Chapter 2

Review of Literature

2.1 BIOFOULING

Biofouling can be defined as the unwanted accumulation of living organisms such as microbes, plants and animals on any immersed surfaces in a common matrix. It is considered to be a tedious problem in the marine ecosystems, predominantly to shipping. The submerged surfaces including underwater pipelines, fishing nets, cables and bridge pillars are often fouled by marine organisms posing adverse effects on maritime industries. Several traditional antifouling coatings have been developed to prevent the adhesion of fouling organisms on marine structures. The biocides present in these coatings have been reported to kill possible microfouling organisms including bacteria, algae, fungi, and macroorganisms such as plants and molluscs (Shan et al., 2011). However, due to the lethal effects of biocides on non-target marine organisms, world-wide legislation has imposed several limitations on its use to combat biofouling.

2.1.1 THE PROCESS OF MARINE BIOFOULING

It has been reported that marine biofouling involves a huge diversity of fouling organisms. The size of the fouling organism ranges from micrometers (bacteria, algal spores, diatoms) to centimeters (barnacles, mussels, oysters, mollusc). Fig. 2.1 shows a range of fouling organisms with respect to their size. Biofouling can be described as a dynamic process, which is typically observed over a period of time scale. Fouling of any new surface in the marine environment can be divided into four phases: formation of a conditioning film by organic molecules, primary colonization by microorganisms such as bacteria and diatoms, secondary colonization by algal spores, and finally attachment of macrofoulers.
2.1.2 CONDITIONING FILM

It often begins with the adsorption of dissolved organic matter to the material surface upon exposure in an aquatic medium. Ions and water molecules present in the aqueous solution form an electric double layer, which eventually affects the surface from being adsorbed by the proteins (Ostuni et al., 2001). The proteins adsorbed on the material surface act as the conditioning film for the attachment of microfouling organisms including bacteria and diatom. The conditioning film changes physical and chemical characteristics of the original surface (Characklis and Cooksey, 1983), which, in turn, affects subsequent primary colonizers. Compere et al. (2001) reported the development of a conditioning film comprising of proteins, polypeptides and polysaccharides on the surfaces immersed in a potential fouling environment before the formation of biofilm and macrofouling.
2.1.3 MICROFOULING

Microfouling refers to the formation and development of biofilm on the immersed surface. It begins with the attachment of bacteria with the conditioning film first. Since bacteria often dominate the aquatic environment by number besides other organisms and they attach to the surface within an hour of immersion. Once the bacteria attach to the surface, they begin to produce a matrix of extracellular polymeric substances (EPS) which plays a vital role in the adhesion and subsequent biofilm formation. Bacterial biofilms constitute different communities that often vary with time and environment. Quorum Sensing has been recently recognized as one of the major factors that decide biofilm formation and maturation. It has also been reported to play a major role in the organization of a proper biofilm.

Quorum sensing regulation basically operates through a genetic circuit which is composed of transcriptional factor (LuxR) and an acyl homoserin lactone (AHL’s) synthetase (LuxI) (Fig. 2.2). Thus the development of biofilm is primarily based on the relative abundance of each type of microfouling organisms. In general, the sequence of attachment in the aquatic environment is as follows: dissolved molecules > bacteria > fungi > diatom, protozoans, and algal spores. In addition to this, physical and chemical characteristics of a new surface, behavior of settling microfoulers also influence the early colonizers. The development of a biofilm is a continual process on any surface since microbial communities will go through continuous succession (Arai et al., 2009). Development of microfouling (biofilm maturation) is influenced by several physical and chemical factors of the external environment. The highly dynamic development and succession/changes of microbial communities in biofilms make it difficult for controlling microfouling processes in nature.
Fig. 2.2 LuxR/I system in *V. fisheri* (Duque et al., 2013).

2.1.4 MACROFOULING

Macrofouling refers to the settlement and growth of marine macroorganisms such as spores of macroalgae and larvae of marine sessile invertebrates on marine structures. Accumulation and settlement of macroorganisms, including seaweeds, oysters, mussels, tube-building polychaetes, clams, and barnacles is the most problematic effect and real concern of marine maritime industry. It is predicted that the biofilm nature differs extensively, and they could cause either positive or negative stimuli towards the settling macroorganisms. One of the widespread characteristic of macroorganism attachment is physiochemical cueing. Jakob et al. (2008) have reported selective settlement of invertebrate larvae on suitable substrates, and their choice is primarily decided by water streaming conditions, surface topography and chemical properties. The chemical cues
springing from the substratum in water columns trigger the larvae either to settle or reject
the settling surface.

