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SELECTIVITY OF COMMERCIAL BIOCIDES ON CONTROL OF MICROBIAL CONTAMINATION




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Abstract :

Microbial contamination is one of the most common problems faced by the pulp and paper mills. The reason being the proleferic growth of microorganism in the mill environment due to favourable conditions of pH, temperature and available nutrients .Microbial contamination is known to cause runnability and quality problems and also microbial induced corrosion in the long run .Consequently all Pulp and Paper mills have microbial control programs for which they largely depend upon the use of biocides.

Today several biocides having different chemical formulations are commercially available. However the effect of these biocides on the microbial population may differ significantly. In the present article, CPPRI evaluated the effectiveness of available biocides in terms of the reduction in microbial count and hence the kill rate. The effect of using chlorine -di- oxide as a biocide was also studied. The studies indicated highest kill rate for two biocides with composition of methyl substituted isothiazolinone and Di-guanidine and quaternized ammonium based biocides. However, to realize a high kill rate an optimum dosage of biocide was necessary.

INTRODUCTION :

Microbial contamination refers to the presence of micro-organisms like bacteria, fungi, yeast and algae in the mills process streams. Near neutral pH, moderate temperature and adequate availability of dissolved nutrients, all favour the growth of micro-organisms in the mills environment. Depending upon the mill conditions, the microbial population comes in the range of 10^4 to10^8 micro-organisms/ml. The presence of micro-organisms in the process stream may be relatively harmless until the time they start to colonize the equipment surfaces resulting in the formation of bio-films. These accumulations not only contaminate equipment surfaces but also interfere with paper making process causing web breaks and paper defects like spots and holes. As a consequence all pulp and paper mill resort to use of biocides to combat the problem of microbial contamination.

Biocides for control of microbial contamination

Biocides are products that control the growth of micro-organisms through various mechanisms. These include interfering with respiration or reproduction process, disrupting nutrient uptake or breaking the cell wall. While different formulations of biocides are available, they are broadly categorized as non-oxidizing and oxidizing. Oxidizing biocides are preferred for their fast killing while non- oxidizing biocides are effective in other aspects and convenience of use.

In a paper mill biocides are generally added to the white water loops where good dispersion is possible. Biocides may also be added to pulp stock where holes and spots appear in the finished products. Biocides may be added in batch or continuously depending upon the initial microbial load and level of control desired

The success of any biocide program adopted by a mill will largely depend on the efficacy of the biocide used. To ensure the adequacy of the mills biocide program, it is important to ascertain the suitability of the biocide for control of microbial population. As the effectively or response of different biocides to total bacterial count may vary greatly.

In view of this, CPPRI conducted a study to evaluate the efficiency of different biocides available commercially. Two biocides being used by paper mills were also evaluated. The efficacies of the biocides were evaluated by measuring the response of biocides to the microbial population in terms of kill rate %.

Materials & Methods

Biocide samples - For conducting the study, samples of biocides having different chemical formulation were procured from various suppliers. Table (1) gives details of these biocides. Three other biocides being used by two paper mills were also procured.

Collection of Mill samples – For the purpose of study, samples of slime , paper machine back water & effluent sample were collected from two nearby mills

Table 1: Biocides Used in the Study

Biocide used	Chemical Formulation
B1	Bromine based
B2	Blended isothiazolenes
B3	Blended carbamate
B4	Bromine based
B5	Chlorine dioxide based
B6	2,2, Dibromo-3- nitrilo- propionamide
B7	Methyl substituted isothiazolenones & bronopol
B8	Methyl substituted isothiazolenes
B9	Di – Guanidine and quartenised ammonium based
BM 1	isothiazolenes
BM2	Unknown
BM3	Unknown

Experimental

Sterilization techniques

All the material used in this study like Petriplates, spatulas, beakers, test tubes, pipettes, distilled water (used for media preparation) and media were sterilized in autoclave at 121ºC for 15 min

Method to determine Kill Rate

Each of the individual samples was serially diluted using standard APHA Method (9215A.4 and 9215A.5). Dilutions were prepared using saline and were subsequently selected for all the samples.

