T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF ENVIRONMENTAL ENGINEERING

BIOETHANOL PRODUCTION FROM *GLOEOCYSTIS VESICULOSA* IN SYNTHETIC WASTEWATER

M.Sc. THESIS

PREPARED BY: Shaymaa Ghaeb AL-BADRI
SUPERVISOR: Assist. Prof. Dr. Melih ONAY

VAN-2021

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**BIOETHANOL PRODUCTION FROM *GLOEOCYSTIS VESICULOSA* IN SYNTHETIC WASTEWATER**" presented by Shaymaa Ghaeb AL-BADRI under supervision of Assist. Prof. Dr. Melih ONAY in the Department of Environmental Engineering has been accepted as a M. Sc. thesis according to Legislations of Graduate Higher Education on 22 / 06 / 2021 with unanimity of votes members of jury.

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on ..……../.........../…..…... with decision number ....

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THESIS STATEMENT

All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition, all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

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Shaymaa Ghaeb AL-BADRI

ABSTRACT

BIOETHANOL PRODUCTION FROM *Gloeocystis Vesiculosa* IN SYNTHETIC WASTEWATER

AL-BADRI, Shaymaa Ghaeb
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Microalgae are used in environmental engineering in many areas from wastewater treatment to bio-fuel generation. In this thesis work, *Gloeocystis vesiculosa* 901 was grown and cultured in synthetic municipal wastewater. *Gloeocystis vesiculosa* 901 was grown in various concentrations of glucose, ammonium, sulfate, sodium and calcium, which are the major components of wastewater, and their theoretical estimation was made with the response-surface methodology (RSM) and the carbohydrate percentage was calculated. The highest biomass concentration was 0.63 g/L at 90 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 35 mg/L of NaCI and 5 mg/L of $CaCI_2$. The maximum carbohydrate concentration was experimentally obtained at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI and 5 mg/L of $CaCI_2$ with 251 mg/L. The highest carbohydrate percentage was 44% at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI and 5 mg/L of $CaCI_2$. In addition, maximum theoretical carbohydrate percentage calculated via RSM was 43.3% at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI and 5 mg/L of $CaCI_2$. The carbohydrate obtained from these components was used in the production of bioethanol. Carbohydrate concentration was approximately 4.36 g/L for 10 g/L of biomass and bio-ethanol content was 2.12 g/L. In conclusion, *Gloeocystis vesiculosa* can be used for bio-ethanol production as large scale in synthetic municipal wastewater in the future.

Keywords: Gloeocystis vesiculosa, Bio-ethanol, Wastewater, RSM, Carbohydrate content

ÖZET

SENTETİK ATIKSUDA *Gloeocystis Vesiculosa*'DAN BİYOETANOL ÜRETİMİ

AL-BADRI, Shaymaa Ghaeb
Yuksek Lisans Tezi, Çevre Mühendisliği Anabilim Dalı
Tez Danışmanı: Dr. Öğr. Üyesi Melih ONAY
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Mikroalgler çevre mühendisliğinde atıksuyun arıtılmasından biyo-yakıt oluşumuna kadar birçok alanda kullanılmaktadır. Bu tez çalışmasında, *Gloeocystis vesiculosa* 901 sentetik belediye atık suyunda büyütüldü ve kültürlendi. Atıksuyu oluşturan büyük bileşenler olan glukoz, amonyum, sülfat, sodyum ve kalsiyumun çeşitli konsantrasyonları içerisinde *Gloeocystis vesiculosa* 901 büyütüldü ve bunların cevap- yüzey yöntemi (CYY) ile teorik olarak tahminleri yapılarak karbonhidrat yüzdesi hesaplandı. *Gloeocystis vesiculosa* 901'in en yüksek biyo-kütlesi 0,63 g/L olarak 90 mg/L glikoz, 100 mg/L NH_4^+, 35mg/L SO_4^{-2}, 35 mg/L NaCI ve 5 mg/L $CaCI_2$ içerisinde hesaplandı. Deneysel olarak en yüksek karbonhidrat içeriği 251 mg/L ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI ve 5 mg/L $CaCI_2$ içerisinde bulundu. Deneysel olarak en yüksek karbonhidrat yüzdesi %44 ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI and 5 mg/L $CaCI_2$ olarak gerçekleşti. Buna ek olarak, cevap yüzey metodu kullanılarak teorik olarak hesaplanan en yüksek karbonhidrat yüzdesi %43,3 ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI and 5 mg/L $CaCI_2$ oldu. Buradan elde edilen karbonhidrat biyo-etanol üretimi için kullanıldı. 10 g/L biyo-kütle içerisindeki karbonhidrat konsantrasyonu yaklaşık olarak 4.36 g/L ve biyo-etanol içeriği de 2.12 g/L olarak hesaplandı. Bu sonuçlara göre *Gloeocystis vesiculosa* gelecekte daha büyük sistemlerde sentetik atıksu içerisinde biyo-etanol üretimi için kullanılabilir.

Anahtar kelimeler: Gloeocystis vesiculosa, Biyo-etanol, Atıksu, CYY, Karbonhidrat içeriği Karbonhidrat içeriği

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This thesis is dedicated to the memory of my late martyr father. Your memories are forever alive with me.

2021

Shaymaa Ghaeb AL-BADRI

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SYMBOLS AND ABBREVIATIONS

Some Symbols and Abbreviations used in this study are presented below, along with Descriptions.

Symbols	Description
cm	Centimeter
mm	Millimeters
°C	Degree Celsius
kg/m^3	Kilogram/cubic meter
S	Standard
Lt	Liter
Kg	kilo gram
GW	Giga Watt
g	Gram
m	meter
mg	Milligram
mL	Milliliter
nm	Nano Meter
GF/C	Glass Micro Fiber Filter
$\mu mol\ m^{-2}s^{-1}$	Micro Mole Per Second and Square Meter
min	Minute
rpm	Revolutions Per Minute

Abbreviations	Description
M	Molarity
P	Phosphorus
N	Nitrogen
NO_3	Nitrate
$NaNO_3$	Sodium Nitrate
HCl	Hydrochloric Acid

Abbreviations	Description
NH_4^+	Ammonium
$S\ O_4^{-2}$	Sulfate
NaCI	Sodium Chloride
$CaCI_2$	Calcium Chloride
CO_2	Carbon Dioxide
$C_{12}H_{22}O_{11}$	Sucrose
$C_6H_{12}O_6$	Glucose
$2C_2H_5OH$	Ethanol
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
HRT	Hydraulic Retention Time
FPBR	Flat Photo-bioreactor
PMMA	Polymethyl Methacrylate
CCALA	Culture Collection of Autotrophic
NAD^+	Nictotinamide Adenine Dinucleotide
RSM	Response Surface Methodology
CCD	Central Composite Design
MSE	Mean Square
RSP	Response Surface Plots
CP	Contour Plots
ANOVA	Analysis of Variance

1. INTRODUCTION

1.1. Energy

We can recognize energy as a feature of a matter that could be transformed into one pattern of the following: radiation, work, or heat as the power that produces from work (Mort, 1996). Energy could be present chemically in two states, potential and kinetic, and can be stocked and transformed to the thermal, electrical. The most vastly used kinds of energy are oil, coal, and natural gases; also, they could be the sources of non-renewable energy (Azad et al., 2015). Oil and its subalterns have the biggest impact on the economy of the world. Their benefactions are around 88% of the energy (Tabatabaei et al., 2011). It is crucial and always very important for development, and this is an essential addition to the economic needs of today's civilization. The primary sources of energy were the wind, sun, water, biomass, renewable energy, and the non-renewable fuel of the fossils (Godfrey et al.,2003). In recent decades, the population has been rapidly incremented so has the necessity of energy for the globe. Currently, fossil fuels like oil, coal and natural gases provide most of the global requirements for energy (Silva et al., 2018). In many territories of life, energy is a fundamental element of daily habits. The essential purpose of the whole country would be dodging environmental problems. A huge percentage of the environmental problems around the globe is the variation of the climate and the pollution of the environment. The extracted fuel from the used oil by us could originate harmful damage to our environment. We want to raise renewable energy employment to replace fossil fuels (Amin, 2009). The chemicals raised the greenhouse gases were emitted into our atmosphere due to the fuel inflammation. The greenhouse gases are prohibiting the balance of the atmosphere, which leads to the warming of the globe (Watanabe and Hall, 1996; Benneman, 1997; Amin, 2009). The abundance of production and raising the requirement of transportation have been incremented the request for fuel. The global consumption of oil is predicted to increase by 40% in 2025 (Ghasemi et al., 2012). The extracted fuels from Fossils have been the source of energy and the emission of greenhouse gases. The investigations for substitute, renewable, economical energy resources are going on

(Nigam and Singh, 2011). As the beginning of the industrial revolution, energy has been turned into a key agent to enduring the economic evolution and high living criteria for peoples. In the future, in 2030, the necessity for extra energy will be rising to 50% comparing to the present day, India and China will be considered for 45% (IEA, 2007; Shahid and Jamal, 2011). Transportation has been exposed to a steady growth according to the incremented number of auto-mobiles. The use of energy for transportation has been predicted to be raised by a rate of 1.8% annually between 2005 and 2035. The sector of transportation is the 2nd biggest sector of consuming energy, following the fabrication sector and takes around 30% of the generated energy, 80% has been considered for the highway transportation (EIA, 2010). The key element of the transportation fuel market around the globe is the evolution of bio-fuels that could be easily substituted by current auto-mobiles (Çelikten et al., 2010). However, biofuels are presently considered for a tiny amount of 1.9% of the consumption fuel for transportation around the globe, and tendencies have introduced to us a triple increment along with the upcoming 20 years. For the industries, the water, wind, biomass, solar and geothermal heat could be the renewable sources if the biomass could have relied on the production of fuel and the chemical industries as a substitute resource (Lynd and Wang, 2003). The fuels extracted from petrol will be substituted by biomass sources like biodiesel, bioethanol, etc., extracted from sugarcane, algae, corn, etc. Biogas has also been reported as a potential motor fuel for organic farms in the short and medium term. The anaerobic digestion of organic matter forms biogas. When used as a biofuel, CO_2 is separated from the gas to increase the energy content, and the gaseous fuel can be stored at high pressure. A potential extract of around 1281 megawatts of biogas made from agro-waste in India (Dhussa, 2004). The yearly manufacturing of methane from organic trash in Sweden is roughly 38 PJ, considered for 11% of the domestic energy demands for transportation in 2007. It is predicted to be suitable to engage the EU 2020 (Lime et al., 2008). Countries worldwide have directed their policies towards the use of biomass to fulfill their energy requirements to decrease carbon dioxide as in the Kyoto Protocol and decrease the reliance on fossil fuels. Although biomass can be an enormous source of transportation fuels, such as bioethanol, biomass is commonly used to generate both power and heat, usually through combustion. Currently, ethanol is vastly utilized liquid as a biofuel for vehicles (Lewis, 1996). According to a diversity of factors, like the warming of the globe and climate variation, the amount of ethanol has been increased. According to the exhaustion of crude oil alimentations, many states worldwide are shifting their sights on renewable resources for output power. There is a requirement for ethanol as a substance in the transport fuel market, replacing gasoline. Even though the bioethanol producing costs are more than the fossil fuel. The global output of bioethanol has been 31 billion liters during 2001 (Berg, 2001). In 2006, the output raised to 39 billion liters, and it is evaluated to pass more than 100 billion liters in 2015 (Taherzadeh and Karimi, 2007). The biggest ethanol manufacturers states are Brazil and the USA, with 62% of the wide world's sources of ethanol (Kim and Dale,2004).

1.2. The Energy Types

There are two kinds of energy, major and minor (Figure1.1). the major energy has been produced or given straight from the atmosphere. However, the electricity or the fuel would be shifted from minor energy to major energy. The major energy could be produced and stored from the atmosphere. Minor energy in the shape of electrical or fuels, major energy is replaced into minor energy, like gasoline, methanol, fuel oil, hydrogen, and ethanol. Mostly, the major energy is the same as the renewable energy resources, like the wind, solar, geothermal, biomass and water current from the electricity or the thermal energy. Fuel and Electrical energy, considered as beneficial energy, is permanent energy. mechanical, thermal, Electrical, and chemical energy are the four types of eventually selected energy (Øvergaard, 2008).

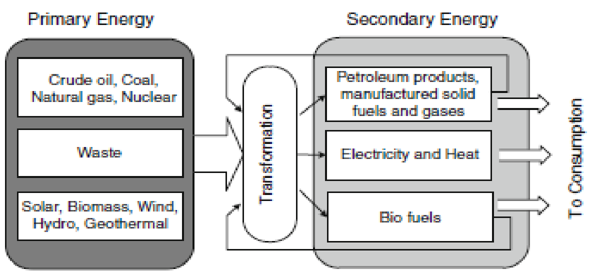

Figure 1.1. The major and minor sources of energy (Øvergaard, 2008).

1.3. The Recourses of Energy

1.3.1. The non-renewable sources

Non-renewable sources have been used by converting them to various sources of energy to contact the necessities. Coal is generally the most utilized fuel of fossil. The common sources are the natural gases since it produces lower pollution comparing with coal. Nuclear energy has a massive metal that could be utilized for electricity-producing (EIA, 2011).

1.3.2. The renewables sources

Renewable energy is gained from the sun or the ground heat produced in the bottoms of the earth. Naturally renewed energy like photochemical, thermal, and photoelectric, the stored wind as biomass, photosynthetic and hydropower energy, or various natural motions and environmental techniques (like tidal and geothermal energy). The consumption of renewable energy sources was nearly 19% in 2008, 13% from traditional biomass, which is majorly utilized for heating, and 3.2% gained from hydropower. Supplementary other renewables nearly 2.7% of a little hydropower, wind power, biomass, solar power, geothermal energy, and biofuels shared about 15% of worldwide electricity derived from the power hydroelectric and 3% from the contemporary sources of renewable energy, the portion of energy that would be renewable in electricity-generating nearly 18%. Costs and state funding boxes participate in raising renewable energy use (EIA,2011). From 2004-2009, renewable energy capacity has been increased with an average from10% to 60% yearly, affecting job and business opportunities available. In four regions: power output, heating of the space, fuel for transportation, and country services of energy, renewable energy substitutes the traditional fuels (Bolinger and Wiser, 2005). Renewable energy has the following benefits:

1-Provides the most efficient substitute to utilizing the energy out of a limited resource.

2-Lower the reliance on the energy that has been imported and the various blend of energy providers by offering local resources for use are available, also adding stability to energy.

3-The deficiency for emission of the carbon-based energy and pollution factors in the environment has been presented in the renewable energy shape.
4- So that (RE) has min. To zero emissions of greenhouse gases, so they would mitigate the temperature variety caused by people.
5-Helping to decrease the damaging effects of airborne pollution.
6-The renewables in developed states have been supported the growth for new planning, managing, manufacturing, and services districts and repairing projects. According to the major reasons, the shifting for the plain sources which rely on geographic positions, the renewable power has no adjustable to any individual community. Utilizing all the renewable technology needs sustainable research that concentrates on the key elements: environmental hazards, outsource costs, and financial. Any of these elements have an important influence on using renewable energy sources (Mann, 2011). A schematic of the renewable energy sources (Figure 1.2).

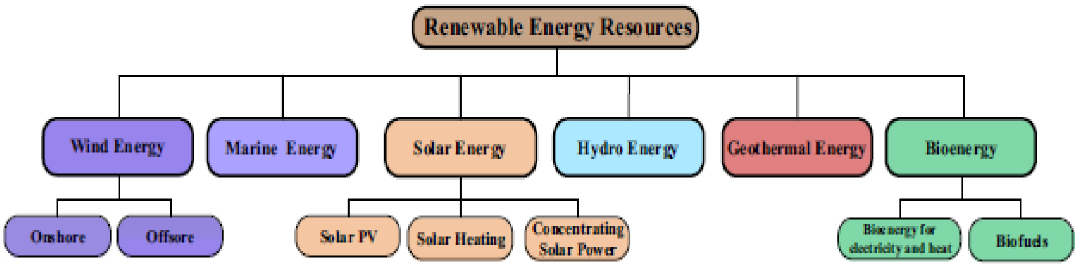

Figure 1.2. Types of Renewable sources of energy (Ellabban et al., 2014).

1.3.2.1. The hydro energy

A neat and durable source of electricity is called hydropower. Most states give prime concern to their growth, economics, technology, and environmental merits of using hydropower. With a full virtual capacity of hydropower with 694GW, China has prosperous hydropower resources globally. It is substantial to reduce the lack of energy and the polluted environment causing by economically developed countries like china in the present century (Huang and Yan, 2009). Hydropower has many merits over the electrical power generated from the other resources. A demonstrated technology, a high implementation, low prices for repair and service, and the potential to fast respond to variations in load give high reliability. Generally, a few hydropower plants were constructed with reservoirs that provide the city raw water, flood prevention, and recreational merits. In addition, the hydropower has no waste, the acidity raindrops with the greenhouse emissions. The obstacles of hydro-energy contain big primary expenses for the facility; the uncontrollable amount of water; a variety of in-stream system impact on fishes, wildlife and plants; over flooding the territory and the wildlife native land and shifting the people who they are living in the reservoir boundary (Yuan et al., 2013).

1.3.2.2. Wind energy

The motion energy of the wind has been converted by a turbine of the wind to the mechanical energy that has been utilized to generate the electricity. It could be generated in many territories, like an agricultural area or a forest, and also the wind is a clean, renewable resource that could originate noise and pollution. It could disturb the residents who were living close to it (Kaygusuz, 2009). Wind energy has many merits, like decreasing greenhouse gas emissions by turbines that operate by wind and producing electricity and energy could lower the price of electricity. Wind is a plentiful, cheap, and renewable source which gives us a sustainable source. The motive to idealize the merits of this source it would be a safer, healthier environment for the globe. Constructing turbines can be considered a profitable investment; technology development and growth could give back a highly potential refund. Recently, the mechanics are attempting to upgrade the power variables to save energy for other times if there is no wind. Wind power is the perfect choice to decrease the draining of fuels and oils and perform in sync with nature to empower social modifying (Bull, 2001). Suppose a huge reservoir of energy or the possible transmission between continents, the mysterious and volatile presence of wind energy could constrain any district's benefaction. Wind turbines are not deep-rooted or amazing because they could produce noise, fuzzy radio, signal interference, and kill migrated birds (Fetter, 2000).

1.3.2.3. Solar energy

Sunlight is a significant power source globally, while it has been used for different purposes, like water heating or the homes' warming in winter. It could be used for power generating, straight by panels of photovoltaic and converting the sunlight to electricity or generating the steam by specific stations that should be considered while utilizing the solar (Byrne et al., 2010). The photovoltaic (PV) panel could be more expensive than the fuels of burning fossils, just 0.04% of the fundamental electricity used by people obtained straight from solar sources. Concentrated researches have been made to study the organic materials for PV implementations not only because the power of the sun has been gained beneficially, but since it does cost lower to generate] the power from materials of organic photovoltaic (OPV) than the rest of PV technologies (Moulé, 2010). Solar energy is non- contaminated, does not produce greenhouse gas, like oil-based energy, or does not leave any waste, like nuclear energy. The solar panels do not have any dynamic portions and required a little maintenance after the usual cleaning. It would be memorized as the only access with the capacity to meet the current requirement of the solar photovoltaic panels (Pogson et al., 2013). The solar panels became almost forbidden according to the expenses, considering the development in technologies. The machines requiring the energy to be stocked for the utilizing could be very costly; in addition, the panel's expense is overpriced.

1.3.2.4. Geothermal energy

The heat of the ground is a great resource of energy, specifically in the vital tectonic territories. It could generate electricity, air conditioning for the buildings in the winter and summer seasons, like the other sources of renewable energy, a neat source. Still, it might cause to generate acid rain in many compounds (Yan et al., 2010). It does provide a stable and trustable source for renewable energy that has no mentioned impacts on its perimeter (Mohtasham, 2015). There are various applications on geothermal energy or produced from the heat spreading out of the core of the globe. Those applications could be split into three divisions: for warming (straight use) devices, producing the power, and pumping the geothermal heat. The geothermal power plant does not have to use electricity, so we consider it renewable and environment friendly. The activities of geothermal energy produce little emissions.

The renewable techniques would lead to the incremented requirement for electricity and permit the advanced technology without hurting the future of the globe, the atmosphere, and the climate. Turkey, for example, owns very wealthy geothermal energy providers. The Resources have graded in the fifth place in globe following

China, Japan, America then Iceland. For promotion and funding the sustainable energy by the authority have been an important push for the geothermal sources (Kılıc and Kılıc, 2013). Accumulation for the geothermal power could be obtained at the plate circumstances; anywhere, volcanoes are clustered with the most frequent earthquakes. Geothermal energy areas are sometimes out of steam for a few months, during which the power plant is unable to produce electricity. The energy obtained from geothermal has been severe to transfer. Geothermal energy factories could supply electricity to only the regions around them. However, steam could be secure and covered; it is possible to produce underground toxic materials like sulfide of hydrogen, ammonia, mercury, then arsenic. Lastly, geothermal power could induce earth tremors. (Mohtasham, 2015).

1.3.2.5. Biomass energy

Biomass includes a variety of substances: biomass, residues of foodstuffs, compost, manure, and plant components which can be reclaimed and used either as fuel for heating or electricity generation. Biomass is an organic element that is produced from plants and living creatures, and micro-organisms. Biomass is a green energy source that operates on the carbon cycle, with some definitions of biomass fuels being trees, seeds, and algae. When the biomass consumes, chemical energy is launched further into biomass as energy. Biodiesel and bioethanol are instances of biomass being produced into some other biofuels.(Milbrandt, 2005). Biomass energy consumption will significantly reduce greenhouse gas emissions, global dependency on oil, industrial waste and potentially help the individual agriculture and forestry sectors. Paper waste, timber debris, and industrial pollution are the main biomass sources of nutrition for power. Production of sustainable energy from crop residues, aspects of seed residues, like wheat straw or maize cookers, and pieces of seed residues, which are not in competition with land-based crop production and sustainably collected timber and woodland remnants. During plant rot, regulated carbon cycling of the ecosystem to the plants then returning to the soils then atmosphere can also be counted as part of the global carbon cycle (Tilman et al., 2009). There are some downsides of it as well, in addition to all the good qualities of biomass oil.

For instance, biomass oil is an insufficient energy source (gasoline vs. ethanol). It may be a perfect opportunity to release greenhouse gases related to the rise and recycling of biomass resources, the transportation of raw material to the power plant, and the burning or gasification of raw materials. For all sources of biomass, emissions from transport and burning are nearly equal. Therefore, it is important to differentiate between those biomass possibilities that help minimize pure carbon releases, which have unknown effects, and raise pure releases. (Spath and Maan, 2004). An additional ecological effect for biomass oil is soil depletion because of loss for verdant plants (Pimentel et al., 1995). Eventually, through land use for the manufacture of resources that are considered sources of biomass energy. Usage of solar energy in line with renewable energy sources (Figure 1.3).

