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EFFECTS OF VARIOUS METAL SUBSTRATA ON ACCUMULATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS AND THE EFFICACY OF MONOCHLORAMINE AS A BIOCIDE

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Monochloramine at 4 $mg \cdot l^{-1}$ was used to treat biofilms of *Pseudomonas aeruginosa* grown in annular reactors on various metal substrata, including stainless steel, mild steel and copper. Disinfection of *in situ* biofilms was most efficient on the stainless steel slides. The treatment reduced viable cells in the biofilm on the stainless steel slides by more than 4 orders of magnitude after 60 min, while the same treatment reduced viable cells in the biofilms on the other slides (mild steel and copper) by only 2 orders of magnitude. Mild steel corrosion products retarded the disinfection of homogenized biofilm cells. The composition of steady state biofilms on the various substrata (before biocide treatment) differed. Biofilm on stainless steel slides had more viable cells but less extracellular polymeric substance (EPS). Biofilms on mild steel and copper slides had fewer viable cells but more EPS. Almost 95% of total cells in the biofilm on the copper slides were dead cells. The lower efficacy of disinfection of *in situ* biofilms on mild steel and copper is hypothesized to be due to the higher EPS content of biofilms on those substrata.

KEYWORDS: biofouling, *Pseudomonas aeruginosa*, monochloramine, stainless steel, mild steel, copper

INTRODUCTION

Biofilm accumulation on equipment surfaces causes many problems in industry. It causes increased heat transfer resistance in heat exchanger tubes and cooling tower fill, reduces water quality in drinking water distribution systems, and accelerates corrosion due to microbial processes at the biofilm-substratum interface (Characklis & Marshall, 1990).

Several methods have been used to cope with the biofouling problem. They include mechanical and chemical treatments (van der Wende, 1991). The treatment which can be applied depends on environmental, process, and economic considerations. Chlorination has been frequently used, but has drawbacks. Chlorination generates trihalomethanes and other by-products that pose hazards to human health. Furthermore, it has been reported that free chlorine reacts with the popular pipeline materials, mild steel and stainless steel, and causes corrosion due to its high oxidizing activity (Ventura *et al.*, 1989; Franklin *et al.*, 1991; Gundersen *et al.*, 1991).

In previous papers (Chen *et al.*, 1993; Griebe *et al.*, 1993), the potential of using monochloramine as a biocide was investigated and its effectiveness compared with that of free chlorine. It was found that monochloramine was more effective than free chlorine for disinfecting biofilm cells of *Pseudomonas aeruginosa*. In addition, it was found that

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diffusional resistance within the biofilm impacted the biocide performance (Chen *et al.*, 1993). In this article, the research has been extended to investigate the effects of three different substrata, stainless steel, mild steel and copper, on biofilm accumulation and the efficacy of monochloramine as a biocide.

MATERIALS AND METHODS

Experimental System

An annular biofilm reactor (RotoTorque) was used for this study. The experimental system has been described previously (Chen *et al.*, 1993). The RotoTorque is a Continuous Flow Stirred Tank Reactor (CFSTR) consisting of two concentric cylinders (polycarbonate), a stationary outer cylinder and a rotating inner cylinder. Twelve removable metal slides form an integral part of the inside wall of the outer cylinder and permit sampling of biofilms accumulated on them. For each experiment, all 12 slides were of the same metal. The metal was changed for different experiments to investigate the effects of these substrata, stainless steel (SS304), mild steel (AISI-SAE 1010-1018 low carbon steel) and copper (Electrolytic Tough Pitch Copper, 99.9 Cu-0.04 O), on biofilm accumulation and monochloramine efficacy as a biocide.

The microorganism used was *Pseudomonas aeruginosa* ERC1 which was an environmental isolate. The bacterium is an early colonizer in waste water systems. The microorganism was grown in the RotoTorque reactor as a batch for 1 d before continuous feeding process commenced. The batch cultivation allows attachment of cells onto the metal surface. The medium used (with glucose and nitrate as the carbon source and nitrogen source, respectively) and the operating conditions for the RotoTorque as well as the inoculation conditions were the same as used previously (Chen *et al.*, 1993). Since the dilution rate in the RotoTorque was so high (3.2 h^{-1}), the influence of planktonic cells could be neglected. Essentially all cells in the bulk fluid were detached biofilm cells.

Biocide Treatment

The disinfection efficacy of monochloramine at 4 $mg \cdot l^{-1}$ against different biofilms was investigated. The treatment was conducted by a pulse addition of concentrated monochloramine solution, resulting in 4 $mg \cdot l^{-1}$ in the bulk, followed immediately by 1 h step feeding of 4 $mg \cdot l^{-1}$ monochloramine (contained in the influent). The influent medium had no monochloramine demand (Chen *et al.*, 1993). The biofilms were at steady state before the treatment. Steady state was attained 7~8 d after continuous operation, as reflected by steady viable cell counts and total cell counts in the bulk fluid (Chen *et al.*, 1993).

Analytical Methods

Effluent (representing the bulk fluid in the RotoTorque) and biofilm samples were taken during monochloramine treatment. Biofilm samples were scraped off the metal slides and homogenized with a Tekmar Tissumizer™ for 2 min with 100% power input. Viable cell counts were performed by triplicate plating of serial dilutions of homogenized samples (effluent and biofilm) on R2A™ agar (Difco). The result was expressed as number of colony forming units $(cfu) \cdot ml^{-1}$. Total cell counts were performed by direct counting of DAPI (4',6-diamidino-2-phenylindole) and AO (acridine orange) stained samples using an Olympus BH2-epifluorescence microscope. Concentrated monochloramine stock

solution was prepared by using 3:1 molar ratio of ammonia (ammonium chloride, Fisher) to free chlorine (pH 9.0). Total organic carbon (TOC) and monochloramine concentration were analyzed using a Dohrmann Carbon Analyzer and a Hach test kit (Hach Co., Model CN-66), respectively. The procedures for these methods were described previously (Chen *et al.*, 1993).

RESULTS AND DISCUSSION

Bulk Phase Disinfection

The effect of substratum material on the disinfection of bulk phase cells is shown in Figure 1. Three RotoTorque systems, each with slides of a different metal, were treated with 4 $mg \cdot l^{-1}$ monochloramine for 60 min. Each data point reported represents the mean of triplicate counts for each sample. Standard deviations for viable cell counts and total cell counts were typically 10% and 15%, respectively. The initial viable cell concentrations ($t = 0$) in the bulk fluid were of the same order of magnitude 10^7 $cfu \cdot ml^{-1}$ for the three different RotoTorque systems. A predicted curve obtained by assuming convection without disinfection for bulk phase cells in the reactor is given in Figure 1 for comparison. The curve was calculated by

$$X(t) = X_o e^{-Dt} \tag{1}$$

where X, X_o, D and t represent viable cell count after treatment, initial viable cell count, dilution rate and treatment time, respectively.

