

**DETERMINATION OF MICROORGANISMS IN PAPER
MACHINE WHITE WATER SYSTEM AND KILLING
EFFECT OF**

**“2, 2 – DIBROMO – 3 NITRILOPROPIONAMIDE, ALKALI DIMETHYL
BENZYL AMMONIUM CHLORIDE AND ALKALI DIMETHYL
AMMONIUM CHLORIDE”**

A DISSERTATION

Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF TECHNOLOGY

IN

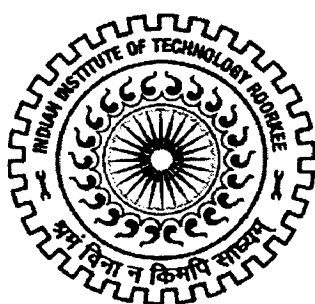
PULP AND PAPER TECHNOLOGY

SUMBITTED BY

NIRDESH KUMAR TYAGI

UNDER THE GUIDANCE OF

**Dr. C.H. Tyagi
Dr. Dharm Dutt**



**DEPARTMENT OF PAPER TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY, ROORKEE
SAHARANPUR CAMPUS, SAHARANPUR**

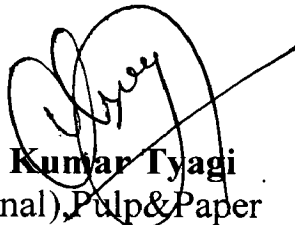
2006 – 07

RP

CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in this dissertation report entitled **“DETERMINATION OF MICROORGANISMS IN PAPER MACHINE WHITE WATER SYSTEM AND KILLING EFFECT OF 2, 2 – dibromo – 3 nitrilopropionamide, alkali dimethyl benzyl ammonium chloride AND alkali dimethyl ammonium chloride”** in partial fulfillment of the requirement for the award of the degree of Master of Technology in Pulp and Paper, I I T Roorkee, is an authentic work of my own efforts carried out, under the supervision of **Dr.Dharmdutt**, Associate Professor and **Dr. C. H. Tyagi**, Associate Professor, Department of Paper Technology, Indian Institute of Technology, Roorkee (Saharanpur Campus).


The work embodied in the dissertation report has not been submitted by me for the award of any other degree.


Nirdesh Kumar Tyagi
M.Tech.(Final), Pulp & Paper

DATE: 28.6.2007

PLACE: SAHARANPUR

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

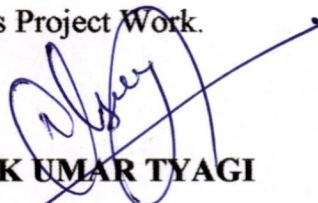

C. H. Tyagi
(Associate Professor)
DPT, Saharanpur
IIT Roorkee

Dharm Dutt
(Associate Professor)
DPT, Saharanpur
IIT Roorkee

ACKNOWLEDGEMENT

I would like to take this as an opportunity to express my profound sense of gratitude to my guide Dr. Dharm Dutt, Associate Professor and Dr. C. H. Tyagi Associate Professor Department of Paper Technology, IIT, Roorkee, Saharanpur campus, Saharanpur for his valuable inspiration and guidance throughout the Dissertation work.

I would like to thank my Prof. Dr. J.S.Upadhayay, Dr.M.C.Bansal, Dr.V.P.Singh, Dr S.P.Singh and all my friends who cooperated all the time during this Project Work.


NIRDESH K UMAR TYAGI

M. Tech. 2nd year

Date: 28.06.2007

TABLES OF CONTENTS

Declaration:	(i)
Acknowledgement:	(ii)
List of Tables:	(v)
List of Figures:	(vii)
Abstract:	(viii)
Chapter 1: Introduction:	
1.1 Precautions to be taken while carrying out (microbiological tests)	(9)
Chapter 2: Materials and Methods:	
2.1 Machine conditions and wet end additives	(13)
2.2 Samples and Sampling	(13)
2.3 Enumeration of microbiological growth	(13)
2.4 Plant trials	(15)
2.4.1 Machine Pre-cleaning / Boil-out using 2, 2-dibromo-3-nitrilopropionamide (A):	(15)
2.4.2 Shock Dose of 2,2-dibromo-3-nitrilopropionamide (A)	(15)
2.4.3 Regular Dose of 2,2-dibromo-3-nitrilopropionamide (A)	(16)
Chapter 3: Observations:	(17)
Chapter 4: Results and Discussions:	(37)
Chapter 5: Conclusions:	(43)
References:	(45)

Appendix:

1:	Total Bacterial Count	(49)
	Requirements	(49)
	Procedure	(49)
2:	Efficacy Test Procedure	(51)
	Using Bactaslides	(51)
	For Control	(51)
	For Biocide at 5ppm Conc.	(51)
3:	Determination of Total Bacterial Count	(52)
	Apparatus	(52)
	Reagents	(52)
	Sterilization	(52)
	Procedure	(52)
4: (1)	Test for Bacterial Counts:	(54)
	(2) Active Matter Testing	(54)

LIST OF TABLES

Table 1-	Quality wise operating parameters for PM ₁ .	17
Table 2-	Quality wise wet end additives for PM ₁ .	18
Table 3-	Quality wise operating parameters for PM ₂ .	19
Table 4-	Quality wise wet end additives for PM ₂ .	20
Table 5-	Quality wise operating parameters for PM ₃ .	21
Table 6-	Quality wise operating parameters for PM ₄ .	22
Table 7-	Quality wise wet end additives for PM ₃ and PM ₄ .	23
Table 8-	Total bacterial colony count of process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides.	24
Table 9-	Microbiological analysis for yeast and mould in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides.	25
Table 10-	Microbiological analysis for pseudomonas in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides.	26
Table 11-	Microbiological analysis of iron bacteria in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides.	27
Table 12-	Microbiological analysis for sulphate reducing bacteria in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides.	28

Table 13-	Plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-1 of Star Paper Mills Ltd., Saharanpur.	29
Table 14-	Cost calculation during plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-1 of Star Paper Mills Ltd., Saharanpur (U.P.).	30
Table 15-	Plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-2 of Star Paper Mills Ltd., Saharanpur.	31
Table 16-	Cost calculation during plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-2 of Star Paper Mills Ltd., Saharanpur (U.P.).	32

LIST OF FIGURES

Figure 1-	Dosage, cost and total bacterial colony count at PM-1 during plant trial.	33
Figure 2-	Dosage, finished production and treatment cost at PM-1 during plant trial.	34
Figure 3-	Dosage, cost and total bacterial colony count at PM-2 during plant trial.	35
Figure 4-	Dosage, finished production and treatment cost at PM-2 during plant trial.	36

ABSTRACT

Microbial problems in paper mills have significantly increased after closing up white water system to reduce fresh water consumption. Environmental constraints also have contributed to the need of reducing the amount of effluents. The recirculation of white water has created serious slime problems because of the growths of microbes in the system. Increased recycling increases the amount of nutrient present in solids or dissolved form. It is quite obvious that due to recycling the concentration of nutrients considerably increases. Besides the microbial activity itself produces organic acids, vitamins, enzymes etc. to the systems. It is note worthy that also chemicals (viz. additives utilized in the production process) in themselves represents an ideal nutrient source (starch) or contain as impurities quite a lot of nutrients for microbes (Kaolin). Filler suspensions and additives used can contain considerably amount of phosphorus and Nitrogen, which along with the carbon source, is the principle nutrients for most microbes.

The aim of this study was to access the microorganism in the white water systems of Star Paper Mill using cellulosic raw materials Viz., Popular deltoids, Eucalyptus Tereticornis and Bamboos aurandacea and manufacturing writing and printing and wrapping and packaging papers. In order to improve product quality and to explore the possibilities of reuse of recycled water different biocides were tested to examine their killing effect on different microorganisms.

The stock preparation sections of paper machines 1-4 contain different composition of various nonfibrous additives. Therefore, the population of different types of bacteria may

vary due to variation in composition of substrate types of bacterial like sulphate reducing bacteria, Iron bacterial, Pseudomonas, yeast and mould in each stock preparation sections. Keeping in view problems like production loss, inferior quality due to slime and minimum profit level various biocides were analyzed in laboratory to study the killing efficiency of different types of bacterial finally, a plant trial was conducted with high spectrum biocides.

The process water of paper machine 1,2,3, & 4 contains total bacterial colony count 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 water of paper machine 1,2,3, & 4 are 49, 42, 44 and 46 cfu/ml respectively. The bacterial colony count of pseudomonas in the process water of paper machines 1,2,3, & 4 with a dilution of 10^{-1} are 36, 32, 29 and 30 cfu/ml respectively. The bacterial colony count of iron bacteria in the process water of paper machines 1,2,3, & 4 are 45, 42, 39 and 36 cfu/ml respectively. The no. of sulphate reducing bacteria in the process water of paper machines 1,2,3, & 4 are 65, 62, 58 and 55 cfu/ml respectively. These values indicate that these bacteria not only fail to assure product and consumers safety but cause production loss also.

In order to eradicate these problems the killing efficiencies of three different biocides i.e. 2,2 dibromo-3 nitrilopropionamide (A), alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C) were tested. The biocide 2,2 – dibromo-3- nitrilopropionamide (A) is wide spectrum for all types of bacteria and fungi and killing efficiency is about 100% in most of the cases.

CHAPTER 1

1.0 INTRODUCTION

Pulp and paper industries are looking at a high degree of closure of the process water or the use of biological waste water treatment plants due to stringent environmental regulations and consumer attitude (1). This has been achieved by increased recycling of process water. In the 1970s, approximately 100 m³ of water was used per metric tone of paper manufactured, but less than 10 m³ of water was 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 problem. (2). Formation of slime deposits is a major problem facing paper industries. Slime problems in the pulp and paper industry were studied in the 1950s and 1960s (3). Microbiological growth in a paper mill may cause loss of production due to breaks, reduction in quality due to appearance of slime spots, odors in product, corrosion, production of malodorous gases, and off-spec and rejected products (4).

The pulp stream, in the course of its progress, receives large inoculations of organisms from growth accumulations within the mill system and, supplying as it does a water solution and suspension of inorganic and organic nutrients (5), rapid development of microorganisms results. Since the ground wood contains almost all the constituents of the wood itself (6), an excellent carbohydrate-rich medium is provided. The rapid growth of organisms in the sulphite system indicates that the sulphite pulp stream furnishes an adequate food supply. Distribution of microorganisms takes place through air dissemination of spores and the continuous circulation of white water and stock. In the case of the ground wood mill, counts ranging from two to three million bacteria per cubic

centimeter of white water and stock have been obtained during slime trouble. Inasmuch as the fresh water supply was adequately chlorinated, the inoculations came directly from bacterial and fungus development within the system.

The masses of growth built up on surfaces over which pulp suspensions flow, are spoken of collectively as slimes. Pulp and paper mill slimes are, therefore, accumulated microbiological growths, composed heterogeneously of microorganisms, products of growth, fiber, and inorganic debris. The heterogeneous nature of slime growths is emphasized by Fritz (5) who states that no fungus, or limited association of fungi, can be held universally responsible for the trouble. This statement is abundantly confirmed in the present investigation.

The ability to form viscous growths or close masses of mycelium on appropriate surfaces is characteristic of the microorganisms developing in pulp and paper mill systems. The types and degrees of viscosity which they reveal on carbohydrate media vary with the different species, from the gelatinous or viscid bacterial growths, similar to the cellulan slimes described by Beijerinck (7), to the doughy and rubbery formations of *Oidium* and certain yeast-like fungi. The compact, matted masses of mycelium formed by *Actinomyces* and other filamentous organisms, introduces another slime factor of far-reaching importance in paper manufacture.

The bacterial slime-formers isolated thus far, may be grouped under the following genera:

- **Achromobacter:** A number of species were studied which, in all probability, should be placed in this genus. The isolations resemble, more or less closely, several of the species described by Bergey (8). Among them are *A. geminum* (*B. Geminus*-Major

Ravenel); *A. Pinnatum* (Ravenel) Bergey; *A. Ambiguum* (Wright) Bergey; *A. Reticularum* (Jordan) Bergey; *A. viscosus* (Adametz) Bergey.