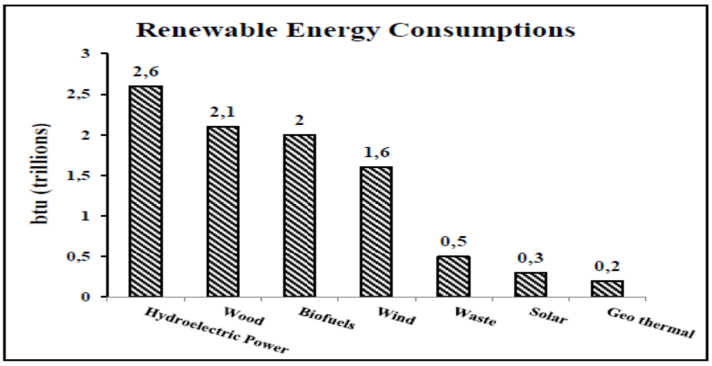

Figure 1.3. Amounts of renewable energy usage in 2013 as trillions of British thermal (EIA, 2013)

1.4. Purpose of Study

In recent decades, the rate of energy use has risen dramatically urbanization and industrialization, which consequently raises the pressure on fossil fuels to fulfill the required energy needs, not only creating environmental inequalities, including climate change but also draining the natural non-renewable energy reserves that take millions of years to refresh. Global passion for green energies, such as bioenergy technologies, is expanding daily to address these environmental problems due to the unsustainable use of fossil fuels. Consequently, natural green energy sources generate a range of biofuels, like bio-diesel, bioethanol, bio-methane, and bio-hydrogen. To satisfy their energy needs, people have studied tools. Microalgae could be used for multiple industrial uses, such as nutritious foods, makeup, chemistry, and the pharmaceutical industry. Many functional and biochemical products like biomass, dietary additives, lipids, polymers, and renewable energy resources can be produced from the massive processing of microalgae. Microalgae get a single cell or basic multicellular form and are procaryotic or multicellular photosynthesizing micro-organisms.

Consequently, they can increase production and survive comfortably under various environments (e.g., seawater, bitter water, and even wastewater). Even so, it poses a downside despite planting costs when staring at the production of micro algal biomass. Nearly 80 kg of nitrogen and 5 kg of phosphorus are required to grow a ton of algae biomass. Using the response surface method (RSM) with required process variables to efficiently develop biomass microalgae and carbohydrate profitability. Increasing microalgae, standardized values of media elements, and developing conditions be calculated.

1.5. Aims and Objectives

This study aims to the determine maximum carbohydrate concentration using RSM in various concentrations of glucose, NH_4^+, SO_4^{-2}, NaCI and $CaCI_2$ for *Gloeocystis vesiculosa* and its bio-ethanol content the association between processing parameters will be studied by the method of response surface and fundamental composite design. One of the mathematical methods is RSM. Microalgae developed in wastewater is a potential source of production of bioethanol. Optimizing fermentation variables such as algal biomass, fermentation period, and yeast volume ratio greatly increases ethanol output. Synchronized optimization of the variables using a statistical tool is a successful way to maximize performance and forecast a model that defines the interaction between these variables and their reaction (RSM).

1.6. The Study's Problem and Significance

As a green feedstock, microalgae offer tremendous potential to generate third-generation biofuels. In sewage treatment, the cultivation of microalgae is theoretically a financially feasible supply for biofuel production. Consequently, mounting biomass could be labeled a vantage to create microalgae biofuel, a trade possibility. Besides that, economic feasibility and environmental protection remain the primary obstacles to the commercial viability of these techniques. Without technical breakthroughs and innovative integration of processes, these challenges are cost-related and can't be answered. Utilizing sewage as a means and incorporating waste water management. The production of bio products based on algae will overcome some of the main challenges described. However, several significant scientific and technological problems must be tackled before large-scale biofuels produced from microalgae could become commercially feasible. In addition, existing sewage treatment systems could be used to operate algae processing facilities, Minimizing the cost of resources and scalability problems. Technological developments, including improvements in the layout of the image bioreactor, the aggregation, drying, and handling of biomass from microalgae, are key areas that will contribute to improved profitability and, thus, the efficient commercial application of the biofuel policy for microalgae. Further investigation into advanced algal biofuel manufacturing techniques based on wastewater is needed to utilize them fully.

2. LITERATURE REVIEW

Reviewing and reading articles with diverse origins are part of this section.

2.1. Renewable Biofuels

Biomass, which has many uses, is one of the renewable suppliers of numerous biofuels, such as bioethanol and biodiesel (Ho et al., 2010). Biofuels are biomass-derived liquid, gas, or solid fuels of different types. They are made of ethanol, hydrogen, biodiesel, methanol then methane. Biofuels are a traditional renewable energy source and are commonly known as main and secondary biofuels (Nigam and Singh, 2011). For electrical refining, cooking, and heating use, such as firewood, wood chips, and other organic substances, main biofuel in its untreated type is widely used. After biomass processing, secondary biofuels are extracted, and the class of secondary biofuels depends on the kind of biomass they are made. Bioethanol is extracted from sugar or starch-containing products to lignocellulosic materials in its components, and bioethanol can be generated from a range of natural products (Nigam and Singh, 2011). In Brazil, a few of the world's largest bio-ethanol suppliers, sugar reed is the primary raw material. The USA depends mostly on maize and sugar beets (Chiaramonti, 2007). There is a well-industrial method of refining ethanol, like beet sugar, cane sugar, and sugar grains, from these first-generation raw products. There are also some of the least complex bio-ethanol processing methods. However, prices can be heavy for raw materials. Another significant problem in using natural products from first-generation biofuels is lowering arable land on land. Competition for food from biofuel crops could contribute to higher food costs, negatively impacting the vulnerable. With the growth in the world's entire population, this issue is becoming more serious and leads to the use of farm land in food and energy states. Other factors, such as the depletion of fertilizers and pesticides in farm fields, crop erosion, Intensive oil crop production, also results from nitrogen loss, reduction of plant biological variation, and degradation of ecosystem resources for greenhouse gas pollution control (Subhadra, 2010). Raw products lignocellulosic biomass, animal starch waste, food, trees, sawdust, sludge, and animal manure are the second generation. They were called inexpensive raw materials. It is necessary to enzymatically decay these materials into fermented sugars and convert them into bioethanol. Some lignocellulosic waste such as crop residues (Kim and Dale, 2004), urban solid waste (Mtui and Nakamura, 2005), waste from forest goods (Kádár et al., 2004), As well as some animal manure research, leaves and garden waste (Lissens et al., 2004). Their ability to decompose lignin from these raw materials, despite these promising raw materials, is hindered by low yields and high costs of biofuel conversion and cannot be fermented because they are not compatible with food stocks. (Harun et al., 2010). Renewables account for 17 percent of Europe's total electricity usage (Camdessus, 2019). If the proportional volumes of hydro, solar, wind, and biofuels were still the same, an extra EUR 35 billion a year would be needed to increase to 51% of renewable electricity for a triple of the biofuel energy demand.

2.1.1. Types of biofuels

Biofuels are classified according to their origins and manufacturing processes into four different generations (Aro, 2016).

1- The first batch of biofuels was generated by sugar with crop oils and need plane treatment to turn them from natural products into the final product. The best instance is refining ethanol from corn. Biodiesel produced from veggie oil with esterification is often a popular first batch of biofuel. They existed for a quiet time then were generated on a trade scale in many states. Basic components utilized in food supplies are important because they add value to disputes and the high cost of production.

2-The second generation of biofuels comes from lignocellulosic agricultural biomass, the plant's total inedible biomass, and the food crop's indigestible residue. Corn waste, maize cob, and wheat straw are all forms of indigestible food leftovers.

3-As from the third generation of biofuels, micro-algae, and microbes are removed. This biofuel version is free from the core limitations of the first and second generations of biofuels, without significant food production and land use (Brennan and Owende, 2010). Raw biofuel products of the third generation produce at least 30 times the energy production of raw materials of the second generation per second-floor component. (Ghasemi et al., 2012).

4-Fourth-generation biofuels wouldn't need biomass degradation for processing. These biofuels provide electric fuels that are liquid or gas fuels that store electricity in their chemical ties and solar fuels that, with the help of solar heat, cause chemical processes. A figure illustrating the biofuel forms is given below. (Figure 2.1)

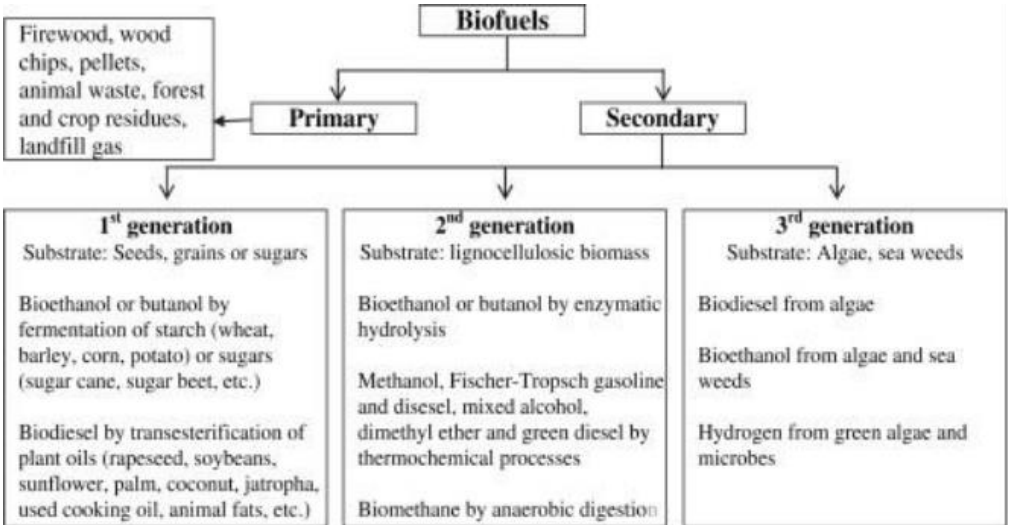

Figure 2.1. Classification of biofuels (Nigam and Singh, 2011).

2.2. Microalgae Biomass Production

2.2.1. Microalgae

Algae are primitive plants that live in diverse habitats which do not have stems, branches, and roots and will not have sterile coverings in their cells. Chlorophyll is being used as optical pigments in which algae absorb Sunlight optical algae, and carbon dioxide and dissolved carbonates are removed from the air. With amounts of 150,000 parts per million of carbon dioxide, they can tolerate and use (Brown et al., 1996). Algae are classified into two parts, macroalgae which microalgae, and are centered on the specimens' size and morphology. Microalgae can also be accused of being microscopic photosynthetic species. All of them were uni-cellular. However, it isn't necessarily valid. Broad algae are bigger and consist of many cells that coordinate to create systems such as greater plant life.

Microalgae are made up of prokaryotic cyanobacteria and eukaryotic algae (Chen et al., 2010). Their simple molecular structure makes them efficient converters of solar energy; they extend photoautotrophic allies and generate about half of the global atmospheric environment by curing carbon dioxide oxygen., It contains approximately half of the global oxygen in the atmosphere. Biologists have broken microalgae into distinct classes, largely based on their tincture, essential cellular structures, and life cycle. These small biological enterprises use Photo-synthesis to harness nature's resources and increase their biomass each couple of hours through their accelerated development era (Chisti, 2007). They can expand speedily and generate biomass that is beneficial forms desirable human utility crops. The fast growth and high duplication rate, and lipid accumulation of up to 50 percent of their biomass are common microalgae properties. The basic method needed to generate all possible bioenergy replacements from photosynthetic micro-organisms is photo-synthesis. It is, nevertheless, a comparatively low-efficiency method in terms of energy transfer compared to the backend synthesis of targeted materials. Around 90% of the photon energy provided to a given photosynthetic footprint can be subsided as heat or fluorescence. Current photosynthetic energy conversion estimates are around 6% of the total light energy of the event (Blankenship et al., 2011; Ort etal, 2011). Maximizing photosynthetic potential is one of the most important and complex hurdles in current efforts to optimize primary performance for bioenergy implementations (Figure 2.2). Any researchers have attempted to make specific use of abundant algae or plants to extract biofuels from the sea or ponds (Scaife et al., 2015). However, it should be recalled that the common use of existing algal biomass (i.e., marine algae) biofuels would destroy the planet's marine ecosystem and change the global atmosphere. However, algae might also be quickly grown and collected in small-scale aquatic industrial systems under optimum conditions due to microalgae' photo-synthesis processes.

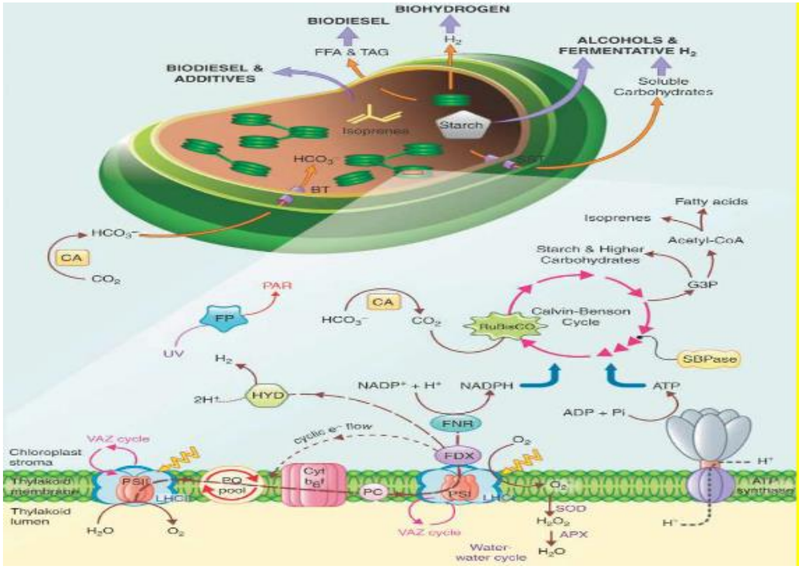

Figure 2.2. Generic chlorophylls green algae, showing the placement and integration into bioenergy production of fuel-relevant main metabolites. The core components of photo-synthesis and carbon stabilization are also depicted, including elements designed to strengthen these pathways (Doan et al., 2011).

2.2.2. The ways of growing microalgae

Microalgae can be cultivated in different ways. Productive and expense-efficient huge planting for microalgae has required the prosperity of microalgae biomass such a sustainable energy nominee. For mass, algal planting, there have been several templates. The cultivation technique might have achieved in sustained, semi-batch, or batch systems. There are two ways in which continuous processes can be carried out, Cultures in turbid conditions or chemo stats. It can be broken down for (a) stranded media like open lakes or sealed reactors, then (b) paralyzed media, like biofilms and combination paralyzed the system. The popular structures used are exterior ponds and closed photo reactors. The features of these two types of structures used for production are substantially different, such as the control of growth variables, there are significantly different pollution, water evaporation effectiveness, final treatment characteristics, and investment costs (Brennan and Owende, 2010). Most microalgae are grown as photoautotrophic and use photo-synthesis from substances found in their atmosphere to produce their nutrition. As a feedstock for the processing of biofuels, this is one of the key advantages of the production of microalgae. It also imposes some limits on the designs to produce modern microalgae processing systems capable of producing biomass (Bux, 2013). For the growth of microalgae, any of the following four systems have been used:

1-Wetlands Detailed.

2-Circular and Raceway Reservoirs.

3-Tubular-shaped Photobioreactors.

4-Fermenters (where algae are grown on organic substrates in the dark) (Borowitzka and Moheimani, 2013).

2.2.2.1. Open lakes

The huge predominant processing systems activity is giant-average algal lakes or raceway lakes. With a paddle wheel that provides algae and nutrient distribution, raceway ponds (102-106 L volumetric capacity range) are accessible and shallow. Raceways are relatively cheap to establish and repair, but they often have low reliability (Chisti, 2007; Mata et al., 2010). Tubular image bioreactors (volumetric capacity range (101–104 L)) which were alone kind of utilized sealed systems in the processing of large-scale algae (Chisti, 2007; Alam et al., 2012). For over 60 years, open raceway lakes have been used, and their operations consist of tremendous knowledge and experience (Tredici and Materassi, 1992). The culture of the raceway lake pond allows sunlight to penetrate successfully is typically less than 30 cm deep (Chisti, 2007) (Figure2.3). While it is typically easy to run, and use open systems as a source of energy for sunshine, they have many drawbacks.

The biggest downside was the shortage of each actual influence along with the observed ecological provisions. The climate is, as an example, not regulated also can mutate from season to season and daily. According to volatility, it could be substantial water depletion according to volatility, the processing of CO_2 and light by media is less efficient than image bioreactor systems. Pollution by vying for algal descents with bacteria is hard to prevent within open systems. In some situations, it is important to utilize extremophilic creatures that could live under provisions that other descents cannot process (e.g., elevated salinity). That puts a major limit on various strains where there are characteristics that need to be observed when selecting algae to grow biofuels in open ponds (Table 2. 1). Usually, open lake productivity is poor due to these limitations. Closed camera bioreactors are costlier to build and maintain, but they have greater power over cultural facilities. These open lakes can be excavated with or without lining and can be used with insulated materials. Unlined lakes are not natural due to algae contamination and soil and environmental requirements (Ghasemi et al., 2012). Open lake systems are commonly used in three types: watercourse pool, oval pool, and angled pool. Rounded lakes are minimum suitable according to the costly concrete building, heavy power usage for tossing, the mechanical complexity of the source of CO_2 and land use incompetence, streams with comparatively low capital and operating costs. There are also downsides to commercial open basin schemes, such as lower productivity, greater harvest prices, lack of evaporative water Decreased success of the use of carbon dioxide (Tan et al., 2018).

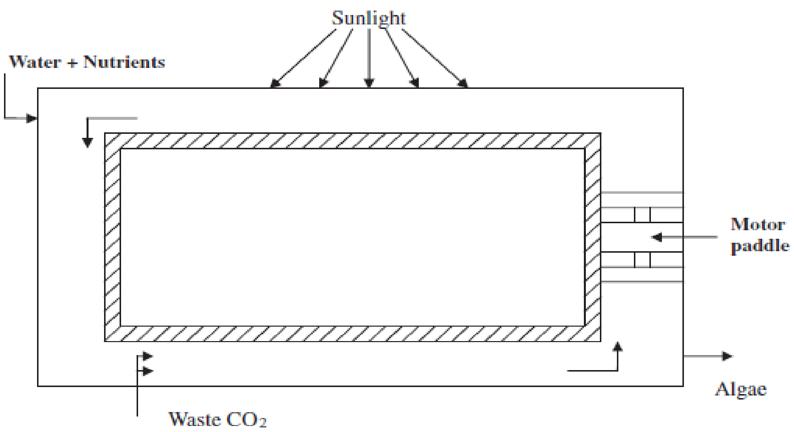

Figure 2.3. Open raceway pond (Demirbas, 2010).

Table 2.1. The suitable characteristics of algae harvesting to open farm ponds for biofuels are manufactured (Osundeko, 2014)

Advantages	Characteristic
Competitive benefits for rival organisms, as well as a reduction in the amount of culture space available	Rapid growth rate
Higher biofuel productivity the higher value of biomass.	Maximum biomass concentration Item quality is high
Exploitation and pollution are reduced (Confirm: Only a small number of organisms can thrive in harsh environments, which can be hard to sustain)	Extreme environments foster development
Reduce Expenses in growing and reservoir production	Auto flocculation
Cultural environments may be regulated more loosely. Seasonal and atmospheric weather patterns affect the development	Wide tolerance of environmental conditions
Bigger CO_2 privatization and residue CO_2 use capability	CO_2 tolerance and uptake
Enables for the use of less expensive pumping and blending systems	Tolerance of shear force
Capability for development of contaminated water and exhaust gas with elevated CO_2, NOx, and SOx levels	Tolerance of contaminants
decreases auto inhibition of development with high biomass densities	No excretion of auto inhibitors

2.2.2.2. Photo bioreactors

Due to the restrictions imposed on open lake systems, shielded light reactors have been installed for algae production in large quantities. There are two major kinds of optical closed reactors sheet and tubular. These were several benefits to a closed structure, such as narrow light paths, wide lighting space, and a comparatively controllable setting; all make optical reactor densities greater than open lake systems with fewer emission problems (Chisti, 2007). Even so, because of the greater price of cell mass production, there are some limited intakes, pH gradients, soluble oxygen and CO_2 along the tubes, shell growth, fouling, hydrodynamic stress, and high expansions to expand their length. (Borowitzka, 2008). Even so, the overall cost of optical reactors remains far higher than that of open lake systems (Brennan and Owende, 2010). Hybrid systems which mix optical reactors with open lake systems have traditionally been investigated. Algae strains are first developed to a density so high in hybrid systems that stress toxicity is not a concern in closed optical reactors, then moved to open lakes Where they are cultivated into the final product (Brennan and Owende, 2010). The system used for the photo reactor could be minor classification like a) longitudinal image reactor, b) a lateral or flat photo reactor, then c) a spiral photo reactor. The helical image reactor is considered the fastest production to scale up. Tubular image bioreactors can provide improved pH, nutrient dosing, and temperature regulation compared to open lakes, better defense against contamination from culture, better mixing, evaluated by comparing to open reservoirs, less evaporative leakage, and higher cell concentrations (Mata et al., 2010). Each process, however, has comparable merits and demerits. Among the biggest problems of rectangular utilizing and cylinders image bioreactors is biomass restoration. Similarly, because of their low building expenses and simplicity of handling, open lake systems are probably selection methods for volume production for microalgae in different designs and variants; as seen in the figure below, photo bioreactors for algal growth have been developed (Figure2.4).

Figure 2.4. Cultivation Systems for Microalgae (Zhou et al., 2014)

Figure 2.4. Cultivation Systems for Microalgae (Zhou et al., 2014)(Continue).