The results indicate that the disinfection of bulk phase cells in the RotoTorque with copper slides was the most efficient. The number of viable cells in the RotoTorque with copper dropped from $2.8(\pm 0.13) \times 10^7$ to $5(\pm 0)$ $cfu \cdot ml^{-1}$, nearly 7 orders of magnitude, after 60 min treatment. The disinfection of bulk phase cells in the RotoTorque with the mild steel slides was the least efficient. The cell number dropped from $1.0(\pm 0.08) \times 10^7$ to $1.5(\pm 0.14) \times 10^6$ $cfu \cdot ml^{-1}$, approximately a one order of magnitude decrease. Disinfection on mild steel occurred in the first 5 min. The numbers of viable cells measured after 25 min treatment were at the same levels as those predicted, calculated assuming no disinfection (Fig. 1). There might have been some detachment of biofilm in the RotoTorque with mild steel, which raised the number of viable cells in the bulk fluid. The disinfection of bulk phase cells in the RotoTorque with stainless steel reduced viable cell numbers from $2.5(\pm 0.13) \times 10^7$ to $8.5(\pm 0.75) \times 10^2$ $cfu \cdot ml^{-1}$, about a 4 order of magnitude reduction, at 60 min. The reduction in viable cell counts seen on copper and stainless steel was mainly due to disinfection as compared with the predicted curve without disinfection.

It should be noted that a second RotoTorque run has also been done for each metal, in which viable cell counts for 2 treatment intervals (last and middle) were measured and compared to the initial cell count to verify the experimental trends. The results indicated the same order of magnitude reductions of viable cells for the 2 intervals for each metal.

Corrosion on mild steel was observed 2 d after continuous operation, as revealed by the appearance of yellowish brown deposits on the metal surface. The chemistry of corrosion products from mild steel have been well characterized by several investigators. In an aerobic system such as this, with or without microorganisms, corrosion products from mild steel contain ferrous and ferric hydroxides (Ulig, 1971; Little *et al.*, 1990). In the RotoTorque with mild steel, corrosion products (dissolved iron) in the bulk were

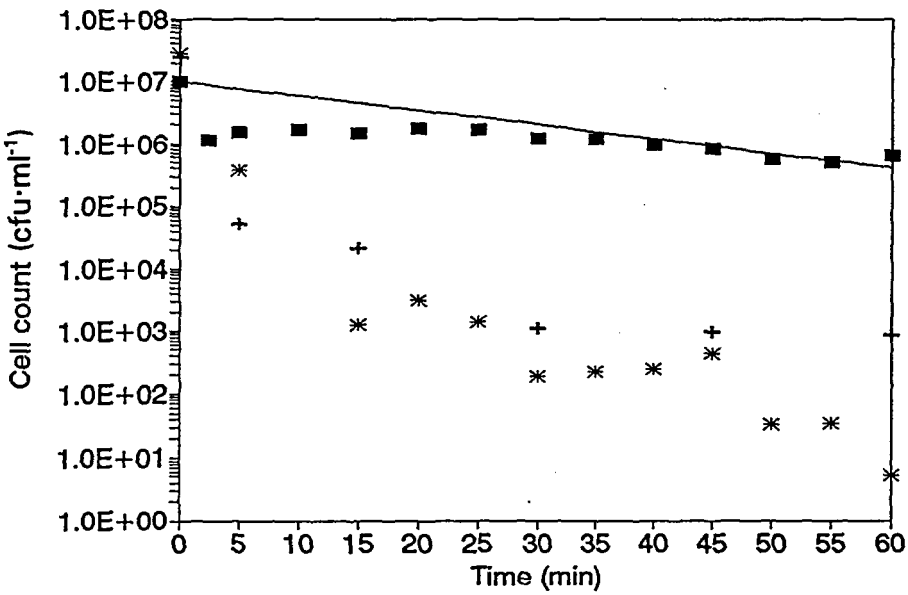

Fig. 1 Disinfection of bulk phase cells in the RotoTorques with different substrata by 4 $mg \cdot l^{-1}$ monochloramine. A predicted curve calculated by Equation (1) assuming no disinfection but convection only is also given for comparison. *Line* = predicted; + = stainless steel; ■ = mild steel; * = copper; time $t = 0$ corresponds to the beginning of biocide addition.

measured to be 6.1 $mg \cdot l^{-1}$ and 1.2~9.1 $mg \cdot l^{-1}$ (using an atomic absorption spectrophotometer, Perkin Elmer, Model 3100) before and during the 1 h treatment, respectively.

In order to investigate whether corrosion on mild steel impacted the biocide performance, corrosion products on mild steel in a sterile RotoTorque reactor (7 d) were scraped off (*via* a sterile cell scraper, Fisher Scientific) and added to homogenized biofilm solutions (biofilm from a RotoTorque with polycarbonate slides; no corrosion). Figure 2 shows the results of batch incubations (25°C) of homogenized biofilm solutions with different initial amounts of corrosion products (solids). The resulting solutions contained $6\sim7\times10^6$ $cfu \cdot ml^{-1}$ viable cells. The monochloramine concentration in each batch was initially set at 3 $mg \cdot l^{-1}$ and showed no change throughout the treatment (pH = 7.0). The results indicate that corrosion products retarded the disinfection of homogenized biofilm cells. The time needed for each culture to reach a specific extent of disinfection increased as the corrosion product concentration increased in the batch (Fig. 2). This effect could explain the lower disinfection efficiency of bulk phase cells (detached biofilm cells) observed in the mild steel RotoTorque, and in a following study, the lower efficacy of disinfection of *in situ* biofilms on mild steel.

