Evaluation of Sodium Salt of Sulfonated Cashew Nut Shell Liquid (CNSL) as an Effective Biocide in Paper Mill

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ABSTRACT

Sodium salt of sulfonated cashew nut shell liquid (SCNSL) has antimicrobial activity and dispersant property; it can be used for slime control in paper mill. This study is already done and published in IPPTA J. (2011) April June issue. The present study has been done to evaluate the potential of SCNSL as a biocide and to provide a complete solution to overcome the acute microbial problems that occur in a paper mill. Problems caused by uncontrolled growth of microorganisms in paper mill are mainly biological slime (bio film or biomass), plugging and fouling, biodeterioration and microbially induced corrosion. The antibacterial and anti oxidant properties of SCNSL synthesised from commercial CNSL (cardanol) by sulfonation, were tested in the laboratory. The antibacterial properties were studied against six types of bacteria responsible for slime production in paper mill. It exhibited minimal inhibitory concentrations (MIC) against *Flavobacterium antarcticus, Bacillus megaterium, Bacillus pumulis, Pseudomonas aeruginosa, Bacillus subtilis and Actinomyces howellii.* Antioxidant studies for ferric reducing antioxidant power and hydroxyl radical scavenging activity were done. *The results confirmed antimicrobial and antioxidant properties of SCNSL and thus confirmed its application in paper mill as a effective biocide.*

KEYWORDS: *Microbial slime, Slime control, Sulfonated CNSL (SCNSL), Biocide, Microbial deposits, Antibacterial activity, Antioxidant power*

Introduction

Most microbiological problems associated with industrial process cooling water systems are caused by a mixed group of microscopic plants or animal like organisms referred to as micro flora. The micro flora is typically composed of algae, fungi and bacteria. Very rarely is a single type of microorganism completely responsible for wide spread operational problem in a system. Each of the different types of microorganisms possesses few unique characteristic as well as many characteristics in common. An insight in to the growth requirement and characteristic of microorganisms helps to indentify and control the problems associated with process cooling water systems[1].

Excessive growth of microorganism in paper mill systems leads to undesirable effects which interfere with normal operation of the mill and sometimes makes the final product unfit for use. The two principal results of excessive microbial activity are slime production and corrosion, but there are many other troubles caused by microbial growth, including degradation of the cellulose , creation of bad odors, and spots/stain on paper. Besides from principal problems of slime formation and corrosion, there are many troubles which may be traced to excessive microbial growth. There are number of wool destroying organisms which attack paper mill felts, causing degradation and loss of tensile strength and there by shortening the life of the felt. Bacteria in pulp system can create a condition which is harmful to rosin sizing[2].

Pulp and paper industry are looking at a high degree of closer of the process water or the use of biological waste water treatment plants due to stringent environmental regulations and consumer attitude. This has been achieved by recycling of process water. In 1970 s, approximately 100 m^3 of water was used per metric ton of paper manufactured, but nowadays less than 10 m^3 of water is used per metric ton of paper[3]. As a result, the paper machine white water has become richer in nutrient salts and degradable carbon, thus contributing to microbial problem. Almost all the paper machines are periodically affected with the problems caused by microscopic organisms, usually referred as slime. There is always some corrosion of metal parts in a paper mill. Many conditions may be responsible for excessive corrosion, and one of these is certain type of bacteria[4].

Bacteria growth is 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 (e.g. *Actinomyces howellii)*. The non spore forming bacteria (particularly *Aerobacter* spp.) are one of the most prolific slime producers.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as a plastic, a synthetic fibre, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions[5]. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols[6].The hydroxyl radicals are most reactive free radical that can damage DNA in its proximity. Some of the dietary phytochemicals are capable of either sequestering Fe^{2+} and/or scavenging HO radicals thus preventing damage to DNA. The antioxidant can donate hydrogen radical to the hydroxyl radical and neutralize them. Antioxidants are used as food additives to help guard against food deterioration. Antioxidants are frequently added to industrial products[7]. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasoline to prevent the polymerization that leads to the formation of engine-fouling residues[8].

Sodium salt of CNSL is obtained from CNSL by sulfonation[9]. The compound is less toxic and biodegradable. It is a surface acting agent[10], found to have good penetrating[11], dispersing[12] and insecticidal properties[13]. 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 slimicide 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[14].

