Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, Stylissa masa

Authors: Suman Mallick
Submitted: 27. July 2023
Published: 14. August 2023
Volume: 10
Issue: 4
Affiliation: Ecology and Environment Laboratory, School of Arts and Sciences, Ahmedabad University, Ahmedabad, India
Languages: English
Keywords: Biodiversity, Marine Biology, Biomimetic Solutions, Conservation
Categories: Demetrios Project, Life Sciences, News and Views
DOI: 10.17160/josha.10.4.926

Abstract:

Though a number of studies in relation to fouling and antifouling have been carried out throughout the world, few studies on antifouling compounds from marine natural sources have been carried out to address fouling in Andaman and Nicobar Islands (Patro et al., 2009). There is a real need for the continuous development of new non-toxic antifouling formulations. An ideal antifouling formulation would have the following properties: permit at least five years of biofouling life cycle control, durable and resistant to damage, repairable, low maintenance, easy to apply, hydraulically smooth, compatible with existing anticorrosion coating, cost-effective, non-toxic to non-target species, and, effective at port and sea. An interesting and promising line of research is inspired by biomimetic solutions. Recently, particular attention has been paid to the physical defences of marine organisms, especially the surface topography of molluscan shells, crustose coralline algae, marine mammals and shark skin. All marine sessile organisms use adhesive materials (with temporary or permanent capabilities) to attach to surfaces. Controlling an organism's settlement could be achieved by physically preventing adhesion. The best anti-adhesive

JOSHA
josha.org

Journal of Science, Humanities and Arts

JOSHA is a service that helps scholars, researchers, and students discover, use, and build upon a wide range of content

Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, *Stylissa masa*

A Dissertation submitted to the Pondicherry University for the partial fulfilment of the M.Sc., Marine biology Degree Examination, 2018

BY:

Suman Mallick

Registration No. 16373033

PONDICHERRY UNIVERSITY

(A Central University)

DEPARTMENT OF OCEAN STUDIES AND MARINE BIOLOGY

BROOKSHABAD CAMPUS, PORT BLAIR – 744112

ANDAMANS

April, 2018

Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, *Stylissa masa*

A Dissertation submitted to the Pondicherry University for the partial fulfilment of the M.Sc., Marine biology Degree Examination, 2018

By:

Suman Mallick

Registration No. 16373033

PONDICHERRY UNIVERSITY

(A Central University)

DEPARTMENT OF OCEAN STUDIES AND MARINE BIOLOGY

BROOKSHABAD CAMPUS, PORT BLAIR – 744112

ANDAMANS

April, 2018

PONDICHERRY UNIVERSITY
(*A Central University*)
DEPARTMENT OF OCEAN STUDIES AND MARINE BIOLOGY
Brookshabad Campus, Port Blair, Andamans – 744 101

Dr. Jayant Kumar Mishra
Professor
jkmishra.omb@pondiuni.edu.in

Tel: 03192- 262337/317(O)/Fax: 262323/9474213264 (M)
E-Mail: jayant64@yahoo.com /

CERTIFICATE

This is to certify that Mr Suman Mallick, student of M. Sc., Marine Biology, IVth Semester has worked under my guidance for her dissertation work entitled "**Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, *Stylissa masa***". This project work has been carried out by him on his own and has not been submitted earlier to this University or any other Institution for award of any degree or diploma.

DATE: 20/04/2018

PLACE: PORT BLAIR

GUIDE:

Prof. Jayant Kumar Mishra
M. Phil., Ph. D., D. Fs.
Dept. of Ocean Studies and Marine Biology
Pondicherry University (Brookshabad Campus)
Port Blair, Andamans - 744 101

HEAD OF THE DEPARTMENT:

Prof. R. Mohanraju Ph.D
Head of the Department
Department of Ocean Studies and Marine Biology
Pondicherry University. Brookshabad Campus
Port Blair- 744 112. Andamans

EXAMINER:

DECLARATION

I, Mr. Suman Mallick, hereby declare that the dissertation work entitled **"Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, *Stylissa masa*"**, submitted by me for the partial fulfilment of my M. Sc. Degree in Marine Biology is of my own work and has not been submitted previously for award of any degree or diploma to this University or any other Institution.

Suman Mallick
(SIGNATURE)

DATE: 20.04.2018
PLACE: PORT BLAIR

ACKNOWLEDGEMENT

I express my sincere thanks to Dr. Jayant Kumar Mishra, Professor, Department of Ocean Studies & Marine Biology for his guidance throughout the study and providing me all the facilities to complete the work.

I would like to express my sincere gratitude to Dr. R.Mohanraju, Associate Professor & Head of the Department of Ocean Studies & Marine Biology. I would like to express a sincere thanks to all the faculty members and Ph.D. scholars for imparting me knowledge about the subject and helping me to complete my work.

Lastly, I would like to express my sincere thanks to my family, elders & friends without whose help, it was impossible for me to complete the dissertation.

CONTENT

LEGEND TO THE TABLES

LEGEND TO THE FIGURES

Sl. No.

Page

LEGEND TO THE PLATES

CHAPTER –I

Inhibition of Microbial Biofilm by the Crude Extracts of Marine Sponge, *Stylissa masa*

General Introduction

The Andaman and Nicobar (A & N) archipelago is one of the mega biodiversity hotspots in the India Ocean consisting of 572 islands surrounded by Bay of Bengal to its west and by the Andaman Sea at its east coast lying between 6°45'-13°45' N and 92°12'-93°57' E in the Indo-Burmese microplate junction. These islands have rocky coast line, which provide suitable habitat for numerous sedentary marine organisms. As it is being observed, on the immersion of any non-reactive solid material into the sea, some kind of inorganic, organic and biotic matter get accumulated on its surface. This process of accumulation is referred as a kind of marine fouling.

This process of fouling is initially the result of physical and chemical properties of the solid-liquid interface, but subsequently involves biological interactions leading to the colonisation of sedentary microorganisms and biofouling. But the initiation of this process always commences with the basic colonisation process by microorganisms by forming a microbial biofilm on the submerged structures in the marine environment, which can be as a kind of molecular fouling. As it is being reported that within few minutes of immersing a clean surface in sea water, it adsorbs a molecular conditioning film or layer of organic molecules and there by changes the physical and chemical properties of the surface (Stal and Brouwer, 2003).

Biofouling as defined is nothing but the attachment of undesirable micro and macro-organisms to living or non-living surfaces submerged in sea water (Emara and Belal, 2004; Yebra et al., 2004; Guenther, 2007; Marechal and Hellio, 2009). Both of these organisms, which are responsible for fouling are principally called as "biofoulers" and include the members from the protozoans to higher invertebrates (Wahl, 1989; Rodriguez et al., 1993).

