THE EFFECT OF 2,2-DIBROMO-3-NITRILOPROPIONAMIDE, 2-(THIOCYANOMETHYLTHIO) BENZOTHIOAZOLE AND ALKALI DIMETHYL BENZYL AMMONIUM CHLORIDE ON MICROORGANISMS IN A PAPER MACHINE WHITE WATER SYSTEM, ON MACHINE RUNNABILITY AND PAPER QUALITY

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An optimum temperature, humidity and ample easily assessable nutrient sources in the paper machine favor the multiplication of bacteria and fungi. As a result, they do not only impair the quality of paper (as per norms for food grade paper), but also reduce the paper production, because of numerous interruptions. The present study aims at determining the various microbes, such as total bacteria, sulphate reducing bacteria, iron bacteria, pseudomonas, yeasts and filamentous fungi present in the white water of the paper machine. The effect of three biocides, namely, 2,2-dibromo-3-nitrilopropionamide, 2-(thiocyanomethylthio) benzothioazole and alkali dimethyl benzyl ammonium chloride on various microbes are studied. It is observed that biocide 2,2-dibromo-3-nitrilopropionamide has a wide spectrum effect on all types of bacteria and fungi, its killing efficiency being, in most cases, of about 100%. A plant trial conducted with 2,2-dibromo-3-nitrilopropionamide indicates that the number of breaks is reduced from 10 to 3 in 24 h, while paper properties get improved.

Keywords: paper machine, white water, microbes, biocides, paper properties, paper breaks

INTRODUCTION

The pulp and paper industries have been looking for efficient system closure of process water or for the use of biological waste water treatment plants, given the stringent environmental regulations and consumer attitude.[1] This has been achieved by an increased number of recyclings of process water. In the 1970s, approximately 100 m^3 of water were used per metric ton of manufactured paper while, at present, less than 10 m^3 of water are used per metric ton of paper. As a result, the paper machine white water has become richer in nutrient salts and degradable carbon, thus contributing to microbial problems.[2] The formation of slime deposits is a major problem faced by paper industries. Microbiological growth in a paper mill might cause loss of production, due to breaks, reduction in quality caused by slime spots, odors in product, corrosion, production of malodorous gases, and off-spec and rejected products.[3] The biofilms seed microbes into the process water, deteriorating the hygienic quality of the product. According to FDA regulations,[4] fibers should be used in food packing materials "only in a way that assures product and consumer safety". At an international level, there are no specific biological standards for such grades, intended to come into contact with food.[5] At a national level, some countries have limited value recommendations, *e.g.* for packing materials used for dairy, butter and bakery products. Depending on the case, the limit values vary from 1 fungal cfu/dm^2 to 250 bacterial cfu/g. Generally, the total accepted counts of yeast, moulds and bacteria must be low and no pathogenic bacteria, including enterobacteria and *Escherichia coli*, should be detected.[6-8]

Also, biofilms clog wires and cause paper discoloration. Usually, the paper machine systems support significant growth of microorganisms, due to congenial and favorable conditions persisting during the manufacture of papers. The unique environmental conditions, *viz.* available low molecular weight carbohydrates, high moisture levels, moderate temperatures, recycling of the process water and pH, transform the paper mills into a perfect breeding ground for microorganisms.[9] The slime might be biological or non-biological in nature. The biological deposits composed of varied microflora along with fibers, fillers and dirt, are the most troublesome. Biofilm formation contributes decisively to the development of paper machine deposits. *Meiothermus silvanus* and *M. ruber* were found in paper and board products with color defects and the connections between deposit-forming microbes and end-product spots were evidenced.[10] Isolated *Pseudomonas*, *Bacillus* and *Pseudoxanthomonas* were found in relatively high ratios in both pulp and slime samples. This was the first time that *Pseudoxanthomonas* strains were isolated from pulp and slime samples on a paper machine.[3] Although biofilm formation has been studied intensively in natural aquatic systems, waste water treatment systems and medical applications,[4-5,11-13] only few studies have been devoted to biofilms in the paper machine environment,[4,6-7] in spite of the fact that paper and paperboard production represent one of the largest industries in the world. Polyphasic characterization of the isolated *Sphaerotilus* strains revealed interesting adaptations of the strains to the environmental paper mill conditions, with regard to temperature tolerance and utilization of cellulose and starch.[8] The slime producing microbes secreted extra cellular polysaccharides, which gummed up the process machinery.[14-15] Biofilm development induced technical problems in the paper machines[16] and reduced the hygienic and technical quality of the produced paper. An alternative technology, known as surface-active biofilm matrix blocker (SAM), was developed to control biofilms/deposits.[17] Differential turbidity (DTM) and automatic pressure drop (PD) measurements proved useful for the measurement of deposit formation in the side stream of a paper machine white water circuit.[18] The phenyl mercuric compounds have been found as the most effective biocides in the pulp and paper industry, yet they cause serious pollution of lake sediments.[19]

