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COMPARISON OF TWO BIOCIDES – CARBAMATE AND GLUTARALDEHYDE – IN THE CONTROL OF FOULING IN PULP AND PAPER INDUSTRY

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ABSTRACT

Formation of fouling deposits is a serious problem facing paper mills. Despite the search for alternative methods, chemical biocides still represent the chief countermeasure to control microbial growth and general fouling buid-up in pulp and paper mills. The purpose of this work was to determine the effect of two biocides (carbamate and glutaraldehyde) on both planktonic cells and fouling layers of a paper machine system. A flow system was used for the study of fouling accumulation in an industrial white water circuit. Both biocides proved to be more effective in reducing the microbial loading of the white water circuit than the deposit accumulated on the stainless steel surfaces. Carbamate, in contrast to glutaraldehyde, had the ability of promoting cell agglomeration since the microbial loading decreased much more when the white water, treated with carbamate, was filtered through a filter-linen. The retention of suspended cells in the cellulose fibres acquires major importance since it is obtained by using an already existing physical process (filtration), which strongly enhances the overall microbial reduction obtained with the addition of the carbamate, without increasing the economic costs. These results also suggest that the use of conventional retention agents in pulp and paper processes can be efficient in controlling unwanted microbial effects.

Keywords: Paper mills; white water; flow cell; fouling; biocides

INTRODUCTION

Paper mill water systems generally offer a favourable environment for the growth of a wide range of microorganisms. Therefore, slime formation on the surfaces of pipes and manufacturing equipment is quite unavoidable. Production breakdown, biocorrosion of machinery, reduction of paper quality, bad odours, health problems, and degradation of the cellulose fibres and additives are very frequent and serious problems, always resulting in substantial economical losses. Production losses are generally related the occurrence of paper breaks, holes, spots, and discoloration in the final product due to the incorporation in the paper of slime detached from the walls of pipes and tanks.

In recent years, the problems caused by the presence of microorganisms in paper manufacture increased in severity and frequency, in part due to the installation of larger, faster and more complex paper machines, the increased reuse of white water, the increased use of chemical additives and secondary fibre sources, the high process water temperature and the sporadic use of effective antimicrobial agents [1].

Due to the more restrictive environmental regulations, the use of less toxic or even non-toxic alternative biofilm control methods should be developed. These alternative approaches must always include a combined strategy between "good housekeeping" [2] based on a "clean system philosophy" and the optimisation of other countermeasures such as environmental friendly biocides, enzymes and bacteriophages.

Nevertheless, biocides still represent the most common and effective practice in eliminating unwanted slime, particularly in the paper making industry. The selection of a suitable biocide for a given system requires the knowledge of the process operational conditions and the prevailing types of biofilm-forming microorganisms [3]. The economic considerations and the possibility of damage to humans and to the environment must also be taken into account and the use of less harmful chemicals should be encouraged. It must

be stressed that biocides can only be applied successfully when their structure, properties and mechanisms of action are well known. Thus, the appropriate choice of a biocide should always be supported by earlier laboratory studies.

In previous laboratory investigations, with suspended microbial cultures, Pereira *et al.* [4] showed that a carbamate-based mild biocide (combination of sodium dimethyl dithiocarbamate and disodium ethylene bisthiocarbamate) induced changes in the surface electrical charge of *Pseudomonas fluorescens* cells from negative to positive or neutral values, depending on the pH value and the biocide concentration used. Hence, besides its traditional use as an antimicrobial agent, this biocide was able to promote aggregation between cells, and of cells to cellulose fibres before the former could produce their slimy polymeric matrix. This chemical characteristic might be explored in pulp and paper processes, since the cell-cell or fibre-cell aggregates could be retained in the pulp by the filter-linen in the wet-end section of the paper machine, thereby minimising the formation of slimy biofilms on the walls of the water recirculating systems and their incorporation in the paper itself, after detachment.

In the present work, the dual advantage of the carbamate being a biocide as well as a retention aid in paper pulp processes was investigated in real industrial systems. This was achieved by developing biofilms on stainless steel surfaces placed within flow cell reactors, through which actual process water circulated, coming directly from the industrial white water circuit of a pulp and paper mill. The efficacy of glutaraldehyde, another usual and more letal biocide, to control biofilm formation in the same conditions was also evaluated, and the performances of the two biocides were compared.

MATERIAL AND METHODS

Reactor operation

The biofilms were formed on several adhesion slides placed within two similar flow cell reactors (which have a semi-circular cross section with a hydraulic diameter of 2.02 cm), where industrial white water flowed under turbulent conditions (*Re* between 5000 and 6000, liquid velocities between 0.512 m s^{-1} and 0.614 m s^{-1}). The adhesion slides (0.6 x 1.8 cm and approximately 0.8 mm thick; average weight of 631±18 mg cm^{-2}) were made of stainless steel (ASI 316) and were glued to rectangular pieces of "Perspex" properly fitted in the apertures of the flow cell. The equipment was connected to a side stream in a paper mill, as schematically represented in Figure 1, so that the flow cells were continuously fed with process water coming directly from the white water circuit. One of the flow cells was used to assess the effect of different concentrations of two biocides on biofilm development. Simultaneously, in the other flow cell, control tests were carried out under the same hydrodynamic conditions as in the first cell, but without the addition of biocides.

