

Harnessing the Power of Halophilic Bacteria for Human Welfare

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I. Introduction

Though the term biotechnology was coined by Karl Ereky in 1919, it has gained momentum only in late 1900s or in early 21st century. This can largely be attributed to the developments in the industrial sector with respect to biology. It was only when we learned to exploit the role of biotechnology for human use in a direct manner, biotechnology gained its importance. These applications could be in respect to fermentation or product formation at large scale using microbes or in the context of raising transgenics for human use or utilizing microbes for environmental sustainability. Thus, while on one hand, development in biotechnology was paving the way for advancements, on the other hand, industrialization was equally crucial to harness the applications for human use. Nevertheless, as we learned to harness the potential of mesophilic microorganisms (the microbes surviving in usual environmental conditions like the ones favourable to humans), the discovery of extremophiles and their role in human applications became evident.

The remarkable adaptability of microorganisms to survive in extreme environments has always been a subject of scientific fascination. Among these resilient life forms, halophilic bacteria have stood out as examples of survival in such severe hypersaline conditions that would usually prove fatal to most other organisms. These microbes have developed intricate biochemical strategies to thrive in saline ecosystems, ranging from salt flats to hypersaline lakes and salt mines. However, in accordance with their extensive evaluation, their significance extends far beyond their ability to survive in these harsh habitats. In recent years, the potential of halophilic bacteria to contribute to various aspects of human welfare has become increasingly evident. Their unique characteristics make them valuable assets in biotechnological applications that encompass fields as diverse as healthcare, bioremediation, industrial processes, biofuel production and even electricity generation. Harnessing the power of halophilic bacteria has opened new

avenues for addressing pressing challenges faced by humanity, from pollution control to the development of novel therapeutic approaches.

This chapter, therefore, delves into two major arenas *viz.* developments in industrial biotechnology in the recent times and multifaceted world of halophilic bacteria and their role in improving human well-being including their pivotal role in almost all arenas of life and beyond ranging from health industry to bioremediation. The concept of ONE-HEALTH APPROACH in recent time has very clearly suggested that human health, animal health and environment sustainability all need to be taken care of together for the success of either of them. Therefore, this chapter is focused on the wide range of applications of halophiles particularly in the context of industrial biotechnology. As we navigate through the fascinating realm of halophilic bacteria, we will uncover how these resilient microorganisms are making significant strides towards enhancing human welfare and sustainable development. However, before we delve deeper into the role of halophiles in this regard, we will focus on the origin and evolution of industrial biotechnology with special emphasis on market share and segmentation in the context of COVID-19 pandemic.

II. Industrial Biotechnology at a glance

The term biotechnology has been defined in multiple ways depending upon the application being targeted and advantages being exploited. One of the most general definitions of biotechnology is provided during “The United Nations Convention on Biological Diversity”, defining the term as *“any technological application that uses biological systems, living organisms, or their derivatives to create or modify products or processes for specific purposes.”* On the contrary, a simpler definition is offered by Wikipedia, describing it as *“A field of applied biology involving the utilization of living organisms and bioprocesses in engineering, technology, medicine, and other areas requiring bioproducts.”* Additionally, the definition adopted by the Polish Ministry of Science and Higher Education, following the OECD (Organization for Economic Co-operation and Development), states that *“Biotechnology is an interdisciplinary branch of science and technology that deals with the transformation of living and non-living matter using living organisms, their components, or products derived from them. It also involves the creation of models of biological processes to generate knowledge, goods, and services.”* Though there have been multiple ways of defining the term with minor variations, the two things that were common are the use of biological systems and certain industrial applications. This forms the basis of the current chapter as to highlighting the role of halophiles in various industrial applications. Before we delve deeper into the specific applications, the chapter provides a brief overview of the current status of industrial biotechnology across the world and India with special reference to effects observed after the COVID-19 pandemic. While there are various classifications of biotechnology, the color-coded classification system is the most widely used owing to its simplicity and clarity. Initially, four major colours were considered: red, green, white, and purple to describe different applications of the field which has now extended to other rainbow colours owing to the recent and novel advancements in the field. However, none of these classifications is accurate and all these are interchangeable. As already stated, that the term focusses majorly on industrial replication, the colour code “white” is specifically targeted in this direction and will therefore be the major highlight of the current chapter. There have been certain deviations in this regard wherein environmental biotechnology was also included as a part of white biotechnology while considering its relevance, it has been segregated as “Grey biotechnology”. As evident from these examples, the color-coded classification of biotechnology is still evolving and cannot be considered absolute. The list of colour codes of biotechnology is given below:

- i. Green biotechnology, which focuses on agricultural development.
- ii. Yellow biotechnology, often referred to as nutritional biotechnology.
- iii. Red biotechnology, dedicated to medicine and human health.
- iv. White biotechnology, primarily highlighting industrial applications.
- v. Grey biotechnology, addressing environmental protection issues.
- vi. Blue biotechnology, concentrating on marine and aquatic regions.
- vii. Brown biotechnology, related to arid and desert regions.
- viii. Gold biotechnology, connected with bioinformatics, computer science, and chip technology.
- ix. Violet biotechnology, dealing with ethical, legal, and philosophical aspects.
- x. Dark biotechnology, associated with bioterrorism and biological weapons.

As already described, it is essential to note that this classification is not exhaustive and does not include certain emerging fields such as nanotechnology, which are undergoing significant development in recent times. Fortunately, there are still some "rainbow colours" available to accommodate new and evolving areas of biotechnology. However, the scope of this chapter from here on lies on industrial biotechnology.

Industrial biotechnology is a term referring to the process of converting raw materials, typically renewable carbon feedstock, into a wide range of products and goods. This transformation is accomplished through the activity of

microorganisms including both naturally existing and genetically modified transgenics within a controlled setting, such as closed bioreactors.

The primary aim of White biotechnology is to shift from oil-based processes to more sustainable ones, thereby reducing environmental impacts. It is estimated that industrial biotechnology can potentially cut carbon dioxide emissions by up to 50%, lower energy consumption by 20%, and reduce water usage by 75%.