2.2.3. Harvesting microalgae

After the algae farms expand, collect the biomass. That is challenging work since algae are always poor in basic density, and removing and extracting them from solvent fluids is difficult and very expensive (Chen et al., 2010). In many aspects, it can be collected: physical, chemical, and mechanical. The efficiency of the carbohydrates of the preferential strain must be high due to generate a significant quantity of biomass with high sugar microalgae to generate bio-ethanol at a low price. Several studies have shown that the sharp increase in fat and carbohydrate production in nitrogen-deficient ecosystems has started developing (Ho et al., 2012; Dragone et al., 2011; Yeh and Chang, 2011). Under nitrogen deficiency conditions, proteins and peptides contained within microalgae cells could be converted into fats or carbohydrates (Ho et al., 2013), however when under strain, the carbon stream within microalgae is directed to high power substances, such as fats and carbohydrates, and there is a competition between the two configurations Both differ in aggregation from strain to strain underneath stress requirements (Ho et al., 2012). Micro-algae are the biggest abundant species on the globe. Those were steadily expanding and can withstand a wide range of conditions varying from freshwater, semi-saline water, seawater, high temperatures, and pH requirements. Because of these properties, microalgae and many other characteristics are the key contenders as a biofuel feedstock. Many microalgae strains can store enormous quantities of carbohydrates, rendering that suitable for the processing of bioethanol. *Chlorella, Chlamydomonas, Dunaliella, Spirulina, and Scenedesmus* were many instances of descents able to store over 50 percent of their dry mass in starch and glycogen (Chen et al., 2010). Cellulose, which could be converted into ethanol, is a portion of the micro-algae content. The processing of ethanol from microalgae has more advantages than from big algae. The first gain is that microalgae, owing to their lower growth rates, have greater sugar yields. Second, the microalgae-produced carbohydrates are marinated more readily. Monosaccharides, which are hard to ferment, are mostly alginate and mannitol (Wargacki et al., 2012).

2.2.4. Energy conversion of microalgae to bioenergy

The operating principle of the microalgae conversion of energy is that biomass materials are transformed into biofuel energy by chemical or biological processes. Thermochemical transfer and biochemical transformation are commonly used as two primary energy transitions in laboratory science and a few industrial productions. Different techniques can produce various forms of bioenergy from microalgae in the operations of energy conversion. Understanding the most successful technical method of micro-algae conversion. It is important to observe the energy transfer. Gasification, desalination, pyrolysis, fermentation, and transesterification are five widely used procedures. Biogas, bio-oil, biodiesel, and bioethanol from microalgae can be developed using these strategies. (Figure 2.5).

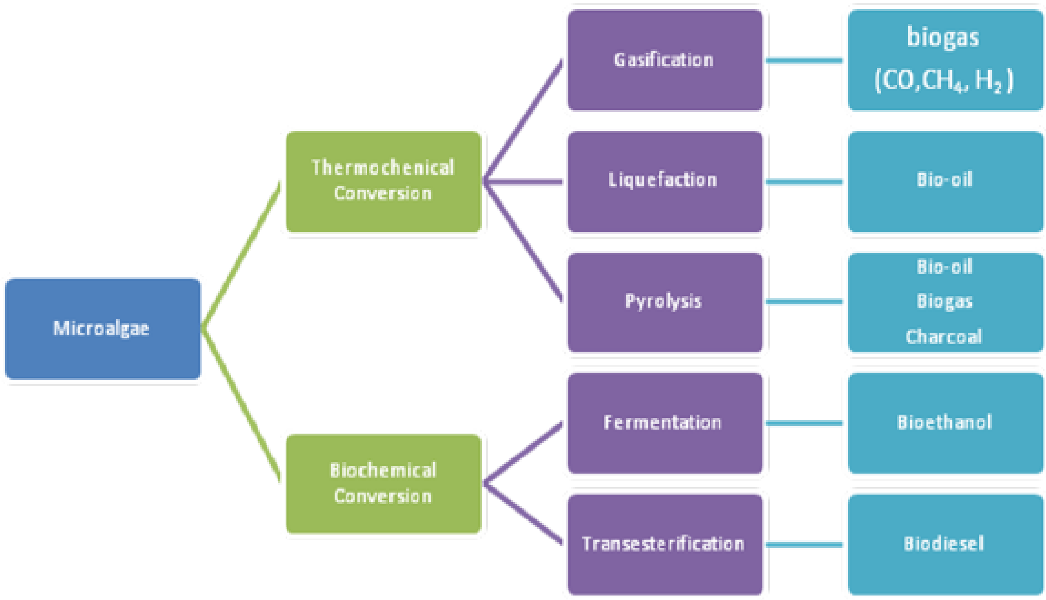

Figure 2.5. Energy conversion processes for microalgae-based biofuel processing (Wang, 2013).

2.2.4.1. Conversion of thermochemical

Thermochemical transformation is a method that refers to the need for chemical processes to turn biomass materials into biofuel under heating conditions. It requires gasification, pyrolysis, and desalination.

2.2.4.2. Biochemical conversion

A biochemical method is used to operate the gas and liquid fuel using the Features of Biomass Input products from a biochemical perspective with the digestion of microbial raw materials. Two traditional biochemical transformation methods could be used as fermentation and transesterification techniques in microalgae energy production. Fermentation leads to a method that transforms sugar into ethanol and gases in a catalyst of yeast (alcohol). Leavening is commonly used to manufacture bioethanol from starch and sugar products (McKendry, 2002). Changing biofuel through the microalgae utilizing the leavening process is also a practical method. Microalgae have a high volume of starch. That it could turn within sugar, the processing methods could be seen in the below phases. A cells' substrate is removed during the first stage. The microalgae used a mechanical or an enzyme method. In the following stage, saccharomyces cerevisiae is applied to the microalgae's natural fermentation substance as the microalgae cells continue to degrade. It will then include carbon dioxide and ethanol. The equations of reactions are:

$C_{12}H_{22}O_{11}$ (sucrose) → $C_6H_{12}O_6$ (glucose) + $C_6H_{12}O_6$ (fructose) ------------ The initial stage

$C_6H_{12}O_6$ + yeast (catalyst) → $2C_2H_5OH$ (ethanol) + $2CO_2$ ↑ ------------------- The final stage

In the final stage, ethanol liquid might be removed from the reservoir and pushed to some other storage reservoir to be filtered. Carbon dioxide could be extracted and reutilized once more (Demirbas, 2010) to produce microalgae.

2.2.5. Biofuels production from microalgae

Algae are one of the oldest forms of life. They exist in all terrestrial ecosystems. For a brief time, micro-algae may contain vast quantities of fats, proteins, and carbohydrates. Algae is often used to remove carbon dioxide from the chimneys of energy factories to minimize releases of greenhouse gases due to its success in stabilizing carbon dioxide. The three key portions of algae (fats, proteins, and carbohydrates) could be transformed into biofuel. (Chen et al., 2011). It is possible to process some very rich sources of fatty acids becoming biofuels. A few varieties contain fats, including those in gasoline, which are hydrocarbons. Others can include lipid-like fats that can be transformed by esterification into synthetic diesel fuel. Carbohydrates could be transformed to ethanol with leavening. In anaerobic metabolism, three elements, including fats, carbohydrates, and protein, can be transformed into methane. In addition, certain algae descents could be changed hereditary or within development provisions to generate main items such as fats or carbohydrates. The different algae strains and the overall composition of the biomass are clarified in the table below (Table 2.2).

Table 2.2. Algae biomass formulation (percent dry wt.) from various strains (Dudenhoeffer, 2016)

Algae	Lipids	Carbohydrates	Protein
Anabaena cylindrical	4.0 –7.0	25.0–30.0	43.0–56.0
Aphanizomenon flos-aquae	3	23	62
Chlamydomonas rheinhardii	21	17	48
Chlorella pyrenoidosa	2	26	57
Chlorella vulgaris	14–22	12–17	51–58
Dunaliella salina	6	32	57
Euglena gracilis	14–20	14–18	39–61
Porphyridium cruentum	9–14	40–57	28–39
Scenedesmus obliquus	12–14	10–17	50–56
Spirogyra sp.	11–21	33–64	6–20
Arthospira maxima	6–7	13–16	60–71
Spirulina platensis	4–9	8–14	46–63
Synechococcus sp.	11	15	63

Algae define biodiesel and bioethanol production; many essential characteristics of algae make it possible to be a wonderful nominee for renewable fuels. The third generation solves the main hurdles of the first and second generations of biofuels. There was a constant effort to produce biofuels with an algae source in the 1970s, with the fuel crisis ever since. In transforming photons, algae are very successful and can absorb and store vast quantities of fuel-producing oils or carbohydrates. Using inexpensive raw materials will do this (Subhadra, 2010). Algae can withstand high amounts of carbon dioxide used to extract carbon dioxide from plant pollution. Decreasing emissions of greenhouse gases and gasoline. The benefits of aqueous microalgae are that structural biopolymers like lignin and hemicellulose are removed, which has been an obstacle for second-batch biofuel raw ingredients algae part of a varied collection of thousands of plants, making for several strains that could be ideal for various uses and ecosystems. Compared to more productive land plants, due to the structural variations among algae and land corps, algae could generate higher averages of storage facilities. Kelp woods in shallow coastal regions are the most active societies on earth, which can be used during the year to grow carbohydrates. The increased supply for ethanol raw materials provides a better reflection (Harun et al., 2010). Based on sunshine, biomass, and a limited amount of other nutrients, algae produce at a high density and are very effective in using it (Dismukes et al., 2008). Algae are associated with a lack of competition in the production of food crops with freshwater because of the likelihood of production in coastal, saltwater, or municipal waste water (Shilton et al., 2008). Algae biomass likewise includes other elements which could be utilized routinely, such as biopolymers and proteins.

2.2.6. Bioethanol production from microalgae

All other sugars are processed into bioethanol by the required ethanol product. There are vast quantities of biomass in algae in the shape of cellulose/starch that could be turned into rapturous sugars. During dark fermentation, some algae may serve as a tiny ethanol processing plant. During cultivation in the dark, some algae can serve as a small manufacturing plant for ethanol. Starch algae are predominantly preserved into biomass that could be routinely collected out of big farms with starch offprint. The starch could be processed by many methods, including mechanical processes or enzymes to degrade cell walls. The starch can be harvested and used for fermentation by removing it with water or an organic solution to generate bioethanol. Once the starch is removed from the biomass of the algae, two methods, heating, and fermentation are used to turn it into ethanol, similar to other raw materials dependent on starch (Rubin, 2008). These two methods can be carried out in one or two steps. Starch involves hydrolysis of simple sugars before fermentation occurs, and this step is called scarification. Acid or enzymatic hydroxylation may be used to turn starch into basic sugars. Fermenting the simple sugars to ethanol with an appropriate yeast strain is the next step. Either amylase generating descent could be utilized for leavening, both processes can be performed simultaneously. This ethanol is then removed from the dissolved alcohol product to eliminate water and all other impurities. Usually, that's 10 to 15 percent ethanol. Condensed ethanol will be combined with fossil fuels or used directly as gasoline (Brennan and Owende, 2010; Nigam and Singh, 2011). *Zygnemataceace*, *Cladophoraceace*, and *Oedogoniales* algal strains are being used to manufacture ethanol. These strains of algae accumulate starch and form colonies or filaments. The algae are generated by aquaculture, and the algae are removed by sedimentation, flocculation, filtration, or centrifugation. Due to a gloomy environment that was anaerobic and aqueous, the biomass then decomposed. The summarized biomass has been brewed with *Saccharomyces uvarum* and *Saccharomyces cerevisiae* to generate ethanol, isolated from the leavening stock. Oil-extracted biomass could be utilized as a base of ethanol output *Chlorococum sp.* About It was tested by fermentation with *Saccharomyces bayanus* as a potential feedstock for bioethanol. Skimmed biomass algal came in as 3.8 g/L ethanol for the ten g/L reacted matter utilized (Harun et al., 2010). Ethanol descents must gain high development scales with great cellulose/starch imports to generate ethanol. The main pillars being used in ethanol production include starch and cellulose. Algae should be grown largely managed ecologically to fulfill big profitability and boost all the elements influencing biomass production (Borowitzka, 2008). Algae should be generated during the year to supply the rising demand for gasoline.

2.3. Wastewater

Water contamination is one of the most critical environmental concerns (Abdel-Raouf, 2012). Industrialization and human activities bring enormous surplus resources, from flowing water, lakes, ponds, and the sea, to various water sources. The main source of these toxins is the discharge of industrial, agricultural, and municipal waste water that has been properly treated (Lim, 2010). They include great concentrations of natural inorganic toxins, intense minerals, pathogens, and poisons. The chemicals that impact flora and fauna for watery life influence people's health across the nourishment spectrum (Iqbal and Edyvean, 2004). Handling wastewater is a crucial stage in eliminating pollution and patho-genetic species to conserve water ecology and fulfill a sustainable water needs of a developing community with limited environmental challenges. Conventional methods have been used for many years to minimize pollutant aggregation and enhance the sewage consistency of drainage until it is released into earth-water nor re-entry into particles of water (Rawat, 2011). These techniques are costly and ineffective at eliminating all sources of toxins and need ultra-power supplies with specialist assistance.

Compared, the traditional solution generates a huge quantity of sediment that demands more dealing with excess pollutants. These limits on conventional treatment plants inspire scientists to seek an alternative solution to handling wastewater. Several improvements to traditional care processes have been adopted over the years to increase patient quality. Nevertheless, many of these improvements have certain disadvantages, such as massive fees and the difficulty of procedures and maintenance, because of their dual role in wastewater bioremediation and the harvesting of biomass from algae, which could be used in the generation of bioenergy (Figure 2.6). Microalgae have recently acquired an interest in pharmaceuticals, herbal fertilizers, and animal feed (Batista, 2015; Cai, 2013). As well as that, algal remediation of wastewater has many affordable and environmentally sympathetic process to the product disposal for inorganic contaminants, coliform bacteria, heavy metals containing nitrogen and phosphorus, and the reduction of the need for biological oxygen (BOD) and chemical oxygen (COD) (Abdel-Raouf, 2012; Cai, 2013; Rawat, 2011). The microalgae are single-cell photo-synthetic species that could realize significant phosphorus quantities, nitrogen with carbon to the oxygen, and development output. Utilizing a wide variety of microalgae like *Phormidium sp.*, *Scenedesmus sp.*, *Chlorella sp.*, *Spirulina sp.*, and *Chlamydomonas sp.*, has been reported to treat municipal waste water technique's effectiveness seems to be encouraging (Chinnasamy, 2010; Olguín, 2003). The microalgae were usually chosen for the bioremediation process because of their high photosynthetic ability, rapid nutrient absorption, combined with a reduced life expectancy with a basic dietary criterion; however, they could be cultivated and vastly increased in both industrial and conditions in the lab (Dwivedi, 2012). Large organic matter volumes, various micro-organisms of pathogenic origin, untreated waste water, inorganic nutrients, and hazardous chemicals are typically found. Eutrophication is caused by insanitary dumpsites, unreleased contaminated water in water sources, and solid waste disposal has often combined to surface water, soil with earth-water run-off, indicating wellness and climate threats. Treatment of drainage is removing wastewater pollutants to produce waste streams or industrial waste compatible with disposal or reemploy. Mechanical, Physical with biological processes were utilized to expel toxins out of sewage. The drainage water management seeks to protect the environment in a manner that is consistent with socio-economic and community health concerns.

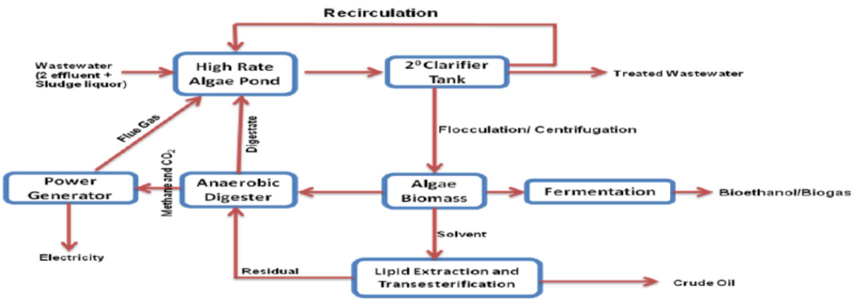

Figure 2.6. A flow diagram shows how microalgae can be stored Integrated intodrainage activities with the dual function of biofuel remediation and development (Osundeko, 2014).

In fact, for the effective management of waste water, algae development is controlled worldwide. The microalgae-founded handling approach was functional like the traditional curing method has many merits (Table2.3). It entails min. Sediment production needs little electricity to prevent the freeing of greenhouse invaders from the handling facility (CO_2). Further, low-cost solid waste is considered more cost-effective and provides usable compost that could be utilized like animal feed, fertilizer with bioenergy output (Rawat; 2011; Mata et al., 2010). Microalgae regulation is designed to cope with various waste water sources, such as household, sludge, and manufacturing waste. Also to additional feeds should be reduced (phosphate, nitrogen), toxic minerals then pathogenetic life forms.

Table 2.3. Benefits of algae-based waste water treatment over conventional Approach (Mata et al., 2010)

Traditional Process	Algal-dependent Treatment for Wastewater
Expensive Solution	Cost of operation low
There is a significant amount of toxic sludge that can generate more kinds of contamination.	There was very little chemical & biological sediment generated
Alloys cannot be retrieved	Able to retrieve precious alloys
It could not be used for any other purposes	Microbial biomasses have various industrial applications.
Less efficient to remove heavy metals	A more efficient method, especially to remove heavy metals.
Releases greenhouse gases like CO_2	Reduces the release of greenhouse gases

Wastewater is used water whose physical, chemical, or biological properties have been altered because of such compounds being introduced. Body waste, hair shampoo, hair, food scraps, fat, laundry powder, clothing conditioners, and micro-organisms (germs) are the contaminants that can make humans unhealthy and harm the ecosystem. The process and equipment used to remove much of the contaminants found in the waste water is wastewater treatment. Wastewater management involves treating waste water to maintain the ecosystem (Metcalf and Eddy, 1991). Over the last half of the 20th century, the population has increased, resulting in demand for already scarce water sources. Urbanization has also moved the agricultural core of many regions. Agriculture is the main recipient of available water by far. Temporal and spatial water storage is still a big concern for the supply of overdrawn groundwater (NRC, 2005). In various aquatic environments, algae may grow, such as urban waste water, fresh, brackish, and marine water. The chemical composition and physical properties of waste water are peculiar since, in addition to other trace elements, there are sufficient concentrations for carbon (chemical or artificial substances), N (ammonium, urea either nitrate), then P. Latest experiments using waste water have demonstrated the huge potential of the mass processing of algal biomass for biofuels (Zhou et al., 2011; Zhou et al.,2014; Wang et al., 2010). Wastewater kinds could be described as below:

1- Domestic Sewage sludge (excreta, vomit, and fecal sludge) and greywater (kitchen and bathing waste water) effluent; institutional sewage, such as hospital water;

2-Industrial sewage, wastewater discharged from industrial operations, e.g., poultry disposal, pharmaceutical industry.

3-Rain run-off, another urban run-off again.

4- Effluent from agriculture, horticulture, and aquaculture, either solubilized or as suspended matter (UN-Habitat, 2010).

In addition, untreated waste water begins with raw ingredients, minerals, and micro-organisms (Rawat et al., 2011). The impact of waste water on the urban hydrological system is important, with about 75% of the overall water returned as waste water from the urban population (Qadir et al., 2010). More than 50 countries where non - treated or diluted waste water is being used for irrigation are estimated to have 20 million hectares of land (FAO, 2003).

2.3.1. Cultivation of microalgae in wastewaters

In the early 1950s, the first research on using microalgae to waste water curing was launched. Development for waste water algae has developed from algae in treating wastewater (Zhou et al., 2014; Oswald et al., 1957). The nutrients have been effectively exhausted in such a symbiotic environment. Treatment of wastewater dependent on algae can remove foods (like P & N). Stable local wastewater is most efficient than conventional operated wastewater processes (Tam and Wong, 1989). It has been researched that the production of microalgae for the clearance of waste water has considerable potential. Microalgae production for the clearance of wastewater has been practiced for at least half a century. The production and utilization of high cultivation costs have limited for similar systems. Only several full-scale wastewater treatment plants for algae have been built. Relevant developments include changes in the screening methods for the growth capacity of multiple types of microalgae and the detection of municipal waste water. The utilization of chemical energy in organic carbon dissolved in wastewater is used as algae are grown inside a wastewater treatment system that could be a good choice. In a 2016 study, it has been shown that industrial waste water could be an efficient replacement for the algae culture medium (Van,2016). For up to 70 percent of their lipid content in some cases, microalgae are the most exciting new source of biomass and biofuels. Algal development and its metabolism mechanisms are important in treating wastewater with so many economic opportunities. Treatment of wastewater with microalgae is particularly desirable because of the photosynthetic potential of algae to convert solar energy into usable biomass and to combine nutrients such as nitrogen and phosphorus that induce eutrophication (De la Noüe and De Pauw, 1988). The most popular use of microalgae in treating wastewater is stabilization lakes (Aravantinou and Manariotis, 2014). Stabilization lakes are low-cost natural reservoirs for the treatment of municipal and industrial waste water. Stabilization lakes are classified as optional, maturation, and high-rate algal lakes. For disposal of organic matter and particles and pathogen containment, optional ponds with normal depths of 1.2 to 2.5 m and HRT 5 to 20 d hydraulic retention is most widely used (Manariotis and Grigoropoulos, 2002). Increased levels of nitrogen reduction can be accomplished at greater atmospheric temperatures in well-designed septic systems, which involve shallow maturation ponds (Silva et al., 1995; Soares et al., 1996). Recently, numerous attempts have been made to resolve the ability of the symbiotic algal-bacterial technique for main effluent treatment (Aravantinou et al., 2016), supplementary effluent (Gonçalves et al., 2016), and other forms of irrigation water (Gonzalez-Fernandez et al., 2011). A large category of photosynthetic micro-organisms are microalgae, comprised of eukaryotic microalgae and prokaryotic cyanobacteria. The accumulation of nitrogen and phosphorus in sewage is a significant factor that directly affects algae growth rates, nutrient removal, and lipid concentration (Aravantinou et al., 2013). Several variables affect the consistency of algal structures, such as nutrient levels, CO_2, pH, aeration intensity, light levels, and temperature (Aslan and Kapdan, 2006; Aravantinou and Manariotis, 2016). Nutrients integrated by algal biomass can be recycled by fertilizer processing, whereas algal biomass could also be used for bioenergy production or pharmaceutical creation substances or foodstuffs (Rawat et al., 2011; Grobbelaar, 2004). Furthermore, their ability to remove heavy metals and certain volatile organic products that avoid secondary pollution makes microalgae a safe sewage treatment choice (Aravantinou et al., 2013). Algal-based wastewater treatment is a realistic alternative to conventional treatment systems for efficiently and sustainably handling wastewater. Using microalgae in wastewater treatment would dramatically enhance the treatment efficiency by eliminating toxins, pathogenic species, biological oxygen demand (BOD), CO_2, emissions, and decreasing oxygenation requirements. Furthermore, microalgae can use sewage with various advantages, like fertilizer, animal nutrition, biofuel, nutraceuticals, then off-spring for the growth of their biomass. The combination of wastewater treatment with biomass generation from microalgae lowers production costs. Therefore, more research is needed for large-scale farming for microalgae within the waste water, environmental with secure hazard evaluation, and the utilization of microalgae as indispensable. Collecting has a big obstacle to the treatment of drainage water for integrated microalgae, as that is a very expensive microalgae treatment method. A few collecting were implemented, but extra. A study was needed to improve different cost-effective collecting methods. The benefits of min.-cost natural materials with no minor emissions for raw materials, algae can be promising for waste purifying water. The utilization of microalgae cultures during biological processing decreases energy demand and creates opportunities to recycle nutrients effectively. Microalgae treatment of wastewater will reduce the need for commercial fertilizers as algae grow incredibly well in waste rich in nutrients (Benemann, 2003).