The mechanism leading to decreased efficacy of monochloramine as a biocide in the presence of iron corrosion products is not clear. Many studies have focused on iron pipe systems with free chlorine as the biocide. Free chlorine is known to react with ferrous iron, a corrosion product, to produce insoluble ferric hydroxide in iron pipelines (White, 1986), which reduces its efficacy as a biocide. White (1986) indicated that if iron is present in a complex form, free chlorine is more effective than combined chlorines in breaking up the iron complex so that ferrous oxidation can proceed. It is likely that biofilm organisms

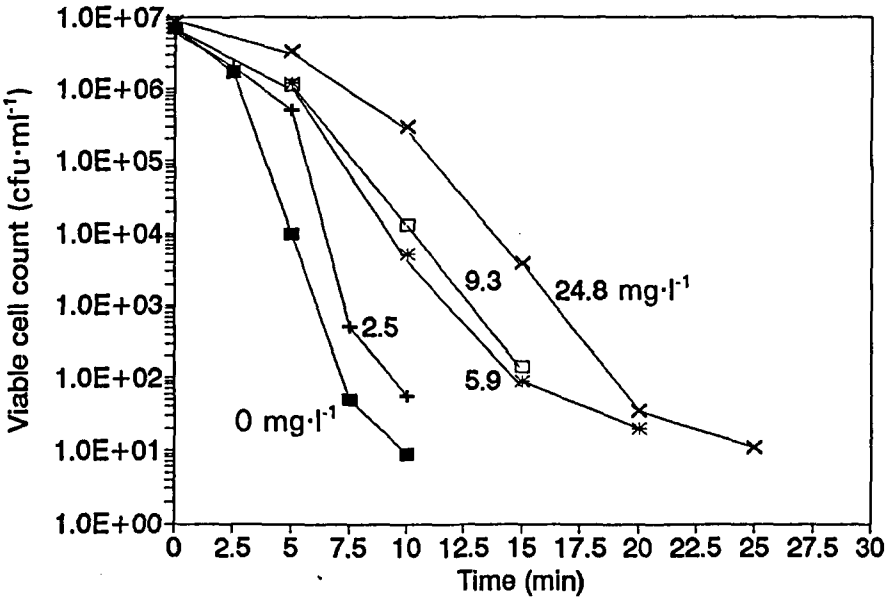

Fig. 2 Effect of corrosion products on the disinfection of bulk phase cells. Batch experiments were performed by incubating homogenized biofilm solution (biofilm from a RotoTorque with polycarbonate slides) with different initial amounts of corrosion products (0–24.8 $mg \cdot l^{-1}$ added as solids; scraped off corroded mild steel slides). Monochloramine remained at a concentration of 3 $mg \cdot l^{-1}$ throughout the treatment (pH = 7.0).

complex ferrous ions from corroded metal surface within the glycocalyx layer. Free chlorine, therefore, not only reacts with extracellular polysaccharide, but liberates ferrous ions which consume the free chlorine residual (White, 1986; LeChevallier, 1991). The addition of corrosion inhibitors can therefore improve the disinfection efficacy of free chlorine against biofilm bacteria on iron pipes (LeChevallier *et al.*, 1990).

In the present experiments, however, negligible reaction of monochloramine with corrosion products was found (data not shown). The residual concentration of monochloramine in the mild steel reactor was high (see later). In addition, monochloramine was found to be non-reactive with polysaccharide (LeChevallier *et al.*, 1988). A possible explanation for the decreased efficacy of monochloramine as a biocide in the presence of corrosion products is the binding of corrosion products (ferrous/ferric ions) to membrane proteins which bear electric charges (Stanier *et al.*, 1986). This could affect the transport of molecules through the cell membrane.

The higher disinfection efficacy of bulk phase cells seen on copper was probably due to copper toxicity. It has been reported that copper as a substratum for biofilm accumulation inhibited cell growth of *P. aeruginosa* on its surface (Mueller, 1992). Domek *et al.* (1984) indicated that low levels of copper (0.007 to 0.54 $mg \cdot l^{-1}$) in chlorine-free distribution water induced injury of coliform populations. Singh and McFeters (1986) showed that exposure of enterotoxigenic *Escherichia coli* strains to a sublethal concentration (0.75 $mg \cdot l^{-1}$) of copper induced cell injury and sensitivity to deoxycholate. Singh *et al.* (1985) and Singh and McFeters (1987) further demonstrated that besides cell injury, a lower pH can cause extensive loss of viability in copper-exposed cells of *Yersinia entercolitica*. It is likely that most cells which detached from biofilms on copper in the present experiments were injured. Therefore, disinfection of bulk phase cells seen on copper was more efficient.

Figure 3 shows the residual monochloramine concentration in the bulk fluid from the three reactors. The residual monochloramine concentration in the RotoTorque with the stainless steel slides was significantly lower than the respective concentrations in the RotoTorques with the mild steel and the copper. The sterile RotoTorque system with mild steel had a monochloramine demand of 0.5 $mg \cdot l^{-1}$ during the treatment period, while sterile reactors with stainless steel or copper exerted no monochloramine demand. The fact that disinfection of bulk phase cells in the RotoTorque with the mild steel slides was less efficient than that in the RotoTorque with the stainless steel slides, even though the residual concentration of biocide was higher, suggests that corrosion products retarded disinfection.

In Situ Biofilm Disinfection

Disinfection of *in situ* biofilms on different substrata is shown in Figures 4, 5 and 6, which present the areal density of viable cells ($cfu \cdot m^{-2}$ biofilm area), the areal density of total cells and the total organic carbon (TOC), respectively. Duplicate slides were taken for the first (t = 0 min), the middle (t = 30 min) and the last (t = 60 min) sampling. The areal density of viable cells in the biofilm on stainless steel dropped from $3.8(\pm 0.37) \times 10^{12}$ to $1.8(\pm 0.12) \times 10^{10}$ $cfu \cdot m^{-2}$ at 15 min, and to $2.4(\pm 0.23) \times 10^{8}$ $cfu \cdot m^{-2}$, about a 4 order of magnitude decrease, after 60 min (Fig. 4). The areal density of viable cells in the biofilm on mild steel decreased from $5.1(\pm 0.42) \times 10^{11}$ to $9.6(\pm 0.35) \times 10^{9}$ $cfu \cdot m^{-2}$ at 60 min, or about one order of magnitude. The areal density of viable cells in

