Final

REPORT OF THE

CESS FUNDED PROJECT

" ENZYMATIC IMPROVEMENT IN DRAINABILITY OF THE WIRE PARTS IN PAPER MACHINE"

SPONSORED BY

INDIAN AGRO PAPER MILLS ASSOCIATION

WORK CARRIED OUT AT

CENTRAL PULP & PAPER RESEARCH INSTITUTE
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1. BACKGROUND OF THE PROJECT

The Indian paper Industry has been slowly awakening to the environmental pressures over the last two decades. The economic reason coupled with tighter limitation being placed on fresh water use and wastewater disposals are forcing the industry to close up their back water system. New regulations and consumer preference are also demanding the use of increased quantities and varieties of waste paper. This has resulted in spiralling concentration of colloidal and soluble substances in waste water system. This factor, coupled with the use of higher filler level, increased chemical additives, more complex and faster running machines have led to the uncontrolled growth of undesirable microbes, leading to formation of slime and giving rise to the problem of paper breaks, increased down time, loss of yield, quality problem due to spots and holes in the paper, higher chemical cost, corrosion and odour which ultimately results in increased maintenance and production cost. The problem is more aggravated in small agro-based mills using increased proportions of agro-residues and recycled waste papers.

Paper mill slime is one of the accretion or accumulation in paper machine caused by certain microorganisms in presence of fibres, fillers and other wet end additives. Felt degradation by slime, next to the cost of raw material has a prominent effect on the over all economics of paper and board making. Woollen felt fibre suffer enzymolysis of the proteinaceous wool fibre through the action of proteolytic enzyme released by certain microorganisms. The problem could be overcome by use of 100% synthetic fibre. However, synthetic fibre in their own right contribute to felt plugging due to low resistance of the fibres to compression causing the felt to become dense. Impregnation of woollen fibres with biocides could reduce the problem of felt degradation, but the felt warping will still occur due to slime build up. The degree of plugging increases, with felt lifetime, therefore the problem is greater for woollen felt, which are desired to last longer. Although it may be true to say that felt plugging would occur due to fillers alone, the pressure of bacterial mass may increase the degree of plugging by 35% as their structure traps and bind fillers and fibres.

Microbiological control has become a necessary part of continuous paper and board production to ensure trouble free running of the paper machine without slime induced paper breaks and the resultant loss in production. By its very nature, microbial control prevents organic growth and has therefore been seen as potentially detrimental to the environment.

Slime growth control techniques using conventional slimicides, being adopted in Indian paper industry have not been ecologically compatible and environmentally acceptable which are based on the use of chlorophenols and mercury based compounds which are found to be mutagenic or carcinogenic and have a serious environmental impact due to high level of residual toxicity and therefore have been prohibited for use. Further due to change in customers consciousness towards environmentally cleaned products, Indian paper industry will be forced to find out alternative slime control additives which are ecologically compatible.

In view of above Indian Agro Paper Mills association now renamed as All India Agro & Recycled Paper Mills Association sponsored a CESS funded project to Central Pulp and Paper Research Institute (CPPRI) entitled "Enzymatic Treatment to Improve the Drainability of the Wire Part" i.e. control of slime in small agro based paper mills using ecologically compatible preferably enzyme based slimicides. Initially the project activities were basically aimed at using enzyme based slimicides (enzyme technology is being environmentally friendly) technology. CPPRI after extensive interaction with leading enzyme manufacturers and chemical companies could observe that enzyme based slimicides could not make a headway in any part of the world for control of slime in paper machine. Interaction with the companies and extensive literature survey revealed that although some of the companies try to develop enzyme based slimicides but the effectivity could not come to the expected levels. CPPRI also formulated an enzyme-based slimicide and studied its activities along with other Eco-friendly slimicides.

Basic objective of the project is to identify and evaluate the globally available environmental friendly and ecologically compatible slimicides to promote them in Indian Paper Industry after evaluating their efficacy under the conditions prevailing in Indian Paper Industry. However before implementing an effective slime control programme in a mill will be essential to make a systematic study in context to identification of the sources and extend of microbial contamination in paper machine white water loop besides other parameters like extend of build up of slime, detailed characterisation of slime and efficacy test of various slimicide against the microbes responsible for formation of slime. Thus the present report basically covers:

- Study on the extent of build up of slime.
- Detailed physico-chemical, biochemical and microbiological characterisation of the samples of slime collected on slime collection unit and from various parts of the paper machine loop and various other samples of white water from trey, back water tank, silo and stock from premachine chest and head box etc. with respect to microbial assay.
- Procurement of samples ecologically compatible slimicides from companies of National and International repute and their evaluation with respect to:
    - Relative population density test against dominating microbes responsible for slime development
    - Efficacy test against sample of white water and pulp stock.

2. SLIME FORMATION

The paper maker is constantly troubled with slimy or gelatinous accumulations which adhere to the inside of the pipe lines, chests and screens and in particular on the exposed spiders of the cylinder machines and along the edge of the wire pit on fourdrinier machines. A slippery feel on any of these surfaces is an indication of slime. The deposit frequently grow to such size that they break loosely from their point of attachment and carried along with the stock to the paper machine where they cause clogging of the felts and wires and causes breaks on the paper machine. Today mills try to keep slime under control through frequent wash up and the use of toxicants. Slime can build up in the paper mill system unnoticed and the first indication may be breaks on the machine and

production of dirty paper. Careful examination of the machine and use of slime measuring boards are the best indications of the slime build up.

As the paper mills find it essential to recycle more white water due to environmental or economic pressures, an increase in biological activity is common. This increase is a direct result of an increased concentration of nutrients, which provides a more favourable environment for biological growth.

As the level of dissolved and suspended solids increases due to white water recycling, the rate of chemical depositions are usually a combination of fibrous material and chemical additives such as alum, titanium dioxide, size etc. An increase in chemical deposition often leads to more sheet holes, breaks and wash-ups.

Increasing the amount of recycled white water also increase the operating temperatures of the machine system. It is not uncommon for the temperature to reach 140^0F or higher. This temperature is high enough to kill or inhibit the growth of most aerobic bacteria. However encapsulated and spore forming bacteria may survive at this high temperature. As a result of increased operating temperature and increased deposition, the growth of anaerobic bacteria is enhanced. Because this type of bacteria lives in the absences of oxygen, it thrives beneath the accumulation of chemical and biological deposits. Anaerobic bacteria causes two of the most common problems associated with recycling white water i.e. corrosion and odour.

The metabolic activity of microorganisms causes the deterioration of metals by corrosion. The most troublesome group of microorganisms associated with corrosion consists of anaerobic sulphate reducing bacteria.

These reducing bacteria play a dual role in corrosion. First, they act as cathodic depolarisers and second they produce corrosive hydrogen sulphide. Bacterial action reduces the sulphate present in the water to form hydrogen sulphide. The hydrogen

sulphide reacts with the dissolved ferrous iron released at the anode. The result is precipitation of ferrous sulphide.

While there is no direct correlation between the number of sulphate reducing bacteria and the degree of corrosion in a given system, the presence of these organisms in a system shows that a potential for biological corrosion does exist. These organisms can be controlled with the use of biocides, dispersants or a combination of the two products. The resulting decrease of biological corrosion improves machine runnability and minimisation of down time for repair.

A malodour in finished paper is often related to spore forming bacteria, both aerobic and anaerobic. Spore produced by these bacteria is able to survive extreme adverse conditions and are often very difficult to control. Species of *Bacillus* and *Clostridium* bacteria are frequently isolated from board samples. These microorganisms produce high levels of acetic and butyric acid, which produce offensive odour when they germinate in finished products. Normally, it is necessary to use an optimised biocide, such as chlorine or hypochlorite in the white water system and a conventional biocide in the stock system, together with additives, to gain control of spore forming bacteria.

Slime varies from soft, gelatinous, rubbery, or stringy matter to horny growth. Usually it is submerged in the pulp system, but the spatter slime, which forms at or above the water line, is also quite common. Ordinarily mill slime consists of a mixture of microorganisms, fibres, fillers and debris all matted together. Slime usually contain some form of hemicellulosic material probably a levulan, galactan, araban or cellulan which swells in water but does not dissolve in water or even dilute alkali and acid. Each of the various groups of slime produces a characteristic form of slime. Some slime is non-microbial. These may contain starch, aluminium hydroxide or other non-microbiological accretions.