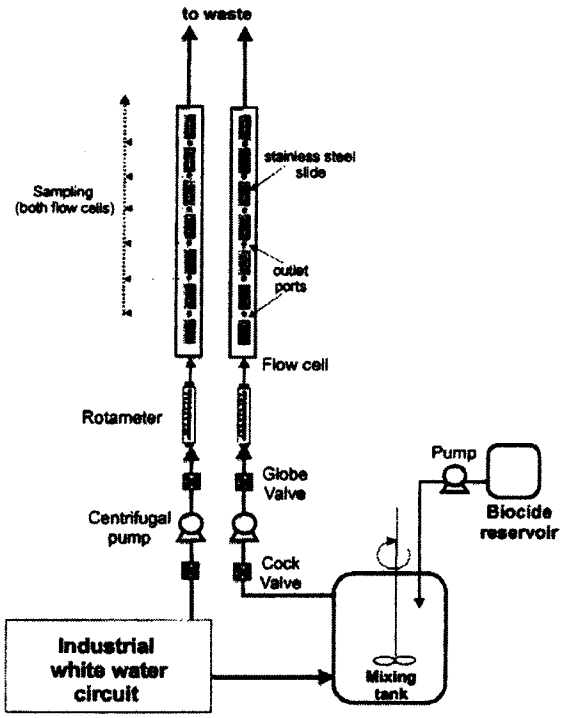

Figure 1. Schematic representation of the experimental set-up.

Every two days, one of the slides of each flow cell was carefully removed and replaced with clean slides and the deposit attached on the slides was collected for further analysis. The removal of the slides was performed without stopping the flow. Each experiment was run for almost fifteen days.

Paper machine operating conditions

The paper machine operated in acid conditions with temperatures from 43 to 50 °C and raw materials consisting of nearly 80% of original wood (pine and eucalyptus) and about 20% of secondary fibre. This paper mill performs exclusively one production process - the kraft liner grade, and does not have a program for the control of the inorganic deposit. Therefore the influence of the inorganic matter is practically constant in all the machine runs.

At the moment, glutaraldehyde is the biocide used for the slime control by the plant operator (15 mg of effective product per ton of paper produced) and it is applied to the white water circuit in intermittent shocks every eight hours. However, only a residual concentration (less than 5 mg l^{-1}) was found in the zone where the flow cells were connected to the main stream. An illustrative example of the characteristics of the white water is shown in Table 1.

Table 1. White water characterisation (illustrative example).

Parameter	
pH, Sorenson scale at 25 °C	4.6-5.8
Temperature, °C	43-50
Total suspended solids, mg l^{-1}	135
Chemical oxygen demand (COD), mg O_2 l^{-1}	1487
Kjeldahl nitrogen, mg N l^{-1}	0.16
Nitrate, mg NO_3 l^{-1}	<2.0
Total iron, mg Fe l^{-1}	0.45
Total phosphorous, mg P l^{-1}	0.45
Sulphate, g SO_4 l^{-1}	1.20
Calcium, mg Ca l^{-1}	229

Biocides

Two non-oxidising biocides were tested in the flow cell experiments: a solution of carbamate (30% w v^{-1}) and a solution of glutaraldehyde (25% w v^{-1}). The carbamate-based biocide was used as a follow-up to the previous laboratory studies with suspended cells [4] the purpose now being its application to biofilms. Glutaraldehyde, another common but stronger industrial biocide, was used as a reference for comparison. The biocide concentrations tested were 100 and 200 mg l^{-1} (respectively, 0.6 and 1.2 mmol l^{-1}) of carbamate and 50 and 100 mg l^{-1} (respectively, 0.5 and 1 mmol l^{-1}) of glutaraldehyde.

The biocides were continuously added to the white water pipe that fed the flow cell selected for biocide experiments (Figure 1) in such a way that each biocide was in contact with white water for 30 min, before it enters the flow cell.

Deposit mass assessment

The effect of each biocide concentration on the deposit formation was assessed by quantification of the mass accumulated on the slides. In the beginning of each experiment, all the stainless steel slides were identified and weighed before being placed in the flow cells. As soon as they were removed from each flow cell (every two days), the mass of the slides plus deposit attached was determined after being air dried to constant mass in similar conditions. The mass of the deposit accumulated on the several stainless steel slides was determined as the difference between the two weights, and expressed in grams per cm^2 surface area of the stainless steel slide. The results are the average of more than three measurements.

The fraction of inorganic solids in the deposit was determined according to Standard Methods [5].

Biofouling curves

The fouling curves were obtained by fitting the experimental data (deposit mass values) to the overall model for biofilm development described by [6] through the following equation:

$$m_t = m_f^{\infty}[(1-\exp(-\beta t)] \quad (i)$$

This equation is equal to the one traditionally used to describe the build-up of inorganic fouling [7].

Chemical analysis of the deposit

The deposits that covered the slides were completely scraped into 5 ml of phosphate buffer, vigorously agitated for 2 min in a vortex and the deposit content in terms of total proteins and total polysaccharides (as an indirect way to assess biomass accumulation) was evaluated. The total proteins were determined using the Lowry modified method (SIGMA-Protein Assay Kit n° P5656) with BSA as a protein standard, and the polysaccharides by the phenol-sulphuric acid method of Dubois et al. [8], with a glucose standard. The total protein and polysaccharide were determined in triplicate with a relative standard deviation lower than 10% and 15%, respectively, of the mean value.

