OPOLE UNIVERSITY OF TECHNOLOGY

DEGREE IN CHEMICAL ENGINEERING

STUDY OF THE SEDIMENTATION OF BIOLOGICAL SUSPENSIONS

FINAL PROJECT

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ABSTRACT

The final project includes description and characteristic of sedimentation process of biological suspensions (algae suspensions) with different initial concentrations. The aim of the work is described sedimentation of biological suspensions, based on experimental research made by other scientists.

This project include description how stage of the growth influence on sedimentation process. The efficiency of the separation cells from liquid as indicator of sedimentation efficiency will be described and compared with the systems where different types of flocculants were used. Based on the above results, a recognition is performed with the possibility of a continuous sedimentation process in a sedimentator with a given design.

Recommendations on the construction of equipment and the use of the appropriate type of flocculants will be presented to improve the efficiency of separating process of suspended solids. Finally, an economic study and comparison of the cost of selected separation methods analysed above will be presented

Index

1. Introduction

1.1. Wastewater treatment

Water is an essential resource for life. All living beings require it, and over time it is becoming an increasingly limited resource. As societies generate wastewater from the activities of population settlements, they are collected by different sewage systems and are ultimately discharged into receiving aquatic bodies.

Increased pollution load to surface water after the introduction of sewers led to a severe decrease in water quality in many surface waters. In 1860 wastewater treatment began with flow fields near the City of London. In the same period, the septic tank was developed to remove solid waste from wastewater. This does not solve the problem, as the effluent of the septic tank was not yet addressed. Frankland (1868) developed a sand and soil filter to treat the effluent of septic tanks, mainly to reduce the areas of soil needed for wastewater disposal.

Currently, wastewater originates in homes, institutions, offices, and industries, and can be diluted with rainwater, groundwater, and surface water. Failure to treat wastewater prior to discharge into the receiving bodies results in harmful effects on human health and the environment, such as odor generation, depletion of dissolved oxygen and release of nutrients, toxic pollutants, and pathogens **[1]**. Therefore, the need arises to eliminate the pollutants causing the contamination present in the wastewater by applying adequate treatment to improve the level of sanitation of a community.

Wastewater treatment is a set of physical operations, chemical and biological processes, which form unit operations and processes respectively [**2]** that aim to eliminate contaminants present in wastewater.

The goal is to accelerate the processes that are generated in the self-depuration of a body of water in nature, thus producing clean or reusable water in the environment. This type of treatment has an essential process for the integral sanitation of a human settlement to avoid public health impacts **[3].** In this way, the quality of the receiving aquatic bodies and their ecosystem is protected, reducing a negative impact on the environment.

Conventional wastewater treatment consists of three stages or levels: primary treatment, secondary treatment, and tertiary treatment. These treatments are classified according to the size of the particles you want to remove. In the primary treatment, larger suspended materials are removed, then secondary treatment removes macromolecules and colloids, eliminating sedimented material and oxidizing organic matter. Finally, the obtained effluent is discharged

into the receiving body, but this effluent contains high nutrient content (phosphorus and nitrogen mainly), which can cause eutrophication to the receiving body. Therefore, tertiary processing should be applied to perform the removal of dissolved materials.

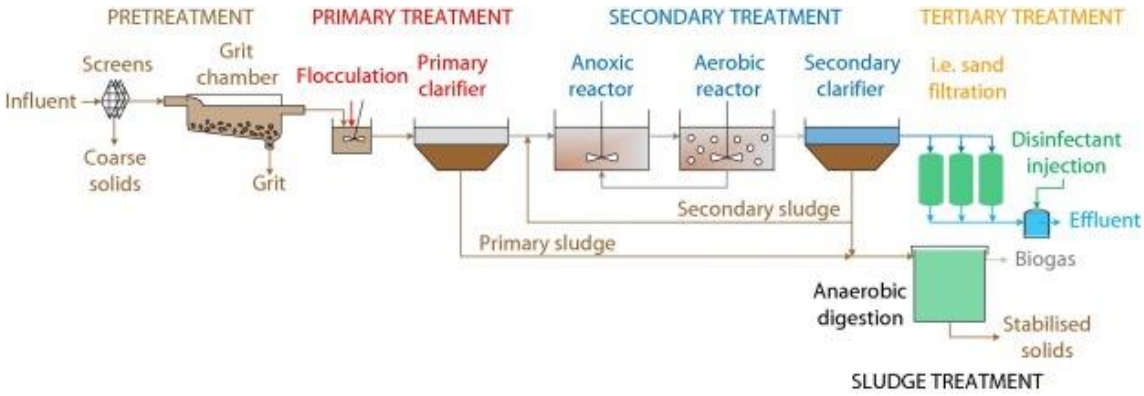


Illustration 1. Scheme of a traditional wastewater treatment plant

As wastewater treatment levels increase, the quality of treated water improves due to the different levels applied during treatment, these are: preliminary, primary, secondary, and tertiary.