2.2 DELETERIOUS EFFECTS OF MARINE BIOFOULING

Some of the adverse effects of marine biofouling include: Increased weight of the
shipping vessel, which ultimately reduces its speed. Increased fuel consumption due to
which toxic and harmful gases is often released into maritime industries. Increase in
incidence of docking operations and loss of maneuverability. Surface coatings are
worsened leading to discoloration and variations of electrical actions across the surface.

2.3 ANTIFOULING TECHNIQUES

A large number of physical parameters viz. light, temperature, salinity, voyage
speed and water depth influence the severity of biofouling. In general, biofouling is more
pronounced in increased water temperatures, because temperature is one of the key factor
that determines breeding periods and growth rates of fouling organisms (Rascio, 2000).

Since these parameters cannot be modified to great extent, a variety of coating system has
been developed and investigated to avoid the attachment of fouling organisms. In
addition to this, some of the metabolites secreted by the microorganisms were found to
inhibit the attachment of macroorganisms (Peter et al., 1997) and extracts of these
metabolites could also be effectively used as antifouling agents. Therefore, in general,
antifouling techniques can be divided into three main categories: physical, chemical and
biological methods.

2.3.1 PHYSICAL METHODS

2.3.1.1 ELECTROLYSIS AND RADIATION

Several physical methods have been employed for preventing biofouling.
Production of hydrogen peroxide (H$_2$O$_2$), bromine (Br$^-$) and hypochlorous acid (HClO)
through electrolysis of seawater are the widespread techniques employed. (Yebra et al.,
2004; Chiang et al., 2000; Matsunaga and Lim, 2000). Among these agents, HClO being
a powerful oxidizing agent could effectively reduce colonization of fouling organisms.
However, the disadvantage of these agents is that, they could build up the deterioration of steel due to voltage drop across the façade. Consequently these agents are not highly efficient. Alternatively, Liang and Huang, (2008) studied the antifouling efficiency of anodic coatings supported by titanium due to their low decomposition tension, low energy consumption, and higher current effectiveness, but the development of these systems has been limited. Microcosmic electrochemical systems, based on direct electron transfer between an electrode and the microbial cells, have also been attained as antifouling system. The antifouling abilities of vibration methods have also been developed (Sanford and Rittscho, 1984), but all these methods are not convenient in use.

2.3.1.2 MODIFICATION OF SURFACE PHYSICAL PROPERTIES

Several antifouling systems that are free of biocides have also been found to impede the attachment or settlement of fouling organisms. Changing the surface physical properties such as surface chemistry or topography may inactivate the adhesive proteins thus making the surface unsuitable for the adhesion or prevents long-term settlement by potential fouling organisms.

2.3.1.2.1 FOULING RELEASE LOW SURFACE ENERGY COATINGS

These coatings principally work by dropping the critical surface tension at the coating surface. There are two main types of fouling release low surface energy coatings viz. fluorinated polymers and silicones. Anderson et al. (2003) investigated the combined effect of both fluorinated polymers and silicone coatings for fouling release properties. Hexafluoroacetonepolyol and polypropylene glycols, which are fluorinated polymers inhibited fouling and decreased the attachment or settlement of fouling organisms effectively. These polymers also presented relatively high surface energy and were found to be effective against the settlement of barnacles. In another study, addition of fluorine (4-10 % w/v) into the coatings improved corrosion protection showing the additional benefit of these fluorinated coatings (Malshe and Sangaj, 2005).

An example of antifouling coating with low surface energy and low elastic modulus is platinum-catalysed silastic T-2™ (poly (dimethylsiloxane) elastomer (PDMSe)). Among these, Sharklet AFT™ topographic surface intended to imitate shark
skin has been reported to inhibit 86 % reduction in the settlement of zoospores of *Ulva* (Carman et al., 2006).

