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A review on Halophiles as the crude oil hydrocarbon degrading bacterial species

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ABSTRACT

Pollution has become a major threatening part of life of each and every species present on planet Earth. One of the most common contributors to pollution is the oil spills. This causes the contamination and degradation of nutrients, minerals and salts present in the soil; it directly enters the food chain of various species present in the environment and can lead to severe life-threatening consequences. Whereas in case of humans, the oil contaminants can cause skin problems, respiratory issues, etc. But the solution to the problem lies in the natural environment; the microbial degradation. The halophilic species better known as extremophiles grows at NaCl concentration of about 15 to 20%. These species have been discovered to produce certain enzymes such as lipases, proteases, alkyl hydroxylases, cellulases which can easily degrade the crude oil hydrocarbons by a natural technique called Bioremediation. As these bacterial strains are halotolerant, for that reason they can easily withstand or resist the extreme conditions, for instance, higher salt concentration, increased temperature, increased pH etc. Because of these unusual properties, *halophilic bacterial* species can become the most suitable way of removing the hydrocarbon contamination from soil, seas, and oceans. This review puts light on another beneficial and more of a convenient way of degrading the crude oil.

Keywords: Crude oil degrading halophiles; Major oil degrading enzymes; Bioremediation by microbial agents

INTRODUCTION

The Chennai Oil Spill: A Lesson for India;s Maritime agencies; The Chennai oil spill is a wakeup call: India needs a better response to maritime ecological disasters. The spill is being seen as one of India's worst ecological disasters ever. As a blanket of toxic sludge envelopes Chennai's beaches, ecologists are confronted with prospect of large-scale destruction of sea life and a ner permanent imbalance of the region. (Source: by Abhijit Singh, February 08, 2017, THE DIPLOMAT, (Source: by Abhijit Singh, February 08, 2017, THE DIPLOMAT).

The changes in the environmental conditions have become more intact. Pollution has increased drastically because of more human activity. Meanwhile, in past few years the problem of oil spills has become one of the major concerns. Extensive research is being done in order to discover more and more solutions to this situation. PAH (polyaromatic hydrocarbons) pollutants have been reported as one of the major cause of pollution (Shekhar et al., 2015). Polyaromatic hydrocarbons originate from anthropogenic sources like mineral oil (Shekhar et al.,2015). They are basically the product of incomplete combustion and can cause the accumulation of fatty tissues inside the body e.g. liver (*Kaushal et al.,2017*). These hydrocarbon pollutants can easily enter the food chain of various species and can cause severe effects. There are number of micro-organisms present in the natural environment which are capable of degrading the crude oil to a limit. The matter of success of the degradation of crude oil completely depends on the potential of the native micro-organism.

A vast amount of residual water having oil and salinity upto10%, is generated by various petroleum industries. Ample amount of water is wasted in the extraction of crude oil. Contaminants such as the poly cyclic and aromatic hydrocarbons act as the major contaminants, present in the production water. But most of the microbial species are unable to grow at high saline conditions as they loose their cell wall integrity, their structural proteins get denatured and their usual osmotic pressure changes. Such conditions leads to the inhibition of normal microbial growth. Meanwhile, halophiles can tolerate extreme conditions and can easily treat the highly saline residual water produced while the crude oil extraction (Abha et al., 2012)(refer to fig.1 for typical hydrocarbons structure).

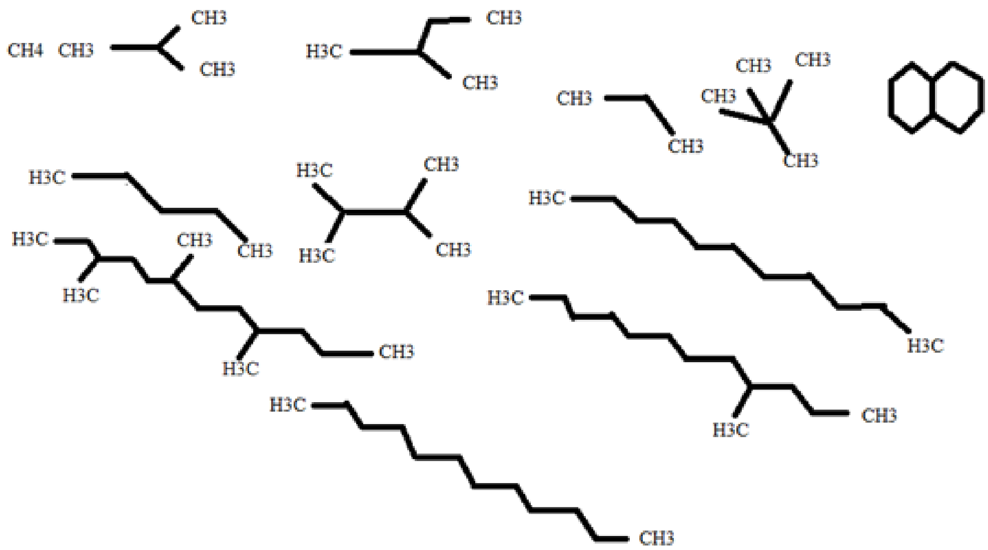

Figure 1; Saturated Branched and Unbranched structure of hydrocarbons

The use of petrol, diesel and crude oil has become a major concern as these can easily degrade the naturally present nutrients and minerals in soil and water. The major constituents of crude oil include paraffin (15-60%), naphthalene (30-60%), aromatics (3-30%) and asphaltic (6%) (Borah et al., 2014).These constituents consist of hydrogen (10-14%), nitrogen (0.1-2%), oxygen (0.05-1.5%), carbon (83- 87%), sulphur (0.05-6%) and metals(less than 0.1%) (*Borah et al. 2014*).