- *Preliminary processing or* preparation: the process of decreasing thicker solids such as sands, gravel, plastics, and other floating materials is carried out by means of a sieve or overflowing grille to retain the solids. The grilles can be thick (> 50mm), media (15-50mm) or thin (8-12mm). Then, the particles that could not be filtered in the previous step, is followed by a sieving process. Filtered water passes through a degreaser-degreaser, where particles of smaller size, but with densities larger than water, collide with a sloping wall due to the movement of water, and are deposited at the bottom. Fats are floated and removed by surface scrapers. The purpose is to protect the facilities and operation of these to reduce undesirable conditions related to the aesthetic appearance of plants
- *Primary treatment*– the separation of suspended solids and a small percentage of organic matter occurs through physical operations such as sieving and sedimentation by a decanter. During the process they are distinguished in two phases: sedimentable solids at the bottom and floating solids on the surface, with the help of coagulation-flocculation to facilitate sedimentation of them.

- *Secondary* treatment: biodegradable organic matter and suspended solids are eliminated by chemical and biological processes. This reduces the concentration of nitrogenous compounds and uniformizes organic loads for subsequent treatments.
- *Tertiary* treatment: nutrients such as nitrogen and phosphorus are altered by a combination of unit processes and operations. This treatment is not installed in the vast majority of EDREs.

Wastewater treatment is a complete process at a high cost to meet the standards. The need for space and funds limits the implementation of advanced biotechnologies. Increasingly stringent environmental regulations require improved treatment facilities and alternative methods of waste flow management.

The new microalgae biotechnologies offer an alternative method for wastewater treatment. In this sense, microalgae are used in purification processes to reduce the costs of purification and improve the quality of discharge of the waters. These microalgae produce a large amount of oxygen, which consumes carbon dioxide and mineral nutrients, reaching oxygen concentrations up to 20 mg $^{L-1}$ in cultivation. Therefore, the use of microalgae and bacteria may be suitable for wastewater treatment. Why microalgae would consume carbon dioxide and mineral compounds produced by bacteria by degrading organic matter, and thus provide the oxygen needed for bacteria that perform degradation [**4-5].** The advantage of the use of microalgae occurs the decrease in volatilization of compounds such as methane and ammonia, due to decreased aeration during the purification process.

1.2. Microalgae

Microalgae are used as an alternative system for treating wastewater. From a biotechnological point of view, the definition of microalgae refers to single-celled microorganisms containing chlorophyll and other similar photosynthetic pigments, capable of performing oxygenic photosynthesis. In this context, cyanobacteria or blue-green algae, prokaryotes have traditionally been considered within the microalgae group. Microalgae include organisms with two cell types: cyanobacteria, which have prokaryote cell structures, and the remaining microalgae with eukaryotic cell structure; however, the term has no taxonomic value **[1-5].**

Plants can harness solar energy in the form of energy through the photosynthesis process so that it is then stored in its biological structures. In the photosynthesis process, large amounts of CO2 are captured atmosphere with the consequent production of oxygen.

However, microalgae, have a different structure, have a greater photosynthetic efficiency, around 10-50 times higher than land plants, so they help significantly reduce CO2 concentration of the atmosphere **[5].** They can also colonize various habitats (sea, sweet and wastewater) and the growth factors of their populations (lighting, temperature, salinity, aeration, pH, etc.) have a wide range of variability. It should also be noted that, depending on environmental conditions and the crop medium used, the composition of microalgae (lipid content, carbohydrates, and proteins) can vary and can therefore be manipulated during growing process.

Microalgae are characterized because they are essential in primary production within the trophic chain, which at the same time is the first organic matter former. The structure of the microalgae has an average size of between 5 and 50 m, which makes them easily digestible by many organisms whose main food source is phytoplankton, possess chlorophyll-to which gives them a greenish appearance similar to that of plants, so they need light to develop and create organic matter. In addition, microalgae are the source of a good number of compounds used in different industries.

