

BIODESALINATION- USING MICROORGANISMS TO REDUCE WATER SALINITY

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CANDIDATE'S DECLARATION

I, Jaspreet Kaur, Roll no- 2K22/MSCBIO/22 student of Master of science (Biotechnology), hereby declare that the project dissertation titled “BIODESALINATION- USING MICROORGANISMS TO TREAT WATER SALINITY” which is submitted by me to the Department of Biotechnology – Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the project dissertation titled “BIODESALINATION – USING MICROORGANISMS TO TREAT WATER SALINITY” which is submitted by Jaspreet Kaur, Roll no. 2K22/MSCBIO/22, Department of biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of master of science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or fully for any degree or diploma to this university or elsewhere.

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ABSTRACT

The global demand for freshwater is rising, exacerbating water scarcity issues affecting nearly half the world's population. The existing freshwater resources are quite unevenly distributed and unsustainably used, making it difficult for them to suffice the growing needs of the population. Therefore, it becomes essential to tap into the large ocean water reserves and make that water potable. Although present in vast amounts, ocean water can't be used for useful purposes due to the immense salt content present in it. Therefore, it is important to devise newer and sustainable technologies for efficient salt removal from brackish and ocean water reserves.

Conventional desalination methods to reduce salt content, though useful, are energy-intensive and have detrimental environmental impacts, making it necessary to explore other alternatives to desalinate water. Such methods should be cost-effective, rapid, and environmentally sustainable for them to surpass the existing technologies. For instance, thermal desalination requires significant amounts of heat energy, often generated from non-renewable sources, contributing to greenhouse gas emissions. Membrane-based techniques, such as reverse osmosis, also consume substantial energy and produce brine, which poses disposal challenges that can harm marine ecosystems.

For this project, I have reviewed various desalination technologies, emphasizing their pros and cons, exploring the historical evolution of desalination, including thermal and membrane-based methods, and introducing a novel approach—biological desalination—using salt-tolerant microorganisms like cyanobacteria and algae. The molecular and physiological aspects of how these microbes facilitate desalination and their potential in reducing salinity have been evaluated. Biological desalination presents a sustainable and energy-efficient alternative, particularly for isolated locations.

Key words- Biodesalination, microbial desalination, sustainable water purification, halophytic organisms.

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CHAPTER1- INTRODUCTION

Approximately 71% of the Earth's surface is covered by water, and the oceans hold about 96.5 percent of all Earth's water (Gleick, 1996). Only about 3.5% of the available freshwater is fit for human use, out of which 70% is found in the poles (Jalihal & Venkatesan, 2019; Kalogirou, 2005). The ever-increasing population and economic growth have posed a constant threat to the existing limited freshwater resources. According to UN Water and UN-Water (2023), there are currently 3.56 billion people on the planet (47% of the Earth's total population) who live in regions where there is at least a monthly shortage of water, and an estimated 15% of people do not have access to potable clean drinking water (*Water Scarcity / Threats / WWF*, n.d.). Mekonnen & Hoekstra (2016) reported that the numbers could be bigger, i.e., 4 billion or 52%. The 2018 edition of the United Nations (UN) World Water Development Report (WWDR) highlighted that global water demand has been increasing at a rate of about 1% per year over the past decades. At this rate, the total population that faces water scarcity for at least a month could increase up to 57% (Wong, 2023).

This growing scarcity can be attributed to the uneven distribution of water resources worldwide, unsustainable use, poor management, and water pollution. Population and economic growth are also significant factors (*Four Billion People Facing Severe Water Scarcity / Science Advances*, n.d.; Wong, 2023). According to the UN Food and Agriculture Organization (FAO) and UN Water, global water use has been growing at more than twice the rate of population increase in the last century. With no sign of a decrease in population growth, the current 1.3% growth rate projects the population to be approximately 9.8 billion by 2050 (*UN POPULATION DIVISION ISSUES 'WORLD POPULATION PROSPECTS: THE 2000 REVISION' | Meetings Coverage and Press Releases*, n.d.), which would increase the anthropogenic loads on available freshwater resources by causing widespread water pollution leading to a shortage of freshwater resources (Ceola et al., 2015; *International Conflict and Cooperation over Freshwater Resources / Nature Sustainability*, n.d.). Thus, the existing conventional sources of water are insufficient to meet the daily needs in most water-scarce regions and pose threats to other developing countries (Dhakal et al., 2022).

Desalination provides an alternative provision for a feasible and potential resource to address the deficit in the water budget and reduce water scarcity (Mohsen & Al-Jayyousi, 1999).

Desalination has been used as an alternative method to treat saltwater in various water-scarce regions to suffice the increasing need for available water (Bremere et al., 2001). Desalination is the process of removing salt content from the salty water, i.e., brackish water or brine water, to obtain potable freshwater. Desalination is a climate-independent method to obtain freshwater from non-conventional sources (Jones et al., 2019). However, some of the methods employed could pose a threat to the environment (Einav et al., 2003).

CHAPTER 2- NOVEL PERSPECTIVES

While biodesalination represents an environmentally sustainable alternative, it is not yet feasible for large-scale industrial application on its own. To achieve the primary goal of enhancing the sustainability of desalination, our comprehensive review suggests that integrating biodesalination with other production processes is the most effective approach for scaling up. By using biodesalination as a preliminary step before reverse osmosis (RO), the energy requirements for RO can potentially be reduced. Additionally, the resulting algal biomass from biodesalination can be utilized for the production of lipids or biodiesel, contributing to a circular economy.

Moreover, combining biodesalination with wastewater treatment offers a dual benefit. It facilitates the detoxification and recycling of industrial wastewater through advanced oxidation processes (AOPs), thereby addressing both water scarcity and pollution issues simultaneously. This integrated approach not only enhances the overall efficiency and sustainability of desalination but also promotes the utilization of renewable resources and waste minimization. Consequently, the integration of biodesalination into existing industrial processes holds significant promise for the future of sustainable water treatment.

CHAPTER 3- VARIOUS APPROACHES TO DESALINATION TECHNOLOGY

The inception of desalination, the process of extracting freshwater from brackish or saline ocean water, is attributed to the Royal Navy of the United Kingdom during the late 18th century. Their pioneering use of this technology was driven by the need to enhance navigational independence while minimizing the burden of water storage aboard ships (Curto et al., 2021). Subsequently, in the late 1950s, the establishment of desalination plants for civilian purposes aimed to address the growing demand for potable water while mitigating production costs (Jalihal & Venkatesan, 2019).

Desalination methods are diverse and can be categorized based on technological and energy sources into **Thermal, Membrane, and Biological desalination technologies**. Each classification represents a unique approach to tackling the challenge of freshwater scarcity through innovative means.

3.1 THERMAL DESALINATION TECHNOLOGIES-

The process of thermal desalination relies on harnessing thermal energy to induce the conversion of salt solution into water vapor. This conversion is achieved through either the manipulation of environmental pressure or the consistent application of thermal energy. Historically, the early desalination facilities predominantly employed thermal energy due to its cost-effectiveness, primarily driven by the accessibility of fossil fuels for energy production (Al-Othman et al., 2019). Among the prevalent techniques within thermal desalination are Multistage Flash Desalination (MSF) and Multieffect Desalination (MED), both of which entail a sequential transfer of latent heat (Jalihal & Venkatesan, 2019). At its core, thermal desalination operates on the principle of inducing a phase change in saline water, causing it to evaporate into vapor while leaving behind the salt content (Gude & Fthenakis, 2020).

