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Nature Based Solution for CO₂ Emissions Offset: Spirulina Microalgae Cultivation

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ABSTRACT

This research provides a literature review on potential applications through Spirulina (microalgae) which can be a nature-based solution in order to tackle several environmental issues that come up during unconventional oil and gas production operations. Examples of such environmental issues are mainly fresh water consumption due to hydraulic fracturing process and carbon dioxide emissions. The objective of this work was to investigate carbon dioxide bio-sequestration potential of Spirulina and other application which can be done once it is grown such as; produced water treatment, pigment extraction and soil amendment.

Initially, growth systems and needed environmental conditions for growing Spirulina were researched. Two main growth systems were found: Open systems for mainly large-scale applications and closed systems for laboratory scale applications. Optimum environmental parameters found were the following: 28-35 °C of temperature, 8-11 of pH, nitrogen, phosphorus, potassium, and carbon feed, agitation of the pond and illumination. Cost analysis regarding a Spirulina cultivation plant was made. Later on, carbon dioxide bio-sequestration potential of Spirulina was searched, as this microorganism grows and proliferates by photosynthesis reaction which requires a carbon source. This carbon source can be organic (carbon dioxide) or inorganic (bicarbonate). The following topic was pigment extraction application. Spirulina is a microalgae, as mentioned before, therefore it contains several pigments with different colors. These pigments can be used in food, cosmetic, pharmaceutical and nutraceutical industries as natural dye and antioxidant. Optimum method for the best yield of pigment extraction from Spirulina with a careful consideration of energy consumption was found. Market analysis for pigment (phycocyanin) was made. Produced water treatment was another topic included into this research, as oil and gas production require hydraulic fraction process. Heavy metal and radioactive material bio-accumulation potential of Spirulina was evaluated and compared to other microalgae strains by taking comparison studies done by researchers as reference. The last application assessed in this work was soil amendment potential of Spirulina. A study was evaluated within that chapter and comparisons in plant height, root length and number of leaves were made between

different plants by using chemical fertilizer, Spirulina (as bio-fertilizer) and without any fertilizer (control group).

Overall, for each application, results were promising. 50 tons/year/ha carbon dioxide bio-sequestration potential have been seen for 10 g/L/day productivity of Spirulina. Productivity increases by the time and with an increase of surface area, as long as needed environmental conditions are provided. Therefore, this number for carbon dioxide bio-sequestration has a tendency to increase. In case of pigment extraction, according to comparison studies, the method for highest yield can be obtained from pigment extraction was freezing and thawing method when it is applied to wet biomass. Because the literature indicates that, drying the biomass causes 50% of pigment loss. Produced water treatment potential of Spirulina was found high as well according to the evaluation of comparison studies. Produced water chemical composition of Total Austral and discharge standards in Argentina was compared and a pilot wastewater treatment plant was designed and cost analysis for such plant was done. Finally, in soil amendment chapter, comparative studies have shown that Spirulina can be an alternative to chemical fertilizers as the results of plant height, number of leaves and root length either greater than the plants grown by chemical fertilizers or similar.

Overall, Spirulina has great potential and showed promising results in diverse applications. Spirulina has proven being a natural solution for different environmental concerns, and must be considered as a possible application for climate change mitigation and sustainable development studies.

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CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Industrialization and human population on the earth creates higher demand for energy as they increase. In order to supply this energy demand, non-renewable energy sources are used to produce energy as it is easier and less costly with fossil fuels. The question rises here is, “What about emissions of these fuels when they are burned?”. CO₂, NO_x and SO_x gases are the main emissions released by burning fossil fuels. NOAA (National Oceanic and Atmospheric Administration) stated that CO₂ levels in atmosphere showed an increasing trend by increasing from 397,5 ppm to 408 ppm only in 4 years which is measured at their Observatory located in Hawaii (NOAA, 2018). Several applications have been studied and implemented CO₂ capture, including growing microalgae. Thanks to its cost effectiveness and naturalness, growing microalgae for CO₂ sequestration has a rising trend recently. Microalgae grow and proliferate by photosynthesis reaction which means it needs a carbon source to live and produce biomass. Injecting CO₂ into microalgae cultivation systems has showed good yields in terms of CO₂ utilization according to many studies evaluated within this study. In order to obtain many advantages from growth microalgae while offsetting CO₂ emissions, other applications are included into the scope of this work. Therefore, specifically Spirulina microalgae is chosen to be evaluated in terms of CO₂ capturing potential and its other promising applications for instance; producing Spirulina as food supplement or bio-fertilizer thanks to its high nutritional value.

Spirulina is a filamentous microalgae, belongs to cyanobacteria familia which has a history of millions of years. It is believed that, these prokaryotic microorganisms were the first photosynthetic organisms on the earth and switch the earth from anoxic form into aerobic form. Even though evaluation of CO₂ capturing potential of Spirulina is the main objective

of this study, several side applications have been assessed and promising results have been found in the literature.

1.2. OBJECTIVES

The objectives of this research are;

- Reviewing the literature in fields of algae, microalgae, cyanobacteria and specifically Spirulina strains in order to evaluate carbon dioxide bio-sequestration potential of this microorganism. The reason why Spirulina is chosen is that, once Spirulina is cultivated under specific conditions where it is suitable for human consumption when it is harvested, it is a good food supplement and so, while offsetting carbon dioxide emissions, sales of Spirulina in food industry make extra revenue. As one of the objectives of this research is creating a business plan for possible investment, Spirulina is a good option thanks to its potential on food market.
- Finding the most efficient technique for pigment extraction from Spirulina by comparing the studies in the literature.
- Analyzing the potential of Spirulina to bio-accumulate heavy metals and NORMs (Naturally Occurred Radioactive Materials) in order to evaluate the possibility of treating produced water from hydraulic fracturing process by cultivating Spirulina in this water.
- Evaluating the literature in order to see the effects of Spirulina on plants when it is used as bio-fertilizer.

The research questions of this study are the following;

RQ1: What is the potential of Spirulina microalgae to capture carbon dioxide, under which conditions it should be grown in order to show the best yield in terms of carbon dioxide bio-sequestration?

RQ2: What is the optimum method of pigment extraction from Spirulina and under which conditions does the pigment must be extracted for the best yield?

RQ3: What is the possibility of produced water treatment by growing Spirulina in this water? What are the needed additional treatment processes and equipment to obtain better efficiency?

RQ4: Is Spirulina a good material for bio-fertilizer production? What is the efficiency of plant growth when it is grown by addition of bio-fertilizer produced from Spirulina?

RQ5: What is the acceptability of these applications by stakeholders?

In order to answer these research questions, a detailed literature review was carried out on cyanobacteria and especially on Spirulina including the topics as history of Spirulina, cultivation conditions, cultivation systems, carbon dioxide bio-fixation potential. Moreover, through this review, optimum technique and conditions for pigment extraction from Spirulina was obtained. Heavy metal adsorption and radioactive material absorption potential of Spirulina was compared to other microorganisms (microalgae strains and bacteria), lastly bio-fertilizer production from Spirulina has been seen as a possible application and included in this research. Comparative studies in the literature were collected and evaluated; improvement in soil quality and plant growth has been assessed for different studies.

CHAPTER 2: LITERATURE REVIEW

2.1. OIL AND GAS INDUSTRY OVERVIEW

2.1.1. OIL INDUSTRY

Oil, so called petroleum, is a mix of hydrocarbons with different molecular weights and some other organic materials. Oil is a kind of liquid material which occurs naturally and can be seen in rock formations. Commonly believed fact about oil is that, it occurs from the dwelled planktons which takes millions of years by the affect of pressure and heat. By the time, this formation starts to be covered by several layers and goes deeper parts of Earth's crust. Finally, this structure becomes reservoir (James G. Speight, 2014).

Petrol production includes five processes: Exploration, extraction, refining, transporting and marketing, respectively. Beside energy production, petroleum is used also in chemical industry for manufacturing of pharmaceutical products, fertilizers, pesticides etc. Oil products has an important role in civilization of countries as it is indispensable for many industries. Especially, considering the energy consumption of the world, as 32-53% of energy demand is supplied by oil in the world. Thirty billion barrels of oil is consumed in the world per year and the highest percentage of this consumption belongs to developed countries such as United States. Considering the value in terms of dollar, petroleum industry is accepted as the biggest industry (Central Intelligence Agency, Country Comparison-Oil Consumption, 2018).

According to Central Intelligence Agency, top ten countries in oil consumption is shown on the Table 2.1.1 below with the amount of oil consumed.

TABLE 2.1.1.: COMPARISON OF COUNTRIES IN OIL CONSUMPTION (CENTRAL INTELLIGENCE AGENCY, COUNTRY COMPARISON-OIL CONSUMPTION, 2018)

Rank	Countries	BBL/day	Date of Information
1	US	19.500.000	2008 estimated
2	EU	14.440.000	2007 estimated
3	China	7.850.000	2008 estimated
4	Japan	4.785.000	2008 estimated
5	India	2.940.000	2008 estimated
6	Russia	2.900.000	2008 estimated
7	Germany	2.569.000	2008 estimated
8	Brazil	2.520.000	2008 estimated
9	Saudi Arabia	2.380.000	2008 estimated
10	Canada	2.260.000	2008 estimated

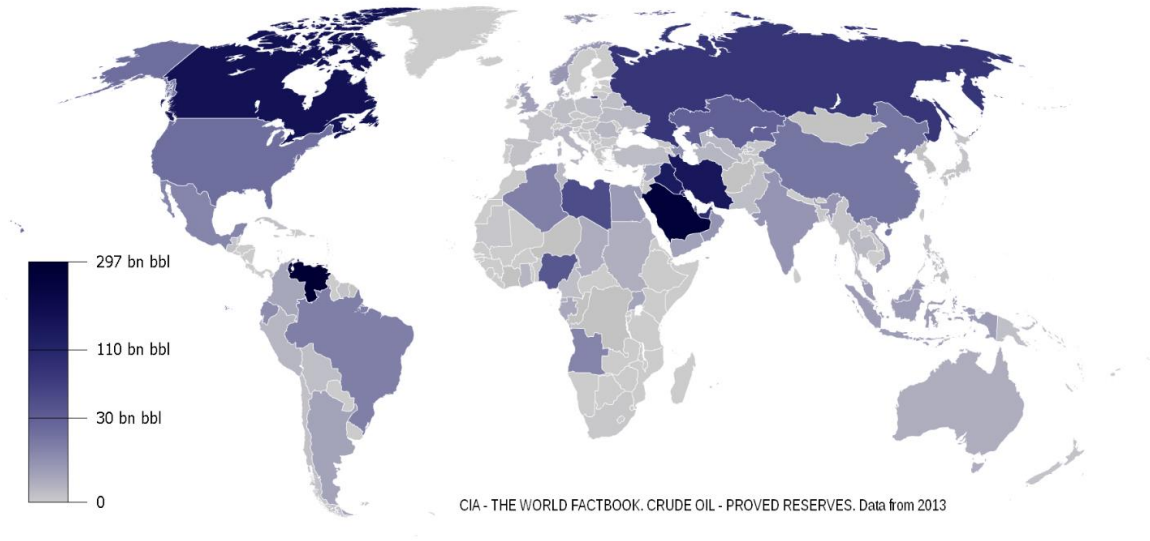


FIGURE 2.1.1.: WORLD OIL RESERVES (CIA, CRUDE OIL PROVED RESERVES, 2013)

Distribution of oil reserves in the world is shown in the figure above. According to this map, Arabian Peninsula and Venezuela in South America oil reserves are very concentrated followed by Canada and Russia after.

2.1.1.1. ENVIRONMENTAL IMPACTS OF OIL PRODUCTION

Oil industry is responsible mainly for water and air pollution because of the production and extraction processes. Water pollution occurs due to oil spillovers, by-products and refining applications. Especially hydraulic fracturing process during extraction results with heavy metal and radioactive material accumulated water which is called produced water (Osborn, Vengosh, Warner, & Jackson, 2011). Hydraulic fracturing application is done for extracting the oil from the perforated rock formations by filling the cracks with water, sand and some lubricant chemicals. As this mix enters the cracks underground, when it is taken back to surface, it brings heavy metals and radioactive materials with it. Leakages from the underground tanks are another source of water pollution created by oil industry as well.

In terms of air pollution, oil industry is responsible for very high levels of volatile organic compounds (VOCs) and greenhouse gases due to the burning process. CO₂, NO_x, SO₂ and CH₄ are the emissions created by oil industry too (EPA, Controlling Air Pollution from the Oil and Natural Gas Industry, 2018). All these greenhouse gas emissions affect climate change negatively. Therefore, renewable energy sources have begun to be a new approach for energy production.

2.1.2. GAS INDUSTRY

Natural gas is a gas mix which is composed of a high amount of methane, alkanes and a little amount of CO₂, N₂, H₂S and He (Energy Tomorrow, 2013). It occurs naturally by the exposition of heat and pressure on the animal and plant residues for millions of years under the Earth's crust. Natural gas is being used with the purposes of electricity, heating, transportation and cooking. As a source of energy for transportation it is used as fuel (EPA, Energy and the Environment, 2013).

Before using natural gas as a fuel for vehicles there is a necessity of purification process which is removing impurities from the extracted gas. By this process, natural gas becomes compatible with the fuel standards. As a result of this application, several by-products are produced such as; H₂S, CO₂, evaporated water, hydrocarbons and compounds like butane, ethane, pentane, propane and may be N₂ and He.

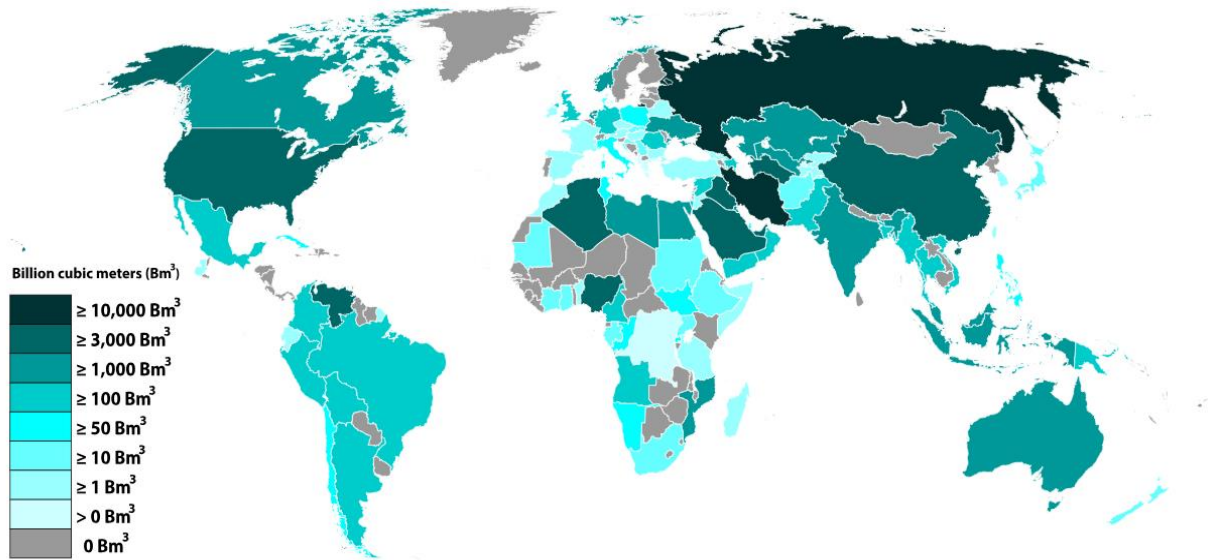


FIGURE 2.1.2.: WORLD GAS RESERVES (CIA, NATURAL GAS PROVEN RESERVES COUNTRY COMPARISON, 2014)

In the figure above, proven natural gas reserves are shown and according to this map, Russia and Iran show the highest potential in the world.

Natural gas consumption levels increase day by day as the population increases. According to Central Intelligence Agency top ten countries in natural gas consumption is shown on the table below:

TABLE 2.1.2.: COMPARISON OF COUNTRIES IN TOTAL GAS CONSUMPTION PER YEAR (CENTRAL INTELLIGENCE AGENCY, COUNTRY COMPARISON-OIL CONSUMPTION, 2018)

Rank	Countries	m ³	Date of Information
1	US	773.200.000.000	2015 estimated
2	EU	428.800.000.000	2016 estimated
3	Russia	418.900.000.000	2015 estimated
4	China	186.200.000.000	2015 estimated
5	Iran	186.000.000.000	2015 estimated
6	Japan	123.600.000.000	2015 estimated
7	Canada	114.800.000.000	2015 estimated

8	Saudi Arabia	102.300.000.000	2015 estimated
9	Germany	81.350.000.000	2015 estimated
10	Mexico	77.930.000.000	2015 estimated

2.1.2.1. ENVIRONMENTAL IMPACTS OF NATURAL GAS PRODUCTION

Natural gas contains mostly methane (CH₄) and when it is released to atmosphere oxidation reaction creates carbon dioxide. Even though lifetime of methane amounts half of lifetime of carbon dioxide, methane traps the heat in the atmosphere more than carbon dioxide, therefore, it has much higher potential of creating global warming compared to carbon dioxide. Leakage of methane into the atmosphere happens due to the activities as; extraction, transportation, storage and finally distribution of natural gas (Stocker & Qin, 2014). Natural gas production produces less carbon dioxide compared to coal and oil, but human activities done by natural gas produces a significant amount of carbon dioxide (EPA, Energy and the Environment, 2013). Comparison of emissions due to oil, natural gas and coal burning is shown on the table below:

TABLE 2.1.3: EMISSIONS FROM OIL, COAL AND NATURAL GAS BURNING (EPA, ENERGY AND THE ENVIRONMENT, 2013)

Pollutant (lb/MMBtu)	Natural Gas	Oil	Coal
Carbon dioxide	117	164	208
Carbon monoxide	0,04	0,033	0,208
Sulphur dioxide	0.01	1,122	2,591
Nitrogen oxides	0.092	0,448	0,457
Particulates	0.007	0,084	2,744
Mercury	0	0,000007	0,000016

2.1.3. TOTAL OIL AND ENERGY COMPANY

Total has started its operations in 1924 with the name of Compagnie Française des Pétroles (CFP) and they were producing oil in Middle East. By the time company started to expand its business and began to operate in gas, refining, petrochemicals and petroleum product marketing, solar power, bioenergy and energy storage. Shale revolution opened a new path for Total as well and Total started to explore and extract unconventional products as shale oil, shale gas and coal bed methane (Total Oil and Energy, 2018). Unconventional operations mean that, extracting the sources which cannot be extracted by conventional extraction methods. Unconventional operations include hydraulic fracturing and horizontal drilling activities. Unconventional production creates a negative environmental impact as conventional production. Carbon dioxide emissions and produced water production are the issues discussed in this project.