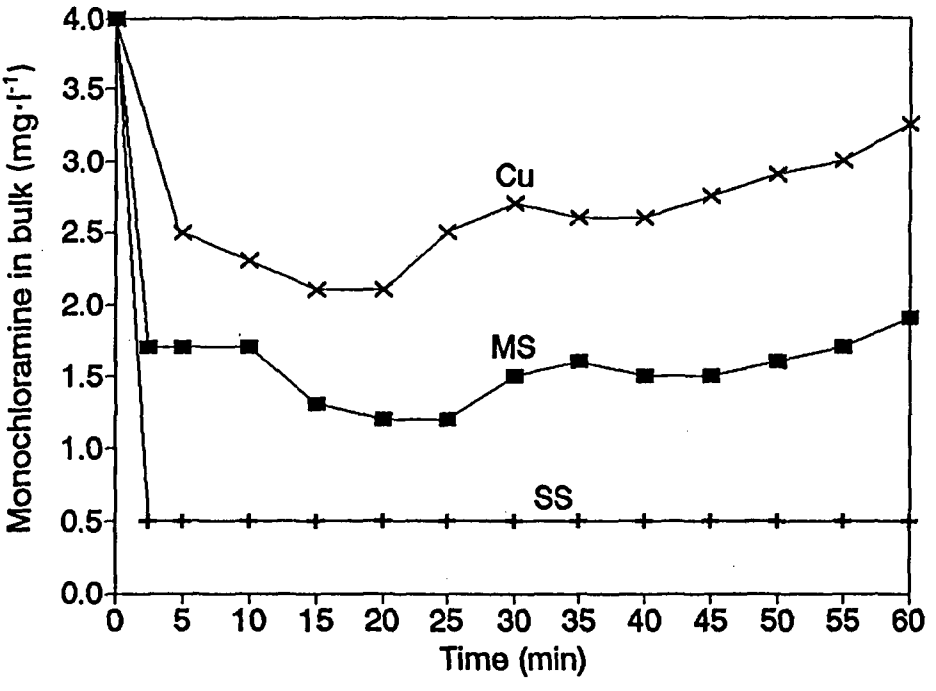

Fig. 3 Residual monochloramine concentrations in RotoTorques with different substrata during 4 $mg \cdot l^{-1}$ monochloramine treatment. SS = stainless steel, MS = mild steel, Cu = copper; time t = 0 corresponds to the beginning of biocide addition.

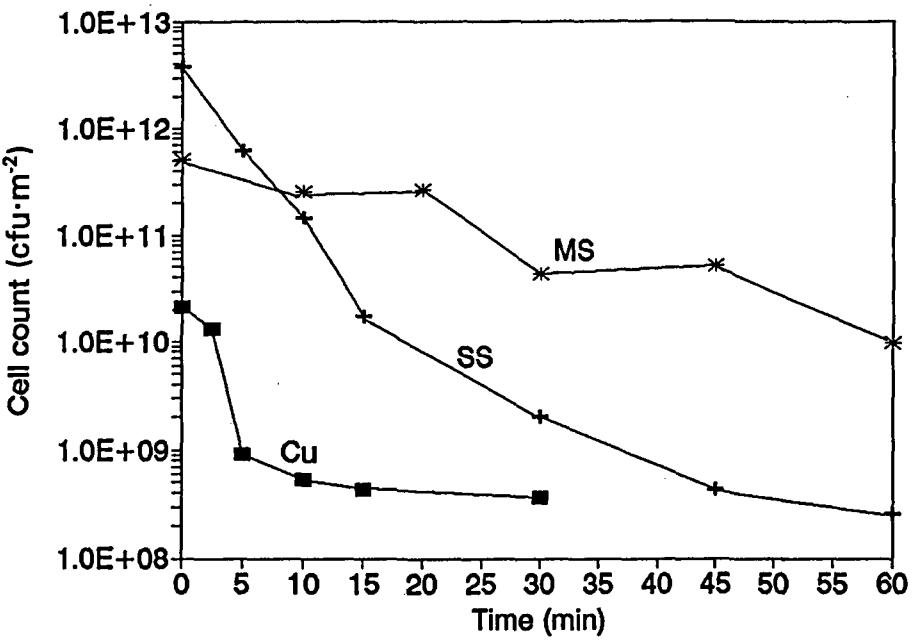

Fig. 4 Areal density of viable cells in the biofilms on different substrata during 4 mg·l^{-1} monochloramine treatment. SS = stainless steel, MS = mild steel, Cu = copper; time $t = 0$ corresponds to the beginning of biocide addition.

the biofilm on copper decreased from $2.1(\pm 0.21)\times 10^{10}$ to $1.3(\pm 0.10)\times 10^{8}$ cfu·m^{-2}, approximately a 2 order of magnitude reduction, at 10 min, and remained steady thereafter (Fig. 4). A second run for each metal indicated the same order of magnitude decrease of areal density of viable cells after treatment. Disinfection of *in situ* biofilm was most efficient on stainless steel.

The areal densities of total cells in the biofilms on stainless steel slides, mild steel slides and copper slides before the treatment ($t = 0$ min) were 4.0×10^{12} cells·m^{-2}, 1.4×10^{12} cells·m^{-2} and 4.2×10^{11} cells·m^{-2}, respectively (Fig. 5). During the 4 mg·l^{-1} monochloramine treatment, the areal densities of total cells in the biofilms on stainless steel slides and copper slides did not change, while the areal density of total cells in the biofilm on mild steel declined by one order of magnitude (Fig. 5). The TOC content of the biofilms on stainless steel, mild steel and copper before treatment ($t = 0$ min) were 375 mg·m^{-2}, 465 mg·m^{-2} and 80 mg·m^{-2} (error 5%), respectively (Fig. 6). During the treatment, the respective TOC's in the biofilms on the three different slides did not show significant changes. The results indicate that there was very little detachment, if any, of biofilms from stainless steel and copper during the treatment, since the TOC and total cell density content of these biofilms did not change significantly. There was some detachment of biofilm cells from mild steel slides, since the areal density of total cells dropped during the treatment (Fig. 5). This is consistent with the previous analysis for bulk phase disinfection seen on mild steel. It has been demonstrated that spalling or sloughing of corrosion products results in the detachment of biofilm patches associated with the corrosion products (Little *et al.*, 1990).

