2. REVIEW OF LITERATURE

Biofouling is one of the serious and most studied operational challenges that affect various maritime activities including shipping industry, fishing, and aquaculture equipments cooling towers of power plants, oil and gas industries, offshore platforms and other structures exposed to seawater worldwide (Nagabhushanan and Alam, 1988; Callow and Callow 2002; Venkatesan et al., 2006 and Pati and Rao, 2012).

During last centuries studies were focused on understanding the biology and physiology of micro and macrofouling species, fouling process, succession, recruitment and community structure of fouling assemblages have been studied (Oshurkov, 1992; Kocak, 2007; Litulo, 2007 and Tremblay et al., 2007).

Concurrently, several attempts have been made to understand the role of substrate characteristics and abiotic and biotic factors that influence the fouling process (WHOI, 1952), Intra and interspecific interactions within microfouling (Davey and Toole, 2000), micro and macrofouling community (Wieczorek et al., 1995; Huang and Hadfield, 2003 and Lau et al., 2003) and mechanisms of attachment of fouling organism Crisp, 1984; Berglin and Gateholm, 2003; Bromley and Heinberg, 2006 and Khandeparker and Anil, 2007) have been studied.

The adhesion mechanism of marine sedentary organism was previously observed by Holm et al. (2006) who have reported that the adhesion mechanisms of marine sedentary organisms in manmade marine submerged surfaces are complex, inconsistent and not clearly understood as previously observed (Vladkova, 2007).
Though, the phenomenon of biological fouling on marine structures has attracted several researchers worldwide from the time man started exploring oceans, the widespread attention on biofilms were gained during the last 70 to 80 years with the introspective research initiated during the 1930’s and 40’s (Zobell, 1943). Even as several earlier reports have indicated the presence of the surface adheherence of bacteria, it was the “bottle effect” observed by Zobell, (1944) who confirmed the tendency of bacteria to adhere to surfaces rather than staying in suspension. This path breaking research finding has paved way for several research initiatives to decipher the cryptic events underlying in the process of biofilm formation which is a challenge faced in the field of medicine (Costerton et al., 1987), process industries (Simoes et al., 2005), water treatment industries (Cho et al., 1999; Korbutowicz et al., 1999; Drews et al., 2006), cooling water towers (Tobin et al., 1981; Rajagopal et al., 1998; Nair, 1999), and a major issue with respect to environmental management (Bayoudh et al., 2005; Simos et al., 2006) especially in maritime operations (boat, Vessels and offshore structures) Marshall et al., 1971; Dempsey, 1981). Numerous studies have been carried out on microfouling of surfaces immersed in temperate and tropical waters (Majumdar et al., 1999).

The widely addressed issues on biofilm formation and its role in the marine biofouling process encompasses several aspects such as understanding the diversity of the organisms (Duraisamy et al., 2004; Finlay and Esteban, 2004; Hedlund and Staley, 2004; Whittfield et al., 2005; Alio, 2006), structure and biology of
these communities (Hamilton, 1987; Costerton et al., 1995; Lappin-Scott and Costerton, 1995; Burchard and Sorongon, 1998; Jones et al., 2007), their adhesion mechanism (Cooksey and Cooksey, 1992); Seasonal variation in their population (Railkin, 2004), effect of environmental factors (Chiu et al., 2006; Moldoveanu, 2012), composition of Biofilms (Decho, 2000; Madielo and Gavilan, 2005; Flemming, 2011), Communications between cells in the biofilm (Gilbert et al., 1997), the benefits to the organisms producing it (Decho, 1990; de Philippis and Vincenzini, 1998; Decho, 2000). The availability of the molecular techniques during the recent past had imparted remarkable momentum to the identification; molecular tools, coupled with the conventional approach had strengthened the process of understanding the Exopolysaccharides (EPS) (Allison 1998). Most of the studies on identification of bacterial strains have vested more interest in understanding the phenotypic diversification and genetic identity of the strains (Dang and Lovell, 2000; Jones et al., 2007; Battin et al., 2007) apart from the conventional tests.

Biofilms were defined as surface associated layers of microbial cells embedded in EPS (Characklis and Marshall, 1989; Stal and Caumette, 1994; McSwain et al., 2005) which form a slime layer loosely attached to the cell surface or secreted into the environment (Madiedo and Gavilan, 2005). The development of advanced microscopic techniques and structural analysis techniques has made the characterization of the organisms and the associated macromolecules at ease.
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(de Beer and Kuhl, 2001). The molecular composition and the diversity of the individual units of the macromolecular assembly of EPS have been reported to be composed of polysaccharides, Polyuronic acids, proteins, nucleic acids and other chemical moieties (Decho, 1990; Decho and Lopez, 1993; Schmidt and Ahring, 1994; Drews et al., 2006).