2.3.1.2.2 POLYMER BRUSHES IN ANTIFOULING

Polymer brushes are molecular level brush-like structures composed of surfaces covered with long chain polymer molecules joined at one end to the surface of the material. They have been extensively utilized in biomaterials and biomedical devices (Neoh and Kang, 2011; Xu et al., 2009a). They can be fabricated with the incorporation of functional groups with specific anti-adhesion, antimicrobial and anticorrosion properties to serve as an alternative to biocide-releasing coatings for inhibiting marine biofouling. Tethering of polymer brushes to material surfaces can be accomplished by two methods i.e. ‘grafting-to’ or ‘grafting-from’ approaches. In the ‘grafting-to’ approach the polymer brushes are attached to material surfaces either through physisorption or covalent bonding. On the other hand, in ‘grafting-from’ approach a dense and stable polymer brush coatings are obtained via polymerization from initiators bound on the material surfaces (surface initiated polymerization). In particular, surface-initiated controlled radical polymerization (SICRP), such as atom transfer radical polymerization (ATRP), is one of the powerful techniques for preparation of well-defined and narrowly dispersed polymer brushes (Fig. 2.3). Chitosan a polymer with natural antibacterial property have been added to the polymer to give an antibacterial surface that resisted colonization of *Escherichia coli* by 60–70 %. However, the validity of using natural barnacle cement in coatings is inadequate for marketing. Kuang and Messersmith (2012) have prepared polysulfobetane methacrylate (PSBMA) polymer brushes using small molecules comprising alkyl bromide group attached to the C-terminus of a tripeptide containing two L-DOPA residues that mimic mussel cement. In another study, antifouling coatings were prepared on glass surfaces modified with the bromomethyl-terminated silane for preventing attachment of algal zoospores, diatoms and barnacle cyprids (Aldred et al., 2010).
Highly efficient coupling reactions, such as azide-alkyne click chemistry, thiol-ene click reactions and activated ester-amine reactions, provide convenient and versatile approaches for efficient polymer synthesis and macromolecular engineering.
Fig. 2.3 Functional polymer brush coatings prepared via SI-ATRP and click chemistry (Yang et al., 2014).
2.3.1.2.3 ANTIFOULING SOL-GEL COATINGS

Sol–gel materials are inorganic/organic hybrids formed from the hydrolysis and condensation of metal alkoxide and organoalkoxysilane precursors [(tetramethoxysilane (TMOS), tetraethoxysilane (TEOS), n-propyltrimethoxysilane (C3-TMOS), n-octyltriethoxysilane (C8-TMOS), 3,3,3-trifluoropropyltrimethoxysilane (TFP-TMOS) or bis [(3-trimethoxysilyl) propyl]etylenediamine (enTMOS)] mixed with water and a solvent in the presence of acid or base catalyst (Wen and Wilkes, 1996). Sol–gel materials can form highly porous solid films, which can be tailored to entrap/immobilize biomolecules such as enzymes and proteins retaining their biological function with potential fouling release functionality, as well as for other applications. The use of different precursors and a range of mixing times for the sol–gel formulations were found to have an effect on the ability of coatings to inhibit macrofouling (Tang et al., 2005). In addition, they are cost effective, require minimal preparation and also offer transparent function to the coatings (Dave et al., 1994). Thus sol-gel coatings are considered to be a potential alternative to existing antifouling paint systems.

2.3.1.2.4 ZWITTERIONIC SURFACES IN ANTIFOULING TECHNOLOGY

Polymer surfaces that could withstand the adhesion of proteins are zwitterionic surfaces. Zwitterionic polymers, such as sulfobetaine, carboxybetaine and phosphorylcholine, contain a mixture of anionic and cationic pendent groups. Due to the presence of positively and negatively charged groups, these polymers exist as neutrally charged surfaces. Zwitterionic polymers have been reported to posses’ better antifouling efficacies.

Aldred et al. (2010) investigated the antifouling properties of PSBMA prepared via SI-ATRP against the settlement of barnacle cyprid larvae (B. amphitrite). The results of cyprids behavior on both the surfaces were quite different. On the PSBMA-modified surface, the cyprids explored the surface but were unable to settle, whereas the cyprids did not attempt exploration and left quickly from the PCBMA-functionalized surface (PCBMA).
2.3.1.2.5 AMPHIPHILIC SURFACES

