1.0 GENERAL INTRODUCTION

Any environmental condition that can perceive beyond the normal acceptable range is an extreme condition (Sathyanarayanan et al., 2005). Extreme environments are found in many parts of the world and all these environments are colonized by microorganisms adapted to these environments. Many extreme environments are found in the earth and the microorganisms inhabiting such environments are termed as extremophiles (Buchalo et al., 1999). Most known extremophiles are microbes. The domain Archaea contains renowned examples, but extremophiles are present in numerous and diverse genetic lineages of both bacteria and archaeans. Furthermore, it is erroneous to use the term extremophile to encompass all archaeans but some are mesophilic. Neither all extremophiles are unicellular; protostome animals found in similar environments include the Pompeii worm, the psychrophilic Grylloblattodea (insects), Antarctic krill (a crustacean), and the "water bear". There are many different classes of extremophiles that range all around the globe, each corresponding to the way its environmental niche differs from mesophilic conditions. The classifications are not exclusive.

Among the three major habitats of the biosphere, the marine realm which covers 70% of the earth’s surface provides the largest inhabitable space for living organisms, particularly microbes (Qasim, 1999). The oceans are the largest bodies of saline water with average salinities ranging from 32-35 ppm. Hypersaline environments, with salinities far more than the normal sea water salinities, originate as a result of evaporation of sea water. Such environments are inhabited by halophiles, the salt loving organisms. Halophiles are distributed in hypersaline
environments all over the world, mainly natural hypersaline brines in arid, coastal and deep sea locations as well as the artificial salterns. Halophiles include prokaryotes and eukaryotes adapted to these hypersaline environments at the highest salt concentrations at or close to the solubility limit of NaCl. Due to the absence of predators, the halophilic communities are denser in high salt concentration zones. They withstand extreme saline conditions and have the capacity to regulate the osmotic pressure which results in the denaturing effects of salt in their environment.

The hypersaline microorganisms comprise a variety of heterotrophic and methanogenic *Archaea*; photosynthetic lithotrophs and heterotrophic bacteria, as well as photosynthetic and heterotrophic eukaryotes (Das Sarma and Arora, 2001). A common phenomenon in hypersaline environment is the occurrence of gradients in salinity as a result of the evaporation of seawater. Hypersaline environments are those with salt concentrations above that of seawater (3.5% total dissolved salts). Based on their origin, they are classified into thalassohaline environments with salt concentration similar to that of seawater: sodium and chloride being the dominating ions and the pH is near neutral to slightly alkaline as these environments result from evaporation of seawater. During evaporation, some changes occur in ionic composition out of precipitation of gypsum (CaSO$_4$.2H$_2$O) or other minerals once their solubility has been exceeded. Sodium chloride saturated thalassohaline brines, such as those found in saltern crystallizer ponds often display a bright red colouration as they harbour a large number of pigmented microorganisms (Oren, 2002). A great diversity of microbial life is observed in such brines of marine salinity upto 3-3.5 M (Caumette *et al*., 1994).
Athalossohaline hypersaline environments, in which the ionic composition differs greatly from that of sea water, are also populated by microorganisms. An example is the Dead Sea, a lake in which the concentration of divalent cations (about 1.9 M Mg\(^{2+}\) and 0.4 M Ca\(^{2+}\)) exceeds that of monovalent cations (1.6 M Na\(^+\) and 0.14 M K\(^+\)) and of which the pH is relatively low (around 6.0). Even such a hostile environment periodically supports dense microbial blooms (Oren, 1988). A number of alkaline hypersaline soda brines also exist e.g., the Wadi Natrun lakes of Egypt, Lake Magadii in Kenya and the Great Basin lakes of Western United States, many of which go intermittently dry. Soda brines lack magnesium and calcium divalent cations because of their low solubility at alkaline pH. Such environments are very dynamic experiencing significant seasonal variations in size, salinity and temperature. In addition to natural hypersaline lakes, numerous artificial solar lakes are man–made for the production of sea salt. Once NaCl precipitates, the concentration of potassium and magnesium chloride and sulphate brines’ (bitterns) remain, are returned to the sea. Hypersaline environments also occur in subterranean evaporate deposits e.g., the Deep Sea basins created by the evaporation and flooding of ancient seas. Deep sea brines are relatively stable as a result of their higher density as reported in the Red Sea and Gulf of Mexico (Mac Donald et al., 1990).