Enumeration of suspended viable cells

During the experiments, samples of the outlet fluid of each flow cell (with and without biocide application) were collected, every two days, for cell counting. The samples were vigorously homogenised in a vortex with 100% power input and the viable cells of each sample were enumerated by performing serial dilutions and triplicate plating on nutrient agar (Merck) using the conventional plate counting method. The plates were incubated at 30±1.0 °C and the number of colonies was counted after 48 h of incubation. The results were expressed in CFU (colony forming units) per millilitre of the sample and presented in log format ($\log_{10}$).

In the assays carried out with 200 mg l^{-1} of carbamate and with 50 mg l^{-1} of glutaraldehyde, representative amounts of the outlet water samples, collected from each flow cell, were passed through a 200 mesh filter-linen. The cell concentration of the filtrated samples obtained was also assessed as mentioned above but without vortexing. In this case, CFU could represent either colonies or isolated bacteria or cell aggregates.

Scanning electron microscopy (SEM) observations

During the experiments some deposit-covered stainless steel slides were observed by SEM. Prior to SEM observations, the biofilm-containing slides were dehydrated through an absolute ethanol series to 100% (10%, 25%, 40%, 50%, 70%, 80%, 90%) and dried in a desiccator for three days. The samples were sputter-coated with gold and examined with a Leica S360 scanning electron microscope at 10-15 kV. The slides were not fixed because the fixation procedures involve

the use of chemicals that tend to react with some of the components of the biological matrix, as documented by Azeredo *et al.* [9], hence modifying the real deposit structure.

RESULTS AND DISCUSSION

The ability of two biocides, carbamate and glutaraldehyde, of preventing the initial formation of fouling deposits in a paper machine environment was investigated.

Figures 2 and 3 show some micrographs of the stainless steel slides after, respectively, 5 and 10 days in contact with the white water stream.

These photomicrographs clearly show that the fouling deposits present a very heterogeneous structure and a wide

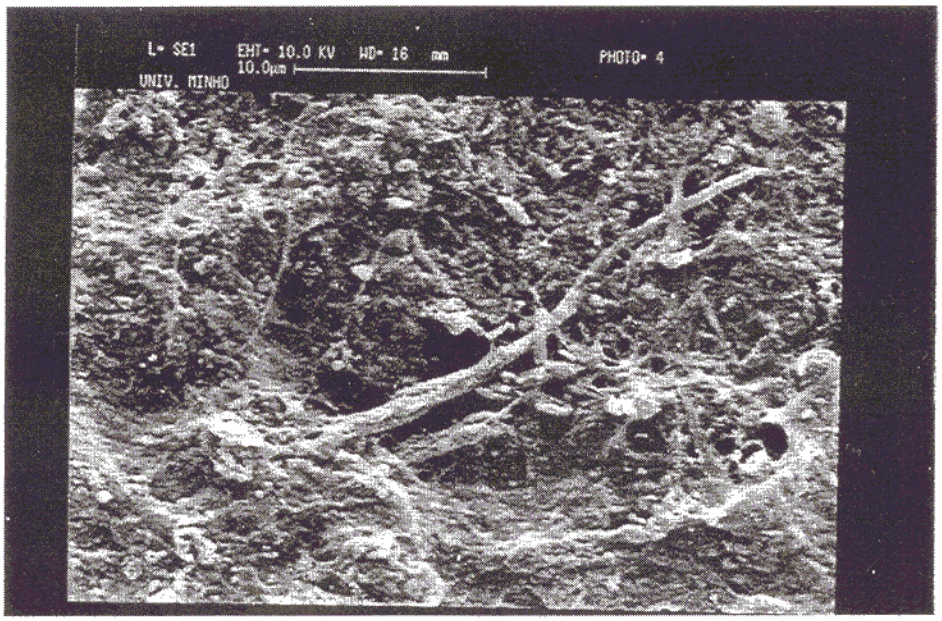

Figure 2. SEM micrograph (7000x) of a deposit-covered stainless steel slide by the circulation of real white water for five days (turbulent flow).

Figure 3. SEM micrograph (3000x) of a deposit-covered stainless steel slide by the circulation of real white water for ten days (turbulent flow).

kind of materials in its composition. A great variety of microorganism morphologies in close contact with extracellular gelatinous material was also observed. This kind of deposit structure and composition was expected. The formation and nature of the deposits are directly related with the characteristics of the aquatic system where they were developed. In the case of the pulp and paper industry, deposits are formed due to the accumulation of diverse microorganisms together with pulp fibres, anionic compounds, particles, etc. [10] [11], *i.e.*, the nature of a fouling deposit in this kind of industry generally involves a nonbiological fraction held together by the biological matrix. Therefore, the information given by the deposit mass may not be *per se* indicative of the efficacy of the biocides. It is known that biocides cause irreversible damage to a vital structure or function of the cell, thereby reducing the microbial activity (disinfection), but they do not necessarily cause the reduction in the fouling layer (detachment). Thus, in this work, besides the determination of the mass of the deposit, measurements of the protein and polysaccharide content of the deposit (as an indirect measure to estimate deposit biomass) were also carried out since these two parameters represent the chief components of a biofilm [12]. It should be emphasised that the polysaccharide and protein contents indicated in Table 3 may include also non-cellular components containing sugars and/or proteins (*e.g.*, the carbohydrates of the cellulose fibres). However, it is reasonable to assume that the biocides exert their effect essentially on the biofilm fraction of the deposit and, therefore, the changes caused by the biocides in the protein/polysaccharide content of the deposit are mainly due to their effect on the biomass.

