REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

A MULTICRITERIA DECISION ANALYSIS FOR THE EVALUATION OF MICROALGAL GROWTH AND HARVESTING

Elifnaz UNAY

MASTER OF SCIENCE THESIS

Department of Environmental Engineering

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Advisor

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A thesis submitted by Elifnaz UNAY in partial fulfillment of the requirements for the degree of MASTER'S is approved by the committee on 20.05.2021 in Department of Environmental Engineering, Environmental Engineering Program.

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Dedicated to my family

and my best friend

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LIST OF SYMBOLS

m^2	Area
d	Day
°C	Degree Celcius
Si+	Distance from Ideal Best
Si-	Distance from Ideal Worst
kWh/m^3	Electric Consumption Measured in Kilowatt Hour per Cubic Meter
V+	Ideal Best Value
V-	Ideal Worst Value
L	Liter
g/L	Measurement of mass concentration as gram per liter
min	Minutes
%	Percentage
Pi	Performance Scores
mPa	Pressure in megaPascal unit
$	Price in Dollars
#	Rank
g	Weight in Grams

LIST OF ABBREVIATIONS

AFDW	ASH Free Dry Weight
CAPEX	Capital Expenses
CF	Concentration Factor
DAF	Dissolved Air Flotation
ECF	Electro-Coagulation-Flocculation
EROI	Energy Return on Investment Rate
GHG	Greenhouse Gas
GWP	Global Warming Potential
LCA	Life Cycle Assessment
MCDA	Multi-criteria Decision Analysis
MCDM	Multi-criteria Decision Making
MUFA	Monounsaturated Fatty Acids
NER	Net Energy Ratio
OPEX	Operational Expenses
PBR	Photobioreactor
PE	Photosynthetic Efficiency
PUFA	Polyunsaturated Fatty Acids
RE	Recovery Efficiency
RED	Renewable Energy Directive
SAF	Suspended Air Flotation
TEA	Techno-Economic Analysis
TOPSIS	Technique for Order Preference by Similarity to Ideal Solution
VAPs	Value Added Products
VFAs	Volatile Fatty Acids

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ABSTRACT

A Multicriteria Decision Analysis for the Evaluation of Microalgal Growth and Harvesting

Elifnaz UNAY

Department of Environmental Engineering

Master of Science Thesis

Advisor: Prof. Dr. Bestami ÖZKAYA

Increasing food and energy demands worldwide, coupled with air and water pollution lead researchers to focus on new renewable, sustainable resources. As microalgae represent, a natural, non-toxic, renewable, and sustainable feedstock for various fields such as dietary supplements, nutraceuticals, biofuels, food colorants, medicinal and pharmaceutical field, animal fodder, cosmetics, etc. microalgal biomass research studies gained interest in recent years. Although they are an environmentally friendly option with rapid growth rates and high biomass yields, microalgae cultivation and harvesting on large scale still have some challenges and limitations. After reviewing the growing methods for phototrophic microalgae and harvesting techniques both suitable for large-scale commercialization current conversion technologies and suitable utilization fields for microalgal biomass are reviewed and discussed in detail with their environmental applications. TOPSIS method is adopted in this study, by using a multicriteria decision-making tool excel spreadsheets are used to evaluate the information that is derived from literature and pilot-scale studies to find the most suitable alternative for microalgae cultivation and harvesting on large scale. With microalgae field having multiple pathways related to each other for a system to be viable, it is important to select the right microalgae species, culturing and harvesting systems, and conversion technologies. As the result of the TOPSIS analysis without over significant dominance tubular (helical) PBR and plate (glass-flat Panel) PBR deemed suitable among other cultivation methods and without over significant dominance bio-flocculation and chemical harvesting using an organic flocculant chitosan were deemed suitable for harvesting, while plastic bag PBR and suspended air flotation were deemed the least acceptable alternatives among others.

Keywords: TOPSIS, microalgae, microalgae utilization, cultivation systems, harvesting techniques

ÖZET

Mikroalg Üretim ve Hasat Yöntemlerinin Çok Kriterli Karar Analizi ile Değerlendirilmesi

Elifnaz UNAY

Çevre Mühendisliği Bölümü

Yüksek Lisans Tezi

Danışman: Prof. Dr. Bestami ÖZKAYA

Dünya genelinde artan enerji ile gıda ihtiyacı ve beraberinde artan çevresel kirlilik araştırmacıları yenilenebilir, sürdürülebilir yeni kaynaklar bulmaya yöneltmiştir. Mikroalglerden elde edilen biyokütlenin, doğal, yenilenebilir, sürdürülebilir ve toksik olmayan hammaddeyi temsil etmesi ve biyoyakıtlar, ilaç sektörü, kozmetik, gıda ve hayvan yemleri gibi birçok endüstride geniş uygulama alanlarına sahip olması nedeniyle bu alanda yapılan çalışmalar son yılllarda büyük ilgi görmeye başlamıştır. Yüksek biyokütle verimleri, hızlı büyüme oranları ile birlikte aynı zamanda çevre dostu bir seçenek olmasına ragmen mikroalglerin büyük ölçekli olarak üretim ve hasat henüz ekonomik olarak uygun ve sürdürebilir bir şekilde gerçekleştirilememektedir. Sistemlerin büyük ölçekli uygulanılmasını etkileyen birden fazla faktör olduğundan dolayı uygun tür, yetiştirme ve hasat yöntemleri ile uygun dönüşüm teknolojilerinin kullanılması bu konuda ayrı bir önem arz etmektedir. Bu çalışmada geniş ölçekli üretime uygun fototrofik mikroalg üretim metotları ve hasat yöntemleri ile elde edilen biyokütlerini kullanım alanları, dönüşüm teknolojileri ve çevresel uygulamaları detaylı bir şekilde incelenmiştir. Literatürden ve pilot ölçekli çalışmalardan alınan veriler, geniş ölçekte mikroalg üretim ve hasat yöntemlerinden en uygun seçeneğini bulmak bir çoklu kriterli karar analizi olan TOPSIS (Technique for Order of preference by Similarity to Ideal Solution) analizi ile değerlendirilmiştir. Uygulanan analiz sonucunda, cam malzeme kullanılan sarmal fotobiyoreaktör ve levha fotobiyoreaktör mikroalg yetiştime sistemleri arasında; chitosan kullanılarak yapılan kimyasal hasat ve biyo-flokülasyon metodları ise hasat yöntemleri arasında en uygun yöntemler olarak belirlenirken plastik torba kullanılan fotobiyoreaktör ve hava flotasyonu (SAF) yönteminin uygun olmadığı sonucuna varılmıştır.

Anahtar Kelimeler: Mikroalg, üretim metodları, hasat yöntemleri, TOPSIS, kullanım alanları

1
INTRODUCTION

1.1 Literature Review

As the population worldwide increases day by day, demand for energy and feedstock along with pollution increase rapidly, this led to develop sustainable alternative resources which are environmentally friendly and economically viable. The microalgae term comprehends the variety of eukaryotic and prokaryotic organism, which can grow autotrophically and heterotrophically. Various research studies showed that microalgae have great potential as a valuable renewable bioresource for substances (lipids, proteins, vitamins, carbohydrates) shown in Table 1.1. These substances can be utilized in various industries such as cosmetics, dietary supplements, biofuels (bioethanol, biodiesel, etc.), bioproducts, cosmetics, human nutrition, and animal fodder besides their important environmental applications such as bioremediation of wastewaters and CO_2 bio-fixation (Khan et al., 2018; Barkia et al., 2019; Leng et al., 2020).

It is stated that nearly 7000 tons of dry algal biomass are produced worldwide and the worth of algae biomass global market value differs from 3.8 to 5.4 billion USD (Tang et al., 2020). The process of extracting valuable bioproducts starts with selecting the right strain and cultivation method for microalgae biomass which is classified as upstream process and once the microalgae reached it mature stage, cultivation is followed by downstream processes such as harvesting, extraction, purification, and conversion technologies to obtain the target products.

Although microalgal biomass offers many benefits by being renewable and non-toxic with derived bio-products having multiple utilization fields, there are still some challenges associated for obtaining the target products from the cultured and harvested microalgae biomass in large-scale commercialization. These challenges include the design of the cultivation and harvesting systems, overall system performances, culture stability, high costs and biomass yields, and the current extraction and conversion technologies being complex and having high costs which make large-scale commercialization impractical.

Table 1.1 Biochemical composition of some microalgae strains on a dry matter basis (%) (Demirbas and Demirbas, 2011)

Species of sample	Proteins	Carbonhydrates	Lipids	Nucleic Acid
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chylamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6-20	33-64	11-21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesium parvum	28-45	25-33	22-38	1-2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46-63	8-14	4-9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43-56	25-30	4-7	-

1.1.1 Microalgae Biology and Species Selection

Microalgae are photosynthetic microorganisms that can grow in diverse aquatic habitats (ponds, lakes, rivers, oceans, wastewater streams, etc.). They can adapt and grow rapidly in a wide range of pH values, different light intensities, temperatures, salinities and can exist as individual cells, in chains, or in small colonies. Their size is dependent on the species and can range from a few micrometers (μm) to a few hundred micrometers (Raja et al., 2014). It is estimated that about 200,000-800,000 microalgae species exist but only a limited number, of around 30,000-50,000 have been studied and described (Grubišić et al., 2019).

The surface of the microalgae cells are negatively charged (−7.5 to −40 mV) therefore, they are suspended in the aqueous medium (Yin et al., 2020). Microalgae produce approximately half of the atmospheric oxygen by using light, water, nutrients (phosphorus and nitrogen as major nutrients) and carbon dioxide to grow photo-autotrophically as seen in the following Equation 1.1 (Falkouski & Raven, 2007):

$$6CO_2 + 12H_2O + \text{Light} + \text{Chlorophyll} \rightarrow (C_6H_{12}O_6) + 6H_2O + 6O_2 \qquad (1.1)$$

Although they do not have roots, stems, or leaves like terrestrial plants; microalgae are more efficient at utilizing sunlight while consuming harmful pollutants, having minimal resource requirements and do not compete with food or agriculture for precious resources. They received attention recently because of their adaptability in new environments, high biomass yields, and multiple utilization fields. The high lipid content of some microalgae strains has a promising potential to produce biofuels while long-chain acids, pigments, and protein contents can be used in the pharmaceutical and nutritional applications (Eleren and Oner, 2019).

Microalgae are classified into different groups according to their chemical nature and pigment composition, morphologic characteristic, cell wall, etc. Among the eukaryotic, green microalgae species class *Chlorophyceae*, those most widely utilized in the commercialization field recently belong to the genera *Chlamydomonas, Chlorella, Haematococcus*, and *Dunaliella* (Figure 1.1).

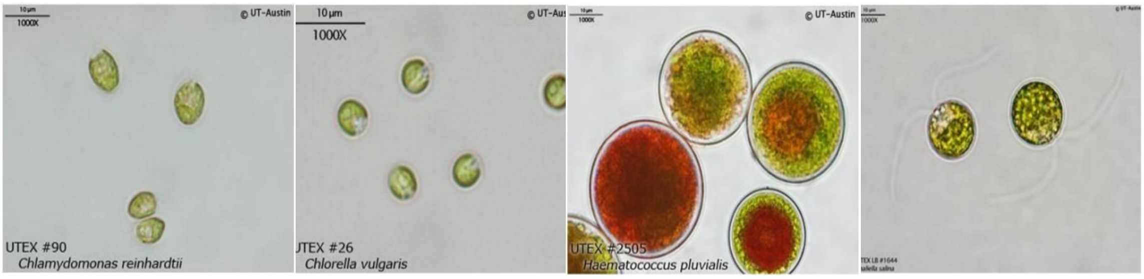

Figure 1.1 Microscopic view of *Chlamydomonas reinhardtii, Chlorella vulgaris, Haematococcus pluvialis* and *Dunaliella salina* respectively. (Culture Collection of Algae, University of Texas at Austin(utex.org))

By changing the abiotic factors like temperature, light, CO_2, and nutrient supply the chemical composition of microalgae can change but the main factor affecting the biochemical composition of cultured microalgae cells is the microalgae strain selection. Therefore, selecting the right strain is as important as selecting the right cultivation and harvesting system for a sustainable and viable system design.

In this study, microalgae species *Chlorella* is selected to evaluate large scale cultivation and harvesting due to its easy and rapid growth potential and bioactive compound content which can be used as a raw material/ resource in multiple fields.

As a single-celled primary green algae, about 2 to 10 μm in diameter and in a spherical shape without flagella, *Chlorella* can multiply rapidly, by photosynthesis requiring small amounts of minerals. Biochemical composition of *Chlorella* contains chlorophyll-a and chlorophyll-b (green photosynthetic pigments), a large amount of lipids, proteins, polysaccharides, vitamins, pigments, and various other bioactive compounds (Saad et al., 2019). A general biochemical composition for *Chlorella* cells can be seen in the Figure 2.1.

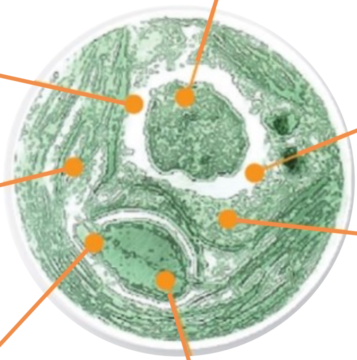

Figure 1.2 General biochemical composition of *Chlorella* (adapted from FDA website)

Chlorella is a feedstock of which can be utilized in pharmaceuticals, cosmetics, health supplements, animal fodders, agricultural purposes, biofuels beside its benefits in wastewater treatment and atmospheric CO_2 mitigation. Some examples of bioactive compounds that can be obtained by culturing and harvesting *Chlorella*;

- Biofuels: biogas, bioethanol, biodiesel, biohydrogen,
- Animal fodder: feed for fish and farm animals
- Human food supplements and food additives: Vitamins A, B1, B2, B12, C, E, folic and pantothenic acid, biotin; Omega 3 fatty acids; proteins; etc.
- Skin care and pharmaceutical products: anti-irritant and antiaging creams, skin regenerating creams, anti-cancer, anti-inflammatory, immune system support

1.1.2 The Potential Use of Products Obtained from Microalgal Biomass

As mentioned in the previous section, microalgae are autotrophs, utilizing light energy to synthesize various bioactive compounds. (proteins, lipids, vitamins, pigments, etc.) After the downstream process for obtaining bioproducts from microalgae the end products which are recognized as safe, environmentally friendly, and non-toxic may vary depending on the microalgae strain selection, cultivation conditions and the selected extraction and purification methods. The obtained end products and environmental applications for microalgal biomass are generally summarized in Figure 1.3 below.

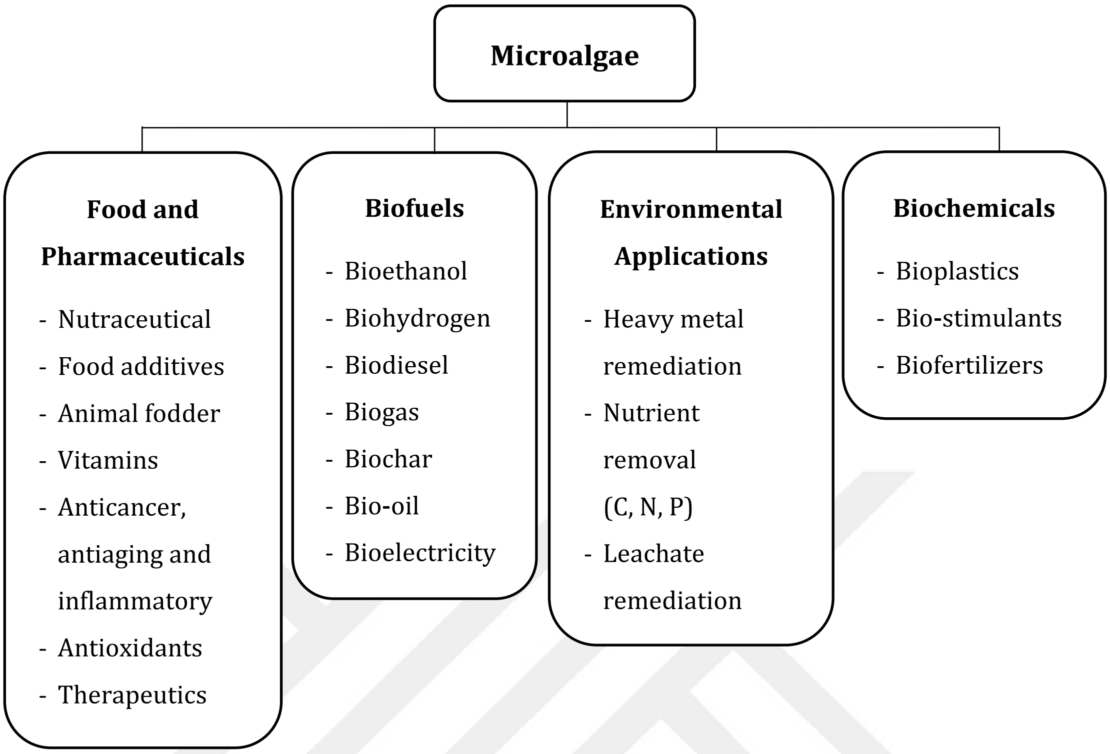

Figure 1.3 Bioproducts and environmental applications of microalgal biomass

Microalgae generally have high lipid contents consisting of polar lipids (polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), non-polar lipids (triacylglycerols molecules) or phytosterols and waxes which can be utilized in biofuels as well as pharmaceutical fields such as immune-stimulatory, detoxifying, antiviral and antibacterial, etc. (Tang et al., 2020). Biofuels is a term used to describe liquid, solid, or gaseous fuels derived from renewable raw materials. Because the growth rate of microalgae is 5-10 times faster than conventional crops and with their high lipid and protein content they are seen as a promising resource for the production of numerous biofuels such as; biodiesel, bioethanol, biogas, bio-hydrogen (Benedetti et al., 2018).

Because of the large quantities of carotenoids and pigments present in microalgae cells, they gained the attention of the food and nutrition industries also the medical and pharmaceutical industries. β-carotene, phycobiliproteins, astaxanthin, and canthaxanthin which can act as a natural colorant and food additive has promising potential in the food industry, they also have some beneficial properties like antioxidant activity. Pigments also have a market in the cosmetic industry replacing synthetic materials with their non-toxic and natural characteristics. The market value of some of the mentioned pigments is shown in Table 1.2.

Table 1.2 Market values of some pigments which can be extracted from microalgae cells (Barkia et al., 2019)

Product	Price (USD/kg)
Astaxanthin	2500-7000
β-carotene	300-1500
Omega-3	80-160

Polysaccharides and proteins are the essential products that play an important role in microalgae structure. They are commonly used as food additives and nutraceuticals also demonstrating significant health benefits. Some noteworthy microalgae species and derived bioactive compounds from them are summarized in Table 1.3 and detailed information about the potential utilization of microalgal biomass will be given in the following sections.

Table 1.3 Some microalgae species and their biotechnological applications for production of bioproducts (Benedetti et al., 2018)

Application	**High value metabolities**	**Dietary supplement and nutraceuticals**	**Biofuel**	**Cosmetics and food colorant**	**Medical and Pharmaceutical Field**
(Bioproduct category) Microalgae species	(Vitamins) *Euglena gracilis* (biotin, α-tocopherol) *Prototheca moriformis* (ascorbic acid) *Arthrospyra platensis, Chlorella spp.* (Proteins) *Arthrospyra platensis, Chlorella spp*	(Polyunsaturated fatty acids, PUFAs) *Parietochloris incise, Porphyridium spp.* (arachidonic acid) *Arthrospyra platensis, Rhodomonas salina, Tetraselmis uecica* (α-linolenic acid) *Chlorella minutissima, Monodosus spp,. Nannochloropsis spp., Neochloris oleoabundans, Pavlova lutheri* (eicosapentaenoic acid) *Crypthecodiuimu spp., Isochrysis galbana, Schizochytrium spp., Thalassiosira spp., Thraustochytrium spp.*	(Oil to biodiesel) *Botryococcus braunii, Chlorella spp., Dunaliella salina, Monoraphidium contortum, Scenedesmus spp.* (Carbohydrate to bioethanol) *Spirogyra spp., Chlorococum spp.* (Bio-hydrogen) *Chlamydomonas reinhardtii*	(Phycobiliproteins) *Arthrospyra platensis* (Carotenoids) *Arthrospyra platensis, Chlorella vulgaris, Haematococcus pluvialis, Chlorella zofngiensis,* (astaxanthin, β-carotene) *Dunaliella salina*	(Polysaccharides) *Chlorella spp.* (bglucans, starch) *Porphyridium cruentum, Netrium digitus* (Phycotoxins) *Amphidinium Dinophysis, Prorocentrum* (Phycobiliproteins) Red algae

1.2 Objective of the Thesis

Microalgae, which has the potential of being an economical and a sustainable raw material for VAPs production, are also considered as the third-generation type of biofuel. Therefore, studies on low cost, eco-friendly microalgae cultivation and harvesting techniques have increased recently to develop an alternative and economically viable system. (Borowiak et al., 2012; Muhammad et al., 2020).

In this study, the cultivation and harvesting techniques for selected microalgae species, *Chlorella*, is evaluated by TOPSIS method, which is a multicriteria decision-making tool, to identify the most acceptable alternatives among others by the current technology. Then the modelling results, current situation are compared with new developments in the microalgae field for future research. Therefore, in the following sections, microalgae characteristics with strain selection, microalgae culturing systems and current harvesting techniques will be discussed together with both advantages and disadvantages and their importance in this field.

1.3 Hypothesis

It is hypothesized that current technologies for microalgae culturing systems and harvesting methods are not suitable for large-scale commercialization because of their high costs and energy needs. Therefore, there is a need for multicriteria decision analyses for evaluating the current microalgae culturing systems and harvesting methods to find the most acceptable option both in an environmental and economical perspective.

2
MICROALGAL BIOMASS GROWTH SYSTEMS

In algal biomass pipeline, many elements contribute to system performance and overall costs. The three main factors to consider are:

- Choice of microalgae production system
- Nutrient supply with mixing and aeration strategies
- Harvesting methods and processing of biomass (Benedetti et al., 2018).