Microorganisms are assigned to different categories according to their salt response (Larsen, 1962; Gibbons and Baxter, 1953; Kushner, 1985). Non-halophiles, which grow best in media containing less than 1% (w/v) total salts. Some are able to tolerate high salt concentrations and are considered halotolerant. Slight halophiles, which grow best in media containing 1-3% (w/v) salt which, include the marine bacteria. Moderate halophiles; grow best in media containing 3-15% (w/v) salt.
Borderline extreme halophiles, which grow best in media containing 9-23% (w/v) salt and extreme halophiles, which grow best in media containing 15-32% (w/v) salt. Both moderate and extreme halophiles are found in hypersaline lakes (Elazari-Volcani, 1940; Brisou et al., 1974; Nissenbaum, 1975; Post, 1977) and in solar salterns (Davis, 1978; Rodriguez-Valera et al., 1981, 1985; Javour, 1984).

1.1 Unique features of halophiles

Most cells cannot survive in concentrated salt solutions, but halophiles are able to accumulate high solute concentrations in the cell cytoplasm. When an osmotic balance with their surroundings is achieved, cell volume is maintained. Some halophiles accumulate amino acids, sugars and polyols as balancing solutes, but most haloarchaea use KCl. The proteins of haloarchaea tend to contain an excess of acidic amino acids. This is important for their salvation and activity at high salt concentrations. In hot dry regions haloarchaea abound to high levels of radiation. So it is not surprising that they contain pigments that are useful in photochemical processes. In low oxygen and high light conditions, some haloarchaea express bacteriorhodopsin, a 1:1 complex of bacterioopsin (a protein) and retinal (a light absorbing chromophore), which builds up on the cell surface to form a “purple membrane”. This acts as a solar powered pump to transport protons from the interior to the exterior of the cell. The resulting electrical potential across the cell wall drives ATP synthesis that supplements the normal heterotrophic pathways.

Halorhodopsin is another protein-retinal complex found in some haloarchaea. Its function is to act as a solar powered chloride pump to regulate electrolyte concentration in the cell. There are also sensory rhodopsins that control phototaxis so
as to optimize the photochemical processes. This involves moving towards beneficial (green) light, and away from harmful shorter wavelength blue and UV light. Rhodopsins are also present in many “true” bacteria and also in eukaryotes, so these pigments may have evolved from some common ancestor that predates the divergence of archaea, bacteria and eukaryotes. Another possibility is that lateral gene transfers might have occurred after divergence. Either way, an interesting hypothesis suggests that chlorophyll-based photochemical processes might have evolved to take advantage of light that was not being absorbed by the established retinal-based processes. The characteristic red colour of many haloarchaea is mainly due to the presence of carotenoid pigments. Their functions are to act as a sunscreen and to assist in the repair of cell damage by UV exposure. One particular carotenoid pigment, β-carotene, also acts as a source of retinal when it is cleaved by oxidation.

Living in concentrated salt solutions means experiencing gradients of all kinds. Salt concentration can vary with depth due to surface evaporation, or an inflow of fresh water, or as a result of gravity. Temperature can change with depth due to insulation and light reflection at the surface. Oxygen concentration is reduced as salt concentration rises. To cope with these environmental gradients, many haloarchaea use flagella to move as conditions change. Some also have gas filled vesicles that confer buoyancy. Floating to the surface increases access to oxygen, or, in low oxygen situations, provide light exposure to power the purple membrane ATP synthesis mechanism.

