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BIOCIDE DOSING STRATEGIES FOR BIOFILM CONTROL

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ABSTRACT

In order to reduce environmental impact of biocide use for the control of biofilm formation in cooling water circuits, "environmentally friendly" biocides have been developed, but they are generally more expensive than the more traditional chemicals. It is imperative therefore, that the minimum quantity of biocide is employed, so that costs are kept to a minimum. To achieve this objective optimum dosing strategies are required. Using apilot plant in conjunction with a monoculture of *Pseudomonas fluorecsens* as the biofouling bacterium, tests were carried out using a proprietary biocide, to investigate the effects of dose concentration, duration and frequency of dosing and fluid mechanics on biofilm control. With four 15 minute applications per day, at a peak concentration of 16.8 mg/l, it was not possible to inhibit biofilm development. Control was effected however, by doubling the peak concentration using a short dosing period. Concentration, as would be expected, was shown to be a critical factor for control. A boicide concentration below that for growth inhibition, seemed to enhance biofilm formation! Increase frequency of dosing is only effective if the concentration employed is biofilm growth inhibiting.

INTRODUCTION

Because of the well known effects of heat exchanger fouling, it is necessary to control biofilm formation in cooling water circuits. The usual approach is to apply a biocide to kill the micro organisms. For many years the preferred biocide has been chlorine, because of its effectiveness and relatively low cost. Since in many cooling water circuits, the water is taken from natural sources such as lakes or rivers, and discharged back to the source, the presence of the biocide represents an environmental hazard. Chlorine, for instance, reacts with organic matter to produce cancer-forming substances, and the chlorine can enter the food chain.

In order to overcome these potential problems for the environment, so called "environmentally friendly" biocides have been developed, but their cost is high. It is therefore imperative that techniques to maximise their effectiveness are employed.

This paper reports work using a proprietary biocide, to illustrate how optimum biocide dosing strategies may be developed.

MATERIALS and METHODS

The laboratory pilot plant used in the work is based on a "feed and bleed" concept and operates for long periods of time. Fig. 1 is a simple schematic sketch of the equipment.

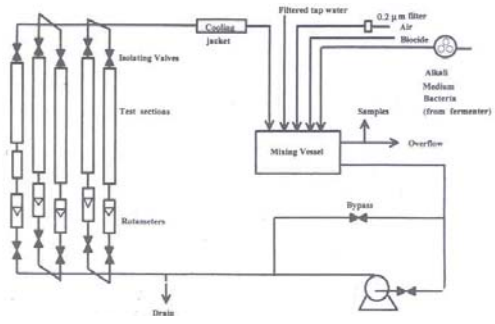

Fig 1. Flowsheet of the pilot plant

A monoculture of *Pseudomonas fluorescens* is grown in a fermenter and a predetermined flow of water laden with micro organisms, is pumped into the mixing vessel. The *Pseudomonas* species is known to produce biofilms in aqueous systems, and in these experiments the species is used as a representative contaminant found in cooling water circuits. The concentration in the

circulating water from the mixing vessel is 10^7 cells /ml. A known rate of nutrient addition and filtered tap water , from which traces of chlorine have been removed by an activated carbon filter, is also fed to the mixing vessel. The filter system removes all particulate matter down to 1μ so that it may be assumed all potential contaminates including micro organisms, have been removed. Tests with no added micro organisms, demonstrated that no biofilms were formed, confirming that the filter system was effective. The nutrient solution is sterilised in an autoclave at 120^0 C for one hour before being fed to the mixing vessel. Automatic alkali addition in response to a pH probe, maintains the pH at 7. *Pseudomonas fluorescens* is aerobic so the mixing vessel is sparged with filtered air to ensure that the circulating water is saturated with oxygen. The simulated cooling water is passed through vertically mounted glass tubes, which act as the test surfaces. The tubes are 1m in length with internal diameters of 9 and 23.1 mm.

