11-2021

BIOLOGICAL TREATMENT OF PRODUCED WATER USING ALGAE: A PROOF OF CONCEPT

Shibin Nadersha

جامعة الإمارات العربية المتحدة
United Arab Emirates University

MASTER THESIS NO. 2022:3

College of Engineering

Department of Civil and Environmental Engineering

BIOLOGICAL TREATMENT OF PRODUCED WATER USING ALGAE: A PROOF OF CONCEPT

Shibin Nadersha

November 2021

United Arab Emirates University

College of Engineering

Department of Civil and Environmental Engineering

BIOLOGICAL TREATMENT OF PRODUCED WATER USING ALGAE: A PROOF OF CONCEPT

Shibin Nadersha

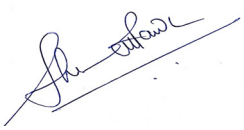
This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Water Resources

Under the Supervision of Dr. Ashraf Aly Hassan

November 2021

Declaration of Original Work

I, Shibin Nadersha, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Biological Treatment of Produced Water Using Algae: A Proof of Concept*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Ashraf Aly Hassan, in the College of Engineering at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature:  Date: 02.01.2022

Advisory Committee

1) Advisor: Dr. Ashraf Aly Hassan

Title: Assistant Professor

Department of Civil and Environmental Engineering

College of Engineering

2) Co-advisor: Dr. Mohamed Hamouda

Title: Associate Professor

Department of Civil and Environmental Engineering

College of Engineering

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

1) Advisor (Committee Chair): Dr. Ashraf Aly Hassan
   Title: Assistant Professor
   Department of Civil and Environmental Engineering
   College of Engineering

   Signature _____ Date 30/11/2021

2) Member: Dr. Muhammad Iqbal
   Title: Assistant Professor
   Department of Chemical and Petroleum Engineering
   College of Engineering

   Signature _____ Date 30/11/2021

3) Member (External Examiner): Dr. Linhua Fan
   Title: Associate Professor
   Department of Chemical & Environmental Engineering
   Institution: RMIT University, Australia

   Signature Munjed Maraqa Date 30/11/2021
   On behalf of Dr. Fan

This Master Thesis is accepted by:

Acting Dean of the College of Engineering: Professor Mohamed Al-Marzouqi

Signature Mohamed AlMarzouqi Date May 09, 2022

Dean of the College of Graduate Studies: Professor Ali Al-Marzouqi

Signature Ali Hassan Date May 9, 2022

Copy ____ of ____

Abstract

Produced water (PW) is the effluent generated during oil mining and extraction. On average, for every barrel of oil, 4 - 5 barrels of PW is generated worldwide. In UAE, 1.22 billion cubic meters of PW was generated in the year 2017. Proper management of PW is thus very important taking into account the large volumes of it being generated. In addition, PW is considered toxic as it contains various contaminants such as aliphatic and aromatic hydrocarbons, inorganic salts, metals, phenols, radioactive materials, and chemical additives. Disposal of untreated PW into oceans and water bodies can cause adverse effects on human health and the environment. Therefore, proper treatment is required before reuse or disposal. The currently used methods of treatment include physical and chemical treatments that are expensive and increase the overall cost of the oil product. Biological treatments are, therefore, recommended as an economical alternative. Microalgae can utilize contaminants in the PW as nutrient sources and thus be used for bioremediation. In this study, microalgae enriched and grown in wastewater were acclimatized to three different produced water samples, obtained from different geographical regions, one being onshore and the other two being offshore. Acclimatization was attained following progressive adaptation in a steadily increasing ratio of produced water. The algae which could adapt and grow in the highest ratio in minimum time was used for bioremediation of produced water at a concentration of 6.25% (v/v). The microscopic examination revealed that the dominant species were *Chlorella*. Treatment was done in batch reactors for seven days. Treatment efficiency was examined by measuring parameters such as pH, COD, TOC, conductivity, salinity, TDS, anions, and alkalinity. COD removal between 22 - 44% and TOC between 4 - 25% were achieved. A reduction of 12 - 18% of EC, 12 - 20% TDS, 13 - 22% salinity, 16 - 25% chloride ion concentration, and an increase of 78 - 85% alkalinity was observed in the three produced water samples over a 7-day treatment period.

Keywords: Produced water, bioremediation, acclimatization, microalgae, removal efficiency.

Title and Abstract (in Arabic)

المعالجة البيولوجية للمياه المنتجة باستخدام الطحالب: دليل على المفهوم

المياه المنتجة هي النفايات السائلة المتولدة أثناء التنقيب عن النفط واستخراجه. في المتوسط ، لكل برميل نفط يتم إنتاج 4-5 براميل من المياه المنتجة في جميع أنحاء العالم. في دولة الإمارات العربية المتحدة، تم إنتاج 1.22 مليار متر مكعب من المياه المنتجة في عام 2017. وبالتالي فإن الإدارة السليمة للمياه المنتجة هام للغاية مع الأخذ في الاعتبار الأحجام الكبيرة منها. بالإضافة إلى ذلك، تعتبر المياه المنتجة سامَّة لاحتوائها على ملوثات مختلفة مثل الهيدروكربونات الأليفاتية والعطرية والأملاح غير العضوية والمعادن والفينولات والمواد المشعة والإضافات الكيميائية. يمكن أن يتسبب التخلص من المياه المنتجة غير المعالجة في المحيطات والأجسام المائية في آثار ضارة على صحة الإنسان والبيئة. لذلك، المعالجة المناسبة مطلوبة قبل إعادة الاستخدام أو التخلص من المياه المنتجة. تشمل طرق المعالجة المستخدمة حاليًا المعالجات الفيزيائية والكيميائية باهظة الثمن والتي تزيد من التكلفة الإجمالية للنفط. لذلك، يوصى باستخدام العلاجات البيولوجية كبديل اقتصادي. يمكن أن تستخدم الطحالب الدقيقة الملوثات في المياه المنتجة كمصادر مغذية وبالتالي يمكن استخدامها للمعالجة الحيوية. في هذه الدراسة، تم أقلمه الطحالب الدقيقة المخصبة والمزروعة في مياه الصرف الصحي على ثلاث عينات مختلفة من المياه المنتجة عن طريق التكيف التدريجي في نسبة الزيادة المطردة للمياه المنتجة تم الحصول عليها من مناطق جغرافية مختلفة، إحداها بريه واثنتان بحريه. تم تحقيق التأقلم بعد التكيف التدريجي في زيادة نسبة المياه المنتجة بشكل مطرد. تم استخدام الطحالب التي يمكن أن تتكيف وتنمو بأعلى نسبة في أقل وقت للمعالجة الحيوية للمياه المنتجة بتركيز 6.25% (حجم/حجم). أظهر الفحص المجهري أن الأنواع السائدة كانت كلوريللا. كما تم الكشف عن وجود أنواع نانوكلوروفسيس. تمت المعالجة في مفاعلات على مدى سبعة أيام. تم فحص كفاءة المعالجة عن طريق قياس العناصر مثل الأس الهيدروجيني، COD، TOC، الموصلية، الملوحة، TDS، الأيونات السالبة والقلوية. تمت إزالة COD بنسبة تتراوح بين 22-44%. كما تمت إزالة 12-18% من EC و12-20% منTDS و13-22% من الملوحة و16-25% من تركيز أيون الكلوريد وتمت زيادة القلوية بنسبه 78-85% في عينات المياه الثلاثة المنتجة على مدى فترة معالجة مدتها 7 أيام.

الكلمات المفتاحية: المياه المنتجة، المعالجة الحيوية، التأقلم، الطحالب الدقيقة، كفاءة الإزالة.

Acknowledgements

Learning is accomplished and learners succeed when they are surrounded by people who unconditionally share their knowledge and experience and when those kind people inspire them with patience, dedication, and motivation

I would like to express my sincere gratitude towards the most dedicated and motivating advisor, Dr. Ashraf Aly Hassan, whose constant guidance and support were priceless throughout the course of this research. His enthusiasm and encouragement always urged me to learn, understand and work more during unsuccessful experiments. Deep gratitude to my Co-advisor Dr. Mohamad Hamouda and my committee for their valuable support and guidance to improve this thesis.

I deeply acknowledge and appreciate the help and timely guidance of lab instructor, Mr. Salem Hegazy. Special thanks to my fellow lab mates, friends, and colleagues especially Abdul Mannan Zafar and Asad Javed for helping me solve daily challenges, who contributed suggestions, and provided deep knowledge about laboratory instruments. I would like to thank the entire faculty and members of the Department of Civil and Environmental Engineering for assisting me throughout my graduate studies.

I owe my utmost gratitude to my husband, children, and family for always being there by my side and to whom I will be forever grateful. Thanks to Almighty for giving me the strength to learn, understand and accomplish!

Dedication

To my most loved husband Nadersha and children Rehan, Mehrin, and Zidan

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BOD	Biochemical Oxygen Demand
BTEX	Benzene Toluene Ethylbenzene and Xylene
CFU	Cyclone Floatation Unit
COD	Chemical Oxygen Demand
EC	Electrical Conductivity
HRT	Hydraulic Retention Time
IC	Ion Chromatography
MBR	Membrane Bioreactor
NORM	Naturally Occurring Radioactive Material
OPEC	Organization of the Petroleum Exporting Countries
PW	Produce Water
TC	Total Carbon
TDS	Total Dissolved Solids
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbons
TSS	Total Suspended Solids

Chapter 1: Introduction

1.1 Overview

Over the past decades, the exponential increase in industrial development and the rise in human-induced activities have resulted in a strong increase in energy demand. Due to its abundance and the variety of products derived from crude oil, the use of fossil fuels in general and especially oil was favored among all the energy production techniques (Dores et al., 2012; Lusinier et al., 2019). Produced water is a by-product of the extraction of crude oil. Produced water comes from three primary sources in the oil and gas industry: a) formation water, b) injection water and c) condensed water. Formation water is either fresh or sea water, which is let out when oil is explored along with the hydrocarbons and is trapped in oil and gas reservoirs. Oil and gas recovery is enhanced by pumping injection water along with production chemicals into reservoirs. The condensed water is present in reservoirs as gas and condenses during a water-oil separation process (Fisher, 1987; Lee & Neff, 2011). Produced water composition varies tremendously depending on the location and the type of production facility (Lee & Neff, 2011). Several factors determine its composition, including the presence of water-soluble components and minerals in the reservoir, chemical additives during oil/gas production, and the type of separation process used (Akob et al., 2015). In addition, surfactants are also added to the reservoir to lower the surface tension between the oil and the water, facilitating easier mobility (Chittick & Srebotnjak, 2017; Skaare et al., 2007). These surfactants, along with other reservoir contaminants (such as salts, metals, hydrocarbons, and radioisotopes), are present as pollutants in the injected water and later become part of the produced water (Danforth et al., 2020).

Thus, produced water is considered toxic due to the complexity of its composition and can cause adverse effects on the environment (Danforth et al., 2020; Horner et al., 2011).

To address those environmental concerns, regulations have been established to minimize the effects of PW discharge on the environment. Before discharging produced water into the sea, an aquifer, or a reservoir, the oil industry employs various treatment methods to meet these regulations (Beyer et al., 2020; Knudsen et al., 2004). These include physical, chemical, and biological methods. The nature of the composition of produced water and the strict discharge standards require proper management and the use of combinations of different treatment methods.

1.2 Statement of the Problem

Oil and gas production produces more polluted water than desirable products. Produced water constitutes 80 percent of all wastes generated by the oil and gas industry, making it the largest waste stream by-product. Around 250 million barrels of produced water are generated each day across the globe compared to 80 million barrels of oil produced. Nearly 40% of these are introduced into the environment, posing a severe threat to the environment (Bakke et al., 2013; Igunnu & Chen, 2014). The ratio of produced water to oil also rises as reservoirs mature and as more oil is extracted (Fakhru'l-Razi et al., 2009). For the oil and gas industry to be sustainable, an environment-friendly discharge of produced water is crucial. The treatment of produced water, however, poses numerous challenges owing to its complexity and toxicity (Lee & Neff, 2011). As a result of such high contamination levels and complex compositions, industries have to invest a great deal in expensive treatment equipment. The profitability of an oil field is largely determined by the cost of

operating the treatment and disposal facilities for produced water. The cost of handling produced water often exceeds the revenue from the well, causing the well to shut down (Ersahin et al., 2018; Wenzlick & Siefert, 2020).

Out of the OPEC countries, UAE is the fourth largest petroleum-producing following

Saudi Arabia, Iraq, and Iran. UAE produced 3.7 million Barrels Per Day (BPD) of oil (petroleum and other liquids) in 2016. According to the data revealed by the Abu Dhabi Department of Energy, the volume of PW during the year 2017 was around 1.22 billion cubic meters. In certain fields of the UAE, such as the Asab oil fields, Abu Dhabi, PW is reinjected into the reservoir using injection wells (Khurshid et al., 2018). However, the salt concentration of PW at Asab ranges from 150,000 to 262,000 ppm (Khurshid et al., 2018). Therefore, effective treatment methods are required for treating PW before disposal. Both physical (e.g., adsorption) and chemical (such as precipitation and coagulation) treatment methods are widely used in removing pollutants from the PW (Al-Ghouti et al., 2019; Igunnu & Chen, 2014; Lusinier et al., 2019). These physical and chemical processes have limitations such as high cost, large footprint, chemical inputs, and low efficiency in pollutants removal (Fakhru'l-Razi et al., 2009). Moreover, these processes cause an increase in the cost of the final petroleum products. Therefore, the biological treatment process may be used to significantly reduce the cost associated with the entire treatment process. The use of microalgae has been proposed as a unique method to bio-remediate PW effectively.

Microalgae can utilize the nutrients present in PW, with minimal extra inputs (Lutzu Giovanni Antonio & Turgut Nurhan Dunford, 2019). Algal treatment is one of the most energy-efficient forms of generating dissolved oxygen. The biomass of algae can provide supersaturated oxygen in water. This makes the process more cost-

effective and productive, as the oxygen can be a natural aeration system providing an alternative to mechanical aeration thereby making the overall system economical in terms of operating cost. Compared to alternative biological treatment methods like the activated sludge process, the algal treatment setup is more compact and less expensive. The studies and available information using algae for bioremediation of PW are limited (Gonçalves et al., 2014; Rahman et al., 2020; Talebi et al., 2016). Therefore, the aim of this study is to develop an effective and economical method for the biological treatment of PW using algae, where microalgae consortia from wastewater will be enriched and acclimatized in several steps in various concentrations and used for the bioremediation of produced water.

Thus, the hypothesis is that the microalgae consortia enriched from wastewater can be acclimatized to high salinity and high oil content of the produced water by progressively increasing the ratio of produced water in which they are grown. These preadapted algae consortia can be introduced into batch reactors containing produced water along with additional balancing nutrients and can be used to metabolize and break down organic and inorganic matters, macro-nutrients, and heavy metals, thereby enabling economic treatment of PW.

1.3 Research Objectives

The objective of this research is aimed at answering the research questions, which are: 1) Is it possible to enrich and grow algae in wastewater and PW? and 2) What is the effect of treatment using algal biomass from wastewater on PW?. Therefore, the specific objectives of the study can be listed as follows:

- To enrich and grow algae that are inherently present in the produced water which can be used for the treatment of produced water.

- To enrich and grow algae inherently available in wastewater by providing nutrient supplements.
- To acclimatize the algae available in wastewater to the high salinity and harsh environment of the produced water in the progressive adaptation process.
- Scale-up and prepare stock culture of preadapted algae for bioremediation of produced water.
- Characterize produced water quality parameters before and after treatment and analyze treatment efficiencies.

1.4 Structure of Thesis

For a more comprehensive overview of the research, a literature review was conducted. Chapter 2 presents a cited summary of this literature review. Chapter 3 includes a detailed description of all the methods used in this experimental research

The summary of the results, graphical illustrations, and discussions of the results are presented in Chapter 4. Chapter 5 provides a summary of conclusions. Directions for future research are discussed in Chapter 6. All references are combined after Chapter 6. Appendix A includes trial experiments and supporting material for future research.

Additional description of analytical methods is included in Appendices B and C. Appendices D, E, and F contains the figures of types of equipment, raw data, and growth curves respectively.

Chapter 2: Literature Review

2.1 Produced Water

Oil mining and extraction is one of the most essential industrial practices of modern society and in addition to fuel production, petroleum has many other uses in the chemical industry (Arriada & Abreu, 2014; Arthur et al., 2005). This operation, however, produces a significant effluent called produced water (Clark & Veil, 2009). Initially produced water is the water that is naturally present in the oil reservoir, also known as formation water. As the extraction progresses, to maintain the hydraulic pressure of the wells and achieve an adequate level of oil and gas extraction from the wells, excess water is pumped into production wells (Hakim et al., 2018; Stephenson, 1992). This mixture of produced water comprises of mixed hydrocarbons and various chemical additives. Various factors such as, oil production pathways, the geological nature of the reservoir, and the composition of oil and gas that mix with water determine the characteristics of PW (Veil et al., 2004). The produced water quality deteriorates in due course due to the presence of various components, such as bacteria, organic and inorganic components dissolved within the produced water (Kondash et al., 2020).

2.2 Constituents of Produced Water

The produced water contains varying levels of impurities, such as inorganic salts, aliphatic and aromatic hydrocarbons, phenols, metals, radioactive elements, and chemical additives used in the extraction line separating water and oil (Figure 1) (Campos et al., 2002). Inorganic constituents include anions and cations which contribute to the salinity of this geologic water (Al-Haleem et al., 2010). These ions

include, Sodium (Na^{+}), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Iron (Fe^{2+}), Barium (Ba^{2+}) Potassium (K^{+}), Strontium (Sr^{+2}), Aluminum (Al^{3+}), Lithium (Li^{+}) among cations and Chloride (Cl^{-}), Sulphate (SO_4^{2-}), Carbonate (CO_3^{2-}) and Bicarbonates (HCO_3^{-}) (Al-Haleem et al., 2010; Alley et al., 2011). Among these the most prevalent are Sodium and Chloride ions. Hence the salinity of produced water can vary from a few to as high as 300,000 mg/L (Lusinier et al., 2019). High salt concentrations could cause severe hindrance to biological processes due to cell-membrane disruptions and hence the proposal to use the microbiota acclimatized to the produced water for treatment. Produced water also contains certain salts such as Calcium carbonate ($CaCO_3$), Iron sulfide (FeS_2), and Calcium sulfate ($CaSO_4$) that may precipitate during changes in pH increasing the turbidity of produced water (Emam et al., 2014; Martinez et al., 2016).

Dispersed or dissolved oil and gas compounds constitute the organics of produced water. The most commonly found are aliphatic hydrocarbons, phenols (Cresol, Xylenol, and Phenol), carboxylic acid (Formic acid, Propionic acid, Butanoic acid, and Naphthenic acid), BTEX (Benzene, Toluene, Ethylbenzene, and Xylene), and also some low molecular weight aromatic compounds are found as dissolved oil compounds (Khosravi & Alamdari, 2009). A significant point that can be noted which is an important factor for biological processes is the imbalance in the C/N/P ratio. PW often has a lack of some of these nutrients and has to be overcome by adding nitrogenous and phosphorous compounds such as ammonium chloride and potassium phosphate to enhance biological processes (Sharghi & Bonakdarpour, 2013).

In order to ensure proper extraction operation, the oil producer may add different chemical additives at various stages of oil production. Some of these may include wax inhibitors, hydrogen sulfide scavengers, antifoam, corrosion inhibitors,

demulsifiers, etc. (Lusinier et al., 2019). In addition, some forms of gasses such as Oxygen, Carbon dioxide, and Hydrogen sulfide are also present in the produced water in a dissolved form due to bacterial activity (Gevertz et al., 2000). Inorganic minerals dissolved in the PW may vary according to well age. Inorganic compounds primarily contain heavy metals, cations and anions, and radioactive materials within the old mining wells. Whereas metals usually include the following compounds such as Iron, Boron, Aluminum, Zinc, Barium, Copper, Cadmium, Lead, Chromium, Lithium, Mercury, Manganese, Strontium, Silver, Titanium, Arsenic, and Beryllium (Fakhru'l-Razi et al., 2009). The presence of these inorganic minerals also affects the conductivity of the produced water.

In addition to all the above constituents, produced water also contains suspended solids. They are generated mainly due to precipitation and corrosion that may occur due to changes in pressure, temperature, and various chemical changes.

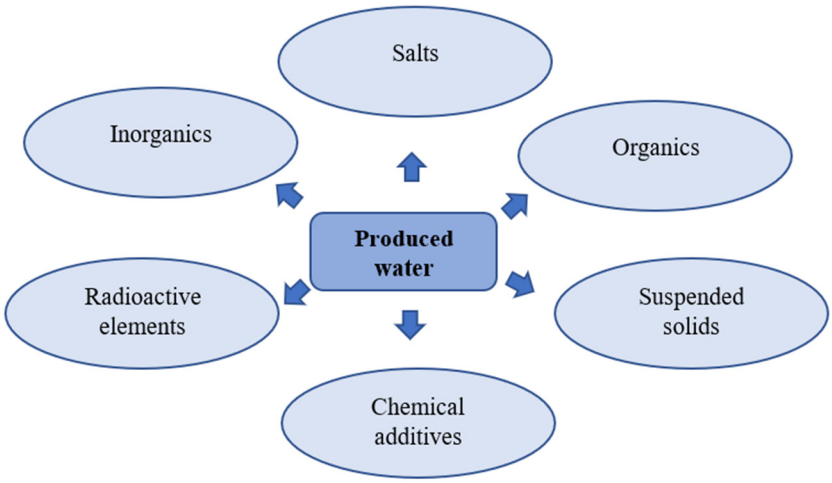


Figure 1: Constituents of produced water

An average of 5 to 15 percent of produced water is produced by a new oil field, but as the oil wells become exhausted the amount of PW rises to 90 percent of the total volume extracted, significantly exceeding the volume of oil generated

(Hansen & Davies, 1994). It was estimated that a total of 250 million barrels of produced water are produced worldwide a day, while the daily production of oil is only about 80 million BPD; which is roughly 3 times greater than the production of oil (Al-Ghouti et al., 2019; Clark & Veil, 2009).

2.3 Need for Treatment of PW

The toxicity of some of the compounds released with the produced water has raised environmental concerns when discharged into the environment (Knudsen et al., 2004; Strømgren et al., 1995). The presence of petroleum hydrocarbons dispersed in produced water can cause serious environmental damage (Grini et al., 2002; Jiménez et al., 2018). The expulsion of the toxic constituents and pollutants into the aquatic environment poses a serious threat to aquatic life and agricultural resources by changing the natural state of the aquatic environment (Middleditch, 1984; Obire & Amusan, 2003; Silva et al., 2017). It was reported that the effect of produced water in oceans is less due to the level of mixing and dilution when compared to the onshore setting where the rate of mixing and dilution is less (Beyer et al., 2020). Global research has shown that the PW effluents exhibit high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) generated from organic compounds. Compared to seawater, produced water has a much higher salinity that could result in freshwater aquatic destruction (Gazali et al., 2017). Gazali et al. (2017) also opined that heavy metals and Naturally Occurring Radioactive Material (NORM) associated with the produced water could be of environmental concern. Drilling mud and wastes were disposed of in a waste pit that could overflow to nearby rivers and streams. The massive amount of produced water discharges, their complex nature, which may be hazardous combined with the lack of information on their potential long-term and

ecological effects, have made produced water disposal the most concerning problem.