Thanks to the internship opportunity given to me, a research has been done for six months in order to find nature-based solutions for offsetting carbon dioxide emissions and produced water treatment.

2.1.4. CARBON EMISSION CONTRIBUTORS CURRENT CARBON CAPTURING, UTILIZATION AND STORAGE (CCUS) TECHNOLOGIES

A study has been done by Richard Heede, director of Climate Accountability Institute, in order to define the majority of carbon emissions contributors. In this study, which is called Carbon Majors, some data has been published:

- 1) There are 100 extant fossil fuel producers which are 41 public investor-owned companies, 16 private investor-owned companies, 36 state owned companies and 7 state producers.
- 2) Between 1854 – 2015, 923 GtCO₂e (carbon dioxide equivalent) as carbon dioxide and methane emitted by operational activities and product related activities which amounts half of the emissions since the industrial revolution.
- 3) 72% of greenhouse gas emissions is emitted by 224 companies (Griffin, 2017).

Figure below shows the greenhouse gas emissions from top 50 fossil fuel producers in terms of production process and product use period.

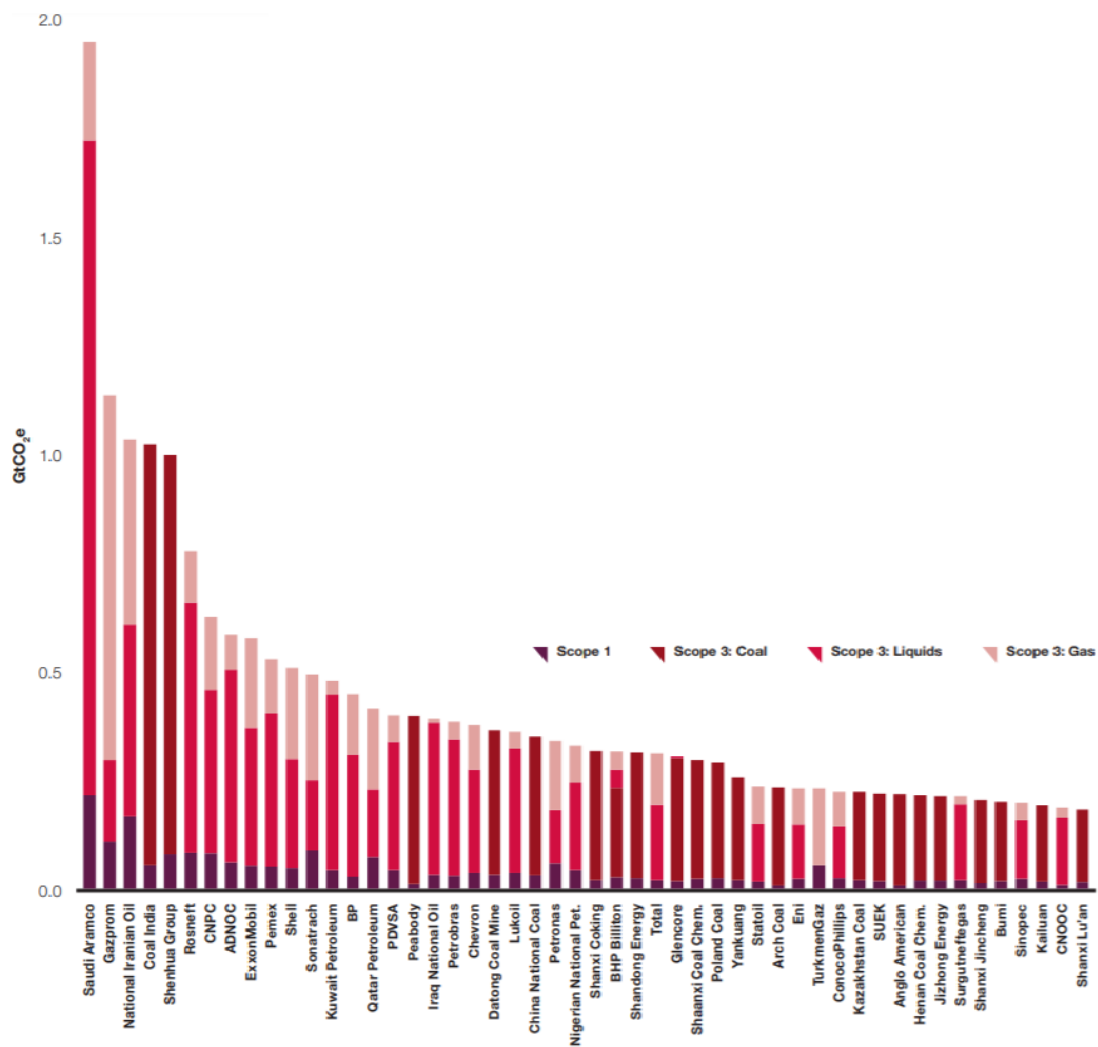


FIGURE 2.1.3: TOP 50 FOSSIL FUEL COMPANIES IN 2015 BY OPERATIONAL (SCOPE 1) AND PRODUCT (SCOPE 3) GREENHOUSE GAS EMISSIONS (50% OF GLOBAL INDUSTRIAL GREENHOUSE GAS EMISSIONS (GRIFFIN, 2017))

According to this figure, in 2015, Total Oil and Energy was on the 29th place in greenhouse gas emissions through operations and product use among 50 fossil fuel companies. In the extraction and production phase of oil and gas by Total, emissions are significantly less compared to many companies in this study. Total emits the most during the consumer use of

liquid fuels and it is followed by consumer use of gas fuels. As Total does not produce energy from coal, there is zero emissions in that phase (Griffin, 2017). Even though Total does not contribute as much as most of its competitors on the market, there is still a huge carbon emissions issue. In order to be more efficient in this sense, top management has decided to put more effort on CCUS technologies and other possible applications for capturing carbon.

Carbon capture, utilization and storage is a way first to extract CO₂ from atmosphere and flue gas, then a recycle process comes after and finally a long-term storage of CO₂. Even though there are technologies to produce product in a carbon efficient way, removing CO₂ from atmosphere is still needed as has a negative impact on climate change. Therefore, carbon capture, utilization and storage methods are indispensable to mitigate climate change. There are several CCU (carbon capture and utilization) methods as capturing and compressing CO₂, then conversion of this CO₂ into some useful chemicals or it can be used for alkaline industrial wastes or it can be utilized for oil extraction. These methods are useful for utilizing carbon, but there is still a huge amount of carbon dioxide in the atmosphere to be removed, that is the reason why storage is an important step of CCUS applications. In order to store CO₂, there are several methods as planting trees for capturing CO₂ by photosynthesis or injection of CO₂ into water or geological compositions. In order to have a successful CCUS application, it must be environmentally friendly, durable and cost effective (Fan, Gibbins, Rubin, Bocarsly, & Petit, 2018).

In this study, *Spirulina* microalgae cultivation for capturing, utilizing and storing carbon as a nature-based solution has been researched. Beside CCUS application, other possible applications for different environmental concerns were investigated.

2.2. MACROALGAE, MICROALGAE AND CYANOBACTERIA

Algae are organisms which are basic, non-blooming, living in aquatic media, comprise unicellular embodiment and seaweeds. Nonetheless, algae do not have vascularity, leaves, a proper trunk nor roots. As algae have variety of forms, it is possible to grow it anywhere on

the earth and are an important source of food as they are primary producers. Algae produce about 70% of the oxygen in the atmosphere. They can be found in sea, lakes, saline waters and wastewaters (LAKNA, 2017).

Algae in aquaculture are a perfect food source for the living creatures as they do photosynthesis and produce biomass and also oxygen while consuming carbon dioxide. According to algae classification there are two groups called microalgae and macro algae. Phytoplankton are microalgae while seaweeds are macro algae. The use of macro algae is mostly as food and also as natural filters in the saltwater aquariums because they are able to remove the wastes contain nitrogen (LAKNA, 2017). With the same idea, both macro and microalgae are useful for wastewater treatment, for instance, wastewater from municipal WWTP (wastewater treatment plant) or even industrial wastewater could be suitable for algae growth as these waters contain high level of nitrogen and phosphorus which are nutrients needed for algae growth (What are algae?, 2017).

2.2.1. MACROALGAE

Macro algae, comparing to microalgae, have bigger cells. As these organisms are autotrophic, they can produce organic substances by using inorganic substances by photosynthesis, which means they are able to produce their own food source. Most of the time macroalgae are named as seaweeds. The significance of macro algae is that it is a food source for the herbivores in the aquatic media. Moreover, the reason why macro algae are called natural filter is because they decrease the nitrogen and phosphorus quantity in the water in which they live (LAKNA, 2017).

2.2.2. MICROALGAE

Microalgae are one-celled microorganisms which can be seen only by microscope. Usually these microorganisms are named as phytoplankton. In general microalgae are autotrophic

like macroalgae are. As mentioned above, microalgae needs carbon, nitrogen and phosphorus to grow. In case there is too much of these nutrients, microalgae would grow more every day and cover the surface of the water. This state of algae growth is called eutrophication which does not allow the livings in that water to get enough sunlight and oxygen (LAKNA, 2017).

2.2.3. CYANOBACTERIA

Cyanobacteria (blue-green algae) are prokaryotic and the first photosynthetic microorganisms on the earth. These microorganisms have 150 different familia with 2000 different types, in diverse forms and dimensions. Numerous sorts of Cyanobacterial cells, cellular forms, and physiological strategies are possible to be seen. They can grow in brackish and also fresh water. Cyanobacteria have a huge beneficial impact on the quality of the water they grow in as they consume nitrogen and phosphorus. Cyanobacteria may have negative impact on water quality as well. They create taste and odor in the water they grow in as almost all micro algal blooms do (W.F. Vincent, 2009). Another issue is that some species of cyanobacteria may produce toxic materials which affect human and animal health. People can be affected by these toxins by drinking or contacting to water. It is important to know that not all cyanobacteria produce toxins but some of its species may produce even more than a single kind of toxin. Problems cyanobacteria create are still under assessment of researchers (EPA, Epidemiology & Health Effects of Cyanobacteria, 2017).

The only prokaryotes which are able to use sunlight as an energy source are Cyanobacteria. They are unique among the prokaryotic cells also because they utilize water to meet their electron need while using the atmospheric air as their carbon source, some species of cyanobacteria are capable of fixing the nitrogen. Cyanobacteria embody several specific aspects such as having high percentage of proteins within their thylakoids (an organelle in the cell) where they produce different pigments, exopolysaccharides and extracellular glycolipids (Heidorn, et al., 2011).

Many Cyanobacteria species are grown naturally in inland water or cultivated in diverse cultivation systems and harvested to be utilized as food sources, animal feeds, fertilizers, and

health products (W.F. Vincent, 2009). Recently one species of Cyanobacteria became very popular as a food source which is called Spirulina.

2.3. SPIRULINA SPECIES

2.3.1. *SPIRULINA* IN THE HISTORY AS A FOOD RESOURCE, GEOGRAPHICAL LOCATIONS WHERE *SPIRULINA* GROW NATURALLY AND TOP COMMERCIAL PRODUCERS WORLDWIDE

As an ancient microorganism which uses dissolved carbon dioxide in the water as their food source Spirulina has a history from 3.5 billion years ago. Spirulina is a photosynthetic blue-green algae (cyanophyte) which is capable to grow under strenuous conditions where most of the microorganisms cannot bear such as high temperature, high alkaline levels, high illumination etc. (FAO, 2008).

500 years ago, when Spanish forces invaded Mexico, they noticed that the habitants of the capital city Tenochtitlan were harvesting an edible green and blue colored substance which is now called Spirulina from the surface water (Sasson, 1999). These edible materials had a special name called “techuitlatl”. After the harvesting period, techuitlatl was used for making colorful cakes. After the sixteenth century, techuitlatl was not spoken of possibly because the waters where Spirulina grew were discharged for agriculture work. What is known currently is that there are still Spirulina living in the Texcoco lake in the city of Transito in Mexico (FAO, 2008).

Later, in 1967, by the International Association of Applied Microbiology, spirulina was already called as “wonderful future food source” (Sasson, 1999). Evaluated nourishing aspects showed that Spirulina consists of 60-70% of its dry mass protein which are really high in qualitative sense. This information was convenient for the organizations to focus on Spirulina in their researches with an industrial point of view as Spirulina would be a low-price protein source. In the same year Institut Français du Pétrole offered a project to the company Sosa-Textcoco Ltd about a bloom of algae occurring in the evaporation ponds of their sodium bicarbonate production facility in a lake near Mexico City. This was the very

first project about growth parameters and morphologic properties of *Spirulina* evaluated in detail. This work was the first step of *Spirulina* cultivation for industrial purposes in large scale was the thesis of a Ph.D. student Zarrouk (1966) (Sasson, 1999).

Today, Kanembu tribe near Lake Chad gather *Spirulina* in a wet form, filter the liquid by fabric sacks then lay out the algae on the coast of the lake to dry it by the heat from the sun. When the algae is half-dried, they place it on their rooftop to dry it even more by the sun (Abdulqader, Barsanti, & Tredici, 2000). Later when *Spirulina* is ready for consumption, they sell the algae in the local bazaar with a name called Dihé. Kanembu tribe prepares a specific food with *Spirulina* which covers 70% of their meal (FAO, 2008). United Nations has predicted at least 250 tonnes (dry weight) sales for each year locally (Henrikson, 2011). The revenues obtained from dihé which is collected from Kossorom Lake per year are over US \$100,000 for around forty tonnes of dihé. This results with a financial contribution to the local economy (Abdulqader, Barsanti, & Tredici, 2000).

Spirulina maxima Geitler and *S. platensis* Geitler are different strains of *Spirulina* which can be found in huge populations in the tropical and semitropical waters with great carbonate and bicarbonate (inorganic carbon source for *Spirulina*) contents and high pH levels (as much as 11) which makes the water more alkaline (Vonshak, 2002). *S. platensis* is more likely to be found in Africa, but it is also possible to find in Asia and South America. Whereas finding *S. maxima* (syn. *S. geitleri*) seems to be in fact limited to Central America. This shows that *S. Platensis* is more widespread compared to *S. Maxima*. However, *S. Maxima* is the major constituent of the phytoplankton in the Texcoco Lake where this species originate (Vonshak, 2002). Likewise, the half-desert Sudan-Sahel zone has lakes with high pH and salinity levels (main ones are Lake Chad and the ones in Eastern Africa) which are covered by *S. platensis* blooms and should be perceived as the originating location of *Spirulina* species (Vonshak, 2002).

Beside naturally growing *Spirulina*, there is an increasing trend of growing *Spirulina* for commercial purposes. Nowadays investors see a good potential in growing *Spirulina* as food grade material and also for its high potential of sequestering carbon dioxide. In a worldwide perspective, top *Spirulina* producers are Earthrise Nutritionals (California, USA) (production capacity of 500 tons/year), Cyanotech (Hawaii, USA) (production capacity of 400 tons/year),

Boonsom Farm (Thailand), Parry Nutraceuticals (India), Hainan Island (China) (with thousands of tons of production capacity the largest Spirulina producer in the world). Spirulina farms can be found in Taiwan, Australia, Cuba, Chile, Vietnam, Israel, Bangladesh, Philippines, Martinique, Peru, Brazil, France, Spain, Portugal, Chad and several more countries as well (Henrikson, 2011).

2.3.2. MORPHOLOGIC PROPERTIES OF SPIRULINA

2.3.2.1. CELL STRUCTURE

Spirulina is a unicellular, filamentous and proliferate by binary fission blue-green microalgae (FAO, 2008). It is perceived as microalgae, because it is a photosynthetic microorganism. However, it has to be taken into account that Spirulina (cyanobacteria) does not possess a nucleus covered by a membrane which makes it possible to be perceived as a bacteria as well (McKnight, et al., 2018). Being photosynthetic means at the same time being autotrophic, more clearly Spirulina can produce its own food by utilizing atmospheric carbon dioxide, water and sunlight. Disk or rod-shaped Spirulina possess different pigments as phycocyanin, chlorophyll 'a' and carotenoid and these pigments give the blue, greenish and orange-yellow color to Spirulina, respectively whilst, some of the Spirulina kinds contains phycoerythrin which gives the color of red-pink to Spirulina (FAO, 2008). Moreover, flamingos around some specific lakes which contains Spirulina with phycoerythrin pigment, live on this bacterium and as a result, Spirulina transfers this pink-red color to flamingos.