Microalgae can be classified according to cell structure, pigmentation, life cycle, chemical nature of storage compounds and cell wall type. Considering these parameters are grouped as follows **[5]**:

- **Prokaryotes**: prokaryotic microalgae or cyanobacteria are characterized by lack of organelles limited by a membrane and have the smallest ribosomes. These are planktonic organisms without their own motility, with different morphology and usually occur in extreme media.

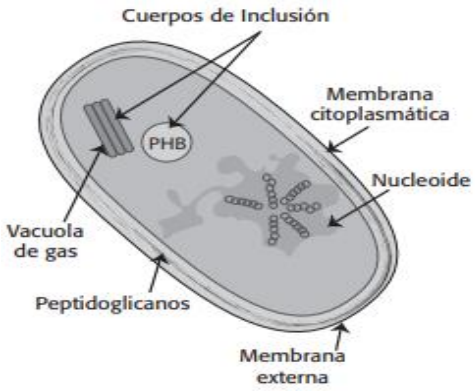


Illustration 2. Prokaryote cell

- **Eukaryotes:** this class of microalgae have a cell wall composed of a microfibrillar layer of cellulose that may be surrounded by another amorphous layer. Within this

classification stand out chlorophylls or green algae whose color is due to chlorophyll, carotenoids and xanthophylls.

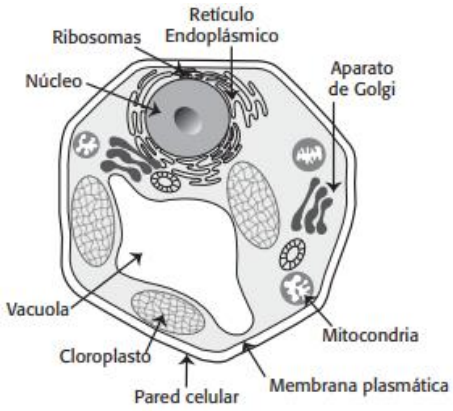


Illustration 3. Eukaryotic cell

However, microalgae are a very diverse group of eukaryotic and prokaryotic single-celled photosynthetic organisms adapted to different habitats such as seawater, sweet, waste and bracken, can be classified according to their diet. Within this classification we have species that usually obtain energy from sunlight except for a few exceptions that can grow using organic matter as an energy or carbon source. Therefore, the following differentiation must be made as to its mode of feeding, distinguishing between **[6]:**

- **Photootophorahs:** corresponds to the microalgae that get all the elements they need to grow carbon from inorganic compounds and energy from light.
- **Photoheterotrophs:** phototrophs microalgae obtain energy from the sun and use organic compounds as a source of carbon.
- **Mixotrophs**: are called mixotrophic microalgae when they have a growth in both autotrophic and heterotrophic conditions, that is, the energy source would be both light and organic matter and carbon is obtained through organic and CO2 compounds. For example, microalgae Spirulina *platensis* or *Chlamydomonas Reinhardtian* would show growth under these conditions.
- **Heterotrophs:** This type of microalgae use organic compounds to obtain both energy and carbon source, allowing them to develop in the absence of light. An example of this type of behavior would be the species Chlorella *prototheorids.*

From this classification you can find various kinds of microalgae in nature. There are microalgae of fresh water, which depend on biotic and abiotic factors with cyclic exchanges that do not close completely. They can be found in different natural habitats, developing in various forms, according to the territory: some of them, for example, developing in aquatic

ecosystems, have physicochemical characteristics, such as *Coccomonas sp. hydrurus sp.,* which inhabit waters that have high calcium content, while in the dystrophic waters and with low pH values, the most developing microalgae are desmidiáceas. In other types of nutrient-rich waters, Volvocales*, Chlorococcals* and *Euglenophilics* predominate.