The EPS which form the basic building unit of the biofilm have drawn a widespread attention among the researchers, univocally, even to this date as it seems to exhibit a diverse compositional as well as organizational variation that remains cryptic (Decho, 2000; Drews et al., 2006; Battin, 2007; Flemming, 2011), while the physiological role of these molecules are yet to established (Madiedo et al., 2005).

Another study by Morales et al. (2007) has shown that the EPS differs in their composition between bacterial strains. The variation in the chemical composition and the physical properties of the polysaccharides in biofilm matrices has been attributed to the type of monomer units, the kind of glycosidic linkages and the occurrence of different organic and inorganic substitutions (Pamp et al., 2007). The poor understanding of the molecular nature of the EPS (Costerton et al., 1995; Decho, 2000; Sutherland, 2001; Parsek and Fuqua, 2004) has been attracting several studies over the last few decades. Furthermore, the biofilm formed on marine structures apart from influencing the settlement and development of other bacterial and invertebrate groups, is reported to influence the corrosion of metals
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(Costerton et al., 1987; Ford and Mitchell, 1990; Majumdar et al., 1999; Lee and Newman, 2003; Beech and Sunner, 2004).

The diatoms are another significant group of community to settle on artificial surfaces during the initial period of exposure (Mithavkar and Anil, 2000). Diatoms which are autotrophic eukaryotes are important primary producers in the biofilm system (Paerl, 1997; Molino and Wetherbee, 2008) and these organisms along with the other microbes and their EPS serve as food for several meiofauna and larvae of various animals (Bhosle et al., 1995; Decho, 1990), influencing their settlement and growth hence play an important ecological role (Jain et al., 2005).

In general, the diatoms are sensitive to chemical, physical and biological changes in the environment (Munda, 2005) and temporal and spatial variations in their community structure have been reported earlier by Cooksey et al. (1984); Devi, (1995); Patil and Anil, (2005).

The biofouling process initiated by the microbes on to the next stage with the settlement of invertebrate larval forms (Callow, 1996). Decades of studies on the microbial influence on macrofouling settlement indicate that the final community structure is determined by the microbes (Bhosale et al., 2002) that form the primary colonizers. The presence of complex interactions between the micro- and macrofouling community in the marine environment have attracted several research work in the recent past that have attempted to study the microbe larval interaction and the role
of microbial community in influencing or determining of larval invertebrates (Henschell and Cook, 1990).

Biofouling in tropical waters is rapid and heavier when compared to temperate regions (Huang et al., 1999) owing to the favourable hydrographical conditions. In India the tropical conditions are moderate (Satheesh, 2006) to highly favourable (Rajagopal, 1991) for marine growth. A survey of literature on biofouling from Indian sub continent indicates that studies on biofouling and biodeterioration were carried out as early as 1930 and 1940 by Erlandson, (1936) and Paul, (1942) appear to represent the earliest work on biodeterioration and biofouling respectively.

Several studies have been conducted along the East and West coasts of India to understand the biology (Kurian, 1953), ecology (Wesley, 1980; Ranganathan et al., 1982), diversity of the biofouling community (Daniel, 1954; Nair et al., 1988; Sasikumar et al., 1993; Rajagopal et al., 1998; Murthy et al., 2004; Marimuthu et al., 2005; Satheesh, 2006; Satpathy et al., 2006), distribution and growth of organisms (Ganapathi et al., 1958) at different depths (Velayudhan et al., 1988), biofouling and corrosion was conducted (Chidambaram, 1990; Maruthamuthu et al., 1993; Eashwar et al., 2008 and Palanichamy et al., 2012).

Similarly, on the west coast of India, studies have been focused on delineating biology of fouling organisms (Karande and Palekhar, 1963; Menon et al., 1977; Rao et al., 1982; Venugopalan, 1987; Venkat et al., 1995;
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Udayakumar and Karande, 1996), ecology of macrofouling organisms (Sawant and Wagh, 1994; Dattesh and Anil, 2005), Seasonal variation (Nair and Meenakumari, 1998), influence of biofilm on macrofouling settlement (Nair and Pillai, 1977; Khandeparker and Kumar, 2011), biofouling on engineered test panels, including wood and metals (Wagh and Swant, 1982; Raveendran et al., 1991; Srinivas et al., 1992; Raveendran and Wagh, 1993; D’Souza and Bhosle 2003; Swami and Udhayakumar, 2004) and biofouling and corrosion on metals (Sawant et al., 1995).

