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RETENTION OF BACTERIA BY CELLULOSE FIBRES AS A MEANS OF REDUCING BIOFOULING IN PAPER PULP PRODUCTION PROCESSES

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The effectiveness of a non-oxidising biocide (a carbamate-based solution) as a retention agent for cells of *Pseudomonas fluorescens* in a paper pulp suspension was studied, as a way to reduce biofouling in pulp and paper production processes. The results indicated that the addition of the biocide not only changed the pH of the paper pulp suspension, but also shifted the surface charges of the bacterial cells from negative to neutral or positive values, depending on the pH value and the biocide concentration. Therefore, since the paper pulp fibres are negatively charged, adhesion of bacterial cells to the fibres is promoted. Hence, it was possible to obtain an increase in cell retention on the cellulose fibres from 45% to 75%, within less than 5 min of contact between the carbamate and the pulp suspension. This effect increased with the concentration of carbamate solution in the fibre suspension (100–300 mg l^{-1}), and was always higher than in the absence of biocide.

Keywords: pulp and paper industry; biofouling; biocide; *Pseudomonas fluorescens*

INTRODUCTION

Biofilms are highly hydrated matrixes attached to surfaces, containing extracellular polymeric substances (EPS), embedded microorganisms and other components, such as small inorganic particles. Inside these matrixes,

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the microorganisms, predominantly bacteria, seem to be protected against external aggressions, such as biocides (Mattila-Sandholm & Wirtanen, 1992).

The working conditions in pulp and paper mills favour the occurrence of biofilms that can cause severe problems such as shut down time, corrosion, lower product quality, bad odours and health problems (Khahre *et al.*, 1997). The environment prevailing in the water circulation systems of paper machines, defined by temperature ranges between 25–50°C, a high content of carbon substrates and micro-nutrients (in part due to the increasing use of recycled paper) and the closure of white water loops, promotes the fast growth of microorganisms. Practical experiments indicate that biofilms in pulp processing equipment detach easily from the surfaces (Nurmiaho-Lassila *et al.*, 1990). This loose slime may eventually become incorporated in the paper, causing holes, which can result in machine breaks and/or rejected paper, decreasing productivity and the operational efficiency of the process. At least, specks and spots may appear in the paper, affecting the final product quality. Moreover, biofilms interact with their support and increase the corrosion rate on stainless-steel alloy systems and other industrial materials. This phenomenon is called "microbial influenced corrosion" (MIC) and is responsible for about 20% of all reported corrosion damage (Flemming, 1996).

In the pulp and paper industry, several types of microorganisms can grow, *viz.* bacteria (slime formers, sulphate-reducing bacteria), fungi, algae and Metazoa (Khahre *et al.*, 1997). One of the bacteria isolated from the slime found in pulp and paper machines is *Pseudomonas* sp. which was used in the present work as a study model. These bacteria are present in the river water used as process water in many pulp and paper production plants.

There are methods of controlling unwanted biofilms which attempt to prevent bacterial adhesion, delay or reduce biofilm formation or remove existing biofilms (Bryers, 1994). This last objective can be achieved by mechanical or chemical cleaning, but is often impracticable and can be costly because it usually involves equipment downtime.

In order to mitigate biofouling, antimicrobial agents are frequently applied, depending on environmental, process, and economic considerations (Chen *et al.*, 1993). They must be used appropriately to avoid risk to humans and to the environment, and must not disturb the pulp manufacturing processes, *i.e.* they should be compatible with the many auxiliary products used in paper making. Also, they should have low toxicity and should not be present in the finished product (Paulus, 1993).

Antimicrobial agents can adversely affect the initial adhesion of microorganisms by changing the surface free energy of the substratum or bacterial wall hydrophobicity (Cheung & Beech, 1996). They also affect cell viability,

by short-circuiting the electron transport system, leading to failure of the bacterial energy system and they can also cause changes to the polymeric matrix that may affect biofilm structure (Nesaratnam & Bott, 1984).

The use of chlorine has been very popular as a biocide, but has important drawbacks. Chlorination generates trihalomethanes and other products hazardous to human health (Chen *et al.*, 1993). Furthermore, the free chlorine reacts with the materials of the pipelines and machines causing additional corrosion problems. It is also important to realise that this and other biocides can react or be adsorbed in the paper pulp and thus become inactivated. In general, biocidal treatments are relatively expensive and their costs tend to escalate with increasing use of the closure of white water loops.

There are other strategies to combat biofouling in pulp production plants. One of these is to remove the microbial contaminants by bleeding the excess white water from the system, although this process is unattractive for environmental reasons. Another way of achieving this result is to remove the bacteria by retention in the pulp before biofilm formation occurs.

In the present work, the effectiveness of a non-oxidising biocide as a retention agent for use in paper pulp mills was studied by simulating the industrial conditions in the laboratory. This type of biocide is believed to be relatively safe from an environmental point of view (its decomposition occurs quickly, increasing with temperature) and it is a broad spectrum microbiocide already used for controlling the growth of bacteria and fungi in paper pulp manufacture. It is also currently applied in the protection of formulations used in papermaking (Paulus, 1993).