The two most important group of microorganism adapted to live in hypersaline habitats are moderately and extremely halophilic bacteria. Halotolerant
and slight halophiles seem to play a minor role in these habitats and at least in habitats with more than 10% salts they constitute a low proportion of the total microbial population (Rodriquez-Valera, 1988). Microbial growth occurs over a wide range of salt concentrations spanning the whole range from fresh water environments to NaCl concentrations of 3-5 M (Oren, 1999). In order to survive in conditions of high salinity, halophilic and halotolerant microorganisms have developed specific cell and cell membrane adaptations. Eubacteria and other halotolerant organisms use organic solutes from the surroundings or synthesize compatible osmolytes to balance the high external osmotic pressure (Oren, 1999). Extremely halophilic archaea maintain osmotic balance by accumulating salts through osmoregulation and have adapted their membranes to have low proton and sodium permeability at high salt concentrations (Van de Vossenberg et al., 1999). In solar salterns, halobacteria colour the brines red in NaCl crystallizer ponds with brine densities of 25-30% (Gonzalez et al., 1978). Extreme halophilic bacteria have been isolated from a broad diversity of hypersaline environments, especially those resulting from the evaporation of sea water (Larsen, 1962). It is likely that these organisms could be dispersed throughout the world via ocean currents (Larsen, 1976).

To live at high salt concentrations, halophilic and halotolerant microorganisms must maintain a cytoplasm that is osmotically isotonic with the outside medium. Two different strategies have been used to achieve this osmotic equilibrium. The first option, used by the aerobic halophilic archaea of the family Halobacteriaceae and the anaerobic halophilic bacteria of the order Haloanaerobiales involves the maintenance of high intracellular ionic concentrations, where K⁺ is the dominant cation instead of
Na\(^+\) and adaptation of the entire intracellular machinery to function in the presence of high salt (Dennis and Schimmin, 1997).

The second option observed in the most halophilic and halotolerant representatives of the bacteria, eukarya and also in halophilic methanogenic Archaea, is the maintenance of cytoplasm lower in salt concentrations and the accumulation of compatible osmotic solutes that serve to achieve osmotic equilibrium while not being too inhibitory to enzymatic activity (Gallinski, 1995 and Ventosa et al., 1998). The concentrations of these osmotic solutes are regulated according to the salt concentration in which the cells are found and can be rapidly adjusted as required when the outside salinity is changed. Adaptation and adaptability of halophilic bacteria depends on the regulation of synthesis of organic osmolytes such as glycine, betaine, ecotine (1, 4, 5, 6-tetrahydro 2-methyl-4-pyrimidine carboxylic acid) and glucosyl glycerol among others (Galinski and Louis, 1998).

Sodium chloride influences many physiological functions of halophilic bacteria. One of the most important function is the protection of bacteria from changes in osmotic pressure between the internal and external environments (Takahashi and Gibbons, 1959). Sodium chloride prevents swelling, deformation, bursting and lysis of halophilic bacteria as a result of osmotic shock. Boring et al. (1963) proposed the lytic processes of halophiles which are controlled by enzymes that are inhibited by Na\(^+\) ions and this inhibition in turn prevents the destruction of the muco peptide portion of the cell wall bacteria. On the other hand, Na\(^+\) ions were found to stimulate the activity of other enzymes of halophiles, for example, permeases (Lanayi, 1974), lipases, amylases (Onishi, 1972). Baxter and Gibbons (1956) stated
that a number of enzymes are inactive in the absence of sufficient concentration of sodium chloride. According to Drapeau et al. (1966) Na\(^+\) ions are necessary for the oxidation of sugars, aminoacids and organic acid by halophilic bacteria.

### 1.2. Applications of halobacteria

Applications (current and potential) of halophilic microorganisms are divided into a number of categories. Centuries-old processes such as the manufacturing of solar salt from seawater and the production of traditional fermented foods, existed long before the nature of the microorganisms involved became known, and little if anything is done to control these microorganisms to improve the production processes; utilization of the salt tolerance of halophilic microorganisms and of enzymes produced by them to catalyze processes in high salt environments.