Therefore, it is important to select an effective microalgae cultivation system to overcome challenges, economical and biological, that remain associated with large-scale microalgae cultivation. The biochemical composition, biomass yields, and growth rate of microalgae are affected by abiotic parameters like CO_2, temperature, pH, nutrient contents, light, etc. Microalgae can be cultivated in four main types of metabolisms which are (Harun et al., 2010; Grubišić, et al., 2019; Tang et al.,2020);

- Phototrophic: It is known that most of the microalgae strains have high growth rates with high photosynthetic efficiency. In this phototrophic metabolite, microalgae cultured commonly in open ponds and enclosed PBRs generates energy and organic matters by the fixation of inorganic carbon, CO_2, and use sunlight as their energy source.
- Heterotrophic: In this metabolite microalgae utilize organic carbon for their growth instead of photosynthetic process. Although heterotrophic cultivation has advantages over phototrophic cultivation such as better control on cultivation system, harvesting biomass at a low-cost and there are limitations for these systems. Limited species selection, contamination from other organisms, using glucose as a substrate for culturing as well as release of CO_2 through respiration are some major limitations for these systems to use in large-scale cultivation.
- Mixotrophic: In these systems, algae which use both pathways, photosynthesize (autotrophically), and utilize organic material

(heterotrophic), for their growth and development are cultured. Microalgae cultured here can survive depending on the availability of organic carbon sources and light intensity.

- Photoheterotrophic: Like mixotrophy, microalgae cultured in this metabolite requires light to use organic compounds as the carbon source. In photoheterotrophy mode microalgae cannot adsorb and metabolize CO_2 as the main difference from mixotrophy.

Among these four main types of metabolisms, only commercially feasible one for large scale biomass production is phototrophic cultivation. Photosynthesis process of a phototrophic microalgae cell can be seen in the Figure1.4. In addition, phototrophic cultivation can act as a potential carbon sink by capturing atmospheric CO_2. (Brennan and Owende, 2010). Therefore, in this study phototrophic microalgae cultivation methods are evaluated and presented in the following section.

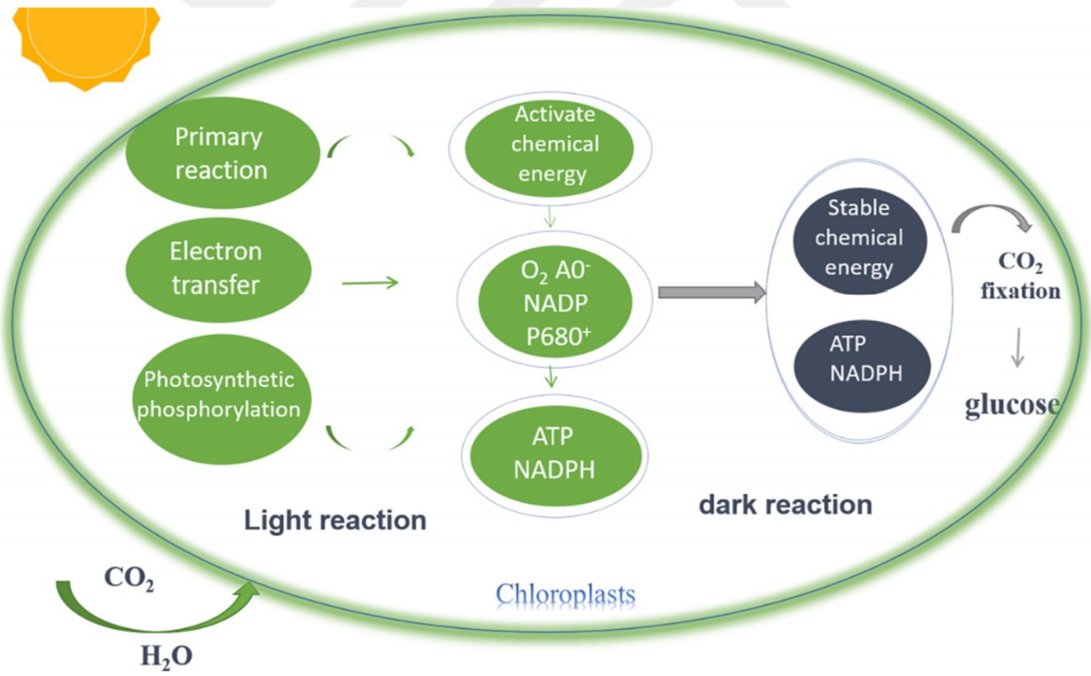

Figure 2.1 Photosynthesis process of a phototrophic microalgae cell (Adapted from Yin et al., 2020)

Microalgae cultivation systems can be classified into two main groups: open pond cultivation systems and closed photobioreactor cultivation systems. Open pond and closed photobioreactor growing systems for microalgae cultivation, each one with their advantages and disadvantages, will be discussed in detail. The comparison between open and closed microalgae PBR systems can be seen in Table 2.1 while a summarized evaluation of advantages and disadvantages for each system can be found in Table 2.3.

Table 2.1 Comparison of Open Pond and Closed Photobioreactor Cultivation Systems for Microalgae (Chen et al., 2011; Park et al., 2019; Yin et al., 2020)

Comparison Factor	Open Pond Cultivation Systems	Closed PBR Cultivation Systems
Algae species	Restricted (resistant strains are selected)	Flexible
Area- to volume ratio	Low	High
Contamination Risk	High	Very low
CAPEX and OPEX	Low	High
Control over growth conditions	Difficult	Easy
Mixing efficiency	Poor/Fair	Fair/Excellent
Biomass quality	Variable	Reproducible
Land requirement	Large	Small
Cleaning	None	Required periodically
Water loss through evaporation	High	None
Light path (utilization efficiency)	Poor	Excellent
Maintenance requirements	None / Simple	Required / Can be complex
Hydrodynamic stress on algae	Very low	Low-to-high
Harvesting costs	High	Lower

2.1 Open Pond Production Systems

This method is the oldest, cheapest and the simplest cultivation system used for large-scale microalgae cultivation. Although regular maintenance, construction, and cleaning of these systems are easy with a low amount of energy required; in these systems, the biomass yields are limited by abiotic growth factors, and large spaces are required (Medipally et al., 2015; Eleren and Oner, 2018). This method is used since the 1950s and there are various types of open pond systems, which vary based on their shape, size, inclination, construction materials, type of agitation. Because open pond systems are very sensitive to contamination, algae strains like *Dunaliella salina, Spirinula sp. and Chlorella sp.* which are resistant and have specific growth conditions are mainly used for commercial production (Jorquera et al., 2010). Because they are less expensive compared to the other production systems, open ponds systems are widely used in the commercial microalgae production (Barkia et al.,2019).

Some common types for open pond systems are:

- Raceways stirred by paddle wheels,
- Circular ponds with rotating arm,
- Unmixed circular ponds
- Cascade systems

Among those mentioned systems raceways and circular ponds stirred with rotating arms are the commonly used ones in commercial production. Therefore, in this study these two cultivation systems will be reviewed and evaluated for large scale *Chlorella* cultivation with TOPSIS method.

2.1.1 Circular Ponds (Stirring with rotating arm)

Circular ponds are typically simple, round concrete dams, which has a rotating arm (moving in axial direction) located in the center to mix the culture. (Figure 2.2). It is commonly used for cultivation of large-scale commercial microalgae, such as *Chlorella* and *Dunaliella* in Asia (El-Baz and El Baky, 2018). They can have a depth in the range of 20-70 cm and the diameter can be up to 50 m. (Lee, 2001). The size of the pond can be a limiting factor because of the poor mixing efficiency so the diameter must not be above 50 m for efficient cultivation. Although the system is cost-effective, less technical in design, easy to clean, and has a large production capacity, there are major disadvantages such as; having no control over pH, light intensity, dissolved oxygen levels, temperature, contamination, water loss due to evaporation and competition by other species. Especially heterotrophic organisms might graze on the autotrophic organisms which will lead to productivity loss (Raja et al., 2014). Biomass concentrations for circular ponds with stirring arms are around 0,5-1 g/L and biomass productivity between 8.5 $g/m^2.d$ and 21 $g/m^2.d$ (El-Baz and El Baky, 2018).

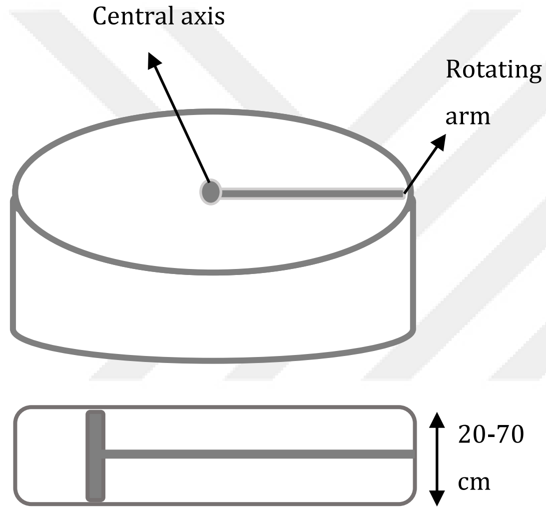

Figure 2.2 Circular pond design with stirring arm

2.1.2 Raceway Ponds

The design of a raceway pond is oval-shaped single or multiple loop recirculation channels built together (Figure 2.3) with a depth of 15-50 cm (Ting et al., 2017) Channels may be built-in low-cost materials such as rubber sheet, compacted earth, concrete, or lined with plastics. Mixing is done by paddlewheels that drive water continuously around the circuit to ensure proper mixing. It also has similar disadvantages to the circular ponds; contamination, seasonal light, pH and temperature changes, water loss due to evaporation. Raceway ponds are commonly used for commercial algal culture because of their low construction and maintenance costs. The water-flow velocity is required to prevent the algae cells from settling. Therefore, the energy need for mixing is the main cost for the system. The optimal velocity of 10 to 20 cm/s was found effective for viable cultivation (Shen et al., 2009). Biomass productivity around 10-25 g/m^2.d (Chen et al., 2011; Davis et al., 2016) and cell concentration up to 1 g/L can be achieved by using raceway ponds (Shen et al., 2009).

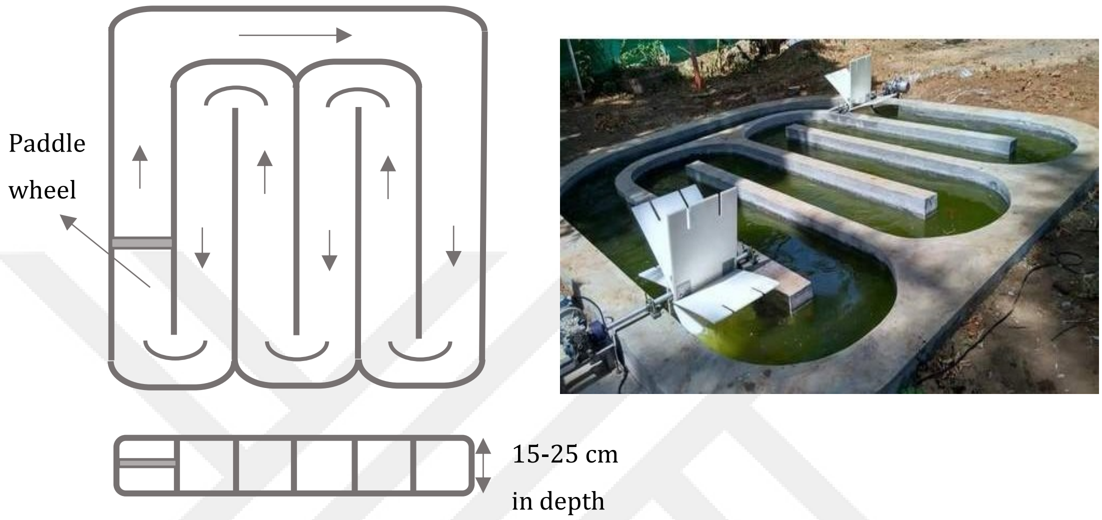

Figure 2.3 Raceway pond design

2.2 Closed Photobioreactor Systems

Reactors that utilize light as energy sources to perform photobiological reactions are called photobioreactors (PBR). To produce large quantities of biomass under controlled conditions photobioreactors with various designs are used in algae cultivation.

Closed PBRs of different configurations and geometrics are adopted for mass microalgal biomass cultivation, to overcome the abiotic limitations associated with open microalgae culture systems. These systems are flexible and can be optimized by many factors such as: geometries, design, operational sustainability, space requirement, configurations, maintenance cost, biological and physical characteristics of microalgae species (Barkia et al., 2019).

Closed PBR systems expands the potential product range by the possibility of growing various types of species with higher yields and they are much easier to control the abiotic parameters like CO_2, temperature, light intensity, and nutrient level without contamination. Biomass concentrations obtained from closed PBRs are around 30-50 g/m^2.d (Brennan and Owende, 2010). Although the capital and operational expenses for closed PBRs are higher than open systems, because the obtained biomass concentrations are higher there is a significant decrease in harvesting costs.

Closed systems include tubular, flat plate, plastic bags, single or in the array, with various types of designs developed according to factors as mentioned before. (Saad et al., 2019) In this research, glass tubular (Horizontal and Helical), flat plate, and plastic bag PBR systems are evaluated for large-scale *Chlorella* cultivation.

Table 2.2 Advantages and disadvantages of closed PBRs over open ponds systems

Advantages of PBRs	**Disadvantages of PBRs**
– Low CO_2 losses – Smaller area requirement – Possibility of temperature regulation – Low risk of contamination and species competition – Reproducible culture – Low environmental influence – Due to excessive sunlight distribution and saturation efficiency microalgae maximum growth efficiency is achieved – Higher cell densities and volumetric productivity	– Difficult to clean – High operational and capital costs – Cooling of the system is needed – Degassing needed for the O_2 that the microalgae produce – Tube materials should be selected carefully, cost effectively due to partial decrease of the sunlight – Due to large size and surface area chemical agents are needed to be sterilized effectively

2.2.1 Tubular (Glass-Helical) Photobioreactor

Helical photobioreactors contain coiled clear and transparent flexible tubes with small diameters connected to a degassing unit having a vertically stacked fence design. The algae culture is utilized to the system by a pump and coupling, fittings and bars provide support for the system structure (Figure 2.4). Light absorption of the tube material is important for higher photosynthetic potential therefore tubes with transparent materials are used in these systems (Singh and Sharma, 2012). It is reported that an increase in the tube diameter in these systems leads to a decrease in surface/volume ratio having a negative impact on the microalgal growth rate. (Shen et al., 2009) Although cooling and pumping systems for effective cultivation increase the costs; this type of PBRs can have high biomass productivity of 40-50 g/m^2.d and cell concentrations of 2 g/L (Clippinger and Davis, 2019).

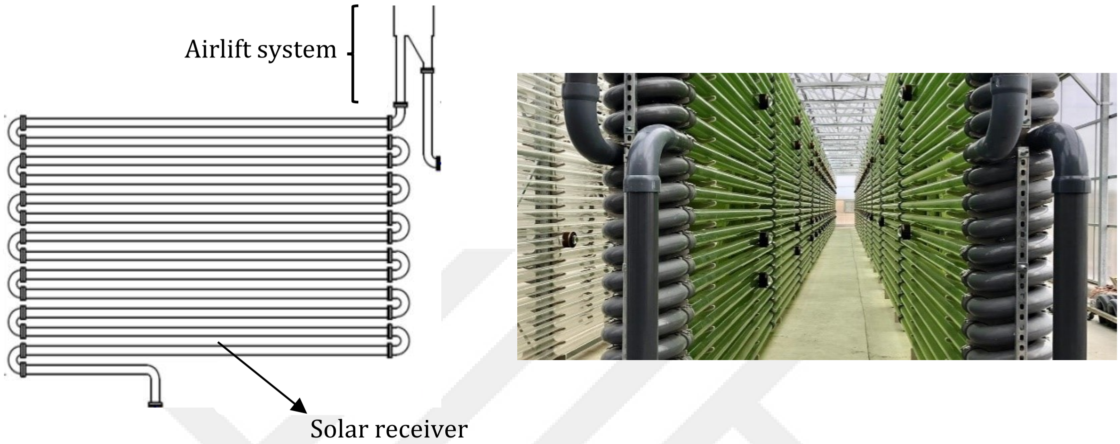

Figure 2.4 Tubular (Glass-Helical) photobioreactor design

2.2.2 Flat Plate (Glass- Flat Panel) Photobioreactor

The flat panel photobioreactors are characterized by their rectangular box shape (Figure 2.5) and high surface area to volume ratio and made from transparent materials such as; glass, polycarbonate, plexiglass, plastic (El-Baz and El Baky, 2018). The algae can be cultivated either outside with direct daylight or inside with artificial light sources. Stainless steel bars provide support for the panels vertically and a pipe within at the bottom of the panel accomplishes gas transfer (Clippinger and Davis, 2019). The light can be easily penetrated into the culture due to its lightweight path and the agitation is driven by air bubbles that are generated from air units. As mentioned for the tubular PBRs cooling units are needed. This cultivation system can have high biomass productivity of 30 g/m^2.d and cell concentration up to 2 g/L due to its homogeneous light exposure (Shen et al., 2009). However, biofilm can develop very quickly, and it is complicated to clean due to its poor hydrodynamic system.

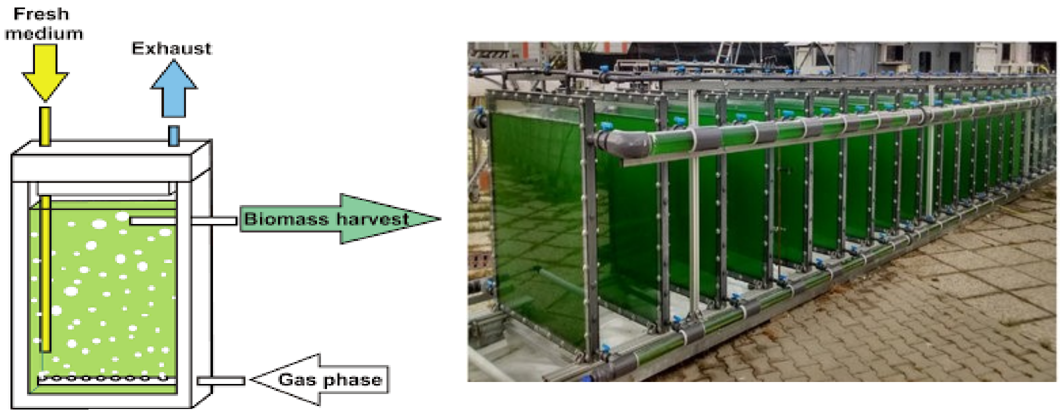

Figure 2.5 Flat Plate (Glass- Flat Panel) photobioreactor design

2.2.3 Plastic Bag Photobioreactor

The high costs of microalgae cultivation systems have led the researchers to develop economically PBR system designs. Plastic bag photobioreactors consist of hanging a transparent plastic bag or vessel with a CO_2 supply pipe and exposing the algae culture to light like flat plate PBRs (Figure 2.6). This method is the cheapest cultivation system by material costs so far (Zhu et al., 2018). Although it is quick to install and easy to operate, the system has a short lifespan therefore this result in overall system costs being high in long-term commercialized cultivation, like the other cultivation systems. The system has limited sustainability as the bags are thrown away and need to be replaced from time to time. Plastic bag PBRs can have biomass productivity of 50 g/m^2.d or more and cell concentrations of 1 g/L (Clippinger and Davis, 2019).

Figure 2.6 Plastic bag photobioreactor design

2.2.4 Tubular (Glass-Horizontal) Photobioreactor

This type of PBRs is the most standard one, placed horizontally, a single or parallel set of tubes in a loop shape (Figure 2.7). This shape gives an advantage in light conversion efficiency in outdoor cultures (Singh and Sharma, 2012).

The gas mixture (CO_2) is added into the tube by a pump and the degassing unit removes the oxygen that is produced by algae during photosynthesis. Spraying water on the surface of the tubes, regulating the temperature by recirculation system, and overlapping the tubes are the adapted cooling methods for this system (Singh and Sharma, 2012). These systems have high energy consumption, due to cooling and gas exchange systems. Another main issue about horizontal PBRs is not all microalgae species are compatible with culturing these in these systems (Shen et al., 2009).

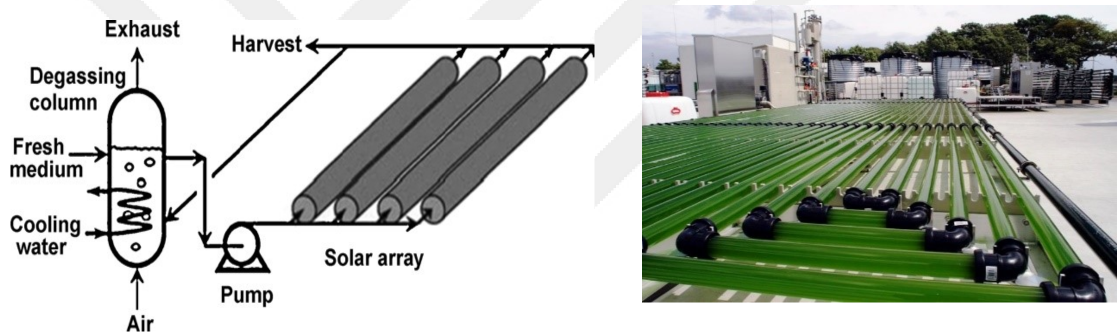

Figure 2.7 Tubular (Glass-Horizontal) photobioreactor design

2.3 Hybrid Production Systems

The hybrid production systems are two-stage cultivation methods that exploits the advantages of two different algal growth systems by combining them, by this combination one overcomes the other system's disadvantages. Usually, growth stages in photobioreactors are combined with open pond systems. In the first stage, it is a controllable photobioreactor where the contamination is minimized, and the cells have continuous division. An open pond system is operated in the second stage in which cells are exposed to nutrient and environmental stress. In hybrid cultivation, higher biomass productivity can be achieved with maintenance and monitoring. However, because of their high costs, large area requirements, and being a batch system, not a continuous one hybrid systems are not suitable for large-scale microalgae production (Singh and Sharma, 2012).