This conditioning substance mostly consists of dissolved organic materials such as colloidal organic molecules, polysaccharides and proteins available in the surrounding sea water medium and become a basis for attracting the microscopic life forms like bacteria, unicellular algae or diatoms and cyanobacteria (Lee et al., 2003; Garg and Bhosle, 2004; Karayanni et al., 2010). Bacteria are usually the first organisms to attach (Corpe, 1970; Sieburth, 1979), which proliferate and produce mucilage i.e. an acidic mucopolysaccharide of fibrous reticular nature that binds them to the surface (Fletcher & Floodgate, 1973) and eventually forms a thick layer (Jones et al, 1969; de Chalain, 1979). Assemblage of these microscopic organisms, which also can be termed as micro-fouler forms a biofilm which is referred as "micro-fouling" or "slime" (Cooksey and Cooksey, 1995; Chiu et al., 2005; Brinkmann et al., 2007). Reports also suggest that the diatoms play a major role in slime formation as it secrets certain extra polymeric substances (EPS) that acts like a matrix in which other foulers are embedded (Sutherland et al., 1998; Underwood, 2005; Patil and Anil, 2005). This initial layer of bacteria, fungi and non-motile small diatoms remains in intimate contact with the submerged substratum.

Later, large motile diatoms, microalgae, filamentous fungi, debris, flagellates and other protozoa attach onto this layer in succession (Fig. 1) to complete the formation of the primary film (Marszalek et al., 1979). This primary film can modify surface structures into ecologically more complex systems, capable of supporting a diverse array of species (Paul et al., 1977; Little, 1984). The sequence of biofouling includes a primary microfouling phase, followed by the secondary macrofouling assemblage. The slime layer provides the source of food as well as attaching platform for the reproductive propagules or larva of macrofoulers (Redekar and Wagh, 2000; Callow and Callow, 2002).

Macrofouling can be categorised as 'soft fouling' or 'hard fouling' depending on the nature of the attached organisms. Soft fouling comprises macro algae and invertebrates such as soft corals, sponges, anemones, tunicates and hydroids, while hard foulers are invertebrates such as barnacles, mussels and tubeworms.

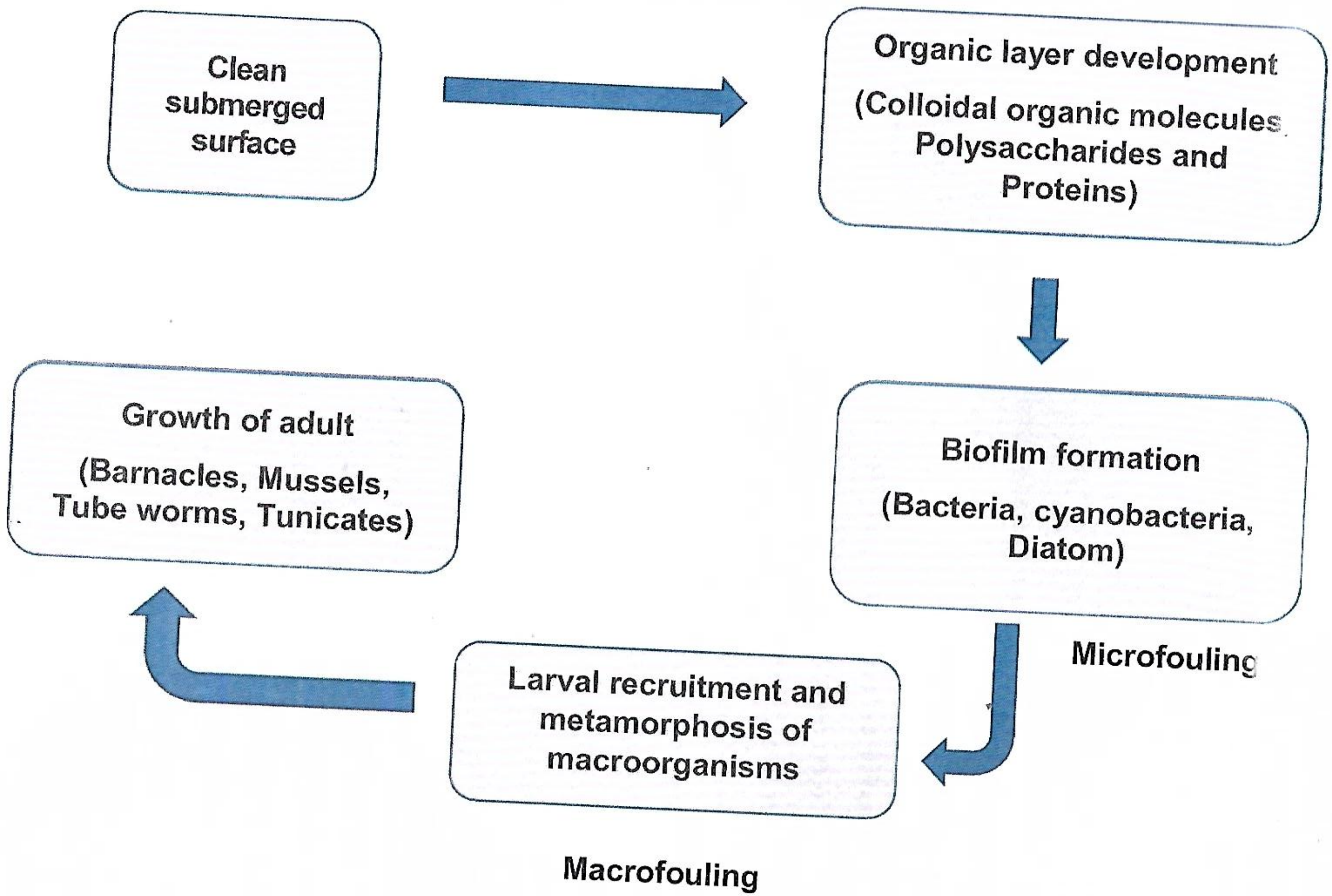

Fig. 1. **Fouling process in the submerged structure in the marine environment.**

Billions of dollars are spent every year to manage the age-old biofouling problems in heat exchangers by the Shipping industries, Power plants, desalination plants and Oil & Gas industries. The prevention and removal of calcified macrofoulants is the most challenging operational and maintenance issues that cost such Industries billions of dollars per year due to increased operating costs (in anti-fouling measures, regular manual tube cleaning and higher energy consumption) as well as reduced operational efficiency (due to shorter run cycle, lower output, throughput disruptions and unscheduled outages).

The use of antifouling devices has been in use since early 700 B. C with the use of lead sheathing to self-polishing copolymer antifouling paints like TBT in the present century (Claire, 2010). But these developments in antifouling processes though promise to prevent fouling, but it has bought significant negative effects on the nonspecific organisms in marine the marine environment. Development of synthetic antifouling agents contains a variety of toxic materials (e.g., copper, lead, mercury, arsenic) were used to control the settlement of fouling organisms (Qian et al., 2010). Later, they were replaced by organotins like Tributyltin (TBT), which had proved to be the most effective antifouling agent known (Willemsen & Ferrarl 1993; Chambers et al., 2006). But due to their non-degradable property and impact on both target and non-target species, they proclaimed as the most toxic biocide ever introduced (Saint-Louis, 1994; Alves de Lima et. al., 2006).