Experimental
I Study of antibacterial activity

Sulfonated CNSL was used to study the antibacterial activity by using agar diffusion method[15, 16]. The Bacteria analyzed were Flavobacterium antarcticus (MTCC 676), Bacillus megaterium (MTCC 428), Bacillus pumulis (MTCC 2296), Pseudomonas aeruginosa (MTCC 7837), Bacillus subtilis (MTCC 736), and Actinomyces howellii (MTCC 3048). The solvent used was water. Standard Antibiotic used was Gentamycin. Concentrations of sulfonated CNSL screened for microbial activity were 50, 100, 150, 200, 250 ppm. Sample solution was prepared by dissolving 5mg of sulfonated CNSL in 2ml of water to get stock sample concentration 2,500 ppm

Media Used (g/l)

For *Flavobacterium antarcticus*- Peptone-5 g, yeast extract-2 g, agar-15 g, soil extract-50 ml.

For *Bacillus megaterium, Bacillus pumulis, Actinomyces howellii*- Beef extract-1g, Yeast extract-2 g, Peptone-5 g, NaCl-5.0 g, agar-15 g.

For *Pseudomonas aeruginosa, Bacillus subtilis*- Peptone-10 g, Yeast extract-5 g, NaCl-10 g, agar-20 g

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hour. The culture of *F. antarcticus* was grown at 15°C. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hour old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. The control wells with Gentamycin were also prepared. All the plates were incubated at 37ºC for 24 hour and the diameter of inhibition zone were noted. The results are presented in table-1and 2 as diameter of inhibition zones in cm. finally photographs were taken for all the petriplates

II Anti-oxidant analysis
1. Ferric Reducing Antioxidant Power[17]

- Various concentrations of SCNSL (10µg, 50µg, 100µg and 500µg) in Dimethyl sulfoxide were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide.
- The mixture was incubated at 50ºC for 20 min. Next, 2.5 ml of 10% (w/v) trichloroacetic acid was added.
- 5 ml of above solution was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride.
- The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant. The results are presented in table-3.

2. Hydroxyl Radical Scavenging Activity[18]

- Various concentrations (10µg, 50µg, and 100µg) of SCNSL in methanol were taken in different test tubes and made up to 250µl with 0.1M phosphate buffer.
- One millilitre of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of dimethyl sulfoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid.
- These reaction mixtures were incubated at room temperature for 15 min.
- The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v).
- Three millilitres of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for colour development.
- The intensity of the yellow colour formed was measured spectrophotometrically at 412 nm against reagent blank.
- Ascorbic acid was used as reference standard.
- The percentage hydroxyl radical scavenging activity was calculated by the following formula:

% hydroxyl radical scavenging activity =

$$\left[1 - \frac{\text{difference in absorbance of the sample}}{\text{difference in absorbance of blank}}\ 100\right]$$

The results are presented in table-4.

Results and Disscution

Gentamycin was taken as a standard compound for the study of antibacterial activity, its dose was fixed to 50 ppm where as for sulfonated CNSL different doses were used. Antibacterial activities were observed for all the bacteria sample under study at different doses (table-2). For Pseudomonas aeruginosa minimal inhibitory concentrations (MIC) is 100ppm, where as for Flavobacterium antarcticus it is 50 ppm. Sulfonated CNSL has shown very good activity against Flavobacterium antarcticus even better than Gentamycin (table-2). From the table -1 and photographs it is clear that the SCNSL has antibacterial activity against all bacteria under study. The active doses for antibacterial activity were found to be in between 50ppm to 250 ppm.

Butylated hydroxyl anisole (BHA) was used as standard compound for the analysis of ferric reducing antioxidant power. Sulfonated CNSL has shown absorbance at all the concentrations but lower than the standard compound. The absorbance values increased with the increase in concentration of SCNSL, clearly suggest that the compound has ferric reducing antioxidant potential. Thus SCNSL is capable of preventing microbially induced corrosion at paper mill.

Ascorbic acid was used as standard compound for the analysis of Hydroxyl Radical Scavenging Activity. Sulfonated

Table -1 Minimal inhibitory concentration (MIC) for antibacterial activity of SCNSL

Organism	Concentration in ppm					
	50	100	150	200	250	MIC
Pseudomonas aeruginosa	0	0.2	1.2	0.7	0.7	100
Actinomyces howellii	0	0	0	0	0.5	250
Bacillus subtilis	0	0	0	0.7	1	200
Bacillus pumulis	0	0	0	0	1.2	250
Bacillus megaterium	0	0	0	0	1.4	250
Flavobacterium antarcticus	0.7	1.5	1.6	2.1	1.3	50

Table -2 Minimal inhibitory concentration (MIC) for antibacterial activity of Gentamycin