Amphiphilic surfaces incorporate both hydrophilic and hydrophobic components result in phase segregation and are expected to be capable of resisting biofouling and releasing of fouling organisms. Inhibition of the algal and barnacle attachment has been reported on amphiphilic polymer networks of photochemically cross-linked hydrophobic PFPE and hydrophilic PEG. The flexible PEG chains have provided enhanced antiadhesion property. The influence of the PEG chain length in the amphiphilic networks on the antifouling performance has also been investigated besides the PEG flexibilities. The amphiphilic PFPE-PEG networks with the highest molecular weight PEG (PEG 1100) has shown to inhibit the settlement of alga (*Ulva*), diatom (*N. perminuta*) and cyprids of barnacle *B. Amphitrite* (Wang et al., 2011). More recently, hyperbranched cross-linked amphiphilic networks have been prepared via ATRP technique. One such example was amphiphilic hyperbranched fluoropolymer (HBFP) with a bis-amino functionalized PEG, which exhibited antifouling as well as fouling release properties against the settlement of cyprids of barnacle *B. amphitrite*. In case of diatoms (*N. incerta*) the fouling release efficiency of HBFP-PEG surfaces was found to be two- to three-times easier than the poly (dimethylsiloxane) (PDMS) elastomer surface. However, in tests with algal spores (*U. linza*), only two formulations, among the four HBFP–PEG surfaces displayed excellent fouling release performance (Imbesi et al., 2012a; Imbesi et al., 2012b; Gudipati et al., 2005).

Surface-active block copolymers (SABC), which are amphiphilic and fluorinated, have been prepared as marine fouling resistant and fouling release coatings (Weinman et al., 2009; Park et al., 2010; Dimitriou et al., 2011; Sundaram et al., 2011). SABC can be immobilized on the material surfaces by employing a primer layer of polystyrene-\textit{block}-poly (ethylene-\textit{ran}-butylene)-\textit{block} polystyrene (SEBS). This bilayer coating approach not only produces sufficiently thick polymer film but also offers good mechanical property with low modulus (Youngblood et al., 2003). In a study, the attachment strength of *U. linza* sporelings was lower on the ‘low modulus’ primer layer than on the ‘high’-modulus primer layer (Weinman et al., 2009). Thus, by incorporation of a low-modulus underlayer the fouling release properties can be enhanced, while the amphiphilic SABC
layer improves the fouling-resistant efficacies. Some of the hydrophobic components, such as hydrocarbons (PDMS and polyesters), have also been employed to prepare amphiphilic polymers instead of fluorinated polymers. Bartels et al. (2011) have prepared amphiphilic polymer networks, based on alkene-modified dendritic polyester and PEG tetrathiol and reported that the settlement densities of zoospores (Ulva) on the amphiphilic surfaces were much lower than that on the PDMS surface.

2.3.2 CHEMICAL METHODS

The chemical methods employed in the antifouling technology can be categorized into two types: Traditional and modern chemical methods.

2.3.2.1 TRADITIONAL CHEMICAL METHODS

In traditional chemical methods, organic tin and its derivative have been extensively used due of its extremely toxic nature towards a broad variety of fouling organisms. Two main organo-tin compounds i.e. tributyltin oxide (TBTO), and tributyltin fluoride (TBTF) have been effectively used as antifoulants in AF coatings. These compounds are fungicides that can entirely hamper the development of the fouling species even at very low concentrations and are considered to be effective biocides (Iwao, 2003a). Based on the chemical nature and solubility in water, the paints comprising these compounds can be divided into two groups’ viz. insoluble and soluble matrices. (Fig. 2.4).

Antifouling coatings with insoluble matrix are made of vinyl or epoxy polymers that are insoluble in water. When immersed in water soluble biocides present in the coatings dissolve, leaving a multiporous structure called leached layer. As the seawater infiltrate deep into the matrices, more lethal biocides dissolves in the water leading to the toxicity (Yeabra et al., 2004). The benefit of this type of coating is that the structure of polymer matrix is instinctively tough and secure to corrosion and photodegradation. Thus very thick coatings can be prepared in order to boost the content of biocides. However, during course of time, the leached layer turn into so thick that water cannot go through any deeper and thus declining the release of biocides that is insufficient for antifouling. Hence the lifespan of these coatings are as short as 12–18 months (Marson, 1969).
Antifouling coatings with soluble matrix have been developed in order to increase the lifespan of coatings. These coatings consist of soluble resins that can readily dissolve when immersed in sea water. Due to the presence of thin leached layer, soluble biocides present deep inside the matrix can be readily exposed to the seawater with increase in sailing speed, thus increasing the duration of these coatings (Yebra et al., 2004). The drawbacks of these coatings are: under static conditions, insoluble salts present in the water could block the pores which significantly reduce the release of biocides (Rascio et al., 1990) and thus favoring the settlement of fouling organisms. In addition to this, the soluble resin present in the matrices is brittle and instable to oxidation.
2.3.2.2. MODERN CHEMICAL METHODS