2.4 Produced Water Treatment Methods

Some environmental regulatory agencies in countries with substantial offshore oil and gas production impose limits on the petroleum concentrations (usually measured as total oil and grease) that may be present in the produced water that is to be discharged into oceans (Neff et al., 2011). For a long time, the government regulated only non-polar Oil In Water (OIW), and very little attention was given to dissolved organics in produced water (Igunnu & Chen, 2014). Current research focuses more on the effects on living organisms of dissolved organic materials, heavy metals, and processing chemicals additives since their long-term environmental effects are not fully documented and understood. A standard regulation for the discharge of produced water into the sea was 40 ppm OIW, but a rise in environmental awareness has led many countries to introduce stricter regulatory standards. Many oil and gas companies across the world are therefore working to ' zero-discharge ' pollutants into produced water (Igunnu & Chen, 2014). In addition to regulations, many water-scarce oilfield countries are looking for ways to enhance their limited freshwater supplies by concentrating on effective and affordable methods of treating produced water, so that it can be diverted to agricultural and industrial uses (Veil et al., 2004).

The basic goals for the treatment of produced water are de-oilage (removal of accumulated oil and grease), desalination, removal of suspended particles and gravel, removal of soluble organic matter, removal of dissolved gases, elimination of Naturally Occurring Radioactive Materials (NORM), disinfection and removal of

excess hardness of water (Arthur, 2005). To achieve these objectives, treatment of PW is usually done through a physical, chemical, and biological process, where the process can function independently or even in combination.

2.4.1 Physical Treatment Methods

One of the most common physical treatment methods for the removal of contaminants from produced water is physical adsorption. Activated carbon, organoclay, copolymers, zeolite, resins are widely used to treat produced water. The combination of activated carbon and organoclays proved to be more efficient in removing Total Petroleum Hydrocarbons (TPH). Copolymers reduce the oil content up to 85%. Zeolites are efficient in removing BTEX compounds. A multi-stage adsorption and separation system was developed, for example, by EARTH Canada Corporation to recover dispersed oil droplets in water, whose size is greater than 2 microns (Plebon et al., 2005).

Sand filters are generally used to remove metals from produced water. A compact Cyclone Floatation Unit (CFU) could remove dispersed oil from 50% to 70% using a centrifugal force. The major drawback of using a cyclone unit is its low efficiency and inability to remove dissolved components (Duraisamy et al., 2013).

Membrane filtration is a physical separation technique that uses pores in a continuous structure to selectively fractionate components from a flowing fluid (Zolghadr et al., 2021). Membrane processes are classified according to the driving force and pore size of the materials used. Different membrane materials are used based on the process used for separation. There are four main pressure-driven

membrane processes, namely, Ultra-Filtration (UF), Micro Filtration (MF), Nano Filtration (NF), and Reverse Osmosis (RO) (Venkatesan & Wankat, 2017). Membrane filtration is an effective technology for removing TDS from PW because of low energy consumption and cost, high rejection rates, and the procedure requiring fewer chemicals (Muggeridge et al., 2014). However, pressure-driven membrane processes such as RO are impractical for treating very high salinity PW because the hydraulic pressure required to overcome the osmotic pressure of high-salinity waters can exceed the permitted pressure of RO membrane material. Desalination of PW with high salinity having TDS concentrations greater than 35 g/L necessitates more energy-intensive technologies (Shaffer et al., 2013; Zolghadr et al., 2021).

2.4.2 Chemical Treatment

One of the predominant methods for the chemical treatment of produced water is the precipitation system. Coagulation and flocculation may reduce the suspended solids and the colloidal particles. Many coagulants such as modified hot lime, FMA (a mixed metal-polymer), Spillsorb, calcite, and ferric ions have been used as coagulants for the treatment of produced water. The drawbacks of this process are its inefficiency for dissolved components and the increased concentration of metals in the formulated sludge.

Chemical oxidation uses a combination of strong oxidants (e.g., O_3 and H_2O_2), irradiation (e.g., UV), and a catalyst (e.g., photocatalyst), which oxidizes the organic components to their maximum stable oxidation condition (Duraisamy et al., 2013). Almost 90% of BOD and COD could be removed from produced water in a short time (of the order of 6 minutes) by the electrochemical method. Sonochemical

oxidation using ozone could destroy BTEX compounds but the process requires high initial and operating costs (Fakhru'l-Razi et al., 2009).

2.4.3 Biological Treatment Methods

Classically, biological processes are the most common and economical techniques used to treat water, especially for removing organic compounds in wastewater treatment plants (Pendashteh et al., 2012; Tong et al., 2013). With the growing concern over the disposal of potentially toxic effluent, such as in produced water, the quantity of which is constantly increasing, biological processes may represent an attractive choice because they are environmentally friendly and have potentially high degradation efficiencies on a wide range of molecules (Pendashteh et al., 2012; Tong et al., 2013). The ability of many microorganisms to grow in highly saline media, such as algae, bacteria, fungi, yeast, etc., makes them ideal for bioremediation of effluents such as produced water (Lu et al., 2009). Aerobic and anaerobic microorganisms have been used in biotreatment studies of produced water. In biological treatment, four sources of microorganisms such as naturally occurring microorganisms, commercial microorganisms, specific groups of microorganisms, and acclimated sewage sludge were examined (Fakhru'l-Razi et al., 2009). For treating produced water under low salinity, traditional techniques such as conventional activated sludge and biological aeration filter were employed. Although some studies showed good efficiencies in the overall removal of COD, such performances were often obtained under low salt concentration and/or high HRT, which could jeopardize the implementation of such processes on oil installations where space availability is restricted. To overcome these new challenges, hybrid biological processes incorporating both free and fixed biomass have shown promising results in high

salinity and low HRT treatment of real-field PW (Lusinier et al., 2019). MBRs (Membrane Bioreactors) have been widely studied over the last decades. This process consists of a suspended sludge aeration tank coupled with a physical separation, usually microfiltration or ultrafiltration cutoff membrane systems. MBRs have attracted interest in urban wastewater, emerging pollutants, as well as industrial wastewater treatment, such as petroleum wastewater (Kraume & Drews, 2010), due to multiple advantages including lower emissions, lower sludge generation or increased effluent consistency in suspended solids.

2.5 Biological Treatment Using Algae

Algae can remove dissolved and complex chemical pollutants from a variety of wastewater sources it lives in, including nutrients, dissolved organic chemicals, and heavy metals (Badrinarayanan, 2017; Talebi et al., 2016). Despite the toxic components present in PW that inhibit algae growth, it also contains several nutrients like nitrogen and phosphorus, in the form of ammonium and phosphate, which are essential for algae (Rahman et al., 2020). Thus, the cultivation of microalgae in PW will efficiently remove nutrients from PW and clean up this effluent. Toxic elements are primarily removed by algae through biosorption and bioaccumulation. It is important to note that the bioremediation capacity of algal cells is directly related to biomass concentration. Therefore, increasing biomass concentration can result in higher absorption capacities (Rahman et al., 2020). Additionally, the algal approach to treat PW can also serve as a more sustainable remediation technique since it produces biomass that may be used as feedstock for biofuel production, bioactive compounds, and nutrient supplements (Cardozo et al., 2007; Selvaratnam et al., 2015).

The wastewater treatment using microalgae has been extensively studied. Treatment of wastewater with various strains has been investigated over the years. Unicellular green algae such as Chlorella and Scenedesmus are widely used, since they colonize naturally, have fast growth rates, and have high nutrient uptake capacities per unit biomass. However, very few experiments have been conducted on the removal of COD and oil from oilfield PW while simultaneously producing biomass using microalgae (Ammar et al., 2018).

2.5.1 Microalgae

A microalga is a single-celled microscopic organism that is mostly photosynthetic. While some of them are single-celled, others can form colonies, that are filaments or spheres. Microalgae fix CO_2 during photosynthesis and are the principal producers of organic matter in aquatic environments. They can thrive in almost all aquatic and terrestrial habitats, even in very harsh conditions.

The size, shape, colors, cell wall composition, and the presence of different photosynthetic pigments form the basis for the classification of microalgae. Microalgae can be spherical, rod-shaped, spindle-shaped, or club-shaped depending on their cell morphology.

Recent studies suggest that eukaryotic microalgae and cyanobacteria could be utilized to produce food and fuel in a sustainable way (Chu, 2012). There are a variety of drugs that can be extracted from marine microalgae, including carotenoids which are used in pharmaceuticals, foods, and cosmetics (Sigamani et al., 2016). A variety of bioactive compounds derived from microalgae exhibit anticancer, anti-inflammatory, antimicrobial, and antioxidant properties (Sigamani et al., 2016). Using microalgae cultures is an ideal solution for wastewater treatment together with

the potential for producing valuable biomass, which can be used for a variety of purposes (biogas, biofuels, composting, animal feed, and fine chemicals). Inorganic nitrogen and phosphorus are used by the microalgae for growth and they can also remove heavy metals and some toxic organic compounds (dyes and antibiotics), reducing also the COD and BOD (Biris-Dorhoi et al., 2016).

2.5.2 Algal Growth Parameters for Cultivation

Microalgal species require special conditions to grow. In addition to nutritional requirements, microalgae cultivation can only be accomplished under four major abiotic conditions, which include the optimum intensity of light, suitable temperature, pH, and mixing of the water (Hakim et al., 2018). The specific conditions that microalgae require may, however, differ from species to species.

2.5.2.1 Light Intensity

Light is one of the most important factors associated with the autotrophic growth of microalgae. As is well known, each molecule of CO_2 requires for its fixation approximately 8 photons of photosynthetically active radiation (roughly 48 percent of the incident solar light) (Amaro et al., 2011; Eriksen, 2008). The high flux density of photons, however, may cause photodamage, reducing photosynthetic efficiency. As a result, a light: dark ratio that is suitable for microalgal production may play an important role. This is because the dark period brings about the repair of photoinduced damage in the microalgae (Eriksen, 2008). Microalgal growth, carbon dioxide capture, and nutrient removal efficiencies were evaluated in a study by (Gonçalves et al., 2014) in relation to light irradiance, light: dark ratio, and microalgal strains. Higher light intensity values and longer light periods resulted in

higher growth rates, higher CO_2 uptake rates, and better nutrient removal (Gonçalves et al., 2014).

2.5.2.2 Optimum Temperature

Microalgal/cyanobacterial growth is easily affected by temperature changes since the metabolic activity of these photosynthetic microorganisms can be inhibited by extreme temperatures (Gonçalves et al., 2016). Temperatures between 10 and 30 degrees Celsius are generally considered appropriate for algal growth. Different species may, however, have their own optimum temperatures (Singh & Singh, 2015). Gonçalves et al., (2016) studied the effect of different temperatures and light intensity on four different species of algae. All the studied species displayed greater biomass production and nutrient removal efficiency at 25°C compared to other temperatures studied.

2.5.2.3 pH

The optimum pH range for most species of microalgae is between 7 and 9. Typically, the pH increases when microalgae grow. This results from the consumption of dissolved CO_2 within the culture medium during cell growth and photosynthesis. There is also evidence to suggest that low pH value inhibits the growth of algae in most cases, whereas different algae have different adaptability to pH value, such as *Chlorella vulgaris* which is capable of growing at its maximum rate in the wide pH range of 6.0 to 9.0 (Zhao et al., 2011; Zheng et al., 2016).

2.5.2.4 Mixing

For the high growth rates of the microalgae cultivation system, it is important that every cell receive appropriate light conditions for photosynthesis. Other benefits

of mixing include degassing the oxygen formed from photosynthesis, improving mass transfer and nutrient uptake between cells and the environment, eliminating thermal stratification, and preventing cells from settling at the bottom (Gao et al., 2015; Ogbonna et al., 1995).

Chapter 3: Materials and Methods

The research design adopted for this research to bioremediate produced water using algae is shown in Figure 2. The experiments were conducted at room temperature of 25°C in the Environmental Engineering Lab, Department of Civil and Environmental Engineering, UAE University.

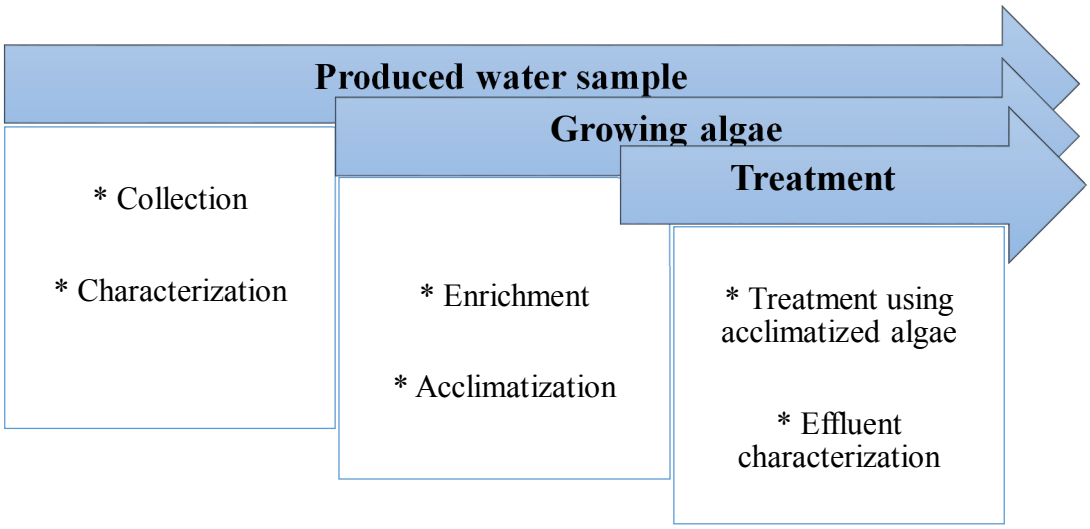


Figure 2: Research design

3.1 PW Sample Collection and Characterization

The PW samples (PW1, PW2, and PW3) were collected from three different sources in UAE and Middle East. Figures 3 and 4 exhibit the major oil fields of UAE and Middle East.

Figure 3: Major oil fields of UAE (Granier et al., 2003)

Figure 4: Oil Fields of the Middle East ("Petroleum," 2008)

The physical characteristics such as pH were analyzed by pH meter (Extech) and salinity, Total Dissolved Solids (TDS), and electrical conductivity using a conductivity meter (Laqua Horiba, EC210) respectively. To determine the hydrogen ion concentration using a pH meter, a three-point standard curve is generated using pH buffer solutions, and a glass electrode is used to measure the emf between the sample and a reference solution. The conductivity meter contains a graphite, four-pole conductivity probe, and conductivity are measured in milli-Siemens/cm. COD was measured using TNT-plus vial tests (Hach, LCK 514). A detailed description of the methodology is given in Appendix B.

Other parameters that were be analyzed are anions and Total Organic Carbon (TOC). TOC is the amount of carbon that is present in an organic compound and is often used as a non-specific water quality indicator. A standard Total Carbon (TC) analysis determines both the total TOC and the Total Inorganic Carbon (TIC), the latter reflecting the quantity of non-organic carbon, such as carbon in carbonate minerals. The Difference between total carbon and inorganic carbon yields TOC. The sampling analysis was done using Analytic Jena, Multi NC 2100 TOC analyzer. Since the TOC concentration of produced water is high, the samples were diluted 500 times before analyzing in a TOC analyzer.

The determination of anions was done using Ion Chromatography (IC). The analysis of samples was done using the Thermo-Scientific Dionex IC system. Since the chloride content of produced water was beyond the measuring range of the IC system, the samples were diluted 2,500 times and the results obtained were multiplied by the dilution factor to obtain actual concentration.

The acid capacity is a measure of its basic characteristics (alkalinity), i.e., its ability to bind acids (H+ ions) and thus produce a buffering effect. Carbonic acid anions (hydrogen carbonate, HCO_3^-, and carbonate, CO_3^{2-}) are primarily responsible for this capacity in natural waters. Since these anions are nearly entirely protonated at pH 4.3, the "acid capacity to pH 4.3", KS4.3 (total alkalinity; acid-neutralizing capacity, ANC) is usually evaluated. The alkalinity of produced water was measured using Hach LCK 362 cuvette test. The method of analysis is described in Appendix C.

3.2 Microalgae Cultivation, Enrichment, and Preadaptation in PW

3.2.1 Phase 1: Growing Algae Inherently Present in PW

This phase was a trial to enrich and grow algae inherently present in the produced water samples. The experiments were done in 5 L Schott bottles. The growth media used for all the experiments were BG-11 media. The composition (in g/L) of each of the reagents and the volume of each of the reagents for the media are given in Tables 1 and 2.

50 mL of each of the reagents and 5 mL of the trace metal solution were added to each of the three bottles and made up to 5 L with the three produced water samples. The bottles were placed on magnetic stirrers with constant stirring. Continuous air supply was provided to enable aeration as well as mixing. Light intensity was maintained at 1500-1700 Lux. The bottles were left undisturbed for 2 weeks to monitor any growth.

3.2.2 Phase 2: Growing Algae Inherently Present in Wastewater

This phase involved enriching algae present in wastewater. The wastewater was collected from the effluent stream of Al Saad wastewater treatment plant, Al

Ain, UAE. Similar to the phase 1 experiment, BG11 reagents for 5 L volume were added to the Schott bottle and made up to 5L with wastewater. The growth parameters such as light and temperature were kept at 25°C with 12 hours of light and dark periods. Both sunlight and artificial light were used to maintain the light and dark cycle with the intensity ranging from 1500-1700 Lux (Mollamohammada et al., 2021). GE plant light kits provided the light source, the light intensity was maintained and monitored using a digital light meter (model 935976, Leaton). Continuous aeration along with a magnetic stirrer was used to maintain a homogeneous culture for a growth period of fourteen days.

Table 1: BG11 composition

#	**Component**	**Stock solution concentration (g/L)**	**Amount**
1	$NaNO_3$	37.5	10 mL/L
2	KH_2PO_4	1	10 mL/L
3	$MgSO_4.7H_2O$	1.875	10 mL/L
4	$CaCl_2.2H_2O$	0.9	10 mL/L
5	Citric acid.H_2O	0.15	10 mL/L
6	Ferric ammonium citrate	0.15	10 mL/L
7	Na_2 EDTA.$2H_2O$	0.025	10 mL/L
8	Na_2CO_3	0.5	10 mL/L
9	BG11 trace metal solution		1 mL/L

Table 2: BG 11 trace metal solution composition

#	Component	Concentration (mg/L)
1	H_3BO_3	2.86
2	$MnCl_2.4H_2O$	1.81
3	$ZnSO_4.7H_2O$	0.22
4	$Na_2MoO_4.2H_2O$	0.39
5	$CuSO_4.5H_2O$	0.08
6	$Co(NO_3)2.6H_2O$	0.05

3.2.3 Phase 3: Growth in PW and Preadaptation

Figure 5 shows the major steps of acclimatization of algae consortia in produced water before treatment. The ratio of produced water to algae was gradually increased in a step-by-step process to acclimatize the algae to the high salinity and toxicity of produced water. Produced water- BG11 mixture was prepared by adding media reagents as per requirement for 500 mL and produced water instead of deionized water. 500 mL of the stock culture of wastewater algae was mixed with 500 mL of the produced water-BG11 mixture to make up to a volume of 1 L in Scott bottles and the ratio of PW: algae being 1:1. The bottles were placed on magnetic stirrers, providing the same conditions of light (1500-1700 lux) and continuous air supply for a growth period of fourteen days. The optical density was monitored every day using a spectrophotometer (DR 3900, Hach Lange). The algae were allowed to grow for fourteen days after which this served as inoculum for a higher ratio of produced water.

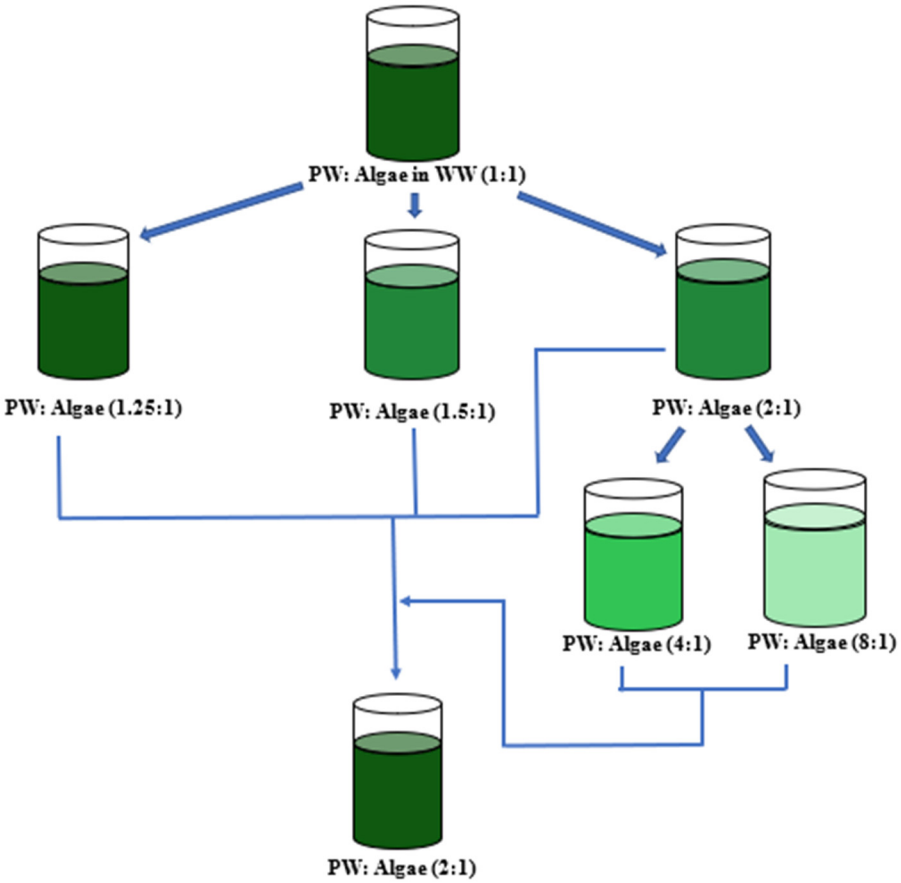


Figure 5: Acclimatization of algae grown in wastewater to produced water

Spectrophotometry is a useful indirect technique to record the optical density value at a specific wavelength. It works on the principle of Beer-Lamberts law of absorbance where optical density is represented in terms of transmittance. Beer-Lambert law correlates the cell concentration with the optical density of a sample based on the attenuation of light as it passes through the sample (Hardesty & Attili, 2010).

Similar to preparing 1:1 ratio sample, produced water-BG11 mixture was initially prepared. This was mixed with 1:1 algae in the ratios (PW: algae) 1.25:1, 1.5:1 and 2:1 keeping in mind the amount of produced water already present in the 1:1 bottle. At the same time, the wastewater inoculum was directly inoculated in the ratios of PW: algae, 1.5:1, 2:1, 4:1, and 8:1. The growth was monitored by measuring the optical density every day and the period each ratio took to reach the stationary

phase was monitored. The volume of the bottles was also being monitored as some volume reduced over time because of aeration and evaporation. The volume was made up to the initial level using deionized water.