It is probably true to say that the water in every cooling water circuit is unique, since each system will draw on water available in the vicinity of the plant. The different sources will display a considerable range of composition depending on the local ecology. The simulated cooling water in the pilot plant, made up as described, is meant to represent a cooling water of a standard composition that is suitable for testing the effects of different treatment strategies. The principal difference between it and a "real" cooling water is that it only contains one organism, whereas a natural source of water will contain a whole spectrum of species. The point of using a monoculture is that the water will be of consistent quality in all respects, so that comparisons of different dosing strategies will be valid.

In the experiments reported in this paper two water velocities were chosen to represent industrial water flows of 0.5 and 1.3 m/s. The Reynolds number was around 12,000 since it has been shown (Pujo and Bott , 1991) that at this value the biofilm growth rate is a maximum. The water temperature was approximately 20^0C. A proprietary biocide containing 2,2 Dibromo-3-nitrilopropionamide was used in the experiments, at different concentrations with different dosing regimes, to determine their effect on for biofilm control. The residence time in the pilot plant was 60 minutes. Variable residence times could affect the quality of the simulated cooling water. The use of a fixed residence time therefore, is to ensure that a consistent quality of simulated cooling water is maintained through out the tests, so that reliable comparisons of the dosing strategies may be made.

The pilot plant is equiped with a non intrusive infrared monitor (Tinham and Bott, 2002).The absorbance of the infrared radiation by the biofilm residing on the surface of the glass test section, is a measure of biofilm accumulation at the point of application of the infrared probe. A comparison of the absorbance recorded under different operating conditions, biocide concentration and application strategy, provides a means of assessing effectiveness of the particular technique.

In strategies where the dosing is not continuous and the application of biocide is over a relatively short time (e.g. 15 minutes) the concentration of the biocide rises to a peak value and then falls away to a level approaching 0 mg/ l. A model of biocide concentration variation with time has been given by Characklis and Marshall (1990). A typical biocide concentration/time profile is presented in Fig. 2.

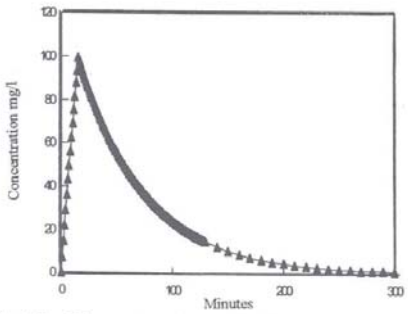

Fig. 2 Biocide concentration profile during and after dosing at 100 mg/l

RESULTS

Continuous Dosing

Continuous dosing of the biocide at concentrations of 100 and 50 mg/l were found to control biofilm formation (Fig 3).

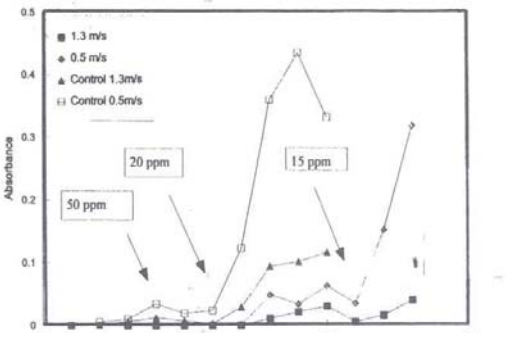

Fig. 3 Effect of continuous dosing of biocide from a starting concentration of 100 mg/l

A reduction of biocide concentration to 20 mg/l showed that there was some biofilm development at this concentration detected by the infrared monitor, but nevertheless there seemed some inhibition of growth at this biocide concentration as the biofilm could not be seen with the naked eye. A further reduction of biocide concentration to 15 mg/l resulted in an increased rate of biofilm growth, particularly noticeable at the lower water velocity of 0.5 m/s. The growth rate of the biofilm at this velocity was similar to the control (no biocide present), indicating that the biocide was exerting very little effect. At the higher water velocity of 1.3 m/s biofilm growth appeared to be under control. Work with a similar biocide (Pujo 1993), demonstrated that continuously dosing at a concentration of 15 mg/l, effectively prevented biofilm formation. Table 1 provides data on the total biocide used daily in the continuous dosing experiments, at the different concentrations employed. On a full scale cooling water system if these concentrations were used, they would represent large quantities and associated costs.

