

**ASSESSMENT OF THE DESALINATION POTENTIALS OF SOME
ALGAL/CYANOBACTERIAL STRAINS ISOLATED FROM BUFFALO RIVER IN THE
EASTERN CAPE PROVINCE, SOUTH AFRICA**



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A dissertation submitted in fulfillment of the requirements for the degree of

MASTER OF SCIENCE (MICROBIOLOGY)



By

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DECLARATION

I, Yolanda Blessings Mnikina-Cibi, hereby attest that, unless otherwise stated, this dissertation, entitled “Assessment of desalination potential of freshwater and marine algal/cyanobacterial strains isolated from Buffalo River in the Eastern Cape Province, South Africa”, which I submitted to the Department of Biochemistry and Microbiology at the University of Fort Hare for a Master of Science in Microbiology, is entirely my original work and has not been submitted to any other university.

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This dissertation titled “Assessment of desalination potential of freshwater and marine algal/cyanobacterial strains isolated from Buffalo River in the Eastern Cape Province, South Africa” complies with the University of Fort Hare's regulations for awarding the Master of Science (MSc) degree and is approved for its contribution to scientific knowledge.

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DEDICATION

I dedicate this dissertation to the wonderful God for His grace upon my life, to my lovely Husband Asanda Cibi, and to family.

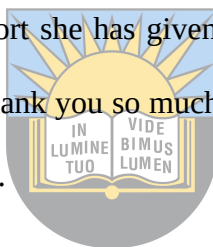


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LIST OF ABBREVIATIONS

AEM	Anion exchange membranes
AEMREG:	Applied and Environmental Microbiology Research Group
ATP	Adenosine Triphosphate
BCM	Buffalo City Municipality
CEM	Cation exchange membranes
CFU:	Colony forming unit
COD	Chemical oxygen demand
DMSO	Dimethyl sulfoxide
DO	Dissolved oxygen
EC	Electrical conductivity
EPS	Extracellular Polymeric Substances
FTIR	Fourier-Transform Infrared
GDP	Gross domestic product
GMRDC:	Govan Mbeki Research and Development Centre
LDH	Lactate dehydrogenase
MDC	Microbial Desalination Cell



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MED	Multiple-effect distillation
MFC	microbial fuel cells
MSF	Multi-stage flash distillation
MSc	Master of Sciences
OD	Optical density
RO	Reverse osmosis
SAMRC	South African Medical Research Council
TDS	Total Dissolved Solids
VC	Vapor-compression evaporation
WHO:	World Health Organization
WWTW	Wastewater treatment water
XPS	X-ray photoelectron spectroscopy



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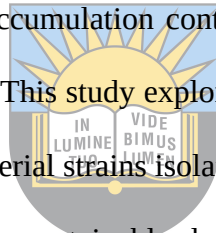
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General Abstract

Water scarcity is a global disaster, especially in arid and semi-arid countries including South Africa. When compared to the global average, South Africa's average rainfall is very low. Furthermore, freshwater scarcity is expected to worsen over the next few decades as the human population and demands grow, and the development of sustainable, energy-efficient desalination technologies could make about 97% of the water in the oceans become a useable substitute. Biodesalination using cyanobacteria is an emerging approach that could be used to achieve this feat. As a source of nutrition for growth, algae and cyanobacteria absorb ions that have a negative impact on water quality, and cyanobacteria can grow in high salt concentrations while producing a low-salt environment. Both biosorption and bioaccumulation contribute to salt removal, with biosorption having the highest salt removal efficacy. This study explores the potential of desalination potential of freshwater and marine algal/cyanobacterial strains isolated from the Buffalo River in the Eastern Cape Province, South Africa. It supports sustainable development goals related to clean water, climate action, and environmental conservation.



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The standard culture-based method was used for the isolation of presumptive algal and cyanobacterial strains from water collected from the Buffalo River. The presumptive cyanobacterial isolates with desalination potential were then sent to Inqaba Biotech for identification. Identification was done based on Next-generation sequencing (NGS). In total, 25 species were identified, comprising 12 cyanobacteria (*Anabaena* sp., MAG: *Pseudanabaena*, *Pseudanabaena* sp., *Nostoc minutum*, *Synechococcus lividus*, *Hydrococcus rivularis*, *Spirulina subsalsa*, *Chroococcus* sp., *Cyanobacterium aponinum*, MAG: *Dolichospermum*, *Dolichospermum* sp., *Chamaesiphon minutus*), diatom (*Nitzschia inconspicua*), and 12 green algae (*Coelastrella* sp., *Chlamydomonas* sp., *Chlorella vulgaris*, *Monoraphidium* sp., *Parachlorella kessleri*, *Chlorella sorokiniana*, *Tetrademus*

obliquus, *Monoraphidium* sp., *Picochlorum costavermella*, *Stichococcus bacillaris*, *Haematococcus lacustris*) isolated from the buffalo River.

Out of the 20 screened isolates, 5 non-axenic (25%) strains exhibited biodesalination potential of more than 40% salinity reduction. These 5 non-axenic cultures named: 3 Wimpy, 12 Grinika, 14 Grinika, 15 Ndevana, 20 Buffalo Pass were further investigated for optimisation of culture conditions for high algal biomass to improve the efficiency of the algae/cyanobacteria biodesalination. The optimum parameters investigated included carbon source, nitrogen source, pH, light source, and effects of different salinity concentration. Salinity reduction was achieved by up to 92% salinity reduction at salt concentrations of 20 ppt seawater. Both sucrose and nitrates were found to be the best carbon and nitrogen sources under high salinity conditions yielding 55.5% and 50.0 % respectively at the regulated optimum parameters of shaker speed (180 rpm), temperature (30 ± 3 °C), 40 Watts cool white, fluorescent bulbs, and pH 7.5.

To prevent the release of salt and cyanotoxins produced by cyanobacteria reverting into the desalinated water, membrane filtration was used to safely separate and recover algal and cyanobacteria strains from desalinated water. Removal of cells from the desalinated water resulted in a significant salinity reduction. To address public health and safety concerns, an *in vitro* toxicology study was conducted using the CyQUANT LDH Cytotoxicity Assay Kit to assess the potential risks associated with desalinated water, which turned out to be negative.

The use of cyanobacteria and photosynthetic organisms in the desalination of seawater has been proven in this study. Algal strains have been shown to produce significant biomass in seawater while at the same time reducing the salt content. Using optimal conditions for algal/cyanobacterial growth, such as light, carbon, and nitrogen sources enables cyanobacteria to grow at high densities and

enables future biological processes for sustainable water treatment applications. However, energy sources were a major challenge in this study. Alternative methods should be investigated for the supply of artificial energy sources. Equally, more stringent monitoring of physiological changes caused by ionic stress should be investigated. Understanding how salinity affects cell growth and viability would enable scaling up cyanobacterial growth to meet the demands of water treatment scenarios.

Keywords: Biodesalination; Cyanobacteria; Freshwater; Optimisation; Seawater; Water-scarcity.



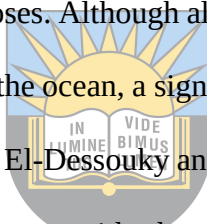
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CHAPTER 1

GENERAL INTRODUCTION

1.1. Background of the study

Global water security is becoming an emerging issue; water resources need to be preserved particularly in the regions of the world that experience water shortage conditions. Freshwater scarcity has been identified as a rapidly rising concern and has been linked to future limited resources because of the increasing population, increase in food production demand, and unprecedented climatic changes (Misra, 2014). There will always be a high demand for clean water which is vital for domestic purposes. Although almost 70% of the earth's surface is made up of water, 97% of which is found in the ocean, a significant amount of that water is frozen in glaciers and snow (Sophia et al., 2016; El-Dessouky and Ettouney, 2002). Only about 3% (v/v) of the total water on earth is clean water, with about 1% usable as drinking water (World Wildlife, 2020).



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One of the major constraints to South Africa's development is lack of freshwater in abundance (WWF, 2013). South Africa is considered as one of the driest countries in the world (South African Government, 2020). It is a semi-arid country with 464 mm of average normal rainfall, leading to a drastic dropping in dam levels in most parts of the country (World Wildlife, 2020). During certain period of the year, some regions in South Africa gets adversely affected by drought; however, approximately 58% of its water is available for drinking and agriculture purposes which may be as a result of climate change, decreased rainfall, and increased population as well as environmental pollution (DWA, 2020). Only 15% of South Africa's 223 river ecosystems are at the right margins, 60% are threatened, and 25% are critically endangered (WWF, 2013; Hedden and Cillier, 2014). Domestic and urban water use account

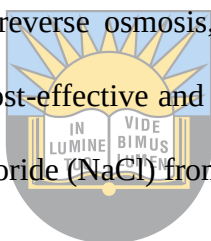
for approximately 11% of total water use, while mining and a few large industries account for approximately 8% (Visser and Verhoog, 2007).

Buffalo River is the major river for urban, rural, and industrial water supply in the Buffalo City Metropolitan Municipality (BCMM). It has a length of 126 km and originates in the Amatole Mountains where it passes through King William's Town to the Indian Ocean in East London (EOH, 2016). Increasing human activities have contributed to significant pollution, with high levels of microbial contamination and heavy metals (Yahaya et al., 2017). Along this river, there are two municipal wastewater treatment plants, namely Zwelitsha and East London wastewater treatment works (WWTW), and industrial discharge of wastewater and leachates from dumping sites in most parts of Buffalo City Municipality (BCM). Agricultural activities of residents of this region, which include farming and animal husbandry, may be high in nitrogen and phosphorus and can promote or cause excessive fertilization, which contributes to the growth of cyanobacterial blooms. Operational spillages and ship repair contribute to increased water pollution in the harbor area of the river (Yahaya et al., 2017). Therefore, South Africa must act urgently to protect its water resources.

Several studies have focused on alternative methods of providing additional fresh water, the results have been replete with challenges. Borehole drilling, dam construction, wastewater reclamation, and desalination facilities are among the measures being implemented to address water scarcity in South Africa. The country's poor water infrastructure and growing population have exacerbated the water issue, forcing citizens to adopt strict lifestyles, resulting in an increase in water-related diseases such as cholera outbreaks. The high energy requirements of desalination facilities, combined with the country's already stressed energy situation, remain a challenge. Prins et al. (2022) noted that, while wastewater reclamation appears to be more feasible because it requires less energy overall, public acceptance poses significant challenges. While biological processes are being advocated many such processes are still not very well

developed and the processes are either slow or cannot easily be optimised. Thus, biodesalination of sea water by cyanobacteria is a novel endeavor in the quest for provision of freshwater in South Africa.

Vast amounts of water trapped in the oceans can only be accessed if cost-effective and environmentally friendly desalination methods are used. Desalination is the process that removes salts of saline water from either seawater or brackish water to obtain freshwater (El-Dessouky and Ettouney, 2002). Desalination has rapidly expanded globally and is now a crucial technique used to obtain useable water from the existing seawater. However, the process uses a lot of energy and has several detrimental social, economic, and environmental effects (Rustum et al., 2020). Desalination techniques being presently employed, such as thermal desalination, solar desalination, and reverse osmosis, are expensive and energy intensive. Hence, alternative methods that are cost-effective and less harmless to the environment must be considered to eliminate sodium chloride (NaCl) from the seawater (Sahle-Demessie et al., 2019).



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As an environmentally friendly technology, Microbial Desalination Cell (MDC) has been receiving a significant consideration for desalination, showing success in removing salinity and hardness from water, remediation of ground and wastewater, and generating electricity and hydrogen gas (Sophia et al., 2016). Photosynthetic bacteria-like cyanobacteria have not been incorporated into conventional water treatment technologies. As a result, it would be interesting and pertinent to develop a novel, low-energy biological desalination process based on cyanobacteria and algae biological membranes.

Biodesalination is the biological technique that uses photo-autotrophic marine organisms as ion-exchangers to reduce sodium concentrations using active radiation from the sun (Apte et al., 1987). The application of cyanobacteria in water treatment, and growing them at high cell

densities, can be sustained through accessibility of available energy sources such as sunlight, carbon dioxide, and minerals (Amezaga et al., 2014). The desalination potential of cyanobacterial strains isolated from both freshwater and marine environments would provide an alternative means of solving the problem of water security in South Africa. Cyanobacteria act as an ion exchanger where sodium chloride moves from high levels of concentration in the sea to low sodium volume through transport proteins and cell membranes (Apte and Thomas, 1997). Thus, it is important to establish the optimum conditions required for high cyanobacteria biomass for the discovery of effective cyanobacterial strains for removing high salt content from seawater.

Cyanobacteria are a group of organisms from the phylum of oxygenic photoautotrophs (Altermann and Kazmeirczak, 2003). The term "blue-green algae" is derived from the coloration (hence "cyan") that many members of this algal division exhibit (Harding and Paxton, 2001). They consist of two pigments, phycobilins, which is a blue photosynthetic pigment, and chlorophyll, a green pigment, both of which are present in phycobilisomes and are crucial for absorbing light for photosynthesis (Abraham and Luque, 2018). Cyanobacteria are single-celled, filamentous, or colonial in form, in contrast to the multicellular eukaryotic cells found in plants and animals. They share characteristics with both prokaryotic and eukaryotic organisms (Gillespie, 2018). Cyanobacteria can adapt to a wide range of habitats due to their long evolutionary history, including hot springs and ice layers in the polar regions, as well as freshwater and marine environments (Harding and Paxton, 2001).

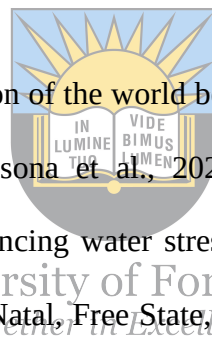
Any use of synthetic biology raises numerous health and environmental concerns, particularly when cyanobacteria are used, resulting in low social acceptance (Amezaga et al., 2014). Many cyanobacteria genera and species such as *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, *Nostoc*, and *Oscillatoria* (Planktothrix) are known to produce toxins and odors that are hazardous to humans and animals alike. These toxins include neurotoxic and

hepatotoxic secondary metabolites (Harding and Paxton, 2001). As a result, this technology must be evaluated to assess the potential risk associated with desalinated water to address the public health issue as well as the risk of low social acceptance of this treatment technology. Understanding physicochemical factors makes it easier to separate cyanobacterial cells from desalinated water while maintaining cell integrity (Amezaga et al., 2014). This research aligns with the United Nations Sustainable Development Goals, particularly SDG 6 (Clean Water and Sanitation), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land). By developing sustainable biodesalination methods, this study contributes to the global efforts to ensure clean water availability, mitigate climate change, and sustainably use marine and terrestrial resources.

1.2. Problem Statement

Water scarcity is affecting every region of the world because of climate change, urbanisation, and natural disasters (Bischoff-Mattsona et al., 2020). Many parts of South Africa are experiencing or will soon be experiencing water stress. Drought disasters were declared in some provinces including KwaZulu-Natal, Free State, North-West, and Limpopo in 2015. In 2020, Mpumalanga was in the final stages of declaring a drought disaster (South African Government, 2020). Regions such as the Western Cape Province and parts of the Eastern Cape Province (Port Elizabeth) have previously experienced severe water shortages (Bischoff-Mattsona et al., 2020). Concurrently, urbanization is expected to increase, with significant implications for water-related vulnerabilities. As a result of these convergent 'trends', urban areas around the world are facing actual or impending disruption of essential water services – a crisis termed 'Day Zero'. (Bischoff-Mattsona et al., 2020; Jiang and O'Neill, 2017).

The Bridle Drift Dam along the Buffalo River in East London has a storage capacity of 98 000 000 m³, but, at some point in 2020 it was at 39.5%, down from 45% in 2019 (DWA, 2020). This demonstrates that if no measures and interventions are implemented to alleviate the water



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scarcity crisis, the Bridle Drift Dam will eventually dry up. As a water-scarce country, South Africa must act quickly to protect water resources and develop and improve water-access technologies.

Traditional desalination techniques, water-recovery technologies such as groundwater remediation, water recycling, and improving the infrastructure of water community can all help to reduce the need for and increase the amount of water available for human use. However, these strategies necessitate long-term investments, and many developing countries suffer from chronic water scarcity (Sahle-Demessie et al., 2019). Hence, desalination comes up as a potential alternative pursuant to addressing the challenges of the water crisis. Notwithstanding, that desalination has negative environmental consequences, it involves use of high energy levels as well as the production of a lot of concentrated brine waste (Lior and Kim, 2018). A way out of this challenge is the use of biological entities to remove salt from the seawater in a process called biodesalination. This study there evaluates the potential of some indigenous algae and cyanobacterial strains isolated from South African aquatic environment for use in the biodesalination of seawater. This research aligns with the United Nations Sustainable Development Goals, particularly SDG 6 (Clean Water and Sanitation), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land). By developing sustainable biodesalination methods, this study contributes to the global efforts to ensure clean water availability, mitigate climate change, and sustainably use marine and terrestrial resources.

1.3. Aim and objectives.

This study aimed to assess the potential of some algal strains isolated from aquatic environment of the Eastern Cape province, South Africa, for the removal of salt in seawater.

1.3.1. Specific objectives

To achieve the above aim, the study had the following specific objectives:

- To isolate and purify the presumptive cyanobacterial isolates from water samples collected from marine and freshwater environments.
- To screen the presumptive cyanobacterial isolates for desalination potential and identify the most promising cyanobacterial isolates with molecular-based techniques.
- To optimise cultivation conditions required for high cell densities culture from the most promising cyanobacterial strains with the high desalination potential.
- To assess the potential risk associated with desalinated water through *in vitro* toxicology study using cell lines.



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CHAPTER 2

LITERATURE REVIEW

2.0. Introduction

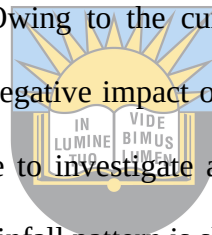
Water scarcity is a global disaster, especially in arid countries like South Africa. When compared to the global average, South Africa's average rainfall is less. Freshwater scarcity is expected to worsen over the next few decades as the human population and demands grow. Only by developing sustainable, energy-efficient desalination technologies will 97% of the water in the oceans be used. Biodesalination using cyanobacteria could be an alternative method for achieving this. Cyanobacteria can grow in high salt concentrations while producing a low salt environment. According to current research, both biosorption and bioaccumulation contribute to salt removal, with biosorption having the highest salt removal efficacy. Hence, freshwater strains have a greater capacity for sodium efflux and may accumulate more net Na⁺ intake than marine strains. Although biodesalination is the most suitable alternative method when compared to conventional technologies, the main challenge of this technology is to safely recover cells from desalinated water to prevent the release of salt and cyanotoxins produced by cyanobacteria from reverting to the desalinated water. This review discusses in detail, the various desalination technologies, including strain selection, desalination mechanism, and molecular basis of desalination. The review also discusses the environmental factors that influence desalination, cell separation, safety, public acceptance, and industrial applications of these processes, as well as potential future research areas such as cyanobacterial optimization.

2.1. The vast abundance of water resources

Water is an essential part of life that ensures environmental sustainability and industrial growth (Kummu and Varis, 2011). Water has become a scarce resource on a global scale, especially in developing countries such as South Africa, where the development of technology remains a

challenge. Most of South Africa is dry or semi-arid, with only a few areas along the southern coast receiving significant rainfall. Sixty-five per cent (65%) of the country receives less than 500 mm of rain per year; 21% receives less than 200 mm, and the remaining gets less than 100 mm (Baiyegunhi, 2015; DWAF, 1994; DWAF, 2013; WHO, 2021). Water-related disasters such as droughts and floods in most regions are a result of seasonal and yearly variations in rainfall patterns, which are caused by climatic changes (Mukheibir, 2010).

There have been significant effects of reliable and clean water in many regions of South Africa, especially in the Eastern Cape and Western Cape provinces, as well as KwaZulu-Natal Province. The country is on the verge of depleting all the freshwater resources available within its borders. South Africa's water resources have already been utilised up to 98% of capacity (Smuts, 2011; UN-Water, 2006). Owing to the current drought, most of the country is experiencing day zero, which has a negative impact on water quality and lowers dam levels and reservoirs. Thus, it is imperative to investigate and utilise additional water sources to supplement the current supply. The rainfall pattern is shown in Figure 2.1 below.



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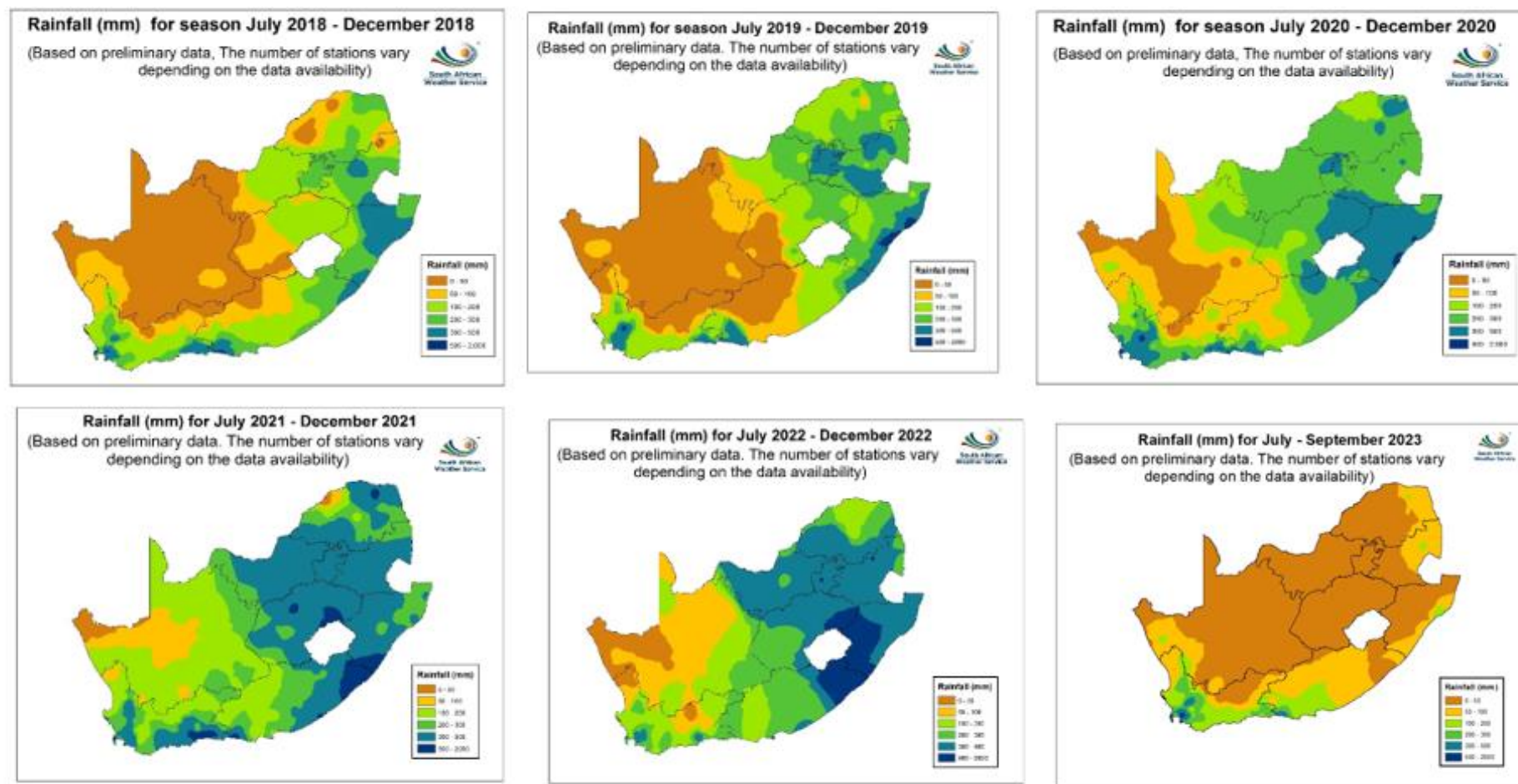


Figure 2.1: South Africa's seasonal rainfall for period 2018-2023 (South African weather Service 2023 (<https://www.weathersa.co.za/home/historicalrain>)).

The lingering pressures on water resources will continue to affect other socio-economic spheres such as agriculture, mining, and industrial sectors, as South Africa's economic growth is directly affected by the scarcity of water (DEA, 2011; Du Plessis et al., 2006). In South Africa, for example, only 2% of the nation's water is utilised to produce energy, and about 60% of the nation's water is used for agricultural purposes, which contribute 17% to the Gross domestic product (GDP). The industrial sector in South Africa uses close to 11% of the nation's water resources although it contributes to about 29% of the nation's GDP (DWAF, 2013; Ndlela et al., 2018), suggesting that municipalities will have to think about desalination to access less-used marine water sources where there are insufficient freshwater resources. Figures 2.2 (a) and (b) below show one of South Africa's largest dams, the Bridle Drift Dam (Grinika) in Mdantsane, and the Laing Dam, one of the Amatola potable water treatment plants in Kwa Qongqotha, a village in King William's Town. These two dams show a drastic drop in water levels with grass and other vegetation now growing where there once was water. These two dams are part of one of the biggest rivers in the Eastern Cape, namely the Buffalo River. The Bridle Drift Dam has a storage capacity of 98.0%; however, projections have shown a constant decline of 21.6%, 39.5%, and 45% for 2021, 2020, and 2019 respectively (DWS, 2022), and it is not only dams, but also some rivers, which have dried out. The South African government has indicated that there is high demand for clean water, and it is expected to worsen over the next few years as the human population and demands grow. A call to build a sustainable seawater desalination plant is a promising option to unravel the water scarcity problem in South Africa. However, the current desalination methods are expensive, and the use of cyanobacterial membranes as a sustainable and energy-efficient desalination technique has been advocated.

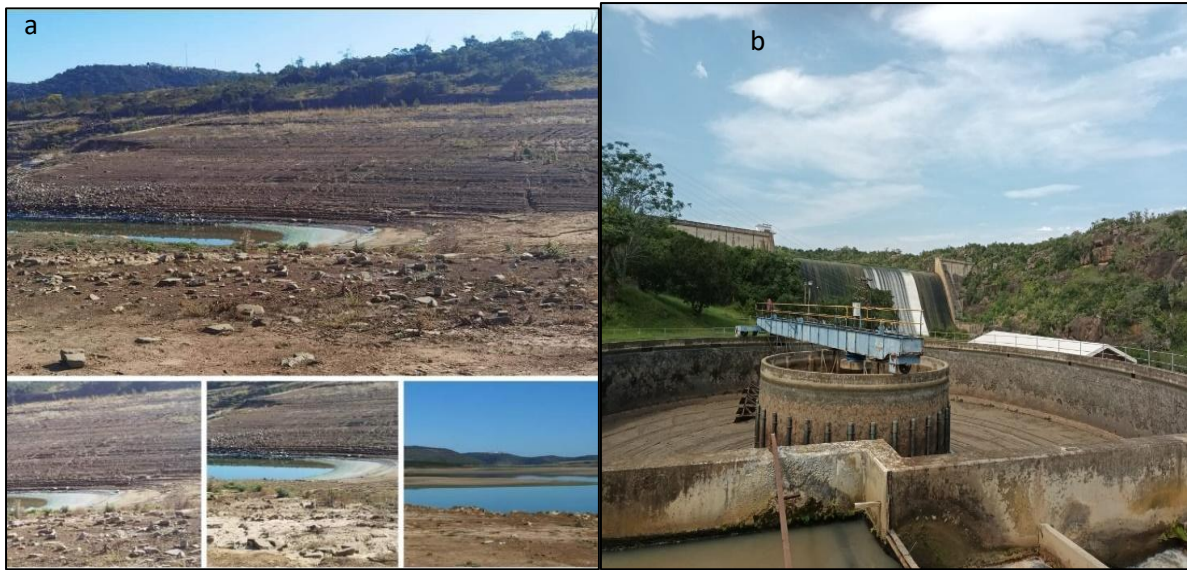


Figure 2.2: A representative image of a drastic drop in water levels in the different points taken from Grinika and Laing Dam water treatment plants along the Buffalo River during data collection. 2(a) was taken from the Briddle Dam (Grinika) in Mdantsane and 2(b) the Laing Dam, one of the Amatola potable water treatment plants, located in one of the villages in King William's Town named Kwa Qongqotha.

2.2. Desalination technologies

The World Health Organization (WHO, 2021) guidelines for drinking water standards allow for a maximum salinity of 500 ppm, with some exceptions allowing for up to 1000 ppm. The country's water supply is insufficient because accessible surface water is salty, with salinities of up to 10,000 ppm (Shatat and Riffat, 2014; WHO, 2021). South Africa has a coastline of over 2500 km, and the oceans are regarded as a plentiful source, but this water cannot be used without sustainable, energy-efficient desalination technologies. The chemical compositions of saltwater are altered by several chemical transport mechanisms, making seawater desalination difficult. The typical salinity of seawater is 36,000 ppm of total dissolved solids (Shatat and Riffat, 2014). Seawater is an aqueous mixture of 96.5% water, 2.5% salts, and 1% dissolved substances such as organic and inorganic compounds, atmospheric gases, and other substances

(Mackenzie, Byrne, and Duxbury, 2022). Also, dissolved solids account for more than 99% of the elements in trace concentrations (White, 2009). Rivers transport chemicals, both dissolved and particulate matter, to the oceanic margins (Byrne, 2007). The salinity of seawater ranges from 3.2% to 4.0% (32 000-40 000 ppm), with Cl^- and Na^+ salt contents of approximately 55% and 31% respectively (Kirtel et al., 2018). These elements are concentrated during the desalination process, which results in the production of various scale forms and other pollutants, leading to the desalination equipment malfunctioning (Du Plessis et al., 2006; Swartz et al., 2006). The 10 operational desalination plants in South Africa produce a limited amount of water that is only suitable for household use as outlined in Table 2.1 below. Although desalination technologies have been used for centuries, thermal and membrane processes are the most popular and successfully commercialised systems for converting saltwater to freshwater (Shatat and Riffat, 2014).



On a global scale, thermal desalination technology is a simple and cost-effective method for producing fresh water from saline water. The main principles of thermal desalination are evaporation, boiling, and condensation. The salty vapor separates and condenses when salinity water boils to generate freshwater (Winter, Pannell, and Mccann, 2002). Salt can also separate from water by freezing the feedwater, which separates freshwater from the saline solution. Vapor-compression evaporation (VC), multi-stage flash distillation (MSF), cogeneration, solar water desalination, and multiple-effect distillation (MED) are common examples of thermal desalination. Thermal distillation plants provide a substantial proportion of the world's capacity for desalinating seawater (Swartz et al., 2006). It is believed to be more robust since it requires less pre-treatment than other desalination processes. However, thermal desalination consumes up to four times more energy than reverse osmosis devices (Du Plessis et al., 2006; Swartz et al., 2006; Winter et al., 2002). Thermal desalination processes are typically not considered for brackish or seawater desalination in South Africa unless there is enough waste

heat or low-cost fuel such as nuclear power generation (Swartz et al., 2006). Currently, no biodesalination technology has been employed in South Africa.