Balanus reticulatus is one of the major fouling organism in tropical Indian water as the species is reported to prefer higher salinity (Fernando, 2006). The experimental stages, settlement behaviour and other features in context to the biofouling pattern is understood to be more common among the species of barnacles though minor changes may be exhibited due to several intrinsic and extrinsic factors.

As on date most research on the adhesion of barnacles has centered on the adult system is attributed to the accessible nature and their continuous production in adults compared to cyprids (Marshall et al., 2009). Focuses of research on barnacle adhesion can be arbitrarily directed into four aspects: 1. Formation of adhesion, (2) characterization of the adhesive interfaces, (3) adhesion depth analysis and (4) failure analysis on interfaces. In the present study, the second and third aspects were studied, since surface dependency of interfacial morphology and adhesion strength has been little studied with respect to metal surfaces.
Several intensive studies (Yule and Walker, 1987; Naldrett and Kaplan, 1997; Berglin and Gatenholm, 2003; Sun et al., 2004; Odling et al., 2006) on the physico-chemical, morphological, mechanical characterization of the barnacle base plate; their mechanics of adhesion & release behaviour on engineered surfaces (Brady and Singer, 2000; Kavanagh et al., 2005; Holm et al., 2006; Meyer et al., 2006; Ramsay et al., 2008; Scardino et al., 2009); genetic control of adhesive properties (Holm et al., 2005) and the characterization of interfacial proteinaceous cement layer (Khandeparker and Anil, 2007) have been made.

Majority of the studies have been carried out with Balanus amphitrite as the test organism, while the most studied adhered being the foul release coatings (Swain and Schultz, 1996; Waterman et al., 1997; Brady, 1999; Kavanagh et al., 2003; Stein et al., 2003; Wendt et al., 2006; Kim et al., 2008) and polymers (Berglin et al., 2001; Berglin and Gatenholm, 2003) with a focus on deciphering the variation in adhesion strength towards antifouling coatings. In India, though many studies have been focused on understanding the chemical nature of the adhesive protein, a few representative investigations alone delineate the properties of interfacial surface, adhesive strength, nano mechanical properties of adhesive cement (Sangeetha et al., 2010a, b; Raman and Kumar, 2011b) and on the construction of exoskeleton (Raman and Kumar, 2011a) of the adult B. amphitrite adhered to substrates such as PMMA, SS and titanium. The paucity of multidisciplinary approach in understanding the complexities of the processes (Sangeetha et al., 2010a) seems to
have left a knowledge gap on the adhesive mechanism of the barnacles on various substrates.

Several studies were oriented to characterize the properties with a view to develop preventive and curative remedies yet another stream of study is focused on the understanding the role of biofouling and the interactions between the organism and the attachment surfaces leading to deterioration of the substrate especially biocorrosion (Aftring and Taylor, 1979; Nickels et al., 1981; Langhammer and Sunderberg, 2005; Wang, 2011).

Satheesh and Wesley, (2011) have investigated the influence of submersion season on the development of test panel biofouling communities at Kudankulam coast. In this study, the authors have reported that test panel submersion season might have a significant effect on the development of fouling communities in coastal waters.

Little attention has been paid to the influence of the different sea water parameters on the performance of chemically active antifouling paints. It has recently been shown that chemical reactions and diffusion phenomena are key mechanisms in the performance of biocide based antifouling paints and that these can be markedly affected by seawater conditions (Kill et al., 2002).

Compere et al, (2001) found that the biofilm foundation and macrofouling are usually preceded by the formation of a conditioning film comprised of adsorbed polysaccharides which form on surface within 1 min of immersion in a potentially fouling environment.
In a study by Compere et al. (2001) in which he immersed stainless steel in natural seawater collected at Brest (France) a nitrogen containing compound (possibly derived from proteins) and carbohydrates were detected on the surface after 5 h of immersion. After 24 h of an immersion, he observed amount of adsorbed molecules species and the proportion of bound carbohydrate increased relative to protein but no continuous film was revealed by the analytical techniques used.