There is a distribution pattern that is predominant for lotic aquatic ecosystems and another for lyantic aquatic ecosystems. The aquatic ecosystems have the characteristic of these in constant movement, so it can generate a constant oxygenation, in addition to continuously renewing nutrients, so it is common the presence of *Fragilaria*sp., *Amphora sp., Cocconeis sp., Spirogyra sp*., *Tribonema sp.,*amongothers. On the other hand, in lotic waters with mineral loads can be found cyanophytes and diatoms, while in the acidic environments predominate chlamydomonas *acidophila, Euglena mutabilis*, *Stichococ minorcus* and *Microspora tumidula* and many more. Finally, in rivers and streams the phytoplannic flora is quite diverse, with predominance of chlorophylls or cyanophytes, diatoms and rhodophyric algae: *Ulotrix sp., Spirogyra sp., Achnantes sp., Oedogonium sp., Tribonema sp., Cymbella sp., Euglena sp., Scenedesmus sp., Chlorella sp., Navicula sp., Nitzchia sp., Zygnema sp.* , among others **[6]**.

Microalgae can survive in isolated conditions or in colonies in the form of cellular aggregates. Approximately thirty thousand species have been found, which can have spherical, elliptical, cylindrical, or spiral forms, all of which actively contribute to the oxygen balance on planet Earth representing almost 50% of the world's photosynthesis. Similarly, microalgae are the basis of the global food chain, with about 70% of total organic matter production. Microalgae can therefore grow and develop in almost all environments, although most belong to marine or freshwater systems, and in woody or wet areas. Your ability to adapt depends. In terms of the growth and development of microalgae, five main phases can be demonstrated:

Table 1. Microalgae development

PHASE	DURATION	FEATURES
Induction	1-3 days	It begins the absorption of nutrients by the cells and the process of adaptation to the environment in which they have developed. During this state, cells do not tend to divide, because there are no appropriate conditions for this process, as adjustments are still needed in terms of the biochemical conditions of the crops.
Exponential	4 days	It starts when the cells have already adapted, and multiplication has occurred. During the exponential phase, cell division is much faster than in the rest of the phases.

Stationary	--------	The algal population becomes constant, without increasing. Its duration tends to be too short to be noticeable.
Relative growth decline	1-2 days	During this phase cell division decreases, unfavorable factors in crops predominate. In addition, the lack of nutrients, pH mismatches, decreased solar irradiations, among others.
Death	--------	The increase in the number of bacteria, fungi, and foams present in the crop. Conditions get worse for the development of microalgae. The death of the crop therefore occurs.

These stages of growth and development of microalgae are linked to the existence of artificial crops, which seek to assimilate as closely as possible to the natural environment but generating the conditions for a controlled and functional crop.

The factors of the environment that influence during the growth of microalgae, which vary depending on the desired composition, need to meet conditions guaranteed by the following parameters:

- **Lighting**: fundamental for the photosynthesis of microalgae, so it cannot be affected by any obstacle that prevents its presence. During the development of microalgae culture systems, it is therefore important to know the absorption spectrum of the species with which it works so that we can select the most appropriate light source. This spectrum depends on the majority pigments present in the body. Among the artificial light typologies, the LED system offers a more efficient and economical option, emitting more than 98% of its light at absorbable and usable wavelengths for growth.
- **Nutrients**: The main nutrients that microalgae take from the environment for their development are:
  - **Carbon:** Auto-engine microalgae use CO2 as a power supply, useful in the atmosphere or from industrial activity. They can tolerate high concentrations of CO2. Following inorganic compounds, these Na2CO3 and NaHCO3 organisms can also be fed as a source of bicarbonate ions.
  - **Phosphorus:** This compound, also essential for the development of microalgae, although in smaller amounts than the previous ones, is taken from the medium in the form of orthophosphates, the concentration of the protonated forms depends on the pH of the medium. Factors such as

at excessively high or low pH, or the absence of ions such as potassium, sodium, or magnesium, slow down the intake of phosphates.

- **Nitrogen:** This is another of the main nutrients for algae growth. These can take nitrogen from the medium usually in the form of urea, nitrate, nitrite, ammonium, nitrogen gas and nitrogen oxides. It has not yet been shown to be the most energy-beneficial form of nitrogen, but if ammonium is opted, it should be noted that algal biomass growth can be limited to high concentrations as the compound inhibits parts of the photosynthetic process and competes with water in oxidation reactions for oxygen production. Depending on the selected species, maximum ammonium concentrations range from 200mg/L for Spirulina to 400mg/L for *Chorella sorokiniana.*

- **Temperature:** Cell phone reproduction is much higher when found at favorable and stable temperatures. Microalgae, almost all their species, grow in temperatures ranging from 10 to 35ºC, although the optimal range is between 16 and 24ºC.