A. From Ancient to Contemporary Industrial Biotechnology

The roots of microbial fermentation stretch back to 7000 BC during Neolithic times when early societies attributed the transformation of food materials into fermented products for divine intervention. The Egyptians credited Osiris for beer brewing, the Greeks revered Bacchus as the god of wine, and in early Japanese breweries, a small shrine received daily reverence. The scientific approach to fermentation began in the 16th century with Antonie van Leeuwenhoek's invention of the microscope, which allowed the identification of small organisms (considered bacteria) through a self-made apparatus with a 266x magnification that remained unmatched for 150 years. In the late 18th century, Lavoisier established the laws of mass conservation, and in the early 19th century, Gay-Lussac defined the chemical formula of sugar transformation into ethanol and CO₂. Cagniard de la Tour, Theodor Schwann, and Frans Kuetzing reported "microbes" reproducing by budding in fermented products, coining the term 'yeast' for these smaller organisms. This discovery sparked a 19th-century debate between proponents of spontaneous generation and scientists like Louis Pasteur, who believed fermentation resulted from living organisms. Pasteur ended the debate with ingenious cultivation techniques, sterilization processes, and microbiological isolation principles. He linked specific microbes to lactic, butyric, vinegar, and alcohol fermentation, distinguishing anaerobic from aerobic fermentation by the role of oxygen. Pasteur's methods laid the foundation for industrial microbiology. Though Antonie van Leeuwenhoek is considered as the father of Microbiology, Louis Pasteur has contributed significantly to the emergence of white biotechnology. However, white biotechnology (WB v1.0) gained momentum in the early 20th century with industrial-scale fermentation of acetone-butanol-ethanol (ABE) by *Clostridium acetobutylicum*, citric acid production by *Aspergillus niger*, and the industrial production of penicillin in the 1940s. In the 1960s, industrial-scale amino acid production began with sodium glutamate using Ajinomoto via *Corynebacterium glutamicum*. Most of these fermentations required aeration, driving technological advancements for sterile air supply. Microbial strains for large-scale fermentation were often selected randomly after chemical mutagenesis. The second phase of white biotechnology (WB v2.0) emerged in the early 1970s with recombinant DNA technology. Molecular biology innovations then integrated with traditional microbial fermentation, leading to biopharmaceutical industries like Genentech, which used genetically modified organisms (GMOs) for drug production. Recombinant DNA technology also contributed to the food and agriculture sectors with engineered products like recombinant crops with better food quality such as Golden rice and robust crops such as BT cotton, Flavr Savr tomato and virus-resistant crops including papaya, some of which have been commercialised as well. However, these advances sparked debates on genetic manipulation's merits and safety. The late 20th and early 21st centuries witnessed automation, miniaturization, and computing tools advancements, giving rise to "Bio Info-Nanotechnologies." Next-generation sequencing and "omics" technologies allowed comprehensive analysis of biological systems. Systems biology emerged, aiming to understand biological systems, emphasizing connectivity and interactions. Synthetic biology followed, deliberately designing, or rewiring biological systems for specific functions. It brought about biotech startups specializing in DNA synthesis, engineered strains, and bio foundries, supporting biological system engineering [2].

B. Global Biotechnology Market Overview

The concept of industrial biotechnology remains incomplete without discussing the economics and market overview. This is the segment bringing the laboratory to land and for societal use. The global biotechnology market reached an estimated value of USD 1.37 trillion in 2022, with a projected compound annual growth rate (CAGR) of 13.96% from 2023 to 2030. This robust growth is driven by several key factors, including government support for regulatory modernization, improvements in approval processes, reimbursement policies, and the standardization of clinical studies. The rise of personalized medicine and an increasing number of orphan drug formulations are also contributing to the market's expansion, attracting emerging and innovative biotechnology companies. This was further enhanced over the last couple of years owing to the COVID-19 pandemic which is separately discussed in the upcoming section.

C. Impact of COVID-19

Though the havoc created by the COVID-19 pandemic has affected the entire world drastically, there has been a positive impact on the biotechnology market. It has led to increased opportunities and advancements in drug development and vaccine manufacturing. In 2021, more than 11 billion doses of COVID-19 vaccines were produced

globally, vaccinating about half of the world's population within a year. The success of mRNA vaccines and accelerated approval processes resulted in substantial revenue generation, with Moderna, Pfizer/BioNTech, and Johnson & Johnson vaccines generating a combined revenue of approximately USD 31 billion in 2021.

D. Role of Fermentation Technology in the field of human health:

As already stated, fermentation technology plays a significant role in the life sciences and healthcare sectors. Advancements in bioreactors, such as simplified and vortex bioreactors, have improved fermentation technology and its adoption. These advancements have extended to wastewater processing, enhancing operational feasibility. The continuous exploration of CAR T and TCR T-cell therapies as potential treatments for chronic viral infections, including HIV, hepatitis B, and SARS-CoV-2, is expected to drive further research and market growth.

E. Treatment of Chronic Diseases

The rising demand for clinical solutions for chronic diseases, including cancer, diabetes, age-related macular degeneration, and various forms of arthritis, is expected to boost the market. Major research groups and firms are actively working towards the development of products in pipeline for the treatment of diseases like Alzheimer's, Parkinson's, various cancers, and cardiovascular conditions.

F. Market Segmentation

Needless to say, advancement in technology will play a key role in both industrial biotechnology and eventually in regulating the market share. Technologies such as DNA sequencing hold a significant market share due to declining costs and government funding in genetic research. Similarly, Nanobiotechnology is expected to expand rapidly, driven by nanomedicine approvals and advanced technology. Another global impact in terms of the healthcare industry is made by Tissue engineering and regenerative medicine due to investments, healthcare spending, and the presence of key players.

When the different applications of biotechnology are evaluated in terms of the market share they hold and the impact they make, it has been observed that Health applications dominate the market, driven by disease burden followed by agri-biotech availability, and technological developments. Bioinformatics applications are expected to increase, especially in industries like food and beverages, due to advancements in AI, machine learning, and big data. On a similar note, the regional Insights in this regard suggest that North America leads the market (41.63%), owing to key players, extensive R&D, and high healthcare expenditure. However, Asia Pacific (18.17% CAGR) is projected to grow the fastest, driven by investments, improved healthcare infrastructure, government initiatives, and expansion strategies by market players.

G. Key Players

- 1) Since it has been observed that the market segmentation is affected by a number of factors including the application, the region of study, etc. Highlighting certain key players in this section is of utmost importance driving the attention to the developments: AstraZeneca, Gilead Sciences, Inc., Bristol-Myers Squibb, Biogen, Abbott Laboratories, Amgen Inc., Novo Nordisk A/S, Merck KGaA, Johnson & Johnson Services, Inc., Novartis AG, Sanofi, F. Hoffmann-La Roche Ltd., Pfizer, Inc., Lonza, and others.
- 2) Gero collaborated with Pfizer to harness mRNA biology modulators for treating various diseases.
- 3) Gilead Sciences, Inc. acquired XinThera, gaining assets focused on oncology and inflammation.
- 4) Gilead Sciences, Inc. acquired MiroBio, enhancing its immune inhibitory receptor agonists portfolio.
- 5) AstraZeneca acquired Neogene Therapeutics Inc., focusing on TCR-Ts for cancer treatment and personalized medicine.

To conclude, the global biotechnology market is experiencing significant growth, driven by factors like government support, advancements in personalized medicine, and the impact of the COVID-19 pandemic. The market encompasses various technologies and applications, with North America leading in terms of market share and Asia Pacific expected to grow rapidly. Key players are making strategic moves through collaborations, acquisitions, and expansions to capitalize on the market's potential.

H. Status of biotechnology in the global market:

Global biotechnology market, valued at USD 1.37 trillion in 2022, is set for substantial growth with a projected CAGR of 13.96% from 2023 to 2030. This growth is underpinned by various factors, including government support for regulatory modernization, streamlined approval processes, reimbursement policies, and standardized clinical studies. The ascent of personalized medicine and the proliferation of orphan drug

formulations are enticing innovative biotech firms. Additionally, the COVID-19 pandemic has spurred drug development and vaccine manufacturing, exemplified by over 11 billion COVID-19 vaccine doses produced in 2021, yielding a combined revenue of approximately USD 31 billion from Moderna, Pfizer/BioNTech, and Johnson & Johnson vaccines. The agricultural sector has witnessed a surge in biotechnology applications such as micro-propagation, molecular breeding, and genetically modified crops. Strong clinical trial pipelines and funding in tissue engineering, along with a growing focus on chronic disease treatment, are further driving market expansion. Fermentation technology, including CAR T and TCR T-cell therapies, plays a vital role in healthcare. Stem cell technology, DNA fingerprinting, and genetic engineering are also on the rise, contributing to the biotech market's growth. Market segmentation highlights DNA sequencing, nanobiotechnology, and tissue engineering as key technologies, with health applications dominating, especially in bioinformatics. Regionally, North America leads with 41.63% market share, while the Asia Pacific is the fastest-growing region, expected to achieve an 18.17% CAGR from 2023 to 2030. Key players like AstraZeneca, Gilead Sciences, and Pfizer are making strategic moves through collaborations, acquisitions, and expansions to tap into this burgeoning market's potential. <https://www.grandviewresearch.com/industry-analysis/biotechnology-market>.