MATERIAL AND METHODS

Microorganism

The microorganism used was *Pseudomonas fluorescens* isolated from river water. This is a good biofilm producer and seems to be favoured when phosphate buffered at pH 7 (Oliveira *et al.*, 1994). The optimum growth temperature is 27°C, and glucose was used as the limiting substrate.

Continuous cultures were performed in 3 l glass fermenters, aerated and agitated. The fermenter, with the medium that contained $5\,g\,l^{-1}$ glucose, $2.5\,g\,l^{-1}$ peptone and $1.25\,g\,l^{-1}$ yeast extract, in phosphate buffer at pH 7 (0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4), was autoclaved at 120°C for 25 min.

When the medium was cold, 300 ml of a culture of *P. fluorescens* in the exponential growth phase was used as inoculum and the bacterial culture was continuously fed with the buffered medium described above, at a dilution rate small enough to prevent biofilm formation on the walls of the fermenter.

Biocide

A non-oxidising biocide solution composed of sodium dimethyl dithiocarbamate (Figure 1a) (15% w/v) and disodium ethylene bisothiocarbamate (Figure 1b) (15% w/v) in water was tested as a retention aid.

Initial Adhesion Assays

The effect of biocide concentration (100, 200 and 300 mg l^{-1}) on the initial adhesion of bacteria to stainless steel (ASI 316) slides was investigated. Stainless steel slides (1.5 cm × 1.5 cm × 1.0 mm) were prepared and cleaned. The slides were polished with sand-paper (Indasa, P-1200) and with a fine grade polishing cream (Nuamel). Finally, the slides were degreased with detergent, rinsed with distilled water and immersed in ethanol.

The initial adhesion of *P. fluorescens* was investigated using epifluorescence microscopy (Zeiss filter set no. 09, excitation BP 450–490 nm, beamsplitter FT 510, emission LP 520), with acridine orange (Sigma no. A-6529) as the fluorescent dye.

Bacterial culture was centrifuged (5000 rpm, 10 min) and washed three times with phosphate buffer and resuspended in the same growth medium in order to obtain a final cell concentration of 1×10^6–3×10^6 cells ml^{-1}. This culture was then divided into several flasks, each containing two suspended stainless steel slides. A known amount of the biocide was introduced into each flask, and after 1 h and 3 h, respectively, one of the slides was removed and gently washed in sterile distilled water to remove reversibly attached cells, and prepared for observation by epifluorescence microscopy.

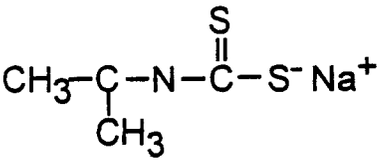

FIGURE 1a Sodium dimethyl dithiocarbamate

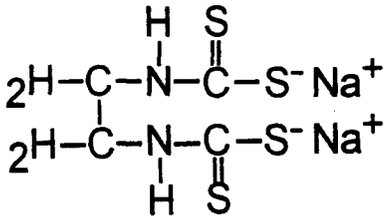

FIGURE 1b Disodium ethylene bisothiocarbamate

The same procedure was also performed without biocide addition and used as the control.

Epifluorescence Microscopy

The slides were stained with 0.002% acridine orange solution for 15 min at room temperature in the dark. Prior to the observation, the slides were gently washed with sterile distilled water to remove excess stain. The coupons were placed on a glass slide and observed in a Zeiss Microscope (400 ×) fitted with fluorescence illumination.

Zeta Potential Measurements

The effect of the biocide on the electrokinetic potential of the bacteria was evaluated at the biocide concentrations stated above.

The cells were prepared as described previously (growth in a continuous fermenter, washed and resuspended) in order to obtain a final concentration of 1×10^9–3×10^9 cells ml^{-1}. The culture was divided into 100 ml flasks and biocide was introduced into each flask to give final concentrations of 100, 200 and 300 mg l^{-1}. The zeta potential of each culture was determined according to Loosdrecht *et al.* (1990) using a Zeiss ZetaMeter system 3.0+ (ZetaMeter Incorporated, New York), by applying a potential between 200 and 300 V across the electrophoresis cell. The results expressed in mV are the average of 20 measurements. In some cases, the zeta potential was repeatedly measured after known time intervals.

The zeta potential of the stainless steel surface was also determined, using the same procedure as described above with stainless steel fillings.

Paper Pulp Assays

During these assays, the operation mode of a pulp-mill was simulated in order to investigate the retention ability of the biocide in paper pulp circuits.

A suspension of paper pulp suitably refined was prepared by fine-mincing sheets of bisulfide paper pulp (using a kitchen Moulinex mincer) in order to obtain a homogeneous aqueous suspension with a final concentration of 6 g l^{-1} of pulp. This suspension was inoculated with a pure culture of *P. fluorescens* in exponential growth, to give a final bacterial concentration of 10^4–10^9 cells ml^{-1}, and divided into several identical agitated (120 rpm) vessels. After 30 min (the time needed to permit adaptation of the bacteria to the pulp suspension), a known amount of biocide was added to each flask, to

obtain different concentrations of the substance. One of the flasks was used as a control, without biocide addition.

At known time intervals, the pulp-bacteria suspensions in each flask were passed through a 200 mesh filter-linen (the pulp fibres could not pass through this filter) and samples of the filtrate were collected. Samples were also taken from each flask prior to the addition of the chemical.