After 16 days, algae from the 2:1 bottle was inoculated into PW in the ratio 4:1 and 8:1. The adaptability was monitored by measuring the optical density at 620 nm. Those bottles which had no green color at all were considered unadaptable.

Even though, some ratios took more time to reach the stationary phase than others, those which showed an increase in optical density at some point of time indicated the ability of algae to adapt to the toxic and saline nature of algae. Since all the attained tolerance could be useful in the bioremediation of produced water, all of it was used to further adaptation steps.

Some had darker green colors while some were lighter in color. However, all of it contained algae that could adapt to the produced water environment. All these adapted algae consortia were mixed. The amount of produced water already present in all the bottles was calculated. The required amount of respective produced water-media mixture solution was further added to each of the bottles to make a final ratio 2:1 PW: algae for PW1 and PW2 (Figure 6).

For PW3 after the initial step of inoculating the wastewater inoculum in 1:1 ratio, the ratio of PW: algae had to be further reduced rather than increase after the observations in color and optical density. PW: algae ratios of 0.5:1 and 0.75:1 were prepared simultaneously during acclimatization of PW1 and PW2. All the bottles were given BG11 media supplements and physical conditions of light and air similar to PW1 and PW2. The growth was monitored by measuring the optical density. At the end of 16 days, all these acclimatized algae were mixed and a final ratio of 0.5:1 PW: algae was

made. These were the final stock culture and scale-up for the bioremediation experiments. It was done in 5L Schott bottles which were placed in the same conditions as the previous experiment, with continuous air supply, magnetic stirring, and 12 hours light and dark period with intensity between 1500 and 1700 LUX. An optical stereo microscope was used to examine the microalgae cells produced during cultivation.

The dry weight of the stock cultures of the three produced water samples which served as the initial inoculum for treatment was obtained by oven drying 25 ml of sample in four different dilutions for 24 hours in a china dish. The difference between the initial and final weights of the china dishes gave the dry weight. The cell concentrations were calculated using the formula:

$$\text{Concentration (g/L)} = \{\text{Difference in weight (g)} / 25\ \text{ml}\} * 1000$$

A calibration curve of Absorbance vs Biomass concentration was plotted. The cell concentrations of algae during adaptation were calculated using this calibration curve for each of the produced water samples.

3.2.4 Growth Kinetics

The sigmoid curve idea is widely acknowledged as the best way to describe the growth of a cell culture over time. The initial phase where the cell population grows relatively slowly is known as the lag phase. As the population increases the rate of growth shows steepness on the curve. This phase when the population approaches half of the carrying capacity is called the log phase or the exponential phase. From this point on, the slope of the curve steadily decreases as the population increases, until it reaches its carrying capacity which is then known as the stationary phase.

Specific growth rate (μ) represents the steepness of this sigmoid curve and is defined as the rate at which a cell population increases per unit of biomass concentration.

It can be determined in batch cultures because the rate of increase in biomass per unit of biomass concentration is constant over time. Therefore, the growth fits a straight line during the log phase and the following straight-line equation can be used:

$$\mu = \frac{(\ln C - \ln C0)}{t}$$

where μ is the specific growth rate, C_0 is the initial biomass concentration and C is the biomass concentration after time, t (Godoy-Hernández & Vázquez-Flota, 2012). The specific growth rates of the different algal cultures were analyzed.

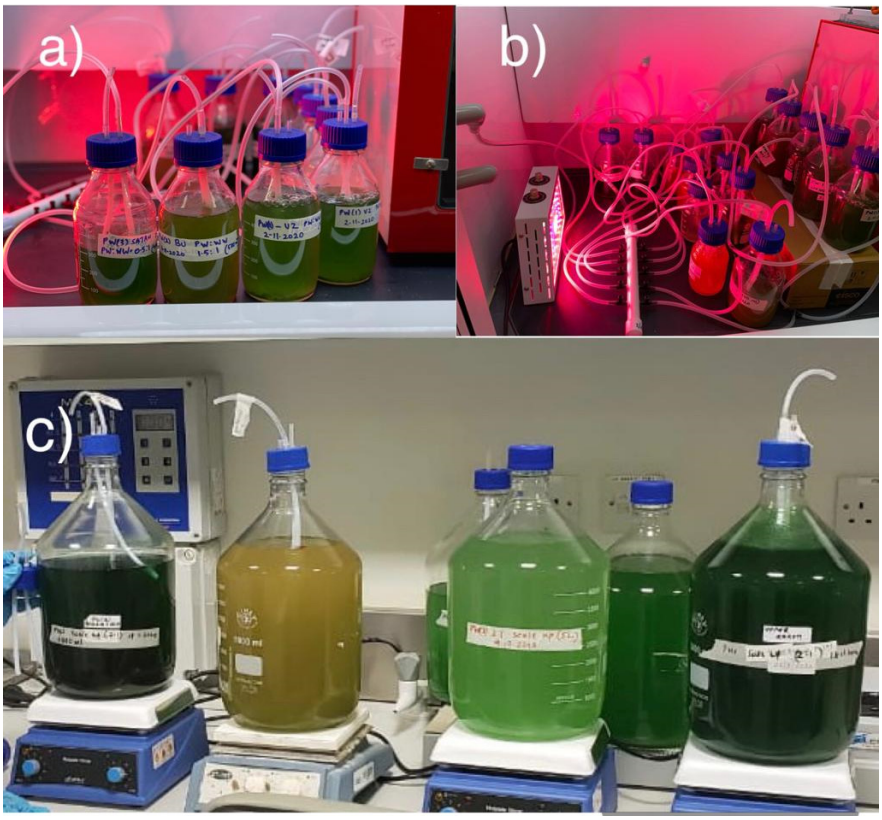


Figure 6: Acclimatization step 2, acclimatization step 3, and Stock culture- scale up. a) acclimatization step 2, b) acclimatization step 3, and c) Stock culture scale-up

3.3 Produced Water Treatment with Algae Consortia

All the experiments were done in 250 ml Schott bottles in triplicates. Algae from the stock solution were centrifuged (SL8 centrifuge, Thermo Scientific) at

7,500 rpm for 10 minutes. The supernatant was discarded, and algal biomass was resuspended with a minimum amount of BG11 media. The optimum amount of algae for treatment was obtained as 6.25% (Mollamohammada et al., 2020).

Therefore, 12.5 mL of the algae was mixed with produced water to a volume of 200 mL. A control for each produced water was prepared without algae. Magnetic stirrer plates were used to enable mixing and light intensity was maintained at 1500 – 1700 LUX with 12 hours of light and dark cycles (Figure 7). The treatment was done for seven days. Parameters such as optical density, pH, Electrical Conductivity (EC), TDS, salinity, COD, TOC, alkalinity, and anions were monitored after one, two, three, five, and seven days of treatments.

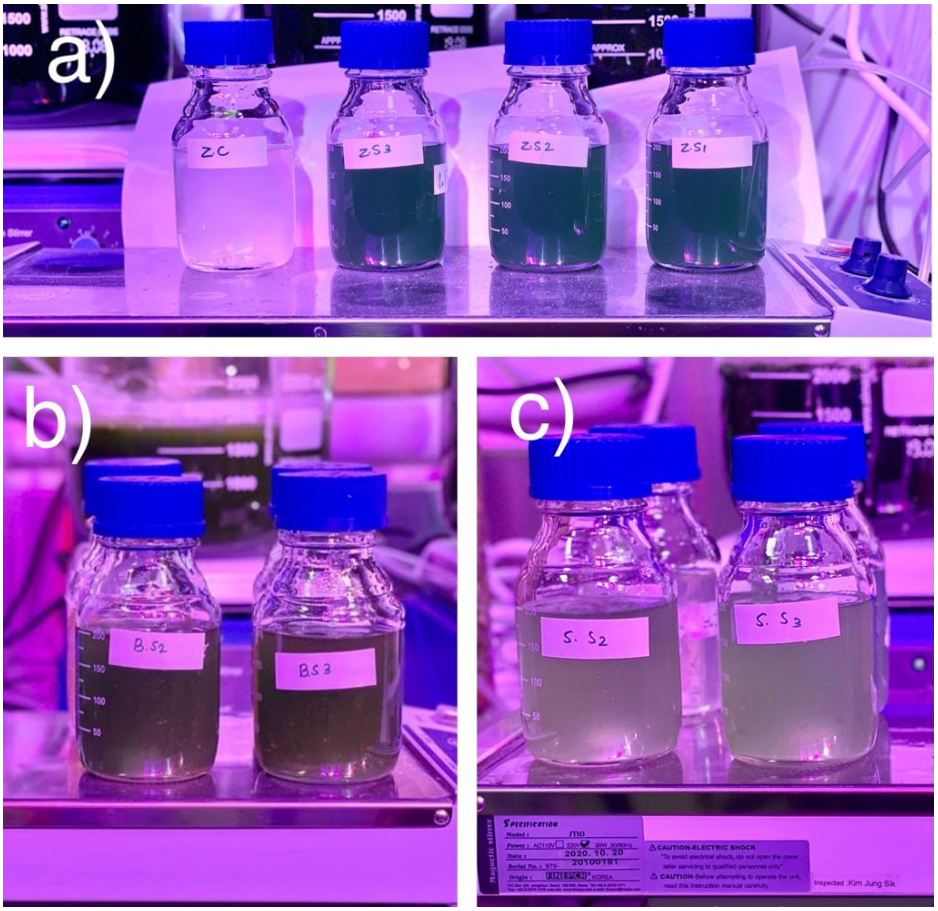


Figure 7: Experiment set up on magnetic stirrer, PW1, PW2 and PW3. a) PW1, b) PW2, c) PW3

Chapter 4: Results and Discussion

4.1 Produced Water Characteristics

The physical and chemical characteristics of the three different PW samples used in this research work were measured and are presented in Table 3.

Table 3: Characteristics of PW1, PW2, and PW3

Parameter	Unit	PW1	PW2	PW3
COD	mg/L	1,520	1,540	1,080
TOC	mg/L	2,600	2,500	2,550
pH	-	6.41	6.4	6.39
EC	mS/s	94.8	104	103.5
TDS	g/L	47.7	52.2	51.7
Salinity	ppt	55.6	61.4	60.3
Chloride	mg/L	42,748	53,074	47,478
Alkalinity	m.mol/l	2.57	4.35	3.65
Sulphate	mg/L	598.6	-	1,705.5

Among the three samples, PW1 and PW3 are from offshore fields while PW2 is from an onshore field. PW2 had a characteristic orange color and was more turbid which settled when kept undisturbed, than the other two samples. Although all three had the characteristic strong odor, PW3 seemed to have a stronger odor. The results of the initial characterization clearly indicate that produced water is highly saline and toxic in nature. Generally, the pH did not differ too much among the three samples.

All pH was in the mildly acidic range. PW3 had the lowest pH of 6.39 and PW1 highest with 6.41. The alkalinity of all three samples was very low, PW1 being the lowest. This indicated that the buffering capacity of all three sample streams was very less. TOC and COD concentrations were significantly high in all the produced water samples. The EC of each of the samples was also significantly high, with the highest being PW2 having 104 mS/cm. The EC values were strongly correlated to the TDS and salinity values. Produced water with the highest EC showed the highest TDS concentrations and salinity and vice versa. Chloride concentration also signifies the high salt concentration and was the major ion contributing to salinity in produced water since calcium and magnesium ions were not detected in IC. Higher salt concentrations create an unfavorable environment for algae growth. Increased ion concentrations in the external environment impair the osmotic balance of cells and their surroundings, causing cell exosmosis (Shetty et al., 2019). Acclimatization helps algae become salt-tolerant, resulting in steady growth (Sahle-Demessie et al., 2019). The number of nitrates and phosphates was below the detectable range of IC. IC also detected a considerable amount of sulphate ions in offshore produced waters PW1 and PW3. However, no sulphates were detected in the onshore-produced water PW2. This confirms the fact that the constituents of produced water vary drastically with the type of reservoir and geographical location (Neff et al., 2011).

4.2 Growing Algae in Produced Water

4.2.1 Phase 1: Growing algae inherently present in PW

The attempt to enrich and grow microalgae inherently present in PW, despite added nutrient media, did not succeed. The initial OD at the start of the experiment were: 0.13, 0.46, and 0.17 for PW1, PW2, and PW3, respectively. For more than

7 days, there was no significant change in the OD in all three samples. PW2 showed mild incremental fluctuation for 3 days after which it decreased to initial values. There were no changes in both other samples. This could be explained by the toxicity of PW, high salinity, characteristics of the PW, lack of micronutrients (e.g., nitrogen and phosphorus), etc. Environmental stress resulting from high salinity is challenging for microorganisms. Unicellular photosynthetic algae are especially vulnerable as they have to deal both with the ionic imbalance and osmotic stress, as well as with ROS (reactive oxygen species) interfering with their photosynthesis (Shetty et al., 2019). Therefore, algae from a different source had to be used for further experiments.

4.2.2 Phase 2: Growing algae inherently present in wastewater

The wastewater used was unfiltered effluent without sludge. A homogeneous solution was maintained by magnetic stirring. Continuous air supply and light provided favorable conditions for photosynthesis and growth. The algal growth was monitored by measuring optical density at 620 nm. It was observed that the growth was rather quick with no lag phase. The first five days showed exponential growth followed by a stationary phase in the following six days. The pH of the wastewater was near-neutral pH (6.85) and the COD concentration of 528 mg/L. This along with BG-11 media, high nitrogen, and phosphorous content significantly enhanced and created favorable conditions for faster algal growth.

4.2.3 Phase 3: Growth in PW and preadaptation

To use the algae grown in Phase 2 for bioremediation of produced water, it was necessary to acclimatize the algae to the highly saline and toxic environment. The salinity of wastewater was 0.5 ppt which is very low as compared to the produced water salinity (>55 ppt). To generate salt-adapted algae, a directed

experimental evolution method has been successfully applied by selecting survivors under increasing stresses (Perrineau et al., 2014). The wastewater algae were subsequently grown in increasing concentrations of produced water to slowly adapt the algae to high salinity and use the compounds present in produced water for its growth.

4.2.4 Growth in PW1

Table 4 shows the data for the calculation of biomass concentration for the calibration curve.

Table 4: Data for estimation of biomass concentration

Sr. no.	Dilution factor	Optical Density (620 nm)	Empty weight (g)	Oven-dried weight (g)	Weight difference (g)	Biomass concentration (g/L)
1	1:1	5.29	38.15	38.77	0.62	24.93
2	1:2.5	2.07	37.35	37.66	0.31	12.41
3	1:50	0.065	36.88	36.89	0.01	0.39
4	1:100	0	0	0	0	0

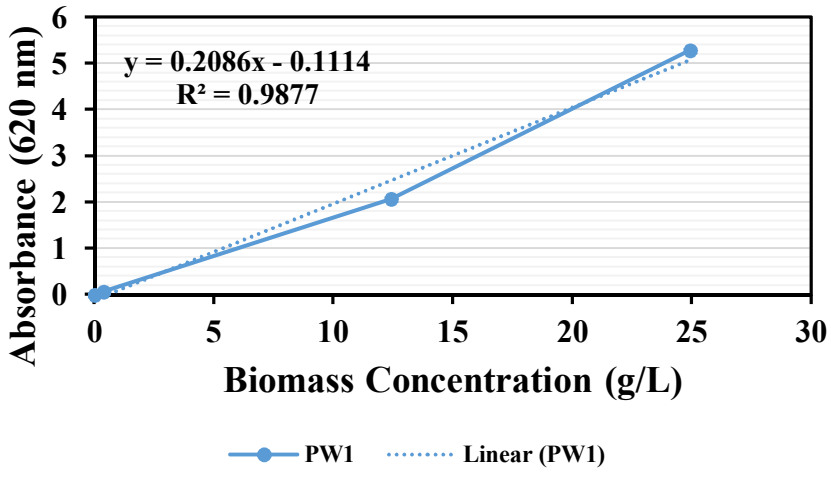


Figure 8: Calibration curve for biomass in PW1

This calibration curve (Figure 8) was used to calculate cell biomass concentration during the adaptation process. Figure 9 shows the biomass concentration over time during the adaptation process.

The first step was to grow the wastewater algae in produced water in a 1:1 ratio of PW: algae, respectively. The growth showed a lag phase up to 6 days and then there was a gradual increase in the biomass indicating the growth phase. This was followed by a stationary phase from day twelve. The algae from the 1:1 ratio were inoculated into three different ratios of PW1: algae, namely 1.25:1, 1.5:1 and 2:1, respectively. The growth curve generated from the optical density measurements is given in Appendix F.

The 1.25:1 and 1.5:1 samples showed a slight decrease in biomass initially. This was followed by a log phase and then a stationary phase. The same pattern was observed by the 2:1 ratio; however, there was a considerable decrease in biomass initially for the first four days followed by a lag phase until day ten. The log phase started after 10 days and reached the stationary phase after 15 days. It was evident that increasing the ratio of produced water led to a longer time for algae to reach the stationary phase. When algae are subjected to high salt concentrations, they undergo multiple morphological and molecular changes that improve survival (Shetty et al., 2019). A gradual increase in salt tolerance leads to steady-state growth following acclimation of algae (Lachapelle et al., 2015; Sahle-Demessie et al., 2019). The algae inoculated from the initial 2:1 to 4:1 ratio of PW: algae did not seem to survive. The 4:1 ratio did not show any increase in biomass and had lost all the green color by day four. The same happened in the 8:1 sample, the color disappearing sooner that 4:1. Subsequently, algae in all the different ratios which had adapted were combined

and further grown in 2:1 ratio as it was observed that 2:1 ratio is the highest possible ratio in which algae could grow in least amount of time.

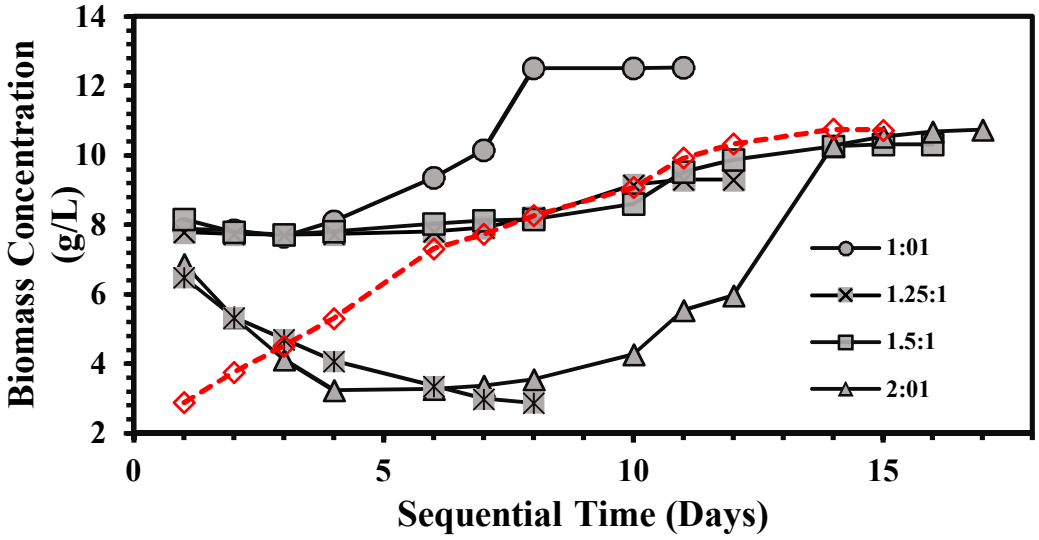


Figure 9: Biomass concentration vs time curve of algae in different ratios of PW1, sock culture implies the final preadapted ratio for treatment

The specific growth rate, μ, for the different ratios of PW was calculated and is shown in Table 5

Table 5: Specific growth rates of algal biomass in different ratios of PW1

Sr. no.	Ratio of PW	Initial concentration, Co (g/L)	Final concentration C (g/L)	Time, t (Days)	Specific growth rate, μ (Day $^{-1}$)
1	1:01	7.8	12.52	15	0.031
2	1.25:1	7.7	9.3	12	0.014
3	1.5:1	8.2	10.3	16	0.015
4	2:01	6.86	10.7	16	0.03
5	4:01	6.49	2.87	8	-0.1
6	2:1 stock	2.89	10.7	15	0.09

The specific growth rate decreased as the ratio of PW increased. This is due to the longer lag phase which was in turn due to the harsh condition to which algae needed to adapt to. Adapting to a new harsher environment required more time and this delayed the growth in the population of algae. The specific growth rate of stock culture, however, was higher because the algae consortia were already adapted to the PW1 environment.

4.2.5 Growth in PW2

Table 6 shows the data for calculation of biomass concentration for calibration curve.

Table 6: Data for estimation of biomass concentration in PW2

Sr. no.	Dilution Factor	Optical Density (620nm)	Empty weight (g)	Oven dried weight (g)	Weight difference (g)	Concentration (g/L)
1	1:1	7.89	37.44	38.22	0.77	31.05
2	1:2.5	2.425	37.35	37.71	0.36	14.42
3	1:50	0.087	36.95	36.97	0.01	0.52
4	1:100	0	0	0	0	0

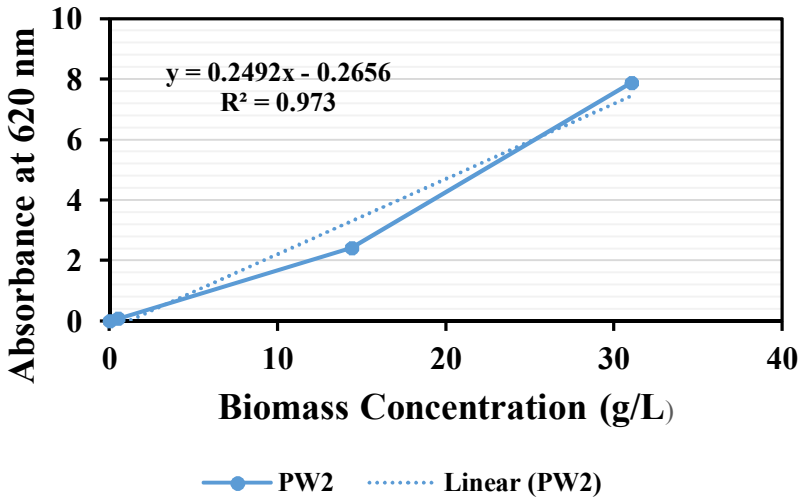


Figure 10: Calibration curve for biomass in PW2

This calibration curve (Figure 10) was used to calculate cell biomass concentration during the adaptation process. Figure 11 shows the biomass concentration over time during the adaptation process.