The present study aims at assessing the various microbes present in the white water system of an integrated paper mill, using mixed pulp furnish of *Populus deltiodes*, *Eucalyptus tereticornis* and *Bambusa aurandacea*, for manufacturing writing, printing, wrapping and packaging papers. To improve product quality and to explore the possibilities of reusing recycled water, different biocides were tested, as to their killing efficiency on different microbes.

MATERIALS AND METHOD

Sample collection

The samples, from 4 different paper machines of Star Paper Mills Ltd., Saharanpur,[1-4] were coded (PM 1-4). The total circulating process water from white water pit under Fourdrinier section of paper machine was collected. All glassware was first washed with soap, followed by thorough rinsing with tap water, then autoclaving at 15 Pa for 15 min and oven-drying at 60 °C for 5-6 h. After sterilization, 9 mL of each sterile saline were added to a set of 4 dilution tubes. 1 mL of white water was poured into the first bottle (10^{-1} dilution) and then transferred to the second bottle, containing a saline solution, after thorough shaking of the contents. This gave a dilution of 10^{-2}. 1 mL of each dilution bottle, numbered as 10^{0}, was transferred into two sterile Petri dishes, the operation being repeated for the plates with 10^{-1} and 10^{-2} dilutions, respectively. After being maintained at an environmental temperature of 45 °C, the contents were transferred into Petri dishes, then swirled, so as to mix the medium with a representative process water sample.

Enumeration of microbiological growth

Total bacterial colony counts in the process water of PM 1-4 were determined by using yeast malt extract peptone dextrose agar (Difco 0712). To isolate the bacterial species present in the white water samples, only low numbers, 100 mg l^{-1} cycloheximide (catidion) (Sigma C-7698), were added to the media to prevent the growth of fungi. For the detection of yeasts and filamentous fungi, rose Bengal chloramphenicol agar medium was used. To prevent bacterial growth, 100 mg 1^{-1} chloramphenicol (Sigma C-0378) and 100 mg 1^{-1} chlorotetracycline (Sigma C-4881) were added by plating, with the exception of chloramphenicol agar, already contained in the selective agent. The pseudomonas bacteria present in the process water of paper machines[1-4] were determined by using cetrimide agar (Himedia M 024). Also, to

prevent the growth of fungi, 100 mg 1^{-1} cycloheximide (catidion) (Sigma C-7698) were added to the media. The iron bacteria occurring in the process water of paper machines[1-4] were determined by using medium iron bacteria (Himedia M 622). To stop the growth of fungi, 100 mg 1^{-1} cycloheximide (catidion) (Sigma C-7698) were added to the media. An SRB broth medium was used for determining the sulphate reducing bacteria in the process water of paper machines.[1-4] To control the growth of filamentous fungi and mould, 100 mg 1^{-1} cycloheximide (catidion) (Sigma C-7698) were added to the media.