Below are several of the standard approaches employed in thermal desalination technologies.

3.1.1 Multistage flash desalination (MSF)-

In the Multi-Stage Flash (MSF) desalination process, heated water is introduced into a chamber that operates under reduced pressure, causing a portion of the water to vaporize. This technique is often used in conjunction with a power supply to enhance efficiency (Einav et al., 2003). In

MSF systems, steam is utilized to heat saline water, which is then introduced into a series of effects or vessels. The lowered pressure within these vessels allows the water to boil rapidly, resulting in a sudden "flash" of steam. This steam is subsequently condensed, gradually yielding fresh water.

3.1.2 Multi- effect desalination-

The multi-effect distillation (MED) technique stands as the oldest method of thermal desalination, wherein salty water undergoes a process of evaporation and condensation to achieve desalination. This method capitalizes on the utilization of latent heat during steam production, resulting in lower energy consumption compared to the multi-stage flash (MSF) distillation process (Al-Shammiri & Safar, 1999).

Furthermore, Darwish et al. (2006) highlighted the significant advantage of MED's capability to operate at lower temperatures, which translates to a reduction in the necessary mechanical and fuel energy. To enhance energy efficiency even further, the desalination plant can be integrated with a solar steam production system. This integration provides the requisite thermal energy to support the plant's operations, potentially covering up to 68% of the plant's total energy requirements (Bataineh, 2016). By leveraging solar energy, the overall cost and environmental impact of the desalination process can be significantly reduced.

3.1.3 Humidification- dehumidification (HDH)-

This innovative technique entails heating saline water to an elevated temperature, causing it to evaporate within a humidification chamber. In this chamber, the air flow moves in the opposite direction to the saline water which is distributed via an overhead liquid distributor. As the heated saline water undergoes evaporation, it transforms into humid air. This moisture-laden air is then transferred from the humidifier to the dehumidifier. Within the dehumidifier, a condenser works to lower the air temperature, resulting in condensation. This condensation process effectively captures the water vapor, converting it into liquid water. The resultant condensed water, due to its high purity, is suitable for drinking. The efficiency of this method is enhanced by the high humidity of the air within the system, ensuring a consistent and reliable production of potable water (Capocelli et al., 2018)

3.1.4 Adsorption desalination-

This desalination process operates through a cycle of adsorption and desorption involving heated saline water. The process begins by heating the salty water until it vaporizes. This vapor is then adsorbed using materials such as silica gel and zeolites. These materials have high adsorption capacities, allowing them to attract and hold water molecules effectively. The adsorption continues until the materials reach their saturation point, at which stage they can no longer hold additional water vapor. Once saturation is achieved, the water molecules are released and condensed into liquid form through a desorption process, which involves heating the materials to expel the water vapor. The condensed water is then collected in a container, completing the desalination cycle. This method is noted for its efficiency and effectiveness in separating salt from water (Hua et al., 2022).

3.1.5 Ocean-based low-temperature thermal desalination (LTTD)-

LTTD is a process that capitalizes on the natural temperature gradient between two distinct bodies of water. This method involves evaporating warmer water at low pressures, a process that is facilitated by the difference in temperature. The vapor produced during this evaporation phase is then condensed using cooler water, effectively converting it into high-quality freshwater. This technique not only harnesses the thermal energy present in the ocean but also provides a sustainable means of addressing freshwater scarcity (Mutair & Ikegami, 2014; Rognoni et al., 2008).

3.1.6 Vapour compression-

Vapour compression encompasses a range of techniques designed to facilitate evaporation by operating under reduced pressure conditions. In mechanical vapour compression, a mechanical device, typically a compressor, is employed to increase the pressure and temperature of the vapour, thereby providing the energy required for the phase change from liquid to gas. On the other hand, thermal vapour compression utilizes a high-pressure steam jet to compress the vapour, leveraging thermal energy for the same purpose. These methods are instrumental in various industrial processes where efficient and controlled evaporation is essential (El-Dessouky et al., 2000)(Ben Amara et al., 2004).

3.1.7 Multieffect humidification-

Multieffect Humidification (MEH) shares similarities with Multi-Stage Flash (MSF) and Multi-Effect Distillation (MED) processes in that it also involves the thermal evaporation of water. However, MEH distinguishes itself from MSF and MED by utilizing the evaporated water differently. Instead of processing the evaporated water directly as vapor or steam, MEH employs this water to humidify a gas stream (Ben Amara et al., 2004).

Thermal desalination represents a significant portion of the global desalination capacity, accounting for 33% (Elimelech & Phillip, 2011). This method is particularly prevalent in the Middle East, where it constitutes a staggering 65% of the region's desalination capacity. The dominance of thermal desalination in this area can be attributed to several key factors. Firstly, the Middle East hosts a vast array of thermal desalination units, which are extensively integrated with power plants. This integration is facilitated by the high availability of fossil fuels, a common and abundant resource in these countries. This combination of abundant fuel resources and reliable desalination technology underscores why thermal desalination is so heavily utilized in the Middle East, significantly contributing to its water production capabilities (*Commercial Desalination Technologies* / SpringerLink, n.d.).

3.2 MEMBRANE DESALINATION TECHNOLOGIES

Membrane-based desalination technologies have transformed the process of obtaining freshwater from saline sources, meeting the increasing global demand for potable water. These technologies function by allowing water molecules to pass through semi-permeable membranes while blocking salts and other impurities, resulting in high-quality freshwater. (Elimelech & Phillip, 2011; *Materials for Next-Generation Desalination and Water Purification Membranes* / Nature Reviews Materials, n.d.).

Below mentioned are various types of membrane based desalination technologies-

3.2.1 Reverse osmosis (RO)-

Reverse osmosis (RO) is the most widely utilized membrane-based desalination technology, characterized by a purely physical process that does not involve a phase change. Instead, RO employs the principle of osmosis, where freshwater is forced through a semi-permeable

membrane by applying pressure higher than the natural osmotic pressure exerted by the saltwater. This pressure differential allows freshwater to pass through the membrane while retaining the salt and other impurities. The process capitalizes on the natural tendency of water to move from a region of lower solute concentration to a region of higher solute concentration, thus overcoming the concentration difference (Younos & Tulou, 2005).

During reverse osmosis, water containing inorganic salts, soluble and insoluble organic matter, gases under pressure, and aquatic microorganisms is separated from the freshwater. These impurities remain on the inlet side of the membrane, effectively being filtered out. The purified, desalinated water passes through the membrane, emerging on the other side ready for use. Typically, reverse osmosis systems can yield between 35% and 60% of the water volume that enters the system, and advanced industrial machines are capable of removing 99.8% to 99.9% of Total Dissolved Solids (TDS), making it an incredibly efficient method for producing high-quality desalinated water (*Hybrid Renewable Energy Systems for Desalination | Applied Water Science*, n.d.).

Currently, RO-based desalination plants account for approximately 70% of the global desalinated water production, a testament to the technology's effectiveness and reliability.