Bioactive ingredients produced by marine organisms as their secondary metabolite have been emerged as a promising alternative to the biocides (Abarzua and Jakubowski, 1995; Qian et al., 2010). Being natural, they are effective to target species and also easily degradable in the environment. Sponges, exclusively are aquatic & mostly marine, are found from the deepest oceans to the edge of the sea. There are approximately 15,000 species of sponges in the world out of which 486 species are found in India.

Technologies have been developed to produce novel natural product from marine sponge which have been found to have potent antifouling property. It has been proved that marine organisms are excellent source of bioactive secondary metabolites &

number of compound have been reported. The natural antifoulants isolated from a wide range of marine organisms of which the major groups are represented by sponges (Wagh et al., 1997; Tsoukatou et al., 2002). The other sources includes soft corals (Wilsanand et al., 1999; Epifanio et al., 2006; Limna-Mol et al., 2010, Raveendran et al., 2011), algae (Bazes et al., 2009; Manilal et al, 2010; Plouguerne et al. 2010), Ascidian (Murugan and Ramasamy 2003), echinoderms (Guenther, 2007) and microorganisms (Holmstrom et al., 1992; Dobretsov and Qian, 2004).

Though numerous natural antifouling compounds with anti-settlement activities have been reported to date, only in a few cases potential compounds which could be used as replacements for toxic, metal-based antifoulants have been discovered. Some of these natural antifoulants have higher activities compared with those of organotin compounds and copper compounds (Raveendran and Limna-Mol, 2009).

Review of Literature

Among the hard fouler , barnacles or cirripedes are known as the most dominant macro fouling organism worldwide (Kumari, 1999; Swain et al., 2000; Pineda et al., 2005; Chan, 2006; Aldred and Clare, 2008; Desai and Prakash, 2009). Because of their unique body shape, they generate maximum frictional drag in ships and therefore are studied in greater details than any other fouler (Desai, 2002). There are nearly 800 species of barnacles recorded worldwide, out of which 25 species are documented as major foulers on manmade structures (Salgado-Barragan and Hendrickx, 2002; Chan, 2006; Anil et. al., 2012). Daniel (1956) reported nearly 280 species barnacles in the Indian Ocean, and out of this around 48 species were recorded from the intertidal zone and 15 species of barnacles were recorded from the intertidal waters of Andaman & Nicobar Islands. Out of this 4 species are of the genus *Balanus* such as *Balanus amphitrite, Balanus madrasensis*, *Balanus tintinnabulum* and *Balanus perforates*. These are well known for their fouling activity. As per the earlier record, *Balanus amphitrite* is the most dominating fouling species in the Andaman and Nicobar Islands (Karande, 1978).

Organotins (TBT) which had proved to be the most effective antifouling agent known (Willemsen & Ferrarl 1993; Chambers et al., 2006). But due to their non-degradable property and impact on both target and non-target species, they proclaimed as the most toxic biocide ever introduced (Saint-Louis, 1994; Alves de Lima et. al., 2006). This led the International Maritime Organization (IMO) to prohibit their application to ships, effective from 17 September 2008 (IMO, 2001). Alternative biocide based antifouling paints such as Irgarol 1051, Diuron, Sea-Nine 211, Chlorothalonil, Dichlofluanid, and Zinc Pyrithione are the most frequently used booster biocides worldwide, and some of these have also been found to accumulate in coastal waters at levels that are deleterious for marine organisms (Raveendran and Limana-Mol, 2009; Guardiola, 2012).

It has been found that the major sources for antifouling compounds are sponges, corals and macro algae and/or their associated microflora and/or symbionts and several active ingredients are being isolated. To date, purification of active products from marine organisms has yielded to around 200 molecules with variable degrees of Antifouling activities against a wide range of marine fouling organisms (Hellio Claire and Maréchal Jean-Philippe, 2009).

Moreover, the marine environment is rich in unexplored species (estimated at 1–2 million) that may have novel biosynthetic capabilities. Data analysis highlighted that Antifouling activity is not driven by latitudinal trends, but rather by phylogenetic constraints. Very promising compounds have been purified from microorganisms, macro algae and sponges. (Maréchal J.P., 2009)

Species of the genus *Laurencia* have been extensively investigated for the production of secondary metabolites and are known to produce ca. 700 natural products, particularly bioactive halogenated compounds (Hellio,C.; Maréchal, J.P.; Da Gama, B.A.P.;Pereira,R.C.;Clare,A.S.,2009). Regarding Antifouling activity, the best compound obtained from this genus is the elatol which is potent against marine bacteria, and invertebrates (*Balanus amphititre* and *Bugula neritina*) at low concentration.

Concerning Phaeophyceae, the most investigated genus are *Bifurcaria* and *Sargassum*. Diterpenes displaying large spectra antifouling activities were isolated from *Bifurcaria bifurcate* (Daoudi, M.; Bakkas, S.; Culioli, G.; Ortalo-Magne, A.; Piovetti, L.; Guiry, M.D.,2001). Interesting compounds from *Sargassum tennerimum* were shown to interfere with larval settlement of *Hydroides elegans* and biofilm formation (Hellio, C.; Thomas-Guyon, H.; Culioli, G.; Piovetti, L.; Bourgougnon , N.; Le Gal, Y., 2001).However, active compounds are quite often produced by the associated microflora (on the surface or within the organisms), which offers a great advantage for the chemical industry as they can be grown in large volume for production of compounds. Even if no Marine Natural Products have made it yet to the Antifouling market, an interesting product was patented a few years ago, called Biojelly, which is a polymer that is formed on a cellulose acetate membrane immersed in seawater and harbour specific bacteria, which inhibits attachment of marine organisms such as algae and barnacles (Hayase, N.; Sogabe, T.; Itou, R.; Yamamori ,N.;Sunamoto, J.,2003). Most of the secondary metabolites rapidly breakdown when, released in the environment and as a consequence their incorporation in paint a formulation is very challenging. Release rate have to be carefully controlled in order to enhance the paint lifetimes. So far, the best method developed to counter this have been perform through microencapsulation of the Bioactive Marine Natural Product (Price, R.R; Patchan, M.; Clare, A.; Rittschof, D., 1992).

A number of studies have been carried out worldwide to understand the variation in abundance of marine fouling community at different geographical locations. Brown and Swearingen (1998) observed the fouling community at Northern Gulf of Mexico. Karayanni et al. (2010) studied their colonization and succession on chromated copper arsenate (CCA) treated wood in Aegean Sea and the distribution of these organisms in three tidal zones such as super tidal zone, inertial zone and sub-tidal zone. The fouling communities in Egypt was reported to be dominated by barnacles (Emara and Belal, 2004; Ramadan et al., 2006) and the same organisms were also observed to be dominant throughout the world (Chan, 2006; Phang et al., 2006; Aldred and Clare, 2008; Desai and Prakash, 2009).