The extremophiles are the most renowned micro-organisms that have been in use since very long. These extremophiles have so many applications, not only in industries but in environment protection as well. Extremophiles are classified into different categories based on their tolerance to the particular condition: Thermophiles; growing in extreme heat conditions like in thermal springs, oil wells, at temperature above 55degree Celsius. Alkaliphiles grows in alkaline conditions usually above pH 9.0. Halophiles is the next category that involves salt tolerant micro – organisms, these can grow in slightly saline, moderately saline or extreme saline conditions (2 – 30% of NaCl concentration). Mostly found in salt lakes, salterns, ponds.

Severe impacts of hydrocarbons contaminants:
Despite the presence of vast number of hydrocarbons in petroleum products, only a small number of the compounds are well characterized for their toxicity (Al-Hawash et al., 2018). These crude oil hydrocarbons can lead to severe distinct levels of toxicity. The chemicals with high toxicity level present in crude oil damage any organ system in the human body like the nervous system, respiratory system, circulatory system, immune system, reproductive system, sensory system, endocrine system, liver, kidney, etc. and eventually cause range of diseases and disorders (Costello, 1979).

People who are living with pre- existing health concerns, new borns, young people and pregnant women easily get exposed to the effects of these hydrocarbons. A study was done by *Singh et al. (2004)* to determine the toxic level of various fuels on mice. The results of the experiment showed that the diesel exhaust had much more toxicity as compared to the forklift engine exhaust (Al-Hawash et al.,2018). Another study was conducted by Kinawy (2009) revealed that (leaded or unleaded) the monoamine neurotransmitters level and other biochemical parameters in different areas of the rats' brains got weaken on inhalation of aromatics and oxygenated compounds of gasoline vapors (Al-Hawash et al.,2018).On the other hand, the crude oil remains pertinent over its release site due to which it can cause major issues for humans and other living species. Crude oil consist of C, N, S,O atoms in its various polar organic compounds which makes them a serious concern for the environment. These can directly enter the soil or aquatic ecosystem and can potentially contaminate them *(Al-Hawash et al.,2018*).

Halophiles; the solution to crude oil hydrocarbons contamination:
The halophilic micro – organisms are having the most peculiar properties that make it able for them to survive in extreme conditions. Halophiles are mostly found in the environment that has high salt concentration such as Great Salt Lake, Owens Lake, and Dead Sea. Mostly prokaryotes have great varieties of species in halophilic category but bacteria and eukaryotes are also there for example, Algae *Dunaleilla salina (The Halohandbook: Dr. Mile Dyall Smith;2009*).
Classification of halophiles according to their required salt concentration:
a. Extreme tolerants: 0 – 5% NaCl
b. Slightly halophilic: 2 – 5% NaCl
c. Moderately halophilic: 5 – 20% NaCl
d. Extremely halophile: 20 – 30% NaCl.

The required growth conditions for halophiles are:

Salt	Most of the halophiles require about 23% NaCl	Example; *Haloarchae hispanica*
Temperature	At 40 and 37 degree celsius	Example; *Bacillus species*
Anaerobic growth	Few strains are capable of fermentative growth and use nitrate as electron source.	Example; *Halococcus*
pH	i. non alkaliphilic; pH 7.5 ii. alkaliphilic; pH 8.5 to 9	Example; *Bacillus cereus*
Substrate	Media with simple carbon source such as glucose or glycerol	Example; *Halomonas, Halobacterium*

The classification of Halophiles is as follows;

Category no.	**Name of the category**	**Characteristics**	**Example**
1.	**Archae**	Most of the extreme halophiles belong to this category. These usually grow at lower degrees of salinity. The diversity of genera increases at lower degree of salinity.	*Halococcus, Halobacterium, Haloarcula*
2.	**Bacteria**	Just like archae, these grow at lower saline conditions. But these may co – exist with archae community.	*Salinibacter*
3.	**Eukarya**	These are usually absent at extreme environments. However, few are moderately or slightly halophilic eukaryotes.	*Debaryomyces hansenii, Hortea werneckii*

ADAPTATIONS OF HALOPHILES:

There is considerable diversity of the halophilic micro – organisms. These halophiles have basically two strategies to adapt according to the saline environment.

Every micro – organism has to maintain the osmotic balance. Therefore, in case of halophiles they have to maintain the turgor pressure so it is necessary for them to be slightly hyper osmotic.

The first strategy needs very less energy in the form of ATP's. In this strategy, the halophiles try keeps the concentration of Na+ ions low inside the cytoplasm rather the K+ and Cl- is high inside the cytoplasm which maintains the osmotic balance. There are very few halophiles that use this type of mechanism. In some Archae, the amount of KCl accumulated inside the cytoplasm is equal to the NaCl concentration in the outside environment.

The second strategy involves the production of osmolytic solutes such as ectoine, glycine – betaine which maintains the osmotic balance by excluding as much salt as they can from the cytoplasm. But this osmolytic solutes mechanism is expensive in terms of ATP's or energy.