3.2.2 Forward osmosis (FO)-

Forward Osmosis (FO) represents a cutting-edge and energy-efficient desalination technique that harnesses the natural osmotic pressure gradient between draw solutions to effectively separate water from dissolved solutes. This method capitalizes on the osmotic pressure difference to drive the movement of water across a semi-permeable FO membrane, leaving behind the salt and other impurities. A landmark study by McCutcheon et al. (2005) demonstrated the potential of FO in desalination by employing an appropriate membrane in conjunction with a robust draw solution composed of highly soluble ammonia and carbon dioxide gases. Their findings indicated that this approach could successfully desalinate saltwater, making it a promising alternative to traditional methods.

3.2.3 Electrodialysis (ED)

Electrodialysis (ED) is an advanced electrochemical process utilized for the separation of ions from one solution to another by leveraging an electrical potential difference as the primary driving force. This technique has demonstrated significant efficacy in the treatment of brackish water. According to research by Strathmann (1995), electrodialysis can achieve the removal of 75% to 98% of total dissolved solids, effectively transforming brackish water into potable water suitable for consumption. Strathmann (2010) also highlights its role in the pharmaceutical industry, where ED is applied in the purification of bioproducts, showcasing the versatility and broad applicability of this technology (Strathmann, 1995, 2010).

3.2.4 Membrane distillation (MD)

Membrane Distillation (MD) is a membrane-based desalination technology that stands out due to its operation at significantly lower temperatures compared to traditional thermal desalination methods (Lawson & Lloyd, 1997). This process harnesses the difference in vapor pressure across a hydrophobic membrane to achieve separation, which enables it to operate efficiently at these reduced temperatures by utilizing low-grade heat sources. Unlike other membrane technologies, MD leverages the vapor pressure differential of volatile compounds across the membrane rather than relying on total pressure differences for the separation process (Yarlagadda et al., 2009).

3.2.5 Nanofiltration (NF)

Nanofiltration (NF) is an advanced pressure-driven membrane filtration process that utilizes the selective permeability of membranes with a pore size around 1 nanometer. These membranes have a molecular weight cut-off typically ranging between 300 and 500 Daltons, making them highly effective for certain separation tasks (Mohammad et al., 2015). Nanofiltration is particularly useful for separating inorganic salts and small organic molecules. One of its distinguishing features is its ability to achieve lower rejection rates for monovalent ions while providing higher rejection rates for divalent ions.

Conventional desalination methods, such as Multi-Stage Flash (MSF) distillation, require substantial and costly machinery to operate efficiently, presenting significant feasibility challenges (Mezher et al., 2011). To address these issues, various renewable energy sources (RES), including biomass, solar, wind, ocean, and geothermal energy, have been extensively

researched and integrated with existing desalination technologies. This integration aims to reduce operational costs and minimize the carbon emissions associated with desalination processes, thereby enhancing their overall feasibility and sustainability (Shokri & Sanavi Fard, 2022). In addition to these advancements, innovative approaches like Freeze Desalination (FD) have been introduced. FD aims to tackle the increasing challenges faced by both thermal and membrane-based desalination methods, providing a promising alternative that could potentially revolutionize the desalination landscape (Kalista et al., 2018).

3.3 BIOLOGICAL DESALINATION TECHNOLOGIES

Biological desalination represents a groundbreaking approach to desalination that circumvents the need for high energy consumption or the application of high-pressure systems. This method leverages the natural processes of salt absorption and adsorption carried out by various salt-tolerant microorganisms (Wei et al., 2020). The concept of biological desalination was first introduced by Cao et al. in 2009, who proposed the innovative use of the Microbial Desalination Cell (MDC). This cell functions by transferring ionic species out of the water, a process driven by the electric current generated by bacterial activity (Cao et al., 2009).

Further advancements in biological desalination have highlighted the potential of specific microalgae (Gao et al., 2021) and photosynthetic cyanobacteria (Minas et al., 2015) in facilitating this process. These organisms contribute to desalination by naturally filtering and removing salts from water through their metabolic activities. The technique stands out for its potential to offer a low-cost, energy-efficient solution for desalination, particularly beneficial for remote and isolated areas suffering from water scarcity. By harnessing biological systems, this method could present a viable and sustainable alternative to conventional desalination techniques, reducing reliance on energy-intensive and environmentally detrimental practices (Gao et al., 2021).

CHAPTER 4- NEGATIVE CONSEQUENCES OF CURRENT DESALINATION METHODS

The rapidly growing demand for potable water has led to a significant increase in the installation of desalination plants. This trend shows no signs of slowing down, and it is anticipated that the number of desalination facilities will continue to rise. As a result, the environment may be pushed beyond its threshold capacity to handle the by-products generated by these desalination processes.

Currently, there are approximately 15,906 desalination facilities in operation worldwide. This vast capacity is divided among several desalination technologies. The most widely used method is Reverse Osmosis (RO), which accounts for 68.77% of the global capacity. Following RO, Multi-Stage Flash (MSF) distillation constitutes 17.66% of the capacity, while Multi-Effect Distillation (MED) makes up 6.89%. Other technologies include Nanofiltration (NF) at 3.44%, Electrodialysis (ED) at 2.44%, and a variety of other less common methods contributing to the remaining 1.0% (Jones et al., 2019).

4.1 Impact on energy efficiency and carbon footprint

The production of 1000 cubic meters per day of freshwater necessitates an annual consumption of 10,000 tons of oil (Kalogirou, 2005), making water desalination the most energy-intensive among water treatment processes. This process accounts for a substantial portion of global energy consumption, utilizing approximately 75 terawatt-hours (TWh) of electricity annually, which constitutes around 0.4 percent of the world's total energy usage, consequently generating 76 million metric tons of CO₂ emissions. With the continual expansion of desalination capacities worldwide, these emissions are projected to rise significantly, reaching an estimated 218 million metric tons by the year 2040 (Shahzad et al., 2017). Thermal desalination methods, such as distillation, though effective in producing freshwater, encounter a considerable challenge in terms of energy efficiency. The energy input required for distillation, approximately 2200 kilojoules per kilogram to evaporate water, far exceeds the theoretical minimum of 3-7 kilojoules per kilogram. Achieving energy efficiency closer to this theoretical minimum would necessitate the implementation of impractically large systems with numerous operating stages, operating across minute temperature differentials to enable extensive energy recovery. Moreover, the

elevated temperatures required for distillation processes increase the risk of scale formation from inversely soluble mineral salts, such as calcium sulfate (Miller, 2003), further complicating the efficiency and maintenance of desalination plants. Multi-Stage Flash (MSF) and Multi-Effect Distillation (MED) are the predominant thermal desalination technologies, each consuming approximately 18 kilowatt-hours per cubic meter and 15 kilowatt-hours per cubic meter respectively (Al-Sahali & Ettouney, 2007), thus significantly contributing to carbon emissions and chemical consumption, highlighting the urgent need for sustainable and energy-efficient desalination solutions to mitigate environmental impacts.