- **Escherichia:** One species was encountered corresponding to the description of *E. Gastrica* (Ford) Bergey. This organism produced a thick, viscous, yellowish growth on potato-glucose agar, while on beef-peptone agar the development was abundant, spreading, and somewhat rogues.
- **Aerobacter :**Organisms typical of *A. Aerogenes* (Kruse) Beijerinck and *A. Cloacae* (Jordan) Bergey occurred prominently.
- **Pseudomonas:** *P. Viscosa* (Frankland) Migula was isolated once.
- **Bacillus:** *B. Vulgatus* Trevisan, isolated from certain tenacious deposits, produced very viscid growths on enrichment media. *B. Vulgatus* was found also in hot ground wood taken directly from the grinder pit. In its descriptive features, this organism is entirely typical. *B. Vulgatus* forms an abundance of levan in the sucrose medium used by Hibbert (9) and it is mentioned by Beijerinck (7) as one of the levulan-forming bacilli. *B. Subtilis* (Ehrenberg) Cohn also appeared among the slime-formers.

In addition to the bacterial slimes there are those formed essentially by *Odium*, *Monilia*, and related yeast-like fungi of doubtful identity. The growths produced by these forms are usually doughy or rubbery, effectually resisting control measures. They develop rapidly on carbohydrate media and possess the ability to build up tenacious growths within the mill system. The cultural features of these organisms have been studied in the laboratory using glycerol and potato decoction media. The growth masses consist of a form of gelatinized cellulose. The importance of filamentous fungi of the mold type is stressed by Fritz (5), who states that the bulk of the slime in her

investigations was composed of these forms. *Aspergillus fumigatus* var. is representative of a considerable number of isolations made at this laboratory whose activities extend to active pulp deterioration, including the production of sliminess, discoloration, and cellulose decomposition. This group includes members of the genera *Aspergillus*, *Acrostalagmus*, *Alternaria*, *Cladosporium*, *Chaetomium*, *Trichoderma*, and *Penicillium*. In a few cases cultures of filamentous fungi were obtained which could not be identified, due to the failure to produce fruit bodies under the cultural conditions provided. Schmid (10) refers to *Trichoderma* and *Cladosporium* as important slime-forming organisms. Reference is made by Pattillo (11) to *Paxillus panuoides* Fries as one of the producers of sliminess and pulp deterioration.

The filamentous bacteria have received considerable attention in this connection. An *Actinomyces* proved to be the predominating form in slime from a section of one mill. The alga-like bacteria have been identified with various similar conditions by Schmid (10), Pattillo (11), Boruff and Stoll (12), and Gesell (13). Members of the *Chlamydoacteriaceae* are conspicuous among the causal species.

If slime is caused by a bacterial organism, then one may speculate for a moment as to its structure and as to certain metabolic requirements of the form necessary in all probability to produce it. The consistency of this material reminds one of a bacterial zoogloea. Thus a capsule producing organism probably must be sought. For maximum building of capsule structure by bacteria, a supply of carbohydrate is essential. Zettnow (14) has indicated stimulation to increased capsule formation by carbohydrate within the medium and Heidelberger and Avery (15) have shown that the addition of glucose to a

culture of pneumococcus increases capsule production many fold. Carbohydrate in such a form as to be usable for bacterial metabolism must therefore be available.

We are thus brought to the point of determining whether carbohydrate in form available for growth of zoogloea producing bacteria is present in white water. If present, it may be in solution within the water itself or it may be in dose contact with the suspended cellulose or other colloidal material. Inasmuch as the various modes of treatment applied to cellulose are attended by a certain degree of hydrolysis, inversion of at least a small portion of the cellulose is to be expected. Indeed Lottermoser and Mathieson (16) have shown a content of 1.4 per cent sugar in certain sulphite liquors and the largest fraction of this carbohydrate content exists as glucose. Moreover, we have shown that if one suspend bleached pulp in distilled water and then add either Fehling or Benedict solution, a definitely positive reaction for the presence of carbohydrate is obtained. Hexoses, therefore, are present here although they may be absent in unbleached pulp as proven by a similar test. Like tests made with white water at Berkeley have given negative results but the samples were at least five days old when received and thus carbohydrates had already doubtless been destroyed by micro-organic fermentation. The above work, however, has proven that carbohydrate in relatively simple form is present within or in contact; with the cellulose fiber and that it results in part at least from hydrolytic activity of certain of the reagents used in processes of manufacture.

The cultural reactions of the organisms constituting the heavy mucilaginous colonies may be determined following purification by plating or streaking upon Sabouraud's agar. They are as follows. Gram-negative, non-sporulating rod, 3 to 4 microns long by 1 micron thick, slightly motile, capsule positive by Gins' method.

Gelatin is not liquefied. Indol production is negative. It grows luxuriantly upon Sabouraud's agar and also to less degree upon all the usual agar formulae utilized in routine laboratory practice. Growth upon media poor in carbohydrate however is not attended by formation of heavy capsular structure. Small amounts of H₂S are produced upon lead acetate medium. Nitrates are reduced to nitrites. The methylred reaction is negative while the Voges-Proskauer test is usually positive. Growth in plain beef broth is attended after some days by formation of a definite and heavy pellicle. Milk becomes acid in five days and some strains cause coagulation. Sugar reactions are the following: "Acid with gas from glucose, sucrose, salicin, maltose, raffinose, mannitol, laevulose, galactose, and dextrin. This last is slow to react". Lactose is negative at first with some strains but becomes positive with attendant gas production after two or three transfers. With other strains, both gas and acid are produced with freshly isolated organisms. Incubation temperatures of 30°C or 37°C are seemingly equally satisfactory. The organism grows best as an aerobe although it proliferates in deep beef agar without added sugar and under a Vaseline seal. Under these conditions, gas is produced with disruption of the agar and it is likely that the carbohydrate content of the medium naturally present is now utilized.

This organism closely resembles *B. aerogenes* but it differs from it in certain details. Many freshly isolated cultures are inactive with lactose although they acquire ability to break down this carbohydrate easily following a few transfers. Slight yellowish pigmentation may appear with recently isolated strains. Capsule production upon carbohydrate-containing medium is very heavy. Nevertheless this form probably may be classified as *B. aerogenes*.

Inasmuch as it has become advisable to attempt to determine the presence of this organism in water which is to be used by pulp and paper mills, a method for enrichment for culture purposes has been elaborated. In its background, it is based upon the technique used for the sanitary examination of water. The procedure follows fifty cubic centimeter amounts of the water to be tested are first centrifugalized at high speed for one-half hour. The resulting sediment is then streaked out upon Sabouraud's medium, since in certain instances this process of concentration is sufficient to reveal the form, if present. It is more important however to place the sediment in glucose broth fermentation tubes with Andrade indicator. These preparations are made in duplicate sets. Of these sets, one is incubated at 37°C while the other is placed in a chamber at 30°C. When acid and gas have been produced, the tube showing this reaction is now streaked out upon a poured plate of Sabouraud's agar which is then incubated. When, and if, the characteristic large thinly mucilaginous colonies develop, they may be picked and cultural reactions determined as outlined above. By this method the slime producing organism has been isolated from raw water taken from the vicinity of pulp mills upon a number of occasions.

The proof that this organism is capable of causing slime in contact with cellulose is shown by the following test. Crude wood pulp is placed in flasks and to this is added Sabouraud's fluid which has the same formula as that used for the semi-solid medium except that the agar is omitted. Sufficient of the fluid is added to moisten the pulp thoroughly together with a little excess. It is of advantage to add a slight amount of powdered calcium carbonate in order to neutralize resulting acidity in part. The material is now sterilized by the intermittent method. The flask thus prepared is inoculated and

then incubated at either 37°C or 30°C. In four or five days the surface of the pulp shows increased reflection of light due to the accumulation of the capsular material and after two or three weeks, the whole mass has become sufficiently gummy so that the smaller particles stick together and pulp dimer is thus produced artificially. For the final step of proof in a manner simulating the requirements of Koch's postulates, the original organism may be isolated from this preparation provided such isolation is made reasonably soon. This bacillus is killed after some days by accumulation of its own acid by-products and thus such preparations become sterile after a period of three, or four weeks, particularly if buffer be absent. For this, experiment, it is advisable to utilize freshly isolated cultures inasmuch as continued culture upon artificial media reduces somewhat ability to form zoogloea.

1.1 PRECAUTIONS TO BE TAKEN WHILE CARRYING OUT MICROBIOLOGICAL TESTS

1. Carry out microbiological testing in a separate lab. If separate lab is not available, then testing should be done in an isolated corner of the main lab.
2. Entry into the micro-lab should be restricted.
3. The lab should be dust free.
4. The working tables and laminar flow table should be cleaned thoroughly with dettol.
5. Nobody should be allowed into the lab while work is going on.
6. Avoid talking, coughing or sneezing while plating.
7. All glassware should be sterilized before use.
8. The glassware should not be very hot while performing the experiment.
9. The tip of the pipettes should not be touched while performing the experiment.
10. The Nutrient medium should not be very hot otherwise the bacteria will die. On the other hand if the medium is cooled below 45°C it will solidify in the flask itself.
11. If the Nutrient medium is to be cooled it should be held under running tap water and the flask should be continuously shaken to avoid Jump formation.
12. After the experiment is over, the tables should again be cleaned with dettol.
13. The lab should be sterilized once in 15-20 days. For this take dilute formaldehyde in a Petri dish and keep it in the micro-lab for 24 hr. Next day, open all windows till the fumes subside, since the fumes will cause irritation to the eyes.
14. Do not open the lid of the Petri dish if not required.
15. Use separate pipette for each dilution. This will increase the accuracy.

16. Do not blow the pipette.

17. Do not keep the UV light on, while working.

18. Keep the Petri dishes in the incubator in an inverted position.

The biofilms seed microbes into the process water, deteriorating the hygienic quality of the product. Biofilms also clog wires and cause discoloration of the paper. Paper machine systems usually support significant growth of micro-organisms due to congenial and favorable conditions persisting during the manufacture of papers. In paper mill plants, unique environmental conditions viz., available carbohydrates, high moisture levels, moderate temperatures, the recycling of the process water and pH make a paper mill system a perfect breeding ground for microorganisms (17). The slime may be biological or non-biological. Biological deposits that are composed of varied microflora along with fibers, fillers and dirt are the most troublesome. Biofilm formation contributes a major role in the development of paper machine deposits. *Meiothermus silvanus* and *M. ruber* were found in paper and board products with colored defects and connection between deposit-forming microbes and end-product spots was shown (18). *Pseudomonas*, *Bacillus*, and *Pseudoxanthomonas* isolates were represented at a relatively high proportion in both pulp and slime samples. This is the first time that *Pseudoxanthomonas* strains have been isolated from pulp and slime samples on a paper machine (4). Although biofilm formation has been studied intensively in natural aquatic system, waste water treatment systems and medical appliances (19-25), only a few studies have made on biofilms in the paper machine environment (26-28) in spite of the fact that paper and paperboard production represents 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 (29). Biofilm formation is a dynamic process that begins with bacterial cells becoming associated with a submerged surface and continues through a series of events that can be divided into five distinct stages (30). Slime producing microbes secrete extra cellular polysaccharides that gum up the process machinery(31-32). Slime contains several components of bacterial polysaccharides in addition to glucose. The uronic acid-containing polysaccharides are responsible for the accumulation of heavy metals in the slime (33). Micro biocides are used as biofilm- and deposit-control agents in the paper industry but can be hazardous to machine operators and/or non-target organisms. The use of biocides is subject to restriction when food quality paper products are made (34-35). *Sphaerotilus* enters the system primarily via fresh water containing low levels of chlorine and is implicated in the appearance of biological slimes (36-37). Biofilm development induces technical problems in the paper machines (37) and reduces the hygienic and technical quality of the paper that is produced. An alternative technology, known as surface-active biofilm matrix blocker (SAM), was developed to control biofilms/deposits (38). Differential turbidity measurement (DTM) and automatic pressure drop (PD) measurement proved to be useful for the measurement of deposit formation on line in the side stream of a paper machine whitewater circuit (39). A carbamate-based biocide was used to modify the surface properties of the microbial cells in order to promote their attachment to the cellulose fibres and to preventing the bacteria from developing biofilms on the equipment. About 75% of the cells were retained by the fibres. The effect of glutaraldehyde was also tested and it was concluded that this biocide did not modify the surface charge of the bacteria

(40). Microbiological activity, which causes lost production, off-spec quality and increased costs, is controlled by feeding the most effective biocidal agent - found through laboratory screening - into the paper system (41). Chlorine dioxide is commonly used for disinfecting potable water and has also been found to be very effective in controlling and removing biofilm (42). The biological removal of sulfate from water systems was shown to be an effective method for controlling the growth and activity of sulfate-reducing bacteria (43). In the presence of 5 ppm of methylene bithiocyanate or 2, 2-dibromo-3-nitrilopropionamide in paper-machine water, 55 strains formed biofilms. Moreover, 39 strains increased biofilm production by 5–753% in the presence of biocide, suggesting that biocide concentrations inhibitory to planktonic but not to surface-attached cells may actually promote biofouling (44). Phenyl mercuric compounds are the most effective biocides in the pulp and paper industry but cause serious pollution of lake sediments (45).