One of the morphologic properties of Spirulina is their filaments, so called trichome, having the spiral form in a length between 50 to 500 μm while the width varies from 3 to 4 μm which can be observed only in liquid surroundings. This aspect allows scientists to cultivate Spirulina in laboratory conditions in a culture medium. Besides having a spiral-shaped filaments, the cell of these species contains vacuoles filled with gas. Thanks to these vacuoles, Spirulina becomes a floating mass when high populations come together (FAO, 2008). Cyanobacterial cell wall is formed by several layers as cell membrane, peptidoglycan layer,

outer membrane, mucoid sheath, capsule and slime coat and all of these layers protect the cell from the osmotic pressure caused by cytoplasm and gives its shape to the cell.

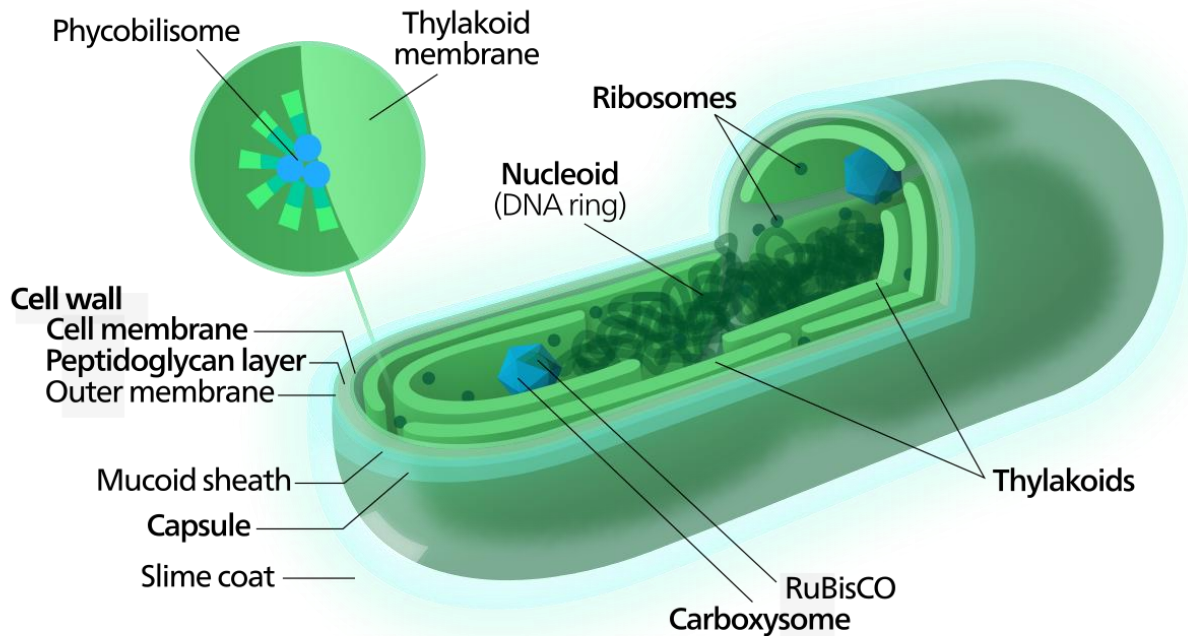


FIGURE 2.3.1: CYANOBACTERIAL CELL STRUCTURE (SONG, 2013)

2.3.2.2. BIOCHEMICAL COMPOSITION

Protein content in Spirulina amounts between 59% and 65% of its dry weight while for instance peanuts are composed 25% or soybeans 35% of protein. As the cell wall of Spirulina does not contain cellulose, human digestion system can digest Spirulina easily and quickly (Sasson, 1999).

As mentioned before, Spirulina is called ‘Super Food’. The reason why this label is given is that Spirulina has high percentage of high-quality proteins, major fatty acids, vitamins,

minerals and photosynthetic pigments. In order to clarify these contents, the biochemical composition of Spirulina is given below (FAO, 2008):

2.3.2.2.1. PROTEIN

Protein content of Spirulina varies between 59% to 65% of its dry mass depending on the nutrients fed which is extraordinarily high comparing to other protein sources (Phang, Miah, Yeoh, & Hashim, 2000). The protein in Spirulina is composed of all of the major amino acids (the smallest units of protein) with a lack of lysine, methionine and cysteine as compared to regular protein sources such as dairy, meat and poultry products. Spirulina has an elevated protein content compared to vegetable-based proteins.

2.3.2.2.2. MAJOR FATTY ACIDS

When hydrogen atoms are absorbed by the carbon chain of a fatty acid, this fatty acid is called unsaturated fatty acid. Moreover, if the valency of the carbon chain of an unsaturated fatty acid has over double or triple bonds for each molecule, these unsaturated fatty acids are called polyunsaturated fatty acid (Princeton, 2003-2008). Having more bonds for a fatty acid implies difficulty in fragmentation. Spirulina contains overall around 6% of fat and 1.3-15% of this lipid belongs to the unsaturated fatty acids. One of the most essential lipids is γ -linolenic acid which covers a percentage of between 30-35% of the overall lipid in Spirulina (Borowitzka, Phang, Kun, & Whitton, 1994).

2.3.2.2.3. VITAMINS

The vitamin content in Spirulina is also high as it contains vitamin C, D, E, and B1, B2, B3, B6, B9, B12 (thiamine, riboflavin, nicotinamide, pyridoxine, folic acid, cyanocobalamin respectively for vitamin B group) (FAO, 2008).

2.3.2.2.4. MINERALS

Spirulina contains the major minerals such as calcium, copper, manganese, zinc, potassium, chromium, phosphorus, selenium, iron, sodium and magnesium in a percentage of 7 for the industrially produced Spirulina powder. This number decreases to 2,75-3,00% for Spirulina cultivated in the laboratories depending on how much of the needed growth parameters are met (FAO, 2008).

2.3.3. CULTIVATION OF SPIRULINA

Growing Spirulina requires attention for different parameters such as temperature, pH, salinity, medium to grow Spirulina in, inoculum size, carbon source and its amount, illumination, agitation method and initial biomass concentration. One of the most important parameter is the growth system. Prior to growing Spirulina in a bigger system, it should be grown till a certain biomass concentration most of the time in a small lab scale system. Commercially bought Spirulina strains

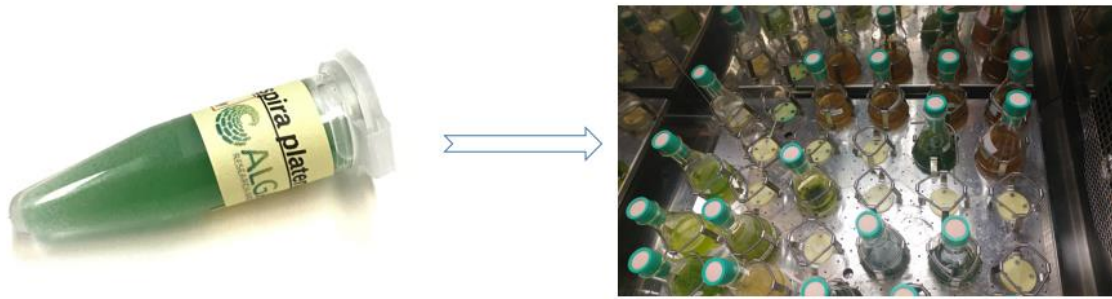


FIGURE 2.3.2: COMMERCIALY BOUGHT SPIRULINA STRAINS (ALGAE RESEARCH SUPPLY: ALGAE CULTURE, ARTHROSPIRA PLATENSIS (SPIRULINA), 2018) AND INCUBATION PROCESS (ALGAEPARC, 2018)

are first kept in flasks (usually 100 ml flask) with nutrient addition and incubated for 5-7 days in 25°C. Later on, when Spirulina reaches desired initial biomass concentration, incubated strains are transferred to laboratory scale cultivation systems such as flat panel PBR. In figure 2.3.3, the flat panel PBR (Algaemist-S) is a lab scale algae cultivation system and is illuminated by fluorescent lamp internally, and normally totally closed and does not receive any light from out. In this system; CO₂ inlet, pH, temperature and light intensity are controlled via a screen on the equipment by dialing up or down.

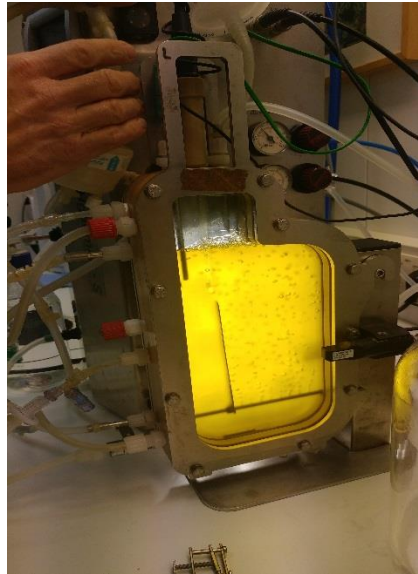


FIGURE 2.3.3: LAB SCALE FLAT PANEL PBR (WAGENINGEN UNIVERSITY, ALGAEPARC PILOT FACILITY) (ALGAEPARC, 2018)

2.3.3.1. CULTIVATION SYSTEMS

There are several systems to cultivate *Spirulina* such as open ponds, lakes as open systems and closed photobioreactors (PBR) as closed systems (Soni, Sudhakar, & Rana, 2017). Open ponds are the most applicable ones for the large-scale commercial production of *Spirulina* because in the closed systems pumps are used for circulation of algae. Pumping *Spirulina* with a high speed damages the filaments and so closed PBR for large scale cultivation is not preferable (AlgaePARC, 2018).

2.3.3.1.1. OPEN PONDS

The open systems can be classified as natural or artificial. Natural open systems are lakes, brackish water, sea etc. Typically for large scale algae production shallow artificial ponds are constructed. Artificial ponds are categorized as heated ponds and not heated open ponds. Heated open ponds have higher initial investment costs and running costs however they allow to control the temperature (Algreen, 2018).

Following the growth of algae to some extent, algae and nutrient are added to ponds. Generally, carbon source of these systems is inorganic carbon from bicarbonate (HCO_3) which is presented in the nutrient blend. Sunlight is used for illumination. Usually paddle wheels are used for agitation. Recently paddle wheels are seen inefficient as they are located on the surface of the pond, deeper part is not well-mixed which creates a heterogeneous culture medium. That is the reason why, researchers are working on new models for agitation in open ponds (Algreen, 2018).



FIGURE 2.3.4: OPEN CULTIVATION POND (CYSEWSKI, 2018)

The open method has advantages as being simplistic in the sense of constructing and operating the plant. Therefore, the production and operation expenses are nominal (Ugwu, Aoyagi, & Uchiyama, 2008). There are disadvantages as well. The most significant disadvantages of the open method are water loss by evaporation, carbon dioxide loss by propagation to the atmosphere, and high land surface demand. In these systems cultivation efficiency depends on the season and the temperature of the geographical area. Another disadvantage is that cells may not receive required sunlight and this problem occurs because of shading effect due to the high biomass concentration or the ineffectiveness of the agitation

method (Cuaresma, Janssen, Vilchez, & Wijffels, 2011). Contamination of the ponds is another disadvantage which should be considered for algae in general (Singh & Sharma, 2012). Greenhouses are an option to mitigate some of these drawbacks (Algreen, 2018). In order to reduce these effects, alternatively closed systems have been developed (Singh & Sharma, 2012). To obtain tenfold efficiency of the open ponds and reduce the risks, Spirulina can be produced in greenhouses, where there is possibility of controlling parameters such as temperature, fed carbon dioxide concentration, humidity/evaporation, illumination and also potential contamination. Therefore, providing the needed conditions for a specific algae would be easier (Sierra, et al., 2008).

2.3.3.1.2. PHOTOBIOREACTORS (PBR)

Photobioreactors are closed systems where algae do not have any contact to the environment while growing. Even though PBR systems are expensive, have several advantages over open systems such as, control on algae cultivation in sense of carbon dioxide feed, illumination, water quantity, pH, temperature, biomass density, contamination, aeration and gas exchange. PBR systems are used in order to overcome the disadvantages of open systems and obtaining higher productivity.

There are different types of closed systems for algae growth such as, vertical tubular PBR, flat-plate PBR, horizontal tubular PBR vertical column PBR. As these systems are closed to environment, in lab scale they need artificial illumination which is generally provided by fluorescent lamps.

In figure 2.3.5, polyethylene bags are used for flat plates PBR in a larger scale than laboratory scale. Algae in the flat plate PBR is homogenous which means they grow equally, therefore in order to harvest the algae, opening the valve on the bottom is enough. Also for taking samples the same valve is used. Illumination is provided by sunlight while heating is provided by pipes inside the bag and aeration by a tube at the bottom of the system.



FIGURE 2.3.5.: FLAT PLATE PBR (WAGENINGEN UNIVERSITY, ALGAEPARC PILOT FACILITY)
(ALGAEPARC, 2018)

Figure 2.3.6 shows a type of vertical tubular PBR. This specific PBR is called vertical stacked tubular PBR in Wageningen University/Netherlands. Algae culture is added to a tank first, then circulated along the tubes by a pump. Sunlight is the illumination method for this system. Neon green columns present on both sides for a homogenous sunlight dispersion. Nutrient is given by a small nutrient pump. Harvesting pump starts to work when the concentration sensor shows a certain maximum level until a minimum level both are defined in advance on the automation system.



FIGURE 2.3.6: VERTICAL STACKED TUBULAR PBR (WAGENINGEN UNIVERSITY, ALGAEPARC PILOT FACILITY) (ALGAEPARC, 2018)

Figure 2.3.7 demonstrates horizontal tubular PBR. In this system, algae medium circulates, and algae are harvested again by pumps. This system is operated in the same way as vertical stacked tubular PBR in the sense of adding algae culture to the system, growing the algae, illumination and aeration methods and harvesting.



FIGURE 2.3.7: HORIZONTAL TUBULAR PBR (WAGENINGEN UNIVERSITY, ALGAEPARC PILOT FACILITY) (ALGAEPARC, 2018)

Comparison between all systems is shown on the Table 2.3.1 below:

TABLE 2.3.1: COMPARISON OF DIFFERENT CULTIVATION SYSTEMS (MADKOUR, KAMIL, & NASR, 2012)

Cultivation System	Dimensions	Specific Growth Rate (μ) (1/hour)	Advantages	Disadvantages
Open Systems	Variable	0.025/hour	Economical, easy to clean, good for mass cultivation of algae	Less controlled environment, difficult to culture algae long time, high land surface requirement, low productivity, contamination
Tubular PBR	Diameter: 3-10cm	0.055/hour	Large illumination surface area, suitable for outdoor cultures, high productivity, relatively cheap	Dissolved oxygen and CO ₂ along the tubes, wall growth, high land surface requirement
Flat plate PBR	Width: 0.07m, Height: 1.5m, Length: 2.5m Productivity: 1g/L/day	N/A	Large illumination surface area, suitable for outdoor cultures, easy to relocate the cultures, high productivity, relatively cheap, easy to clean	Difficulty in scaling up, wall growth

2.3.3.2. GROWTH PARAMETERS

Spirulina productivity and growth rate depends on several factors, as mentioned before, such as; temperature, pH, culture medium, initial culture, nutrient feeding method, agitation and aeration methods and illumination.

2.3.3.2.1. TEMPERATURE AND PH

Optimum temperature for growing Spirulina is between 30-35°C (Soni, Sudhakar, & Rana, 2017). While what was reported by Soni, Sudhakar, & Rana (2017) it can still grow between 20-37°C, below 17°C the growth rate is nil, however above 38°C growth of Spirulina is inhibited. Bacteria are dangerous for Spirulina in sense of contamination and the major contamination is caused by protozoan and paramecium under humid conditions. pH for a good productivity, ranges between 9 and 11. Spirulina is unique as it can grow under high alkaline conditions because usually other microorganisms cannot grow under these high alkaline conditions which decreases the level of contamination (Soni, Sudhakar, & Rana, 2017).

2.3.3.2.2. CULTURE MEDIUM

Culture medium is one of the most important parameters to be considered. Water where Spirulina grow in and nutrient source composes the culture medium. Spirulina is able to grow in high alkaline, saline waters. However, it is better to filter the water before growing it. Also, hard water would create mud. Because of this reason, water treated by reverse osmosis is the best option to grow Spirulina (Soni, Sudhakar, & Rana, 2017). The other component of culture medium is the nutrient blend. There are several mediums available while Zarrouk's medium is the most suitable for growing Spirulina.