4.2 Brine discharge and marine ecosystem disruption

Effluent disposal remains a complex and multifaceted challenge, influenced by both technological capabilities and economic considerations. The discharge of effluent, characterized by its persistently high salt content, continues to present significant concerns for marine ecosystems and related human activities. With concentrations ranging from 45 to 80 grams per liter, the discharged brine accumulates densely on the seabed, forming saline layers that can adversely affect flora, fauna, and various human endeavors. This phenomenon, as documented by Algoul et al. (2022), underscores the potential ecological ramifications, necessitating careful management strategies. Moreover, the discharge of effluents contributes to the proliferation of algae within marine environments, a phenomenon observed by Narasimha Rao and Pragada (2010). Such algal growth, particularly harmful algal blooms (HABs), poses a direct threat to the operational efficiency of desalination plants. Al-Rawajfeh et al. (2023) highlight the compounding effects of HABs, including increased energy consumption and membrane fouling in reverse osmosis desalination processes (M. K. Shahid et al., 2023). These intertwined challenges underscore the critical importance of mitigating effluent discharge impacts through innovative solutions and robust environmental management practices (Seubert & Caron, 2018).

4.3 Land use and coastal development

Land Use and Coastal Development encompass a multitude of considerations and consequences, extending beyond mere technical aspects to encompass broader environmental impacts. For instance, the establishment and operation of a desalination plant introduce a plethora of challenges, including but not limited to noise pollution, heightened energy consumption, and potential repercussions on groundwater quality. Furthermore, the construction of such plants necessitates the utilization of natural resources and land, thereby amplifying concerns regarding environmental exploitation and habitat disruption. The magnitude of these risks varies depending on factors such as the specific type of desalination technology employed and the characteristics of the water source. Additionally, the disposal of brine effluent from desalination processes emerges as a significant concern due to its hazardous nature, posing a substantial threat to marine ecosystems. Brine effluent, characterized by high salinity levels and the presence of various toxic substances, has been identified as a potential agent of ecological harm by numerous studies (Panagopoulos et al., 2019). Therefore, the implications of land use and coastal development extend far beyond the immediate vicinity of the desalination plant, encompassing a spectrum of environmental, social, and economic considerations that demand careful assessment and management.

4.4 Pretreatment chemical use and disposal

The utilization of membranes within desalination processes mandates meticulous maintenance protocols to ensure optimal performance. Among the critical components of this maintenance regime are the chemical cleaning treatments essential for addressing fouling issues and sustaining membrane efficiency over time. However, the discharge of effluent streams resulting from these cleaning procedures poses a significant environmental challenge, particularly concerning marine ecosystems. The chemical constituents present in these waste streams, along with their elevated temperatures and saline concentrations, can exert detrimental effects on nearby aquatic environments if not properly managed. The potential disruption to sensitive marine habitats directly receiving these effluent outfalls from desalination facilities is a pressing concern (Tularam & Ilahee, 2007). Consequently, the implementation of robust management strategies is imperative to minimize the adverse impacts of such discharges on marine biodiversity and ecosystem resilience. Through careful monitoring and proactive measures, the

negative consequences of desalination-related effluent discharge can be mitigated, ensuring the long-term sustainability of both water treatment processes and marine ecosystems alike.

CHAPTER 5- MICROBIAL DESALINATION- EXPLORING MOLECULAR AND PHYSIOLOGICAL ASPECT

Microorganisms present in the environment offer promising alternatives for sustainable biodesalination techniques. These organisms can effectively desalinate water without the need for electrical energy input or the application of high water pressure. Instead, they utilize organic substances as an energy source to drive the desalination process. This approach not only reduces the reliance on conventional energy sources but also promotes an eco-friendly method of water purification, as demonstrated by Cao et al. in their 2009 study. By harnessing the natural capabilities of these microorganisms, we can develop efficient and sustainable methods for producing fresh water from saline sources (Cao et al., 2009).

5.1 Cyanobacteria

Cyanobacteria, a type of photosynthetic bacteria, play a crucial role in the global ecosystem by contributing significantly to oxygen production. They thrive in extensive blooms that occur in both freshwater and marine environments, thanks to their minimal nutrient requirements, making them highly adaptable and widespread (Minas et al., 2015). These microorganisms have been extensively studied for their unique biological processes. Notably, research by Blumwald et al. (1983) revealed that cyanobacteria, when subjected to ionic stress, accumulate osmolytes either intracellularly or extracellularly. This accumulation facilitates the transfer of Na⁺ ions through diffusion, demonstrating their ability to adapt to varying environmental conditions (Blumwald et al., 1983).

This remarkable biological process has inspired the development of innovative technologies, such as the cyanobacteria-based biodesalination plant. This plant leverages the natural capabilities of cyanobacteria to create a low-salt biological reservoir within saline water, enabling efficient ion exchange (Amezaga et al., 2014). The biodesalination method hinges on modifying cyanobacteria to accumulate sodium within their cells rather than exporting it. This

modification is achieved by strategically starving the bacteria, which blocks their natural sodium export pathways. Consequently, the cyanobacteria respond to the induced ionic stress by allowing sodium influx through light-driven mechanisms (Blumwald et al., 1983).

The potential applications of cyanobacteria extend far beyond their natural ecological roles. By harnessing their biological processes, scientists and engineers can develop environmentally friendly technologies to address some of the world's pressing challenges. For instance, biodesalination offers a sustainable solution to the problem of freshwater scarcity by using biological methods to desalinate seawater. This approach not only reduces the energy costs associated with traditional desalination techniques but also minimizes the environmental impact.

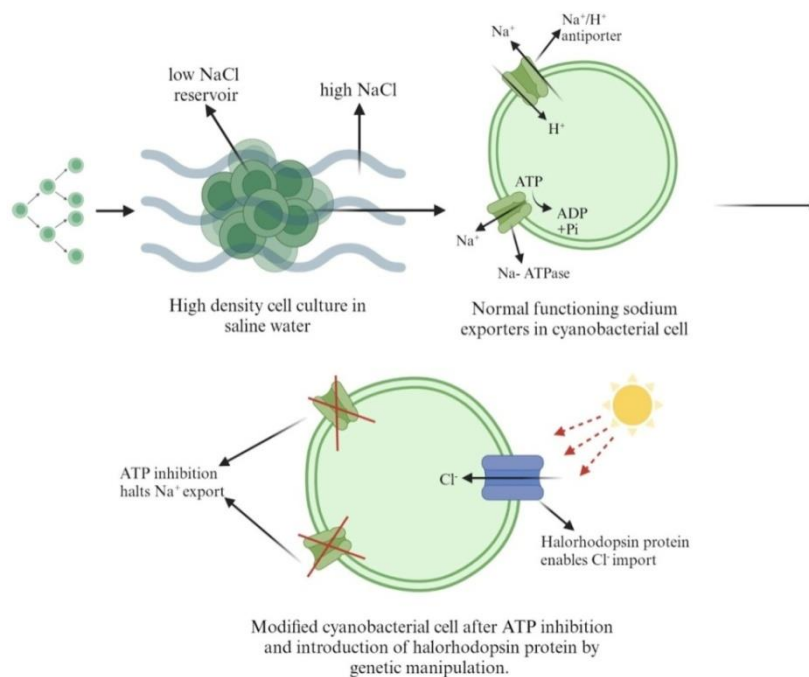


Fig. 1- mechanism of salt accumulation by cyanobacteria

(Kaur et al., 2024)

The given diagram provides a detailed illustration of the complex mechanism by which genetic manipulation of the cyanobacterial membrane can alter its natural function of sodium ion export. This intricate process begins with the inhibition of the Sodium-Adenosine Triphosphatase (Na-

ATPase) pump, as well as other sodium-exporting mechanisms embedded in the membrane, through the targeted blockage of Adenosine Triphosphate (ATP). Simultaneously, an additional protein, known as Halorhodopsin, is introduced into the membrane. This protein is crucial for importing chloride ions, facilitating a significant shift in the cell's ion transport dynamics.