Organism	Concentration in ppm					
	50	100	150	200	250	MIC
Pseudomonas aeruginosa	1.7	1.9	2.1	2.2	2.4	50
Actinomyces howellii	2.0	2.2	2.4	2.6	2.9	50
Bacillus subtilis	1.3	1.7	2.1	2.5	2.9	50
Bacillus pumulis	1.9	2.1	2.4	2.7	3.0	50
Bacillus megaterium	1.7	2.1	2.3	2.5	2.6	50
Flavobacterium antarcticus	0.3	0.6	0.8	1.3	1.5	50

Table-3 Absorbance at 700 nm for ferric reducing antioxidant power

(µg of sample)	SCNSL	BHA
10	0.014	0.096
50	0.068	0.289
100	0.099	0.479
500	0.14	*

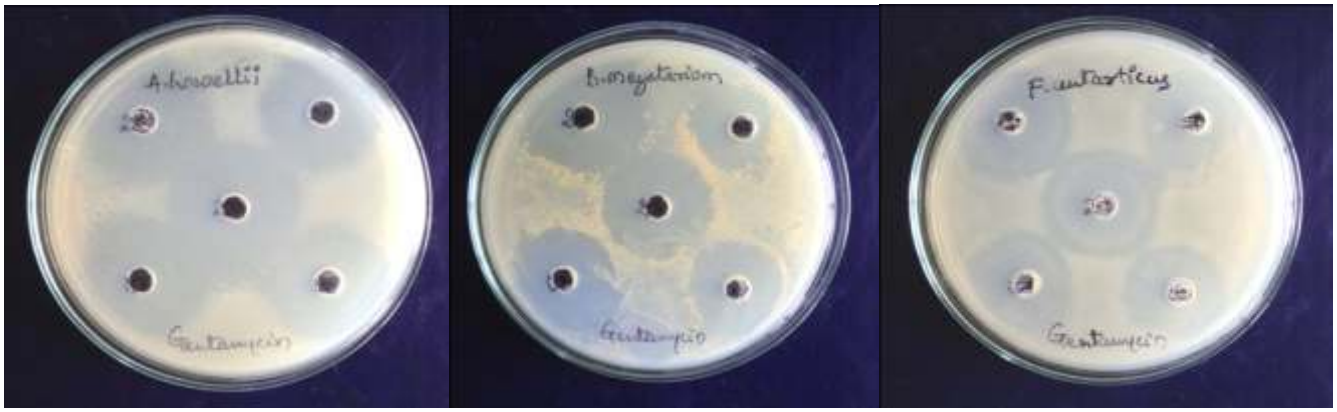
* Beyond measurable range: Much higher activity

Table -4 Percentage hydroxyl radical scavenging activity

µg of sample	SCNSL	Ascorbic acid
10	3.23	18.26
50	10.51	30.35
100	18.38	45.86

Photographs of petriplates for antibacterial activity using gentamycin as standard

Pseudomonas aeruginosa *Bacillus pumulis* *Bacillus subtilis*



Actinomyces howellii *Bacillus megaterium* *Flavobacterium antarcticus*

Photographs of petriplates for antibacterial activity using sulfonated CNSL

Pseudomonas aeruginosa *Bacillus pumulis* *Bacillus subtilis*

Actinomyces howellii *Bacillus megaterium* *Flavobacterium antarcticus*

CNSL has shown absorbance at all the concentrations but lower than the standard compound. The % hydroxyl scavenging activity values increased with the increase in concentration of SCNSL; clearly suggest that the compound has hydroxyl radical scavenging activity.

The antibacterial activity of sulfonated CNSL is mainly because of its constituents. Sulfonated CNSL is a complex mixture of sulfonated cardanol & 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 antioxidant potential is mainly because of the presence of phenolic group in the molecule.

Conclusion

In the light of results obtained in the present study it is suggested that sulfonated CNSL can be used for slime control in paper mill. It is easy to handle at paper mill between the concentrations of 200-250 ppm in aqueous medium[14]. The penetration effect is more for sulfonated CNSL as it is a surface active compound. The compound has antibacterial as well as antioxidant properties thus it is capable of protecting cellulose from degradation. It can also be used as a biocide to protect felt from degradation and loss of tensile strength. SCNSL is capable of preventing microbially induced corrosion at paper mill because of its antioxidant property. Thus this study confirms that sulfonated CNSL is an effective biocide. This study gives rise to a less toxic, biodegradable, safe and ecologically compatible biocide than conventional synthetic chemicals used in paper mill as biocide.

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