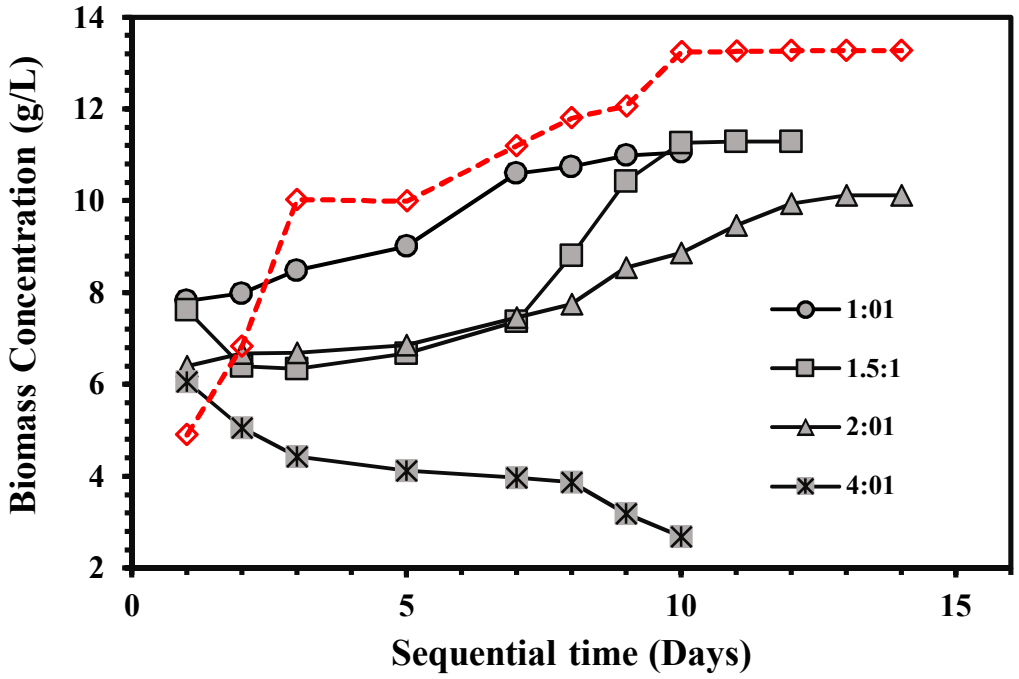


Figure 11: Biomass concentration Vs Time curve of algae in different ratios of PW2, Stock culture implies the final preadapted ratio for treatment

Wastewater algae showed better adaptation to PW2 compared to PW1. All the tested ratios reached the stationary phase sooner than PW1. However, the adaptation steps followed the same trend as observed in PW1. The 1.25: 1 ratio was not tested for PW2. The growth curve generated for the different acclimatization ratios is given in Appendix F. The 1.5: 1 ratio showed a mild decrease in biomass initially, followed by lag phase, and reached stationary phase by day seven. The Initial 2:1 ratio reached the stationary phase by day twelve. The algae in the 4:1 ratio maintained the green color of the biomass until day eight, after which the green color

disappeared. The algae in the 8:1 ratio lost all the green color by the fourth day which indicated there were no viable cells in the solution. Conclusively, the least dilution in which microalgae flourished in minimal time in PW2 was chosen to be the 2:1 ratio. The final 2:1 ratio stock culture was prepared by mixing all the adapted algae to a final ratio of PW: algae as 2:1. The specific growth rate, μ, for the different ratios of PW was calculated and is shown in Table 7.

Table 7: Specific growth rates of algal biomass in different ratios of PW2

Sr. no.	**Ratio of PW**	**Initial Concentration, Co (g/L)**	**Final concentration, C (g/L)**	**Time, t (Days)**	**Specific growth rate, μ (Day $^{-1}$)**
1	1:01	7.82	11.05	10	0.034
2	1.5:1	7.62	11.29	12	0.032
3	2:01	6.39	10.11	14	0.032
4	4:01	6.06	2.68	10	-0.081
5	2:1 stock	4.91	13.27	14	0.071

The specific growth rate decreased as the ratio of PW increased. However, the decrease was not significantly high. This could justify that the lag phase in PW2 was shorter compared to PW1 and PW3. The specific growth rate of stock culture, however, was similarly higher because the algae consortia were already adapted to the PW2 environment.

4.2.6 Growth in PW3

Table 8 shows the data for the calculation of biomass concentration for the calibration curve.

Table 8: Data for estimation of biomass concentration in PW3

Sr. no	Dilution Factor	Optical Density (620nm)	Empty weight (g)	oven-dried weight (g)	weight difference (g)	Biomass concentration (g/L)
1	1:1	2.03	38.15	38.65	0.50	20.16
2	1:2.5	1.11	37.23	37.43	0.21	8.22
3	1:50	0.03	36.88	36.89	0.01	0.24
4	1:100	0	0	0	0	0

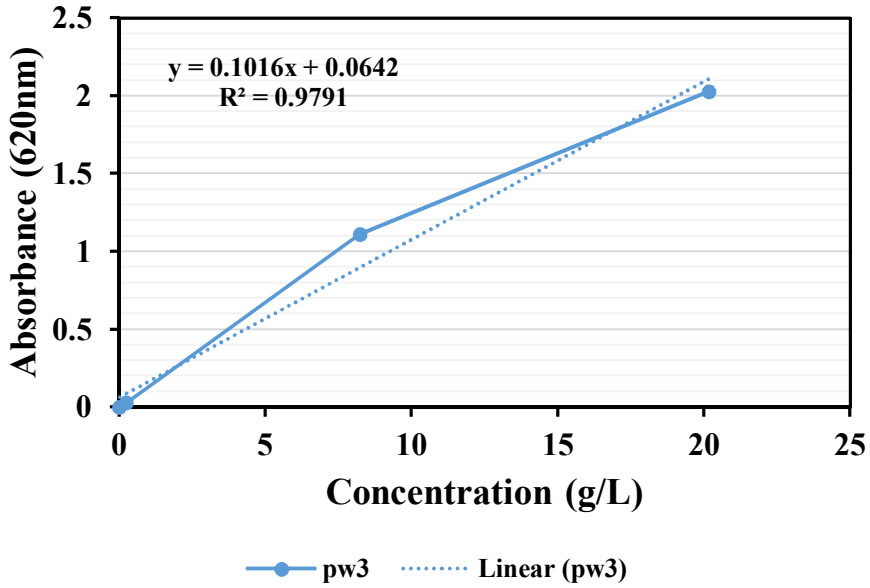


Figure 12: Calibration curve for biomass in PW2

This calibration curve (Figure 12) was used to calculate cell biomass concentration during the adaptation process. Figure 13 shows the biomass concentration over time during the adaptation process.

PW3 exhibited a toxic environment for algal growth. There happens to be some undetected factor that inhibits the growth of algae. The analyzed parameters such as pH, EC, COD, salinity, TDS, etc. of PW3 were similar to that of PW1 and PW3. However, the biomass concentration of the 1:1 algae reduced over time. Even though the biomass concentration at day seven was 9 g/L, the green color had completely disappeared. Hence ratios lower than 1:1 had to be tried to adapt algae for the treatment of PW3. Ratios of 0.5:1 and 0.75:1 tried. In both the trial samples, biomass concentration initially reduced, then slowly increased from the fourth day in 0.5:1 and it reached stationary phase by day ten. The 0.75 ratio bottle showed irregular OD. However, it showed an optical density of 2.85 on the twenty-seventh day. This showed the ability of the algal culture to develop tolerance to the toxicity and salinity of produced water over time. It was concluded that the least dilution of PW3 in which microalgae can adapt in minimal time was 0.5:1. The growth curve generated for the different ratios are shown in Appendix F.

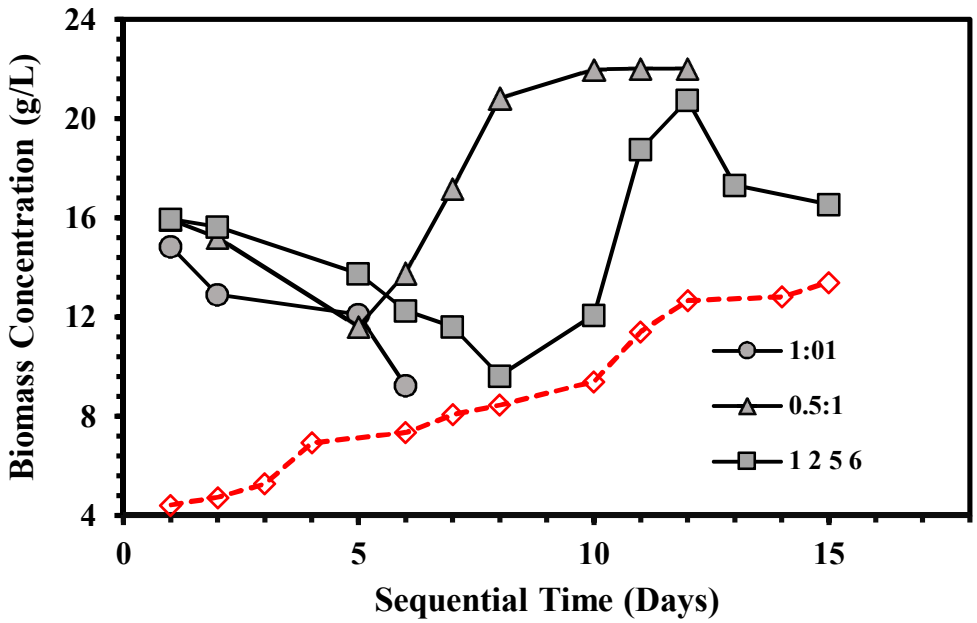


Figure 13: Biomass concentration Vs Time curve of algae in different ratios of PW3, Stock culture implies the final preadapted ratio for treatment

The specific growth rate, μ, for the different ratios of PW was calculated and is shown in Table 9.

Table 9: Specific growth rates of algal biomass in different ratios of PW3

Sr no:	Ratio of PW	Initial Concentration, Co (g/L)	Final concentration, C (g/L)	Time, t (Days)	Specific growth rate, μ (Day $^{-1}$)
1	0.5:1	15.92	21.99	12	0.027
2	0.75:1	15.93	16.53	15	0.0024
3	1:01	14.84	9.24	6	-0.078
4	0.5:1 stock	4.42	13.4	15	0.074

The specific growth rate value was smaller compared to PW1 and PW2. This confirms that PW3 provided a very harsh condition for algal growth. As seen in the case of the other two produced water, the growth rate decreased as the ratio of PW increased. The lag phase was therefore very long in the case of PW3. Adapting to a new harsher environment required more time and this delayed the growth in the population of algae in PW3 further. The specific growth rate of stock culture, however, was similarly higher because the algae consortia were already adapted to the PW3 environment.

Figures 14, 15, and 16 show the images of the algae grown in produced water 1, 2, and 3 samples respectively. Most cells were identified as *Chlorella* species. The presence of *Nannochloropsis* species was also detected. There were other types of cells that were unidentified. Some colonies were spherical while some were filamentous (Figure 17 b, d). The cell density was maximum in PW2 and minimum in PW3.

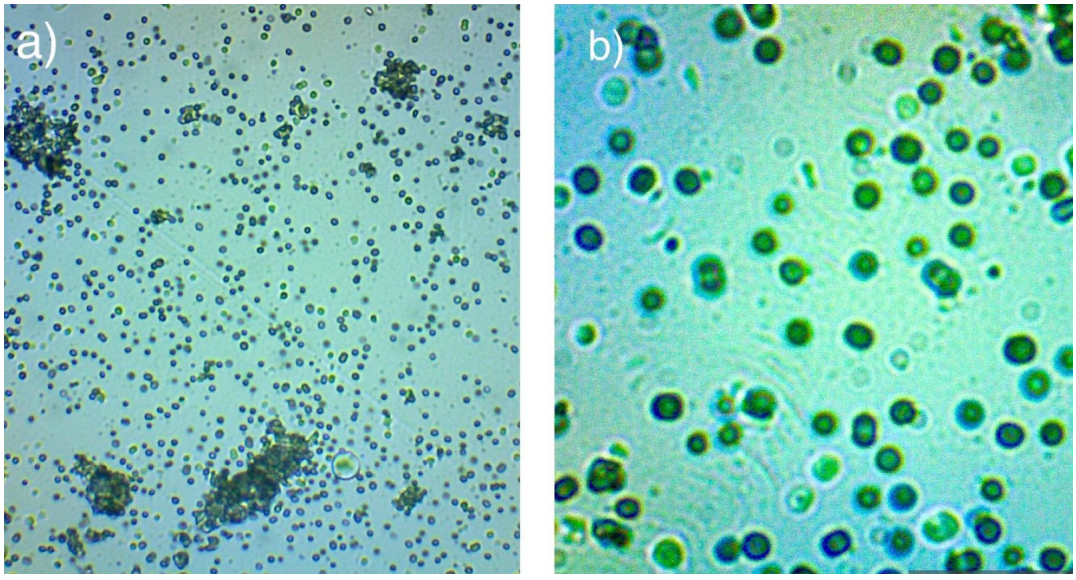


Figure 14: Microscopic images of PW1 algae in 10x and 40x: a) 10x b) 40x

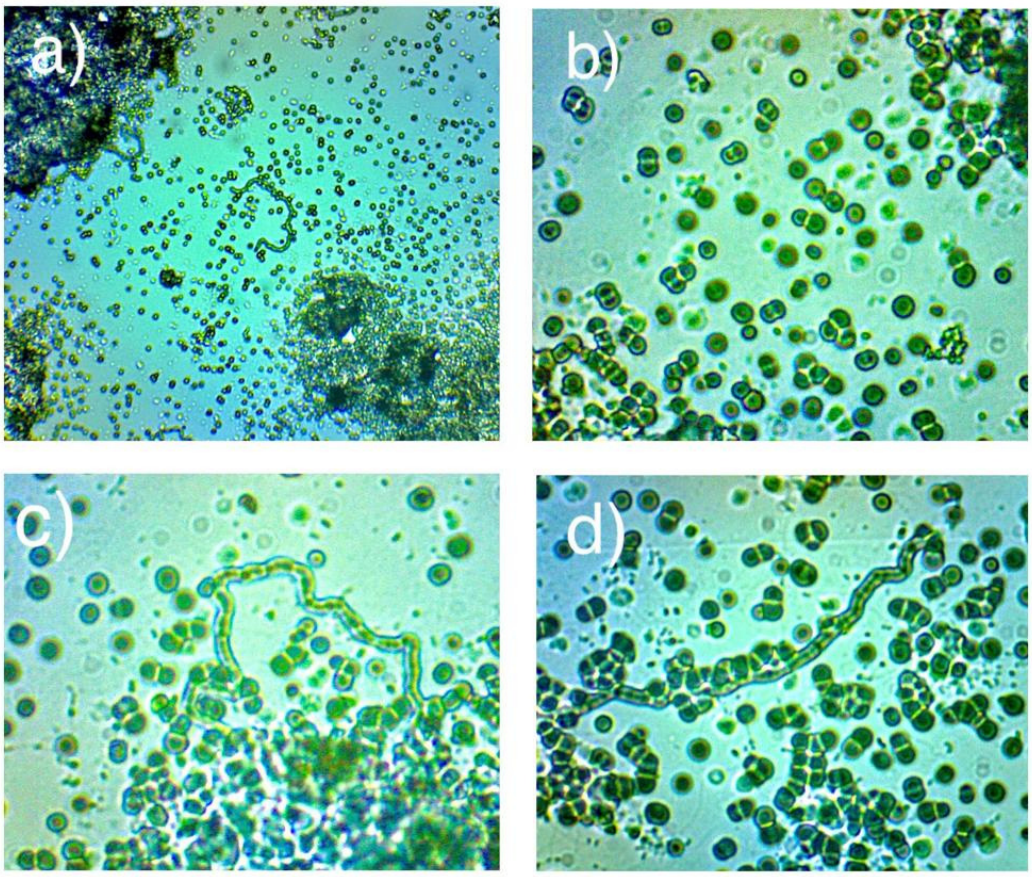


Figure 15: Microscopic images of PW2 algae in 10x and 40x: a) 10x; b) c), and d) 40x

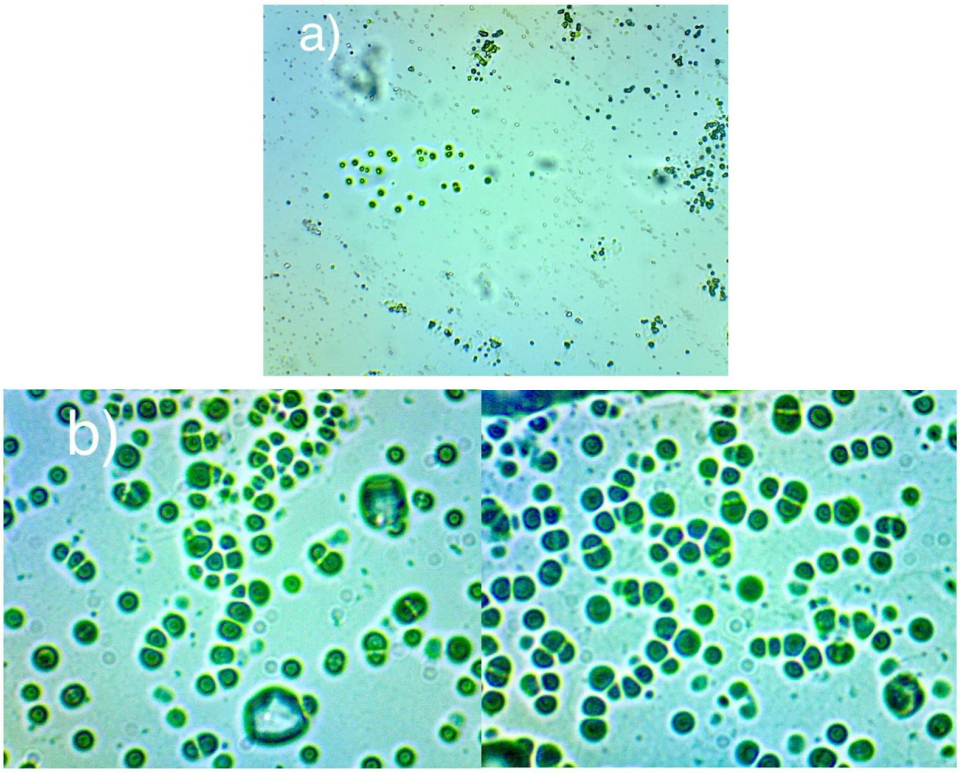


Figure 16: Microscopic images of PW3 algae in 10x and 40x: a) 10x b) 40x

4.3 Treatment of PW Using Microalgae

During treatment, parameters such as COD, pH, electrical conductivity, salinity, TDS, optical density, alkalinity, and anions were monitored on days one, two, three, five, and seven. These parameters were compared with the initial day zero (before treatment) values. The concentration of nutrients in the samples with microalgae culture decreased over time. This indicates that the microalgae are absorbing the nutrients during photosynthesis.

4.3.1 COD and TOC Removal Efficiency

The initial COD concentrations in PW1, PW2 and PW3 were 1,520 mg/L, 1,540 mg/L and 1,080 mg/L, respectively. Figure 17 and Table 10 show the decrease

in COD in the produced water samples and their respective controls over the seven days treatment period. Maximum removal was found to be during the first 48 hours. In the first 48 hours, about 25%, 30%, and 10% COD was removed in PW1, PW2, and PW3, respectively. The overall removal efficiency for the seven-day treatment was maximum in PW2 with 44%. PW1 showed 39% removal and PW3 least with 23% (Figure 18). This confirms the ability of the microalgae consortia in decomposing and utilize the organic compounds in the toxic produced water for their growth. The COD of PW2 control remained more or less the same throughout the treatment. There was a considerable decrease in COD of PW1 and PW3 controls during the second day. However, the removal percentage in the PW1 control was very less (0.6%) as compared to 2.1% of PW2 and 7.7% of PW3. COD removal in PW3 control indicates the presence of bacteria, nonetheless, this did not serve to be beneficial for overall COD removal during algal treatment.

Table 10: Change in COD (mg/L) of PW1, PW2, PW3 and their respective controls over 7 days treatment period

Sequential time (days)	**PW1 with algae**	**PW2 with algae**	**PW3 with algae**	**PW1 control**	**PW2 control**	**PW3 control**
0	1,520	1,540	1,080	1,520	1,540	1,080
1	1,307	1,183	1,043	1,497	1,580	1,170
2	1,139	1,070	975	1,386	1,520	960
3	1,130	978	947	1,540	1,533	1,011
5	1,009	932	913	1,466	1,530	1,087
7	932	856	832	1,511	1,507	996

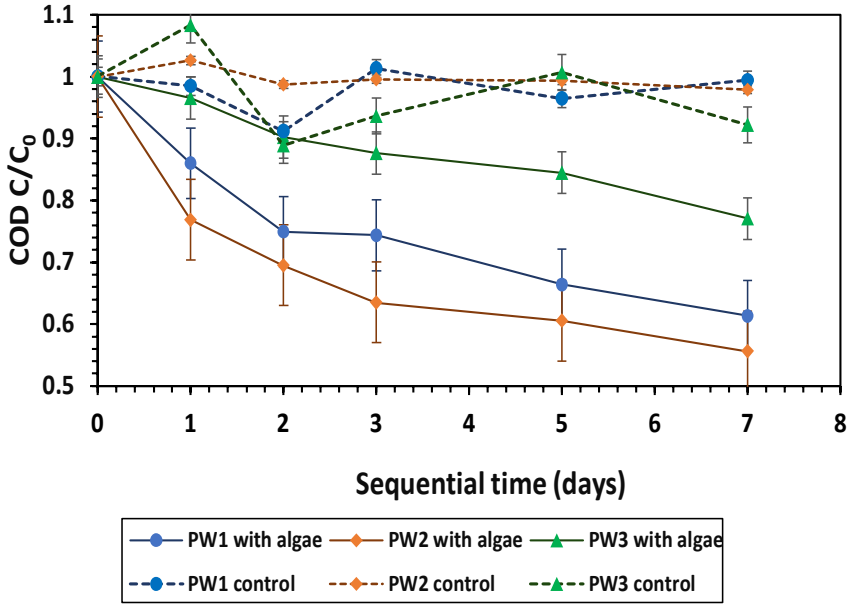


Figure 17: Variations in COD in the three produced water and their respective controls over a period of 7 days

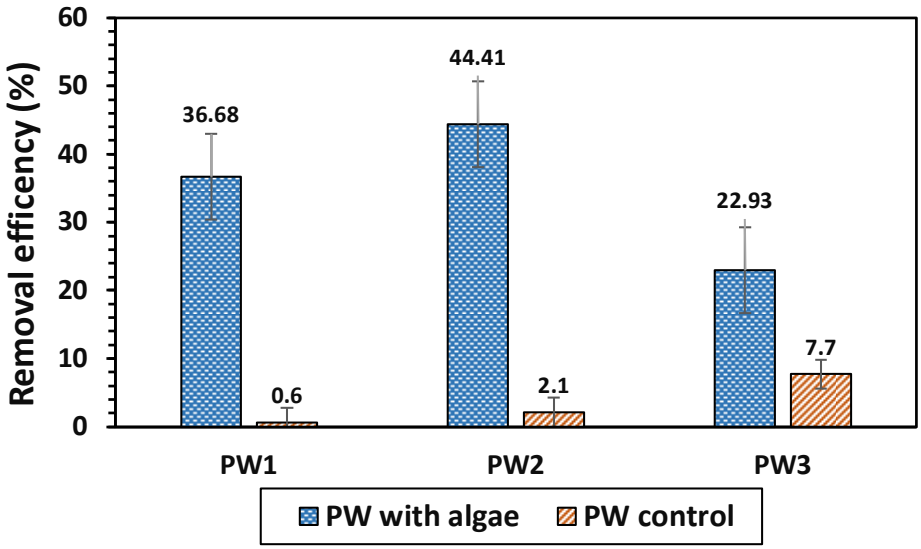


Figure 18: COD removal efficiencies of PW1, PW2, and PW3 with algae and their respective controls over the 7 days treatment period.