2.3.2. Techniques for algal output from sewage

In recent years, multiple experiments were carried out for increasing microalgae for various forms of wastewater (spin from agriculture, urban, and manufacturing disposal sources, as well as cultivated animal nutrition processes). It was clearly stated as findings for these papers concentrated on the potency of strains of the microalgae chosen. A variety of species of micro-algae, including *Micractinium sp., Scenedesmus sp., Chlorella sp., Hindakia sp., Heynigia sp., Actinastrum sp., Pediastrum sp., Dictyosphaerium sp., Botryococcus sp., Chlamydomonas sp.* accompanied the *Coelastrum sp.* N with P and other trace elements in the wastewater have been checked and demonstrated to be used and removed (Zhou et al., 2014). In addition, biomass may be used to manufacture biofuels with many items like medications, meats, fish/animal nutrition, fertilizers addendums, and the low-cost algal was processed as an ideal feedstock (Zhou et al., 2014). These experiments would be of considerable value in producing a microalgae cultivation system based on waste water. Because of the nutrient profile deficit, the lack of a few crucial track components, with the prevalence of inhibitory/ venomous substances within drainage water sources, microalgae strains are typically vulnerable to numerous forms of wastewater.

Only a limited number of strains within a few organisms (e.g., *Chlorella sp.* and *Scenedesmus sp.*) can respond well to different wastewater conditions (Zhou et al., 2014). There was a strong requirement to choose extra firm microalgae descents, indulgent for an interesting form of waste water. Many experiments have shown the microalgae have adjusted to crop situations like anywhere they have been located and have usually evolved better than those purchased from algae banks (Zhou et al., 2014). Microalgae can either be grown at the secondary and tertiary levels wastewater treatment phase by an open or closed method. Microalgae biomass can be integrated into wastewater processing sludge for methane and CO_2 production (Kumar et al., 2010). In an algae lake that has a carbon source, CO_2 can be filtered. For cost-effective and renewable biofuels production, the high biomass and lipid productivity of microalgae grown in waste water reported in many studies must be transformed to a large industrial scale. Industrial waste water has been used to cultivate algae, and there is a complex composition of waste water released from an industrial facility. Nitrogen and phosphorus are two key nutrients that can aid in algae growth in agricultural waste water, but carbon is inadequate. However, the production of microalgae within commercial water will pose a few obstacles because Variables items can be found throughout this water. Because manufacturing sewage is widely thought of as unfit for algae farming according to the inherent features for comparatively feeding imbalance profiles with extremely harmful substances, several types of research have shown that microalgae grown in different industrial wastewaters can generate algae biomass. For example, a method containing waste water from waste chemicals and pigments from carpet factories utilized within grinders with various chemical materials. Metal ratios with typically comparatively low P and N levels. In a revised BBM media, they analyzed biomass development with the lipid development of couple descents for freshwater microalgae. They found that it had been TAI-2 *Chlamydomonas sp.* were greater growth of biomass with bigger out puts of lipid comparing with TAI-1

Desmodesmus sp. Ideal development with lipid deposition for *Chlamydomonas sp.* was checked. The BBM medium adjusts TAI-2 within various CO_2 and nitrogen ratios with the enlightenment time. They also found that *Chlamydomonas sp*. When maximal CO_2 aeration was 5 percent, TAI-2 reached full lipid aggregation under continuous illumination. When the medium has been used for industrial drainage, they observed that *Chlamydomonas sp.* $NO_3^{\ -}$ - N (3.1 mg/L) and $NH_4^{\ +}$-N 100% (38.4 mg/L) and PO_4^{3-}-P 33% (44.7 mg/L) reaching to 18.4% lipid accumulation have been omitted from TAI-2. Nearly 90% of the total fatty acids that have been used to generate biodiesel are 14:0, 16:0, 16:1, 18:1, and 18:3 fatty acids (Wu et al., 2012).

2.4. Response Surfaces Methodology (RSM)

The response surface methodology (RSM) is well known and is a revamped approach to constructing approximation models related to physical tests, computational experiments (simulations), and confirmed observations (Box et al., 1978; Montgomery, 2017). Box and Wilson created RSM, the response surface methodology, a blend of mathematical and computational strategies to construct the experimental model. The goal is to improve the reply (production constrain) effected with multiple separate constrain by thoughtful test design (input constraints). The experiment is a series of tests called runs, in which adjustments in the input variables are set to explain the effects of changes in the output variable (Montgomery and Runger, 1994). RSM contains two basic values:

1- Choosing an approximate prototype.

2- Experimental strategy where it is necessary to measure the response.

A sequence of replies often defines the accuracy of a produced product. Overall, these reactions were connected and deliberated across a special measuring tool. Thus, to maximize each answer, the decision-maker should solve the problem of parameter choice. Subject to different response parameters, this problem is a multiple response efficiency issues. Most traditional strategies were imperfect; an answer constrain has described as essential and reinforced by complying with the other set of standards constraints., several probabilistic methodologies were developed to solve the problem of multiple choices. Khuri and Cornell addressed the multiple response problem using the solution surface approach (Khuri and Cornell, 1987). An answer was weighted to solve the problem (Tai et al., 1992). To optimize the multiple answer problem, use square to create an estimated lower bound, combine the divergence from the objective and the uncertainty (Pignatiello, 1993).

A mechanism is introduced that can concurrently consider three functions: the measured loss function, usefulness, and the function of distance (Layne, 1995). Tech experimenters want to create the conditions that achieve a process for optimized results to obtain optimal results. A minimum or maximum function of the specification parameters may be optimum. One strategy for achieving efficiency is response surface Software. A valuable mathematical and numerical tool for solving these problems for analyzing and simulation engineering issues is the solution surface methodology. The response surface technique defines the interaction between controllable input variables and the response layers that have been obtained. The protocols for the construction of the methods for the reply layer are as below

(i)The design for a set of experimentations to correctly consistently test the reply for significance.

(ii) Building a design statistical on the final class reaction face and the correct attachments.

(iii)To achieve the maximum or minimum target value, look for the optimum set of experimental parameters.

(iv) Demonstrating the specific with reacting results of 2-dimensional with 3-dimensional plot phase variables.

If it is presumed that all variables are observable, the reply face could be represented as below:

$$"Y = F (x_1, x_2 ... x_k)" \quad (2.1)$$

The aim was to idealize the vector y for the response. Through experiments with marginal errors, the independent parameters are believed to be constant and controllable. It is necessary to look for a suitable estimation of the actual operational link for two individual's parameters with the reply face. For reaction surface methodology, a second-order model is usually used.

$$"y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i \hat{}2 + \sum_{i=1}^{k} \beta_{ij} x_i x_i + \varepsilon \;" \quad (2.2)$$

Where ε is a spontaneous mistake, in the second-order model, the β coefficients to be determined are derived by the least square procedure. It is normally possible to write (2.2) in the composite structure.

" Y = E + bX " (2.3)

As Y was described as a calculated valuation, composite X is defined as an independent matrix of parameters. The E and b matrices are composed, respectively, of factors with mistakes. By the matrix method, an answer for (2.3) could be acquired.

2.5. Examples of Response Surfaces Methodology (RSM) About Microalgae

The response surface methodology (RSM) has drawn researchers' interest in the microalgae sector. The technique of reaction surface methodology (RSM) has been used in several experiments.

First example: Mohamed et al. (2013) used the marine microalga *Tetraselmis sp.* FTC209 had its growth and lipid synthesis augmented via mixotrophic metabolism. In this analysis, to enrich the W-30 algal starter culture, the fifth-grade three-element design of central composite (CCD) was studied. Response surface methodology (RSM) was used to model the impact on biomass concentration, max, and lipid production of three medium variables, namely glucose (organic C source), $NaNO_3$ (primary N source), and yeast release (supplementary N, amino acids and vitamins). The RSM functionality has been measured versus the network of artificial neural (ANN) strategy to determine a composition that would result in maximum lipid productivity, PR lipid. A quadratic RSM transformation with a Marquardt - Levenberg trained ANN for ten secret neurons ultimately yielded relative outcomes, although higher response output values were reported in the ANN formulation. Finalized amounts of $NaNO_3$ (4.70 g/L), glucose (24.05 g/L) with ferment derivative (0.93 g/L) influenced a max rise to 12.38 g/L with lipid then currently 195.77 mg/g of aggregation. It has participated in the lipid output throughout two-week cultivation at 173.11mg/L per day. The figure shows the topologies of response surfaces that characterize the influence of the intermediate three components on lipid output in engagement. Any plot has a bunker surface.

Nonetheless, in terms of nonlinearity in the output variable, such graphs imply a recognizable elongate contour, suggesting a diagrammatic complexity. (Figure.a) reveals that lipid efficiency improves while $NaNO_3$ was set with the medium grade (5.0 g/L) as the ferment take off with glucose were ratcheted up to a confirmed grade prior eventually lowering for the additional information for those elements. The interaction with glucose and $NaNO_3$ (Figure. b) with the reciprocity with ferment take out with $NaNO_3$ (Figure. c) shows a similar pattern.

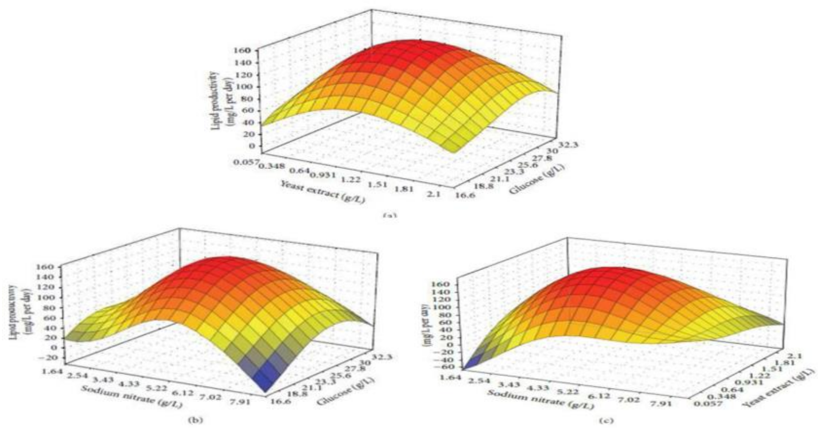

Figure 2. 7. Response to lipid usability surfaces showing associations between (a) glucose and yeast extraction, (b) glucose with $NaNO_3$, then (c) with ferment take out of $NaNO_3$ as represented by the network of neural (Mohamed et al., 2013).

Second example: Kim et al. (2018) carried out research, the impact of a couple of main ecological variables on photoautotrophic cultivation on the biomass and lipid surface productivity of *Ettlia sp.*, light strength, and CO_2 concentration. The current rising system (LE) grain with an inoculation face concentration of 2.5 g/m^2 is used as the optimal initial conditions for associated cultivation. The predicted answers were established and schemed like a 3D face with an outline diagram based on the expected quadratic model (Figure 2.8 a, b). However, and a reduction in low frequency with CO_2 intensities for lipid amount for biomass improved, the ideal value for ultimate lipid face output converted to lower light intensity and CO_2 intensity out of the ultimate biomass ground output. In related production methods, the reported lipid productivity is approximately 1 to 3 g/m^2/day 36, 35, 11. even though the attached lipid amount for the overripe *Ettlia sp*. According to the max, compared with other oleaginous species released, YC001 is relatively low, and the maximum lipid surface impacts this study's reliability. Biomass face profitability is the maximum lipid face efficiency recorded so away. Overall biomass face efficiency of about 28 ±1.5 g/m^2/day was obtained of 730 μE/m^2/s also 8 percent CO_2 by enhancement. Total lipid face efficacy was 4.2±0.3 g/m^2/day of 500 μE/m^2/s and 7 percent CO_2. Changes in the structure of greasy acids involving environmental variables have improved biodiesel efficiency at a maximum light concentration with ultimate CO_2 intensities. Farming for *Ettlia sp.* linked to comparative min. Energy light costs with max. CO_2 usage, YC001 has generated lipids and biomass efficiently with a max. output average.

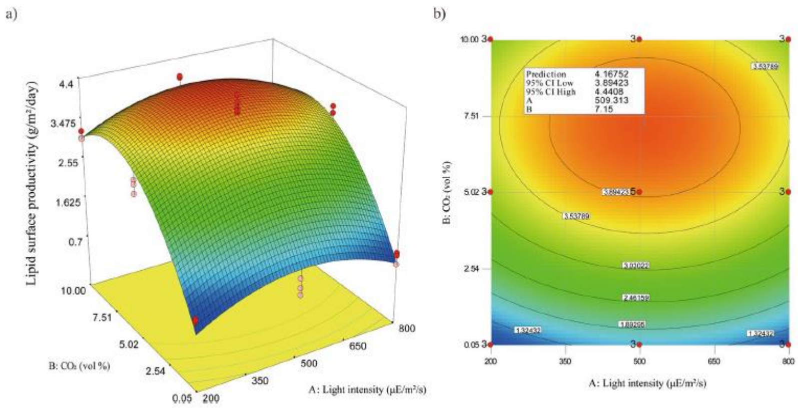

Figure 2.8. The response of the lipid surface profitability surface in the attached *Ettlia sp.* From YC001., (a) 3-dimensional diagram and (b) highlighter chart are created based on the projected lipid layer efficacy (Kim et al., 2018).

Third example: Bajwa et al. (2019) used the Box-Behnken architecture of the answer surface technique to evaluate the right levels of specific variables on Chlorella *sp.*, An experimental project has been carried out. Considering the prime iron, phosphorus, then nitrogen content like different factors. Chlorophyll, biomass, lipid, protein then carbohydrate content have been analyzed for answer constraints. Different biochemical - physic properties have been analyzed in the many levels of nourishing in media of BG_{11}, nitrate ($NaNO_3$ 11-751 mg mL^{-1}), iron (ferrous citrate ammonium,3.1-9 mg mL^{-1}), phosphate (K_2HPO_4, 41-121 mg mL-1), lipid ratio (36.1 dc percent) then biomass average (1.88 g L^{-1}) Nitrogen, phosphorus, and iron were collected under nutrient stress conditions to determine the exact prospect of *Chlorella pyrenoidosa* during conditions of photo-trophic nutritional deficiency. The lipid aggregation and biomass average of *Chlorella pyrenoidosa* through ferric citrate ammonium, K_2HPO_4, then $NaNO_3$ is revealed in 3-dimensional charts (Figure 2. 9). It indicates higher lipid aggregation of three variables under limited nutrient demand A Prob>F amount for >0.051 and < 0.051 respectively demonstrated. Variables were meaningful and were not model terms. The suitable model did undergo R^2 amounts of reach to 96.36% of lipids then 94.12% of biomass; proteins have identical results, carbohydrates with chlorophyll. The FAME outline for *Chlorella pyrenoidosa* included oleic ($C_18:_1$), palmitic ($C_16:_0$, stearic acid), linolenic ($C_18:_3$), then linoleic ($C_18:_2$) that presented the *Chlorella pyrenoidosa* had a suitable oily acid side view which could be used effectively to generate biodiesel.

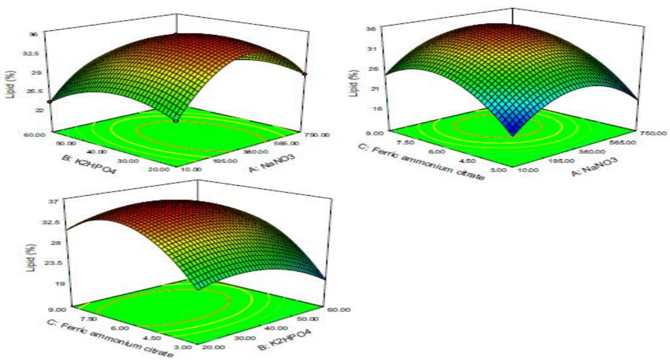

Figure 2.9. *Chlorella pyrenoidosa* lipid aggregation and biomass yield under various initial $NaNO_3$, K_2HPO_4, and ferric ammonium citrate values. (Bajwa et al., 2019).

2.6. Factors of the Environment

Light, temperature, pH, zooplankton parasitism, pathogens (including microbes, fungi, and viruses), and competition from invasive species are the key environmental variables. A major challenge that reduces algal production in large-scale farming environments was its ample lighting power to cause photo-synthesis. The control of temperature is another critical element in the successful mass production of microalgae (Benemann, 2008; Van Harmelen and Oonk, 2006). Viruses contamination and fungus predation could dramatically decrease the algal inhabitance through a couple of days (Kagami, 2007; Park., 2011). with different sources of wastewater, the effect of the microbial population is complex and involves more Study.

2.7. Principal Obstacles

Two main problems are facing the algae biofuel processing industry, and these were high biomass output from algae with algae collecting. It is important to extract the algae to require downstream refining to manufacture biofuels and other by-products. For algae development, the obtainable for major dietary fibers plus supplements is required. While gas trading was ineffective, the algae media could be carbon restricted. Photo-synthesis inhibitory oxygen products will reach (Carvalho et al., 2006). The limiting factor can also be light emission at high cultural densities in shape for photo-synthetic actively radiator (PAR) (Zijffers et al., 2008). Growing growth environment management can improve productivity, but there are additional costs involved. The most critical challenge is harvesting and downstream handling of the algae (Uduman et al., 2010).

There are three primary nutrients and many micronutrients (carbon, nitrogen, and phosphorus), such as magnesium, silica, calcium, etc. For algal biomass growth, manganese, iron, potassium, sulfur, cobalt, copper, then zinc was needed. However, where wastewater is used, the micronutrients needed of trial quantities of algal development are used with the important micro-alimentary seldom restrict algal development (Knud-Hansen, 1998). If macro and micronutrients are absent from the water supplies or the algal growth water does not have adequate nutrients, the inclusion of commercial fertilizers would significantly increase production costs. Wastewater, thus, is an attractive resource for algae production. Algal growth requires sufficient gas exchange, all of which involve appropriate carbon dioxide transport to the cells and proper removal of oxygen (Pittman et al., 2011). Some algae can be cultivated heterotrophically, but the autotrophic potential of algae to facilitate the process environmentally and economically viable must be used by utilizing inorganic carbon as the source of carbon. Algae can specifically use carbon dioxide and bicarbonate between those major sources for lignin dissipated carbon but could typically not use carbonate (Knud-Hansen et al., 1998). Algal farming was vulnerable to pollution while attempting to conduct single farming of dietary additions or different bio-products.

Single farming for unlikely algae within drainage water curing plants (Abu-Rezq et al., 1999). Finding solutions to accomplish the good handling for rising ecological despite incurring unjustified expenses a problem. A large amount of land with a usable source of water requires large-scale algal biomass production. The provocation could be faced while algae could be produced in wastewater curing utilities.

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3. MATERIAL AND METHODS

3.1. Growth of *Gloeocystis vesiculosa* 901

Gloeocystis vesiculosa 901 was obtained from the Culture Collection of Autotrophic Organisms (CCALA) in Czechia. *Gloeocystis vesiculosa* 901 was cultivated at 24 ± two °C. Firstly, microalgae were grown in 200 mL of working volume at 300 mL of the flask. Then, *Gloeocystis vesiculosa* 901 was transferred to FPBR, and the cultivation of microalgae was progressed. FPBR was illuminated at 130 μmol $m^{-2}s^{-1}$ and 16:8 (L/D). Cultivation of *Gloeocystis vesiculosa* 901 in flasks was given in Figure 3.1.

Figure 3.1. Cultivation of *Gloeocystis vesiculosa* 901 in flasks.

3.2. Design of Photo-bioreactor

The flat photo-bioreactor (FPBR) used in this study was designed in the previous study. FPBR was built in polymethyl methacrylate (PMMA), and the total and working volumes were 1,3L and 1L, respectively. Cultivation of *Gloeocystis vesiculosa* 901 in FPBR was given in Figure 3.2.

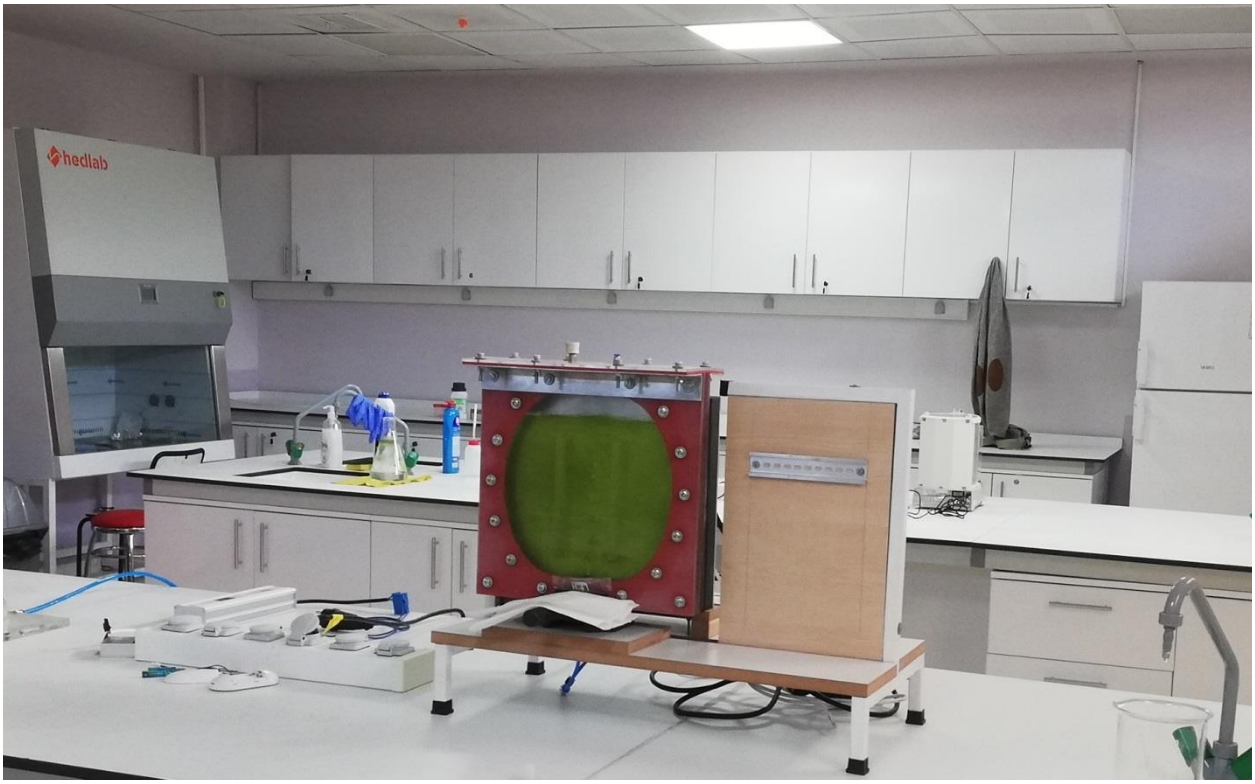

Figure 3.2. Cultivation of *Gloeocystis vesiculosa* 901 in FPBR.