2.3.2.2.1 TBT self-polishing copolymers (TBT-SPCs) as antifoulants

These paints are based on acrylic polymers (usually methyl methacrylate) where, the backbone of the polymers is bounded to TBT groups by an ester linkage. The soluble pigment particles dissolve when immersed in the sea water, but the hydrophobic TBT methacrylate and methyl methacrylate polymers stops the sea water from infiltrating the coatings. Furthermore, hydroxylation of the TBT linkage occurs in slightly alkaline environments (seawater), resulting in the breakdown of TBT portions from the copolymer, thus discharging the biocides into the water. The partially reacted brittle polymer backbone can be easily washed off by the moving seawater, eventually exposing a fresh coating surface when numerous TBT portions have been cleaved, (Iwao, 2003a; Kiil, 2001).

Some of the advantages of these TBT-SPC paints are: the polymer chemistry can be manipulated to control the polishing rate, thus increasing the possibility of balancing efficiency and extended duration of the coatings. Ships working under different sailing speed and circumstances can therefore be tailored by these systems. Increased antifouling performance was noticed due to the constant release of TBT with sailing speed even under static conditions. Moreover, these paints with high mechanical strength, high stability to corrosion, convenient maintenance was found to be cost effective. Hence, the TBT-SPC antifouling coating have been broadly applied in the shipping industry prior to it prohibition.

2.3.2.2.2 Tin-free SPC technology.

The International Maritime Organization (IMO) conference in 1998 has restricted the use of TBT due to its damaging effect on non target species. Therefore, the use of TBT-SPC coatings in antifouling has been prohibited from January, 2008. Consequently, tin free coatings have been initiated in antifouling technology (Anna, 2009). Tin-free SPCs mostly contain acrylic copolymer and non-tin metals which hydrolyze soluble acidic polymer. The Zn\(^{2+}\) ions releases into the water and act as antifoulant leaving the polymers. Next to zinc, copper and silver have been extensively used in many of the tin-
free antifouling paints (Wisniewski and Reichert, 2000). The main disadvantage of paints containing copper and silver are, they could target only particular organisms unlike tin based antifouling paints (Voulvoulis et al., 1999). In order to increase the antifouling properties of these coatings, booster biocides such as Irgarol 1051 and Diuron (Iwao, 2003a; Anita et al., 2009), copper pyrithione and isothiazolone (Shtykova et al., 2009) have been added. However there are concerns about the controlled release of copper containing paints and booster biocides into the marine atmosphere (Anita et al., 2009).

2.3.3 BIOLOGICAL METHODS

2.3.3.1 MARINE DERIVED NATURAL PRODUCTS AS ANTIFOULANTS

In order to develop safe and environmentally benign antifouling agents, marine organisms which were found to be free of fouling on their facade have been widely explored due to the presence of natural chemical defenses in their systems (Pawlik, 1992; Abarzua et al., 1999; Piazza et al., 2011; Dobretsov et al., 2013). Several antifouling compounds have been dug out and characterized from marine seaweeds and invertebrates. In order to overcome the difficulty of obtaining these compounds in huge quantities, a diverse group of organisms including marine fungi, bacteria, aquatic plants, and terrestrial plants have been explored (Dahms et al., 2006; Dobretsov et al., 2006).

2.3.3.2 COMPOUNDS FROM MARINE MICROORGANISMS

Bioactive compounds from marine microorganisms were found to be more gifted. Fig. 2.5 shows the new antifouling compounds extracted from microorganisms dwelling in the marine ecosystem. *Aureobasidium* sp. (marine fungus) with new ester-aureobasidin-7 was reported to inhibit the settlement of *B. amphitrite* larvae (Abdel-Lateff et al., 2009).