I. Indian biotechnology market

Halophiles have made rapid progress in the field of biotechnological applications and have thus created a vast global market, and India's biotechnology sector holds significant promise for substantial growth. This growth potential can contribute significantly to India's role in the global industrial biotechnology landscape. India is currently one of the world's fastest-growing trillion-dollar economies, ranking fifth overall with a GDP of \$2.94 trillion. Biotechnology (BT) and information technology (IT) are pivotal drivers of this growth, accounting for approximately 5% of the country's annual GDP. Within Indian biotechnology, notable areas include biopharmaceuticals, bio-industrial, bio-services, bio-agricultural, and bioinformatics. Among these, the bio-industrial sector stands out as particularly promising and advanced.

Indian industrial biotechnology is exploring innovative approaches, such as utilizing microorganisms to produce valuable bioactive components like industrial enzymes, organic acids, bulk chemicals, and single-cell proteins. These developments have played a significant role in advancing biotechnology beyond biopharmaceuticals. Consequently, India has become one of the world's top 12 destinations for biotechnology.

J. Role of halophile in industry

The biotechnological importance of halophilic archaea and bacteria for mankind is multifaceted and encompasses various fields of application. One significant area of interest is the potential management of neurodegenerative diseases, such as autism spectrum disorder (ASD). ASD is a complex neurodevelopmental disorder characterized by language impairment, deficits in socialization, and repetitive stereotypic behaviours. The prevalence of ASD has been on the rise, with approximately 1 in 59 children diagnosed with the condition, according to the United States Centres for Disease Control and Prevention (CDC (Centres for Disease Control)).

One of the key factors contributing to ASD is excessive oxidative stress and a decreased capacity for antioxidant defence within the body. This imbalance leads to mitochondrial dysfunction and oxidative damage, particularly in the brain, which is highly susceptible due to its constant need for oxygen, low antioxidant defences, and high levels of omega-3 polyunsaturated fatty acids. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels surge in individuals with ASD.

Recent research suggests a significant connection between gut microbiota and brain activity, emphasizing the importance of the gut-brain axis (GBA) in modulating oxidative stress levels. Halophilic bacteria play a role in maintaining metabolic homeostasis by accumulating compatible solutes and producing short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate. These substances can influence the level of oxidative stress by affecting prooxidant components and antioxidant enzyme capacity.

Furthermore, the production of biofuels is another critical application of halophilic microbes. As traditional fossil fuels become scarcer and less environmentally friendly, there is a growing need for renewable and sustainable biofuels. Halophiles are directly involved in the production of biofuels like ethanol and butanol through sugar fermentation techniques in hypersaline environments. Various bacterial strains have been identified that can produce biofuels and enzymes essential for their production.

Enzymes derived from halophilic microorganisms are highly stable under extreme conditions, such as high salinity and pH. These enzymes have significant industrial applications in biotechnology, offering promising solutions for various processes, including biofuel production and other biocatalytic applications.

Halophilic microorganisms also play a role in remote sensing through the production of pigments. These pigments protect their cells from external damage and can be monitored through satellite remote sensing systems, helping track environmental changes and circulation patterns.

Another fascinating application is the potential use of halophiles in metal bioremediation through the synthesis of nanoparticles. Halophiles can produce biogenic nanoparticles capable of remediating metal contaminants, offering a potential solution to metal pollution in various environments.

Additionally, small peptides, including antimicrobial peptides (AMPs), produced by halophiles have shown promise as potential biomedicines. These peptides have demonstrated antimicrobial, antifungal, and even anticancer activities, suggesting their potential in the development of alternative treatments for various diseases.

Furthermore, halophiles have been explored for the formulation of new-generation liposomes, which have applications in drug delivery and vaccination.

Lastly, halophilic bacteria have been utilized in the repair of concrete cracks caused by road salt. These bacteria, such as *Sporosarcina pasteurii*, contribute to biomineralization by converting calcium ions from soil into calcium carbonate, effectively sealing cracks in concrete.

In summary, halophilic archaea and bacteria offer a wide range of biotechnological applications, from addressing neurodegenerative diseases to biofuel production, enzyme stability, metal bioremediation, and more. Their unique adaptations to extreme environments make them valuable assets in various industries and scientific endeavours.

III. Applications of halophiles in biotechnology

It has clearly been stated the chapter is focused on two major aspects: industrial biotechnology and its growth in recent times followed by the role of halophiles in this regard. Since the first segment is clearly well discussed in the previous sections, the chapter now focuses on the second aspect *viz.* the role of halophiles in multiple industrial applications. The major applications discussed in the upcoming sections include bioremediation, agriculture, drug development and biomedical innovations, electricity generation and biofuel production. Figure 1 represents applications of halophilic bacteria.

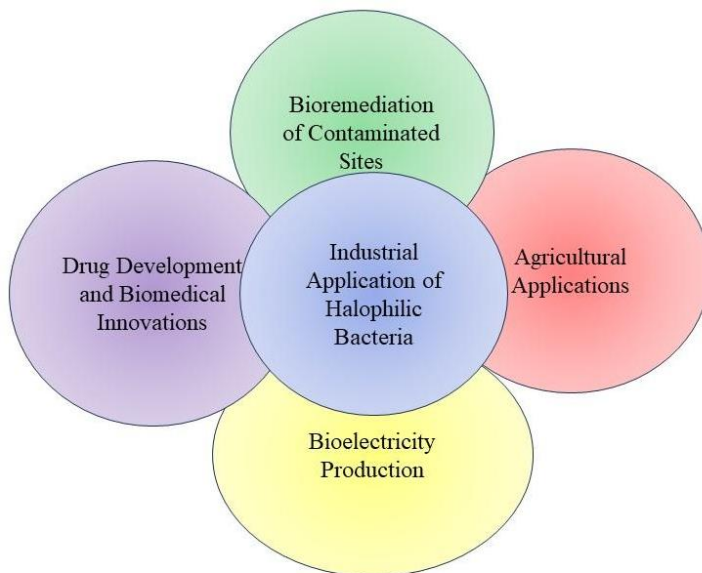


Figure 1: Different Applications of Halophilic Bacteria; Types of industrial application where halophilic bacteria can be used

A. Bioremediation and Environmental Cleanup

Bioremediation is a branch of biotechnology that employs the use of living organisms, like microbes and bacteria, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments. The following are the advantages of using halophilic bacteria in bioremediation:

First, high salinity greater than 1% in wastewater can decrease the microbial diversity of activated sludge which is resolved either by adapting the biomass to high salt concentration or using halophilic bacteria; Second, a high salt content can decrease organic compound solubility in water by a salting out effect, and therefore decrease the bioavailability of the organic compounds. This problem could be naturally overcome by the biosurfactant production

from halophilic hydrocarbon-degrading bacteria or by adding substances to increase the pollutant solubility, such as 2-hydroxypropyl β -cyclodextrin; Third, high concentration of salinity decreases the oxygen amount in water, thus limiting the action of oxygenase, and therefore the aerobic biodegradation rate (Castillo-Carvajal et al., 2014). However, it has been reported that extreme halophilic archaea *Haloferax*, *Halobacterium*, and *Halococcus* have higher biodegradation rates at 2.2 mg L⁻¹ than at 5.3 mg L⁻¹ dissolved oxygen (Al-Mailem et al. 2010). Though the role of halophiles is extensive in bioremediation, the major highlights discussed here include hydrocarbon degradation and treatment of industrial effluents with high salinity.