2.2.1. Membrane desalination

Synthetic membrane applications were first restricted to municipal water desalination to provide clean water to island and coastal communities (Amezaga et al., 2014a; Sahle-Demessie et., 2019a). Over the years, there have been new membrane developments to produce fresh water by reverse osmosis (RO). They have been expanded and are now used in high separation processes such as beverage purification, enzyme concentration, and chemical separations, in addition to the water industry (Mackenzie et al., 2022; Thimmaraju et al., 2018). Membrane desalination is time-saving and cost-effective and emits far fewer greenhouse gases than traditional water processes like thermal desalination (ELGA, 2014; Goga, Friedrich, and Buckley, 2019; Swartz et al., 2006). Membrane technology involves several processes to separate salts or particulate matter from water, which include a pre-treatment stage, membrane filtration using either micro-filters, nano- or ultra-filters, and the post-treatment stage as shown in Figure 2.3 below. The primary difference in membrane desalination is the size of retained particles or particles allowed to pass through the membranes (Smuts ,2011; Thimmaraju et al., 2018; UN-Water, 2006). Furthermore, most seawater desalination plants discharge the waste concentration of salt back into the sea because the seawater can dissolve several times its normal salt concentration and the large volume of available water will dilute the salt concentration (Paerl, Tucker, and Bland, 1983; Schoeman and Steyn, 2003).

The high-capacity distillation plants in South Africa have an upper capacity limit of about 100 000 m³/d for RO desalination of seawater (Goga, Friedrich, and Buckley, 2019; Mgeni Water, 2022). One of the most recent additions to Ashkelon, Israel is a large-scale seawater RO desalination plant with a capacity of 330 000 m³/d (Goga, Friedrich, and Buckley, 2019; Du

Plessis et al., 2006; Schoeman and Steyn, 2003). It is challenging to achieve the average capacity needs of potential water denitrification and desalination plants in South Africa owing to the country's restricted access to power. If there are no feasible, inexpensive technologies, reverse osmosis (RO) would be the preferred technology. As a result, well-established South African seawater, and brackish water desalination plants such as Umgeni Water, Quality Filtration, and the Mpumalanga Mine-water Reclamation Plant use membrane technologies to achieve the desired separation. However, the amount of potable water produced by these desalination plants is insufficient. The Umgeni Water Treatment Plant is designed only to produce 150 megalitres/day (ML/d) of potable water; however, the plant produces, on average, 70 ML/d (Goga, Friedrich, and Buckley, 2019; Mgeni Water, 2022). The Mpumalanga Mine-water reclamation plant is designed to recycle wastewater of contributing mines in the eMalahleni coal fields to potable water standards. The plant desalinates 30 megalitres of water a day and has supplied 22 billion litres of water to the local municipality (Natalia and Iwa, 2008). Mossel Bay's daily capacity is 15 ML/d. Knysna produced 2 ML/day (currently shut down). The desalination plant in Plettenberg Bay produces 2 ML/d, which accounts for about 7% of the municipality's potable water needs. The municipality of Ndlambe has two desalination plants, namely Bushman's River Mouth, which produces 1.8 ML/day, and Cannon Rocks, which produces 0.75 ML/day. Lamberts Bay has a daily capacity of 1.7 ML. Richards Bay has a desalination plant that was built to produce 10 ML/day of water, but it only produces 6 ML/day. The by-products of these traditional sources outweigh the generated water by a factor of three to five (Sahle-Demessie et al., 2019a). As shown in Table 2.1, traditional sources require a significant amount of thermoelectric energy; specifically, it takes 4 to 8 kWh of electrical power per m^3 to produce freshwater, and more than 1.8 kg is emitted into the atmosphere per m^3 , in addition to the concentrated brine being released back into the ocean (Raluy et al., 2004; Sahle-Demessie et al., 2019a). Hence, this study proposes cyanobacteria-

based biodesalination as an alternative method to unravel the water scarcity problem in South Africa by growing in high salt concentrations while producing a low salt environment. Figure 2.3 below is a modified block flow diagram for desalination process proposed by Burke et al. (2016).

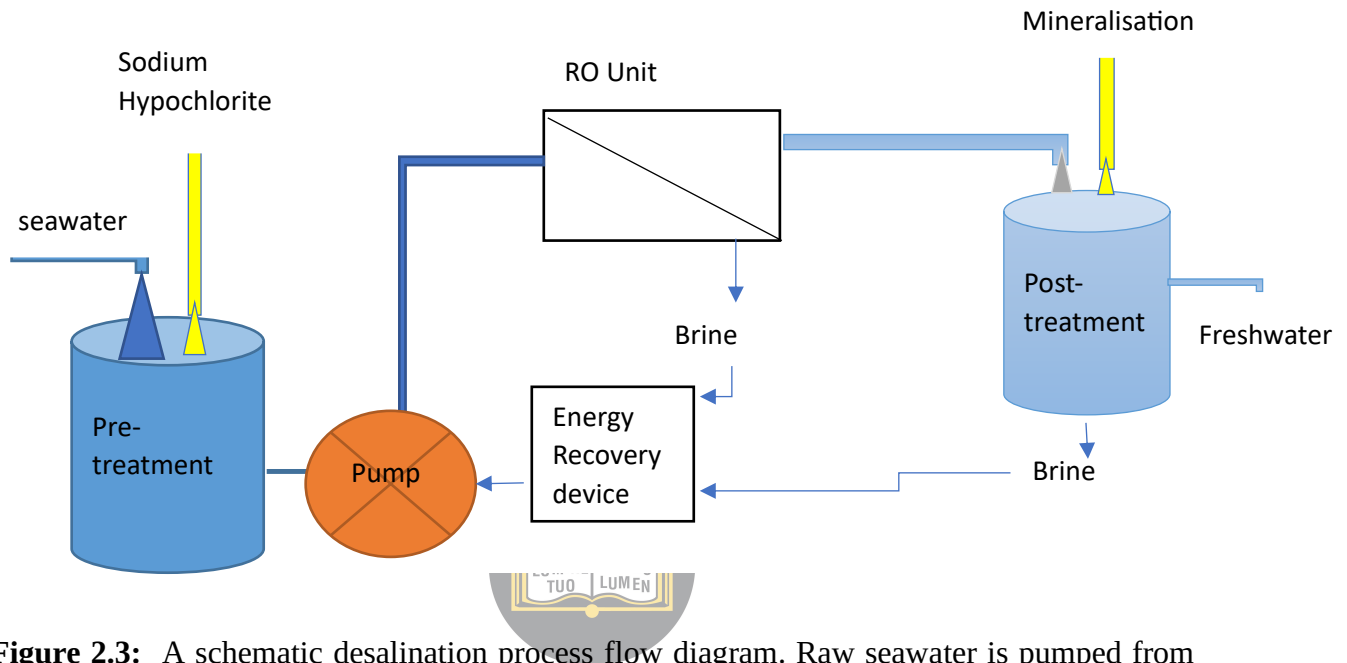


Figure 2.3: A schematic desalination process flow diagram. Raw seawater is pumped from the ocean to the pre-treatment facility. Sodium hypochlorite is used to remove particles and biological organisms that could clog the RO membranes before they are pumped to high pressure. Reverse osmosis occurs in two banks of spiral wound membrane modules, namely a) The brine is directed to an energy recovery unit, which extracts mechanical energy from the compressed concentration and pumps the high concentration seawater back into the ocean. b) To meet drinking water requirements, the filtered reverse osmosis product is transported to posttreatment and remineralised (Burke et al., 2016).

Table 2.1. Comparison of different desalination technologies (Taheri et al., 2016) and seawater desalination plant success in South Africa (Goga, Friedrich, and Buckley, 2019; Mgeni Water, 2022; Sahle-Demessie et al., 2019a).

Desalination technology	Required energy (kWh.m ⁻³)	CO ₂ emissions (kg CO ₂ .m ⁻³)	Potential energy gain (kWh.m ⁻³)
Thermal desalination			
Multi-stage flash Distillation	72.94 - 88.33	20.4-25.3	~0
Multiple-Effect Distillation	43.17 - 66.11	11.8-17.6	~0
Vapor-Compression Evaporation	7.5 - 13.00	0	~0
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Membrane desalination			
Reverse Osmosis	4.00-8.00	1.4-2.79	~0
Biodesalination			
Photosynthetic microbial desalination cells	~0	~0	4.21
MDC			
Successful desalination plants in South Africa	Region	Volume treated	

turnkey RO seawater desalination plant	Mossel Bay - Western Cape	150 Ml/d
RO seawater desalination plant-Cannon Rocks & Boknes Communities	Ndlambe- Eastern Cape	0.75 Ml/d
RO seawater desalination plant	Lamberts Bay - Western Cape	1.7 Ml/d
RO treatment plant is designed	Umgeni water	150 Ml/d
seawater desalination plant	Plettenberg Bay - Eastern Cape	2 Ml/d
Mpumalanga Mine-water reclamation plant	Mpumalanga	30 Ml/d
Seawater desalination plant	Knysna - Western Cape	2 Ml/d
Seawater RO desalination-Quality Filtration	Eastern Cape Port Alfred	2 Ml/d
		3 Ml/d
RO desalination	Richards Bay	6 Ml/d

2.2.2. Biodesalination

The technologies discussed above are expensive and they negatively affect the environment. Other technological developments should therefore be considered to reduce the system's energy and chemical consumption while also improving the environment. Biodesalination, however, is more efficient, cost-effective, and less harmful to the environment, and it can be used as an alternative method to remove sodium chloride, dissolved solids, and gases from the oceans only by using photosynthetic organisms (Minas et al., 2014a; Sahle-Demessie et al., 2019a). Biodesalination uses macro- and micro-algae, cyanobacteria, and other living microorganisms

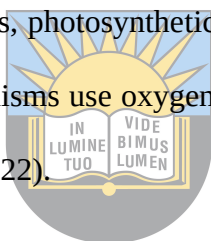
such as bacteria and zooplanktons to remove salt from halophilic environments. In a biodesalination application, the active export of ions is stopped by starving the cells of energy. The intracellular sodium (Na^+) accumulation replaces the Na^+ export to generate cells (Minas et al., 2014a). Light-driven sodium flux facilitated by molecular units into the cells is expressed while endogenous Na^+ export is inhibited. Biological switches build a robust control system that expresses the salt accumulating molecular units correctly (Amezaga et al., 2014a). Biodesalination relies on two fundamental mechanisms, namely biosorption and bioaccumulation (Kumar et al., 2021). Biosorption is a passive process that adsorbed metal ions on the surface of dead biomass, while bioaccumulation is an active process that uses living organisms and accumulate ions within the cell (Goa et al., 2021; Kumar et al., 2021, Leong and Chang, 2020). The biosorption and bioaccumulation processes of microalgae for desalination have not been extensively studied, and the majority of the current data available focuses on heavy metals and other persistent pollutants. It has been noted that the microalgae, among other microorganisms, have exceptional biological properties that are critical in remediation of heavy metals due to their high binding affinity, abundance of binding sites, and large surface area (Leong and Chang, 2020). Therefore, the fundamental goal of biodesalination is to cultivate microorganisms to achieve maximal salt removal from brackish or saline water.

Wei et al., (2020) investigated the mechanisms of algae-based desalination using fresh and dried microalgae *Scenedesmus obliquus*. They discovered that dry algae removed NaCl and desalinated at a slightly higher rate than fresh algae; however, as salinity increased, fresh algae adsorption of NaCl increased more than dry algae. They concluded that there are two possible explanations for this phenomenon. Firstly, because dried algae lose water inside their cells, fresh algae have a greater surface area and more accessible free sites for ion adsorption. Secondly, fresh algae may also absorb salts intracellularly. Therefore, instead of using living biomass, dead biomass has the advantages of requiring no nutrients or energy sources, having

a higher sorption capacity, faster processes, no harmful effects from contaminants, and being easier to recover contaminants, allowing for biomass regeneration. In addition to these advantages, the desorption process enables the reuse of dead microbial biomass (Leong and Chang, 2020).

2.2.2.1. Photosynthetic organisms in biodesalination

Bacteria have been used in municipal and industrial wastewater treatment plants to purify water (Singh et al., 2016a). Most species are harmed by ionic, osmotic, and oxidative stressors, and high salinity is lethal to organisms that use light for photosynthesis (Pade and Hagemann, 2014). However, photosynthetic bacteria from salty environments such as aqueous solutions and soils have removed sodium and enhanced the rates of seed germination (Minas et al., 2014a). Because of its unique qualities, photosynthetic desalination has drawn the interest of several scholars. Photosynthetic organisms use oxygen produced during photosynthesis as an electron acceptor (Shaikhoun et al., 2022).



2.2.2.2. Algae-based desalination

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Algae-based desalination is an emerging desalination method that has received less attention than prior technologies. Algae and cyanobacteria absorb ions that have a negative impact on water quality as a source of nutrition for growth. Halotolerant cyanobacteria and algae are common in halophilic environments like saline marshes and salt lakes (Kirkwood et al., 2008). Cyanobacteria take up ions and minerals through different metabolic processes, such as biosorption and bioaccumulation (Wei et al., 2020). Extracellular polymeric substances (EPS), functional groups, and various charges all contribute to the binding of ions to the outer cell walls of living organisms during biosorption (Sahle-Demessie et al., 2019a; Wei et al., 2020). By means of ionic transport and diffusion, cyanobacteria consume ions during the bioaccumulation process, which causes the ions to progressively build up inside the cells.

However, in biosorption the presence of different charges, extracellular polymeric substances (EPS), and functional groups, allows ions to bind to the outer cell walls of living organisms (Zafar et al., 2021). Algae/cyanobacteria biodesalination efficiency can be increased by considering growth parameters such as species selection, light intensity, nutritional media, air, and carbon dioxide (Singh and Singh 2015; Gao et al. 2021) as discussed in sections 2.3 and 4. Previous research has shown that the use of cyanobacteria as well as the photosynthetic organisms as listed in Table 2.1 using direct injection or suspensions have produced salt removal results of >5% under various time and condition parameters. Costs of the algae-based desalination process are likely to be high because of extra nutrients and carbon required to sustain the entire life cycle of cyanobacteria. There are various ways that can be used to minimise the costs, including:

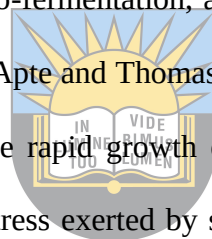
(1) The biomass generated during desalination can be used to make a variety of other industrial products such as biofuels, cosmetics, and animal feed; therefore, profit generated from these industrial products can help reduce the high costs.

(2) Wastewater is rich in nutrients such as nitrates, ammonia, phosphate, etc. needed by cyanobacteria for growth; therefore, wastewater can be supplemented as an additional source of nutrition to the cyanobacterial desalination suspension. However, even though cyanobacteria are nitrogen fixing organisms, impact assessments need to be evaluated to establish if the high saline concentrations do not affect nitrogen cycle.

2.2.2.3. Selection of desalination strains

In response to ionic stress, cyanobacteria have shown accumulation of intra- or extra-cellular osmolytes (Apte and Thomas, 1997a). The body of cyanobacteria has a collection of sodium ion exporter proteins. Sodium ion export occurs either directly via sodium ion pumping ATPases or indirectly via hydrogen pumping ATPases since they get their energy from ATP

(Maiti, 2019). Additional research demonstrates that the bound extracellular polymeric substances (EPS) from two strains of the *Anabaena* genus – a freshwater strain (*Anabaena* L-31) and a brackish strain (*Anabaena torulosa*) – accumulated more than 90% of salt, while the remaining were osmotically active and internalised. The intake of net Na^+ was lower in the brackish strain than in the freshwater strain. This may be caused by the higher sodium efflux capacity of the freshwater strains (Apte and Thomas, 1983a, 1997a; Reed et al. 1985). Environmental conditions such as alkaline pH, extracellular potassium, and the presence of ammonia change with the magnitudes of sodium influx and efflux, as demonstrated by the sodium influx reduction in these two strains (Reed et al., 1985). Cyanobacteria accumulate compatible solutes that protect the cells against salt stress (Carrieri et al., 2010). Furthermore, cyanobacteria are also capable of auto-fermentation, and internally stored carbohydrates can be catabolised, including osmolytes (Apte and Thomas, 1983). Enough light and nutrients are thermodynamically favourable for the rapid growth of cyanobacteria; the sugars stored as energy allow the cell to handle the stress exerted by salt. In addition, cells with insufficient energy or that may have depleted their energy are not able to export Na^+ (Minas et al., 2014a).

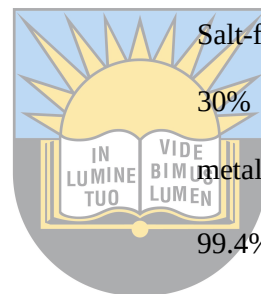


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Table 2.2: Successful biodesalination projects using photosynthetic organisms.

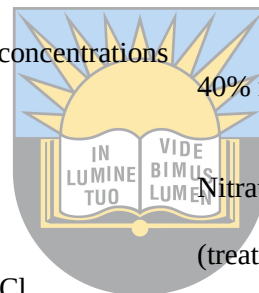
Desalinating Species	Media Used	Desalination Efficiency	Reference
<i>Synechocystis</i> (Cyanobacteria)	PCC7002 ATCC medium 957	More than 50% salinity reduction	(Azmi et al., 2018)
<i>Synechocystis</i> cyanobacteria	PCC6803 BG-11	5% salinity reduction after 10 minutes	(Minas et al., 2014a)
<i>Synechococcus</i> (cyanobacteria)	PCC7002 BG-11 Seawater (BG11-SW)	5% salinity reduction after 10 minutes	(Minas et al., 2014a)
<i>Anabaena</i> (Cyanobacteria)	<i>torulosa</i> Five-fold nitrogen-free Cyanophycean medium	90% salt accumulation to EPS	(Apte and Thomas, 1997a)
<i>Anabaena</i> (Cyanobacteria)	<i>L-31</i> Five-fold nitrogen-free Cyanophycean medium	9.6 µg/Na ⁺ N uptake	(Apte et al., 1987)
<i>Avecennia marina</i> and <i>Vibrio alginolyticus</i>	NA and NB medium with 15 ‰ and 25 ‰ NaCl.	49.16% and 40.58% salinity removal	(Chimayati and Titah, 2019)

<i>Rhizophora mucronata</i> and <i>Vibrio alginolyticus</i>		NA and NB medium with 15 ‰ and 25 ‰ NaCl	64.68% and 40.18% salinity removal	(Chimayati and Titah, 2019)
<i>Scenedesmus</i> (Algae)		<i>species</i> Biofilter-Media supplement with 20 g/l salt	More than 200 mM accumulation of Na ⁺ and Cl ⁻ in cells	(Sahle-Demessie et al., 2019a)
<i>Chlorella</i> (Algae)		<i>vulgaris</i> Biofilter-Media	More than 200 mM accumulation of Na ⁺ and Cl ⁻ cells	(Sahle-Demessie et al., 2019a)
<i>cf. Peridia tenuis</i>		bucket of seawater	Salt-free	(Sahle-Demessie et al., 2019a)
Shrimp ponds and purple non-sulfur bacteria (NW16 and KMS24)		GM broth	30% removal of salts and heavy metals	(Panwichian et al., 2010a)
<i>Synechocystis</i> (Cyanobacteria)	PCC 6803	Simulated saline	99.4% removal efficiencies of ammonium and phosphate	(Abedi et al., 2019)
		BG-11 media	97.5% ammonium and phosphate removal	
<i>Desmodesmus subspicatus</i> and <i>D. armatus</i>		Seawater + BG-11 media	Seawater salinity reduced 37.5 to 26.25 g/ L	(Ahamefule et al., 2021)
<i>Dictyosphaerium</i> spp.		Seawater + BG-11 media	Seawater salinity reduced 37.5 to 30.00 g/L	(Ahamefule et al., 2021)



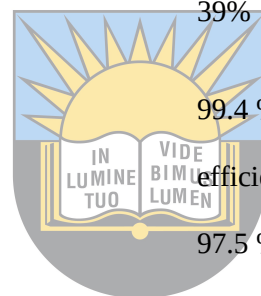
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<i>S. Phormidium</i> (Cyanobacteria)	<i>keutzingianum</i>	BG-11+	46%	(Zafar et al., 2022a;
		2885	50%	
			29%	
			40%	
<i>Chlorella vulgaris</i> (Algae)		M.BG-11 with NaCl concentrations about 1000 ppm	80% reduction efficiency	(Barahoei et al., 2021a)
		M.BG-11 with NaCl concentrations about 5000 ppm	40% reduction efficiency	
			Nitrate removal from 68.2 (treatment) to 97.9% .	
		BBM + 10 000 mg/ L NaCl	Phosphate removal from 25.8 to 43%	
<i>Coelastrum morus</i> (Algae)			Conductivity reduction 47.4 to 69.7%	(Figler et al., 2021)
		BBM + 5000 mg/ L NaCl	Chlorine Removal from 23.5 to 55.7%	



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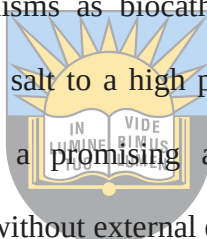
<i>Scenedesmus obliquus</i> (Algae)	BG-11 media+ NaCl	15% reduction efficiency 20% reduction efficiency	(Wei et al., 2020)
<i>Chlorella vulgaris</i> (Algae)	BBM	27% reduction efficiency	(Figler et al., 2019)
<i>Scenedesmus obliquus</i> (Algae)	f/2 medium	30%	(Sahle-Demessie et al., 2019)
<i>Scenedesmus obliquus</i> (Algae)	BBM	39%	(Figler et al., 2019)
<i>Synechocystis</i> PCC6803	Simulated saline + BG11 media	99.4 % ammonium removal efficiency 97.5 % phosphate removal efficiency	Abedi et al. (2019)
<i>Dictyosphaerium</i> spp.	Seawater + BG-11 media	Seawater salinity reduced 37.5 to 30.00 g/l	Ahamefule et al. (2021)



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2.2.3. Microbial Desalination Cell (MDC)

Microbial Desalination Cell (MDC) is a novel sustainable technology that can generate electricity, desalinate water, and remove contaminants from industrial or residential wastewater (Ponnusamy, and Mohamed, 2022). It addresses both energy demand and environmental sustainability. MDC is derived from modified microbial fuel cells (MFC). A pair of anion exchange membranes (AEM) and cation exchange membranes (CEM) are installed between the anode and cathode, which are filled with desalinated water (Cao et al., 2009). This method of desalination is like electrodialysis, except that electricity is generated while the water is being desalinated, and an external electrical energy source is used to supply the energy for separating the ionic species. Several researchers looked at the notion of a microbial desalination cell (MDC), which uses microorganisms as biocathodes. Various lab-scale studies have demonstrated that MDCs may reduce salt to a high proportion (>90%). MDC has attracted great attention because it provides a promising approach for desalinating water and simultaneously generating electricity without external energy (Al-Mamun et al., 2018).



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2.3 Cyanobacteria

Cyanobacteria are a large group of microorganisms; found in diverse habitats on the earth's surface (Stal and Moezelaar, 2006a). These species can grow in different environments such as light intensity, temperature, pH, salinity, and nutrients, mainly carbon, nitrogen, and phosphorus source (Whitton, 2006). They are prominently found in marine and limnic environments and conditions that do not promote the growth of other microalgae (Apte and Thomas, 1997). They belong to the phylum of photoautotrophic that can combine oxygenic photosynthesis like chloroplast with features of typical prokaryotic photosynthesis (Nubel et al., 2000; Whitton and Potts, 2000). Although oxygenic photosynthesis is carried out by all cyanobacteria, some species use sulphide as an electron donor to switch to a normal bacterial anoxygenic photosynthesis. Certain strains require minimum nutrients because they fix the

atmospheric dinitrogen (Bergman et al., 2006) resulting in reduced pH, electron conductivity, and sodium in the surrounding environment (Singh and Dhar 2010).

Cyanobacteria have a unique cell that resembles gram-negative bacteria in structure. They are single-cell organisms that grow in colonies or filaments, and their cell division can take place via binary fission or invagination of the plasma membrane and peptidoglycan layer. *Microcystis* sp. cells that grow in colony form are packed in a mucilaginous sheath and the cells growing in filaments grow as free-floating strands or in floating mats (Hitzfeld, Höger, and Dietrich, 2000). Anagnostidis and Komárek (1988) propose and recognise four of the most extensively accepted systems of cyanobacterial taxonomy orders which may have, with or without a sheath, whether in a single trichome or bundles form, either thick or thin. *Chroococcales* (unicellular species), *Oscillatoriales* (simple unbranched filaments), *Nostocales* (heterocyst-containing filaments), and *Stigonemetales* (branched filaments with heterocysts) (Hitzfeld et al., 2000). The outer membrane of the cyanobacterial cell is combined with lipopolysaccharides but has an oxygen-evolving photosynthetic system like that of higher plants and eukaryotic algae (De Philippis and Vincenzini, 2003a) and the cells are surrounded by polysaccharidic structures in some cyanobacterial strains (Sun et al., 2017). As shown in Figure 2.4 below, cyanobacteria are called blue-green algae because the blue pigment phycocyanin masks the presence of chlorophyll-a, giving it a blue-green colour. These pigments are light-harvesting structures for photosystems found in phycobilisomes (Mur, Skulberg, and Utkilen, 1999).

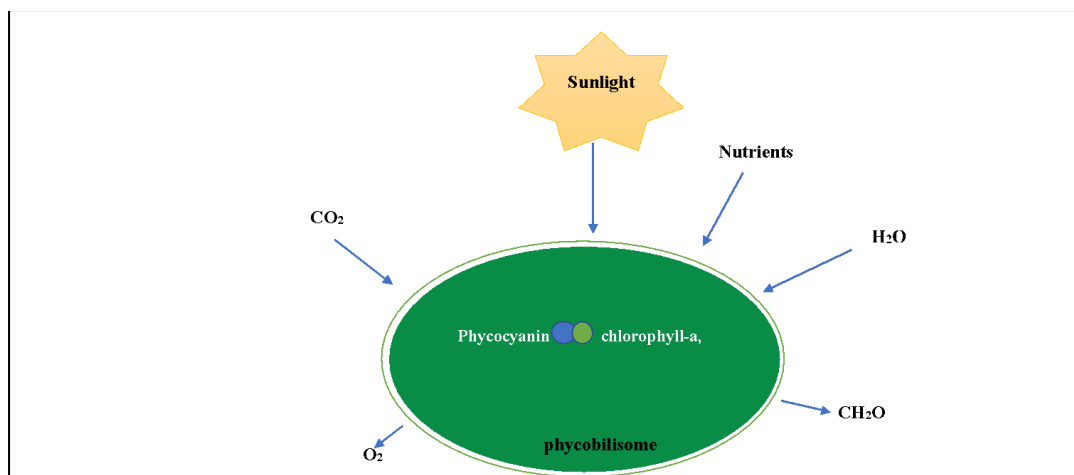


Figure 2.4. The schematic illustrating the photosynthetic reaction carried out by cyanobacteria: the blue pigment phycocyanin masks the presence of chlorophyll-a, giving cyanobacteria a blue-green colour (Mur, Skulberg, and Utkilen, 1999).

Microalgae desalination results in a desalination activity that is cost-effective and energy-efficient when compared to conventional desalination technology as shown in Table 2.1. Innovative biodesalination techniques, particularly the use of cyanobacteria, can desalinate not only saline water, but are also widely used for phytoremediation and can generate electricity and biofuel without harming the environment (Gao et al., 2021a; Minas et al., 2014a). Furthermore, this proposed technology harvests the enormous energy stored in autotrophic marine life, biological membranes, and ion gradients, with obvious societal benefits such as reducing greenhouse gas emissions, improving soil fertility, detoxifying heavy metals, and extracting bio-compounds for cosmetics and medicine (Gao et al., 2021a; Kumar et al., 2021; Singh and Singh, 2015; Wei et al., 2020; Yensen, 2006b; Zafar, Javed, and Aly Hassan, 2022a). Despite the appealing promise of this technology, there are several health concerns associated with the use of synthetic biological methods for desalination, particularly the use of cyanobacteria, which has a known toxicity risk (Amezaga et al., 2014a; Hunter et al., 2012).

General concerns about cyanobacteria in drinking water are their ability to release toxins, unpleasant taste, and odour, which greatly impair water quality and endanger human and

animal health (Chow et al., 1999; Drikas et al., 2001; Şengül, Tüfekçi, and Aktan, 2016); therefore, environmental safety needs to be addressed (Bubela, Hagen, and Einsiedel, 2012; Hunter et al., 2012). One well-known toxin called microcystin activates the liver tumour promoter and inhibits specific enzyme systems that protect internal organs, resulting in selective damage to the gut and liver (Chow et al., 1999). Cyanotoxins can be encased in cell walls, exist intracellularly in the cytoplasm, or be released into the environment as extracellular cyanotoxins through excretion, cell lysis, or cell breakage (Amezaga et al., 2014a; Westrick et al., 2010). Strategies on the various methods tested to reduce the chance of contamination of cyanobacteria in drinking water are the major focus of many researchers around the world.

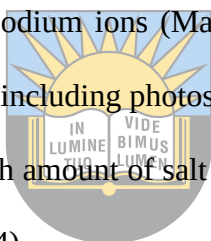
Toxins and cyano metabolites increase chemical demand, the formation of disinfection by-products, and microbial growth in the distribution system. One of the most important factors influencing cyanotoxin removal/inactivation in water is determining whether the cyanotoxin is extracellular or intracellular (Westrick et al., 2010). This necessitates cell separation of cyanobacteria cells from desalinated water and an *in vitro* toxicology investigation to assess the potential risk associated with desalinated water. Owing to the similar density of water and cyanobacterial cells, as well as their colloidal behaviour, it is difficult to remove cyanobacterial cells from an aqueous solution (Minas et al., 2014a). We will be able to investigate ion mobility between saline media and viable cells more thoroughly if we develop an analytical methodology that detects traces of Na^+ and Cl^- in the water to avoid cell lysis, reversing salts, and toxins, as well as to characterise cyanobacterial cells when subjected to desalinated water.