Table 2.3 Summarized evaluation of advantages and disadvantages for cultivation systems

Cultivation System	Advantages	Disadvantages
Circular Pond	• Good for mass cultivation • Cheap • Easy to construct and operate • No energy requirements • No O_2 building • No need for cleaning and cooling systems	• Evaporation loss • Contamination risks • Affected by climate • Low yield • Large area requirements • Limited strains • Poor mixing
Raceway Ponds	• Relatively cheap • Easy to construct and maintain • Low energy requirements (for pumps) • Good for large scale cultvation • No O_2 build-up	• Poor biomass productivity • Large are required • Limited Strains • Contamination risks • High evaporation loss • Temperature and pH dependent on climate
Tubular Photobioreactors	• High biomass productivity • Cheap • Less prone to contamination • Can be controlled outdoors • Water saving • High surface-volume ratio	• High investment costs • High cooling costs • Energy requirements • Wall growth • Needs cleaning periodically (hard to clean need chemicals)
Flat Plate Photobioreactors	• High biomass productivity • Smaller light path • Less prone to contamination • Easy to clean • Lower O_2 build-up	• Large area required • Difficult temperature control • Wall growth • Material intensive • Cooling system needed
Plastic Bag Photobioreactors	• Cheap material costs • High biomass productivity • Less prone to contamination	• Bag replacement needed • Not sustainable • Cooling system needed • Energy requirements

3
MICROALGAL BIOMASS HARVESTING METHODS

As mentioned in the previous sections, harvested microalgal biomass can be used in numerous products such as biofuels, animal feed, food supplements, and skincare products as mentioned before. The harvesting step refers to the separation of microalgae from its growth medium. The cell densities of microalgae in the culture can be estimated by microalgae cell count, chlorophyll content, optical density dry weight, and ash-free dry weight and usually have low rates around 0.1 to 5 g/L ash-free dry weight (Brennan and Owende,2010; Pahl et al., 2013). The concentration of the algal biomass must be increased to suit the requirements of downstream processes. The effectiveness of a harvesting process can be described by recovery efficiency (RE) (Equation 3.1) and concentration factor (CF) (Equation 3.2) :

$$Recovery\ Efficiency\ (RE) = \frac{mass\ of\ cells\ removed}{mass\ of\ cells\ in\ initial\ culture} \tag{3.1}$$

$$Concentration\ Factor\ (CF) = \frac{concentration\ of\ algae\ in\ final\ product}{initial\ concentration\ of\ algae\ in\ culture} \tag{3.2}$$

Nearly 20-30% of the total costs for microalgal growth and processing systems are covered by the harvesting step (Brennan and Owende, 2010). The main difficulties and high costs for the harvesting step is associated with microalgae size, specific gravity, surface charge and morphology of the cells and the high capital and operational equipment costs. Therefore, to achieve sustainable and economical product production from microalgal biomass, the harvesting techniques must be selected or designed less costly and less energy intensive (Shelef et al., 1984; Singh and Patidar, 2018). There are many different methods for solid-liquid separation which can be employed to harvest the cultured microalgae. Current harvesting processes can be classified as physical methods (sedimentation, disc stack centrifugation, decanter centrifugation, cross-flow filtration, vacuum filtration, pressure filtration, dissolved air flotation, micro bubble generation, dispersed air flotation) chemical methods (flocculation using organic polymers or flocculation using inorganic polymers), biological methods (auto-flocculation and bio-flocculation) and electrical methods (electrolytic coagulation, electrolytic flotation and electrolytic flocculation) (Christenson and Sims, 2011).

Because the harvesting biomass slurry can contain 5-15 % dry solid content it cannot be stored and must be processed immediately after harvest. Therefore, in some fields there might be a need for a dehydration/dewatering process to extend to viability of the target products. The techniques which are commonly used are sun drying, low-pressure drying, spray drying, freeze-drying, and drum drying. Drying temperature and used technique affect microalgal biochemical composition therefore it affects the lipid, carbohydrate, pigment, and other bioactive compound extraction from algal biomass. Among these methods, sun drying is the cheapest dewatering method, but the major disadvantages are a long time need and large surface requirements. To obtain high value-added products, the spray drying method is commonly used but it is an expensive method that can cause some deterioration of some pigments in algal cells if used on large scale. Freeze-drying provides extracting lipids without cell disruption, but it is also an expensive method for large-scale microalgae operations. Therefore, if there is a need, establishing a balance between the dewatering efficiency of algal biomass and the cost-effectiveness of the methods is important (Brennan and Owende, 2010).

If the microalgae biomass will be processed after the harvesting, the methods used for harvesting large scale microalgae must be;

- able to handle large volumes
- highly reliable
- have low operational and capital expenses
- easily constructed
- easily managed and flexible for environmental and physical changes.

Currently, there is no proven best method to meet these standards for microalgae harvesting in the literature. Therefore, in this section, the harvesting methods will be evaluated among themselves and their suitability for large-scale *Chlorella* cultivation. The advantages and disadvantages of different harvesting techniques for microalgae derived from literature studies are presented in Table 3.5.

3.1 Sedimentation

Sedimentation technique is one of the simplest forms of liquid-solid separation. Algae suspension is separated into a concentrated slurry by using sedimentation tanks or lamella separators. While removing particles with reasonable settling velocity from the suspension gravity sedimentation is satisfactory. However, particles with small diameters may need flocculants to form larger particles and to increase the rate of sedimentation (Shelef et al., 1984). Sedimentation is an inexpensive but slow process that needs large volumes and without adding flocculants, the reliability of this process can be low. The density and radius of the microalgae play an important role for this method (Terminal velocity of the particles are related to Stoke's Law.); it is found that microalgal particles which have low density and are smaller than 70 μm in size do not settle well (Sharma et al., 2013). Sedimentation tanks with or without lamella separators are commonly used in this technique are presented in Figure 3.1.

The major advantages of sedimentation processes are:

- Low operational cost with little power consumption

- Simple designs with low costs

The major disadvantages of sedimentation processes are:

- Large area requirements

- High time requirements because of slow sedimentation rates

- Receiving low solids concentrations

In summary, this method is not attractive for large scale algae production of microalgae that are small in radius like *Chlorella* which is commonly 2 to 10 μm in diameter and can be outcompeted by flocculation technique.

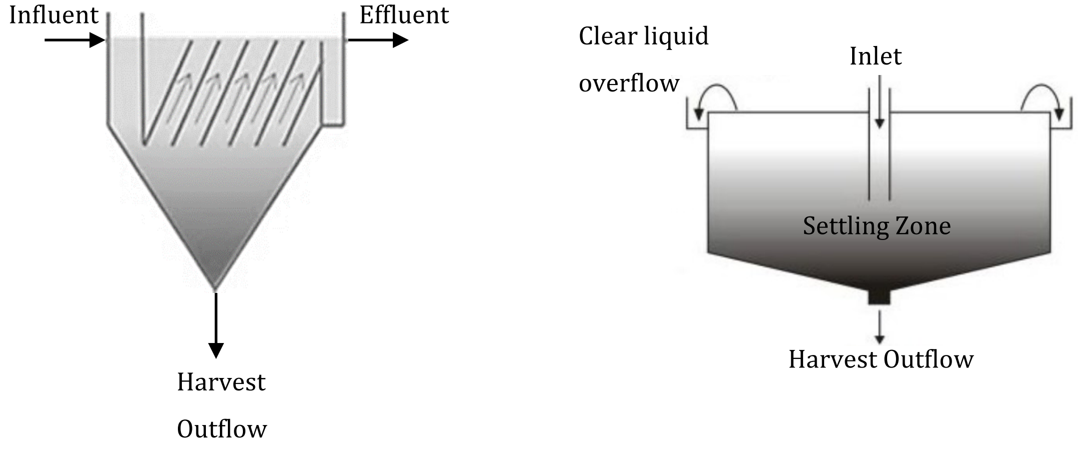

Figure 3.1 Sedimentation tanks with or without lamella separators

3.2 Cross-Flow Filtration

In filtration method, a permeable medium is used, so while allowing the liquid to pass through it can retain the algal biomass with efficiency rates ranging from 20% to 90%. The pressure difference across the filter is required to force the fluid across the membrane (Al-hattab et al., 2015). While there are various types of filter designs used in the industry, the most common ones are filter presses, belt filters, tangential (cross) flow membrane filters and drum filters (Singh and Patidar, 2018). However, vacuum and pressure types of filters are not suitable for all microalgae types such as *Chlorella* (Chen et al., 2011). Therefore, only Cross Flow Membrane Filtration will be considered as the filtration harvesting method in this study.

The membrane filters can be classified based on the size of the pores; reverse osmosis (less than 0.001 μm), ultra-filtration (between 0.02 to 0.20 μm), micro-filtration (between 0.1 to 10 μm), and macro-filtration (greater than 10 μm). Cross Flow Filtration (Figure 3.2) is a method by using micro-filtration or ultra-filtration to retain particles. In this technique, suspension flows or pumped through, tangentially across a membrane; the particles that are larger than the membrane pore size is retained/rejected (retentate), the particles that are smaller than the pore size of the membrane pas through with the liquid(permeate) (Gerardo et al., 2015).

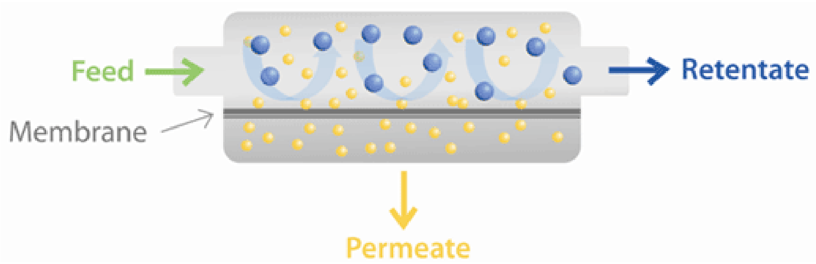

Figure 3.2 Cross-flow filtration design

There can be shear force created by the parallel flow parallel to the membrane surface to keep the membrane clean. However, the high shear force can cause cell damage. Also, in long-term use membrane fouling can occur, which will result in reduced permeate flux, membrane life, and product quality. To prevent clogging filter aids can be used (Pahl et al., 2013).

The energy consumption for cross-flow membrane filtration depends on the membrane characteristics, feed, system design, etc. and can range from 3 kWh/m^3 to $10kWh/m^3$ (Rossignol et al., 1999).

This method is advantageous over sedimentation, centrifugation, and flocculation because it is rapid and results in complete removal of the biomass. The costs for this method are associated with membrane replacements due to clogging and pumping the suspension. However, in the use of large-scale the frequent replacement of the membranes due to fouling and clogging can limit this technique.

3.3 Centrifugation Using Disc Stack

The centrifugation method relies on the centrifugal force that acts radially and causes the particles which are denser than the liquid to migrate outwards. The particles which are not denser than the liquid migrates inwards. Therefore, the efficiency is stated around 80-90% in the literature (Chen et al., 2011) and is based on the centrifugal force and type of the centrifuge. There are five basic types of centrifuges, one of the commonly used types on an industrial scale is centrifugation with disc stack. Disc stack centrifuge consists of a stack of closely spaced metal discs that rotate, which form a shallow cylindrical bowl (Figure 3.3). The suspension is placed on the center of the stack discs and the lighter mixture by the density remains on the inside while the denser phase migrates outwards to under the discs (Fasaei et al., 2018). This type is efficient for the use of separating materials that are in the range of 3-30 μm (Singh and Patidar, 2018). The energy need stated for disc stack centrifuge varies from 0.53 kWh/m^3 to 5.5 kWh/m^3 in the literature (Al-hattab et al., 2015).

The advantages of disc stack centrifuges as harvesting methods for algal biomass are:

- High removal efficiency
- Suitable for all types of microalgae
- Can be used as a continuous, batch or semi-batch process
- Rapid process (Christenson and Sims, 2011).

However due to its mechanical complexity, high operational and capital cost as well as energy requirements and small space between stacks which requires chemicals to clean are some disadvantages of this method. Although it is a promising process for microalgae harvesting high costs of this process and the cell damage that can be caused by high gravitational and shear forces can limit this technique for use of large scale (Harun et al., 2010).

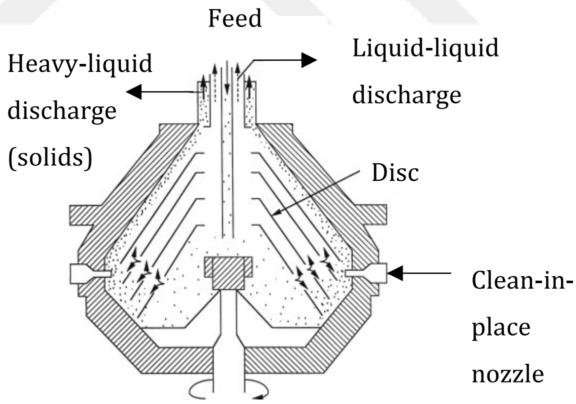

Figure 3.3 Centrifugation using disc stack

3.4 Centrifugation Using Decanter

Another commonly used centrifuge type on an industrial scale is centrifugation with decanter. Decanting or scroll centrifuge is based on two concentric rotating bowls (tanks) surrendered by a case of cover (Pahl et al., 2013). The outer and inner bowls rotate at different speeds which leads to solids that are entering the decanter are conveyed to the discharge bowl (Figure 3.4) while the liquid is discharged from the opposite side using the gravitational forces (Shelef et al., 1984). The efficiency of the decanter centrifuge can be up to 90% (Singh and Patidar, 2017) and energy varies from 1.3 kWh/m^3 to 8 kWh/m^3 in literature (Sharma et al., 2013).

The advantages of decanter centrifuges as harvesting methods for algal biomass are;

- The biomass obtained from decanter centrifuge is more concentrated than disc stack.
- Can handle particle size which are greater than 15 μm
- Rapid process (Gerardo et al., 2015).

This method is one of the most promising one as it can operate continuously, rapidly, effectively with high capacity and low maintenance (Najjar and Abu-Shamleh, 2020). However, it has high capital and operational costs as well as high energy demands which can limit the large-scale applications. And high-speed spinning of the algae cells can cause cell damage or disruption (Al-hattab et al., 2015).

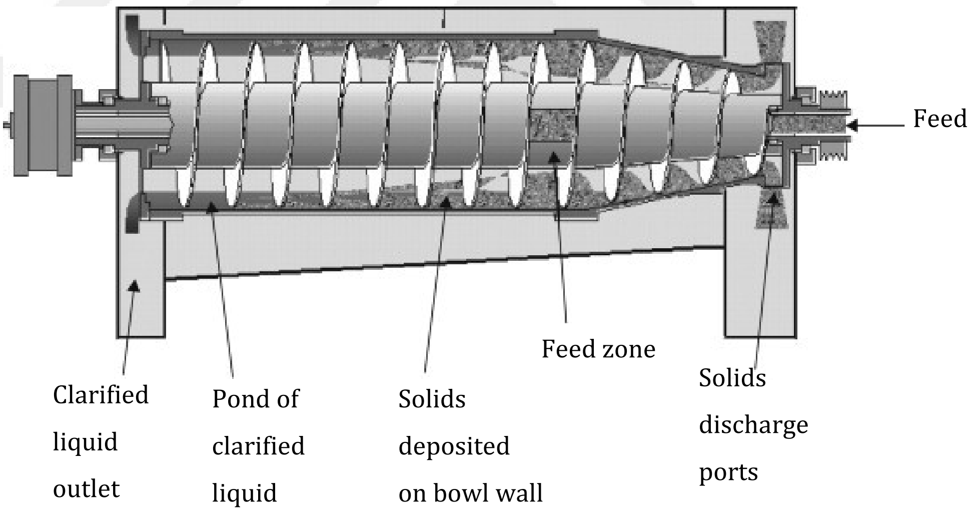

Figure 3.4 Centrifugation using decanter

3.5 Dispersed Air Flotation

Flotation is a gravity separation process where bubbles are ejected into the system for separation. It is classified according to the generated bubble size (Al-hattab et al., 2015). Dissolved air flotation uses the advantage of the low density of microalgae; by generating bubbles of 700 to 1500 μm by air passing through a porous mechanical agitator then injected into the system. The bubbles that pass through the algal suspension causes the microalgae to float to the surface (Figure 3.5). The particles that float to the surface are skimmed off (Pahl et al., 2013). The efficiency of this method is dependent on the particle size, pH of the medium, air-particle contact, and instability of the particle (Liu et al., 2006). The smaller particles will be lifted easily, high air-particle contact, and the lower instability increases the efficiency. This system consumes less energy (less than dissolved air flotation) but it requires costly equipment to create the bubbles (Yin et al., 2020). The efficiency of overall process can be up to >90% improved by using surfactants. For dispersed air flotation to be effective, the particles need to be hydrophobic, that's why there are multiple studies with surfactant adding into the system to boost the efficiency (Singh and Patidar, 2018). Compared to harvesting of microalgae by sedimentation, dispersed flotation method is much faster and effective. However, high cost and energy requirements of this system can limit suitability of this method for large scale commercialization.

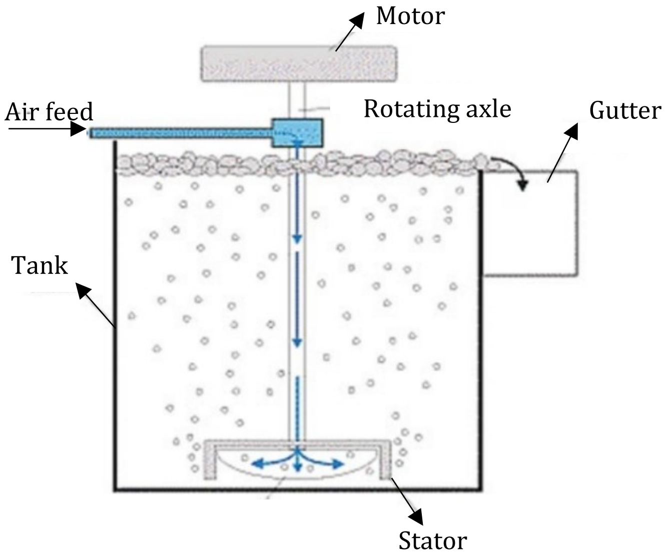

Figure 3.5 Dispersed air flotation design

3.6 Dissolved Air Flotation

In dissolved air flotation method, bubbles sizes range from 10 to 100 μm are generated from the air is dissolving in water under very high pressure with generators are used (Chen et al., 2017). Then the bubbles injected into the system carry the microalgae to the surface while passing through the suspension (Figure

3.6). The biomass floating at the surface is skimmed off and collected (Gerardo et al., 2015). The system efficiency depends on bubble size, pressure difference, particle size (Niaghi et al., 2015). This method is usually combined with chemical flocculants to have a more consistent and sustainable harvesting systems with an efficiency that can be up to 80 to 95% (Singh and Patidar, 2018). The clarified liquid suspension can be saturated with air and recycled into the system. To prevent backflow or pressure drops the flow rate must be consistent (Pahl et al., 2013). DAF units are unfortunately energy-intensive (high pressure required to dissolve air in water) and with their high costs, it may cause limitations for large-scale use even though they are effective systems for microalgal biomass harvesting (Hanotu et al., 2011).

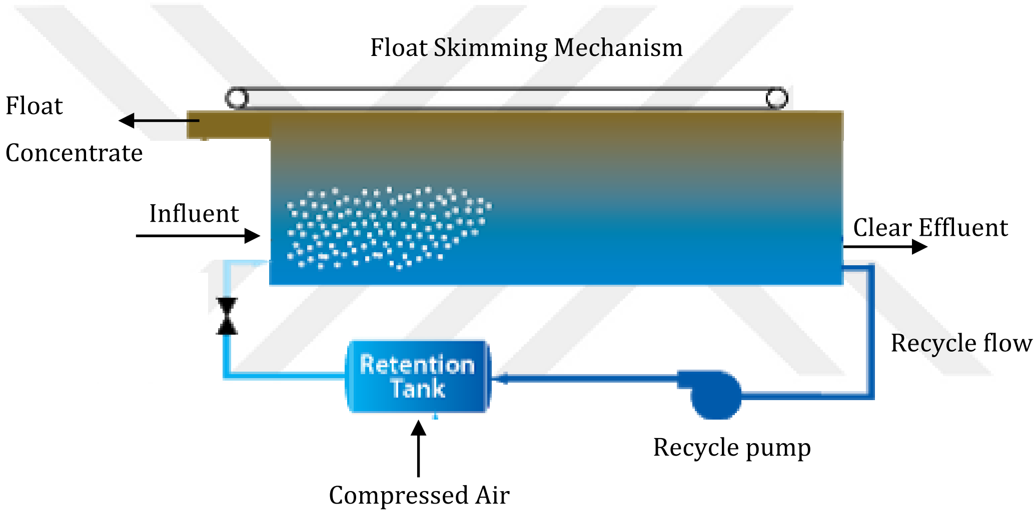

Figure 3.6 Dissolved air flotation design (Adapted from Komline-Sanderson, 2015)

3.7 Fluidic Oscillation (Micro-flotation)

Fluidic oscillation as known as micro-flotation is a new, fast and efficient microalgae harvesting method achieved by generating micro-bubbles through a fluidic oscillator. It works by supplying continuous air supply into oscillatory flow in a specific frequency and generating bubbles that are the size of the pores. By this method, the bubbles formed are ten times smaller than the other air two flotation methods. The methodology of the system is like the DAF system: microalgae cells attaching to the bubbles and floating to the surface. pH, bubble size, and flow rate are the important parameters that affect the fluidic oscillation process. Some studies reported a recovery rate of >95% using microbubble generating with add-off coagulants (Al-hattab et al., 2015). Although the method has high efficiency and uses less energy than conventional DAF systems, because the system is not economical for large-scale harvesting of microalgal biomass there are limited research studies, been performed using this technique (Hanotu et al., 2011).

3.8 Chemical Harvesting Using Inorganic Compounds

Due to other methods that demand high energy chemical flocculation is a promising technique for large-scale use. Chemical harvesting with inorganic compounds works by charge neutralization; using inorganic flocculants such as ferric chloride, ferric sulfate, and aluminum chloride, positively charged metal ions, can be seen in Table 3.1 to form cationic hydrolysis products (polyhydroxy complexes) (Li et al., 2020). Because the microalgae surface is negatively charged positively charged flocculants are required to bond the cells and through charge neutralization. Negatively charged microalgal cells are adsorbed by inorganic flocculants (Figure 3.7) and the process is followed by settlement of the algae to the bottom due to increasing density.

Table 3.1 Comparison of flocculation efficiency of different inorganic flocculants with *Chlorella* (Li et al., 2020)

Microalgae Specie	Microalgae Concentration	Flocculants	pH	Time (min)	Dosage (mg/L)	Efficiency (%)
Chlorella sp.	0.12 g/L dry weight	$FeCl_3$	8.1	40	143	10
Chlorella sp.	1.7 g/L	$FeCl_3$	6.43	90	560	100
Chlorella vulgaris	20 x 10^6 cells/mL	$FeCl_3$	-	5	50	90
Chlorella sp.	1.52 g/L	$Fe_2(SO_4)_3$	5.3	-	900	98
Chlorella sp.	1.7 g/L	$Fe_2(SO_4)_3$	6.43	90	960	100
Chlorella vulgaris	0.25 g/L	$Mg(OH)_2$	10.3	60	7.5 mM	95
Chlorella vulgaris	0.5 g/L dry weight	$Mg(OH)_2$	10.5	60	0.15 mM	95
Chlorella zofingiensis	1 g/L dry weight	$Mg(OH)_2$	10.5	25	219	94
Chlorella sp.	0.12 g/L dry weight	$Al_2(SO_4)_3$	6	60	152	100
Chlorella vulgaris	1.2 g/L	$Al_2(SO_4)_3$	-	10	2500	92.4
Chlorella vulgaris	1.5 g/L	PAC	6.5	60	5	90
Chlorella sp.	1.5 g/L	Aminoclay-conjugated TiO_2	-	10	3000	85

Concentration of the used flocculent, pH, and the surface charge of the flocculent are some of the many factors affecting inorganic flocculation efficiency (Pahl et al., 2013). The concentration of the flocculants and optimal pH range varies on the microalgae species (Wyatt et al., 2011). The amount of the flocculent available to bridge the algae cell to one another highly affects the overall system efficiency. It is stated that flocculants with higher charge density results better flocculation effects that is why in the research which aluminum and iron salts used as flocculants resulted with better efficiency rates around >90% (Al-hattab et al., 2015).