In all cases, growth was monitored at 30 °C by varying the incubation period from 3 to 14 days, except for yeasts and filamentous fungi detection, where growth was monitored at 25 °C by varying the incubation period from 3 to 14 days. The results are reported in Tables 1-5.

The killing efficiency of 2,2-dibromo-3-nitrilopropionamide, 2-(thiocyanomethylthio) benzothioazole and alkali dimethyl benzyl ammonium chloride on total bacteria, yeasts and filamentous fungi, pseudomonas bacteria, iron bacteria and sulphate reducing bacteria in the process water of PM 1-4 was determined.

Plant trial

To validate the positive laboratory results, a plant trial was made during the manufacture of maplitho paper of 70 g/m^2. The bleached pulp contained bamboo (*Bambusa aurandacea*), eucalyptus (*Eucapyptus tereticornis*), poplar (*Populus deltoides*) and veneer waste (*Populus deltoides* and *Populus ciliata*) in a ratio of 20:15:15:50. Pulp brightness was of 87% (ISO). The pulp was beaten at a beating level of 28±2 ^{o}SR and mixed with non-fibrous additives, such as 20% soap stone powder, 2% alkyl ketene dimer (AKD), 1% melamine formaldehyde, 2% starch, 2% wax emulsion, 1% retention aids, on oven dry pulp basis. The final ^{o}SR at head box and first pass retention were of 45 and 80.5%, respectively. The paper was surface-sized with starch (3%) and Cartafluor CFI liquid (oleophobic and hydrophobic sizing chemicals 3.0% as such on paper basis). The paper samples were conditioned at 27±2 °C and relative humidity of 65±2%, being evaluated for basis weight (T410 om-03), breaking length (T494 om-01), double fold numbers (T423 cm-98), wax pick test (T459 om-03), internal bond strength (Scott type) (T569 pm-00) and tear index (T414 om-98). The number of breaks within 24 h was observed during plant trial; the results are reported in Table 6.

RESULTS AND DISCUSSION

Star Paper Mills Ltd., Saharanpur, with 4 paper machines located at the foothills of Shivalik, Western Uttar Pradesh (India), produces writing and printing, packaging and wrapping, absorbent and some specialty paper grades. Depending on the quality of the paper to be manufactured, the quantity of non-fibrous additives utilized per ton of paper varies: starch – 10-25 kg, alkyl ketene dimer (AKD) – 14-17 kg, polyaluminum chloride – 45-50 kg, dispersed rosin – 10-55 kg, fiber lock (retention aids) – 1-2 kg, soapstone powder – 60-190 kg, optical whitener – 2-6 kg, refiner aids (refiner rich) – 5 g, TiO_2 – about 2 kg, melamine formaldehyde resin – 1-3.5 kg, wax emulsion – 1.0-3.0 kg, cartaflex (glazing agent) – 1.0-2.0 kg, dyes and some specialty chemicals – based on oven dry pulp basis.

The deposition of these non-fibrous additives and the optimum conditions for bacterial growth favor the growth of various types of bacteria, causing production losses, due to machine downtime, and paper defects due to microbiological growth. Table 1 shows the total bacterial colony counts in the process white water of PM 1-4 and the killing efficiency of different biocides. The total counts of bacteria in the white water system of PM 1-4 are nearly the same, but a total bacterial colony count in the process water of PM 2 was double, *i.e*. $7.12X10^5$ cfu/mL. The conditions for bacterial growth are the following: an incubation period of 48 h, a temperature of 30 °C and a medium consisting of yeast, malt extract, peptone, dextrose agar. 100 mg/L cycloheximide (catidion) were added to isolate the bacterial species and to prevent the growth of fungi. The killing efficiency of three examined biocides, *viz.* 2,2-dibromo-3-nitrilo-propionamide (A), 2-(thiocyanomethylthio) benzothioazole (B), and alkali dimethyl benzyl ammonium chloride (C) was analyzed at a shock dose of 5 ppm. The killing efficiency of 2-(thiocyanomethylthio) benzothioazole (B) was the highest, compared to other biocides, a poor killing efficiency being recorded for alkali dimethyl benzyl ammonium chloride (C).