Cyanobacteria are particularly amenable to such genetic alterations due to their relatively simple and accessible genetic makeup, which allows for straightforward manipulation. The Halorhodopsin protein is of particular interest because it utilizes solar energy, rather than relying on ATP, to drive the import of chloride ions into the cell. This solar-powered process is not only energy-efficient but also aligns with the natural photosynthetic capabilities of cyanobacteria.

The incorporation of Halorhodopsin ultimately leads to the internal accumulation of sodium chloride (NaCl) within the cyanobacterial cell. This process highlights a remarkable example of how genetic engineering can be employed to modify cellular functions for specific applications. The ease with which these membrane modifications can be performed in cyanobacteria opens up a wide range of potential biotechnological applications, particularly in areas that require precise regulation of ion transport and accumulation within cells. This capability could be harnessed for various purposes, including bioremediation, bioenergy production, and the synthesis of valuable biochemical products.

5.2 Algae

Salt removal from saline water by microorganisms such as algae occurs through two primary methods: biosorption (adsorption) (Veglio' & Beolchini, 1997) and bioaccumulation (absorption) (Wei et al., 2020). Both processes play crucial roles in the biodesalination of saline environments, offering a sustainable approach to address water salinity.

Biosorption is a rapid and reversible process where ions from water bind to functional groups on the surface of biomass. This process is independent of the microorganism's metabolic activities (Michalak et al., 2013). During biosorption, salts can be eliminated through the formation of complexes on the surface of algal cells due to interactions with functional groups, involving covalent coordination and electrostatic attraction (Brinza Tepes et al., 2007). Specifically, salts can adsorb onto the algal cell surface through various molecular interactions between salt molecules and algal cell wall components. One notable mechanism is ion exchange, where

sodium ions replace other cations bound to the algal surface (Crist et al., 1990). Research indicates that sodium ions can effectively replace manganese ions bound to algal cell surfaces, highlighting the efficiency of ion exchange in this context (Rajfur et al., 2014). Additionally, micro precipitation might occur when the algal cell surface becomes oversaturated with sodium ions, leading to the formation of salt precipitates on the cell surface (González et al., 2011).

Given that biosorption accounts for more than 80% of salt uptake by algae, and relies solely on the functional groups and cell surface proteins present on algal cells, dead or inactivated algal cells can also be utilized for this purpose. Studies comparing the metal removal capacities of dead and living algal biomass have demonstrated a clear advantage in using dead biomass. Inactivated cells can endure toxic metals and do not require additional nutrients or light, making them easier to maintain (Mehta & Gaur, 2005). However, inactivated cells cannot perform bioaccumulation and are challenging to harvest after treatment, which limits their large-scale application (Zafar et al., 2023). Despite these limitations, the use of inactivated cells offers a viable option for specific applications where maintenance simplicity and resistance to toxic environments are critical.

On the other hand, bioaccumulation is a slower, energy-dependent process occurring in living cells through salt uptake or accumulation. This process involves two stages: the initial absorption stage, where salts migrate to the surface of microalgae cells from the bulk solution, and the deposition stage, where salts are actively transported to the surface of the algae through mechanisms like ion exchange, micro precipitation, and complexation (Mrvčić et al., 2012). Studies have shown that bioaccumulation plays a relatively minor role in desalination, requiring a significantly longer time to effect change compared to biosorption, which constitutes approximately 75% of total desalination (Wei et al., 2020). Despite its slower rate, bioaccumulation is vital for understanding the full scope of salt removal processes and for developing comprehensive biodesalination strategies that can maximize efficiency and effectiveness. By leveraging both biosorption and bioaccumulation, a more complete and nuanced approach to biodesalination can be achieved, potentially enhancing the capacity for sustainable water treatment solutions.

CHAPTER 6- METHODOLOGY

Below is a generalized step-by-step guide for performing biological desalination in a laboratory setting.

6.1 MICROBIAL SCREENING

The first crucial step in initiating a biodesalination process is to select a suitable strain of microorganism—either bacteria or algae—that can act as a natural agent for removing salt from seawater. This selection is pivotal because the effectiveness of the entire desalination process hinges on the capabilities of the chosen microorganism. The microorganism must exhibit high salt tolerance and a robust capacity for salt accumulation. Furthermore, it must be capable of growing in large populations to ensure that the desalination process is efficient and scalable. These microorganisms need to be meticulously screened based on their ability to not only survive but also reproduce in highly saline environments, alongside their efficiency in removing salts from these environments.

Salt uptake by microorganisms, particularly microalgae, is influenced by two main processes: absorption and adsorption. Among these, adsorption is the primary mechanism that drives salt removal. The cell membranes of microalgae have specific functional groups that facilitate rapid adsorption, enabling faster and more efficient salt removal compared to the slower process of absorption, which contributes minimally to the overall salt removal (Wei et al., 2020). This distinction underscores the importance of selecting microorganisms with superior adsorption capabilities.

Therefore, the key criteria for screening microbes include their salt tolerance, salt removal capability, and the adsorption of ions on the cell surface (Wei et al., 2020). To identify the most suitable microorganisms, halotolerant microbes are cultured and grown in media with progressively increasing salt concentrations over a predetermined number of days. Throughout this period, their growth rate and changes in the conductivity of the medium are meticulously measured. This indicates levels of salinity. Microorganisms that demonstrate normal growth rates in high salinity conditions and a significant reduction in medium conductivity are deemed suitable candidates for biodesalination.

Additional screening criteria are also considered to ensure the selected species are optimally suited for practical applications. These criteria include selecting species that are preferably native to the local environment, ensuring compatibility with the design of the experimental system, requiring minimal additional nutrients, being easy to separate from the medium post-desalination, having potential for use in the production of alternative commercial products, and being amenable to genetic manipulation for further enhancement of their desalination capabilities (Gao et al., 2021). By adhering to these comprehensive screening and selection criteria, researchers can identify and cultivate the most effective microorganisms for biodesalination, paving the way for innovative and sustainable solutions to address the global challenge of freshwater scarcity.

Several potential candidates for the process, identified through numerous laboratory research and pilot-scale studies, include the following:

6.1.1 Halophytic algae (Sahle-Demessie et al., 2019)

M.J. Khan and D.J. Weber, in their book "Ecophysiology of Highly Saline Tolerant Plants," describe how certain salt-tolerant algae can accumulate salt in their vacuoles at concentrations higher than that of the surrounding saline water, thereby reducing the salt content of the surrounding water (Khan & Weber, 2006). For instance, the microalga *Scenedesmus obliquus* has been shown to effectively remove 30% of NaCl from water while also producing valuable lipids as a byproduct (Gan et al., 2016). Additionally, *Chlorella vulgaris*, although primarily a freshwater alga, can thrive in NaCl concentrations up to 20 g/L and can reduce the salinity of the water by up to 26% (Sahle-Demessie et al., 2019).