Chemical flocculation is a rapid and effective harvesting process by the characteristics of the flocculants used. However, there are some disadvantages such as the end products may be contaminated by the metal salts and the used inorganic compounds may be toxic for microalgae cells or the environment; so separating the metal salts after the process is necessary (Matter et al., 2019). Although it is a promising technique with using chemical flocculants which are less expensive than organic flocculants there are also major drawbacks such as;

- There is a need of large concentrations of inorganic flocculants for system to be effective, therefore the price maybe higher than expected also with large quantity of sludge (Chen et al., 2011)
- The process is highly sensitive to pH
- Not all the compounds used is effective for every microalgae specie (Sharma et al., 2013).

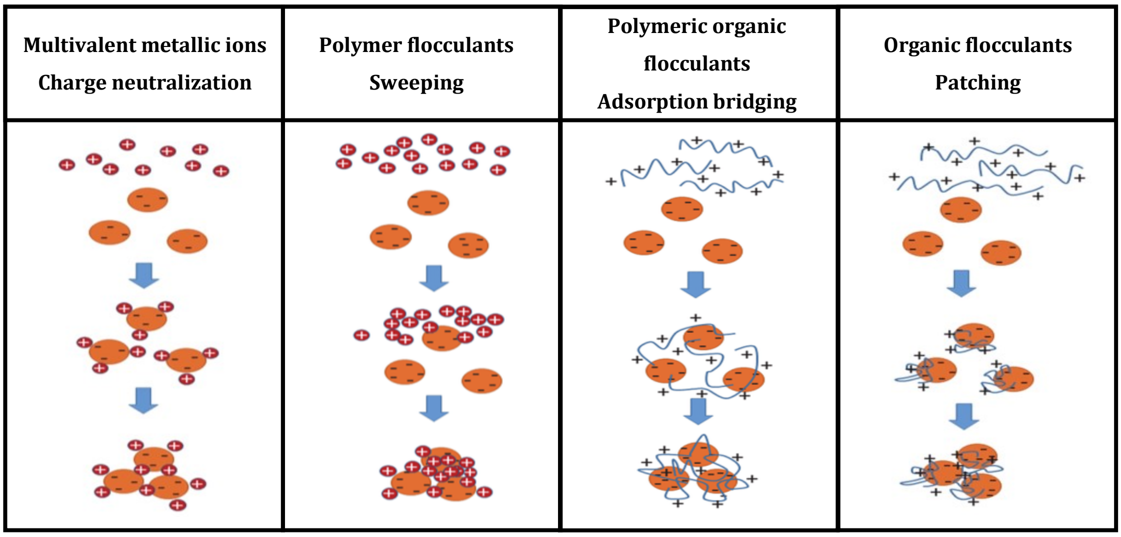

Figure 3.7 Chemical harvesting mechanism of different organic and inorganic flocculant types

3.9. Chemical Harvesting Using Organic Compounds

In chemical harvesting with organic compounds like chitosan or organic polymers like polyelectrolyte flocculants (polymeric, synthetic) such as polyelectrolyte polyamine are used. These particles consist both non-ionic and ionic particles and the method works by combining charge neutralization and particle bridging which can be seen in Figure 3.7 (Pahl et al., 2013). The polymers attach onto the microalgae cells through chemical or electrostatic forces that are;

- Coulombic Force: Polymers leads the microalgae cells attach to one another,
- Dipole-Dipole Force: The partially negative portion bonds to the partially positive one when two polar molecules approach one another,
- Van der Waals Force: Intermolecular forces attraction,
- Hydrogen Interaction Force: A type of dipole-dipole bonding when a highly electronegative molecule is bonded to a hydrogen atom. The polymer attaches on the surface and leaves its tail out to form loops which allows to attach other cells by forming bridges between. (Al-hattab et al., 2015).

Organic flocculants that are used in this technique are generally considered to be non-toxic and commercially available by varying molecular weight and charge density (Chen et al., 2011). While organic flocculants are more expensive than inorganic ones, the amount of flocculant need in the process is less therefore the cost of chemical harvesting with inorganic or organic compounds is nearly equal (Gerardo et al., 2015).

The efficiency of this method depends on polymer charge, dosage, pH, and salinity. Over or under the amount of these factors can result in failure to forming a bridge to connect microalgal cells together which will decrease the process efficiency (Sharma et al., 2013).

Although other factors are important it must be noted that the major factor which affects the efficiency is the degree of microalgae cells covers the polymer. If the attachment degree is below the optimum amount, it can result in agitation by cannot standing the shear forces; if the degree of the cover is higher than the optimum amount it can cause static hindering of the bridging process. The recent studies stated that flocculation using chitosan and other polyelectrolytes resulted in 95 to 100% efficiency under controlled pH levels (Al-hattab et al., 2015).

Table 3.2 Comparison of flocculation efficiency of different organic flocculants for *Chlorella* (Li et al., 2020)

Microalgae Specie	Microalgae Concentration	Flocculants	Dosage (mg/L)	Efficiency (%)
Chlorella vulgaris	1.2 g/L	Chitosan	250	91.9
Chlorella vulgaris	1 g/L dry microalgae	Chitosan	120	95
Chlorella vulgaris	1095 mg/L dry weight	Chitosan	30	>90
Chlorella sp.	1.32 g/L	Cationic locust bean gum biopolymer	50	96.68
Chlorella sp.	0.89 g/L	Cationic guar gum	40	94.5
Chlorella sp.	0.12 g/L	Cationic cassia gum	35	93
Chlorella protothecoides	0.12 g/L	Cationic starch	40	98
Chlorella vulgaris	0.35 g/L	Cellulose nanocrystal	40	97

3.10 Bio-flocculation

The bio-flocculation method is based on adding microorganisms to the microalgae culture (bacteria, algae, fungi, yeast, etc.) and causing the weight to increase by bridging and patching. This results in the settlement of the microalgae cells to the bottom (Matter et al., 2019). The selection of the microorganism culture can vary on the microalgae species that is going to be harvested (Table 3.3). The efficiency of this process is affected by pH, bio-flocculent concentration, and microorganism selectivity (Kurniawati et al., 2014). Bio-flocculation has a non-toxic nature compared to other methods and intermediate product that forms during the degradation are not secondary pollutants also it does not have high energy demands and easy to operate. Li et al. stated that harvesting with fungi or bacteria did not significantly change the bio-oil and C, H, N, S contents in the harvested microalgal biomass (Li et al., 2020). The main costs for this process include the equipment maintenance, nutrient medium sources, cleaning and light costs. It is mentioned that replacing light sources with natural sunlight and carbon source with wastewater can greatly reduce the costs but the use of wastewater as a nutrient medium might cause some limitations in the product utilization for microalgal biomass (Gerardo et al., 2015). Because it is an effective and feasible practice with low energy requirements bio-flocculation recently attracted a lot of attention. But the microorganisms that are used are species-specific can be time-consuming and not all bio-flocculants will be efficient for all varying types of microalgae (Singh and Patidar, 2018). Also, recycling and recovery of these microorganism can be difficult and because there may be microbiological contamination, all the products must be assessed before using in the food products (Al-hattab et al., 2015). Therefore, there are not many successful studies applied rather than lab scale.

Table 3.3 Harvesting *Chlorella* by co-cultivation with microorganism (Matter et al., 2019)

Bio-flocculant	Bio-flocculant dosage	Bio-flocculated algae (Cell Density, Volume or Amount)	Time	Flocculation efficiency (%)
Fungus	*A. fumigatus*	*C. protothecoides*	24 h	~90
	Penicillium cells (1.92 g)	*Chlorella sp.* (3.84 g)	150 min	~98
	Penicillium spores (1.1×10^4 cells/mL)	*Chlorella sp.* (3.84 g)	28 h	~99
Yeast	Extracellular protein of *S. bayanus* (0.1 g/L)	*C. reinhardtii* (10 mL)	3 h	95
	S. bayanus (1:1, v/v)	*C. reinhardtii* (10 mL)	6 h	80
	S. pastorianus (0.4 mg/g cell)	*C. vulgaris* (5 g/L)	70 min	90
Bacterium	*Flavobacterium, Terrimonas, and Sphingobacterium*	*C. vulgaris* (6×10^6 cells/mL)	24 h	94
Algae	*Phormidium sp.*	*Chlorella sp*	5 min	100

3.11 Electrocoagulation - Flocculation

Electro-coagulation-flocculation (ECF) is an alternative combined technique of metal flocculant and microbubbles in-situ for recovering the microalgae by decreased electric energy consumption. There are several attractive studies that investigated the use of ECF as a microalga harvesting method. (Singh and Patidar, 2018). In ECF, by electrolytic oxidation, sacrificial anode releases aluminum or iron ions (Figure 3.8). Although no anions, such as chlorine, are passed into the process water which is an advantage compared to the other methods, electricity is required for electrolytic oxidation of the anode (Richardson et al., 2014). If an aluminum anode is used in ECF, the following reactions will occur (Al-hattab et al., 2015).

$$Al \rightarrow Al^{3+} + 3e^- \tag{3.3}$$

$$x\, Al^{3+} + y\, OH^- \rightarrow Al_x(OH)_y^{z+} \tag{3.4}$$

If an iron anode is used in ECF, following reactions will occur:

$$Fe \rightarrow Fe^{2+} + 2e^- \tag{3.5}$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2 \tag{3.6}$$

or

$$Fe \rightarrow Fe^{3+} + 3e^- \tag{3.7}$$

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3 \tag{3.8}$$

It is not clear whether ferrous or ferric ions are formed during ECF and in the presence of oxygen oxidizing can occur.

For both Al and Fe anode reactions, oxygen is produced by the side reaction of water being oxidized. The reduction of water and the formation of hydrogen gas are the main reactions that occur in the cathode. The cations that are released from the sacrificial anode reduces the negative surface charge, destabilizes microalgae.

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \tag{3.9}$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \tag{3.10}$$

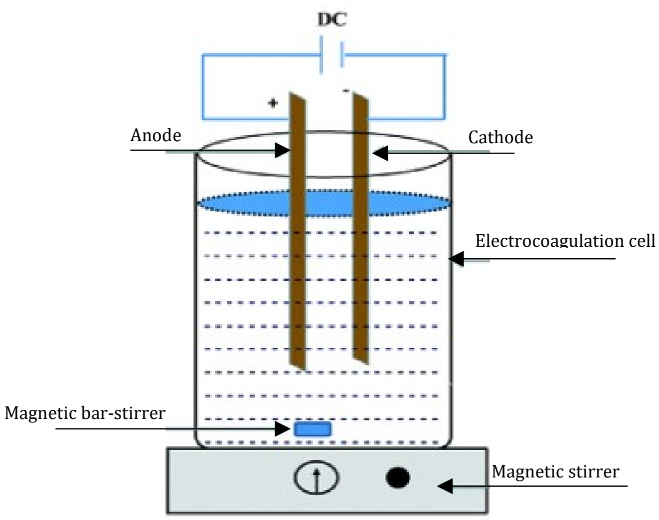

Figure 3.8 Electrocoagulation system mechanism

Among research studies, aluminum was a better electrode than the other materials. The flocs formed may sink to the bottom or float to the surface by attaching to the hydrogen bubbles generated at the cathode by depending on the ECF system design. ECF is an easy and effective process (removal efficiency 80 to 95%) without the use of chemicals which can be applicable to all microalgal species (Chen et al., 2011). The electrode material, pH, composition of the microalgal suspension, and electrolysis time affects the efficiency of the process. Some studies about factors affecting the microalgae recovery in ECF processes can be seen in Table 3.4 (Li et al., 2020). It is a promising technology having significant cost savings over other methods, such as centrifugation, without much research performed; however, having high equipment and operational costs (change of metal anode) as well as the metal contamination that can occur in the final product, temperature increase in the algal suspension are major drawbacks for this technique (Pahl et al., 2013).

Table 3.4 Performance of electrocoagulation-flocculation on microalgae recovery (Li et al., 2020)

Microalgae specie	Microalgae concentration	Power input	pH	Time	Efficiency (%)
Nannochloris oculata	1 g/L	0.75 A	4	10 min	95
Chlorella vulgaris	0.5 g/L	6.7 mA/cm^2	-	60 min	99
Dunaliella salina	0.88 g/L	0.5 A	7.2	3 min	94.5
Chlorella vulgaris	0.3–0.6 g/L	12 mA/cm^2	8	10 min	95
P. tricornutum	0.3–0.6 g/L	3 mA/cm^2	8	10 min	85
M. aeruginos	1.2–1.4 $\times 10^9$ cells/L	5 mA/cm^2	5	10 min	100

3.12 Suspended Air Flotation

Suspended Air Flotation (SAF) technique is like DAF method, system works by creating small bubbles that cause microalgae cells to surface on the water. The difference is that in SAF units, smaller bubbles are created with chemicals /surfactants, without a compressor or saturator (Wiley et al., 2009). Because of the chemical usage, the medium cannot be recycled back into the system. By having fewer mechanical components SAF is a promising technology for harvesting by being less energy-intensive, cost-effective, and requires less space than DAF units (Pahl et al., 2013).

Table 3.5 Summarized evaluation of advantages and disadvantages for harvesting systems

Harvesting Technique	Important Harvesting Conditions	Advantages	Disadvantages
Sedimentation	• Sedimentation velocity • Cell viability	• Low energy consumption • No need for skilled operators • Low investment and operation costs • Suitable for large scale	• Time consuming • Open for contamination • Specie specific • Not reliable and effective • Low slurry recovery
Centrifugation (Disc Stack) (Decanter)	• Size of microalgae • Acceleration factor	• High harvesting efficiency • Rapid • Suitable for almost all species • Easy to operate and reliable	• High capital and operational costs • Can cause cell damage • Contains freely moving parts • Preferred for small scale-lab applications
Filtration (membrane)	• Size of microalgae • Flow rate • Temperature • Transmembrane pressure difference	• Wide range of membrane types • No chemical use • Water can be recycled • High recovery rate • Cost effective	• Membrane clogging and fouling can occur • High operational and maintenance costs • Not suitable small sized algae • Replacement of the membrane can be needed
Flocculation (using organic polymers-chitosan)	• Molecular weight • pH • Charge density • Chain length of the polymer	• Wide specie range • Wide range of flocculant / coagulants • No energy input • Flocculant requirement is less than inorganic polymers • Easy to operate	• Highly sensitive of pH • Flocculant/coagulants may be costly • Large space needed
Flocculation (using inorganic polymers- ferric salts)	• Molecular weight • pH • Charge density • Chain length of the polymer	• Wide specie range • Flocculant/coagulants are less expensive than organic polymers • Suitable for large scale • No energy input • Easy to operate	• Highly sensitive of pH • More flocculant/coagulants needed • Large space needed • Used chemicals are toxic • Used chemicals can contaminate the product
Flotation (Dispersed Air Flotation) (Dissolved Air Flotation) (Suspended Air Flotation) (Fluidic Oscillation)	• Size of bubbles • Airborne particle contact • Instability of the suspended particle	• Prone to harvest mass culture • Less expensive than others • Suitable for large scale • Short operation time • Less space requirements • Good flexibility	• Specie specific • Depends on bubble distribution • Need surfactants • Pumps can be costly • Can be energy intensive • May require chemical flocculants

Table 3.5 Summarized evaluation of advantages and disadvantages for harvesting systems (continued)

Bio-flocculation	• Culture pH • Culture concentration	• Fast and easy to apply • No need for energy and chemicals • Low costs • Efficient process • Biodegradability • Avoids secondary pollution	• Microorganism can contaminate product • Specie specific • Unclear mechanism that needs to be explored • Slow growth and harvesting periods • Unstable
Electro flocculation and coagulation	• Voltage	• Reliable • Wide variety of species • Do not require chemicals • Energy efficient	• Costly for large scale use • Cathode fouling and harvesting system damage

4
CONVERSION TECHNOLOGIES, UTILIZATION AND ENVIRONMENTAL APPLICATIONS OF ALGAL BIOMASS

Due to microalgae phylogenic diversity, the biochemical composition of the algal cells varies greatly which makes them attractive in the research field of new resources for biofuels and bio-products. If the microalgae is cultivated using freshwater, after production and harvesting steps, it can be directly sold as food and nutrient supplements or the desired metabolites can be extracted using solvents and with a purification process, the products can be sold in the marketplace. But for microalgae to be used as a third-generation feedstock for biofuel there is a need for conversion process, to extract the desired content (protein, lipid, carbohydrate) and to transform it to the target product such as biodiesel, bioethanol, biogas, etc. Utilization fields and bioproducts obtained from microalgal biomass can be seen in Figure 4.1.

If the microalgae are culturing using wastewater, the utilization of the produced biomass can be limited due to the heavy metals, toxicant, colorants, etc. in the wastewater stream and strict safety regulations. On the other hand, the combined production of renewable energy and feedstock for multiple fields with an environmental application such as CO_2 mitigation and wastewater bioremediation supports sustainability and overall system economics in the microalgae field.

Therefore, in this section conversion technologies, utilization, and environmental application of microalgal biomass will be mentioned and discussed in detail.

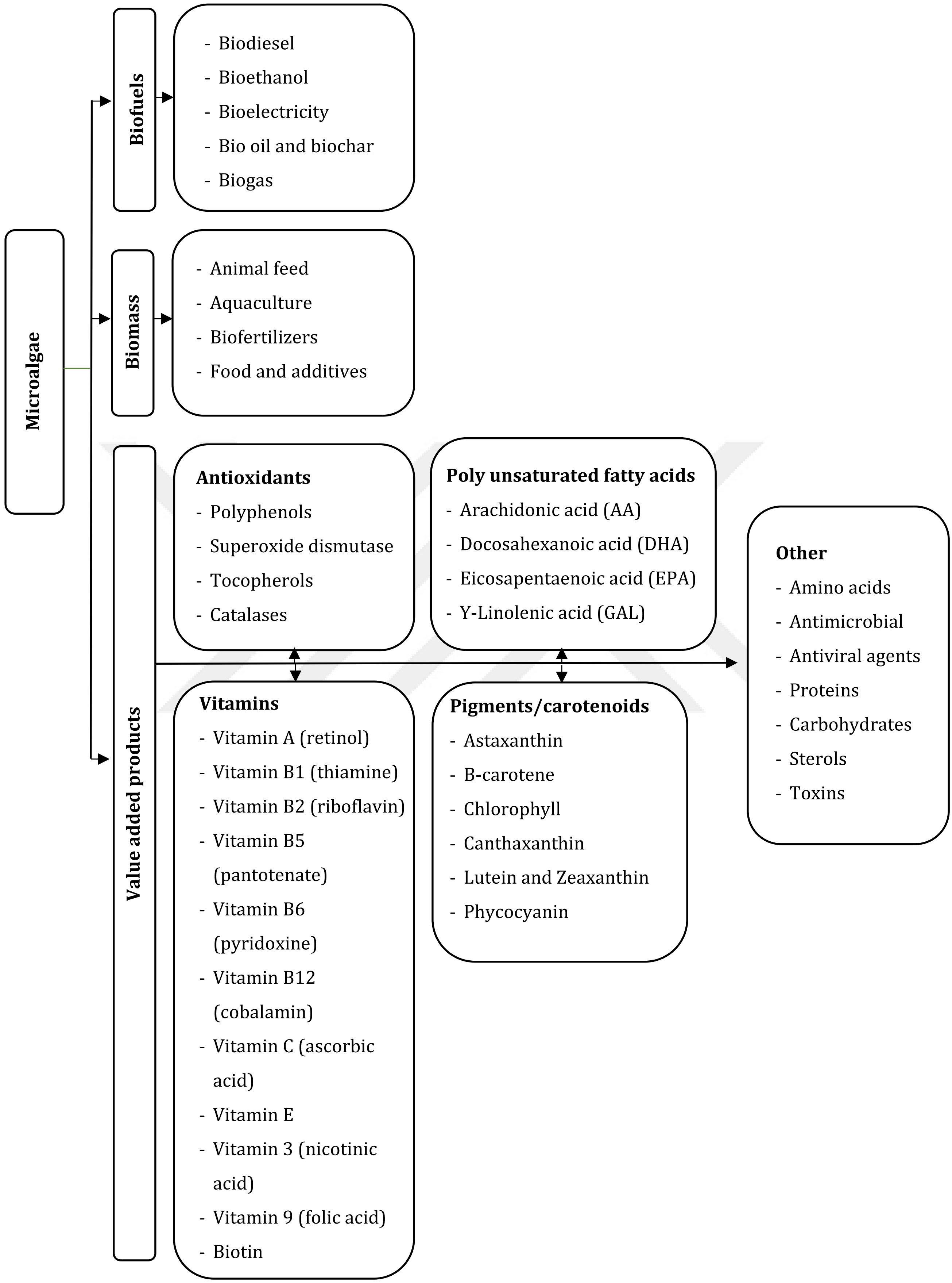

Figure 4.1 Utilization fields and bioproducts obtained from microalgal biomass

4.1 Microalgal Biomass Conversion Technologies for Biofuels

Because of their high growth rates, biochemical characteristics (high carbohydrate and lipid contents), and biomass yields, microalgae have a promising potential as been the third generation of biofuel feedstock. (Saad et al., 2019). Biodiesel, biomethane, bio-syngas, bioethanol, biohydrogen, and biochar are valuable biofuels that can be produced from microalgae biomass with different experimental conditions and conversion methods (Table 4.1). Algae carbohydrates can be used for producing bioethanol while the lipid contents can be used for biodiesel production. The residual biomass can be used for biomethane of fuel oil production as well as nutraceuticals, fertilizers, protein supplements, cosmetics. Different methods can be used to achieve desired target product. For example, to produce biodiesel physical methods are used while chemical and enzymatic pretreatment methods are used to produce bioethanol. (Zhang et al., 2014). It is known that the pretreatment processes can be a costly step for biomass production so feasible and economical methods need to be developed to optimize the process (Adams et al., 2018).

