Study Of Antimicrobial Properties Of Sodium Salt Of Sulfonated Cashew Nut Shell Liquid (CNSL) For Slime Control In Paper Mill

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ABSTRACT

The major in-mill microbiological problem of the paper industry is "*Slime*". Slime is the accumulation of microbiological growth, composed heterogeneously of microorganisms, by-products of microbiological growth, fiber and various organic and inorganic components. Microbial 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. The commonly used effective slimicides are chlorophenols and mercury based compounds. These compounds possess a negative environmental threat to their effluent streams, contain particularly persistent toxic compounds. Consequently there became public awareness of the long term effect of organochlorine compounds and the need to look for alternatives. This leads to the development of alternative source of protectants from natural materials, which involves finding more environmentally acceptable substances like wattle tannis and cashew nut shell liquid (CNSL). These are less toxic than the conventional compounds. Sodium salt of CNSL is obtained from CNSL by sulfonation. The compound is less toxic and biodegradable. It is a surface acting agent, found to have good penetrating, dispersing and insecticidal properties. The compound was used to study the killing efficiency on microorganisms present in white waters of hard wood based paper mill. The study included dose fixation of the compound as a slimicide, comparison with other slimicides and killing efficiencies in different white waters of paper machines. The killing efficiencies are found to be in the range of 70-90% in different paper machine white waters. This will provide an eco-friendly slimicide to paper mills.

KEYWORDS: *Microbial slime, Slime control, Sulfonated CNSL, Slimicides, Microbial deposits, Plate count*

Introduction

The latest trends in the paper industry are towards manufacturing by a neutral or alkaline process, greater consumption of secondary fibers and the closing up of process water systems. Paper machine systems usually support significant growth of microorganisms due to congenial and favorable conditions there. The pH, elevated temperatures, high nutrient levels and increased reuse of process water makes a paper mill system a perfect breeding ground of microorganisms. Almost all paper machines are periodically affected with problems caused by microscopic organisms, usually referred as '*Slime.*' Microbial deposits that are composed of varied micro flora along with fibers, fillers and dirt are the most troublesome. Slime producing microbes secrete extracellular polysaccharides that gum up the process machinery. Uncontrolled microbial growth in the system results in reduced product quality, odors problem, safety issues and lost production[1]. The presence of slime affects the quality of finished product by introducing defects such as slime spots, discoloration, pinholes and poor sheet formation. Slime related problems are a huge economic drain. Slime deposits are mainly due to bacteria and fungi. In most of the cases the slime causing deposits are bacteria. Most of the slime occurs in the neutral pH condition. Usually at lower pH levels, slime might be caused by the growth of fungi or moulds.

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 substance in the pulp system in to slimy material. This material may diffuse away from the organism and produce slime in other locations. Usually rod shaped bacteria are responsible for slime, although the round or cocci forms may also contribute. Some of the genera frequently produce slime are *Aerobactor, Bacillus, Pseudomonas, Flavobacterium, Alcaligenes, Cellulomonas, Achromobactor,* and the filamentous bacteria[2]. The non spore forming bacteria (particularly *Aerobacter* spp.) are one of the most prolific slime producers.

Fungal slime

Slime is produced by fungi of both the mould type and yeast type. The slime deposits produced by fungi are the results of tangled masses of filaments which entrap other ingredients, such as fillers, fibers and undispersed bits of coating[2]. Fungal slimes are ordinarily loose, bulky, and stingy in contrast to the firm, tough slimes produced by bacteria. Bacteria ordinarily produce more slime trouble than fungi. Among the most important types of fungi found in paper mill slime are *Oidium, Monilla, Trichoderma, Aspergillus, Penicillium, etc.*

Mill conditions have pronounced effect on the amount and type of microbiological growth. Some of the important mill conditions are fibrous furnish, non fibrous furnish, surface of

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attachment and methods of handling white water. The types of microorganisms found in paper mill systems depend upon the fiber furnish. One of the most important factors is the amount of available food substance. Ground wood pulp is very rich in food substance; straw, semi-chemical and waste paper pulp are also rich in food substance. Chemical pulps are relatively low in food substance. However, nearly all pulp systems contain enough food to support a variety of microorganisms. The addition of non-fibrous materials such as starch, glue, and vegetable gums to furnish is often a source of contamination.