Furthermore, several green microalgal species, including *Chlorella*, *Chlorococcum*, *Desmodesmus*, *Scenedesmus*, and *Monoraphidium*, have been tested for their biodesalination capabilities. These species were observed to significantly decrease the conductivity of saline water and reduce the chloride ion concentration by up to 39% (Figler et al., 2019). This ability to lower both conductivity and chloride ion levels demonstrates the potential of these microalgae as effective agents for biological desalination, offering a promising solution to the challenge of reducing salt content in saline water.

6.1.2 Cyanobacteria

Cyanobacteria offer distinct advantages over algae in various applications, primarily owing to their remarkable growth rate coupled with minimal nutrient requirements and the ease of genetic manipulation. When subjected to osmotic stress, Cyanobacteria demonstrate an efficient mechanism utilizing ATP to expel Na ions from the cell, a process crucial for their survival. However, disrupting their energy balance can impede this sodium export mechanism, allowing for facile manipulation of their membranes to express photo-driven Chloride pumps, thus facilitating ion import. Research conducted on two prominent strains, namely *Synechococcus sp.* Strain PCC 7002 and *Synechocystis sp.* Strain PCC 6803, revealed their capability to significantly reduce salinity in a short span. For instance, a mere 1-liter volume of cyanobacterial cells could effectively decrease the salinity of 2 liters of water within 10 minutes, translating to a noteworthy 5% reduction in NaCl concentration from the original seawater composition (Minas et al., 2015). Consequently, the integration of cyanobacterial membranes as an ion exchange mechanism holds promise for seawater desalination by establishing a low salt concentration pool within the seawater matrix.

Moreover, introducing the Halorhodopsin protein from *Natronomonas pharaonis* (NpHr) into cyanobacterial membranes can further enhance desalination efficiency through light-facilitated import of positively charged ions such as sodium (Amezaga et al., 2014). This innovative approach underscores the potential of harnessing cyanobacterial systems for sustainable solutions in water treatment and resource management.

6.1.3 Purple non- sulfur bacteria (PNB)

Purple non-sulfur bacteria (PNB) have garnered attention for their unique capabilities, particularly in environments with high salinity and heavy metal contamination. Two distinct strains, MW16 and KMS24, were isolated from shrimp ponds in Thailand, exhibiting remarkable halotolerance and resistance to heavy metals. Studies conducted by Panwichian et al. (2010) revealed that these PNB strains not only thrive in saline conditions but also play a crucial role in mitigating salinity levels by reducing NaCl concentration by up to 31%. Additionally, their proficiency extends to heavy metal remediation, where they efficiently remove significant amounts of these pollutants from contaminated water sources. This dual functionality

underscores the potential of PNB strains as valuable assets in environmental remediation strategies, offering sustainable solutions for addressing challenges associated with salinity and heavy metal contamination in aquatic ecosystems (Panwichian et al., 2010).

6.2 PREPARATION OF ARTIFICIAL SEAWATER AND CULTURE MEDIA

Artificial seawater serves as a pivotal resource in experimental settings, offering precise control over salt concentrations and mineral compositions. Prepared using instant ocean salt mixtures, it provides researchers with a standardized medium that can be easily replicated, ensuring consistency across experiments (Sahle-Demessie et al., 2019). This controlled environment proves particularly advantageous when compared to natural seawater, where variability in composition can pose challenges for reproducibility.

In studies exploring the behavior of microalgae, both artificial seawater and natural seawater have been employed. Immobilized bead experiments conducted with real seawater have revealed insights into chloride ion reduction over time, with notable findings such as the highest reduction rate observed in *Chlamydomonas sp.* after 15 days (Zafar et al., 2023). Immobilization techniques offer distinct advantages, including cell protection and enhanced salt tolerance. However, when utilizing natural seawater, thorough characterization of its chemical properties is imperative, encompassing parameters such as pH, ion concentrations, organic compounds, and mineral content, determined through appropriate analytical methods.

While seawater alone may lack sufficient nutrients to support microbial growth, supplementation with specialized growth media becomes essential. Various formulations such as BM, Alga-Gro, F/2, and BG-11 are commonly employed to provide microalgae with essential nutrients (Minas et al., 2015; Rippka et al., 1979; Sahle-Demessie et al., 2019). Moreover, optimization of pH, temperature, and carbon dioxide levels within the growth medium is crucial to create an environment conducive to the specific needs of the microbial species under investigation. By meticulously tailoring these parameters, researchers can cultivate ideal conditions for the study and cultivation of microorganisms in experimental settings.

6.3 MICROBIAL GROWTH MEASUREMENT

Microorganisms undergo an incubation process within photobioreactors, maintaining a temperature of 25°C and a CO₂ concentration ranging from 0.5% to 5%, which serves to optimize their growth and photosynthetic activity (Sahle-Demessie et al., 2019). Following this incubation period, meticulous attention is directed towards assessing cell proliferation and viability within the nutrient-rich medium, often sourced from seawater. A variety of methodologies are employed to gauge the extent of microbial growth within the culture milieu. These include traditional techniques such as microscopic cell enumeration, as well as more sophisticated methods such as monitoring turbidity alterations, which manifest as changes in light absorbance due to fluctuations in medium turbidity. Furthermore, measurements of chlorophyll content utilizing fluorescence or spectrophotometric analysis represent additional avenues for evaluating microbial growth dynamics (Butterwick et al., 1982; Sahle-Demessie et al., 2019).

6.4 POST- CULTURE SALINITY MEASUREMENTS

The determination of salinity within a given medium can be achieved through the quantification of either sodium or chloride ions present. This assessment is particularly crucial when evaluating the impact of microbial addition on seawater salinity, necessitating measurements both before and after microbial introduction. While the most precise method involves comprehensive chemical analysis or titration utilizing silver nitrate, as outlined by Kjerfve (1979) and Strickland & Parsons (1972), this approach can be labor-intensive. Consequently, expedited outcomes are often sought through indirect methodologies, which establish correlations between salinity and physical properties such as conductivity, refractive index, or density (Kjerfve, 1979; Strickland & Parsons, 1972).

Ion chromatography stands as a standard technique for quantifying anions like chloride ions, offering insights into the salinity of the water body. As elucidated by Sahle-Demessie et al. (2019), the relationship between chloride ion concentration and salinity allows for direct measurement using chloride probes. Moreover, the conductivity of the medium serves as a reliable indicator of salinity, given that higher salinity levels correspond to elevated concentrations of conductive sodium and chloride ions. However, it's imperative to note that conductivity is influenced by temperature variations, necessitating concurrent measurements of both parameters (Sahle-Demessie et al., 2019). Instruments such as conductivity meters or

salinometers, as highlighted by Kjerfve (1979), facilitate the simultaneous assessment of conductivity and temperature (Kjerfve, 1979).

Ideally, the cultivation of halotolerant microbial species in seawater should result in decreased conductivity levels, indicative of ion internalization by the microbes to mitigate salinity levels. Additionally, the process of measuring salinity pre and post-microbial intervention serves not only to monitor the effectiveness of microbial adaptation but also to inform strategies for optimizing microbial growth conditions in saline environments. This comprehensive approach to salinity measurement ensures accurate evaluation and management of aquatic ecosystems and microbial biotechnological applications therein.