The Archae: methanogens have been found which uses the accumulation of osmotic solutes to maintain the osmotic balance e.g. *Natronococcus ocultus*, *Natrialba magadii.*

The biodegradation of hydrocarbons by halophiles:

The biodegradation of crde oil is one of the most difficult processes. As there are very trivial number of micro-organisms preset in the nature that can degrade the crude oil or PAH, it leads to the restriction of the biodegradability of oil contaminants. In earlier studies various enzymes, for instance, oxidases, cellulases, amylases, and etc. produced by distinct micro-organisms have been found to degrade the crude oil with the help of bioremediation.

A. Hydrocarbons of petroleum;

Earlier, there were few reports that gave evidence of the microbial degradation of crude oil by halophilic species. This occurred because in most of the cases, as the salt concentration increased the metabolic activity of the microbes decreased (Abha et al., 2012). But later on, it was observed that after long time exposure, the degradation of hydrocarbons increased in saline conditions and (Kleinstuber et al., 2006, Riis et al., 2003, Abha et al.,2012).

Table 1: The growth of different halophilic species at distinct salt concentrations leading to the degradation of hydrocarbons according to the research reports has been discussed;

Halophilic species	**Salt concentration required (%)**
Marinobacter sp., Erwinia ananas, Bacillus sp.	0 – 22%
Cellulomonas sp., Bacillus marisflavi, Dietzia maris, Halomonas eurihalina	17.5%
Halomonas, Ditzia, Ralstonia	0 – 20%
Idiomarina	7.5%
Alcanivorax, Marinobacter	15%
Caulobacterse	15- 20%
Cyanobacterial rich mats	21%
Rhodococcus, Micrococcus, Arthrobacter	0.5- 25%
Halobacterium, H. salinarium, H. volcanii, H. distributum	30%
Streptomyces albiaxalis	Upto 30%

These various halophilic species are able to degrade the prtroleum hydrocarbons, diesel oil, paraffin, crude oil etc. Other then the above species of halotolerant; *Sphingomonas* (PAH degrader), *Janibacter* (a xenobiotic-degrading actinomycete) and the *Ectothiorhodospiraceae* and *Methylophilaceae* family, *Desulfobacula, Desulfosporosius*, *Halomonas, Ralstonia* and *Dietzia* (Hydrocarbon degraders).

B. Aromatic Hydrocarbons;

These are the hydrocarbons that are recognized by the presence of an aromatic ring and carboxylic group.

A huge amount of aromatic acids is present in the Earth's atmosphere, mostly originated from the plants and human activities. Some of the aromatic acids like, syringic acid, vanillic, ferulic, cinnamic, p – coumaric and 4 hydroxybenzoic acids are present in plants. These hydrocarbons are used in pharmaceutical and agrochemical-based industries as raw material. Aromatic hydrocarbons can enter the environment directly due to biodegradation of natural polymers, for instance lignin and tannin, or pollutants such as PAH.

They can easily reach the soil, therefore becomes a problem for the soil and groundwater as well.

The first reported archeon which had the potential of metabolizing the aromatic acids under aerobic conditions was the *strain D1227* of *Haloferax species.* This strain metabolized the benzoic, 3 phenylpropionic and cinnamic acids. This particular species was isolated from U.S.A, near Gran Rapids, Mich, from the soil contaminated with petrol (Abha et al.,2012). It requires NaCl concentration of about 2M. 3 phenylpropionic acid has been studied in depth for its degradation pathway.

As mentioned above, the D1227 strain was not able to degrade other aromatic acids except the 3 phenylpropionic acid, another strain was reported which degraded the 4 hydroxybenzoic acid. The *strain D1 of Haloarcula species* was the first to degrade 4HBA. It uses 4HBA as its carbon source and degrades the source by following gentinase pathway (refer to figure 2 and 3).