Table 1 Total daily use of biocide at different continuously dosed concentrations

Dose concentration mg/l	100	50	20
Daily total Mg	28,000	14,000	5,760

At the higher water velocity of 1.3 m/s, the infrared absorbance of the biofilm was lower than that observed with a water velocity of 0.5 m/s. It has been demonstrated (Bott 1995), that at low velocities mass transfer plays an important role in biofilm development; growth increasing with increasing mass transfer of nutrients as velocity is raised up to about 1m/s. At velocities above 1m/s the effect of the increased shear on the biofilm is greater than the effect of the associated increased mass transfer of nutrients. As a result the thickness of the biofilm is reduced.
These results confirm the findings of previously published work (Bott and Taylor, 1997).

Shock treatment

Shock treatment involved subjecting the system to a relatively short "burst" of biocide over a period of 15 minutes.

The first tests used a concentration of 100mg/l of biocide, since this concentration was found to be effective on a continuous basis, but the results indicated that shock dosing at this concentration did not prevent biofilm growth (Fig.4).

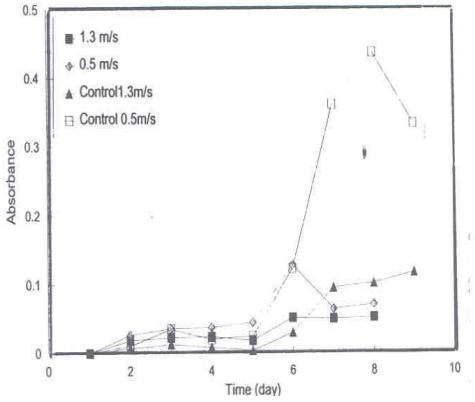

Fig. 4 Effect of daily shock dosing 100 mg/l biocide

After two days biofilm could be observed on the test surfaces at the lower water velocity of 0.5 m/s. For the first 6 days biofilm growth was similar to the control (no biocide present), i.e during the lag phase and the beginning of the exponential phase. Development began after 2 days and 5 days at water velocities of 0.5 and 1.3 m/s respectively. Beyond the lag phase there did appear to be some inhibition of biofilm growth, although it has to be reported that in the parts of the pilot plant e.g. the mixing vessel and pipe work where the velocity was particularly low, significant biofilm could be seen.

The conclusion of this experiment was that a shock dose of 100 mg/l of biocide was not an effective treatment for biofilm control at the water velocities tested, although there was an indication of inhibition of biofilm growth.

Since this level of shock treatment was not effective, it was decided to go to the other extreme and give a massive shock dose, based on the total amount of biocide used per 24 hours for continuous treatment, as presented in Table 1. On this basis 28800mg of biocide was pumped into the system in a period of 15 minutes, resulting in a peak biocide concentration of 2133 mg/l. The results are given in Fig.5, demonstrating that this shock treatment was a failure.

As can be seen from Fig.5 biofilm appeared and could be observed on the fourth day after commencing the biocide treatment. A sudden growth of thick biofilm was observed on the test surfaces at both water flow velocities (0.5 and 1.3 m/s). There was no difference in the time taken for the onset of biofilm growth. In contrast to normal experience, biofilm growth

at the higher velocity regime was greater than at a water velocity of 0.5 m/s.

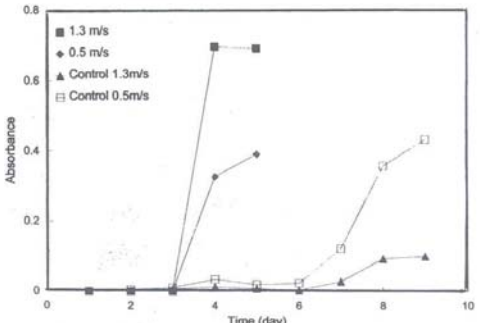

Fig. 5 Effect of daily shock dosing a total of 28,800 mg biocide

A possible explanation of this observation is that, following the "wash out" of the biocide from the system, the higher mass transfer, afforded by the higher water velocity, provided micro organisms and nutrients at a greater rate than at the lower velocity. The experiment was not repeated.