More research on cell separation techniques is required to address the water quality issues caused by extensive cyanobacterial growth, as well as the potential hazards caused by cyanobacterial release of toxins and odour into desalinated water. This is done to find a new solution to South Africa's water security problem. EPS characterisation and the creation of a regulated cell-aggregation process to safely extract cyanobacterial cells from desalinated water

without compromising cell integrity and return the salt to the desalinated water are examples of cell removal methods. Previously removed cells can be re-inoculated into the growth chamber to restart the process.

2.4. Mechanism of desalination process by algae/cyanobacteria

Sodium-ion (Na^+) is required for the development of cyanobacteria and the action of nitrogenases in nitrogen-fixing species such as *Anabaena torulosa*, *Anabaena* L-31, and *Plectonema boryanum* (Apte, Reddy, and Thomas, 1987; Chimayati and Titah, 2019). Adverse effects of sodium ion concentration differ in different strains depending on the environmental factors, and the organism's internal factors (Marin et al., 2004). Photosynthesis depends upon counter anions that are carrying necessary chemicals. Besides cell division, pH homeostasis in the basic environment also requires sodium ions (Maiti, 2019; Miller, Turpin, and Canvin, 1984). However, cellular metabolism, including photosynthesis, transcription, and translation, in some strains, is inhibited by the high amount of salt concentration in the cytoplasm (Miller et al., 1984; Pade and Hagemann, 2014).



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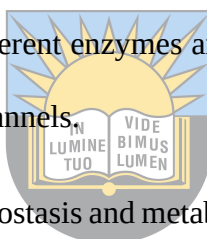
Acclimatisation to high salinity is critical, and genes play a vital role in salt adaptation and regulation in cyanobacteria. Under hypersaline conditions, sodium enters cells along an electrochemical gradient. Many metabolic processes in both eukaryotic and prokaryotic cells are regulated by Na^+ . Sodium is the most common inorganic cation in oceans and other saline environments (Hagemann, 2011a; Whitton, 2006). Exceeding intracellular sodium ion concentration brings harmful effects such as destabilising fatty acid balance in the cell membrane, and termination of autotrophic growth (Bhargava et al., 2003). As per Figure 2.5, salt-shocked cells export Na^+ very quickly to keep balance inside the cell and prevent the cells from shrinking or lysing (Blumwald, Mehlhorn, and Packer, 1983). Cells experience a salt shock and immediately lose water and rapidly shrink when exposed to excessive amounts of

NaCl. The rapid influx of external salts, though, allows water to return to the cell and reverse the shrinkage (Reed et al., 1985). Gao et al., (2021a) define this process in three stages, namely:

(1) Adaptability in morphology – In response to the high salt content in their medium, algae quickly adjust their turgor pressure (organism with a cell wall) or volume (well-less cells). This is caused by the cytoplasmic outflow of water and a variety of other solutes.

(2) Advanced osmoregulation – By maintaining their turgor or volume and metabolism, algae need advanced osmoregulation to survive and adapt to hypersaline circumstances. Advanced osmoregulation involves ion exchange (ion uptake/release), osmolyte accumulation/deprivation (exchange of organic and inorganic osmolytes), activation/inactivation of different enzymes and transport proteins, and regulation of pertinent water and solute channels.

(3) Re-establishment of homeostasis and metabolism – Algae gradually adapt to a new steady state in which homeostasis and metabolism are restored after surviving the initial salt shock and continuing to thrive in very saline surroundings.



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2.4.1. Biosorption and bioaccumulation

Biosorption is a reversible, metabolically energy-independent process that occurs on the surface. Two mechanisms are involved in the process of microalgal biosorption, namely:

(1) Ion exchange - when Na^+ , which is bound to the reactive groups on the algal cell surface, exchanges with other cations (Kumar et al., 2021). Ion-exchange or diffusion technique is employed when salt accumulates inside the cell. The concentration gradient causes salt to diffuse slowly into the cell with the help of ATPase enzymes or ATP as an energy source (Timková, Sedláková-Kaduková, and Pristaš, 2018).

Cyanobacteria, though, have ATP-independent light-activated biological batteries known as ‘‘halorhodopsin’’ proteins (Amezaga et al., 2014a; Sahle-Demessie et al., 2019a). Cl⁻specific pumps in halorhodopsin remove chloride ions from brackish or hypersaline water (Shaikhoun et al., 2022a).

(2). Complexation – is when salt ions bond with reactive groups. These adsorptions primarily rely on reactive groups that adsorb salt ions via carboxyl, hydroxy, thiol, amino, and phosphate actions (Kumar et al., 2021). Bioaccumulation, an energy-dependent biological process in living cells, causes salt and other ions to build up inside the cell (Chojnacka, 2010; Fal et al., 2022). It is a two-step process that begins with a quick passive salt adsorption followed by continuous intracellular diffusion on the cell's surface. Bioaccumulation takes time since salt absorption does not begin until the cells are active.

Bacteria regulate osmosis pressure within cells using an osmoregulation mechanism to reduce toxicity from high levels of Na⁺ concentrations in their cytoplasm (Taheri et al., 2016; Yensen 2006b). The accumulation of compatible solutes and the active export of inorganic ions maintain the osmotic balance within the cell (Pandhal, Wright, and Biggs, 2008). These compatible solutes are protein synthesis independent, responsible for establishing and maintaining the turgor pressure of salt-stressed cells (Hagemann, 2011a). Poor salt tolerance strains accumulate carbohydrates such as glucosylglycerol, trehalose, or sucrose, whereas moderate salt-tolerant strains accumulate glucosylglycerol, and sometimes, a second metabolite called glucosylglycerate; glycine betaine and glutamate betaine are major intracellular organic osmoticum, synthesised by highly halotolerant strains. The freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 (Syn7942) rapidly accumulates sucrose after the enzyme sucrose-phosphate synthase is activated, which indirectly increases ion concentration (Liang et al., 2020). The osmoprotectant glycine betaine, for example, is

accumulated by *Methanohalophilus portucalensis* via the glycine betaine transporter. *Halobacillus halophilus* is a moderate halophile that has developed a novel hybrid mechanism to adapt to changes in salinity in its environment (Taheri et al., 2016).

2.4.2. Osmoregulation

Osmoregulation is a natural mechanism used by halophilic bacteria such as cyanobacteria to channel salts out of the cell (Pade and Hagemann 2014). One of the important strategies to maintain the intracellular ion balance against salt stress of cells is to express related transporters (Bhunia et al., 2018). Different Na^+/H^+ antiporters, Mrp-systems, and Na^+ -pumping ATPase transporters have been discovered in some cyanobacterial strains. The K^+ gradient is maintained in bacteria that have adapted to saltwater conditions by a combination of Na^+/H^+ antiporters and K^+ uniporters. Bacteria with an efficient Na^+/K^+ pump system accumulate primarily organic compounds such as sugars, amino acids, and their derivatives when subjected to osmotic stress (Cui et al., 2020; Minas et al., 2014a). Except for the unicellular *Anacystis nidulans*, which have an active, energy-dependent cation extrusion, the ionic relations of cyanobacteria are poorly understood (Apte et al., 1987; Chinyama et al., 2016; Hagemann, 2011a).

Evidence shows that a turgor-sensitive K^+ influx occurs during salt stress and that K^+ accumulation occurs before organic solute accumulation. The halotolerant cyanobacterium *Aphanothece halophytica* was found to have a putative F₀F₁-type Na^+ -pumping ATPase, and *Acaryochloris marina* MBIC11017 and *Synechococcus* sp. PCC 7002 were also found to have a homologous operon; however, their role in salt tolerance is still unknown, and more research is thus required (Apte and Thomas, 1997a; Cui et al., 2020; Soontharapirakkul et al., 2011).

Figure 2.5 below illustrates a cyanobacterial biodesalination mechanism during a culture's growth phase. Many marine species use biodesalination to ensure their survival by creating a

salt-free or low-salt biological zone within saline water that acts as an ion-exchanger (Maiti, 2019). Cells inside the salty environment create a reservoir with low salt concentration by using endogenous transport proteins across the plasma membrane via active Na^+ export (Amezaga et al., 2014a). Na^+ is physiologically used by halophilic bacteria to generate electrochemical gradients and improve membrane stability. They are getting their energy from ATP and therefore sodium ion export happens directly through sodium ion pumping ATPases or indirectly through hydrogen pumping ATPases (Maiti, 2019). The harmful ions are activated and expelled from the cell's metabolically active region via Na^+ export mechanisms (Whitton, 2006). To regulate the internal pH, highly pH-dependent Na^+/H^+ antiporters or ATP (Na^+ -ATPases) are energised by cytochrome oxidase activity. The H^+ gradient is also in charge of removing Na^+ from the cell (Singh et al., 2016a). As a result, Na^+ export from the cytoplasm is dependent on ATP and the proton motive force. The salt tolerance of *Anabaena torulosa* and *Anabaena* sp. strain L-31 at alkaline pH appear to be related to Na^+ transport. This was demonstrated by reduced Na^+ influx at pH 8 (Apte et al., 1987). The proton motive force is created from the energy generated by the light source and captured by two ATP-synthases in the thylakoid, namely photosystems and chemiosmosis.

In the desalination phase, photosynthetic ATP production is inhibited to prevent Na^+ export, and halorhodopsin uses light energy to directly pump chloride into the cells. The negatively charged cell results in a potentially negatively charged membrane. The Na^+ is drawn to the negative membrane and is channelled into the cell through a Na^+ -permeable channel (Amezaga et al., 2014a). Na^+/H^+ antiporters catalyse the generalised transport reaction as follows: Na^+ (in) + 2H^+ (out) $\leftrightarrow$ Na^+ (out) + 2H^+ (in). The mechanism of desalination process by cyanobacteria is shown in Figure 2.5 below.

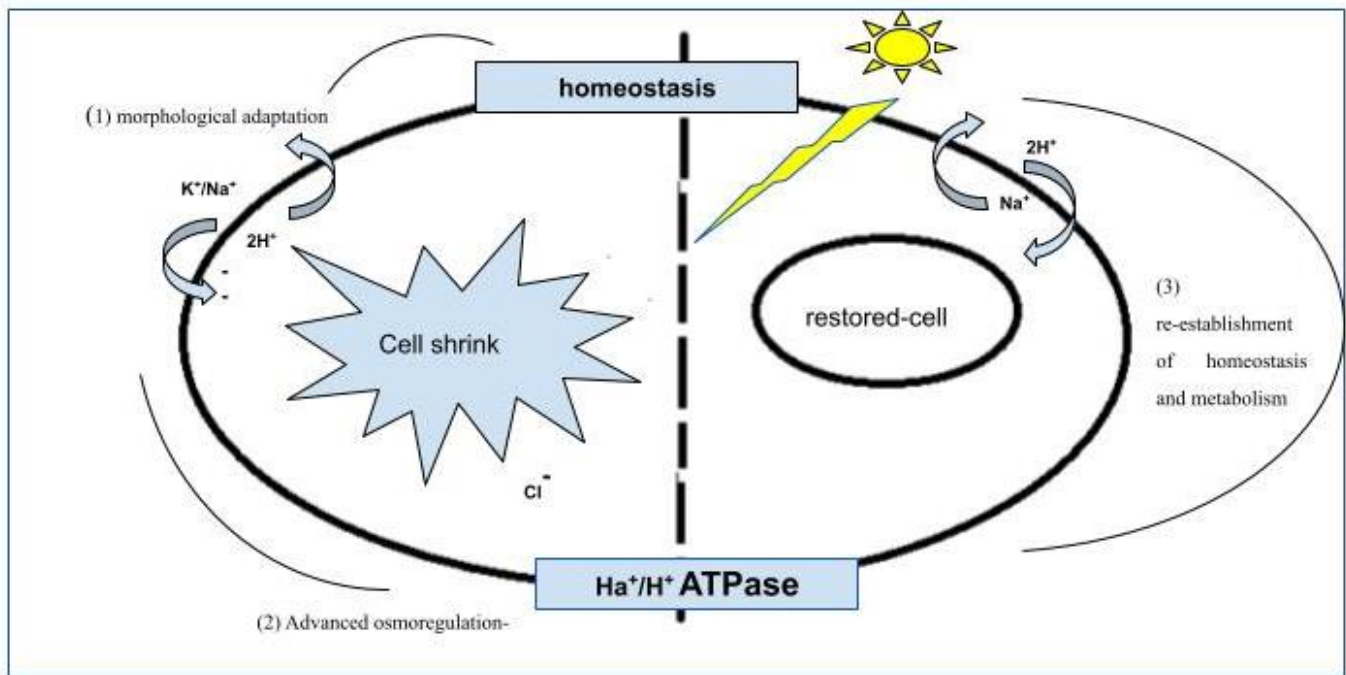


Figure 2.5. Mechanism of desalination process by microalgae (Amezaga et al., 2014; Gao et al., 2021).

Cells experience a salt shock and immediately lose water and rapidly shrink when exposed to high amounts of NaCl. Salt-shocked cells export Na^+ very quickly to keep balance inside the cell and prevent the cells from shrinking or lysing. The rapid influx of external salts, however, allows water to return to the cell and reverse the shrinkage.

Even though *Anabaena* sp. Has been reported to withstand a wide range of salinities; *Anabaena* is one of the toxic cyanobacterial strains (Al-Mamun et al., 2018; Cao et al., 2009; Pade and Hagemann, 2014). The most used species in published data is *Synechococcus* sp., particularly the freshwater strain (*Synechocystis* sp. Strain PCC 6803) and the marine strain (*Synechococcus* sp. Strain PCC 7002). This is because of their tolerance for higher salt concentrations, extensive sequencing, and effectiveness across the desalination spectrum. The entire genomes of both strains have been sequenced, and they have both undergone extensive research as model organisms for cyanobacterial photosynthesis and ionic stress tolerance (Azmi et al., 2018; Chen, Cheng, and Chen, 2009; Marin et al., 2004; Apte, Reddy, and Thomas, 1987, 2006;

Miller et al., 1984; Minas et al., 2014a; Vinnemeier and Hagemann, 1999). Minas et al. (2014a) and Azmi et al. (2018) successfully desalinated seawater using *Synechocystis* sp. Strain PCC 6803 and *Synechococcus* sp. Strain PCC 7002 *Synechocystis* sp. PCC 6803 has been tested in cyanobacterial salt acclimation by a moderately halotolerant strain. After an hour of salt addition, Hagemann, (2011a) observed fast ion transport pathways in *Synechococcus* 6301 and *Synechocystis* 6714 cells, as well as an immediate increase in internal Na^+ content and a rapid decrease to levels comparable to long-term salt-acclimated cells.

The function of a gene family of Na^+/H^+ antiporter is analysed in two strategies, namely gene inactivation and gene expression in *E. coli* mutants. The activity of the Na^+/H^+ antiporter in *Synechocystis* 6803 expressed in a salt-sensitive *E. coli* mutant revealed three true antiporters (NhaS1, NhaS3, and NhaS4), with NhaS3 having the highest transport activity and restoring Na^+ tolerance, whereas gene inactivation had no effect on the mutant's salt and pH (Hagemann, 2011a; Vinnemeier and Hagemann, 1999). The adaptation of *Synechocystis* sp. PCC 6803 in high NaCl concentrations is facilitated by the *ntpJ* gene, also known as the slr1509. When cells are exposed to sodium chloride and undergo hyperosmotic shock, and/or when Na^+ is taken up via bicarbonate symport, the *ntpJ* gene acts as the sodium efflux gene (Singh and Dhar, 2010). To activate glucosylglycerol (GG) synthesis, gene expression must be strong and rapid, as observed on the cell of *Synechocystis* sp. PCC 6803, which accumulated an osmoprotective compound glucosylglycerol as the major compatible solute. This cyanobacterial strain's salt-shocked cells showed slightly increased expression, where the biochemical and gene (*ggpS*) expression levels were activated by the key enzyme glucosylglycerolphosphate synthase (*GgpS*) (Marin et al., 2006).

The cyanobacterial genome contains *mrp* operons from different environments. *Mrp*-systems are significant in hypersaline habitats (Cui et al., 2020). Four salt-regulating genes have been

identified in *Synechocystis*: (1) *np60* – which is a gene that encodes a molecular chaperone GroEL; (2) an *IsiA* gene – which encodes for a chlorophyll-binding protein; (3) *slr0082* genes – responsible for encoding a protein of unknown function, and lastly, the *slr0083*, responsible for a putative RNA helicase (Vinnemeier and Hagemann, 1999). In addition, Marin *et al.*, (Marin et al., 2004) report the *slr0689* gene as the major gene that exports Na⁺ from the saline environment.

Even though cyanobacteria could be a game-changer for desalination, both freshwater and seawater cyanobacterial strains can generate a low-salt reservoir of brackish and seawater by manipulating transport proteins in the cell membrane to operate as an ion exchanger. The biggest issue with this method, however, is producing biomass on a large scale. As previously stated, *Synechococcus* spp. are the most used species; hence, further research necessitates the identification of other suitable desalinating species. Looking into a systematic biodesalination (Figure 2.6) that details the steps of the desalination process, including the pre-treatment phase, as well as how to use cyanobacteria as effectively as possible overall, would help. This can be achieved by mixing the growth medium with saltwater in a 1:1 ratio to avoid cells experiencing salt shock and rapidly losing water and shrinking when exposed to high levels of NaCl, allowing for cell growth and viability, and enabling scaling up cyanobacterial growth to satisfy the technology's requirements. Cyanobacterial cells produce secondary metabolites and make use of the sodium ion concentration in the environment when nutrients become limited. In this regard, more robust monitoring of physiological changes induced by ionic stress would improve the applicability of biodesalination methods.

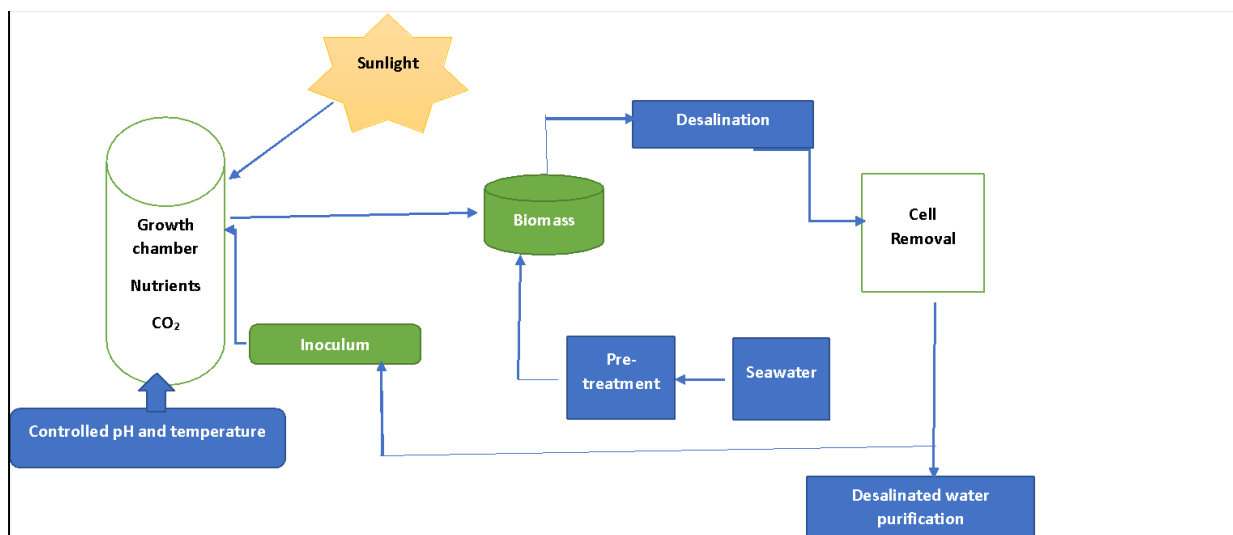


Figure 2.6. A proposed systematic outline for biodesalination stages to be followed when implementing a desalination plant.

2.5. Environmental factors influencing desalination by cyanobacteria.

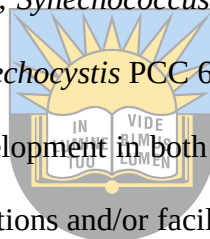
The five essential abiotic factors essential for the autotrophic growth of photosynthetic organisms are light, pH, temperature, carbon dioxide, and nutritional supplements (Al-Mamun et al., 2018; Kumar et al., 2021; Pade and Hagemann, 2014; Singh and Singh, 2015). The presence of cyanobacteria in the water supply has been identified in South African reservoirs, as evidenced at the Hartbeespoort, Rietvlei, and Mockes dams, leading to the classification of the Vaal Dam reservoir as eutrophic based on total in-lake phosphorus and chlorophyll-a (chl-a) content (Chinyama et al., 2016; Harding, 2008). Cyanobacteria have been used as biofertilizers, biocontrol agents, salt-affected soil reclamation, bioremediation, and as a source of bioenergy or biofuel (Singh et al., 2016a). However, there is no evidence that these species have been used to provide water in South Africa. The bioenergy, food, and precious chemicals industries, as well as saltwater desalination, have necessitated large production of cyanobacteria and other microalgae. However, economic sustainability is the most important factor determining the success of adequate biomass production for various commercial uses.

The slow growth rates of cyanobacteria pose a substantial challenge for this method. It is critical to avoid any growth inhibition and optimise the light supply to develop optimal biomass (Minas et al., 2014a; Sahle-Demessie et al., 2019a). As a result, the following parameters are discussed to provide insight into finding solutions to this problem. These environmental parameters are explored in the context of a desalination spectrum to obtain high bacterial biomass. The following environmental parameters were investigated in a controlled saline water inoculation with standardised cyanobacteria culture to find an appropriate adjustment of each parameter to obtain optimal culture conditions.

2.5.1. Effect of light

Cyanobacteria are principally phototrophic in nature and light is essential to their multiplication (Singh et al., 2016a). To grow, cyanobacteria require light energy to use inorganic carbon into organic molecules, such as converting carbon dioxide into carbohydrates (Al-Mamun et al., 2018; Quesada et al., 1998; Singh and Singh 2015). The photosynthetic system that is responsible for energy conversion becomes limited or inhibited at low irradiance (Quesada et al., 1998). The green pigment, which is chlorophyll-a contained in cyanobacteria, harvests light and conducts photosynthesis. The cyanobacterial cell also has phycobiliproteins, which contain other pigments such as a blue pigment called allophycocyanin, phycocyanin which is also blue, and sometimes a red pigment called phycoerythrin (Whitton and Potts, 2000). Cyanobacteria contain phycobiliproteins and chlorophyll-a, which efficiently harvest light energy in green, yellow, and orange pigments on a portion of the 500-650 nm spectrum that is rarely used by other phytoplankton species. According to research, red light promotes cell growth in *Nostoc* sp. and *Synechococcus* sp. strains while blue light inhibits cell growth (Polyzois et al., 2020). A relatively increasing production of carotenoids protects these cells from photoinhibition (Paerl et al., 1983).

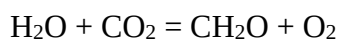
Light intensity, photoperiod, and light quality are all important factors in the growth and metabolism of photosynthetic organisms such as cyanobacteria (Bi and Hu, 2004; Ge et al., 2014; Polyzois et al., 2020; Singh and Singh, 2015). As a result of cyanobacteria being photoautotrophic, they respond to the amount and the quality of light. High light intensity is detrimental to most cyanobacteria when exposed to prolonged periods, and they can lead to the production of cyanotoxins; for instance, the increase of light photoperiod also increases geosmin production in *Phormidium* sp. (Polyzois et al., 2020a). Light intensities greater than $180 \text{ E m}^{-2} \text{ s}^{-1}$ were found to inhibit the growth of *Planktothrix agardhii*, formerly known as *Oscillatoria* (Van Liere and Mur, 1980). Cyanobacteria bloom formed at the surface rivers and reservoirs can tolerate high light intensity up to the reach of $700\text{-}1,000 \mu\text{E m}^{-2} \text{ s}^{-1}$ (Bergström 2010; Paerl et al., 1983). Despite this, *Synechococcus* PCC 7002 appears to grow at a lower light intensity of $25 \text{ mE}^{-2} \text{ s}^{-1}$ than *Synechocystis* PCC 6803, which grows faster at $95 \text{ mE}^{-2} \text{ s}^{-1}$. Intermittent light enables overall development in both strains, which may also allow time for light-independent photosynthesis reactions and/or facilitate carbon concentrating systems that increase photosynthesis by capturing CO_2 (Azmi et al., 2018).



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2.5.2. Effect of carbon source

Cyanobacteria have evolved specialised structures called carboxysomes which contain several other enzymes that speed up the reaction rate when concentrating the CO_2 (Kaplan and Reinhold, 1999). In the presence of light, cyanobacteria mainly fix carbon from sugars through the Calvin-Benson-Bassham cycle for growth and energy storage in the form of glycogen or carbohydrates (Rosgaard et al., 2012). They can use the energy of light to produce organic matter (CH_2O) and oxygen (O_2) out of the water (H_2O) and carbon dioxide (CO_2) through photosynthesis (Stal and Moezelaar, 2006b) as per the formula below, and fermentation can be carried out under anoxic conditions and during the dark (Abraham and Alanís, 2013a; Saad and Atia, 2014; Stal and Moezelaar, 2006b).



In dark conditions, the oxidative pentose phosphate pathway, glycolysis pathway (glycolysis), and the Entner-Doudrofff pathway break down the carbohydrate's storage, generating phosphorylated hexose intermediates, ATP, and reducing equivalents (Chen et al., 2016; Chen et al., 2009). The effects of various carbon and nitrogen sources on cell growth and EPS production in *Aphanothece halophytica* were evaluated by Saad and Atia (2014). Nitrate was found to be the most effective nitrogen source for cyanobacterial cell growth and EPS. Similarly, sodium acetate is the most efficient carbon source for EPS production, and natural CO₂ promotes cell growth (Saad and Atia, 2014).

2.5.3. Effect of nitrogen and phosphate

Nitrogen is one of the best sources that can be used in the growth of cyanobacterial cells and some cyanobacteria are nitrogen-fixing heterocyst (Pang et al., 2018a). Cyanobacteria use different organic and inorganic nitrogen sources for their growth which include urea, nitrate, ammonium, nitrite, amino acids, cyanate, and several species that can fix dinitrogen (N₂) gas to meet their cellular N demand (Herrero, Muro-Pastor, and Flores, 2001). Urea is found to be an excellent and preferred nitrogen source for many cyanobacterial species (Erratt, 2017a), the same as nitrate and ammonium. Inorganic nitrogen assimilation is always converted into ammonium (Zhang et al., 2018a).

The level of natural phosphorus and/ or nitrogen from the lakes and rivers also has a positive impact on the growth of cyanobacterial cells. The rate at which phytoplankton grow in reservoirs and lakes is affected by the concentrations of these nutrients (Bergström, 2010). Phosphorus is recognised as the primary factor influencing phytoplankton productivity in inland waters (Figler et al., 2021). Previous studies have shown a proportional growth relationship between the concentrations of phosphorus in reservoirs and cyanobacterial

abundance (Chaffin et al., 2011), where the trophic state of lakes and rivers is also increased by the increase in total nitrogen and total phosphorus concentrations (Zhang et al., 2016a).

2.5.4. Effects of temperature

Most algae have less optimum growth temperature as compared to cyanobacteria. Green algae and diatom are favoured by low temperatures compared to cyanobacteria that grow well at temperatures over 25 °C (Zhang et al., 2016a). Paerl (2014a) also report that cyanobacteria have an optimum temperature that is higher than 25 °C. This temperature overlaps the temperature of green algae, which is between 27 °C and 32.8 °C, but it differs from that of diatoms and dinoflagellates, i.e., 17–27 °C and 17–22 °C, respectively. Cyanobacteria are insignificant in temperature, ranging from 0-20 °C, only less than 1% of the cyanobacterial biomass is observed (Konopka and Brock, 1978). It was discovered that freshwater cyanobacterial strains have a higher optimal temperature of 30.0-32.5 °C than marine cyanobacterial strains, which have a growth temperature of 20-27.5 °C (Crettaz-Minaglia et al., 2020; Lürling et al., 2013). Even though incubation time varies from strain to strain, *Microcystis*, *Anabaena*, and *Aphanizomenon* thrive at 25 °C, while *Cynlindrospermopsis raciborskii* and *Planktothrix agardhii* thrive at 27.5 °C (Apte et al., 1987; Apte and Thomas, 1983a, 1997a; Crettaz-Minaglia et al., 2020; Konopka and Brock, 1978). Temperatures that are equal to or higher than 25 °C and less than 37 °C may lead to an increase in the rate of cyanobacterial growth, leading to an excessive population of cyanobacteria in controlled water bodies (Kumar et al., 2021; Paerl, 2014a).

2.5.5. Effect of pH

pH has an essential role in the distribution and determination of algal flora composition and its establishment (Roger and Kulasooriya, 1980). The pH of the culture media ranges between 7-10 for optimum cyanobacterial growth (Azmi et al., 2018). Cyanobacteria grow best in neutral

to alkaline environments under natural conditions, which explains the correlation between the pH of the water and cyanobacterial number, soil pH and cyanobacterial growth in paddies (Apte et al., 1987; Apte and Thomas, 1983a; 1997a). Organisms that require a pH optimum above 9 and salt for growth are called halophilic or alkalophilic. Those that require high salt concentrations and an optimum pH growth that is less than 9 but can tolerate pH above 9 are called halotolerant (Madsen et al., 2021). These organisms are stressed under acidic conditions, and they do not grow at pH that is lower than 5, whereas eukaryotic algae grow well under these conditions. Only a few reports confirm the growth of cyanobacteria in acidic conditions. Dense cyanobacterial bloom has been reported in the soil pH of 5.5 in Japan, acid-bog lands in Swedish soils and at pH 3.5 in Kerala (Dominic and Madhusoodanan, 1999; Nayak and Prasanna, 2007).