Exploitation of the properties of specific compounds produced by certain types of halophiles enables them to withstand the high salt concentrations in their medium (ectoine, glycerol and others). Applications of unique compounds made by some halophiles are not directly connected with their life in high salt environments. The prime example is bacteriorhodopsin in the purple membrane of *Halobacterium salinarium*, which is not essential for its growth, but is stable and active even in the absence of salt. Compounds such as β-carotene, poly-β-hydroxyalkanoate, exopolysaccharides, etc. found in some halophiles are also made by many other microorganisms. Sometimes there is a clear advantage to using halophiles for their production; in most other cases, however, the superiority of the halophiles as producers of such compounds is yet to be proven.
In times in which fossil fuels are getting depleted and the world is searching for alternative sources of energy, biofuel is a fashionable alternative. Although halophilic microorganisms may not be the most obvious source from which such fuels may be commercially produced, they still may be of interest. Some industrial processes generate highly saline waste-water. For the biological treatment of industrial wastewaters with salt concentrations up to 10%, such as the brines generated by the pickling industry, aerobic treatment systems have been developed based on aerated percolators or rotating discs to improve aeration and mixing. The halophilic fermentative bacterium *Haloanaerobium lacusrosei* was successfully used in an anaerobic packed bed reactor operating at salt concentrations up to 10% (Kapdan and Erten, 2007; Kapdan and Boylan, 2009). Bacteriorhodopsin is a 25-kDa protein that carries a retinal group bound by means of Schiff-base to lysine-216. It is a highly unusual protein within the proteome of *Halobacterium* and is commercially manufactured in the form of purple membrane patches prepared from *Halobacterium salinarum* (Oren, 2010).

### 1.3. Biotechnological potential of halobacteria

Bacteriorhodopsin is used as halographic storage in which interference patterns are registered as purple or yellow areas. As the transitions are reversible, a bacteriorhodopsin holographic matrix can be used repeatedly (Barnhart *et al.*., 2004 and Birge *et al.*, 1999). It is used for the construction of ‘bioelectronic’ elements of computer memories and information processing units. Optical switching is based on the $B \rightarrow M$ and $M \rightarrow B$ transitions. A high density of information storage is possible and both permanent optical image storage, data storage and transient optical image storage by bacteriorhodopsin is possible. It is used for the ultrafast light detection,
construction of artificial retinas, detection of motion (Song, 2005). It is used for the nanotechnology applications such as the construction of molecular transistors, molecular motors, artificial retinas and molecular sensors (Pandey, 2000). Compatible solutes have gained increasing interest for biotechnological applications as stabilizers for biomolecules (enzymes, DNA, membranes) and whole cells, salt antagonists or stress-protective agents. Ectoines produced from extremely halotolerant bacteria is used to retain and stabilize the activity of enzymes such as amylase, lipase, cellulose or protease. One of the most promising applications is the use of cetoine as stabilizers in the PCR (Sauer and Galinski, 1998) Betaine are the typical compatible solutes of halophilic phototrophic bacteria (Galinski, 1995).