All samples were assayed for total protein using the Lowry modified method (SIGMA-Protein Assay Kit no. P5656) as an indirect measure of biomass concentration. (The conventional plate count method was not used to enumerate bacterial cells due to the phenomenon of cell aggregation.) The measurements were used to calculate the retention of cells in the paper fibres, using the equation:

$$\% \text{ Cell Retention} = \frac{(\text{Protein concentration})_{\text{suspension}} - (\text{Protein concentration})_{\text{filtrate}}}{(\text{Protein concentration})_{\text{suspension}}} \times 100.$$

RESULTS

Figures 2–6 show the effect of biocide concentration on the initial adhesion (up to 3 h) of *P. fluorescens*. The bacteria can be identified by the orange/yellow colour.

As can be seen in Figure 2, in the absence of biocide, the bacterial cells were isolated and randomly distributed on the metal slides. In the presence of 100 mg l^{-1} of biocide, the pattern of distribution was different; apart from dispersed cells, some aggregates were also observed (Figures 3 and 4). Figures 5 and 6 show the distribution of cells on the surface, at biocide concentrations of 200 and 300 mg l^{-1}, respectively. In these cases, the distribution pattern was similar to that obtained without biocide, but a higher number of cells were observed.

Table I shows the zeta potential of *P. fluorescens* suspensions (with and without biocide) as a function of pH.

These results indicate that the presence of the biocide may cause a shift in the surface electrical charge of the bacteria towards the positive range, depending on the pH and on the biocide concentration. In the absence of the biocide, the zeta potential was always negative between pH 5.5 and 8.3, and similar to values published previously for the same system (Pinheiro *et al.*, 1988). When the concentration of biocide was 100 mg l^{-1}, the surface charge of the bacteria became neutral up to pH 6.7, and was the same as the previous

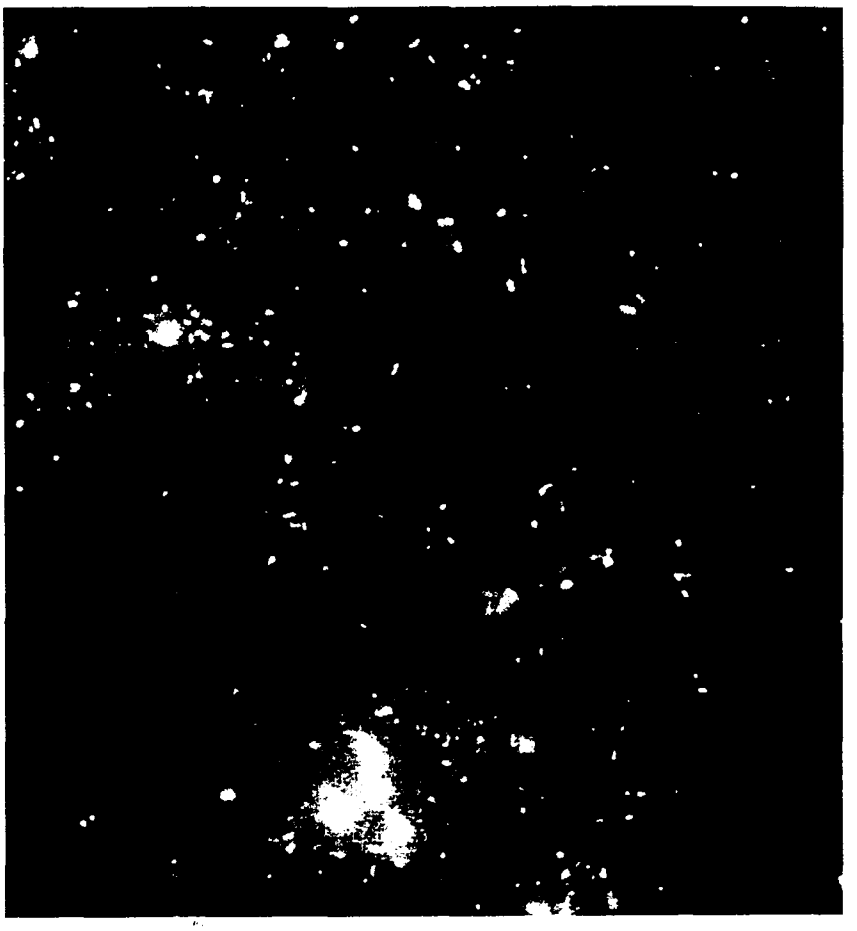

FIGURE 2 Epifluorescence micrograph of a stainless steel coupon with some bacteria attached, in the absence of biocide after exposure for 1 h to a bacterial suspension. × 400 (See Colour Section.)

case when the pH was 8.3. When the concentration was raised to 200 mg l^{-1}, the charge did not change if the pH was maintained at 8.3, but became neutral at pH 6.7 and positive if the pH was 5.5. Using 300 mg l^{-1} of biocide, the bacterial surface charge became positive at pH 5.9 and 6.7, and negative at pH 8.3.