2.5.6. Effect of salt

Microbes require the uptake of sodium for their growth (Madsen et al., 2021; Singh and Dhar, 2010). The growth of most phytoplankton such as green algae and other eukaryotic phototrophs is very poor at salt concentrations beyond 10%. Only members of cyanobacteria have adapted to high concentrations of salt from phytoplankton species (Oren, 2015). Cyanobacteria need salt for their metabolism. Apte and Thomas (1983a) report that both the freshwater and brackish water nitrogen-fixing cyanobacterium require a minimum level of about 20–25 mM Na⁺ for growth. The metabolism of cyanobacteria adapted to high salt concentrations during evolution, i.e., special structures developed in proteins for cyanobacterial cells to remain active in a salty medium (Müller and Oren, 2003; Pade and Hagemann, 2014). Several cyanobacteria's salt tolerance has been used successfully in the reclamation of brackish soils including *Anabaena* sp. Adaptation of *Nostoe muscorum* to salty medium requires the intercellular changes in thylakoids, in which *Nostoe muscorum* developed a specific physiological capacity to withstand NaCl (Blumwald, Mehlhorn, and Packer, 1983b). Eukaryotes and most prokaryotes



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including cyanobacteria use a salt-out mechanism for acclimation to high or salt-changing concentrations. This mechanism is based on keeping the internal ion concentrations at low levels. This is achieved by two processes; first, at high salt concentrations, the compatible solutes do not interfere with the metabolism of the organisms and, secondly, the inorganic ions are exported gradually and diffuse on electrochemical gradients into the cytoplasm (Brown, 1976; Pade and Hagemann, 2014). The affinity (K_m) of freshwater and brackish water differs, most likely because of the metabolic requirement of Na^+ . Both *Anabaena* L-31 and *A. torulosa* demonstrate rapid Michaelis-Mentor kinetic uptake. *A. torulosa* saturates Na^+ quickly and has a higher affinity for Na^+ than *Anabaena* (Amezaga et al., 2014a; Apte et al., 1987; Apte and Thomas, 1983a; 1997a). Most cyanobacteria are halotolerant, i.e., saline environments favour them, although some require salinity for their survival, i.e., they are known to be halophilic organisms. Marine cyanobacteria are viewed as an excellent source for natural-derived molecules and novel nutraceuticals (Singh et al., 2014b).

2.6. Cell separation from desalinated water

Salt-free soil was observed after cyanobacterial mats were removed by rainfall (Apte and Thomas, 1997a); thus, cells must be separated from desalinated water for efficient salt removal. However, maintaining cell integrity when removing cells from desalinated water and not reverting the salt into the water are the main challenges of this technology (Amezaga et al., 2014a; Westrick et al., 2010). The difficulty is caused by (a) comparable density of the cell and water; (b) organic colloids made by small inorganic particles make the removal of cyanobacterial EPS from conventional water treatment processes difficult, and (c) charged surface stabilises the cell suspensions (Maiti, 2019). Therefore, coagulation and flocculation-based processes are viewed as energy and cost-effective procedures to remove cyanobacteria in conventional drinking water treatments. Inorganic metal salts have been used successfully for effective cyanobacteria separation by neutralising the surface charge; however, cell damage

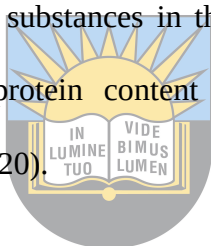
and intracellular components release are associated with the treatment of inorganic salts (Lam and Lee, 2015; Maiti, 2019; Sun et al., 2013). Interestingly, auto-flocculation is an outstanding alternative between the extracellular polymeric material of cyanobacteria and heterotrophic cells (Maiti, 2019; Markou, Chatzipavlidis, and Georgakakis, 2012). In environmental conditions, the cells are exposed without affecting cell membrane integrity (Lee, Lewis, and Ashman, 2009). Cyanobacterial cell density increases when cells absorb the coagulants and the EPS forming insoluble complexes resulting in sediments. The coagulation process has a removal efficiency of up to 80-90% without compromising the cyanobacterial cells (Sun et al., 2013).

2.6.1. Chemical methods

Pre-oxidation is one of the effective pre-treatment techniques, and it is relatively established to control cyanobacterial bloom (Ma et al., 2014; Zhou et al., 2019). The coagulation efficiency of cyanobacteria has been achieved using potassium permanganate, ozone, and chlorine pre-oxidation and these pro-oxidants have been used broadly to oxidise the EPS (Chen, Yeh, and Tseng, 2009). In comparison, chlorine is a strong oxidant; the lower oxidant dose can rapidly degrade most of the EPS; however, it also has the potential to reduce cell activity and rupture the cell structure or kill the organism (Sun et al., 2017). Although potassium permanganate is a better choice, factors such as motility, structure, and EPS influence its performance. The bound EPS consumes more pre-oxidants and weakens oxidation. However, an excessive dose of pre-oxidants will rupture the cell structure and release intracellular toxins (Svane and Eriksen, 2015). Aluminium sulphate flocculation was reported by Drikas et al. to remove 99.9% of a cell using a jar-test apparatus dosed with aluminium sulphate 99.9% without releasing *microcystin* (toxin) in the finished water (Drikas et al., 2001).

2.6.2. Centrifugation

Centrifugation is a physical method, commonly used to remove EPS in water, but it cannot efficiently remove cells without the additional physical and chemical auxiliary forms (Sun et al., 2020). High-speed centrifugation of $10\,000\times g$ efficiently removes cyanobacterial EPS (Flaibani, Olsen, and Painter, 1989). However, there is possible leakage of endotoxins and disruption of the cyanobacterial cell if no careful attention is paid to the process of centrifugation force (Sun et al., 2020). Su *et al.* (2008) observes that the combined 80°C water bath method with low-speed centrifugation effectively separated soluble and bounded EPS of the cyanobacterial cells. Xu *et al.* (2014) combined a 60°C water bath with a buffer extraction method to remove EPS using variable-speed centrifugation. Nevertheless, this technique needs to be monitored to prevent chemical substances in the buffer extraction solution and high temperatures from denaturing the protein content of the organism and rupturing the cyanobacterial cell wall (Sun et al., 2020).



2.6.3. Ultrasonic pre-treatment

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Ultrasonic pre-treatment is an effective method to control cyanobacteria blooms, particularly *Microcystis aeruginosa*, and is more environmentally friendly than other conventional treatments, but implementation conditions of this method are still questionable (Sun et al., 2017). The fundamental principle of ultrasonic pre-treatment is that the gas vesicles are destroyed inside the cyanobacterial cell, and they create sediment (Liang et al., 2009). However, cyanobacterial cells are separated, and the adverse effects of cyanobacterial EPS are weakened because of the clump of cyanobacterial groups at low-power ultrasonic treatment (Sun et al., 2020). The release of intracellular toxins into the desalinated water may be caused by the disruption of the cyanobacteria cell structure if subjected to high-power ultrasonic treatment for a long time (Liang et al., 2009; Zhang et al., 2006).

2.6.4. Activated carbon.

Membrane separation technology has developed rapidly and become the most advanced technology in water treatment facilities to purify drinking water over the years (Sun et al., 2017). Activated carbon mainly filters out particles, bacteria or algae, and other organic pollutants such as EPS. The micro-pore structure and other functional groups on the surface of activated carbons, such as hydroxyl radicals, allow cyanobacterial EPS to combine and remove organic matter (Ni et al., 2018; Sun et al., 2013, 2020; Wang et al., 2021). On a pore size of 30 μm , the activated carbons have more than 98% removal efficiency with a destruction rate of less than 2% on a membrane (Gijssbertsen-Abrahamse et al., 2006). However, the concentration of cyanobacteria is reflected in the adsorption saturation period; the adsorption effect is reduced in the high presence of cyanobacterial cells and EPS. Dramatic reduction of flux results from increased transmembrane pressure and it causes the adhesion of organic particles with remaining cyanobacterial EPS and cells on the surface of the membrane (Teixeira and Rosa, 2005). Besides, the applicability of the cyanobacterial cells and their athletic ability allow growth on the surface of the activated carbon with a high organic concentration in some parts of the cyanobacteria (Shao et al., 2012). Therefore, populated cyanobacterial cells and EPS and cells reduced the efficiency of activated carbons, making the application of this technique redundant (Sun et al., 2017).

2.7. Characterisation of extracellular polymeric substances

Extracellular polymeric substances (EPS) are mainly made up of a complex mixture of nucleic acids, protein (proteoglycans), polysaccharides, and lipids (glycolipids) with functional groups, including hydroxyl, amino, carboxylic, and phosphoric groups (Pereira et al., 2009). Most bacteria can synthesise and secrete both the release EPS into the surrounding environment and covalently linked EPS to the surface of the cell (Neu and Marshall, 1990; De Philippis and Vincenzini, 2003a; Rossi and De Philippis, 2015). These extracellular polymeric substances

produce biofilms and protect the cell against damage, penetration of toxic metals, phage attack, and phagocytosis. Exopolysaccharides have two groups, namely homopolysaccharides, and heteropolysaccharides (Sutherland, 2001). Sucrose synthesise homopolysaccharide by the action of sucrose and has only one type of monosaccharide, whereas heteropolysaccharide is synthesised by different sugar residues of high-molecular-mass hydrated molecules such as glycosyltransferases (De Vuyst et al., 2001). The EPS of cyanobacteria is a heteropolysaccharide in their anionic nature owing to inorganic substituents (phosphate and sulphate), anionic organic (acetyl and pyruvil), and uronic acid (glucuronic acid) (Okajima, Sornkamnerd, and Kaneko, 2018). The cyanobacterial EPS has a molecular weight ranging from 10 kDa to 2 MDa and attaches hundreds to thousands of cyanobacterial cells with diameters ranging from 4~6 μm , resulting in amorphous aggregated particles (Rossi and De Philippis, 2015). Cyanobacterial EPS holds higher complexity among microbes. There is no current agreement regarding EPS classification in cyanobacteria (Delattre et al., 2016). However, cyanobacterial EPS falls between two classes, namely bound EPS or unbound EPS. Bound EPS is associated with cell-bound polysaccharides according to their thickness, consistency, and appearance comprising capsules, sheaths, and slimes (Pereira et al., 2009). The second classification, unbound polysaccharides, is polysaccharides which are released into the surrounding environment (Amezaga et al., 2014a).

The effects of light intensity, pH, temperature, salinity, and growth time also influence the morphology and the formation of the EPS of cyanobacteria (Bi and Hu, 2004). Accumulation of EPS of *Cylindrotheca closterium* is promoted by pH <8 (Sun et al., 2017). The influences of environmental factors, inclusive of temperature, pH, and light intensity play a key role in the reduction of sodium (Azmi et al., 2018). The EPS yield of *Scytonema javanicum* increases with increasing temperature and salinity in a controlled range. The EPS yield of *Nostoc* is also increased by light intensity (Ge et al., 2014). It is also reported that the protein content in the

EPS is increased by continuous exposure to light intensity, whereas monosaccharides remain unaffected. This indicates that the distribution of EPS components and EPS yield are affected by light (Sun et al., 2020; Svane and Eriksen, 2015). The kinetics of *Microcystis flosaquae* EPS synthesis revealed that available nutrients in a system are closely related to the EPS growth rate (Svane and Eriksen, 2015). EPS synthesis was observed at a higher rate of up to 76 mg/(gday) during the exponential growth phase but decreased six times to 12 mg/(gday) during the stationary phase. This suggests that the C/N ratio is closely related to the increase of formed EPS. Heavy metals also have a strong impact on the EPS of the algae and cyanobacteria; the growth of algal EPS is inhibited by the heavy metal Cd^{2+} (Sun et al., 2017).

During aggregation, different functional groups on the cell surface impact the cell. After examining FTIR spectra, three different algae species (*Microcystis* sp., *Chodatella* sp., and *Chlorella vulgaris*) have been found to share a functional group with components that resemble proteins and polysaccharides (Ni et al., 2017; Okajima et al., 2018). According to the results of X-ray photoelectron spectroscopy (XPS) analysis by Minas et al. (2014a), the cell surfaces of freshwater strains and marine strains differ. The surface of the freshwater strain *Synechocystis* PCC 6803 is rich in polysaccharides, whereas the surface of the marine strain *Synechococcus* PCC 7002 is mostly composed of proteins. *Microcystis* has tryptophan-like substances in the bound EPS, whereas *M. aeruginosa* growth has humic- and tryptophan-like substances in the released EPS (Ni et al., 2017).

2.8. Public acceptance

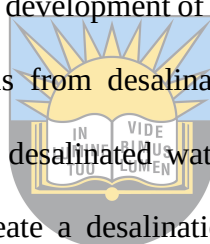
Only a few countries have adopted and operated desalination facilities as a technique of producing water for human consumption due to widespread scepticism of alternative water sources (Dawound, 2005). Social acceptance varies from country to country and issues of public acceptance have been undertaken in countries where there is need for conventional desalination plants as alternative methods for water supply, mainly in countries with serious

droughts. A person who has received formal education has more knowledge and is more likely to accept this technology. It is well-known that levels of acceptance of recycled water are influenced by education, knowledge, financial status, and age (Dolnicar, Hurlimann, and Grun, 2011; Dolnicar and Schäfer, 2006). Optimisation and adjustment of contamination of cyanobacteria are carried out in most water treatment plants. However, the regulation done on the cyanobacterial EPS is not enough because the control strategies focus mainly on eliminating the cyanobacterial cells and toxins degradation (Sun et al., 2020). The problems emerging during the studies of the cyanobacterial EPS are: (1) the contamination of cyanobacterial EPS has high organic content, and its contamination control methods and mechanism are unclear; (2) flocculation performance and other water treatment processes reduce the extracellular wrapping of EPS when cyanobacterial cells are combined with flocculants, and (3) the degradation technology of the EPS is still inefficient and not safe because the residual cyanobacterial EPS in the pipe network are a major contributor to bacteria breeding (Arrington et al., 2003), which consequently brought this technology to low social acceptance risk and lack of public acceptance (Dolnicar et al., 2011). Therefore, methods for detecting the biological effects of organic micropollutants and toxicological *in vitro* methods are required and useful for identifying and detecting toxins in a complex mixture of substances in an event this technique is employed for public health and safety.

2.9. Chapter Summary and future outlooks

The use of natural resources as nutrients for cyanobacterial growth, such as sunlight, carbon dioxide, and minerals, enables cyanobacteria to grow at high densities and opens the door to future biological processes for sustainable water treatment applications. Cyanobacterial strains from both freshwater and saltwater have been shown to form brackish and seawater low-salt reservoirs capable of producing significant biomass. Cyanobacteria can be used as an ion exchanger by manipulating transport proteins in the cell membrane. A pre-treatment stage and

cyanobacteria optimisation. In this regard, more stringent monitoring of physiological changes caused by ionic stress would increase the applicability of biodesalination processes. Understanding how salinity affects cell growth and viability will enable scaling up cyanobacterial growth to meet the demands of water treatment scenarios. There is need for in-depth research of ion mobility in saline media and viable cells, as well as how environmental conditions and nutrient availability can be used in large-scale biodesalination. More investigation into cell separation methods is required to address water quality issues associated with large-scale cyanobacterial growth, as well as the potential risk associated with the cyanobacterial release of toxins and odour into desalinated water while attempting to provide an alternative solution to South Africa's water security problem. These cell separation methods must include EPS characterisation, the development of a controllable cell-aggregation protocol to safely remove cyanobacterial cells from desalinated water without compromising cell integrity, and the revert salt into the desalinated water. Systematic biology and bioreactor technology must be combined to create a desalination prototype based on statistical data generated by various biodesalination stages. Finally, before making desalinated water available to the public, it should be thoroughly tested for toxicity to ensure public health and safety.



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MATERIALS AND METHOD

3.1. Ethical clearance

The ethical clearance # OKO031SMNI01 for this research was obtained from the University of Fort Hare Research Ethics Committee.

3.2. Description of the study area

Buffalo River is the major river for water supply for urban, rural and industry in the Buffalo City Metropolitan Municipality (BCMM). It is 126 km long and originates in the Amatole Mountains where it passes through King William's Town to the Indian Ocean in East London (EOH, 2016). Increasing human activities have contributed to severe pollution with elevated levels of heavy metals and bacterial contamination. Along the river, there are two municipal wastewater treatment plants, namely Zwelitsha and East London wastewater treatment works (WWTW) and industrial discharge of wastewater and leachates from dumping sites in most parts of Buffalo City Municipality (BCM). Agricultural activities of residents of this region, which include farming and animal feed, may be high in nitrogen and phosphorus and can promote or cause excessive fertilization, which contributes to the growth of cyanobacterial blooms. Operational spillages and ship repair contribute to increased pollution in the harbor area of the river (Yahaya et al., 2017).

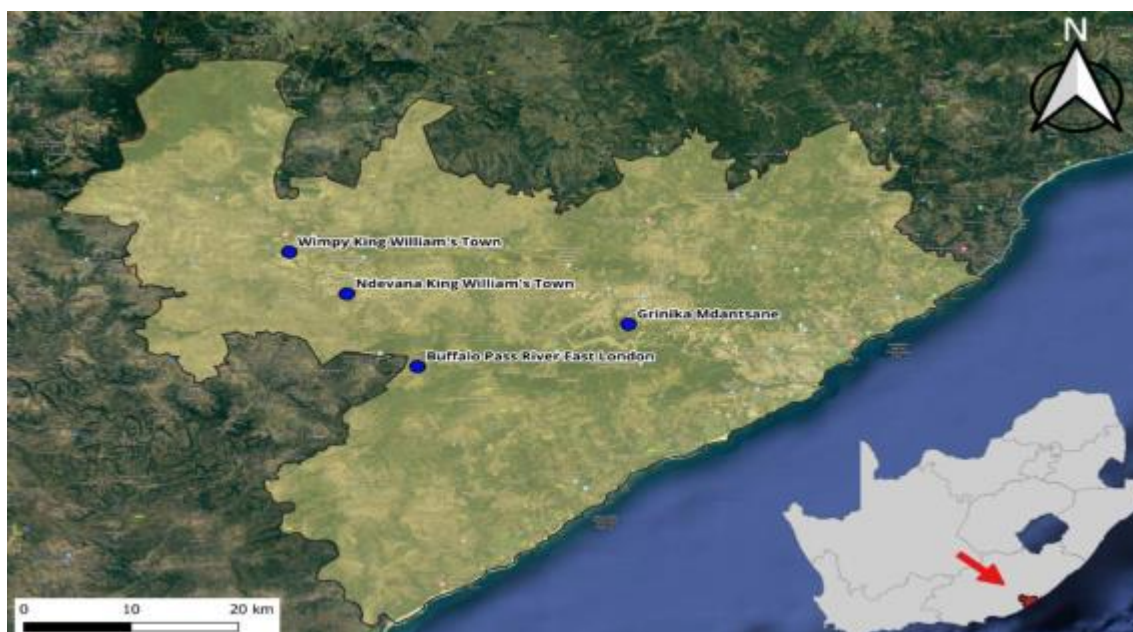


Figure 3.1: Four different sampling points along the Buffalo River (East London Buffalo Pass River, Grinika, Ndevana, and King William's Town), extending from King William's Town to East London were mapped on Quantum Geographic Information System (QGIS).

3.2. Sample collection

Water samples were collected randomly from different points of the Buffalo River, East London, South Africa (i.e., East London Buffalo Pass -33.005998,275139 in East London, Grinika -32.960392,27512 in Mdantsane, Ndevana -32.927617,27.442924.4 in rural areas on King William's Town, and Wimpy -32.882488,27.335204 in King William's Town) using 100 ml sterile containers for the isolation of cyanobacterial isolates. Afterwards, the samples were transported in ice packs to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory for processing and they were stored at room temperature under illuminating conditions.

3.2.1. Sample processing and media preparation

BG-11 Broth Preparation

BG-11 broth (100x concentrate sterile filtered, Cat. No.73816) used for isolation of indigenous cyanobacteria species was prepared according to the manufacturer's instruction (Merck, 2021), by dissolving to a 1:100 dilution (i.e., 10 ml were added to 1000ml sterile water) and its pH adjusted, under aseptic conditions, with 1M NaOH to 7.5. When compared to other bacteria, cyanobacteria have high metal requirements for optimal growth, owing to metal cofactors in the oxygenic photosynthetic electron transfer apparatus, such as cytochromes, plastocyanin, and chlorophyll rings. Hence, 1ml of the Trace Metal Mix A5 with Co was included in the medium



BG-11 Agar Preparation

The dehydrated bacteriological agar (15g/l) was prepared and sterilized by autoclaving and allowed to cool to 50 °C. Thereafter, 10 ml of the prepared BG-11 medium 100x concentrate sterile filtered (Cat. No.73816), was aseptically added to the agar base medium and pH was adjusted, under aseptic conditions, with 1M NaOH to 7.5. One millilitre of sterile trace metal Mix A5 solution was then added to the prepared BG-11 agar. The medium was kept on a 2-8 °C refrigerator for further analysis.

Sample Processing

To prepare a ten-fold serial dilution, 10 ml of each homogenised water sample were added into 90 ml of 8.5% saline solution. The diluted solution was agitated for 30 s using a vortex and further diluted before plating on BG-11 agar to obtain isolated cyanobacterial colonies.

3.3. Isolation of the presumptive cyanobacteria isolates.

Ten millilitres of the sample were inoculated into 90 ml of BG-11 broth, supplemented with 50 mg/l of cycloheximide to eliminate contaminating eukaryotes (Ferris and Hirsch, 1991) and Vitamin B₁₂ and incubated on the rotary shaker (180 rpm) at 30 ± 3 °C for 7 days, under 40 Watts cool white fluorescence bulbs for inoculation preparation.

After incubation, the modified standard procedure of membrane filtration as described by Abraham and Alanís, (2013) was followed to isolate and purify cyanobacteria from water samples. Briefly, collected samples were prepared by a serial dilution and 100 ml of a sample were filtered on 0.45 µm pore size polycarbonate filter membrane using a Vacuum manifold filter system. The filter was placed with the grid side up on solidified BG-11 agar plates and incubated at 30 ± 3 °C for 7 days with 40-Watt fluorescent cool white bulbs. After seven days of incubation, single colonies showing characteristic algal/cyanobacterial colour and morphology were streaked aseptically to a new agar plate, supplemented with 50 mg/l of cycloheximide to eliminate contaminating eukaryotes (Ferris and Hirsch, 1991) and incubated at 30 ± 3 °C for 7 days and at 40 Watts cool white, fluorescent bulbs.

3.4. Screening for biodesalination potential for the presumptive cyanobacterial isolates.

The modified method of Azmi et al., (2018) was used to screen the isolates for desalination potential. Briefly, each presumptive isolate was sub-cultured in 200 ml BG-11 broth contained in 250 ml flask and on the rotary shaker (180 rpm) at 30 ± 3 °C and at 40 Watts cool white, fluorescent bulbs for 7 days. After incubation, the cyanobacteria cells were harvested by centrifugation at 8 000 rpm for 15 min at 25 °C. After centrifugation, cells were washed twice with distilled water and suspended in 100 ml seawater sample in a 250 ml flask maintained on the rotary shaker (180 rpm) at 30 ± 3 °C and at 40 Watts cool white, fluorescent bulbs, and pH

7.5. One flask was left uninoculated as a control. The initial salinity of the uninoculated seawater sample was monitored for each sample and recorded for 7 days. A per cent of salinity reduction was calculated as per the formula below:

$$\text{salinity reduction} = \frac{(\text{initial salinity} - \text{final salinity})}{\text{initial salinity}} \times 100$$

3.5. Identification of presumptive cyanobacterial strains with high desalination potential.

All presumptive isolates that show desalination potential were preserved in 5% (v/v) Dimethyl sulfoxide (DMSO) stock and stored at -80°C freezer. In brief, 1 ml of each culture suspension was transferred to a centrifuge tube and spun at 10,000 rpm for 5 minutes at room temperature, following the modified procedure of Ali et al., (2021). The pellet tube was submerged in crushed ice after the supernatant was discarded. To resuspend the pellet, 1 ml of fresh BG-11 broth was added, followed by an equal volume of 1 ml of 5% DMSO at 0.1 ml intervals. After vortexing the cell suspension, they were transferred into 1 ml cryovials, incubated for 30 minutes at 4 °C before being stored in a -80°C freezer.

The presumptive cyanobacterial isolates with desalination potential were then sent to Inqaba Biotech for identification. Identification was done based on Next-generation sequencing (NGS). Briefly, the genomic DNA was extracted and quantified using Qubit HS dsDNA Assay and qualified on Tapestation using genomics screentape. The tapestation revealed that the typical fragment size was ~7-10Kb, eliminating the requirement for shearing. SMRTbell libraries were performed using PacBio's Microbial Multiplexing workflow. The resulting libraries were quantified using Qubit HS dsDNA Assay and qualified on Tapestation using genomics screentape. Libraries were then prepared for sequencing following the online SMRTlink guided protocol (Binding kit 3.2 and Control 1.0, Sequel II Sequencing plate 2.0 and SMRT cell 8M.). Sequencing was done on the Sequel IIe platform. The resulting data was

processed on SMRTLINK v11 Genome Analysis module and Dada2 for dereplication and clustering of the amplicon sequence variants [ASV (s)]. The generated ASV(s) were blasted against the Algae DataBase for taxa classification.

3.6. Optimisation of culture condition for high bacteria biomass

3.6.1. Standardisation of seed culture

The five best desalinating isolates were pre-cultured in 100 ml BG-11 broth in a 250 ml flask on a rotary shaker (180 rpm) at 30 ± 3 °C with 40 Watts cool white, fluorescent bulbs for inoculation preparation and incubated for 7 days. After 7 days of incubation, the suspension was adjusted to an optical density 0.1 (OD_{750}) using BG-11 broth employing the modified method of Okaiyeto et al., (2015).



3.6.2. Effects of salinity

To assess the impact of cyanobacterial biomass on salinity, a linearity test was carried out as described by the modified procedure of Sahle-Demessie et al., (2019). Ten milliliters (10 ml) of standardised seed culture were inoculated into a 100 ml growth seawater solution (seawater and BG-11 at a 1:1 ratio), which was combined with NaCl to achieve salinity ranges of 1.5, 2, and up to 4% (w/v). The salinity reduction was monitored over 15 days.

3.6.3. Effects of Carbon and nitrogen Sources.

Sources of carbon and nitrogen are essential to cyanobacterial growth. A modified method described by Spiller and Shanmugam, (1987) was used to evaluate the effects of the carbon and nitrogen sources on cyanobacterial biomass. The seed culture was inoculated into a growth medium supplemented with a different carbon source (10 mM of sucrose, acetate, sodium bicarbonate, and carbonic acid) in a separate 250 ml Erlenmeyer flask. The mixture was then

incubated for 15 days at 30 ± 3 °C and 40 Watts cool white, fluorescent bulbs on a rotary shaker (180 rpm).

In the case of nitrogen source, aspartate, asparagine, glycine, and urea were used for the tests.

3.6.4. Effects of pH

The modified method of Azmi et al., (2018) was used to assess the effects of pH on biodesalination. Briefly, a 100 ml flask containing a 1:1 growth seawater solution (seawater and BG-11) was adjusted to a pH of 3 using 0.1 M HCl. A standardized seed culture was inoculated, and pH was measured using a calibrated pH meter.

3.6.5. Evaluation of optimum condition and Light on Salinity.

All the best optimum parameters obtained in this study were combined with the effects of light to assess environmental factors that promote biodesalination. Briefly,

Light Conditions: a cool white, fluorescent light was used as a source of light. According to the modified Minas et al., 2014, the culture was placed in dark, continuous light, and 12h dark:12h light conditions (12:12h) of 40 Watts cool white, fluorescent lights. Temperatures at 30 ± 3 °C were measured for 20 days.

Composition of culture medium: the composition of the medium was determined using the optimum parameters obtained in this investigation. Each seed culture was inoculated into a 250 ml flask containing a 1:1 growth seawater solution (seawater and BG-11) adjusted to 2% salinity, 10 mM sucrose, and 10 mM nitrate at pH 7.5. The suspension was incubated for 20 days at 30 ± 3 °C on a rotary shaker at 180 rpm under the light conditions specified above.

3.7. Removal of cells from desalinated water

3.7.1. Membrane Filtration

The desalination cultures were filtered through a 0.45 µm pore size polycarbonate filter membrane using a Vacuum manifold filter system using standard filtration method (APHA, 2005). The filtered water was collected for assessment of potential risk associated with desalinated water.

3.8. Assessment of potential risk associated with desalinated water through *in vitro* toxicology study.

Before commencement with toxicology study the below buffers and reagents were prepared according to the manufacturer's instructions (Thermofischer, 2023):

The Lysis buffer and Stop Solution were warmed to room temperature for approximately 20 minutes. The Substrate Stock Solution was then prepared by slowly stirring 11.4 ml of diH₂O into the Substrate until it was dissolved. The assay buffer stock solution was thawed to room temperature and protected from light with foil. To make the Reaction Mixture, combined 600 µl of Assay buffer Stock Solution with 11.4 ml of Substrate Stock Solution. Store away from light until needed. Lastly, the 1X LDH Positive Control was prepared by diluting 1.5 µl with 1 ml of 1% BSA in PBS.

Toxicology assay

In a 96-well tissue culture plate, 10 µl of filtered desalinated water was pipetted into 100 µl of 1% BSA in PBS medium. After incubating the plate overnight at 37°C with 10% CO₂, 10 µl of 10X Lysis buffer was gently added into the suspension. The plate was then incubated for 45 minutes at 37 °C with 10% CO₂. Each sample mixture was added to a new sterile, 96-well plate

in duplicate, with 50 μL of each mixture. To conduct an LDH Positive Control assay, 50 μl of serially diluted LDH Positive Control was plated. Wells 1, 3, 5, 7, and 9 were spiked with a 50 μl 10^{-4} dilution of LDH Positive Control. Each sample well was gently mixed with 50 μL of the Reaction Mixture. The plate was incubated at room temperature for 30 minutes, with foil protecting it from light. After incubation, a 50 μl of a Stop Solution was added and the absorbance was measured at 490 and 680 nm. The LDH activity was calculated by subtracting the 680-nm absorbance from the 490-nm absorbance. Thereafter, plotted the graph.