3.3. Preparation of Wastewater

For this study, synthetic municipal wastewater was prepared. It was prepared with a few modifications, according to Abdelrahman (Abdelrahman, 2018). Glucose (45-720 mg/L), ammonium (10-150 mg/L), sulfate (10-200 mg/L), sodium chloride (10-200 mg/L) and calcium chloride (1-30 mg/L) concentrations changed. On the other hand, concentrations of starch (200 mg/L), urea (75 mg/L), monopotassium phosphate (40 mg/L) and sodium carbonate (150 mg/L) remained stable.

3.4. Optical Density Measurements

Gloeocystis vesiculosa 901 was grown in PBR, and optical densities of samples were measured at 680 nm at stationary phase spectrophotometrically. Cultivation of *Gloeocystis vesiculosa* 901 was terminated when they reach the stationary phase.

3.5. Biomass Concentration of *Gloeocystis vesiculosa* 901

Samples of *Gloeocystis vesiculosa* 901 were centrifuged at 3000 g at four °C for 10 min. Then, the samples were washed three times with distilled water and filtered utilizing filter paper (1.2 µm GF/C; 47 mm). Next, filtered samples were dried at 105 °C overnight. Dried samples were weighted by balance gravimetrically. Samples were kept at -20 °C for analytical experiments.

3.6. Hydrolysis of *Gloeocystis vesiculosa* 901

In this study, *Gloeocystis vesiculosa* 901 was hydrolyzed by chemical hydrolysis. Samples were mixed with 1M of HCI. *Gloeocystis vesiculosa* 901 samples were autoclaved at 121°C for 20 min. Then, they were cooled, and samples were centrifuged at 3500 g for 15 min. The supernatant samples were used for the calculation of carbohydrate concentrations (Onay, 2019).

3.7. Calculation of Carbohydrate Concentration

Carbohydrate concentrations were carried out by the anthrone method (Zhao et al., 2013). Glucose was used for standard. Carbohydrate concentration was measured at 620 nm spectrophotometrically.

3.8. Fermentation of *Gloeocystis vesiculosa* 901

Baker’s yeast, *Saccharomyces cerevisiae*, maintained the fermentation of *Gloeocystis vesiculosa* 901. The culture of fermentation was prepared according to Harun (Harun et al., 2010; Harun et al., 2011). Fermentation with microalgae samples was examined at 35 °C at 140 rpm for 48h anaerobically.

3.9. Bio-ethanol Production from *Gloeocystis vesiculosa* 901

Bioethanol concentrations were measured according to Bonnichsen and Theorell with modifications (Bonnichsen and Theorell, 1951). The concentration of bio-ethanol in microalgae was determined as the ethanol-dependent reduction of NAD^+ at 340 nm spectrophotometrically (Rizza et al., 2017).

3.10. Response Surface Methodology (RSM)

Central composite design (CCD) was examined on carbohydrate concentrations with various variables such as glucose (X_1), ammonium (X_2), sulfate (X_3), sodium chloride (X_4), and calcium chloride (X_5) from *Gloeocystis vesiculosa* 901. The five effects were carried out on lower, zero, and higher levels to determine the maximum variable effect. A total of 36 samples were applied to carbohydrate concentration. MATLAB and SIMULINK (R2015a) were used for these experiments.

$$Y = \beta_\circ + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{n} \beta_{ij} X_i X_j \qquad (3.1)$$

The response values (Y) were calculated for each test.
In this study, Y is the response value. B_o is the intercept, and X is the independent variable.

4. RESULTS AND DISCUSSION

4.1. Determination of Variables for Biomass and Carbohydrate Concentration

Municipal wastewater content changed for the determination of maximum biomass and carbohydrate concentration. Different concentrations of glucose, NH_4^+, SO_4^{-2}, NaCI and $CaCI_2$ were carried out and the variables used for this study were given in Table 4.1.

Table 4.1. The factors affecting biomass and carbohydrate concentrations

Factors (mg/L)	-2 (-∝)	-1	0	1	2 (+∝)
X_1: Glucose	45	90	180	360	720
X_2: NH_4^+	10	20	50	100	150
X_3: SO_4^{-2}	10	35	70	100	200
X_4: NaCI	10	35	70	100	200
X_5: $CaCI_2$	1	5	10	15	30

4.2. Biomass Concentration of *Gloeocystis vesiculosa* 901

Biomass concentrations of *Gloeocystis vesiculosa* 901 were calculated at different conditions, and the results were given in Table 4.2

Table 4.2. Biomass concentration of Gloeo-cystis vesiculosa 901 at different conditions

Exp. Order	Glucose (mg/L)	NH_4^+ (mg/L)	SO_4^{-2} (mg/L)	NaCI (mg/L)	$CaCI_2$ (mg/L)	Biomass concentration (g/L)
1.	90	20	35	35	15	0.38
2.	90	20	35	100	5	0.37
3.	90	20	100	35	5	0.43
4.	90	20	100	100	15	0.32
5.	90	100	35	35	5	0.63
6.	90	100	35	100	15	0.53
7.	90	100	100	35	15	0.59
8.	90	100	100	100	5	0.55
9.	360	20	35	35	5	0.43
10.	360	20	35	100	15	0.33
11.	360	20	100	35	15	0.37
12.	360	20	100	100	5	0.35
13.	360	100	35	35	15	0.59
14.	360	100	35	100	5	0.57
15.	360	100	100	35	5	0.61
16.	360	100	100	100	15	0.55
17.	45	50	70	70	10	0.47
18.	720	50	70	70	10	0.48
19.	180	10	70	70	10	0.55
20.	180	150	70	70	10	0.56
21.	180	50	10	70	10	0.46
22.	180	50	200	70	10	0.51
23.	180	50	70	10	10	0.54
24.	180	50	70	200	10	0.53
25.	180	50	70	70	1	0.55
26.	180	50	70	70	30	0.51
27.	180	50	70	70	10	0.57
28.	180	50	70	70	10	0.55
29.	180	50	70	70	10	0.56
30.	180	50	70	70	10	0.57
31.	180	50	70	70	10	0.55
32.	180	50	70	70	10	0.56
33.	180	50	70	70	10	0.55
34.	180	50	70	70	10	0.56
35.	180	50	70	70	10	0.57
36.	180	50	70	70	10	0.55

The maximum biomass concentration was obtained at 90 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 35 mg/L of NaCI, and five mg/L of $CaCI_2$ 0.63 g/L. The lowest biomass concentration was 0.32 g/L at 90 mg/L of glucose, 20 mg/L of NH_4^+, 100 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and 15 mg/L of $CaCI_2$. Generally, biomass concentration increased up to 180 mg/L of glucose. In addition, biomass concentrations were higher than other biomass concentrations at the 100 mg/L of NH_4^+. At lower concentrations of glucose (90 mg/L) and NH_4^+ 10, 20 and 50 mg/L), biomass concentrations decreased. Middle concentrations of SO_4^{-2}, NaCI, and $CaCI_2$ such as zero factor had more tolerable biomass concentrations such as 0.55 g/L, 0.56 g/L, and 0.57 g/L at 180 mg/L of glucose, 50 mg/L of NH_4^+, 70 mg/L of SO_4^{-2}, 70 mg/L of NaCI, and ten mg/L of $CaCI_2$. Microalgae can grow under different conditions. Changing the medium content causes the microalgae to produce a variety of biomass. *Chlamydomonas* generally are grown in the TAP medium, and NH_4^+ is the primary nitrogen source in this medium (Morais Junior et al., 2020). In another study, *Chlorella pyrenoidosa* was cultivated as heterotrophic growth, and glucose was a primary carbon source with 40 g/L (Wu et al., 2009). *Chlorella sorokiniana* Mb-1 was cultivated as mixotrophic growth, and the primary carbon source was acetate with six g/L (Chen et al., 2016). Microalgae can also be grown in a variety of wastewater. *Chlorococcum sp.* RAP13 was grown in dairy effluent, and lipid productivity was 42% (Beevi and Sukumaran, 2014). *Auxenochlorella protothecoides* UMN 280 was cultivated in municipal wastewater, and lipid productivity was 28% (Zhou et al., 2012). In another study, *Scenedesmus obliquus* was studied at 30 ^oC. Phosphorus source was PO_4^{3-}. Its limitation and absence (0–10.1 mg L^{-1}) were carried out. All samples showed different growth rates and kinetic parameters such as Ks = 0.25 μM, Ki = 0.955.72 μM, and μm = 0.0471 h^{-1} (Martinez et al., 1999). *Ceratium hirundinella* was studied at different concentrations (0.014-0.061 mol) of nitrogen and μm and Q min values, kinetic parameters, were 0.26 d^{-1} and 0.033 mol (Sommer, 1991).

4.3. Carbohydrate Concentrations and Productivities of *Gloeocystis vesiculosa* 901

Carbohydrate concentrations and productivities of *Gloeocystis vesiculosa* 901 were examined at various conditions of medium components and the results were given in Table 4.3 and Table 4.4, respectively.

Table 4.3. Carbohydrate concentration of Gloeocystis vesiculosa 901 at various conditions

Exp. Order	Glucose (mg/L)	NH_4^+ (mg/L)	SO_4^{-2} (mg/L)	NaCI (mg/L)	$CaCI_2$ (mg/L)	Carbohydrate concentration (mg/L)
1.	90	20	35	35	15	67.6
2.	90	20	35	100	5	74.0
3.	90	20	100	35	5	61.5
4.	90	20	100	100	15	49.6
5.	90	100	35	35	5	166.3
6.	90	100	35	100	15	198.7
7.	90	100	100	35	15	128.6
8.	90	100	100	100	5	132.0
9.	360	20	35	35	5	147.5
10.	360	20	35	100	15	116.8
11.	360	20	100	35	15	110.3
12.	360	20	100	100	5	112.0
13.	360	100	35	35	15	246.6
14.	360	100	35	100	5	251.0
15.	360	100	100	35	5	234.9
16.	360	100	100	100	15	216.7
17.	45	50	70	70	10	60.6
18.	720	50	70	70	10	138.2
19.	180	10	70	70	10	114.9
20.	180	150	70	70	10	161.3
21.	180	50	10	70	10	133.0
22.	180	50	200	70	10	127.0
23.	180	50	70	10	10	147.4
24.	180	50	70	200	10	145.2
25.	180	50	70	70	1	159.0
26.	180	50	70	70	30	144.3
27.	180	50	70	70	10	164.7
28.	180	50	70	70	10	159.5
29.	180	50	70	70	10	161.3
30.	180	50	70	70	10	165.3
31.	180	50	70	70	10	159.0
32.	180	50	70	70	10	161.0
33.	180	50	70	70	10	159.3
34.	180	50	70	70	10	163.0
35.	180	50	70	70	10	164.8
36.	180	50	70	70	10	159.6

Table 4.4. Carbohydrate productivities of *gloeocystis vesiculosa* 901 at various conditions

Exp. Order	Glucose (mg/L)	NH_4^+ (mg/L)	SO_4^{-2} (mg/L)	NaCI (mg/L)	$CaCI_2$ (mg/L)	Carbohydrate productivity (%)
1.	90	20	35	35	15	17.8
2.	90	20	35	100	5	20.0
3.	90	20	100	35	5	14.3
4.	90	20	100	100	15	15.5
5.	90	100	35	35	5	26.4
6.	90	100	35	100	15	37.5
7.	90	100	100	35	15	21.8
8.	90	100	100	100	5	24.0
9.	360	20	35	35	5	34.3
10.	360	20	35	100	15	35.4
11.	360	20	100	35	15	29.8
12.	360	20	100	100	5	32.0
13.	360	100	35	35	15	41.8
14.	360	100	35	100	5	44.0
15.	360	100	100	35	5	38.5
16.	360	100	100	100	15	39.4
17.	45	50	70	70	10	12.9
18.	720	50	70	70	10	28.8
19.	180	10	70	70	10	20.9
20.	180	150	70	70	10	28.8
21.	180	50	10	70	10	28.9
22.	180	50	200	70	10	24.9
23.	180	50	70	10	10	27.3
24.	180	50	70	200	10	27.4
25.	180	50	70	70	1	28.9
26.	180	50	70	70	30	28.3
27.	180	50	70	70	10	28.9
28.	180	50	70	70	10	29.0
29.	180	50	70	70	10	28.8
30.	180	50	70	70	10	29.0
31.	180	50	70	70	10	28.9
32.	180	50	70	70	10	28.8
33.	180	50	70	70	10	29.0
34.	180	50	70	70	10	29.1
35.	180	50	70	70	10	28.9
36.	180	50	70	70	10	29.0

The highest carbohydrate concentration was 251 mg/L at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and five mg/L of $CaCI_2$. The lowest carbohydrate concentration was 49.6 mg/L at 90 mg/L of glucose, 20 mg/L of

NH_4^+, 100 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and 15 mg/L of $CaCI_2$. Carbohydrate concentrations (246.6 mg/L, 251 mg/L, 234.9 mg/L and 216.7 mg/L) increased at 360 mg/L of glucose, 100 mg/L of NH_4^+. However, below 360 mg/L of glucose and 100 mg/L of NH_4^+, carbohydrate concentrations (67.6 mg/L, 74 mg/L, 61.5 mg/L and 49.6 mg/L) decreased. We calculated the carbohydrate yield better to see the effects of nutrients on the carbohydrate content. The maximum carbohydrate productivity was 44 % at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and five mg/L of $CaCI_2$. The minimum carbohydrate productivity was 12.9 % at 45 mg/L of glucose, 50 mg/L of NH_4^+, 70 mg/L of SO_4^{-2}, 70 mg/L of NaCI, and ten mg/L of $CaCI_2$. Carbohydrate productivity (41.8 and 44 %) enhanced at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}. On the other hand, carbohydrate productivity diminished at 90 mg/L of glucose, 20 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}. As can be seen from these results, glucose, ammonium, and sulfate significantly affected carbohydrate production. Bremauntz et al. studied carbohydrate concentration utilizing osmotic shock with 0.1 M NaCI for *Scenedesmus sp*. Carbohydrate content enhanced from 22 to 650 mg/sugar/g of biomass in this situation (Bremauntz et al., 2014). In another study, *Scenedesmus quadricauda* was cultivated at 1 M NaCI. Carbohydrate concentration increased from 0.04884 mg/mL to 0.06941 mg/mL compare to control group (Kirrolia et al., 2011). Hiremath and Mathad similarly examined the NaCI effect on carbohydrate concentration for *Chlorella Vulgaris*. Carbohydrate concentration increased from 24 μg/GFW in 10 days to 50 μg/GFW in 30 days at 0.4 M NaCI (Hiremath and Mathad, 2010). *Scenedesmus sp.* CCNM 1077 was studied for carbohydrate productivity at various concentrations of bicarbonate. The results showed 25.56% of carbohydrate productivity obtained at 0.6 g/L of sodium bicarbonate. Nitrogen starvation and bicarbonate supplementation maintained the highest carbohydrate productivity with 54.03% for *Scenedesmus sp.* CCNM 1077 (Pancha et al., 2015). In another study, *Chlorella sorokiniana* was cultivated in aquaculture wastewater. The highest carbohydrate productivity was 172.91 $mg^{-1}d^{-1}$ at 400 mg^{-1} of sodium nitrate in wastewater (Guldhe et al., 2017).

4.4. Response Surface Methodology (RSM)

Carbohydrate concentrations with various variables such as glucose (X_1), ammonium (X_2), sulfate (X_3), sodium chloride (X_4), and calcium chloride (X_5) from *Gloeocystis vesiculosa* 901 were carried out through RSM, and the results were given in Table 4.5.

Table 4.5. RSM Carbohydrate concentrations from various variables via RSM

Exp. Order	Glucose (mg/L)	NH_4^+ (mg/L)	SO_4^{-2} (mg/L)	NaCI (mg/L)	$CaCI_2$ (mg/L)	RSM Carbohydrate (%)
1.	90	20	35	35	15	16.2
2.	90	20	35	100	5	17.8
3.	90	20	100	35	5	15.1
4.	90	20	100	100	15	14.3
5.	90	100	35	35	5	25.3
6.	90	100	35	100	15	34.9
7.	90	100	100	35	15	21.3
8.	90	100	100	100	5	23.1
9.	360	20	35	35	5	34.1
10.	360	20	35	100	15	34.1
11.	360	20	100	35	15	30.3
12.	360	20	100	100	5	32.3
13.	360	100	35	35	15	41.3
14.	360	100	35	100	5	43.3
15.	360	100	100	35	5	38.5
16.	360	100	100	100	15	38.9
17.	45	50	70	70	10	17.0
18.	720	50	70	70	10	29.1
19.	180	10	70	70	10	21.3
20.	180	150	70	70	10	30.9
21.	180	50	10	70	10	33.6
22.	180	50	200	70	10	24.7
23.	180	50	70	10	10	26.3
24.	180	50	70	200	10	28.3
25.	180	50	70	70	1	28.6
26.	180	50	70	70	30	29.1
27.	180	50	70	70	10	28.9
28.	180	50	70	70	10	28.9
29.	180	50	70	70	10	28.9
30.	180	50	70	70	10	28.9
31.	180	50	70	70	10	28.9
32.	180	50	70	70	10	28.9
33.	180	50	70	70	10	28.9
34.	180	50	70	70	10	28.9
35.	180	50	70	70	10	28.9
36.	180	50	70	70	10	28.9

The highest theoretical carbohydrate percentage was 43.3% at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and five mg/L of $CaCI_2$. This result was in line with the experimental results (44%). The lowest theoretical carbohydrate percentage was 14.3% at 90 mg/L of glucose, 20 mg/L of NH_4^+, 100 mg/L of SO_4^{-2}, 15 mg/L of NaCI, and 14.3 mg/L of $CaCI_2$. Carbohydrate percentage had higher values (41.3%, 43.3%, 38.5% and 38.9%) at 360 mg/L of glucose, 100 mg/L of NH_4^+ than carbohydrate percentage values of other nutrient concentrations. The equation related to the empirical model was given below:

$$\mathbf{Y} = -0.5448 + 0.1321 X_1 + 0.2592 X_2 - 0.0053X_3 + 0.0629 X_4 + 0.0471 X_5 - 0.00012 X_1X_2 + 0.00011 X_1X_3 - 0.00011 X_1X_4 - 0.00084 X_1X_5 - 0.00061 X_2X_3 + 0.000525 X_2X_4 + 0.0033 X_2X_5 - 0.00058 X_3X_4 - 0.0040 X_3X_5 + 0.0038 X_4X_5 + 0.000131 X_1X_1 - 0.0012 X_2X_2 + 0.00024 X_3X_3 - 0.00026 X_4X_4 - 0.00097 X_5X_5$$

For the model, the mean square (MSE) value was 4.5103. In addition, the R^2 value was 0.9615, and the adjusted R^2 value was 0.9102. An advanced investigation of the variable was given in ANOVA results.

3D response surface plots (RSP) and related 2D contour plots (CP) related to the commitment status of glucose (X_1) and NH_4^+ (X_2) were displayed in Figure 4.1 and Figure 4.2, respectively.

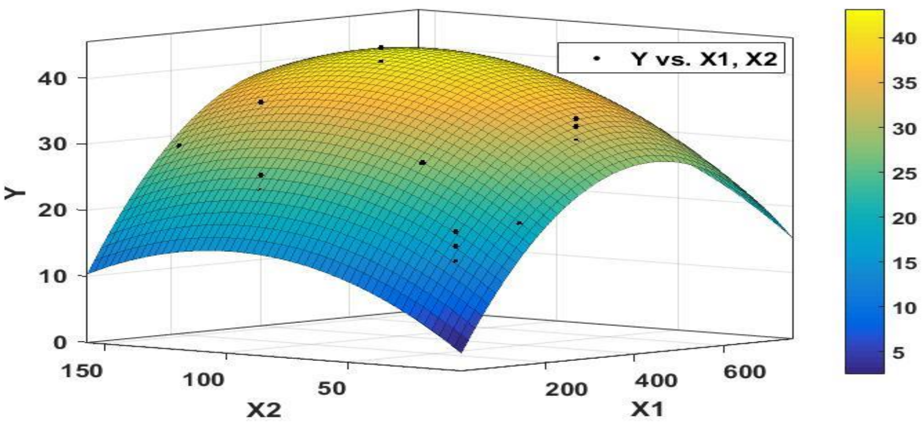

Figure 4.1. 3D response surface plot (RSP) about glucose (X_1) and NH_4^+ (X_2).

In the figure, while glucose concentration and NH_4^+ concentration increased from level -2 to 1, carbohydrate concentration increased. From level 1 to level 2, the carbohydrate amount decreased slightly.

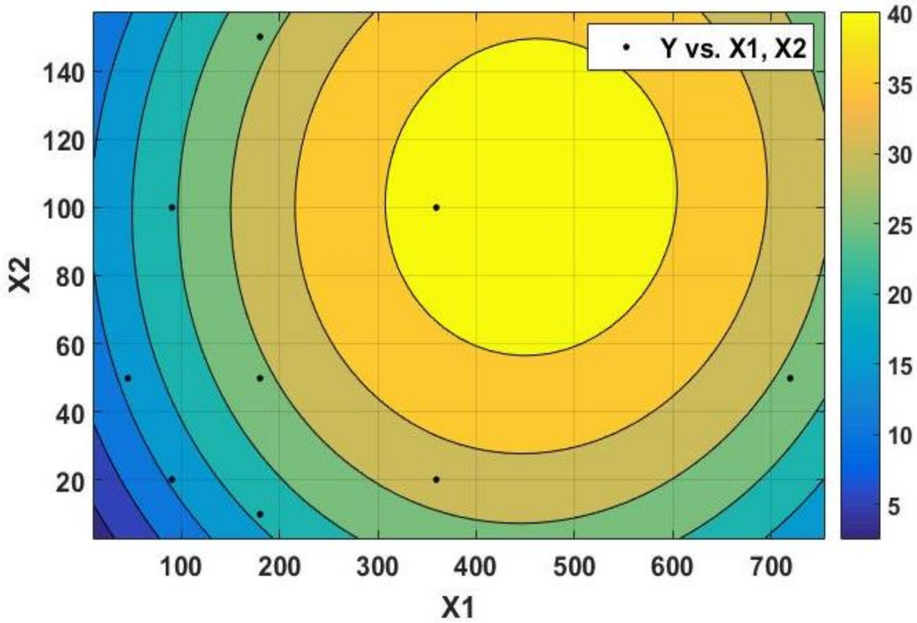

Figure 4.2. 2D contour plot (CP) about glucose (X_1) and NH_4^+ (X_2).