Table 2 illustrates the microbiological analysis for yeast and mould in the process white water of PM 1-4, and the killing efficiency of the above-mentioned biocides. Rose Bengal chloramphenicol agar medium was used for the detection of yeasts and filamentous fungi. 100 mg/L chloramphenicol and 100 mg/L chlorotetracycline were added by plating, with the exception of chloramphenicol agar,

which already contained the selective agent to prevent the growth of bacteria. The culture was incubated at 25 °C and growth was monitored from 3 to 14 days. The mould and fungi were observed to be of 49 cfu/plate, in the process white water of PM 1, while mould and fungi varied between 42-46 cfu/plate, in the process white water of PM 3-4. The killing efficiencies of 2,2-dibromo-3-nitrilopropionamide (A) and 2-(thiocyanomethylthio) benzothioazole (C) were of 100%, at a shock dose of 5 ppm. On the contrary, the killing efficiency of alkali dimethyl benzyl ammonium chloride (B) was of about 92%.

Table 1
Total bacterial colony count in the process white water of PM 1-4 of Star Paper Mills Ltd., and the killing efficiency of different biocides

Particulars	Total bacterial counts/mL, cfu/mL				Killing efficiency, %			
	PM 1	PM 2	PM 3	PM 4	PM 1	PM 2	PM 3	PM 4
Blank	$4.20x10^6$	$7.12x10^5$	$3.84x10^6$	$3.31x10^6$	–	–	–	–
A	$9.35x10^4$	$3.34x10^3$	$5.65x10^4$	$4.25x10^4$	97.77	99.53	98.52	98.72
B	$8.90x10^5$	$8.54x10^4$	$7.76x10^5$	$9.22x10^5$	78.81	88.00	79.79	72.15
C	$3.04x10^5$	$4.21x10^4$	$4.46x10^5$	$4.32 x10^5$	92.76	84.08	88.38	96.94

Conditions: reaction volume: 100 mL; dosing of 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C): 5 ppm, respectively; reaction time: 4 h

Table 2
Microbiological analysis for yeast and mould in the process white water of PM 1-4 and the killing efficiency of different biocides

Particulars	Dilution no.	Cfu/plate				Killing efficiency, %			
		PM 1	PM 2	PM 3	PM 4	PM 1	PM 2	PM 3	PM 4
Blank	Undiluted	51	45	48	47	–	–	–	–
	Undiluted	47	39	40	42				
	Mean	49	42	44	46				
	10^{-1}	02	01	02	03	–	–	–	–
	10^{-1}	05	03	03	02				
	Mean	04	02	03	03				
A	10^{-0}	Nil	Nil	Nil	Nil	100	100	100	100
	10^{-0}	Nil	Nil	Nil	Nil				
B	10^{-0}	6	4	3	2	91.84	92.22	93.75	92.55
	10^{-0}	2	3	3	5				
C	10^{-0}	Nil	Nil	Nil	Nil	100	100	100	100
	10^{-0}	Nil	Nil	Nil	Nil				

Conditions: quality manufactured: writing and printing; GSM: 60 g/m^2; pH of back water: 6.5; paper production: 40 TPD; reaction volume: 100 mL; dosing of 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C): 5 ppm, respectively; medium used: Rose Bengal chlorophenicol; reaction time: 4 h

Table 3 shows the microbiological analysis for pseudomonas in the process white water of PM 1-4 and the killing efficiency of the above-mentioned biocides. Cetrimide agar medium was used for the culture of pseudomonas. 100 mg/L cycloheximide (catidion) were added to the media, to prevent the growth of fungi. The incubation temperature was of 30 °C and growth was monitored for 24 h. The maximum bacterial colony counts for pseudomonas were of 36 cfu/plate in PM 1. The killing efficiency of 2,2-dibromo-3-nitrilopropionamide (A) was the highest for the process water of PM 1-4, while a poor killing efficiency was observed for 2-(thiocyanomethylthio) benzothioazole (B).