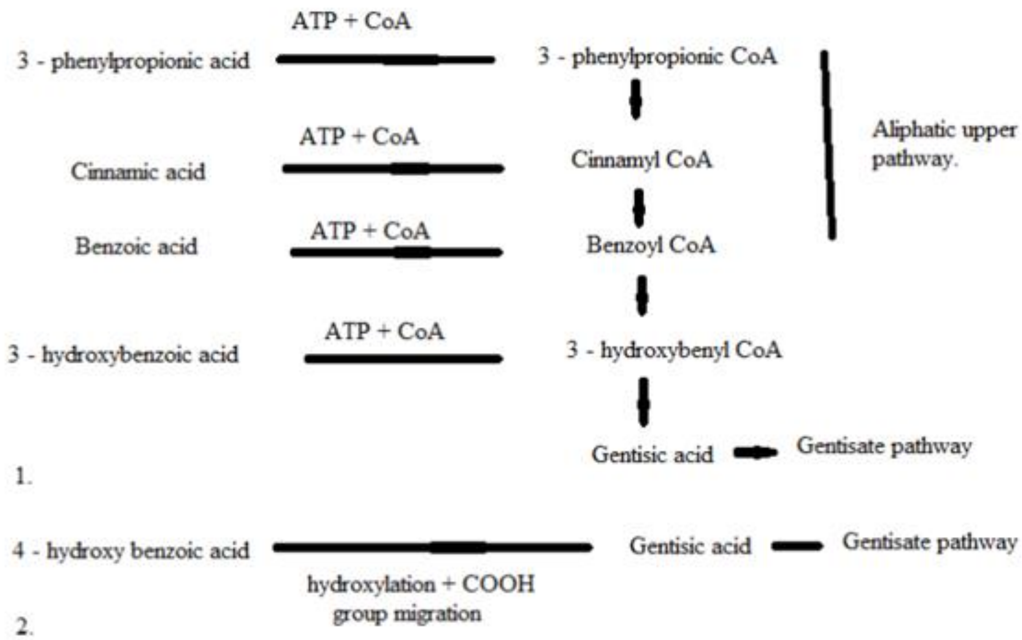

Figure2; 1. 3- Phenylpropionic acid degradation by *Haloferax sp. D1227*.
2. 4 HBA degradation by *Haloarcula, D1*

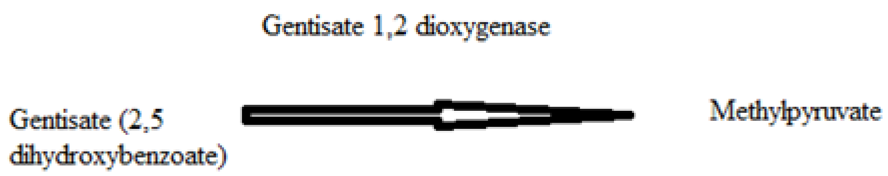

Figure 3; The gentisate degradation pathway

C. Phenols and Phenolic compounds;

These compounds are the major contributors to the pollution, produced as effluents by the coke converting industries, pharmaceuticals, oil refineries etc.

Hinetreggar and Schreiber (1997) studied the strain of *Halomonas sp.* isolated from the Great Salt Lake. *Halomonas* species has the capability to degrade phenol (Abha et al.,2012). The strain utilized phenol as an energy and carbon source. The growth of this strain was observed at NaCl concentration of about 3 to 5%. In 13 hrs, about 0.1 g of phenol per litre was degraded. The *cis, cis mucconic acid* was produced as an intermediate during the phenol degradation by *ortho – cleavage* pathway.

Another scientists Alva and Peyton in 2003, discovered that the halophilic species *Halomonas campisalis* was capable of degrading both catechol and phenol in presence of 150 g of NaCl and the mineralization of phenol into Carbondioxide as observed.

The isolation of *EF11 strain* from *Halomonas* species by Maskow and Kleinstuber (2004), depicted the degradation of phenol by *meta pathway* in case of high C/N ratio but in case of low carbon to nitrogen ratio, both *ortho and meta pathways* were followed. (refer to figure 4)

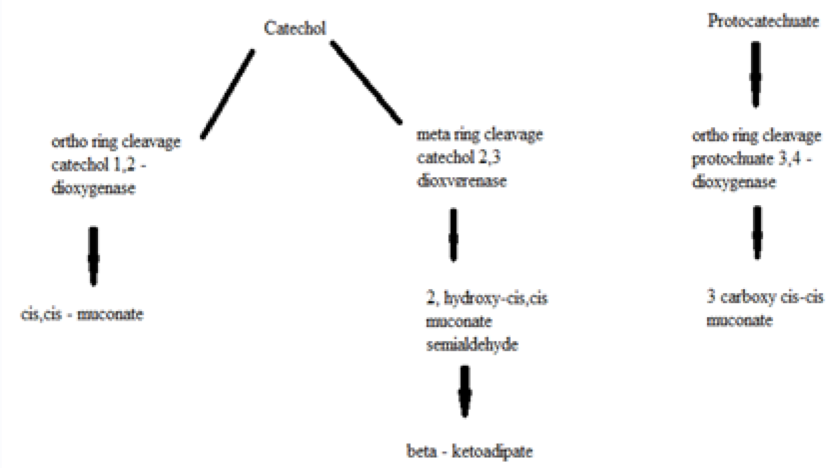

Figure 4; Phenol and Protocatechuate degradation pathway.

Other then these halotolerants, the *Thalassobacillus devorans and Halomonas organivorans* were able to degrade phenol at 7.5 to 10% NaCl concentration. The strain of Bacillus species, YAS1, is able to transform p – hydroxyphenylethanol or tyrosol which is one of the major effluent from olive oil mills, into p – hydroxyphenylacetic acid. This strain grows at 50g of NaCl and 1g yeast extract per litre. Marinnobacter species, as reported by Nicholson and Fathepure, 2004, at 145g/l NaCl concentration did the degradation of major pollutants BTEX (Benzene, Toluene, Ethylebenzene and xylene).

D. Hydrocarbons with halogens;

These are the aliphatic and aromatic compounds in which one or more hydrogen atom is replaced by halogen group such as chlorine, fluorine etc. These are of major concern as they are very much toxic in nature and well known to affect the ozone layer. Chlorinated hydrocarbons are used widely due to their anti – fungal, anti- herbicidal properties *Maltseva et al. in 1966*, reported one of the bacteria belonging to the Halomonadaceae family of halophiles that was capable of degrading 2, 4 Dichlorophenoxyacetic acid (widely used as herbicide) in presence of 0.6 to 1M NaCl. *Nocardioides sp. strain M6*, degraded 2,4–dichlorophenol, 2,4,5 and 2,4,6 trichlorophenol (the widely used preservative for woods). *Halobacterium, Haloarcula and haloferax* are capable of partially decomposing DDT and lindane (Abha et al., 2012).