2.a. Bacterial Slime:

Bacteria are one of the principal causes of slime in the paper mill system and both the spore forming and non-spore forming bacteria are guilty. The slime-producing bacteria convert excess food substances in the pulp system into slimy material. This material may diffuse away from organism to produce slime in other locations.

Usually rod shaped bacteria is responsible for slime although the round or cocci forms may also contribute. Some of the genera frequently found in slime are *Aerobacter, Bacillus, Pseudomonas, Flavobacterium, Alkaligens, Cellulomonas, Achromobacter* and the filamentous bacteria. The non-spore forming bacteria are prolific slime producers but fortunately they are easily controlled by toxicants. The spore forming bacteria do not multiply as fast, but they form very tough rubbery slime, which is extremely resistant to heat, and chemicals. The latter are likely to cause trouble in board mills. Aerobacter tend to produce gelatinous films, strands or viscous masses of growth.

The filamentous bacteria produce extremely bulky slimes. The filamentous iron bacteria secrete ferric or manganese oxide within their capsule, thus giving rise to coloured slime which often breaks away to cause staining in the paper. Iron can be precipitated by regular bacteria such as the Siderocapsaceae, but the filamentous type are the most troublesome. Four genera that cause trouble are the *Sphaerotilus, Clonothrix, Leptothrix* and *Crenothrix*. Chlorination of the fresh water is the best means of controlling the iron bacteria.

Pink slime is particularly troublesome in some mill systems. Under severe conditions it will result in pink spots in the paper, pink slime can be cause by the bacteria such *as Serratia marcescens*, or special strains of *Bacillus subtilis* caused by the yeast like fungi or even by mould type fungi.

3. WHY AND HOW THE SLIME (*BIOFILMS*) IS FORMED?

Bacteria become attracted to surfaces for a number of reasons. One may be gravity, microorganisms may just settle out and end up resting on a surface, or bacteria may attract to the positive charges on some inorganic surfaces. But there is evidences that biofilm formation is much more than physical force. May surfaces attract and concentrate nutrients, and many types of bacteria have the capacity to detect and move towards high concentration of nutrients, an ability called chemotaxis.

* How do bacteria develop into a teeming, active community?

Some cells are able to produce copious amount of polysaccharides, which acts as mucus layer and hold the cell to the surface. These are called the primary coloniser. These external slime captures other bacteria called secondary coloniser, which live and grow off the waste products produced by the primary colonisers. There is an extensive and complex microbial community, all tangled up inside the polysaccharide called the biofilm.

* The advantage of living in a biofilm.

Protection from antibiotics/ chemicals.

Scientists have shown that much higher concentrations of antibiotics are needed to kill bacteria in biofilms, compared to free-living bacteria. Originally, it was assumed that the biofilm provides a physical barrier against the antibiotic. This may play a role in providing the protection. However there is evidence that the nature of the colonies themselves provides protection. By growing in microcolonies, the outer cell protect the inner cell from the antibiotic that does not penetrate the biofilm, allowing the inner cells to grow and multiply.

Concentration of nutrients

Because negatives charges are often associated with the biofilm matrix, many nutrients particularly cations are attracted to the biofilm surface. In addition, nutrients with negative charges can exchange ions on the surface. This provides the bacteria cell within the biofilm with food from the surrounding water.

Microbial communities.

No bacterium is an island- that is nearly all bacterium lives with, and depends on, other microorganisms for energy, carbon and other nutrients. The biofilm physically contains substances that are released by microorganisms making them more available to other microbes in the biofilm.

A classic example of this is provided by the degradation of cellulose. Cellulolytic microbes are able to break the cellulose into sugar monomers, which fermenting bacteria can use, giving off smaller organic acids, carbon-dioxide and hydrogen gas, which methanogens or sulphate reducers use for their carbon and energy. In addition to food and energy sources, genetic material can be more easily exchanged within the confines of the biofilm. This increases the potential that new, better-adapted, strains of bacteria will evolve within the biofilm.

4. NATURE OF SLIME AND FACTORS RESPONSIBLE FOR THE SLIME FORMATION

Paper mill slime may be defined as an accretion or accumulation caused by certain microorganisms in the presence of pulp fibre, fillers, dirt and other materials mixed in varied proportion having variable physical characteristics and accumulating at continuously changing rates. Slimes are composed of gelatinous, sticky, viscous, pasty or leathery mass of anaerobic and aerobic bacteria, yeast and moulds, commonly referred as fungi. These may in turn associate with each other in one of the four ways.

1.Synergestic
2.Multi-symbiotic
3.Antagonistic symbiotic
4.Commensalistic

It is this association, which play an important role in the development and selection of microorganisms found in the paper mill system. Factors such as pH, temperature and level of organic nutrients also play a significant role in slime development pattern. Bacterial growth occur in three main stages

The (L) or lag stage is non-exponential that is to say cell division is slow and irregular. In this stage bacteria metabolise nutrients in preparation of growth. The (G) or growth stage is exponential and the following (M) or mature stage reflects the maximum bacterial population that a particular environment can support. It is the last stage that the greatest slime formation occurs.

The area most susceptible to slime formation are those of reduced flow such as the under side of the suction box, joints and pipes. In other areas were flows of pulp is rapid and the abrasive nature of fillers prevents substantial development of slime.

The form of micro-fauna found in the mill environment are also those that occur in natural system. An area difficult to control is that of infection from pulp mass. Pulp is ready source of contamination. Broke system are another difficult area to control along with recirculating white water which account for up to 50% of the microbiological contamination encountered in the mill. Water recycling offers attractions such as heat recovery, increased fibre reclamation, reduced water intake, and reduced wastewater and solid discharge. Unfortunately the high water temperature incurred by closing up the system encourages the rise of more resilient bacteria species. Anaerobic species are favoured at higher temperature due to the reduction of dissolved oxygen present in the water and this causes increased odour and corrosion problem. The high temperature increases the metabolic rate of organisms present in the mill system, accelerating the life cycles and increasing the slime production.

pH also effects the adsorption of the bacteria on to the surface as well as affecting the change of organic and inorganic compounds present in the system. It has been seen that under alkaline pH, cations such as Fe^{3+}, Al^{3+} and Ca^{2+} will coagulate the suspended solids including bacteria, which can be adsorbed on to the particle surfaces. These coagulated materials form insoluble gels, which are involved in more than 90% of all undesirable deposits.

5. STAGES OF SLIME (*BIOFILM*) FORMATION

Development of slime is a complex process involving various stages of formation, which are as follows: -

Stage-I The first stage is the formation of a conditioning layer, which form from reversible and irreversible adsorption of organic and inorganic substances that are present in the water.

Stage-II The next stage is bacterial attachment to the surface, or colonisation. The initial contact of bacterial cells to the surface has been shown to be reversible, but the longer cell stays in place, the harder it is to dislodge as extracellular polymer substances are produced to secure the cell to the surface.

Stage-III Stage three is growth and early biofilm formation. After cells become firmly associated with the surface, they continue to reproduce and formation of the biofilm begins.

Stage-IV In stage four, the biofilm matures and conditions allow particles in the water, such as fillers, fibre and pitch to become trapped by the biofilm matrix.

Stage-V In stage five, once a biofilm reaches a certain thickness, its growth and material discharge tend to be in balance. Studies have demonstrated that single cells or large clumps are routinely, but intermittently, eliminated from the biofilm. The thicker the biofilm the greater the probability that large clumps will be sloughed. An obvious function of sloughing is for cells to be dispersing within a system so other site can be colonised and additional biofilm can be formed.

6. PROBLEMS OF SLIME IN VARIOUS SEGMENTS OF PAPER INDUSTRIES:

The severity of the problem of slime formation in paper making differ greatly depending upon the composition of the raw material employed, process conditions and quality of the end product. One of the segments of the paper Industry producing mechanical grade of pulp for newsprint & magazine paper is severely affected due to the problem of formation of slime since during production of mechanical pulp, nearly 10kg/tp of low molecular weight sugar material is dissolved in the process water which act as a best substrate for the microbial growth.

Another segment of paper mills using recycled waste paper, as raw material will also face a severe slime problem as the recycled waste paper is heavily contaminated especially if it is not properly stored. Further the dissolved carbohydrate, particularly the hemicellulose dissolved during recycling becomes a good substrate for the growth of microbes.