The average initial and final concentrations of TOC in the three produced water samples are given in Table 11. PW1 showed maximum removal efficiency with 25.6%. TOC removal by PW2 and PW3 were 13% and 4.12%, respectively (Figure 19).

Mixotrophy is the ability of certain microalgae to use dissolved organic carbon as a carbon source in the presence of light (Burkholder et al., 2008). It is possible that the strains in the algae consortia were mixotrophic, and hence some of the TOC was subsequently removed from the produced water due to consumption. The minor percentage removal of TOC in control where no algae were added could be because of the presence of a bacteria community present in the produced water.

Table 11: TOC Concentration before and after the 7 days treatment.

Sample	TOC Initial (mg/L)	TOC Final (mg/L)
PW1	2,600	1,960
PW1 control	2,600	2,485
PW2	2,500	2,500
PW2 control	2,500	2,425
PW3	2,550	2,550
PW3 control	2,550	2,505

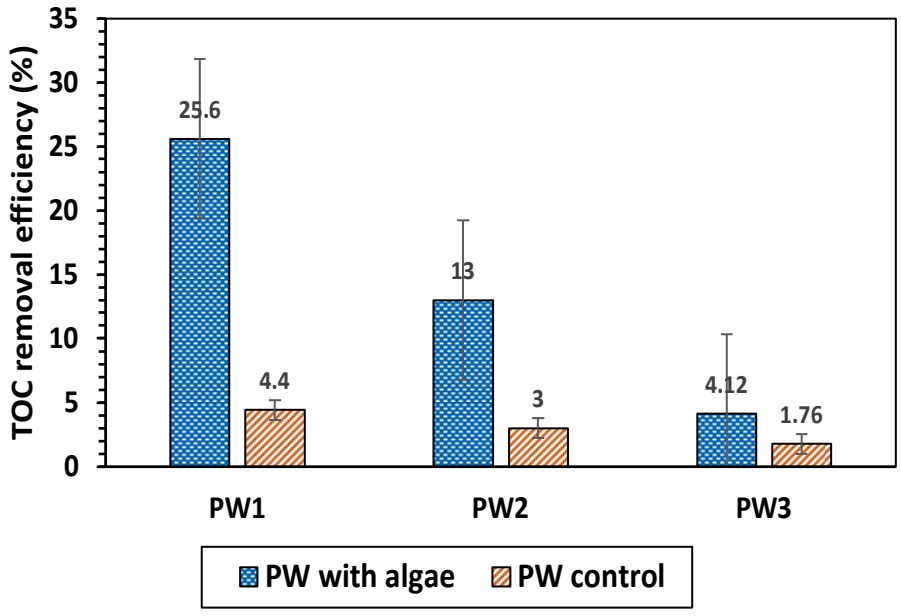


Figure 19: TOC removal efficiencies of PW1, PW2, and PW3 with algae and their respective controls over the 7 days treatment period.

4.3.2 Electrical Conductivity, Salinity, and Total Dissolved Solids

The EC and TDS are also indicators of the salinity of aqueous solutions. Solids dissolved in water, such as chloride, calcium, magnesium, potassium, sodium, bicarbonates, sulfates, and organic matter generally account for the TDS (Peng et al., 2020; Zhang et al., 2017). The algal cells take up TDS species, absorbing them as nutrients and minerals to support their physiology and metabolism thereby reducing TDS in the water (Lutzu et al., 2019; Peng et al., 2020).

Table 12: Change in Electrical conductivity of PW1, PW2, PW3 and their respective controls over 7 days treatment period

Sequential time (days)	PW1 with algae	PW2 with algae	PW3 with algae	PW1 control	PW2 control	PW3 control
0	95	104	103.5	94.8	104	103.5
1	91	100.4	98.7	93.7	103	104.8
2	89.5	98	95	94.1	102.4	103.4
3	89.5	95	94.4	96	100.7	99.9
5	86.7	93.5	91.4	94.3	103.8	100.6
7	77.2	87.2	91	93.8	103.6	101.2

All three parameters showed decreasing trend during the 7 days treatment (Tables 12, 13, and 14). Figure 20 depicts the variations in these parameters over the period and Figure 21 shows the overall removal efficiencies. The initial EC of the three samples were 94.8 mS/cm, 104 mS/cm, and 103.5 mS/cm in order. PW1

showed an EC reduction of 18.56% with a final conductivity of 77.2 mS/cm. PW2 and PW3 showed a reduction percentage of 16.1% and 12.17% respectively with final readings of 87.2 mS/cm and 90.966 mS/cm. The consistent reduction during the 7 days was achieved as the algae were in their growth phase. The results suggest that acclimatization helped in halotolerance and in the bioaccumulation of salts by algae.

Table 13: Change in TDS of PW1, PW2, PW3 and their respective controls over 7 days treatment period

Sequential time (days)	**PW1 with algae**	**PW2 with algae**	**PW3 with algae**	**PW1 control**	**PW2 control**	**PW3 control**
0	47.7	52.2	51.7	47.7	52.2	51.7
1	45	50	49.4	47	51.2	49.6
2	44.8	49	47.6	47.1	51.1	51.7
3	44.5	47.5	47.2	47.5	50.6	50.1
5	43.2	46.8	45.4	47.2	52.1	50
7	38	43.4	45.6	46.4	51.9	50.1

The EC, TDS, and salinity removal efficiencies of PW1, PW2, and PW3 are illustrated graphically in Figure 23.

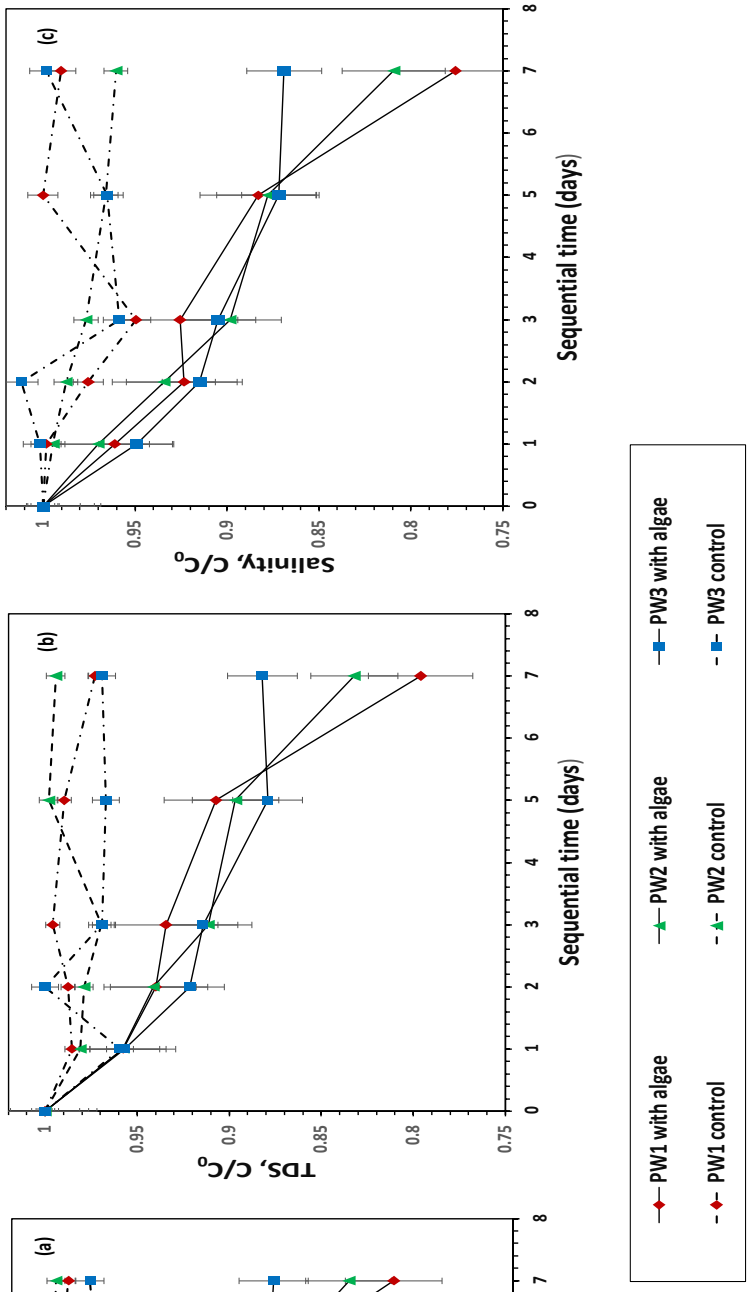


Figure 20: Variations in a) Electrical conductivity, b) TDS, c) salinity in the three produced water and their respective controls over 7 days treatment period.

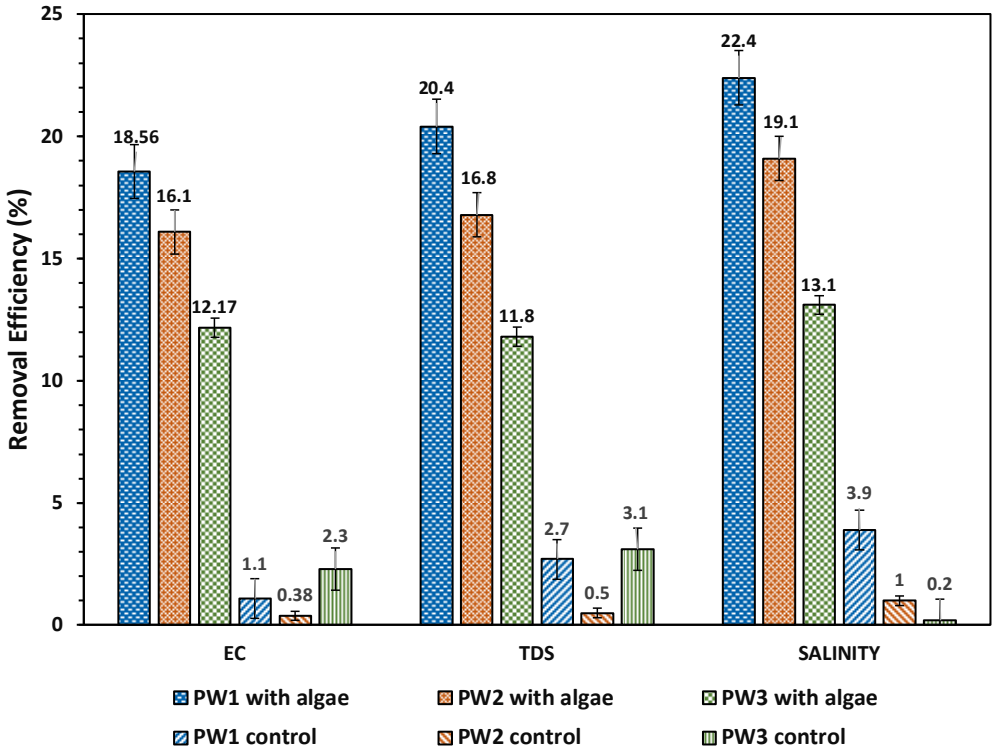


Figure 21: EC, TDS, and salinity removal efficiencies of PW1, PW2, and PW3 with algae and their respective controls over the 7 days treatment period.

The TDS removal rate for PW1 and PW2 was almost the same during the first 48 hours. Thereafter, removal was slower for PW1 until day 5 and then the TDS decreased rapidly from day 5 to day 7, with an overall removal of 20.4%. PW1 showed the highest removal of 12.25%, in the last two days of treatment. The overall removal efficiency of PW2 was 16.8 %. PW3 showed greater removal efficiency during the initial three days. Thereafter the decrease in TDS was gradual and at the end of the treatment period, the efficiency was only 11.8%. On the contrary, the PW3 control shows a greater decrease in TDS of 3.1%. PW2 control remained in a steadier state with a 0.5% decrease from the initial value and PW1 control removed 2.7% TDS (Figure 21).

Table 14: Change in Salinity of PW1, PW2, PW3 and their respective controls over 7 days treatment period.

Sequential time (days)	PW1 with algae	PW2 with algae	PW3 with algae	PW1 control	PW2 control	PW3 control
0	55.6	61.4	60.3	55.6	61.4	60.3
1	53.4	59.6	57.2	55.3	61.3	60.4
2	51.3	57.4	55.1	54.9	59.9	61
3	51.4	55.2	54.5	54.3	58.3	57.8
5	49.1	53.9	52.5	53.7	61.4	58.2
7	43.1	49.7	52.4	53.4	60.8	60.2

The decrease in salinity showed a similar trend as TDS and EC, with maximum removal efficiency by PW1 (22.4%) followed by PW2 (19.1%) and PW3 (13.1%).

The ions that constituted in TDS of the three produced water and that detected by IC were chloride and sulphate. PW1 also showed the presence of bromide. Produced water has high chloride content because of its highly saline nature. Since all the three produced water contained chloride as the major ion, the removal of the chloride ion was examined. Table 15 and Figure 22 shows variation in chloride ion concentration with time. The highest removal rate and removal efficiency (Figure 23) was shown by the produced water which had the highest concentration of chloride, that is, PW2 which had 53.1 g/L of chlorides showed a removal efficiency of 24.89%. Similarly, the other two produced water samples showed the same trend. PW3 which had an initial chloride concentration of 47.5 g/L showed removal of 18.77% whereas, PW1 with an initial chloride concentration of 42.75 g/L showed the least removal efficiency of only 16.1%. It should also be noted that there was considerable removal of chloride ion concentration

in the controls as well. The microalgae may also have an ability to adapt to the high chloride concentration. This could justify the fact that PW2 algae reached the stationary phase earlier than the other two produced water samples during the acclimatization stage.

Table 15: Change in Chloride ion concentration of PW1, PW2, PW3 and their respective controls over 7 days treatment period.

Sequential time (days)	PW1 with algae	PW2 with algae	PW3 with algae	PW1 control	PW2 control	PW3 control
0	17.1	21.2	19	17.1	21.2	18.9
1	16.4	18.2	18.9	18.5	19.6	20.5
2	14.4	16.5	15.8	15.7	17.9	18.8
3	14.6	17.2	16	16.5	18.7	17.3
5	14.3	16.4	18.9	17.5	18.3	21.1
7	14.2	16	15.4	15.1	18.8	18.5

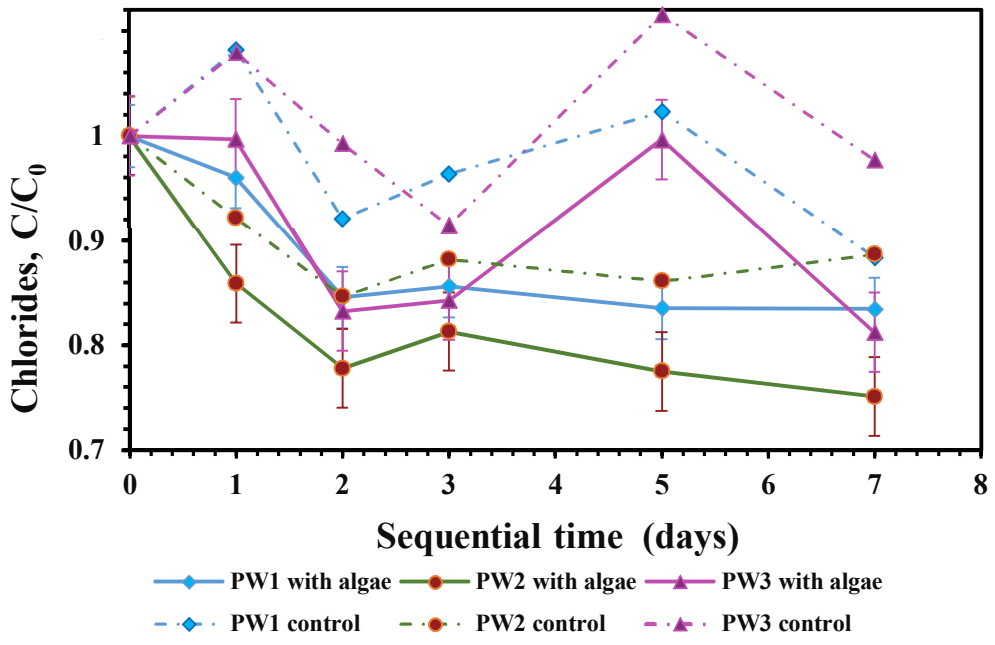


Figure 22: Variations in chloride concentration in the three produced water and their respective controls over 7 days treatment period.

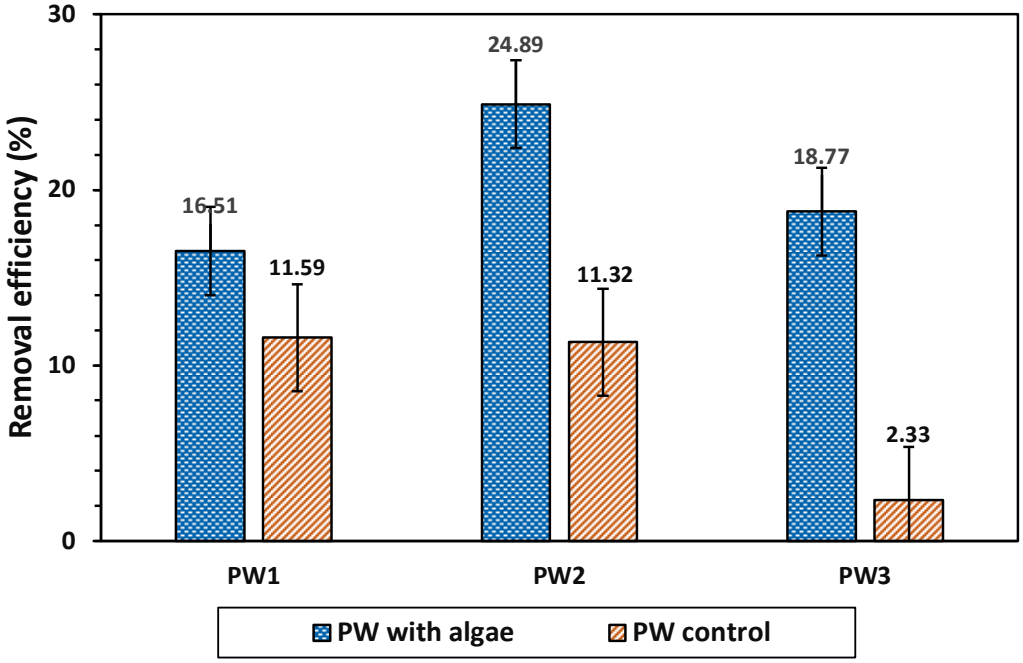


Figure 23: Chloride removal efficiencies of PW1, PW2, and PW3 with algae and their respective controls over the 7 days treatment period.

4.3.3 Change in pH and Alkalinity

The pH and alkalinity are closely related parameters. While pH indicates how acidic or alkaline a solution is, alkalinity indicates the ability of water to maintain stable pH. Table 16, 17 and Figure 24 a, b shows the average changes in pH (a) and alkalinity (b) of the produced water samples during the seven days treatment. All the three produced water showed an increase in pH during the treatment period from an initial pH of 6.41, 6.4, and 6.39 respectively to 7.44, 7.49, and 6.98. During its growth, algae absorb carbon dioxide from the solution for its growth. This causes an increase in pH (Freire et al., 2001). The pH of the controls did not show significant change.

Table 16: Change in pH of PW1, PW2, PW3 and their respective controls over 7 days treatment period

Sequential time (days)	PW1 with algae	PW2 with algae	PW3 with algae	PW1 control	PW2 control	PW3 control
0	6.4	6.4	6.39	6.4	6.4	6.39
1	7	6.8	6.8	6.9	6.5	6.49
2	7.1	6.97	6.8	6.9	6.5	6.4
3	7.1	6.9	6.8	6.9	6.6	6.4
5	7.4	7.1	6.9	6.9	6.6	6.41
7	7.4	7.5	6.9	6.9	6.6	6.45

Table 17: Change in alkalinity of PW1, PW2, PW3 and their respective controls over 7 days treatment period.

Sequential time (days)	PW1 with algae	PW2 with algae	PW3 with algae	PW1 control	PW2 control	PW3 control
0	2.57	4.35	3.65	2.57	4.35	3.6
1	10.7	8.6	10.6	1.88	4.57	5.9
2	14.6	12.4	13.6	2.46	4.8	3.6
3	15.6	16.1	15.1	2.75	4.3	3.7
5	16.5	17.6	17.4	3.04	4	3.35
7	17.8	20.4	19.1	2.62	3.53	3.72

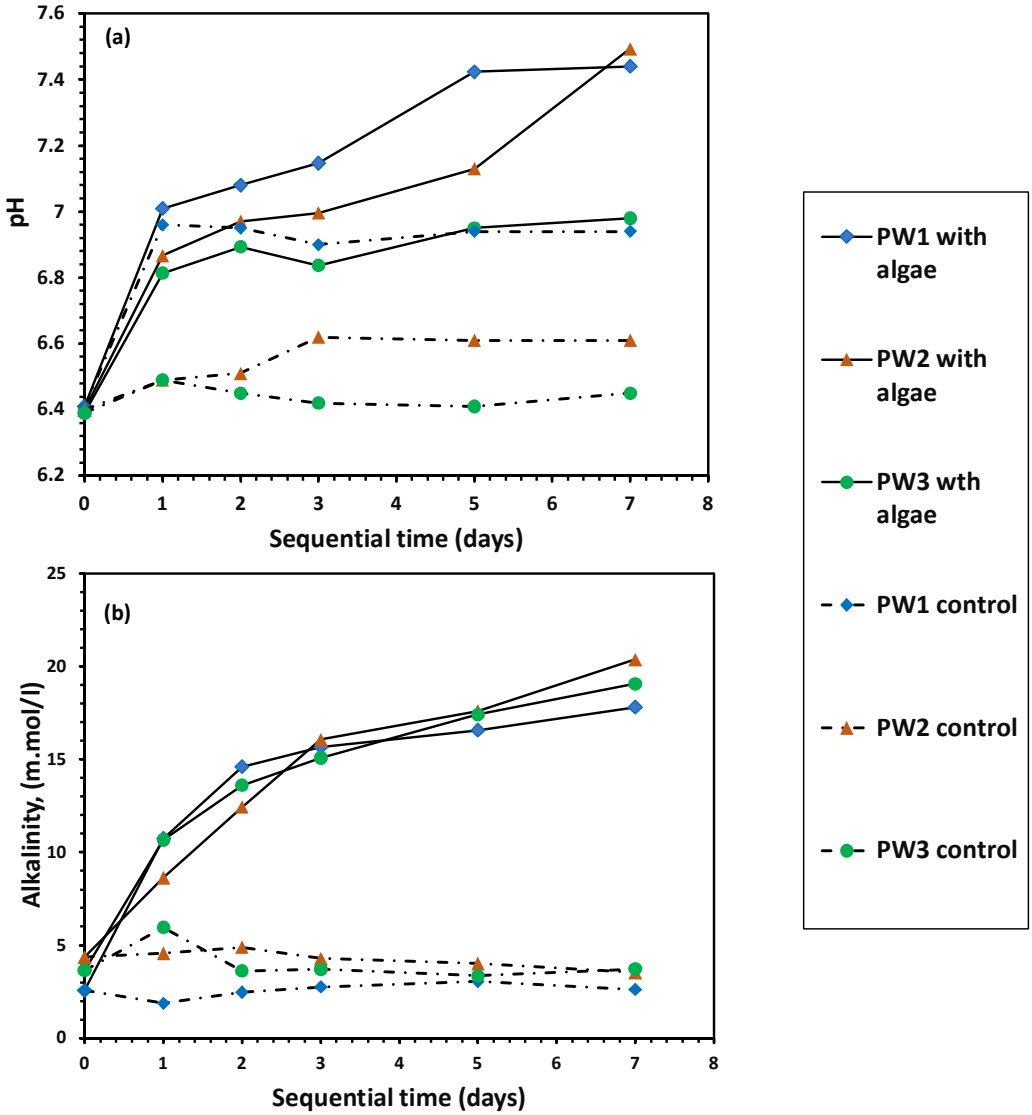


Figure 24: Variations in a) pH and b) alkalinity in the three produced water and their respective controls over 7 days treatment period.