Bhattarai et al. (2006) have isolated two compounds, 2-hydroxymyristic acid (HMA) and cis-9-oleic acid (COA) from the marine bacterium, *Shewanella oneidensis* SCH0402. In the laboratory scale experiments, both HMA and COA inhibited the growth of fouling bacteria when compared to commercial antifouling compound tributyltin oxide (TBTO).
Antifouling activities of five structurally similar compounds isolated from the crude extract of a marine *Streptomyces* have been studied against major fouling organisms (Xu et al., 2010). The functional moiety responsible for antifouling activity was found to be present in the 2-furanone ring. In another study, Xu et al. (2009b) studied the anti-larval settlement activity of 12-methyltetradecanoid acid (branched-chain fatty acid) isolated from *Streptomyces* sp. UST040711-290. This species produced the highest yield of 12-MTA which strongly inhibited the settlement of the polychaete (*Hydroides elegans*) larvae. Diketopiperazines (bacterial and fungal metabolites) which are known to inhibit bacterial quorum sensing have been isolated as antifouling compounds. Sterigmatocystin, methoxysterigmatocystin and 6, 8-di-O-Me versiconol have been isolated from *Aspergillus* sp. (derived from corals). The first two compounds demonstrated tremendous antifouling activities against *B. amphitrite* (Li et al., 2013).
2.3.3.3 ANTIFOULING COMPOUNDS FROM AQUATIC PLANTS

Seaweeds constitute an important group of plants that has been actively explored for antifouling compounds. On the discovery of brominated furanones and elatol in seaweeds as potent antifouling agents (Fusetani, 2004), antifouling activity of several seagrasses and mangrove plants have been screened. Zosteric acid, a seagrass metabolite which was found to be non-toxic antifoulant has been isolated by Xu et al. (2005). Fig. 2.6 shows some of the recently isolated antifouling compounds from aquatic plants and seaweeds.
Culioli et al. (2008) have isolated three mero-diterpenoids (16–18) from *Halidryssiliquosa* (brown alga), which inhibited the attachment of *B. amphitrite* cyprids. The first two terpenoids demonstrated noticeable toxicity (*LD*$_{50}$ at 5 µg/ml), whereas the third compound did not exhibit toxicity to the nauplli (*LD*$_{50}$ > 100 µg/ml). In another report three of the dolastane-diterpenoids from the brown alga (*Canistrocarpus cervicornis*) was found to hinder byssal production in mussels (Bianco et al., 2009). In a similar study, primarene (diterpene 23) extracted from the mangrove plant *Ceriopstagal* has been employed in anti-settlement assay against cyprids of *B. albicostatus*, which has been proved to be an excellent antifouling agent (Chen et al., 2008). Qi et al. 2008a investigated the inhibitory effect of antifouling agent (Luteolin-4’-glucuronide (26)), extracted from the seagrass (*Enhalusacoroides*) against the larvae of *B. neritina*.

### 2.3.3.4 COMPOUNDS FROM MARINE INVERTEBRATES

Metabolites derived from marine invertebrates have long been known for their non-toxic AF compounds (Fusetani, 2004). Several non-toxic AF agents such as isocyano terpenoids (Nogata and Kitano, 2006), 3-alkylpyridinium salts (Chelossi et al., 2006; Elersek et al., 2008) have been derived from sponge metabolites and halogenated 1-methylgramines from a bryozoan (Kawamata et al., 2006). (Braekman and Daloze, 2004).

Analogs of Avarol (30) extracted from marine sponge *Dysideaavaara* at a concentration of 0.45 to 26 µg/ml demonstrated excellent antifouling activity against *B. amphitrite* cyprids. In particular 3’-(p-chlorophenylamino) avarone (31) and 4’-propylthioavarone (32), which demonstrated good therapeutic ratios (*LC*$_{50}$/EC$_{50}$ > 100) were found as potential candidates (Tsoukatou et al., 2007). In a similar study, Hellio et al. (2005) have isolated 14 terpenoids from mediterranean sponges in order to evaluate their antifouling activity against larvae of barnacles. Among the studied isolated compounds, hydroquinone A acetate (33) and dihydrospongin II (34) were found to be more potential than others (Fig. 2.7).
Fig. 2.6 Compounds extracted from seaweeds and aquatic plants showing antifouling activity (Qian 2010).
Similarly, manoalide (38) and its analogs, sester terpenoids, derivatives of *Luffariella variabilis* (sponge) also reported to hamper bacterial quorum sensing in organisms associated with coral reefs of great barrier (Skindersoe et al., 2008). Many new antifouling compounds have been reported from the genus of *Sinularia* over the past five years. Pavidolide C (37) and pavidolide D (38) new diterpenoids, isolated from *S. pavida* successfully inhibited larval attachment of *B. amphitrite* (ED$_{50}$ values of 11.92 and 6.92 μM).