Media used for pour plating was Nutrient Agar Media. The samples were Pour Plated (APHA Pour Plate Method 9215 B) which acted as control (A). Another set (B) was run with same conditions consisting of biocide with desired concentration. The total microbial count was measured after 24 hours of incubation at 37ºC. The colonies were counted using quadrant method

Calculation

The CFU/ml (Colony Forming Unit/ ml) was calculated using the formula:

$$\frac{Cfu}{ml} = \frac{Number\ of\ Colonies * Dilution\ Factor}{Volume\ of\ sample}$$

The Kill rates of the biocide were calculated using the formula:

$$Kill, \% = \frac{(A - B)}{A}$$

Where:
A = count of control after 24-h incubation
B = count of test with biocide after 24-h incubation.

Results & Discussion

Evaluation of commercial biocides

Table 2a and 2 b gives the relative population density and microbial count and kill rates in control and samples after addition of commercial biocide. Microbial kill rates were determined for individual biocides after addition of 0.5 % concentration of biocide to the slime scratch sample.

For biocides samples B1 to B6 the relative population density decreased from $1.56x10^7$ to not less than$7.9x10^6$. Microbial counts also dropped from 156 to 79 only .Consequently Kill rates were low and did not exceed more than 50% showing that none of these biocides were capable of arresting the microbial growth at this concentration Low kill rates therefore indicate that higher concentrations of biocides will be required to achieve kill rate of over 90 %. It was also observed that efficiencies of B4 and B5 bromine and Chlorine dioxide based biocide respectively were especially low.

Table 2a : Biocidal Activity of Commercial Biocide

Sample : PMslime scratch   Biocide conc : 0.5%   Dilution plated : 10^4 Incubation time 24 hours

S.no.	Sample	Relative population Density (Cfu/ml)	Microbial count	Kill Rate %
1	Control	$1.56x10^7$	156	-
2	B1	$8.4x10^6$	84	46.75%
3	B2	$8.5x10^6$	85	45.15%
4	B3	$7.9x10^6$	79	49.3%
5	B4	$1.02x10^7$	102	34.61%
6	B5	$1.04x10^7$	104	33.33%
7	B6	$8.8x10^6$	88	43.58%

In case of Biocides B7, B8 AND B9, at similar biocide concentration, high kill rates were obtained. Biocide B8 was observed to be most efficient resulting in a kill rate of 100% with microbial count dropping from 135 to zero. The performance of B7 was also satisfactory with a kill rate of approximately 95 %.

The performance of these biocides were however greatly reduced at a lower concentration Table 2(c)

Table 2b : Biocidal Activity of Commercial Biocide

Sample- PM slime scratch   Biocide conc- 0.5%   Dilution plated: 10^4 Incubation time 24 hours

S.no.	Sample	Relative population Density (Cfu/ml)Cfu/ml	Microbial Count	Kill Rate %
1	Control	$1.35x10^7$	135	-
5	B7	$7x10^4$	7	94.81%
6	B8	-	-	100%
7	B9	$2x10^4$	2	89.5%

Table 2C : Biocidal Activity of Commercial Biocide

Sample- PM3 slime scratch   Slimicide conc- 0.05%   Dilution plated: 10^3 Incubation time 24 hours

S.no.	Sample	Relative population Density (Cfu/ml)Cfu/ml	Microbial count	Kill Rate
1	Control	$1.14x10^7$	114	-
2	B7	$3x10^5$	30	73.68%
3	B8	$2x10^5$	20	82.45%
4	B9	$2.4x10^5$	24	78.94%

These results indicated that amongst all biocides that were studied, Methyl substituted isothiazolenones & bronopol and Di – Guanidine and quartenised ammonium based biocides were most suitable biocides. But an optimum dosage is required to achieve the desired kill rate of 100 %.In view of their good performance, biocide B7, B8 and B9 were selected for further study.