In order to verify whether the effect of the biocide on the bacterial cell surface was dependent on the contact time, the zeta potential of the bacteria was determined as a function of time. Table II presents the results for zeta potential under two situations, similar to those displayed in Table I. In the first the zeta potential did not change significantly when the biocide was added (100 mg l^{-1}), and in the second a shift in the electrical charge occurred in the presence of biocide (300 mg l^{-1}). It appears that time is not a relevant factor, at least after the first 5 min of exposure to the biocide. In both cases, the main effect of the biocide, *i.e.* to induce (or not) a change in the surface

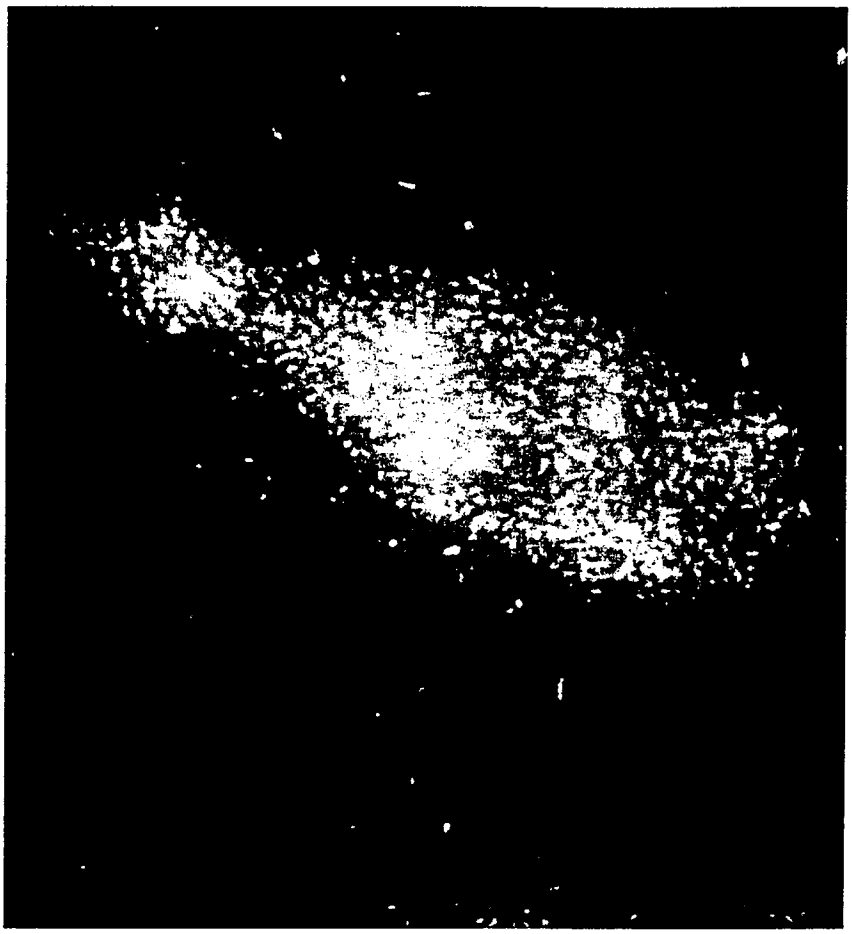

FIGURE 3 Epifluorescence micrograph of a stainless steel coupon after exposure for 1 h to a bacterial suspension treated with 100 mg l^{-1} of biocide. × 400 (See Colour Section.)

TABLE I Zeta potential of *P. fluorescens* without biocide and in the presence of different concentrations of biocide at several pHs

System	*pH*	*Zeta potential* (mV)	*SD* (mV)
P. fluorescens	5.5	−27.400	7.156
	6.7	−20.988	6.906
	8.3	−26.235	7.906
P. fluorescens + 100 mg l^{-1} biocide	5.9	0	0
	6.7	0	0
	8.3	−25.633	5.948
P. fluorescens + 200 mg l^{-1} biocide	5.5	18.687	3.103
	6.9	0	0
	8.3	−29.547	5.076
P. fluorescens + 300 mg l^{-1} biocide	5.9	17.905	2.953
	6.7	12.241	6.093
	8.3	−28.463	6.423