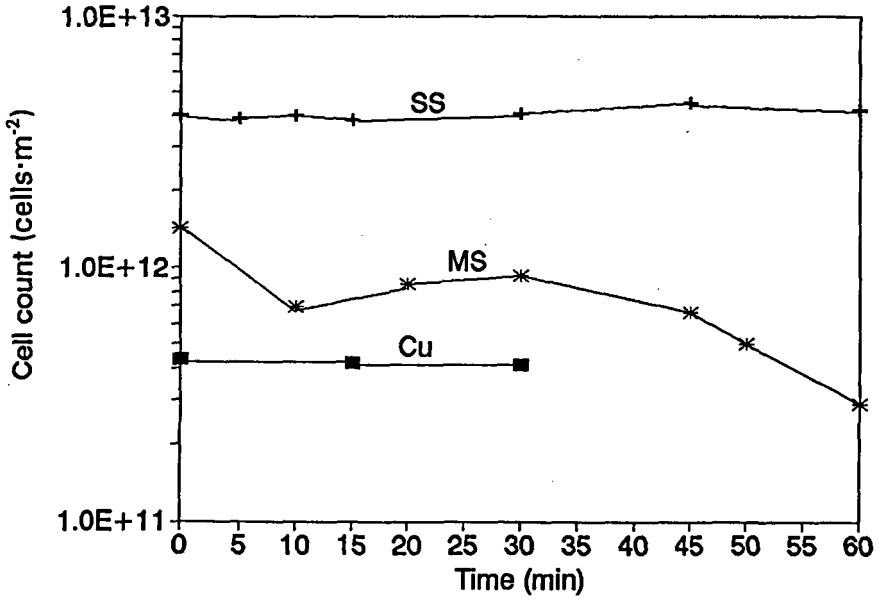

Fig. 5 Areal density of total cells in the biofilms on different substrata during 4 mg·l^{-1} monochloramine treatment. SS = stainless steel, MS = mild steel, Cu = copper; time $t = 0$ corresponds to the beginning of biocide addition.

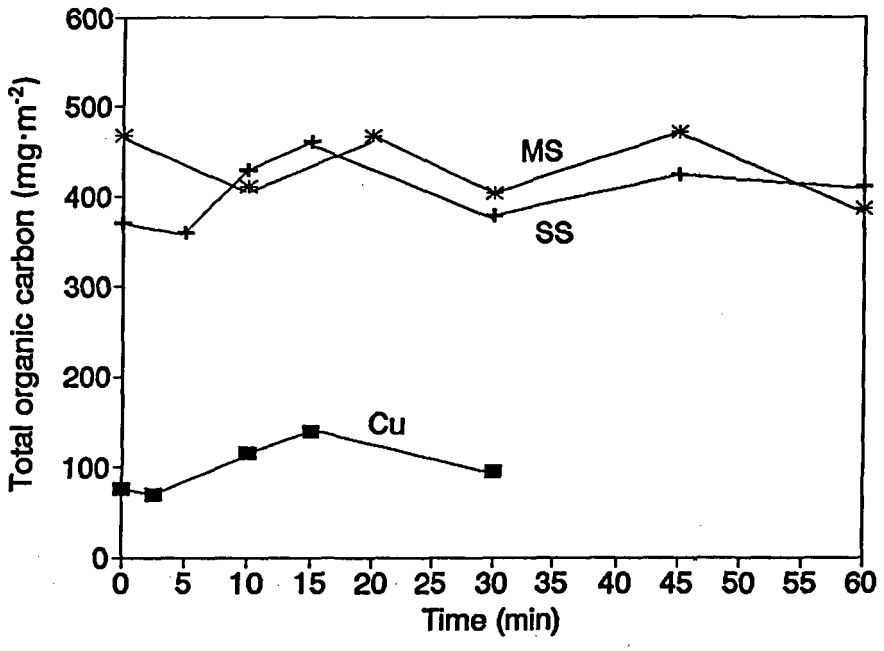

Fig. 6 Total organic carbon content of the biofilms on different substrata during 4 mg·l^{-1} monochloramine treatment. SS = stainless steel, MS = mild steel, Cu = copper; time $t = 0$ corresponds to the beginning of biocide addition.

In Situ *Biofilm Composition*

It has been shown that the substratum affects biofilm accumulation and the extracellular polysaccharide of biofilm organism protects biofilm cells against disinfection by biocide. In studies of biofilm accumulation on the substrata, Al-Brass, Cu90Ni10 and pure Ti, de Sánchez (1992) found that the metals under study affected chemotaxis of the *Pseudomonas* strain H35 ATCC with the highest density of bacteria attached on Ti. Learn *et al.* (1987) reported that the extracellular polysaccharide of a mucoid clinical isolate of *P. aeruginosa* scavenges hypochlorite. In addition, the extracellular polysaccharide, alginate, of mucoid *P. aeruginosa* strains protects the organism against tobramycin by forming a barrier and inhibiting the antibiotic diffusion (Nichols *et al.*, 1988, 1989).

In order to investigate whether *in situ* biofilm disinfection was related to biofilm structure, biofilm composition was analyzed. Table 1 shows the biofilm composition for the three different biofilms, expressed in terms of the ratio of areal density of viable cells to areal density of total cells, and the ratio of TOC to areal density of total cells. Data from $t = 0$ min in Figures 4–6 were taken for the calculation of these ratios. The composition of *in situ* biofilms was affected by the type of substratum used. The biofilms on the different substrata were also visually distinguishable for their thickness and texture. Biofilms on stainless steel were thick, white and homogeneous, whereas biofilms on mild steel were thin, yellowish and patchy. Biofilms on copper were even thinner than those on mild steel, and were also patchy. The thickness of steady state biofilms on stainless steel has been measured by previous experiments to be 35 μm (±20%; Bakke & Olsson, 1986; Siebel & Characklis, 1991).

Table 1 *In situ* biofilm composition

Substratum	areal density of viable cells / areal density of total cells	TOC / areal density of total cells ($\times 10^{10}$ (mg/# of cells))
Stainless steel	0.95	0.94
Mild steel	0.36	3.32
Copper	0.05	1.90

The results indicate that nearly all cells in the biofilm on the stainless steel slides were viable (95% of total cells) and that the biofilm had less extracellular polymeric substance (EPS), as reflected by the lower TOC-to-total cell density ratio, than the other biofilms (Table 1). The biofilm on mild steel had 64% of total cells which were dead, while 95% of total cells in the biofilm on the copper were essentially dead cells. It is possible that EPS protects cells from disinfection. The higher EPS content of biofilms on mild steel and copper would be consistent with the lower biocide efficacy observed on these biofilms if this were the case.

CONCLUSIONS

(1) Disinfection of *in situ* biofilms on stainless steel slides was more efficient than on mild steel and copper slides.

(2) The type of substratum used affected the biofilm composition, altering the viable cell fraction and relative EPS content.
(3) Corrosion products from mild steel retarded disinfection of homogenized biofilm cells.