Liposomes are used in medicines and cosmetics for the transport of compounds to specific target sites in the body. Ether-linked lipids from archaeal halophiles have a high chemical stability and resistance against esterases and thus have a higher survival rate than liposomes based on fatty acid derivatives (Galinski and Tindall, 1992; Gambacorta et al., 1995). Halophilic exopolysaccharide (EPS) producers are also an interesting source for microbialy enhanced oil recovery (MEOR) where polymers with appropriate properties (high viscosity, high temperature, pseudoplasticity, resistance to slat and thermal degradation) act as emulsifiers and mobility controllers (Kulichevskaya et al., 1992). Lectins, selective sugar – binding proteins, are useful tools for cell typing and cell-surface research. Lectins from halophilic archaea might be useful for archaeal typing and analysis of their cell-surface carbohydrates.
Polyhydroxyalkanoates (PHA) are intracellularly accumulated bacterial storage compounds. Properties of some PHAs are comparable to those of polyethylene and polypropylene. Poly β-hydroxyl butyric acid (PHB) recovery is simplified in halophiles because the exposure of the halophile to high salt concentrations causes cell lysis (Ventosa and Nieto, 1995). Another advantage of the halophile is that it can be cultivated easily in a simple saline open pond without risk of contamination. Many enzymatic activities of halophilic archaeae have been characterized, including enzymes of potential application interest, such as amylases, proteases and nucleases. Many halophilic enzymes also function at elevated temperatures. Halophilic bacteria are metabolically more versatile than the archaea, and their enzymatic activities are more diverse. Moreover, most haloarchaeal enzymes require atleast 10-15% salt both for stability and activity, while bacterial enzymes generally do not show such a strict salt requirement (Kamekura, 1986).

1.4. Industrial applications of halophiles

Extremophilic microorganism, supply industries with a variety of biotechnology tools like outstanding stable enzymes which remain active in a wide range of pH, temperature and extreme saline concentrations. Extreme environments include conditions such as high salinity, acidity, alkalinity and extreme temperature, which are predominant conditions in a variety of industrial processes. Microbial population that can live and reproduced in this kind of environment are called extremophiles, and its microbiology its being widely studied around the world, since they produce enzymes able to work under such conditions and can be used in biotechnological and industrial potential application (Hashim and Suhaila, 2004; Ventosa et al., 1998).
Halotolerant and halophilic microorganisms grow in environments with high salinity concentrations. Halophilic bacteria are found in different environments such as salt lakes, saline soils and salted foods (Ventosa et al., 1998). The majority of halophilic microorganisms studied so far produce compounds with great potential in industrial processes and they have physiological properties that facilitate their use with commercial aims. Enzymes produced by halophilic microorganisms have developed particular features that confer them stability and solubility at high salt concentration, thus, low water concentration. Halophiles produce exoenzymes such as amylases, proteases and nucleases of potential commercial values (Alqueres et al., 2007; Gomes and Steiner, 2004; Gupta et al., 2005). Halophilic proteins are distinguished from their homologous proteins by exhibiting remarkable instability in solutions with low salt concentrations and by maintaining soluble and active conformations in high concentrations of salt up to 5 M NaCl (Hough and Danson, 1999, Nascimento and Martins, 2006). There are a number of enzymes of this type produced by some halophilic microorganisms that have optimal activity at high salinities and could therefore be used in many harsh industrial processes where the concentrated salt solutions used would otherwise inhibit many enzymatic conversions (Doida et al., 2006, Gupta et al., 2005, Namwong et al., 2006).

Halophilic and halotolerant bacteria secrete a wide range of hydrolytic enzymes into their surrounding environments. Several of these enzymes which include amylases, proteases, xylanases and cellulose display polyextremophilic properties. They are generally haloalkaliphilic and thermotolerant which renders them amenable to an array of industrial processes, normally performed at extreme conditions of temperature and pH. However, only a limited number of these enzymes have been
well characterized and only few of them are exploited commercially, mainly because research has largely focused on microbial diversity in hypersaline environments rather than the industrial potential of halophiles. In order to fully reap the benefits of newly described bacterial species, it is necessary to understand their metabolic and physiological properties. This will allow the generation of valuable information and the definition of the repertoire of extreme enzymes that has the potential to open new biotechnological applications. Therefore, it is necessary to expedite research on the sequence analyses, characterization of halophilic enzymes so that the potential of these enzymes for industrial applications can be explored.