The aim of this study was to assess the microorganisms in the white water systems of an integrated paper mill using cellulosic raw materials viz., *Populus deltoides*, *Eucalyptus tereticornis* and *Bambusa aurandacea* and manufacturing writing and printing and wrapping and packaging papers. In order to improve product quality and to explore the possibilities of reuse of recycled water different biocides were tested to examine their killing effect on different microorganisms.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Machine conditions and wet end additives: The quality wise operating conditions and wet end additives according to paper grades are to be manufactured of paper machines 1-4 are reported in Tables 1-7.

2.2 Samples and Sampling

The coding of samples from four different paper machines of Star Paper Mills Ltd., Saharanpur (A-C) is set out. A total of circulating process water from white water pit under Fourdrinier section of paper machine was collected. All the glassware were first washed with soap followed by thorough rinsing with tap water, then autoclaved at 15 Pa for 15 minutes and were dried in oven at 60 °C for 5-6 h. After sterilization 9 ml each of sterile saline is added to a set of 4 dilution tubes. Add one ml of white water to the first bottle (10^{-1} dilution). Shake the bottle well and transfer one ml from the first bottle to the second bottle containing saline solution. This gives a dilution of 10^{-2} . Transfer one ml each from the dilution bottle numbered 10^0 in to two sterile Petri dishes. Similarly, repeat this for plate 10^{-1} and 10^{-2} dilutions respectively also. Cool the medium to approximately 45 °C and add it to the Petri dishes, swirling the plates while addition so as to mix the medium and process water sample.

2.3 Enumeration of microbiological growth

Total bacterial colony count in process water of paper machines (1-4) were determined by using yeast malt extract peptone dextrose agar (Difco 0712). In order to isolate species present in the samples only low numbers, 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media to prevent the growth of fungi. The

incubation temperature was 30 °C and the growth was monitored from 3 to 14 d. The results are reported in Table 8.

For the detection of yeasts and filamentous fungi rose Bengal chloramphenicol agar medium was used. To prevent the growth of bacteria, 100 mg/l chloramphenicol (Sigma C-0378) and 100 100 mg/l chlorotetracycline (Sigma C-4881) were added by plating, except to chloramphenicol agar which already contained the selective agent. The incubation temperature was 25 °C and the growth was monitored from 3 to 14d. The results are reported in Table 9.

Pseudomonas bacteria in the process water of paper machines (1-4) were determined by using cetrimide agar (Himedia M 024). In order to prevent the growth of fungi 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation temperature was 30 °C and the growth was monitored from 3 to 14 d. The results are reported in Table 10.

The iron bacteria in process of paper machines (1-4) were determined by using medium iron bacteria (Himedia M 622). To vanish the growth of fungi 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation temperature was 30 °C and the growth was monitored from 3 to 14 d. The results are reported in Table 11.

The sulphate reducing bacteria in the process water of paper machines (1-4) SRB broth medium was used. To control the growth of filamentous fungi and mould 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation temperature was 30 °C and the growth was monitored from 3 to 14 d. The results are reported in Table 12.

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

2.4 Plant trials

In order to validate laboratory results, a plant trial was conducted on paper machines 1 and 2. The following steps are involved in order to conduct plant trial:

2.4.1 Machine Pre-cleaning / Boil-out using 2, 2-dibromo-3-nitrilopropionamide (A)

Prior to commencement of plant trial at PM 1 and 2 with 2,2-dibromo-3-nitrilopropionamide (A), the machine system (starting from mixing chest) should be pre-cleaned / taken for boil-out, incorporating bio-dispersant; into the water. The typical dosage of 2,2-dibromo-3-nitrilopropionamide (A) is 1 liter product, per m³ of water taken for pre-cleaning. 2,2-dibromo-3-nitrilopropionamide (A) is to be added into the water in the mixing chest. The capacity of mixing chest is 40 m³ of PM1 and 80 m³ of PM2 therefore, 40-80 liters of 2,2-dibromo-3-nitrilopropionamide (A) will be required for the boil-out. A boil-out is recommended once in 30-40 days in order to keep the system clean.

2.4.2 Shock Dose of 2,2-dibromo-3-nitrilopropionamide (A)

2,2-dibromo-3-nitrilopropionamide (A) is a blend of unique stabilized bromine compounds and powerful dispersant for the control of microbiological fouling due to various bacteria in paper mill systems. 2,2-dibromo-3-nitrilopropionamide (A) is to be added as a shock dose (double the regular dose) for the first 24 hours of production. The

treatment starts after about 2 hours of commencement of paper production. The shock dose would be approximately 350 ml per MT of paper produced. The necessary quantity is added in 4 equal lots maintaining a time gap of 6 hours. The entire quantity for each addition must be added at once (slug dose and not drip dose), without dilution.

2.4.3 Regular Dose of 2,2-dibromo-3-nitrilopropionamide (A)

The regular dose is half the shock dose, i.e. approximately 175ml per MT of paper. Just like the shock dose, the product is to be added as it is without dilution after every 6 hours. The results of plant trials were reported in Tables 13-16 and Figures 1-4.

Table 1— Quality wise operating parameters for PM.

S.No	Quality	Substance GSM	Speed RPM	Head mm	Slice mm	Couch Vacuum mm Hg	Section Vacuum mm Hg	loading (+ 20%)			Calendar 1 st Kg/cm ²	2 nd Kg /cm ²	Steam (± 20%)		Riser Loading (± 20%)	
								Size Press, kP	Fabric Press kP	Size Press, kP			Pre-dryer Kg/cm ²	Post-dryer Kg/cm ²	SW/DDR	0 SD/M
1.	MARLITHO	70	189	606	10	380-430	250-350	13-18	35-40	3-5	40-60	40-60	1.3-1.7	0.5-1.0	220-260	400-500
2.	SS MAP / SUPERB NS	80-120	182-123	560-254	10	390-450	DO	13-18	35-40	3-5	40-60	40-60	1.2-2.0	0.8-1.5	260-300	400-500
3.	STARMAP NS HI DLX	65	182	560	10	360-390	220-280	13-18	35-40	3-5	40-60	40-60	1.0-1.5	0.5-1.0	220-260	400-500
4.	SS MAPLITHO	58-64	181-186	550-580	10	360-390	220-280	13-18	35-40	3-5	50-70	50-70	1.0-1.5	0.5-1.0	240-280	400-500
5.	DO	68-80	189-182	606-560	10	390-450	250-350	13-18	35-40	3-5	50-70	50-70	1.2-2.0	0.5-1.0	240-300	400-500
6.	DO	81-140	182-103	560-180	10	390-450	250-350	13-18	35-40	3-5	50-70	50-70	1.2-2.0	0.8-1.5	260-300	400-500
7.	MAP SUPER DLX	58-64	182-186	550-580	10	360-390	220-280	13-18	35-40	3-5	40-60	40-60	1.0-1.5	0.5-1.0	220-260	400-500
8.	DO	68-80	189-182	606-560	10	390-450	250-350	13-18	35-40	3-5	40-60	40-60	1.2-2.0	0.5-1.0	240-300	400-500
9.	DO	81-120	182-123	560-254	10	390-450	250-350	13-18	35-40	3-5	40-60	40-60	1.2-2.0	0.8-1.5	240-300	400-500
10.	STAR APTURE LAIDS/DLX	60-70	168-163	480-500	10	360-430	230-280	13-18	35-40	3-5	40-60	40-60	1.0-1.8	0.5-1.0	220-260	400-480
11.	DO	71-80	175-171	510-370	10	390-450	250-350	13-18	35-40	3-5	40-60	40-60	1.0-2.0	0.8-1.3	240-300	400-480
12.	DO	81-105	154-133	400-300	10	DO	250-350	13-18	35-40	3-5	40-60	40-60	1.2-2.0	0.5-1.5	240-300	400-480
13.	SS A/C BK WHITE	81-95	170-146	490-360	10	DO	250-350	13-18	35-40	3-5	60-80	60-80	1.2-2.0	0.8-1.5	240-300	400-500
14.	SS A/C BOOK YEL/D YEL	68-80	175-180	510-550	10	380-450	250-350	13-18	35-40	3-5	60-80	60-80	1.0-2.0	0.8-1.3	240-300	400-500
15.	DO	81-95	170-146	490-360	10	DO	250-350	13-18	35-40	3-5	60-80	60-80	1.3-2.0	0.8-1.5	240-300	400-500
16.	MF STEFFNER CVR	130	104	180	10	380-480	250-350	13-18	35-40	3-5	40-60	40-60	1.3-2.0	0.8-1.5	240-300	400-500
17.	SS MAP H.Br.	64-70	186-189	580-606	10	360-430	230-280	13-18	35-40	3-5	50-70	50-70	0.7-1.8	0.5-1.3	220-260	400-500
18.	DO	71-80	186-182	580-560	10	390-450	250-350	13-18	35-40	3-5	50-70	50-70	0.8-2.0	0.8-1.5	260-300	400-500
19.	DO	81-100	182-148	560-375	10	DO	250-350	13-18	35-40	3-5	50-70	50-70	1.3-2.0	0.8-1.5	260-300	400-500
20.	DO	101-120	148-120	370-245	10	DO	250-350	13-18	35-40	3-5	50-70	50-70	1.3-2.0	0.8-1.5	260-300	400-500
21.	MAP SUP. HIGH BULK	90-140	167-103	475-180	10	DO	250-350	13-18	35-40	3-5	40-60	40-60	1.3-2.0	0.8-1.5	260-300	400-500
22.	STAR COPIER 2K	68-80	175-177	510-520	10	360-430	250-350	13-18	35-40	3-5	-	20	1.5-2.0	0.9-1.6	240-300	400-500
23.	SS SUPER PRINT	68-80	189-182	510-520	10	390-450	250-350	13-18	35-40	3-5	50-70	50-70	1.0-2.0	0.8-1.5	240-300	400-500
24.	SS SUPER PRINT	81-90	182-167	520-475	10	390-450	250-350	13-18	35-40	3-5	50-70	50-70	1.3-2.0	0.8-1.5	260-300	400-500

1) 2 TO 4 Tw of Starch solution is to be maintained at size press in MSD, SS/M, and MAP SUPERB (HB) etc. varieties
 2) In SS A/C BOOK varieties STAR COPIER and SS MAP (HIGH Br.) 4.5 - 6.0 Tw of starch solution is to be maintained at size press
 3) No addition of Dyes and whitening agents in Natural Shade varieties.
 4) 2 to 3 kg / T BASOPLAST added in all varieties.
 5) Defoamer @100 gm / T is added in all varieties.
 6) Defoamer @ 100 gm / T is added in all varieties.
 7) Biocide added to white water @ 2 kg / day
 8) Krotha Flocculants added @ 2 kg / day in save-all.

Table 2—Quality wise wet end additives for PM1.