Figures 4, 5, 6, and 7 show the effect of different concentrations of carbamate and glutaraldehyde on the total mass, as a function of time. The results were statistically tested in order to assess whether the differences between the deposit mass values obtained by the application of the biocides and the ones achieved in the corresponding control test could be considered significant. Because the dependent variables, in each assay, are two related samples, a paired

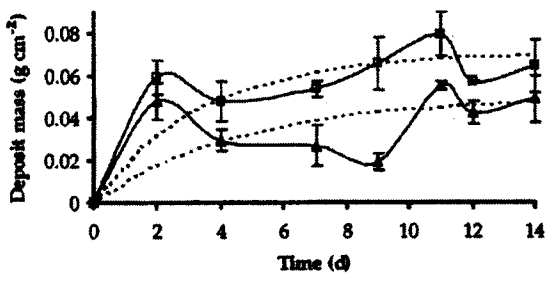

Figure 4. Effect of 100 mg l^{-1} (Δ) of carbamate on deposit mass; (□) the control assay, *i.e.*, without carbamate addition; (—) experimental line, (---) trend line obtained by fitting equation (i) (the bars represent the standard deviation from the mean).

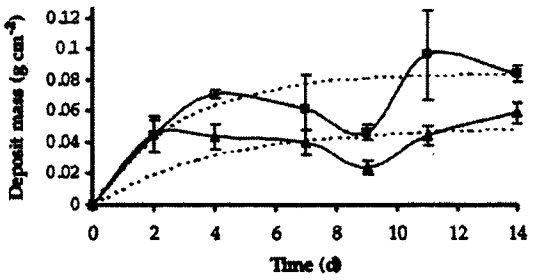

Figure 5. Effect of 200 mg l^{-1} (▲) of carbamate on deposit mass; (□) the control assay, *i.e.*, without carbamate addition; (—) experimental line, (---) trend line obtained by fitting equation (i) (the bars represent the standard deviation from the mean).

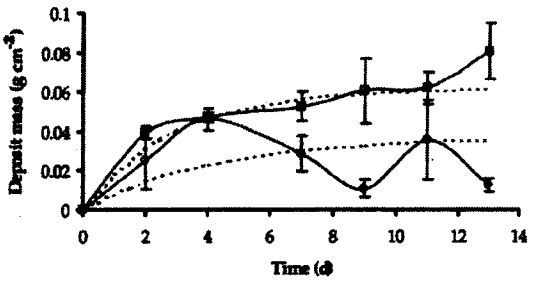

Figure 6. Effect of 50 mg l^{-1} (○) of glutaraldehyde on deposit mass; (□) the control assay, *i.e.*, without glutaraldehyde addition; (—) experimental line, (---) trend line obtained by fitting equation (i) (the bars represent the standard deviation from the mean).

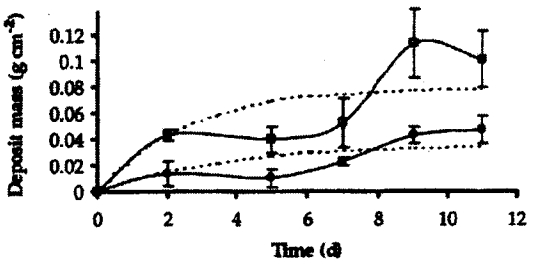

Figure 7. Effect of 100 mg l^{-1} (●) of glutaraldehyde on deposit mass; (□) the control assay, *i.e.*, without glutaraldehyde addition; (—) experimental line, (---) trend line obtained by fitting equation (i) (the bars represent the standard deviation from the mean).

comparison test using Student t test distribution based on differences [13] was performed. The conclusions demonstrated that the differences between the paired values are statistically significant since confidence levels higher than 95% were obtained ($P<0.05$ for n=6 when 50 mg l^{-1} of glutaraldehyde was added; $P<0.01$ for n=5 when 100 mg l^{-1} of glutaraldehyde was added; $P<0.01$ for n=7 when 100 mg l^{-1} of carbamate was added; $P<0.02$ for n=7 when 200 mg l^{-1} of carbamate was added). These values mean that the biocides applied to the white water loop affect the deposit mass accumulated on the metal slide, in every case.

The experimental data follow typical deposit growth curves both in the control tests (in the absence of the biocides) and in the tests performed with the addition of the biocides.

It should be stressed here that all tests were carried out under industrial operating conditions which could not be fully controlled, mainly as regards possible fluctuations in the flow rates. This may explain the significant deviations from the overall fouling model described by Equation (i), although some of the fluctuations can also be attributed to natural phenomena such as erosion and sloughing off of the deposit. However, since the asymptotic trend is consistently displayed in all figures, it was decided to fit the simple model to the data (the fitted curves are drawn in each figure). Table 2 presents the values of parameters m_f^∞ (maximum amount of deposit, at pseudo-steady state) and $1/\beta$ (resistance of the deposits to detachment) obtained by this fitting process.