1.4.1. Lipases

Lipolytic enzymes represent a hydrolase group which specifically works over carboxylic ester. Lipases are defined as a carboxylesterases which catalyses the hydrolysis and synthesis of long-chain acylglycerols with trioleoylglycerol being the standard substrate. Lipases are able to develop hydrolysis, esterification, perhydrolisis, and aminolysis reactions. Lipases shape a versatile group of enzymes, due to a big amount of catalyzed reactions, therefore a high potential of applications such as detergent, flavor development, paper recycle, chemical systems, racemic mixture and so on (Jaeger et al., 1999). A variety of microbiological origin lipases with different properties and substrate specificity has been isolated and characterized so far. Lipases and esterases have been recognized as very biocatalysts due to its wide-ranging versatility in industrial applications. Practical use of microbial lipases has developed a great interest concerning the improvement of both the producing strains and the biochemical properties of lipolytic enzymes (Prim et al., 2003).
Biotechnological potential of lipases, due to their esterosepecificity, is enormous and they attracted a high interest for food, agricultural, chemical, pharmaceutical, medical and cosmetic industry among other areas. Most of catalyzed reactions by lipases show a selectivity and efficiency, and they occur under middle conditions. These reactions occur without added cofactor and with low energy requirements, these properties contribute to reduce industrial conversion cost and justify the growing interest in lipases (Kouker and Jaeger, 1986).

1.4.2. Proteases

Microbial proteases are one of the most extensively studied enzymes and they are widely applied in industrial processes. They are commonly used as additives in laundry detergents, food processing, pharmaceuticals, leather and diagnostic reagents, waste management as well as in silver recovery units (Amoozegar *et al.*, 2007). Halophilic proteases have been isolated and characterized from several bacterial species including *Bacillus sp.*, *Salicola sp.* and *Filobacillus sp.* (Hiraga *et al.*, 2005). These enzymes display optimal activity in the presence of NaCl and maintain stability over a wide pH range (pH 5-10), in addition these enzymes are active at temperatures of 40-75°C. While some of the enzymes display an absolute requirement of NaCl for activation, the protease from *Chromohalobacter* was reported to retain 100% stability in the absence of NaCl (Vidyasagar *et al.*, 2009).

Halophilic microorganism plays an essential role in various fermentation processes that occur in the presence of salt. The high salt tolerance of extreme halophiles enables their cultivation under non sterile and thus cost-reducing conditions. So far, several well known proteases such as bromelain, papain, and
pepsin have been used as biocatalysts in fish sauce fermentation (Beddow and Ardeshir, 1979; Gildberge, 1989). However, most of these proteases are not sufficiently stable in the presence of high salt concentration. Halophilic organism have evolved in saline environment and are able to overcome the deleterious effect of salt and their enzyme require salt, retaining activity in salt upto saturating concentration. Some halophilic proteases have been purified and characterized which showed maximum activity at neutral pH at a temperature ranging from 55-66°C (Kamekura and Seno, 1990; Studdert et al., 2001). Halophilic proteases have wide application in the processing of food, leather and detergent (Alqueres et al., 2007; Hough and Danson, 1999).

1.4.3. Amylases

Amylases are a class of hydrolases which catalyse the degradation of starch polymers to produce dextrins and different glucooligosaccharides of variable lengths. Amylase are widely employed in different biotechnological applications including the food industry where they are used extensively in bread and baking industry to improve the volume of dough, colour and crumb softness. Amylases are also applied in detergents to promote stain removal and are utilized in the paper and pulp industry for the modification of starches for coated paper (Gupta et al., 2003). Halophilic amylases, commonly cyclomaltodextrinases have been produced from bacteria such as Micrococcus halobius (Onishi and Sanoda, 1979), Halomonas meridiana (Coronado et al., 2000). These enzymes generally display broad pH optima and stability and they remain active at temperature above 50°C. For instances, the amylases from Halobacillus and Chromohalobacter were found to be stable at pH 7-10. Some of the enzymes such as the Chromohalobacter maintain their stability in the
presence and absence of NaCl. Halophilic amylases display molecular weights ranging between 50-75 kDa. The stability of these enzymes at extremes of pH and NaCl, as well as their ability to function optimally at elevated temperature make them attractive candidates for hydrolysis of starch in industrial processes which are commonly performed at low water activity such as the production of syrups and also in the treatment of saline water or waste water solutions containing starch residues in the presence of high salt (Margesin and Schinner, 2001). In addition, some of the halophilic enzymes such as the amylases form a marine *Streptomyces sp.* remain stable in the presence of commercial detergents and would therefore, be attractive additives in laundry detergents (Chakraborty *et al.*, 2009). Currently, only a few halophilic amylases encoding genes have been sequenced.