Sl.	UNIT →	A.K.D.	PAC	Dis. Rosin	Fib. Lock	Soap ST.	Whitener	RGASAL VOIL	E. green	Retine Rich	STARCH	TO ₂
		Kg/T	Kg/T	Kg/T	Kg/T	Kg/T	Kg/T	gm/T	gm/T	gm/T	kg/T	kg/T
1.	M/APLITHO ARSR	14-17	50	18	1-2	160-190	---	---	---	5	20-24	---
2.	SS MAP/ SUPERB NS	DO	DO	DO	DO	140-160	---	---	---	5	20-24	---
3.	STARMAP NS HL DLX	DO	DO	DO	DO	120-140	---	---	---	5	20-24	---
4.	SS MAPLITHO	DO	DO	DO	DO	120-140	4+4	80-150	---	5	20-24	---
5.	DO	DO	DO	DO	DO	140-160	4+4	80-150	---	5	20-24	---
6.	DO	DO	DO	DO	DO	160-190	4+4	80-150	---	5	20-24	---
7.	MAP SUPER DLX	DO	DO	DO	DO	120-140	3+3	70-140	---	5	20-24	---
8.	DO	DO	DO	DO	DO	140-160	3+3	70-140	---	5	20-24	---
9.	DO	DO	DO	DO	DO	160-190	3+3	70-140	---	5	20-24	---
10.	STAR AZURE LAIDS/S DLX	---	45-50	18-24	DO	140-160	---	AURAMINE 300-350	B. GREEN 25/30	---	10-15	---
11.	DO	---	DO	DO	DO	160-180	---	DO	DO	---	10-15	---
12.	DO	---	DO	DO	DO	160-180	---	DO	DO	---	10-15	---
13.	SS A/C BK WHITE	---	DO	DO	DO	160-180	---	40-45	---	5	20-25	---
14.	SS A/C BOOK YEL/D YEL	---	DO	DO	DO	140-180	---	D. YEL. 3/5, 10/12	D. SCRLFT 5/10	---	20-25	---
15.	DO	---	DO	DO	DO	140-160	---	DO	DO	---	20-25	---
16.	MF STIFFNER CVR	---	DO	10-15	DO	160-180	---	---	---	5	---	---
17.	SS MAP H.Br.	---	DO	10-13	DO	140-160	5+5	15-25	---	5	20-25	2+2
18.	DO	---	DO	DO	DO	140-160	5+5	15-25	---	5	20-25	2+2
19.	DO	---	DO	DO	DO	160-180	5+5	15-25	---	5	20-25	2+2
20.	DO	---	DO	DO	DO	160-180	5+5	DO	---	5	20-25	2+2
21.	MAP SUP. HIGH BULK	---	DO	DO	DO	160-180	4+4	15-25	5-10	5	---	---
22.	STAR COOPER 2K	---	DO	DO	DO	140-160	5+5	100-150	---	5	20-25	---
23.	SS SUPER PRINT	---	DO	DO	DO	160-180	5+5	130-200	---	5	20-25	2+2
24.	SS SUPER PRINT	---	DO	DO	DO	160-180	5+5	130-200	---	5	20-25	2+2

Table 4—Quality wise wet end additives for PM.

Parameters	TDR	0/1 STD	JORDAN	ALUM	FOR ROSIN	DIS. ROSIN	FIB. LOCK	PAC	SOAP ST.	WHITENER	MET. VOIL.	MF RESIN	CARTAF.	ANUF 400	SOD. SIL.
MF STEFFNER (HIGH BK.)	amp. A	amp. 400-650	amp. LY	kg/T -	kg/T -	kg/T 18-20	kg/T 1.5-2.0	kg/T 50-35	kg/T 40-80	kg/T -	gm/T 15-40	kg/T -	kg/T -	kg/T -	kg/T 10-15
MF F.N.S.	S	400-650	RE	-	-	23-26	1.5-2.0	55-60	120-160	2-4	15-40	1.0-1.5	1.0-2.0	-	-
MG Cover/NS	P	400-650	QU	-	-	23-26	1.5-2.0	55-60	120-160	2-4	15-40	1.0-1.5	1.0-2.0	-	-
SS MAP/LTHO	R	400-650	IR	-	-	18-20	1.5-2.0	50-55	120-180	5-7	15-40	-	1.0-2.0	-	-
MAP BASE DAP/NS	E	400-650	ED	-	-	18-20	1.5-2.0	50-55	70-130	5-7	15-40	-	1.0-2.0	-	-
MF BD BASE/NS	Q	400-650	ON	-	-	18-20	1.5-2.0	50-55	60-100	2-4	15-40	-	1.0-2.0	-	-
STAR S S SUPER PRINT	U	400-650	IF	-	-	23-26	1.5-2.0	55-60	60-110	2-4	15-40	-	1.0-2.0	-	-
MG CVR BASE/NS	I	400-650	RE	-	-	23-26	1.5-2.0	55-60	60-120	2-4	15-40	-	1.0-2.0	-	-
MG P BD BASE/NS	R	400-650	QU	-	-	18-20	1.5-2.0	50-55	100-130	2-4	15-40	-	-	-	-
MAP BRAILLE/NS	E	400-650	IR	-	-	18-20	1.5-2.0	50-55	100-140	2-3	15-40	-	-	-	-
MF P BD RE/NS	D	400-650	ED	-	-	18-20	1.5-2.0	50-55	100-160	2-3	5-20	-	-	-	-
STAR CART. CARD WEFT	R	400-650	JDC	-	-	18-20	1.5-2.0	50-55	100-180	4-6	15-40	-	-	-	-
MSD	R	400-650	JDC	-	-	18-20	1.5-2.0	50-55	100-180	4-6	15-40	-	-	-	-
KRAFT PLAIN	E	600-780	200-250	80-90	13-15	-	-	-	-	-	-	-	1.0	8-10	-
KR PL. (EVE)	F	600-780	ON	80-90	13-15	-	-	-	-	-	-	-	-	8-10	-
KRAFT BRAILLE PAPER	I														
KR PL. ABS.	N	600-780	QU	10	-	-	-	-	-	-	-	1.0-3.0	-	-	-
STAR AB KRAFT	N	600-780	IR	10	-	-	-	-	-	-	-	1.0-3.0	-	-	-
KR PL. ABS (SG)		600-780	ED	10	-	-	-	-	-	-	-	3.5	-	-	-

Table 5—Quality wise operating parameters for PM.

S. no	Quality	Subst Rang e	Spee d	Head mm	COU CH Vaeu um	LOADING (+ Or - 20%)		Steam MG Dye t	REINER LOADING (+ Or - 20%)				CHEMICALS											
						SUC MG	1 st MG		2 nd MG	MG Dye t	TDR Amp.	SDM Amp.	kg/T	kg/T	Alu m	For. Ros.	Dis. rosin	wax EML	Gum/ ANU F	Soap St.	White ener	Met VOI L	MF Resin	CARTA FL
1.	MG POSTER NS/WH	50- 95	190- 109	508- 180	150- 200	220	700	600	2.0- 3.5	80- 130	400- 580	250-320	50- 55	---	26- 28	1-3	2.0- 3.0	50- 80	2.0- 4.0	20-40	---	1.0	100	---
2.	MG CVR NS/WH	100- 120	103- 86	200- 150	180- 220	220	700	600	3.5- 4.0	80- 130	400- 580	250-320	50- 55	---	26- 28	1-3	2.0- 3.0	80- 130	2.0- 4.0	20-40	1-2	1.0	100	---
3.	MG POS. BASE NS/WH	51- 90	180- 108	450- 180	150- 200	220	700	600	2.0- 3.0	80- 130	400- 580	250-320	50- 55	---	26- 28	1-3	2.0- 3.0	50- 80	2.0- 4.0	20-40	---	1.0	100	---
4.	SS PLAIN KRAFT	48- 60	206- 192	600- 450	130- 200	220	700	600	2.0- 3.5	80- 130	400- 620	250-340	50- 55	12- 15	26- 28	---	3.0- 10.0	---	---	---	---	1.0	---	---
5.	KRAFT PLAIN	65- 120	179- 95	450- 150	180- 220	220	700	600	2.5- 4.2	80- 130	400- 760	---	80- 85	12- 15	---	---	10	---	---	---	---	1.0	---	---
6.	ABS. KRAFT	65- 80	163- 133	390- 270	150- 200	220	700	600	2.0- 4.0	80- 130	400- 760	---	10	---	---	---	---	---	---	---	1-3	1.0	---	---
7.	SS KRAFT ITC	60	192	510	200	220	700	600	3.5	80- 130	400- 760	---	80- 92	12- 15	---	---	3.0- 10.0	---	---	---	---	1.0	---	---

1) SS Kraft ITC - Pulp brightness to be maintained 43 - 46 0 PV.
 2) No addition of whitening agent in MG Poster / cover NS variety.
 3) Cartaflex is being used as and when required except absorbent Kraft.
 4) Dyes for colour Poster / cover varieties: -

Varities: -	A. PINK	D. FAST	D. RED	kg/T
B. YELLOW	D. SCARLET	15.0-40.0	kg/T	
	D. YELLOW	3.0-5.0	kg/T	
C. GREEN	B. GREEN	0.5-0.8	kg/T	
	AURAMINE	0.25-0.50	kg/T	
D. BLUE	M. BLUE	0.30-0.50	kg/T	
	M. VIOLET	15.0-40.0	kg/T	

Table 6— Quality wise operating parameters for PM.

Sl. No.	Quality	Substance Range	Speed	Head ± 10%	Bagley Box Vac. ± 10%	SUC Press	LOADING (+ or - 20%)		Steam MG Dryer (+ or - 20%)	REFINER LOADING (+ or - 20%)		
							1 st MG Touch Roll	kg/Cm		TDR amp	O/I SDM amp	S/W Ref amp
	UNIT →	GSM	Mfr->A/min	mm	mm Hg	kg/Cm	kg/Cm	kg/Sq Cm				
1	SS PLAIN KRAFT	33-60	158-115	350-190	150-250	60	100	2.0-4.50	80-130	400-600		
2	SS RIBBED KRAFT	38-60	149-110	120-170	150-250	60	100	2.0-4.50	80-130	400-600		
3	SS RIB KRAFT PINK	40-64	158-97	350-150	160-250	60	100	2.0-4.50	80-130		250-340	
4	KRAFT PAPER	38	149	320	180	60	100	2.0-4.0	80-130	400-600		
5	M G POSTER	26-32	165-160	390-350	150-180	60	100	2.0-3.0	100-130	400-500	260-320	
6	M G POSTER	34-61	159-105	350-160	180-250	60	100	2.5-4.0	100-130	400-500	260-320	
7	M G POS. RIB DEEP PINK	32	145	300	180	60	100	2.0-3.0	120	400-500	260-320	
8	MANILA PINK	35	144	300	180	60	100	2.5-4.0	120	400-500	260-320	
9	TDI POSTER DLX	32-50	149-128	350-230	200-250	60	100	3.0-4.5	120	400-500	260-320	
1) Shade of Poster ARSK and Poster ARSK (HI) are same as MG Poster. 2) SS Ribbed Kraft Pink to be manufactured with Semi Bleached Pulp (Brightness of Pulp 43 - 46 OPV 3) No addition of whitening agent MG Poster NS variety. 4) SCPSPF is to be used as and when required. 5) Cartaflex is being used in Unbleached and Bleached varieties as and when required. 6) Dyes for Coloured Varieties: -												
					A. Manila Pink			Acid Orange	1.0-3.0 kg/T			
					B. MG Poster Rib Deep Pink			Pargasol Red	4.0-7.0 kg/T			
								Rodamine Acid	1.0-3.5 kg/T			
					C.S.S. Rib Kraft Pink			Orange	16-22 kg/T			

Table 7---Quality wise wet end additives for PMs and PM.