Agro-based pulp and paper mills producing bleached variety of paper also suffers from slime problems which affects the quality of the products due to spots and holes in the paper besides other problems of paper breaks and down time. Paper mills producing packaging grade of paper from unbleached pulps and recycled waste paper are also effected due to slime formation in respect of product quality.

7. CONTROL OF SLIME FORMATION IN PAPER MACHINE:

During the course of study, it has been experienced that it is essential for the mills to adopt various measures to control formation of slime in paper machine. The measures are: -

- design of the paper machine system
- good house keeping
- use of slimicide

7.1. Design of the Paper Machine System:

Equipment surfaces having high smoothness and where the flow speed is high will have lower risk for growth of microbes. Moreover, piping & other equipment should be designed to facilitate mechanical & chemical cleaning. Introduction of mixing into the back eddies and dead ends help in reducing in the growth of microbes. Chests & silos having slow mixing and long dwell time tend to increase the formation of slime. Thus designing of the paper machine system plays an important role in control of microbial growth.

7.2. Good House Keeping:

Regular, systematic & planned cleaning of the paper machine also help in controlling the formation of slime to a great extent by-way-of keeping the biological activity at low level and reduce the requirement of slimicide dosages.

7.3. Use of slimicides

One of the effective measures for control of slime growth has been the use of slimicides under optimised dosing levels.

7.4. Slimicide/dispersants approach

Although many species of microorganisms have been isolated from slime deposits, the actual deposits are usually a combination of microbiological and non-microbiological accumulation. The composition of a deposit will very dependent upon existing conditions at a particular time and the area of deposition in the machine system.

For many years, slime control programme has been based on the use of biocides. In an effort to optimise the cost –effectiveness of slime control programme, dispersant have been incorporated. Although the concept of dispersant in slime control has long been

recognised, it has only recently been emphasised. Recent research work has shown that the ability of a biocide to prevent or remove slime deposit can be improved or enhanced when used in combination with a specific dispersant.

Most slime causing bacteria adhere to the surface by means of a specialised coating, comprising protein and/or starch, referred to as a capsule. The capsule enables the bacteria to adhere firmly to surfaces and prevents the cell from being swept away in the normal process water. Further cell attachment continues and multiplication of these bacteria causes a slime mass to accumulate on the surface. As the mass grows, various forms of inorganic and organic material are trapped.

Biodispersants are typical chemical dispersant, which have been screened and selected to be highly effective against biological deposits. Some have a filming effect when added to a system. This film disrupts the contact between the biofilm and surface. In addition the biocides/ dispersant penetrate the deposit, weakening the structure and making it easier to erode and remove the biofilm with the normal flow of water throughout the system. Biodispersant have very low toxicity, so they are readily acceptable in environmentally sensitive system. They are easy to apply and can provide both prevention and cure. They can be used alone in place of biocides or to supplement biocides. In either case use of biocides in minimised and treatment are more efficient. A dispersant aids the biocide performance in two ways: by wetting the various surfaces, thus interfering with the attachment of bacteria, and by allowing the biocide to penetrate the deposit and the cell at a faster rate, thereby minimising slime accretions.

7.5. Enzyme approach

Enzyme based slimicides acts as biodispersants and consists of stabilised enzymes. The enzymatic slimicide attacks the pilli with which bacterial attach themselves to surface and to each other. Degradation of these structures weaken the structural strength of the biofilm and allow it to be flushed away by the shearing force of the water. Enzyme may have the ability to catalyse the breakdown of a microcapsular. component. Theoretically if a

reduction can be made in the formation of bacterial capsule, then the ability of some bacteria to adhere to the machine surface and to other part can be reduced.

Having advantage of being environmentally friendly and non toxic in nature, the application of enzyme suffer with a setback of its specific nature, which limits its effect to a particular component (capsule varies greatly within species as well as between species). However, recently developed enzyme based slimicides by few of the enzyme/ slimicides manufacturing companies, have not proved to be effective in control of slime.

8. EVALUATION OF SLIME CONTROL PROGRAMME

8.A. MONITORING THE BEGINNING OF BIO FOULING

(a) Visual method

Daily visual observation can be made, of two or three accessible surfaces in the pulp and paper mill systems that are in contact with white water to determine the amount of slime growth on the surface. The slime can be removed at each observation and an estimate made whether the growth is increasing, decreasing or remaining about the same. When there is any doubt about whether microorganisms are primarily responsible for the deposit, microscopic examinations can be made. It may be noted that small amount of capsulated microorganisms can bind together rather large amount of other materials.

(b) Plate counting method

Dilute white water is cultivated on standardised nutrient media, and the colonies are counted. The breeding time of the total number of colonies is 48 hours, for yeast and mildews 5 days. The disadvantage is that this method shows only the biological life in the white water, not that on the side walls. The long evaluation time makes it unsuitable to support decisions for defensive action.

(c) The ATP method

With the ATP measurement the content of Adenosine Tri Phosphate in the white water is measured. ATP is responsible for the energy transmission in the cells. As dead cells give off their ATP relatively quickly through autolysis, the content of ATP in a sample is directly proportional to the number of total living cells. ATP is transferred by luciferine and luciferase, and light is set free. This is then measured in the luminometer, giving a good approximation value for the total number of colonies.

The disadvantage of this method is the expensive measuring instrument and exact operation is essential. The advantage is that results are obtained within approx. 30 minutes.

(d) The test tube method

In this method test tubes are installed in a bypass between the dilution water pump and the white water-1 chest. As the cross section of the pipe and the amount of water through flow are constant and known, the flow characteristics in the test tube can be adapted to those of the problem zones of the paper machine.

The test tube can be opened at any time during production. The surface is then checked for beginning of the bio fouling. This can be done in a qualitative manner by scraping off the film, and then weighing it in g/m^2 in relation to the area. The biofilm sample can also be checked by biologist as to its specific composition. Slime fighting can then be optimised by applying the concerted measures against the main causes.

The quantitative evaluation- mass per area and time also allows a concerted judgement of slime fighting measures for the individual paper machine and production programme. The effectiveness of boil outs can also be checked at the test tube.

(e) Use of slime measuring unit

This method involves the use of a slime-measuring unit of the type shown in fig.-2. this unit could help in determining the trend of slime accretion in a pulp and paper mill. Determinations are made of the amount of accretion of slime in a given period of time on certain area of wooden panel mounted in a stainless steel housing which is preferably connected to a source of unfiltered white water returning to the fan pump. The unit mounted with the top of the housing level in both directions and at a place, which is readily accessible for taking the readings and cleaning the unit.

Before recovering the panel from the unit, the inlet valve should be cleaned and the drain valve opened. When the panel compartment is empty, the wooden panel is recovered from the unit. After the panel is recovered from the unit, it should be held in a vertical position until the water ceases to run in a steady stream and begins to drip. The wet slime consisting of micro-organisms, entrapped fibres, fillers and water, etc. then is thoroughly scrapped from the sides and edges of the panel in to a beaker by means of a sterilised rubber or wooden spatula and weighted. The accumulation of wet slime determined in this manner and is expressed in gm/m^2 of the surface per 24 hour.

In most instances, it will be desirable to determine the amount of slime on the wooden panel once a day, however when the rate of slime accretion is less than 5 gm/m^2/day it may be advantageous to determine the amount every 2 days or once a week or even for more time depending on the situation.

The method for measuring slime is simple and reliable compared to other methods such as bacterial colony count or ATP etc.

8.B. INTEGRATED APPROACH FOR CHARACTERISATION OF BIOFILM (SLIME) COLLECTED FROM AN AGRO-BASED PAPER MILL:

Before an effective slime control programme could be implemented in a paper mill, it is essential to know the source, extent of the contamination and the nature of the slime. Other factors such as the correct dosage and frequency of the biocide, temperature and the pH of the system are also important to the success of any biocide treatment programme. This requires detailed study in respect of physico-chemical, biochemical and microbiological characterisation of slime and white water.

For characterisation and identification of sources of slime, a slime collection unit as shown in fig.1 was fabricated and put up in an agro-based mill producing writing and printing grade of paper. Various samples of white water, pulps and slimes from paper machine white water loop were collected for characterisation with respect to physicochemical, biochemical and microbiological assays. An integrated approach adopted for characterisation of the biofilm samples collected on slime collection unit and from various points in paper machine is shown in fig.1.