TABLE 2.3.2: COMPOSITION OF ZARROUK'S MEDIUM (ZARROUK, 1966)

Ingredient	Zarrouk's Media (gms/l)
NaHCO ₃	16.80
K ₂ HPO ₄	0.50
NaNO ₃	2.50
K ₂ SO ₄	1.00
NaCl	1.00
MgSO ₄ ·7H ₂ O	0.20
EDTA	0.08
CaCl ₂ ·2H ₂ O	0.04
FeSO ₄ ·2H ₂ O	0.01
H ₃ BO ₃	2.86
MnCl ₂ ·4H ₂ O	1.180
ZnSO ₄ ·7H ₂ O	0.222
Na ₂ MoO ₃ ·	0.015
CuSO ₄ ·5H ₂ O	0.074
NH ₄ VO ₃	22.9
NiSO ₄ ·7H ₂ O	47.8
NaWO ₂	17.9
Ti ₂ (SO ₄) ₃ ·6H ₂ O	4.4
Co(NO ₃) ₂ ·6H ₂ O	4.4

Even though Zarrouk's medium is very suitable, it is an old model and recent commercial applications are done by preparing ad-hoc medium blends. The reason why is that, Zarrouk's medium contains high amount of bicarbonate which is way more than needed amount, so for large scale production this medium is not recommended. Also preparing customized medium blend is more economic than paying for Zarrouk's medium (Algreen, 2018). While preparing the medium; phosphate, magnesium and calcium should be added in low concentrations (in order to prevent to create a hard water), potassium could be increased but concentration of potassium should be one-five of sodium concentration maximum (Soni, Sudhakar, & Rana, 2017). Algreen start-up company stated that they prepare their medium and composition of their medium is 2 g/L of nitrate (NO₃⁻), 1 g/L of sulphate (K₂SO₄), 0.8 g/L of phosphate (PO₄⁻³), 0.2 g/L of magnesium (MgSO₄), 10 gram of bicarbonate (HCO₃), iron and trace elements. Wastewaters or organic wastes can be used as medium as well because these materials consist of high level of nitrogen and phosphorus as they are organic matters.

Several studies have been done about using pure CO₂, compressed air containing a certain percentage of CO₂ (depending on the study, strain and other parameters) and flue gas as carbon source by replacing bicarbonate with these sources.

2.3.3.2.3. INITIAL CULTURE

Prior to cultivation, *Spirulina* strains must be totally grown, and color must be green, straight filaments must compose the most 25% of all strains and the rest must be coiled filaments. Another important point is lipid content of initial culture. The strain must contain 1% of gamma-linolenic acid (fatty acid) of its dry weight while specific growth rate is more or less 30% under optimum conditions. Initial concentrated culture can be supplied from the floating part of a culture which contains grown algae or by diluting an already filtered culture. The least biomass concentration is better for beginning (Soni, Sudhakar, & Rana, 2017). In order to obtain high productivity and better quality of production, strains with high lipid content should be chosen for cultivation (AlgaePARC, 2018). Researchers at Algaeparc Research Facility have developed a new technology as a result of their strain improvement studies which allows to detect the strain with the highest lipid content. In this technology one kind of dye is injected into strains and the strain with high lipid content tends to have a denser color compared to others.

2.3.3.2.4. NUTRIENT FEEDING METHODS

There are three methods to feed the culture with a medium (nutrient source); semi-continuous system, continuous system and batch system (Barsanti & Gualtieri, 2010).

In semi-continuous systems, medium is delivered by opening a valve until needed amount of medium is delivered to culture. Once the needed amount of medium delivery is completed the valve is closed and algae is left to grow for 24 hours and the same procedure is done all over again. This system extends the time to use of cultures in big tanks as the culture is harvested partially and by immediately replenished medium, algae grow continually (Barsanti & Gualtieri, 2010).

Continuous systems are other alternative which give an opportunity of delivering endless nutrients to culture. These systems work automatically. According to the growth rate of algae, nutrients are delivered in a certain amount which means; when algae grow till desired

concentration, harvesting system starts to work while nutrient pump delivers nutrients to compensate volume of harvested biomass. Growth rate chosen for automatic harvest to start is almost the maximum growth rate. This system provides a sustainable growth for algae as nutrients will be supplied to the cultures whenever needed (Barsanti & Gualtieri, 2010).

Batch method has a closed system where nutrients are not provided infinitely, algae concentration increases till a limiting factor appears that means, algae consume nutrients by the time until one of the critical nutrients finished in the medium which interrupts algae growth if they are not provided with nutrients. So, algae are supplied with nutrients at regular intervals. Batch systems are low cost and simple systems which makes them the most popular culture feeding method. There are six phases of algal growth for batch systems which are shown on the Table 2.3.3 below (Barsanti & Gualtieri, 2010):

TABLE 2.3.2: SIX PHASES OF ALGAL GROWTH (BARSANTI & GUALTIERI, 2010)

Growth Phase	Growth Rate Interpretation	Description
Lag phase	Null	Physiological adaptation of the inoculum to changing conditions
Acceleration phase	Increasing	Trivial
Exponential phase	Constant	Population growth changes the environment of the cells
Retardation phase	Decreasing	Effects of changing conditions appear
Stationary phase	Null	One or more nutrients (or light) are exhausted down to the threshold level of the cells
Decline phase	Negative	The duration of stationary phase and rate of decline are strongly dependent on the kind of organisms

2.3.3.2.4.5. AGITATION AND AERATION METHODS

Agitation is a significant process in order to obtain a homogenous culture in sense of growth. For an equal illumination and homogenous spread of nutrients all over the medium mixing system choice is critical (Soni, Sudhakar, & Rana, 2017).

Aeration is another important process which supplies carbon dioxide and oxygen to medium usually blended in compressed air with a certain percentage in case of using carbon dioxide as carbon source. Carbon dioxide percentage in supplied compressed air depends on each study for laboratory scale or production process for commercial scale.

Usually in PBR systems agitation is provided by aeration tubes which ensures mixing the medium while delivering CO₂ and O₂. However, in open raceway ponds, there is no need for a specific aeration method as usually in raceway ponds carbon source is bicarbonate; while in a study done by Costa, et al., 2015 sprinklers are placed on the bottom of the raceway pond to aspire carbon dioxide to the system. Agitation method for open raceway ponds is paddlewheels but, as mentioned before, this system is not seen very efficient as it is difficult to mix bottom of the pond by paddlewheels.

2.3.3.2.4.6. ILLUMINATION

Spirulina grows by photosynthesis reaction utilizing light energy, water and carbon dioxide and releasing oxygen and producing glycose (biomass) as all the photosynthetic organisms. In order to obtain a good quality of biomass and high growth rate, illumination intensity, surface area and duration of illumination is crucial. Higher the light intensity and illumination duration, higher the productivity to some extent. These variables should be applied in optimum levels. For instance; high light intensity for cultivation results in high growth rate and productivity while low light intensity results in cultivation of strains which contains more pigments and proteins compared to the former. Another important point for cultivation is the illumination period which depends on the geographical area for outdoor systems which utilize sunlight for growth. For artificial illumination, shading effect must be considered (high biomass concentration) as it diminishes growth rate (Soni, Sudhakar, & Rana, 2017).

2.3.4. HARVESTING METHODS

There are several harvesting techniques such as; filtration, centrifugation, precipitation, ultrasonic vibration, ion exchange, flotation. Filtration by a filter at 50 microns for a good yield of collection is the first step which is essential in order to separate grown biomass from medium however 50% of filtrated part is the culture medium. Therefore, in order to remove and attain Spirulina, concentration of the biomass is required after filtration (Soni, Sudhakar, & Rana, 2017).

Centrifugation is an expensive method, that is the reason why in large scale commercial Spirulina production, filtration systems are used to concentrate the biomass. Two different equipment is used as filtration which are, vibrating and inclined screens. Both systems are able to harvest between 10-18 m³ Spirulina /hour. Advantage of vibrating screens over inclined screens is that inclined screens require three times more filtration area than vibrating screens which is around 3 m²/unit. As filamentous structure of Spirulina is sensitive, vibrating systems can damage Spirulina (Soni, Sudhakar, & Rana, 2017). However, Algreen start-up company uses vibrating bath system for harvesting and states that it is one of the most efficient methods as it is more economic than several systems and yields high biomass concentration (Algreen, 2018).

The most suitable harvesting time of the day is early morning because of several motives such as; having time left to dry Spirulina under sun and high protein content of Spirulina in that time of the day (Soni, Sudhakar, & Rana, 2017).

2.3.5. DRYING METHODS

Consuming Spirulina is possible when it is fresh and also dried. Following harvesting period, if it is preferred to consume it fresh, it must be consumed in six hours while, in order to conserve it longer time, drying procedure must be applied. Soni, Sudhakar, & Rana (2017) indicates that “Different drying methods include sun drying, freeze drying, spray drying,

drum drying and cooking.” The most suitable method is sun drying as it is better for cell structure of Spirulina. However, sun drying may cause chlorophyll loss and results with a blue biomass. Even though freeze drying gives better results, it is a costly method. As a result of that, for industrial purposes spray drier is being used (Soni, Sudhakar, & Rana, 2017).

2.3.6. CARBON DIOXIDE SEQUESTRATION POTENTIAL OF SPIRULINA

Theoretically, microalgae utilize around 9% of solar energy and is able to produce 280 tons of biomass/ ha per year and fixes 513 tons of CO₂ (Bilanovic, Andargatchew, Kroeger, & Shelef, 2009). As a photosynthetic microorganism, Spirulina needs a carbon source to grow. This carbon source can be organic or inorganic. Usually carbon dioxide is used as an organic carbon source while bicarbonate is used as an inorganic source. Cultivating Spirulina by carbon dioxide has an advantage of mitigating climate change by reducing carbon dioxide in the atmosphere beside producing biomass. Many laboratory experiments have been done up to date in order to identify carbon dioxide bio-sequestration potential of Spirulina.

2.3.6.1. LABORATORY SCALE CULTIVATION

Laboratory scale cultivation of Spirulina usually is done in photobioreactors. In this kind of small-scale applications PBR can be more suitable as it does not require high surface area compared to open ponds. Moreover, efficiency of PBR is higher in sense of carbon dioxide bio-fixation and productivity.

During the experiments cell concentration must be measured every day, before and after harvesting, by spectrometers. Spectrometers measure optical density and according to optical density results, concentration can be converted into mg/L unit. According to the results of cell concentrations, productivity is calculated by the formula;

$$P = (X_t - X_0) / t \quad (\text{g/L/day})$$

Where X_t is the biomass concentration measured at time t , X_0 is the initial biomass concentration.

Maximum specific growth rate must be calculated with linear regression of biomass concentration change in a specific time frame with the following formula;

$$\mu = (\ln(X_t - X_0)) / (t - t_0) \quad (\text{day}^{-1})$$

Where X_t is the biomass concentration measured at time t , X_0 is the initial biomass concentration, t_0 is equal to 0.

Doubling or generation time is the time frame where microalgae doubles its biomass concentration. Shorter the doubling time, higher the productivity. It has an inverse relation with specific growth rate;

$$\text{Doubling time} = 1/\mu_{\max} \quad (\text{day})$$

Maximum carbon dioxide bio-fixation rate is calculated with the equation below:

$$R_{\text{CO}_2} = P_X \cdot X_{\text{cbm}} \cdot (M_{\text{CO}_2} / M_C) \quad (\text{g/L/day})$$

Where; R_{CO_2} is bio-fixation rate, X_{cbm} is carbon fraction in the biomass, M_{CO_2} and M_C are the molecular weights of carbon dioxide and carbon respectively.

(Shabani, Sayadi, & Rezaei, 2016).

In a study (Morais, Silva, Henrard, & Costa, 2015) 2L volume of vertical tubular PBR and modified Zarrouk medium (replacing bicarbonate with carbon dioxide) was used to cultivate *Spirulina*. Carbon dioxide inlet concentration was 12% of compressed air added to culture with different initial biomass concentrations (200, 400, 600, 800 and 1000 mg/L). The highest bio-fixation rate was obtained when the initial biomass concentration was 600 mg/L which is 186.8 ± 73.1 mg/L/d while maximum productivity was calculated as 85.9 ± 6 mg/L/d while in another experiment with 2L of vertical tubular PBR (Rosa, Moraes, Cardias, Souza, & Costa, 2015) and using modified Zarrouk medium, a chemical absorbent was used in order to evaluate effect of chemical absorbent on *Spirulina* growth and carbon dioxide capturing potential. Results demonstrate that maximum carbon dioxide bio-fixation rate was 104

mg/L/day and maximum productivity was 0.0621 g/L/day. Specific growth rate of Spirulina in this experiment was 0.324 / day. This study showed that chemical absorbent addition did not make a significant change in bio-fixation rate and productivity levels.

As mentioned before Spirulina is able to grow in saline water. Researchers who decided on carrying out an experiment to see the differences between productivity, bio-fixation rate and growth rate of Spirulina under different salinity levels (Shabani, Sayadi, & Rezaei, 2016) indicated that, among three different salinity levels (pure water, natural water and artificial sea water with conductivities 3, 1500 and 34000 $\mu\text{s}/\text{cm}$ respectively) natural water filled medium has the highest potential of bio-fixation as the maximum bio-fixation rate obtained is 0.49 g/L/day while maximum productivity is 0.098 g/L/day. This experiment has the highest result in bio-fixation rate among all other experiments evaluated during this internship however, productivity is strikingly low. The reason behind this can be the concentration of injected carbon dioxide or illumination levels or even the quality of the cultivated strain. This comment shows that productivity, specific growth rate and bio-fixation rate highly dependent on input parameters.

2.3.6.2. LARGE SCALE CULTIVATION (COMMERCIAL PURPOSES)

Recently, Spirulina production became very popular thanks to its health benefits and pigment content. Usually cultivation system recommended for Spirulina in large scale is open systems. Greenhouses are also very common as it has the advantage of controlling the environment during cultivation.

Spirulina market is getting larger every day because there is a high potential in this business in the sense of revenues. The market is becoming more concentrated by the time as start-up companies begin to invest in this field.

One of the start-up companies in the Netherlands called Algreen BV started their business in 2015. Two students from Wageningen University who founded this company are working as consultants for other firms who are willing to operate in this market. One of the plants which

founders of Algreen designed in Italy has four open ponds: two heated, two non-heated. Each pond has 224 m² surface area while heated ones have 20 cm of depth and non-heated ones have 25 cm of depth. Temperature of heated pond is between 34-35 °C and pH of both ponds is between 10-10.3. Carbon source of this system is bicarbonate and nutrient source is not purchased but prepared by their own. Self-prepared nutrient blend contains; 2 g/L of nitrate, 1 g/L of sulphate, 0.8 g/L of phosphate, 0.2 g/L of magnesium sulphate, 10 g/L of bicarbonate, iron and trace elements. As agitation method self-developed current breaker system is used in order to prevent negative effects of paddlewheels. In this system paddlewheels are embedded into the water which results with higher mixing efficiency. Medium is being recycled in order to use less water and nutrients. Productivity of heated pond is 12 g/m²/day and operation duration is 270days/year while non-heated one has productivity levels as 10 g/m²/day with operation duration as 200 days/year (Algreen, 2018).

Assuming that these productivity levels obtained by using carbon dioxide as carbon source, with a simple calculation as follows:

Molecular weight of HCO₃ (bicarbonate) is 61 g/mole and of carbon is 12 g/mole. In this production plant 10g/L of HCO₃ is used. With a linear correlation calculation if 61 gram of HCO₃ contains 12 g of carbon, 10 gram of bicarbonate contains around 2 g of carbon. In order to use 2 g of carbon in an organic form, 7.3 g of carbon dioxide would be needed. In literature, per each ton of biomass produced, around 1.8 – 2 tons of carbon dioxide is captured by algae (FAO, 2008). According to this statement, by adding 7.3 g/L carbon dioxide to this system instead of 10 g/L of bicarbonate, with the same productivity levels, around 4,7 tons CO₂ would be captured per year by only these four ponds.

Overall, considering that in many studies, productivity of microalgae cultivation by using bicarbonate as carbon source is less compared to productivity of microalgae biomass cultivated by using carbon dioxide. Therefore, captured carbon dioxide levels can be higher in case of using carbon dioxide as carbon source. In order to clarify the number given, 4,7 tons CO₂ / year/ 1000m² is equal to almost 50tons CO₂ /year/ha which is a very promising result considering the emissions from oil and gas industry.

2.3.7. MARKET ANALYSIS FOR SPIRULINA

Today, the size of Spirulina market is around 90 mln US dollars and it is expected to reach 129.2 mln dollars by 2023 with a CAGR (compound annual growth rate) of 7.54% between the years 2018 – 2023. There are many drivers for Spirulina market such as its popularity as super food, nutritional value, health benefits etc. Besides, it can be also used as livestock and poultry feed or as bio-fertilizer, nutraceuticals, pharmaceuticals and personal care. Different applications are being discovered every day and it increases the number of new entrants to the market which decreases the market concentration. Customer segments for the product would be e-commerce companies, retailers and wholesalers, end users as vegetarians, vegans, healthy life lovers, sport people, business people who are always in hurry and do not have time to eat properly, so spirulina can be consumed by these customers as food supplement. Spirulina market segments are capsule, powder or liquid. Powder segment has the highest proportion in the market compared to other segments. Leaders of the market are Earthrise, Cyanotech Corp., DIC Corp. etc (Market Research Future, 2017).