Impact of barnacles as a fouling organism has brought the attention of researchers towards studies related to their distribution, ecology, life cycle and settlement mechanism (Mishra and H. Kitamura, 1999; Mishra et al., 2000; 2001; Mishra, 2002). Similarly, Zullo (1979) reported the fouling cirripedes from North-eastern United States. Chan (2006) investigates and reviews the ecology and distribution of intertidal barnacles in Hong Kong, Taiwan and Japan. Chan et al. (2009) reported the morphology and distribution of acorn barnacle *Tetraclita reni* in Madagascar and adjacent waters.

Crisp (1954) reported the breeding pattern of barnacle *Balanus porcatus* from Irish Sea. Similarly, the spatio-temporal variation in the settlement and recruitment of the intertidal barnacle *Semibalanus balanoides* in Northern Europe was reported by Jenkins et al. (2000). In another study, Hung et al. (2007; 2008) reported the effect of types of biofilm and substrate on settlement of *Balanus amphitrite* and concluded that differences in biofilms help in biofouling adhesion strength when experimented on three known silicone formulations and an epoxy control at seven static immersion sites located in California, Florida, Hawaii, Hong Kong, India, Italy and Singapore.

The initial event in biofouling on marine surfaces is the adsorption of an organic layer which is usually followed by the formation of biofilm with the help of microorganisms such as bacteria and diatoms (Cooksey and Cooksey, 1995). Chiu et al. (2005) reported the effect of season, salinity and temperature over qualitative and quantitative composition of a biofilm. This was followed by Briand et al. (2012), who studied the biofilm forming community on an artificial substrate in French Mediterranean coast site and found *Licmophora gracilis* and *Cylindrotheca closterium* as the most dominant fouling diatoms. Whereas, Munda (2005) studied the seasonal fouling of diatoms at different depths at Gulf of Trieste, Northern Adriatic and also Molino and Wetherbee (2008) reported the biology of fouling diatoms and their role in development of the biofilm signifying the importance of biofouling research in the world.

In the Indian context, a number of studies focusing on marine fouling have been carried out to understand the fouling communities of Indian tropical waters. Rajagopal et al. (1990) studied the seasonal fouling by using teakwood panels in Edaiyur backwaters and reported Polychaetes (*Hydroides elegans*, *Polydora* sp., *Ficopomatus* sp. and *Mercierella enigmatica*), barnacles (*Balanus* sp.), green mussel (*Perna viridis*), oyster (*Crassostrea madrasensis*), brown mussel (*Modiolus* sp.) and algae as the dominant foulers of the region. Rao and Balaji (1988) used timber and glass panels to study the biological fouling at port of Kakinada in Godavari estuary and reported polychaete (*Mercierella enigmatica*), barnacle (*Balanus amphitrite*), and the bryozoans (*Electra bengalensis*, *Membranipora amoyensis*, *Alderina arabianensis* and *Victorella pavida*) as the most dominant foulers. Raveendran and Wagh (1993) studied the biofouling at Mormugao harbor by using different species of Indian timbers and results suggested that barnacle (*B. amphitrite*), tunicate (*Diplosoma* sp.) and bryozoans (*Hippoporina indica, Alderina arabianensis* and *Electra bengalnensis*) are the major foulers.

Study on the settlement and succession of macro fouling community on teakwood panels carried out in Kalpakam coastal waters suggests that green mussel (*Perna virirdis*), barnacles (*Balanus* sp.), hydroid (*Obelia* sp.), ascidians (*Didemnum psammathodes* and *Lissoclinum fragile*) and sea anemones (*Setularia* sp. and *Aiptasia* sp.) are the most dominating fouling species (Sahu et al., 2011). Srinivas et al. (1992) studied about the fouling on stainless steel and aluminium panels at Vijaydurg harbor and reported the settlement of barnacles (*Balanus* sp.) throughout the year. Swami and Udhayakumar (2010) studied the influence of season on settlement, distribution and diversity of fouling organisms on Perspex test coupons at Mumbai harbour and reported that the species settlement in pre-monsoon period is higher than monsoon and post-monsoon.

In another significant study, Daniel (1956) studied the barnacles of Indian Ocean and reported nearly 280 species out of which 15 species are inhabited in the intertidal waters of Andaman and Nicobar Islands. As per the earlier reports, *B. amphitrite* is the major fouler in India and has been reported from both east and west coast (Karande, 1978; Rao and Balaji, 1988; Srinivas et al., 1992; Sahu et al., 2011).

Importance of Present Study

Marine biofouling is of major economic concern to all marine industries. The shipping trade is particularly alert to the development of new antifouling strategies, especially green antifouling paint as international regulations regarding the environmental impact of the compounds actually incorporated into the formulations are becoming more and stricter. It is also recognised that vessels play an extensive role in invasive species propagation as ballast waters transport potentially threatening larvae. It is then crucial to develop new antifouling solutions combining advances in marine chemistry and topography, in addition to a knowledge of marine biofoulers, with respect to the marine environment.

Biofouling can lead to significant increase in the cost of maritime transportation. The globalization of production and trade are concomitant as one cannot function without the other. The scale, volume and efficiency of the international trade all have continued to increase since the 70s. The importance of maritime transportation in the global freight trade is unmistakable, particularly in terms of tonnage as it handles about 90% of the global exchange (Kemp, J.F.; Boon, J.P., 1994).

Due to high fouling pressure in tropical waters due to major shipping route, synthetic ant biofouling paints prove to be very much detrimental for the environment as tributyl tin (TBT) in seawater were identified as inducing serious biological damage to marine invertebrates(Clark, E.A; Steritt, R.M.; Lester, J.N.,1988). There is a real need for the continuous development of new non-toxic antifouling formulations by understanding the various chemical cues of sedentary marine organisms which are successfully preventing epibionts by releasing some secondary metabolites.

Objective of the Study

Though a number of studies in relation to fouling and antifouling have been carried out throughout the world, but few studies on antifouling compounds from marine natural sources have been carried out to address fouling in Andaman and Nicobar Islands (Patro et al.,2009). There is a real need for the continuous development of new non-toxic antifouling formulations. An ideal antifouling formulation would have the following properties: permit at least five years biofouling life cycle control, durable and resistant to damage, repairable, low maintenance, easy to apply, hydraulically smooth, compatible with existing anticorrosion coating, cost effective, non-toxic to non-target species, and, effective at port and sea. An interesting and promising line of research is inspired by biomimetic solutions. Recently, particular attention has been paid to the physical defences of marine organisms, especially the surface topography of molluscan shells, crustose coralline algae, marine mammal and shark skin. All marine sessile organisms use adhesive materials (with temporary or permanent capabilities) to attach to surfaces. Controlling organism's settlement could be achieved by physically preventing adhesion. The best anti-adhesive properties have been observed with the use of silicones as polymers, which on top have the advantage of being very durable. These compounds could be used as active ingredients in antifouling formulations. However, keeping the promising bio-resources of Andaman Sea with probable antifouling property, the present study was focused at investigating anti biofilm effect of sponge crude extracts on the micro and macro fouling organisms along the coast of Port Blair, South Andaman.

