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Biocidal Efficiency of Surfactants on *Sargassum swartzii* (SS) - Zn^{2+} System for Microbiological Corrosion Prevention

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S. Manimegalai*[1] and P. Manjula[2]

*[1]*Assistant Professor, Post Graduate and Research Department of Chemistry,*
Arulmigu Palaniandavar College of Arts and Culture, Palani- 624601, Tamilnadu, India.
[2]*Associate Professor, APA College for Women, Palani- 624601, Tamilnadu, India.*

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ABSTRACT

Seaweed is the Kingdom of Protista and may belong to one of several groups of multicellular algae - the red algae, green algae and brown algae. In the study, the combined effect of inhibitor system such as *Sargassum swartzii* (SS) - Zn^{2+} and surfactants (CTAB and SDS) used to control microbial corrosion. The methanolic extract of *Sargassum swartzii* possesses the large spectrum of anti-microbial activity and environmentally non toxic. The study concluded that brown seaweed, *Sargassum swartzii* has an anti-microbial ability to control microbial corrosion in an eco-friendly way.

INTRODUCTION

Microbiologically influenced corrosion (MIC) is metal deterioration as a result of the metabolic activity of various microorganisms. Microbiologically influenced corrosion is also known as biological corrosion and microbial corrosion. There is a wide range of bacteria in water known to provoke MIC in such materials as stainless steel, carbon steel, copper alloys, and aluminum alloys. Biological organisms present in the aqueous medium have potential to increase or decrease oxygen transport to the surface; consequently, these organisms have a role in increasing or decreasing to form general corrosion. Microscopic organisms also tend to settle on metal surfaces in the form of discrete colonies or at least spotty, rather than continuous films. Biological organisms fall under two groups based on the type of corrosion they engender: (a) anaerobic corrosion, (b) aerobic corrosion. Sulfate reducing bacteria (SRB) from the genera Desulfovibrio are a typical example of anaerobic MIC. Aerobic sulfur oxidizing bacteria of the type Thiobacillus can create an environment of up to 10 percent sulfuric acid, thereby encouraging rapid corrosion. The mechanism of actions of microbial corrosion is as shown in Fig.1.

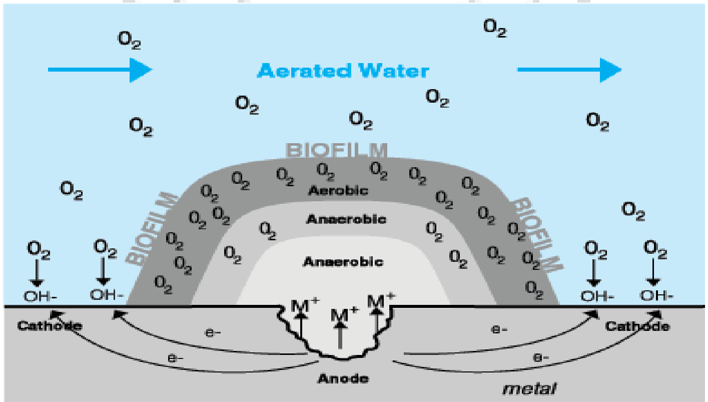

Fig. 1. Mechanism of Microbial corrosion

Various industries are affected by this type of corrosion include:

- Offshore and onshore gas and oil industries.
- Water flood and mothball systems as well as gas and oil handling systems.
- Industries related to chemical processing: flanged joints, tanks made of stainless steel, specifically in welded portions.
- Water treatment facilities such as piping and heat exchangers.

- Metal working facilities where increased wear, emulsions and machining oils may be present.
- Nuclear power plants with stainless, carbon steel tanks and piping as well as aluminum bronze, and brass cooling tubes.

MIC can be prevented through the following methods:

- Periodic mechanical cleaning.
- Chemical treatment using biocides to prevent bacteria population.
- Dry storage and total drainage.

MIC can occur in any aqueous environments, and because of nature of microbes in fluid systems, MIC is due to the presence of microbes, adequate nutrients, and corrosive byproducts. A number of metals, such as structural steels, copper alloys etc. tend to corrode generally over the entire surface in the absence of crevices or galvanic effects. In such cases, corrosion is determinate by the rate at which dissolved oxygen can be delivered to the metal surface.