From Table 2 it is possible to conclude that: i) the maximum amount of deposit is always reduced when biocides are applied to the system; ii) glutaraldehyde causes a greater reduction in the final mass of deposit than carbamate; iii) the presence of the biocides seems to increase the mechanical resistance to detachment of the remaining deposits. Points i) and ii) were expected and give a clear indication of the contribution of biofilm structures to mixed deposits such as the ones formed in the experiments. Cells and the polymeric network that they excrete play an important role in the overall amount and stability of the deposit. Point iii) suggests that deposits containing a small fraction of biological material (which was supposedly removed in part by the action of the biocides) will be physically more stable than those with a high biological content.

The effect of each biocide on the maximum protein and polysaccharide content of each deposit (final values) can be seen in Table 3. The amounts of proteins and polysaccharides

Table 2. Effect of the biocides on the maximum mass – m_f^∞ (in brackets the standard deviation from the mean) and resistance to detachment ($1/\beta$) of the deposits.

Biocide	Biocide concentration (mg l^{-1})	Maximum mass (m_f^∞) (g cm^{-2})	Resistance to detachment ($1/\beta$) (d)	Reduction in m_f^∞ (%)
Carbamate	0 (control)	0.071 (±0.008)	3.33	29.6 (±11.3)
	100	0.050 (±0.006)	4.55	
	0 (control)	0.085 (±0.016)	2.86	41.2 (±16.2)
	200	0.050 (±0.010)	4.00	
Glutaraldehyde	0 (control)	0.063 (±0.011)	2.86	41.3 (±17.6)
	50	0.037 (±0.009)	4.00	
	0 (control)	0.080 (±0.022)	2.50	53.8 (±18.7)
	100	0.037 (±0.011)	3.70	

Table 3. Effect of biocides on the maximum (final) protein and polysaccharide contents of the deposits (in brackets the standard deviation from the mean value).

Biocide	Biocide concentration (mg l^{-1})	Maximum protein content (mg cm^{-2})	Reduction in protein content (%)	Maximum polysaccharide content (mg cm^{-2})	Reduction in polysaccharide content (%)
Carbamate	0 (control)	0.25 (±0.05)	44.0 (±13.8)	0.35 (±0.09)	37.1 (±17.8)
	100	0.14 (±0.02)		0.23 (±0.02)	
	0 (control)	0.27 (±0.07)	51.9 (±12.6)	0.37 (±0.11)	43.2 (±17.1)
	200	0.13 (±0.004)		0.21 (±0.01)	
Glutaraldehyde	0 (control)	0.56 (±0.12)	64.3 (±10.5)	0.58 (±0.04)	41.4 (±6.6)
	50	0.20 (±0.04)		0.34 (±0.03)	
	0 (control)	0.75 (±0.12)	66.7 (±6.0)	0.70 (±0.21)	64.3 (±11.5)
	100	0.25 (±0.02)		0.25 (±0.03)	

in the deposit are, in fact, reduced when biocides were introduced into the system.

Glutaraldehyde is more effective in reducing the organic content than carbamate. This fact supports the previous assumption that the biocides act mainly on the biofilm fraction of the deposit, because glutaraldehyde is known to be a much stronger biocide than carbamate (acting on the cell membrane [14] and on the polymeric matrix [15]). It is interesting to note that the overall final mass of the deposit is not affected by the increase in biocide concentration (Table 2). This stable tendency may reflect some loss of sensibility of the remaining deposits to the antagonistic action of the biocides, inducing the development of a biological resistance of the deposit. Moreover, these deposits contain significant nonbiological fractions (up to 30% of the total deposit mass) that seem to be strongly adhered to the steel surfaces considering their higher mechanical resistance to detachment (Table 2). In the industrial environment, this fact acquires major importance because such kind of deposits represent an increased difficulty to the mechanical cleaning of the paper machine and piping.

In general, the values displayed in Table 3 corroborate the fact that the doubling of the concentration of both biocides did not bring about considerable increases in protein and polysaccharide removal.

The results suggest that anti-fouling measures should focus on preventing the presence of biofilm precursors such as microorganisms in the water circuit instead of just simple cleaning. With this in mind, the effectiveness of the two biocides against the planktonic populations of the white water circuit was also monitored in the outlet stream of the flow cells. Figures 8, 9, 10 and 11 show the suspended cell concentrations obtained with the application of, respectively, 100 and 200 mg l^{-1} of carbamate, and 50 and 100 mg l^{-1} of glutaraldehyde, as a function of time. Table 4 summarises the reduction in the suspended viable bacteria (as Δlog) due to the action of carbamate and glutaraldehyde.

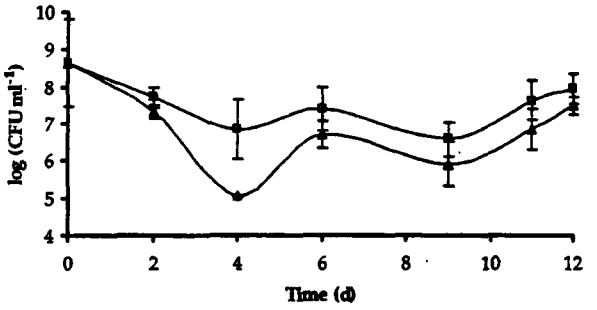

Figure 8. Effect of 100 mg l^{-1} (Δ) of carbamate on the suspended viable bacteria in the outlet fluid; (□) the control test, *i.e.*, the cell concentration without carbamate addition. (the bars represent the standard deviation from the mean).