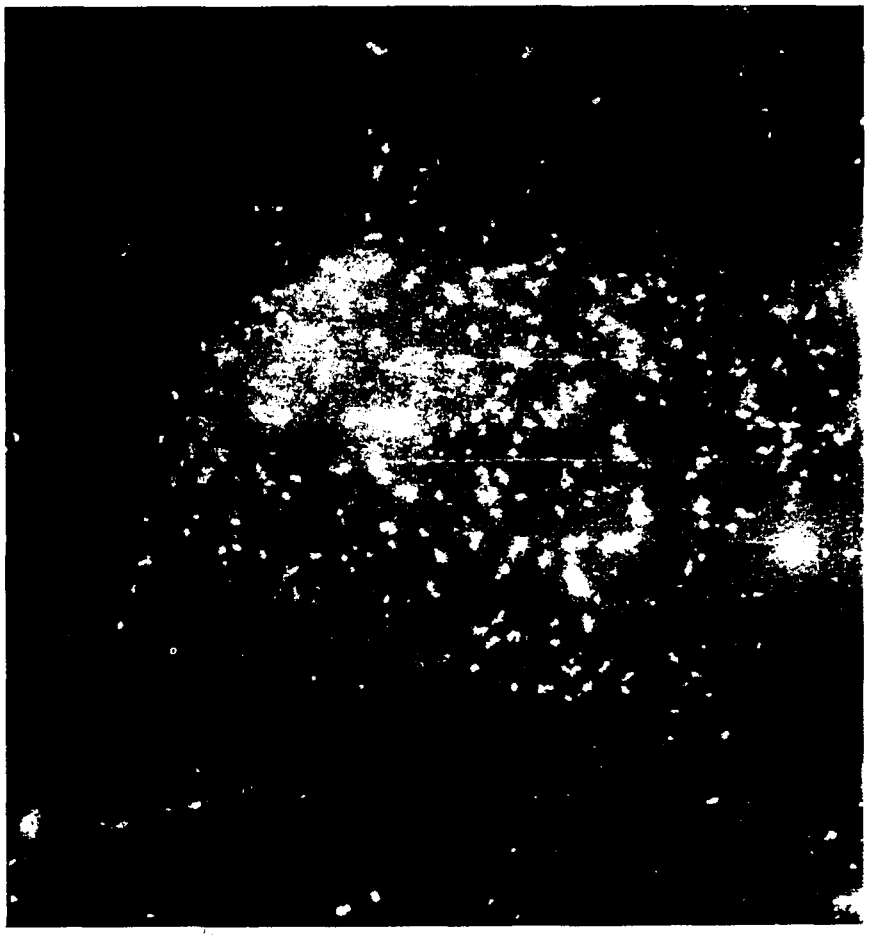

FIGURE 4 Epifluorescence micrograph of a stainless steel coupon after exposure for 3 h to a bacterial suspension treated with 100 mg l^{-1} of biocide. × 400 (See Colour Section.)

TABLE II Zeta potential data for *P. fluorescens* treated with different concentrations of biocide as a function of time

System	*pH*	*Time* (min)	*Zeta Potential* (mV)	*SD* (mV)
P. fluorescens + 100 mg l^{-1} biocide	8.3	5	−24.892	6.281
		15	−24.696	4.406
		25	−25.774	7.156
P. fluorescens + 300 mg l^{-1} biocide	5.9	15	16.721	2.501
		30	17.721	1.759
		40	18.651	1.591
		60	21.420	4.317

electrical charge occured in less than 5 min. No other shift during the remaining time of the experiment was observed.

Table III demonstrates the effect of the biocide (300 mg l^{-1}) on the electrical charge of a stainless steel surface. The charge retains its negative value in the presence of the biocide.

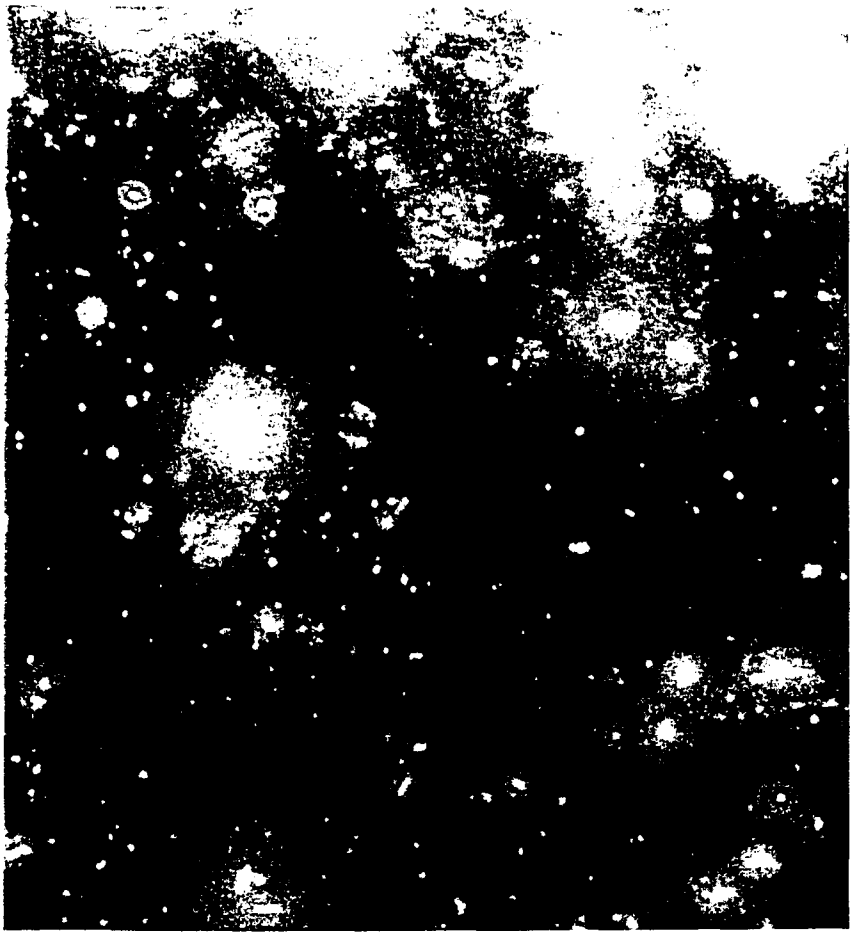

FIGURE 5 Epifluorescence micrograph of a stainless steel coupon after exposure for 1 h to a bacterial suspension treated with 200 mg l^{-1} of biocide. × 400 (See Colour Section.)