Aquatic plant constituents have been described as potential tool to control the growth of several microorganisms involved in biocorrosion [1-10]. The main aim of this study suggests *Sargassum swartzii* (SS) may be employed in biocorrosion control. The objective of the study deals with i) to analyse the antimicrobial activity of *Sargassum swartzii* (SS) and ii) to prevent / control the growth of microorganism by combined effect of inhibitor system, *Sargassum swartzii* - Zn^{2+} and biocides (CTAB and SDS) in aqueous medium containing 60 ppm Cl^{-} ion.

MATERIALS AND METHODS

Screening for Antimicrobial activity assay by Disc diffusion Method

The dried powder of methanolic extract of *Sargassum swartzii* (SS) was used in the assay of antimicrobial analysis [11]. Pathogenic microorganisms such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* were selected for the present investigation. The microorganisms were originally obtained from Microbial Germ Plasma Culture collection RMMCH Medical lab, Annamalai University. The antimicrobial activities of the *Sargassum swartzii* extract were tested against the selected bacterial strains. The 20 ml of sterilized agar medium was poured into each sterile Petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5 cm was made in the medium by using a sterile cork borer, 150 μl of each methanolic extract of *Sargassum swartzii* were transferred into separate wells. After these plates was incubated at 37^0C for 48 hours. After incubation period, the diameter of inhibition zone around the each well was measured and the results were observed.

Biocidal Efficiency (BE) Analysis

Biocidal efficiency of various surfactants such as N-Cety1-N,N,N – trimethyl ammonium bromide (CTAB) (cationic surfactants) and Sodium dodecyl sulphate (SDS) (anionic surfactant) on BE were studied in this investigation. Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants (sanitizers) act as a biocidal agent (i.e.) bacterial resistance.

(i) N-Cety1-N,N,N – trimethyl ammonium bromide (CTAB)

CTAB is used an effective antiseptic agent against bacteria and fungi. It is a cationic surfactant. It is used as buffer solution for the extraction of DNA. It has been widely used in synthesis of gold nanoparticles (e.g., spheres, rods, and bipyramids), mesoporous silica nanoparticles (e.g., MCM-41) and in hair conditioning products.

(ii) Sodium Dodecyl Sulfate (SDS)

Sodium dodecyl sulfate (SDS or NaDS), sodium laurel sulfate or sodium lauryl sulfate (SLS) is an anionic surfactant, used in many cleaning and hygiene products. The salt is of an organo sulfate consisting of a 12-carbon tail attached to a sulfate group, giving the material the amphiphilic properties required for a detergent. Derived from inexpensive coconut and palm oils, it is a common component of many domestic cleaning products.

SS - Zn^{2+} formulation in aqueous medium (60 ppm Cl^- ion) which offered the best corrosion inhibition efficiency was selected. The biocidal efficiency of biocides such as CTAB and SDS were determined. Various concentrations of CTAB and SDS in the range of 10 – 100 ppm were added to the formulation consisting of the inhibitor system. Polished and degreased mild steel specimens in triplicate were immersed in aqueous environment for a period of 9 hours. After 9 hours immersion, one ml each of test solutions from the environment was pipette out into sterile Petri dishes containing 20 ml of sterilized Zobell medium kept in a sterilized environment inside the laminar flow system. Then the Petri dishes were kept in an incubator at 30^0 C in an inverted position for 2 days. Distinct colonies of bacteria were developed. Such colonies may be easily isolated. The numbers of colony forming units (CFU/ml) present in the above formulations were determined by step dilution technique [12]. Each organism grows and reproduces itself. The number of colonies was analyzed [13].

RESULTS AND DISCUSSION

Antimicrobial activity

The dried extract of *Sargassum swartzii* possesses antimicrobial activity. The antimicrobial activity of methanolic extract of *Sargassum swartzii* were inspected against the selected experimental pathogens such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* by disc diffusion methods and tabulated in Table - 1. The methanol extract was exhibited maximum zone of inhibition against *E. coli* (27 mm), *Bacillus subtilis* (23 mm), and *Staphylococcus aureus* (20 mm) at 500 µg *Sargassum swartzii* (Table -1). The antimicrobial study confirmed that flavonoids and hydroxylated phenolic substances synthesized from *Sargassum swartzii* act as antimicrobial substances against a wide array of microorganisms.