E. Organic solvents;

Methane oxidizing halophilic species, Methylomicrobium N1 strain, from a soda lake having pH 9-10 was reported by *Fuse (1998),* able to oxidize the industrial solvent TCE (trichloroethylene; a chlorinated hydrocarbon) in a medium consisting 2-6% of NaCl. (Khemelina et al., 1997; Sorokin et al., 2000). Another halophilic bacteria, *Methylobacterium* and *Methylobacter alcaliphilus species* growing upto 1.1 to 1.5 M NaCl concentration, reported to be responsible for methanol metabolization. N, N_-dimethylformamide (DMF) is a one of the unique solvent that is usually involved in the textile and pharmaceutical companies, for a reason that it is soluble in aqueous as well as organic solvents. Aerobic bacterial species are well adapted to degrade about 200 mg/l of DMF with NaCl concentrations (up to 7%) (Bromley-Challenor et al., 2000), making it very efficient for treatment of the industrial effluents contaminated with DMF. Sorokin et al. (2007) isolated a moderately halophilic bacterial species, *Natronocella* *acetinitrilica* from soda lake sediments. This bacteria efficiently used acetonitrile and propionitrile as a source for carbon, energy and nitrogen at moderate salinity of 0.6 M and high pH of 10.

F. Organophosphorus compounds;

The thiol or ester derivatives of phosphonic, phosphoric and phosphoramidic acids are called as the organophosphorus compounds. These are widely used as pesticides which leads to the contamination in ecosystem. The isolate, designated JD6.5, tentatively identified as an obligate halophile, *Alteromonas* species. This particular species was found out to be able to hydrolyze the ester bonds in case of the organophosphorus compounds and the isolated enzymes were observed to be active against diiso-propylfluorophosphate, *p* nitrophenylmethyl and ethyl (phenyl) phosphinate, diethyl *p* –nitrophenyl-phosphate (paraxon)(Abha and Singh, 2012). *Halobacterium salinarum, an archaeon* was reported to consist an enzyme alkaline *p* -nitrophenyl phosphatase, hydrolyzes *p* -nitrophenylphosphate in reverse micelles in organic solvent (Marhuenda-Egea et al., 2002).

Chromohalobacter marimortui/ Pseudomonas beijerinckii, a Gram-negative halophile, utilized phosphonoacetate, 2-aminoethyl-, 3-aminopropyl-, 4-aminobutyl-, methyl- and ethyl-phosphonates as phosphorus sources for growth at NaCl concentration of 10% (Hayes et al., 2000).

G. Nitrogenous compounds;

These compounds are very commonly used in food preservation, poisons manufacturing, fertilizers etc. A well renowned herbicide called phenylurea is used worldwide as a pesticide.

The phenyl ring degradation by various microbes in nitrogenous compounds is quite a slow process (Sørensen et al., 2003). The *Marinobacter* sp. (moderately halophilic) isolates from a contaminated ephemeral desert stream bed in the Negev Desert, Israel (Sørensen et al.,2002) did the potential degradation of 1,3-diphenylurea (DPU) .This isolate completely mineralized the DPU, where aniline acted as an intermediate. This pathway involved the breakage of urea bridges of phenyl structures. The growth was observed with NaCl (0.51M) and temperature at 35° C.

The denitrifying bacterium which is strictly halophilic, was able to degrade trimethylamine (TMA) isolated from coastal sediments and the contaminated wastewater (Kim et al., 2003). TMA is a very evil smelling pollutant usually present in effluents of fish meal processing industries that causes abnormality in physiological development of animal embryos. The 16S rRNA genes sequence analysis depicted that these isolates get combined on a branch from other genera in the alpha-Proteobacteria and the nearest homologs were *Roseobacter* species. At NaCl concentration about 0.25–0.50 M. These facultative anaerobic bacteria species was demonstrated they can potentially degrade TMA under the denitrifying and anaerobic conditions. The degradation of TMA with the process involving conversion of dimethylamine to ammonia. These bacterial species consist TMA monooxygenase and TMA dehydrogenase for the oxidation purpose of TMA under aerobic conditions.

H. Azo dyes;

These are the compounds that consist of R–N=N–R_ in their structure, where R and R_ can
alkyl or aryl. The N=N group is called an azoor di-imide and the derivatives with two aryl groups are the most stable azo dyes. The dye manufacturing industries, releases these dyes in their effluent wastewater (Abha and Singh, 2012).

The disobedient nature of azo dyes compounds could be prevailed over by the utilization of anaerobic-aerobic co-cultures (Field et al., 1995). However, N=N groups has a electron withdrawing nature this breakage of the N=N double bond leads to the disappearance of color. (Guo et al. 2007) have described the strain GTW of *Halomonas* genus, isolated from coastal sediments in Dalian Bay (China), reported to decolorize azo dyes under the anaerobic conditions. Optimal decolorization occurred at NaCl concentration of 10 to 20%, pH 6.5 to 8.5 and temperature 30 degree Celsius. It has been reported by (*Asad et al. 2007*) that the newly isolated strains of *Halomonas* species use azo dyes as their carbon source. These strain were capable of decolorizing azo dyes at distinct concentrations of NaCl (up to 20%) and pH (5–11), under the anaerobic conditions only and in static cultures. The decolorization of azo dyes occurred through azo bonds reduction. Then, further the reduced bonds get cleaved to produce aromatic amines as analysed by the HPLC.