TABLE III Zeta potential data for stainless steel treated with 300 mg l^{-1} of biocide

System	*pH*	*Zeta potential* (mV)	*SD* (mV)
Water + stainless steel fillings	8.3	−15.211	5.093
	8.9	−24.801	6.614
	10.9	−35.320	8.937
Water + stainless steel fillings + 300 mg l^{-1} biocide	9.5	−38.104	3.515

In order to evaluate the effect of the biocide on bacterial retention by the paper pulp suspension, a set of assays was performed using different concentrations of the biocide. Tables IV–VI present the retention values of *P. fluorescens* by paper pulp for different contact times, for biocide concentrations of 100, 200 and 300 mg l^{-1} respectively, and in the absence of biocide (control).

FIGURE 6 Epifluorescence micrograph of a stainless steel coupon after exposure for 1 h to a bacterial suspension treated with 300 mg l^{-1} of biocide. × 400 (See Colour Section.)

TABLE IV Retention values obtained in assays with paper pulp suspensions in the presence of 100 mg l^{-1} of biocide (SD in brackets)

Biocide contact time (min)	*Protein concentration* (mg l^{-1})				
	Control	*Flask A*	*Flask B*	*Flask C*	*Flask D*
0	70.082 (±0.795)	71.284 (±6.256)	70.964 (±3.750)	72.897 (±1.486)	67.421 (±2.053)
5	67.684 (±2.251)	40.266 (±0.626)			
15	77.534 (±9.732)		33.681 (±0.205)		
30	73.301 (±1.372)			36.155 (±2.271)	
90	69.270 (±2.003)				43.321 (±0.716)
Retention (%)	—	43.5 (±0.158)	52.5 (±0.013)	50.4 (±0.133)	35.7 (±0.054)

TABLE V Retention values obtained in assays with paper pulp suspensions in the presence of 200 mg l^{-1} of biocide (SD in brackets)

Biocide contact time (min)	*Protein concentration* (mg l^{-1})				
	Control	*Flask A*	*Flask B*	*Flask C*	*Flask D*
0	72.493 (±1.141)	65.225 (±1.230)	63.333 (±4.114)	66.096 (±2.917)	69.321 (±2.073)
5	71.606 (±3.301)	24.083 (±0.800)			
15	65.621 (±2.686)		25.785 (±0.804)		
30	69.200 (±0.339)			23.093 (±3.194)	
90	70.542 (±2.039)				42.598 (±0.205)
Retention (%)	—	63.1 (±0.011)	59.3 (±0.177)	65.1 (±0.416)	38.5 (±0.049)

TABLE VI Retention values obtained in assays with paper pulp suspensions in the presence of 300 mg l^{-1} of biocide (SD in brackets)

Biocide contact time (min)	*Protein concentration* (mg l^{-1})				
	Control	*Flask A*	*Flask B*	*Flask C*	*Flask D*
0	62.548 (±2.995)	69.940 (±3.738)	70.963 (±3.977)	68.641 (±4.741)	69.094 (±1.850)
5	69.360 (±1.471)	17.140 (±3.441)			
15	66.889 (±6.515)		16.167 (±6.572)		
30	73.625 (±2.978)			11.958 (±2.330)	
90	68.248 (±1.879)				17.546 (±1.265)
Retention (%)	—	75.5 (±0.848)	77.2 (±1.801)	82.6 (±1.187)	74.6 (±0.303)

The results indicate that the retention values increased with increasing biocide concentration, for contact times up to 30 min. However, the retention values decreased with longer contact times. Figure 7 summarises the values presented in Tables IV–VI.

Figure 8 presents the pH profiles obtained during the paper pulp assays described above. In the presence of the biocide, there was a sharp increase in the pH after 5 min contact with the biocide and then a less steep rise in the pH values to the alkaline range, the final value being dependent on the biocide

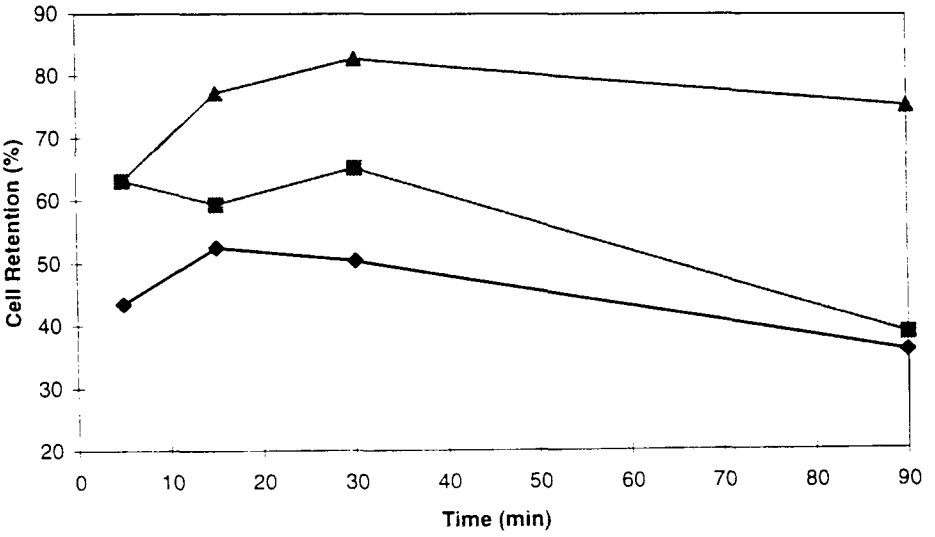

FIGURE 7 Cell retention (%) as a function of time, obtained in assays with pulp suspensions, in presence of 100 mg l^{-1} (◆), 200 mg l^{-1} (■), and 300 mg l^{-1} (▲) of biocide.