Kelly et al. (2003) have isolated Oroidin (43), (an alkaloid from sponges) which vigorously repressed the attachment of bacteria has lead to the development of more active oroidin based suppressants. Ascidian larval attachment and its metamorphosis has been completely inhibited by Haliclonacyclamine A (46) derived from the marine sponge *Haliclona* sp. (Roper et al., 2009). Ortlepp et al. (2008) have investigated the AF activity of polybrominateddiphenyl ethers, which are metabolites of marine sponges (*Dysidea*), along with their synthetic analogs against the barnacle larvae. The results reveal that the compound 48 with an EC$_{50}$ of 1.26 μM against the larvae also repressed adhesion of mussels and diatoms, thus was found to be the most potent AF compound.
Fig. 2.7 (a), (b) Antifouling compounds from invertebrates of marine origin (Qian 2010).
2.3.3.5 COMPOUNDS FROM TERRESTRIAL SOURCES

Various terrestrial plants have been explored as potential sources of AF compounds during 1980s, but due to huge investigations on marine organisms they have been superseded in 1990s (Fig. 2.8). Feng et al. (2009a) have isolated antifouling compounds (methoxykurarinone (58) and matrine (59)) from *Sophora flavescens*, a Chinese medicinal plant, which showed inhibitory effect on *B. albicostatus* cyprid settlement and their lethality was found to be moderately low (LC$_{50} > 250$ µg/ml, respectively).

Fig. 2.8 Compounds from terrestrial sources with antifouling activity (Qian 2010).
Angarano et al. (2009) evaluated the inhibitory effect of 19 capsaicin-related compounds against the zebra mussel attachment, among which capsaicin (60) showed the most promising AF activity with EC$_{50}$ value of 13.7 µM. Juvenoids which are analogs of insect juvenile hormones has also been investigated as promising antifoulants. Tannins are extensively dispersed in plants and demonstrate a diversity of biological activities. Stupak et al. (2003) studied the inhibitory role of tannins against the settlement of B. amphitrite naupliar larvae.

2.4 NANOTECHNOLOGY AND NANOPARTICLES

Nanomaterials could considerably solve several technological and environmental issues especially in the areas of catalysis, water treatment, medicine, solar energy conversion etc. Metal nanoparticles (MNPs) especially AgNPs and ZnO-NPs with tunable physical and chemical properties have been attracted intensely in various fields of science. Furthermore they were considered to be less toxic and safe antibacterial agents to higher animals.

2.4.1 SYNTHESIS OF AgNPs AND ITS APPLICATIONS

Silver is one of the soft transition metal having high electrical and thermal conductivity. In ancient period, it was used in many forms such as coins, vessels, lotions, foils, ointments etc. It has also long been known for its therapeutical application in medicine for the treatment of several infectious diseases and surgical infections. Silver nanoparticles have received much attention in nanobiotechnological research due to their distinct properties. It has several important applications in diverse areas including catalysis, optics, electronics, biomedicine etc (Bhainsa and Souza, 2006).

2.4.1.1 CHEMICAL METHODS

Chemical methods employed for the synthesis of AgNPs includes: Chemical reduction (Jiang et al., 2006), photochemical method, electrochemical method (Lim et al., 2006), and pyrolysis (Qiaoxin et al., 2009). Morphology of the nanoparticle strongly depends on the temperature adopted during the synthesis process. Abubakar et al. (2007) synthesized nanospheres of Ag$^+$ (average size ~11 nm) by reducing silver nitrate in
polyolsolution and in the presence of PVP and KNO$_3$ employing electrochemical method. Spray pyrolysis is one of the chemical methods used for AgNPs synthesis. Although chemical methods produce pure and well-defined AgNPs, the chemicals used in the synthesis process are highly toxic to the environment. In addition, these methods are energy consuming, expensive, and not suitable for biological applications.

2.4.1.2 PHYSICAL METHODS

Physical methods employed for AgNP synthesis includes: physical vapor condensation (PVC) and Arc-discharge method. In PVC technique, target materials are vaporized by using external heat source and then allowed to rapidly condense. A novel technique for preparing nanosilver is arc-discharge method, which operates without using any surfactants and stabilizers.