This business has a relatively low sunk cost but high initial investment cost, however with a good market analysis and a detailed technical research, it has a huge potential as it is very popular and has a great possibility of scalability. A study has been funded by United Nations indicates the cost allocation of a pilot Spirulina farm must be as below;

TABLE 2.3.4: COST ESTIMATION FOR A PILOT SPIRULINA FARM IN WESTERN KENYA (500 M2) (IN EUROS) (PICCOLO, 2012)

LAND COST	
Price per acre	8000
Number of acres	0.25
Total price of land	2000
LEGAL TRANSFER COSTS 10%	1000
BUILDING COSTS	
Boundary wall	3000

Landscaping	1000
Security lighting	500
INDEPENDENT PRODUCTION UNITS	
Raceway ponds (500m ²)	10000
Galvanized super structure for covers	1000
UV. Proof covers	500
1000w geared motor	200
Paddle wheel and fittings	650
Pumps and piping	350
Harvesting station	200
Drying Screens (each)	100
Solar Dryer	500
Total Independent Production Units	13500
SHARED STRUCTURES	
Fresh Water Treatment pond	1000
Small and Medium culture ponds	2000
Testing and monitoring laboratory	10000
Administrative building	10000
Storage / Packaging Unit / Grinding	5000
Washrooms	6000
Guard Unit	2000
PV (Photovoltaic) Solar Power Unit	3000
Total Shared Structures	39000
TOTAL SETUP COSTS	60000
OPERATIONAL COSTS	
Culture cost	100
Per pond inoculation costs	

Water 100 m ³ x 3.5	150
Medium preparation fertilizer	200
Total for 1 pond	350
Labor	
Farm manager	200
Lab operator	150

Packing manager	120
Unit Staff 2 per unit (100 x 2 x 1)	200
Gardner / groundskeeper	100
Guard	150
Total Labor Cost p/m	920
Total Operation Cost per Month	1370
PRODUCTION COST	
Direct cost - water/ fertilizer / day	50
PROFIT	
Total expected output	10g/m2/day
500 m2	5 kg per day
Wholesale price per kg	50
Total expected production x wholesale price/ day	250
Gross Profit / day	200
GP per month (average 25 days)	5000
Less Monthly Expenses	1370
Total Expected Net profit per month	3630

According to CEO of Algreen BV, Stefano Canziani, cost structure of a Spirulina farm must be composed of initial investment cost and running costs (consumables, labor cost, depreciation). Initial investment cost of the farm constructed by Algreen was between 150.000-250.000 euros without considering land cost. Then heated ponds cost more than traditional ponds and difference is between 40.000-60.000 euros for two heated ponds. Consumable costs depend on that specific geographical location where Spirulina farm is located. For instance; Algreen farm is located in Cremona/Italy and for that location labors are paid according to agricultural full-time employment in 25% percentile. Overall, one kilogram of Spirulina production costs 25 euros while sales price is 400 euros per kilogram. Profit from one kilogram of Spirulina sale is 375 euros. Considering initial investment costs, running costs and profits of this farm, payback time is calculated as 5-6 years (Algreen, 2018).

2.3.8. PIGMENT EXTRACTION FROM SPIRULINA

Spirulina contains several pigments such as; chlorophyll a, phycocyanin, carotenoid, allophycocyanin and phycoerythrin. Chlorophyll a gives green color and phycocyanin gives blue color, because of this spirulina belongs to blue-green algae familia. Moreover, carotenoid is reddish-orange color pigment, allophycocyanin is light blue color pigment (Christaki, Bonos, & Florou-Paneri, 2015) while phycoerythrin is purple color pigment (S.Sivasankari, Naganandhini, & Ravindran, 2014). Phycocyanin, phycoerythrin and allophycocyanin pigments are three main groups of phycobiliproteins which present in an organelle of cyanobacteria called phycobilisome (BA & LS, 1986). These phycobiliproteins are light-harvesting pigments and also called accessory pigments as they protect cyanobacteria from high solar radiation and oxidation (S.Sivasankari, Naganandhini, & Ravindran, 2014). Another important mission of phycobiliproteins is capturing the sunlight and send it to chlorophyll to complete photosynthesis reaction because phycobiliproteins can capture light in wavelengths where chlorophyll cannot absorb the light (Christaki, Bonos, & Florou-Paneri, 2015). The maximum wavelengths that phycocyanin, phycoerythrin and allophycocoyanin can absorb the light are between 610 -620 nm, 540-570 nm and 650-655 nm respectively (Reis, Mendes, Lobo-Fernendes, Empis, & Novais, 1998).

Phycobiliproteins are water-soluble, highly fluorescent pigments (Christaki, Bonos, & Florou-Paneri, 2015), produced and used with commercial purposes for food and cosmetic industries as natural dye and thanks to its health benefits such as; anti-tumor, anti-inflammatory, anti-oxidant and anti-aging impacts it is also used in pharmaceutical industry (S.Sivasankari, Naganandhini, & Ravindran, 2014). In order to use these pigments they should be extracted from microalgae then purified. Aromatic amino acids can absorb the light at a wavelength of 280 nm which is shown in figure with a peak, and absorbance at this wavelength gives protein concentration in the solution (Phycocyanin included). Considering this information, ratio between A_{620}/A_{280} is equal to purity of the extracted pigment with respect to most forms of contaminating protein (Sobiechowska-Sasim, Ston-Egiert, & Kosakowska, 2014).

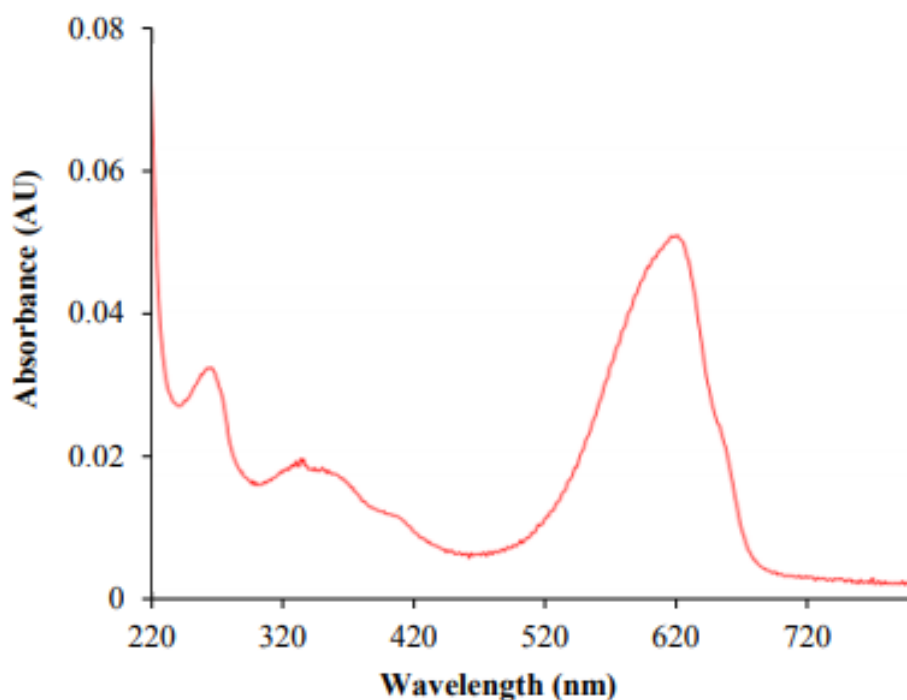


FIGURE 2.3.8: ABSORPTION SPECTRUM OF C-PHYCOERYTHRIN (SURESH, S.K.M., & DAS, 2009)

Phycocyanin purity is an important parameter as its applications differ according to its purity level. If A_{620}/A_{280} ratio is between 0.5 – 1.5 this phycocyanin is acknowledged as food grade pigment, whereas purity level of between 1.5 - 2.5 is cosmetic grade, 2.5 – 3.5 is reagent grade and above 4 is analytical level (Guan, 2016).

Pigment extraction is done basically by disrupting the cell wall of microalgae and taking pigment out of cell. Extracting pigments from Spirulina is difficult compared to many other microorganisms because Spirulina cell wall is composed of several membranes, therefore Spirulina cell is highly resistant to be ruptured (Hellebust & Craigie, 1978). There are many different extraction methods being applied currently, however, finding the optimum method for the highest efficiency is the challenge in pigment extraction applications.

2.3.8.1. PIGMENT EXTRACTION TECHNIQUES

2.3.8.1.1. ULTRASONICATION

Ultrasonication is a method for extracting pigments from biomass by using sound energy. Basically it is used for jolting particles in a solution in order to extract some materials from macro algae, microalgae and even plants (M, G, J.V, & T, 2017), usually with a frequency higher than 20 kHz (Ultrasonication, 2018). In laboratory scale practices, this method generally used with an ultrasonic bath. This method requires attention on magnitude of frequency and duration of exposure.

2.3.8.1.2. INORGANIC AND ORGANIC ACID EXTRACTION

Inorganic or organic acid extraction method is based on penetration of inorganic or organic acid solvent into microalgae cell wall and breaking down the cell membrane by solving the lipids and proteins within the membrane (Jeffrey, Mantoura, & Wright, 1997). Inorganic or organic acid solvents must be chosen depending on the target pigment desired to be extracted. For instance; for phycocyanin extraction organic acid solvents is not useful but inorganic acid solvents result with a high yield as phycocyanin is a water-soluble pigment. Subsequent to organic or inorganic solvent choice, which type of acid solvent desired to be used and its molar concentration are critical

2.3.8.1.3. LYSOZYME TREATMENT

Lysozyme is an enzyme which is used as antibacterial agent because it breaks bacterial cell walls down. As mentioned before, Spirulina has a peptidoglycan layer in its cell membrane and this layer is the exact structure where lysozyme attacks. It disrupts cell wall by hydrolysis reaction. Lysozyme can be found in sweat, tears, saliva and many more body fluids (Paul, 2017).

2.3.8.1.4. FREEZING AND THAWING

Freezing and thawing method is based on freezing biomass below 0°C and thawing usually in room temperature. Freezing temperature can be chosen as -4°C, -20°C or even -50°C, however tricky part here is choosing temperature with the least energy consumption and highest extraction efficiency. Therefore, prior to large scale applications, laboratory scale applications must be done in order to find optimum temperature.

Freezing biomass leads to dilation of the liquid inside the cell, damaging the cell wall as the liquid has been solidified. Damaged cell wall creates more space for liquid when thawed and during the next freezing process frozen liquid becomes more dilated. Usually applying five or six cycles of freezing-thawing as described yields with high pigment concentration.

2.3.8.1.5. MORTAR AND PESTLE

Homogenization by mortar and pestle is a traditional method for many different application, and for pigment extraction as well. In this method, biomass in a solvent is mashed by mortar and pestle. In order to increase the efficiency, some additional material can be used as neutral sand (S.Sivasankari, Naganandhini, & Ravindran, 2014) or diatomaceous earth (Moraes, Sala, Cerveira, & Kalil, 2011).

2.3.8.2. LABORATORY SCALE PIGMENT EXTRACTION

Laboratory scale pigment extraction can be done through many different methods and there are several studies have been done in order to compare these methods in terms of efficiency and pigment concentration. Concentration of C-Pyhcocyanin (C-PC) is usually calculated by the method which is demonstrated in a study (Bennett & Bogorad, 1973) as following:

$$P_C = (A_{620} - 0.474A_{652})/5.34 \quad (1)$$

Where P_C is C-PC concentration (mg/ml), A_{620} is the absorbance of the sample at 620 nm of wavelength, A_{652} is the absorbance of the sample at 652 nm of wavelength. In order to calculate the yield of extraction, following formula is usually used which is demonstrated from the same study:

$$Y (\%) = (P_C \times V) / DB \quad (2)$$

Where Y is extraction yield (mg C-PC/g dry biomass), P_C is C-PC concentration (mg/ml), V is solvent volume (ml) and DB is the dry biomass (g).

In one of the comparative studies where soaking, ultrasonication, freezing and thawing, combination of soaking and ultrafine shearing and combination of soaking, ultrafine shearing and ultrasonication methods were analyzed, yield of pigment extraction from dry biomass was calculated for each method. In soaking experiment; samples were soaked into phosphate buffer with a pH 6.5 for different durations like; 2, 4, 8, 12, 24 and 48 hours. Ultrasonication experiment was carried out by using six different samples in order to find out the effect of duration on pigment extraction and so; samples were exposed to ultrasonication for 2, 4, 6, 8, 10 and 12 minutes in a mode: 2 sec on, 5 sec off) following four hours of soaking at 4°C. Freezing and thawing method applied includes freezing the sample in -20°C for four hours and thawing it in a water bath with 25°C for 20 minutes. It has been applied for 6 cycles. Combination of soaking and ultrafine shearing has been applied as follows: Samples were soaked for 0, 4, 8, 12 and 24 hours then for each solution, seven samples have been crushed by ultrafine food shearing equipment with a duration of 2, 3, 4, 6, 8, 10 and 12 minutes. Lastly, combination of soaking, ultrafine shearing and ultrasonication methods has been applied and sample has been soaked into the phosphate buffer for 2, 4, 8, 12, 24 and 48 hours then subjected to ultrafine food shearing equipment with a duration of 10 minutes, finally solution taken from ultrafine shearing application has been exposed to ultrasonication for ten minutes (Yu, 2017).

Results of this experiment demonstrates that the highest yield obtained is 9.02% from combination of soaking, ultrafine shearing and ultrasonication which is followed by 8.89%, 8.62%, 8.43% and 8.34% by combination of soaking and ultrafine shearing, ultrasonication, soaking and freezing and thawing methods respectively (Yu, 2017).

Another study which is done by using wet biomass shows that the highest yield obtained was 4.38% by sonication method which applied with glass pearls, whereas 1.8% by freezing and thawing, 2.4% by mortar and pestle and 3.8% by inorganic acid extraction methods were obtained (Moraes, Sala, Cerveira, & Kalil, 2011).

One of the studies shows that oven drying causes pigment loss by 35% (Guroy, Karadal, Mantoglu, & Cebeci, 2017), moreover drying process releases carbon dioxide and leads to energy consumption. Therefore, extracting pigment from wet biomass is a better approach in terms of extraction efficiency, environmental-friendly way of applications and less energy consumption.

The comparative study carried out by S. Sivasankari, Naganandhini & Ravindran (2014) indicates that the highest yield (76%) attained from wet biomass was by freezing and thawing method which resulted with a concentration of 0.38 mg C-PC/ml solvent among the other methods which are homogenization, sodium phosphate buffer, organic acid extraction, inorganic acid extraction and sonication and yielded with 12%, 50%, 13.6%, 57.2% and 8% respectively (duration for each experiment was 48 hours). Pigment concentrations for other methods applied in this experiment are as following; 0.06 mg C-PC /ml solvent for homogenization, 0.25 mg C-PC /ml solvent for sodium phosphate buffer, 0.068 mg C-PC /ml solvent for organic acid extraction, 0.286 mg C-PC /ml solvent for inorganic acid extraction and finally 0.04 mg C-PC /ml solvent for sonication method.

Several methods have been applied during the experiments above, and results for the same techniques were not similar which may be a result of effects of different parameters on pigment extraction yield. For instance; duration of exposure for any method is critical. Moreover for ultrasonication method, amplitude is a factor that can affect the yield, if amplitude is too high and if it is not supported by a cooling system, the heat may damage the protein structure and extraction may yield really low. On the other hand, freezing and thawing method must be examined with suitable duration and temperature in order to disrupt cell wall and extract pigment with a good efficiency.

According to many laboratory and large scale applications, pigment extraction from wet biomass by freezing and thawing method with right temperature and duration may be the most suitable approach.

2.3.8.3. MARKET ANALYSIS FOR PIGMENT EXTRACTION

Spirulina cultivation is a cost-effective application as production of Spirulina provides a good profit considering the production and initial investment costs. Taking into account that Spirulina production is economical, phycocyanin business has an increased trend as this pigment can be a good source for cosmetic, pharmaceutical, food and nutraceutical industries. However dyes are the most strictly controlled additives in food industry by regulations worldwide (Research, 2017). For instance, according to European Union regulatory framework on food additives, there are many steps for a food colorant to be approved for human consumption (Lehto, et al., 2017). Therefore; governments in developed countries support natural sources for food grade goods and this can be accepted as a driver for phycocyanin market.

Phycocyanin production for pigment manufacturing is widespread mostly in North and Latin America, Europe, Middle East, Africa and Asia Pacific regions. United States is leading the market in North America while in Asia Pacific China and India are leaders. Between 2017 and 2027, Europe market is predicted to be larger. Worldwide top players are Earthrise, Cyanotech, Parry Nutraceuticals, Prozyme, Ecofuel Laboratories etc.

Current size of phycocyanin market showed a value as 112.3 mln dollars (Future Market Insights, 2018). This market has grown steadily between the years 2013 and 2016 increasing by 26.39% from 15.6 mln US dollars to 31.5 mln dollars and further increase is predicted for 2021 as it is expected to expand to 186.7 mln dollars worldwide (Market Research Store, 2017).

A study where phycocyanin was extracted simply by phosphate buffer solvent has shown the phycocyanin production cost around 250 dollars/kg where the tax rate as 30%, loan will be paid in 10 years and loan interest rate is 7.5% per year, 2% of CAPEX is calculated as

maintenance and utility cost and finally 15% discount rate was assumed. Raw material is Spirulina and cost estimated around 11 dollars per kg, while phycocyanin selling price considered as 500 dollars per kilogram. Economic analysis and financial assumptions have been showed in Table 2.3.5 and Table 2.3.6, respectively. Considered production steps were adding 15 grams of biomass to 1.5 L of phosphate buffer with 100 mM concentration (solid to liquid ratio was 1/100 (w/v)), keeping the blend in room temperature while agitating it for 4 hours, centrifugation, micro and ultrafiltration. Assuming that phycocyanin production facility is located near Spirulina production farm, phycocyanin facility will run for ten years 300 days/year, 16 hours/day; Spirulina contains 15% of phycocyanin and extraction yield is 67%, annual production capacity is 600 kg of food grade phycocyanin which corresponds to 6 tons of Spirulina biomass production capacity and 600 m³ of sulphate buffer solvent.