Table 4.1 Different experimental conditions and conversion methods to obtain biofuels from microalgal biomass (Saad et al., 2019)

Biofuel Type	Algal Species	Experimental Conditions	References
Biodiesel	*Chlorella sp.*	60 ∘C, 4 h, H_2SO_4 , MeOH	(Ehimen et al., 2010)
	Chlorella pyrenoidosa	90 ∘C, 2 h, H_2SO_4 , MeOH	(Huan et al., 2015)
	Desmodesmus quadricaudatus and *Chlorella sp.*	70 ∘C, 180 min, H_2SO_4 , MeOH	(Saad et al., 2018)
Bio-oil	*Chlorella sp.*	300 ∘C, 90 min	(Barreiro et al., 2013)
	Chlorella vulgaris	300 ∘C, 60 min	(Biller et al., 2012)
Methane	*Chlorella vulgaris*	308 ∘C, 30 days, Batch culture, pretreatment (Thermal 40 min/alkali)	(Buxy et al, 2013)
	Scenedesmus obliquus	306 ∘C, 20 days, Anaerobic Membrane Bioreactor, no pretreatment	(Zamalloa et al., 2012)

Table 4.1 Different experimental conditions and conversion methods to obtain biofuels from microalgal biomass (Saad et al., 2019) (continued)

Oil /Gas /Char	*Chlorella protothecoides*	Slow pyrolysis, tubular reactor, 550 ∘C	(Rizzo et al., 2013)
	Chlorella sp.	Pyrolysis, fixed bed reactor, 450 ∘C	(Babich et al., 2011)
	Chlorella vulgaris	Closed tubular photobioreactor. Fast pyrolysis, fluidized bed, 500 ∘C	(Wang et al., 2015)
Syngas	*Chlorella vulgaris*	450 ∘C, 30 min, Batch reactor	(Onwudili et al., 2013)
	Saccharina latissimi	450 ∘C, 30min, NaOH, Ni Batch reactor	

The conversion technologies for utilizing microalgae biomass as biofuels are divided into two main categories as thermochemical and biochemical conversion. The factors affecting the choice of the conversion process are biomass type, desired product, and energy, economic and feasible options, and biomass quantity (Brennan and Owende, 2010). It is stated that biochemical conversions are more environmentally friendly and have fewer energy requirements however, their low efficiency and reaction steps might cause limitations for large-scale production. The technically viable options for the conversion of microalgal biomass are considered in this section.

4.1.1. Thermochemical Conversions

Thermochemical processing is the use of heat/oxidation to decompose the organic components in biomass to produce biofuels including liquid, gaseous, and solid phases. It is considered the simplest way for microalgal to biofuel conversion. It can be classified as; gasification, liquefaction, direct combustion, and pyrolysis based on their temperature, pressure, duration of the heating time differences (Table 4.2).

Table 4.2 Thermochemical conversion technologies classification

Process	Temperature (°C)	Pressure (MPa)
Pyrolysis	300-1000	0.1-0.5
Direct Combustion	700-1400	≥0.1
Gasification	500-1300	≥0.1
Liquefaction	250-330	5-20

Gasification

In gasification the biomass is converted into a combustible gas mixture by a partial oxidation at high temperatures around 500-1300 °C. It is a biomass to energy pathway that can produce syngas (a low calorific gas mixture of CO_2, H_2, CH_4, CO, and N) with the calorific content of around 4 to 6 MJ/m^3 from various types of feedstocks (Saad et al., 2019). There are two gasification processes; conventional gasification process with a temperature around 800-1000°C and pressure of 1-10 bar where dry algal biomass reacts with the oxidizer and supercritical gasification process with a temperature around 300-600°C and pressure of 20 bar where wet algal biomass is directly used (Cho et al., 2020). In some studies, it is stated that conversion of microalgal biomass by gasification at 1000°C resulted in 0.64g of methanol production from 1 g of biomass, the highest theoretical yield, and the residual nitrogen component of microalgae can be converted into fertilizers (Brennan and Owende, 2020). Although gasification is a promising technology, it has been studied by several researchers, and reliable literature data is scarce, therefore this area needs more research.

Pyrolysis

The pyrolysis process occurs at 350-1000°C in the absence of air. The biomass thermally degrades bio-oils, gaseous products, and solid residues (biochar) (Brennan and Owende, 2010). The aim of pyrolysis is to obtain biofuels with a medium to low calorific value compared to others. It can be carried with/without catalyst and depending on the operating parameters like heating rate and residence time, the pyrolysis process is divided into three modes; conventional (slow) pyrolysis, fast pyrolysis, and flash pyrolysis which can be seen in Table 4.3 (Saad et al., 2019).

Table 4.3 Pyrolysis modes and their characteristics (Saad et al., 2019)

Pyrolysis Mode	Temperature(°C)	Heating Rate (°C /min)	Residence Time (seconds)
Slow	400	0.1-1.0	>10
Fast	580-1000	10-200	0.-10
Flash	700-1000	>1000	<0.5

Biomass is heated slowly in the slow pyrolysis process, so the residence time can vary depending on the desired product from minutes to hours resulting in low liquid products and a large quantity of solid residue. Obtained biochar can be also used for power generation, adsorbent, and biofertilizer. While in fast pyrolysis heating rates are much faster resulting in higher liquid product yields (70 to 80%) for bio-oil than conventional systems (Yang et al., 2019). And in flash pyrolysis, high temperatures with short heating times and fast heating rates high syngas yields are obtained. Obtained syngas can be used as a feedstock for methanol production. Therefore, fast and flash pyrolysis has a promising future for replacing fossil fuels with biofuels with high biomass-liquid conversion rates. It is stated to have a promising potential for mass production which can be competitive with petroleum-based fuels (Brennan and Owende, 2010). Although it has great advantages and a promising future, there are still challenges remain of pyrolysis oils because they are unstable, contain solids, and acidic compared to other technologies for biomass to biofuel conversion. Therefore, there is a need for catalyst addition, hydrogeneration, and deoxygenation processes to produce biofuels with the same properties as fossil fuels (Cho et al., 2020).

Liquefaction

The liquefaction process occurs at low temperatures around 250-400°C and at high pressures (40–200 bar) aided by a catalyst, it converts wet algal biomass into bio-oil (liquid fuel), gas, and biochar in the presence of hydrogen (Saad et al., 2019). It is stated the bio-oil produced from the liquefaction process is a more stable product than the pyrolysis process with lower oxygen and moisture contents. Because microalgae have a high moisture content (%80) this technique is very suitable as a conversion process without requiring drying (Cho et al., 2020). However, like the pyrolysis process, the products also contain oxygen and nitrogen which can cause high viscosity; therefore, a pretreatment process is required. Although studies indicate that the liquefaction process is a viable obtain for the conversion of wet algal biomass to energy; the reactors and systems for the liquefaction process are expensive and complex (Brennan and Owende, 2010).

Direct Combustion

Direct combustion is a process, which occurs above 700°C and low pressures (≥0.1MPa) in the presence of air, in a steam turbine, where the biomass is burnt, and the biomass energy is converted into hot gases (Saad et al., 2019). Although any type of biomass can be used, the moisture content needs to be high, above 50% of dry weight, to achieve a feasible process. The heat produced cannot be stored, must be used immediately and the pre-treatment processes for wet algal biomass lead combustions systems to have high costs as a disadvantage. There is limited data in this type of conversion process therefore more research on this field is required (Brennan and Owende, 2010).

4.1.2. Biochemical Conversion

Anaerobic Digestion

The anaerobic digestion process is the conversion of the organic wastes into biogas which consists primarily CH_4. Anaerobic digestion occurs in three sequential stages:

1) Hydrolysis: The bonds in complex organic compounds are broken with the use of water and energy is released while obtaining simple monomers such as soluble sugars and amino acids
2) Fermentation: A metabolic process to convert soluble sugars into acetic acid, volatile fatty acids (VFAs), $H_{2(g)}$, $CO_{2(g)}$, and alcohols by fermentative bacteria
3) Methanogenesis: After the volatile fatty acids are oxidized to acetate substrates methanogen bacteria convert them to methane (60-70%) and carbon dioxide (30-40%).

The wet algal biomass is appropriate for use in the anaerobic digestion process because of the high moisture content (80-90%) need. The residual biomass from this process is a nutrient-rich product that can be utilized in other fields such as fertilizers or recycled into the nutrient medium (Choo et al., 2020).

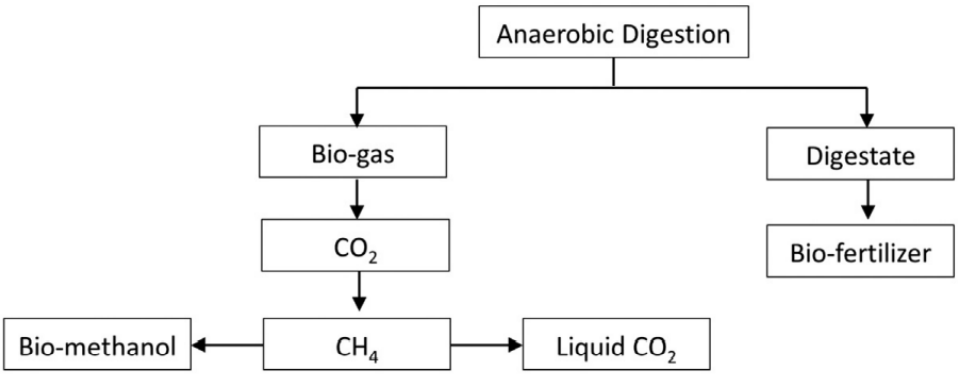

Figure 4.2 *Anaerobic digestion pathway of microalgae biomass*

The extracted nitrogen and carbon contents of microalgae result in a release of methane in the anaerobic digestion process. Therefore, lipid, protein, carbohydrate contents of microalgae are one of the major factors which directly affect the process. Some microalgae have high protein content in their biochemical composition which can result in a low C/N ratio that affects the anaerobic digester performance and increased ammonium production that can inhibit microorganisms. But these problems may be solved with adapted biomass use, with the addition of high C/N ratio products. (Brennan and Owende, 2010).

Transesterification

The transesterification process the lipid compounds of the biomass are reacted with short-chain alcohol in the presence of an acid or base catalyst or biocatalysts(enzymes) to produce biodiesel (Figure 4.3) in the form of fatty acid methyl esters (Choo et al., 2020). There are also byproducts that occur like glycerol, excess alcohol, water, etc. in the transesterification process (Makareviciene and Skorupskaite, 2019). The reaction is highly dependent on catalysts type, molar ratio, and alcohol type and it is important to reduce algal viscosity while increasing fluidity to mix with petroleum diesel to apply directly to engines. (Saad et al., 2019) To achieve the standard, it is necessary to remove the byproducts and there is a need to combine this process with other techniques to increase the yield (up to 90%). There is also direct transesterification (in situ transesterification) of microalgal biomass; a simple and rapid approach by integrating transesterification and extraction into a single step. In direct transesterification, wet or dry biomass is processed with a mixture of base catalyst and methanol in a single reactor to produce fatty acid esters (Brennan and Owende, 2010). It is faster than the conventional transesterification process also less organic solvent amounts are used therefore this process is a less costly conversion technology with higher yields. Although it offers promising results with less time and lower costs this process needs further study to improve the factor which affects the overall process efficiency (Karim et al., 2020).

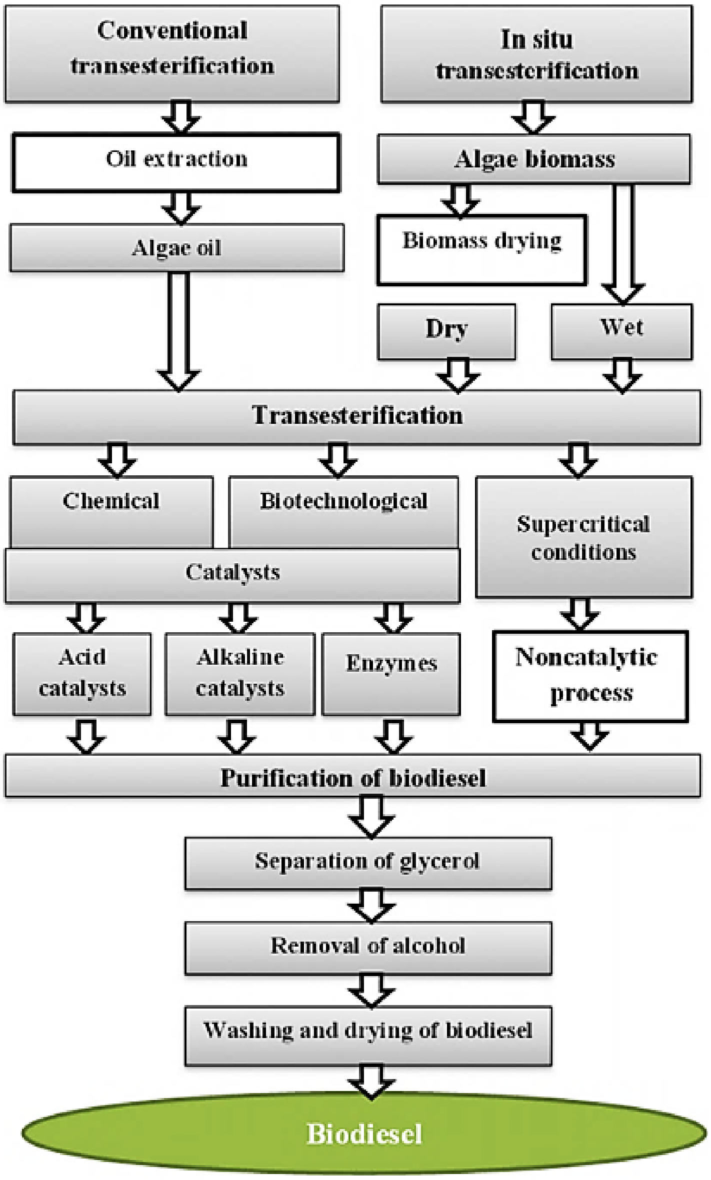

Figure 4.3 Transesterification process to obtain biodiesel (Adapted from Makareviciene and Skorupskaite, 2019)

Alcoholic Fermentation

In alcoholic fermentation process biomass that contains sugar, cellulose, or cellulose converted into ethanol via microbial activities. The starch is converted into sugar and in the fermenters tank it is mixed with yeast and water where the yeast breaks down the sugars in warm temperatures and converts the biomass into ethanol (Choo et al., 2020). Organic material with high carbohydrate content is suitable for this process, therefore microalgae with high carbohydrate content can be used to obtain ethanol. It must be noted that the bio-composition of microalgae can also be modified by controlling the culturing conditions. (nutrients, light level, etc.) After removing the water, alcohol, and other impurities by distillation, a purification process, bioethanol product with 95% purity in liquid form is suitable for use as biofuel. There is some solid residue that occurs from this process and it is stated that it can be used as a feed thermochemical process or can be added into microalgae culture (Brennan and Owende, 2010). In the research studies, *Chlorella vulgaris* is stated as an ideal microalgae species for the fermentation process with its high starch content which is around 37 to 65% (Choo et al., 2020).

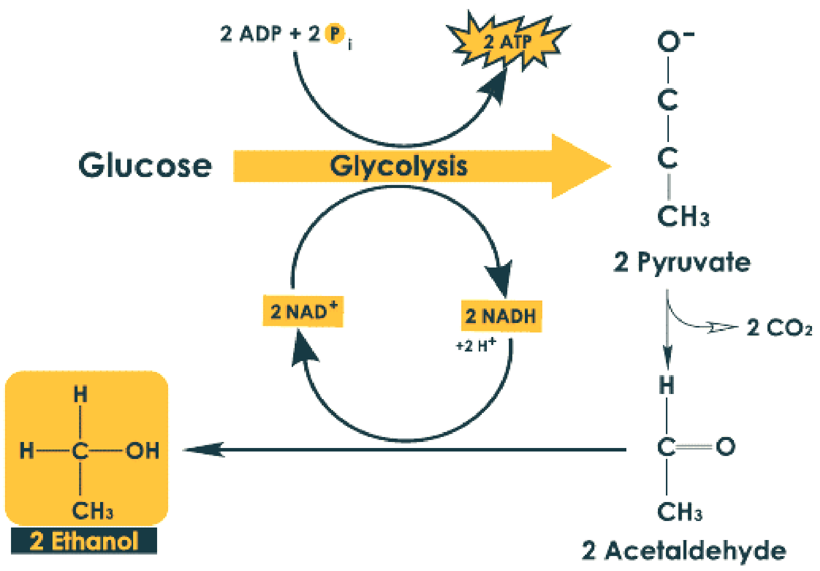

Figure 4.4 Alcoholic fermentation process to obtain biodiesel

4.2 Environmental Application Potentials and Useful Commodities from Microalgal Biomass

With the increasing demand for energy, water, food, and other resources due to global population growth, GHG emissions are increasing. Therefore, the use of microalgal biomass, wastewater treatment, and GHG emission mitigation as well as utilization in multiple fields such as biofuels, pharmaceuticals, cosmetics, health supplements, feed and food industry are major hallmarks of microalgal research which attracted the attention of the researchers worldwide in recent years.

In this section environmental applications of microalgae and useful commodities that are obtained from microalgal biomass has been evaluated.

4.2.1 Environmental Applications: CO_2 fixation

The global CO_2 emission rate is expected to rise by around 1.6% per year. As CO_2 acting as a principal GHG effect contributing to global warming, one of the current major global challenges, it is recommended to obtain 15% of energy from renewable energy sources to reduce greenhouse gas emissions (GHG) up to 20% by 2050 by European Union Renewable Energy Directive (RED) (Tomei and Helliwell, 2015; Milana et al., 2016). As carbon is a key resource for efficient microalgae cultivation (C consists of 36-65% of the dry matter of microalgae), CO_2 fixation by microalgae culturing has drawn attention as a sustainable and environmentally friendly strategy for CO_2 mitigation. Due to low CO_2 concentration in air (360 ppm), adapting photobioreactor systems using flue gasses in microalgae cultivation is a better option having CO_2 concentrations up to 20% (Brennan and Owende, 2010; Bhola et al 2014).

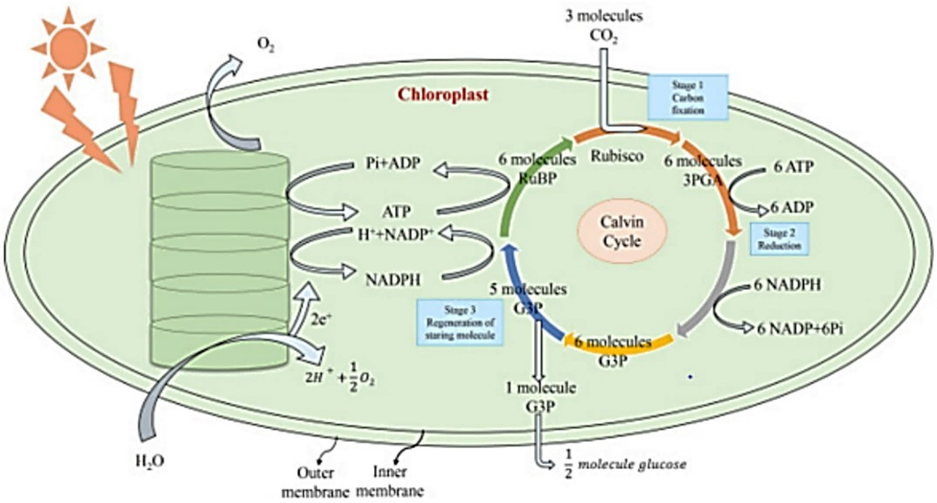

Figure 4.5 CO_2 fixation by a microalgae cell (Jana 2019)

While CO_2 is fixated by photosynthesis; the ability of CO_2 uptake can vary among different algal species therefore selection of the species with high CO_2 uptake might be technically challenging and low solubility rates of CO_2 in water can result in high costs for these systems. But the flue gases need to be cooled down before injecting into the cultivation system and not every microalgae strain is tolerant to high levels of NO_x and SO_x, heavy metals, unburned carbohydrates (C_xH_y) that can be present in flue gasses which can be toxic to some microalgae, therefore the selection of suitable microalgae strains has a significant effect on the efficiency of the overall process and costs (Zhang et al., 2014). There are some research studies that quantified the potential of microalgae use for carbon capture under different operational and nutrient stream conditions with an average efficiency of 60% such as; *Chlorella vulgaris* use on a steel plant wastewater discharge (Yun et al, 1997), reduction of CO_2 in flue gas using *Chlorella sp.* (Doucha et al, 2005), CO_2 bio-fixation using *Spirulina* and *Scenedesmus* (de Morias and Costa, 2007). Therefore, designing a suitable PBR system for microalgae cultivation with CO_2 fixation from flue gases has high a potential for being an environmentally friendly option and needs more research to be an economically viable option.

4.2.2 Environmental Applications: Wastewater Treatment

While produced microalgae using freshwater has many utilization fields, because there are high requirements of CO_2 inlet, nutrient steams, and water the system is stated as not economically viable. There is a nitrate need as 6-8 tons/ha and water need of 3908.3 billion m^3 per year for freshwater microalgae cultivation (Ravindran et al., 2016). Therefore, the potential of microalgae cultivation with the use of wastewater streams combined with utilization of the obtained biomass to produce VAPs is assessed by several authors. As the wastewater stream is rich in carbon, phosphorus, and nitrogen elements which are essential for microalgae production and sludge production are less than conventional systems this combination reduces the costs making the system economically viable (Zhang et al., 2014).

Although lipid amount of microalgal mass cultivated under controlled operating conditions makes them attractive for the third generation of biofuel it must be noted that selection of wastewaters as nutrient media raises potential conflict with some utilization options because industrial wastewaters can contain a high level of toxicants, heavy metals (arsenic, mercury, cadmium), pathogens, color, etc. Although microalgae are exceptional at absorbing these toxic elements or be resistant to pathogens the residual products cannot be utilized in food, animal fodder, fertilizers and can limit biofuel production (Saad et al., 2019). And it is known that algal wastewater treatment plants require large lands for open ponds therefore capital costs for these photobioreactor systems might be high.

There are some studies which have promising results for treating wastewaters and sewage using microalgae such as nutrient removal from various types of wastewater with different algal strains can be seen in Figure 4.6.