2.4.1.3 BIOLOGICAL METHODS

2.4.1.3.1 MICROBE MEDIATED SYNTHESIS OF AgNPs

AgNPs synthesized by *Bacillus flexus* resulted in spherical (12 nm) and triangular (61 nm) shaped particles (Priyadarshini et al., 2013). Jain et al. (2010) synthesized AgNPs of mixed morphology (cubic and hexagonal) using the spore crystal mixture of *Bacillus thuringiensis*. The synthesized nanoparticles were of 15 nm size. The size of AgNPs was found to be influenced by different parameters like pH, temperature, and concentration of AgNO$_3$ in recent studies (Gurunathan et al., 2009; Minaeian et al., 2008). Shape and size of the AgNPs depend on the interaction of silver ions with bacteria.

Similar to bacterial synthesis, several reports are available on the AgNPs synthesis using fungi as potential source. Cell free filtrate of *Helminthosporium tetramera* produced polydispersed spherical AgNPs of size range within 17-33nm and the synthesized AgNPs exhibited significant antibacterial activity (Shelar and Chavan 2014). Extracellular formation of AgNPs has been reported by Syed et al. (2013) using thermophilic fungus *Humicola* sp. The fungus *Bipolaris nodulosa* produced different shapes of AgNPs (spherical, semipentagonal, and hexahedral) with the average size 10–
60 nm (Saha et al., 2010). *Fusarium acuminatum* isolated from infected ginger produced extracellular AgNPs (5–40 nm) within 15–20 min. The possible role of nitrate dependent reductase enzyme in the reduction of the silver ions has been demonstrated and the resulted AgNPs showed significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus epidermidis* (Ingle et al., 2008). Extracellular synthesis of well-dispersed AgNPs has been reported by Bhainsa and D’Souza, (2006) using cell free supernatant of *Aspergillus fumigates*. Similarly, Mukherjee et al. (2001) reported the reduction of silver ions by the enzymes present on the surface of *Verticillium*.

2.4.1.3.2 PLANT-MEDIATED SYNTHESIS OF AgNPs

Plants have been found to be one of the potential sources for the production of AgNPs. In recent years, AgNPs were obtained using plant extracts of *Myrmecodia pendants* (10–20 nm) (Zuas et al., 2014), *Syzygium cumini* (10–15 nm) (Mittal et al., 2014), *Thevetia peruviana* (10–30 nm) (Rupiasih et al., 2013), *Citrus maxima* (2.5–5.7 nm) (Sarvanangala et al., 2013), *Lycopersicon esculentum Mill* (30–40 nm) (Asmathunisha and Kathiresan 2013) *Piper pedicellatum* (2-3 nm) (Tamuly et al., 2013), *Centella asiatica* L. (30–50 nm) (Rout et al., 2013), *Boswellia serrata*, Neem leaves (43 nm) (Kora et al., 2012), *Murraya koenigii* (10–25 nm) (Christensen et al., 2011), Pomegranate seed (30 nm) (Chauhan et al., 2011), and so forth as capping agents.

2.4.2 SYNTHESIS OF ZnO-NPs AND ITS APPLICATIONS

ZnO-NP, an important semiconductor having a direct band gap (3.37 eV) and a large exciton-binding energy (60 meV) is considered as a potential material not only in optoelectronic applications but also recognized as safe and approved for biological applications. Hence it is accepted to be one of the multitasking metal oxides with unique physicochemical properties for wide applications. Several physical (Sharma et al., 2011) and chemical (Yadav et al., 2006; Azam et al., 2010) methods have been employed for the synthesis of ZnO-NPs, which have several adverse effects leading to toxicity in the environment. In this regard, biosynthesis of ZnO-NPs using microorganism and plants have emerged as a novel, non-toxic, eco-friendly alternative to the existing conventional
methods. Although biosynthesis of ZnO-NPs by plants such as *Calotropis procera* (Vidya et al., 2013) *Aloe barbadensis Miller* (Sangeetha et al., 2011) *Ocimum basilicum* L. (Salam et al., 2014), *Borassus flabellifer* L. (Vimala et al., 2014), *Hibiscus sabdariffa* (Bala et al., 2015) have been reported, the potential of plants as biological materials for the synthesis of ZnO-NPs is yet to be fully explored.