S. NO.	Quality	ALUM	FOR ROS	Dis. Rosin	WAX EML	SOAP ST.	Whitener	Met. Voil.	ARRANIL	CARTAFEL	DSR	SCPSF	Sod. Hexa Meta pH	Rutile	Ti O2	Amalase	Others
	UNIT-->	kg/T	kg/T	kg/T	kg/T	kg/T	kg/T	gm/T	kg/T	kg/T	kg/T	kg/T	kg/T	kg/T	kg/T	kg/T	kg/T
1	SS PLAIN KRAFT	80-85	16-Dec	---	---	---	---	---	---	---	3.0-4.0	---	---	---	---	---	---
2	SS RIBBED KRAFT SS RIB KRAFT	80-90	16-Dec	---	---	---	---	---	---	---	3.0-4.0	---	---	---	---	---	---
3	PINK	80-90	20-22	---	---	---	---	---	---	---	3.0-4.0	---	---	---	---	---	---
4	KRAFT PAPER	80-85	16-Dec	---	---	---	---	---	---	---	3.0-4.0	---	---	---	---	---	---
5	MG POSTER	50-55	---	26-28.5	1.0-3.0	0-30	2.0-4.0	20-40	100	1.0-2.0	3.0-4.0	---	---	---	---	---	---
6	MG POSTER	50-55	---	26-28.5	1.0-3.0	0-60	2.0-4.0	20-40	100	1.0-2.0	3.0-4.0	---	---	---	---	---	---
7	MG POS. RIB DEEP PINK	80-85	20-26	26-28.5	1.0-3.0	---	---	---	100	1.0-2.0	3.0-4.0	---	---	---	---	---	---
8	MANILA PINK	80-85	20-26	---	1.0-3.0	---	---	---	100	1.0-2.0	3.0-4.0	---	---	---	---	---	---
9	TDL POSTER DLX	50-55	20-24	50-55	1.0-3.0	40-80	2.0-4.0	20-40	100	1.0-2.0	3.0-4.0	---	0.2	30-40	5.0-10.0	---	---

Table 8— Total bacterial colony count of process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides

Particulars	Total Bacterial Count/ml, cfu/ml				Killing efficiency, %			
	PM-1	PM-2	PM-3	PM-4	PM-1	PM-2	PM-3	PM-4
Blank	4.20×10^6	7.12×10^5	3.84×10^6	3.31×10^6	—	—	—	—
A	9.35×10^4	3.34×10^3	5.65×10^4	4.25×10^4	97.77	99.53	98.52	98.72
B	8.90×10^5	8.54×10^4	7.76×10^5	9.22×10^5	78.81	88.00	79.79	72.15
C	3.04×10^5	4.21×10^4	4.46×10^5	4.32×10^5	92.76	84.08	88.38	96.94

Conditions: Reaction volume:100 ml, dosing of 2,2-dibromo-3-nitrilopropionamide (A), Alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C): 5 ppm respectively and reaction time 04 hrs.

Table 9— Microbiological analysis for yeast and mould in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides

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	100	100	100	100
B	10 ⁻⁰	6	4	3	2	91.8	92.22	93.75	92.55
	10 ⁻⁰	2	3	3	5	4			
C	10 ⁻⁰	Nil	Nil	Nil	Nil	100	100	100	100
	10 ⁻⁰	Nil	Nil	Nil	Nil	100	100	100	100

Conditions: Reaction volume:100 ml, dosing of 2,2-dibromo-3-nitrilopropionamide (A), Alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C): 5 ppm respectively, medium used: Rose Bengal Chlorophenicol and reaction time 04 hrs.

Table 10— Microbiological analysis for pseudomonas in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides

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	99.44	99.53	96.64	96.66
	10 ⁻⁰	Nil	1	1	1				
	Mean	2	1.5	0.5	1				
		0.2	0.15	0.05	0.1				
B	10 ⁻⁰	36	34	27	31	90.97	89.53	90.34	89.33
	10 ⁻⁰	29	33	29	33				
	Mean	32.5	33.5	28	32				
	Cfu/ml	3.25	3.35	2.8	3.2				
C	10 ⁻⁰	12	17	20	18	96.11	95.00	92.59	93.5
	10 ⁻⁰	16	15	23	21				
	Mean	14	16	21.5	19.5				
	Cfu/ml	1.4	1.6	2.15	1.95				

Conditions: Reaction volume:100 ml, dosing of 2,2-dibromo-3-nitrilopropionamide (A), Alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C): 5 ppm respectively, medium used: Cetrimide agar and reaction time 04 hrs.

Table 11— Microbiological analysis of iron bacteria in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides

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: Reaction volume: 100 ml, dosing of 2,2-dibromo-3-nitropropionamide (A), Alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C): 5 ppm respectively, medium: Isolation medium for iron bacteria and reaction time 04 hrs.

Figure 12— Microbiological analysis for sulphate reducing bacteria in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides

Slimicides	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
	5	1	2	4	2	1	3	5	2	4	2	1	65	62	58	55	—	—	—	—
	0	0	0	0	0	0	0	0	0	0	0	0	Nil	Nil	Nil	Nil	100	100	100	100
	0	0	1	0	2	0	1	0	2	1	0	1	2	1	3	2	96.9	98.4	94.8	96.9
	0	0	0	0	0	0	0	0	0	0	0	0	Nil	Nil	Nil	Nil	100	100	100	100

Conditions: Reaction volume: 100 ml, dosing of 2,2-dibromo-3-nitropropionamide (A), Alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C): 5 ppm respectively, medium used: SRB broth and reaction time 04

Table 13—Plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-1 of Star Paper Mills Ltd., Saharanpur

Date	Dosage (1 L/day)	TBC (cfu/ml)	Finished Prod. (MT)	Slimicide Cost (Rs.)	Treatment Cost / Ton (Rs.)
1	7	3x10 ⁴	45.00	1496.04	33.25
2	7		39.70	1496.04	37.68
3	7		41.40	1496.04	36.14
4	7		38.10	1496.04	39.27
5	7		41.80	1496.04	35.79
6	7		42.50	1496.04	35.20
7	7		39.40	1496.04	37.97
8	7	4x10 ⁵	43.60	1496.04	34.31
9	7		44.10	1496.04	33.92
11	7		40.40	1496.04	37.03
12	7		43.80	1496.04	34.16
13	7		39.70	1496.04	37.68
14	7		39.47	1496.04	37.90
15	7		59.59	1496.04	25.11
16	7		37.10	1496.04	40.32
17	7	4x10 ⁴	40.00	1496.04	37.40
18	3		21.70	641.16	29.55
19	7		45.70	1496.04	32.74
20	7		44.69	1496.04	33.48
22	7		37.50	1496.04	39.89
23	7		42.60	1496.04	35.12
24	7		44.80	1496.04	33.39
25	7		35.90	1496.04	41.67
26	7		37.33	1496.04	40.08
27	7		33.40	1496.04	44.79
28	7		36.70	1496.04	40.76
29	7		32.24	1496.04	46.40
30	7		37.75	1496.04	39.63
31	7	3x10 ⁴	42.43	1496.04	35.26
	199		1168.40	42530.28	1065.90

Avg. cost/ton of paper = Rs. 36.76

Table 14—Cost calculation during plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-1 of Star Paper Mills Ltd., Saharanpur

Date	Dosage (lts)	Cost / ton (Rs.)	TBC ($\times 10^5$ cfu/ml)
01-03-07	7	33.24	3
08-03-07	7	34.31	4
17-03-07	7	37.40	4
31-03-07	7	35.25	3
Avg.	7	36.76	3.5

Table 15—Plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-2 of Star Paper Mills Ltd., Saharanpur

Date	Dosage (1 lts/day)	TBC (cfu/ml)	Finished Prod. (MT)	Slimicide Cost (Rs.)	Treatment Cost / Ton (Rs.)
1	6	4x10 ⁵	82.00	659.22	8.04
2	6		76.70	659.22	8.59
3	6		54.00	659.22	12.21
4	6		54.10	659.22	12.19
5	6		75.60	659.22	8.72
6	6		79.30	659.22	8.31
7	6		84.00	659.22	7.85
8	6	8x10 ⁵	82.60	659.22	7.98
9	6		73.70	659.22	8.94
10	6		70.70	659.22	9.32
11	6		80.30	659.22	8.21
12	6		73.30	659.22	8.99
13	6		73.70	659.22	8.94
14	6		85.50	659.22	7.71
15	3		39.84	329.61	8.27
16	6		83.00	659.22	7.94
17	6	9x10 ⁵	86.70	659.22	7.60
18	6		66.60	659.22	9.90
19	6		97.40	659.22	6.77
20	6		88.20	659.22	7.47
22	6		100.90	659.22	6.53
23	6		105.40	659.22	6.25
24	6		93.30	659.22	7.07
25	6		92.00	659.22	7.17
26	6		98.80	659.22	6.67
27	6		87.10	659.22	7.57
28	6		81.80	659.22	8.06
29	6		94.80	659.22	6.95
30	6		89.10	659.22	7.40
31	3	8x10 ⁵	38.70	329.61	8.52
	174		2389.14	18787.77	246.16
Avg. cost/ton of paper – Rs. 8.49					

Table 16—Cost calculation during plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) at PM-2 of Star Paper Mills Ltd., Saharanpur

Date	Dosage (lts)	Cost / ton (Rs.)	TBC ($\times 10^5$ cfu/ml)
01-03-07	6	8.04	4
08-03-07	6	7.98	8
17-03-07	6	7.60	9
31-03-07	3	8.52	8
Avg. (month)	6	8.49	7.25

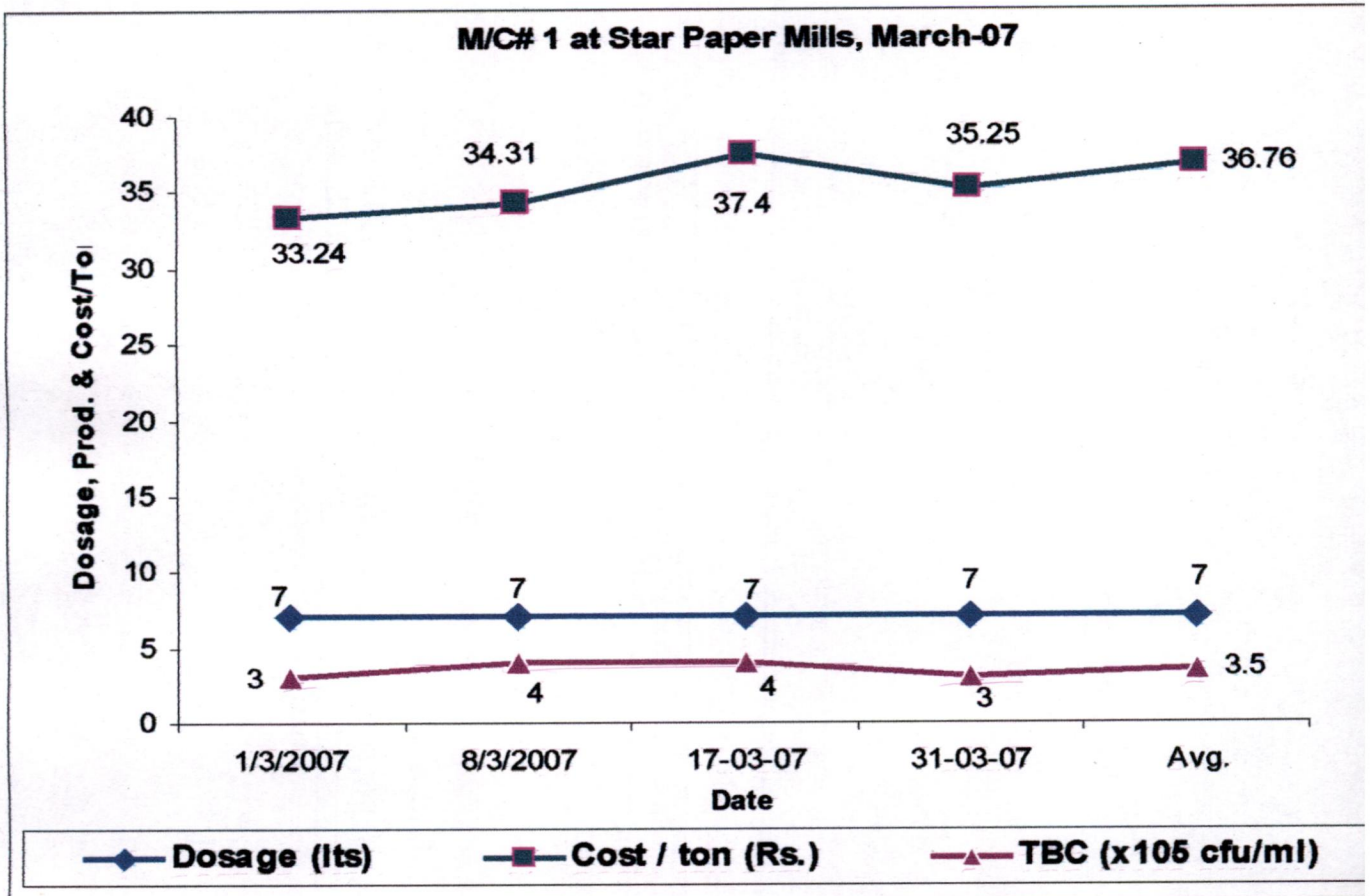


Figure 1— Dosage, cost and total bacterial colony count at PM-1 during plant trial