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CHAPTER 4

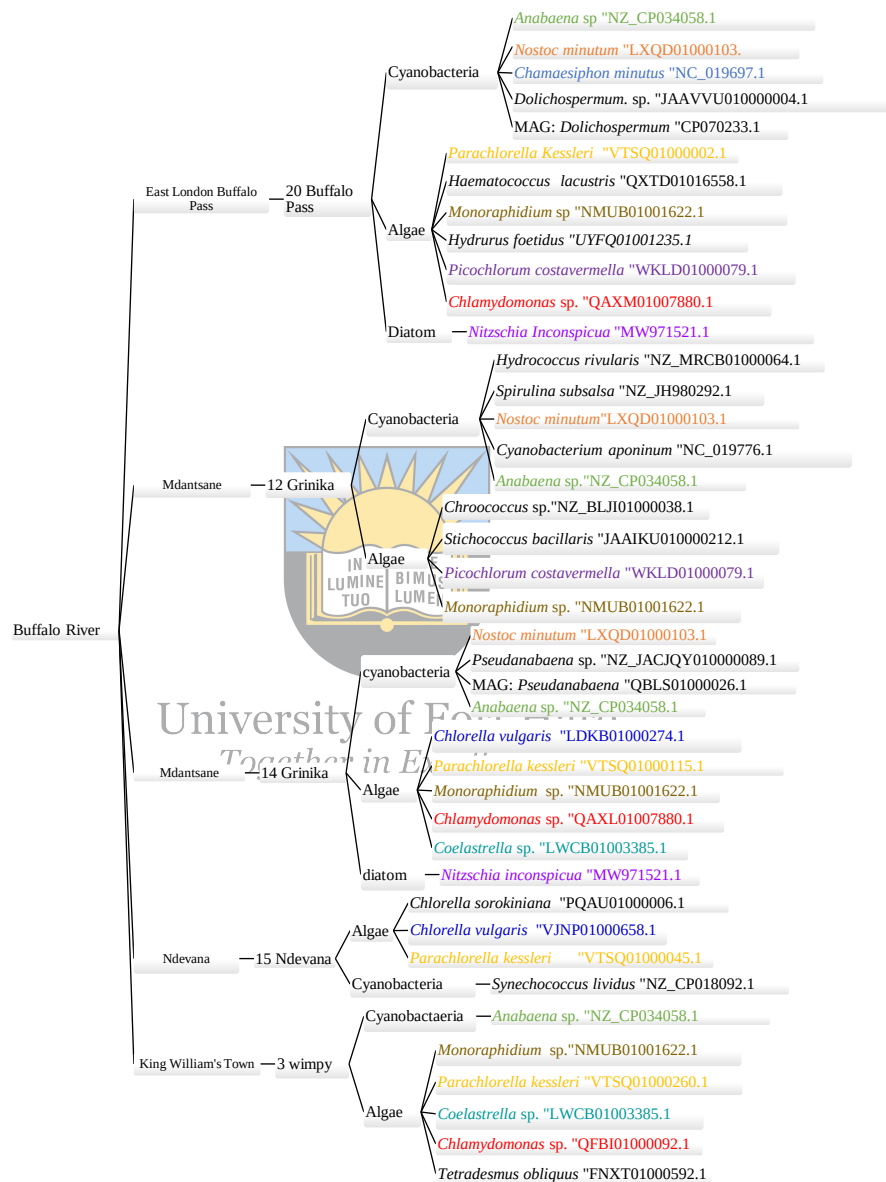
RESULTS AND DISCUSSION

4.1. Isolation and Identification of the Cyanobacterial isolates.

Presumptive indigenous cyanobacterial strains were isolated from the Buffalo River and characterized. Some of the presumptive isolates were of filamentous, cocci, and irregular in shapes with pigmentation ranging from green to blue-green as presented in Table S1. The external surface of the colonies on agar plates were mostly moist and sticky, suggesting that cyanobacterial strains released extracellular polymeric substances (EPS), enabling it to readily adhere to various materials, charges, or ions (Amezaga et al., 2014b; Zafar et al., 2022b). Furthermore, identification of the presumptive cyanobacterial strains in accordance with the data presented in Tables S 3-7 indicates that the five isolates used in this experiment were non-axenic cultures. The major contributing factor for these isolates to be non-axenic strains is that cyanobacterial strains frequently cohabit with heterotrophic bacterial partners in their phycosphere, also known as the "cyanosphere" (Halary et al., 2022). Thus, some green algae persisted in growing even after contaminating eukaryotes were eliminated using 50 mg/l of cycloheximide. Halary et al., (2022) also observed that the interaction of algal species is encouraged by mutualistic interactions, which is made possible by the exchange and recycling of vital nutrients and resources.

In total, 25 species were identified, comprising 12 cyanobacteria (*Anabaena* sp., Metagenome-Assembled Genome (MAG): *Pseudanabaena*, *Pseudanabaena* sp., *Nostoc minutum*, *Synechococcus lividus*, *Hydrococcus rivularis*, *Spirulina subsalsa*, *Chroococcus* sp., *Cyanobacterium aponinum*, MAG: *Dolichospermum*, *Dolichospermum* sp., *Chamaesiphon minutus*), 1 diatom (*Nitzschia inconspicua*), and 12 green algae (*Coelastrella* sp., *Chlamydomonas* sp., *Chlorella vulgaris*, *Monoraphidium* sp., *Parachlorella kessleri*,

Chlorella sorokiniana, *Tetrademus obliquus*, *Monoraphidium* sp., *Picochlorum costavermella*, *Stichococcus bacillaris*, *Haematococcus lacustris*). The data summarized in Figure 4.1, reveals that the most prevalent species in the Buffalo River were two cyanobacteria species, *Anabaena* sp., *Nostoc minutum* as well as a green-algae, *Parachlorella Kessleri*.



- **Figure 4.1:** The identification of Algal and Cyanobacterial isolates on isolate-3 Wimpy, 12 Grinika, 14 Grinika, 15 Ndevana, 20 Buffalo Pass. These isolates were obtained from the Buffalo River (i.e., East London Buffalo Pass River, Grinika, Ndevana, and King William's Town). Color-coding was used to represent the

distribution and concentration of various strains along different sampling points. *Anabaena* sp. and *Nostoc minutum* are the most dominant cyanobacterial species found in Buffalo River.

4.2. Initial screening for biodesalination potential cyanobacterial isolates.

The initial screening for biodesalination potential using the cyanobacterial/algal isolates is represented in Table 4.1. According to the table, all the experimental isolates including the seawater control showed salinity reduction except for one isolate which was isolated from King William's Town (i.e., 1-Wimpy isolate). Isolates 1, 10, and 18 system displayed an increase in salinity as highlighted in red in Table 4.1. The salinity increase may be due to cell lysing and shrinking from being exposed to high salinity concentrations. Seawater consists of several halophilic microorganisms that have a distinct metabolic profile and can adapt to high salinity. These salt-tolerant bacteria can concentrate more salt in their cells than in their surroundings (Amezaga et al., 2014; Sahle-Demessie et al., 2019). To evaluate the impact of these halophilic organisms on algal biodesalination, the seawater control was employed as a positive control. Table 4.1 revealed that the sodium efflux capacity of these marine strains is comparatively lower than that of algal strains. This was also observed by Apte and Thomas, (1997) with two strains of *Anabaena*; a freshwater strain (*Anabaena* L-31) and a brackish strain (*Anabaena torulosa*) accumulated more than 90% of salt, while the remaining were osmotically active and internalised. The brackish strain had a lower net Na⁺ intake than had the freshwater strain. Furthermore, it was observed that while salinity reduction continued to improve, there was no adverse interference caused by these organisms' interactions during algal biodesalination. It was also noted that the biodesalination process occurred during day 3 and 7 of the growth phase. Chimayati et al., (2018) argued that the rate of ionic absorption correlates with the rate at which algae species adapted to high salinity. Therefore, algae require more salt intake to

survive since saltwater was deficient in nutrients. Out of the 20 screened isolates, 5 (25%) strains showed salinity reduction of more than 40%. These 5 non-axenic cultures named: 3 Wimpy, 12 Grinika, 14 Grinika, 15 Ndevana, 20 Buffalo Pass were further investigated in the present study.

Table 4.1: Results for biodesalination potential for the presumptive cyanobacterial isolates.

Sampling site	Isolates	Salinity								%desalination
		0	1	2	3	4	5	6	7	
King William's Town	Control SW	35.03	35.0	35.03	35.0	35.0	35.0	34.8	34.05	2.8
			3		3	2	0	7		
	1 Wimpy	35.03	35.0	35.09	35.0	35.0	35.0	35.6	35.61	-1.7
			9		9	9	9	7		
	2 Wimpy	35.03	35.0	34.89	34.2	34.2	34.2	34.2	33.62	4.0
			1		6	6	6	6		
	3 Wimpy	35.03	34.9	33.50	31.5	29.7	27.4	24.4	20.41	41.7
			3		7	8	1	1		
Mdantsane	4 Wimpy	35.03	33.0	30.03	29.0	27.8	25.0	23.4	21.33	39.1
			2		9	7	8	5		
	5 Wimpy	35.03	35.0	35.03	35.0	31.1	30.9	29.8	29.58	15.6
			3		0	8	2	7		
	6 Wimpy	35.03	35.0	35.02	35.0	35.0	35.0	35.0	35.00	0.1
			2		2	2	2	2		
	7 Grinika	35.03	34.8	34.80	33.9	33.0	32.5	32.0	30.59	12.7
			1		7	3	9	3		
	8 Grinika	35.03	35.0	35.03	31.5	29.8	28.0	27.1	24.85	29.1

			3		9	9	3	6		
	9 Grinika	35.03	35.0	34.98	34.5	32.7	31.2	29.2	27.81	20.6
			2		4	6	9	4		
	10 Grinika	35.03	35.0	33.00	31.4	33.0	33.4	32.0	30.72	12.3
			2		0	0	0	7		
	11 Grinika	35.03	35.0	33.41	31.1	30.9	27.8	25.8	24.96	28.7
			1		4	2	7	3		
	12 Grinika	35.03	34.8	32.87	29.5	27.5	25.9	19.5	17.51	50.0
			7		2	1	5	2		
	13 Grinika	35.03	35.0	34.98	34.0	32.2	30.1	29.0	28.85	17.6
			3		3	4	1	6		
	14 Grinika	35.03	35.0	32.76	31.2	27.1	24.8	18.9	13.08	62.7
			0		4	6	5	9		
Ndevana	15 Ndevana	35.03	33.6	31.95	31.6	30.9	29.9	24.9	19.98	43.0
			7		3	2	8	2		
	16 Ndevana	35.03	35.0	35.03	35.0	34.8	33.9	31.4	30.07	14.2
			3		2	1	8	0		
	17 Ndevana	35.03	35.0	33.49	30.9	30.0	29.7	27.8	27.62	21.2
			0		6	7	7			
East London	18 Buffalo	35.03	34.9	33.00	32.9	32.8	31.7	30.8	32.72	6.6
	Pass		8		6	1	2	1		
	19 Buffalo	35.03	34.8	31.20	30.8	29.5	27.0	25.5	22.06	37.0
	Pass		2		8	2	6	2		
	20 Buffalo	35.03	34.9	33.79	31.8	28.2	25.8	21.5	18.06	48.4
	Pass		6		2	0	8	2		

4.3. Optimisation of culture condition for high bacteria biomass.

Algal biomass growth has a significant impact on the performance of algae-based desalination systems. Algae cannot effectively remove salts unless the conditions are optimal. In response to salt stress, the algae undergo a multistage process known as acclimatisation, which involves readjustment of ionic and osmotic potentials as well as more general physiological changes such as turgor adjustment (Sahle-Demissie et al., 2019). Consequently, certain isolates may be connected to cell death and/or lysing, as Table 4.1 illustrates. This necessitates the search for methods to optimise the culture conditions for high biomass of bacteria. Furthermore, the complexity and difficulty of scaling up the biodesalination process provide technological challenges when attempting to use it on a large scale (Minas et al., 2014).

4.3.1. Standardisation of seed culture

The five seed cultures shown in Figure 4.2 were standardized to an optical density of 0.1 at OD₇₅₀ to optimize culture conditions for high bacteria biomass. This was done to ensure that there was an equal concentration of organisms inoculated throughout the study.

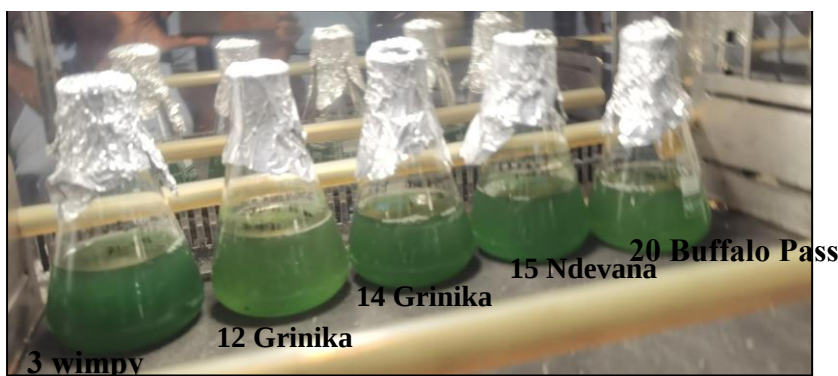


Figure 4.2: The photographic image of seed culture of algal strains with high desalination potential after 7 days incubation.

4.3.2. Effects of salinity

As represented in Figure 4.3, the effects of algae growth on salinity changes were monitored using a linearity test at 15 ppt, 20 ppt, and 40 ppt over a 15-day period. Salinity reduction was observed at all salinity values tested from 15 to 40 ppt. However, growth of these halotolerant microalgae was restricted at 40 ppt and best at 20 ppt. The addition of nutrients from BG-11 broth to seawater reduced the salinity by up to 92% while preserving the integrity of the cells in response to salt stress and affecting the growth rate of algae species. This was done in order to reduce the possibility of cell lysing, as was previously mentioned in Table 4.1, where some strains were lysed after being injected into saltwater without sufficient nutrition. It was observed in this study that the algal species can survive under salinity stress if supplemented with appropriate nutrients. Higher algal biomass production rates at increasing salinities lead to an increased ion uptake and nutrient supply in growth media. However, Zafar et al., (2021) states that, it is vital to determine which algae species can absorb ions under specific conditions. At 15 ppt: 3 Wimpy reduced salinity by 40.3%, 12 Grinika (45.3%), 14 Grinika (34.1%), 15 Ndevana (58.1%), and 20 Buffalo Pass (47.4%). At 20 ppt: 3 Wimpy reached salinity reduction of 60.7 %, 12 Grinika (50.5%), 14 Grinika (64.8 %), 15 Ndevana (92.0%), and 20 Buffalo Pass (48.5%). However, at increasing salinities of 40 ppt, ion uptake reduced; 3 Wimpy reached salinity reduction of 27.7 %, 12 Grinika (46.3%), 14 Grinika (13.9%), 15 Ndevana (45.2%), and 20 Buffalo Pass (29.8%). In overall, isolate 15 Ndevana was the most resistant salt isolate across salt concentrations.

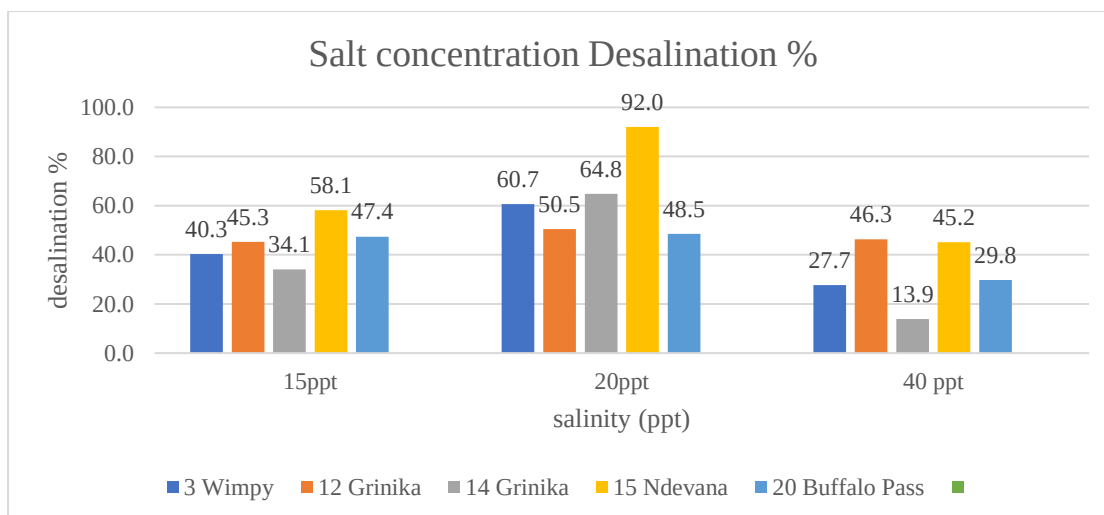


Figure 4.3: Algae growth was monitored using salinity changes over a 15-day period. The figure depicts the salinity reduction at different concentrations of 15 ppt, 20 ppt, and 40 ppt.

Photosynthetic bacteria from salty environments such as aqueous solutions and soils have been reported to remove sodium, which has also enhanced the rates of seed germination (De Philippis and Vincenzini, 2003b). Algae, like plants, can absorb Na^+ ions by lowering their absorption of K^+ ions to maintain the K^+/Na^+ ratio (Minas et al., 2024). Photosynthetic desalination has drawn the interest of several scholars, and this because of its unique qualities. Photosynthetic organisms use oxygen produced during photosynthesis as an electron acceptor (Shaikhoun et al., 2022b). Some species, such as those described by Gao et al., (2021b), Shaikhoun et al., (2022b), and Zafar et al., (2021b), not only tolerate high salinity but also store salts intracellularly. Bergi and Trivedi, (2020) and Jhala, Panpatte, and, Vyas, (2017) have proved this theory that *Anabaena oscillarioides*, *Anabaena Aphanizomenoides*, and *Microcystis aeruginosa* are some of the few cyanobacterial species that can tolerate salt concentrations of 7 to 15 g/L. Furthermore, *P. keutzingianum* is one of the halophytic strains that can survive at a salinity of 70 g/L (Zafar, Javed, and Aly Hassan, 2022b). The vacuole of algae, cf. *Pheridia tenuis* tissue was grown in seawater to remove salt and converted saline water to salt-free water (Yensen, 2006b). Two non-sulfur bacteria *Rhodobium marinum* and

Rhodobacter sphaeroides identified as NW16 and KMS24, respectively, removed 30% salinity in shrimp ponds. The study also showed that isolate NW16 was more resistant to saline environments than strain KMS24 (Panwichian et al., 2010). When used for brackish water desalination, *C. vulgaris*, a freshwater species that can adapt to saltwater conditions, attained an 80% salt removal rate in water with a salinity of 1 g/l (Barahoei, Madaripur, and Afsharzadeh, 2021b).

4.3.3. Effects of Carbon and nitrogen Sources

Since cyanobacterial/algal growth depends on sources of both carbon and nitrogen, the effects of these sources on biodesalination were evaluated, as Figures 4.4 and 4.5 illustrate. Table 4.2 summarises and presents the findings for optimum carbon source, which differed between isolates. Briefly: Sucrose: 3 Wimpy reduced salinity by 54.8%, 12 Grinika (55.5%), 14 Grinika (45.8%), 15 Ndevana (46.1%), and 20 Buffalo Pass (48.2%). Acetate: 3 Wimpy reached salinity reduction of 42.9%, 12 Grinika (47.2%), 14 Grinika (48.9%), 15 Ndevana (46.0%), and 20 Buffalo Pass (42.6%). Sodium bicarbonate: 3 Wimpy reached salinity reduction of 48.3%, 12 Grinika (31.4%), 14 Grinika (49.2%), 15 Ndevana (45.4%), and 20 Buffalo Pass (46.6%). When supplemented with sodium carbonate, ion uptake was reduced: 3 Wimpy experienced a 32.8% salinity reduction, 12 Grinika (27.6%), 14 Grinika (26.8%), 15 Ndevana (35.0%), and 20 Buffalo Pass (33.7%). Overall, sucrose was found to be the best carbon source for desalination conditions, while sodium carbonate was the least desalinating carbon source. Cyanobacteria accumulate compatible solutes that protect the cells against salt stress (Carrieri et al., 2010). Sucrose is identified as one of the compatible solutes that are protein synthesis independent, responsible for establishing and maintaining the turgor pressure of salt-stressed cells (Hagemann, 2011a).

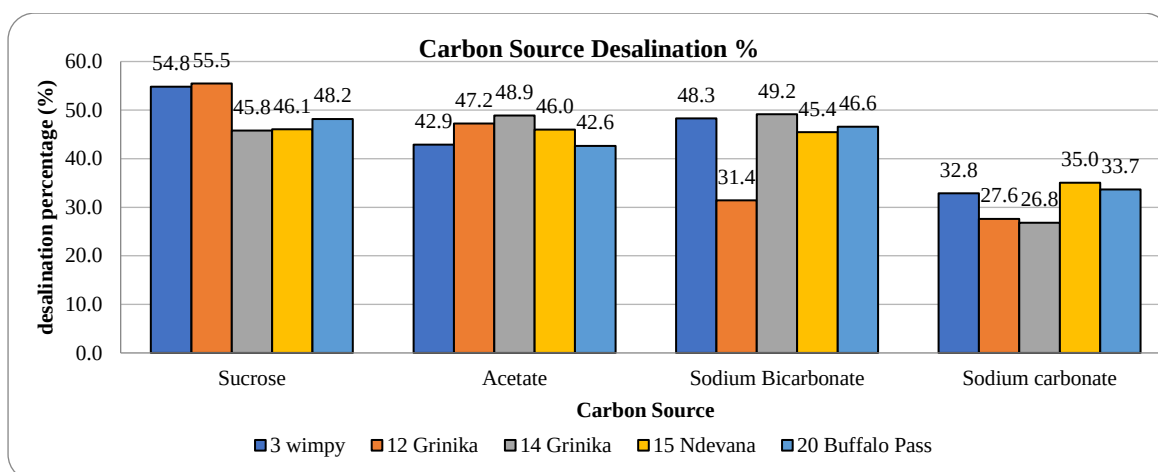


Figure 4.4. Effects of carbon sources on biodesalination.



Table 4.2: Summary report for optimum carbon source observed in this study.

Isolates	carbon Source				
3 Wimpy	Sodium carbonate	<	Acetate	<	Sodium bicarbonate < Sucrose
12 Grinika	Sodium carbonate	<	Sodium bicarbonate	<	Acetate < Sucrose
14 Grinika	Sodium carbonate	<	Sucrose	<	Acetate < Sodium bicarbonate
15 Ndevana	Sodium carbonate	<	Sodium bicarbonate	<	Acetate < Sucrose
20 Buffalo Pass	Sodium carbonate	<	Acetate	<	Sodium bicarbonate < Sucrose

Similarly, nitrogen is one of the key nutrients for the growth of cyanobacterial cells and some cyanobacteria are nitrogen-fixing heterocyst (Pang et al., 2018b). Urea is found to be an excellent and preferred nitrogen source for many cyanobacterial species (Erratt, 2017b), which is also the case with nitrate and ammonium. However, in this study, as shown in figure 4.5, nitrates followed by ammonia were observed to be the preferred nitrogen source in most species when compared to urea and asparagine. Asparagine was found to be detrimental in all the isolates. The results for optimum nitrogen source were summarised and presented in Table 4.3.

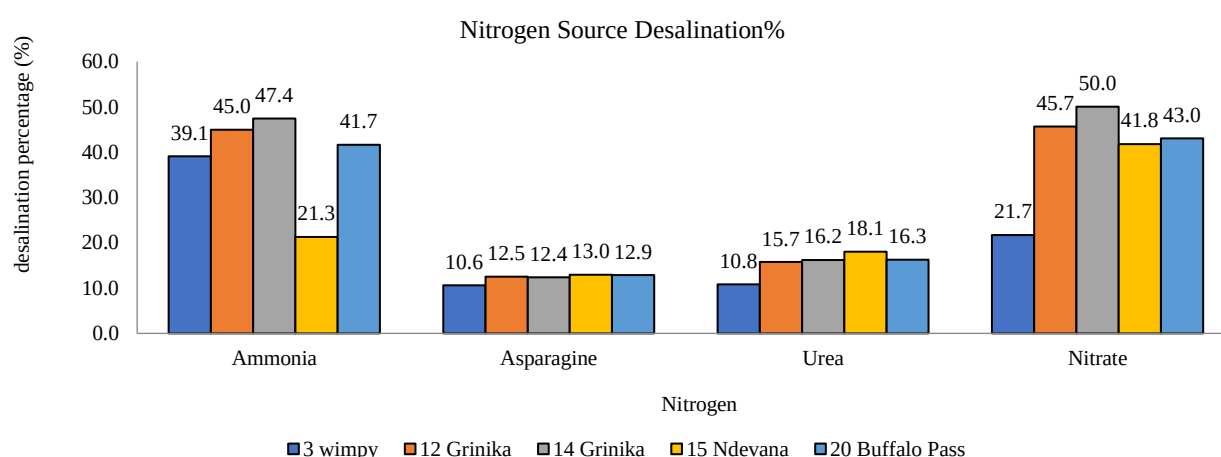
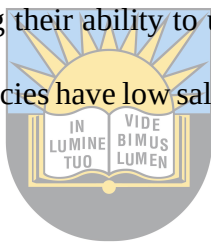


Figure 4.5: Effects of Nitrogen Sources on biodesalination.

Table 4.3: Summary report for optimum nitrogen source observed in this study.

Isolates	NITROGEN SOURCE				
3 Wimpy	Asparagine	< Urea	<	Nitrate	< Ammonia
12 Grinika	Asparagine	< Urea	<	ammonia	< Nitrate
14 Grinika	Asparagine	< Urea	<	Ammonia	< Nitrate
15 Ndevana	Asparagine	< Urea	<	Ammonia	< Nitrate
20 Buffalo Pass	Asparagine	< Urea	<	Ammonia	< Nitrate

Algae use different organic and inorganic carbon and nitrogen sources for their growth which include bicarbonate, CO₂, urea, nitrate, ammonium, nitrite, amino acids, cyanate, and several species that can fix dinitrogen (N₂) gas to meet their cellular N demand (Herrero, Muro-Pastor, and Flores, 2001b). The absence of organic carbon and nitrogen sources in the medium limits the growth algae. As a result, introducing carbon and nitrogen sources into the seawater medium reduced salinity while enhancing algae biomass. However, when the medium was supplemented with energy sources i.e., nitrogen and carbon sources, the salinity percentage was less compared to when algae species were grown directly on seawater or under the effects of salt. This shows that microalgae consumed sufficient nutrients from the growth medium to survive at increased salinities, limiting their ability to utilise Na⁺ and Cl⁻ or rather ions in the seawater. Therefore, supplemented species have low salt influx compared to directly inoculated species to seawater.



Inorganic nitrogen assimilation always results in the formation of ammonium (Zhang et al., 2018b). The cyanobacteria used the NO₃⁻ ions as a source of nitrogen to grow. In cyanobacteria, nitrate ions act as a dietary supplement to provide nitrogen in the face of high salinities (Rai and Abraham 1995). Nitrates enter cells through the membrane and nitrite reductase enzymes convert NO₃⁻ ions into nitrite ions (NO₂⁻). Nitrite is further broken down into ammonium ions, and the cell stroma digests them to form amino acids, which are then converted into proteins within the cell, and utilised in various metabolic processes (Larsdotter et al., 2007; Sommer, 1983; Zafar et al., 2021). Mohapatra et al., (1998) studied *Scenedesmus bijugatus* in high salt concentrations and reported that it grows faster in nitrogen and phosphorus-rich environments.

4.3.4. Effect of pH, temperature, and light on salinity

Algal and cyanobacterial photosynthetic activity frequently results in pH rises due to electron transfer from photosystem II (PSII) to photosystem I (PS) (Shaikhoun et al., 2022b; Singh et al., 2016b). Table 4.4 indicates that there was an instantaneous rise in pH following the seed culture's inoculation in seawater. Due to this increase in pH from the standardized pH of 3.3 to 5.3, 6.3, 5.9, 6.1, and 4.9 values for 3 Wimpy, 12 Grinika, 14 Grinika, 15 Ndevana, and 20 Buffalo Pass, respectively, the neutral and alkaline pH could not be evaluated. This may be due to fact that the current experiment was carried out in seawater, a hyper-saline environment, the Na^+/H^+ anti-porters had easy access to large amounts of Na^+ ions. Singh et al., (2016b) explains that during photosynthesis, cyanobacteria exchange electrons, ions, and carbon. Na^+ homeostasis and cell pH are significantly influenced by the Na^+/H^+ anti-porters, which are important channels in eukaryotic and prokaryotic Na^+ and H^+ circulation. This necessitated adjusting the pH to 7.5, the pH used throughout this study.

Table 4.4: Effects of acidic pH on algal/cyanobacterial biodesalination.

Isolates	pH before inoculation	pH after inoculation
3 Wimpy	3.3	5.3
12 Grinika	3.3	6.3
14 Grinika	3.3	5.9
15 Ndevana	3.3	6.1
20 Buffalo Pass	3.3.	4.9

Light intensity, photoperiod, and light quality are all crucial factors in the growth and metabolism of photosynthetic organisms. Photosynthesis principles dictate that optimal light should be obtained at bi-light. However, the optimal energy source was determined under

continuous light, as shown comparatively, in Figure 4.6, which summarises that more light can lead to increased photosynthesis. Under dark conditions, photosynthetic organisms could not capture enough energy source for their growth and metabolism, hence desalination was slow. As a result of cyanobacteria being photo autotrophic, they respond to the amount and the quality of light.

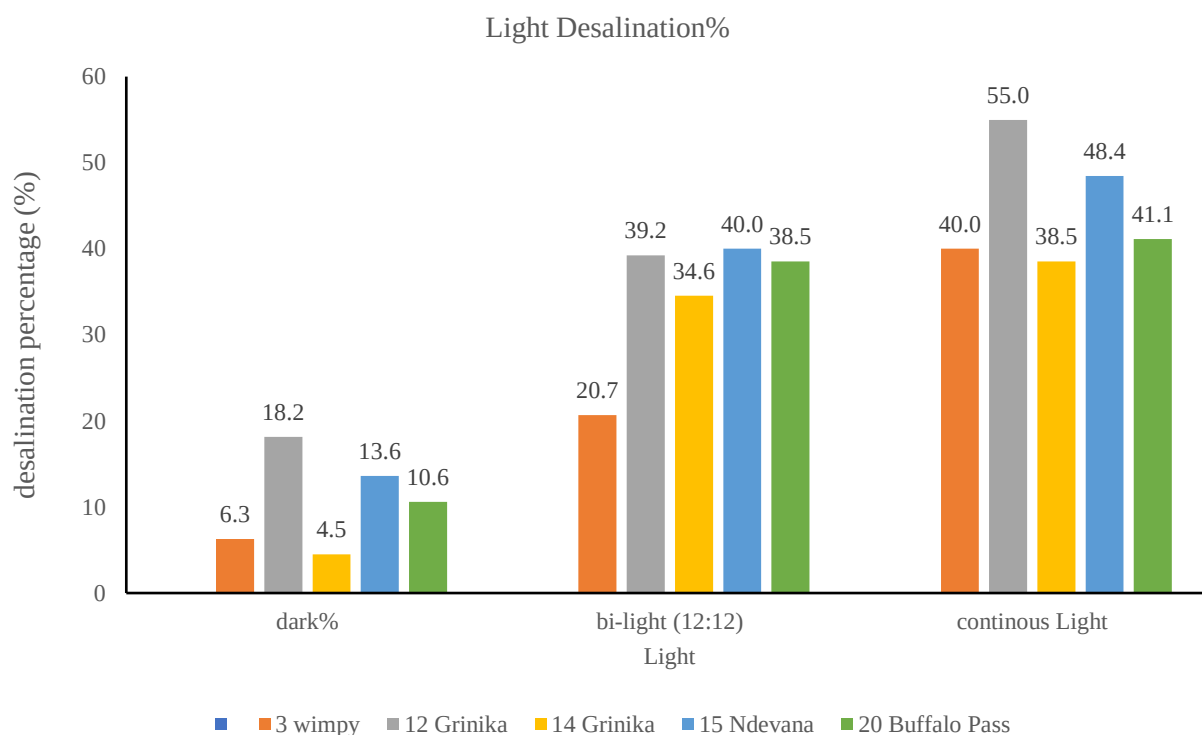


Figure 4.6: Effects of light on biodesalination.