TABLE 2.3.5: VALUES AND DESIGN PARAMETERS USED FOR ECONOMIC ANALYSIS IN THE STUDY

Items	Cost / units (US\$ 1 = THB 32.30)	Required quantity for phycocyanin
Equipment:		
Mixing tanks (500 L)	4489.16 US\$/set	3 set
Mixing tanks with temp. control	5417.96 US\$/set	–
Continuous centrifuge (5000L/h)	160,990.71 US\$/set	1 set
Storage tank (500 L)	3560.37 US\$/unit	2 unit
Cooling system (5000 L/h)	6191.95 US\$/set	1 set
Freeze Drier (40kg/day)	157,894.74 US\$/set	1 set
Rotary evaporator	139,318.89 US\$/set	–
Filtration system: (4000 L/h)		
MF (1&0.2 µm) and UF 50kDa	236,842.11 US\$/set	1 set
UF 30 kDa	8,359.13 US\$/set	–
Plant Assumption:		
Building (1600m ²)	13,931.89 US\$/unit	1 unit
Plant life	10 years	
Working day	300 days/year	
Working hour	16 hours/day	
Productions capacity:		
Phycocyanin production (kg/year)		600
Extraction vol. of phycocyanin (L/year) (2000 L/day; 1000L/batch × 2 batches/day)		600,000
Arthrospira biomass (kg/year)	10.84 US\$/kg	6000
Lipid production (kg/year)		–
Polysaccharide production (kg/year)		–
Material assessment:		
K ₂ HPO ₄ (ton/year)	5.26 US\$/kg	6.38
KH ₂ PO ₄ (ton/year)	3.87 US\$/kg	3.17
Water (m ³ /year)	0.40 US\$/m ³	600
Ethanol (m ³ /year)	1.15 US\$/L	–
Electricity (kW.h/year)	0.09 US\$/kW.h	26,400
Labor (persons/year)	12.08 US\$/day	4
Utility & Maintenance at 2% of capital cost		

TABLE 2.3.6: FINANCIAL ASSUMPTION FOR THIS STUDY

	Only phycocyanin	2 products	3 products
Revenue:			
Sale revenue (US\$/year)	300,000.00	309,000.00	317,750.00
Expenses:			
Land rental (US\$/year)	1547.99	1547.99	1547.99
O&M cost (US\$/year)	140,018.02	181,927.52	211,946.18
Total investments (US\$)	596,439.63	741,176.47	754,953.56
(loan 50%)	(298,219.81)	(370,588.24)	(377,476.78)
Debit financing (US\$/year)	29,821.98	37,058.82	37,747.68
Depreciation (US\$/year)	59,643.96	74,117.65	75,495.36
Replacement cost of membrane (US\$/time)	29,411.76	29,411.76	29,411.76

A practical method for food grade pigment extraction from Spirulina by freezing and thawing method has been developed in a study where ultrafiltration, longer retention time and spray dryer processes eliminated, the system has been made simpler, less costly and more efficient. Experimental analysis of this study consists of comparison of extraction methods (lysozyme treatment, freezing and thawing and shearing methods), different durations for freezing and thawing (1h, 2h, 3h, 4h and 5h), different solvents (potassium sulphate buffer, HEPES and distilled water), g biomass/ml solvent ratio (1:1, 1:15, 1:20 and 1:25), activated carbon concentrations for purification (60 g/L, 70 g/L, 80 g/L, 90 g/L, 100 g/L), stirring duration after activated carbon addition (10min, 20min, 30min). The highest yield was obtained from application of freezing and thawing for 3 hours at -20 °C by using 1:15 potassium sulphate buffer solvent and purifying with 80 g/L of activated carbon and stirring it for 10 min. The extraction and purification processes consist of 30 ml of potassium sulphate buffer addition with 1:15 solid to liquid ratio to 2 grams of Spirulina powder, then leaving the blend to freeze at -20°C for 3 hours, thawing the frozen biomass at room temperature, centrifugation in order to obtain supernatant, 80 g/L of activated carbon addition for purification, vacuum filtration (membrane with a pore size 0.22 µm) for separation of the extract and residue and finally drying the extract to produce phycocyanin powder. Traditional process takes around 5 days whereas this practical procedure takes only 10 hours (Guan, 2016).

According to that study, shearing, freezing - thawing and lysozyme methods cost 0.3 dollars/L, 2.8 dollars/L, 7.5 dollars/L respectively. The reason why freezing-thawing method

was chosen as optimal is that cost – efficiency trade off resulted better for freezing and thawing method (Guan, 2016).

Within that study a pilot plant has been designed where production capacity is 1 ton per day, which requires 3 tons of Spirulina powder, 45 m³ of potassium sulphate buffer solvent and 3.6 tons of activated carbon. As the working volume is 60 m³, 100 m³ of biomass can be frozen in 2 shifts per day in the cold storage part. Total equipment cost was calculated as 85.000 dollars considering cold storage room (for freezing the biomass), centrifuge, vacuum pump, freeze dryer, tube and pump. Running costs were calculated daily and resulted in total as 27.135 dollars considering spirulina powder, potassium sulphate buffer solvent, activated carbon, electricity, labor and unforeseen costs. According to these assumptions, cost of phycocyanin would be 27.1 dollars/ kilogram (24 euros) (Guan, 2016).

Nowadays phycocyanin can be bought for between 135 – 480 dollars/kg in a powder form which equals to 120 – 430 euros per kilogram (www.alibaba.com, 2018). This difference between production cost and selling price makes this business very profitable and attractive.

2.3.9. PRODUCED WATER TREATMENT BY SPIRULINA

2.3.9.1. UNCONVENTIONAL OIL AND GAS PRODUCTION

Unconventional oil and gas extraction is a new and alternative approach to conventional oil and gas production. Typical unconventional sources are shale gas, shale oil, tight gas, tight oil and coalbed methane (Jiménez, M.M.Micó, M.Arnaldos, F.Medina, & S.Contreras, 2018). Shale is a type of sedimentary rock and a natural gas or oil source (Energy Information Administration, 2010). In unconventional gas extraction operations two processes are significant: Horizontal drilling and hydraulic fracturing. These two processes are combined in order to obtain unconventional resources.

2.3.9.1.1. HORIZONTAL DRILLING

Horizontal drilling process is composed of vertical drilling the formations until the kick off point which is located on top of the aimed reservoir, then diverging the route of well bore around a curve until the entry point ends where well bore continues drilling within the horizontal layer. (Helms, 2018).

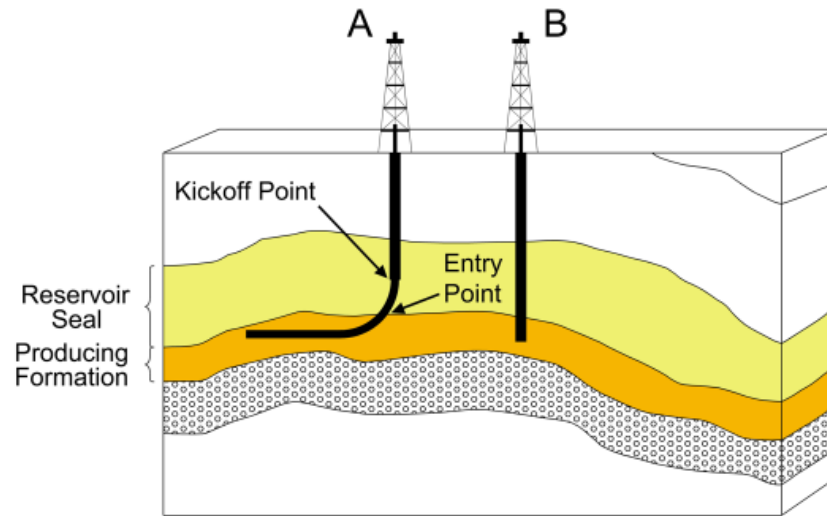


FIGURE 2.3.9: HORIZONTAL DRILLING (A) AND VERTICAL DRILLING (B) (ENERGY INFORMATION ADMINISTRATION, 2010)

2.3.9.1.2. HYDRAULIC FRACTURING

In order to extract shale gas and tight gas, fracturing of sedimentary rocks is needed in order to release the trapped gas from these rocks. Prior to hydraulic fracturing, perforation of the geologic formations must be done. Then water with some chemical additives and sand is pumped through the tubing into created reservoir with a high pressure. It fills the breakages created by perforation process and keeps the fractures open. The water reaches underground in a depth which varies for each well, and turns into a water which carries heavy metals, potentially naturally occurred radioactive materials (NORM) and other chemicals as these matters exist underground and in ground water already.

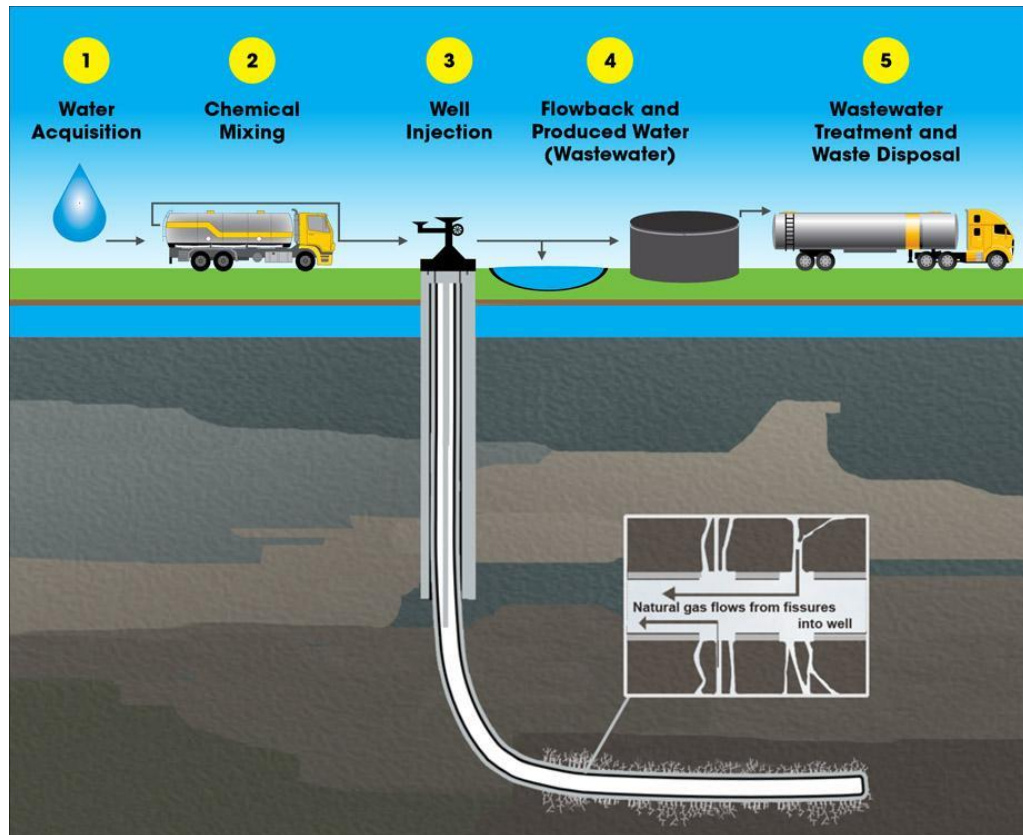


FIGURE 2.3.10: HYDRAULIC FRACTURING IN A HORIZONTAL DRILLING OPERATION (BROWNSTEIN, 2015)

Produced water can be disposed, re-used for fracturing or treated and discharged. Disposal is the most common solution for produced water challenge, but it does not solve the problem permanently. Re-use of produced water in hydraulic fracturing process is another environmentally friendly option and better than disposing the water. Produced water treatment must be considered thanks to its benefits for environment, but economic sustainability is a concern for this application. Economically feasible treatment applications can be the best solution for produced water problem. Based on this approach, treating produced water by microalgae can be an ideal option as it has a great potential of removing heavy metals and NORM from produced water, and it is potentially more economic application compared to traditional applications as well.

2.3.9.2. HEAVY METAL BIOACCUMULATION POTENTIAL OF MICROALGAE

2.3.9.2.1. PROSPECTS OF HEAVY METALS

Heavy metals are naturally occurred elements with high atomic weight and high density. It is assumed that there is a relation between having a high atomic weight and toxicity and according to this assumption metalloids, a group involved in heavy metals, are toxic materials even at low concentrations, for instance, arsenic (Tchounwou, Yedjou, Patlolla, & Sutton, 2012).

Heavy metals exist in environment with a low concentration, and so they are assumed as trace elements (Kabata-Pendias & Pendias, 2001). Biological (biochemical adaptation, characteristics of species where heavy metals exists), physical (adsorption, temperature) and chemical factors affect availability of heavy metals in the environment (Hamelink, Landrum, Bergman, & Benson, 1994).

The effect of heavy metals on biological forms such as; organelles like lysosome, mitochondria, cell wall, cell membrane etc. is reported (Wang & Shi, 2001). Arsenic (As), mercury (Hg), cadmium (Cd), chromium (Cr) and lead (Pb) are acknowledged as the most five toxic heavy metal for human health (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). On the other hand there are some heavy metals such as; copper (Cu), cobalt (Co), manganese (Mn), nickel (Ni) and zinc (Zn) are needed nutrients for growth of plants (Gaur & Adholeya, 2004).

Heavy metals cannot be biodegraded as they are natural compounds of soil and earth crust, therefore they are bio-accumulated (Herrera-Estrella & Guevara-García, 2009).

2.3.9.2.2. TRADITIONAL AND DEVELOPING TECHNIQUES FOR HEAVY METAL REMOVAL

Traditional heavy metal removal methods are precipitation in presence of a chemical additive (composite creation from heavy metal and chemical material), oxidation and reduction reactions, adsorption (using a material to adsorb heavy metal such as activated carbon), coagulation process, ion exchange, reverse osmosis etc. These methods are usually either expensive or results with an ineffective removal rate (Rich & Cherry, 1987). They also have several drawbacks such as; less tolerance for change in pH (Ahluwalia & Goyal, 2007), high energy and reagent need (Ahalya, Ramachandra, & Kanamadi, 2003), suitability for only high or low heavy metal concentrations (Antunes, Luna, Henriques, & Costa, 2003) and high initial investment cost requirement (Oboh, Aluyor, & Audu, 2009). Whereas biological approaches have low cost, and a better yield. In order to overcome disadvantages of traditional methods, biological heavy metal removal methods became popular and showed an increasing trend. Certainly, there are several factors affect efficiency of biological heavy metal removal processes, such as; prospects of the metal in an ion form (ready to give or receive electrons to form a compound), temperature, pH, exposure time, biomass density, in case of microalgae; chosen strain and cultivation parameters (Wang & Chen, 2006). In case of microalgae use for heavy metal removal as a bio-sorption method, thanks to the mechanisms microalgae consists, heavy metal adsorption capacity of microalgae is higher compared to of activated carbon or ion-exchange resin (Doshi, Ray, Kothari, & Gami, 2006). Moreover, microalgal bio-sorption provides less energy and time consumption, faster adsorption, eco-efficiency, reuse – recycle, ease in operation, shorter generation time compared to higher plants, binding around 10% of its biomass, possibility to use for waters with high heavy metal concentrations (Monteiro, Castro, & Malcata, 2012).

There are two ways of application of microalgal bioaccumulation method: using living organisms or dead biomass. Dead (passive) microalgae does not need attention on any cultivation parameters, can be applied to anaerobic and aerobic systems and it can remove heavy metals from solutions with presence of multiple heavy metals. Whereas alive (active) microalgal biomass requires oxygen, carbon dioxide, sunlight and nutrients in order to grow (Figueira, B. Volesky, & Ciminelli, 2000). Strains with heavy metal affinity have a higher

potential in treatment of heavy metal carrying wastewaters (de-Bashan & Bashan, 2010), moreover, in a study, microalgae strains as *Phaeodactylum tricornutum*, *Stigeoclonium tenue*, *Stichococcus bacillaris*, *Chlamydomonas reinhardtii*, *Scenedesmus subspicatus*, *Scenedesmus quadricauda*, *Scenedesmus abundans*, *Chlorella salina*, *Chlorella sorokiniana*, *C. vulgaris*, *Chlorella miniata*, *Chlorococcum* spp., *Cyclotella cryptica*, *Lyngbya taylorii*, *Porphyridium purpureum*, *Spirulina platensis* and *Spirogyra* spp. were defined as strains which have a good potential as bio-sorbent (Brinza, Dring, & Gavrilesco, 2007). A comparative study on cadmium (Cd^{+2}), cobalt (Co), chromium (Cr^{+3} , Cr^{+6}), copper (Cu^{+2}), ferrous (Fe^{+3}), mercury (Hg^{+2}), nickel (Ni^{+2}), lead (Pb^{+2}) and zinc (Zn^{+2}) bioaccumulation potential of different microalgae strains shows that for cadmium removal (Cd^{+2}) live *Chaetoceros Calcitrans* strain pH at 8 yielded the highest in heavy metal uptake with 1055.27 mg heavy metal/g harvested biomass, followed by live *Tetraselmis Chuii* with a yield of 292.6 mg/g, live *Planothidium Lanceolatum* at pH 7 with the yield of 275.51 mg/g and finally with immobilized *Chlorella Sorokiniana* at pH 5 obtained yield was 192 mg/g. Live *Spirulina Platensis* resulted with 44.56 mg/g whereas non-living *Spirulina platensis* resulted with a yield of removal as 98.04 mg Cd/g biomass. Uptake of cobalt was resulted the best by dead *Oscillatoria Angustissima* at a pH of 4 with 15.32 mg/g and followed by dead *Spirogyra Hyalina* and the yield was 12.82 mg/g. Cobalt uptake by dead *Spirulina* spp. was observed and removal yield was 0.01 mg/g. The best results for Cr^{+3} removal was obtained by alive *Spirulina* and yield was between 167-306 mg/g, whereas maximum Cr^{+3} binding potential of dead *Chlorella* was 98 mg/g. Cr^{+6} and Cr^{+7} uptake experiments showed similar results in terms of strains, as alive *Spirulina* has the highest yield in Cr^{+6} biosorption with 333 mg/g and dead *Spirulina* resulted with 143 mg/g of yield followed by dead *Dunaliella* spp. with 58.3 mg/g of yield. Similarly, for Cr^{+7} uptake, live *Spirulina* showed a yield as 226 mg/g, while *Chlorella* spp showed a yield of 104 mg/g. Strain with the greatest copper uptake potential was shown by live *Spirulina* sp and the yield was 576 mg/g and this was followed by alive *Chlorella* spp. which was yielded 220 mg/g and 108 mg/g for two different experiments. Ferrous adsorption potential of dead *Chlorella* was the highest among the other strains and the yield was 24.52 mg/g at a pH of 2. In order to remove mercury, immobilized *Chlamydomonas Reinhardtii* is the best option, according to the reference study as it showed a yield of 106.6 mg/g. *Spirulina* was not efficient enough to use for mercury removal, because

the yield of dead Spirulina was 1.34 mg/g. Biosorption of nickel by alive Spirulina resulted as; 1378 mg of Ni⁺² adsorbed per each gram of harvested Spirulina and for another experiment with live Spirulina, this number was 1108 mg/g followed by dead Chlorella spp. with a yield of 183 mg/g. Immobilized Chlamydomonas Reinhardtii showed a great potential for lead bioaccumulation and yielded with 380.7 mg/g at pH 6. Living Spirulina Platensis had a yield less than Chlamydomonas Reinhardtii and showed a potential of adsorbing 188 mg of lead per one gram of biomass, pH at 7. Dead Chlorella Vulgaris had a yield of 127 mg/g at a pH of 7. Finally, during zinc adsorption experiments, passive Desmodesmus Pleiomorphus gave the highest result and yielded 360.2 mg/g, followed by passive Cyclotella Cryptica which showed a result of 242.9 mg/g. The highest potential of zinc adsorption by Spirulina among the other experiments with Spirulina was much lower than Desmodesmus Pleiomorphus as it yielded 7.36 mg/g with a passive biomass whereas the highest yield obtained from dead Chlorella Vulgaris was 43.41 mg/g (Kumar, Dahms, Won, Lee, & Shin, 2014).