1) Degradation of hydrocarbons by halophiles

Halophiles can be used to treat wastewater containing hydrocarbon as petroleum and salinity are coexisting factors. In other words, the presence of oil in any place is usually observed with high salt concentrations. As a result, it would not be surprising that the presence of halophiles could be seen in different parts of the oil industry. Crude oil is mainly formed near highly saline areas with high evaporation rates and its formation is associated with hypersaline brines. Furthermore, drilling operations increase the exposure risk of oily brines into the environment. Moreover, high salt amounts are added to the drilling muds to maintain an osmotic barrier and to prevent inflation in drilled rocks, that can finally be discharged into the environment.

Considering economic aspects, oil wells are exploited to the highest possible extent. In the first stage, the drilling process leads to the oil outflow due to the natural pressure of the well. Following the pressure reduction, in the second stage of oil recovery, water or gas is injected into the well to maintain the pressure. One-third to one-half of crude oil could be recovered by these two phases. The petroleum formation, source rock has a mainly porous structure. Therefore, large amounts (one-half to two-thirds) of oil penetrate the capillary pores of reservoir, access to which is impossible even by water flooding or gas injection. To overcome this, chemical surfactants or polymer could be added in the water-flooding step to increase the sweeping efficiency of flooded water. However, chemical surfactants and polymers are not cost-effective and eco-friendly. Therefore, the third stage of oil recovery have been done by exploiting microorganisms or their bioproducts and is named MEOR (Microbial Enhanced Oil Recovery) (Gudin[~]a et al., 2012; Brown, 2010). They could be applied for the bioremediation of oil-polluted terrestrial and aquatic environments (Liu et al., 2010; Sarkar et al., 2005). Haloarchaea can also degrade hydrocarbons; however, their degradation is more efficient for low molecular-weight hydrocarbons. The degradation of naphthalene, phenol, p-hydroxybenzoic acid, and 3-phenyl propionic acid, and oxychlorides like perchlorate or chlorate by haloarchaea also make it an attractive choice for wastewater treatment (Martínez-Espinosa et al., 2015; Mukherji et al., 2020; Li et al., 2021).

2) Treatment of saline industrial effluent

Various industrial activities such as tannery (Lefebvre et al., 2005), agro-food production, chemical manufacturing, and oil and gas production (Woolard and Irvine, 1995) generate saline wastewaters. Saline effluents are conventionally treated by physio-chemical methods as the biological treatment is strongly inhibited by high salts, mainly NaCl (Lefebvre and Moletta, 2006). However, since the costs of physio-chemical treatments are particularly high and the efficiency of biological treatments is relatively low, microorganisms for the treatment of saline wastewater are warranted for further research (Zhuang et al., 2010).

Several biotechnological-based processes can be benefited from the use of haloarchaea. Thus, the use of whole cells in wastewater treatments and bioremediation of brines and salty soils has been recently revealed as a promising tool. Hypersaline wastewater is a common byproduct of industrial processes. Hence, cost-effective treatment of hypersaline wastewater is necessary for sustainable development. Biological wastewater treatment has been considered a more economical approach, but mesophilic microorganisms used so far for this purpose cannot be used in the biological treatment of polluted brines of wastewater containing high salt concentration. In this context, recent research findings revealed the role of haloarchaea in the treatment of salty wastewater and brine generated as final residues in water desalination plants (Moopantakath et al., 2023).

Application of halophilic bacteria can improve the removal efficiency of COD from saline wastewater. Tannery wastewater-adapted bacteria *Pseudomonas aeruginosa*, *Bacillus flexus*, *Exiguobacterium homiense*, and *Staphylococcus aureus* showed 80% decrease in COD at 8% salinity (Sivaprakasam et al. 2008). Introducing halophilic bacteria in textile effluents is a prominent approach for treatment of synthetic dyes, where other microorganisms are not able to degrade the same (Pourbabaee et al. 2011). In a study, *Halomonas* sp. strain IP8 showed decolorization of dye from 50 to 20 mg/L, during 16–24 hours at 1–1.5 M NaCl salt concentration, at temperature range of 25–45°C (Pourbabaee et al. 2011). Presence of high salt concentrations in heavy metal and hydrocarbon contamination sites

also arises great demand of halophilic microorganisms for biotreatment of these sites (Sharma et al., 2016). Table 1, includes list of halophilic bacteria degrading different pollutants.

Table 1: Type of pollutant degraded by halophilic bacteria- This table include pollutant degraded by halophilic bacteria

Halophilic Bacteria	Pollutant degraded	Reference
<i>Haloarcula spA 235</i>	<i>Phenol</i>	<i>Acikgoz et al., 2016</i>
<i>Halomonas campisalis</i>	<i>Phenol, catechol</i>	<i>Alva et al., 2003</i>
<i>Haloferax alexandrinus st. KCTC</i>	<i>Benzoate</i>	<i>Bonfa et al., 2011</i>
<i>Halomonas sp. GT-83</i>	<i>Vanadate (Heavy metal)</i>	<i>Ghazvini et al., 2009</i>
<i>Halophilic coccus TM-1</i>	<i>Crude oil and low molecular weight organic subs</i>	<i>Hao et al., 2009</i>
<i>Halomonas sp</i>	<i>Diesel Fuel</i>	<i>Kleinsteuber et al., 2006</i>

B. Agricultural Applications

The demand for biotechnology tools in agriculture has expanded significantly. Applications such as micro-propagation, molecular breeding, tissue culturing, and the development of genetically modified crops have driven market growth. Genetically modified crops, herbicide-tolerant, and insect-resistant seeds are gaining popularity, especially in the agricultural sector. Tissue culture technology is being used to produce novel rice variants and disease- and pest-free banana varieties in regions like South Asia and Africa. Therefore, this section focuses on the role of halophiles and archaeobacteria in the agriculture particularly in context of plant growth promoting microbes and rhizobacteria (PGPR), PGPRs affecting phytopathogens, ACC-deaminase producing PGPRs and role of halophiles in phosphate solubilization,

1) Plant Growth Promoting Microorganisms for Use in Soil Desalinization

Soil salinity is one of the grave concerns in respect to the soil fertility thereby affecting agricultural practices in almost all parts of the world. The salinity in soil is inherently present in natural environment but is eventually aggravated by the excessive use of chemicals fertilizers, and inappropriate ways of irrigation (over-irrigation and poor drainage). The salinity affects nutrient uptake by plants and therefore, utilizing halotolerant plant growth promoting (PGP) bacteria by which cultivation in saline soil can be improved needs to be explored (Mishra et al., 2017). The microbiomes that can be used for this purpose are usually the ones isolated from crops growing in saline ecosystems. The plant microbiomes have been reported as epiphytic, endophytic, and rhizospheric and have been characterized for plant growth promotion under and *in vivo* condition of salinity stress. The PGP microbes belonged to different genera such as *Achromobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Methylobacterium*, *Micrococcus*, *Micromonospora*, *Pantoea*, *Pseudomonas*, and *Streptomyces* as epiphytic microbes; *Achromobacter*, *Bacillus*, *Burkholderia*, *Microbiospora*, *Micromonospora*, *Nocardioides*, *Pantoea*, *Planomonospora*, *Pseudomonas*, *Streptomyces*, and *Thermomonospora* as endophytic microbes; and *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Paenibacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium*, and *Serratia* as rhizospheric microbes from crops growing in diverse agro-ecosystem (Yadav et al., 2018).