I. Sulphur compounds;

Mercaptanes, carbonyl and methylsulfides are included in this category of compounds. These compounds play a crucial role in sulfur cycle, acid precipitation and global warming.

The *Methanohalophilus* genus of Alkaliphilic halophilic and methylotrophic methanogens are capable of catabolizing DMS and can also grow in a medium in presence of a methanogenic substrate, such as methanol or trimethylamine (Mathrani et al., 1988). Another strain of *Methanohalophilus* was able to grow over 1.2 to 2.9 M NaCl concentration. They used the substrates such as methanol, trimethylamine and DMS for methanogenesis as described by *(Robertson et al., 1992*). The bacterial strain used osmotic solutes in the regulation of the increased NaCl concentration in external environment.

– AC 1 organic sulfur compound, Thiocyanate (SCN) is produced during biological cyanide detoxification processes and commonly found coke and metal plants wastewater.

Thioalkalivibrio paradoxus and *Thioalkalivibrio thiocyanoxidans* (Sorokinet et al., 2002); *Thialkalivibrio thiocyanodenitrificans* can show optimal growth either aerobically or anaerobically with the help of the electron donor thiocyanate and electron acceptor nitrate or nitrite (Sorokin et al., 2004) and *Thiohalophilus thiocyanoxidans* again using the same electron acceptors and donors can grow potentially under anaerobic, hypersaline conditions (2–4 M NaCl) (Sorokin et al., 2007). These species are able to use thiocyanate as an energy source. Other then these Halomonas species were able to degrade the thiocyanate by utilizing it as nitrogen source.

Major oil degrading Enzymes produced by Halophiles:

Enzymes are the substances that help in accelerating the rate of a chemical reaction without undergoing any physical or chemical changes in it. The enzyme molecule consists of amino acids joined together with polypeptide chains. An enzyme may lose its activity if any kind of variations in the temperature or pH occurs. The Halophilic bacteria consist of following enzymes:

1. Proteases

These are also called as proteinases or peptidases. Any enzyme that breaks the protein molecules into peptides, and then to amino acids are known as proteases. Most of the protease enzyme exists inside the digestive tract system. Similar to other enzymes, this particular enzyme has its role in food and detergent industries. On the basis of catalysis of peptide chain; these enzymes are categorized as *(Proteolytic Enzymes: Encyclopedia Brittanicca*)*:*

a. Endopeptidases; acts inside the inner region of peptide chain
b. Exopeptidases; acts at the terminal amino and carboxyl region.

On the basis of the source, proteases are classified in Table 2.

2. Lipases

Common in blood, intenstinal juices, adipose tissues and pancreatic secretions (Karigar et al., 2011). It basically perform the breakdown of the fats into fatty acids and glycerol. These enzymes are oftenly used as emulsifying agents in various food and cosmetics industries. Lipases are the most useful indicators for hydrocarbon degradation in soil.

Table 2: Proteases a classification

Source	Name of the source organism	Enzymes present
By organism	Animal Plant Bacteria Fungus	Chymosin, trypsin, pepsin Bromelain, papain, ficin Subtilisin, bacillopeptidases Aspergillopepsin
By proteolytic mechanism	Serine proteases Threonine proteases Cysteine proteases Aspartic proteases Metallo proteases Glutamic proteases	

The structural chemistry of proteases;