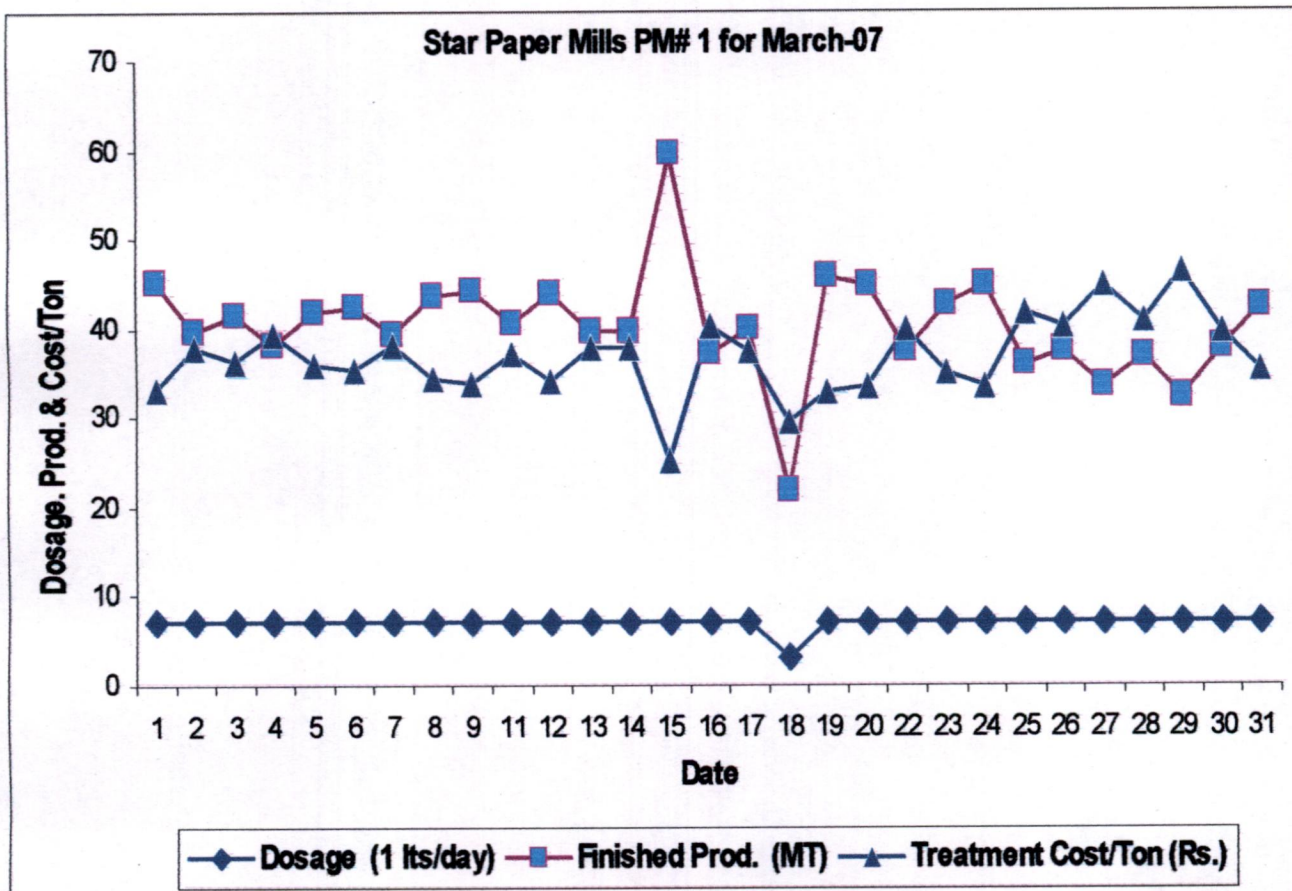


Figure 2- Dosage, finished production and treatment cost at PM-1 during plant trial

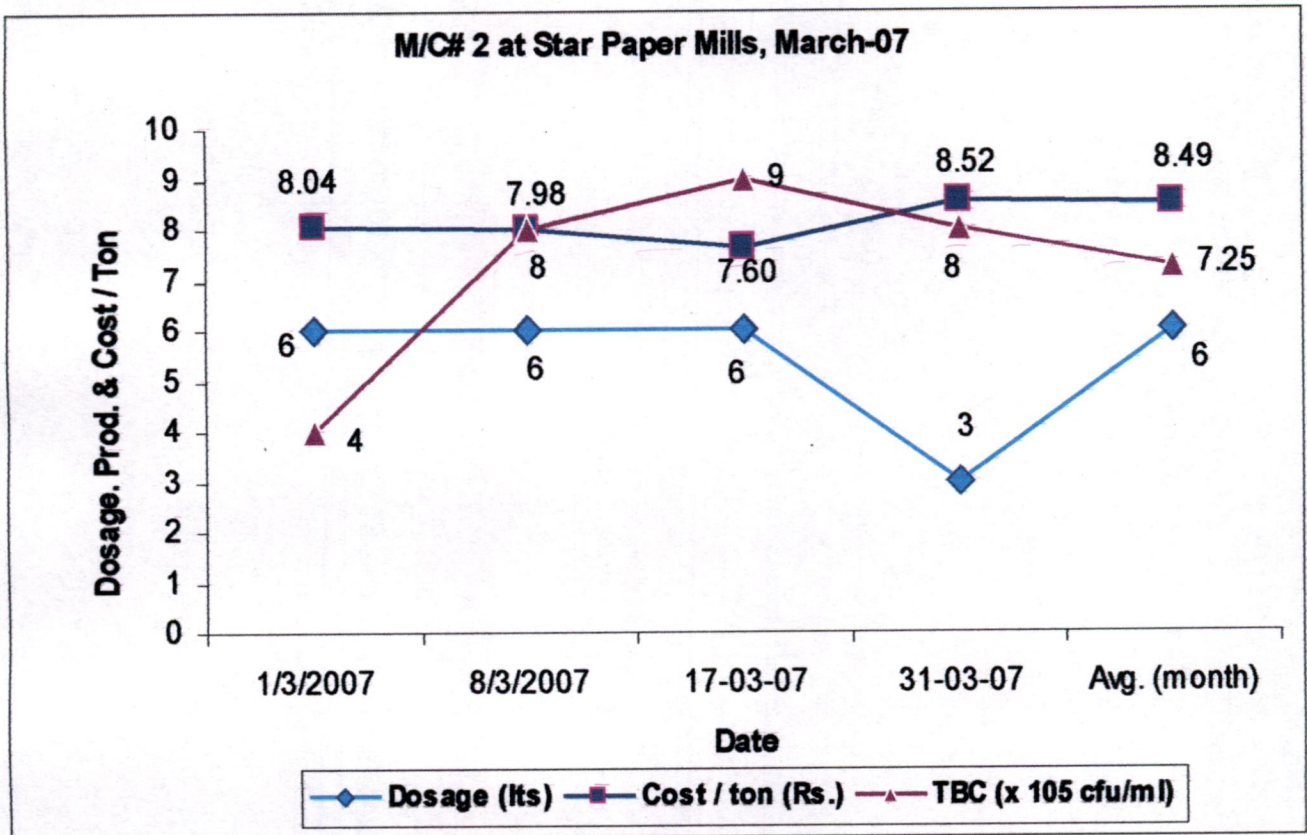


Figure 3- Dosage, cost and total bacterial colony count at PM-2 during plant trial

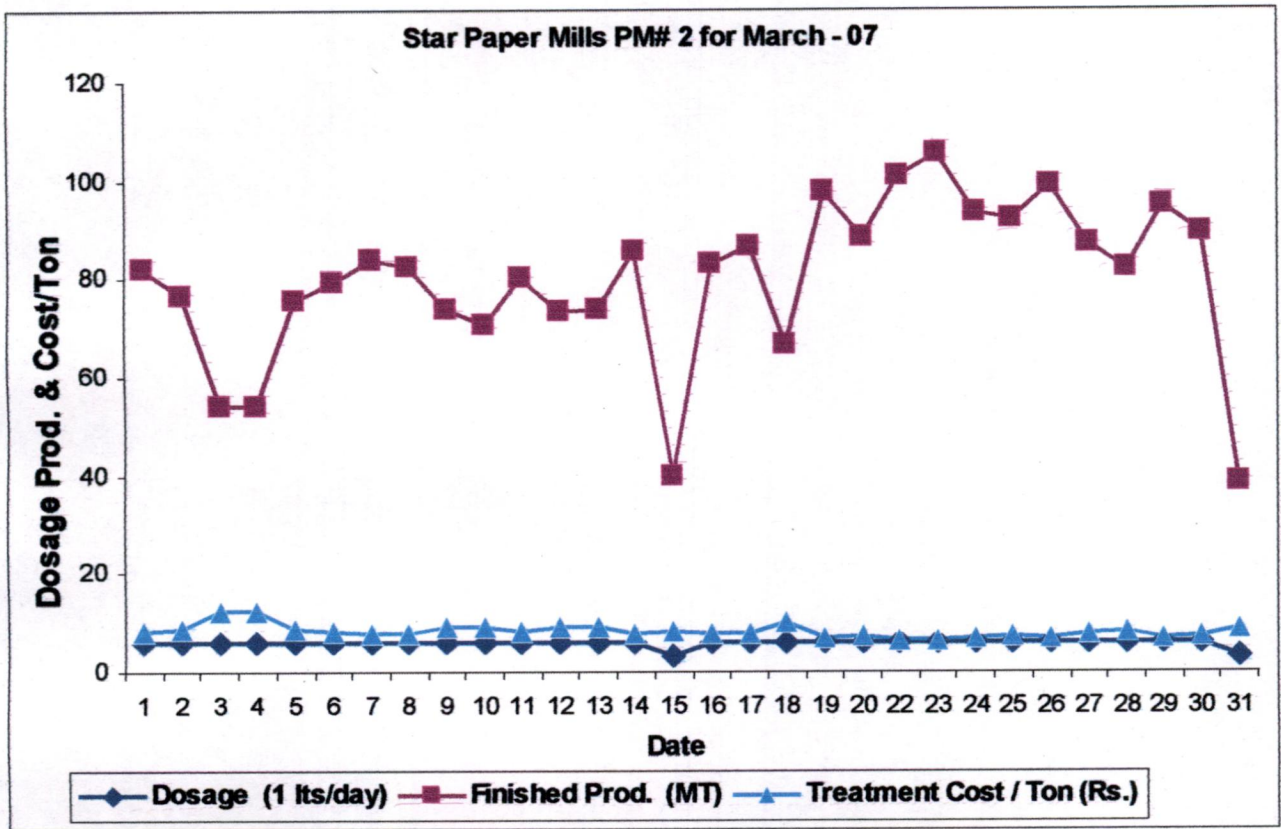


Figure 4— Dosage, finished production and treatment cost at PM-2 during plant trial

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS

Table 1 and 2 reveal the quality wise parameters of paper machine-1. Mostly, writing and printing grades of GSM varied from 58 to 120 g/m² are being manufactured. Starch solution of 2 to 4 °TW is added at size press in Maplitho Super Deluxe, Surface Sized Maplitho and Maplitho Super High Bright are added. On the other hand, starch solution of 4.5 to 6.0 °TW is to be maintained at size press of paper machine-1 for Surface Sized Account Book varieties, Star Copier and Maplitho High Bright. All the paper qualities as mentioned in table 2 contains starch solution at size press ranging between 10-25 kg/tonne of paper. In addition to starch solution other non fibrous additives like, whitening agent, basoplast (wet end bonding additives), defomers and flocculants are added at size press of paper machine-1. The problem of paper breaks is more severe in case of Surface Sized Account Book varieties, Star Copier and Maplitho High Bright than that of Maplitho Super Deluxe, Surface Sized Maplitho and Maplitho Super High Bright. The only reason is that starch solution which contains amyloplast and amylopectin is the principle source of food for the growth of bacteria. The higher concentration of starch solution may cause more down time in dryer section due to microbiological activities. Most of the qualities as mentioned in table 2 contains starch solution ranging between 10-25 kg/tonne of paper, AKD 14-17 kg/tonne of paper, Ploy aluminum chloride 45-50 kg/tonne of paper, dispersed rosin 10-24 kg/tonne of paper, fiber lock (retention aids) 1-2 kg/tonne of paper, soap stone powder 160-190 kg/tonne of paper, optical whitener 3-5 kg/tonne of paper, refiner aids (refiner rich) 5 g/tonne of paper and TiO₂ about 2 kg/ tone of paper with dyes as per qualities to be manufactured at

in stock preparation. These non-fibrous additives are the principle source for sulphate reducing bacteria, iron bacteria, pseudomonas and yeast and mould. They may cause breaks at pressing section. It is also observed that breaks at pressing are 2/3rd times more compared to dryer section of paper machine 1.