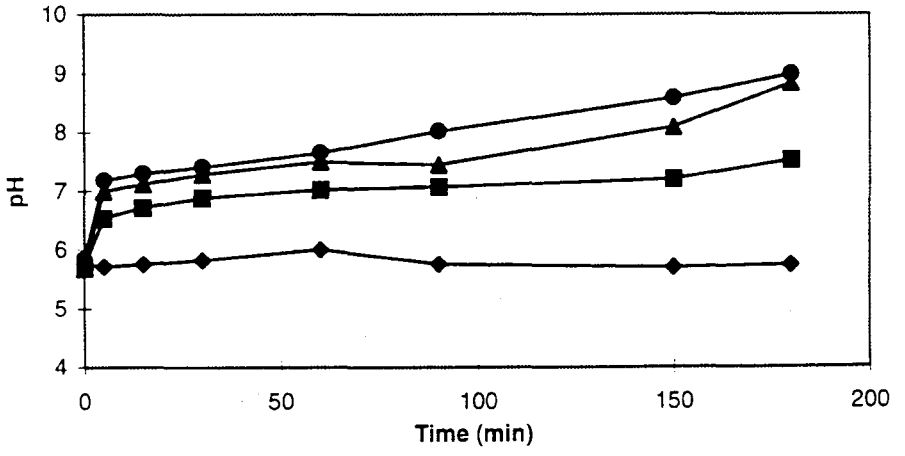

FIGURE 8 pH of pulp suspension, as a function of time, in the presence of 100 mg l^{-1} (■), 200 mg l^{-1} (▲), and 300 mg l^{-1} (●) of biocide. ◆ = control.

concentration. No significant difference in pH values was observed for the control suspension.

DISCUSSION

A biocide can interfere with biofilm formation in different ways, as stated in the Introduction. One of these is modification of the attachment process of bacteria to surfaces. If the process of bacterial adhesion is considered in terms of approach to the surface, the effects of long- and short-range forces,

and the interactions between bacterial and substratum surface properties (Marshall & Blainey, 1990), then any interference to one of these steps can prevent or enhance the process.

If a bacterium is regarded as a colloidal particle, the DLVO theory can explain its reversible adhesion at a surface (Oliveira, 1992). According to this theory, the attraction between two negatively charged bodies (cell and substratum) can occur at the secondary attraction minimum, resulting from the interaction between the van der Waals attraction forces and the electrical repulsion forces in the overlapping double layers surrounding the negatively charged surfaces (Marshall & Blainey, 1990). The bacteria and surface are of different chemical composition, therefore, the van der Waals interactions established between bacterium/bacterium and bacterium/substratum may be expected to be of different magnitudes. This means that the Hamaker constants for each type of interaction have different values (Visser, 1972). Thus, negatively charged bacteria can be attached to a negatively charged surface, but scattered, due to their mutual repulsion effects. This can explain the results shown in Figure 2.

In the presence of 100 mg l^{-1} biocide and for a non-alkaline pH (according to Table I the bacterial surface has a neutral charge), there is no repulsion between bacteria, nor between bacteria and surfaces, meaning that the prevailing forces are the van der Waals forces of attraction. This clearly accounts for the occurrence and predominance of cell aggregates on the surface (Figures 3 and 4). When the biocide concentration is raised and the bacterial cell surface becomes positive (Table I) some repulsion between the cells is expected to occur (as in the absence of biocide), but the adhesion between cells and surface is promoted, since they have opposite charges, thus, the occurrence of many scattered cells (Figures 5 and 6). Again, it is important to emphasise that the results discussed above are a consequence of a shift in the bacterial surface charge and not of a change in the surface charge of stainless steel. Table III indicates that the surface charge of stainless steel does not change in the presence of biocide.

The effect of the biocide on the bacterial cell surface was evaluated as a function of biocide concentration, pH and time of exposure. The action of biocides such as glutaraldehyde on bacterial metabolism is known to be dependent on pH (Paulus, 1993; Bott, 1995). The intensity of the action of glutaraldehyde increases with pH, being more effective in alkaline media (Paulus, 1993).

In the present study, the biocide had an immediate effect on the cells and this effect depended more on biocide concentration than on the time of

exposure to the biocide. It may be possible that the biocide used reacts with the thiol groups of the bacteria (*i.e.* with sulphur containing aminoacids such as cystein), causing alteration in the cell surface charge. The thiol groups can be oxidised by the action of an oxidising agent, and a covalent disulfide bond between the side chains of neighbouring cysteine residues in a protein can be formed.