The general conclusion of these experiments is that short shock treatment times on a relatively infrequent basis, even with high biocide concentrations, is not effective in the control of biofilm formation.

Pulse dosing

In effect, pulse dosing may be taken as shock treatment on a more frequent application. Since a continuous dose of 20mg/l of biocide appeared to restrict biofilm growth, it was decided to use the total biocide used over a 24 hour period, and dose this quantity in 4x15 minute pulses evenly spaced throughout 24 hours. The total dose in 24 hours given in Table 1 is 5760 mg, which gave a peak biocide concentration of 106.19 mg/l. As can be seen from Fig.6 this pulse dosing completely prevented biofilm growth, and the monitor indicated almost zero accumulation of biofilm on the test surfaces.

Treatment with 5760 mg/day in 8x15 minute doses gave a peak concentration of 53.1 mg/l for each dose. The dosing regime, as can be seen from Fig.7, prevented biofilm growth at both velocities tested. As with the previous experiment, the infrared absorbance monitor recorded zero at the test surfaces. Biofilm deposits were seen in other parts of the equipment, where the water velocity was low. It could be stated that both these pulsed dosing strategies were successful and in comparison with the continuous dosing treatment with 20mg/l biocide concentration, yield better control.

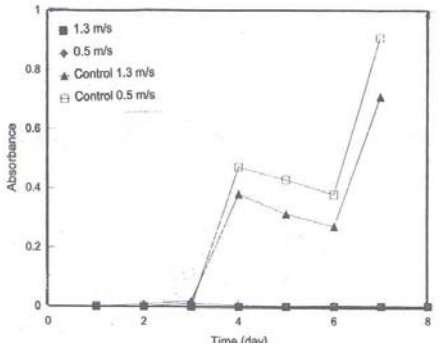

Fig. 6 Effect of pulse dosing biocide 4×15 minutes/day at a peak concentration of 106 mg/l

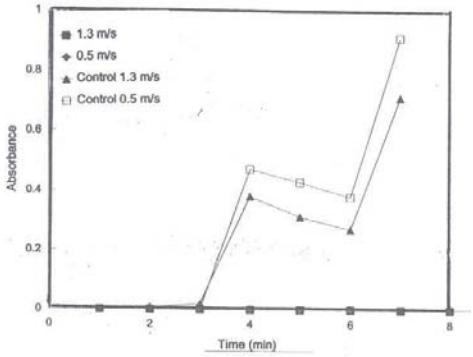

Fig. 7 Effect of pulse dosing biocide 8×15 minutes/day at a peak concentration of 53.1 mg/l

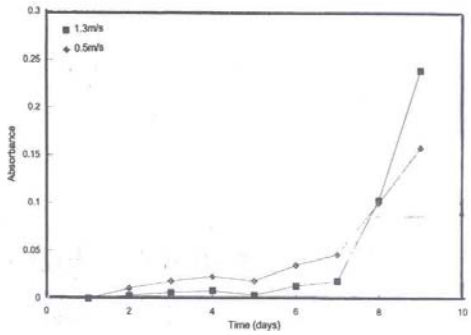

Fig. 8 Effect of pulse dosing biocide 8×15 minutes/day at a peak concentration of 8.43 mg/l

Taylor(1995) using the same biocide but with 4x30 minute doses in 24 hours at a peak concentration of 15mg/l, demonstrated that biofilm formation was prevented at a water velocities of 1.27 and 0.86 m/s, i.e. similar to the present study with a Reynolds number of 14000. It was calculated that under this treatment, the total biocide consumption would be 914.9 mg/day, and this figure was used as the basis for treatment using 15 minute pulses. The peak biocide concentrations associated with the three regimes are given in Table 2.