Slimicides

Conventional slimicides used in paper mills are chlorine, chlorinated phenols, chloramines, organic mercuric compounds. There are many other substances which can be used in paper mill systems to control microbial activity. These include copper sulfate, potassium permanganate, zinc sulfide, sodium salt of dihydroxy dichlorodiphenylmethane, etc. Slimicides may act in the following ways to control the growth of microorganisms:

1. Coagulation of proteins
2. Inactivation of enzymes
3. Disruption of cellular lipids
4. Damage to genetic apparatus
5. Destruction of DNA
6. Damage to cell walls

Conventional slimicides used in past have not been found ecologically compatible and environmentally acceptable. Today, due to changing customer's consciousness towards environmentally clean products industry is forced to find alternative slime control additives, which are environmentally acceptable[3].

Natural CNSL is reported to have useful insecticidal, fungicidal, antibacterial, antitermite and medicinal applications[4, 5], but it cannot be used as slimicide because of its oily nature. Therefore, it was decided to make use of a compound of CNSL which can be conveniently used for the purpose. Cashew nut shell liquid (CNSL) is a byproduct of cashew industry. CNSL is a complex mixture of anacardic acid, cardanol, cardol and 2-methyl cardol. Commercially, natural CNSL is vacuum distilled to get cardanol. It contains 60-70% of cardanol, 10-15% of cardol and 15-30% of polymeric residol. Residol is separated out and remaining mixture is sulfonated at low temperature to get sulfonated compound[6].The sulfonated CNSL is then treated with NaOH to get sodium salt. It is a surface active substance. This compound retains its antimicrobial properties as that of the parent compound (CNSL). Unlike CNSL, sodium salt of sulfonated CNSL can be conveniently used as slimicide because of its non oily nature. Sodium salt of sulfonated CNSL is an emulsion, buff in color with soapy smell. The compound has following physico-chemical properties (table 1).

Table 1
Physico-chemical properties of sodium salt of sulfonated CNSL

[illegible]	Values
Color	Light brown
Specific gravity (26 ^{0}C)	0.9916
Moisture content (%)	25
pH	5.0-6.0
Surface tension (Dyne/cm)	34.94
Viscosity (cP)	7390
Iodine value (SL I_2/100g)	180-200
HLB value	6-8

Sodium salt of sulfonated CNSL finds its application as penetrating aid, in Kraft pulping process. It increases the rate of delignification[7]. It is also found to be effective as a dispersant in coating of paper and as additive in neutral rosin sizing. These applications in paper industry are mainly due to its surface acting, penetrating, dispersing and solubilisation properties, which are being studied elaborately in the laboratory.

Experimental :
Materials

Petridishes (10 x 1.5 cm), 1 ml pipette, 10 ml pipette, transfer pipette, measuring flasks (100 ml), glass beaker, glass bottles (1.0 l capacity), digital colony counter, agar (dry powder) and sodium salt of sulfonated CNSL-10% suspension in water.

Methods

Nutrient agar medium was prepared by dissolving 2.8 gm in 100 ml distilled water, plugged the conical flask with cotton plug. Water and nutrient agar medium were steam sterilized at 3 kg/cm^2 pressure for 20 minutes at 120-125 ^{0}C. All the glasswares were first washed with soap followed by thorough rinsing with tap water, then autoclaved at 3 kg/cm^2 pressure for 20 minutes and dried in oven at 60 ^{0}C for 5-6 hours. Circulating process water from white water pit under Fourdrinier sections of paper machine was collected. Experiments were carried out by pour plate method.[8]

Machine conditions and wet end additives

The quality wise, operating conditions and wet end additives on paper machine 1, 2, 3, 4 and 5 are given below

Paper machine-1

Mostly writing and printing grades (GSM varied from 70 to 270) are being manufactured. Starch solution of 6% to 9% is added at size press in surface sized maplitho, pulp board UHB. On the other hand, starch solution of 3% to 5% is to be maintained at size press of paper machine-1 for surface sized deluxe varieties. All the paper qualities contain starch solution at size press ranging from 18-22 kg/tonne of paper. Specialty papers such as MICR cheque paper, parchment and Azure laid TSAD are manufactured. White water is acidic in nature.