### 1.4.4. Cellulase

Cellulases are mainly applied in textile industry for biopolishing of fabrics and production of stonewashed denims, as well as in laundry detergents for fabric softening and brightening (Aygan and Arikan, 2008). Interest in cellulase is also increasing in the production of bioethanol as the enzymes are used to hydrolyse pretreated cellulosic material to fermentable sugar (Wang *et al*., 2009). Currently, halophilic and halotolerant cellulases are derived from *Bacillus sp.* This particular enzyme is thermostable, halostable and alkalostable thus making them as ideal candidates for various industrial applications.

### 1.4.5. Xylanase

Xylanases play a pivotal role in the degradation of xylan. They are widely used in the baking industry to improve the properties of dough and also for the past two
decades the potential use of xylanases in biobleaching of paper and pulp has been growing perpetually (Mamo et al., 2009). However efficient application of xylanases in biobleaching requires them to be alkalophilic and thermotolerant. Halophilic organisms are the most likely sources of enzymes with such properties although research in this arena is currently limited. Only a few halophilic/halotolerant organisms have been described which include enzymes derived from marine and hypersaline bacteria such as Glaciecola mesophila (Guo et al., 2009). Some of these enzymes display stability at wide pH (6-11), remains active at temperature above 60°C and may display an absolute requirement for NaCl (Guo et al., 2009).

Halotolerant microorganisms play an important role in various fermentation processes that occur in the presence of salt. These organisms catalyze the fermentation, thereby producing various compounds that give the characteristic taste, flavour, and aroma to the resulting products. β-carotene is used in the food industry as a natural food colourant. As a precursor of vitamin-A, it is of importance as additive in cosmetics, multivitamin preparations and health food products. The ability of halophiles/halotolerants to oxidize hydrocarbons in the presence of salt is useful for the biological treatment of saline ecosystems contaminated with petroleum products. Successful bioremediation of oil spills has been observed in marine, Arctic and Antarctic environments (Delille et al., 1998; Margesin and Schinner, 1999). Several halophilic isolates produce organophosphorus acid anhydrases (OPAA) with hydrolytic activity against several organophosphorous chemicals and their related compounds such as sarin and soman. These enzymes have considerable potential for the decontamination and demilitarization of chemical warfare agents (De Frank et al., 1993). Hydrogen is considered to be a likely future energy source because it is easily
converted to electricity and easily combustible. Halophilic culture systems would enable cost-saving H₂ production from biomass by means of the use of sea water as a basal culture medium (Margesin and Schinner, 2001).

1.5 Description of the Study area

Cape Comorin coast having a stretch of about 67.69 km is situated on the southern extremity of the Indian Peninsula (Lat. 08° 04’ and 08° 21’ N; Long. 77° 26’ and 77° 30’ E). The coast receives both the southwest monsoon from June to September and northeast monsoon from October to December. Considering the geomorphological, anthropogenic and ecological factors, 2 different saltpan stations viz. Puthalam and Thamaraikulam were selected for the present study. The sampling stations have an average distance of 10 km, along the Cape Comorin coast, which is the trijunction of Arabian sea, Bay of Bengal and Indian Ocean.
1.5.1 Puthalam saltworks