Evaluation of Mill Biocides

Biocides from two pulp and paper mills were evaluated for their effectivity towards microbial control. The kill rates were then determined at different concentrations and compared with those identified earlier in the study

Mill-I

The effectively of the biocide BM1 used by mill 1 was tested on the mills paper machine backwater at different biocide concentrations. The results in

S.No.	Sample	Microbial Count			Relative population Density (Cfu/ml)Cfu/ml			Kill Rate		
Concentration		0.25 %	0.5 %	1 %	0.25%	0.5%	1%	0.25%	0.5%	1%
1	Control	296				$2.96x10^6$			-	
2	BM1									
3	B9	1	0	0		-	-	99.6%	100%	100%
4	B8	9	1	0		$1x10^4$	-	96.69%	99.6%	100%

Table 3 indicated that where B7 , B8 and B9 consistently resulted in kill rates close to 100% , the biocide BM1 was less effective. In case of BM1, kill rates however increased with increasing biocide concentration. Also , oxidizing biocides B4 and B5 showed less than satisfactory performance.

Table 3: Comparison of Biocidal activity of Commercial and Mill- 1 Biocides at various biocide concentrations

Sample – Paper machine Back Water Biocide concentration - 0.25%, 0.5%, 1%. Dilution plated: 10-2

S.No.	Sample	Microbial Count			Relative population Density (Cfu/ml)Cfu/ml			Kill Rate		
1	Control	296			$2.96x10^6$			-		
Concentration		0.25 %	0.5 %	1 %	0.25%	0.5%	1%	0.25%	0.5%	1%
2	BM1	147	71	31	$1.47x10^6$	$7.1x10^5$	$3.1x10^5$	50.6%	76.01%	89.5%
3	B9	1	0	0	$1x10^4$	-	-	99.6%	100%	100%
4	B8	9	1	0	$9x10^4$	$1x10^4$	-	96.69%	99.6%	100%
5	B7	20	7	0	$2.0x10^5$	$7x10^4$	-	93.24%	97.63%	100%
6	B4	159	89	51	$1.59x10^6$	$8.9x10^5$	$5.1x10^5$	46.28%	69.9%	82.7%
7	B5	186	101	73	$1.86x10^6$	$1.01x10^6$	$7.3x10^5$	37.16%	65.87%	75.3%

Mill-II

Table (4) gives the comparison of 2 biocides BM2 And BM3being used by mill 2 with B9. The performance of BM2 was visibly lower compared to BM3 . However both the biocides showed rising trend in kill rates with increasing biocide concentration. The major disadvantage of use of higher biocide concentration is that they may interfere with the papermaking process and also add to the cost of mills microbial control program .In contrast B9 resulted in kill rate of 99.5 % even at low biocide concentration.

Table 4 : Comparison of Biocidal activity of Commercial and Mill- 2 Biocides at various biocide concentrations

Sample –Effluent Biocide concentration- 0.25%, 0.5%, 1%. Incubation time 24 hours

S.No.	Sample	Microbial Count			Relative population Density (Cfu/ml)CFU/ml			Kill Rate %		
1	Control	200			$2x10^5$			-		
Concentration		0.25 %	0.5 %	1 %	0.25%	0.5%	1%	0.25%	0.5%	1%
2	BM2	170	59	30	$1.7x10^5$	$5.9x10^4$	$3x10^4$	15%	29.5%	85%
3	BM3	57	30	21	$5.7x10^4$	$3x10^4$	$2.1x10^4$	71.5%	85%	89.5%
4	B9	1	0	0	$1x10^3$	-	-	99.5%	100%	100%

Conclusion

The study gives a general overview of the fact that different biocides can exhibit different biocidal activities. In the study, compared to oxidizing biocides, non oxidizing biocides resulted in higher efficiencies in terms of kill rate. Both Di-guanidine and quarternised ammonium based biocide and those having methyl substituted isothiazoles and bronopol were found to be most effective towards control of microbial activity giving kill rates of 95 and 100% at biocide concentration of 0.5 %. Other non oxidizing biocides having carbamate and propionamide formulations showed moderate biocidal activity with kill rates around 45 to 50 %. For oxidizing bromine and chlorine di- oxide based biocides, kill rates did not exceed 35 %

Further, the performance of the biocides used by the mills was relatively poor compared to the biocide used in the study indicating a need to find a suitable biocide based on response to reduction of microbial population.

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