Table 4 illustrates the microbiological analysis of iron bacteria in the process water of PM 1-4 and the killing efficiency of the above-mentioned biocides. The culture of iron bacteria is grown on a medium of iron bacteria. To prevent the growth of fungi, 100 mg/L cycloheximide (catidion) were added.

The incubation temperature was of 30 °C and growth was monitored for 24 h. The maximum iron bacteria colony counts were of 45 cfu/plate in PM 1, whereas the iron bacteria colony counts varied between 36-42 cfu/plate in PM 3-4. The killing efficiency of 2,2-dibromo-3-nitrilopropionamide (A) was the highest for PM 1, whereas that of 2-(thiocyanomethylthio) benzothioazole (B) was twice lower, and that of alkali dimethyl benzyl ammonium chloride (C) was of only 75%.

Table 5 reveals the microbiological analysis for sulphate reducing bacteria in the process water of PM 1-4 and the killing efficiency of the above-mentioned biocides. An SRB broth medium for sulphate reducing bacteria was used. 100 mg/L cycloheximide (catidion) was added to control the growth of filamentous fungi and mould. The incubation temperature was of 30 °C and growth was monitored for 24 h. The number of SRB bacteria per 100 was maximum, almost similar in the process white water of PM 1-2, and slightly lower in the process water of PM 3-4.

Table 3
Microbiological analysis of pseudomonas in the process white water of PM 1-4 and the killing efficiency of different biocides

Particulars	Dilution no.	Cfu/plate				Killing efficiency, %			
		PM 1	PM 2	PM 3	PM 4	PM 1	PM 2	PM 3	PM 4
Blank	10^{-1}	33	30	28	31				
	10^{-1}	39	34	30	29				
	Mean	36	32	29	30	—	—	—	—
	10^{-2}	4	4	2	2				
	10^{-2}	1	2	2	2				
	Mean	2.5	3.0	2	1.5	—	—	—	—
	cfu/mL	$3.6x10^2$	$3.2x10^2$	$2.9x10^2$	$3.0x10^2$	—	—	—	—
A	10^{-0}	2	2	Nil	1				
	10^{-0}	Nil	1	1	1				
	Mean	2	1.5	0.5	1	99.44	99.53	96.64	96.66
		0.2	0.15	0.05	0.1	—	—	—	—
B	10^{-0}	36	34	27	31				
	10^{-0}	29	33	29	33				
	Mean	32.5	33.5	28	32	90.97	89.53	90.34	89.33
	cfu/mL	3.25	3.35	2.8	3.2	—	—	—	—
C	10^{-0}	12	17	20	18				
	10^{-0}	16	15	23	21				
	Mean	14	16	21.5	19.5	96.11	95.00	92.59	93.5
	cfu/mL	1.4	1.6	2.15	1.95	—	—	—	—

Conditions: quality manufactured: writing and printing; GSM: 60 g/m^2; pH of back water: 6.5; paper production: 40 TPD; reaction volume: 100 mL; dosing of 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C): 5 ppm, respectively; medium used: Cetrimide agar; reaction time: 4 h

Table 4
Microbiological analysis of iron bacteria in the process white water of PM 1-4 and the killing efficiency of different biocides

Particulars	Mean cfu/plate				Killing efficiency, %			
	PM 1	PM 2	PM 3	PM 4	PM 1	PM 2	PM 3	PM 4
Blank	45	42	39	36	—	—	—	—
A	04	04	03	02	91.10	95.24	92.31	94.44
B	19	18	16	15	57.78	57.14	58.97	58.33
C	11	13	14	13	75.55	69.05	64.10	63.89

Conditions: quality manufactured: writing and printing; GSM: 60 g/m^2; pH of back water: 6.5; paper production: 40 TPD; reaction volume: 100 ml; dosing of 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C): 5 ppm, respectively; medium: isolation medium for iron bacteria; reaction time: 4 h