Table 1. Antimicrobial activity of methanolic extract from *Sargassum swartzii against* clinical pathogens expressed as MIC (µg/ml)

Bacterial strains	Gram	100 µg	200 µg	300 µg	400 µg	500 µg
E. Coli	-	6	9	16	19	27
B. Subtilis	+	7	10	19	21	23
S. aureus	+	5	8	14	17	20

Microbiological corrosion inhibition of *Sargassum swartzii* (SS) - Zn^{2+} system by using biocides (CTAB and SDS) in aqueous medium.

Green inhibitor, *Sargassum swartzii (SS)* (brown algae) is a potential tool of antimicrobial activity of some microorganisms as described in previous section. This study was carried to determine the biocidal efficiency of cationic surfactant (CTAB) and anionic surfactant (SDS) in absence and the presence of *Sargassum swartzii* (SS) -Zn^{2+} system in aqueous medium after suspending the specimen samples for 9 hours.

Influence of SS-Zn^{2+}system on the biocidal efficiency of CTAB

The biocidal efficiencies of CTAB [14-15] in the presence and absence of *Sargassum swartzii* (SS)– Zn^{2+} formulation in aqueous medium containing 60 ppm $C1^-$ ion , after suspending the mild steel for 9 hours is given in Table-2. The visuals of the bacterial colonies formed in the presence and absence of inhibitor system are shown in Fig. 2. The number of colony forming units (CFU/ml) as a function of concentration of CTAB (0, 10, 25, 50 and 75 ppm) in the presence of SS - Zn^{2+} system is shown in Table-2. Mild steel widely used in the fabrication of cooling water pipes and heat exchanger tubes [16-17]. When 10 ppm and 25 ppm of CTAB is added, the total number of bacterial count is 15 x 10^2 CFU/ml and 7 x 10^2 CFU/ml respectively. When 50 ppm of CTAB is added, no bacterial count, which imply, the biocidal efficiency is 100%. Hence, the optimum concentration of CTAB is ≥ 50 ppm. Thus it could be seen from Table -2, the formulation consisting of 60 ppm Cl^-, 120 ppm of SS, 100 ppm of Zn^{2+} and 50 ppm of CTAB has 100% biocidal efficiency. The 100% biocidal efficiency as evidenced by reported results in earlier research works [18-20] proved that the cationic surfactant like CTAB along with other organic and inorganic inhibitors might be applied as an excellent biocide in cooling water systems.

Table 2. Biocidal efficiencies of CTAB in various environments

Inhibitor system: *Sargassum swartzii* (SS) – Zn^{2+}–CTAB. Immersion time: 9 hours

Cl^- ppm	SS ppm	Zn^{2+} ppm	CTAB ppm	Total bacterial count (CFU/ml)	Biocidal Efficiency (%)
60	0	0	0	$50x10^2$	-
60	0	0	10	$15x10^2$	70
60	0	0	25	$7x10^2$	86
60	0	0	50	0	100
60	120	100	0	$32x10^2$	36
60	120	100	10	$10x10^2$	80
60	120	100	25	$5x10^2$	90
60	120	100	50	0	100
60	120	100	75	0	100

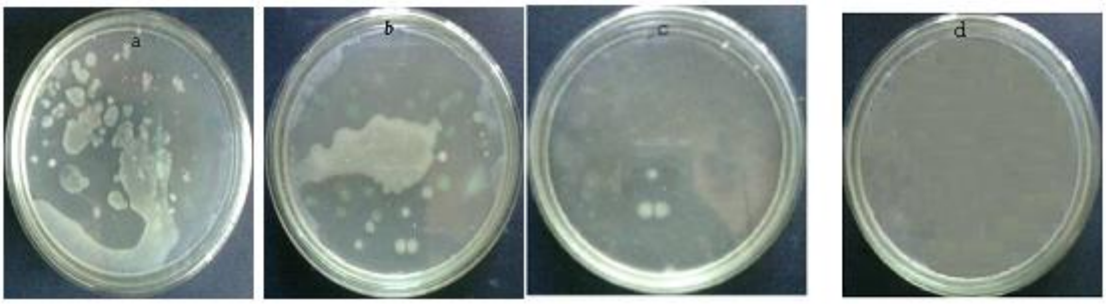

Fig. 2. Bacterial colonies formed in

a) Cl^- ion (60 ppm) b) 120 ppm SS + 100 ppm Zn^{2+} + 60 ppm Cl^- ion

c) 10 ppm CTAB d) 120 ppm SS + 100 ppm Zn^{2+} + 60 ppm Cl^- + 50 ppm CTAB

CFU/ml: Biological unit to count number of bacterial colony forming units in one ml of an incubated sample solution.

