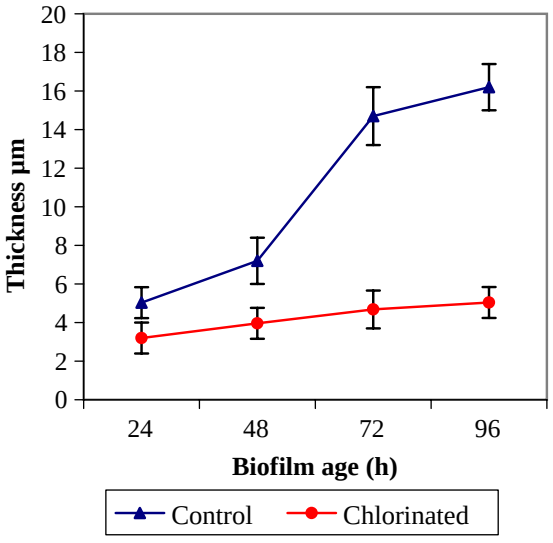

Fig. 1. Biofilm thickness on heat exchanger.

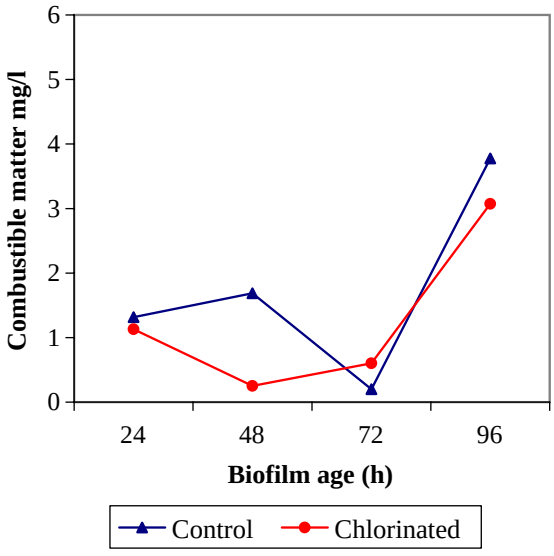

Fig. 3. Combustible organic matter in biofilms on heat exchanger plates.

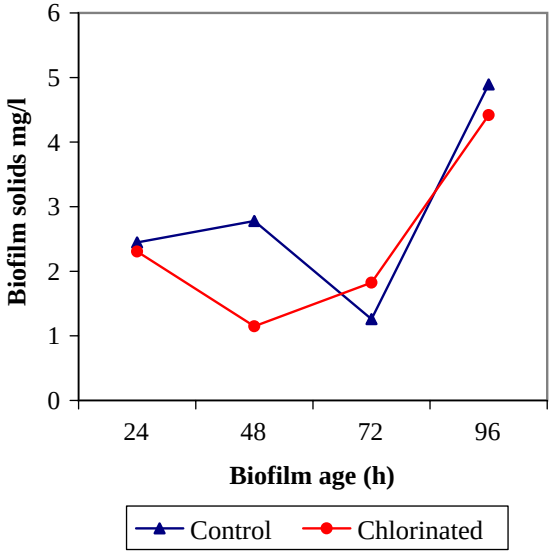

Fig. 2. Biofilm solids on heat.

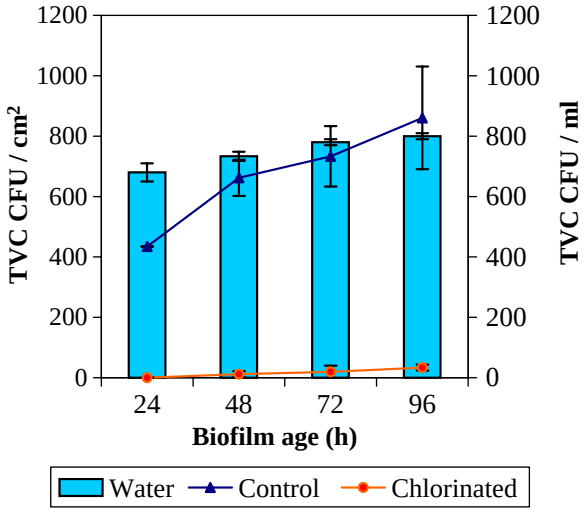

Fig. 4. Total viable bacterial counts in biofilms.

chlorinated biofilms, organic matter showed a gradual increase with time.