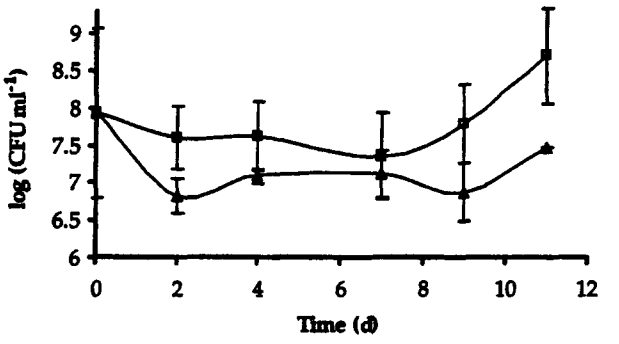

Figure 9. Effect of 200 mg l^{-1} (▲) of carbamate on the suspended viable bacteria in the outlet fluid; (□) the control test, *i.e.*, the cell concentration without carbamate addition. (the bars represent the standard deviation from the mean).

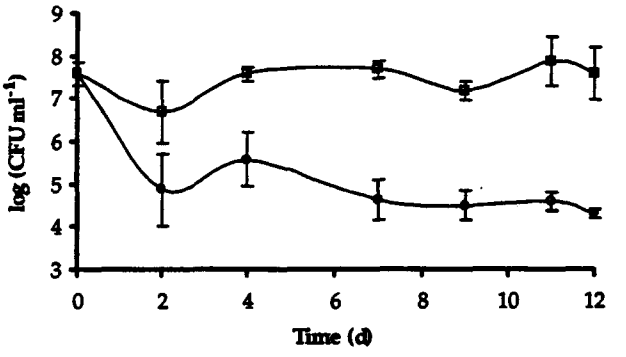

Figure 10. Effect of 50 mg l^{-1} (○) of glutaraldehyde on the suspended viable bacteria in the outlet fluid; (□) the control test, *i.e.*, the cell concentration without glutaraldehyde addition. (the bars represent the standard deviation from the mean).

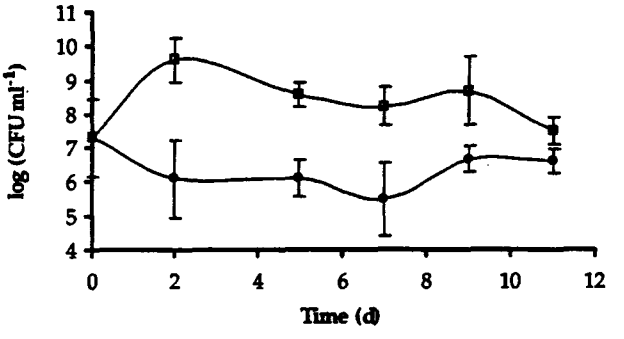

Figure 11. Effect of 100 mg l^{-1} (●) of glutaraldehyde on the suspended viable bacteria in the outlet fluid; (□) the control test, *i.e.*, the cell concentration without glutaraldehyde addition. (the bars represent the standard deviation from the mean).

Table 4. Reduction in suspended viable bacteria caused by carbamate and glutaraldehyde, as a function of time (in brackets the standard deviation from the mean value).

Time	Reduction in suspended viable bacteria (Δlog_{10})			
(d)	100 mg $l^{-1}$of carbamate	200 mg $l^{-1}$of carbamate	50 mg $l^{-1}$of glutaraldehyde	100 mg $l^{-1}$of glutaraldehyde
2	0.4 (±0.3)	0.8 (±0.6)	1.8 (±1.1)	3.5 (±1.3)
4	1.8 (±0.8)	0.5 (±0.4)	2.0 (±0.6)	2.5 (±0.6)
7	0.7 (±0.6)	0.2 (±0.7)	3.1 (±0.5)	2.8 (±1.2)
9	0.7 (±0.8)	0.9 (±0.8)	2.7 (±0.4)	2.0 (±1.1)
11	0.8 (±0.3)	1.2 (±0.8)	3.3 (±0.6)	0.9 (±0.5)
12	0.4 (±0.4)	*	3.3 (±0.6)	*

* not determined

The results show that, in all assays, the planktonic viable bacteria found in the white water circuit without biocide application were very high and varied with time in the range between 10^4 – 10^5 CFU ml^{-1}. Values in the same order of magnitude are reported by Raaska *et al.* [16]. Although these values were high, they are not surprising since a paper machine is an open system where it is impossible to operate in sterile conditions. Moreover, the increasing use of secondary fibres and the reuse of the process water also contribute to the dissemination of microorganisms. The results also demonstrate that suspended viable bacteria always decreased with the application of the biocides. Once again, the suspended bacteria reduction obtained with glutaraldehyde was significantly greater than the one achieved in the presence of the carbamate. In general, the presence of glutaraldehyde in the white water circuit reduced viable suspended cells by 3 orders of magnitude, while carbamate reduced by only about 1 order of magnitude. There does not seem to be a clear effect of the biocide concentration on the reduction of viable bacteria.