2) Halophytes and PGPRs that Control Phytopathogens

Soil salinity not only disrupts nutrient uptake, plant physiology and morphology but also makes the plant prone to pathogens (Besri, 1993). Though agricultural chemicals have been employed to get rid of these plant pathogens, PGPRs (Plant Growth Promoting Regulators) have shown promising results in this regard via multiple mechanisms. Production of PGPRs acting as antimicrobial metabolites by *Bacillus* and *Pseudomonas* (Etesami et al., 2018) or fungal cell wall degrading enzymes including lipase and protease are certain mechanisms (Olanrewaju et al., 2017).

3) Halophytes and ACC Deaminase-Producing PGPRs

Ethylene, one of the crucial plant hormones, is responsible for the senescence, ripening and abscission of plants and is thus produced by all plants. However, its production is alleviated in response to environmental stress conditions including drought and salinity. These heightened levels reduce plant growth by inhibition of root growth via reduction in nitrogen fixation and thus the ethylene levels need to be contained to maintain the appropriate plant growth. In this regard, ACC deaminase producing PGPRs have shown promising results as they metabolize ACC, ethylene precursor, to α -ketoglutarate and ammonia thereby reducing ethylene production. Therefore, PGPRs producing ACC deaminase have been found associated with extended root growth and high resistance to salinity. Also, these PGPRs have been known to affect other enzymes of ethylene production pathway such as ACC synthase and ACC oxidase (Etesami et al., 2018).

4) Phosphate (P) Solubilization

Phosphorous is the major nutrient required for plant growth as it is an integral part of the major biomolecules present in the cell such as nucleic acids, nucleotides, phospholipids, and phosphoproteins. In most cases, salinity is known to decreased P accumulation in plant, thereby developing P deficiency symptoms (Parida and Das 2005). Phosphorus exists in two forms in soil, as organic and inorganic phosphate, and, like other nutrient elements such as potassium, iron, zinc, and copper, possesses limited mobility in the soil (Hayat et al. 2010). The conversion of insoluble phosphate compounds (both organic and inorganic) in a form accessible to the plant is an important trait of PGPB (Plant Growth Promoting Bacteria) strains. This is achieved through the acidification, chelation, ion-exchange reactions, and production of low-molecular-weight organic acids such as gluconic acids. Halotolerant PGPBs have been proved to be vital for circulation of plant nutrients in many ways, thereby reducing the need for chemical fertilizers. In addition to production of phytohormones and ACC-deaminase activity, multiple bacterial strains can affect plant growth directly by solubilizing inorganic phosphate, improving nutrient uptake, and mineralizing organic phosphate (Sharma et al., 2016). Diby et al. (2005) reported enhanced nutrient mobilization in the rhizosphere of black pepper and significant uptake of nitrogen (N) and phosphorus (P) in the PGPR-treated black pepper vines that resulted in root proliferation and enhanced plant growth. Strains of rhizobacteria that have efficient phosphate solubilizing ability even under high saline (60 g L⁻¹ NaCl) conditions have been reported (Upadhyay et al. 2011).

C. Exploring Halophiles for Drug Development and Biomedical Innovations

Halophiles have been obtained in all three domains of life: eukaryotes, prokaryotes and archaeobacterial. Adaptation is necessary to survive in environmental changes and for proliferation of microbes. The developing activity by organism in its habitat is an evolutionary process is called adaptation (p-4) (Gohel et al., 2015). Halophiles produce saline-tolerant proteins and metabolites, such as carotenoids, which assist in tolerance toward salinity. Haloarchaeal carotenoids have industrial interest due to their antioxidant, anticancer, antimicrobial, anti-inflammatory, food colorant, and several other biomedical applications (Verma et al., 2020). Bacterioruberin is also commonly used in cosmetics and drug encapsulation (Serrano et al., 2022). Haloarchaea have been extensively explored for bioactive metabolites with anticancer, antimicrobial, and antioxidant activities. Among the several haloarchaeal secondary metabolites, carotenoids gained attention due to their multi-faced applications in cosmetics, food, and biomedical sectors (Serrano et al., 2022). Carotenoids have multiple roles in bacteria, plants, and archaea. Carotenoid helps photosynthetic plants and microbes to increase light absorption in the blue-green region through the singlet-singlet energy transfer (Hashimoto et al., 2015). It also has photoprotective effects against excessive light and reactive oxygen species through the triplet energy transfer of chlorophylls to carotenoids (Maoka, 2020). They are crucial in structural stabilization and render tolerance to hypersalinity.

1) Antimicrobials by halophiles

Antimicrobial resistance is continuously emerging owing to misuse and overuse of such agents which has further increased during the pandemic; therefore, novel antimicrobials are needed to combat the same. The carotenoids synthesized by *Haloarchaeal* are less explored as compared to other carotenoids. However, the potential of haloarchaeal carotenoids as antimicrobials was demonstrated against several pathogens (Gómez-Villegas et al., 2020). According to a study conducted by Sahli et al. in 2022, it has been revealed that carotenoids derived from *Halogeometricum* sp. (ME3), *Haloarcula* sp. (BT9), and *Haloferax* sp. (ME16) exhibit antimicrobial properties when tested against some of the most infectious pathogens including *Vibrio anguillarum*, *Pseudomonas aeruginosa*, and *Pseudomonas anguilliseptica*, respectively. Haloarchaea are known to produce another potent antimicrobial compound called halocins. One such halocin, derived from *Haloferax larsenii* HA1, has demonstrated remarkable antimicrobial efficacy in a study by Kumar and Tiwari in 2017. Previous research on halocins from

haloarchaea has suggested that their antimicrobial activity could be linked to competitive interactions between different species within similar niches, involving competition for resources, food, or habitat. For example, *Halobacterium salinarum* ETD5, isolated from the solar saltern in Sfax, Tunisia, exhibited antagonistic effects against other haloarchaea inhabiting similar niches, including *Halorubrum* sp. (strains ETD1, ETD2, ETD6, ETR7), *Halorubrum chaoviator* sp. (strains ETD3, ETR14, and SS1R17), and *Halobacterium salinarum* ETD19, as detailed in the study conducted by Ghanmi et al. in 2016. Additionally, it was found that the C50 carotenoid pigment sourced from *Natrialba* sp., M6, demonstrated significant potential in effectively combating hepatitis C virus (HCV) and hepatitis B virus (HBV) within human blood mononuclear cells, underscoring its potent antiviral properties, as outlined in the study by Hegazy et al. in 2020. Halocins, on the other hand, are proteinaceous bacteriocins produced by extremely halophilic bacteria and archaea. These halocins have been categorized into two primary types: (1) Microhalocins, characterized by their smaller size, typically less than 10 kDa, and (2) protein halocins, which possess a larger size, generally exceeding 10 kDa, according to research presented by Mukhtar et al. in 2019.