4.3.5. Evaluation of optimum condition on salinity

All optimum conditions obtained in this study were combined to evaluate environmental factors contributing to biodesalination and effects of light on biodesalination were assessed simultaneously as presented in Figure 4.7. In contrast to the results of the initial desalination screening in section 4.2 and the effects of salts in section 4.3.1, where desalination was achieved from day 3, salinity started to drop on day 9 and continued to decline significantly on day 19. This shows that, even though the optimisation of culture conditions for high bacteria

biomass have improved the efficiency of algae/cyanobacteria biodesalination and desalination of seawater has been proven successful, desalination was prolonged, and the obtained results were less than those achieved previously in section 4.3.1. This suggests that the release of secondary metabolites by algae/cyanobacteria is prolonged because of excess nutrients in the experiment. It was also noted that, without the optimal operating conditions, algae cannot remove salts effectively i.e., when the experiment was supplemented with nutrients, no cell lysing was observed. This implies that specific growth conditions are necessary for photosynthetic organisms used in biodesalination. The efficiency of an algae-based desalination system is highly dependent on the growth of algal biomass (Gao et al., 2021). The ability of algae/cyanobacterial cells to adjust to their surroundings, as well as the period of salt exposure, are critical for successful salt acclimation (Amezaga et al., 2014b; Hagemann, 2011b).

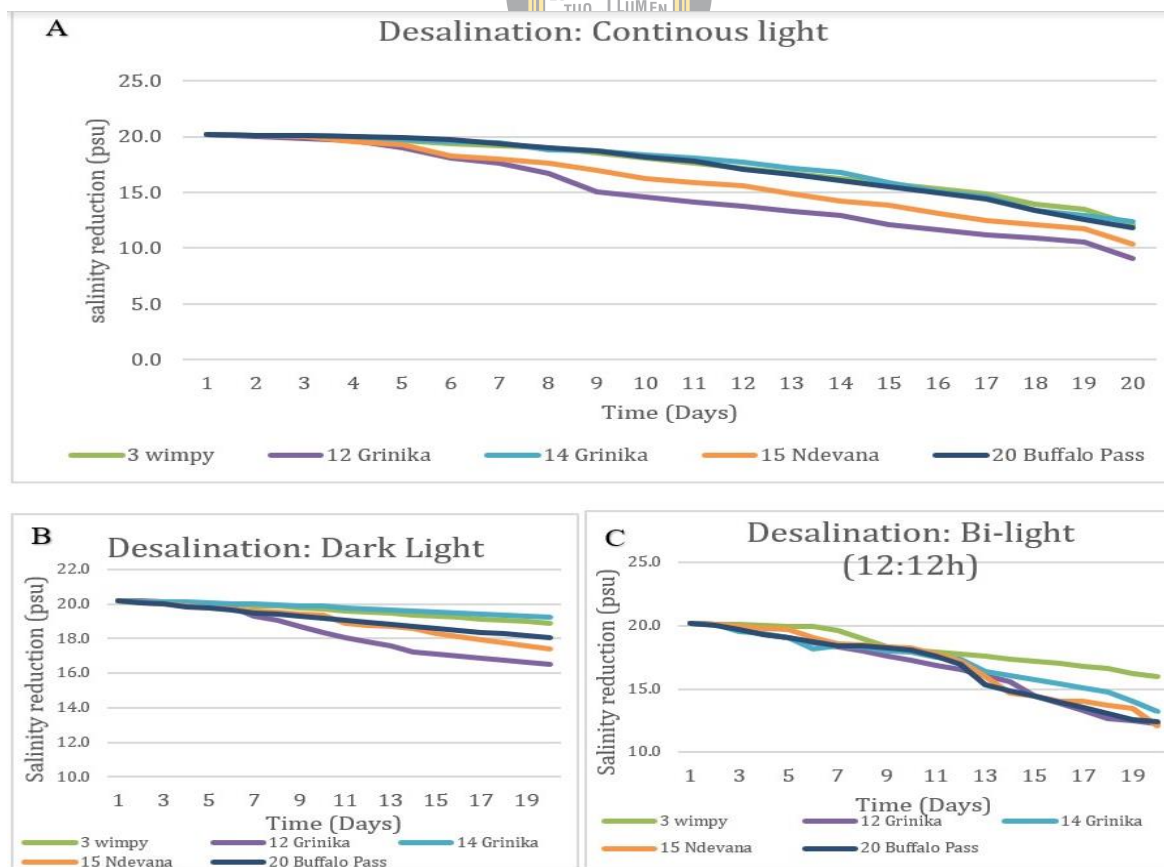


Figure 4.7: Optimal condition summary test of algal/cyanobacterial species on optical environmental conditions of this study. A. Operated under continuous conditions; B. operated under Dark light conditions, and C. operated under Bi-light conditions (12h:12h).

4.4. Removal of cells from desalinated water

The main challenge with this technology is successfully recovering cells from desalinated water owing to the cyanobacteria's small size, negative surface charge, decreased cell density, and unusually high moisture content. Thus, to minimise the release of cytotoxins from lysed cells due to aggregation formation, the membrane filtering technique was used in this study to remove cells from desalinated water. The typical size of cyanobacteria is between 1 and 10 μm , and the polycarbonate filters that were used have pore sizes of 0.45 μm , which allows the cells to be trapped and prevented from passing through with desalinated water while maintaining the integrity of the cells during removal as shown in Figure 4.8. This was done in compliance with Minas et al.'s (2014) findings, which stated that using common metal salts as coagulants to promote aggregation formation results in extensive cell death and lysis throughout the separation process. The salinity reduction percentage increased significantly when the cells were removed from or separated from the desalinated water: for 3 Wimpy, 12 Grinika, 14 Grinika, 15 Ndevana, and 20 Buffalo Pass, it went from 40,0%, 55.0%, 38.5%, 48.4%, and 41.1% to 55.1, 70.8, 60.3%, 77.2%, and 72.8%, respectively (Table 4.5). This suggested that salt was accumulating within the cells. Yensen, (2006) and Khan and Weber, (2006) have demonstrated that the halophytic alga *Pheridia tenuis* accumulates salt in its cell vacuoles, supporting this notion. Algae cell walls are primarily composed of proteins, polysaccharides, and lipids, resulting in a negatively charged and sticky cell wall that attracts and binds to water cations. Furthermore, microalgae can remove metal ions from water via biosorption or bioaccumulation. Biosorption absorbs about 80% of cations (Gao et al., 2021b; Zafar et al., 2021b). Thus, removing cells from desalinated water also reduces the salt content.

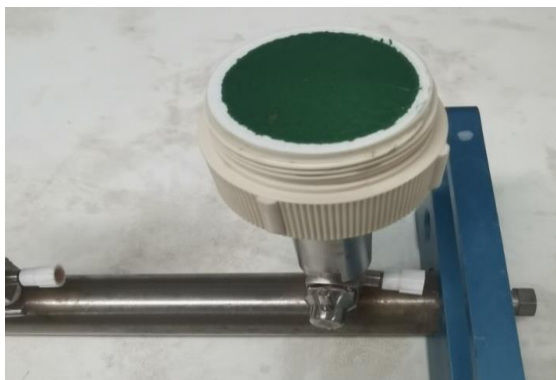


Figure 4.8: Recovered algal/cyanobacterial cells from passing through with desalinated water by membrane filtration method.

Table 4.5: Salinity reduction after removal of cells from desalinated water

Isolates	% Desalination after cell removal		
	Initial	Final	Salinity reduction%
3 Wimpy	20.17	9.05	55.1
12 Grinika	20.17	5.88	70.8
14 Grinika	20.17	8.01	60.3
15 Ndevana	20.17	4.6	77.2
20 Buffalo Pass	20.17	5.48	72.8

4.5. Assessment of potential risk associated with desalinated water through *in vitro* toxicology study.

This technology is linked with potential risk associated with the cyanobacterial release of cytotoxins and odour into desalinated water. To address these public health and safety concerns, an *in vitro* toxicology study was conducted using the CyQUANT LDH Cytotoxicity Assay Kit to assess the potential risks associated with desalinated water. The CyQUANT LDH

Cytotoxicity assay is a colorimetric approach that uses tetrazolium salt (INT) to identify toxins in a complex mixture of chemicals. Lactate dehydrogenase (LDH), which is generated by dead or dying cells or injured cells, transforms lactate to pyruvate by reducing NAD^+ to NADH. NADH converts INT to a red formazan product. The amount of fluorescence or LDH is directly proportional to the number of damaged cells.

In the current investigation, samples in columns 1, 3, 5, 7, and 9 of a 96-well microplate was spiked with a 10^{-3} dilution of the positive control, while samples in columns 2, 4, 6, 8, and 10 were not spiked. All the cytotoxin-containing wells were expected to be red to pink in colour. The desalinated water filtered from a complex mixture of cyanobacterial and algae species had no significant influence of producing cyanotoxins. The non-spiked samples in Figure 4.9b do not show any colour change, indicating that the organisms did not release any cytotoxins into the desalinated water.

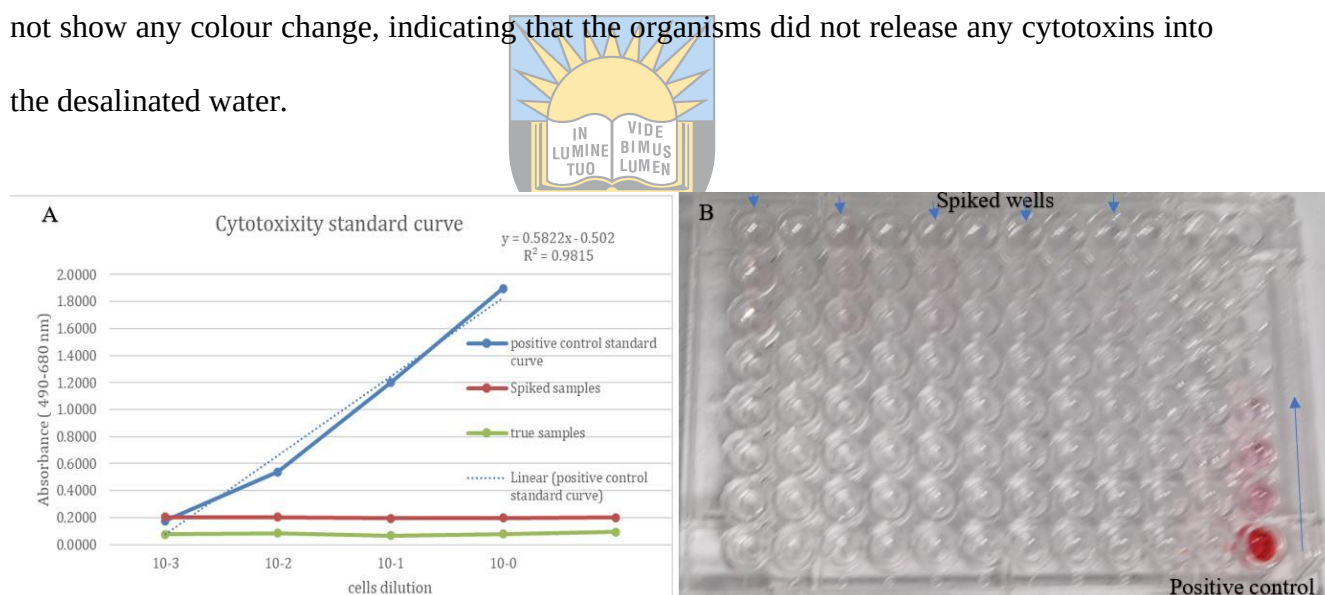


Figure 4.9: An *in vitro* toxicology study carried out using the CyQUANT LDH Cytotoxicity Assay Kit to evaluate the possible risks connected with desalinated water. A. shows the cytotoxicity standard curve at 0.98 regression. B. Shows the CyQUANT LDH Cytotoxicity assay on microtiter plate; the resulting NADH reduces INT to a red formazan product (column 1,3,5,7, and 9 are spiked samples while 2,4,6,8,10 are non-spiked samples. Positive control was inoculated on row 10 of the 96-well microplate as indicated by the arrows).

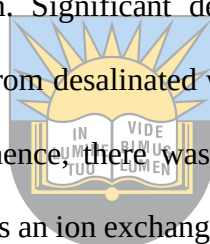
Cyanotoxins are a chemically diverse category of bioactive compounds that have traditionally been divided into four types based on their primary target organ: dermatotoxins and irritants, neurotoxins, hepatotoxins, and general cyanotoxins. Cyanobacteria are known to produce a wide range of bioactive compounds and chemically diverse secondary metabolites (Li et al., 2023). The most well-documented cyanobacterial strains are from the genera *Microcystis*, *Dolichospermum* (previously *Anabaena*), *Aphanizomenon*, *Planktothrix*, and *Oscillatoria* (Dulić et al., 2022). The results achieved above contrast with Khanavi et al., (2012) findings, which showed that the chloroform fraction of algae had high cytotoxic effect on normal cell lines than cancer cells. This suggest that removal of algal cells from desalinated influenced the negative cytotoxicity activity results obtained. Membrane filters could sufficiently trap different types of microbes and particles found in desalinated water, including chloroform, which could aggravate the cytotoxicity effect.

Furthermore, it could be because direct cytotoxicity is limited in the current study because it occurs outside of the host's immune milieu and tumor microenvironment (Guedes et al., 2013). The study was performed on Bovine Serum albumin (BSA). BSA is a soluble monomeric protein produced in the liver that regulates extracellular fluid volume and transports a wide range of small molecules in serum, including steroids, fatty acids, and thyroid hormones. BSA protein can act as an antioxidant by scavenging potentially hazardous free radicals (Thermofischer 2023). To identify toxins in a complex mixture of chemicals, comparison studies on in vivo assays must be conducted before this method is used as an alternative technology to desalinate seawater.

4.7. Chapter Summary

The utilisation of cyanobacteria and photosynthetic organisms in the desalination of seawater has been proven successful. Buffalo river is having high algal/Cyanobacterial population.

Microalgae cultivation from the exponential phase to the stationary phase is approximately 7 days. The use of natural resources as nutrients for cyanobacterial growth, such as carbon and nitrogen source, light and pH enable cyanobacteria to grow at high densities and opens the door to future biological processes for sustainable water treatment applications and maintains the cell integrity from lysing. As a result, as the salinity of the water increases, so does the ability of microalgae to remove NaCl. Algal/cyanobacterial strains have shown to form low-salt reservoirs and can produce significant biomass on high salinity densities. Optimisation of culture conditions for high bacteria biomass has improved the efficiency of algae/cyanobacteria biomass on biodesalination. However, the microalgae used showed better tolerance to extreme salt stress up to 92%, compared to being supplemented with carbon and nitrogen sources for nutrition during culture optimisation. Significant desalination reduction was noted after removal of algal/cyanobacterial cell from desalinated water. This suggests that the algal cells accumulated salt inside their cells; hence, there was reduction of salinity after cells were removed. Cyanobacteria can be used as an ion exchanger by manipulating transport proteins in the cell membrane. The findings of this study have significant implications for sustainable development. The development of biodesalination methods directly supports SDG 6 by providing innovative solutions for clean water. Additionally, by reducing the environmental impact of desalination processes, this research contributes to SDG 13 and SDG 14, promoting climate action and the sustainable use of marine resources.



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CHAPTER 5

GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1. General Discussion

Given that water scarcity is currently one of humanity's most pressing issues, developing a novel, low-energy biological desalination process is an interesting and novel approach. The currently employed desalination technologies consume huge amounts of energy and require high capital investment. The biodesalination from algal community provides an alternative means of solving water security problems in South Africa. Algae and cyanobacteria have drawn the interest of several scholars for their ability to thrive in high and fluctuating salinity biotopes. These halophilic microorganisms have a distinct metabolic profile on their membranes and can concentrate more salt in their cells than in their surroundings (Amezaga et al., 2014; Sahle-Demessie et al., 2019; Shaikhoun et al., 2022). They act as an ion exchanger where sodium chloride moves from high levels of concentration from the sea to low sodium volume through transport proteins and cell membranes (Amezaga et al., 2014). Algae and cyanobacteria have been extensively studied as salt tolerance species, and it appears that high salinity has a significant influence, potentially resulting in lower growth and survival of many freshwater species. Some species, though, not only survive but also accumulate salts intracellularly in high saline conditions (Gao et al. 2021; Shaikhoun et al. 2022; Zafar et al. 2021). The cell wall of algae is negatively charged and sticky, which allows it to absorb cations. Algae absorb cations through surface deposition, physical and biosorption, active transport, and passive diffusion (Sahle-Demessie et al., 2019). It occurs through two mechanisms, namely biosorption and bioaccumulation, with biosorption having the highest salt removal efficacy (Gao et al., 2021).

Nonetheless, most researchers have used bioaccumulation; and salinity reduction has been studied in relation to uptake of chemical oxygen demand (COD), ion removal on heavy metal, reduction of total dissolved solids (TDS), and reduction of electrical conductivity (EC). The vacuole of algae, cf. *Pteridion tenuis* tissue, was grown in seawater to remove salt and convert saline water to salt-free water (Yensen, 2006). *Anabaena oscillarioides*, *Anabaena Aphanizomenoides*, and *Microcystis aeruginosa* are some of the cyanobacterial species that can tolerate salt concentrations of 7 to 15 g/L (Singh et al. 2014). In addition, *P. keutzingianum* is one of the halophytic strains that can survive at a salinity of 70 g/L (Zafar et al., 2022). Two non-sulfur bacteria *Rhodobium marinum* and *Rhodobacter sphaeroides* identified as NW16 and KMS24, respectively, removed 30% salinity in shrimp ponds. The study also showed that isolate NW16 was more resistant to saline environments than strain KMS2 (Panwichian et al., 2010). *C. vulgaris*, a freshwater species that can adapt to saline water, achieved an 80% salt removal rate with 1 g/l in brackish water desalination (Barahoei et al., 2021). The genetic modifications of two euryhaline strains, *Synechocystis* sp. strain PCC6803; a freshwater strain, and the marine origin strain called *Synechococcus* sp. strain PCC7002 are widely used species because they have been fully sequenced and have shown an increase in the bioaccumulation capacity of Cl^- (Minas et al., 2014; Singh and Singh, 2015).

Even though microalgae could be a game-changer for desalination, the biggest issue with this method is producing biomass on a large scale (Minas et al., 2014). The application of biodesalination in water treatment can be sustained through accessible of available energy sources such as the source of energy, and minerals for their survival to grow at high cell densities (Amezaga et al., 2014). Higher rates of algal biomass production increase ion uptake. Thus, it is important to establish the optimum conditions required for high cyanobacteria biomass for the discovery of effective cyanobacterial strains for the removal of high salt contents from seawater. The five essential abiotic factors essential for growth of photosynthetic

organisms are light, pH, temperature, carbon dioxide, and nutritional supplements (Al-Mamun et al., 2018; Kumar et al., 2021; Pade and Hagemann, 2014; Singh and Singh, 2015), as explored and discussed in Chapter 4. Most species grow best at alkaline pH. The salt tolerance of *Anabaena torulosa* and *Anabaena* sp. strain L-31 at alkaline pH appeared to be related to Na^+ transport. This was demonstrated by reduced Na^+ influx at pH 8 (Apte et al., 1987).

The best temperature range for the algae strains to grow in, according to Minas et al., (2014), is between 20 and 40 °C. This would allow the selected strains to be used under ambient conditions in a wide range of countries. Meanwhile, Paerl, (2014) reports that cyanobacteria have an optimum temperature that is higher than 25 °C. The effects of temperature on this study were kept at optimum temperature of 30 ± 3 °C. It has also been discovered that *Microcystis*, *Anabaena*, and *Aphanizomenon* thrive at 25 °C, while *Cynlindrospermopsis raciborskii* and *Planktothrix agardhii* thrive at 27.5 °C (Apte and Thomas, 1983; Zhang et al., 2016). Temperatures that are equal to or higher than 25 °C and less than 37 °C may lead to an increase in the rate of cyanobacterial growth, leading to an excessive population of cyanobacteria in controlled water bodies (Paerl, 2014).

As much as this technology is appealing, maintaining cell integrity when removing cells from desalinated water and not reverting the salt into the water remains a challenge (Amezaga et al., 2014; Westrick et al., 2010). The difficulty is caused by comparable density of the cell and water; organic colloids made by small inorganic particles make the removal of cyanobacterial EPS from conventional water treatment processes difficult, and charged surface stabilises the cell suspensions (Maiti, 2019). Synthetic and organic polymers have been used to replace inorganic metal slats for coagulation and flocculation processes. Cationic polymers such as polyethyleneimine and polyacrylamide can flocculate cyanobacterial cells with excellent efficiency without affecting the cell membrane (Arrington et al., 2003). In addition to synthetic polymers, chitosan and chemically modified chitosan also help the flocculation, but cell death

has been observed (Shao et al., 2012). Therefore, the use of a flocculation-based process is inefficient as the surface characteristics of the cells and extracellular polymeric materials are also determining factors (Teixeira et al., 2010). It is for this reason that membrane filtration using filters was utilised to safely recover cells from desalinated water, hence preventing cell lysing and salt and cytotoxins reverting in desalinated water. Cell removal from desalinated water not only reduces the risk of toxicity release, but it also increases the likelihood of salt removal, as cells accumulate salts and ions through the bioaccumulation process.

The main disadvantage of algae-based biodesalination is its slow growth rate; however, this could be addressed through genetic manipulation of cyanobacteria for enhanced salt uptake. Manipulating engineer cyanobacteria, cis-genetic alteration via genome engineering is the most reliable method. This is due to the susceptibility of many cyanobacterial strains to genetic mutation, insertion, or deletion. Two popular model cyanobacteria are *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 has been transformed using this technique, which allows for targeted chromosomal alterations to express heterologous genes and synthetic biosynthetic pathways (Govindasamy et al., 2022). Given *E. coli*'s rapid growth rate as an example, cyanobacteria strains can be modified with an *E. coli* plasmid containing the desired gene, marker gene, and flanking regions that correspond to the desired genomic loci. When the two *E. coli* strains mix, the conjugative plasmid transfers to the strain that contains the cargo and helper plasmids. The latter can conjugate with the added cyanobacterial strain and transfer the cargo plasmid to the new host (Amezaga et al., 2014; Govindasamy et al., 2022).

5.2. Conclusion and Recommendations

The use of cyanobacteria and photosynthetic organisms in the desalination of seawater has been proven successful. Algal strains have been shown to be capable of producing significant biomass on seawater and reducing salt content. The use of optimal conditions for

algal/cyanobacterial growth, such as light, carbon, and nitrogen sources enable cyanobacteria to grow at high densities and opens the door to future biological processes for sustainable water treatment applications. However, energy sources were a major challenge in this study. Available energy source in South Africa remains a challenge. South Africa is facing “electric” loadshedding, in which alternative methods should be investigated for supply of artificial energy source such as solar. In addition, more stringent monitoring of physiological changes caused by ionic stress should be investigated. It would be vital to assess the functional groups in microalgae responsible for desalination using qualitative methods such as nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) analysis, and scanning electron microscopy (SEM). Understanding how salinity affects cell growth and viability will enable us to scale up cyanobacterial growth to meet the demands of water treatment scenarios. There is also need for in-depth research on EPS characterisation to neutralise the sticky property that binds to surroundings to effectively purify cyanobacterial strains on solid media, and the development of a controllable cell-aggregation protocol. Novel approaches to optimizing biodesalination could involve modifying cyanobacteria through genetic engineering to increase their capacity to absorb salt or modeling microbial interactions in saline environments using bioinformatics tools. Lastly, while attempting to provide an alternative solution to South Africa's water security problem, desalinated water should be thoroughly tested for toxicity to ensure public health and safety before making it available to the public.

In conclusion, this research advances the field of biodesalination and supports the broader goals of sustainable development. By addressing critical issues related to water scarcity, climate change, and environmental sustainability, this study contributes to the achievement of several United Nations Sustainable Development Goals. Currently, this technology is only proposed to be used as a pre-treatment stage on desalination. Further purification stages are still required to address water quality issues.

References

- Abedi S., Astaraei F.R., Ghobadian B., Tavakoli O., Jalili H., Greenwell H.C., Cummins I., Chivasa S. 2019. Decoupling a novel *Trichormus variabilis* *Synechocystis* sp. interaction to boost phycoremediation. *Sci. Rep.* 9, 2511.
- Abraham, P. and Luque A. 2013. *Isolation, Characterization and Identification of Microalgae from the Red Sea*.
- Ahamefule C.S., Ugwuodo C.J., Idike, P.O., Ogbonna J.C. 2021. Application of photosynthetic microalgae in the direct desalination pretreatment of seawater. *Water Environ. J.* 35 (2), 657–669. <https://doi.org/10.1111/wej.12659>.
- Ahmed M. E., Zafar A. M., Hamouda M. A., Aly Hassan A, and Arimbrathodi S. 2022. “Biodesalination Research Trends: A Bibliometric Analysis and Recent Developments.” *Sustainability* 2023, Vol. 15, Page 16 15(1);16. doi: 10.3390/SU15010016.
- Al-Kharabsheh, S., and D. Yogi Goswami. 2003. “Analysis of an Innovative Water Desalination System Using Low-Grade Solar Heat.” *Desalination* 156(1–3):323–32. Doi: 10.1016/S0011-9164(03)00363-1.
- Al-Mamun A., Ahmad W., Mahad S. B., Mohammad K., Bipro R. D. 2018. “A Review of Microbial Desalination Cell Technology: Configurations, Optimization and Applications.” *Journal of Cleaner Production* 183:458–80. Doi: 10.1016/J.JCLEPRO.2018.02.054.
- Ali P., Fucich D., Shah A. A., Hasan F., and Chen F. 2021. “Cryopreservation of Cyanobacteria and Eukaryotic Microalgae Using Exopolysaccharide Extracted from a Glacier Bacterium.” *Microorganisms* 9(2):1–14. doi: 10.3390/microorganisms9020395.
- Amezaga J. M., Amtmann A., Biggs C. A., Bond T., Gandy C. J., Honsbein A, Karunakaran E, Lawton L, Mary Ann Madsen M. A., Minas M, and Templeton M. R. 2014. “Biodesalination:

A Case Study for Applications of Photosynthetic Bacteria in Water Treatment.” *Plant Physiology* 164(4):1661–76. Doi: 10.1104/pp.113.233973.

Anagnostidis, K., and Komárek J. 1988. “Modern Approach to the Classification System of Cyanophytes. 3 – Oscillatoriales.” *Algological Studies/Archiv Für Hydrobiologie, Supplement Volumes* 327–472.

APHA 2005. Standard Methods for the Examination of Water and Wastewater. 22nd Ed. Washington DC: American Public Health Association/American Water Works Association/Water Environment Federation,.

Apte, K. S., Reddy R. B., and Thomas J. 1987. *Relationship between Sodium Influx and Salt Tolerance of Nitrogen-Fixing Cyanobacteria Downloaded From*. Vol. 53.

Apte, K. S, and Thomas J. 1983. “Impairment of Photosynthesis by Sodium Deficiency and Its Relationship to Nitrogen Fixation in the Cyanobacterium *Anabaena Torulosa*.” *FEMS Microbiology Letters* 16(2–3):153–58. Doi: 10.1111/j.1574-6968.1983.tb00277.x.

Apte, S. K., and Thomas J. 1997. “Possible Amelioration of Coastal Soil Salinity Using Halotolerant Nitrogen-Fixing Cyanobacteria.” *Plant and Soil* 189(2):205–11. Doi: 10.1023/A:1004291830391.

Arrington, S. A., Margo J. Z., Donald W. O., and Lu K. J. 2003. “Effects of Polyethyleneimine on Cyanobacterium *Anabaena Flos-Aquae* during Cell Flocculation and Flotation.” *Enzyme and Microbial Technology* 32(2):290–93. Doi: 10.1016/S0141-0229(02)00294-6.

Azmi, A. S., Fazrizatul S.S., Fathilah A., and Maizirwan M. 2018. “Interactive Effect of Temperature, PH and Light Intensity on Biodesalination of Seawater by *Synechococcus* Sp. PCC 7002 and on the Cyanobacteria Growth.” *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 52(1):85–93.

- Baiyegunhi, L. J. 2015. “Determinants of Rainwater Harvesting Technology (RWHT) Adoption for Home Gardening in Msinga, KwaZulu-Natal, South Africa.” *Water SA* 41(1):33–40. Doi: 10.4314/WSA.V41I1.6.
- Barahoei, M., Mohammad S. H., and Saeed A. 2021. “Direct Brackish Water Desalination Using *Chlorella Vulgaris* Microalgae.” *Process Safety and Environmental Protection* 148:237–48. Doi: 10.1016/J.PSEP.2020.10.006.
- Bergi J., and Trivedi R. 2020. “Bioremediation of Saline Soil by Cyanobacteria.” *Undefined* 447–65. doi: 10.1007/978-981-15-1812-6_17.
- Bergman B., J. R. Gallon, A. N. Rai, and L. J. Stal. 2006. “N₂ Fixation by Non-Heterocystous Cyanobacteria.” *FEMS Microbiology Reviews* 19(3):139–85. Doi: 10.1111/j.1574-6976.1997.tb00296.x.
- Bergström A. 2010. “The Use of TN:TP and DIN:TP Ratios as Indicators for Phytoplankton Nutrient Limitation in Oligotrophic Lakes Affected by N Deposition.” *Springer* 72(3):277–81. Doi: 10.1007/s00027-010-0132-0.
- Bhargava S., Saxena R. K., Pandey P.K., and Bisen P. S. 2003. “Mutational Engineering of the Cyanobacterium *Nostoc Muscorum* for Resistance to Growth-Inhibitory Action of LiCl and NaCl.” *Current Microbiology* 47(1):5–11. Doi: 10.1007/s00284-002-3921-4.
- Bhunja B., Uday U. S., Oinam P., Mondal G. A., Bandyopadhyay T. K., and Tiwari O. N. 2018. “Characterization, Genetic Regulation and Production of Cyanobacterial Exopolysaccharides and Its Applicability for Heavy Metal Removal.” *Elsevier* 179:228–43. Doi: 10.1016/j.carbpol.2017.09.091.
- Bi Y. H., and Hu Z. Y. 2004. “Influence of Temperature, Nutrients and Light Intensity on the Growth of *Nostoc Flagelliforme*.” *Chinese Journal of Process Engineering* 4(3):249–54.