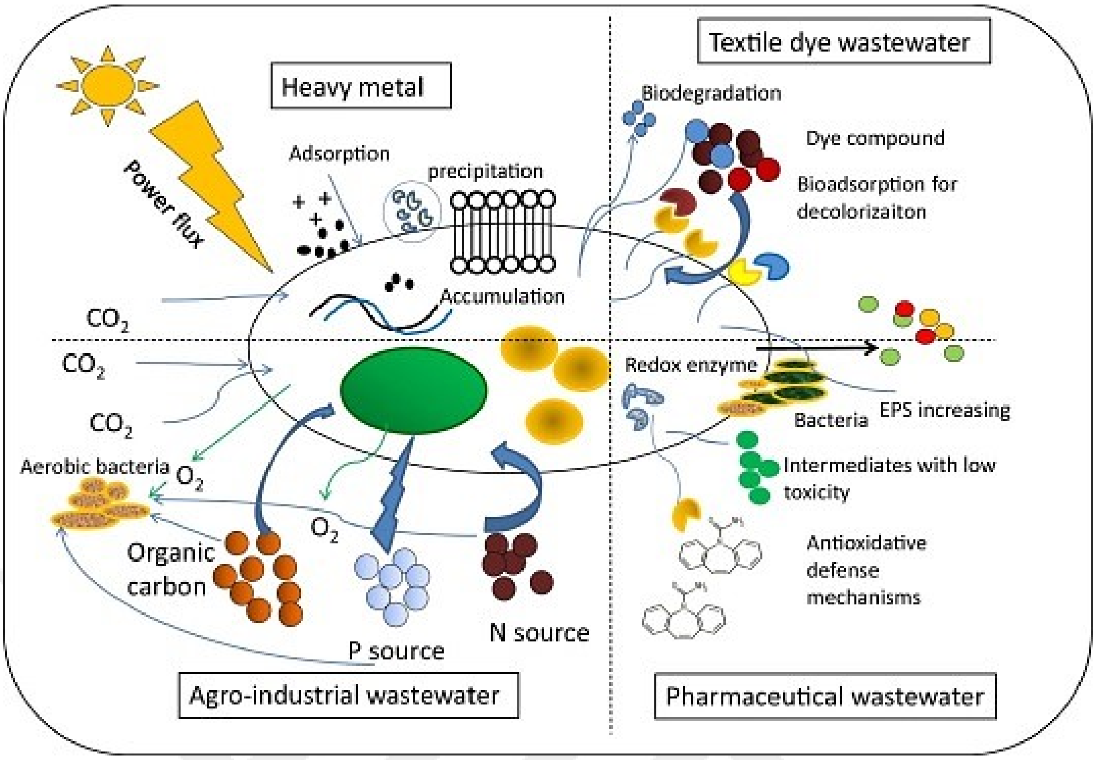

Figure 4.6 Mechanisms of various industrial wastewater treatments using microalgae and bacteria (Wang et al., 2016)

Although using microalgae is effective in treating wastewaters from multiple fields, domestic and industrial, as well as removing toxic metals while producing high concentrations of dissolved oxygen with cleaner effluents it must be noted that endogenous bacteria in wastewater streams can affect microalgae growth. Large land requirements and capital costs as well as because of the characteristics of various wastewaters this method is currently used at a laboratory scale (Cabanelas et al.,2013; Alvarez-Diaz et al., 2013; Cai et al., 2013; Brennan and Owende, 2010).

4.2.3 Useful Commodities: Biofuel Production

It is known that current reserves are not sufficient for the increasing demand for fossil fuels and have major disadvantages such as pollution, global warming, and high oil prices therefore microalgae represent a potential source of sustainable and renewable third generation type of biofuel resource while being an alternative energy source for fossil fuels. They are suitable for biofuel production because of their rapid growth, high biomass yields, high carbohydrate, and lipid contents. As mentioned before the biochemical composition of microalgae can vary with species

(Table 1.1), culture operational and geographical conditions (Ravindran et al., 2017) Therefore, microalgae can contain different amounts of lipids, proteins, vitamins, and bioactive compounds. Some pilot-scale studies show that some microalgae species can have up to 50% of lipid content which is suitable for good quality biofuel production (Grubisic et al., 2019).

The advantages of using biofuels obtained from microalgal biomass are;

- All year-round and high yield production with rapid growth potential
- Less land and water need than terrestrial crops while having higher yields
- Minimal environmental impacts while not requiring herbicides or pesticides
- Produced biofuels are non-toxic and highly biodegradable
- CO_2 fixation, bioremediation of wastewaters, different types of biofuel production and co-products (Brennan and Owende, 2010).

As mentioned in the previous section, there are many biofuels that can be obtained with different conversion technologies (Figure 4.7).

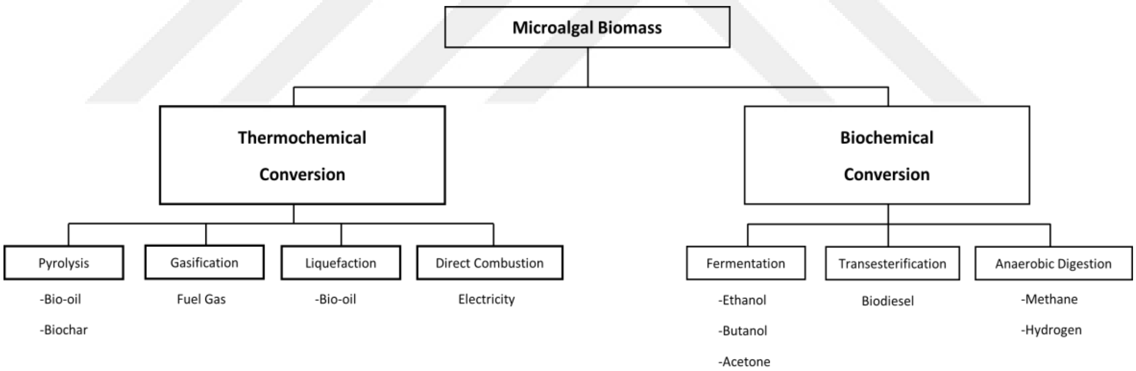

Figure 4.7 Biofuels that can be obtained from microalgal biomass with different conversion technologies

Methanol, hydrogen (anaerobic digestion) and ethanol, butanol, acetone can be produced through biochemical conversion processes while bio-oil, charcoal, and fuel gas can be produced from thermochemical conversions. With direct combustion steam or electricity can be obtained while with the chemical transesterification reaction produces biodiesel.

Biodiesel derived from biomass, is a mixture of long-chain fatty acids, by transesterification with alcohol, is an alternative for conventional diesel fuels.

Selecting the right strain is important to achieve suitable growing conditions to obtain a desired fatty acid composition which directly affects the quality and quantity of biodiesel. Also, biodiesel derived from microalgal biomass must match the properties of International Biodiesel Standards for Vehicles- EN12214 (Brennan and Owende,2010). Apart from these, microalgal biodiesel has many advantages over petroleum diesel; being renewable and biodegradable, non-toxic with reduced levels of particulates are some of them (Tiwari and Kiran, 2017).

It is stated that conversion of microalgal biomass, leaving nutrient-rich residual biomass that can be recycled, into biogas can recover as much energy as the derived energy from the lipid extraction from cells. But there is also a need for co-digestion because the C/N ratio of microalgae feedstock is usually around 10:1. In conclusion, since it does not require lipid extraction producing biogas from microalgal biomass represents an advantage over other biofuel technologies. But to ensure a convenient biogas yield, the factors, such as C/N ratio and pH, which affect the anaerobic digestion process must be considered (Tiwari and Kiran, 2017; Saad et al., 2019;).

There is a promising potential in the production of biohydrogen and bioethanol from microalgae, but the system must be combined with co-products or other biofuels to ensure economic feasibility. More research on this field is needed to analyze the full-scale of the system (Greenwell et al.,2009; Tiwari and Kiran,2017). The details of the conversion processes and obtained biofuels are discussed in detail in the previous section, therefore no additional information will be given in this section.

It is stated that microalgae can use light more efficiently and with their rapid growth they can produce 2 to 15 times higher lipids than terrestrial plants like corn, soybean, etc. (Brennan and Owende, 2010). Although they grow in aqueous media, they require less water and capable of all-year-round production with oil productivity that exceeds the yields of other oilseed crops. A comparison of microalgae and other oilseed crops that are used as a first, second, and third type of biodiesel feedstock can be found in Table 4.4 (Saad et al., 2019).

Table 4.4 Comparison of microalgae and other oil seed crops that are used as biodiesel feedstock (Saad et al., 2019)

Feedstock source	Oil content (% oil by wt. in biomass)	Oil yield (oil litres/ha/year)	Biodiesel productivity (kg biodiesel/ha/year)
Oil palm	36	5366	4747
Maize	44	172	152
Physic nut	41-59	741	656
Caster	48	1307	1156
Microalgae with low oil content	30	58,700	51,927
Microalgae with medium oil content	50	97,800	86,515
Microalgae with high oil content	70	136,900	121,104

Despite their high potential in the biofuel field, there are still some challenges that need to overcome to become be viable biofuel resources. The biofuels that are derived from microalgae:

- Must be competitive or less costly than fossil fuels
- With no additional water or land usage during production, harvesting, and conversion systems,
- Should enable environmental quality improvements such as; effective CO_2 mitigation, valuable product production, etc. while meeting up the primary energy demand.

One of the bottlenecks for biofuel production is the small size of microalgae causing energy requirements and costs to increase. Compared to agricultural practice, energy extraction from microalgae farming is more costly than other oil plant farming (Christenson and Sims, 2011).

An overall assessment of biofuel production from microalgae is usually constrained by high operational and capital expenses that challenge the economic feasibility of large-scale production. The co-production of high value-added products, pigments, bioactive compounds, antioxidants, biopolymers, fertilizers that can increase the profitability of the process must be considered to achieve sustainable performance. By improving harvesting and culturing systems, reducing overall costs, achieving continuous production, or applying biorefinery concepts which will be discussed in the following sections a variable and sustainable biofuel production from microalgal biomass can be achieved (Harun et al., 2010).

Another measure for sustainability is the energy return on investment rate (EROI), which can be defined as the ratio between an obtained energy from a certain energy source and the exergy spent to obtain it. If EROI <1 the system is undoubtedly sustainable with requiring energy to obtain the fuels is higher than the energy which presents in the fuel and its co-products. The energy required for microalgal biofuel production systems include the construction of the facility, chemicals, cooling systems, CO_2 pipelines, filters, pumps, harvesting techniques, conversion technologies, transportation, storage, etc. The estimated algal biofuel EROI is between 0.13 to 0.71, therefore making it unsustainable for the current situation. The desirable rate for EROI is >3 for the fuel to be a sustainable energy source. It is known that sustainability is the key to natural resource management, therefore it must involve operational, social, economic, and environmental considerations. (Saad et al.,2019). The summarized impacts of large-scale microalgae production on different dimensions can be found in Table 4.5.

Table 4.5 Summarized impacts of large-scale microalgae production on different dimensions

Dimensions	Issues
Environmental	- Water and land usage - Environmental impacts and co-products - Sustainability - Life cycle assessments - Interruption of animal habitats - Risk of algal blooms, biological invasion and eutrophication
Economical	- High capital and operational expenses - Energy requirements - Commercialization - Maintenance costs

Table 4.5 Summarized impacts of large-scale microalgae production on different dimensions (continued)

Social and Cultural	- Safety concerns about microalgae culturing - Effects on local wildlife and people
Political	- Government policies - Financial incentives
Technical	- Selection of suitable culturing and harvesting methods - Performance evaluation of overall system

4.2.4 Useful Commodities: Food colorant, nutraceuticals, dietary supplements for animal and human nutrition

Besides biofuels, a wide range of commercially valuable products (shown in Table 4.6) such as vitamins, essential amino acids, starch, cellulose, pigments, etc. can be extracted from microalgal biomass, which increases their potential for use in human nutrition and animal feed industries. It is stated that biofuel derived from microalgae is cost-effective unless coupled with obtaining high value-added co-products (functional foods and nutraceuticals) with health benefits (Barkia et al., 2019). Therefore, a coupled concept of biofuel and bioproducts extraction is needed for sustainable development in microalgal utilization. It is known that many green algae species have been utilized as food sources from ancient times. Studies show that commercial production of microalgae has started in the 1960s with *Chlorella*, in Japan and Taiwan and continued to develop with the mass production of *Arthrospira, Dunaliella salina* and *Haematococcus pluvialis* in Australia, China, USA, and Israel in the 1980s (Barkia et al, 2019). And currently many countries such as Taiwan, USA, India, Australia has expanded the production of microalgae for the dietary supplements, nutraceuticals, food colorants, and animal feeds.

Table 4.6 Commercially valuable products derived from microalgae biomass (Jha et al., 2017)

Type of Nutraceutical	Product name	Producing microalgae	Industrial application
PUFA's	EPA	*Pavlova sp., Nannochloropsis sp., Monodus sp., Phaeodactylum sp.*	Nutritional supplement and aquaculture feed constituent
	DHA	*Crypthecodiuimu sp., Schizochytrium sp.*	Nutritional supplement and constituent of infant and aquaculture feeds
	GLA (g-linolenic acid)	*Spirulina sp.*	Nutritional supplement
	AA	*Porphyridium sp.*	Nutritional supplement
Phycobiliproteins	Phyocyanin	*Spirulina platensis*	Natural colorant for food and cosmetological products, antioxidant
	Phycoerythrin	*Porphyridium cruentum*	Diagnostic fluorescence agent
Carotenoids	β-Carotene	*Dunaliella salina*	Natural food colorant, antioxidant, anti-cancer properties
	Astaxantin	*Haematococcus pluvialis*	Pigment and antioxidant
	Echinenone, Zeaxanthin	*Dunaliella sp.*	Food colorant
	Lutein	*Chlorella sp., Chlamydomonas sp.*	Food colorant

Table 4.6 Commercially valuable products derived from microalgae biomass (Jha et al., 2017) (continued)

Aminoacids	MAA (mycosporine-like amino acids)	*Aphanizomenon flos-aquae*	Sunscreen agent
Polysaccharides	Carragenan/aliginate	*Porphyridium cruentum*	Viscosifier, lubricant, flocculant, antiviral agent

The high nutritional value of algal products is sold in pure form as extracts, capsules, tablets, and as food additives/agents for several products (Khan et al., 2018). Therefore, as a rapidly growing market some of the dominating products and producers for dietary supplements and nutraceuticals can be found in Table 4.7 (Barkia et al, 2019).

Table 4.7 Dominating products and producers for dietary supplements and nutraceuticals worldwide (Barkia et al., 2019)

Microalgae (form)	**Major Producer**	**Origin**	**Annual Production (tons/year)**	**World Production (tons/year)**
Arthrospira (whole, dried microalgae)	Earthrise Nutraceuticals Cyanotech Corporation Hainan DIC Microalgae Japan Algae Co., Ltd. Parry Nutraceuticals FEMICO Nan Pao International Biotechnology Co. Ltd. Biorigin TAAU Australia Pty Ltd.	USA USA China Japan India Taiwan Taiwan Switzerland Australia	450 360 350 30-100 >175 50-150 70 60 50-60	4100-6700

Table 4.7 Dominating products and producers for dietary supplements and nutraceuticals worldwide (Barkia et al., 2019) (continued)

Chlorella (whole, dried microalgae)	Taipei Roquette Klötze Blue Biotech Earthrise Nutritionals	Taiwan Germany Germany USA	400 130-150 * *	2000
β-carotene from *Dunaliella* (as oleoresin)	Cognis Nutrition & Health Co. Nature Beta Technology Ltd.	Australia Israel	* *	1200
Astaxanthin from *Haematococcus* (as oleoresin)	Cyanotech Corp. Mera Pharmaceuticals Inc. Fuji Chemical Industries BioReal AB	USA USA Japan Sweden	* * * *	300

Currently *Arthrospira sp., Chlorella sp.* are used in yogurts and cheese to promote probiotics viability and to increase hardness (Caporgno and Mathys, 2018; Varga et al., 2002). *Arthrospira platensis, Chlorella vulgaris and H. pluvialis* are used in cookies to boost the fiber and protein contents while increasing the antioxidant capacity (Singh et al., 2015; Hossain et al., 2017). As well as derived pigments and carotenoids from mentioned microalgal biomass and many others such as are used as additives, colorings, and protein ingredient in snacks, sports drinks, and pasta (Lucas et al., 2018). Also, oil extracted from microalgae are rich in high-value polyunsaturated fatty acids (PUFAs) and has a similar composition to vegetable oils (De Marco et al., 2014; Fradique et al., 2013; García et al., 2017).

It is also known that *Chlorella, Scenedesmus,* and *Spirulina* species have beneficial aspects on animals such as improved fertility and immune response, healthier skin, and coat. Also, algae can be used as fish feed in aquacultures, coloring for farmed salmons, enhancing the immune systems, etc. (Grubisic et al., 2019). However, the concentration of the algae in the animal fodder and human nutrition must be controlled, to not cause any detrimental effects and microalgae biomass must be cultured under strict food safety regulations. Therefore, it must be noted that microalgae cultured using wastewaters streams are not suitable for utilization for nutrition. In conclusion, microalgae represent a promising future for a reliable and sustainable substitute for commercialized commodities for human and animal nutrition. However, to date, a limited number of microalgae species have been utilized for commercial production; therefore, extensive developments and research is needed in this field including genetic engineering modifications to enhance the yield.

4.2.5 Useful Commodities: Biofertilizers

As mentioned above sections, residual biomass from some conversion technologies such as pyrolysis and anaerobic digestion has potential as a biofertilizer and bio stimulants in agricultural applications and are considered an environmentally friendly alternative for synthetic fertilizers and plant protection products. They contain large amounts of N, P, and other nutrients present in microalgal biomass which is essential for crop growth; therefore, they can be considered as organic fertilizers (Ronga et al., 2019). The wastewater grown microalgae can only be considered as biofertilizer if algal biomass has no heavy metals or other toxic non-biodegradable compounds which can be found in industrial waste streams. Spirulina species in wastewater streams are investigated for bioremediation of wastewaters and nutrient sources for biofertilizers in recent studies compared to organic and synthetic fertilizers are shown in Table 4.8 shows promising results for whole-cell biomass is suitable for use of biofertilizers (Mahapatra et al., 2018).

Table 4.8 Experimental results for whole cell biomass for use of biofertilizers comparing organic and chemical fertilizer characteristics (Mahapatra et al., 2018)

Macromolecular composition		Spirulina sp. (cultivated)	Spirulina sp. (wastewater-fed lakes)	Organic fertilizer	Chemical fertilizer
Lipids (%)		11	9.8	-	-
Carbohydrate (%)		22	18.2	-	-
Protein (%)		48.2	45	-	-

Table 4.8 Experimental results for whole cell biomass for use of biofertilizers comparing organic and chemical fertilizer characteristics (Mahapatra et al., 2018) (continued)

Nitrogen (%)	7.7	7.2	1.09	12.4
Phosphorus (%)	0.88	0.71	0.7	6.6
Potassium (%)	1.76	2.44	1.27	12.5
Calcium (%)	0.67	0.52	0.48	0.1

Algalization, which is a term developed in the 1970s; the use of chlorophytes and cyanophytes as biofertilizers has a high potential to enhance productivity and plays an important role in a variety of agricultural and ecological situations. In several studies, microalgal extracts showed beneficial effects for plants, such as improved nutrient uptake, crop yields, and quality, nitrate reduction potential (L Oritz-Moreno et al., 2019). However, agronomic, chemical, physiological studies are needed to be addressed to better understand the effects of microalgal extracts for agricultural sustainability.

4.2.6 Useful Commodities: Pharmaceutical and Cosmetic Industries

Vitamins, polysaccharides, bioactive compounds, essential amino acids, fatty acids, and commercially important pigments which are commonly used in the pharmaceutical and skincare industry can be extracted in various types of microalgal biomass. Bioactive compounds and commercially important pigments (β-carotene, chlorophyll, carotenoids, astaxanthin, etc.) have antioxidant, antiviral effects and are used in treatments for optical diseases, tumorigenesis, and neuronal disorders. A summary of potential health benefits of bioactive compounds obtained from microalgal biomass is shown in Table 4.9 (Barkia et al., 2018).

Table 4.9 Summary of potential health benefits of bioactive compounds obtained from microalgal biomass to be utilized in pharmaceutical and medical industry (Barkia et al., 2018)

	Bioactive compounds	Source	Health Benefits
Caretonoids	B-carotene	*Dunaliella salina*	Antioxidant, pro-vitamin A, anti-allergic, anti-inflammatory
	Astraxanthin	*Haematococcus pluvialis, C. zofingiensis*	Antioxidant, anti-inflammatory
	Lutein	*Scenedesmus spp., Muriellopsis sp., C. sorokiniana*	Antioxidant, anti-inflammatory
PUFAs	Arachidonic acid (AA)	*Porphyridium purpureum, P. cruentum, Parietochloris incisa*	Improves normal growth, visual and functional development in infants
	Eicosapentaenoic acid (EPA)	*Nannochloropsis sp., Phaeodactylum tricornutum, Porphyridium cruentum*	Cardiovascular benefits, mental development and support, anti-inflammatory, protection against atherosclerosis
	Docosahexaenoic acid (DHA)	*Crypthecodinium cohnii, Schizochytrium spp., Ulkenia spp.*	Cardiovascular benefits, improves nervous system development and function of the brain
Other metabolites	Peptides	*Chlorella pyrenoidosa, Nannochloropsis oculata*	Antioxidant, anti-inflammatory, anticancer, antihypertensive
	Phenolics	*Arthrospira maxima, Tetraselmis suecica, Botryococcus braunii, Isochrysis sp., Chlorella vulgaris, Nannochloropsis sp.*	Antioxidant
	Phycocyanin	*Arthrospira platensis*	Antioxidant, anti-inflammatory
	Sulfated polysaccharides	*C. pyrenoidosa, C. stigmatophora, Porphyridium sp., Phaeodactylum tricornutum*	Antioxidant, anti-inflammatory antiviral, immunomodulatory
	Water soluble extract	*Chlorella stigmatophora, Phaeodactylum tricornutum, Graesiella sp*	Anti-inflammatory, analgesic, antioxidant, antiproliferative

Algal fatty acids are among the essential fatty acids for human development and have high nutritional, medicinal, and therapeutic effects for cardiac diseases and asthma. There are new research fields about identifying the bioproducts that can be obtained from microalgae and can be used in biological and medicinal activities. Sterols that are produced by microalgae (phytosterols) are a promising source for beneficial health effects for anti-cancer, anti-inflammatory, neurological diseases, and also can be used in nutraceuticals. Peptides and amino acids have therapeutic effects on cells and tissues and microalgae is also a rich source of essential vitamins (E, C, A) for humans (Tang et al.,2020; Barkia et al., 2019). In conclusion, there is a need for further research on pharmaceutical and medical fields, which is currently a multibillion-dollar industry, of bioactive compounds that are derived from microalgal biomass. Products aimed to improve and protect the structure and appearance of skin are called cosmetics. Currently, there are emerging health issues and allergic reactions which arise from synthetic compounds that are used in cosmetic products. Microalgae are naturally exposed to oxidative stress and develop various types of bioactive compounds during their growth. These bioactive compounds can have benefits for the skin, promotes blood circulation, moisturizing skin, repair damaged skin, renewing cells, hair care, inhibit inflammation and have anti-aging effects, etc. and can be used in the cosmetic industry (Wang et al., 2015; Ariedea et al., 2017). Representing a natural and non-toxic resource for the cosmetic industry, some of the bioactive compounds that can be utilized are shown in Table 4.10.