Paper machine- 2

Mostly writing and printing, soap wrappers, MG packaging, MG color pulp boards and natural shades of GSM 80-300 are being manufactured. White water is acidic in nature.

Paper machine-3

Different grades of paper being manufactured at paper machine-3 are Creamwove, surface sized maplitho classic, B2B Copier, MCPHHBCG, and Azure wove of GSM 52 to 89. White water is alkaline in nature.

Paper machine-4

Different grades of paper being manufactured at paper machine-4 are Duplex Board, Grey back, White back with LWC and HWC of GSM 230-480. White water is neutral in nature.

Paper machine-5

Different grades of paper being manufactured at paper machine-5 are Duplex board, Grey back, and Kraft back with LWC and Mill wrapper of GSM 200 to 650. White water is neutral in nature.

Results

I. Dose fixing of sodium salt of sulfonated CNSL

For dose fixing, white water of paper machine-3 was collected. To 1 liter of white water sample, different concentrations (25 to 225 ppm) of sodium salt of sulfonated CNSL were added. The bottles were shaken well and then kept for 45 minutes. After 45 minutes, added 1ml of this treated white water to 99ml sterilized water (10^{-2} dilution). Shaken the flask well and transferred 1 ml from the first flask to the second flask containing 99ml of sterilized water (10^{-4} dilution). Shaken the flask well and transferred 10ml from the second flask to the third flask containing 90ml of sterilized water (10^{-5} dilution). Shaken the flask well and transferred 1ml each from the dilution flask numbered 10^{-2} into 3 sterile petridishes, similarly repeated the same for 10^{-4} and 10^{-5} dilutions also. To these petridishes then added cooled agar medium by swirling the plates while addition so as to mix the medium and treated white water sample. One plate was kept as control containing only medium. Allowed the medium to set and kept the plates in an inverted position at 35 ^{0}C for 48 hours of incubation. After 48 hours of incubation, colonies were counted from the plates of all the dilutions. Mean of these counts were calculated and expressed the results in Cfu/ml (table 2).

II. Comparative study of sodium salt of sulfonated CNSL with other slimicides

A comparative study was done by using some popular slimicides generally used in paper mills. Slimicides chosen were non-oxidizing type biocides. Killing efficiencies of the slimicides analyzed were, (A) sodium salt of sulfonated CNSL, (B) alkali dimethyl benzyl ammonium chloride and (C) tetra kis (hydroxy methyl) phosphonium sulphate, with dosing of 200, 40 ppm respectively. For this study, white water of paper machine-3 was used. Results are tabulated in table 3.

III. Study of effect of slimicide for different machine white waters

In this study, circulating process waters from white water pit under Fourdrinier sections of paper machines 1, 2, 3, 4 and 5 were collected and whole experiment was repeated as above, using a dose of 200 ppm for sodium salt of sulfonated CNSL. In this experiment for machines 1and 2 white waters with dilutions of 10^{-2} and 10^{-3} were prepared and used. Results are tabulated in table 4.

Discussion

- ❖ It is clear from table 2 that sodium salt of sulfonated CNSL has antimicrobial activity and this activity increases as the concentration increases. At 200 ppm dosing, highest killing efficiency (93.75%) is observed and it remains constant on further increase in concentration. Therefore, a dose of 200 ppm was fixed for slimicidal activity of sodium salt of sulfonated CNSL.
- ❖ The results of table 3 clearly show that sulfonated CNSL has quite comparable killing efficiency to other slimicides, commonly used in paper mills. Dosing of sulfonated CNSL is high in comparison with slimicides B and C.
- ❖ From table 4 it is clear that sulfonated CNSL is an effective slimicide for the white waters of all the machines. This shows that the effective pH range for sulfonated CNSL as a slimicide is 3.0 to 8.0.
- ❖ Different values of killing efficiency are observed for white waters of different machines at a constant dosing of 200 ppm of sodium salt of sulfonated CNSL. This is mainly because of the variation in the properties of white waters collected from different machines. According to the qualities of paper to be produced at these machines, different types of wet end additives are being added at the stock preparation sections. Thus, the white water samples collected from different machines contain different compositions of fibrous and non-fibrous additives. Therefore, the population of different types of bacteria may vary due to variation in composition of substrate. Wet end additives of organic and inorganic nature provide nutrients for microbial growth.