2.3.9.3. RADIOACTIVE MATERIAL BIOACCUMULATION POTENTIAL OF MICROALGAE

Radioactive materials in produced water are the forms which are naturally occurred in formations underground. They are called Naturally Occurred Radioactive Materials (NORM). Due to hydraulic fracturing process, water pushed underground, takes these materials in its body and brings them to the surface as dissolved elements. Barium, ²²⁶Ra and ²²⁸Ra are the forms of NORM which are more commonly found in produced water (Veil, Puder, Elcock, & Jr, 2004).

Intercellular polysaccharides within microalgae bodies play a role as activated carbon in terms of absorption of radionuclides from water. More specifically, as microalgae comprise protein, carbohydrate and lipid, it has a great potential of bioaccumulation of radioactive materials. There are challenges in using living microalgae for this purpose, for instance; α , β and γ rays have a strong effect and can damage DNA structure of living organisms. Therefore, the challenge here is finding a α , β and γ resistant or at least tolerant strain. Table 2.3.7 is a

comparison table of several studies which demonstrates diverse strains and their tolerance to different radioactive isotopes (Sukla, Subudhi, & Pradhan, 2018). *Deinococcus radiodurans* is the most evaluated species in studies in terms of radioactive-resistance however, microorganisms as *Thermococcus gamma-tolerans*, *Halobacterium* sp., *Alternaria alternata*, *Pyrococcus furiosus* and *Chroococcidiopsis* sp. are also highly resistant to radioactive isotopes (Rivasseau, et al., 2016). As there are challenges in this application, there are also advantages for example; due to fast biomass production, surface for radioactive material absorption increases with a high speed (Sukla, Subudhi, & Pradhan, 2018).

TABLE 2.3.7: MICROALGAE STRAINS AND THEIR TOLERANCE TO DIFFERENT RADIOACTIVE MATERIALS (SUKLA, SUBUDHI, & PRADHAN, 2018)

Algae	Tolerance to different radioactive isotopes
<i>Closterium moniliferum</i>	^{90}Sr
<i>Coccomyxa actinabiotis</i>	^{110}Ag , ^{60}Co , ^{58}Co , ^{124}Sb , ^{51}Cr , ^{65}Zn , ^{54}Mn , ^{137}Cs , ^{238}U , ^{14}C
<i>Chara</i> asp., <i>Nitella</i> sp., <i>Pistia</i> sp., <i>Jussia</i> sp., <i>Eichornia</i> sp., <i>Hydrilla</i>	^{226}Ra
<i>Polysiphonia fucoides</i> , <i>Furcellaria lumbricalis</i>	^{51}Cr , ^{54}Mn , ^{57}Co , ^{60}Co , ^{65}Zn , ^{85}Sr , ^{109}Cd , ^{110}Ag , ^{113}Sn , ^{137}Cs , ^{241}Am
<i>Ulva</i> sp., <i>Ecklonia radiata</i> (macroalgae)	^{131}I
<i>Deinococcus radiodurans</i>	Radioactive waste mixed with Hg (II)
<i>Chroococcidiopsis</i>	Artificial ionization radiation from tungsten disk
<i>Jania longifurca</i> , <i>Cystoseira</i> , <i>Sargassum vulgare</i>	^{137}Cs , ^{210}Po , ^{210}Pb
Chlorophyceae	Artificial γ -rays and fast neutrons

In one of the studies about bio-mineralization through microalgae, it has been stated that presence of vacuoles within microalgal body helps capture of Strontium (Sr) and Barium (Ba) and precipitation of these two radioactive nuclides. As cyanobacteria sp. contains vacuoles,

Sr and Ba precipitation through cyanobacteria is an application with a high potential if it is applied through *Closterium Moniliferum* (Krejci, et al., 2011). This claim was also a topic during a meeting of American Chemical Society and cyanobacteria *Closterium Moniliferum* was found as a microorganism with a high potential in accumulating ^{90}Sr and precipitating with it and can be a remediation technique especially for accidents like Fukushima Nuclear Plant in Japan (Sukla, Subudhi, & Pradhan, 2018). As mentioned before, *Spirulina* is a cyanobacteria and contains vacuoles, therefore it has a potential in accumulating radioactive materials as it does it for heavy metals. In one of the studies which has been done on removing radionuclides using microalgae with a duration of six days, four strains were chosen as the most efficient strains among many others with radionuclide absorption potential: *Chlorella vulgaris*, *Chlorella sorokiniana*, *Spirulina platensis*, *Dunariella tertiolecta*. This study demonstrates that *Chlorella sorokiniana* and *Chlorella Vulgaris* are effective strains for Cesium (^{137}Cs) and Strontium (^{90}Sr) removal. Viability of these strains under high and low concentrations of Cs, Sr, and U (Uranium) was evaluated and results show that *Chlorella Vulgaris* is able to survive under high concentrations of these three radionuclides the most compared to other strains, even though its viability decreases by the time, cell concentration catches a steady trend from the second day. *Chlorella sorokiniana* loses its viability at the end of the sixth day in presence of Uranium with high concentration, while it also loses viability in presence of other radionuclides as well but shows less efficiency with high uranium concentration. *Dunariella tertiolecta* shows a sharp decrease in viability from the end of the first day until the second day and decelerates after the second that until the end. *Spirulina platensis* shows this sharp decrease in cell concentration from very beginning and from the second day its cell concentration becomes steady until the sixth day. However, cell concentration is related to growth parameters as well, therefore suitable medium, Radionuclide uptake capacity of each strain was also examined within this study. *Chlorella Vulgaris* was the most efficient strain in uptake of each radionuclides. *Chlorella sorokiniana*, *Spirulina platensis* and *Dunariella tertiolecta* strains were not very effective in radionuclide uptake for each of these isotopes. The only radionuclide which *Chlorella vulgaris* was not efficient in uptake was Uranium with high and low concentration (Lee, et al., 2015).

2.3.9.4. TOTAL AUSTRAL PRODUCED WATER TREATMENT

Total started its operations in Argentina in 1978, it was not only exploration and production operations but also transmission and distribution of gas and oil. Specifically, in Total Austral in Argentina, oil and gas exploration operations are being done, gas supply and marketing is under control of another branch of Total. Onshore field being operated by Total Austral is Ara-Canadon Alfa while offshore fields under control of Total Austral are Hidra, Kaus, Carina, Aries and Vega Pleyade, and 51% of oil and gas production of Total Austral is provided by offshore fields. Gas exploration activities of Total in Argentina covers 27% of country's gas production, 30% of cars in Argentina supply their need from Total. Currently Total Austral employs 1100 contract people and had 225 kbpd operated production capacity in 2017.

Total Austral started to operate in unconventional field first in 2008 in Aguada Pichana with horizontal drilling process in order to produce tight gas, then later initiatives of pilot plants for shale gas production have been undertaken in Vaca Muerta, Aguada Pichana and Rincon La Ceniza – La Escalonada. Finally, large scale shale gas production has been started in Aguada Pichana Este and 20 wells were planned to be drilled per year. As mentioned before, hydraulic fracturing process requires water in order to fill fractures created underground for drilling. Number of stages varies according to each well, and as 20 wells have been planned to be drilled for the first year, produced water becomes an important issue for unconventional oil and gas production. Taking the water discharge standards in Argentina into account, some comparisons have been made between chemical composition of Total Austral produced water discharge standards. According to these comparisons, some of the parameters of Total Austral produced water need to be improved in order to be discharged. Discharging the produced water even though it is treated well, is not an option for oil and gas industry for protecting the brand image. Because, such application may create a public resistance as PW discharge to any water body may cause heavy metal accumulation where PW is planned to be discharged., because water treatment cannot guarantee 100% of heavy metal treatment.

TABLE 2.3.8: GRID AND FRESH WATER DISCHARGE STANDARDS IN ARGENTINA (PULIAFITO, MORANDINI, BRANDI, & MUÑOZ)

PARAMETERS	GRID DISCHARGE WATER STANDARDS	FRESH WATER DISCHARGE STANDARDS	MARINE DISCHARGE STANDARDS
Aluminum	5 mg/L	5 mg/L	5 mg/L
Ammonia			
Hardness (CaCO ₃)			
Ferrous	25 mg/L	10 mg/L	
Sulfates	400 mg/L	1000 mg/L	
Sulfides	1 mg/L	0.5 mg/L	0.5 mg/L
Zinc	10 mg/L	5 mg/L	10 mg/L
Barium	5 mg/L	2mg/L	5 mg/L
Vanadium	5 mg/L	5 mg/L	
Fluorides		5 mg/L	5 mg/L
Manganese	10 mg/L	2 mg/L	
Nitrates	40 mg/L (Total Nitrogen)	15 mg/L (Total Nitrogen)	40 mg/L (Total Nitrogen)
Nitrites			
Silver	0.5 mg/L	0.1 mg/L	0.1 mg/L
Nickel	2 mg/L	2 mg/L	2 mg/L
Selenium	0.5 mg/L	0.1 mg/L	0.2 mg/L
Arsenic	0.1 mg/L	0.1 mg/L	0.5 mg/L
Boron	2 mg/L	2 mg/L	
Cadmium	0.02 mg/L	0.02 mg/L	0.2 mg/L
Cobalt	0.5 mg/L	0.5 mg/L	0.5 mg/L
Chromium (Cr ⁺⁶)	0.2 mg/L	0.5 mg/L	0.5 mg/L
Conductivity			

Copper	1 mg/L	1 mg/L	1 mg/L
Mercury	0.005 mg/L	0.005 mg/L	0.01 mg/L
Cyanide	1 mg/L	0.1 mg/L	0.2 mg/L
Chlorides	350 mg/L	1000 mg/L	
Hydrocarbons	20 mg/L	20 mg/L	20 mg/L
Lead	0.5 mg/L	0.2 mg/L	0.5 mg/L
TSS	220 mg/L	100 mg/L	100 mg/L
pH	5 – 9	5 – 9	6 – 9
Oil and grease	100 mg/L	0.3 mg/L	3 mg/L

2.3.9.4.1. DESIGN OF A PILOT PLANT FOR PRODUCED WATER TREATMENT IN TOTAL AUSTRAL

Produced water treatment by microalgae growing may need a pretreatment regarding to the stage before microalgae pond in order to treat materials other than heavy metals and NORM. For instance; big solid materials, smaller solid materials, particulate materials, settleable matters, oil-grease, salinity, H₂S, total suspended solids (TSS) etc. In order to obtain a clean water and treat each of these matters, pilot plant must consist of pretreatment which is composed of physical and chemical treatment and advanced treatment which comprises biological treatment.

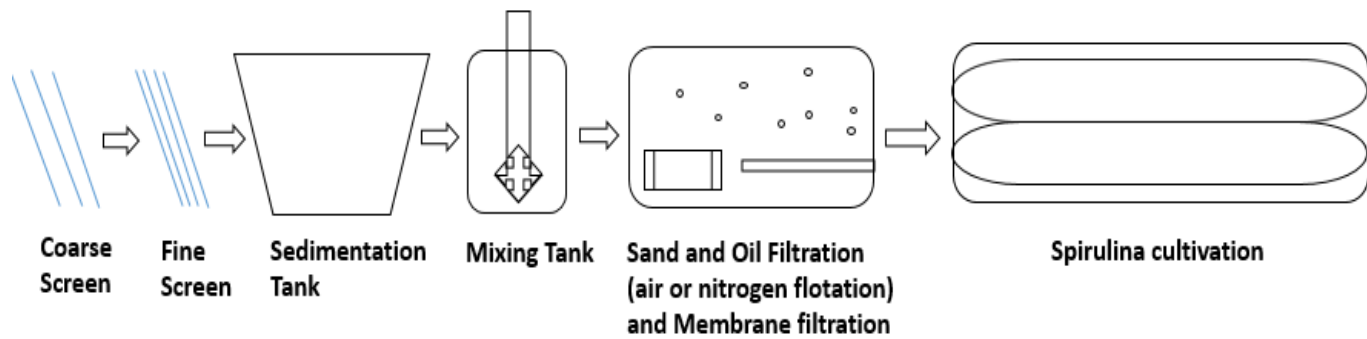


FIGURE 2.3.11: DEVELOPED PILOT PLANT DESIGN FOR TOTAL AUSTRAL PRODUCED WATER TREATMENT

Physical treatment begins with coarse screen and fine screen which are chosen in case of PW containing solid matters with different sizes, while sedimentation tank is used for removing settleable solids by gravity. Shape of this tank eases settling process, settled matters can be removed from the bottom of sedimentation tank easily. Mixing tank is recommended in order to remove H_2S , as turbulence is required for H_2S removal. Sand filtration is used for sand removal while oil filtration is used for oil and grease removal. Principle of oil filtration is aeration of tank from bottom by a blower, creation of air bubbles which then will adsorb oil and grease and rise up until surface, removal of oil from surface by sweeping it out. In order to remove salinity and materials in small size a membrane filtration technology is integrated in aeration tank. There are different membranes according to pore size such as; particle filtration (PF), microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) from bigger pore size to smaller respectively. Figure 2.12 shows pore sizes for these filtration systems and which materials can be filtered with these processes.

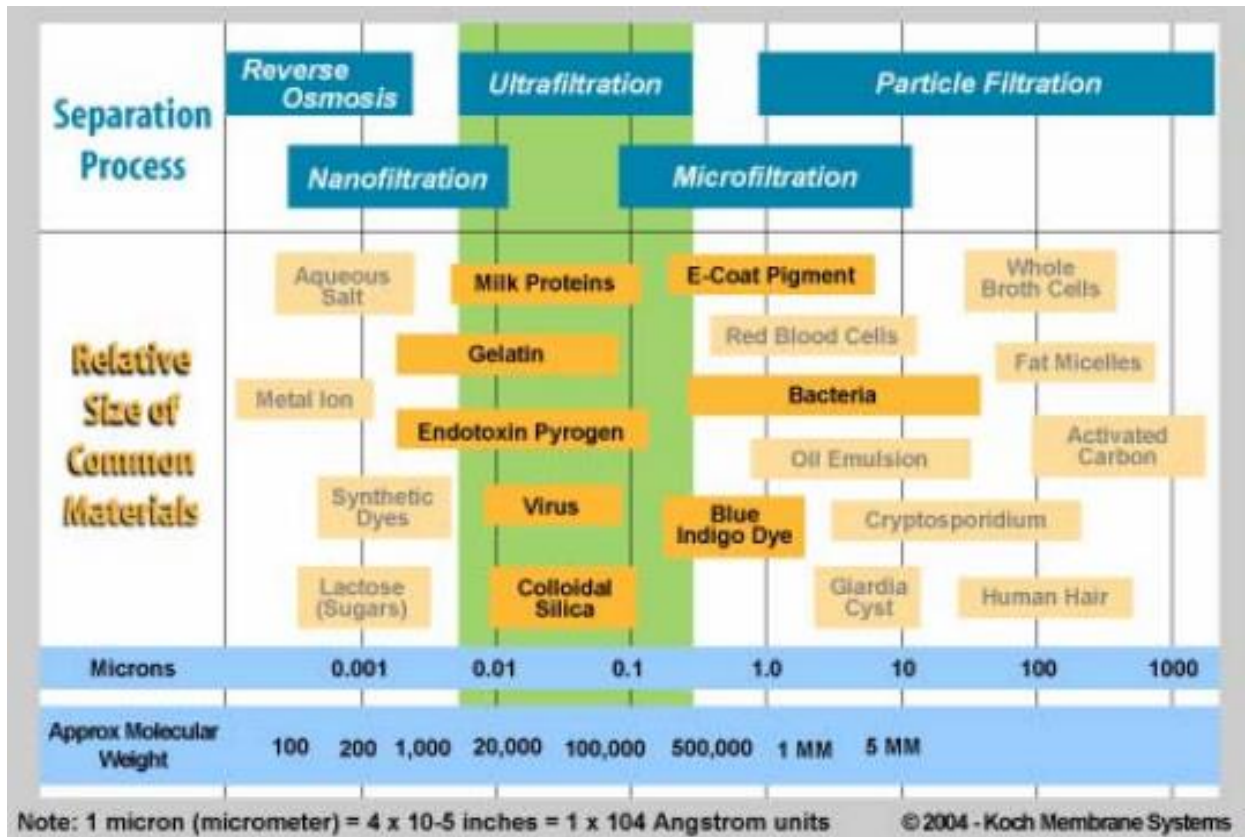


FIGURE 2.3.12: SIZE OF MATERIALS THAT ARE REMOVED BY VARIOUS SEPARATION PROCESSES (SDWF, 2017)

Following oil and sand filtration, spirulina cultivation pond must be built for heavy metals, NORM, nitrogen, phosphorus, potassium, sulfides and carbon removal.