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References

Bakke R, Olsson P Q (1986) Biofilm thickness measurements by light microscopy. *J Microbiol Methods* **5**: 93–98

Characklis W G, Marshall K C (1990) Biofilms: a basis for an interdisciplinary approach. In: Characklis W G, Marshall K C (eds) *Biofilms*, John Wiley & Sons, Incorporated, New York, pp 3–15

Chen C -I, Griebe T, Characklis W G (1993). Biocide action of monochloramine on biofilm systems of *Pseudomonas aeruginosa*. *Biofouling*. **7:** 1–17

de Sánchez S R (1992) The effect of polarization on the chemotaxis of marine bacteria to different metals. *Proc 1st Pan-Am Corrosion and Protection Cong*, Mar del Plata, Argentina, NACE, PP 305–312

Domek M J, LeChevallier M W, Cameron S C, McFeters G A (1984) Evidence for the role of copper in the injury process of coliform bacteria in drinking water. *Appl Environ Microbiol* **48**: 289–293

Franklin M J, Nivens D E, Vass A A, Mittelman M W, Jack R F, Dowling N J E, White D C (1991) Effect of chlorine and chlorine/bromine biocide treatments on the number and activity of biofilm bacteria and carbon steel corrosion. *Corrosion* **47**: 128–134

Griebe T, Chen C -I, Srinivasan R, Stewart P S (1993) Analysis of biofilm disinfection by monochloramine and free chlorine. In: Geesey G G, Lewandowski Z, Flemming H -C (eds) *Biofouling/Biocorrosion in Industrial Systems*, Lewis Publishers, Incorporated, Chelsea, Michigan. in press

Gundersen R, Johansen B, Gartland P O, Fisksdal L, Vintermyr I, Tunold R, Gagen G (1991) The effect of sodium hypochlorite on the electrochemical properties of stainless steels in seawater with and without bacterial films. *Corrosion* **47**: 800–807

Learn D B, Brestel E P, Seetharama S (1987) Hypochlorite scavenging by *Pseudomonas aeruginosa* alginate. *Infect and Immun* **55**: 1813–1818

LeChevallier M W, Cawthon C D, Lee R G (1988) Factors promoting survival of bacteria in chlorinated water supplies. *Appl Environ Microbiol* **54**: 649–654

LeChevallier M W, Lowry C D, Lee R G (1990) Disinfecting biofilms in a model distribution system. *J Am Water Works Assoc* **82**: 87–99

LeChevallier M W (1991) Biocides and the current status of biofouling control in water systems. In: Flemming H -C, Geesey G G (eds) *Biofouling and Biocorrosion in Industrial Water Systems*, Springer-Verlag Berlin, Heidelberg, pp 113–132

Little B J, Wagner P A, Characklis W G, Lee W (1990) Microbial corrosion. In: Characklis W G, Marshall K C (eds) *Biofilms*, John Wiley & Sons, Incorporated, New York, pp 662–668

Mueller R F (1992) Characterization of initial events in bacterial surface colonization by two *Pseudomonas* species using image analysis. *Biotechnol and Bioeng* **39**: 1161–1170

Nichols W W, Dorrington S M, Slack M P E, Walmsley H L (1988) Inhibition of tobromycin diffusion by binding to alginate. *Antimicrob Agents and Chemother* **32**: 518–523

Nichols W W, Evans M J, Slack M P E, Walmsley H L (1989) The penetration of antibiotics into aggregates of mucoid and non-mucoid *Pseudomonas aeruginosa*. *J Gen Microbiol* **135**: 1291–1303

Siebel M A, Characklis W G (1991) Observations of binary population biofilms. *Biotechnol and Bioeng* **39**: 778–789

Singh A, LeChevallier M W, McFeters G A (1985) Reduced virulence of *Yersinia enterocolitica* by copper-induced injury. *Appl Environ Microbiol* **50**: 406–411

Singh A, McFeters G A (1986) Recovery, growth, and production of heat-stable enterotoxin by *Escherichia coli* after copper-induced injury. *Appl Environ Microbiol* **51**: 738–742

Singh A, McFeters G A (1987) Survival and virulence of copper- and chlorine-stressed *Yersinia enterocolitica* in experimentally infected mice. *Appl Environ Microbiol* **53**: 1768–1774

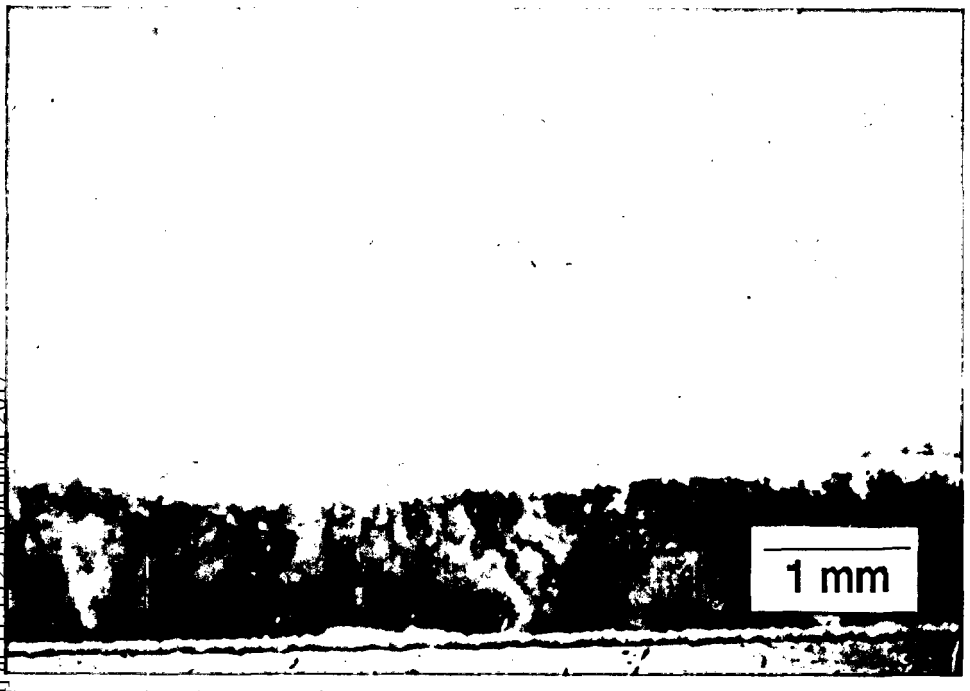
Stanier R Y, Ingraham J L, Wheelis, M L, Painter P R (1986) *The Microbial World*. 5th edn, Prentice-Hall, Englewood Cliffs, New Jersey, pp 148–149