CHAPTER - II

Materials Methods

Study Area:

Study was conducted within the near shore area of Port Blair at Panighat jetty (N 11 ° 36.432' & E 92 ° 45.089') along South Andaman coast (Fig. 2). The area was selected as a fixed station as it has close proximate to the harbour with high frequency of movement of ships from different parts of the world with the probability of an array of intercontinental fouling organisms. Also the jetty selected was easily accessible, less anthropogenic interference. Again the site was ideal for suspending both the control and sponge crude extract coated test panels in the sea water. Also at the study site, attachment of *B. amphitrite* was noticed on structures such as pillars, jetty and walls respectively. Being jetty area where water depth always remains as three to five meters, which was very much conducive for suspending the panels into the water to keep it submerged both in the lowest low and highest high tide period.

Fig. 2. Map showing the Study area at Panighat Jetty, Port Blair, South Andaman

Preparation of Sponge Crude Extract

Sponge species, *Stylissa masa* (Plate – 1) was collected from Kodiyaghat (11°31'16.8" N; 92°43'02.8"E) from the rocky intertidal zone during low tide period and brought to the laboratory.

Plate - 1. *Stylissa masa*

Sponge sample was washed thoroughly under tap water followed by distilled water to remove any associate epiphytes and detritus material. 35 g of sponge sample was taken in a 500 ml sterilised glass beaker, and 200 ml of methanol was added to it and then the sponge sample was finely crushed with a mortar & pestle. The crushed methanol extract was then filtered using whatman filter paper in conical flask. The crude extract then concentrated with a Rotary Evaporator (Rotava-) up to approximately 50 ml. The crude extract at his stage became like a thick sticky solution.

Preparation of Test Panel

Test panels of 10 X 5 mm were prepared by cutting PVC plates of 4 mm thickness and holes were made at both end of the plates to tag it in a row by using the nylon ropes (Plate – 2) for the convenience of immersing it in the sea water during study. Each test panel was coated with freshly prepared sponge crude extract (coated test panel) by immersing the plates overnight in the extract solution followed by sun drying. Similarly untreated panel (control panel) was made without ant crude extract treatment. Then plates were arranged in an order by placing the untreated panel at the top followed by treated panels and gap of 15 cm was kept in-between two consecutive panels. The plates were then put into the seawater by hanging vertically from the jetty with the help of nylon rope and weight was attached at the bottom of the last panel to keen the panels straight in the water column without much disturbance. Care was taken to keep panels suspended below the low water mark always to avoid exposure of these panels during low tide. For the study a total of five sets of test panel were prepared and placed in the water column during the study period. The total study period was 75 days and each set of panel was removed after every 15 days for analysis of micro biofilm formation (fouling) inhibition effect of sponge extracts and anti-colonisation effect towards the settlement of fouling organisms.

Analysis of Test Panel

Once removed from the seawater, test panels were brought to the laboratory in ice boxes to avoid the damage to the biofilm during transportation. The test panel were examined under light microscope using external light source by keeping it in a petriplate along with seawater. The organisms attached to the plate were noted. For a clear observation of fouling organisms, any attached detritus material on the plates were cleaned by using a fine brush.

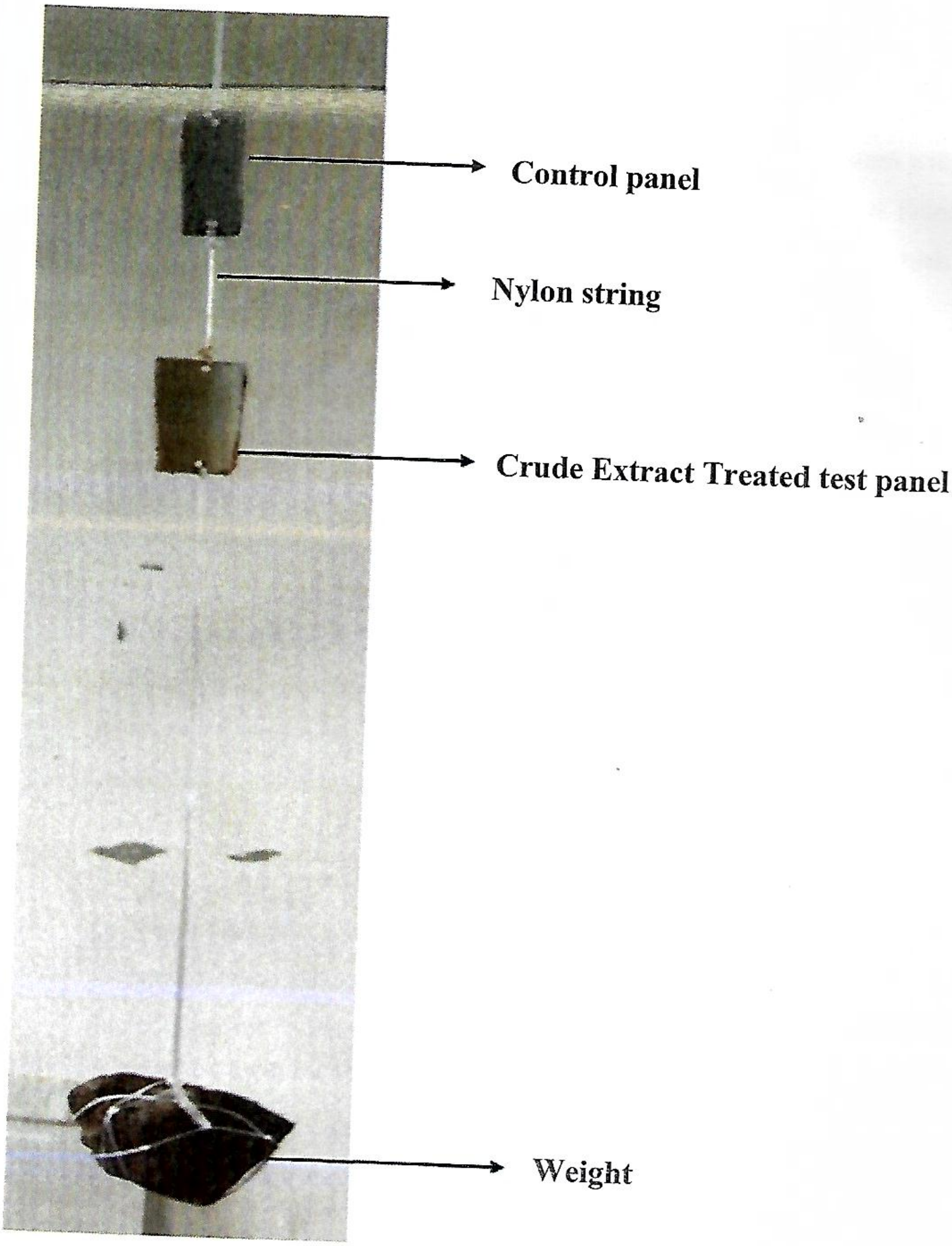

Plate – 2. Schematic diagram of Test panel.