In general, with the carbamate treatment, the deposit accumulated on the slides was reduced by about 30-40% whereas a 0.2-1.8 log_{10} decrease in suspended microbial concentration was observed (70-95%). Glutaraldehyde reduced the planktonic cell loading in a range between 80 and 99% (0.9-3.5 log_{10} decrease), while the deposit mass attached to the slides was reduced only by about 40% to 55%. These findings highlight that the action of both biocides is more marked in disinfecting the white water circuit than in detaching the fouling layers.

Another interesting phenomenon - the capacity of the two biocides to promote aggregation between cells and cells to fibres in this industrial environment - was investigated by filtrating the white water samples in a 200 mesh filter-linen. Tables 5 and 6 summarise the effects of both biocides (carbamate and glutaraldehyde) on the viable bacteria numbers in the white water before and after it was filtered through the filter linen. The data related with the control assays, *i.e.*, without biocide addition, are also presented. The presence of 200 mg l^{-1} of carbamate together with filtration procedure (Table 5) caused an improvement in the reduction of the planktonic populations of the white water circuit since

Table 5. Reduction in suspended viable bacteria obtained by the filtration of the samples, treated with 200 mg l^{-1} of carbamate, through a 200 mesh filter, as a function of time; (in brackets the standard deviation from the mean value); (*) not determined.

Time	Control (no biocide)			With 200 mg $l^{-1}$of carbamate		
	Viable bacteria log_{10}(CFU ml^{-1})		Retention	Viable bacteria log_{10} (CFU ml^{-1})		Retention
(d)	Sample	Filtered sample	(%)	Sample	Filtered sample	(%)
2	7.6 (±0.4)	*	-	6.8 (±0.5)	6.6 (±0.5)	61.0
4	7.6 (±0.5)	7.5 (±0.2)	19.7	7.1 (±0.1)	6.7 (±0.03)	61.5
7	7.4 (±0.6)	7.3 (±0.4)	12.5	7.1 (±0.3)	6.8 (±0.2)	44.2
9	7.8 (±0.6)	8.1 (±0.8)	-	6.9 (±0.6)	6.7 (±0.2)	-
11	8.7 (±0.8)	8.7 (±1.0)	-	7.5 (±0.1)	6.9 (±0.8)	37.9

The retention was not calculated using "log numbers", but with the actual viable bacteria numbers (in CFU ml^{-1}), according to the equation:

$$\%\text{Cell Retention} = \frac{|\text{Cell}|_{suspension} - |\text{Cell}|_{filtrate}}{|\text{Cell}|_{suspension}} x100$$

Table 6. Reduction in suspended viable bacteria obtained by the filtration of the samples, treated with 50 mg l^{-1} of glutaraldehyde, through a 200 mesh filter, as a function of time; (in brackets the standard deviation from the mean value); (*) not determined.

Time	Control (no biocide)			With 50 mg l^{-1} of glutaraldehyde		
	Viable bacteria ml^{-1})	log_{10} (CFU	Retention	Viable bacteria ml^{-1})	log_{10} (CFU	Retention
(d)	Sample	Filtered sample	(%)	Sample	Filtered sample	(%)
2	6.7 (±0.7)	6.6 (±0.3)	24.6	4.9 (±0.8)	5.4 (±0.4)	-
4	7.6 (±0.2)	7.8 (±0.7)	-	5.6 (±0.6)	5.5 (±0.9)	33.5
7	7.7 (±0.2)	*	-	4.6 (±0.5)	*	-
9	7.2 (±0.2)	7.4 (±0.1)	-	4.5 (±0.4)	4.6 (±0.4)	-
11	7.9 (±0.6)	6.2 (±1.0)	44.6	4.6 (±0.2)	4.7 (±0.3)	-
12	7.6 (±0.6)	6.7 (±1.2)	18.2	4.3 (±0.1)	4.2 (±0.2)	14.0

The retention was not calculated using "log numbers", but with the actual viable bacteria numbers (in CFU ml^{-1}), according to the equation:

$$\%\text{Cell Retention} = \frac{|\text{Cell}|_{\text{suspension}} - |\text{Cell}|_{\text{filtrate}}}{|\text{Cell}|_{\text{suspension}}} \times 100$$

an additional 0.2-0.6 log_{10} decrease in cell concentration was observed. This reduction appears to be due to the formation of cell aggregates which can be retained by the filter-linen, hence reducing the potential for biofilm formation in the white water pipes and tanks.

In the case of glutaraldehyde (Table 6), there does not seem to be any reduction in the suspended viable bacteria after filtration. Only for the samples collected on days 4 and 12, some cell density reduction was detected (0.1-log_{10}), but these values are not meaningful because they are in the same range as the ones obtained without glutaraldehyde application.

The percentages of retention obtained by filtration with the application of 200 mg l^{-1} of carbamate to the industrial stream (38-62%) are similar to those achieved by Pereira *et al.* [4] with the application of the same biocide concentration in laboratory conditions to a pure cell culture of *Pseudomonas fluorescens* (39-65%). These findings were, in a certain way, unexpected since it is well known that in a paper machine environment a great variety of microorganisms can be commonly found [11] [17], each species with its own characteristics and resistance. The similarity of the results suggests that carbamate may also be able to modify the surface electrical charge of other microorganisms than *P. fluorescens*, present in the white water system.

The retention of suspended cells as discrete agglomerates on the filter-linen and paper sheet with the aid of the carbamate acquires major importance since it is obtained through a single physical process (filtration) that leads to an overall microbial reduction without increasing the economic costs (note that the wet-end section of the paper machine already contains a "filter-liner").