Lau et al. (2003) has reported that both bacteria and diatoms can influence the settlement of much larger fouling organisms. For example the polychaete, *H. elegans* settles in response to cues from biofilms of diatoms. He also reported some diatoms have an individual effect such as *Achnanthes* sp. and *Nitzschia constricta*, whilst others such as *Amphora tenerrima* and *Nitzschia frustulum* do not affect the settlement of *H. elegans* larvae and or viability of the diatoms.

2.1. Identification of biofilm bacteria

Over the years, marine microbiologists inclined to study the bacterial systematic have employs several methods pertaining to their cultural, morphological, physiological and biochemical characteristics and based on their inhibitory, serological and the chemotaxonomic tests; at the same time the advances in protein and nucleic acids techniques allowed genotypic and phylogenetic analysis (Busse et al., 1996). The development of molecular biology based techniques more specifically the nucleotide analysis resulted in the restoration of the identification methods. The wide spread application of the 16s rRNA methods after the initial
investigation by Woese and Co-workers in 1970s saw many attempts to characterize and identify the bacterial strains through 16S rRNA sequences (Woese et al., 1990; Busse et al., 1996). Moreover, while the evolutionary studies prior to mid 1970 have been restricted to multicellular organisms (Woese et al., 1990), the sequencing revolution have made researchers accessible to the evolutionary history of the microbes through their molecular sequences (Zuckerkandl and Pauling, 1965; Woese et al., 1990).

Several studies have been carried out all over the world including Indian subcontinent to identify and characterize the marine microorganisms isolated from seawater (Giovannoni et al., 1990; Fuhrman et al., 1993), marine sediments (Gray and Herwig, 1996), especially the marine Biofilms formed on seagrass (Merina et al., 2011), acrylic coupons (Lau et al., 2002), Concrete Pier (Lee et al., 2003), marine pipelines (Miguel et al., 2006); glass (Kwon et al., 2002), Polystyrene (Chiu et al., 2006), Various metal panels (Sonak and Bhosle, 1995; Bragadeeswaran et al., 2011), Polymers (Muthukumar et al., 2011; Lakshmi et al., 2012), Wood panels (Satheesh, 2006), and on the hull of ships Dhanasekaran et al., 2009; Inbakandan et al., 2010).

Over the years recognizing the presence of chimers in PCR amplified products that may give an enormous picture of the bacterial diversity (Ashelford et al., 2005), several application programmes, such as Bellerophon (Huber et al., 2004), Chimera Checker (Cole et al., 2009; Nilson et al., 2010), UCHIME (Edgar et al., 2011),
Mallard (Ashelford et al., 2006), and Chimera Slayer (Hass et al., 2011), Perseus (Quince et al., 2011) and Pintail (Ashelford et al., 2005) have been developed to screen for the presence of chimerical sequence.

Das et al. (2006) has observed the marine bacterial diversity is imperative to understand their community structure and distribution pattern. Silverman and Roberto, (2007) have observed the mechanism of mussel attachment in the marine fouling. In this study, he has observed the mussel attachment via a thread and plaque structure which is initiated in the foot of the organism via a channel known as the byssal grove. After release from the foot the adhesive threads cross link and a curing reaction occurs. The process involved in curing include enzymatic oxidation of tyrosine residues to DOPA o quinine, complexation of DOPA with metal ions and formation of cross links between protein molecules by reaction of o quinine moieties with byssal residues.

Schultz, (2007) has developed a detailed formula to analyze the impact of biofouling on fuel consumption, which models the effect of varying degrees of fouling, derived from data obtained using a laboratory-scale model of a frigate, on frictional resistance and increased propeller power (required to keep the vessel at a comparable speed to a ‘clean’ control). The consequence of heavy calcareous fouling on the frigate resulted in an increase in required propeller power of 86% in comparison to a non-fouled ‘clean’ control as observed (Schultz, 2007). Such analysis characteristically indicates that if no antifouling treatments are used on vessels there
may be a 40% increase in the utilization of fuel and a reduction in speed by 10% (Kohli, 2007; Schultz et al., 2011).

A survey of literature shows that antifouling efforts leading to practical applications fall into three main categories such as (1) detachment by mechanical force (2) use of biocides and chemicals to kill the organisms and (3) modification of surface to get low – or non-stick surfaces (Vladkova, 2007). Among the methods, application of biocides had been widely practiced and owing to its effectiveness against all fouling species. Many of the biocide based antifouling paints have been efficient but were harmful to the non-target organisms. TBT based antifouling paints were one of the highly efficient but equally harmful paints developed, so far which was subsequently banned in 2008 (Hadj et al., 2012).