Ulig H H (1971) *Corrosion and Corrosion Control*. 2nd edn, John Wiley & Sons, New York, pp 93–96

van der Wende E (1991) Biocide action of chlorine on *Pseudomonas aeruginosa* biofilm. Ph D dissertation, Department of Civil Engineering, Montana State University at Bozeman

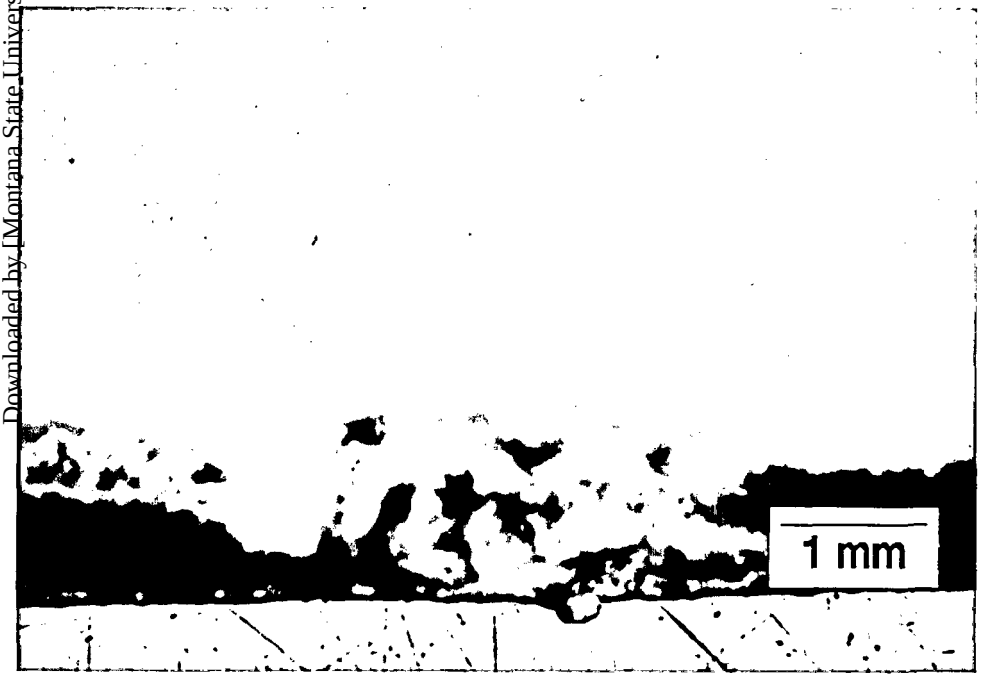
Ventura G, Traverso E, Mollica A (1989) Effect of NaClO biocide additions in natural seawater on stainless steel corrosion resistance. *Corrosion* **45**: 319–325

White G C (1986) *Handbook of Chlorination*. 2nd edn, Van Nostrand Reinhold Company, New York, pp 368–369



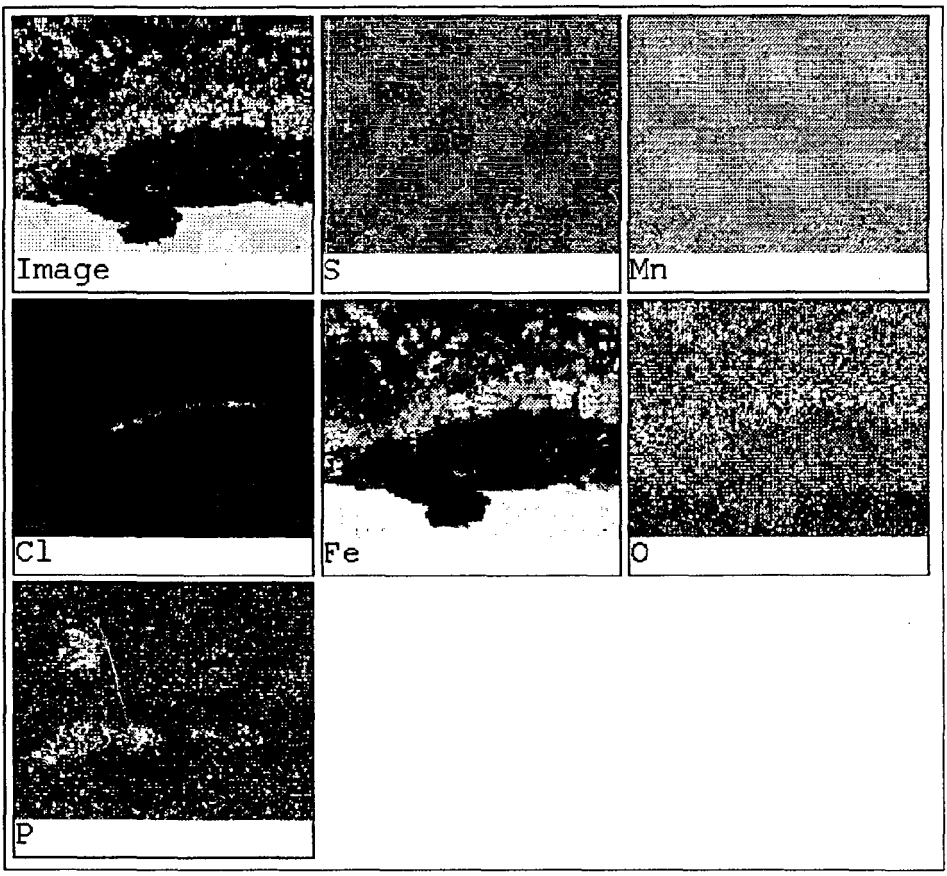
Colour Plate 1 (see Fig. 7. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 7 Optical micrograph of a general corroded steel coupon and fouling deposits at the second week based on cross sectional observation.