The fact that glutaraldehyde is more deleterious to the environment than carbamate also favours the application of the methodology here proposed.

These findings open up the possibility of using other non-biocidal compounds capable of inducing changes in the surface properties similar to the ones caused by carbamate.

CONCLUSIONS

The action of two biocides (carbamate and glutaraldehyde) against planktonic cell concentration and biofilm formation was tested under real industrial conditions in a pulp and paper factory. The efficacy of both biocides against the deposits accumulated on the steel surfaces (in terms of percentage of reduction of mass) was lower than that found for planktonic viable bacteria (in terms of percentage of reduction of viable cells). This fact can be due to the mixed nature of the deposits, where the biological matrix is not the sole dominant fraction. Glutaraldehyde proved to be more effective against paper mill strains than carbamate. However, when coupling the biocidal effect with a filtration procedure, the use of carbamate led to a substantial reduction in the concentration of suspended microorganisms in the water stream, due to the ability of carbamate to induce the formation of cell-cell and cell-fibre aggregates that can be separated from the liquid by filtration (through the "filter linen" of the wet-end section of the paper machine, together with the paper pulp) and be entrapped in the forming paper sheet. Such methodology (use of carbamate or, possibly, use of conventional retention agents together with filtration) is environmentally preferable because it reduces the potential for biofilm accumulation without having to introduce a strong toxic compound into the water. This effect was not obtained when glutaraldehyde was used.

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NOMENCLATURE

Re Reynolds number
t time (T)
m_t mass of deposit at any time t (ML^{-2})
m_t^{∞} maximum mass of the fouling layer, at steady state (*i. e.*, the asymptotic value of m_t when $t \rightarrow \infty$)
$1/\beta$ mechanical resistance of the deposit to detachment (T^{-1})
$\Delta\log$ reduction in the suspended viable bacteria of the white water circuit

REFERENCES

1. Salzburger W., Paper and additives. In: *Microbially Influenced Corrosion of Materials*, Flemming H-C., Sand W., Heitz E. (eds). Springer-Verlag, Heidelberg, Germany, pp. 415-427 (1996).
2. Flemming H-C. and Schaule G., Measures against biofouling. In: *Microbially Influenced Corrosion of Materials*, Heitz E., Flemming H-C., Sand W. (eds), Springer-Verlag, Heidelberg, Germany, pp. 121-139 (1996).
3. Cloete T. E., Jacobs L. and Brozel V. S., The chemical control of biofouling in industrial water systems. *Biodegradation*, **9**, 23-37 (1998).
4. Pereira M. O., Vieira M. J., Beleza V. M. and Melo L. F., Retention of bacteria by cellulose fibres as a means of reducing biofouling in paper pulp production processes. *Biofouling*, **13**, 1-18 (1998).
5. Clesceri L. S., Greenberg A. E. and Trussel R. R. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington D. C. (1989).
6. Melo L. F. and Vieira M. J., Physical stability and biological activity of biofilms under turbulent flow and low substrate concentration. *Bioprocess Eng.*, **20**, 363-368 (1999).
7. Bott T. R. *Fouling of Heat Exchangers*. Elsevier, Amsterdam (1995).
8. Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F., Colorimetric method for determination of sugars and related substances. *Analytical Chem.*, **28**, 350-356 (1956).
9. Azeredo J. A., Lazarova V. and Oliveira R., Methods to extract the exopolymeric matrix from biofilms: a comparative study. *Water Sci. Technol.*, **39**, 243-250 (1999).
10. Pellegrin V., Juretschko S., Wagner M. and Cottenceau G., Morphological and biochemical properties of *Sphaerotilus sp.* isolated from paper mill slimes. *App. Environ. Microbiol.*, **65**, 156-162 (1999).
11. Klahre J., Lustenberger M. and Flemming H-C., The achilles heel of paper production: microbial problems. In: *Proc. of Wet and Chemistry Conference & COST Workshop*. May 1997, Gatwick. Pira International, Leatherhead, UK, (1997).
12. Lazarova V. and Manem J., Biofilm characterisation and activity analyses in water and wastewater treatment. *Water Res.*, **29**:, 2227-2245 (1995).
13. Daniel W. W., *Biostatistics: a Foundation for Analysis in the Health Sciences*. Wiley and Sons, New York (1987).
14. Russel A. D., Glutaraldehyde: its current status and uses. *Infect. Control and Hospital Epidemiol.*, **15**, 724-733 (1994).
15. Pereira M. O. and Vieira M. J., Effects of the interactions glutaraldehyde-polymeric matrix on the efficacy of the biocide on *Pseudomonas fluorescens* biofilms. *Biofouling*, (accepted). (2001)
16. Raaska L., Alakomi H-L., Salkinoja-Salonen M. and Mattila-Sandholm T., Antagonistic activity of *Staphylococcus siderophores* and chemical biocides against *Bacillus subtilis* in a paper-machine environment. *J. Ind. Microbiol. & Biotechnol.*, **22**, 27-32 (1999).
17. Eagon R. G., Paper, pulp and food grade paper. In: *Handbook of Biocide and Preservative Use*, Rossmoore H. W. (ed), Blackie Academic and Professional, Glasgow, UK, pp. 83-95 (1995).