2D contour plot showed spherical shape. This situation shows us that both glucose and NH_4^+ concentrations do not synergistically affect carbohydrate percentage. Carbohydrate percentage reached maximum value when Glucose and NH_4^+ concentrations increased to 360 mg/L and 100 mg/L, respectively. In contrast, carbohydrate concentration decreased importantly when NH_4^+ concentrations decreased. The figure did not have an important effect when both carbohydrate and NH_4^+ concentrations decreased or increased jointly on carbohydrate percentage. In addition, we drew a 3D response surface plot about glucose (X_1) and SO_4^{-2} (X_3). 3D response surface plots (RSP) and related 2D contour plots (CP) related to the commitment status of glucose (X_1) and SO_4^{-2} (X_3) were displayed in Figure 4.3 and Figure 4.4, respectively.

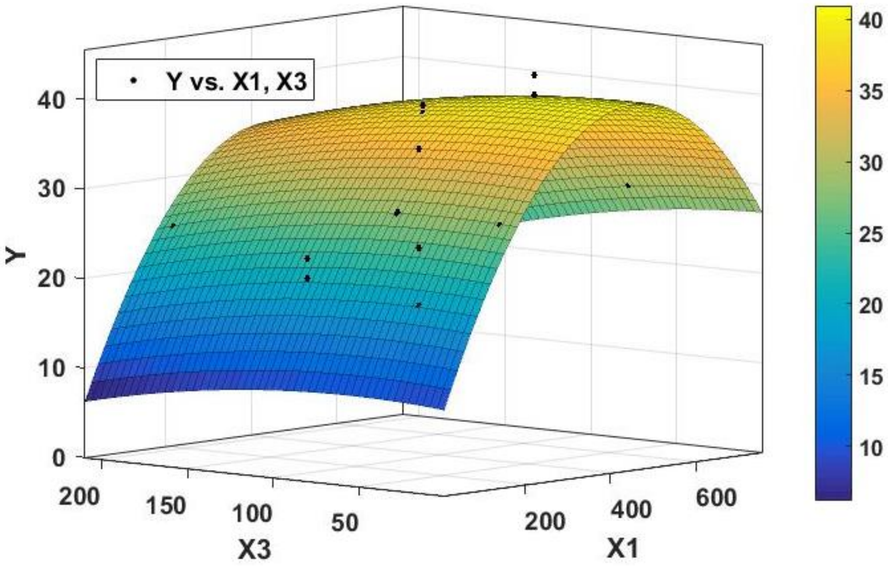

Figure 4.3. 3D RSP about glucose (X_1) and SO_4^{-2} (X_3).

Carbohydrate percentage increased between level-2 and level -1 when concentrations of glucose and SO_4^{-2} enhanced. Then, the variables such as glucose and SO_4^{-2} exhibited independent effects beyond these points.

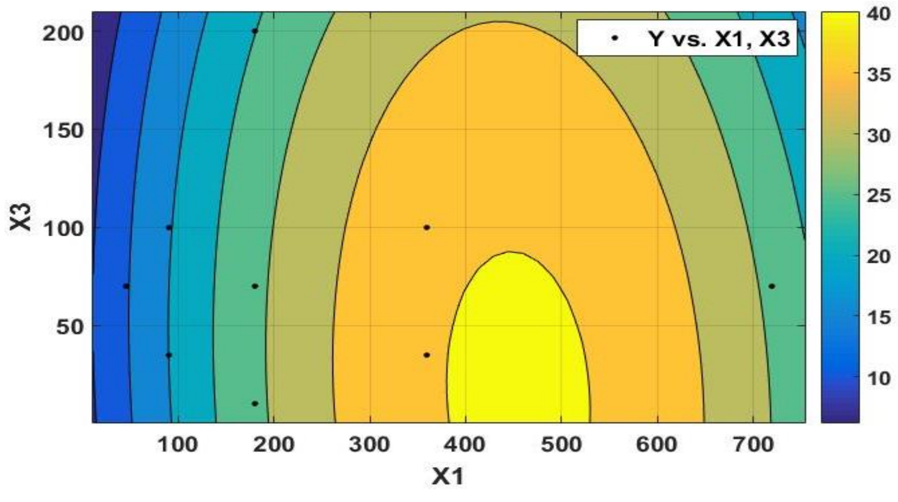

Figure 4.4. 2D contour plot (CP) about glucose (X_1) and SO_4^{-2} (X_3).

2D contour plot showed the half-elliptical contour plot. This displayed interaction between glucose and SO_4^{-2}. The relationship was made up of connections between levels. The maximum carbohydrate percentage (orange color) was seen at 35 mg/L of SO_4^{-2} and 360 mg/L of glucose according to the 2D contour plot. Also, we drew a 3D response surface plot about glucose (X_1) and NaCI (X_4). 3D response surface plots (RSP) and 2D contour plots (CP) related to the commitment status of glucose (X_1) and NaCI (X_4) were shown in Figure 4.5 and Figure 4.6, respectively.

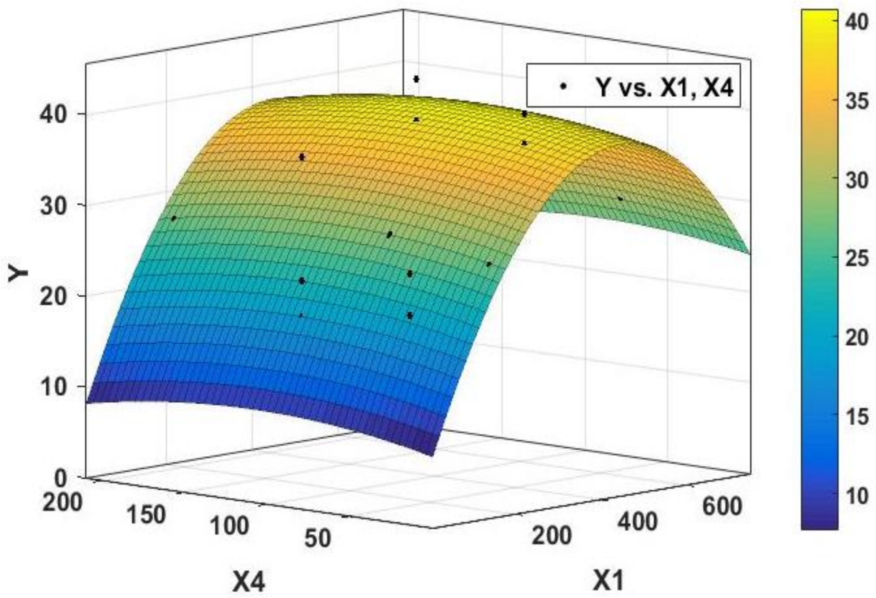

Figure 4.5. 3D RSP about glucose (X_1) and NaCI (X_4).

Carbohydrate percentage increased until concentrations of glucose and NaCI reached level 1. At level 2, carbohydrate concentration decreased. Maximum carbohydrate percentages (yellow color) were obtained at 360 mg/L of glucose and 100 mg/L of NaCI. Minimum carbohydrate percentages (blue color) were found at 90 mg/L of glucose and 35 mg/L of NaCI. In addition, middle carbohydrate percentages were located in green color in Figure 4.5. These values were presented at 180 mg/L of glucose and 70 mg/L of NaCI.

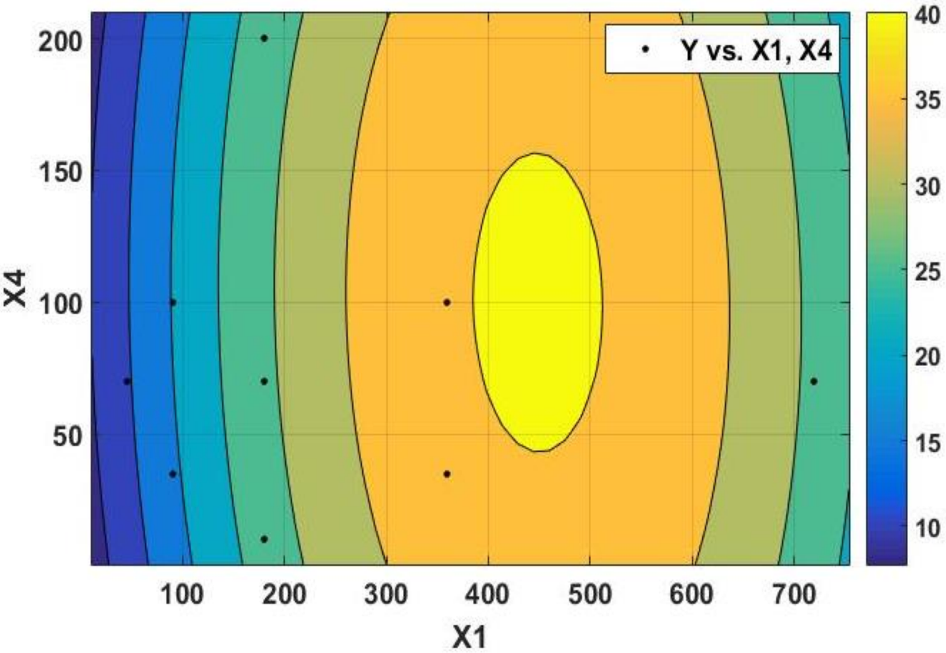

Figure 4.6. 2D CP about glucose (X_1) and NaCI (X_4).

2D contour plot displayed the elliptical contour plot. Between glucose and NaCI, this maintained interaction. The relationship was made up of connections between level -2 and level 1. According to Figure 4.6, the maximum carbohydrate concentration was calculated independently of sodium chloride for the interaction of glucose and NaCI. Carbohydrate percentage (orange color) was 34.1% at 360mg/L of glucose and 35 mg/L and 100 of NaCI. Carbohydrate percentages (green color) were 15.1% and 14.3% at 90 mg/L of glucose and 35 mg/L and 100 of NaCI, respectively. This result showed that interaction was disrupted at the lower concentrations of glucose (90 mg/L).

Then, we plotted a 3D response surface plot about glucose (X_1) and $CaCI_2$ (X_5). 3D response surface plots (RSP) and 2D contour plots (CP) related to the commitment status of glucose (X_1) and $CaCI_2$ (X_5) were shown in Figure 4.7 and Figure 4.8, respectively.

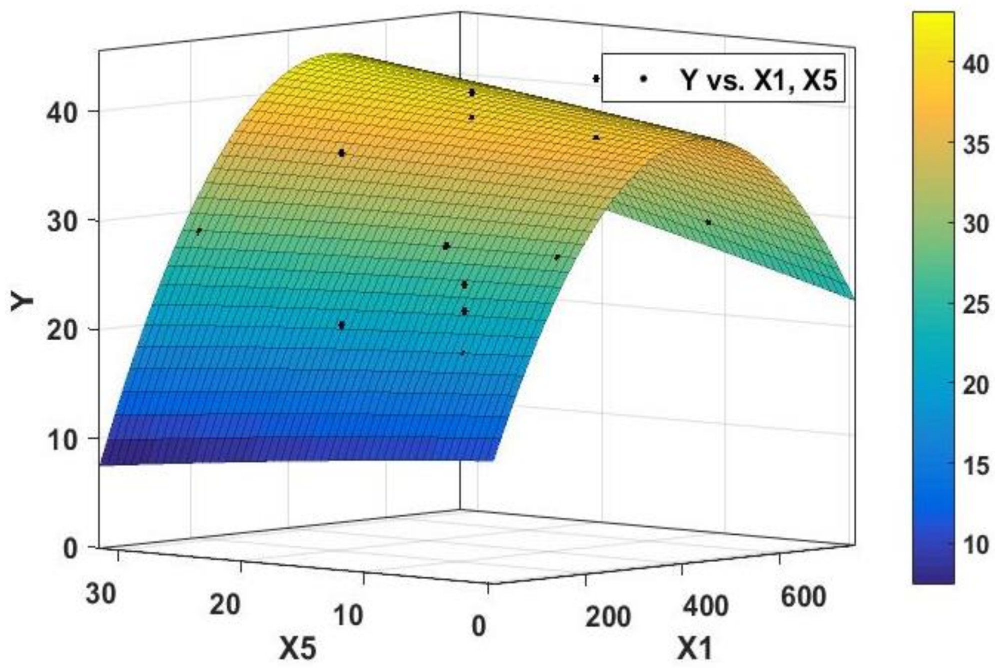

Figure 4.7. 3D RSP about glucose (X_1) and $CaCI_2$ (X_5).

Carbohydrate percentage increased until concentrations of glucose and $CaCI_2$ reached level -1. After level -1, carbohydrate concentration showed an irregular increase between concentrations of glucose and $CaCI_2$. Carbohydrate percentages (yellow color) were 38.5% and 38.9% at 360 mg/L of glucose and five mg/L and 15 mg/L of $CaCI_2$, respectively. In addition to this, Carbohydrate percentages (blue and green color) were 17% and 29.1% at 45 mg/L and 720 mg/L of glucose and ten mg/L of $CaCI_2$, respectively. These results showed that glucose had a higher effect than $CaCI_2$ under the same concentrations for carbohydrate percentage. Also, carbohydrate percentages (green color) were 28.6 and 29.1 at 180 mg/L of glucose and 1 and 30 mg/L of $CaCI_2$, respectively. Carbohydrate percentage was nearly stable under the same glucose concentrations and various concentrations of $CaCI_2$. Finally, concentrations of glucose cause a higher contribution than concentrations of $CaCI_2$ for carbohydrate percentage.

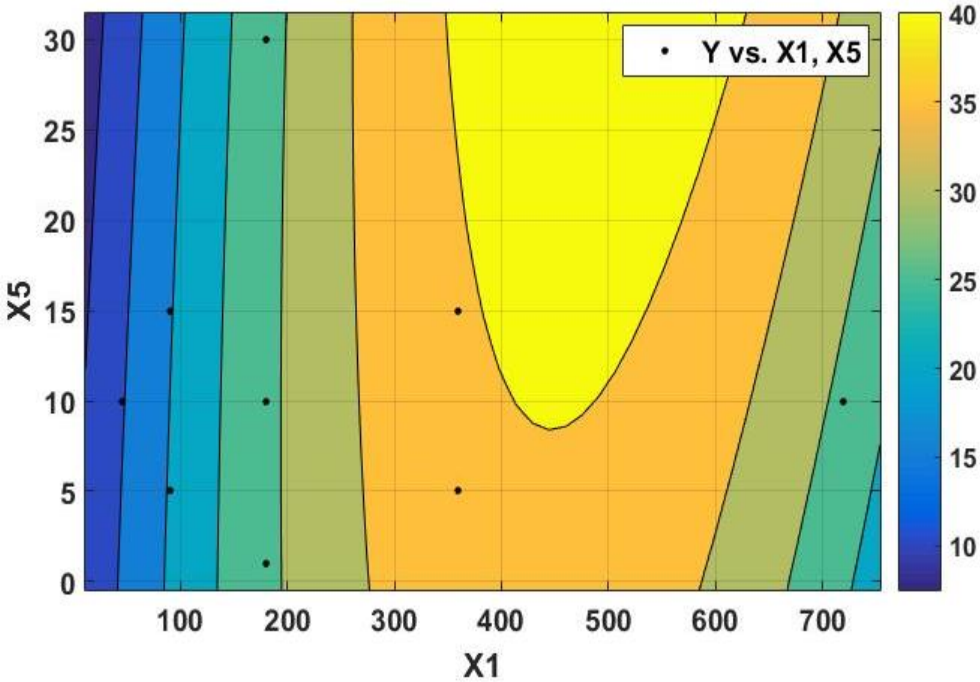

Figure 4.8. 2D CP about glucose (X_1) and $CaCI_2$ (X_5).

2D contour plot showed the irregular contour plot. Interaction between glucose and $CaCI_2$ was the maximum level. Carbohydrate percentage changed utilizing various concentrations of glucose and $CaCI_2$. Carbohydrate percentages were nearly the same at 180 mg/L of glucose and 1, 10, and 30 mg/L of $CaCI_2$. However, carbohydrate percentages enhanced significantly when both concentrations of glucose and $CaCI_2$ increased.

Then, we plotted a 3D response surface plot about NH_4^+ (X_2) and NaCI (X_4). 3D response surface plots (RSP) and 2D contour plots (CP) related to concentrations of NH_4^+ (X_2) and SO_4^{-2} (X_3) were displayed in Figure 4.9 and Figure 4.10, respectively.

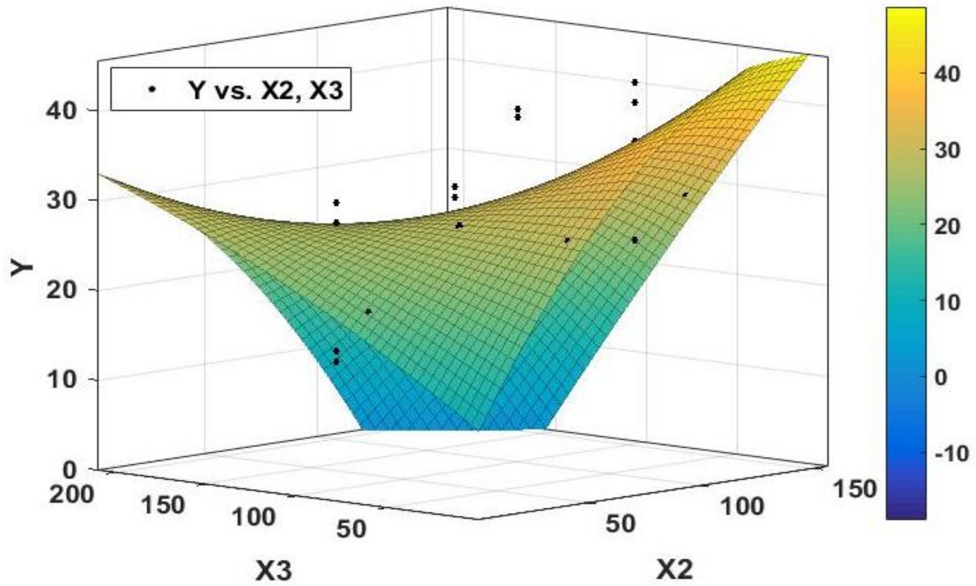

Figure 4.9. 3D RSP about NH_4^+ (X_2) and SO_4^{-2} (X_3)

Carbohydrate percentage increased between level -2 and level 1 when concentrations of NH_4^+ and SO_4^{-2} enhanced. Then, the variables such as NH_4^+ and SO_4^{-2} exhibited independent effects beyond these points.

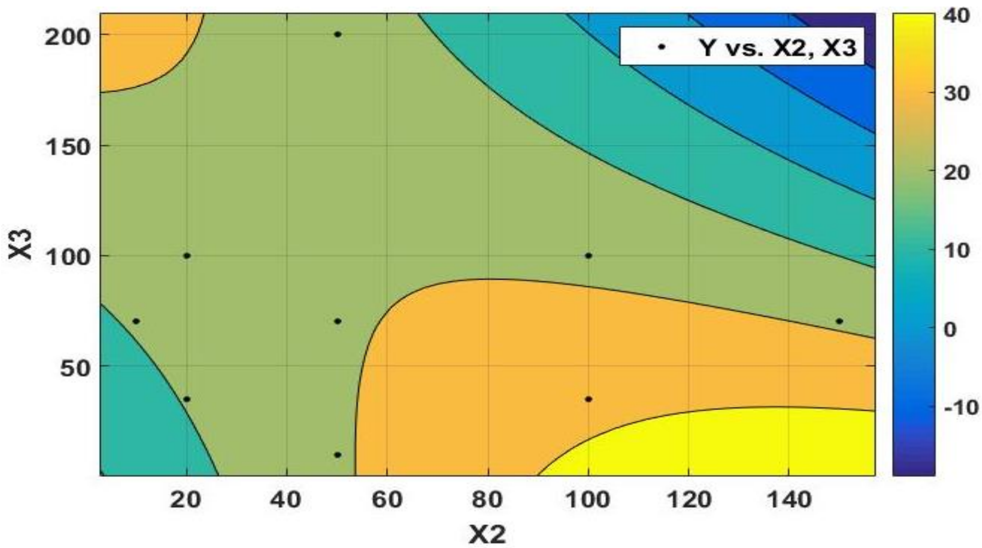

Figure 4.10. 2D CP about NH_4^+ (X_2) and SO_4^{-2} (X_3).

2D contour plot displayed the irregular contour plot. NH_4^+ and SO_4^{-2} had a higher degree of interaction. The relationship was made up of connections between level -2 and level 1. According to Figure 4.10, carbohydrate percentages (green color) were 33.6%, 24.7% and 26.3% at 10 mg/L, 200 mg/L and 70 mg/L of SO_4^{-2} and 50 mg/mL of NH_4^+, respectively. The critical point was carbohydrate percentage at 50 mg/mL of NH_4^+. Carbohydrate percentage significantly increased when the concentration of NH_4^+ was enhanced.

Then, we plotted a 3D response surface plot about NH_4^+ (X_2) and NaCI (X_4). 3D response surface plots (RSP) and 2D contour plots (CP) related to concentrations of NH_4^+ (X_2) and NaCI (X_4) were shown in Figure 4.11 and Figure 4.12, respectively.