Microbiological analysis of biofilms revealed four different types of bacterial colony to be predominant and the easiest bacteria to isolate, viz. *Vibrio*, *Flexibacter*, *Pseudomonas* and *Aeromonas*. Total viable bacteria (Fig. 4) in untreated controls showed a trend to increased counts with the age of the biofilm. There was a highly significant difference ($p = 0.0001$) between chlorinated and control biofilms. When chlorination was 100% effective, during the first 24 h, no bacterial colonies were detected in these biofilms, but with the increase in age of the biofilm the effectiveness was found to decrease. The percentage reduction in bacterial counts in chlorinated biofilms relative to controls is shown in Table 2, and accumulation of cells in biofilms from bulk fluid in Table 3. Chlorination resulted in a decrease in the sessile population compared to the levels in ambient water (Table 4). A similar trend was observed with the heterotrophic bacterial counts (HPC), the counts in control biofilms increasing with respect to the age of the biofilm (Fig. 5). A significant difference ($p = 0.013$) in heterotrophic plate counts was observed between control and treated biofilms. The concentration of slime formers, including *Pseudomonas* sp. and *Aeromonas* sp., also showed a gradual increase with time, both in control and chlorinated biofilms. *Pseudomonas* sp. was absent during the early stages (24 h) in chlorinated biofilms (Fig. 6). Estimation of density of *Pseudomonas* sp. in chlorinated biofilms revealed a marginal increase in their levels compared to ambient water. *Aeromonas* sp. counts in control biofilms

Table 2
Percentage reduction of sessile bacterial counts in chlorinated biofilms compared to controls

Time (h)	Bacterial counts				
	TVC	HPC	PS	AE	SRB
24	100	100	100	100	100
48	95.3	95.1	80.0	100	100
72	93.0	93.8	66.6	82.3	100
96	89.7	94.0	72.0	80.7	100

TVC—total viable counts; HPC—heterotrophic plate counts; PS—*Pseudomonas* sp. counts; AE—*Aeromonas* sp.; SRB—sulphate reducing bacteria.

Table 3
Percentage increase in sessile bacterial density in untreated biofilms compared to ambient water

Time (h)	Bacterial counts				
	TVC	HPC	PS	AE	SRB
24	125	151	260	0	0
48	177	340	1566.1	451	58.6
72	184.6	463	800	566.6	66.5
96	211.2	485.5	1100	714.2	110.2

Table 4
Percentage reduction in sessile bacterial density in chlorinated biofilms compared to ambient water

Time (h)	Bacterial counts				
	TVC	HPC	PS	AE	SRB
24	100	100	100	100	100
48	91.8	83.6	0	100	100
72	87.1	71.4	0	0	100
96	78.7	71.1	0	37.54	100

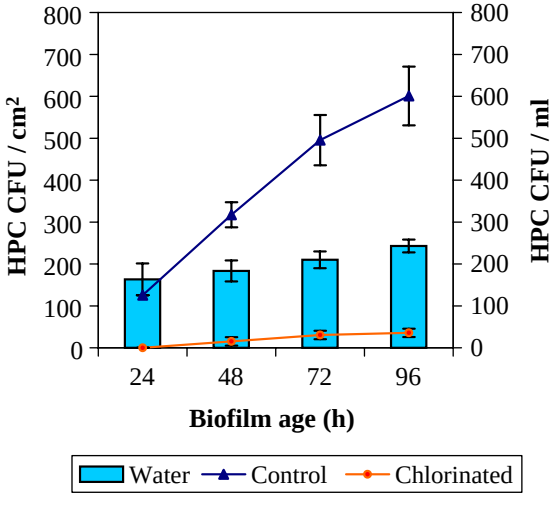

Fig. 5. Culturable heterotrophic bacteria in biofilms.

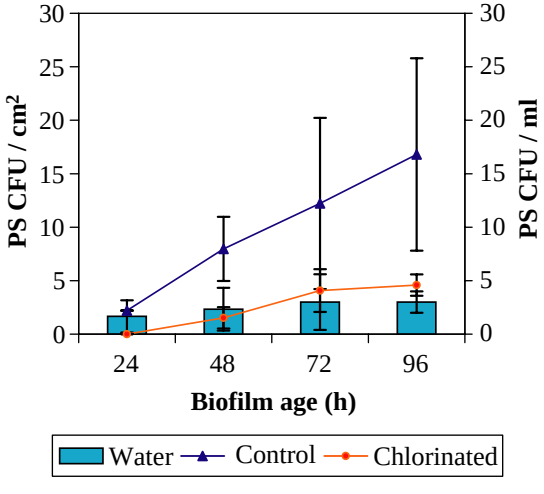

Fig. 6. *Pseudomonas* sp. bacterial counts in biofilms.