2) Anti-cancer compounds

Haloarchaea and their metabolic products are garnering increased attention in the context of treating various cancer types. Specifically, a carotenoid pigment sourced from *Halobacterium halobium*, which was isolated from a saltern in Tunisia, demonstrated anti-cancer effects against the HepG2 cell line (Abbes et al., 2013). Furthermore, the carotenoid pigment extracted from *Halogeometricum limi* exhibited approximately 23% anti-cancer activity against HepG2 cells when applied at a concentration of 720 µg/l (Hou and Cui, 2018). Similarly, haloarchaea with a high carotenoid production capability (0.98 g/l), such as *Natrialba* sp. strain M6, which thrives in environments with 25% NaCl and a pH of 10.0, displayed a notable 50% anti-cancer activity against MCF-7, HepG2, and HeLa cells at low concentrations (Hegazy et al., 2020). These results suggest that role of these bacteria need to be further explored.

3) Antioxidant compounds

The free radicals are produced by human body during metabolic processes, which erect oxidative stress and leads to inflammation and lifestyle diseases. During metabolic activities, the human body generates free radicals, which lead to oxidative stress, inflammation, and lifestyle diseases. Due to their free radical scavenging abilities at lower concentrations, which are significantly better than the typical reference compounds like ascorbic acids, haloarchaeal compounds are currently receiving a lot of interest. 13 C-C units make up the conjugated structure of bacterioruberin, which has a high capacity for scavenging free radicals. When tested using the DPPH (2,2-diphenylpicrylhydrazyl) and ABTS (2,2'-azino-bis (3- ethylbenzothiazoline-6-sulfonic acid) assays, the carotenoid from *Haloferax* sp. shown strong antioxidative activity. In the DPPH and ABTS assays, the carotenoid compound's IC50 values were 56.69 and 39.66 g/ml, respectively (Sahli et al., 2022). Similarly, acetone extracts from the *H. hispanica* HM1 showed 88% (ABTS) and 82% (Ferric ion reducing power) activity (Gómez-Villegas et al., 2020). Similarly, carotenoids from *H. volcanii*, Hgn. rubrum, and Hpl. inordinate have highlighted higher (80%) free radical scavenging properties at a concentration of 10 µg/ml (Hou and Cui, 2018). Zalazar et al. (2019) reported a genetically modified *Haloferax volcanii* strain (HVLON3) with high antioxidative activities (EC50 = 4.5 × 10⁻⁵ mol/l). Thus, haloarchaeal compounds can serve as a prominent source of antioxidant molecules in the future. Similar results were obtained using acetone extracts from the *H. hispanica* HM1: 88% (ABTS) and 82% (Ferric ion reducing power) activity (Gómez-Villegas et al., 2020). Similarly, at a concentration of 10 g/ml, carotenoids from *H. volcanii*, Hgn. rubrum, and Hpl. inordinate have shown stronger (80%) free radical scavenging activities (Hou and Cui, 2018). A genetically altered strain of *Haloferax volcanii* (HVLON3) with significant antioxidative properties (EC50 = 4.5 10⁵ mol/l) was reported by Zalazar et al. in 2019. So, studies so far suggest that haloarchaeal chemicals could be a significant source of antioxidant molecules in the future.

4) Biocompatible polymers

One of the key global research goals is to find eco-friendly biopolymers to be used in manufacturing of biomedical devices and for combating global plastic pollution (Simó-Cabrera et al., 2021). According to their intended use, biodegradable polymers have different desirable qualities in biomedicine, such as high degradative potential for surgical mesh and low degradative potential for bioengineered skin. Biopolymers such polyglycolic acid (PGA) and polylactic acid (PLA) can be synthesised specifically

for these uses (Han et al., 2015). However, these biopolymers made synthetically are linked to problems including inflammation and biocompatibility. Polyhydroxyalkanoates (PHAs) from haloarchaea are among the natural biopolymers with intriguing physicochemical features that can be exploited as bioplastic for packaging or biomedical purposes. PHAs have the benefits of being simple to mechanically customise, biodegradable, and biocompatible (Han et al., 2015). PHAs have therefore been the subject of in-depth investigation for use in medical implants, drug delivery, tissue replacement, etc. Under stressful circumstances, bacteria and archaea store PHAs, which are made up of hydroxyalkanoate monomers, as a source of carbon and energy. The physical characteristics (stiff or elastic) of the PHAs depend on the kind of hydroxyalkanoate monomer used. The most popular PHAs are poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) and poly(3-hydroxybutyrate). PHB and a monomer of 3-hydroxyvalerate (3HV) make up PHBHV. According to Ahmed et al. (2010), PHBHV has more exceptional biocompatibility and biodegradation, does not generate hazardous byproducts upon decomposition in the body, and aids in the formation of fibroblasts, mesenchymal stem cells, etc.

D. Electricity generation by halophiles

The treatment of organic pollutants in saline wastewater, a pressing environmental concern, demands urgent attention. Industries like fisheries, textiles, petroleum, food processing, and coastal cities employing seawater for toilet cleaning collectively contribute to approximately 5% of global wastewater being saline. Traditional approaches to address this issue involve costly and energy-intensive physicochemical methods, including coagulation-flocculation, evaporation, ion exchange, and membrane processes. A more economically viable and energy-efficient alternative involves biological processes, with a special focus on the use of halophilic bacteria in microbial fuel cells (MFCs). In MFCs, microorganisms capable of extracellular electron transfer convert chemical energy from substrates into electricity. These MFCs consist of an anodic and a cathodic chamber separated by a proton exchange membrane, facilitating oxidation and reduction reactions. Halophilic bacteria, uniquely adapted to high-salinity environments, offer promise in efficiently treating saline wastewater while concurrently generating electricity. This approach addresses both environmental concerns in context of saline wastewater treatment and the potential for sustainable energy production, providing a comprehensive solution to a critical global challenge (Logan et al., 2006). These are the certain advantages to use halophiles in microbial fuel cell are:

- 1) **Enhanced Electron Transfer:** Halophiles, adapted to saline environments, often possess unique electron transfer capabilities. They can facilitate the transfer of electrons from organic substrates to the anode (the electrode where oxidation occurs) in microbial fuel cells more effectively than non-halophilic microorganisms. This improved electron transfer efficiency can enhance the overall performance of the MFC (Microbial Fuel Cell) (Vijay et al., 2023).
- 2) **Salinity Gradient MFCs:** Some MFC designs take advantage of salinity gradients, such as those found in estuaries or wastewater treatment plants. Halophiles can be used in the saline (brine) side of these MFCs, while non-halophilic microbes are used in the freshwater side. This creates a natural ion flow that enhances electricity generation (Torres et al., 2012).
- 3) **Wastewater Treatment:** Halophiles can be employed in MFCs for the treatment of saline or hypersaline wastewater. These microorganisms can metabolize organic contaminants present in saline wastewater, simultaneously generating electricity during the process. This makes MFCs a sustainable and energy-efficient option for wastewater treatment in salt-rich environments. These kinds of microbial fuel cells can be used to treat effluent of different industries such as Color industry wastewater, Seafood processing wastewater, tannery wastewater.

The possible application of MFCs for recalcitrant petroleum hydrocarbon decontamination is of particular interest for the development of economical treatment techniques. However, the salinity effect must be taken into consideration since petroleum hydrocarbon contamination is common in saline environments. Adelaja et al. utilized a two chamber MFC, with Pt-based cathodes, for the treatment of phenanthrene and benzene. Electrochemical and degradation performance were improved up to 10 g L⁻¹ NaCl (~1 mW m⁻², COD removal 79.1%), and good results were maintained up to 15 g L⁻¹ of NaCl. Further increase in salinity (25 g L⁻¹ NaCl) resulted in detrimental effects, possibly due to adverse effects on the physiology of the anaerobic microbial consortia. Similarly, Mousavi et al. utilized a single-chamber MFC, equipped with Pt-based cathode, for the treatment of phenol contaminated solutions (Grattieri et al., 2017).