2.3.9.4.2. ECONOMIC ANALYSIS REGARDING PILOT PRODUCED WATER TREATMENT PLANT

Assuming that PW treatment plant will be placed in the plant where oil-gas is separated from water, which does not create additional land cost. While designing a wastewater treatment plant, flow rate of wastewater must be known in order to design each process with an accurate size. Produced water flow back amounts almost 30% of the water injected and this percentage

and amount of water injected vary depending on each plant. Therefore estimating produced water flow rate is not a right approach. Because of this reason, taking into account that volume of produced water will not be as much as volume of wastewater in a municipal or industrial wastewater treatment plant, produced water treatment plant is designed with an assumption of having a small amount of produced water, equipment with small volume are chosen according to these assumptions.

The first step of designed wastewater treatment plant is coarse bar screen and Mechanical Trash Rack Raked Bar Coarse Screen is chosen for this step which costs around €500 with three years of use guarantee. Depending on wastewater flux, number of coarse screen can be increased.



FIGURE 2.3.13: MECHANICAL TRASH RACK RAKED BAR COARSE SCREEN (ALIBABA, MECHANICAL TRASH RACK RAKED BAR COARSE SCREEN, 2018)

Mechanical Trash Rack Raked Bar Coarse Screen consists of pedestal, tines in shape of plow, rake plate, lift chain and motor reducer. Working principle of this equipment is the following: Rake plate is assembled into lift chain and works clockwise which makes lift chain move and take residue from lower part to upper part. When residue reaches to the top of bar screen, it gets discharged by the effect of gravity, rake tines move down under the equipment, next

round starts and equipment works continuously (Alibaba, Mechanical Trash Rack Raked Bar Coarse Screen, 2018). Even though there are bigger sized screens with longer lifetime, for such small application smaller screens are convenient enough.

Sedimentation tank which is made of FRP/SST material costs between €10.000 - €15.000 with a height of 2.7 m and a diameter of 1.2 m and water flux which passes through sedimentation tank is between 10 – 15 m³ / h. This tank is produced for wastewater treatment operations specifically for pretreatment, while it has a three years of use guarantee (Alibaba, Water treatment sedimental tank for pre filtration, 2018).



FIGURE 2.3.14: WATER TREATMENT SEDIMENTAL TANK FOR PRE-FILTRATION (ALIBABA, WATER TREATMENT SEDIMENTAL TANK FOR PRE FILTRATION, 2018)

Metal mixing tank can be purchased with a very low price as the most important part of this step is mixer itself. HP Electric Direct Drive Clamp Mount Mixer is chosen for this process

and Figure 2.3.15 demonstrates whole equipment and its impeller. As shown in the figure, mixer works with a high power electric motor and costs €500 (www.mixerdirect.com, 2018).



FIGURE 2.3.15: HP ELECTRIC DIRECT DRIVE CLAMP MOUNT MIXER (WWW.MIXERDIRECT.COM, 2018)

A metal aeration biological tank can be purchased with a low price as well and major cost of this step is due to blower, aeration mechanism, membrane-filtration and oil scrapers.

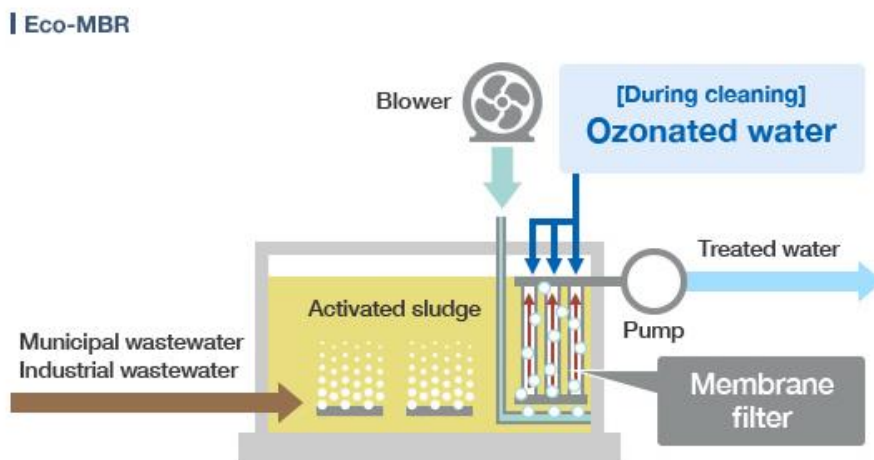


FIGURE 2.3.16: TYPICAL MEMBRANE BIOREACTOR SYSTEM FOR WASTEWATER TREATMENT (MITSUBISHI ELECTRIC, 2018)

A study on cost of wastewater treatment demonstrates that using membrane for filtration costs between \$0.533-0.682\$ per each m^3 of water treated by considering the daily wastewater flow rate is $3785.4 \text{ m}^3/\text{day}$. Total capital cost of this investment was calculated as between \$1.419.000-\$2.330.000 while annual operating and maintenance cost was calculated between \$218.000-\$302.000. The greatest contribution to operating and maintenance cost was energy costs by 34% followed by membrane replacement (28%) and equipment repair and replacement (19%). Another study with a lower daily flow rate which is $1.89 \text{ m}^3/\text{day}$ shows that for such small amount of water flow, capital investment cost of needed MBR system (membrane bioreactor) is \$54.000 and operation and maintenance cost is \$600 per year (Guo, Englehardt, & Wu, 2014). Figure 2.3.16 describes working principle of an MBR system.

The last step of produced water treatment plant is integration of Spirulina cultivation pond. As mentioned earlier, capital investment cost of four not heated ponds with a surface area of 1000 m^2 was between 150.000-250.000 euros. Produced water treatment plant does not require such large application, therefore capital investment would cost around 20.000 euros. Considering variable costs, one kilogram of wet Spirulina production costs around 6-7 euros according to CEO of Algreen start-up company (Algreen, 2018).

Overall capital investment cost for such plant would be around 65.000 euros, considering equipment above, pumps and tanks. Variable costs are electricity, O&M cost, membrane replacement, chemicals for membrane cleaning and chemical material in order to lessen foaming on top of the biological treatment tank. According to flow rate of produced water, variable costs change in Spirulina cultivation pond, as it depends on the produced biomass concentration.

2.3.10. SOIL AMENDMENT

Food demand increases in direct proportion to increase in population, therefore food supply security has become an important concern and fertilizers have a key role in this concept. Fertilizers are natural or processed materials in solid or liquid form which are produced to

apply to soil in order to improve plant health and growth. These products provide additional nutrients for soil, improve chemical and physical quality of soil and so plant growth and quality. There are different types of fertilizers and vary accordingly ingredients it consists of. Chemical fertilizers, organic fertilizers and bio-fertilizers are the major types on the market (Gowariker, Krishnamurthy, Gowariker, Dhanorkar, & Paranjape, 2009).

Chemical fertilizers, also called synthetic or inorganic fertilizers, contain ammonium nitrate, ammonium phosphate, potassium sulfate and produced based on by-products of petroleum industry and majority of chemical fertilizers do not include micronutrients which are needed for improved health and quality of plants. Aggressive use of chemical fertilizers may damage the plant root and soil, therefore following the instructions while using them is essential (www.maximumyield.com, 2018).

Organic fertilizers are produced from natural materials such as plant-based sources, animal manures or by-products of agriculture industry. Nutrients in organic fertilizers depend on which material is used for fertilizer production. However, in general, amount of phosphorus and nitrogen is less compared to synthetic fertilizers. As organic fertilizers contains high level of humid, transportation of this kind of fertilizers have high cost therefore, producing and using them locally is a more cost-effective approach (Davis, 2015).

Bio-fertilizers are natural fertilizers and are produced from living bacteria, fungi or algae solely or combination of these three living microbial inoculants. They boost the supply of primary nutrients to plants by fixing nitrogen and solubilizing phosphorus. Major microorganisms which are being used to produce bio-fertilizer are Rhizobium (soil bacteria), Azotobacter (arable soil bacteria), Azospirillum, Azolla (water fern), Cyanobacteria etc. (Kumar, Kumawat, & Sahu, 2017).

Besides fixing inorganic nitrogen (N_2) by converting it into organic nitrogen which increases health and growth of plants and quality and fertility of soil when applied as fertilizer (Wang, Peng, & Huang, 2015), blue green microalgae has a good potential to be used as bio-fertilizer thanks to its anti-fungi activity (Prakash & Nikhil, 2014). Compatibility of microalgal bio-fertilizers for paddy fields have been studied by several researchers and resulted with high yields of rice fields. One of the studies on microalgal bio-fertilizer use on rice plants shows

almost 20% of increase in yield at the end of four years, while another study proves 17% of yield increase (Wang, Peng, & Huang, 2015). A study on potential of Spirulina when used as fertilizer showed very promising results. Spirulina was cultivated with batch cultivation method in glass bottles with a volume of 2 liters filled with fish water, at 28-30°C temperature. Four pots were filled up with 500 g of soil; first pot was control group and does not consist any additional fertilizer material, 5 g of Spirulina was added to second pot, 0,3 g of chemical fertilizer was added to third pot each week and finally last pot was containing 5 g of Spirulina and 0,3 g of chemical fertilizer per week was added. Pak Choy, Arugula and Bayam Red plants were grown in these four pots in order to see the efficiency of each group and compare them. According to results of this experiment, for Bayam Red plant in the soil enriched with combination of Spirulina and chemical fertilizer (S+CF) showed the highest results in number of leaves, plant height and dry weight while in soil with addition of Spirulina alone (S) showed the greatest efficiency in root length. Chlorophyll content was the highest with chemical fertilizer (CF) which was slightly less in S group. Arugula plant grown in S group gave the highest results in plant height compared to other groups, while CF group had the best efficiency in number of leaves, root length, chlorophyll content, fresh weight and dry weight which were all slightly higher than S group. Lastly for Pak Choy plant, S group showed the best results in root length (equal to the result of S+CF group) and chlorophyll content, while in comparison of number of leaves CF group showed the highest efficiency, followed by S group (Wuang, Khin, Chua, & Luo, 2016).

Another study where *Vigna Radiata* plant was presoaked into water enriched with different concentrations of Spirulina and also into pure water as control group, changes in the leaf area, growth rate, maximum dry weight and yield were observed. Among 1 g/L, 3 g/L, 5 g/L, 7 g/L and 9 g/L concentrations of Spirulina where the seeds of *Vigna Radiata* were soaked, the highest results in leaf area, growth rate, maximum dry weight and yield were obtained with the seeds germinated after being soaked into solution where 7 g/L of Spirulina was used. Presoaking in Spirulina with any concentration showed better results compared to control group (Aung, 2011).

In conclusion, Spirulina can be a great opportunity as bio-fertilizer and increase the plant health and soil quality and alternative to chemical fertilizers as well.

CHAPTER 3: CONCLUSION AND RECOMMENDATIONS

3.1. CONCLUSION

The aim of this research was to do a literature review on carbon dioxide bio-sequestration potential of Spirulina microalgae, as greenhouse gas emissions became a significant concern due to industrialization and anthropogenic activities. Moreover, Spirulina has many potential applications once it is grown, therefore pigment extraction techniques and possible areas of use of these pigments was another implication which had been researched through this study. As produced water through hydraulic fracturing process is an issue to be considered carefully for oil and gas production produced water treatment potential of Spirulina was researched thanks to its ability of adsorbing heavy metals and absorbing radioactive materials. Finally, soil amendment possibility of Spirulina by producing bio-fertilizer was investigated as it has high nutritional value and ability to fix nitrogen and solubilize phosphorus which are needed elements for growth of plants. Comparison of different studies was made to evaluate potential of each application, economic and financial assessment and cost structure of possible investments were mapped. Furthermore, interviews with a start-up company called Algreen and a research facility called Algaeparc were carried out in order to understand the process of growing microalgae clearly by seeing the procedure on site.

The research questions of this study are shown below:

RQ1: What is the potential of Spirulina microalgae to capture carbon dioxide, under which conditions it should be grown in order to show the best yield in terms of carbon dioxide bio-sequestration?

RQ2: What is the optimum method of pigment extraction from Spirulina and under which conditions does the pigment must be extracted for the best yield?

RQ3: What is the possibility of produced water treatment by growing Spirulina in this water? What are the needed additional treatment processes and equipment to obtain better efficiency?

RQ4: Is Spirulina a good material for bio-fertilizer production? What is the efficiency of plant growth when it is grown by addition of bio-fertilizer produced from Spirulina?

RQ5: What is the acceptability of these applications by stakeholders?

Carbon-dioxide bio-sequestration potential of Spirulina was calculated as 50 tons/year/ha when the productivity of ponds of Algreen was taken into account. Considering that this company is new in the market and using smaller ponds compared to incumbents of this market, productivity level of Spirulina ponds may be even higher in case of building bigger ponds hence, captured carbon dioxide would be greater than 50 tons/year/ha.

Pigment extraction is possible with several methods solely or even with combination of different methods. According to the results of this research for the best extraction yield, freezing and thawing method must be applied to wet biomass, as drying the biomass results with 50% phycocyanin pigment loss.

Microalgae in general has ability of bio-accumulating many elements such as; heavy metals and radioactive materials. Results of many comparison studies and experiments on different microalgae strains which are accumulated with diverse heavy metals and radioactive materials showed that for specific heavy metals, Spirulina has a great accumulation potential and cyanobacteria has a good potential in radioactive material absorption.

Finally, for a good quality in soil, it must contain some nutrients such as; nitrogen and phosphorus. Experiments demonstrate that when Spirulina is added to soil as fertilizer, it shows similar results compared to chemical fertilizers in terms of plant height, number of leaves and root length. There are already several companies who produce fertilizer from Spirulina, and so this market is expected to be expanded in the future.

3.2. RECOMMENDATIONS

3.2.1. CARBON DIOXIDE BIO-SEQUESTRATION AND SOIL AMENDMENT

The validity of carbon dioxide capture by microalgae is still under evaluation by governments as it is not considered as a solution by Kyoto Protocol. However, even though the aim of this study was evaluating the acceptability of carbon dioxide bio-sequestration through Spirulina by stakeholders, improving the environmental performance of oil and gas production operations is considered as major objective despite the lack of validation of these methods by governments to date. Considering this; recommendations of this research are conducted by taking carbon capturing potential, energy efficiency, surface efficiency, water efficiency and soil improvement into account. Accordingly, the most efficient method was chosen by combining two objectives: Carbon dioxide bio-sequestration and improving soil quality.

What is recommended here is that growing Spirulina in an area close to the field where afforestation project may be implemented in Argentina, producing bio-fertilizer from harvested Spirulina and using it for planting trees which will be planted for carbon dioxide capture. As captured carbon dioxide by Spirulina would be released back to the atmosphere in case of consuming it as a food supplement, this approach would create a closed loop by trapping carbon, which Spirulina contains, into soil and growing trees with this improved soil and therefore capturing carbon dioxide again by the trees.

In order to be more energy efficient, for harvesting Spirulina, use of settling ponds and in case of using dry Spirulina, sun drying is recommended. In order to increase the velocity of algal settlement, flocculant may be used however, effects of this matter on algal growth and quality of harvested algae must be studied in detail. Bio-fertilizer production process contains a digesting step, which also can be completed in an energy efficient way by using landfill digester method. Digesting microalgae results with methane and pure carbon dioxide production. Methane can be used or sold as biogas whereas, pure carbon dioxide can be recycled back to Spirulina growing pond or sold as it has a great monetary value thanks to its purity.

3.2.2. PRODUCED WATER TREATMENT

Microalgae has a great potential in bio-accumulation of elements, as mentioned earlier. Therefore, Spirulina can be a great source for treatment of produced water thanks to its adsorption and absorption potential. Considering this, a produced water treatment plant was designed and shown in this report. This plant can be a great application for wastewater produced by the operations in Total Austral in Argentina or other temperate climates. However, the question here is, what should be done with accumulated Spirulina by heavy metals and radioactive materials? As it contains hazardous materials, it cannot be used as fertilizer or for human consumption. Throwing this waste away would not be acceptable either. Therefore, a potential recommended solution is producing biofuel from this heavy metal and NORM accumulated microalgae. As microalgae has a high lipid content it is considered as a good potential raw material for biofuel production. However, technical feasibility of this application must be researched in detail.

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