CHAPTER - III

Results and Discussions

The biodiversity of an ecosystem is always influenced by its environmental and geographical factors and the A & N Islands are an unique ecosystem with rich marine diversity, especially in the intertidal environment. The rocky coasts found in the A & N Islands harbour a rich diversity of flora and fauna, most of which are fouling (sedentary) in nature.

In the present study, a total of 17 species were noted, which represent a heterogeneous group of macro foulers including, bryozoans (1 species), polychaetes (9 species), crustaceans (2 species), molluscans (3 species), echinoderms (1 species) and chordata (1 species) (Table – 1).

The highest species richness were found among polychaete (Fig. 1). But the dominant fouling species was *Balanus amphitrite* followed by the molluscs attached to the test panel. The presence of dominant species such as *B. amphitrite*, and *Saccostrea cucculata* in the test panels indicated the community of hard foulers in the study area.

The treated plate having the coating of sponge crude extract successfully prevented the growth of foulers at initial stage which was noted in Table 2 .The Antifouling coat of sponge *Stylissa masa* successfully inhibited the formation of biofilm mainly by microorganisms and diatom species (*Nitzschia sp., Navicula sp., Cocconeis sp.*).

Sl. No	Phylum/Class	Macrofoulers	15-Days	30-Days	45-Days	60-Days	75-Days
I.	**ANNELIDA**						
1.	Polycheate	*Capitella sp.*		+	+		+
2.		*Chaetopterus variopedatus*	+		+	+	
3.		*Chloeia flava*	+		+		+
4.		*Eunice pennata*			+	+	+
5.		*Eurythoe complanata,*	+	+			+
6.		*Pomatoceros triqueter*		+	+	+	+
7.		*Turbocavus secratus*			+	+	+
8.		*Questa ersei*	+	+		+	
9.		*Hesione sp.*			+	+	+
II.	**ARTHROPODA**						
10.	Crustacea	*Balanus amphitrite*		+		+	+
11.		*Cheiriphotis trifurcata*				+	+
IV.	**MOLLUSCS**						
12.	Gastropoda	*Littorina scabra*		+	+	+	+
13.		*Brachidontes exustus*			+		+
14.	Bivalvia	*Saccostrea cucullata*				+	+
IV.	**ECTOPROCTA (BRYOZOA)**						
15.		*Bagula neritina*	+	+	+	+	+
V.	**ECHINODERMATA**						
16.		*Ophiocoma sp.*			+		+
VI.	**CHORDATA**						
17.	Ascidiacea	*Lissoclinum fragile*			+	+	+

Table – 1. Macrofoulers of Panighat Jetty area, Port Blair, South Andaman coast on control panel

Total Days of immersion of Plates in SW	Control Plate	Plate with Crude extract coated
15	Plate became coated with slime layer formed by microfoulers including diatoms. Large amount of Sediments were obtained which was home for various polychaetes (*Q.eresei, E.complanata*) and bryozoans (*B.neritina*).The weed fouling (*Ectocarpus sp.*) was observed on the edge of plates.	Plate was free from slime layer of microfoulers but included the weeds on the edge of the plate along with large quantity of sediments.
30	Slime coated plates gave rise to succession of fouling community from micro to macro foulers. Sediments covered the major part of the plate. Some tubeworm (*P. triqueter, Capitella sp.*) on the surface was observed along with gastropod sp.(*L.scabra*).The larval stage of *Balanus sp.* was observed.	Sediment was the major constituent in this plate along with the growth of brown coloured weed growth (*Ectocarpus sp.*) and green microalgal growth (*ulva sp.*) on the edge of the plate.
45	Plates showed a hierarchy of fouling members starting from the microalgal growth to dominating adult barnacle species (*Balanus sp.*). It also consist of various polychaete sp. (*Capitella sp., Hesione sp., C.variopedatus, C.flava*) and gastropod sp. (*B.exutus*) along with echinoderm (*ophiocoma sp.*).It also included tunicates. (*L. fragile*).	The plates showed large quantity sediment along with the microalgal growth on the boundary of the plates. This plate showed very few members members of polychaetes (*Hesione sp.*) along with the sediments and successfully prevented the growth of other fouling species.
60	Plates consist of various diverse group of fouling organisms, right from polychaete (*E.pennata,Q.ersei*), tubeworm (*P.triqueter*) ,Gastropod(*L.scabra*),bivalve(Saccostrea sp.) & ascidians. The edge was populated by vibrant growth of *ectocarpous sp.*	Micro algal growth along with weeds & sediment were the major component on the edge of the plate. Few species of gastropods were occasionally found. The successfully prevented growth of hard & dominant macro foulers like barnacles.
75	The plate showed all the groups of micro and macro foulers from micro algal growth to weeds polychaetes ,gastropods ,bivalves, tunicates, echinoderms along with sediments .it also showed growth of amphipod sp.(*C. trifurcate*) along with goose barnacle.(*Lepas sp.*)	The plate showed hard fouling attachment of few barnacle larvae (*Balanus sp.*) and its metamorphosis. It contained micro algae and weed growth along with large amount of sediments. It also consist of few polychaete and tube worm as well as ascidians. It failed to inhibit the growth of the foulers.

Table 2:- Condition of Control & Treated Plate after at an interval of every 15 days