Table 5
Microbiological analysis for sulphate reducing bacteria in the process white water of PM 1-4 and the killing efficiency of different biocides

Particulars	No of positive SBR broth tubes												No of SRB bacteria/ 100 mL				Killing efficiency,%			
	PM 1			PM 2			PM 3			PM 4			PM 1	PM 2	PM 3	PM 4	PM 1	PM 2	PM 3	PM 4
Blank	5	1	2	4	2	1	3	5	2	4	2	1	65	62	58	55	—	—	—	—
A	0	0	0	0	0	0	0	0	0	0	0	0	Nil	Nil	Nil	Nil	100	100	100	100
B	0	0	1	0	2	0	1	0	2	1	0	1	2	1	3	2	96.9	98.4	94.8	96.4
C	0	0	0	0	0	0	0	0	0	0	0	0	Nil	Nil	Nil	Nil	100	100	100	100

Conditions: quality manufactured: writing and printing; GSM: 60 g/m^2; pH of back water: 6.5; paper production: 40 TPD; reaction volume: 100 mL; dosing of 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C): 5 ppm respectively; medium used: SRB broth; reaction time: 4 h

Table 6
Effect of treatment with 2,2-dibromo-3-nitrilopropionamide on paper machine efficiency and paper quality

Parameters	Control	Treatment with 2,2-dibromo-3-nitrilopropionamide (A)
Basis weight, g/m^2	70.1	70.2
Wax pick number	13A clear	15A clear
Scott bond, J/m^2	285	310
Breaking length, m	6550	6780
Tear index, mN.m^2/g	6.1	6.3
Double fold number	66	70
Number of breaks in 24 h	10	03

The SRB killing efficiencies of 2,2-dibromo-3-nitrilopropionamide (A) and alkali dimethyl benzyl ammonium chloride (C) were of 100%, while the killing efficiency of 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C) was slightly lower.

Table 6 reveals that wax pick improves from 13A to 15A, Scott bond – from 285 to 310 J/m^2, breaking length – from 6550 to 6780 m, and the double fold numbers – from 66 to 70. The control tests of paper were made at points where slime spots were present. Microbes weaken or destroy paper at several points, which causes reduction in mechanical strength properties and more paper breaks. A plant trial with 2,2-dibromo-3-nitrilopropionamide indicates that paper breaks are reduced from 10 to 3 within 24 h.

CONCLUSIONS

The total bacterial colony counts in the process white water of PM 1-4 gave the following results: 4.20×10^6, 7.12×10^5, 3.84×10^6 and 3.31×10^6 Cfu/mL, respectively. The yeast and mould in the process white water of PM 1-4 were found to be of 49, 42, 44 and 46 Cfu/mL respectively. The pseudomonas bacterial colony counts in the process white water of PM 1-4, with a dilution of 10^{-1}, were of 36, 32, 29 and 30 Cfu/mL, respectively. The iron bacterial colony counts in the process white water of PM 1-4 were of 45, 42, 39 and 36 Cfu/mL, respectively. The number of sulphate reducing bacteria in the process white water of PM 1-4 was of 65, 62, 58 and 55 Cfu/mL, respectively. These values indicate that such bacteria do not only fail to assure product and consumer safety, but may also cause production loss.

To eradicate these problems, the killing efficiencies of three different biocides, *i.e.* 2,2-dibromo-3-nitrilopropionamide (A), 2-(thiocyanomethylthio) benzothioazole (B) and alkali dimethyl benzyl ammonium chloride (C) were tested. Biocide 2,2-dibromo-3-nitrilopropionamide (A) has a wide spectrum effect on all types of bacteria and fungi, with a killing efficiency of about 100%, in most cases. The use of 2,2-dibromo-3-nitrilopropionamide does not only improve the quality of the paper, but also reduces machine downtime.

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