Bacteria community analysis revealed clear differences between the original anaerobic community and the MFC adapted microbial consortium, with the selection of novel bacteria belonging to Bacteroidetes and Proteobacteria. MFCs operated at no salinity ensured stable decolorization and COD removal over 90% during 50 days of operation, reaching a maximum power density of $\sim 15 \text{ mW m}^{-2}$. The addition of NaCl and Na₂SO₄ in a ratio 1:1, up to 25 g L^{-1} TDS was investigated. 10 g L^{-1} of salts resulted in increased kinetic rates for de-colorization and COD removal, reaching a power output up to 22 mW m^{-2} . Higher salinity decreased the performance, with a drastic loss for the maximum salt content. Accordingly, a maximum salt content of 20 g L^{-1} was suggested to maintain reasonable de-colorization and COD removal, together with good power output. The reported results showed that azo dye contaminated wastewater can be successfully treated under industrially relevant conditions (i.e., moderate salinity and temperature $\sim 30 \text{ }^\circ\text{C}$). However, the authors used Pt as a catalyst for oxygen reduction and air was continuously supplied in the catholyte, increasing the operational cost of the device (Grattieri et al., 2017).

Seafood processing produces wastewater characterized by salinity between 30 and 50 g L^{-1} NaCl. You et al. explored the possible treatment of real seafood wastewater in a U-shaped MFC. A COD removal efficiency of 80.1% and a power density of 8.9 W m^{-3} were achieved, when a hydraulic retention time (HRT) of 16.7 h was used. Reduction of the HRT to 4.2 h allowed increasing the power density, up to 16.2 W m^{-3} , at the cost of drastically decreasing the COD removal efficiency to 28.2%. The decrease in COD removal was attributed to the overload of the treating capacity of the biological anoxic/oxic system due to increased organic loading rate at short HRTs. The optimization of HRTs for best COD removal efficiency and power output appear critical in order to develop systems applicable in real conditions. Sun developed a single chamber MFC with a cobalt tetramethoxyphenylporphyrin (CoTMPP) based oxygen reduction catalyst. Seafood wastewater collected from a seafood restaurant and characterized by a salinity of 30 g L^{-1} , was treated, achieving a power density of 390 mW m^{-2} and COD removal higher than 80% (Sun et al., 2012).

1) From lab to real application in saline and hypersaline environments

As the devices would be subjected to harsh and unstable conditions, particularly in saline and hypersaline environments, the development of MFCs for use in real situations, i.e., in the field, requires careful consideration of electrode materials and cell configurations. In this regard, a marine floating MFC was created by Erable et al. and used for six months in the Atlantic Ocean's coastal waters. The dimensionally stable anode (DSA®) and 254SMO stainless steel (Fe 56.1%, Cr 19.9%, Ni 17.8%, Mo 6%, N 0.2%) used in the floating MFC had dimensions of 30 cm in diameter and 40 cm in height. During use, the distance between the electrodes remained constant (Grattieri et al., 2018). This set up can be considered as a real model used in the society and the advantages and limitations of the same can be used for further modifications. Such models are actually crucial for technology transfer so that the research done in ideal lab conditions can be brought to real life societal situations. Therefore, this set up is described here in great detail as a case study.

The development of MFCs to be applied in real conditions, i.e., in the field, require a careful selection of electrode materials and cell setups, as the devices will be exposed to aggressive and unstable conditions, especially in saline and hypersaline environment. Erable et al. developed a marine floating MFC that was applied in the Atlantic Ocean coastal water during a six-month period. The floating MFC was 30 cm in diameter and 40 cm high, equipped with dimensionally stable anode (DSA®), and 254SMO stainless steel (Fe 56.1%, Cr 19.9%, Ni 17.8%, Mo 6%, N 0.2%) as cathode material. The distance between the electrodes was fix during operation (Grattieri et al., 2018).

The seawater's salinity in the location where the Microbial Fuel Cell (MFC) was employed ranged from 36 to 39 grams per liter (g L^{-1}) of Total Dissolved Solids (TDS). In a controlled laboratory setting, an electroactive anodic biofilm was cultivated using a natural marine biofilm as the initial culture. This was achieved by applying a voltage of -0.06 V versus Ag|AgCl in a seawater solution to polarize the anodes. On the other hand, the cathodic biofilm was developed directly at the experimental site, which was the coastal waters of the Atlantic Ocean. This was accomplished by polarizing the stainless-steel electrodes at -0.2 V versus Ag|AgCl in the surrounding seawater.

The anodic compartment was sealed off, and a proton exchange membrane was positioned at the compartment's bottom. The stainless-steel cathodes were placed outside the anodic compartment. The floating MFC demonstrated its ability to generate a maximum power density of 21.25 milliwatts per square meter (mW m^{-2}) during the summer and achieved an approximate Current Efficiency (CE) of 15%. It's noteworthy that the MFC's performance was

significantly influenced by seawater temperature, with the power density exhibiting an eightfold increase between the winter and summer seasons.

Additionally, the membrane was prone to fouling, likely caused by inorganic waste, bacteria, and other factors, which could lead to an increase in the MFC's internal resistance. Through a modeling study, the researchers demonstrated that maintaining a distance between the anode and cathode of more than 1 meter would substantially reduce the power output. This supported the rationale for employing a floating marine MFC with a fixed electrode distance (Grattieri et al., 2017).

D. Biofuel Production

Biofuels are derived from renewable organic materials, including organic waste, making them a significant alternative for replacing fossil fuels in the medium and long term. They contribute to the preservation of economic and environmental sustainability. Biofuels can be categorized into two main types: gaseous, which include methane, hydrogen, and liquid, which encompass ethanol, butanol, and diesel. These liquid biofuels are among the primary products obtained through white biotechnology [4].

In recent years, the world has grappled with dwindling fossil fuel reserves and escalating environmental concerns, driving the need for alternative, eco-friendly, and renewable energy sources. Biofuels have emerged as a promising alternative, generating increasing interest in converting biomass into usable energy. Biofuels fall into two main categories: first-generation biofuels, derived from food sources or crops like sugar cane and corn, and second-generation biofuels, produced from non-food materials, particularly lignocellulosic substances, offering advantages in terms of reduced competition with food production and improved environmental and economic sustainability. Despite these benefits, first-generation biofuels retain their significance.

Biofuels encompass five primary types: bioethanol, biobutanol, biogas, hydrogen, and biodiesel, with biodiesel and bioethanol dominating the industrial-scale biofuel market, constituting over 90% of total biofuel production. Their success hinges on meeting specific chemical and physical criteria. Various chemical and thermochemical processes can be employed for biofuel production, but microbial conversion of biomass into biofuel has gained significant attention due to its cost-effectiveness. During this process, the harsh conditions, such as increased pH and salt concentrations, mimic those found in alkaline and saline environments. Consequently, microorganisms adapted to thrive in these conditions emerge as potential candidates for biomass degradation and biofuel production.

Halophiles, inhabitants of hypersaline environments like saline lakes, salt pans, salt marshes, and saline soils worldwide, encompass a diverse group of microorganisms found in all three domains of life: Archaea, Bacteria, and Eukarya. Halotolerant microorganisms display adaptability to both high and low salt concentrations, with their tolerance sometimes influenced by environmental and nutritional factors. This adaptability renders halophiles and halotolerant microorganisms' ideal candidates for industrial applications, particularly in biofuel production.