Table 3 and 4 reveal the quality wise operating parameters of paper machine-2. Mostly, writing and printing, absorbent grades, wrapping and packing papers of GSM varied from 68-225 g/m² are being manufactured. Stock preparation section of paper machine-2 contains alum 80-90 kg/tonne of paper, dispersed rosin 18-26 kg/tonne of paper, fiber lock (retention aids) 1.5 to 2.0 kg/tonne of paper, ploy aluminum chloride 50-60 kg/tonne of paper, soap stone powder 60-180 kg/tonne of paper, optical whitener 2-6 kg/tonne of paper, M. F. resin 1-3.5 kg/tonne of paper, cartaflex (glazing agent) 1.0-2.0 kg/tonne of paper, anionic urea formaldehyde 8-10 kg/tonne of paper depending upon the qualities are being to be manufactured. These additives of organic and inorganic in nature provide nutrients for microbiological growth. The problem of slime is more serious in paper machine 2 compared to other paper machines. Therefore, the number of breaks is more in paper machine 2 than that of paper machines 1, 3 and 4 as observed.

Table 5 and 6 reveal the quality wise operating parameters of different grades of paper being manufactured at paper machine 3 and 4. MG kraft and MG poster papers in the basis weight range of 48-120 g/m² and plain kraft, ribbed kraft, MG poster, manila pink and TDL poster in the basis weight range of 28-64 g/m² are being manufactured on paper machine 3 and 4 respectively. Table 7 lists the quality wise wet end additives added for paper machines 3 and 4. Most of the grades of paper contain with alum 80-90 kg/tonne of paper, fortified rosin 20-26 kg/tonne of paper, dispersed rosin 26-55 kg/

7

7

7

7

tonne of paper, wax emulsion 1.0-3.0 kg/tonne of paper, soapstone powder 40-80 kg/tonne of paper, optical whitener 2-4 kg/tonne of paper, cartaflex (glazing agent) 1.0-2.0 kg/tonne of paper and other additives depending upon the qualities are being to be manufactured. The deposition of these non-fibrous additives may cause the growth of various types of bacteria which increase machine downtime due to microbiological growth. It is clear that stock preparation sections of paper machines 1-4 contain different compositions of various non-fibrous additives. Therefore, the population of different types of bacteria may vary due to variation in composition of substrate. Therefore, it is very much essential to analyze different types of bacteria like sulphate reducing bacteria, iron bacteria, pseudomonas and yeast and mould in each stock preparation sections. Keeping in view problems like production loss, inferior quality due to slime and minimum profit level various biocides were analyzed in laboratory to study the killing efficiency of different types of bacteria. Finally, a plant trial was conducted with high spectrum biocides.

Table 8 shows the total bacterial colony count in the process water of paper machines 1, 2, 3 & 4 of Star Paper Mills Ltd., and killing efficiency of different biocides. The total counts of bacteria in the white water system of paper machines 1, 3 & 4 are nearly the same but total bacterial colony count in the process water of paper machine 2 was just double i.e. 7.12×10^5 cfu/ml for a incubation period of 48 hours and temperature 30°C using yeast, malt extract, peptone, dextrose agar (Difco 0712). In order to isolate species present in the samples only low numbers, 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media to prevent the growth of fungi. The incubation temperature was 30°C and the growth was monitored from 24 hours. The

killing efficiency of three different biocides viz., 2,2-dibromo-3-nitrilopropionamide (A), alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C) were analyzed at a shock dose of 5 ppm. The killing efficiency of 2,2-dibromo-3-nitrilopropionamide (A) is the highest among other biocides for the white water systems of paper machines 1, 2, 3&4 and minimum killing efficiency with alkali dimethyl benzyl ammonium chloride (B).

Table 9 reveals the microbiological analysis for yeast and mould in the process water of paper machines 1, 2, 3 &4 of Star Paper Mills Ltd., and killing efficiency of different slimicides. For the detection of yeasts and filamentous fungi rose Bengal chloramphenicol agar medium was used. To prevent the growth of bacteria, 100 mg/l chloramphenicol (Sigma C-0378) and 100 mg/l chlorotetracycline (Sigma C-4881) were added by plating, except to chloramphenicol agar which already contained the selective agent. The incubation temperature was 25 °C and the growth was monitored from 3 to 14d. The mould and fungi is 49 cfu/plate in the process water of paper machine 1. Whereas, mould and fungi varies between 42-46 cfu/plate in the process water of paper machines 3-4. The killing efficiencies of 2,2-dibromo-3-nitrilopropionamide (A) and alkali dimethyl ammonium chloride (C) was 100 % in the process water of paper machines 1-4 at a shock dose of 5 ppm.. The killing efficiency of alkali dimethyl benzyl ammonium chloride (B) is about 92%.

Table 10 reveals the microbiological analysis for pseudomonas in the process water of PM- 1, 2, 3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides. Pseudomonas bacteria in the process water of paper machines (1-4) were determined by using cetrimide agar (Himedia M 024). In order to prevent the growth of

fungi 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation temperature was 30 °C and the growth was monitored from 24 hours. The maximum bacterial colony count for pseudomonas was 36 cfu/plate in process water of paper machine 1. The killing efficiency of 2,2-dibromo-3-nitrilopropionamide (A) is the highest for process water of paper machines 1-4 while the lowest killing efficiency was observed for alkali dimethyl benzyl ammonium chloride (B).

Table 11 reveals the microbiological analysis of iron bacteria in the process water of PM-1, 2, 3 & 4 of Star Paper Mills Ltd., and killing efficiency of different slimicides. The iron bacteria in process of paper machines (1-4) were determined by using medium iron bacteria (Himedia M 622). To vanish the growth of fungi 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation temperature was 30 °C and the growth was monitored from 24 hours. The maximum iron bacteria colony count was 45 cfu/plate in the process water of paper machine 1. Whereas, iron bacteria colony count varies between 36-42 cfu/plate in the process water of paper machine 3-4. The killing efficiency of 2,2-dibromo-3-nitrilopropionamide (A) is the highest for process water of paper machines 1 while killing efficiency was just half when alkali dimethyl benzyl ammonium chloride (B) was used and the killing efficiency of alkali dimethyl ammonium chloride (C) was 75%.

Table 12 reveals the Microbiological analysis for sulphate reducing bacteria in the process water of PM-1,2,3&4 of Star Paper Mills Ltd., and killing efficiency of different slimicides. The sulphate reducing bacteria in the process water of paper machines (1-4) SRB broth medium was used. To control the growth of filamentous fungi and mould 100 mg/l cycloheximide (catidion) (Sigma C-7698) was added to the media. The incubation

temperature was 30 °C and the growth was monitored from 24 hours. Number of SRB bacteria/ 100 is maximum and almost similar in the process water of paper machine 1 and 2 and slightly on lower side in the process water of paper machines 3 and 4. The SRB killing efficiencies of 2, 2-dibromo-3-nitrilopropionamide (A) and alkali dimethyl benzyl ammonium chloride (C) were 100% while killing efficiency of alkali dimethyl benzyl ammonium chloride (B) was slightly on lower side.

The killing efficiency of different biocides i.e. 2,2-dibromo-3-nitrilopropionamide (A), alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C) for different types of bacteria i.e. sulphate reducing bacteria, iron bacteria, pseudomonas and yeast and mould were analyzed in laboratory. Keeping their killing efficiency in view a plant trial was conducted with 2,2-dibromo-3-nitrilopropionamide (A) at paper machine 1. The total bacterial colony count at a dose of seven liter per day was reduced to 3×10^4 for a period of one month trial. The finished production for a month was 1168.40 MT. The slimicide and treatment costs for one month were Rs. 42530.24 and Rs. 1065.90 respectively. The average cost of chemical per tone of paper was about Rs 36.76 (Tables 13-14 and Figures 1-2).

A plant trial with slimicide (Alkali dimethyl benzyl ammonium chloride) was conducted at paper machine 2. The total bacterial colony count at a dose of six liter per day was reduced to 8×10^5 for one month trial. The finished production for a month was 2389.14 MT. The slimicide and treatment costs for a period of one month were Rs. 2389.14 and Rs. 246.16 respectively. The average cost of chemical per tone of paper was about Rs 8.49 (Tables 15-16 and Figures 3-4).

5.0 CONCLUSIONS:

On an international level, there are no specific biological standards for such grades which intended to come in to contact with food (46). According to FDA regulation (47), fibers must be used in food packing materials "only in a way that assures product and consumer safety". At a national level, some countries have limit value recommendations e.g., for packing materials used for diary, butter and bakery products. Depending upon the case, the limit values vary from 1 fungal cfu dm⁻² to 250 bacterial cfu g⁻¹. In general, the total counts of yeast, moulds and bacteria accepted must be low and no pathogenic bacteria, including enterobacteria and *Escherichia coli*, must be detected (48-50).

The stock preparation section contains various organic and inorganic additives like, starch, low molecular weight polysaccharides, fillers like., soapstone powder and TiO₂ etc., alum, wet end bonding additives which have the possibilities to deposit in chest corroded pipe lines etc. The optimum temperature, humid atmosphere and ample easily assessable nutrients for the growth of micro organisms may provide atmosphere for bacteria, and fungi multiplication. As a result they not only smear the quality of paper (as per norms for food grades paper) but decrease the production due to more breaks. The process water of paper machines 1, 2, 3 & 4 contains total bacterial colony counts 4.20x10⁶, 7.12x10⁵, 3.84x10⁶ and 3.31x10⁶ Cfu/ml respectively. The yeast and mould in the process water of paper machines 1, 2, 3 & 4 are 49, 42, 44 and 46 Cfu/ml respectively. The bacterial colony count of pseudomonas in the process water of paper machines 1, 2, 3 & 4 with a dilution of 10⁻¹ are 36, 32, 29 and 30 Cfu/ml respectively. The bacterial colony count of iron bacteria in the process water of paper machines 1, 2, 3 & 4 are 45,

42, 39 and 36 Cfu/ml respectively. The number of sulphate reducing bacteria in the process water of paper machines 1, 2, 3 & 4 are 65, 62, 58 and 55 Cfu/ml respectively. These values indicate that these bacteria not only fail to assure product and consumers safety but cause production loss also.

In order to eradicate these problems the killing efficiencies three different biocides i.e., 2,2-dibromo-3-nitrilopropionamide (A), alkali dimethyl benzyl ammonium chloride (B), and alkali dimethyl ammonium chloride (C) were tested. The biocide 2,2-dibromo-3-nitrilopropionamide (A) is wide spectrum for all types of bacteria and fungi and killing efficiency is about 100% in most of the cases.

REFERENCES

- 1 Suihko M Land Skyttä, A study of the microflora of some recycled fibre pulps, boards and kitchen rolls, *Journal of Applied Microbiology*, **30**(1997)199-207.
- 2 Väisänen O M, Nurmiäho-Lassila E-L, Marmo S A & Salkinoja-Sai M S, Structure and Composition of biological slimes on paper and board machines, *Applied and Environmental Microbiology*, **60** (1994) 641-653.
- 3 Appling J W, Cruickshank G A, DeLong R F, Humiston C G, Martin R B, Sanborn J R and Wiley A J, *Microbiology of pulp and paper graph series no 15*, Technical Association of the Industry, Neyork, 1955.
- 4 Purvis M R & Tomlin J L, Microbiological growth and control in the papermaking process, *Tappi Chemical Processing Aids Short Course (Seattle)*, April 10-12, 1991, pp 69-77.
- 5 Fritz, C.W., *Pulp and Paper Magazine of Canada*, **31**(1931), 99.
- 6 Sutermeister, E., *Chemistry of Pulp and Paper Making*, (1929) 299, New York.
- 7 Beijerinck, Van M.W., *Folia Microbiologica*, **1**(1912), 377.
- 8 Bergey, D. H., *Manual of Determinative Bacteriology*, Baltimore (1930).
- 9 Jibbert, H. and Brauns, F., *Can. Jour. Res.*, **4**(1931), 596.
- 10 Schmid, W., *Zellstoff, U. Papier*, **10**(1930), 870.
- 11 Pattillo, D.K., *Pulp and Paper Magazine*, **31**(1931), 551.
- 12 Boruff, D.S., and Stoll, K.E., *Jour. Ind. Eng. Chem.*, **24**(1932) 398.
- 13 Gesell, W.H., *Paper Industry*, **14**(1932), 297.
- 14 Zettnow, Klein Beitrage Sur Morphologie der Bakterien, *Zeit., F., Hyg., U., Infek.*, **85**(1918), 17-32.
- 15 Heidelberger, M., and Avery, O.T., The soluble specific substance of *Pneumococcus*, *Jour., Exp., Med.*, **38**(1923), 73-79.
- 16 Lottermoser, A., and Methiesen, E., Uber Zucker in der Sulfitablauge Tech., u., *Chem. D., Papier u. Zellstoff-fabr.*, **26**(1929), 37-42.