- Blumwald, E., R. J. Mehlhorn, and L. Packer. 1983a. "Studies of Osmoregulation in Salt Adaptation of Cyanobacteria with ESR Spin-Probe Techniques." *Proceedings of the National Academy of Sciences* 80(9):2599–2602. Doi: 10.1073/pnas.80.9.2599.
- Brown, A. D. 1976. *Microbial Water Stress*. Vol. 40.
- Bubela, T, Gregory H, and Edna E. 2012. "Synthetic Biology Confronts Publics and Policy Makers: Challenges for Communication, Regulation and Commercialization." *Trends in Biotechnology* 30(3):132–37.
- Burke L., Chen C., Jamil O., and Majewska N. 2016. *Desalination-Team B From Processdesign Reverse Osmosis Desalination Plant in Durban, South Africa*.
- Byrne R. H. 2007. "Seawater. Encyclopædia Britannica." Retrieved May 8, 2021 (<https://www.britannica.com/science/seawater>).
- Cao X., Xia H., Peng L., Kang X., Zhou Y , Zhang X, and Bruce E. L. 2009. "A New Method for Water Desalination Using Microbial Desalination Cells." *Environmental Science & Technology* 43(18):7148–52. Doi: 10.1021/ES901950J.
- Carrieri D., Dariya M., Ian A. B., Gennady A., Oliver L., Donald A. B, and Charles G. D.. 2010. "Boosting Autofermentation Rates and Product Yields with Sodium Stress Cycling: Application to Production of Renewable Fuels by Cyanobacteria." *Applied and Environmental Microbiology* 76(19):6455–62. Doi: 10.1128/AEM.00975-10.
- Chaffin Justin D., Thomas B. Bridgeman, Scott A. Heckathorn, and Sasmita Mishra. 2011. "Assessment of Microcystis Growth Rate Potential and Nutrient Status across a Trophic Gradient in Western Lake Erie." *Journal of Great Lakes Research* 37(1):92–100. Doi: 10.1016/j.jglr.2010.11.016.

- Chen J. J., Hsuan H. Y, and Cheng I. T. 2009. “Effect of Ozone and Permanganate on Algae Coagulation Removal – Pilot and Bench Scale Tests.” *Chemosphere* 74(6):840–46. Doi: 10.1016/j.chemosphere.2008.10.009.
- Chen X, Schreiber X., Appel J, Makowka A., Fähnrich B., Roettger M., Mohammad R. H., Sönnichsen F. D, Schönheit P., Martin W. F., and Gutekunst K. 2016. “The Entner-Doudoroff Pathway Is an Overlooked Glycolytic Route in Cyanobacteria and Plants.” *Proceedings of the National Academy of Sciences of the United States of America* 113(19):5441–46. Doi: 10.1073/pnas.1521916113.
- Chen Z., Cheng H., and Chen X. 2009. “Effect of Cl⁻ on Photosynthetic Bicarbonate Uptake in Two Cyanobacteria *Microcystis Aeruginosa* and *Synechocystis PCC6803*.” *Chinese Science Bulletin* 54(7):1197–1203. Doi: 10.1007/s11434-009-0148-9.
- Chimayati R. L., and Titah H. S. 2019. “Removal of Salinity Using Interaction Mangrove Plants and Bacteria in Batch Reed Bed System Reactor.” *Journal of Ecological Engineering* 20(4):84–93. Doi: 10.12911/22998993/102792.
- Chinyama A., Ochieng G. M., JSnyman J., and Nhapi I. 2016. “Occurrence of Cyanobacteria Genera in the Vaal Dam: Implications for Potable Water Production.” *Online) = Water SA* 42(3):415–20. Doi: 10.4314/at.v42i3.06.
- Chojnacka K. 2010. “Biosorption and Bioaccumulation – the Prospects for Practical Applications.” *Environment International* 36(3):299–307. Doi: 10.1016/J.ENVINT.2009.12.001.
- Chow, C. W. K., Drikas M., House J, Burch M. D., and Velzeboer R. M. A. 1999. “The Impact of Conventional Water Treatment Processes on Cells of the Cyanobacterium *Microcystis Aeruginosa*.” *Water Research* 33(15):3253–62. Doi: 10.1016/S0043-1354(99)00051-2.

- Crettaz-Minaglia M., Fallico M, Aranda O, Juarez I, Pezzoni M, Costa C, Andrinolo D, and Giannuzzi L. 2020. "Effect of Temperature on Microcystin-LR Removal and Lysis Activity on *Microcystis Aeruginosa* (Cyanobacteria) by an Indigenous Bacterium Belonging to the Genus *Achromobacter*." *Environmental Science and Pollution Research* 27(35):44427–39. Doi: 10.1007/s11356-020-09901-y.
- Cui J., Sun T., Chen L, and Zhang W. 2020. "Engineering Salt Tolerance of Photosynthetic Cyanobacteria for Seawater Utilization." *Biotechnology Advances* 43:107578.
- Dawound M. A. 2005. "The Role of Desalination in Augmentation of Water Supply in GCC Countries. Desalination." *Elsevier* 186(1–3). Doi: 10.1016/j.desal.2005.03.094.
- De Vuyst L., De Vin F., Vaningelgem F., and Degeest B. 2001. "Recent Developments in the Biosynthesis and Applications of Heteropolysaccharides from Lactic Acid Bacteria." Pp. 687–707 in *International Dairy Journal*. Vol. 11.
- DEA, Department of Environmental Affairs. 2011. "Department of Environmental Affairs (DEA). Inland Water -." Retrieved May 8, 2021 (www.environment.gov.za).
- Delattre C., Pierre G., Laroche C., and Michaud P. 2016. "Production, Extraction and Characterization of Microalgal and Cyanobacterial Exopolysaccharides." *Biotechnology Advances* 34(7):1159–79.
- De Philippis R.; Vincenzini, M. 2003. "Outermost Polysaccharidic Investments of Cyanobacteria: Nature, Significance and Possible Applications. Recent Research Developments in Microbiology." 7(17):13–22.
- Dolnicar S., A. H., and Grun B. 2011. "What Affects Public Acceptance of Recycled and Desalinated Water?" *Elsevier* 45(2):933–43. Doi: 10.1016/Fj.watres.2010.09.030.

- Dolnicar S., and Schäfer A. 2006. "Public Perception of Desalinated versus Recycled Water in Australia." *Faculty of Commerce – Papers (Archive)*.
- Dominic T. K., and Madhusoodanan P.V. 1999. "Cyanobacteria from Extreme Acidic Environments." *JSTOR* 77(8):1021–23.
- Drikas M., Chow C. W. K., House J., and Burch M. D. 2001. "Using Coagulation, and Settling to Remove Toxic Cyanobacteria." *Journal / American Water Works Association* 93(2):100–111. Doi: 10.1002/j.1551-8833.2001.tb09130.x.
- Du Plessis J. A., Burger A. J., , Swartz C. D., and Musee N. 2006. *A desalination guide for South African Municipal Engineers Department of Water Affairs and Forestry Water Research Commission*.
- DWA- Department of Water Affairs 2020. "Drought Interventions: Home." Retrieved January 31, 2022 (<http://www.dwa.gov.za/drought/default.aspx>).
- DWAF- Department of Water Affairs and Forestry 2013. "National Water Resource Strategy – 2nd Edition 2013." *Water Resources* 2013.  *Together in Excellence*
- DWAF- Department of Water Affairs and Forestry. 1994. *Water Supply and Sanitation Policy White Paper Water-an Indivisible National Asset*.
- DWS. 2022. "Weekly State of Dams." Retrieved October 17, 2022 (<https://www.dws.gov.za/Hydrology/Weekly/Province.aspx>).
- ELGA. 2014. The Effect of Increasing Reverse Osmosis (RO) Recovery. Pp 327847." 44(327847):327847.
- EOH Coastal & Environmental Services. 2016. *BUFFALO RIVER ESTUARY SITUATION ASSESSMENT REPORT Prepared for: Department of Environmental Affairs: Oceans and Coasts*.

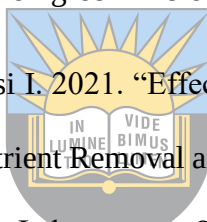
Erratt K. 2017. “Urea as an Effective Nitrogen Source for Cyanobacteria.” *Electronic Thesis and Dissertation Repository*.

Fal S., Aasfar A., Rabie R, Smouni A, and EL Arroussi H. 2022. “Salt Induced Oxidative Stress Alters Physiological, Biochemical and Metabolomic Responses of Green Microalga *Chlamydomonas Reinhardtii*.” *Heliyon* 8(1). Doi: 10.1016/J.HELİYON.2022.E08811.

Ferris M. J., and Hirsch C. F. 1991. “Method for Isolation and Purification of Cyanobacteria.” *Applied and Environmental Microbiology* 57(5):1448–52. doi: 10.1128/AEM.57.5.1448-1452.1991.

Figler, A., B-Béres, V., Dobronoki, D., Márton, K., Nagy, S.A. and Bácsi, I., 2019. Salt tolerance and desalination abilities of nine common green microalgae isolates. *Water*, 11(12), p.2527.

Figler A., Márton K., B-Béres V., and Bácsi I. 2021. “Effects of Nutrient Content and Nitrogen to Phosphorous Ratio on the Growth, Nutrient Removal and Desalination Properties of the Green Alga *Coelastrum Morus* on a Laboratory Scale.” *Energies* 14(8):2112. Doi: 10.3390/EN14082112/S1.



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Flaibani A., Olsen Y., and Painter T. J. 1989. “Polysaccharides in Desert Reclamation: Compositions of Exocellular Proteoglycan Complexes Produced by Filamentous Blue-Green and Unicellular Green Edaphic Algae.” *Carbohydrate Research* 190(2):235–48. Doi: 10.1016/0008-6215(89)84128-X.

Gao L., Zhang X., Fan L., Gray S., and Li M. 2021. “Algae-Based Approach for Desalination: An Emerging Energy-Passive and Environmentally Friendly Desalination Technology.” *ACS Sustainable Chemistry and Engineering* 9(26):8663–78. Doi: 10.1021/ACSSUSCHEMENG.1C00603/ASSET/IMAGES/MEDIUM/SC1C00603_0008.GIF

- Ge H., Xia L, Zhou X., Zhang D, and Hu C. 2014. “Effects of Light Intensity on Components and Topographical Structures of Extracellular Polysaccharides from the Cyanobacteria *Nostoc* Sp.” *Journal of Microbiology* 52(2):179–83. Doi: 10.1007/s12275-014-2720-5.
- Gijsbertsen-Abrahamse, A. J., C., and Heijman S. G. J. 2006. “Removal of Cyanotoxins by Ultrafiltration and Nanofiltration.” *Journal of Membrane Science* 276(1–2):252–59. Doi: 10.1016/j.memsci.2005.09.053.
- Goga T., Friedrich E., and Buckley C. A. 2019. “Environmental Life Cycle Assessment for Potable Water Production – A Case Study of Seawater Desalination and Mine-Water Reclamation in South Africa.” *Water SA* 45(4):700–709. Doi: 10.17159/at/2019.v45.i4.7552.
- Govindasamy R., Gayathiri E., Sankar S., Venkidasamy B., Prakash P., Rekh K., Savaner V., Pari A., Thirumalaivasan N. and Thiruvengadam M. 2022. Emerging trends of nanotechnology and genetic engineering in cyanobacteria to optimize production for future applications. *Life*, 12(12), p.2013.
- Hagemann M. 2011. “Molecular Biology of Cyanobacterial Salt Acclimation.” *FEMS Microbiology Reviews* 35(1):87–123.
- Halary S., Duperron S., Demay J., Duval C., Hamlaoui S., Piquet B., Reinhardt A., Bernard C., and Marie B. 2022a. “Metagenome-Based Exploration of Bacterial Communities Associated with Cyanobacteria Strains Isolated from Thermal Muds.” *Microorganisms* 10(12). doi: 10.3390/microorganisms10122337.
- Harding W. R. 2008. *The Determination of Annual Phosphorus Loading Limits for South African Dams*.
- Herrero A., Muro-Pastor A. M., and Flores E. 2001. “Nitrogen Control in Cyanobacteria.” *Journal of Bacteriology* 183(2):411–25.

- Hitzfeld B. C., Höger S. J., and Dietrich D. R. 2000. "Cyanobacterial Toxins: Removal during Drinking Water Treatment, and Human Risk Assessment." *Environmental Health Perspectives* 108(1):113–22.
- Hunter P. D., Hanley N, Czajkowsk M., Mearns K., Tyler A. N., Carvalho L., and Codd G. A. 2012. "The Effect of Risk Perception on Public Preferences and Willingness to Pay for Reductions in the Health Risks Posed by Toxic Cyanobacterial Blooms." *Science of the Total Environment* 426:32–44. Doi: 10.1016/j.scitotenv.2012.02.017.
- Jhala Y. K., Panpatte D. G., and Vyas R. V. 2017. "Cyanobacteria: Source of Organic Fertilizers for Plant Growth." Pp. 253–64 in.
- Kaplan A, and Reinhold L. 1999. "CO₂ CONCENTRATING MECHANISMS IN PHOTOSYNTHETIC MICROORGANISMS." *Annual Review of Plant Physiology and Plant Molecular Biology* 50(1):539–70. Doi: 10.1146/annurev.arplant.50.1.539.
- Kirkwood A. E., Buchheim J. A., Buchheim M. A., and Henley W. J. 2008. "Cyanobacterial Diversity and Halotolerance in a Variable Hypersaline Environment." *Microbial Ecology* 55(3):453–65. Doi: 10.1007/S00248-007-9291-5/METRICS.
- Kırtel O., Versluys M., Van den Ende W., and Öner E. T. 2018. "Fructans of the Saline World." *Biotechnology Advances* 36(5):1524–39.
- Konopka A., and Brock T. D. 1978. "Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota." *Applied and Environmental Microbiology* 36(4):572–76. Doi: 10.1128/aem.36.4.572-576.1978.
- Kumar P., Yi Sheng Tseng A, Singhania R. R., Chen C. W., JiChang J. S., and Di Dong C. 2021. "Novel Application of Microalgae Platform for Biodesalination Process: A Review." *Bioresource Technology* 337:125343. Doi: 10.1016/J.BIORTECH.2021.125343.

- Kummu M, and Varis O. 2011. "The World by Latitudes: A Global Analysis of Human Population, Development Level and Environment across the North-South Axis over the Past Half Century." *Applied Geography* 31(2):495–507. Doi: 10.1016/j.apgeog.2010.10.009.
- Lam M. K., and Lee K. T. 2015. "Bioethanol Production from Microalgae." Pp. 197–208 in *Handbook of Marine Microalgae: Biotechnology Advances*. Elsevier Inc.
- Lee A. K., Lewis D. M., and Ashman P. J. 2009. "Microbial Flocculation, a Potentially Low-Cost Harvesting Technique for Marine Microalgae for the Production of Biodiesel." *Journal of Applied Phycology* 21(5):559–67. Doi: 10.1007/s10811-008-9391-8.
- Leong, Y.K. and Chang, J.S., 2020. Bioremediation of heavy metals using microalgae: recent advances and mechanisms. *Bioresource technology*, 303, p.122886.
- Liang H., Nan J., He W.J., Li G. B. 2009. "Algae Removal by Ultrasonic Irradiation-Coagulation. Desalination." 239:191-197.
- Liang Y., Zhang M, Wang M., Zhang W., Qiao C., Luo Q., and Lu X. 2020. "Freshwater Cyanobacterium *Synechococcus elongatus* PCC 7942 Adapts to an Environment with Salt Stress via Ion-Induced Enzymatic Balance of Compatible Solutes." *Applied and Environmental Microbiology* 86(7). Doi: 10.1128/AEM.02904-19.
- Lürling M., Eshetu F., Faassen E. J., Kosten S., and Huszar V. L. M. 2013. "Comparison of Cyanobacterial and Green Algal Growth Rates at Different Temperatures." *Freshwater Biology* 58(3):552–59. Doi: 10.1111/j.1365-2427.2012.02866.x.
- Ma M., Liu R., Liu H, and Qu J. 2014. "Mn(VII)–Fe(II) Pre-Treatment for *Microcystis aeruginosa* Removal by Al Coagulation: Simultaneous Enhanced Cyanobacterium Removal and Residual Coagulant Control." *Water Research* 65:73–84. Doi: 10.1016/J.WATRES.2014.07.011.

- Mackenzie FT., Byrne R. H., and Duxbury A. C. 2022. “Seawater | Composition, Properties, Distribution, & Facts | Britannica.” Retrieved October 17, 2022 (<https://www.britannica.com/science/seawater>).
- Madsen M. A, Hamilton G., Herzyk P., and Amtmann A. 2021. “Environmental Regulation of PndbA600, an Auto-Inducible Promoter for Two-Stage Industrial Biotechnology in Cyanobacteria.” *Frontiers in Bioengineering and Biotechnology* 8. Doi: 10.3389/fbioe.2020.619055.
- Maiti C. K. 2019. “The Transformer in Water Treatment: Cyanobacteria for Biodesalination.” (7):359–65.
- Marin K. Y., Kanesaki, Los D. A., Murata N., Suzuki I., and Hagemann M. 2004. “Gene Expression Profiling Reflects Physiological Processes in Salt Acclimation of *Synechocystis* Sp. Strain PCC 6803.” *Plant Physiology* 136(2):3290–3300. Doi: 10.1104/pp.104.045047.
- Marin K., Stirnberg M., Eisenhut M., Krämer R., and Hagemann M. 2006. “Osmotic Stress in *Synechocystis* Sp. PCC 6803: Low Tolerance towards Nonionic Osmotic Stress Results from Lacking Activation of Glucosylglycerol Accumulation.” *Microbiology* 152(7):2023–30. Doi: 10.1099/mic.0.28771-0.
- Markou G, Chatzipavlidis I., and Georgakakis D. 2012. “Carbohydrates Production and Bio-Flocculation Characteristics in Cultures of *Arthrospira* (*Spirulina*) *Platensis*: Improvements Through Phosphorus Limitation Process.” *Bioenergy Research* 5(4):915–25. Doi: 10.1007/s12155-012-9205-3.
- Merck 2021, <https://www.sigmaaldrich.com/ZA/en/product/sial/73816> (accessed 03 March 2021)
- Mgeni Water. 2022. Potable Water Production Increases at Hazelmere Water Treatment Plant.” (April):2022.

- Miller A. G., Turpin D. H., and Canvin D. T. 1984. "Na⁺ Requirement for Growth, Photosynthesis, and PH Regulation in the Alkalotolerant Cyanobacterium *Synechococcus Leopoliensis*." *Journal of Bacteriology* 159(1).
- Minas K., Karunakaran E., Gandy C., Honsbein A., Madsen M., and Amezaga J. 2014. "Newcastle University Eprints L. Biodesalination: An Emerging Technology for Targeted Removal of Na⁺ and Cl⁻ from Seawater by Cyanobacteria. Desalination and Water Treatment 2014, (e-Pub Ahead of Print)." Doi: 10.1080/19443994.2014.940647.
- Mukheibir P. 2010. "The Potential Economic Impact of Climate Change on Equitable Water Access in Small Towns: A South African Case Study Energy Sensitive Urban Water Planning in Developing Countries View Project Supporting Community Adaptation to Water Shortages in Kiribati View Project the Potential Economic Impact of Climate Change on Equitable Water Access in Small Towns: A South African Case Study." *Article in International Journal of Water* 5(3):223–45. Doi: 10.1504/IJW.2010.030588.
- Müller V., and Oren A. 2003. "Metabolism of Chloride in Halophilic Prokaryotes. Extremophiles." *Springer* 7(4):261–66. Doi: 10.1007/s00792-003-0332-9.
- Mur R.; Skulberg O.M.; Utkilen H. 1999. *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management Chapter 2. CYANOBACTERIA IN THE ENVIRONMENT.*
- Natalia B., and Iwa S. 2008. "The Emalahleni Water Reclamation Plant in South Africa, Pp 2008." 1–3.
- Nayak S., and Prasanna R. 2007. "Soil PH and Its Role in Cyanobacterial Abundance and Diversity in Rice Field Soils." *Applied Ecology and Environmental Research* 5(2):103–13. Doi: 10.15666/aeer/0502_103113.

Ndlela L. L., Oberholster P. J., Van Wyk J. H., and Cheng P. H. 2018. “Bacteria as Biological Control Agents of Freshwater Cyanobacteria: Is It Feasible beyond the Laboratory?” doi: 10.1007/s00253-018-9391-9.

Neu T. R., and Marshall K. C. 1990. “Bacterial Polymers: Physicochemical Aspects of Their Interactions at Interfaces.” *Journal of Biomaterials Applications* 5(2):107–33. Doi: 10.1177/088532829000500203.

Ni L., Li D, Rong S., Su L., Zhou W., Wang P., Wang C., Li S., and Acharya K. 2017. “Characterization of Extracellular Polymeric Substance (EPS) Fractions Produced by *Microcystis Aeruginosa* under the Stress of Linoleic Acid Sustained-Release Microspheres.” *Environmental Science and Pollution Research* 24(26):21091–102. Doi: 10.1007/s11356-017-9540-1.



Ni Z. Y., Li J. Y, Xiong Z., Li Hua Cheng L. H., and Xu X. H. 2018. “Role of Granular Activated Carbon in the Microalgal Cultivation from Bacteria Contamination.” *Bioresource Technology* 247:36–43. Doi: 10.1016/J.BIORTECH.2017.07.079.

University of Port Hare
Together in Excellence

Nubel U., Garcia-Pichel F., Clavero E., and Muyzer G. 2000. “Matching Molecular Diversity and Ecophysiology of Benthic Cyanobacteria and Diatoms in Communities along a Salinity Gradient.” *Environmental Microbiology* 2(2):217–26. Doi: 10.1046/j.1462-2920.2000.00094.x.

Okaiyeto K., Nwodo U.U., Mabinya L. V., Okoli A. S., and Okoh A. I. 2015. “Characterization of a Biofloculant (MBF-UFH) Produced by *Bacillus* Sp. AEMREG7.” *International Journal of Molecular Sciences* 16(6):12986–3. doi: 10.3390/IJMS160612986.

Okajima K., Sornkamnerd S, and Kaneko T. 2018. “Development of Functional Bionanocomposites Using Cyanobacterial Polysaccharides.” *Chemical Record* 18(7):1167–77. Doi: 10.1002/tcr.201700074.

- Oren A. 2015. "Cyanobacteria in Hypersaline Environments: Biodiversity and Physiological Properties." *Biodiversity and Conservation* 24(4):781–98.
- Pade N., and Hagemann M. 2014. "Salt Acclimation of Cyanobacteria and Their Application in Biotechnology." *Life* 5(1):25–49. Doi: 10.3390/life5010025.
- Paerl H. W. 2014. "Mitigating Harmful Cyanobacterial Blooms in a Human-and Climatically-Impacted World." *Mdpi.Com*.
- Paerl H. W., Tucker J, and Bland P. T. 1983. "Carotenoid Enhancement and Its Role in Maintaining Blue-Green Algal (Microcystis Aeruginosa) Surface Blooms1." *Limnology and Oceanography* 28(5):847–57. Doi: 10.4319/lo.1983.28.5.0847.
- Pandhal J., Wright P. C., and Biggs C. A. 2008. "Proteomics with a Pinch of alt: A Cyanobacterial Perspective." *Saline Systems* 4(1). Doi: 10.1186/1746-1448-4-1.
- Pang K., Tang Q., Chen L., Bin Wan, Changtai Niu, Xunlai Yuan, and Shuhai Xiao. 2018. "Nitrogen-Fixing Heterocystous Cyanobacteria in the Tonian Period." *Current Biology* 28(4):616-622.e1. doi: 10.1016/J.CUB.2018.01.008.
- Panwichian S., Kantachote D., Wittayaweerasak B., and Mallavarapu M. 2010. "Isolation of Purple Nonsulfur Bacteria for the Removal of Heavy Metals and Sodium from Contaminated Shrimp Ponds." *Electronic Journal of Biotechnology* 13(4). Doi: 10.2225/vol13-issue4-fulltext-8.
- Pereira Sara., Zille A., Micheletti E., Moradas-Ferreira P., De Philippis R, and Tamagnini P. 2009. "Complexity of Cyanobacterial Exopolysaccharides: Composition, Structures, Inducing Factors and Putative Genes Involved in Their Biosynthesis and Assembly." *FEMS Microbiology Reviews* 33(5):917–41.

- Polyzois A., Kirilovsky D, Dufat T. H., and Michel S. 2020. “Effects of Modification of Light Parameters on the Production of Cryptophycin, Cyanotoxin with Potent Anticancer Activity, in Nostoc Sp.” *Toxins* 12(12). Doi: 10.3390/toxins12120809.
- Prakash S., Ponnusamy K., and Mohamed S. N. 2022. “An Insight on Biocathode Microbial Desalination Cell: Current Challenges and Prospects.” *International Journal of Energy Research* 46(7):8546–59. Doi: 10.1002/ER.7748.
- Prins F. X., Etale A., Ablo A. D., and Thatcher A. 2022. “Water scarcity and alternative water sources in South Africa: can information provision shift perceptions.” *Urban Water Journal*, 20 (10), 1438–1449. <https://doi.org/10.1080/1573062X.2022.2026984>
- Quesada A., Nieva M., Leganés F., Ucha A., Martín M., Prosperi C., and Fernández-Valiente E. 1998. “Acclimation of Cyanobacterial Communities in Rice Fields and Response of Nitrogenase Activity to Light Regime.” *Microbial Ecology* 35(2):147–55. Doi: 10.1007/s002489900069.
- Raluy R., Serra G. L., Uche J, and Valero A. 2004. “Life-Cycle Assessment of Desalination Technologies Integrated with Energy Production Systems.” *Desalination* 167(1–3):445–58. Doi: 10.1016/j.desal.2004.06.160.
- Rai A. K., and Abraham G. 1995. “Relationship of Combined Nitrogen Sources to Salt Tolerance in Freshwater Cyanobacterium *Anabaena Doliolum*.” *Journal of Applied Bacteriology* 78(5):501–6. doi: 10.1111/J.1365-2672.1995.TB03092.X.
- Reed R. H., Warr S. R. C, Richardson D. L, Moore D. J., and Stewart W. D. P. 1985. “Multiphasic Osmotic Adjustment in a Euryhaline Cyanobacterium.” *FEMS Microbiology Letters* 28(3):225–29. Doi: 10.1111/j.1574-6968.1985.tb00796.x.

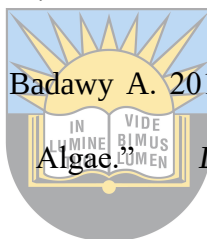
Roger P. A., and Kulasooriya S. A. 1980. "Blue-Green Algae and Rice. IRRI, Los Banos." *Laguna* 112(9).

Rosgaard L., de Porcellinis A. J., Jacobsen J. H., Frigaard N, and Sakuragi Y. 2012. "Bioengineering of Carbon Fixation, Biofuels, and Biochemicals in Cyanobacteria and Plants." *Journal of Biotechnology* 162(1):134–47. Doi: 10.1016/j.jbiotec.2012.05.006.

Rossi F., and De Philippis R. 2015. "Role of Cyanobacterial Exopolysaccharides in Phototrophic Biofilms and in Complex Microbial Mats." *Life* 5(2):1218–38.

Saad A., and Atia A. 2014. "Review on Freshwater Blue-Green Algae (Cyanobacteria): Occurrence, Classification and Toxicology." *BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA* 11(3):1319–25. Doi: 10.13005/bbra/1522.

Sahle-Demessie E., Hassan A. A., and El Badawy A. 2019. "Bio-Desalination of Brackish and Seawater Using Halophytic Algae." *Desalination* 465:104–13. Doi: 10.1016/j.desal.2019.05.002.



Schoeman J. J., and Steyn A. 2003. "Nitrate Removal with Reverse Osmosis in a Rural Area in South Africa." *Desalination* 155(1):15–26. Doi: 10.1016/S0011-9164(03)00235-2.

Şengül A. B., Tüfekçi N, and Aktan S. 2016. "The Use of Alum as Coagulant for Removing Cyanobacterial Cells in Drinking Water." *Desalination and Water Treatment* 57(53):25610–16. Doi: 10.1080/19443994.2016.1153982.

Shaikhoun S., Zafar A. M., Wu Y. H., and Hassan A. A. 2022. "Biodesalination Performance of Phormidium Keutzingianum Concentrated Using Two Methods (Immobilization and Centrifugation)." *Arabian Journal of Chemistry* 15(11). Doi: 10.1016/J.ARABJC.2022.104282.

- Shao J., Wang Z, Liu Y, Liu H., Peng L., Wei X., Lei M., and Li R. 2012. “Physiological Responses of *Microcystis Aeruginosa* NIES-843 (Cyanobacterium) under the Stress of Chitosan Modified Kaolinite (CMK) Loading.” *Ecotoxicology* 21(3):698–704. Doi: 10.1007/s10646-011-0828-5.
- Shatat M., and Riffat S. B. 2014. “Water Desalination Technologies Utilizing Conventional and Renewable Energy Sources.” *International Journal of Low-Carbon Technologies* 9(1):1–19. Doi: 10.1093/ijlct/cts025.
- Singh J. S., Kumar A., Rai A. N., and Singh D. P. 2016. “Cyanobacteria: A Precious Bio-Resource in Agriculture, Ecosystem, and Environmental Sustainability.” *Frontiers in Microbiology* 7(APR):529–529.
- Singh N. K., and Dhar D. W. 2010. “Cyanobacterial Reclamation of Salt-Affected Soil.” Pp. 243–75 in. Springer, Dordrecht.
- Singh P., Kaushik M. S., Srivastava M, and Mishra A. K. 2014a. “Phylogenetic Analysis of Heterocystous Cyanobacteria (Subsections IV and V) Using Highly Iterated Palindromes as Molecular Markers.” *Physiology and Molecular Biology of Plants* 20(3):331–42. Doi: 10.1007/s12298-014-0244-4.
- Singh, S. P., and Priyanka Singh. 2015. “Effect of Temperature and Light on the Growth of Algae Species: A Review.” *Renewable and Sustainable Energy Reviews* 50:431–44. Doi: 10.1016/J.RSER.2015.05.024.
- Smuts Basson M. 2011. *Water Development in South Africa*. UN-Water.
- Soontharapirakkul K., Promden W, Yamada N., Kageyama H., Incharoensakdi A., Iwamoto-Kihara A., and Takabe T. 2011. “Halotolerant Cyanobacterium *Aphanothece Halophytica* Contains an Na⁺-Dependent F₁F₀-ATP Synthase with a Potential Role in Salt-Stress Tolerance*.” *Journal of Biological Chemistry* 286(12):10169–76. Doi: 10.1074/jbc.M110.208892.