8.B.1. Physico-chemical and biochemical characterisation of Slime samples.

Total Biofilm amount analysis

Total amount was estimated from dry weight of biofilm developed, on slime collecting unit fabricated at CPPRI and put up in the paper machine white water circuit in an agro based mill, at fixed time intervals.

Biofilm components

- **Estimation of total inorganic and organic contents**

Total inorganic and organic were measured by standard methods by making ash at 650°C for one hour in muffle furnace.

- **Extraction of extracellular polysaccharide (ECPs) and protein**

ECPs that surround the microorganisms in biofilms require extraction before analysis. Biofilm was suspended in 10 ml of 8.5% NaCl containing 0.22% formaldehyde. The solution was chilled and mixed in homogeniser, during which time the ECPs of the bacteria extracted in to the solution. After removing the residuals at high-speed centrifuge at 12,000 rpm for 30 min, the supernatant was ready for polysaccharide and protein analysis.

- **Estimation of Extra Cellular Polysaccharides (ECPs)**

Quantitative estimation of ECPs was carried out using phenol-sulphuric acid method of Dubois et. al. Into a thick walled test tube of 16 to 20 mm dia pipette 1.0 ml of sample containing the equivalent of 20-100 μg glucose , reagent blank containing 1 ml of distilled water and a set of glucose standard are prepared at the same time. To all tubes 1 ml of 5% phenol is added and mixed by means of a fast flowing pipette. 5 ml of concentrated sulphuric acid is added and mixed in a cyclo mixture. The test tubes are placed in a water bath at 25^0C for 10 to 20 min before reading are taken. The absorbency of the characteristic yellow colour is measured at 488 nm.

- **Estimation of Total Proteins**

Cell mass was indirectly quantified by measuring total protein according to Lowry *et. al.*
To 0.5 ml of suitably diluted sample 2.5 ml of alkaline reagent was mixed, the content were thoroughly mixed and allowed to stand for 10 min. 0.25 ml of Follins reagent was added mix it with cyclo mixture immediately and allow to stand at room temperature for 30 min. The blue colour developed was measured by taking absorbency at 660 nm against a blank of 0.5 ml sample buffer processed under identical conditions as protein content was determined from a standard curve of bovine serum albumin.

8.B.2. Biological characterisation of slime and white water samples

- **Microbial assay of various white water and other samples**

Total microbial assay of various samples of white water, pulp stocks and slime was carried out by standard pour plate method in nutrient agar (NA) medium carried out by serially diluting the samples in normal saline solution to a appropriate concentration in triplicates. The procedure consists of diluting the organisms with a series of sterile normal saline solution. From an appropriate diluted bottle (may be 1: 100000 or 1: 1000000) measured amount of (generally 1 ml) diluted organisms are transferred into empty sterile petriplates, nutrient agar, cooled to 50^0C is then poured into each petriplates. After the nutrient agar has solidified, the plate are incubated for 24 hours to 48 hrs and examined. A plate which has between 30 to 300 colonies is selected for counting. From the count it was calculated the number of organisms per millilitre of the original culture. Experiments were done in duplicates and average was considered for final number calculation in original culture.

- **Relative population density test**

Seven slimicides each of 20 ppm concentration were studied for the relative population density test. Each slimicide was added to distilled water of 50 ml to a final concentration of 40 ppm and added to 50 ml of double strength nutrient broth so as to maintain a final concentration of 20 ppm. Inoculum of 1.0 ml from 24-hour-old culture flask was added to the flask of different slimicides. The flasks were stirred continuously in a rotary shaker cum incubator at 230 rpm. Samples were drawn intermittently and analysed for their viable cell count and expressed as colony forming units.

9. RESULTS AND DISCUSSION:

Formation of slime in paper machine takes place as the white water is enriched with substrate and environmental conditions as temperature, pH etc. are favourable for microbial growth which thereby become a continuous and uncontrollable phenomena.

- **Slime build up trend in paper machine**

Slime build up in paper machine and white water loop is a dynamic process and depends upon various factors like raw materials, fillers used, temperature, pH, etc of the white water. One of the mills was selected by IAPMA and studied for its slime deposit trend in the paper machine by using slime collection unit shown in fig.-2. The deposits on wooden panel were collected and quantified on a particular area which are presented in fig.-3 which shows the trend in slime build up in paper machine. The figure shows that the build up trend is more or less a sigmoid curve. There is a steady increase of slime formation from 0.4 gm/m^2 at 3rd day up to 1.4 gm/m^2 on 25th day onwards, slime deposition does not grow further with time. Studies shows that, maximum growth takes place between 21-28 days which then starts sloughing up of the slime to the white water system with the shear force of the white water. Even after a complete and effective caustic boil out programme, slime deposit starts from the 3rd day.

- **Microscopic examination of slime**

The slime deposited on the wooden panel was collected and studied for is microscopic observation and isolation of bacteria responsible for slime formation. Fig.–4 shows the filamentous bacteria responsible for slime deposition. The different microbial colonies isolated from the slime deposited on the wooden panel are shown in fig.-5.

- **Characterisation of slime and white water samples**

Table-1 shows physico-chemical, biochemical and microbiological analysis of the slime sample collected from the wooden panel of the slime collection points like paper machine wall, wall of krofta, silo etc. It is clear from the result that though the organic and

inorganic composition of the slime collected from slime collection unit is similar in composition with that of mixed slime, the ECPs level is almost double in case of mixed slime as compared to slime of slime collection unit. It is established that these mixed slime are matured and old deposits and have more secretion of ECPs which is the main cause of agglomeration of fibres, fillers and microorganisms. This is the problem-creating agency in paper web on the machine resulting in frequent paper breaks.

Microorganisms reach from one part to the other part of the white water loop through backwater, so it is essential to study the degree of contamination through microbial count in the different parts of the paper machine section. Table-2 shows the microbial count of white water collected from different points of the white water loop as well as process water for papermaking. Results shown in Table-2 clearly indicate that the process water contains as many as 3.3×10^2 numbers of viable microorganisms per millilitre and this number is too high and causative reason of continuous addition of microorganisms to the system. Samples from white water storage tank and premachine chest shows a very high enumeration of 1.1×10^6 and 5.8×10^6 respectively, this may be due to improper cleaning and stagnant of stock/ white water. This high microbial stock could work as the seed of slime formation.

- **Determination of efficacy of various slimicides**

Before going for slime control programme, it is essential to understand the active compounds present in the slimicide. The same was tested and has been tabulated along with their active compounds and shown in Table-3.

Table-4 & Table-5 shows the relative population density test and percentage reduction in the microbe using different slimicides at different time periods (Slcd-1 is being excluded as it is not found to be environmental friendly). The significance of relative population density test implies the effectivity of the test slimicides against the tested (predominant microorganisms present in the white water as well as slime samples) microorganism. The test being performed in nutrient broth medium, tested microorganism gets ample of balanced nutrient required for its growth and regeneration, however the effect of

slimicides can be interpreted from the rate of kill. The table shows Slcd-7 & Slcd-4 have a very effective antibacterial activity against the test microorganism (both are THPS based slimicides), where as Slcd-3 & Slcd-5 have better killing activity at 2.5 hr to 5.0 hr. In comparison, the biocidal activity of the Slcd-5 is best as it has a very consistent activity and at 24 hr also, its action is more in comparison to other slimicides.

The efficacy test of selected slimicides was carried out against the consortium of microorganisms present in the white water system. This test was carried out under dynamic conditions of shaking at 130 rpm and 35^0C in a rotary shaker cum incubator after addition of 20 ppm of slimicide to the test sample of white water collected from the mill to simulate the mill conditions. Since the mill white water contains a number of microorganisms of different groups, this test is very much effective and necessary to study the action of slimicide as broad spectrum, which is effective against diversified group of microorganisms. Table-6 and Table-7 shows Slcd-5 is highly effective and reduces 99.3 % of the microbial count within 15 minute of time period, whereas Slcd-9, Slcd-2 and Slcd-3 reduce 90-91 % within 15 minute of time period.