- 17 Gupta Abha, Kulkarni A G, Mathur R M & Jain R K, Microbial slime in papermaking operations-problems, monitoring and control practices, *IPPTA Conventional Issue 2003*, pp 121-125.
- 18 Jaakko Ekman, Mirva Kosonen, Sanna Jokela, Marko Kolari, Päivi Korhonen and Mirja Salkinoja-Salonen, Detection and quantitation of colored deposit-forming *Meiothermus* spp. in paper industry processes and end products, *J Indus Microbiol and Biotechnol*, published online on 28 November 2006.
- 19 Costerton J W, Cheng K-J, Geesey G G, Ladd T i, Nickel J C, Dasgupta M and Marrie T J, Bacterial biofilms in nature and disease, *Annu Environ Microbiol*, **41** (1987)435-464.
- 20 Eighmy T T, Maratea D and Bishop P L, Electron microscopic examination of waste water biofilm formation and structural components, *Annu Environ Microbiol*, **45** (1983)1921-31.
- 21 Ferris F G, Fyfe W S, Witten T, Schultze S and Beveridge, Effect of mineral substrate hardness on the population density of epilithic microorganisms in two Ontario rivers, *Can J Microbiol*, **35** (1989)744-747.
- 22 Ford T E, Walch M, Mitchell R, Kaufman M J, Vestal J R, Ditner S A, and Lock M A, Microbial film formation on metals in an enriched arctic river, *Biofouling*, **1**(1989)301-311.
- 23 Jacques M, Marrie T J, and Costerton J W, Review: microbial colonization of prosthetic devices, *Microb Ecol*, **13**(1987)173-191.
- 24 Lappin-Scott H M and Costerton J W, Bacterial biofilms and surface fouling, *Biofouling*, **1**(1989)323-342.
- 25 Ridgway H F, Kelly A, Justice C and Olson B H, Microbial fouling of reverse-osmosis membranes used in advanced waste water treatment technology: chemical, bacteriological and ultrastructural analyses, *Appl Environ Microbiol*, **45** (1983) 1066-1084.
- 26 Ford T E, Walch M, Mitchell R, Kaufman M J, Vestal J R, Ditner S A, and Lock M A, Microbial film formation on metals in an enriched arctic river, *Biofouling*, **1**(1989)301-311.
- 27 Jacques M, Marrie T J, and Costerton J W, Review: microbial colonization of prosthetic devices, *Microb Ecol*, **13**(1987)173-191.
- 28 Lappin-Scott H M and Costerton J W, Bacterial biofilms and surface fouling, *Biofouling*, **1**(1989)323-342.

- 29 Véronique Pellegrin, Stefan Juretschko, Michael Wagner and Gilles Cottenceau, Morphological and Biochemical Properties of a *Sphaerotilus* sp. Isolated From Paper Mill Slimes, *Appl Environ Microbiol*, **65**(1) (1999) 156–162.
- 30 Bunnage William J, Singleton, Fred L and Cross, Kevin, Non-biocidal programs for control of biofilm, *54th Appita Annual Conference*, **1**(2000) 181-187.
- 31 Chaudhary A, Gupta L K, Gupta J K & Banerjee U C, Levanases for control of slime in paper manufacture, *Biotechnol Adv*, **16** (5-6) (1998) 899-912.
- 32 Gaudy E, Wolfe R S, Composition of an extracellular polysaccharide produced by *Sphaerotilus natans*, *Appl Microbiol*, **10**(1962)200–205.
- 33 Väisänen O M, Nurmiäho-Lassila E-L, Marmo S A & Salkinoja-Sai M S, Structure and Composition of biological slimes on paper and board machines, *Applied and Environmental Microbiology*, **60** (1994) 641-653.
- 34 Code of Federal Regulations, Title 21, Food and Drugs, Parts 170 to 199, Revised as of April 1, 1989, The Office of the Federal Registrar, National Archives and Administration, U S Government Printing Office, Washington D C (1989).
- 35 Franck R, Kunststoffe im Lebensmittelverkehr, Empfehlungen des Bundesgesundheitsamtes, 39, Lieferung, Stand: März 1990, Carl Heymanns Verlag K G, Cologne, Germany (1990).
- 36 Blanco M A, Negro C, Gaspar I and Tijero J, Slime problems in the paper and board industry, *Appl Microbiol Biotechnol*, **46**(1996)203–208.
- 37 Harju-Jeanty P and Väättänen P, Detrimental micro-organisms in paper and cardboard mills, *Pap Puu*, **3**(1984)245–259.
- 38 Schenker Achim, Singleton Fred and Davis, Christopher, New recipe for biofilm control, *Pulp and Paper Europe*, **4**(2) (1999)3.
- 39 Klahre Joachim and Flemming H-C, Monitoring of biofouling in papermill process waters, *Water Res*, **34**(14) (2000) 3657-3665.
- 40 Vieira M J, Beleza V M and Melo L F, Use of a mild biocide to reduce biofouling in paper and pulp production processes, *Proceedings of the 1998 7th International Conference on Biotechnology in the Pulp and Paper Industry. Part 3*, C193-C196, 1998.
- 41 Gould Ian, Non-biocidal methods of biofilm control, *Paper Technology*, **42**(1) (2001)41-45.
- 42 Wadsworth, J and Simpson G, Control of Biofilm in Alkaline White-Water Systems with Chlorine Dioxide, *1997 Engineering & Papermakers: Forming Bonds for*

Better Papermaking Conference, Nashville , TN , sponsored by TAPPI Press, Atlanta, GA, United States, October 6 (1997) 1095-1106.

- 43 Mueller R F, Biofilm Formation in Water Systems and Their Industrial Relevance, *Biological Sciences Symposium, Minneapolis , MN , sponsored by TAPPI, Atlanta, GA, United States, 195-202, October 6, 1994.*
- 44 M Kolari, J Nuutinen, F A Rainey, M S Salkinoja-Salonen, Colored moderately thermophilic bacteria in paper-machine biofilms, *J Indus Microbiol and Biotechnol*, **30**(4) (2003)225-238.
- 45 World Health Organization, Mercury-Environmental aspacts, IPCS International Programme on Chemical Safetyon Environmental Health Criteria 86, World Health Geneva, 1989.
- 46 K. Svensson, A. Hallikainen, L. Hammarling, T. Hellstorm, L. Lillemark, P. A. Nielsen, *Tamanord 1994: 649, Nordic Council of Ministers, Copenhagen.*
- 47 Annon, Categorization of pathogens according to Hasrad and categories of containment, 2nd edition, Advisory Committee on dangerous pathogens, London, HMSO.
- 48 Lubieniecki v. Schelhorn, M, *Verpackungs-Rundschan*, 24, 27-43 (1973).
- 49 K. T. Witter, *Coating* 12, 159-162 (179).
- 50 G. Cerny, *Allgeneinc Papier Rundschan*, 33 331-335 (1982).

Appendix

Appendix- 1: Total Bacterial Count

Requirements:

1. Nutrient Agar Medium (Hi-Media M001 or M012)
2. NaCl
3. Glass bottles (100 to 200 ml capacity)
4. Conical Flasks (250 ml capacity)
5. Pipettes (10 ml, 2 ml and 1ml capacity)
6. Petra plates (standard)

Procedure:

Prepare nutrient agar medium (Hi-media M001) by dissolving 2.8 gms in 100ml DW, plug the conical flask with cotton plug. Similarly prepare 0.9 % saline by dissolving 0.9 gms of NaCl In 100ml DW. Sterilize all necessary glasswares, Nutrient Agar medium and saline at 15 pound per square inches pressure for 15 mins in Autoclave.

Collect sample of BW from suitable source like BW tank or BW tank in clean and sterile glass bottle. Add 9 ml previously sterilized and cooled saline in 4 or 5 clean and sterile bottles/ screw cap tubes/ test tubes. Add 1ml of Backwater sample to the 1st bottle containing 9ml sterile saline (10^{-1} dilution) shake the bottle well & transfer 1ml from the 1st bottle to the 2nd bottle containing 9 ml saline solution. This gives 10^{-2} dilution, similarly transfer 1ml from 2nd bottle to 3rd bottle. Thus prepare dilution of 10^{-1} , 10^{-2} , 10^{-3} & 10^{-4} . Transfer 1ml from appropriate dilution bottle in two sterile Petrio, salines.

Cool the Nutrient Agar Medium to approx. 45°C & pour into the plates swirling the plates while addition so as to mix the medium & sample. Also, keeps 1 plate as

control containing only the medium. Allow the medium to set & keep the plates in an inverted position at 37°C for 48 hours.

After 48 hours of incubation count colonies from plates and express result in cfu/ml.

E. g at dilution No -10^{-3} if plate shows 66 colonies than count is 6.6×10^4 cfu/ml.



Appendix- 2: EFFICACY TEST PROCEDURE

USING BACTASLIDES:

For Control:

Dip a Bactaslide in 50 ml plain Backwater sample for 1 min. Incubate the slide at around 30-37°C for 24-48 hr.

For Biocide at 5ppm Conc.:

- (1) Prepare 0.1% stock solution of the given Biocide solutions.

0.1 ml → 100 ml D/W.

0.1% = 1000ppm (1 ppm = 1 mg/L)

Required Conc. of Biocide = 5ppm.

Given Conc. = 0.1% (1000ppm).

Total Vol. = 50ml.

Reqd. conc. / given conc. X Total vol.

5ppm / 1000ppm X 50ml. = 0.25ml.

- (2) Add 0.25 ml of the required Biocide from 0.1% prepared stock, solution to 50ml Backwater sample. Incubate at Room Temperature/ shaker for 4-5 hr.
- (3) Dip a Bactaslide into the Backwater sample containing the biocide for 1 min. Incubate the slide at around 30- 37°C for 24-48 hr.

Appendix- 3: DETERMINATION OF TOTAL BACTERIAL COUNT

The viable plate count is an empirical method for estimating the density of living bacteria in a sample of water. The test consists in plating a known volume or if necessary, making a suitable dilution of the water sample and incubating for a specified period to permit the bacteria to form visible colonies.

Apparatus:

- 0.1. Autoclave - Oven
- 0.2. Dilution bottles or tubes
- 0.3. Pipettes - 10 ml and 2 ml (8-10 nos.)
- 0.4. Petridishes - The bottom of the petridish should be free of bubbles and scratches and should be flat so that the medium spreads uniformly throughout the plate.
- 0.5. Incubator

Reagents:

01. Nutrient Agar (HI-MEDIA)
02. Sterile saline solution (0.85% NaCl in D.W.)

Sterilization:

Keep pipettes and petridishes in respective boxes. Prepare the medium saline solution. All glassware medium and dilute is to be sterilized at 15 -18 psi for 15 minutes.

Procedure:

After sterilization, 9 ml each of sterile saline is added to a set of 4 dilution bottles or tubes. Add 1 ml of the water sample to the first bottle (10^{-1} dilution). Shake the bottle well and transfer 1 ml. from the first bottle to the second bottle containing the saline solution. This gives a dilution of 10^{-2} . Similarly transfer 1 ml from the second bottle to the third bottle. Thus prepare dilutions of 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} . Transfer 1 ml each from the dilution bottle numbered 10^{-3} into two sterile petridishes. Similarly plate 10^{-4} dilution

also. Cool the medium to approx 45 °C and add it to the petridishes, swirling the plates while addition so as to mix the medium and water sample. Also keeps 1 plate as control containing only the medium. Allow the medium to set and incubate the plates in an inverted position at 37°C for 48 hours count the number of colonies developed on each plate.

Dilution	No. of colonies	No. of bact./ ml
10^{-3}	X	$X * 10^3$
10^{-4}	Y	$Y * 10^4$

Avg. = ----- / ml.