The alkalinity values of the samples were initially low. The initial alkalinities of PW1, PW2, and PW3 were 2.57, 4.35, and 3.65, respectively. During the seven-day treatment period, the alkalinity of the sample increased rapidly. Figure 24 b) shows the percentage increase in alkalinity of PW1, PW2, and PW3 during the seven days. The alkalinity of PW1 increased 80.56%, while PW2 and PW3 showed a 78.7% and 80.85% increase in alkalinity. Figure 25 shows the PW1 and PW3

controls did not show significant changes while the PW2 control showed a decrease in alkalinity of 23.23%. This could be due to the presence of the bacterial population in the produced water.

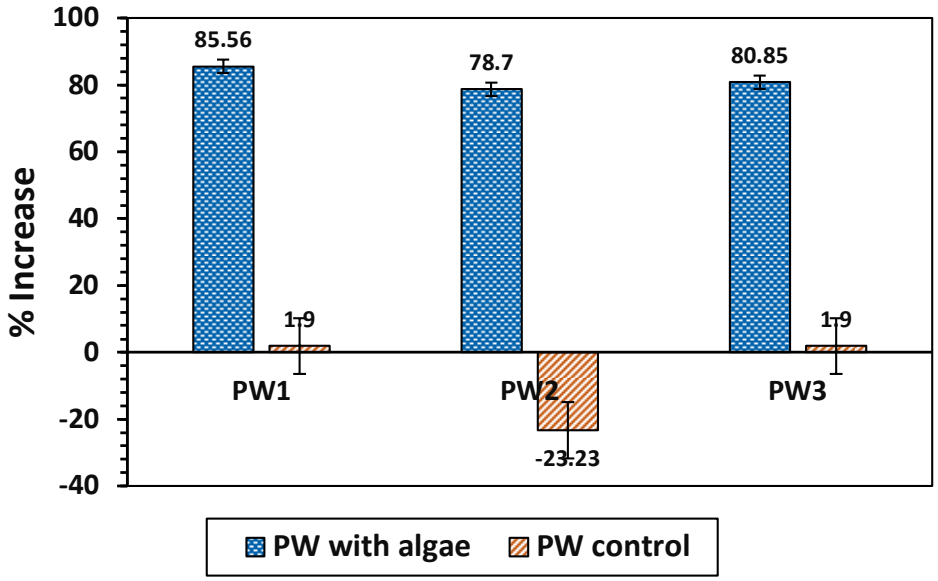


Figure 25: Increase in alkalinity values of PW1, PW2 and PW3 with algae and their respective controls over the 7 days treatment period

Chapter 5: Conclusions

The main conclusions from the research are given below:

- Microalgae enriched from wastewater could adapt to the high salinity and toxicity of three different produced water. When the ratio of produced water increased, the duration of the lag phase was higher. That is, the adaptation time increased when the algae were exposed to higher ratios of produced water. The pre-adaptation process helped in increased tolerance of algae to high salinity and toxicity of produced water and thereby increasing the efficiency of treatment.
- Produced water from different wells and regions are different in characteristics and chemical composition and provide a contrasting environment for algal growth. Algae showed higher adaptability in the produced water from the onshore field, PW2. This was evident from the fact that the algae in PW2 had a much shorter lag phase in all the progressive adaptation steps. PW1 from the offshore field showed better adaptability when compared to PW3 which was also generated in an offshore oil field.
- The least dilution in which microalgae flourished in minimal time was chosen to be the 2:1 ratio (PW1 and PW2) and 0.5:1 ratio (PW3). This was decided based on the length of the lag phase and the number of days the algae took to reach the stationary phase. Algae exhibited the shortest lag phase and least time to reach the stationary phase in PW2 in the 2:1 ratio. PW1 had a longer lag phase, even so, the algae showed adaptability in the 2:1 ratio. However, PW3 happened to be highly toxic and unfavorable for algae to adapt to. Therefore, the highest possible ratio for algae to adapt in PW3 was 0.5:1.

- The adapted algae consortia were used to treat the respective produced water and exhibited excellent treatment efficiencies. The results showed surprisingly good removal of COD, better than foreseen results in removal of salinity, conductivity, TDS, and chlorides. The pH was increased to neutral, and the buffering capacity was increased to a large extend.

- PW1 showed the following removal efficiencies: COD- 36 %, TOC- 26%, EC- 18%, TDS- 20%, salinity- 22%, chloride- 16%. The average increase in alkalinity was 85% and pH- 16%. PW1 showed the highest removal of TOC EC, TDS, and salinity as well as the highest increase in alkalinity.

- PW2 showed the following removal efficiencies: COD- 44.41 %, TOC- 13%, EC- 16%, TDS- 17%, salinity- 19%, chloride- 25%. The average increase in alkalinity was 78% and pH- 17%. PW2 showed the highest removal of COD and chloride

- PW3 showed the following removal efficiencies: COD- 22.93 %, TOC- 4.12%, EC- 12%, TDS- 19%, salinity- 13%, chloride- 18%. The average increase in alkalinity was 81% and pH- 8%. Removal efficiencies were least in PW3.

- Algal treatment research data of produced water is limited as compared to other wastewater streams. The results and findings of this research are therefore expected to be a significant contribution to effective PW treatment research.

Chapter 6: Future Research Directions

The composition of produced water which determines toxicity and salinity had a strong impact on the growth of microalgae. Acclimatization steps in this research helped in achieving the treatment efficiencies during the experiments. Studies with a longer exposure period are needed to determine how well microalgae cultures adapt to different environmental conditions. Since algae in the higher ratios of produced water showed an increase in optical density after twenty-seven days, longer exposure enhances adaptability and brings about beneficial variations in future generations that have a higher tolerance to salinity. In addition, it is necessary to further investigate microalgae acclimatization to enhance pollutants removal efficiency. Furthermore, the produced water quality after treatment in sequencing batch reactors is a promising direction of research in the future.

For developing the algal treatment of PW, it requires further understanding and investigation of the many factors associated with the treatment system, for example, the algae species present, the impact of various components in the PW that can inhibit the bioactivities, longer-term treatment performances, suitable bioreactor configurations to achieve cost-effective remediation and optimization.

Recovering or separating algal biomass from the treated water is one of the main and functional limitations of algal bioprocesses. To recycle wastewater effectively, algal biomass must be removed as efficiently as possible. Therefore, to circumvent the harvest problem as well as to retain the high-value algal biomass for further processing, algal cells can be used in immobilized form for water treatment (Eroglu et al., 2015; Mallick, 2002; Moreno-Garrido, 2008). As compared to

conventional suspension systems, immobilization technology can provide a more flexible reactor design when applied to algal wastewater treatment.

Therefore, treatment of produced water using immobilized acclimatized algae in a suitable matrix in a sequencing batch reactor would be the future direction of this research.

References

Akob, D. M., Cozzarelli, I. M., Dunlap, D. S., Rowan, E. L., & Lorah, M. M. (2015). Organic and inorganic composition and microbiology of produced waters from Pennsylvania shale gas wells. *Applied Geochemistry*, *60*, 116–125.

Al-Ghouti, M. A., Al-Kaabi, M. A., Ashfaq, M. Y., & Da'na, D. A. (2019). Produced water characteristics, treatment and reuse: A review. *Journal of Water Process Engineering*, *28*, 222–239.

Al-Haleem, A. A., Abdulah, H. H., & Saeed, E. A.-J. (2010). Components and treatments of oilfield produced water. *Al-Khwarizmi Engineering Journal*, *6*(1), 24–30.

Alley, B., Beebe, A., Rodgers Jr, J., & Castle, J. W. (2011). Chemical and physical characterization of produced waters from conventional and unconventional fossil fuel resources. *Chemosphere*, *85*(1), 74–82.

Amaro, H. M., Guedes, A. C., & Malcata, F. X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*, *88*(10), 3402–3410.

Ammar, S. H., Khadim, H. J., & Mohamed, A. I. (2018). Cultivation of Nannochloropsis oculata and Isochrysis galbana microalgae in produced water for bioremediation and biomass production. *Environmental Technology & Innovation*, *10*, 132–142.

Arriada, A. de A., & Abreu, P. C. (2014). Nannochloropsis oculata growth in produced water: An alternative for massive microalgae biomass production. *Brazilian Journal of Petroleum and Gas*, *8*(3).

Arthur, J. D., Langhus, B. G., & Patel, C. (2005). Technical summary of oil & gas produced water treatment technologies. *All Consulting, LLC, Tulsa, OK*.

Badrinarayanan, I. (2017). Using Produced Water to Grow Microalgae. *Russel School of Chemical Engineering the University of Tulsa: Tulsa, OK, USA*, 20.

Bakke, T., Klungsøyr, J., & Sanni, S. (2013). Environmental impacts of produced water and drilling waste discharges from the Norwegian offshore petroleum industry. *Marine Environmental Research*, *92*, 154–169.

Beyer, J., Goksøyr, A., Hjermann, D. Ø., & Klungsøyr, J. (2020). Environmental effects of offshore produced water discharges: A review focused on the Norwegian continental shelf. *Marine Environmental Research*, 105155.

Biris-Dorhoi, E.-S., Tofana, M., Mihăiescu, T., Mihăiescu, R., & Odagiu, A. (2016). Applications of microalgae in wastewater treatments: A review. *ProEnvironment Promediu*, *9*(28).

Bouabidi, Z. B., El-Naas, M. H., & Zhang, Z. (2019). Immobilization of microbial cells for the biotreatment of wastewater: A review. *Environmental Chemistry Letters*, *17*(1), 241–257.

Burkholder, J. M., Glibert, P. M., & Skelton, H. M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, *8*(1), 77–93.

Campos, J. C., Borges, R. M. H., Oliveira Filho, A. M. de, Nobrega, R., & Sant'Anna Jr, G. L. (2002). Oilfield wastewater treatment by combined microfiltration and biological processes. *Water Research*, *36*(1), 95–104.

Cardozo, K. H., Guaratini, T., Barros, M. P., Falcão, V. R., Tonon, A. P., Lopes, N. P., Campos, S., Torres, M. A., Souza, A. O., & Colepicolo, P. (2007). Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *146*(1–2), 60–78.

Chittick, E. A., & Srebotnjak, T. (2017). An analysis of chemicals and other constituents found in produced water from hydraulically fractured wells in California and the challenges for wastewater management. *Journal of Environmental Management*, *204*, 502–509.

Chu, W. L. (2012). Biotechnological applications of microalgae. *IeJSME*, *6*(1), S24–S37.

Clark, C. E., & Veil, J. A. (2009). *Produced water volumes and management practices in the United States.* Argonne National Lab.(ANL), Argonne, IL (United States).

Cohen, Y. (2001). Biofiltration–the treatment of fluids by microorganisms immobilized into the filter bedding material: A review. *Bioresource Technology*, *77*(3), 257–274.

Danforth, C., Chiu, W. A., Rusyn, I., Schultz, K., Bolden, A., Kwiatkowski, C., & Craft, E. (2020). An integrative method for identification and prioritization of constituents of concern in produced water from onshore oil and gas extraction. *Environment International*, *134*, 105280.

De-Bashan, L. E., Moreno, M., Hernandez, J.-P., & Bashan, Y. (2002). Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae Chlorella vulgaris coimmobilized in alginate beads with the microalgae growth-promoting bacterium Azospirillum brasilense. *Water Research*, *36*(12), 2941–2948.

Dores, R., Hussain, A., Katebah, M., & Adham, S. (2012). Using advanced water treatment technologies to treat produced water from the petroleum industry. *SPE International Production and Operations Conference & Exhibition*.

Duraisamy, R. T., Beni, A. H., & Henni, A. (2013). State of the art treatment of produced water. *Water Treatment*, 199–222.

Emam, E. A., Moawad, T. M., & Aboul-Gheit, N. A. (2014). Evaluating the characteristics of offshore oilfield produced water. *Petroleum & Coal*, *56*(4), 363–372.

Eriksen, N. T. (2008). The technology of microalgal culturing. *Biotechnology Letters*, *30*(9), 1525–1536.

Eroglu, E., Smith, S. M., & Raston, C. L. (2015). Application of various immobilization techniques for algal bioprocesses. In *Biomass and biofuels from microalgae* (pp. 19–44). Springer.

Ersahin, M. E., Ozgun, H., Kaya, R., Mutlu, B. K., Kinaci, C., & Koyuncu, I. (2018). Treatment of produced water originated from oil and gas production wells: A pilot study and cost analysis. *Environmental Science and Pollution Research*, *25*(7), 6398–6406.

Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L. C., Biak, D. R. A., Madaeni, S. S., & Abidin, Z. Z. (2009). Review of technologies for oil and gas produced water treatment. *Journal of Hazardous Materials*, *170*(2–3), 530–551.

Fierro, S., del Pilar Sánchez-Saavedra, M., & Copalcua, C. (2008). Nitrate and phosphate removal by chitosan immobilized Scenedesmus. *Bioresource Technology*, *99*(5), 1274–1279.

Fisher, J. B. (1987). Distribution and occurrence of aliphatic acid anions in deep subsurface waters. *Geochimica et Cosmochimica Acta*, *51*(9), 2459–2468.

Freire, D. D. C., Cammarota, M. C., & Sant'Anna, G. L. (2001). Biological treatment of oil field wastewater in a sequencing batch reactor. *Environmental Technology*, *22*(10), 1125–1135.

Gao, X., Kong, B., & Vigil, R. D. (2015). Characteristic time scales of mixing, mass transfer and biomass growth in a Taylor vortex algal photobioreactor. *Bioresource Technology*, *198*, 283–291.

Gazali, A. K., Alkali, A. N., Mohammed, Y., Yaba, D., Mohammed, D. D., & Kodomi, M. (2017). Environmental impact of produced water and drilling waste discharge fromthe Niger Delta petroleum industry. *IOSR J. Eng.*, *7*, 22–29.

Gevertz, D., Telang, A. J., Voordouw, G., & Jenneman, G. E. (2000). Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. *Applied and Environmental Microbiology*, *66*(6), 2491–2501.

Godoy-Hernández, G., & Vázquez-Flota, F. A. (2012). Growth measurements: Estimation of cell division and cell expansion. In *Plant Cell Culture Protocols* (pp. 41–48). Springer.

Gonçalves, A. L., Pires, J. C., & Simoes, M. (2016). The effects of light and temperature on microalgal growth and nutrient removal: An experimental and mathematical approach. *RSC Advances*, *6*(27), 22896–22907.

Gonçalves, A. L., Simões, M., & Pires, J. C. M. (2014). The effect of light supply on microalgal growth, CO2 uptake and nutrient removal from wastewater. *Energy Conversion and Management*, *85*, 530–536.

Granier, B., Al Suwaidi, A., Busnardo, R., Aziz, S., & Schroeder, R. (2003). New insight on the stratigraphy of the" Upper Thamama" in offshore Abu Dhabi (UAE). *Carnets de Géologie*, *A05*, 1–17.

Grini, P. G., Hjelsvold, M., & Johnsen, S. (2002). Choosing produced water treatment technologies based on environmental impact reduction. *SPE International Conference on Health, Safety and Environment in Oil and Gas Exploration and Production*.

Hakim, M. A. A., Al-Ghouti, M. A., Probir, D., Abu-Dieyeh, M., Ahmed, T. A., & Aljabri, H. M. S. (2018). Potential application of microalgae in produced water treatment. *Desalination and Water Treatment*, *135*, 47–58.

Hansen, B. R., & Davies, S. R. (1994). Review of potential technologies for the removal of dissolved components from produced water. *Chemical Engineering Research & Design*, *72*(2), 176–188.

Hardesty, J. H., & Attili, B. (2010). Spectrophotometry and the Beer-Lambert Law: An important analytical technique in chemistry. *Collin College, Department of Chemistry*.

Horner, J. E., Castle, J. W., & Rodgers Jr, J. H. (2011). A risk assessment approach to identifying constituents in oilfield produced water for treatment prior to beneficial use. *Ecotoxicology and Environmental Safety*, *74*(4), 989–999.

Igunnu, E. T., & Chen, G. Z. (2014). Produced water treatment technologies. *International Journal of Low-Carbon Technologies*, *9*(3), 157–177.

Jiménez, S., Micó, M. M., Arnaldos, M., Medina, F., & Contreras, S. (2018). State of the art of produced water treatment. *Chemosphere*, *192*, 186–208.

Khosravi, J., & Alamdari, A. (2009). Copper removal from oil-field brine by coprecipitation. *Journal of Hazardous Materials*, *166*(2–3), 695–700.

Khurshid, I., Hossain, M. M., Alraeesi, A., Fares, A., Albalushi, F., & Alhammadi, A. (2018). Desalination of Produced Water of Asab Oil Field Abu-Dhabi to Enhance its Oil Recovery and Water Injectivity. *SPE International Conference and Exhibition on Health, Safety, Security, Environment, and Social Responsibility*.

Knudsen, B. L., Hjelsvold, M., Frost, T. K., Svarstad, M. B. E., Grini, P. G., Willumsen, C. F., & Torvik, H. (2004). Meeting the zero discharge challenge for produced water. *SPE International Conference on Health, Safety, and Environment in Oil and Gas Exploration and Production*.

Kondash, A. J., Redmon, J. H., Lambertini, E., Feinstein, L., Weinthal, E., Cabrales, L., & Vengosh, A. (2020). The impact of using low-saline oilfield produced water for irrigation on water and soil quality in California. *Science of the Total Environment*, *733*, 139392.

Kraume, M., & Drews, A. (2010). Membrane bioreactors in waste water treatment–status and trends. *Chemical Engineering & Technology*, *33*(8), 1251–1259.

Lachapelle, J., Bell, G., & Colegrave, N. (2015). Experimental adaptation to marine conditions by a freshwater alga. *Evolution*, *69*(10), 2662–2675.

Lebeau, T., Robert, J.-M., & Subba Rao, D. (2006). Biotechnology of immobilized micro algae: A culture technique for the future. *Algal Cultures, Analogues of Blooms and Applications. Science Publishers, Enfield*, 801–837.

Lee, K., & Neff, J. (2011). *Produced water: Environmental risks and advances in mitigation technologies*. Springer.

Lu, M., Zhang, Z., Yu, W., & Zhu, W. (2009). Biological treatment of oilfield-produced water: A field pilot study. *International Biodeterioration & Biodegradation*, *63*(3), 316–321.

Lusinier, N., Seyssiecq, I., Sambusiti, C., Jacob, M., Lesage, N., & Roche, N. (2019). Biological treatments of oilfield produced water: A comprehensive review. *SPE Journal*, *24*(05), 2–135.

Lutzu Giovanni Antonio & Turgut Nurhan Dunford. (2019). Growing Algae in Produced Water Generated During Oil and Gas Production Using Hydraulic Fracturing Technique. *Chemical Engineering Transactions*, *74*, 1261–1266. https://doi.org/10.3303/CET1974211

Mallick, N. (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. *Biometals*, *15*(4), 377–390.

Martinez, A., Melo, O., Resendiz, T., Aguilar, J., Vazquez, J., & Molero, N. (2016). Developing a Marginal Field Combining Stimulation and Water Control to Limit Scaling. *SPE International Conference and Exhibition on Formation Damage Control*.

Middleditch, B. S. (1984). Ecological effects of produced water effluents from offshore oil and gas production platforms. *Ocean Management*, *9*(3–4), 191–316.

Mollamohammada, S., Aly Hassan, A., & Dahab, M. (2021). Immobilized algae-based treatment of herbicide-contaminated groundwater. *Water Environment Research*, *93*(2), 263–273.

Mollamohammada, S., Hassan, A. A., & Dahab, M. (2020). Nitrate removal from groundwater using immobilized heterotrophic algae. *Water, Air, & Soil Pollution*, *231*(1), 1–13.

Moreno-Garrido, I. (2008). Microalgae immobilization: Current techniques and uses. *Bioresource Technology*, *99*(10), 3949–3964.

Moreno-Garrido, I., Campana, O., Lubián, L. M., & Blasco, J. (2005). Calcium alginate immobilized marine microalgae: Experiments on growth and short-term heavy metal accumulation. *Marine Pollution Bulletin*, *51*(8–12), 823–829.

Muggeridge, A., Cockin, A., Webb, K., Frampton, H., Collins, I., Moulds, T., & Salino, P. (2014). Recovery rates, enhanced oil recovery and technological limits. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *372*(2006), 20120320.

Neff, J., Lee, K., & DeBlois, E. M. (2011). Produced water: Overview of composition, fates, and effects. *Produced Water*, 3–54.

Obire, O., & Amusan, F. O. (2003). The environmental impact of oilfield formation water on a freshwater stream in Nigeria. *Journal of Applied Sciences and Environmental Management*, *7*(1), 61–66.

Ogbonna, J. C., Yada, H., & Tanaka, H. (1995). Effect of cell movement by random mixing between the surface and bottom of photobioreactors on algal productivity. *Journal of Fermentation and Bioengineering*, *79*(2), 152–157.

Pendashteh, A. R., Abdullah, L. C., Fakhru'l-Razi, A., Madaeni, S. S., Abidin, Z. Z., & Biak, D. R. A. (2012). Evaluation of membrane bioreactor for hypersaline oily wastewater treatment. *Process Safety and Environmental Protection*, *90*(1), 45–55.

Peng, J., Kumar, K., Gross, M., Kunetz, T., & Wen, Z. (2020). Removal of total dissolved solids from wastewater using a revolving algal biofilm reactor. *Water Environment Research*, *92*(5), 766–778.