Table 4.10 Bioactive compounds that can be utilized in cosmetics industry (Tang et al., 2020)

Microalgae	Function in skin care products
Monodus sp., Thalassiosira sp., Chaetoceros sp., and Chlorococcum	Integrated into anti-aging products for collagen stimulation
Phaeodactylum tricornutum	Improve skin elasticity and firmness as well as to protect the skin against UV exposure
Chlamydocapsa sp. (snow algae)	Hydrating the skin and increase the longevity of the skin following exposure to dryness, UV radiation or cold
Spirulina platensis	Avoid the harmful activity in the dermis and epidermis by radicals

Table 4.10 Bioactive compounds that can be utilized in cosmetics industry (Tang et al., 2020) (continued)

Chlorella vulgaris and Spirulina maxima	Produce vitamins that help to get rid of dark circles, refresh and purify the skin Skin moisturizing agent
Aurantiochytrium sp., Thraustochytrium sp. and Schizochytrium sp.	Produce squalene that used in moisturizing lotions to provide ideal skin properties by stimulating emollient and antistatic actions
Haematococcus pluvialis *Anabaena vaginicola*	Antioxidant and sunscreen protection UV protection
Spirulina platensis *Porphyridium cruentum*	Antioxidant and pigment for eye-liner, eye shadow and lipsticks
Chlorella vulgaris	To mask odors in dentifrices and deodorants Collagen repair

Currently, the effects of bioactive compound products derived from microalgal biomass are tested in different studies worldwide. However, more stability, compatibility, and toxicological studies need to be considered to evaluate the commercial potential of microalgae used in the cosmetic industry to eliminate undesirable effects (Ariedea et al., 2017).

5
MATERIALS AND METHODS

There are still some challenges and limitations from mass cultivation and harvesting of microalgae to be a feasible source for biofuels and bioactive compounds. There is a need to develop a flexible techno-economic model to overcome the challenges associated with microalgal systems and to upgrade cultivation and harvesting from pilot-scale to commercial level.

Since the 1960s, MCDM (Multiple-criteria decision making) also known as MCDA (Multi-criteria decision analysis) is an actively used term in research studies to describe and solve problems that involve multiple choices and alternatives. There are different techniques available for MCDM which can be adopted and used, such as IPV, AHP, TOPSIS, etc. which can change the complexity of the problem that wants to be solved. Because environmental decisions are often complex with many variables and can rely on many different models, analyzes and tools there can be a need for a systematic and understandable framework to organize and analyze the collected data. In this study, there is a need for organizing and analyzing the collected data from the literature, pilot-scale studies, and experiments in a systematic and understandable framework. Because there are many parameters affecting the growing and harvesting of microalgae on a large scale, TOPSIS method is selected and carried out in excel spreadsheets to evaluate the selected categories and determine the most acceptable alternatives, among others.

5.1 TOPSIS Method

To identify the solution to finite alternatives based upon having the shortest geometric distance from the ideal positive solution and the longest geometric distance from the ideal negative solution, the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) is developed in 1981 by Ching-Lai Hwang and Yoon. In the TOPSIS technique, different attributes can be incorporated by assigning relative weights to each attribute based on its importance in the study assessment category. The weights, only subjective input needed, can be selected, or assigned by the decision-makers based on their importance in the study assessment category. TOPSIS allows a trade-off between criteria where a poor result in one criterion can be negotiated by a good result in another criterion and the non-linear relationship between single dimension scores and distance ratios produces smoother tradeoffs.

Some other MCDM models were not suitable for the selected field of study, which can lead to inconsistencies in ranking, sensitive to inconsistent data or requiring any cases, difficulty to develop and can need simulations and their results may not reflect the real situation. Unlike the other models, TOPSIS being a holistic decision-making tool, provides a clear choice of direction and one of the most used analysis methods in environmental engineering decisions. TOPSIS approach enables the qualitative analysis using a combination of subjective and objective information and an analytical decision is made based on collected data. This application of the TOPSIS method reveals that decision-makers can identify the most acceptable alternative in a more scientific way instead of relying on intuition or engineering judgment. Also, TOPSIS provides a more realistic form of modeling from other non-compensatory models which include or exclude different alternative solutions based on hard cut-offs. The TOPSIS analysis is carried out as the following steps (Esfandiari and Rizvandi, 2014):

Step 1: Creating the normalized decision matrix

As the first step of this method, create an evaluation matrix that consists m category and n criteria. Therefore, the matrix is presented as $(X_{ij})_{mxn}$ and intersection of each criteria and alternative is given as: X_{ij}, (x = {x_{ij}, i, = 1, 2, 3, …., m;})

Where;

m = alternatives(categories)

n = attributes (criterias)

x_{ij} = the value of the column i and criteria j

Step 2: Normalizing the matrix

In the second step, the matrix is normalized by changing the attributes from dimensional to non-dimensional by using following normalization method:

$R = (r_{ij})_{mxn}$

$i = 1, 2, 3, \ldots, m \qquad j = 1, 2, 3, \ldots, n$

$$R_{ij} = \frac{x_{ij}}{\sqrt{\Sigma_{i=1}^{m} x_{ij}^{2}}} \tag{5.1}$$

R_{ij} = the value in row i and criteria j of the normalized decision matrix

It must be stated that in the reviewed literature data for MCDM and TOPSIS method analysis, vector normalization method is a commonly used normalization method used to normalize the matrix. According to the data from literature (Ozdagoglu, 2013) different normalization methods were evaluated and it is stated that vector normalization method is a reliable method which can be used in the decision-making process in TOPSIS analysis.

Step 3: Calculation of the weighted normalized matrix

All the criteria used in the matrix has a determined criteria weight (wj); which is given to point out the importance of each criteria in the analyses. Each criteria in the normalized matrix (R_{ij}) is multiplied by the assigned weight.

$$V_{ij} = R_{ij} \text{ x } w_j \tag{5.2}$$

Step 4: Determining the ideal best alternative (A_b) and ideal wors alternative (A_w)

$A_b = (v_1^+, \ldots, v_j^+, \ldots, v_n^+) = \{ (\text{max\_j } V_{ij} \mid j = 1, 2, \ldots, n | i = 1, \ldots, m|$

$A_w = (v_1^-, \ldots, v_j^-, \ldots, v_n^-) = \{ (\text{min\_j } V_{ij} \mid j = 1, 2, \ldots, n | i = 1, \ldots, m|$

Where;

$J^+ = \{ j = 1, 2, \ldots, n | i = 1, \ldots, m| \}$ in A_b is associated with the criteria having a positive impact,

$J^- = \{ j = 1, 2, \ldots, n | i = 1, \ldots, m| \}$ in A_w is associated with the criteria having a negative impact.

Step 5: Calculation of the distance of each measure from the ideal

$$S_i^+ = \sqrt{\sum_{J=1}^{n}(v_j^+ - v_{ij})^2} \quad (5.3)$$

$$S_i^- = \sqrt{\sum_{J=1}^{n}(v_j^- - v_{ij})^2} \quad (5.4)$$

S_i^+ = the ideal distance between the best condition Ab and the alternative i

S_i^- = the ideal distance between the worst condition A_w and the alternative i

Step 6: Calculation of the relative closeness of each measure to the ideal

$$c_i = \frac{S_i^-}{S_i^+ + S_i^-} \quad (5.5)$$

Where; $0 \leq c_i \leq 1$

Step 7: Ranking the alternatives according to c_i where $i = 1,2,..,m|$

In the ranking the alternative that is he option closest to 1 is the most preferred and the farthest to 1 is the less preferred alternative among others.

5.2. Data Collection

5.2.1 Selection of Category

Following six commonly used microalgae cultivation systems, suitable for *Chlorella* species, are selected as the set of categories for evaluation of microalgae cultivation methods. Each system is selected among pilot-scale studies and literature review and studied in detail in the previous sections.

- Circular Pond (with stirring arm)
- Raceway Pond (with paddle wheel)
- Tubular (Glass-Helical) PBR
- Tubular (Glass-Horizontal) PBR
- Plate (Glass-Flat Panel) PBR
- Plastic Bag PBR (Leidos)

Following twelve commonly used microalgae harvesting techniques, suitable for *Chlorella* species due to its special characteristics such as cell radius, are selected for evaluation of microalgae harvesting methods. Each method is selected among pilot-scale studies and literature review and studied in detail in the previous sections.

- Sedimentation
- Centrifugation (Decanter)
- Centrifugation (Disc Stack)
- Dispersed Air Flotation
- Dissolved Air Flotation
- Fluidic Oscillation
- Cross-Flow Filtration
- Chemical Harvesting (Organic Compound- Chitosan)
- Chemical Harvesting (Inorganic Compound- Ferric Chloride)
- Electrocoagulation- Flocculation
- Bio-flocculation
- Suspended Air Flotation

5.2.2 Selection of Criteria's and Determining Weightages

As mentioned before various pilot-scale studies and literature about microalgae cultivation and harvesting methods are reviewed while selecting the set of criteria in this section. Each criteria in the matrix are multiplied by their assigned criteria weights and an analytical hierarchy process is performed to decide the set of weights according to their importance in environmental engineering perspective and reviewed studies are shown in Table 5.1 for microalgae cultivation and Table 5.2 for microalgae harvesting.

Criteria that are selected to evaluate *Chlorella* cultivation are;

- Capital Cost ($): include buildings, vehicles, land purchase expenses equipment's, etc. which are one-time expenses to make the project status commercially operable.

- Operation Cost ($/ton AFDW): include salaries, rents, energy costs, maintenance expenses, etc. which are the day-to day expenses to run a facility.
- Biomass Production ($g/m^2.d$) : is the total amount of biomass that is produced daily in the facility. To achieve a suitable production model, it is important to know the amount of biomass production.
- Biomass Selling Price ($/ton AFDW): is the selling price of the produced biomass per unit. The cultivation system design and microalgae quality are the major factors that affect the selling price.
- Cultivation Area (m^2): is the area that the microalgae culturing system (ponds, PBR's, etc.) cover.
- Facility Area (m^2): is the area that the facility buildings, roads, cultivation systems, etc. cover.
- Volumetric productivity (g/L): is the biomass production rate, reported in grams per liter.

Criteria selected to evaluate *Chlorella* harvesting techniques are;

- Energy Need (kWh/m^3): is the consumed energy consumed to harvest the cultured microalgae in the facility. The energy need is an important factor to achieve an economical model and differs by each harvesting method.
- Cost (OPEX, CAPEX, Chemical Costs) ($): includes chemical costs, operational costs, capital costs, maintenance costs, etc. that equals the total amount of expenditures incurred by harvesting operations.
- Time (min): is the time by minutes that are required to perform the harvesting method efficiently. Time is dependent on the selected technique.
- Efficiency (%): equals to the amount of biomass that is recovered using the selected harvesting technique. To achieve an economically viable harvesting technology, efficiency is the most important criteria

Table 5.1 Determined criteria weights for microalgae cultivation matrix

Criteria	Unit	Criteria Effect	Assigned Weightage
Capital Cost	($)	Non Benefit	0.135
Operation Cost	($/ton AFDW)	Non Benefit	0.135
Biomass Production	($g/m^2.d$)	Benefit	0.242
Biomass Selling Price	($/ton AFDW)	Benefit	0.135
Cultivation Area	(m^2)	Non Benefit	0.073
Facility Area	(m^2)	Non Benefit	0.04
Volumetric productivity	(g/L)	Benefit	0.24

Table 5.2 Determined criteria weights for microalgae harvesting matrix

Criteria	Unit	Criteria Effect	Assigned Weightage
Energy Need	(kWh/m^3)	Non Benefit	0.23
Cost (CAPEX, OPEX, Chemical Costs, etc.)	($)	Non Benefit	0.23
Time	(min)	Non Benefit	0.12
Efficiency	(%)	Benefit	0.42

It can be very challenging to identify a technology that has low costs and can achieve a stable culture with minimum energy consumption. To obtain a technical point of view and to assess each current technique in terms of overall system performance and volumetric productivity, biomass production, energy consumption, and time is selected as criteria.

The capital and operational expenses and the facility and cultivation area are essential to analyze and reveal the financial investment for the cultivation methods of microalgal biomass. Because the feasible development of large-scale commercialization depends on economics as well as technologies and obtained products, the costs for each process are added as criteria.

Because they represent the efficiency of the process biomass production and volumetric productivity are marked as the most important beneficial criteria in the microalgae cultivation matrix followed by biomass selling price, capital and operational expenses, which are beneficial and non-beneficial criteria, respectively.

Depending on the facility, energy use, materials type, etc. operational and capital costs can vary. Therefore, expenses are one of the major bottlenecks for economically viable microalgae cultivation system designs and considered among the important criteria. In the conducted matrix for microalgae cultivation the facility and cultivation are marked less important than the other criteria.

Some of the selected microalgae harvesting methods have limited performed studies therefore while determining the TOPSIS matrix for microalgae harvesting methods the factors affecting the systems to be viable in the reviewed pilot-scale studies and literature are selected as the criteria. Efficiency is the most important and only beneficial criteria in this model followed by energy need and overall costs which are non-beneficial criteria. Each harvesting step has different costs depending on the cost of chemicals, the quantity of the chemicals, used equipment, system investment, ad operational costs. And although in harvesting microalgae time is stated as an important it is marked as less important than the other selected criteria according to the conducted analytical hierarchy process.

5.2.3 Determining the Matrix

To evaluate the microalgae culturing and harvesting techniques separately two TOPSIS models are designed and performed with selected categories and criteria sets. Literature and pilot-scale study results are placed into the relevant fields in the matrix. Table 5.3 shows the determined TOPSIS matrix for microalgae cultivation and Table 5.4. shows the determined TOPSIS matrix for microalgae harvesting methods.

Table 5.3 Determined matrix for microalgae cultivation

	Capital Cost ($)	Operation Cost ($/ton AFDW)	Biomass Production (g/m^2.d)	Biomass Selling Price ($/ton AFDW)	Cultivation Area (m^2)	Facility Area (m^2)	Volumetric productivity (g/L)	REFERENCES
Plate Photobioreactor (Glass-Flat Panel)	510617	478	52.5	1793	982612	1675053	2	(Ahmad et al., 2014); (Borowiak et al., 2021); (Chandra et al., 2020); (Chen et al., 2011); (Christenson and Sims, 2011); (Clippinger and Davis, 2019); (Davis et al., 2016); (Garcia et al., 2020); (Hanotu et al., 2011); (Hinterholz et al., 2019); (Mohan et al., 2014); (Münkel et al., 2013); (Perez-Garcia and Bashan, 2015); (Shen et al., 2009);); (Singh and Sharma, 2012); (Singh et al., 2017); (White et al., 2015); (Zhu et al., 2018)
Tubular Photobioreactor (Glass-Helical)	377892	497	42.5	1737	1214100	1945393	2	
Raceway Pond with paddle wheel	33042	214	25	494	2023500	2904128	0.5	
Circular Pond with stirring arm	33042	214	25	400	2023500	2023500	0.5	
Plastic Bag PBR (Leidos)	971124	340	52.5	639	982612	1282090	1	
Tubular Photobioreactor (Glass-Horizontal)	437748	377	25	708	2083396	4226687	2	

Table 5.4 Determined matrix for microalgae harvesting

	Energy Need (kWh/m^3)	Cost s (OPEX, CAPEX, Chemical Costs) ($)	Time (min)	Efficiency (%)	REFERENCES
Sedimentation	0.1	50	60	65	(Ahmad et al., 2012); (Ahmad et al., 2014); (Al-hattab et al., 2015); (Chen et al., 2011); (Christenson and Sims, 2011); (Coward et al., 2015); (Edzwald and Wingler, 1990); (Fasaei et al., 2018); (Fayad et al., 2017); (Gerardo et al., 2015); (Gujar et al., 2019); (Kurniawati et al., 2014); (Lei et al., 2015); (Manheim and Nelson, 2013); (Matter et al., 2019); (Najjar and Abu-Shamleh, 2020); (Ndikubwimana et al., 2016); (Oh et al, 2001); (Papazi et al., 2010); (Patyna et al., 2018); (Rakesh et al., 2014); (Richardson et al., 2014); (Sanyano et al., 2013); (Sharma et al., 2013); (Shelef et al., 1984); (Sim et al., 1988); (Singh et al., 2017); (Wang et al., 2019); (Wang et al., 2013); (Wyatt et al., 2011); (Zhang et al., 2010); (Zhu et al., 2018)
Cross-Flow Filtration	2.06	200	10	92.5	
Centrifugation using disc stack	5.5	500	10	90	
Centrifugation using decanter	8	500	10	80	
Dispersed Air Flotation	10	450	10	93.6	
Dissolved Air Flotation	7.6	450	10	95	
Fluidic Oscillation	10	350	10	95	
Chemical Harvesting using ferric chloride	4	300	60	90	
Chemical Harvesting using chitosan	4	300	30	99	
Bio-flocculation	1	250	20	95	
Electrocoagulation- Flocculation	3	400	20	90	
Suspended Air Flotation	8	350	10	77	

5.2.4 Data Evaluation

The determined matrix and sets are evaluated by TOPSIS analysis steps which is mentioned in the previous sections. Each determined matrix is normalized by vector normalization method and multiplied by their weightage sets. After calculating the weighted normalized decision matrix for each analysis ideal beast and ideal worst alternatives are determined which is shown in the Table 5.5 for microalgae cultivation methods and Table 5.6 for microalgae harvesting techniques.

Table 5.5 Determined ideal best and ideal worst values for microalgae cultivation matrix

	Capital Cost ($)	Operation Cost ($/ton AFDW)	Biomass Production (g/m^2.d)	Biomass Selling Price ($/ton AFDW)	Cultivation Area (m^2)	Facility Area (m^2)	Volumetric productivity (g/L)
Plate PBR (Glass-Flat Panel)	0.05554	0.07105	0.13250	0.08811	0.01797	0.01078	0.13064
Tubular PBR (Glass-Helical)	0.04111	0.07387	0.10726	0.08536	0.02220	0.01252	0.13064
Raceway Pond (with paddle wheel)	0.00359	0.03181	0.06309	0.02427	0.03700	0.01869	0.03266
Circular Pond (with stirring arm)	0.00359	0.03181	0.06309	0.01965	0.03700	0.01302	0.03266
Plastic Bag PBR (Leidos)	0.10563	0.05053	0.13250	0.03140	0.01796	0.00825	0.06532
Tubular PBR (Glass-Horizontal)	0.04761	0.05603	0.06309	0.03479	0.03809	0.02720	0.13064
Ideal best value (V+)	0.00359	0.03181	0.13250	0.08811	0.01797	0.00825	0.13064
Ideal worst value (V-)	0.10563	0.07387	0.06309	0.01965	0.03810	0.02720	0.03266

Table 5.6 Determined ideal best and ideal worst values for microalgae harvesting matrix

	Energy Need (kWh/m3)	Cost (OPEX, CAPEX, Chemical Costs) ($)	Time (min)	Efficiency (%)
Sedimentation	0.0010698	0.0091058	0.073485	0.088551
Cross-Flow Filtration	0.0220369	0.0364232	0.012247	0.126015
Centrifugation using disc stack	0.0588363	0.0910579	0.012247	0.122609
Centrifugation using decanter	0.0855801	0.0910579	0.012247	0.108986
Dispersed Air Flotation	0.1069752	0.0819521	0.012247	0.127513
Dissolved Air Flotation	0.0813011	0.0819521	0.012247	0.129421
Fluidic Oscillation	0.1069752	0.0637405	0.012247	0.129421
Chemical Harvesting using inorganic compound- ferric chloride	0.0427901	0.0546347	0.073485	0.122609
Chemical Harvesting using organic compound- chitosan	0.0427901	0.0546347	0.036742	0.134870
Bio-flocculation	0.0106975	0.0455289	0.024495	0.129421
Electrocoagulation- Flocculation	0.0320926	0.072846	0.024495	0.122609
Suspended Air Flotation	0.0855801	0.0637405	0.012247	0.104899
Ideal best value (V+)	0.0010698	0.0091058	0.012247	0.134870
Ideal worst value (V-)	0.1069752	0.0910579	0.073485	0.088551

Then the distance from the ideal best (Si+) and ideal worst (Si-) for each value is calculated with the given Euclidean formula and the performance scores (Pi) are calculated, ranks are stated and shown in the following Table 7.1 for culturing methods and Table 7.2 for harvesting techniques. The results and ranking of the performed TOPSIS analyses will be discussed in the following section.

6
RECOMMENDATIONS FOR FUTURE RESEARCH DIRECTIONS

6.1. Biorefinery Concept

Biorefining means sustainable processing or breaking down of obtained biomass into marketable bio-based products. (fuels, feed, chemicals, materials, food, etc.) There are different flow diagrams of biorefineries varying on the target product. Developing a microalgae biorefinery will include extracting value from bioactive lipids, proteins, pigments, carbohydrates, and compounds which will be produced by microalgae during their growth time. Because the microalgal biorefinery concept combines all technologies required for harvesting, extracting, purification, and conversion technologies with intermediate recovery and recycling pathways it enables maximizing the final product amount while minimizing the energy inputs and waste generation. But unlike other products that are established in refinery processes biological compounds have very definite shell-life therefore they cannot be stored.

As mentioned in the previous sections phototrophic microalgae species are currently used in multiple industries to produce several products. At present most relevant microalgae species cultivated are Cyanobacterium, Arthrospira platensis, Haematococcus pluvialis, Chlorella vulgaris, Dunaliella salina. However, currently, these algae are dedicated to the production of a single specific product on large scale, meaning while one component is extracted (pigments) the other possibly valuable components are discarded (lipids, proteins, carbohydrates, etc.) And although a biorefinery approach looks like a great solution to improve the large-scale commercialization of microalgae application it is crucial for achieving a complete fractionation and valorization of the microalgal biomass by a multiproduct biorefinery approach the cost and energy requirements are still high due to the lack of mild technologies for harvesting and extracting the bioactive compounds from microalgal biomass. To reduce the costs, simplified or hybrid processes need to be developed for main unit operations. (Lam et al., 2018) Therefore, to achieve a sustainable microalgae biorefinery a complete understanding of the microalgae stream process is needed. As mentioned in the previous sections cultivation and harvesting methods for microalgae each have their own advantages and disadvantages. It is important to select the right method to achieve a scalable, cost-effective, and energy-efficient process. The microalgae utilization can be classified into two main stages as upstream (USP) and downstream (DSP) process.

The efficiency of USP involves culturing of the microalgae is affected by:

- Microalgal strains
- Nutrient availability and concentration (as N and P)
- Light intensity and photoperiod
- CO_2 supply

For most algae species temperature between 20-30 C is considered suitable for culturing but other culturing conditions may differ by the species. Also, some physical adaptations for the operational conditions can have negative impact on the biorefinery process, for example; the decrease of the quantity of unsaturated fatty acids with temperature increase.