showed an increase with biofilm age. (Fig. 7). In chlorinated biofilms they were not detected during the first 48 h. SRB were analyzed in order to follow the onset of anaerobic conditions in the biofilm. The SRB were absent during the first 24 h, but were detected in biofilms at 48 h and later. Their numbers in biofilms were however comparable to the

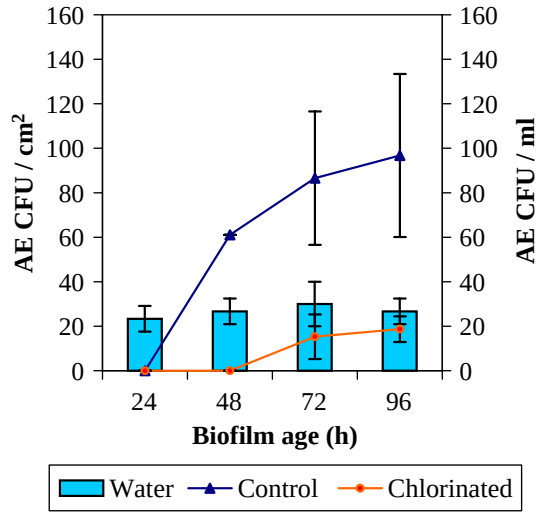

Fig. 7. *Aeromonas* sp. counts in biofilms.

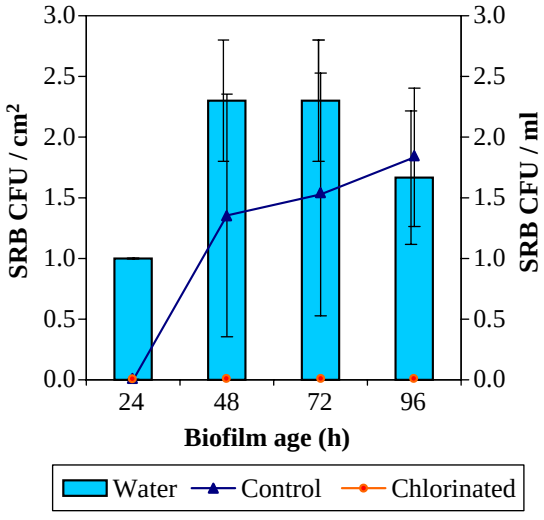

Fig. 8. Sulfate-reducing bacterial counts in biofilms.

levels in water. SRB were absent in chlorinated biofilms (Fig. 8).

Accumulation of corrosion products was observed on both the chlorinated and control plates. Analysis of biofilms for dissolved iron content revealed that the concentration of iron increased with increase in age of biofilm, the concentrations being equivalent to 0.19 μg l^{-1} at 24 h, 0.39 μg l^{-1} at 48 and 72 h, and 7.2 μg l^{-1} at 96 h. In chlorinated biofilms the corresponding concentrations were 0.36, 0.18 , 0.36 and 0.54 μg l^{-1}, respectively.

4. Discussion

Chlorine is widely used as a biocide for microbial fouling control through cooling water systems, where large volumes of water are used. Based on studies by Bitton (1994), the biocidal action of chlorine on bacteria may be classified into two types, disruption of cell permeability and damage to nucleic acids and enzymes. The effectiveness of chlorine is dependent on its reaction with the biofilm. Several factors are known to influence the rate and extent of chlorine-biofilm reaction (Characklis, 1981). These are (a) turbulence intensity, (b) chlorine concentration at the water-biofilm interface, (c) composition of fouling film, (d) fluid shear stress at the water-biofilm interface, and (e) pH. Bacteria in biofilms are generally more resistant to antimicrobial biocides than their planktonic counterparts (Costerton et al., 1987). The reason for this observed effect is that the biofilm matrix constitutes a diffusion barrier to biocides or that the biocides interact with biofilm constituents such as microbial cells/EPS resulting in biocide neutralization (D'beer et al., 1994). In addition the effectiveness of the biocide may be explained as depletion of the biocide within the interior of the biofilm through reaction-diffusion interactions on the periphery (Huang et al., 1995). Microbial biofilms are known for their recalcitrance towards treatment with antibiotics, biocides and/or disinfectants that would adequately control the same organism growing in the planktonic mode (Allison and Gilbert, 1995).