Perrineau, M.-M., Zelzion, E., Gross, J., Price, D. C., Boyd, J., & Bhattacharya, D. (2014). Evolution of salt tolerance in a laboratory reared population of C hlamydomonas reinhardtii. *Environmental Microbiology*, *16*(6), 1755–1766.

Petroleum. (2008, August 26). *Jordan Science and Engineering*. https://jordanscience.wordpress.com/2008/08/26/petroleum/

Plebon, M. J., Saad, M., & Fraser, S. (2005). Further Advances in Produced Water De-oiling Utilizing a Technology that Removes and Recovers Dispersed Oil in Produced Water 2 micron and Larger. *12th International Petroleum Environmental Conference, Houston, TX, Nov*, 8–11.

Rahman, A., Agrawal, S., Nawaz, T., Pan, S., & Selvaratnam, T. (2020). A review of algae-based produced water treatment for biomass and biofuel production. *Water*, *12*(9), 2351.

Ruiz-Marin, A., Mendoza-Espinosa, L. G., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology*, *101*(1), 58–64.

Sahle-Demessie, E., Hassan, A. A., & El Badawy, A. (2019). Bio-desalination of brackish and seawater using halophytic algae. *Desalination*, *465*, 104–113.

Selvaratnam, T., Pegallapati, A., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Lammers, P. J., & Van Voorhies, W. (2015). Feasibility of algal systems for sustainable wastewater treatment. *Renewable Energy*, *82*, 71–76.

Shaffer, D. L., Arias Chavez, L. H., Ben-Sasson, M., Romero-Vargas Castrillón, S., Yip, N. Y., & Elimelech, M. (2013). Desalination and reuse of high-salinity shale gas produced water: Drivers, technologies, and future directions. *Environmental Science & Technology*, *47*(17), 9569–9583.

Sharghi, E. A., & Bonakdarpour, B. (2013). The study of organic removal efficiency and halophilic bacterial mixed liquor characteristics in a membrane bioreactor treating hypersaline produced water at varying organic loading rates. *Bioresource Technology*, *149*, 486–495.

Shetty, P., Gitau, M. M., & Maróti, G. (2019). Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae. *Cells*, *8*(12), 1657.

Sigamani, S., Ramamurthy, D., & Natarajan, H. (2016). A review on potential biotechnological applications of microalgae. *J. Appl. Pharm. Sci*, *6*, 179–184.

Silva, T. L., Morales-Torres, S., Castro-Silva, S., Figueiredo, J. L., & Silva, A. M. (2017). An overview on exploration and environmental impact of unconventional gas sources and treatment options for produced water. *Journal of Environmental Management*, *200*, 511–529.

Singh, S. P., & Singh, P. (2015). Effect of temperature and light on the growth of algae species: A review. *Renewable and Sustainable Energy Reviews*, *50*, 431–444.

Skaare, B. B., Wilkes, H., Vieth, A., Rein, E., & Barth, T. (2007). Alteration of crude oils from the Troll area by biodegradation: Analysis of oil and water samples. *Organic Geochemistry*, *38*(11), 1865–1883.

Stephenson, M. T. (1992). A survey of produced water studies. In *Produced water* (pp. 1–11). Springer.

Strømgren, T., Sørstrøm, S. E., Schou, L., Kaarstad, I., Aunaas, T., Brakstad, O. G., & Johansen, Ø. (1995). Acute toxic effects of produced water in relation to chemical composition and dispersion. *Marine Environmental Research*, *40*(2), 147–169.

Talebi, A. F., Dastgheib, S. M. M., Tirandaz, H., Ghafari, A., Alaie, E., & Tabatabaei, M. (2016). Enhanced algal-based treatment of petroleum produced water and biodiesel production. *RSC Advances*, *6*(52), 47001–47009.

Tong, K., Zhang, Y., Liu, G., Ye, Z., & Chu, P. K. (2013). Treatment of heavy oil wastewater by a conventional activated sludge process coupled with an immobilized biological filter. *International Biodeterioration & Biodegradation*, *84*, 65–71.

Veil, J. A., Puder, M. G., Elcock, D., & Redweik Jr, R. J. (2004). *A white paper describing produced water from production of crude oil, natural gas, and coal bed methane.* Argonne National Lab., IL (US).

Venkatesan, A., & Wankat, P. C. (2017). Produced water desalination: An exploratory study. *Desalination*, *404*, 328–340.

Wenzlick, M., & Siefert, N. (2020). Techno-economic analysis of converting oil & gas produced water into valuable resources. *Desalination*, *481*, 114381.

Zhang, C., Zhang, W., Huang, Y., & Gao, X. (2017). Analysing the correlations of long-term seasonal water quality parameters, suspended solids and total dissolved solids in a shallow reservoir with meteorological factors. *Environmental Science and Pollution Research*, *24*(7), 6746–6756.

Zhao, B., Zhang, Y., Xiong, K., Zhang, Z., Hao, X., & Liu, T. (2011). Effect of cultivation mode on microalgal growth and CO2 fixation. *Chemical Engineering Research and Design*, *89*(9), 1758–1762.

Zheng, Q., Martin, G. J., & Kentish, S. E. (2016). Energy efficient transfer of carbon dioxide from flue gases to microalgal systems. *Energy & Environmental Science*, *9*(3), 1074–1082.

Zolghadr, E., Firouzjaei, M. D., Amouzandeh, G., LeClair, P., & Elliott, M. (2021). The Role of Membrane-Based Technologies in Environmental Treatment and Reuse of Produced Water. *Frontiers in Environmental Science*, *9*, 71.

Appendices

Appendix A: Treatment of Produced Water Using Immobilized Algae

Introduction

Microalgal treatment is an effective method for the bioremediation of produced water when acclimatized algal biomass is used for the treatment. However, harvesting this acclimatized high-value algal biomass for reuse and recycle, and for the reuse or disposal of produced water is difficult and challenging. Thus, the immobilization of microalgae into polymer matrices will be beneficial in solving both problems. Immobilized algal cells have been used in water purification processes and pollutant removal for decades (Cohen, 2001; Moreno-Garrido, 2008). Algal cells in an immobilized state occupy less space and are easier to handle (Mallick, 2002).

Different polymers, both natural and synthetic are used as matrices for immobilizing cells, however, they must meet a number of criteria, including photo-transparency, nontoxicity, cellular viability retention, and stability in the culture medium (Lebeau et al., 2006; Mallick, 2002). The most common and widely used polymer matrix for the immobilization of algae is alginate. It is transparent and nontoxic, both of which allow photosynthesis and growth of autotrophic organisms like microalgae (Bouabidi et al., 2019). A number of studies have utilized immobilized algal cells to remove nutrients from wastewater (De-Bashan et al., 2002; Moreno-Garrido et al., 2005; Ruiz-Marin et al., 2010).

Chitosan is a linear copolymer of D-glucosamine and N-acetyl-D-glucosamine and is a polysaccharide coagulant. Due to its unique physicochemical properties, it

has excellent interactions with many contaminants, including particulate matter and dissolved substances (Fierro et al., 2008). It is obtained primarily from chitin, the second most abundant organic compound in nature as it forms the exoskeleton of crustaceans and other organisms. Immobilization studies with chitosan are very few. (Fierro et al., 2008) studied the applicability of chitosan to immobilizing *Scenedesmus* species cells for determining viability, growth, and nitrate/phosphate uptake.

In this study, experiments were done with both alginate and chitosan matrices to immobilize preadapted microalgae for the treatment of produced water and evaluate the stability of the matrix in produced water and the treatment efficiency.

Materials and methods

Preparation of immobilized algae beads

The algae acclimatized to the three produced water served as the stock culture for the preparation of immobilized algal beads. The immobilized algae treatment experiments were conducted with two different matrices namely alginate and chitosan.

Separation of biomass

The stock culture algae were centrifuged at 7500 rpm for 7 minutes. The supernatant was discarded, and the algal biomass was resuspended using a minimum amount of BG11 media.

Alginate beads preparation

Sodium alginate (5%) solution was prepared by dissolving 25 g sodium alginate in 500 ml deionized water. The resuspended algal biomass was mixed with the alginate solution in the ratio 4:1 alginate: algae. 2% calcium chloride solution

was prepared separately for producing algae-alginate beads and blank alginate beads. The algae-alginate mixture was dropped into calcium chloride solution using a syringe manually. Blank beads were prepared similarly using alginate. This was left overnight for bead formation. The beads were then rinsed thoroughly and stored in DI water for further use in experiments. Figure 26 shows the bead formation in calcium chloride solution.

Chitosan bead preparation

Chitosan solution was prepared by mixing 4gm chitosan powder in 100 ml 0.5% acetic acid. The resuspended algae were mixed with the chitosan solution in 4:1 ratio Chitosan: algae. This mixture was dropped into 0.1N NaOH solution manually using a syringe. Chitosan- cell mixture was allowed to drip for 2 minutes, followed by 3 minutes of agitation and then picking them up with a mesh. The beads were then washed several times to clear off NaOH. This method of dripping-agitating-washing was done to reduce the contact time of the algae with NaOH. The process was repeated until the chitosan-algae mixture was completed. Blank beads were prepared without algae (Figure 28).

Treatment using immobilized algae

The treatment experiments were done in 250 ml beakers. Cylindrical wire mesh was placed inside the reactors to keep the beads to the sides of the beakers so that every bead gets exposed to light and the whole bead surface is in contact with produced water.

In each reactor, 25 mL of the beads were mixed with produced water to a volume of 200 mL. A control for each produced water was prepared with blank

beads. Light intensity was maintained at 1500 – 1700 LUX with 12 hours of light and dark cycles. The beakers were covered with perforated aluminum foil to enable air passage from and to the reactor. The treatment was done for seven days. Parameters such as optical density, pH, electrical conductivity, total dissolved solids, salinity, COD, TOC, alkalinity, and anions were monitored before and after one, two, three, five, and seven days of treatments (Figure 27 and 29).

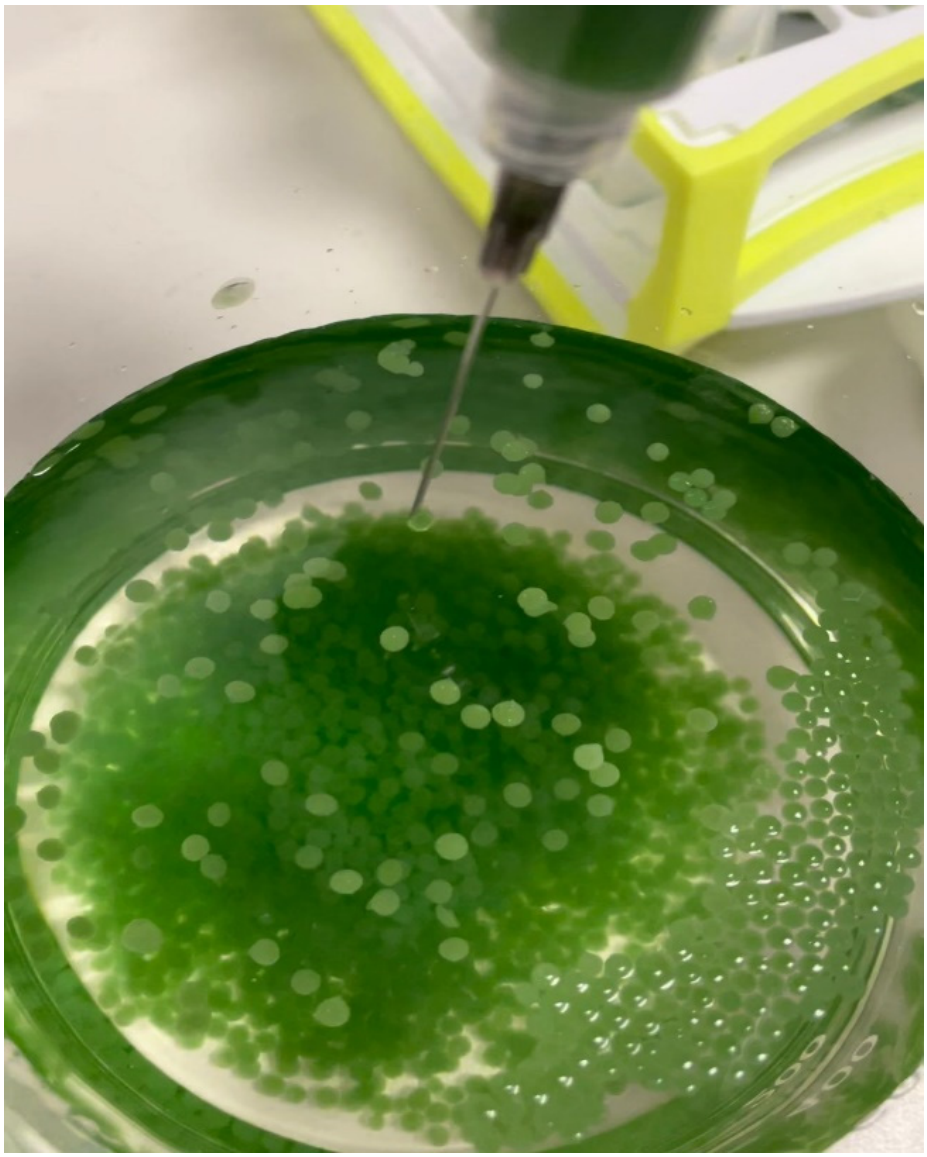


Figure 26: Preparation of immobilized algae beads with alginate.

Figure 27: Treatment of PW2 with algae immobilized in alginate beads

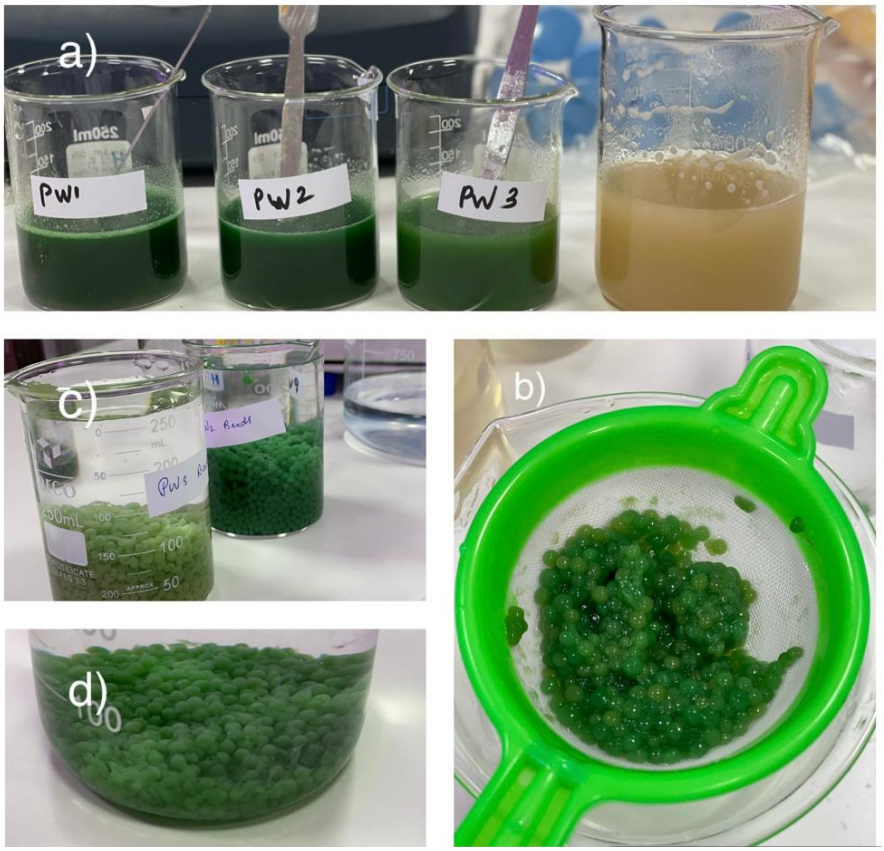


Figure 28: a) Chitosan solution with and without algae b) algae immobilized in chitosan beads c), d) immobilized algae in chitosan beads

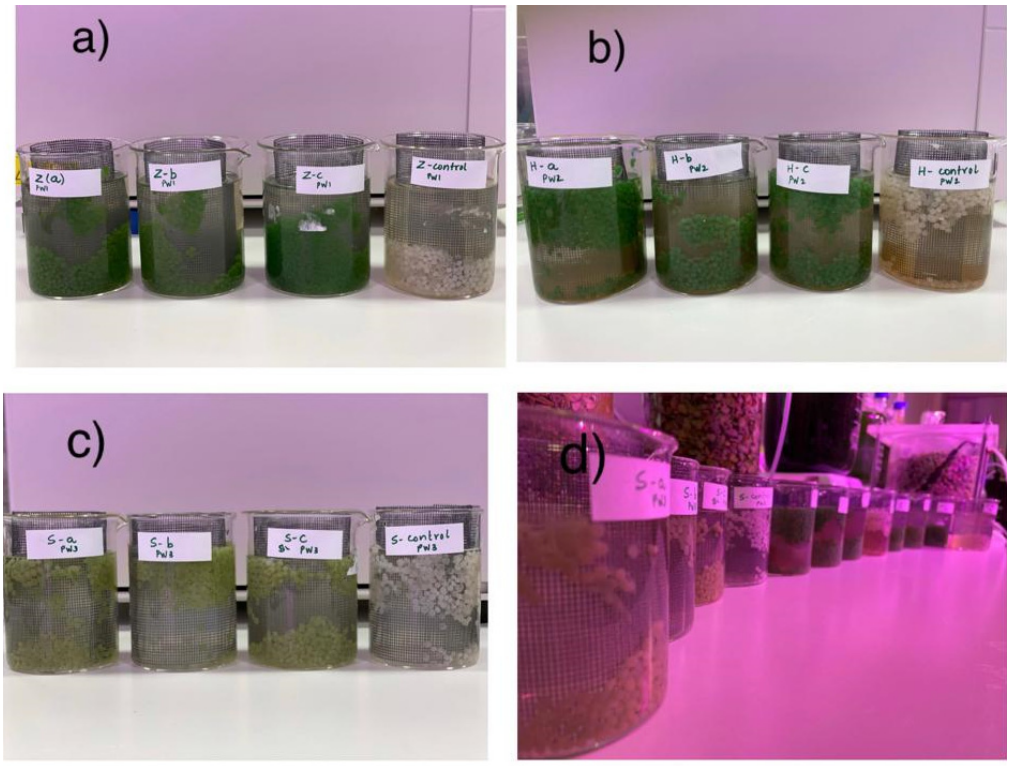


Figure 29: Immobilized algae in reactors a) PW1, b) PW2, c) PW3 d) Experimental set up

Results and Discussions

a) Treatment of produced water with algae immobilized in alginate

Among other parameters, TOC and TSS were closely observed. The pH of the samples increased from an initial 5.7 to an average of 6.74 on day one and to 7.3 on day 3. The conductivity decreased on day one and then showed an increase on day 3. However, the most important observations were the TSS and TOC which showed a consistent increase on day 1 and day 3, not only in the reactors with algae but also in the control reactor. TOC increased from an initial 2500 mg/L to 4687 mg/L after 24 hours of treatment and to 7650 mg/L on day 3. The reason for an increase in TOC and TSS in reactors including the control could be due to the dissolution of alginate in produced water.

b) Treatment of produced water with algae immobilized in chitosan

Since alginate was not stable in produced water, chitosan beads were chosen as the next option for immobilizing algae for the treatment of produced water. The experiments with all three samples of produced water showed results similar to alginate. The TSS of the reactors with algae as well as the controls increased drastically over the days indicating the dissolution of chitosan in produced water. The TSS was much higher in reactors with algae than in the controls. This could be due to the presence of free algal cells in the solution. Therefore, chitosan could not be used as an immobilization matrix for the treatment of produced water.

Conclusion

Produced water is highly toxic for the stability of alginate and chitosan matrices. A more stable matrix has to be determined and experimented with for immobilizing algae and treatment of produced water.

Appendix B: COD Analysis

Method: Using Hach LCK 514 Cuvette test

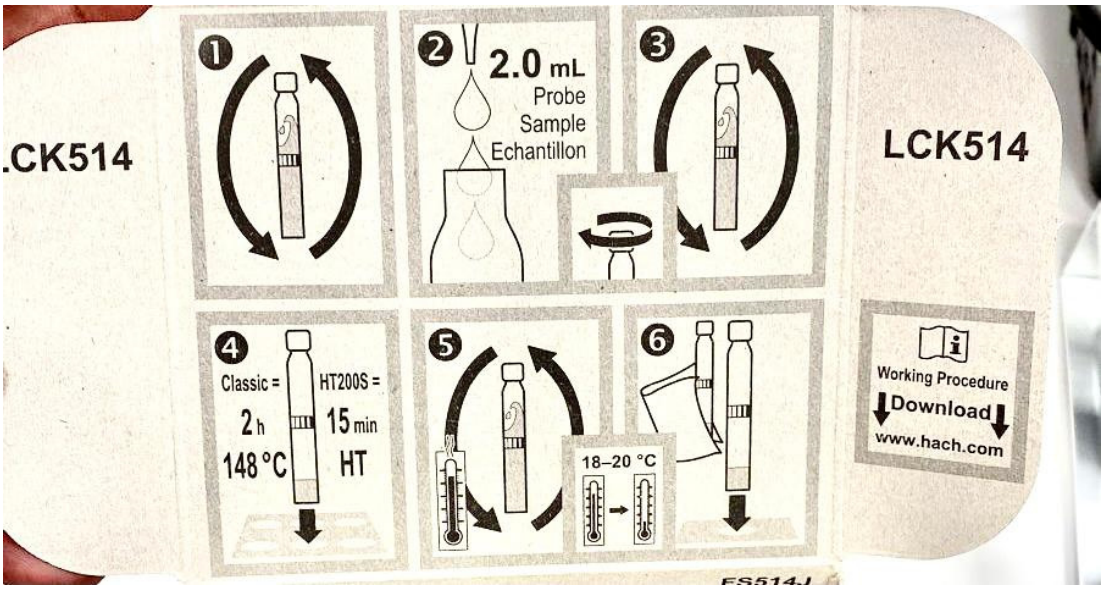


Figure 30: COD analysis method

Procedure

- The method illustrated inside the LCK 514 box (Figure 30) was carefully followed to do the test.
- The sample for the test is prepared by diluting the produced water five times to get the results within the range of LCK 514.
- The cuvette for the test is inverted or shaken thoroughly to suspend the sediments that are settled at the bottom.
- Carefully, 2 mL of the diluted sample is pipetted out and added to the cuvette, and mixed well.
- The cuvette is placed in an HT digester in the standard HT program for 15 minutes.

- After digestion and the lock of the digester opens, the cuvette is inverted twice and left to completely cool to around 20ºC.
- The cuvette is placed into the cell holder of the DR3900 spectrophotometer which recognizes the barcode and displays COD in mg/L.