Table 2.Biocide peak concentration equivalent to 914.9 mg/day consumption

Dosing regime	8×15 min	4×15 min	2×15 min
Peak conc. mg/l	8.43	16.8	33.6

Fig.8 shows the results of using 8x15 minute doses in 24 hours, with the peak concentration of 8.43 mg/l. There was a measure of control for about 5 days after which biofilm development began. It would appear that this treatment regime simply extended the lag phase. The exponential phase started after the sixth day at both water velocities, and by the eighth day biofilm was greater at the higher water velocity of 1.3 m/s.

The results from a treatment regime of 4x15 minute doses every 24 hours are shown on Fig.9. The growth was accelerated for both water velocities compared to the control (no biocide present), the lag phase being reduced by approximately half.

The effect of halving the dosing period from 30 to 15 minutes, even though the total amount of biocide was the same, seemed to stimulate growth at the water velocities studied, with surprisingly, to a greater effect at the higher velocity.

The results of the last test with 2x15 minute treatments in 24 hours with a peak biocide concentration of 33.6 mg/l, was not successful in the prevention of biofilm formation as shown on Fig.10.

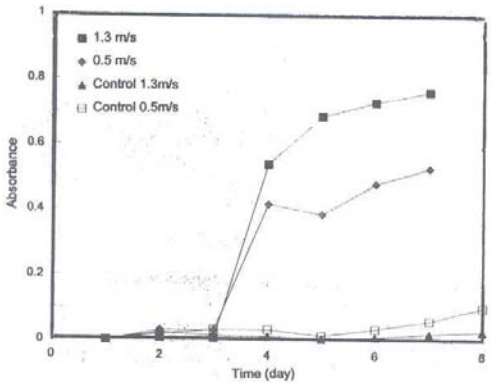

Fig. 9 Effect of pulse dosing 4×15 minute/day at a peak concentration of 16.8 mg/l

Biofilm was detected after two days and growth remained in the lag phase for a further five days before the exponential phase was initiated. The extended lag phase may be noted for each of the water velocities. On the eleventh day of the experiment, biofilm accumulation was again greater in the test section with water flowing at 1.3 m/s.

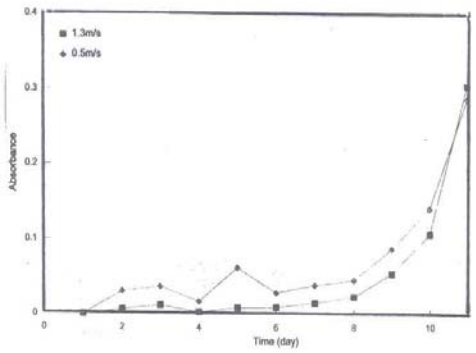

Fig. 10 Effect of pulse dosing biocide 2×15 minute/day at a peak concentration of 33.6 mg/l.

In the light of these results based on the use of 914.9 mg biocide per day, where no control was achieved, it was decided to repeat two of the tests, but using double the daily consumption i.e. 1829.8mg. Because of the particularly poor result of using only two pulses per day in the earlier experiments, the tests were based on four and eight pulses each 24 hours. The peak concentrations are given in Table 3.

Table 3. Biocide peak concentration equivalent to 1830 mg per day consumption

Dosing regime	8×15 min	4×15 min
Peak conc. mg/l	16.8	33.6

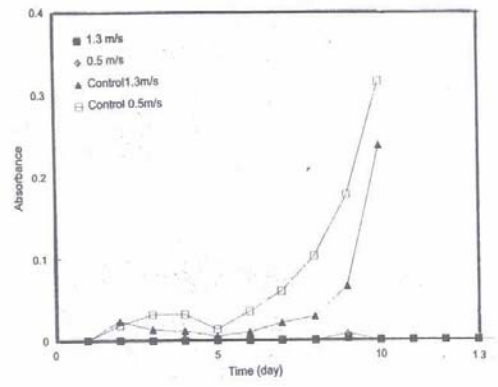

Fig. 11 Effect of pulse dosing biocide 8×15 minute/day at a peak concentration of 16.8 mg/l

Fig. 11 provides the results of the test for eight 15 minute pulses of biocide at a peak concentration of 16.8 mg/l, demonstrating that biofilm growth is controlled under this regime.