Influence of SS-Zn^{2+} system on the biocidal efficiency of SDS

The biocidal efficiencies of SDS in presence and absence of *Sargassum swartzii* (SS) – Zn^{2+} system in aqueous medium containing 60 ppm $C1^-$ ion after immersion of mild steel for 9 hours are shown in Table - 3. It is found that SDS has very good biocidal efficiency in a wide concentration range from 10 ppm to 100 ppm. The number of bacterial colonies formed as a function of concentration of SDS in the presence of SS-Zn^{2+}-SDS system is shown in Fig. 3. When 10 ppm of SDS is added, the total number of bacterial colony formed is 39×10^2 CFU/ml.

When 75 ppm of SDS is added, no bacterial colony is found, inferring biocidal efficiency as 100%. It proves that a mixture of 60 ppm Cl^-, 120 ppm SS, 100 ppm Zn^{2+}, and 75 ppm SDS, provides 100% biocidal efficiency. This is due to the complete destruction of microorganism. The 100% biocidal efficiency is evidenced by reported results in earlier research works [21-22] proved that the anionic surfactant SDS along with other organic and inorganic inhibitors may be used as an excellent biocide in cooling water systems .Hence the optimum concentration of SDS is ≥ 75 ppm.

Table 3. Biocidal efficiencies of SDS in various environments

Inhibitor system: *Sargassum swartzii* (SS) – Zn $^{2+}$– SDS. Immersion time: 9 hours.

Cl^- ppm	SS ppm	Zn^{2+} ppm	SDS ppm	Total bacterial count CFU/ml	Biocidal efficiency (%)
60	0	0	0	50 x 10^2	-
60	0	0	10	39 x 10^2	26
60	0	0	25	12 x 10^2	90
60	0	0	50	9 x 10^2	96
60	0	0	75	0	100
60	120	100	0	32 x 10^2	36
60	120	100	10	28 x 10^2	44
60	120	100	25	17 x 10^2	66
60	120	100	50	10 x 10^2	80
60	120	100	75	0	100
60	120	100	100	0	100

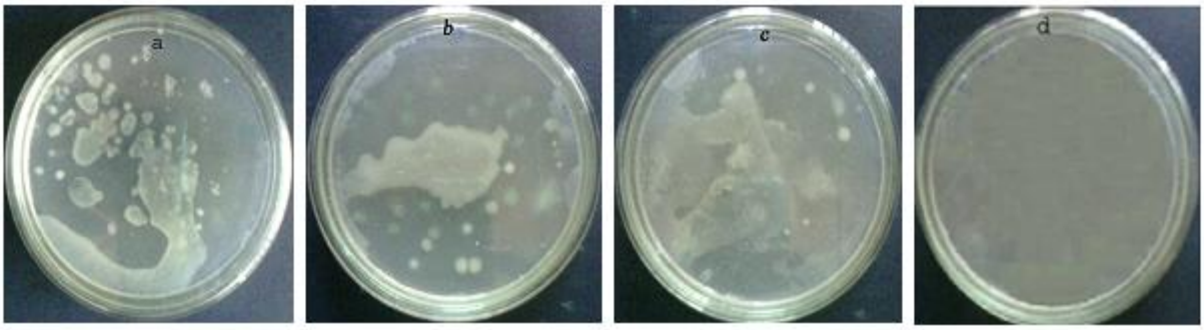

Fig. 3. Bacterial colonies formed in

a) Cl^- ion (60 ppm) b) 120 ppm SS + 100 ppm Zn^{2+} + 60 ppm Cl^- ion

c) 10 ppm SDS d) 120 ppm SS + 100 ppm Zn^{2+} + 60 ppm Cl^- ion + 75 ppm SDS.

CONCLUSION

The study concluded that *Sargassum swartzii* possesses the large spectrum of antimicrobial properties and environmentally non-toxic. The biocidal activity of *Sargassum swartzii* (SS) – Zn^{2+} system increased with increasing concentration of biocides (CTAB and SDS). Hence, the combined effect of SS -Zn $^{2+}$- CTAB and SS - Zn $^{2+}$-SDS were used to be effective in the control of microbial corrosion. Thus the brown seaweed, *Sargassum swartzii has* an anti-corrosive and antimicrobial ability to control microbial corrosion in an eco-friendly way.

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