This site is situated south to Puthalam village, which is 3.5 km away from Manakudy beach and adjoining Manakudy estuary (Lat. 08° 04’ 36" N; Long. 77° 28’ 36″E). The total area of the saltwork is 8.09 ha. The saltwork receives saline bore water (20 – 25 g.l⁻¹) and stored in reservoir ponds of 2 ha. When the salinity reaches 30 g.l⁻¹ within 2 – 3 days, the water was allowed to pass into the condenser pond I having an area of 280 m² with 15 cm height. The water was retained for 9 days. The initial height of the water was 10 cm and on the final day, the height of the water was reduced to 5.6 cm and the salinity was raised to 100 g.l⁻¹. The crystallizer pond is also having the same size as condenser pond (280 m²). There were 72 condenser and 72 crystallizer ponds with varying size with the height of 12 cm. The water retained on crystallizer ponds for 9 days at a level of 8 cm. On the ninth day, the salinity of the water was raised to 250 g.l⁻¹ and the height of the water was reduced to 2.5 cm, which is suitable for the formation of raw table salt (Fig.1).

1.5.2 Thamaraikulam saltworks

Thamaraikulam saltworks (category I) is situated at 13 km southeast coast of Nagercoil and 5 km north to Kanyakumari (Lat. 08° 06’ 48"N; Long. 77° 28’ 0.2″E). This is the largest salt producing saltworks in Kanyakumari district during summer. Seawater supplemented with ground water has been used for salt extraction. The total area of the saltpan is 15.21 ha. The saltpan is situated very close to the Manakudy estuarine water. Here, the sub-soil water was pumped into the condenser I pond (male pond) directly which has a total area of 6.475 ha.
Each pond has an area of 200 m$^2$ with 20 cm height and a salinity of 30 ppt. On the $7^{th}$ day, the height of water was reduced to 8.2 cm and the salinity was increased to 135 ppt. Then the water was passed into the condenser II called ‘Attupathi’. The area of the condenser was 202 m$^2$ and a height of 18 cm. The water was kept for 7 days, allowing the salinity to rise to 185 ppt. The saline water from the condenser II pond was passed to the crystallizer pond for 7 days having the same area with 15 cm height. On the $7^{th}$ day, the height of the water was reduced to 2.5 cm and the salinity was raised to 275 ppt and then salts were formed. High calcium content in this saltpan resulted in the production of salt related products like CaCl$_2$ and CaSO$_4$ (gypsum). These saltpan ponds are reported to have much plankton diversity (Reginald, 2003) (Fig. 1).

1.6 Aim and Objectives

The present investigation has been undertaken to study the halobacterial diversity along the beaches and saltpans of Cape Comorin coast and further to check the possible utility in trace metal bioremediation through the following aspects:

1. Isolation of halobacteria from the Puthalam and Thamaraikulam saltpan environs along Cape Comorin coast.

2. Molecular identification of isolated halobacterial strains through 16s rRNA sequencing

3. Standardisation of optimum physico-chemical parameters *viz.*, pH, salinity and temperature on the growth and carotenoid production of identified halobacterial strains

4. Standardisation of optimum level of carbon sources *viz.*, glucose, sucrose, maltose and mannose on the growth and carotenoid production of identified halobacterial strains
5. Standardisation of optimum level of nitrogen sources *viz.*, beef extract, ammonium nitrate and sodium nitrate on the growth and carotenoid production of identified halobacterial strains

6. Standardisation of optimum level of mineral sources *viz.*, copper sulphate, ferric chloride and manganous chloride on the growth and carotenoid production of identified halobacterial strains

7. Standardisation of optimum level of aminoacids *viz.*, proline, glutamine and aspartic acid on the growth and carotenoid production of identified halobacterial strains

8. Analysis of antimicrobial property of extracted carotenoids from *Halomonas* *sps.* against chosen bacterial pathogens

9. Analysis of biochemical and physiological characteristics of *Halomonas* *sps.*