FIG- 1

INTEGRATED APPROACH FOR BIOFILM (SLIME) CHARACTERISATION

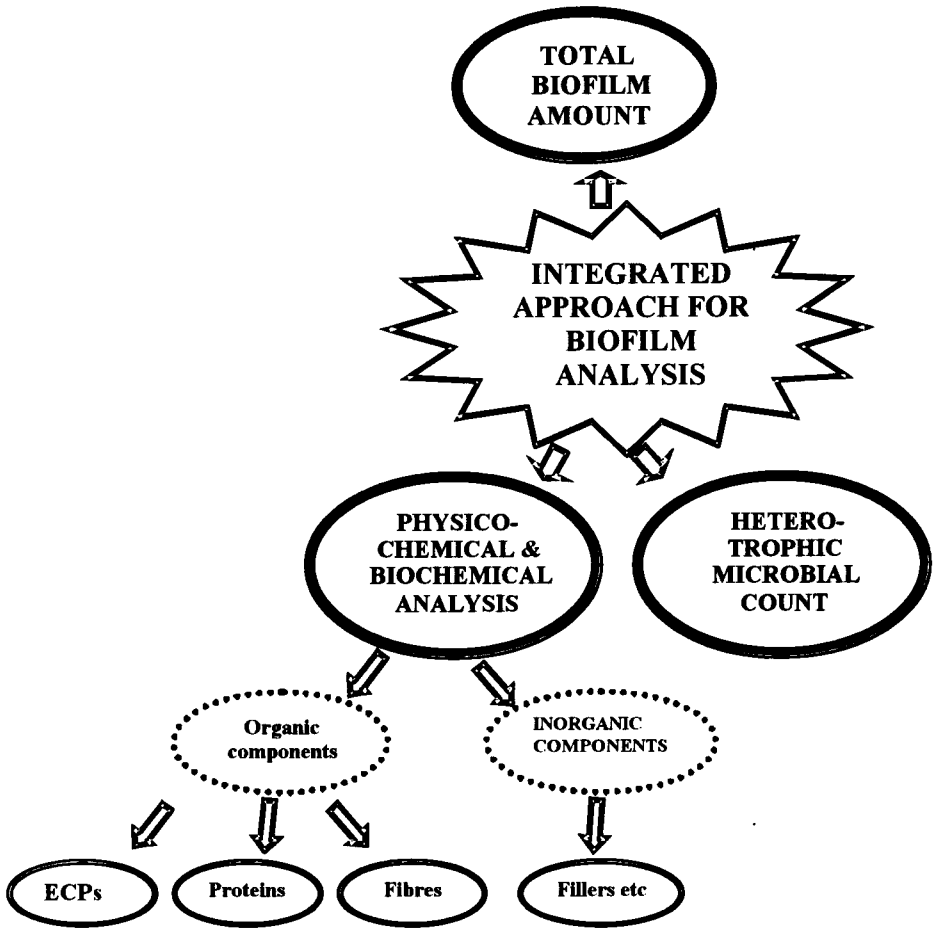

FIG. 2

SLIME COLLECTION UNIT

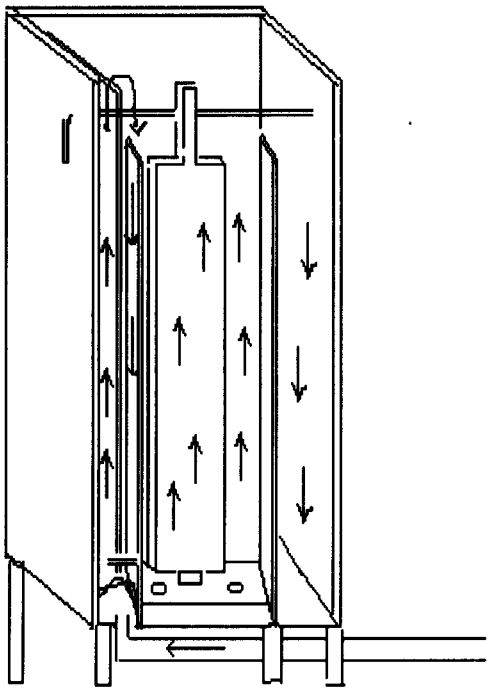

FIG. 3

TREND OF SLIME BUILD UP IN PAPER MACHINE IN AN AGRO BASED MILL PRODUCING BLEACHED VARIETY OF PAPER

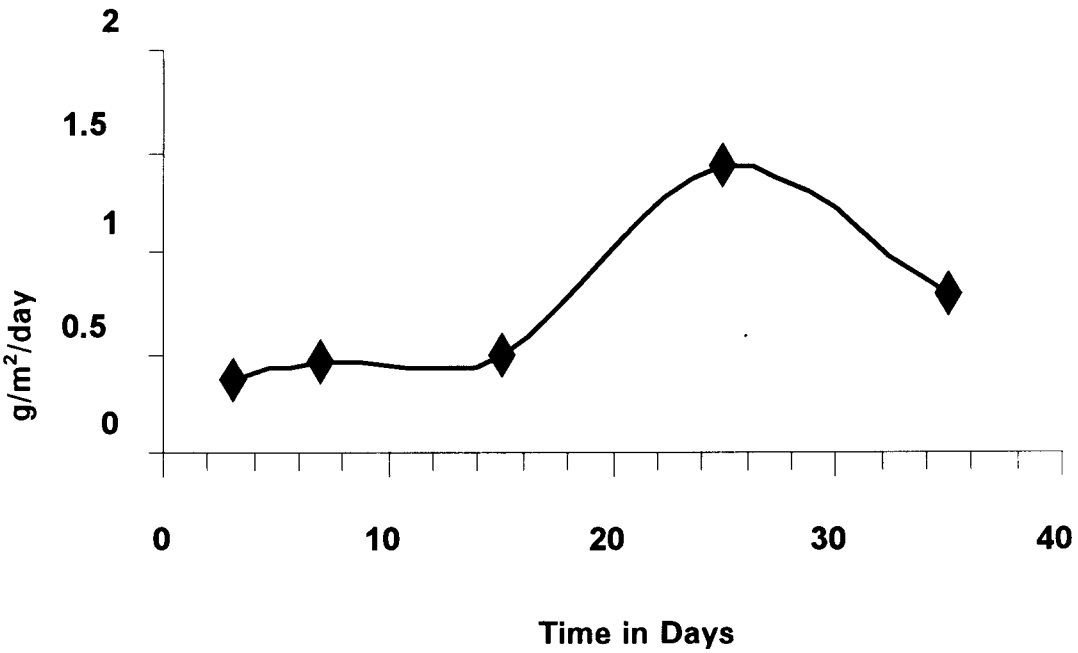

FIG.4

FILAMENTAOUS BACTERIA RESPONSIBLE FOR SLIME FORMATION

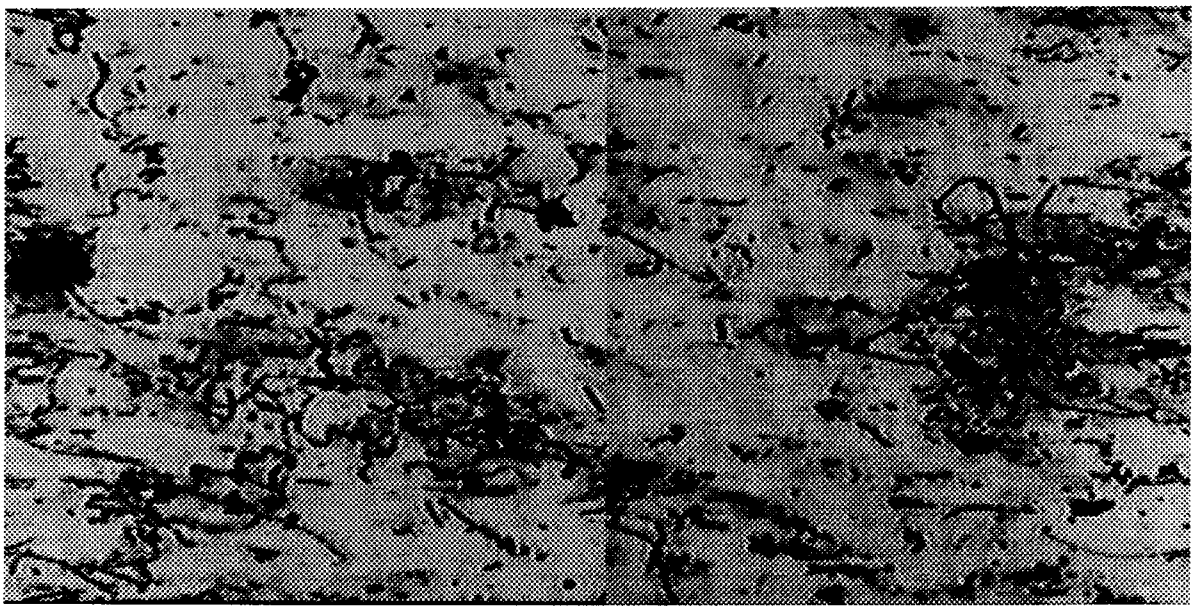

FIG. 5

BACTERIAL COLONIES ISOLATED FROM SLIME SAMPLE COLLECTED FROM THE WOODEN PANEL

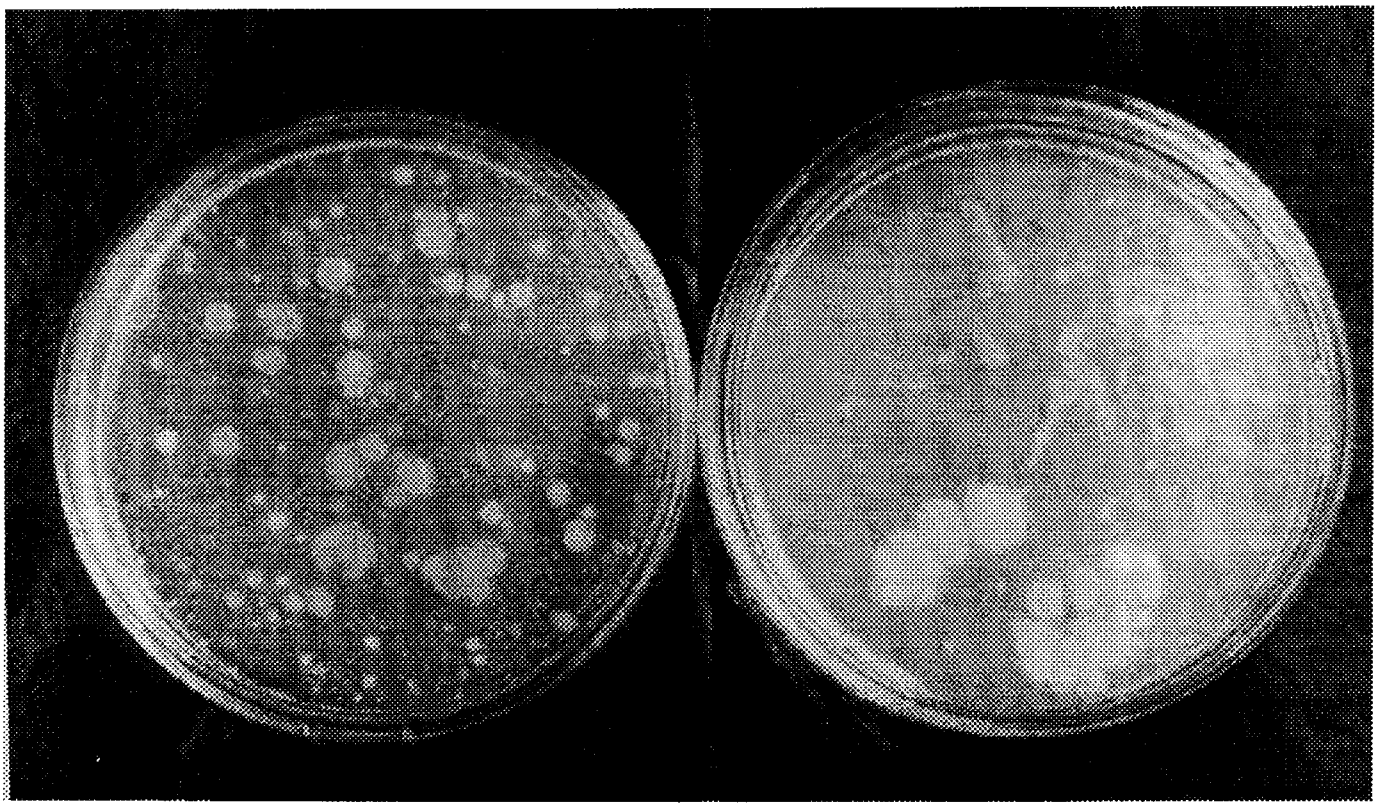

TABLE – 1. PHYSICOCHEMICAL, BIOCHEMICAL & MICROBIAL ANALYSIS OF SLIME SAMPLES			
Parameters	Collection points		
	Slime from Slime collection unit	Mixed slime	Back water
Total organics, % (w/w)	31.00	32	-----
Total inoganics, % (w/w)	69.00	68	-----
Fibres, % (w/w)	29.2		
ECPs,% (w/w)	0.325	0.62	150 μg/ml
Proteins, % (w/w)	0.226	0.21	255 μg/ml

TABLE-2. MICROBIAL ASSAY OF WHITE WATER/ SLIME SAMPLES AT VARIOUS POINTS IN AN AGRO-BASED PAPER MILL		
SL. No.	**Sample collection points**	**Heterotrophic viable microbial count (CFU)**
1	Slime collection unit	1.56×10^7/ gm
2	White water storage tank	1.1×10^6/ ml
3	Krofta	3.2×10^5/ ml
4	Pre machine chest	5.8×10^6/ ml
5	Head box	3.1×10^5/ ml
6	Wire part	9.5×10^5/ ml
7	Process water	3.3×10^2/ ml

TABLE-3. SOME OF THE IDENTIFIED SLIMICIDES AND THEIR ACTIVE PRINCIPLES	
Slimicide code	**Active ingredient**
Slcd-1	Glutaraldehyde