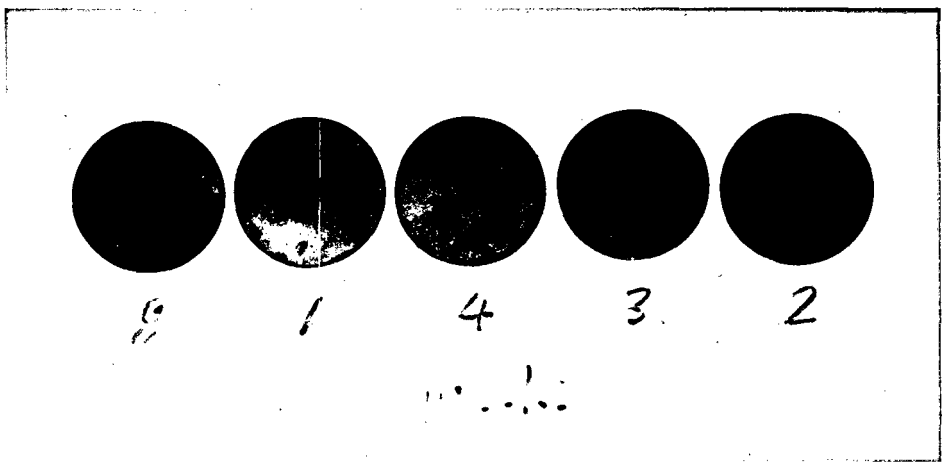
Colour Plate 2 (see Fig. 8. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 8 Optical micrograph of a pitted steel coupon and fouling deposits at the second week based on cross sectional observation.



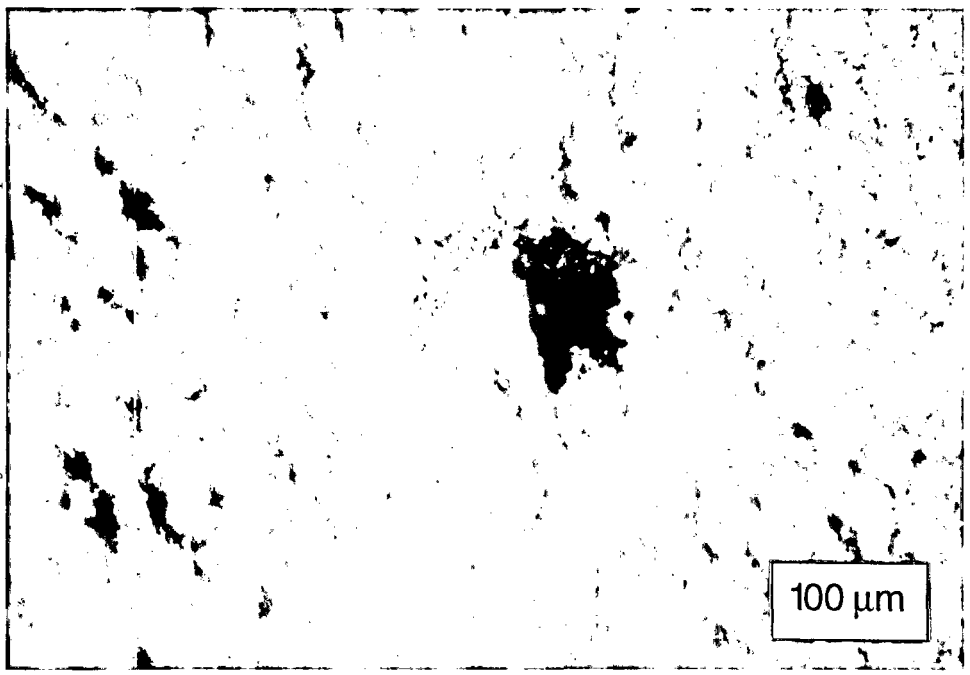
Colour Plate 3 (see Fig. 9. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 9 X-ray map of Figure 8, showing the elemental distribution within a tubercle.



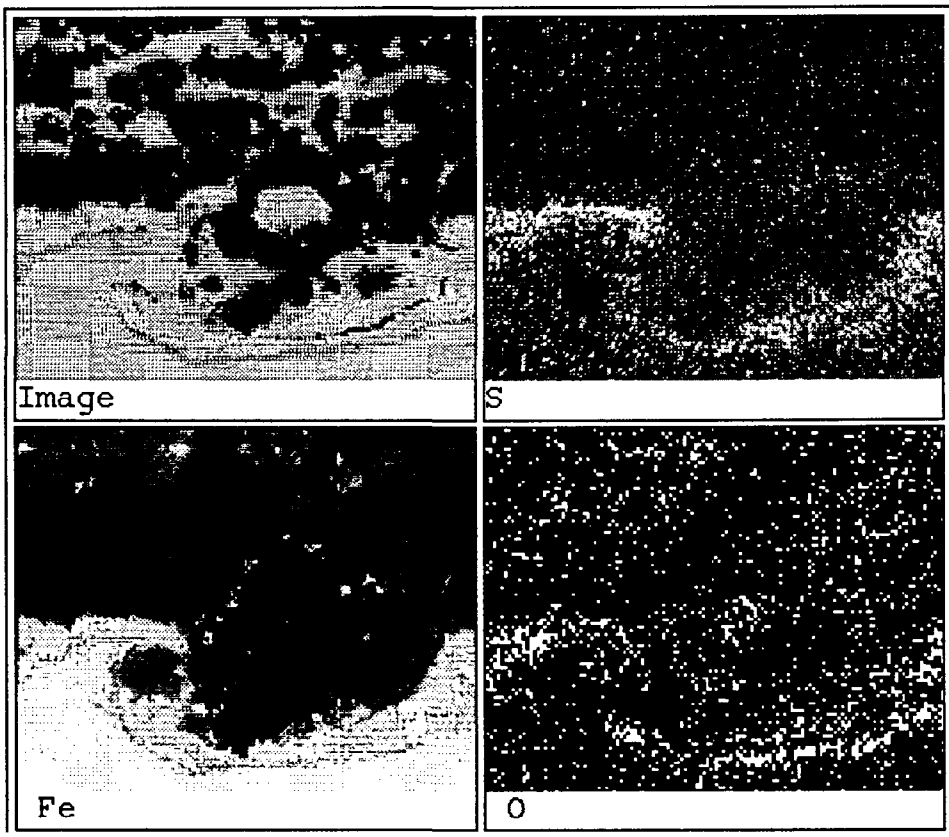
Colour Plate 4 (see Fig. 10. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 10 Optical micrograph of corroded steel coupons after biofilm and corrosion products have been removed at different time periods. Black areas indicated the sulfide attack.



Colour Plate 5 (see Fig. 12. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 12 Optical micrograph at the end of the sixth week of pitted steel in the sulfide-attacked areas caused by SRB.



Colour Plate 6 (see Fig. 13. "Corrosion of Mild Steel ... Part II ... " by W Lee *et. al.*)

Fig. 13 X-ray map of a cross sectional coupon which showed a conductive iron sulfide film around the localized corrosion area at the end of the eighth week.