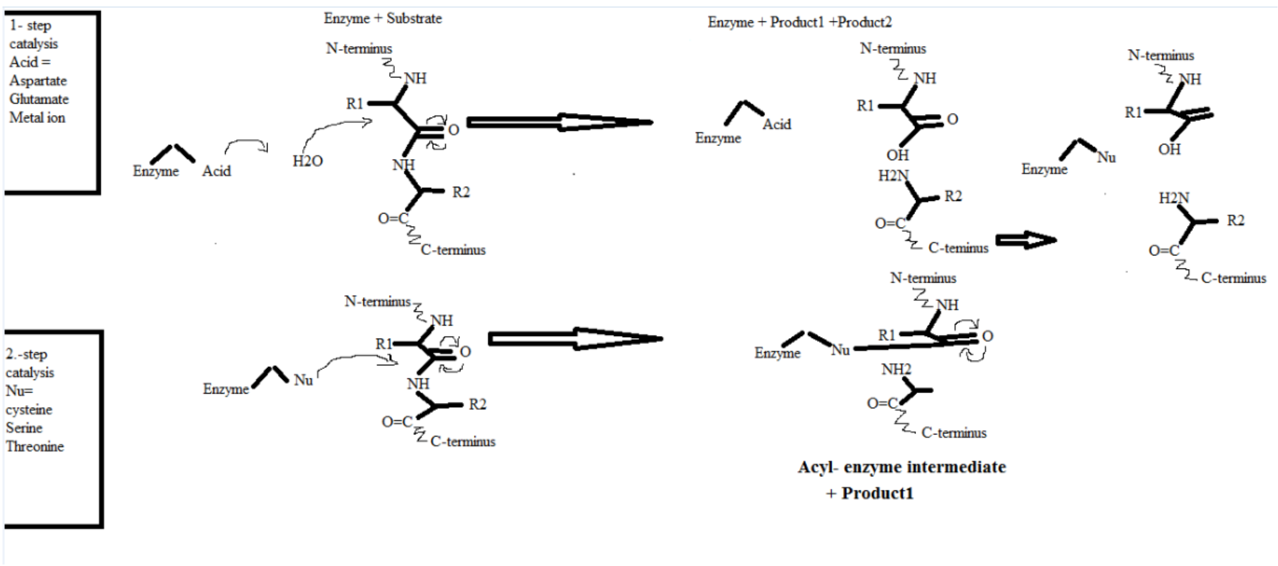

Figure 5; The mechanism of protease activity

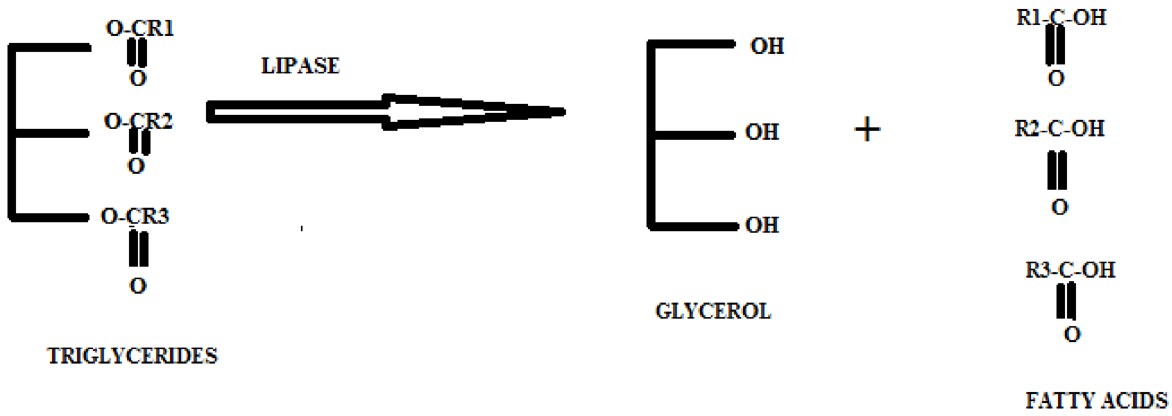

Figure 6; The mechanism of lipase activity, hydrolysis of triglycerides.

Lipases get absorbed on the oil – water interface (Karigar et al., 2011). Then, the breakdown of ester bonds occur due to which monolein, dilein and glycerol are produced. The glycerol formed have hydrophillic nature due to which it easily get dissolved in liquid phase.

3. Nitrate reductase:

Nitrate reductase belongs to the oxidoreductases catagory that does the reduction of nitrate to nitrite. Nitrate acts as a crucial enzyme for the growth of various plants and it is also the nitrogen source in case of soils. Mostly this particular enzyme is used in case of agricultural field.

The common groundwater contaminants include the gasoline soluble components such as benzene, xylenes, ethylbenzene and toluene. Out of these all the most dangerous one is benzene as it is carcinogenic (Burland et al., 1999). The biodegradation of benzene occurs under aerobic conditions whereas that of toluene under the anaerobic conditions. But recent studies have discovered that the biodegradation of benzene can be linked to Nitrate reduction (Burland et al.,1999).

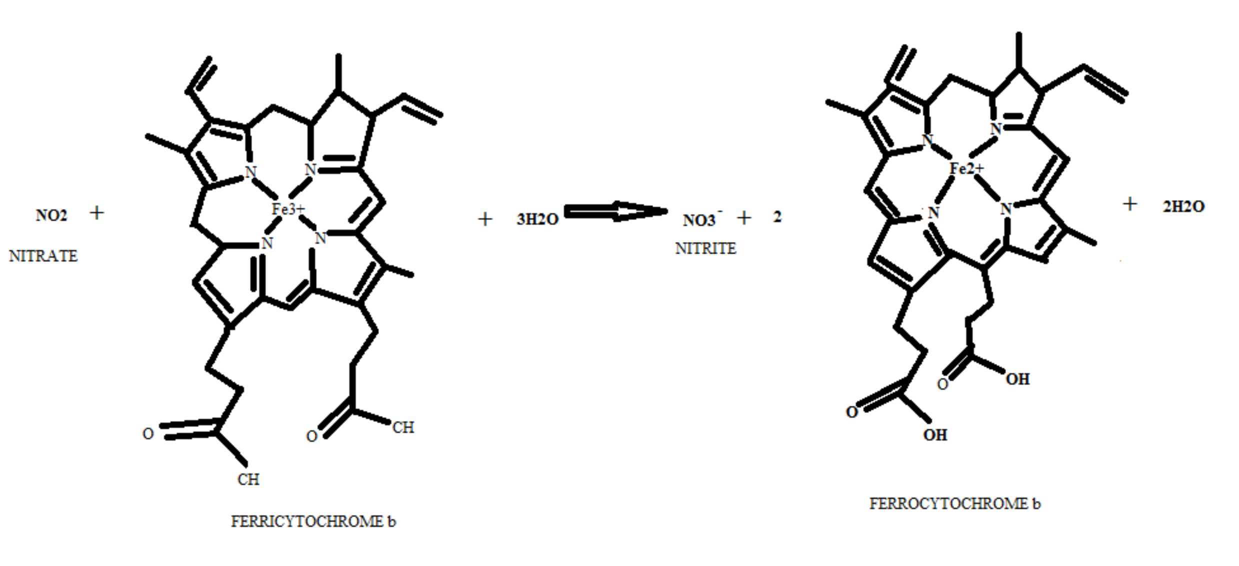

Figure 7; The mechanism of Nitrate Reductase

4. Catalases

It is an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. This belongs to oxidoreductases class of enzyme classification. It is a tetramer consisting of 4 polypeptide chains, each having 500 amino acid, and 4 porphyrin heme or iron groups that makes catalases capable of reacting with the hydrogen peroxide (Kaushal et al., 2018).