Slcd-2	2, 2, Dibromo, 3-Nitrilo-Propionamide
Slcd-3	5-Chloro-2 Methyl- 4 Isothiozolin-3-One +2, Methyl,4, Isothiazolin-3-One
Slcd-4	2, Bromo-2 Nitropropane-1,3 Diol
Slcd-5	Tetrakis Hydroxymethyl Phosphonium Sulphate (THPS)
Slcd-6	Methylene Bis Thiocyanate (MBT)
Slcd-7	MBT
Slcd-8	THPS + MBT
Slcd-9	Unknown
Slcd-10	Unknown

TABLE-4.
RESULTS OF RELATIVE POPULATION DENSITY TEST OF IDENTIFIED SLIMICIDES AT DIFFERENT TIME PERIODS

Slimicides	Time in Hour					
	0	0.5	2.5	3.5	5	24
	Bacterial enumeration in CFUs					
Slcd-2	182×10^5	35×10^5	26.5×10^5	35.1×10^5	47×10^5	95.2×10^5
Slcd-3	182×10^5	40×10^5	5.7×10^5	1.15×10^5	1.9×10^5	67.2×10^5
Slcd-4	182×10^5	55×10^5	3.6×10^5	1.5×10^5	0.6×10^5	10.8×10^5
Slcd-5	182×10^5	15×10^5	7.5×10^5	18.2×10^5	47.5×10^5	64.8×10^5
Slcd-6	182×10^5	84×10^5	3×10^5	6.2×10^5	18×10^5	72×10^5
Slcd-7	182×10^5	85×10^5	2.55×10^5	6.5×10^5	15.5×10^5	61.6×10^5
Slcd-8	182×10^5	30×10^5	4.7×10^5	8×10^5	4.5×10^5	84.8×10^5
Slcd-9	182×10^5	60×10^5	3.6×10^5	6.1×10^5	9.5×10^5	76.8×10^5

TABLE-5.
PERCENT REDUCTION IN RELATIVE POPULATION DENSITY TEST OF BACTERIA IN RESPONSE TO IDENTIFIED SLIMICIDES AT DIFFERENT TIME PERIOD

Slimicides	Time in Hour					
	0	0.5	2.5	3.5	5	24
Slcd-2	0	80.7	85.3	80.7	74.1	47.6
Slcd-3	0	78.0	96.8	99.3	98.9	63.0
Slcd-4	0	69.7	98.0	99.1	99.6	94.0
Slcd-5	0	91.7	95.8	90.0	73.9	64.3
Slcd-6	0	53.8	98.3	96.5	90	60.4
Slcd-7	0	53.2	98.6	96.0	91.4	66.0
Slcd-8	0	83.5	97.4	95.6	97.5	53.4
Slcd-9	0	67.0	98.0	96.0	94.7	57.8