In India several antifouling strategies such as mechanical cleaning, use of toxic metal, Chemical and plant based biocide treatment and application of antifouling paints have been followed for long (Venkatesan et al., 2006) but initiatives on the identification of marine natural products and development of eco-friendly antifouling technologies comparatively recent and belongs to last few decades.

Consequent to the ban on TBT based paints, the use of copper based antifouling paints has increased tremendously (Finnie, 2006; Dafforn et al., 2011) which is further supplemented with booster biocides (Readman et al., 1993; Liu et al., 1997; Konstantinou and Albanis, 2004; Yebra et al., 2004;
Turley et al., 2005) which are also harmful to the environment especially to the non-target organisms (Khandeparker and Kumar, 2011).

Recognizing the harmful nature of these toxic paints several laboratories world-wide have initiated research on natural product based antifoulants (Clare, 1996; Rittschof, 2001; Marechal et al., 2004), especially from marine sources (Faulkner, 2001; Fattorusso et al., 2012). Owing to the variety of new structures and inherent antifouling properties, studies spanning over half-a-century have yielded many novel as well as potential bioactive compounds, while a few of them have already found application in biomedical and pharma industry (Azevedo, et al., 2008; Joseph and Sujatha, 2011).

Several authors have reported that the control of marine biofouling on artificial structures is vital to maintain operational effectiveness and to minimize associated costs (Armstrong et al., 2000a, b; Rittschof, 2001; Yebra et al., 2004). Toxicant-based or biocides based coating systems be capable of provide effective fouling control but those based on organotins are now expelled from being applied to submarine structures due to environmental concerns and regulations (Giacomazzi and Cochet 2004 and Yebra et al., 2004).

The need of an effective antifouling agent which prevent the settlement and growth of marine organisms on submerged structures such as oil rig supports, buoys, fish cages and ships’ hulls is recognized universally (Boxall et al., 2000).
Marine benthic organisms are constantly exposed to colonization by bacterial communities and larvae of fouling organisms. Tan et al. (2010) has observed that some of the benthic organisms can able to develop various strategies to counteract the settlement of fouling organisms, such as the production of antifouling chemicals or physical defenses.

Eighteen antifouling compounds are currently used as biocides were reported (Yonehera, 2000; Thomas, 2001). Nine of them (Chlorothalonil, Dichlofluanid, Diuron, Irgarol 1051, Seanine 211, TCMS pyridine, TCMTB, Zin pyrithione and Zineb) are approved by Health and safety Executive (HSE) in amateur and professional antifouling products marketed in the UK (HMSO, 1988 and Voulvoulis et al., 2002a). As a result, the important coastal concentration has been found in areas of high yachting activity, particularly in marinas and sportive harbors. Voulvoulis et al. (1999a, b) has reviewed 11 alternative antifouling biocides and concluded that there was not enough information on such chemical to perform a sound environmental risk.

Some of the organisms have identified recently been with effective antifouling and antimicrobial effects include soft coral (Mol et al., 2009; 2010a, b), sponge (Selvin and Lipton, 2004; Manilal et al., 2010; Iyapparaj et al., 2012), ascidians (Murthy, 1999; Mohanram et al., 2006), mollusks (Ramasamy and Murugan, 2006).
Yebra et al. (2004) in their review have drawn a detailed sketch on the
development of antifouling technologies through the two millennia across the world.
A survey of their review and the other literature (Venkatesan et al., 2006; Almeida et al., 2007) shows the prominent use of toxic material such as lead and
copper apart from other metals and non-metal such as antimony, tin, tar, pitch, etc., on
wooden ships. Besides, with the advent of ships with iron hull during the 18th century
the use of copper seems to have been less prominent owing to their corrosive effects
on iron leading to loss of antifouling activity, instead zinc, lead, nickel, arsenic,
galvanized iron and alloys of antimony, zinc and tin were used followed by wooden
sheathing which was then coppered (Callow, 1990; Yebra et al., 2004).

Marine natural products identified to possess several bioactive compounds
have crossed over 30,000 compounds (Fattorusso et al., 2012) with over 145
compounds showing potential antifouling activity (Raveendran and Mol, 2009).