6.5 SEPARATION OF SALT LADEN MICROBES FROM WATER

Following the completion of the biodesalination process, a pivotal step involves the separation of salt-laden cells from the water matrix, necessitating meticulous attention to preserve their membrane integrity and prevent any potential leaching of salts back into the water. This imperative task is compounded by the inadequacy of conventional separation techniques such as sedimentation and filtration, which not only demand substantial energy inputs but also undermine the overarching objective of establishing an energy-efficient desalination process (Lam & Lee, 2012).

Microbial separation presents a formidable challenge due to the inherent similarity in surface charges among cells, resulting in mutual repulsion and impeding individual cell isolation owing to their infinitesimal size. Thus, the aggregation of cells into larger flocs becomes indispensable to facilitate their collective separation. Alkaline flocculating agents emerge as a promising solution by effectively neutralizing identical charges, thereby promoting cell agglomeration. Moreover, the application of ultrasonic radiation has been demonstrated to enhance flocculation efficiency, albeit concerns persist regarding the potential toxicity of flocculants (Lam & Lee, 2012; Schlesinger et al., 2012).

In addition to these methods, immobilization of microalgae presents another avenue for effective separation. Here, cells are encapsulated within a matrix of alginate gel, providing a conducive environment for growth within immobilized beads. Upon reaching a stationary phase, these beads settle, simplifying separation through filtration. Notably, this approach not only

significantly reduces energy requirements but also boasts a non-toxic profile, positioning it as a promising strategy for sustainable biodesalination practices (Lam & Lee, 2012).

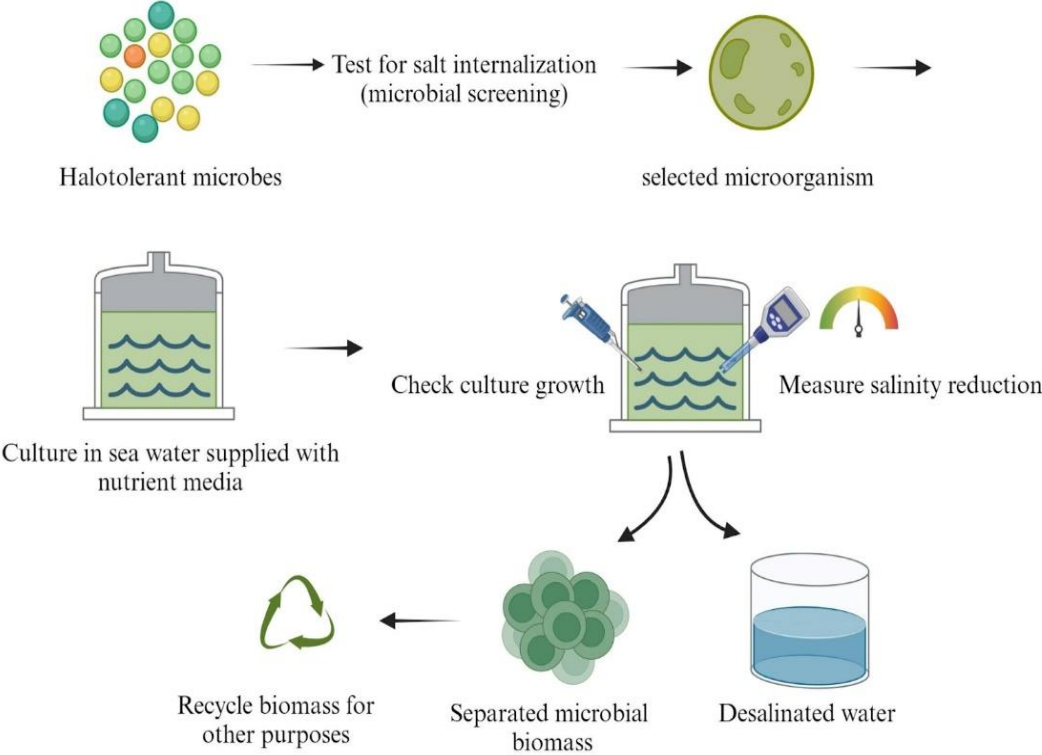


Fig.2- simplified view of biodesalination process

(Kaur et al., 2024)

CHAPTER 7- ADVANTAGES OF BIODESALINATION

In light of the escalating scarcity of water resources globally, there is a burgeoning interest in exploring innovative solutions such as microbial desalination cells (MDCs) and harnessing the resilience of microorganisms capable of thriving in highly saline environments (Taheri et al., 2016). This avenue of research holds immense promise, offering captivating avenues for addressing the pressing challenges posed by water scarcity. Biodesalination, a novel approach, capitalizes on the inherent abilities of photosynthetic microbes and algae to sequester salts, presenting a sustainable and energy-efficient alternative to traditional high-energy desalination techniques.

By leveraging the natural processes of these microorganisms, biodesalination not only facilitates the desalination of water without the consumption of additional energy but also presents ancillary benefits. For instance, it aids in the removal of pollutants from wastewater, mitigating environmental contamination. Moreover, it represents a departure from conventional desalination methods, notorious for generating copious amounts of brine waste, by significantly reducing such harmful byproducts.

Furthermore, the integration of engineered algae and cyanobacteria in seawater desalination processes showcases a multifaceted approach. Beyond water purification, the biomass generated through this method holds potential for the production of biodiesel oil and electricity, offering a sustainable energy source (Zafar et al., 2023). Additionally, these microorganisms serve diverse functions, ranging from biofertilization and dietary supplementation to disinfection and soil bioremediation, thereby enriching agricultural practices and environmental sustainability (Du et al., 2018; Stapleton & Banuelos, 2009).

The evolution of biodesalination technology is also marked by innovative bioengineering endeavors, such as the incorporation of bio cathodes in MDCs. This advancement not only enhances the efficiency of desalination but also enables the generation of electricity concurrently, presenting a paradigm shift in sustainable energy production (*An Insight on Biocathode Microbial Desalination Cell: Current Challenges and Prospects - Prakash - 2022 - International Journal of Energy Research - Wiley Online Library*, n.d.).

Moreover, the affordability and efficiency of biodesalination render it particularly advantageous for regions grappling with water scarcity, especially in developing nations (Esmaeili et al., 2023)(Ng et al., 2013). Its low-cost nature signifies a potential solution that can alleviate the burden of water scarcity without imposing undue strain on the environment.

While still in the realm of active research, biodesalination holds tremendous promise as a transformative solution capable of enhancing global access to freshwater resources. Its renewable nature aligns with sustainable development goals, offering a pathway towards improved living standards and heightened resilience, particularly in arid regions. The compelling environmental and economic benefits underscore the imperative of continued research and development efforts to realize the full potential of biodesalination as a viable and scalable solution (Esmaeili et al., 2023).

CHAPTER 8- FUTURE PROSPECTS

The potential of biodesalination in addressing water scarcity is boundless, thanks to the continuous advancements in technology. With a keen eye on the cost implications of desalination processes, this eco-friendly alternative holds promise for enhanced efficiency. Studies have revealed that the expense associated with desalination is approximately three times greater than that of traditional water sourcing methods (Ziolkowska, 2015). Despite notable technological progress reducing the costs of desalination via reverse osmosis (RO) in recent decades, the escalating expenses of conventional energy production could potentially reverse this downward cost trend (Shokri & Sanavi Fard, 2023). Hence, embracing sustainable and economically viable alternatives, such as those discussed in the referenced paper, presents an auspicious avenue for mitigating water scarcity challenges.