In the present case, Table I shows that the bacterial cell charge is negative, in the pH range studied. According to Bradley and Pritchard (1990), who found similar results for bacterial cells of *Desulfovibrio desulfuricans*, this can be attributed to the anionic lipopolysaccharide (LPS) on the cell surface. However, it can also be due to the proteins present on the bacterial surface. In the presence of the biocide, the bacterial surface charge becomes neutral or positive, depending on both concentration and pH. According to Hunter and Evans (1990), sodium ethylene bisdithiocarbamate (EBDC), known as nabam, has fungitoxicity due to the formation of ethylene diisothiocyanate (EDI), which will combine unspecifically with many cell constituents such as thiol (-SH) containing enzymes. Although zineb (zinc-EBDC) and nabam were used as fungicides (Kaars-Sijpesteijn, 1983), they have been shown to substantially reduce slime (comprising bacteria, diatoms and cyanobacteria) on panels exposed to sea water.

The results presented so far suggest that carbamate solution has potential as a retention aid, besides its traditional application in the pulp and paper industry as a biocide. A retention aid is required to aggregate the dispersed bacterial cells, forming clusters before they produce slime. These aggregates can become incorporated in the paper pulp without causing damage, decreasing bacterial cell number in the water recirculating systems and thus reducing biofilm formation in the circuit.

As mentioned in the Results section, for short contact times, the retention of bacteria on paper pulp was higher in the presence of biocide (for all concentrations studied) than without biocide. It must be stressed, however, that some retention occurred even without the addition of biocide.

When introduced into water, cellulose (the main constituent of paper pulp), becomes negatively charged due to the ionisation of the carboxyl groups on the surface and specific ions from solution (Gama *et al.*, 1997). On the basis of electrostatic forces (although other forces may be involved), bonds between the negatively charged bacteria and the cellulose fibres are not promoted.

With biocide addition, the pH of the paper pulp suspension is raised to values that depend on the concentration of biocide (Figure 8). The gradual

increase in pH is believed to indicate that besides the acid-basic equilibrium established in the first 5 min due to the addition of a solution with higher pH, some reaction occurs between the fibres and the carbamate and dithiocarbamate.

The interpretation of the data presented in Tables IV–VI should be carried out with the help of Table I and Figure 8. For 100 mg l^{-1} of biocide, the retention values obtained after 5 min or after 1.5 h (Table IV) are similar. According to Figure 8, during that period of time, the pH values of the pulp suspension (when 100 mg l^{-1} was added) were lower than pH 7. In this situation, the bacterial cell surface is always neutral (Table I); hence, aggregation of cells is expected to occur, without electrostatic interactions between the cells and the fibres. Therefore, these bacterial clusters may be retained in the fibres and included in the paper.

For 200 mg l^{-1} of biocide (Table V), after 5 min (according to Figure 8 the pH of the pulp suspension is around 7), the cell surfaces become neutral (Table I) (aggregation and no electrostatic interactions). After 1.5 h the pH of the pulp suspension changed from 5.7 to around 7.5 (Figure 8) and the cell surface charge shifted from a positive to a negative value (Table I). In this case, repulsion both between the cells and between the cells and the fibres occurred, decreasing the retention.

For 300 mg l^{-1} of biocide (Table VI), the cells are positively charged (Table I), so, attraction between the cells and the fibres is promoted. After 1.5 h, the pH of the pulp suspension was around 8.6 (Figure 8) and the cells became negative (Table I) thus adhesion between the cells themselves and the cells and the fibres is not promoted, and hence retention decreases.

Comparing the values obtained in the first minutes, for the three biocide concentrations studied, higher retention values were obtained for 300 mg l^{-1}, when the cells were positively charged, decreasing as the concentration was reduced to 200 mg l^{-1} and 100 mg l^{-1}. Nevertheless, in every case, retention was higher than in the absence of biocide.

In papermaking, the interval of time between the biocide application point and the head box is very important. The results obtained suggest that the best interval of time is 30 min, which is compatible with the residence time observed in paper manufacturing.

CONCLUSIONS

The present study showed that a mild biocide (a combination of sodium dimethyl dithiocarbamate and disodium ethylene bisthiocarbamate) can be

used to retain bacterial cells on fibres in the paper pulp processes, thus avoiding the formation of slimy biofilms in the water recirculating systems and on the paper itself.

Assays were performed to determine the effect of the carbamate solution on bacterial cell surface charge, as a function of biocide concentration, pH and time of exposure to the biocide. The results indicated that biocide addition had an immediate effect on the pH of the paper pulp suspension and on the bacterial surface charges, which became shifted from negative to neutral or positive values. Thus, since the paper pulp fibres are negatively charged, adhesion of bacterial cells (treated with the biocide) to these fibres is promoted. These results suggested the potential for using carbamate solution as a retention aid in the paper industry, in addition to its conventional use as a biocide.

This approach showed that it was possible to enhance cell retention by pulp fibres from 45% to 75%, within less than 5 min of contact between the carbamate and the pulp suspension. This effect increased with increase in the biocide concentration ($100-300\,mg\,l^{-1}$) and was always higher than in the absence of biocide. For longer contact times (>30 min) it is highly probable that cell retention decreases when 200 and $300\,mg\,l^{-1}$ of biocide are applied.

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