A survey of the literature shows that the sponges (Munro et al., 1999; Faulkner, 2002; Hellio et al., 2005; Clavico et al., 2006; Ortlepp et al., 2007) and soft
corals (Willemesen and Ferrari, 1993; Clare, 1996; Wilsanand et al., 2001; Pereira et al., 2002) from major groups apart from seaweeds (de Nys et al., 1995;
Clare, 1996; Dobretsov and Qian, 2002; Periera et al., 2003; Selvin and Lipton 2004),
tunicates (Davis and Write, 1990; Kijjoa and Sawangwong, 2004), mangroves
(Prabakaran et al., 2012), bryozoans and seagrasses (Davis et al., 1989; Clare, 1996),
gorgonians (Qi et al., 2008) and microorganisms (Burgees et al., 2003). A survey of
literature on sponges and seaweeds, the major reservoirs secondary metabolites indicates that the compounds identified so far ranged from derivatives of aminoacids and nucleosides to macrolides, porphyrins, terpenoids, steroids, aliphatyic cyclic peroxides, in sponges (Parameshwaran et al., 1989; Sarma et al., 1993; Parameshwaran et al., 1994; Faulkner, 1995; Scheuer, 1995; Parameshwaran et al., 1997; Fontana et al., 2000; Tilvi et al., 2004; Mol et al., 2009) while the seaweeds have been found to harbor terpenoids, acetogenins, polar phenolics, carotenoids, furanones, alkaloids, lactones (Hay, 1996; Fusetani, 1997; Clare, 1998; Pereira et al., 2003; Almeida, 2007; Raveendran and Mol, 2009).

Priya et al. (2013) has studied antifouling activity of prodigiosin from Serratia marcescens CMST 01 an isolate from estuarine. In her findings she has reported the possibility of using bacterial pigments as the source of antifouling compounds for controlling the fouling problem in the marine environment. Likewise, environmentally benign antifouling potential of triterpene-glycosides have been isolated from Streptomyces fradiae an isolate of mangrove (Praksh et al., 2015).

The search for eco-friendly antifouling technology as an alternative for chemical biocides has been previously reported (Clare, 1998; Yebra et al., 2004; Sipkema et al., 2005; Raveendran et al., 2008; Mol et al. 2009). During the past 2 to 3 decades were also focused on the secondary metabolites that maintain the surface of sessile marine organisms free from epiphytic organisms (Omae, 2003).
Silkiana et al. (2012) studied the comparative efficiency of macro algal extracts from *Sargassum muticum* and *Ceramium botryocarpum* and booster biocides as antifouling agents against the growth of three marine diatoms such as *Fragillaria pinnata*, *Cylindrotheca closterium* and *Thallassiosira pseudonana*. In this work she has reported the ethanol extract of *S. muticum* and *C. botryocarpum* inhibited all the three marine diatoms with the effective concentration 4.74 and 5.3 µg/ml respectively.

Silkiana et al. (2009) compared the antifouling activity of macroalgal extracts on *Fragilaria pinnata* with diuron. In the study, she has reported that the crude extract of *Sargassum muticum* and *Ceramium botryocarpum* displayed clean antifouling properties against the marine pinnate diatom *F. pinnata*. These natural antifouling compounds affected the growth and pigment content of the diatom.

Hellio et al. (2001) has studied the extracts of marine macroalgae on the inhibition of marine biofouling bacteria. In this observation she has tested 30 macroalgal species from the coast of France and concluded that 20% of the extracts were more effective and recorded the absence of toxicity on the development of oyster and sea urchin larvae and to mouse fibroblast growth.

According to a recent report about 0 of the 145 natural product antifoulants alone has been implicated to higher activity and lower toxicity compared to the available biocides (Raveendran and Mol, 2009) availability of various number of organisms that are not yet screened had a promising prospect for antifouling research.
The antifouling activity of marine macroalgal compounds is generally assayed using extracts in various organic solvents (Liao et al., 2003). Previous works have shown that ethanol/water and ethanol/dichloromethane extracts from *Sargassum muticum* (Heterokonta, Sargassaceae) and *Ceramium botryocarpum* (Rhodophyta, Ceramiaceae) presented antifouling properties against representative marine organisms such as marine bacteria, phytoplankton, and spores of macroalgae (Bazes et al., 2006, 2009; Silkina et al., 2009).

Assays to identify potential antifouling compounds are performed with representative test organisms in the laboratory under controlled conditions or through field assays mainly the target organisms include biofilm bacteria (Salvador et al., 2007) microalgae (Targett et al., 1983; Wilsanand et al., 2001; Hellio et al., 2004; Pettitt et al., 2004; Statz et al., 2006), macroalgae (Hattori et al., 1998).