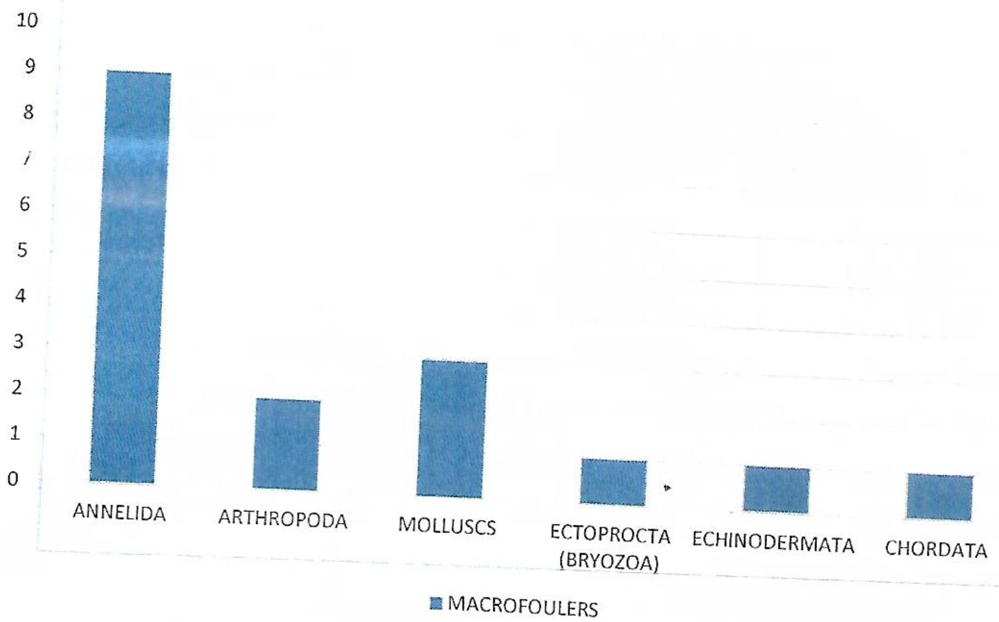

Fig. 3. Graph of species abundance of macrofoulers

The present study gave a positive result to successfully prevent the fouling organism to accumulate on the treated plate for a duration of 2 months, after which some hard foulers (*Balanus sp.*) started to attach to the plate inactivating the effect of the sponge extract. This experiment proves that sponge extract could be a temporary source of anitifouling agent.

Now let us discuss the condition of every plates in context with the foulers and degree of activity of the treated plate to inhibit such fouling.

15 Days Plate:

Both the treated and the untreated plate were full of sediments and detritus which needs to be cleaned by fine brush. Both the plates shows the growth of weeds (*Ectocarpus*) at the edge of the plate. *Ectocarpus* is the most common brown coloured fouling growth having a branched structure.

The control plate consist of a slime layer formed by microfoulers,which is mainly dominated by diatom species(*Nitzschia sp., Navicula sp., Cocconeis sp.*) along with microalgae(*ulva sp.*),bryozoans(*Bagula sp.*) and polychaetes(*Q.eresei, E.complanata*).

The treated plate was free from micro as well as macrofoulers but contained weeds and epiphytic growth at the edge of the plate and sediment and debris were the major constituent.

30 Days Plate

Sediments were the major component along with weeds at the margin of both the treated and control plates were observed.

The Control plate showed a species diversity of polychaetes (*Capitella sp., Eurythoe complanata, Questa ersei)* along with the tube dwelling polychaetes. The tube-building annelid worms (*Pomatoceros triqueter*) have a specialised operculum that blocks the entrance of their tubes when they withdraw into the tubes. They are filter feeders that secrete tubes of calcium carbonate. The gastropod (*Littorina scabra*) along with the *Balanus sp.* was found to be present.

The treated plate successfully prevented the soft foulers by inhibiting the slime layer formation by the diatoms and micro algae. The plate consist of weeds at its border, and several micro algal growth at its periphery.

45 Days Plate

Sediments and weeds were densely populated in both the treated as well as control plates on the edge of the plate.

The Control plate showed a spectacular degree of succession of fouling organisms right from the slime layer formation of microfouling to hard foulers of macrofouling.It algal mat formation at its edge which helped various polychaete sp. (*Capitella sp., Hesione sp., Chaetopterus variopedatus, Chloeia flava*), gastropods sp. (*Littorina scabra, Brachidontes exustus).* Amphipods (*Cheiriphotis trifurcata*) along with Echinoderms (*Ophiocoma sp.*) was also found to be associated with this community in the plate. Marine invertebrate, Tunicates also known as sea-squirt (*Lissoclinum fragile*) having sack-like body plan, distributed globally, usually found in shallow waters. They are mostly sessile animals and filter feeders.

The treated plate showed a little degree of divergence as few members of polychaete species *(Hesione sp.)* was found to be present. *Hesione sp.* are active carnivores that feed by everting the pharynx and sucking up their prey feeding on flora associated with the algal mats on the edge of the plate. This plate showed the majority of growth of sediments and along with elaborate algal mats at its edge.

60 Days Plate

Sediments, debris along with the weeds (*Ectocarpus sp.*), algal biofilm (*Ulva sp.*) were massively found on the edges of both Control plate and treated plate.

The Control panel comprises of wide fouling community which include various species of polychaete (*E.pennata, Q.ersei, Turbocavus secratus, Hesione sp.*), tubeworm (*P.triqueter*), Gastropods (*L.scabra*), bivalves (*Saccostrea* sp.) & ascidians (*Lissoclinum fragile).* Tunicates having a sac like body plan was also observed to be responsive member of fouling community along with the dominant *Balanus sp.*

The Treated plate showed a positive response in preventing the fouling organisms by curtailing the attachment by the release of bioadhesives, which help to organism to stick firmly to the solid substratum.as the sponge extract inhibited the microbial biofilm formation, attachment to the plates became a bit difficult thereby preventing fouling. Gastropods (*L.scabra)* were occasionally found but very few in number but it successfully prevented the hard foulers.

75 Days Plate

As the plates were immersed in sea water for a longer time, it was over populated by the weed species (*Ectocarpus)*, algal mats (*Ulva sp,)* and sediments, silt and debris.

The Control plate consist of wide diversity of fouler community holding a particular niche in the plate. The plate showed all the groups of micro and macro foulers from micro algal growth to weeds, polychaetes ,gastropods ,bivalves, tunicates, echinoderms along with sediments .It also showed growth of amphipod sp.(*C. trifurcate*) along with goose barnacle.(*Lepas sp.*).The dominant barnacle species (*B.amphitrite*) was majority in the fouling population

The Treated plates failed to inhibit the attachment of hard fouling. Hard foulers dominated by *Balanus sp.* & gastropod species (*Brachidontes exustus, Saccostrea cucullata*) were found on the Treated plates along with polychaetes (*Turbocavus secratus, Hesione sp.*)

Summary

The present study was carried out with the objective of assessing the effect of chemical compounds from marine sponge *Stylissa masa* in inhibiting the microbial biofouling, which act as the precursor for attracting macro-fouling organism to any submerged structure in the marine environment.

Attempts have been made to develop microbial biofouling inhibition mechanisms on some structures such as ship's hull, underwater pipelines, turbines, ropes and any other structure immersed in water. As it is observed, some marine organisms do not get fouling on its body, as they can naturally deter the fouling organisms by some kind of repelling mechanism in term of releasing some kind of chemical compounds, may be metabolites as in case of corals, sponges, sea weeds, horseshoe crab, sea cucumber, crab etc.

So, the objective lies to develop such antifouling paint of natural origin which would be environmentally safe, eco-friendly and persistent and efficient in preventing the colonisation of unwanted organisms on the immersed surface. The marine sponge *Stylissa masa* is known to have some antifouling property as it inhabits in the intertidal or rocky substratum by releasing some secondary metabolites which prevents settlement of foulers on its body surface.