While microalgae offer a promising biological approach to desalination, their ability to remove salts from water is often incomplete. To overcome this limitation and improve the overall efficiency of the water purification process, a multi-stage approach can be employed. Here, algal biodesalination acts as a pre-treatment step before utilizing reverse osmosis (RO) for final purification (Sahle-Demessie et al., 2019).

This two-stage system offers significant advantages. By utilizing microalgae first, a large portion of the salts are removed, transforming the seawater into brackish water. Brackish water RO operates at considerably lower energy consumption compared to traditional seawater RO systems (Ismail et al., 2018). This translates to substantial cost savings in the desalination process.

However, a crucial challenge exists. Research suggests that using untreated algal biomass after biodesalination can harm and clog RO membranes, hindering overall efficiency (Caron et al., 2010; Jamaly et al., 2014). Therefore, efficient and sustainable methods for removing algal biomass post-desalination are paramount.

An ideal solution would involve recycling the removed algal biomass for other beneficial purposes, creating a closed-loop system. This could involve converting the biomass into biofuels or other valuable products, maximizing resource utilization and minimizing waste generation. Developing such a system would be a significant step forward in making algal biodesalination a truly sustainable and cost-effective method for water purification.

One promising avenue for innovation lies in merging desalination technologies with wastewater treatment processes. Wastewater, rich in organic compounds, can serve as a valuable source of nutrients and growth media for microalgae cultivation. This symbiotic relationship offers a double benefit: microalgae thrive on the nutrients in wastewater, while simultaneously removing pollutants through various mechanisms like biosorption and biodegradation (A. Shahid et al., 2020). Studies have shown the effectiveness of this approach, with positive environmental and economic implications (Amezaga et al., 2014). For instance, research by Mirzaei et al. (2024) demonstrated that microalgae species like *Arthrospira platensis* and *Dunaliella salina* can flourish in wastewater generated from salmon aquaculture. These algae effectively reduced both salinity and pollutant levels in the wastewater, with *A. platensis* achieving a 45% decrease in electrical conductivity, a measure of dissolved salts (Mirzaei et al., 2024).

However, challenges do exist. The presence of toxic materials in wastewater can hinder algal growth. Factors like low light penetration due to high turbidity, and the presence of specific contaminants, can be detrimental to the process (Amenorfenyo et al., 2019). Industrial wastewater, particularly from sectors like textile manufacturing, often contains highly toxic and carcinogenic contaminants. One such example is Toluene Diamine (TDA), a harmful byproduct

of dye production. Fortunately, advancements in wastewater pre-treatment offer solutions. Research by Sanavi Fard demonstrates the effectiveness of using advanced oxidation processes (AOPs) like UV/H₂O₂ treatment to degrade TDA in industrial wastewater (Sanavi Fard et al., 2023). Similarly, successful application of electro-peroxone (EP) processes for the degradation of azo dyes like Acid Red 182 (AR182) can also be used (Shokri & Sanavi Fard, 2023).

By integrating these advanced oxidation and electrochemical AOP (EAOP) pre-treatment methods with biodesalination systems, we can potentially develop a comprehensive solution for detoxifying industrial wastewater and making it suitable for desalination and subsequent reuse. This approach holds significant promise for creating a more sustainable and efficient water management system.

Researchers like Gan et al. (2016) have shown that algae can be a valuable resource after desalination for more than just clean water. Their study using *Scenedesmus obliquus* in brackish water found that slightly salty environments (8.8 g/L NaCl) actually boosted lipid production in the algae, reaching up to 21%. This salt tolerance suggests a way to improve biodiesel production, a process known for its high costs. By cultivating algae in brackish water, we can reduce the need for extensive desalination while still harvesting the algae for lipid production. Combining biodesalination with biofuel production in this way could significantly lower the cost and environmental impact of both processes (Kaur et al., 2024).

Microbial cells can be engineered to accumulate more salt through a combination of genetic and environmental manipulation. One approach involves a "starvation strategy" where researchers limit nutrient intake to just what the microbes need to grow. This mild starvation restricts their energy (ATP) and hinders them from pumping sodium ions back out of the cell. Additionally, genetic engineering can be used to insert salt-tolerant genes, creating "halotolerant" microbes. While this improves salt tolerance, it doesn't necessarily guarantee efficient salt accumulation. A more promising approach might be manipulating eukaryotic cells, as their vacuoles can act as natural storage compartments for excess salts (Gao et al., 2021). Amezaga et al. (2014) successfully combined both genetic and environmental manipulation to create a light-powered salt accumulator using cyanobacteria. This model serves as a valuable reference for further development in this field (Amezaga et al., 2014).

CHAPTER 9- CONCLUSION

Microbial desalination shows promise as a sustainable and energy-efficient alternative to traditional methods. However, large-scale application remains a hurdle.

- **Advantages:** Biodesalination consumes less energy (e.g., HRAPs use 0.06-0.08 kWh/m³) (Arashiro et al., 2018) and is environmentally friendly due to low greenhouse gas emissions and minimal harmful brine discharge. Additionally, the process operates at ambient temperatures, reducing operational costs. Integration with wastewater treatment or biomaterial production from the algae (biofuel, fertilizer, etc.) further enhances sustainability by utilizing wastewater nutrients and the resulting biomass.
- **Challenges:** Scaling up is difficult. Desalination rate depends on algae growth, requiring significant biomass for large-scale treatment. Maintaining this biomass necessitates constant mixing, which consumes energy. Salt removal from microorganisms is slow, extending treatment times. Cost analyses (e.g., \$1.22-\$2.65/m³ for algae-based wastewater treatment) (Acién Fernández et al., 2019) highlight the need for biomass reuse to improve economic viability. Efficiently removing salt without harming the microbes for reuse remains a challenge, and the viability of salt-laden biomass is uncertain.

In conclusion, research on microbial desalination's potential needs to bridge the gap between lab-scale success and large-scale implementation for a truly sustainable desalination solution.

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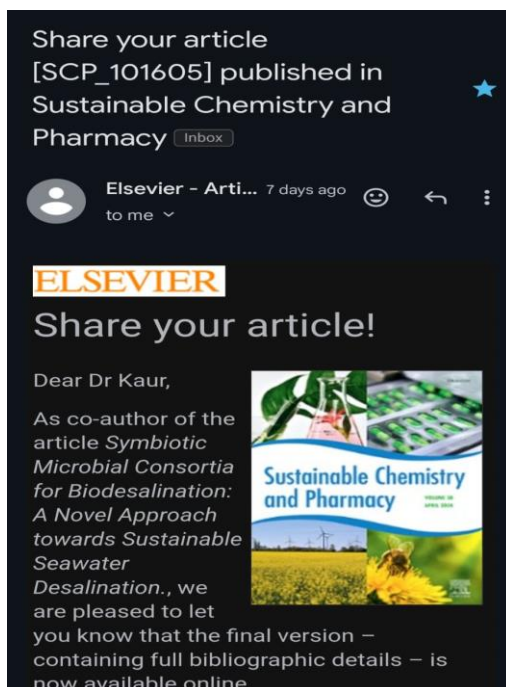
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