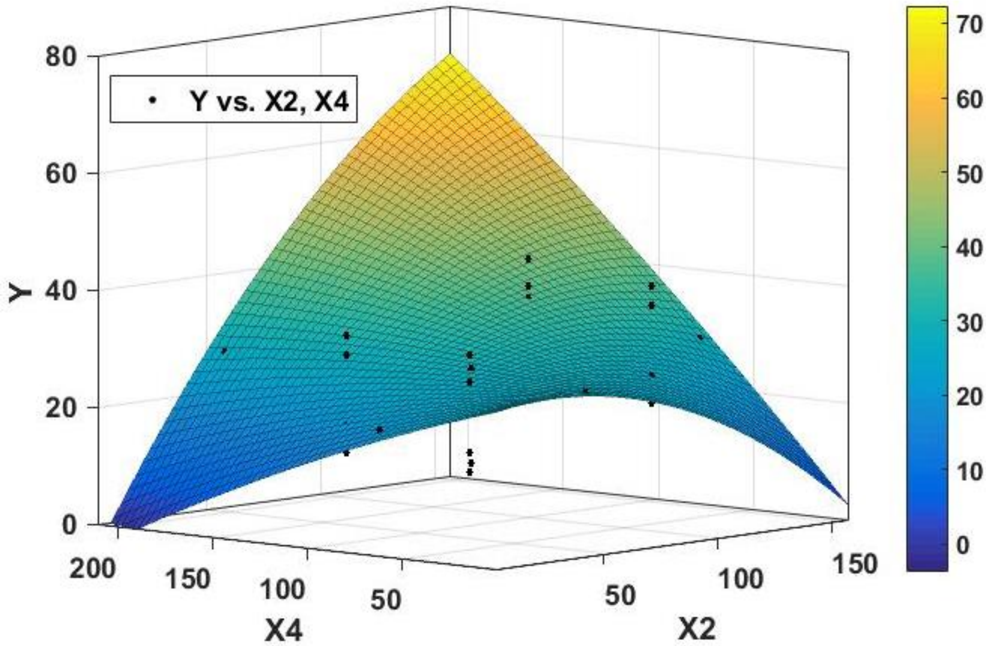

Figure 4.11. 3D RSP about NH_4^+ (X_2) and NaCI (X_4).

Carbohydrate percentage increased between level -2 and level 1 when concentrations of NH_4^+ and NaCI enhanced. Then, the variables such as NH_4^+ and NaCI exhibited independent effects beyond these points.

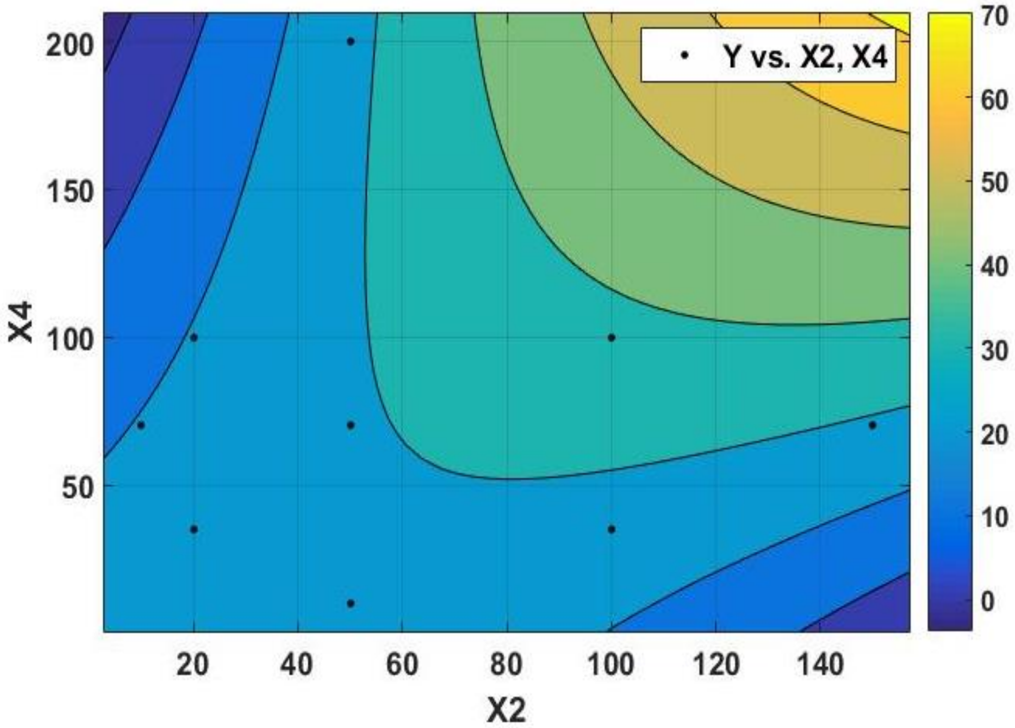

Figure 4.12. 2D CP about NH_4^+ (X_2) and NaCI (X_4).

2D contour plot displayed the irregular contour plot. Similarly, NH_4^+ (X_2) and NaCI (X_4) had a higher degree of interaction. According to Figure 4.12, carbohydrate percentages (light blue color) were 24.7%, 26.3% and 28.3%at 70 mg/L, 10 mg/L and 200 mg/L and 50 mg/mL of NH_4^+, respectively. In these variables, the critical point was carbohydrate percentage at 50 mg/mL of NH_4^+. Carbohydrate percentage significantly increased when the concentration of NH_4^+ was enhanced.

Then, we drew a 3D response surface plot about NH_4^+ (X_2) and $CaCI_2$ (X_5). 3D response surface plots (RSP) and 2D contour plots (CP) related to concentrations of NH_4^+ (X_2) and $CaCI_2$ (X_5) were shown in Figure 4.13 and Figure 4.14, respectively.

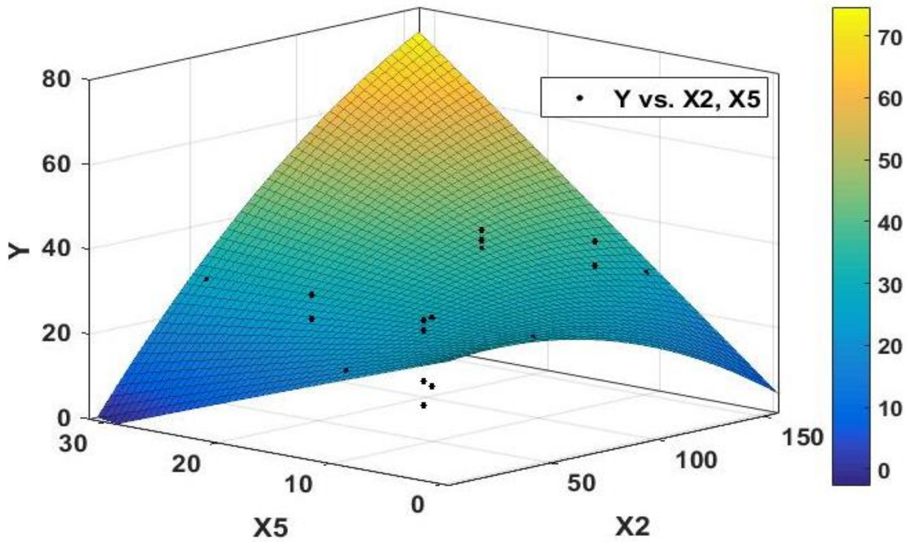

Figure 4.13. 3D RSP about NH_4^+ (X_2) and CaCI2 (X_5).

Carbohydrate percentages (blue color) were 20.9% and 28.8% at 10 mg/L and 150mg/L of NH_4^+ and 10 mg/L of, respectively. In addition, carbohydrate percentage (green color) was 34.1% at 5mg/L and 15 mg/L of $CaCI_2$ and 360 mg/L. This result showed us that increasing the amount of $CaCI_2$ did not change the carbohydrate percentage when NH_4^+ was stable at its high concentration.

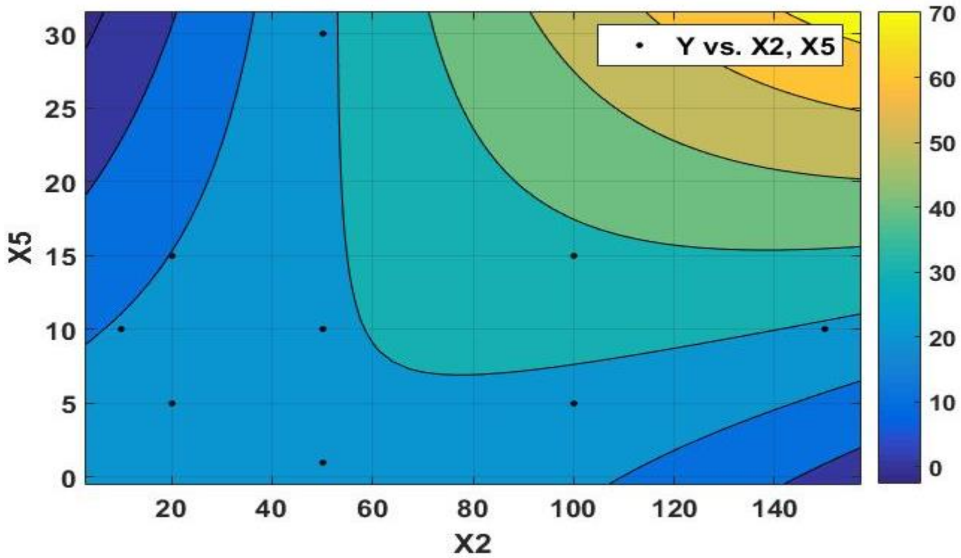

Figure 4.14. 2D CP about NH_4^+ (X_2) and $CaCI_2$ (X_5).

2D contour plot showed the irregular contour plot. Similarly, (X_2) and (X_5) had a higher degree of interaction. According to Figure 4.14, carbohydrate percentages (light blue color) were 28.6% and 29.1% at 28.9% at 1 mg/L and 30 mg/L and 10 mg/L of $CaCI_2$ and 50 mg/L of NH_4^+, respectively. As a result of this, when the amount of NH_4^+ was constant, increasing the amount of $CaCI_2$ did not change the carbohydrate percentage much. On the contrary, when the amount of $CaCI_2$ was constant, and the amount of NH_4^+ increased, the percentage of carbohydrate increased significantly. When the amount of both $CaCI_2$ and NH_4^+ was increased, the amount of carbohydrate increased too.

Next, we plotted a 3D response surface plot about SO_4^{-2} (X_3) and NaCI (X_4). 3D response surface plots (RSP) and 2D contour plots (CP) related to concentrations of SO_4^{-2} (X_3) and NaCI (X_4) were shown in Figure 4.15 and Figure 4.16, respectively.

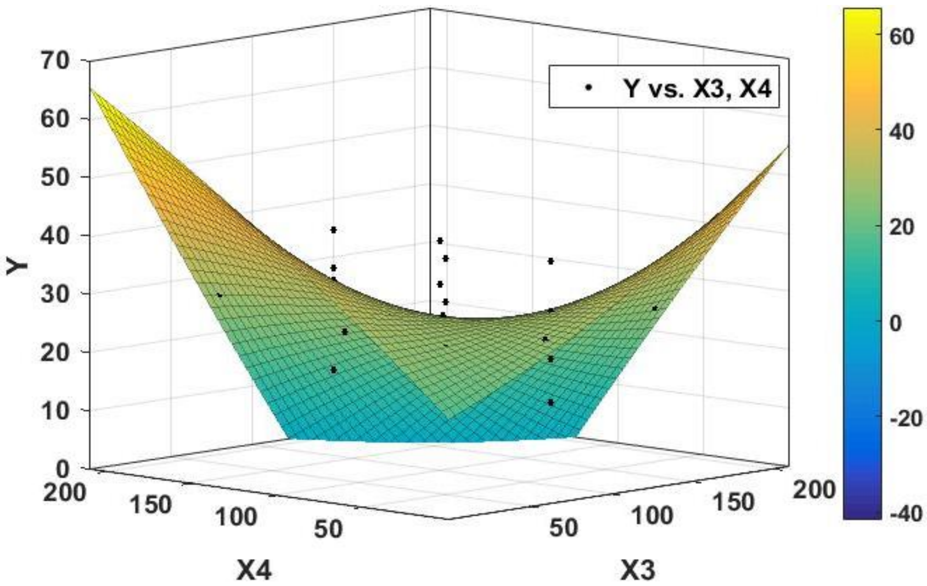

Figure 4.15. 3D RSP about SO_4^{-2} (X_3) and NaCI (X_4).

Carbohydrate percentages (orange color) were 30.3% and 32.3% at 35 mg/L and 100 mg/L of NaCI and 100 mg/L of SO_4^{-2}, respectively. As the amount of both increased, the number of carbohydrates increased.

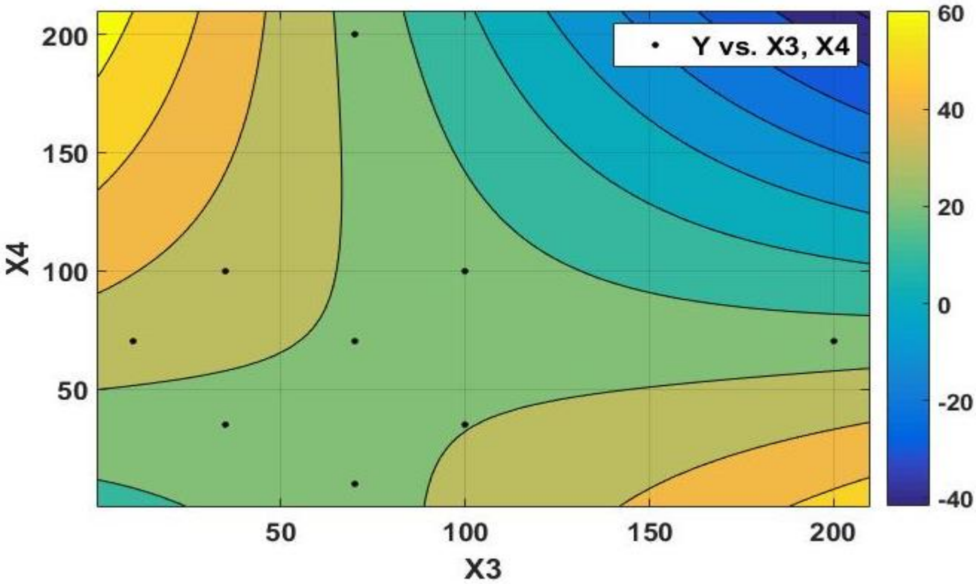

Figure 4.16. 2D CP about SO_4^{-2} (X_3) and NaCI (X_4).

2D contour plot showed the irregular contour plot. (X_3) and (X_4) had a higher degree of interaction. According to Figure 4.16, carbohydrate percentages (green color) were 26.3% and 28.3% at ten mg/L and 200 mg/L of NaCI and 70 mg/L of SO_4^{-2}, respectively. The increase in the amount of NaCI did not change the carbohydrate percentage much when the amount of SO_4^{-2} was constant. But, it contributed about two percent.

Then, we drew a 3D response surface plot about SO_4^{-2} (X_3) and $CaCI_2$ (X_5). 3D response surface plots (RSP) and 2D contour plots (CP) related to concentrations of SO_4^{-2} (X_3) and $CaCI_2$ (X_5) were shown in Figure 4.17 and Figure 4.18, respectively.

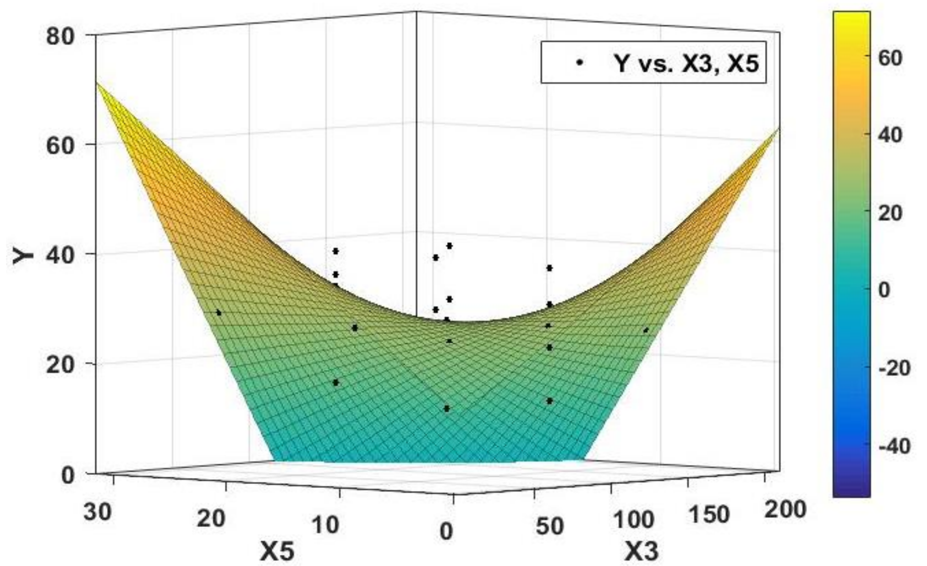

Figure 4.17. 3D RSP about SO_4^{-2} (X_3) and $CaCI_2$ (X_5).

Carbohydrate percentages increased when concentrations of SO_4^{-2} and $CaCI_2$ increased up to level -1. Out of this level, carbohydrate percentages decreased. Changing these two variables had a positive or negative effect on carbohydrate percent. Carbohydrate percentages (green color) were 28.6%, 29.1% and 28.9% at 1mg/L, 30 mg/L and 10 mg/L of $CaCI_2$ and 70 mg/L of SO_4^{-2}, respectively.

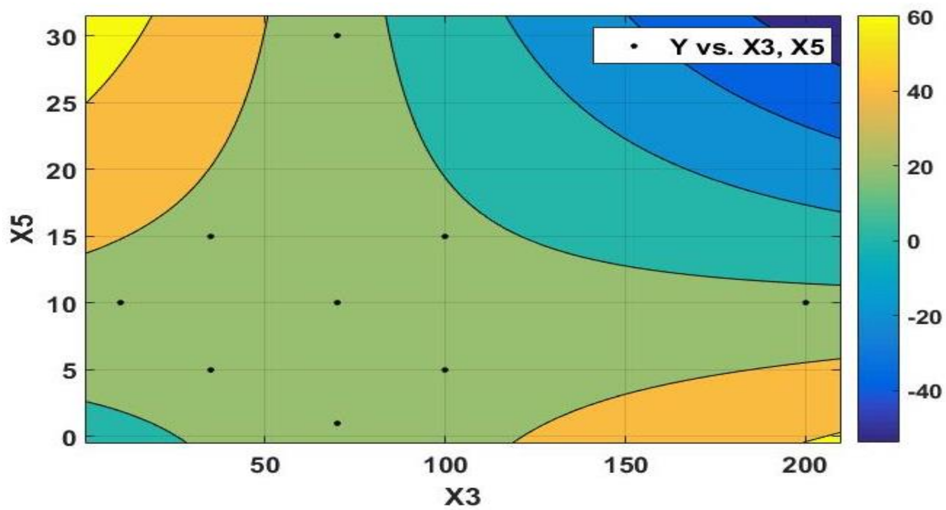

Figure 4.18. 2D CP about SO_4^{-2} (X_3) and $CaCI_2$ (X_5).

2D contour plot displayed the irregular contour plot. (X_3) and (X_5) had a higher degree of interaction. According to Figure 4.18, carbohydrate percentages (green color) were 33.4%, 24.7% and 26.3% at 10 mg/L, 200 mg/L and 70 mg/L of SO_4^{-2} and 10 mg/L of $CaCI_2$, respectively. In conclusion, the percentage of carbohydrates changed with the change in the amount of SO_4^{-2}.

Then, we plotted a 3D response surface plot about NaCI (X_4) and $CaCI_2$ (X_5). 3D response surface plots (RSP) and 2D contour plots (CP) related with concentrations of NaCI (X_4) and $CaCI_2$ (X_5) were shown in Figure 4.19 and Figure 4.20, respectively.

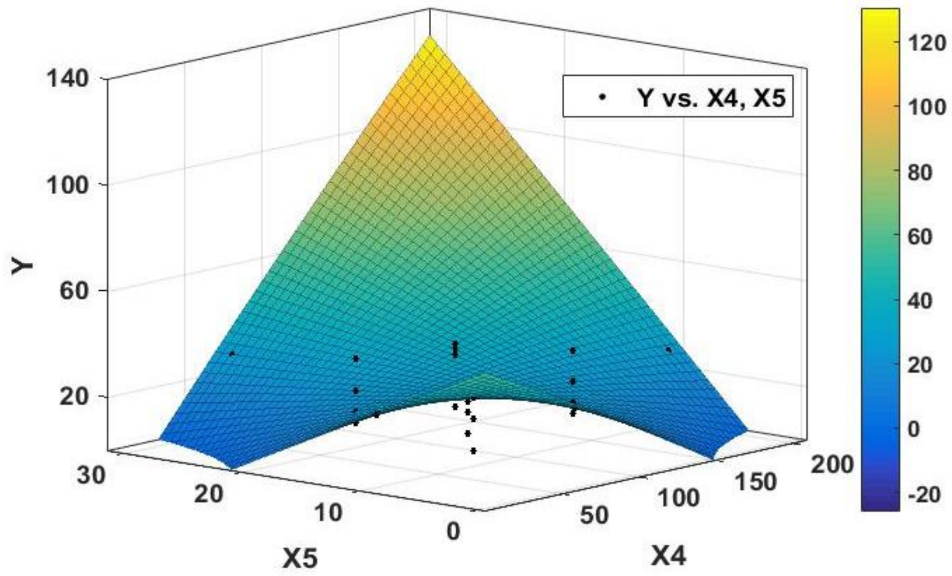

Figure 4.19. 3D RSP about NaCI (X_4) and $CaCI_2$ (X_5).

Carbohydrate percentages increased when concentrations of NaCI and $CaCI_2$ increased up to level -1. Carbohydrate percentages (light blue color) were 28.6%, 29.1% and 28.9% at 1 mg/L, 30 mg/L and 10 mg/L of $CaCI_2$ and 70 mg/L of NaCI. When the amount of $CaCI_2$ increased while the NaCI concentration was constant, the carbohydrate percentage did not change significantly.

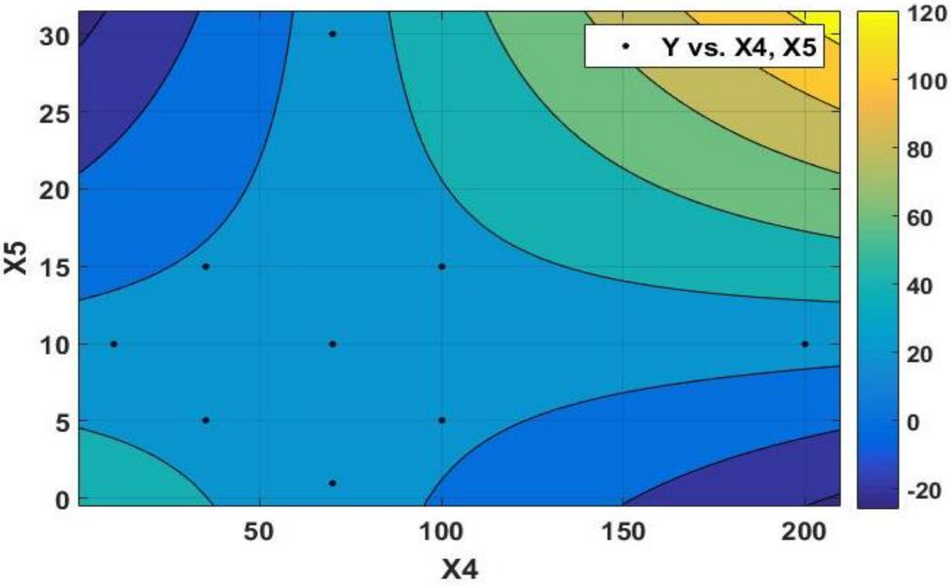

Figure 4.20. 2D CP about NaCI (X_4) and $CaCI_2$ (X_5).