Appendix C: Alkalinity Analysis

Method: Using Hach LCK 362 Cuvette test

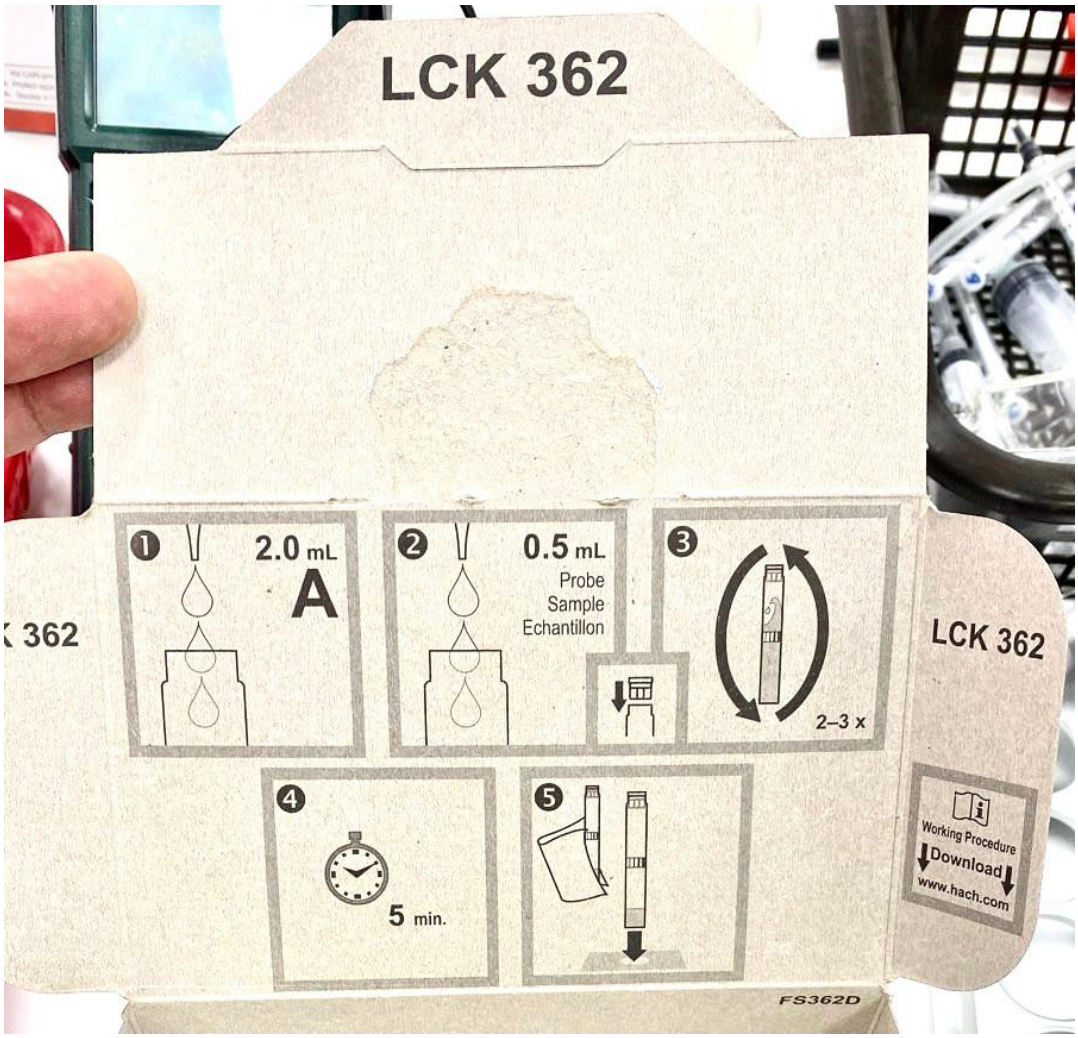


Figure 31: Alkalinity analysis method

Procedure

- The method illustrated inside the LCK 514 box (Figure 31) was carefully followed to do the test.
- The day 0 samples were used directly without dilution. From day 1, all samples were diluted 5 times with DI water as the alkalinity exceeded the range of the test kits.

- 2 mL of the reagent A provided along with the cuvettes and 0.5 ml of the sample solution is pipetted into the cuvette.
- The cuvette is closed and inverted a few times to dissolve all the freeze-dried contents of the cuvette.
- Thereafter the cuvette is allowed to rest for 5 minutes and then placed in the cell holder of the DR3900 spectrophotometer which recognizes the barcode and displays alkalinity in mmol/L.

Appendix D: Images of Instruments and Chemicals Used for Analysis

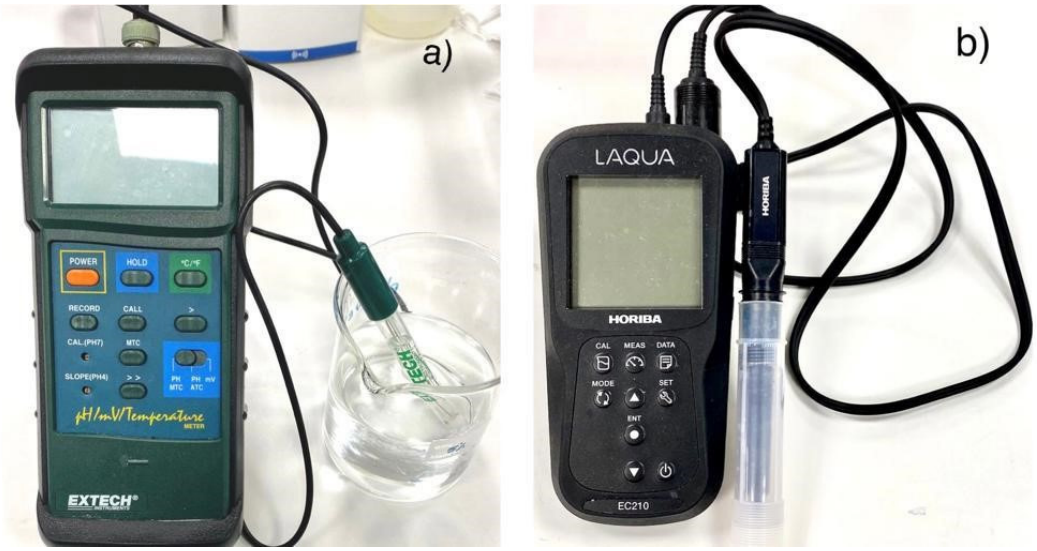


Figure 32: Instruments used for water quality analysis: a) pH meter b) Conductivity

Figure 33: a) Hach COD vial b) Hach vial to measure alkalinity

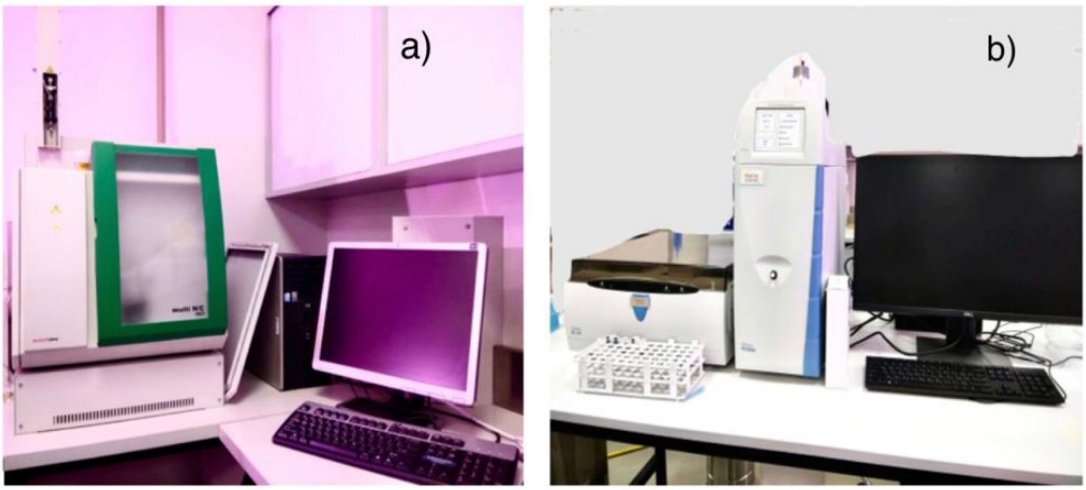


Figure 34: Equipments used for water quality testing: a) TOC Analyzer, b) Ion

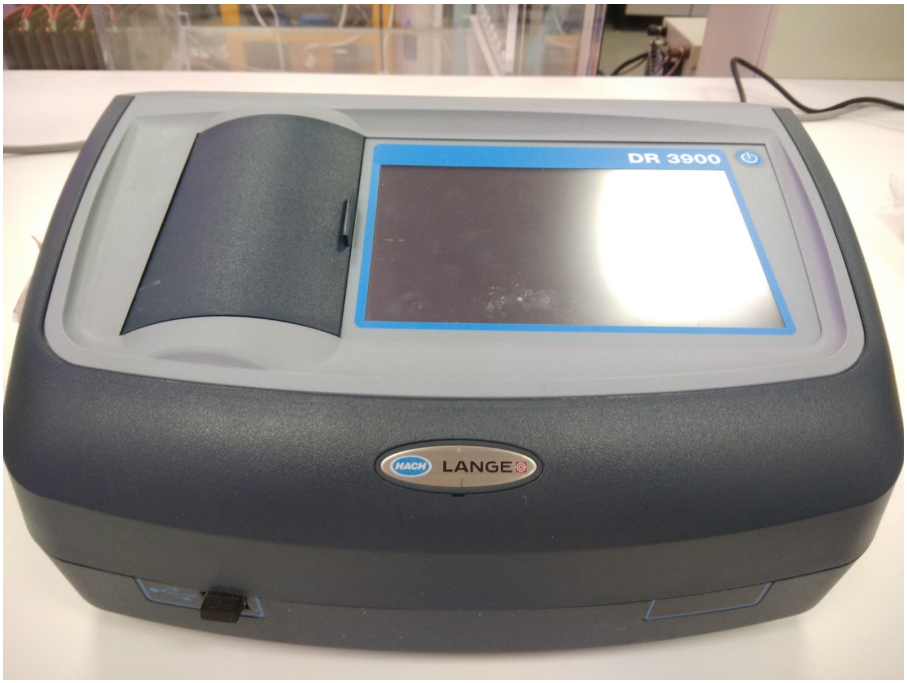


Figure 35: Spectrophotometer

Figure 36: Centrifuge

Appendix E: Raw Data

All the experiments were done in triplicates and control without algae inoculum. The following tables 18-26 depict the raw data whose average were used.

Table 18: PW1 sample 1, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.41**	**94.8**	**47.7**	**55.6**	**1,520**	**2.57**	**2.1**	**17.09**
1	7.02	94.6	46.9	54.5	1,310	9.65	2.1	17.95
2	7.05	93.2	46.7	53.5	1,040	15.4	2.2	14.95
3	7.22	89.3	44.5	51	1,210	14.4	2.1	14.42
5	7.41	87.3	43.8	49.2	972	15.1	2.04	13.34
7	7.52	75.6	38.6	43.1	839	15.5	1.9	13.7

Table 19: PW1 sample 2, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.41**	**94.8**	**47.7**	**55.6**	**1,520**	**2.57**	**2.19**	**17.1**
1	7.05	90.6	45.4	53.1	1,390	11.2	2.2	15.81
2	7.06	87.4	43.9	50	1,180	13.1	2.19	13.51
3	7.05	92.3	46.1	53.2	1,090	16.4	2.19	14.68
5	7.5	87.1	43.5	49.6	1,060	17.3	2.01	14.41
7	7.38	78.7	38.6	42.8	970	19.8	2.17	13.97

Table 20: PW1 sample 3, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.41**	**94.8**	**47.7**	**55.6**	**1,520**	**2.57**	**2.146**	**17.09**
1	7	89.5	44.7	52.7	1,220	11.35	2.12	15.50
2	7.03	87.9	43.9	50.5	1,197	15.3	2.07	14.91
3	7.17	87.1	43.1	50.2	1,090	16.2	2.26	14.81
5	7.36	85.7	42.5	48.5	996	17.3	2.07	15.11
7	7.42	77.3	36.7	43.5	987	18.1	2.20	15.15

Table 21: PW2 sample 1, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.4**	**104**	**52.2**	**61.4**	**1,540**	**4.35**	**2.75**	**21.23**
1	6.74	100.3	50.4	59.9	1,100	8.85	2.22	18.01
2	6.95	100.1	50.3	58.3	1,060	17.7	2.38	16.85
3	7	94.3	47.4	55.1	970	18.6	2.56	17.16
5	7.15	92.9	46.7	53.7	945	20	2.55	16.37
7	7.56	88.2	44.5	50.7	885	21.8	2.22	15.39

Table 22: PW2 sample 2, Chloride concentration at 2500 dilution

B2	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.4**	**104**	**52.2**	**61.4**	**1,540**	**4.35**	**2.53**	**21.23**
1	6.96	100.7	50.5	59.4	1,170	7.9	2.46	18.12
2	6.98	97.5	48.4	57.5	1,080	11.76	2.22	15.58
3	6.99	95.1	47.5	55.1	956	13.9	2.45	17.58
5	7.13	94.3	47.1	54.5	941	16.5	2.47	16.07
7	7.46	87	42.8	49.1	856	19.9	2.39	16.01

Table 23:PW2 sample 3, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.4**	**104**	**52.2**	**61.4**	**1,540**	**4.35**	**2.49**	**21.22**
1	6.9	100.2	49.1	59.4	1,280	9.15	2.41	18.57
2	6.98	97.4	48.7	56.3	1,080	13.1	2.51	17.1
3	7	95.2	47.8	55.3	1,007	15.7	2.48	17.03
5	7.11	93.5	46.6	53.5	923	16.3	2.4	16.91
7	7.46	86.4	43	49.3	827	19.4	2.29	16.42

Table 24: PW3 sample 3, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.39**	**103.5**	**51.7**	**60.3**	**1,080**	**3.65**	**1.287**	**18.9**
1	6.76	100.1	50.1	57.8	1,010	10.6	1.24	17.53
2	6.95	95.6	47.6	55	976	13.2	1.29	15.9
3	6.85	94.6	47.5	54.8	947	14.7	1.54	15.96
5	6.99	92.2	46.6	53.1	940	16.8	1.5	17.75
7	7	91.5	46	52.5	829	18.7	0.97	14.86

Table 25: PW sample 2, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.39**	**103.5**	**51.7**	**60.3**	**1,080**	**3.65**	**1.301**	**18.991**
1	6.8	99.3	49.7	57.5	1,130	10.6	1.208	19.5385
2	6.84	96.1	48.2	55.9	974	13.3	1.663	16.0001
3	6.88	94.6	47.4	54.5	946	15.3	1.414	16.3798
5	6.89	91.3	45.8	52.8	940	18.7	0.966	19.72
7	6.96	90	45.1	52	931	18.8	0.989	15.478

Table 26: PW3 sample 3, Chloride concentration at 2500 dilution

Days	pH	EC (mS/cm)	TDS (g/l)	Salinity (ppt)	COD (mg/l)	Alkalinity (m.mol/l Ks4,2)	OD at 620 nm	Chloride (mg/L)
0	**6.39**	**103.5**	**51.7**	**60.3**	**1,080**	**3.65**	**1.33**	**18.99**
1	6.88	96.7	48.6	56.4	988	10.8	1.19	19.72
2	6.89	94.1	47.1	54.6	974	13.9	1.8	15.53
3	6.78	94	46.9	54.4	948	15.2	1.92	15.67
5	6.97	90.7	45.1	51.8	885	16.7	1.35	19.29
7	6.98	91.4	45.7	52.7	737	19.7	0.72	15.94

Appendix F: Growth Curve of Algae in the Three PW Samples

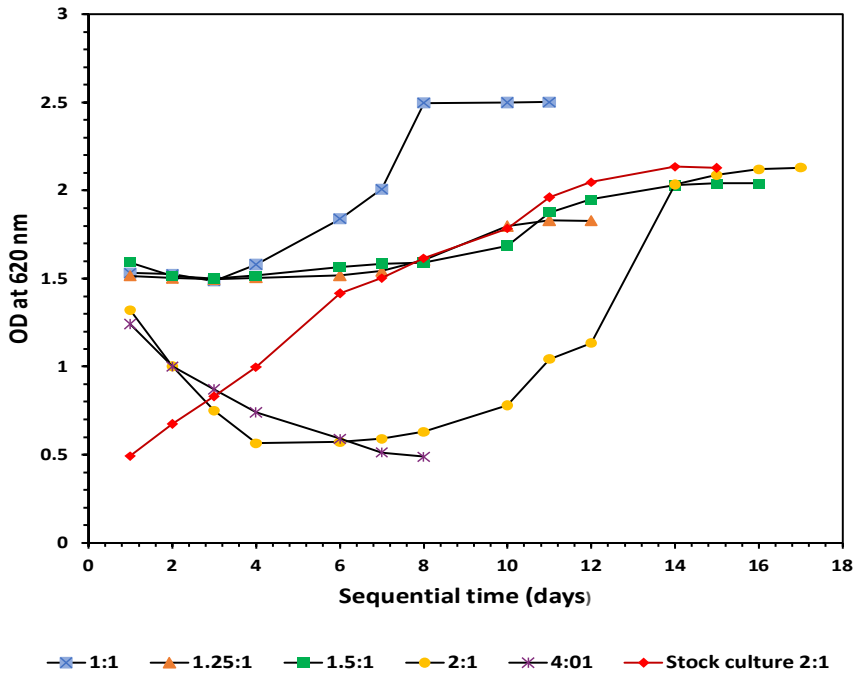


Figure 37: The growth curve of algae in different ratios of PW1, Stock culture implies the final preadapted ratio for treatment.

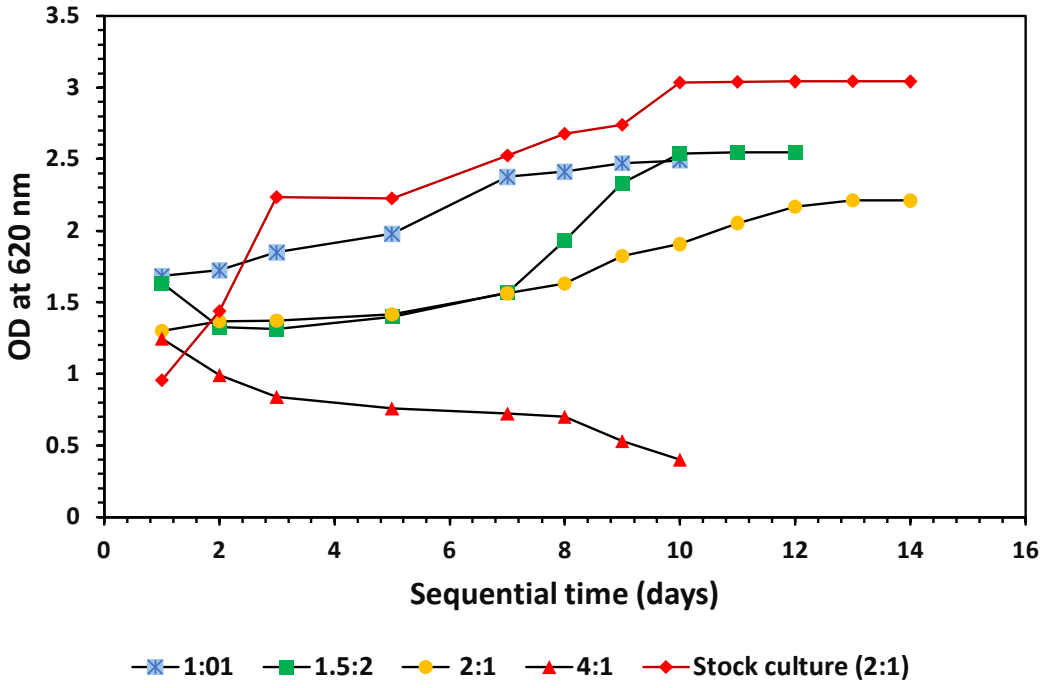


Figure 38: The growth curve of algae in different ratios of PW2, Stock culture implies the final preadapted ratio for treatment.

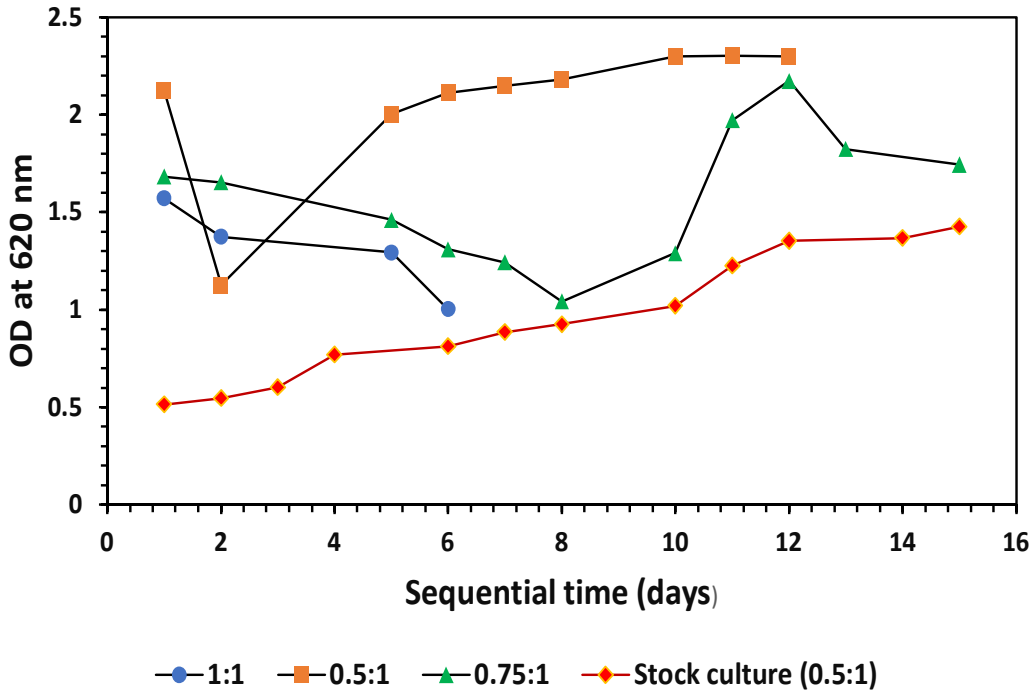


Figure 39: The growth curve of algae in different ratios of PW3, Stock culture implies the final preadapted ratio for treatment.

UAEU

جامعة الإمارات العربية المتحدة
United Arab Emirates University

UAE UNIVERSITY MASTER THESIS NO. 2022:3

Indigenous microalgae consortia present in domestic wastewater, were acclimatized to three real PW samples collected from different oil fields. Progressive adaptation mechanism was applied for acclimatization in a steadily decreasing dilutions of PW. After acclimatization, algae consortia were used for treatment of PW. Treatment was done in batch reactors for seven days. Treatment efficiency was examined. Maximum COD and TOC removal of 44% and 25% were achieved, respectively. An average reduction of 15% of EC, 16% TDS, 18% salinity, 20% chloride ion concentration, and an increase of 82% alkalinity was observed in the three produced water samples over a 7-day treatment period.

Shibin Nadersha received her Master of Science in Water Resources from the Department of Civil and Environmental Engineering, College of Engineering at UAE University, UAE. She received her Bachelor degree in Biotechnology Engineering from Sahrdaya College of Engineering and Technology, Calicut University, India.

www.uaeu.ac.ae