It is known that microalgae grow in aqueous media water is need another important parameter in cultivation. As mentioned in the utilization and the discussion section it is not economically viable for use of freshwater resources for culturing media. The combination of wastewater streams into the system will not only reduce the overall nutrient costs also the bioremediation of various industrial or domestic effluents. And coupling the CO_2 mitigation using the flue gas as a supply in these systems are seen as sustainable options. It must be stated once more, although coupling wastewater streams and flue gasses into microalgae culturing systems have gained attention and seem suitable; the outputs for the biorefinery process can be affected due to heavy metals, toxicants, colors, and safety regulations must be strictly followed.

After the upstream process, the conventional downstream techniques of microalgae biomass including extraction, purification, and harvesting to obtain the target products is the major bottleneck for the microalgae biorefinery concept with their high system costs. Therefore, the target products should be viable for industrial scale to achieve a sustainable system. After an effective harvesting step, the first focus should be the cell disruption to release and make the contents in microalgae available for extraction. When the contents are released from the microalgae cells extraction and separations need to be done. The extraction technique may differ on the microalgae strain therefore, it is important to select the right flexible technique for different species. Or selecting microalgae species without a cell wall would skip the cell disruption step and would make the overall DSP less energy intensive. The proposed procedure for DSP goes as (Vanthoor-Koopmans et al., 2013);

1) After the suitable harvesting specific cell disruption using mild technology (if needed)
2) Extraction of the different fractions (protein, lipids, carbohydrates) to achieve functional components
3) Further separation of the different factions to obtain specific target products such as omega-3 fatty acids.

Therefore, when more products can be obtained from a single microalgae production process, the value of the microalgae will increase, and the process will be economically viable.

Since the microalgae biorefinery research is a relatively new field there can be many improvements and adaptations to increase the number of outputs as well as improving production and extraction efficiency.

There are companies and researchers actively working on this fields recently published life cycle assessments (LCA) and techno-economic evaluations about microalgal biorefineries. (Lam et al., 2018) Evaluation of LCA is conducted on the basis of Global Warming Potential which is quantified by the amount of CO_2 emitted per unit energy and Net Energy Ratio which is the total energy flow of the process. But it must be stated that the conducted LCA and TEA analyzes in various studies only include biofuel production, no further product extraction is evaluated.

Ruiz et al evaluated the complete biorefinery approach for the outputs of all biomass components. In their prediction DSP costs for obtained value-added products were about 60% of the overall process costs. And with specific processes requiring more unit operations, purification, costs are also higher. Therefore, it is stated that an economically feasible biorefinery concept for microalgal biomass cannot be achieved with traditional downstream processes. Rethinking of processes, coupling, technology, and simplification is required. It can be said that the selection of application technology for microalgae biorefinery depends on required energy, costs, and available current technology. (Figure 6.1)

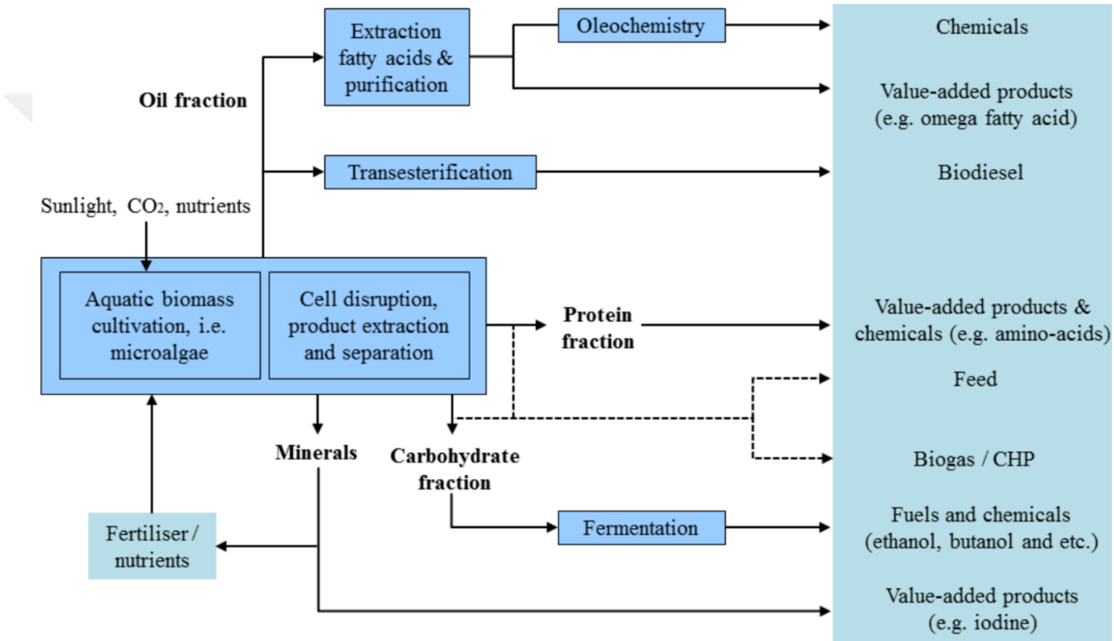

Figure 6.1 Biorefinery concept for microalgae biomass to obtain value-added products (Retrieved from Chew et al., 2017)

Mitra and Mishra evaluated the feasibility of coupling wastewater streams and flue gasses into biorefinery concepts for microalgal biomass to lower the production costs. As the result, the whole microalgal biomass product cannot be used for food or feed production because the used nutrient media is wastewater. After the purification of the microalgal biomass valuable compounds like fatty acids and pigments can be used after appropriate testing according to the regulations while the residual biomass can be used for biogas production. (di Visconte et al.,2019)

Based on the research studies a single extraction from microalgal biomass does not result in positive environmental outcome and there is a need for further evaluation for processing of the residual microalgae feedstock to extract VAPs. It is stated that this coupling can reduce the overall system costs by 30% (Park et al., 2019)

In conclusion, because the TEA and LCA foundation tools can analyze the alternative processing technologies and feasibility of the products there is a need for TEA and LCA for microalgae biorefinery approach for selected algal strains and multiple bioproduct outputs.

6.2. Genetic Engineering

As mentioned in the previous sections microalgae like other plants collect, converts, and storages solar energy in biochemical form. For autotrophic microalgae strains which are currently cultured commercially photosynthetic efficiency (PE) is an important parameter for obtaining VAPs.

$$6CO2 + 12H2O + photons \rightarrow C6H12O6 + 6O2 + 6H2 \qquad (7.1)$$

PE is the fraction of light energy that is stored as biochemical energy during photoautotrophic growth of the green-like plants. The photosynthetic active radiation (PAR) of 400-700nm wavelengths which represent nearly 50% of the total light energy of light spectrum can be captured and used in the Calvin Cycle for production, utilizing CO2 and H2O shown in Equation 7.1.

However, the theoretically, max PE is estimated as 13% and does not account for other factors which can reduce the PE level in green-type plants. (photoinhibition) Due to this terrestrial plant obtains 1 to 2 % PE levels but for microalgae, having a simple structure, PE levels are higher than terrestrial plants and can be up to 20%. (Brennan and Owende, 2010) Therefore, microalgae represent an efficient biomass resource or bioproduct production. As mentioned in the cultivation methods section strain selection is an important factor for overall success for production. According to the research studies, ideal strain should have high biochemical content levels (lipids, proteins, etc.), must be able to survive extreme conditions, have high CO2 sinking capacity, and provide valuable co-products, with rapid growth potential and high PE and with limited nutrient requirements must dominate the wild strains if the open pond system is used. There are no algal strains meeting all these requirements currently and with site-specific adaptation being one of the key elements for commercial products genetic and metabolic engineering on microalgae cells are likely to have an impact on the overall system importance. Reducing photosynthetic inhibition, manipulation of metabolic pathways and other genetic engineering strategies can increase the potential of microalgae as tiny green cell factories (Figure 6.2).

Chylamydomonas reinhardtii is the very first microalgae to have its genome sequenced and used as a model organism to develop new tools. With genetic engineering becoming more accessible many other strains such as; *Chlorella*

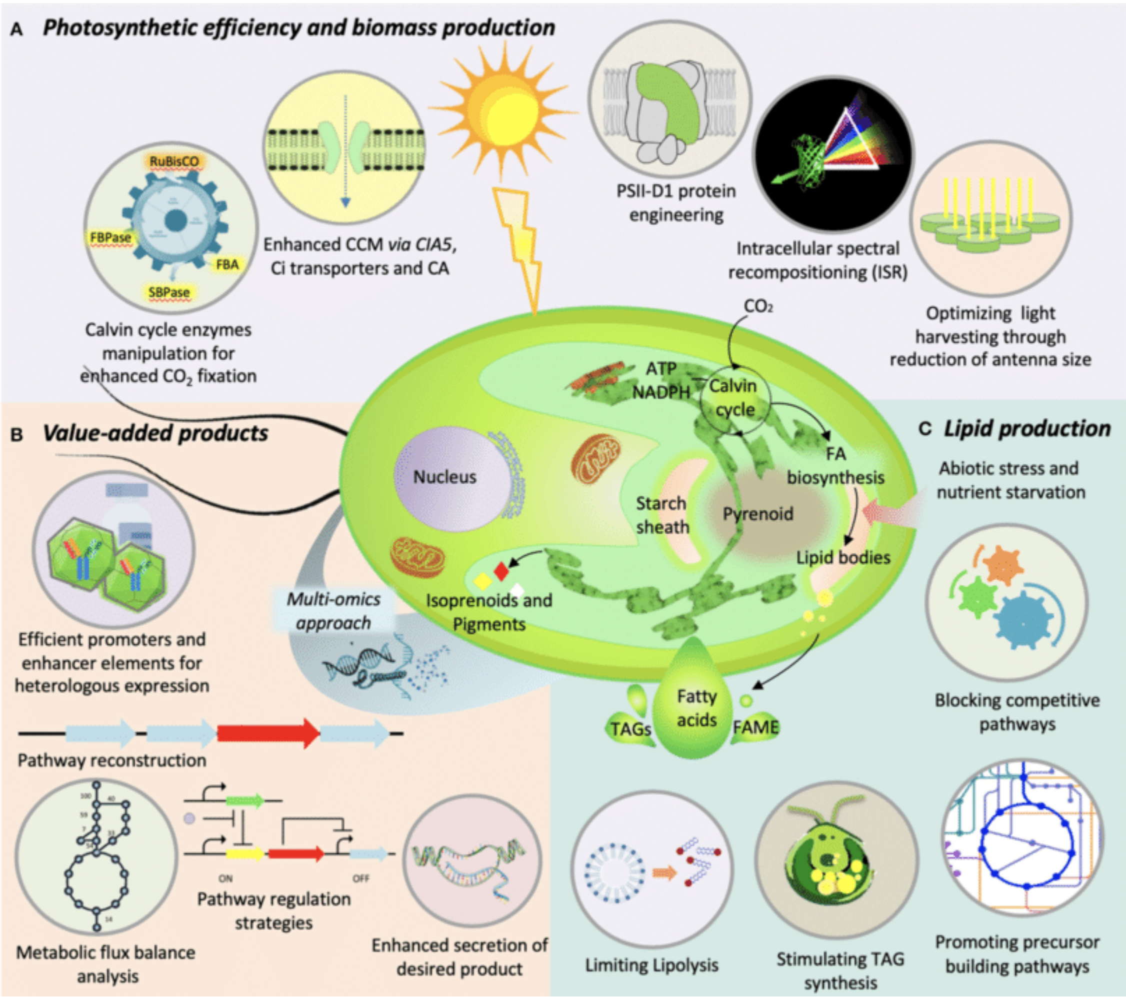

Figure 6.2 Genetic modification ways for microalgae (Retrieved from Kumar et al.,2020)

vulgaris, Fragilariopsis cylindrus, Botryococcus braunii, Pseundonitzschia, Thalassiosira rotula, Dunaliella salina, Porphyra purpurea, Galdieria sulphuraria are being sequenced (di Visconte et al., 2019).

Even though these applications represent a promising potential for the microalgae field there may be some risks and problems faced after the genetic transfer. One of the main problems is genetic stability as most of the microalgae recognize and degrade foreign DNA to protect themselves. Or if open pond culturing systems are used, the genetically modified microalgae can go airborne from the cultivation site and pose many serious commercial risks. Also, there are major drawbacks such as high costs of strain development, regulatory affairs, and negative public opinions about genetically modifying.

With having drawbacks, the major advantage of genetically modified microalgae technology is improving the overall economics of the systems due to improved growth and the efficiency with high molecule production. (Liang et al., 2019)

In conclusion, for use of genetically modified microalgae strains to culture under strict regulations, monitoring, and environmental protection have crucial importance. Because it is a new field many developments can be made for system improvements and biosafety therefore, more research is needed.

7
RESULTS AND DISCUSSION

7.1 Modeling Results

In the first step, the matrix formed for microalgae cultivation methods, given in Table 5.3 to determine the most acceptable alternatives from reviewed microalgae cultivation pilot-scale studies and literature. Matrix was formed by using the following selected criteria; CAPEX, OPEX, biomass selling price and production, volumetric productivity, facility area and cultivation of selected six different microalgae cultivation method. The results obtained by conducted TOPSIS model among six different alternatives are presented in Table 7.1.

Table 7.1 Results and ranks for TOPSIS analysis of microalgae cultivation systems

Cultivation Method	Si +	Si -	Pi	Rank #
Glass-Flat Panel Photobioreactor	0.06515	0.14932	0.69622	2
Glass-Helical Tubular Photobioreactor	0.06211	0.14318	0.69746	1
Raceway Pond with paddle wheel	0.13771	0.11080	0.44585	4
Circular Pond with stirring arm	0.13960	0.11128	0.44355	5
Plastic Bag (Leidos) Photobioreactor	0.13508	0.08562	0.38796	6
Glass-Horizontal Tubular Photobioreactor	0.10464	0.11625	0.52627	3

According to TOPSIS analysis results, the most suitable microalgae cultivation system was glass-helical shaped tubular photobioreactor without significant dominance over glass-flat panel plate photobioreactor and plastic bag photobioreactor system resulted ranking last, by having the maximum distance from the positive ideal the meaning it is the least acceptable suitable alternative among the other cultivation methods. Efficient CO_2 absorption and better gas exchange can be achieved in helical photobioreactors due to getting CO_2 or air into the reactor from the bottom (Chandra et al., 2020) and it has lower capital expenses from the other closed photobioreactor systems. In many different studies it has been stated that helical PBRs are efficient reactors with their improved fluid dynamics but helical PBRs have one of the highest operational costs among all other six systems. Therefore, the operational costs need to be reduced (Hinterholz et al., 2019; Garcia et al., 2020).

The flat panel PBR ranked in second place even though it has the highest biomass selling price and efficiency. The reason for this is that these reactors have the second-highest capital costs a non-beneficial criteria in performed TOPSIS analysis. The resulting no significant dominance between helical and flat-panel PBR from this analysis shows that biomass selling price which is a beneficial criteria might have affected the decision analysis.

In terms of appearance and operating principle tubular horizontal and helical PBR are very similar to each but according to reviewed pilot-scale studies, their efficiency was quite different from each other. The reason for the difference is because the diameter of horizontal PBR is high but the helical PBR is low. Due to the shortness of the light path, the reactors with low diameter have high efficiency. (Clippinger et al., 2019).

Although plastic PBR has one of the highest biomass production rates, because of its high capital and operational costs that are among non-beneficial criteria, might be because of material life and type, it ranked in the last place.

The matrix representation is generated as the second step to determine the most acceptable microalgae harvesting alternative among the reviewed studies were given in Table 5.4. The matrix for selected microalgae harvesting techniques was formed using time, overall costs, energy need, and efficiency criteria because there were limited studies performed in some of the selected studies.

The most acceptable microalgae harvesting alternatives without showing significant dominance were chemical harvesting with organic compound chitosan, bio-flocculation, and cross-flow filtration respectively, according to the TOPSIS analysis results presented in Table 7.2. The centrifugation with decanter resulted in ranking in the last place among others, by having the maximum distance from the positive ideal, which makes it the least acceptable alternative.

Table 7.2 Results and ranks for TOPSIS analysis of microalgae harvesting systems

Harvesting Method	Si +	Si -	Pi	Rank #
Sedimentation	0.27474	0.27886	0.50373	10
Cross-Flow Filtration	0.01004	0.05141	0.83661	3
Centrifugation using disc stack	0.02231	0.04013	0.64263	9
Centrifugation using decanter	0.03974	0.02464	0.38274	11
Dispersed Air Flotation	0.02388	0.07266	0.75266	5
Dissolved Air Flotation	0.01719	0.04536	0.72515	4
Fluidic Oscillation	0.01965	0.04537	0.69776	6
Chemical Harvesting using ferric chloride	0.01982	0.03950	0.66586	8
Chemical Harvesting using chitosan	0.00441	0.05312	0.92328	1
Bio-flocculation	0.00702	0.05461	0.88612	2
Electrocoagulation- Flocculation	0.01744	0.04240	0.70859	7
Suspended Air Flotation	0.04010	0.02130	0.34694	12

One of the most striking points according to Table 7.2, was although the methodology is similar in both chemical harvesting methods, one carried out with organic chemical chitosan addition resulted in one of the most acceptable harvesting systems, while the other one carried out with inorganic chemical ferric chloride addition resulted in ranking eight. This might be the result of the difference between their efficiency which is the most important and only beneficial criteria. The main reason for the difference between their efficiency might be because while the organic flocculants form a physical linkage with algal cells, the inorganic flocculants adsorb negatively charged microalgal cells through charge neutralization (Yin et al., 2020). Also, it is stated that, although inorganic flocculant costs are lower than organic flocculant, the needed amount is higher which makes their cost almost the same (Al-hattab et al., 2015). It must be noted that organic compounds are non-toxic to the environment, requires fewer amounts but with higher costs while the inorganic compounds can be toxic and have a negative impact on microalgae cells and their utilization area.

Although, disk stack centrifugation and decanter centrifugation, have similar methodology and both successful methods not only to separating solids/liquids but also liquids/liquids from each other by using very centrifugal forces because decanter centrifugation has higher energy needs and expensive compared to disc stack centrifugation with less efficiency (Singh and Patidar, 2018; Najjar and Abu-Shamleh, 2020) it resulted in ranking lower compared to disc stack centrifugation.

The results in Table 7.2 also show that bio-flocculation is one of the most acceptable alternatives among the other although is a newer method. With having fewer expenses than the other methods bio-flocculation is also an environmentally friendly harvesting technique with high efficiency that attracts the attention of the researchers in microalgae field (Ummalyma et al., 2017).

The suspended air flotation, which is the alternative having one of the lowest efficiency and high costs resulted ranking in the last place while harvesting methods having a similar methodology with higher costs and efficiency, dispersed air flotation and dissolved air flotation, resulted ranking in the fourth and fifth place, respectively.

According to the results which has no significant dominance over three acceptable alternatives in the Table 7.2, the efficiency, which is the most important and only beneficial criteria in the second TOPSIS analysis might have affected the decision analysis.

7.2 Discussions

The aim of this study is to determine the most suitable and acceptable alternatives among the cultivation and harvesting techniques that are suitable for Chlorella species with data set derived from pilot-scale studies and literature.

Even though the microalgae culturing, harvesting, and utilizing field is renewable and has a promising potential in various industries; there are still improvisations required and challenges remain to obtain a sustainable process. Developing hybrid cultivation or harvesting technologies, designing different process options for cultivation in accordance with target products, optimizing the operational parameters, selecting the right strain, introducing the genetically modified microalgae or developing other biotechnological innovation in this field can lead to viable commercialization and overcome the limitations. These topics will be discussed in detail in the next section.

It is mentioned in the previous sections that there are several studies that assessed the potential of wastewater treatment combined with microalgae cultivation and utilization of the obtained biomass to produce multiple value-added products.

Although this combination reduces the sludge production and the cost of the nutrient media while treating domestic and industrial wastewater stream with the advantage of mitigating CO_2 from the flue gasses seems to be suitable for a sustainable system development there are also some challenges remain such as the temperature of the flue gasses must be reduced before injecting to the system, the toxicants and heavy metals can limit the utilization area of biomass, the microalgae cultivation system must be installed near the industries. Also, SO_x and NO_x concentrations can be found in flue gasses can limit the microalgae growth since not all the strains are resistant to them. Therefore, more research on wastewater and microalgae combination systems must be performed on lab scale before developing a large-scale commercialization system as well as obtaining biofuels and VAPs from the microalgal biomass.

Making up nearly 30% of the total costs the harvesting step is the major bottleneck for sustainable microalgae. Combining different harvesting techniques the high energy costs can be decreased which will lead to overall process improvements such as flocculation for the first step to concentrate the algal biomass then using centrifugation as the second step a viable harvesting process can be achieved.

The conversion, extraction, and purification technologies are not included in TOPSIS analysis because they can vary depending on the desired product and algae strain While utilizing the microalgae there can be a need for some pretreatment process or extraction or dewatering process for converting the algae into useful commodities in some industries. Aside from being costly, it is important to select or develop a suitable conversion processes for the desired product (Molina Grima et al., 2003).

Multistage algal bioprocess with multiple target products or using cost reduced nutrient media, such as wastewater streams and CO_2 from flue gasses must be considered for boosting the commercial application resulting derived VAPs and obtaining bioenergy from microalgae must be combined with multiple biofuels or co-products through a complete biorefinery approach is essential to enhance economic and environmental benefits for microalgal based technologies.

Some parameters used in conducted TOPSIS analysis can depend on selected cultivation systems, algae strains, desired products, conversion technology, and nutrient media. In conclusion, microalgae-based different value-added products have an enormous market value in multiple industries also, can reduce the dependency on fossil fuels. But with current technology and scarce research, there is still a need for improvements in various areas to obtain a large-scale commercial level microalgae cultivation and harvesting with VAP production which is also economically viable and sustainable.

Because the TOPSIS method ranks the alternatives according to the selected criteria and defined weights, although the weights are determined via an analytical hierarchy process, this analysis can have some limitations and the results can vary on different locations, specific operational parameters, etc. However, TOPSIS models can be adopted in flexible techno-economic models to make decisions for selected microalgae species, cultivation systems and harvesting techniques with the desired target products. Further work for this study can include a life cycle analysis of microalgae-based products with a biorefinery approach in a selected field of industry, with specific cultivation system designs and harvesting methods. Wider criteria set including political, economical, technical, social parameters as well as operating conditions, locational factors, and target products can be used to rank the alternatives and find the most suitable option among the others.

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PUBLICATIONS FROM THE THESIS

Papers

1. Unay, E., Ozkaya, B., & Yoruklu, C. H. (2021) A multicriteria decision analysis fot he evaluation of microalgal growth and harvesting. *Chemosphere,* VSI, 279C, 130561
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