It is found in organisms living under the oxygen presence and protects the organelles of those organisms from any sort of damage that can be caused by peroxide. These enzymes are used as food preservatives in food industries and also used for the manufacturing of beverages. On a commercial scale, catalase is often used for the wastewater treatment. This enzyme has been employed as an indicator for the crude oil degradation where it assays the amount and properties of the crude oil (Kaushal et al., 2018)

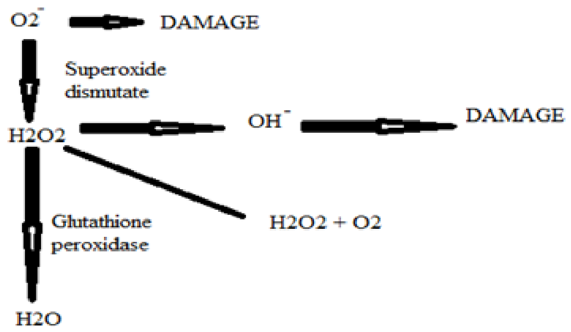

Figure 8; The mechanism of catalase enzyme

5. Amylase

Starch is a carbohydrate that basically consists of two main constituents that are amylase and amylopectin. These are bonded together by the 1, 4-alpha glycosidic linkages. Amylase production can be observed very well in case of fungi. This enzyme test is implemented in order to check the ability of a particular micro-organism to degrade the starch. The iodine is flooded over the media with bacterial colony and if it gives a zone of clearance around the bacterial colony then it determines that starch has been hydrolyzed by the bacteria. On the other hand, if the whole media turns blue as Iodine gets entangled inside the helical structure of starch that depicts the starch is still present. (Figure 9).

DISCUSSION

The problem of pollution is becoming a serious concern with each passing day. There are various factors responsible for this severe effect and one of them is the use of crude oil. The oil leakages or oil spills not only pollute the soil but also causes harmful health issues to the native organisms and humans as well. Therefore, to this problem one of the solution is to use a natural technique in order to not contribute to the already existing above the level pollution rate. So the answer lies in the nature itself; the halotolerants like *Alconivorex, Halomonas, Sphingomonas* and so on. In recent studies, there have been number of new halophilic species being reported for their potential of microbial degradation of petroleum and diesel oils. These species not only degrades the PAH

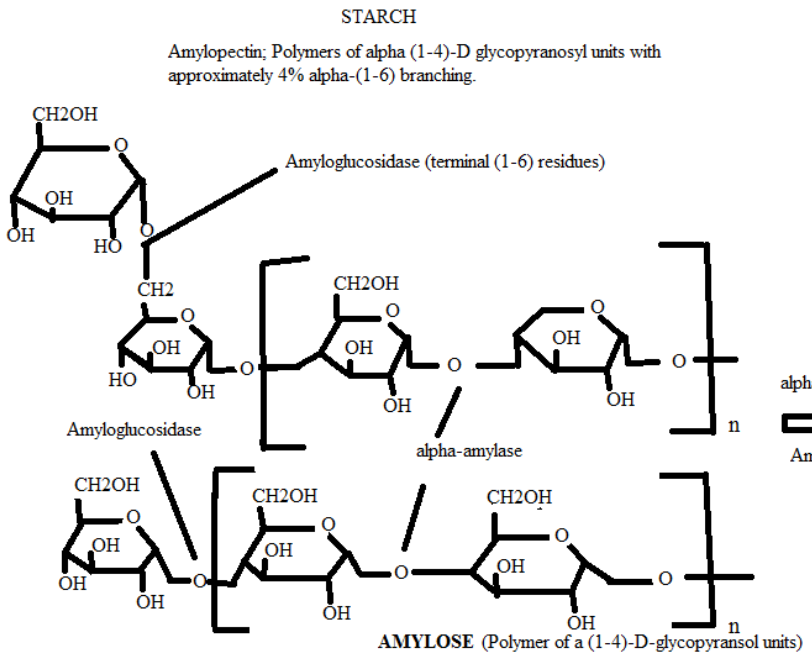

Figure 9; The activity of amylase enzyme.

containing crude oils but also with a process called bioremediation. Halophilic bacteria consist of certain enzymes which help them to do their job, for example, nitrate reductases, catalases, lipases, amylases and etc. The major property of halophiles that is to withstand the extreme saline conditions makes it able to be more of a productive method. It is very important to explore and discover new microbes that can actually do the bioremediation of crude oil contaminants. The whole scientific field needs to find more of cheap, easy and sustainable methods of removing the pollutants while advancing their technologies.

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