TABLE-6.
RESULTS OF EFFICACY TEST OF IDENTIFIED SLIMICIDES AT DIFFERENT TIME PERIOD

SLIMICIDE	TIME				
	0HR	15 MIN	45 MIN	2 HR	24 HR
	Bacterial enumeration in CFU				
Slcd-2	1.1×10^6	1.1×10^5	1.9×10^4	7.0×10^3	3.5×10^4
Slcd-3	1.1×10^6	1.0×10^5	1.0×10^3	6.0×10^3	1.3×10^3
Slcd-4	1.1×10^6	7.0×10^3	1.8×10^4	4.5×10^3	7.0×10^2
Slcd-5	1.1×10^6	3.9×10^5	3.4×10^5	7×10^4	3.1×10^4
Slcd-6	1.1×10^6	1.8×10^5	5.2×10^4	1.6×10^4	2.3×10^3
Slcd-7	1.1×10^6	1.7×10^5	4.5×10^4	1.7×10^4	2.0×10^3
Slcd-8	1.1×10^6	1.7×10^5	0.6×10^5	1.0×10^4	4.9×10^3
Slcd-9	1.1×10^6	1.0×10^5	1.7×10^4	4.6×10^4	9.0×10^2
Slcd-10	1.1×10^6	2.0×10^5	7.9×10^3	1.3×10^3	1.85×10^4

TABLE-7.
PERCENT REDUCTION DURING EFFICACY TEST OF IDENTIFIED SLIMICIDES AT DIFFERENT TIME PERIOD

SLIMICIDE	TIME				
	0HR	15 MIN	45 MIN	2 HR	24 HR
Slcd-2	0	90.0	98.2	99.3	96.8
Slcd-3	0	90.9	99.9	99.4	99.8
Slcd-4	0	99.3	98.3	99.5	99.9
Slcd-5	0	64.5	69.0	93.6	97.1
Slcd-6	0	83.6	95.2	98.5	99.7
Slcd-7	0	84.5	95.9	98.4	99.8
Slcd-8	0	84.5	94.5	99.0	99.5
Slcd-9	0	90.9	98.4	95.8	99.9
Slcd-10	0	81.8	99.2	99.8	98.3

M/s Sikka Paper Mils, Muzaffarnagar

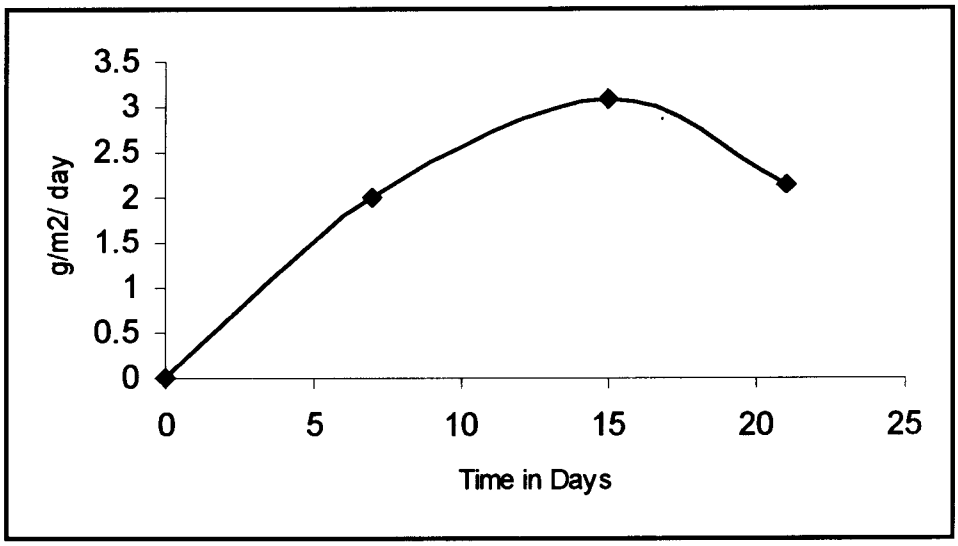

TABLE – 1. PHYSICOCHEMICAL, BIOCHEMICAL & MICROBIAL ANALYSIS OF SLIME SAMPLES		
Parameters	Collection Points	
	Slime from S/C unit	Mixed Slime
Total organics, % (w/w)	42.1	48.27
Total inoganics, % (w/w)	57.9	51.7
ECPs,% (w/w)	0.26	0.26
Proteins, % (w/w)	0.13	0.05

Sampling time in days	CFU in S/C Unit	CFU in M/C	CFU in Back Water	Total Wt, g (OD)	ECP in % (w/w)	Protein, % (w/w)	Organic , % (w/w)	Inorganic, % (w/w)
7	3×10^4	4.5×10^4	13×10^4	1.8	0.26	0.13	30.2	69.8
15	7.8×10^5		4.9×1^0	5.5	0.26	0.05	42.1	57.9
21	1.83×10^4	4.3×10^4	3×10^4	5.7	0.31	0.13	48	52

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TABLE-1(B). MICROBIAL ASSAY SAMPLES FROM VARIOUS POINTS		
SL. No.	**Sample collection points**	**Heterotrophic viable microbial count (CFU)**
1	SR Box layer-1	2.9×10^6
2	SR Box layer-2	2.9×10^6
3	SR Box layer-3	2.7×10^6
4	SR Box layer-4	1.8×10^6
5	Composite Slime sample	8.1×10^6
6.	White water	16.8×10^7

TABLE-2(B) PHYSICOCHEMICAL, BIOCHEMICAL & MICROBIAL ANALYSIS OF SLIME SAMPLES	
Parameters	**Value**
Microbial Count, CFU	**8.1×10^6**
Total organics, % (w/w)	**84.8**
Total inoganics, % (w/w)	**15.2**
ECPs,% (w/w)	**0.84**
Proteins, % (w/w)	**0.31**

TABLE-3. (B) RESULTS OF EFFICACY TEST OF IDENTIFIED SLIMICIDES AT DIFFERENT TIME PERIODS							
Slimicides	**Time in Hour**						
	0	**0.25**	**0.5**	**2**	**3**	**4**	**24**
	Bacterial Colony in CFUs						
Slcd-2	16.8×10^7	85.0×10^4	37.5×10^3	16.2×10^3	5.3×10^3	6.3×10^3	8.7×10^3
Slcd-4	16.8×10^7	67.0×10^4	35.0×10^3	12.4×10^3	8.3×10^3	3.55×10^3	5.9×10^3
Slcd-5	16.8×10^7	98.0×10^4	45.5×10^3	13.6×10^3	10.3×10^3	7.45×10^3	8.5×10^3
Slcd-9	16.8×10^7	80.5×10^4	26.0×10^3	19.9×10^3	12.3×10^3	4.3×10^3	6.3×10^3
Slcd-10	16.8×10^7	72.5×10^4	30.5×10^3	15.0×10^3	15.3×10^3	6.2×10^3	8.2×10^3
Slcd-11	16.8×10^7	73.5×10^4	24.5×10^3	15.7×10^3	15.9×10^3	16.0×10^3	9.7×10^3

TABLE-4. (B) RELATIVE POPULATION DENSITY TEST OF SLIMICIDES AGAINST A PREDOMINANT BACTERIA RESPONSIBLE FOR SLIME FORMATION						
	Time in hr					
Code	0	0.25	1	3	5	24
	Microbial count in CFU					
Slcd.-2	126×10^4	1.5×10^4	9.0×10^4	32.0×10^4	40.5×10^4	2.0×10^6
Slcd.-4	126×10^4	0.5×10^4	34.5×10^4	17.0×10^4	31.0×10^4	2.0×10^6
Slcd.-5	126×10^4	7.5×10^4	10.5×10^4	38.0×10^4	63.0×10^4	7.0×10^6
Slcd.-9	126×10^4	56.5×10^4	40.0×10^4	51.0×10^4	26.0×10^4	82.0×10^6
Slcd.-10	126×10^4	36.0×10^4	22.5×10^4	40.5×10^4	27.0×10^4	73.0×10^6
Slcd.-11	126×10^4	39.5×10^4	47.0×10^4	47.0×10^4	24.0×10^4	68.0×10^6

Samples procured from M/s Bindalas Buplex shows a very high presence of microbial count which are responsible for slime growth as shown in table-1 (B) and 2 (B). Whereas the slime analysed for is characterisation of microbiological and biochemical shows a thick layer of ECPs and proteinaceous material which may subsequently corrodes the paper-machine metallic portions.