The crude extract of the sponge were taken as the antifouling coating on the PVC plate to test for the antifouling property. The extract was successfully able to prevent the initial biofilm formation as it deter the attachment of micro foulers (diatoms and bacteria) to the treated plate curtailing the slime formation. Eventually the attachment of larval settlement and its metamorphosis could not take place for the fouling community to strengthen the growth of macrofoulers. As a result the treated plate negative impact on fouling and thus we can take a positive cue from this for the development of antifouling paint which is biologically selected, evolutionary specified to bind easily with the biomolecules without harming the marine ecosystem.

There is an urgent need for such eco-friendly anti fouling paints as the shipping Industry is spending lakhs of money to address the global concern by environment friendly manner as the chemical paints (organotins,TBT) has brought a large ecological destruction especially in the marine world by dispersing native species, affecting non

target organisms as well as marine world. So, the modern scientific research is searching for such compounds which address the global problem of antifouling in a strong & efficient manner.

CONCLUSIONS

The crude extract of marine sponge *Stylissa masa* was found to be efficient and actively inhibit the growth of micro as well as macrofoulers for stipulated period of time. The micro foulers (Bacteria, cyanobacteria, algae, diatoms) which forms primary slime layer providing the basis for the succession of the macrofoulers in the form of hard fouling (*Balanus sp.,Saccostrea sp.*) was successfully curtailed in the treated plate which consist of the coating of crude extract of marine sponge *Stylissa masa*.

Hence the current study was done to establish a strong relationship that solution for the global concern of fouling lies in the marine world itself. The crude extract of the sponge *Stylissa masa* successfully deter the growth of dominant macrofoulers which include *Balanus amphitrite, Saccostrea cucullata, Bagula neritina* for a specific period of 60 days.

Hence our current study was to throw some light on the natural source of antifouling agent which may have its antifouling effect for short periodicity but can effectively curtail the colonisation process of foulers. The marine sponge *Stylissa masa* proves to be excellent candidate for the extraction of such antifouling agent from our current study.

REFERENCES

Abarzua, S. and Jakubowski, S., 1995. Biotechnological Investigation for prevention of biofouling, I. Biological & biochemical principles for prevention of biofouling. Marine and Ecological Progress Series. 123: 301-312.

Berntsson, K. M and Jonsson, P., 2003. Temporal and spatial patterns in recruitment and succession of a temperate marine fouling assemblage: a comparison of static panels and boat hulls during boating season. Biofouling. 1(9): 187-195.

Claire, H., 2010. The potential of marine biotechnology for the development of new antifouling solutions. J. Sci. Hal. Aquat. 2(1): 35-41.

De Chalain T.M.B, I979.An investigation into aspects of biological fouling on selected artificial substrate at sub-littoral depths in Simonsbay. J. Gen. Microbiol, 7(4): 325-334.

Dehmordi, L. M., Karami, L., Safarpor, N. and Alesadi, B., 2011. Taxonomic identification and distribution of biofouling organisms in Deilam port in Iran. J. Ecol. Nat. Environ., 3(14): 441-445.

Delort, E., Watanabe, N., Etoh, H., Sakata, K. and Ceccaldi, H. L., 2000. Analysis of initial fouling process in coastal environment: Effects of settlement, attachment, and metamorphosis promoters. Marine Biotechnology, 2(3): 224-230.

Daoudi, M.; Bakkas, S.; Culioli, G.; Ortalo-Magne, A.; Piovetti, L.; Guiry, M.D., 2001. Acyclic diterpenes and sterols from the genera *Bifurcaria* and *Bifurcariopsis* (*Cystoseiraceae, Phaeophyceae*).Biochem. Syst. Ecol., 2(9): 973–978.

Goodbody, I., 1961. Inhibition of the development of a marine sessile community. Nature, 1(9):282-283.

Grosberg, 1981.Competitive ability influences habitat choice in marine invertebrates. Nature, 2(8):700-702.

Goodbody, I., 1961. Inhibition of the development of a marine sessile community. Nature, 1(9): 282-283.

Grosberg, I. 1981. Competitive ability influences habitat choice in marine invertebrates. Nature. 2(9): 700-702.

Hadfield, M.G., 1986.Settlement and recruitment of marine invertebrates: a perspective and some proposals. Bull Mar Sci., 3 (9):418-425.

Henschel, J.R. and Cook P. A., 1989. The Development of a marine fouling community in relation to the primary film of microorganisms, Biofouling., Vol. 2, pp. 1-11.

Daniel, A., 1955.The primary film as a factor in settlement of marine foulers. Jou. of Coast Env. 2(5):189-200

Hadfield, M. G., 1986. Settlement and recruitment of marine invertebrates: a perspective and some proposals. Bull. Mar. Sci. 3(9): 418-425

Hayase, N.;Sogabe ,T.;Itou,R.;Yamamori,N.;Sunamoto,J.,2003 Polymer film produced by a marine bacterium. J. Biosci. Bioengineering.9 (5): 72–76.

Hellio, C., 2010. The potential of marine biotechnology for the development of new antifouling Solutions. J. Sci. Mar. Sci., 2(9): 35-41.

Hellio, C.; Maréchal, J.-P.; Da Gama, B.A.P.; Pereira, R.C.; Clare, A.S.,2009 Natural marine products with antifouling activities. In: Advances in Marine Antifouling Coatings and Technologies .Springer. pp. 572–622.

Hellio, C.; Thomas-Guyon ,H.; Culioli, G.; Piovetti, L.; Bourgougnon, N.;Le Gal, Y, 2001. Marine antifoulants from *Bifurcaria bifurcata (Phaeophyceae, Cystoseiraceae*) and other brown macroalgae. Biofouling, 1(7): 189–201.

Henschel, J.R. and Cook, P. A., 1989. The Development of a marine fouling community In relation to the primary film of microorganisms, Biofouling. 2(5): 1-11.

Karande, A. A. & Swami, B. S., 1988. Importance of test coupons in the assessment of marine biofouling community development in coastal waters of Bombay, India. Indian J. Mar. Sci., 1(7): 317-321.

Knight-Jones, E. W., Crisp, D. J., 1953. Gregariousness in barnacles in relation to the fouling of ships and to anti-fouling research. Nature, 1(7): 1109-1110

Lau, S.C.K. and Qian, P.Y., 1997.Phlorotannins and related compounds as larval settlement inhibitors of the tube-building polychaete *Hydroides elegans.* Mar. Ecol. Prog. Ser., 15(9): 219–227

Nair, N. U., 1965. Marine fouling in Indian waters. J. Sci. Ind. Res., 2(4), 483-488

Price,R.R.;Patchan,M.;Clare,A.;Rittschof,D.;Bonaventura,J.,1992.Performance enhancement of natural antifouling compounds and their analogs through microencapsulation and controlled release. Biofouling, 6(1): 207–216.

Richmond, M. D. and Seed, R., 1991. A review of marine macrofouling communities with special reference to animal fouling. Biofouling, 3(20): 151-168.