The infrared monitor recorded near zero absorbance for both velocities.

The results for four x 15 minute pulses of biocide at a peak concentration of 33.6 mg/l are given on Fig.12 Growth was not visible to the naked eye but the monitor detected a small accumulation of biofilm. For a period of fourteen days this biofilm growth control was not so effective under this regime when compared to the eight 15 minute pulse regime of Fig.8 with the higher peak biocide concentration.

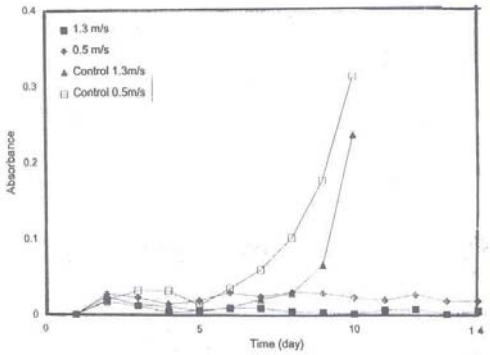

Fig. 12 Effect of pulse dosing biocide 4×15 minute/day at a peak concentration of 33.6 mg/l

DISCUSSION

The results of the experiments suggest that dosing an insufficient amount of biocide may cause an increase in the biofilm accumulation. The studies showed greater biofilm growth in the presence of the biocide, at higher water velocity. The enhanced growth only seemed to occur in the exponential phase of biofilm growth.

It is difficult to appreciate why in the presence of the biocide, growth is stimulated. Extracellular polymer material that forms the "substance" of biofilms , can act as an exchange resin. It may adsorb biocide thereby preventing the biocide from reaching the actual cells in the biofilm (Le Chevallier et al, i988). At the higher water velocity of 1.3 m/s, it is likely that relatively large amounts of biocide reach the biofilm during the lag phase, compared with the situation at the lower water velocity of 0.5m/s, due to the greater mass transfer at the higher turbulence level associated with the higher water velocity. Under the conditions of the experiments there may have been insufficient biocide at the water /biofilm interface to kill the surface colonisers. Exposure to the biocide may have stimulated a degree of resistance to the biocide promoting the production of extracellular polymer that would mop up the biocide as it reached the biofilm. At the lower water velocity (0.5 m/s) the mass transfer of the biocide would be less so that the opportunity for the development of biocide resistance would be reduced.

Many investigators and plant operators have noted a rapid resumption of biofouling following biocide application, particularly in respect of chlorine addition (Characklis 1990). Pujo (1993) also observed this phenomenon which she termed "regrowth" when the application of chemical biocides ceased. This may also be partly responsible for the rapid regrowth seen in this study. Characklis (1990) suggested that regrowth may be due to the following:

The remaining biofilm contains enough viable cells to preclude any lag phase in biofilm accumulation(as observed on clean surfaces)

The remaining biofilm imparts a relative roughness to the surface increasing convective transport and sorption of cells and debris to the surface.

Biocide may preferentially remove the extracellular material and not the microbial cells thus exposing the cells to the nutriants in the system on dosing cessation.

The surviving cells rapidly produce extracellular material as a protective to the biocide

CONCLUDING REMARKS

From this work it is clear that careful attention to the strategy of biocide application is important. Not only does the technique of treatment affect the extent of the control of the biofilm growth, it also has a direct bearing on the cost of the treatment. Plant trials are likely to provide the best opportunity to obtain an optimum dosing regime. It has to be stated however, that prior to such an investigation, it would be necessary to carry out preliminary work in a pilot plant in order to reduce the risk of difficulties occurring during plant trials, that in themselves could prove to be expensive. An alternative to pilot plant studies would be to use a side stream from the full scale plant itself. Frequent reappraisal of the strategy would be necessary to counteract changes in the ecology of the cooling water facility that could develop over a period of time.

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