The Relative population density test and efficacy test of microorganism present in the white water is shown in Table-3(B) and 4 (B). The efficacy test of six identified slimicides against the microorganism present in the white water system shows the effectiveness of the slimicide on the microorganisms. It is distinct that slimicide code Slcd. 1 & 2 have most efficient slimicidal action against the slime forming microbes.

DRAINABILITY TEST OF THE WIRE PART OF PAPER MACHINE TO PROVE EFFECTIVITY OF THE SLIMICIDES.

Experiment-I

To prove the effect of the slime on drainability test, experiment was performed using Dynamic drainability test following Tappi standard procedure- T-261-cm90 with slight modification using wire part of the paper machine fitted in Dynamic drainage jar.

Sample of pulp stock:

Samples of pulp stock from head box of the paper machine collected from an agro-based mill was used during the experiment, which was treated as control sample. Composite sample of slime collected from various part of the paper machine was premixed and stirred in pulp stock. This sample represented sample –2 i.e. pulp stock with slime sample. Sample –3 represents the sample of pulp stock with slime treated with an ecologically compatible slimicide.

Drainability test:

Conditions as per Tappi standard method T-261-cm 90 for dynamic drainability test were followed to find out the effect of slime on drainability. In this experiment a constant stirring of 500rpm, counter clockwise direction using 500 ml of stock sample was taken in Brit. Jar. The volume of the drained filtrate collected in 30 seconds is noted down and compared with the control sample of pulp stock, pulp stock with slime and after treatment with appropriate slimicide. The ECPs were also measured in all the samples.

TABLE-8 EFFECT OF SLIMICIDE ON DRAINABILITY OF WIRE PART OF PAPER MACHINE

Particulars	Sample-I Pulp stock from Head box (control)	Sample-II Pulp stock from Head box with slime	Sample-III Pulp stock from Head box with slime after treatment with slimicide
Drainage rate (ml./min.)	290.6	262.6	273.0
ECPs as reducing sugar (mg/lit)	50.76	69.2	68.3

From the results indicated in the table it is observed that with addition of slime in the stock of paper machine Head box, the drainability became poorer as drained volume of the filtrate was reduces from 290.6 ml (in control sample without slime) to 262.6 l (stock with slime). Now with treatment of this slime rich pulp stock with appropriate dosages of identified ecologically compatible slimicides, there was improvement in drainage as indicated from increased volume of the filtrate from 262.2 ml to 273.0 ml. Determination of the exo-polysaccharide (ECPs) were indication of increased concentration in pulp stock with slime.

Experiment-II

To study the effect of slimicide on the wire part of paper machine on improvement in drainability experiments were performed using the Dynamic Drainage Jar, Tappi standard method T-261-cm 90 and pulp stock from Head box.

Slime development on the wire part:

In order to test the effectiveness of slimicides on drainability of the wire part, experiments were planned in laboratory using wire part of the paper machine collected from the mill. Dynamic drainage test was employed during the study as per the Tappi standard method T-161 cm 90 using paper stock from paper machine Head box of the mill. Before starting of the drainability experiment, wire part was cut in to the circles of the size to be used in Dynamic drainage jar/ Brit. Jar and these pieces of circles were thoroughly washed.

Slime was allowed for 15 days to build up on the wire part drawn from paper machine and was used in the experiment, which are kept dipped in the paper machine back water drawn from the mill and conditions were kept dynamic during the build up of slime just to simulate the paper machine conditions as far as possible. These wire parts after slime development on it, were treated with selected slimicides and enzyme combination of Cellulase: α-amylase: protease. 2:1:1 (w/w) prepared in our laboratory with predetermined optimised dosages for specified time period of 1 hour. During the treatment conditions were allowed to be dynamic and temperature was maintained 37^0C. Drainage conditions, as per Tappi standard method T-261-cm 90 for dynamic drainability test were followed to find out the effect of slimicide/enzyme treatment of wire part on drainability wherein under constant stirring at 500rpm, counter clock wise using 500ml sample of stock taken in Brit. Jar, the volume of the drained filtrate collected in 30 seconds is noted down and compared among the control sample of pulp stock, pulp stock with slime and after treated with appropriate slimicide/enzyme.

TABLE-9 EFFECT OF SLIMICIDE ON WIRE PART OF PAPER MACHINE TO IMPROVE THE DRAINABILITY		
SL. No.	**Particulars**	**Drained volume in 30 seconds**
1.	Control wire build up with slime	164 ml
Slimicide treated wire build up with slime for 1 hr.		
2.	Slcd-2	179 ml
3.	Slcd-9	180 ml
4.	Enzyme treated	166 ml
5.	Caustic washed	180 ml

It can be observed from the table that wire with slime build up have a very low drainage capability as drained volume is 164 ml. The drainage volume increases to 180 ml when it is treated with caustic, the same 180 ml drainage is also obtained when it was treated with slcd-9. The enzyme treated wire part shows apparently no improvement in drainability i.e. 166 ml as against 164 ml in case of slimed wire.

PROPOSED PLAN FOR MILL SCALE TRIAL USING IDENTIFIED ECOLOGICALLY COMPATIBLE SLIMICIDES IN AN AGRO-BASED MILL

Proposed to conduct mill scale trials for a month time to monitor the effect of the slimicide application.

Activities:

- **Procurement of identified biocide/biodispersant** from the identified companies.

- **Evaluation of effectivity of the sample of biocides** at CPPRI to be procured in bulk quantities against mills collected slime/white-water before mill scale trial.

- **Selection of biocide dosages and dosing points**

Based on laboratory evaluation, doses of the biocide to be decided which will vary from 15 ppm – 20 ppm. Initially, after the caustic boil out and scrapping of the side walls of various equipment/ tanks of the paper machine white water loop, shock doses with higher concentration i.e. 20 ppm or 1 kg./tp is advisable later on when the CFUs are under control gradually the doses might get reduced from 1 kg/ tp to 0.3 kg/ tp. or less. This will depend upon determination of the microbial load during continuous running of the paper machine. Further alternate use of two or more identified biocides with different active molecule shall be used in order to avoid resistance of the microbes against any particular biocide over prolonged time period.

- **Biocide/biodispersant approach**

It is advisable to supplement a biodispersant along with biocide, which has the ability to penetrate the biofilm caused by microbes and help to increase the effectivity of the biocide.

- **Dosing points**

Proposed dosing points for biocide and in combination of biodispersant are shown in process flow diagram of the approach flow system of the paper machine.

1. **Biocide:** Addition to long circuit i.e. in let to fan pump/silo, machine chest and broke pulper.
2. **Biodispersant**: Addition to the thick stock in machine chest, silo.

- **Monitoring parameters**

1. **Qualitative /quantitative**:

I. Visual observation by touching

II. Slime builds up trend at slime measuring unit

2. **Physicochemical/biochemical**: Determination of colony forming unit i.e. CFU in thick stock and white water to be collected from various points during trial.

Paper Machine performances: Runnability of the paper machine in respect of:

- Paper break due to slime
- Spots on the paper sheets, if any
- Improved drainability

Table-3. Some of the identified slimicides And their active principles		
Code	**Trade Name**	**Active ingredient**
1.	**Nalcon 7334**	glutaraldehyde
2.	**Nalcon 7649**	2, 2, dibromo3 nitrilo-propionamide
3.	**Nalcon 7647**	5 chloro-2 methyl- 4 isothiozolin-3-one +2, methyl,4, isothiazolin-3-one
4.	**Excel solid**	2, bromo-2 nitropropane-1,3 diol
5.	**Precexcel-3013**	THPS
6.	**Finor-CWT 302**	Methy bis thiocyanate (MBT)
7.	**Antimucine p-95-08 Liq**	MBT
8.	**Antimucine TBT Liq**	THPS + MBT
9.	**Bussan 888**	
10.	**Precexcel-3013**	
11.	**Treline**	Methay iso thiozoline