The concentration of biocide used often determines whether it functions as a bacteriostat or bacteriocide (Atkinson et al., 1979). The most common methods of biocide dosing are (a) low level continuous dosing, more effective for the prevention of biological fouling, and (b) high level, short-duration dosage, better suited for removing established films (Rippon, 1979). A low concentration of the biocide may only inhibit bacterial multiplication, i.e. a bacteriostatic effect, whilst a higher concentration completely kills the bacteria. Further, before choosing a biocide, it is important to identify the type of microorganism present in the biofilm (Atkinson et al., 1979).

The observation of thinner films with increasing concentration of biocide is in agreement with the findings of earlier workers (Nesaratnam and Bott, 1984). Earlier studies conducted nearshore using a modified Pederson's device revealed that macrofouling could be controlled by maintaining a continuous residual above 0.8 ppm. However bacterial slime was observed on the plates necessitating a higher dose. Results of the present study reveal that a concentration of 1.2 ppm applied intermittently (2 h on and 2 h off), is required for controlling biofilm growth (decreasing thickness and bacterial density). This may be attributed to a better capacity of the biocide to attack and penetrate the biofilm barrier. Higher concentrations of NaOCl would allow for the compensation of available chlorine used in breaking down or reacting with the extracellular products. The dose-effect relationship for chlorine is generally log-linear. However, it has been demonstrated that, even with concentration of several mg l^{-1}, fixed bacteria are more difficult to inactivate than planktonic cells in suspension in the bulk phase (LeChevallier et al., 1988; Camper et al., 1986).

Epifluorescence microscopy of biofilms on surfaces revealed a patchy and non-uniform distribution. Such distributions are representative of low-shear, laminar conditions (Brading et al., 1995). The biofilms were heterogeneous, consisting of cell clusters with EPS and particulate matter separated by interstitial voids. In the present study, accumulation of corrosion products was observed on the plate heat exchangers, which originated from the corroding mild steel pipe upstream of the heat exchangers. Discolouration of the plates with time was observed both in control and chlorinated sections. Similarly, particulate matter accumulation in biofilms was found to increase with age of the film. This may be attributed to the entrapment of particulate matter exposed to rust from an upstream steel pipe and the extracellular polymer matrix produced by the bacteria. Polymeric coated pipelines would eliminate this problem.

Chlorination was very effective in controlling the sessile bacterial population during the initial stage of biofilm formation (up to 24 h). Subsequently, the sessile bacterial population in chlorinated biofilms showed an increasing trend with age. This was observed with the different bacterial groups, including *Pseudomonas* and *Aeromonas*. The failure of microorganism to succumb to antimicrobial treatment may arise through (i) an inherent insusceptibility to the agents employed; (ii) the acquisition of resistance by previously susceptible strains, either by genetic mutation or by transfer of genetic material from another species or genus; or (iii) the emergence of pre-existing but unexpressed resistant phenotypes (Allison et al., 2000). However, none of these processes alone can provide a complete explanation for the observed levels of resistance *in situ*. Hence current control strategies involve the design of antimicrobial agents that are specifically targeted at cells growing within the biofilms. These include molecules with high diffusion-reaction ratios and agents targeted at slow or non-growing cells (Allison et al., 2000). In addition, results of that study revealed that daily application of the biocide at one-tenth of the dose has virtually no effect on biofilm formation and that the greater the initial concentration of biocide the greater the effect on biofilm.

5. Conclusions

The prescribed intermittent chlorination regime was found to be effective for plate heat exchangers at the offshore site. Viable bacterial counts in chlorinated biofilms were reduced some 94% compared to controls. Biofilms were patchy, non-uniform and predominantly bacterial. Diatoms and other algal forms were not observed. As the biofilm aged, corrosion products were found to accumulate on the plates, which in turn led to entrapment of suspended particulate matter from the bulk fluid. This was further compounded by the adsorption of organic matter onto the particles, leading to the formation of a barrier between the biofilm cells and the bulk fluid. It is possible that the barrier may consume most of the free chlorine before it is available for killing the cells. This may synergistically be responsible for the observed decrease in the biocidal efficiency of chlorine in older biofilms (48 h onwards), which showed bacterial regeneration and growth. From the present study it is evident that the use of a biodispersant along with the biocide is imperative. This would aid in removing the dead cells and the polymer matrix from the surface, thus facilitating the infusibility of the biocide and its activity. Chlorination was effective in controlling the levels of sulphate reducers.

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