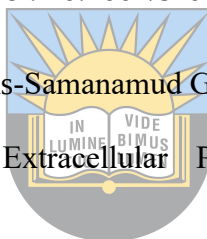
South African weather Service. 2023. “Historical Rain – WeatherSA Portal.” Retrieved October 23, 2023 (<https://www.weathersa.co.za/home/historicalrain>).

Spiller H., and Shanmugam K. T. 1987. “Physiological Conditions for Nitrogen Fixation in a Unicellular Marine Cyanobacterium, *Synechococcus* Sp. Strain SF1.” *Journal of Bacteriology* 169(12):5379–84. doi: 10.1128/JB.169.12.5379-5384.1987.

Stal L. J., and Moezelaar R. 2006a. “Fermentation in Cyanobacteria.” *FEMS Microbiology Reviews* 21(2):179–211. Doi: 10.1111/j.1574-6976.1997.tb00350.x.

Su J., Jia S, Chen X, and Yu H. 2008. “Morphology, Cell Growth, and Polysaccharide Production of *Nostoc* Flagelliforme in Liquid Suspension Culture at Different Agitation Rates.” *Journal of Applied Phycology* 20(3):213–17. Doi: 10.1007/s10811-007-9221-4.

Sun F, Pan R., Qian A., Cong H., and Lamas-Samanamud G. R. 2017. “Consideration on the Water Contamination of Cyanobacterial Extracellular Polymeric Substances (EPS).” Doi: 10.29199/ESAR.101013.



University of Fort Hare

Sun F, Yan Pei H, Hu W. R, Li X. Q, Ma C. X., and Pei R. T. 2013. “The Cell Damage of *Microcystis Aeruginosa* in PACl Coagulation and Floc Storage Processes.” *Separation and Purification Technology* 115:123–28. Doi: 10.1016/j.seppur.2013.05.004.

Sun F, Zhang H., Qian A., Yu H., Xu C., Pan R., and Shi Y. 2020. “The Influence of Extracellular Polymeric Substances on the Coagulation Process of Cyanobacteria.” *Science of the Total Environment* 720. Doi: 10.1016/j.scitotenv.2020.137573.

Sutherland I. W. 2001. “Microbial Polysaccharides from Gram-Negative Bacteria.” Pp. 663–74 in *International Dairy Journal*. Vol. 11. Elsevier.

- Svane R., and Eriksen N. T. 2015. "Exopolysaccharides Are Partly Growth Associated Products in *Microcystis Flos-Aquae*." *Journal of Applied Phycology* 27(1):163–70. Doi: 10.1007/s10811-014-0307-5.
- Swartz C. D., J. A. Du Plessis A. J. B., and Offringa G. 2006. "A Desalination Guide for South African Municipal Engineers." *Water SA* 32(5 SPEC. ISS.):641–47. Doi: 10.4314/at.v32i5.47845.
- Taheri R., Razmjou A., Szekely G., Hou J., and Ghezelbash G. R. 2016. "Biodesalination – On Harnessing the Potential of Nature’s Desalination Processes." *Bioinspiration and Biomimetics* 11(4):041001.
- Teixeira M. R., Sousa V., and Rosa M/ J. 2010. "Investigating Dissolved Air Flotation Performance with Cyanobacterial Cells and Filaments." *Water Research* 44(11):3337–44. Doi: 10.1016/j.watres.2010.03.012.
- Thermofisher. 2024. "Document Connect." Retrieved January 7, 2024 (https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2Fmanuals%2FMAN0018500_CyQUANT-LDH-Cytotoxicity-Assay-Kit_PI.pdf).
- Thimmaraju M., Sreepada D., Babu G. S., Dasari B. K., Velpula S. K., and Vallepu N. 2018. "Desalination of Water. Desalination and Water Treatment." 333–47.
- Timková I., Sedláková-Kaduková J., and Pristaš P. 2018. "Biosorption and Bioaccumulation Abilities of Actinomycetes/Streptomyces Isolated from Metal Contaminated Sites." *Separations* 2018, Vol. 5, Page 54 5(4):54. Doi: 10.3390/SEPARATIONS5040054.

- Turki Y., Mehri L., Lajnef R., Rejab A. B., Khessairi A., Cherif H., Ouzari H., Hassen A. 2016. Biofilms in bioremediation and wastewater treatment: characterization of bacterial community structure and diversity during seasons in municipal wastewater treatment process. *Environmental Science and Pollution Research*, Volume 24, pp. 3519-3530.
- UN-Water. 2006. "UN World Water Development Report 2006 | UN-Water." Retrieved October 15, 2022 (<https://www.unwater.org/publications/un-world-water-development-report-2006>).
- Van Liere L., and Luuc R. Mur. 1980. "Occurrence of *Oscillatoria Agardhii* and Some Related Species, a Survey." Pp. 67–77 in *Hypertrophic Ecosystems*. Springer Netherlands.
- Vinnemeier J., and Hagemann M. 1999. "Identification of Salt-Regulated Genes in the Genome of the Cyanobacterium *Synechocystis* Sp. Strain PCC 6803 by Subtractive RNA Hybridization." *Archives of Microbiology* 172(6):377–86. Doi: 10.1007/s002030050774.
- Visser J, Verhoog S. A. 2007. Water management and planning in a semi-arid country: The South African situation, WIT Press.
- Wang X., Ding S, Song W., Li H., Zhang Y, Ren W., Li M., Lu J., and Ding J. 2021. "A Collaborative Effect of Algae-Bacteria Symbiotic and Biological Activated Carbon System on Black Odorous Water Pretreated by UV Photolysis." *Biochemical Engineering Journal* 169:107983. Doi: 10.1016/J.BEJ.2021.107983.
- Wei J., Gao L., Shen G., Yang X., and Li M. 2020. "The Role of Adsorption in Microalgae Biological Desalination: Salt Removal from Brackish Water Using *Scenedesmus Obliquus*." *Desalination* 493:114616. Doi: 10.1016/J.DESAL.2020.114616.
- Westrick Judy A., Szlag D. C, Southwell B. J., and Sinclair J. 2010. "A Review of Cyanobacteria and Cyanotoxins Removal/Inactivation in Drinking Water Treatment." *Analytical and Bioanalytical Chemistry* 397(5):1705–14. Doi: 10.1007/s00216-010-3709-5.

- White W. M. 2009. "Trace Elements Geochemistry W. M. White Chapter 7: Trace Elements Geochemistry." 260–71.
- Whitton B.A.; Potts, M. 2000. "Introduction to the Cyanobacteria. The Ecology of Cyanobacteria." 1–11.
- Whitton B. A. 2006. "Soils and Rice-Fields." Pp. 233–55 in *The Ecology of Cyanobacteria*. Kluwer Academic Publishers.
- WHO. 2021. "WHO/EU Drinking Water Standards Comparative Table." Retrieved May 8, 2021 (<https://www.lenntech.com/who-eu-water-standards.htm>).
- Winter T, Pannell D. J, and Mccann L. 2002. *The Economics of Desalination and It's Potential Application to Australia*. Doi: 10.22004/AG.ECON.125611.
- World Wildlife W. (2020). Available at: <http://www.worldwildlife.org/threats/water-scarcity> [Accessed 20 January 2020].
- Xu H., Zhong J, Yu G., Wu J., Jiang H., and Yang L. 2014. "Further Insights into Metal-DOM Interaction: Consideration of Both Fluorescent and Non-Fluorescent Substances." *PloS ONE* 9(11). Doi: 10.1371/journal.pone.0112272.
- Yahaya A, Okoh O.O., Okoh A. I., and Adeniji A. O. 2017. "Occurrences of Organochlorine Pesticides along the Course of the Buffalo River in the Eastern Cape of South Africa and Its Health Implications." *International Journal of Environmental Research and Public Health* 14(11). doi: 10.3390/IJERPH14111372.
- Yensen N. P. 2006. "Halophyte Uses For The Twenty-First Century." Pp. 367–96 in *Ecophysiology of High Salinity Tolerant Plants*. Springer Netherlands.
- Zafar A. M., Muhammad Asad Javed M. A, and Aly Hassan A. 2022. "Unprecedented Biodesalination Rates–Shortcomings of Electrical Conductivity Measurements in Determining

Salt Removal by Algae and Cyanobacteria.” *Journal of Environmental Management* 302:113947. Doi: 10.1016/J.JENVMAN.2021.113947.

Zafar A. M., Javed M. A., Aly Hassan A., Mehmood K., and Sahle-Demessie E. 2021. “Recent Updates on Ions and Nutrients Uptake by Halotolerant Freshwater and Marine Microalgae in Conditions of High Salinity.” *Journal of Water Process Engineering* 44. Doi: 10.1016/j.jwpe.2021.102382.

Zafar A. M., Javed M. A., Aly Hassan A., Sahle-Demessie E., and Harmon S. 2022. “Biodesalination Using Halophytic Cyanobacterium *Phormidium Keutzingianum* from Brackish to the Hypersaline Water.” *Chemosphere* 307:136082. Doi: 10.1016/J.CHEMOSPHERE.2022.136082.

Zhang C. C., Zhou C. Z., Burnap R. L., and Peng L. 2018. “Carbon/Nitrogen Metabolic Balance: Lessons from Cyanobacteria.” *Trends in Plant Science* 23(12):1116–30. Doi: 10.1016/j.tplants.2018.09.008.

Zhang G., Zhang P., Wang B., and Liu H. 2006. “Ultrasonic Frequency Effects on the Removal of *Microcystis Aeruginosa*. Ultrasonics Sonochemistry.” *Elsevier* 13:446–50. Doi: 10.1016/j.ultsonch.2005.09.012.

Zhang M., Qin B., Yu Y., Yang Z., Shi X., and Kong F. 2016. “Effects of Temperature Fluctuation on the Development of Cyanobacterial Dominance in Spring: Implication of Future Climate Change.” *Hydrobiologia* 763(1):135–46. Doi: 10.1007/s10750-015-2368-0.

Zhou J., Zhao Z, Liu J., Peng W., Peng X., Han Y., and Xiao P. 2019. “Removal of *Microcystis Aeruginosa* and Control of Algal Organic Matters by Potassium Ferrate (VI) Pre-Oxidation Enhanced Fe(II) Coagulation.” *Korean Journal of Chemical Engineering* 2019 36:10 36(10):1587–94. Doi: 10.1007/S11814-019-0358-Z.

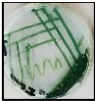
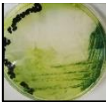
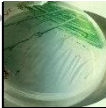


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Supplementary data

Table S1: Presumptive cyanobacterial isolates and morphology from different sampling points.

No.	Isolate(s)	Texture	Elevation	Pigmentation	Shape	Picture
1	KWT	Dry	Flat	Blue Green	Irregular/filamentous	
2	KWT	Dry	Bent	Green	Irregular/filamentous	
3	KWT	Moist	Flat	Blue Green	Single colonies/ cocci	

4	KWT	Dry	Curved	Green	Single colonies/ cocci	
5	KWT	Dry	Flat	Blue Green	Single colonies/ cocci	
6	Grinika	Dry	Flat	Green	Irregular/filamentous	
7	Grinika	Dry	Flat	Green	Irregular/cocci	
8	Grinika	Moist	Flat	Green	Irregular/cocci	
9	Grinika	Moist	Flat	Blue Green	Irregular/ filamentous	
10	Grinika	Moist	Bent	Blue Green	Irregular/ filamentous	
11	Grinika	Moist	Bent	Green	Irregular/ filamentous	



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12	Grinika	Moist	Bent	Blue Green	Irregular/ filamentous	
13	Grinika	Moist	Bent	Blue Green	Irregular/ filamentous	
14	Ndevana	Moist	Flat	Green	Irregular/ filamentous	
15	Ndevana	Dry	Bent	Green	Irregular/ filamentous	
16	Ndevana	Dry	Flat	Green	Irregular/green	
17	Buffalo Pass	Moist	bent	Green	Irregular/ filamentous	
18	Buffalo Pass	Dry	Bent	Blue Green	filamentous/cocci	
19	Buffalo Pass	Dry	Bent	Green	Filamentous/irregul ar	



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20	Buffalo	Moist	Falt	Green	Irregular/	
	Pass				filamentous/	

Table S2: Identified species isolated from East London Buffalo Pass River in East London (i.e., isolate 20 Buffalo Pass).

Featur	Max	Query	E-	Ident	Accessi	Genus	Species	Phylu
e_ID	score	cover	value		on			m
OTU_1	73.75	11.69%	2.94E-10	93.62%	"MW971521.1	<i>Nitzschia</i>	<i>Inconspicua</i>	Diatom
OTU_1	150.66	28.36%	2.07E-33	89.47%	"QAXM01007880.1	<i>Chlamydomonas</i>	<i>sp.</i>	Green Algae
OTU_2	248.71	51.49%	6.28E-63	87.44%	"WKL D01000079.1	<i>Picochlorum</i>	<i>costavermella</i>	Green Algae

OTU_3	325.62	95.03%	4.43E-86	82.72%	"UYFQ 010012	<i>Hydrur</i>	<i>foetidus</i>	Golden
						<i>us</i>		Algae
					35.1			
OTU_3	323.7	91.05%	1.68E-85	83.33%	"NMU B01001	<i>Monora</i>	<i>sp.</i>	Green
						<i>phidiu</i>		Algae
					622.1	<i>m</i>		
OTU_3	319.85	94.28%	2.42E-84	82.59%	"NZ_C P03405	<i>Anabae</i>	<i>sp.</i>	cyanob
						<i>na</i>		acteria
					8.1			
OTU_5	508.28	95.52%	4.59E-141	89.58%	"QXTD 010165	<i>Haemat</i>	<i>lacustri</i>	Green
						<i>ococcus</i>	<i>s</i>	Algae
					58.1			
OTU_5	344.85	95.27%	7.22E-92	82.25%	"LXQD 010001	<i>Nostoc</i>	<i>minutu</i>	cyanob
						<i>m</i>		acteria
					03.1			



OTU_6	425.6	98.26%	3.55E-116	85.32%	"NC_019697.1	<i>Chamaesiphon</i>	<i>minutus</i>	cyanobacteria
OTU_6	381.38	98.01%	7.27E-103	84.26%	"VTSQ010000	<i>Parachlorella</i>	<i>Kessleri</i>	green algae
OTU_6	375.61	97.76%	3.96E-101	83.21%	"CP070233.1	MAG: <i>Dolichospermum</i>		cyanobacteria
OTU_6	369.84	97.76%	2.16E-99	82.95%	"JAAV000004.1	<i>Dolichospermum</i>	<i>sp.</i>	cyanobacteria



Table S3: Identified species isolated from Grinika Buffalo River in Mdantsane (i.e., isolate 12 Grinika).

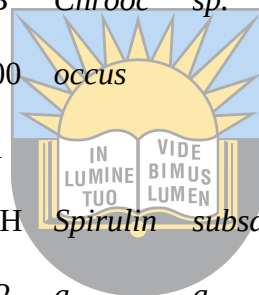
Feature	Max	Query	E-value	Ident	Accessi	Genus	Species	Phylum
_ID	score	cover			on			

OTU1	323.7	91.05%	1.68E-	83.33%	"NMUB	<i>Monora</i>	<i>sp.</i>	green
			85		010016	<i>phidium</i>		algae
					22.1			
OTU1	319.85	94.28%	2.42E-	82.59%	"NZ_C	<i>Anabae</i>	<i>sp.</i>	cyanoba
			84		P03405	<i>na</i>		acteria
					8.1			
OTU2	371.77	99.50%	5.70E-	82.75%	"WKLD	<i>Picochl</i>	<i>costaver</i>	green
			100		010000	<i>orum</i>	<i>mella</i>	algae
					79.1			
OTU2	329.47	90.05%	3.08E-	82.87%	"JAAIK	<i>Stichoc</i>	<i>bacillar</i>	green
			87		U01000	<i>occus</i>	<i>is</i>	algae
					0212.1			
OTU2	325.62	94.28%	4.43E-	81.53%	"WKLD	<i>Picochl</i>	<i>costaver</i>	green
			86		010001	<i>orum</i>	<i>mella</i>	algae
					09.1			
OTU3	379.46	87.81%	2.76E-	86.69%	"NC_01	<i>Cyanob</i>	<i>aponinu</i>	cyanoba



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			102		9776.1	<i>acteriu</i>	<i>m</i>	cteria
						<i>m</i>		
OTU3	371.77	98.26%	5.70E-	83.80%	"LXQD	<i>Nostoc</i>	<i>minutu</i>	cyanoba
			100		010001		<i>m</i>	cteria
					03.1			
OTU3	366	87.81%	3.11E-	85.55%	"NZ_B	<i>Chrooc</i>	<i>sp.</i>	cyanoba
			98		LJI0100	<i>occus</i>		cteria
					0038.1			
OTU3	362.15	87.81%	4.46E-	85.84%	"NZ_JH	<i>Spirulin</i>	<i>subsals</i>	cyanoba
			97		980292.	<i>a</i>	<i>a</i>	cteria
OTU3	362.15	88.81%	4.46E-	85.15%	"NZ_M	<i>Hydroc</i>	<i>rivulari</i>	cyanoba
			97		RCB01	<i>occus</i>	<i>s</i>	cteria
					000064.			
					1			



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Table S4: Identified species isolated from Grinika Buffalo River in Mdantsane (i.e., isolate 14 Grinika).

Feature ID	Max score	Query cover	E-value	Ident	Accession	Genus	Species	Phylum
OTU_1	246.79	63.93%	2.38E-62	83.27%	"NZ_CP034058.1	<i>Anabaena</i>	<i>sp.</i>	cyanobacteria
OTU_1	214.11	50.75%	1.65E-52	84.80%	"LWCB010033.85.1	<i>Coelastrum</i>	<i>sp.</i>	green algae
OTU_4	73.75	11.69%	2.94E-10	93.62%	"MW971521.1	<i>Nitzschia</i>	<i>inconspicua</i>	Diatom
OTU_4	150.66	28.36%	2.07E-33	89.47%	"QAXL010078.80.1	<i>Chlamydomonas</i>	<i>sp.</i>	green algae
OTU_5	327.54	88.56%	1.17E-	83.99%	"QBLS	MAG:	<i>Pseudomonas</i>	cyanobacteria

			86		010000	<i>nabaen</i>	acteria
					26.1	<i>a</i>	
OTU_5	312.16	88.81%	4.99E-	83.19%	"LDKB	<i>Chlorell</i>	<i>vulgaris</i> green
			82		010002	<i>a</i>	algae
					74.1		
OTU_5	310.24	88.56%	1.89E-	83.15%	"NZ_JA	<i>Pseuda</i>	<i>sp.</i> cyanoba
			81		CJQY0	<i>nabaen</i>	acteria
					100000	<i>a</i>	
					89.1		
OTU_7	600.57	88.81%	7.59E-	95.80%	"NMUB	<i>Monora</i>	<i>sp.</i> green
			169		010016	<i>phidium</i>	algae
					22.1		
OTU_7	471.75	100.25	4.56E-	87.35%	"VTSQ	<i>Parachl</i>	<i>kessleri</i> green
		%	130		010001	<i>orella</i>	algae
					15.1		
OTU_7	439.06	96.52%	3.15E-	87.11%	"LXQD	<i>Nostoc</i>	<i>minutu</i> cyanoba

120	010001	<i>m</i>	acteria
	03.1		

Table S5: Identified species isolated from Ndevana Buffalo River in King William's Town (i.e., isolate 15 Ndevana).

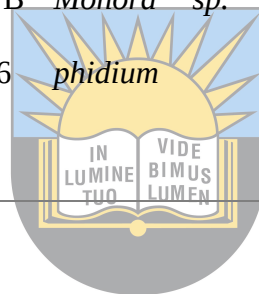
Feature	Max	Query	E-value	Ident	Accessi	Genus	Species	Phylum
_ID	score	cover			on			
OTU_1	773.61	100.00	0	100.00	"VTSQ	<i>Parachl</i>	<i>kessleri</i>	green
		%		%	010000	<i>orella</i>		algae
					45.1			
OTU_2	767.84	100.00	0	99.75%	"VJNP0	<i>Chlorell</i>	<i>vulgaris</i>	green
		%			100065	<i>a</i>		algae
					8.1			
OTU_5	612.1	79.10%	2.55E-	100.00	"PQAU	<i>Chlorell</i>	<i>sorokini</i>	Green
			172	%	010000	<i>a</i>	<i>ana</i>	Algae
					06.1			
OTU_7	358.31	94.03%	6.42E-	84.39%	"NZ_C	<i>Synecho</i>	<i>lividus</i>	cyanoba

96	P01809	<i>coccus</i>	acteria
2.1			

Table S6: Identified species isolated from Wimpy Buffalo Pass River in King William's Town (i.e., isolate 3 Wimpy).

Feature	Max	Query	E-value	Ident	Accessi	Genus	Species	Phylum
_ID	score	cover			on			
OTU_2	110.28	19.40%	2.96E-	91.03%	"FNXT	<i>Tetrad</i>	<i>obliquu</i>	green
			21		010005	<i>smus</i>	<i>VIDE</i>	algae
					92.1	<i>LUMINE</i>	<i>BIMUS</i>	
						<i>TUO</i>	<i>LUMEN</i>	
OTU_2	110.28	19.40%	2.96E-	91.03%	"QFBI0	<i>Chlamy</i>	<i>sp.</i>	green
			21		100009	<i>domona</i>		algae
					2.1	<i>s</i>		
OTU_3	214.11	50.75%	1.65E-	84.80%	"LWCB	<i>Coelast</i>	<i>sp.</i>	green
			52		010033	<i>rella</i>		algae
					85.1			
OTU_5	289.09	75.12%	4.40E-	83.78%	"VTSQ	<i>Parachl</i>	<i>kessleri</i>	green

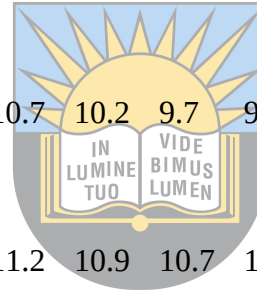
			75		010002	<i>orella</i>		algae
					60.1			
OTU_5	291.01	74.88%	1.16E-	83.39%	"NZ_C	<i>Anabae</i>	<i>sp.</i>	cyanoba
			75		P03405	<i>na</i>		cteria
					8.1			
OTU_5	304.47	81.59%	1.03E-	83.23%	"NMUB	<i>Monora</i>	<i>sp.</i>	green
			79		010016	<i>phidium</i>		algae
					22.1			



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Table S7: Effects of 15ppm (1.5%) salinity concentrations

isolates	% Desalination over 15 days															%Desalination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
3	15.1	14.2	13.9	13.0	13.0	12.9	12.7	12.4	12.3	11.5	11.3	10.6	10.1	9.5	9.0	40.3
Wimpy																
12	15.1	15.0	14.1	13.8	12.9	11.9	11.9	11.8	11.7	10.7	10.2	9.7	9.2	8.7	8.3	45.3
Granika																
14	15.1	14.3	14.0	12.8	12.4	12.1	11.9	11.6	11.3	11.2	10.9	10.7	10.4	10.2	9.9	34.1
Grinika																
15	15.1	14.8	13.9	12.5	12.0	11.8	11.7	10.9	10.0	9.4	8.8	8.2	7.6	6.9	6.3	58.1
Ndevana																
20	15.1	14.5	13.4	13.4	13.1	12.9	12.1	12.0	10.6	10.1	10.0	9.4	8.9	8.4	7.9	47.4
Buffalo																
Pass																



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Table S8: Effects of 20 ppm (2%) salinity concentrations

isolate	% Desalination over 15 days															%Desalination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
3	20.3	19.4	18.6	17.7	16.8	15.9	15.0	14.2	13.3	12.4	11.5	10.6	9.8	8.9	8.0	60.7
Wimp																
y																
12	20.3	20.0	19.5	18.8	17.6	16.8	15.9	14.3	13.1	12.7	12.7	11.9	11.3	10.8	10.1	50.5
Grani																
ka																
14	20.3	19.4	18.4	17.5	16.6	15.6	14.7	13.7	12.8	11.9	10.9	10.0	9.0	8.1	7.2	64.8
Grinik																
a																



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15	20.3	19.1	17.4	16.3	14.9	13.4	11.9	10.5	9.0	8.2	7.5	6.0	4.6	4.1	3.6	82.1
Ndeva																
na																
20	20.3	20.1	19.3	18.9	17.9	16.9	15.9	15.4	14.9	14.4	13.2	12.6	11.9	11.2	10.5	48.5
Buffal																
o Pass																



Table S9: Effects of 40 ppm (4%) salinity concentrations

isolates	% Desalination over 15 days															%Desalination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
3	40.1	40.0	39.8	37.7	35.6	34.5	33.5	33.0	29.9	29.6	29.5	29.3	29.2	29.2	29.0	27.7
Wimpy																
12	40.1	39.9	37.7	31.4	30.6	29.5	28.9	27.8	26.7	24.0	22.6	22.1	21.9	21.6	21.5	46.3
Grinika																

14	40.1	40.1	39.7	38.0	37.0	36.4	35.0	34.9	34.0	33.9	34.0	34.9	35.0	34.5	34.5	13.9
Grinika																
15	40.1	39.1	38.9	36.6	36.1	35.1	34.8	33.3	33.2	31.8	30.0	28.5	25.5	24.2	21.9	45.2
Ndevana																
20	40.1	40.1	39.8	38.0	36.2	34.6	33.0	31.6	31.9	30.7	30.6	29.9	29.3	28.7	28.1	29.8
Buffalo																
Pass																



Table S10: Summary optimal tests of optimal conditions under dark light intensity.

Isolates	Light Intensity: dark																				%Desalination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	n
3	20.	20.	20.	20.	20.	20.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	18.	6.3
Wimpy	2	2	1	1	0	0	9	9	8	7	6	5	5	4	3	2	1	1	0	9	
12	20.	20.	20.	20.	20.	19.	19.	19.	18.	18.	18.	17.	17.	17.	17.	17.	16.	16.	16.	16.	18.2
Grinika	2	2	1	0	0	8	3	0	7	4	0	8	6	2	1	0	9	7	6	5	

14	20.	20.	20.	20.	20.	20.	20.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	19.	4.5
Grinika	2	2	1	1	1	0	0	9	9	9	8	7	7	6	6	5	4	4	3	3	
15	20.	20.	20.	19.	19.	19.	19.	19.	19.	19.	18.	18.	18.	18.	18.	18.	18.	17.	17.	17.	13.6
Ndevan	2	1	0	9	8	7	6	5	4	3	9	8	7	6	3	1	0	8	6	4	
a																					
20	20.	20.	20.	19.	19.	19.	19.	19.	19.	19.	19.	18.	18.	18.	18.	18.	18.	18.	18.	18.	10.6
Buffalo	2	1	0	8	8	6	5	4	3	2	1	9	8	7	6	5	4	3	1	0	
Pass																					



Table S11: Summary optimal test of optimal conditions under bi-light intensity (12:12h)

Isolates	Light Intensity: bi-light (12:12h)																				%Desalination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	n
3	20.	20.	20.	20.	20.	19.	19.	19.	18.	18.	18.	17.	17.	17.	17.	17.	16.	16.	16.	16.	20.7
Wimpy	2	2	1	0	0	9	6	0	3	1	0	8	6	4	2	0	8	6	2	0	

12	20.	20.	19.	19.	19.	18.	18.	18.	17.	17.	16.	16.	16.	15.	14.	13.	13.	12.	12.	12.	39.2
Grinika	2	1	8	3	1	7	3	0	6	3	9	6	1	6	4	8	3	7	5	3	
14	20.	20.	19.	19.	19.	18.	18.	18.	18.	17.	17.	17.	16.	16.	15.	15.	15.	14.	14.	13.	34.6
Grinika	2	1	6	4	1	2	5	4	0	9	6	4	4	1	7	4	1	7	1	2	
15	20.	20.	20.	19.	19.	19.	18.	18.	18.	18.	17.	17.	15.	14.	14.	14.	14.	13.	13.	12.	40.0
Ndevan	2	1	0	8	7	0	6	5	3	3	8	2	9	7	5	0	0	7	5	1	
a																					
20	20.	20.	19.	19.	19.	18.	18.	18.	18.	18.	17.	17.	15.	14.	14.	14.	13.	13.	12.	12.	38.5
Buffalo	2	1	7	3	1	7	4	4	3	1	6	0	4	9	4	0	5	1	6	4	
Pass																					



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Table S12: Summary optimal Test of optimal conditions under continuous light intensity

Isolates	Light Intensity: continuous light																				%Desalinatio
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	n

3	20.	20.	20.	20.	19.	19.	19.	19.	18.	18.	17.	17.	16.	16.	15.	15.	14.	14.	13.	12.	40.0
Wimpy	2	1	1	0	7	3	2	0	5	1	6	2	7	2	8	3	9	0	5	1	
12	20.	20.	19.	19.	19.	18.	17.	16.	15.	14.	14.	13.	13.	12.	12.	11.	11.	10.	10.	9.1	55.0
Grinika	2	0	9	7	0	1	6	7	0	6	2	7	3	9	1	7	2	9	6		
14	20.	20.	20.	20.	19.	19.	19.	18.	18.	18.	18.	17.	17.	16.	15.	15.	14.	13.	13.	12.	38.5
Grinika	2	1	1	1	7	6	5	9	7	4	1	8	1	8	9	0	6	4	0	4	
15	20.	20.	20.	19.	19.	18.	18.	17.	17.	16.	15.	15.	14.	14.	13.	13.	12.	12.	11.	10.	48.4
Ndevan	2	1	0	6	3	3	0	7	0	3	9	6	9	2	8	1	4	1	8	4	
a																					
20	20.	20.	20.	20.	19.	19.	19.	19.	18.	18.	17.	17.	16.	16.	15.	15.	14.	13.	12.	11.	41.1
Buffalo	2	2	1	0	9	8	4	0	8	2	8	1	6	1	5	0	4	4	6	9	
Pass																					



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