Table 2 Dose fixation of sodium salt of sulfonated CNSL using white water of paper machine-3

Dose (ppm)	Counts (Cfu/ml)	Blank (Cfu/ml)	Killing efficiency (%)
25	225 x 10^5	325 x 10^5	30.77
50	210 x 10^5	325 x 10^5	35.38
75	155 x 10^5	325 x 10^5	52.30
100	110 x 10^5	325 x 10^5	66.15
125	40 x 10^5	125 x 10^5	68.00
150	35 x 10^5	125 x 10^5	72.00
175	15 x 10^5	125 x 10^5	88.00
200	5 x 10^5	80 x 10^5	93.75
225	5 x 10^5	80 x 10^5	93.75

Table 3 Comparison of sodium salt of sulfonated CNSL with different slimicides

Slimicides	Counts (Cfu/ml)	Killing efficiency (%)
Blank	140 x 10^5	
A	10 x 10^5	92.85
B	19 x 10^5	85.71
C	30 x 10^5	78.57

Table 4 Study of effect of sodium salt of sulfonated CNSL as slimicide for different machine white waters

Paper machine	Counts (Cfu/ml)	Blank (Cfu/ml)	Killing efficiency (%)
1	12 x 10^2	120 x 10^2	90.00
2	15 x 10^2	135 x 10^2	88.88
3	5 x 10^5	80 x 10^5	93.75
4	110 x 10^5	480 x 10^5	77.08
5	13 x 10^5	90 x 10^5	85.55

- The biological activity of CNSL clearly shows that it has antibacterial [9-11] and antifungal [12-16] properties. The ability of CNSL to inhibit bacterial, fungal, protozoan and parasite growth depends on its interaction with cytoplasmic protein and its membrane disturbing properties[17].
- Sulfonated CNSL is a complex mixture of sulfonated cardanol and cardol. The compound has SO_3H group on its benzene ring along with OH group and a long alkyl side chain $C_{15}H_{31-n}$. The physicochemical properties (table 1) show that it is a nonionic surfactant. The slimicidal activity of the compound is mainly due to the sulfonated phenolic lipids present in it. This study shows that the antimicrobial activity is retained in the molecule even after decarboxylation and sulfonation of the parent compound (CNSL).The high optimum dose for slimicidal activity (200 ppm) indicates a slightly lower antimicrobial activity than CNSL itself.
- The current trend in slime control is toward preventive measures, more general control of troublesome deposits, flexible applications and use of carefully formulated products which can be readily employed in complete programs. Emphasis on killing action alone is no longer the only objective. Mills use products which perform various functions well. In addition to wide control spectrum, a compound should possess positive penetrating and dispersing properties in order to enable the chemical to establish intimate contact with the living cell and to keep both organisms and deposit forming particles moving through the system[18].
- Thus a compound with surface acting and antimicrobial properties i.e. sulfonated CNSL is a better choice than the synthetic chemicals for slime control formulation. The objective of this study was to provide a slimicide, which has natural origin and is environmentally acceptable.

Conclusion

Sodium salt of sulfonated CNSL has antimicrobial activity and dispersant property also, as it is a surface active compound. A surface active slimicide provides superior killing or inhibition action. Therefore, the use of sodium salt of sulfonated CNSL as a slimicide will provide a slimicide combination with dispersant activity to achieve optimal slime control program in terms of efficiency. This investigation gives rise to a less toxic, biodegradable, safe and ecologically compatible slimicide than conventional synthetic slimicides.

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