Additionally, enzymes produced by halophilic microorganisms exhibit optimal activity under extremely high salt concentrations, such as KCl concentrations of ~4 M or NaCl concentrations exceeding 5 M. These enzymes possess extra amino acids that impart an extensive negative charge to their surfaces, enhancing their enzymatic efficiency during biofuel production.

In summary, the growing demand for renewable energy sources has led to increased interest in biofuels, particularly biodiesel and bioethanol. Halophiles, adapted to thrive in high-salinity environments, offer a promising avenue for biofuel production due to their unique abilities to function effectively under extreme salt conditions.

These microorganisms have yielded valuable metabolizing enzymes such as amylases, lipases, cellulases, and chitinases, with some being extremozymes known for their broad tolerance to temperature, pH, and salinity. This extensive arsenal of extreme enzymes holds immense promise for various industries, especially biotechnology and biofuel production.

The utilization of microorganisms is a critical component of biofuel production, and the incorporation of halophiles can further optimize this process. Halophilic microorganisms have the potential to contribute to various stages of biofuel production. Certain halophiles are well-suited for producing bioethanol, biobutanol, biohydrogen, and biodiesel from feedstocks. Several hydrolytic enzymes derived from halophilic microorganisms play a pivotal role in biomass degradation for subsequent bioethanol or biodiesel production.

As illustrated in great detail, halophiles offer a wide array of hydrolytic enzymes with exceptional properties that could significantly impact biofuel production. Halophiles are gradually finding their place in the fermentation stages of bioethanol and biobutanol production, with ongoing research expected to expedite their integration. In the context of biofuel production from lignocellulosic materials, there is potential to discover novel cellulases capable of

degrading biomass without the need for pretreatment. Furthermore, halophilic microorganisms possess unique abilities to contribute to biohydrogen and biogas production. In summary, halophilic microorganisms hold immense promise for advancing biofuel production, and further research is essential to harness their vast potential in this field.[5]

IV. Challenges and Future Directions

Like any other technology, exploiting the applications of halophiles also faces certain challenges which has limited the role in real life as of today. Some of the most common limitations are highlighted in the upcoming section:

- 1) **Feedstock Selection** When selecting feedstock, numerous factors must be considered, including the composition of sugars and lignin, seasonality, abundance, and transport costs. Feedstocks with lower lignin content are preferable as they require milder pretreatment conditions, reducing both capital and operational expenses. The abundance and seasonal availability of feedstock are crucial, especially for continuous bioprocesses that are vulnerable to supply shortages. Furthermore, if feedstock is abundant but scattered over a large area, transportation costs and environmental impacts can significantly increase. Various waste-derived feedstocks, such as brewer's spent grain, sugar beet pulp, and sugarcane bagasse, offer promising options due to their low cost and steady availability.
- 2) **Inhibitory Compounds in Feedstock:** Lignocellulosic materials require pretreatment to release fermentable sugars, but this process can produce unwanted inhibitory compounds. These compounds, including phenolic compounds and furan derivatives, can hinder cellular metabolism and affect productivity. Similarly, non-lignocellulosic feedstocks like crude glycerol may contain inhibitory substances. Removing these inhibitors is more challenging in continuous processes, as it can increase downtime and operational costs.
- 3) **pH Value Fluctuations:** Significant pH variations occur throughout the biorefinery process, from pretreatment to fermentation. Enzymatic hydrolysis requires an optimal pH, but the initial pH often deviates from this range. Adjusting pH consumes water and chemicals, which can be expensive and time-consuming. Continuous processes are particularly susceptible to unexpected pH fluctuations, potentially disrupting the entire production.
- 4) **Water Demand:** Biorefineries consume substantial amounts of freshwater, especially with irrigated crop-based feedstocks. Water is vital for various stages, including pretreatment, fermentation, and downstream processing. Efforts to reduce water usage, such as recirculating cooling water, are essential. One potential solution is using halophilic microorganisms in the fermentation stage, allowing saline water usage and reducing freshwater demand.
- 5) **Metabolic Balance:** Traditional multiple-unit operations are resource-intensive, leading to research interest in consolidated bioprocessing (CBP). CBP combines substrate hydrolysis and fermentation in one step, but optimizing a single strain for both tasks is challenging. Co-culture CBP systems with specialized microorganisms may provide a solution.
- 6) **Product Inhibition:** Product toxicity can impair cellular viability and productivity when the bio-product concentration becomes too high. Traditional metabolic engineering of a single strain may not fully address this issue, necessitating alternative solutions.
- 7) **Continuous Cultivation of Microorganisms:** Continuous fermentations offer advantages but require higher initial capital investment and are prone to contamination and genetic instability. These challenges need to be addressed for successful industrial biotechnological applications.[3]

The challenge in using halophilic bacteria is the requirement of fermenter which would be corrosion free, as high salinity can lead to corrosion. The yield of production of compounds produced by them is low, the optimization and growth parameters of halophiles must be maintained. In MFC, bacteria generate such low amounts of electricity that it needs to be further optimized to bring it to usable levels.

E. Conclusion

In conclusion, this chapter is divided into two sections one is about industrial biotechnology and another one is about industrial application of halophilic bacteria. The journey of biotechnology, spanning from ancient civilizations to contemporary innovation, reflects an awe-inspiring evolution. It has evolved from attributing fermentation to divine forces to the sophisticated field it is today. The global biotechnology market is experiencing rapid growth, driven by government support, streamlined approvals, personalized medicine, and

the impact of the COVID-19 pandemic. Fermentation technology remains crucial, offering solutions for chronic diseases and cutting-edge therapies. Halophiles, thriving in extreme salt environments, are emerging as invaluable assets in biotechnology. They contribute to diverse applications, from managing neurodegenerative diseases to biofuel production and environmental remediation. India's biotechnology sector is on a robust growth trajectory, particularly in bio-industrial applications. As the biotechnology landscape continues to evolve, it holds immense potential. With ongoing technological advancements, increased investments, and innovative applications, biotechnology is poised to play a pivotal role in addressing global challenges and advancing human health and well-being. It represents a bridge between ancient wisdom and modern science, offering promising solutions for the future.

The another section concludes different types of application of halophilic bacteria in agriculture, electricity generation, cleaning of environment, biofuel generation and most importantly production of pharmaceuticals and biomedical products. These bacteria are important as they work in harsh conditions of salinity. The problem of desertification can be solved by forming halotolerant PGPR and biocontrol agents. In addition to this, halophile can be used to create bioelectricity by using bioelectrochemical system and this would facilitate treatment of wastewater characterized by high salinities as bacteria have the potential to use organic compounds present in wastewater. Additionally, the materials utilized for the development of the MFCs should take into consideration with their possible applicability at the industrial scale in terms of scalable manufacturing, cost, and stability. In conclusion, the fields of saline and hypersaline MFCs have seen impressive improvements in the last five years, but more research is needed before the successful inclusion of this technology in the real world. Above all, halophiles can be used in adverse conditions to make environment healthy by hydrolysing filthy environments consisting of hydrocarbons such as petroleum and diesel. With cleaning the environment halophiles produce important bioproducts such as pharmaceuticals: antimicrobial compounds, antioxidant compounds and anti-cancer compounds. With this, they also produce biopolymers which can be used in surgery.

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