2D contour plot displayed the irregular contour plot. (X_4) and (X_5) had a higher degree of interaction. According to Figure 4.20, carbohydrate percentages (light blue color) were 24.7%, 26.3% and 28.3% at 70 mg/L, 10 mg/L and 200 mg/L of NaCI and 10 mg/L of $CaCI_2$, respectively. The changes in both variables contributed to the carbohydrate percentage as a blue color.

There are many studies on RSM in the literature. The application of this to microalgae has recently contributed to the literature. In the literature, *Chlorella minutissima* was studied in a 1500 L raceway open pond. Temperature, methanol ratio, microwave power, and reaction time versus lipid productivity were carried out response surface methodology. Also, transesterification yield was investigated. Lipid percentage was 19.98% under semi-continuous mode. Biodiesel yield was 96.6% at 65 ^oC, 11:1 of alcohol to oil ratio, 800 W, and 7 min (Sharma et al., 2021). *Coccomyxa sp.* KJ was carried out for evaluation of flocculation performance via RSM. Acrylamide-methacrylic acid ester-acrylic acid was used for flocculation. pH and dosage were determined. Stirring time for optimization. The maximum flocculation efficiency was 98% at 339.3, pH 9, and 28.54 mg/L of acrylamide-methacrylic acid ester-acrylic acid with RSM (Wang et al., 2021). In another study, *Spirulina platensis* and *Scenedesmus obliquus* were examined to optimize biomass yield and biochemical components in domestic wastewater through RSM. *Scenedesmus obliquus* had higher light intensity than *Spirulina platensis*. Also, *Scenedesmus obliquus* had higher growth, chlorophyll, and lipid content in domestic wastewater than those of *Spirulina platensis* at the optimized light regimes. In addition, *Spirulina platensis* had higher concentrations of proteins and carbohydrates than those of *Scenedesmus obliquus* (Fan et al., 2020). Barahona et al. studied the direct use of *Chlorella Vulgaris* for brackish water desalination by RSM. The content of the BG-11 medium changed with calcium, nitrate, and other minerals. Microalgae growth, salt exclude efficiency was affected positively in modified BG-11 medium. Electrical conductivity was diminished up to 80% between 1000 and 5000 ppm of NaCI (Barahoei et al., 2021). Parameters of a second-generation magnetically induced membrane vibration were determined by RSM. 4 min of cycle time, and 50% vibration rate was the maximum harvesting parameters for *Dictyosphaerium sp* (Zhao et al., 2020). Tork et al. studied the harvesting efficiency of *Chlorella Vulgaris* using cationic starch nanoparticles utilizing RSM. Microalgae concentration, cationic starch nanoparticle, and pH were determined as variables for harvesting efficiency. Microalgae concentration, cationic starch nanoparticle, and pH were 0.75 g/L, 7.1 g/L, and 11.8 for maximum harvesting efficiency, respectively (Tork et al., 2017). In another study, Spirulina platensis was studied to increase bio-oil utilizing recycled biochar-supported catalysts. In this study, RSM was used, and the maximum bio-oil efficiency was 33% at 250 ^oC for 30 min in 0.2 g of biochar-supported catalyst. The energy yield was 65.34% (Kandasamy et al., 2021). Bio-cement was produced via *Chlorella kessleri* from cement kiln dust utilizing RSM. Maximum $CaCO_3$ productivity was 25.18 g at 23 ^oC and pH 10.63 in 9 d (Irfan et al., 2019). Khalid et al. demonstrated the elemental composition of *Chlorella sorokiniana* in agricultural wastewater via RSM. Light intensity, inoculum size, and photoperiod were determined as variables. The nutrient removal efficiency was calculated with these variables. Ammonium and phosphate removals were 93.36% 94.50% at 200 mmol photon m^{-2} s^{-1}, photoperiod of 12h, and inoculum size of 28%, respectively. These values were maximum (Khalid et al., 2019). In another study, *Nannochloropsis oculata* was studied for biodiesel production using $ZnOMn^{2+}$ nano-catalyst. Optimum conditions were determined with oil to methanol molar ratio, temperature, catalyst amount, and time. The maximum biodiesel yield was 87.5% at 1:15 of oil: methanol ratio, 60 ^oC, 3.5% of catalyst percentage for four h (Raj et al., 2019).

4.5. ANOVA Analysis

Estimation of carbohydrate concentration of *Gloeocystis vesiculosa* 901 was carried out using ANOVA. *F* and *P* values, degrees of freedom (DF), the sum of squares (SS), and mean square (MS) were calculated for the determination of carbohydrate concentration. ANOVA results for this model were given in Table 4.6.

Table 4.6. ANOVA results for carbohydrate concentration of *Gloeocystis vesiculosa* 901

Source	Carbohydrate Percentage		(%)			
	DF [a]	SS [b]	MS [c]	F-value	P-value	Significance
Model	20.0000	1689.3446	84.4672	18.7277	0.0001	Significant
Residual	15.0000	67.6543	4.5103			
Total	35.0000	1756.9989				

F and *P* values were 18.7277 and 0.0001, respectively. P-value was < 0.05. It means that model is important. In addition, ANOVA results for the estimated coefficients of carbohydrate concentration of *Gloeocystis vesiculosa* 901 were given in Table 4.7. R^2 was 0.9615, and the adjusted R^2 value was 0.9102.

Table 4.7. ANOVA results for the estimated coefficients

Source	Carbohydrate percentage (%)				
	Coefficient	StdErr[a]	t value[b]	P value[c]	Significance
(Intercept)	-0.54475	6.6172	-0.082324	0.93548	
X_1	0.1321	0.017322	7.6263	1.5442e-06	Significant
X_2	0.2592	0.066564	3.894	0.0014386	Significant
X_3	-0.0053043	0.068123	-0.077864	0.93897	
X_4	0.06291	0.068123	0.92348	0.37038	
X_5	0.047116	0.44656	0.10551	0.91737	
X_1X_2	-0.00011592	9.7094e-05	-1.1939	0.25107	
X_1X_3	0.00010971	0.00011912	0.92101	0.37162	
X_1X_4	-0.00011283	0.00011912	-0.94721	0.35854	
X_1X_5	-0.00084047	0.00077464	-1.085	0.29506	
X_2X_3	-0.00060575	0.00040477	-1.4965	0.15527	
X_2X_4	0.00052567	0.00040477	1.2987	0.21367	
X_2X_5	0.0033179	0.0026321	1.2605	0.22673	
X_3X_4	-0.00058413	0.00050187	-1.1639	0.26264	
X_3X_5	-0.0039932	0.0032647	-1.2231	0.24015	
X_4X_5	0.0037761	0.0032647	1.1566	0.26551	
X_1X_1	-0.0001305	1.2338e-05	-10.577	2.3769e-08	Significant
X_2X_2	-0.0012319	0.00028408	-4.3366	0.00058704	Significant
X_3X_3	0.0002377	0.00015984	1.4871	0.15769	
X_4X_4	-0.00026184	0.00015984	-1.6381	0.12219	
X_5X_5	-0.00097698	0.0069952	-0.13966	0.89078	

Intercept had a negative value (-0.54475). X_1 and X_2 had positive effects on Y value (carbohydrate percentage). Furthermore, X_1 and X_2 showed a significant effect on carbohydrate percentage because P values were < 0.05. X_3 had a negative coefficient value. But, this negative effect can be ignored because P-value was 0.93897, and the effect on Y was insignificant. On the contrary, X_4 and X_5 had negative coefficient values, and P values were higher than 0.05. Variables were unimportant for the equation. Also, mean square effects were calculated. X_1X_1 and X_2X_2 had negative effects on carbohydrate concentration, and the coefficients of variables were negative values. P values were < 0.05, and coefficient contributions were important. X_3X_3 displayed a positive coefficient value. However, it did not contribute significantly to the Y value due to $P>0.05$. Similarly, X_4X_4 and X_5X_5 were insignificant.

4.6. Bio-ethanol Content

After carbohydrate concentration was obtained at the maximum level, bio-ethanol was produced according to Bonnichsen and Theorell (Bonnichsen and Theorell, 1951). Carbohydrate content was produced at 360 mg/L of glucose, 100 mg/L of $NH4^{+}$, 35 mg/L of $SO4^{-2}$, 100 mg/L of NaCI, and five mg/L of $CaCI_2$. The concentration of bio-ethanol in *Gloeocystis vesiculosa* 901 was determined as the ethanol-dependent reduction of NAD^{+} at 340 nm spectrophotometrically. (Rizza et al., 2017). Carbohydrate concentration was approximately 4.36 g/L for ten g/L of biomass. Bio-ethanol content was 2.12 g/L.

5. CONCLUSION

Microalgae have started to work on wastewater treatment and bio-energy in environmental engineering in recent years. Bioethanol is one of the most preferred types of bio-fuel due to the properties they contain. Bio-ethanol and carbohydrate content potential of *Gloeocystis vesiculosa* was investigated due to its good growth properties and resistance to contamination under municipal wastewater. In this study, various concentrations of glucose, NH_4^+, SO_4^{-2}, NaCI, and $CaCI_2$ were examined to determine their maximum carbohydrate concentration using RSM. The highest biomass concentration was 0.63 g/L at 90 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 35 mg/L of NaCI, and 5 mg/L of $CaCI_2$. In addition, the maximum carbohydrate concentration was experimentally obtained at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and 5 mg/L of $CaCI_2$ with 251 mg/L. The highest carbohydrate percentage was 44% at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and five mg/L of $CaCI_2$. The maximum theoretical carbohydrate percentage was 43.3% at 360 mg/L of glucose, 100 mg/L of NH_4^+, 35 mg/L of SO_4^{-2}, 100 mg/L of NaCI, and 5 mg/L of $CaCI_2$. According to these results, the carbohydrate percentage calculated theoretically with the RSM method could be estimated with a very close value. Moreover, we examined the interactions of the medium components with each other. X_1, X_2, X_1X_1, and X_2X_2 had a significant effect on the model. The carbohydrate obtained from these components was used to produce bioethanol, and carbohydrate concentration was approximately 4.36 g/L for ten g/L of biomass. Bio-ethanol content was 2.12 g/L. In conclusion, *Gloeocystis vesiculosa* can be used for bio-ethanol production as large scale in synthetic municipal wastewater in the future.

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(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

SENTETİK ATIKSUDA *Gloeocystis Vesiculosa*'DAN BİYOETANOL ÜRETİMİ

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ÖZ

Mikroalgler çevre mühendisliğinde atıksuyun arıtılmasından biyo-yakıt oluşumuna kadar birçok alanda kullanılmaktadır. Bu tez çalışmasında, *Gloeocystis vesiculosa* 901 sentetik belediye atık suyunda büyütüldü ve kültürlendi. Atıksuyu oluşturan büyük bileşenler olan glukoz, amonyum, sülfat, sodyum ve kalsiyumun çeşitli konsantrasyonları içerisinde *Gloeocystis vesiculosa* 901 büyütüldü ve bunların cevap-yüzey yöntemi (CYY) ile teorik olarak tahminleri yapılarak karbonhidrat yüzdesi hesaplandı. *Gloeocystis vesiculosa* 901'in en yüksek biyo-kütlesi 0,63 g/L olarak 90 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 35 mg/L NaCI ve 5 mg/L $CaCI_2$ içerisinde hesaplandı. Deneysel olarak en yüksek karbonhidrat içeriği 251 mg/L ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI ve 5 mg/L $CaCI_2$ içerisinde bulundu. Deneysel olarak en yüksek karbonhidrat yüzdesi %44 ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI and 5 mg/L $CaCI_2$ olarak gerçekleşti. Buna ek olarak, cevap yüzey metodu kullanılarak teorik olarak hesaplanan en yüksek karbonhidrat yüzdesi %43,3 ile 360 mg/L glikoz, 100 mg/L NH_4^+, 35 mg/L SO_4^{-2}, 100 mg/L NaCI and 5 mg/L $CaCI_2$ oldu. Buradan elde edilen karbonhidrat biyo-etanol üretimi için kullanıldı. 10 g/L biyo-kütle içerisindeki karbonhidrat konsantrasyonu yaklaşık olarak 4.36 g/L ve biyo-etanol içeriği de 2.12 g/L olarak hesaplandı. Bu sonuçlara göre *Gloeocystis vesiculosa* gelecekte daha büyük sistemlerde sentetik atıksu içerisinde biyo-etanol üretimi için kullanılabilir.

Anahtar kelimeler: Gloeocystis vesiculosa, Biyo-etanol, Atıksu, CYY, Karbonhidrat içeriği Karbonhidrat içeriği

1. GİRİŞ

1.1. Enerji

Enerji iş yapabilme yeteneğidir ve kimyasal olarak kinetik ve potansiyel olarak iki durumda bulunabilir. Bu enerji bir formdan diğerine kimyasal, termal ve elektrik olarak dönüştürülebilir ve depolanır (Azad et al.,2015).

1.2. Enerji kaynakları

1.2.1. Yenilenemez enerji kaynakları

Fosil yakıtlar yenilenemez enerji kaynaklarıdır ve sürdürülemez şekilde oluşan enerji kaynaklarına verilen isimdir.

1.2.2. Yenilenebilir enerji kaynakları

Kullanıldıktan sonra başka bir forma dönüştürülerek tekrar oluşturulabilen enerji kaynaklarına yenilenebilir enerji kaynağı denir. Bunlara örnek olarak rüzgâr, hidrolik, solar, jeotermal ve biyokütle kaynaklarından elde edilebilen enerji türleri verilebilir.

2. KAYNAK BİLDİRİŞLERİ

2.1. Yenilenebilir Biyoyakıtlar

Biyokütle yenilenebilir enerji kaynağı olarak kullanılabilir. Biyokütleden elde edilen kaynaklar daha sonra biyoetanol ve biyodizel gibi yakıtlara dönüştürülebilirler (Ho et al.,2010). Bu yakıt tipleri katı, sıvı ve gaz halinde olabilirler. Biyoetanol ve biyodizel sıvı yakıt türleri iken biyometan ve biyohidrojen gaz yakıt türleridir (Nigam and Singh, 2011).

2.2. Mikroalglerden Biyokütle Üretimi

Mikroalgler gerçek kök, gövde ve yaprakları olmayan tek veya çok hücreye sahip olabilen canlılardır. Yeşil olarak bilinen türleri klorofil üretebilirler (Brown et al, 1996). Bu türler inorganik karbondan organik madde üretebilme kapasitesine sahiptir.

2.3. Mikroalglerin Üretim Yolları

Mikroalgler çeşitli yollar ile üretilebilirler. Bu yollar sürekli, kesikli veya yarı kesikli sistemler olabilirler. Bunların dışında açık havuzlarda da üretimi sağlanabilir. Fotobiyoreaktörde olanları daha kontrollü fakat az miktarda biyokütle üretimi sağlarlarken, açık havuzda büyütülenler kontaminasyona karşı daha fazla maruz kalırlar ama biyokütlenin daha yüksek miktarları üretilir (Tredici and Materassi, 1992). Bunun yanında mikroalgler besiyeri denilen özel ortamlar ile atıksu gibi organik besin kaynağı olan çeşitli ortamlarda da büyütülebilir. Bunların büyüme hızı tamamen büyütülen alg türüne göre değişebilir.

2.3.1. Fotobiyoreaktörler

Fotobiyoreaktörler çeşitli tiplerde olabilirler. Düz panel, kolon, tüp şeklinde reaktörler mikroalglerin kültürlenmesi için kullanılırlar. Bunların birleşimi ile oluşan hibrid sistemler de mikroalglerin büyütülmesi için uygundur (Brennan and Owende, 2010).

2.3.2. Açık Havuzlar

Mikroalglerin yüksek biyokütle ile üretilmesi için açık havuzlar diğer uygun seçenek olabilir. İyice büyüme düzenleri bilinen mikroalgler açık havuzlarda sanayi için yetiştirilebilir. Bunlarda ki en önemli problem ışığın ortamın her yerine özellikle dip kısımlarına iletilmesindeki sıkıntılar ve kontaminasyon sorunları gösterilebilir (Ghasemi et al., 2012).

2.4. Mikroalglerin Hasatlanması

Mikroalgler çeşitli yöntemler ile hasatlanabilir. Bu yöntemler kimyasal, fiziksel veya mekanik metotlar olabilirler.

2.5. Mikroalglerin Enerjiye Dönüşümü

Mikroalgler enerjiye termokimyasal ve biyokimyasal yöntemlerle olmak üzere iki kısımda dönüştürülebilirler. Termokimyasal dönüşümde gazlaştırma, piroliz ve tuzdan arındırma metotları sıklıkla kullanılır. Biyokimyasal dönüşümde ise genelde mikroorganizmalar kullanılarak üretim yapılır (McKendry, 2002).

2.6. Mikroalglerden Biyoetanol Üretimi

Mikroalglerden ilk önce biyokütle eldesi sağlanır. Daha sonra mikroalglerden çeşitli kimyasal veya enzimatik yollarla şeker üretimi yapılarak fermantasyon basamağı sonunda biyoetanol üretimi yapılabilir (Brennan and Owende, 2010).

2.7. Mikroalglerin Atıksuda Kullanımı

Sanayi, tarımsal ve belediye atıkları ile birlikte çok miktarda atıksu oluşmaktadır. Bu atık sular yoğun miktarda organik ve inorganik bileşikler içerirler. Bu organik ve inorganik bileşikler mikroalgler tarafından kullanılabilir. Bu sayede mikroalgler hem büyüyebilir hem de atıksuyun arıtımı sağlanabilir (Edyvean and Iqbal, 2004). 1950lerin başından beri mikroalglerin atıksuda büyütülmesi çalışmaları devam etmektedir (Zhou et al., 2014). Bu sayede suda ki toksin maddeler, fosfor, azot gibi kimyasallar ve suyun arıtılması işlemleri kolaylıkla yapılabilir.

3. MATERYAL ve YÖNTEM

3.1. *Gloeocystis vesiculosa* 901'in büyütülmesi

Bu alg türü Çekyadan Culture Collection of Autotrophic Organisms (CCALA) biriminden elde edilmiştir. Alınan örnekler 24 ± 2 °C de kültürlendi. Bu işlem için örnekler ilk olarak 300 mL'lik flaskler içerisinde 200 mL besi yerinde büyütüldüler. Daha sonra 1L lik fotobiyoreaktöre alınarak ışık şiddeti 130 µmol m-2s-1 ayarlanarak büyütüldüler. Bu çalışmada atık su olarak sentetik belediye atık suyu kullanıldı.

3.2. Optik yoğunluk ölçümü

Optik yoğunluk ölçümleri 680 nm de spektroskopik olarak gerçekleşti. Durağan faza gelen örnekler alınarak deneyler için kullanıldı.

3.3. Biyokütle konsantrasyonlarının ölçümleri

Örnekler 3000g de 4 °C de ve 10 dakika boyunca santrifüj edilerek çöktürüldüler. Daha sonra üç defa saf su ile yıkanarak filtre ile süzüldüler. 105°C de fırında bir gece bekletildiler ve biyokütle ölçümü için tartıldılar.

3.4. Örneklerin hidrolizi

Bu çalışmada örnekler asit hidroliz ile kimyasal hidrolize tabi tutuldular. Bunun için 1M HCI kullanıldı ve örnekler parçalandı (Onay, 2019).

3.5. Karbonhidrat konsantrasyonunun hesaplanması

Karbonhidrat miktarı anthrone metoda göre yapıldı (Zhao et al., 2013). Glikoz standart olarak kullanıldı ve ölçümler 620 nm de yapıldı.

3.6. Örneklerin fermantasyonu

Fermantasyon işlemi için Saccharomyces cerevisiae kullanıldı. Kültür ortamı Harun metoduna göre hazırlandı (Harun et al., 2010; Harun et al., 2011). Fermantasyon ortamı 35 °C, 140 rpm ve 48 saat olarak belirlendi.

3.7. Biyoetanol üretimi

Biyoetanol Bonnichsen and Theorell metoda göre yapıldı (Bonnichsen and Theorell, 1951).

3.8. Cevap-yüzey metodu

Bu çalışmada glikoz, amonyum, sülfat, NaCI ve CaCI2 olmak üzere 5 değişken belirlendi ve bu ortamlarda örneklerin karbonhidrat içerikleri belirlenildi.

4. BULGULAR ve TARTIŞMA

4.1. Biyokütle konsantrasyonu

En yüksek biyokütle konsantrasyonu 90 mg/L glikoz, 100 mg/L NH4+, 35 mg/L SO4-2, 35 mg/L NaCI ve 5 mg/L CaCI2 şartlarında 0,63 g/L olarak bulundu.

4.2. Karbonhidrat konsantrasyonu

En yüksek karbonhidrat konsantrasyonu 251 mg/L olarak 360 mg/L glikoz, 100 mg/L NH4+, 35 mg/L SO4-2, 100 mg/L NaCI ve 5 mg/L CaCI2 şartlarında bulundu.

4.3. Cevap-yüzey metodu sonuçları

En yüksek teorik karbonhidrat yüzdesi %43,3 ile 360 mg/L glikoz, 100 mg/L NH4+, 35 mg/L SO4-2, 100 mg/L NaCI ve 5 mg/L CaCI2 şartlarında gerçekleşti.

4.4. ANOVA sonuçları

Model p-değeri 0,0001 olarak bulundu ve önemli olarak belirtildi. R2 0,9615 ve ayarlanmış R2 değeri 0,9102 idi. X1 ve X2 değişkenleri önemli bulundu. X1X1 ve X2X2 diğer önemli iki değişken idi.

4.5. Biyoetanol içeriği

Biyoetanol içeriği 2,12 g/L olarak 10 g/L biyokütlede 4,36 karbonhidrat konsantrasyonlarında bulundu.

5. SONUÇ

Gloeocystis Vesiculosa gelecekte daha geniş yelpaze de biyoetanol üretimi için kullanılabilir.

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