- **pH:** has direct influence on the chemical form of nutrients in dissolution. In the process, the fixation of CO2 and pH of the medium increases due to the accumulation of hydroxyl groups, and due increase can cause the elimination of part of nitrogen in the form of ammonia into the atmosphere and the phosphorus available by precipitation of the same.
- **Salinity:** Most species of marine microalgae breed in salinities close to 30% and include all salts that are dissolved in the middle, in addition to carbonates and bicarbonates.
- **Aeration: allows nutrients found in the medium to** be more diffuse, in addition to preserving the suspension of microalgae and supplying carbon dioxide (CO2), which serves as a source for photosynthesis and pH stability in crops **[7]**.

- **Agitation:** a system is needed that keeps the level of agitation in the middle in an appropriate range to prevent sedimentation and accumulation of algae, homogenizes pH and distribution of nutrients and light. The turbulent flow is typical for moderate density crops, but sometimes strong agitation should be damage to algae that are sensitive to hydrodynamic stress.

1. Objectives

The overall objective of this end-of-grade work is the study of suspended biological sedimentation using a microalgae culture, based on experimental research conducted by other scientists.

Based on the results of the study, an improvement in the design of a sedimentator will be presented with recommendations on the construction of equipment and the use of the appropriate type of flocculants to improve the efficiency of the suspension solids separation process.

Finally, an economic study and a comparison of the cost of the selected separation methods will be presented.

2. Literature review

Over the past few decades, the use of microalgae has gained interest. These have been used as a biological model in fundamental research for their metabolic flexibility and physiological characteristics. The development of microalgal biotechnology has allowed not only the commercial development of crops of new species, but the extension of the list of applications of these microorganisms and the substances of chemical, pharmaceutical, and industrial interest that are extracted from them.

In 1980, Dutch microbiologist Beijerick established pure crops of a freshwater microalgae: *Chlorella vulgaris.* A little later, Otto Warburg (1919) obtained in the laboratory dense cultures of Chlorella *and* introduced the idea of using these crops as a working tool in the study of photosynthesis. Microalgae cultures have been studied by numerous researchers, observing that, under suitable growing conditions, especially at saturation intensity, they are much more productive than the upper plants or photoautotrophic cells isolated from them **[1-2]**.

The concept of mass microalgae production was first carried out in Germany during World War II, aimed at lipid production, for which the microalgae *Chlorella pyrenoidosa* and Nitzchia *palea* were used **[11].**

After World War II microalgal biomass began to be considered as an important supplement and even able to replace conventional animal or plant proteins for direct consumption of livestock or man, shortening the inefficient protein food chain.

In the 1950s, in West Germany, work began on the cultivation of *Scenedesmus acutus,* which included the use of CO2 produced in the industrial region of the Rhur. These works were

continued by Soeder and his group, who made important contributions to the massive cultivation of microalgae. Also, in the early 1950s, Oswald and collaborators at the University of California, Berkeley, suggested the use of massive microalgae crops for wastewater treatment and protein production, simultaneously (1975). Oswald and Golueke (1960) also developed systems for the mass production of algae for the bioconversion of solar energy in methane.

In the 1960s, the work carried out on the mass production of microalgae in Tr'bon (Czech Republic) was highlighted. In the 1980s, numerous industries were established for the production of microalgae, mainly *Spirulina* and Dunaliella*,* in Taiwan, Thailand, California, Australia, Hawaii and Israel. The production of Dunaliella appears as one of the most promising, for its content in carotene and its therapeutic properties **[8].**

In recent years, technological developments for the mass production of microalgae have been significant around the world. The practical result of this massive crop, in the context of the production of microalgae for food, is the development of a flourishing *Chlorella* industry in Japan and Taiwan. These microalgae are used for the manufacture of tablets, extracts and other dietary foods, for which there is a well-established market in Japan.

The development of algal crop technology has come a long way to this day, and Cuba has integrated into these studies, considering the benefits that these generally bring to the development of aquaculture. It is known that the development of aquaculture depends, to a large extent, on a successful production of seed sowing, i.e. Cher fry and precious larvae, where the use of microalgae, directly or indirectly, has been of great importance.

Photosynthetic microorganisms have traditionally been grouped into the categories of photosynthetic bacteria and microalgae. Cyanobacteria are one of the main contributions to microalgal biotechnology; therefore, the term microalgae makes no taxonomic sense, and within it can include organisms with two cell types: cyanobacteria, which have prokaryotic cell structure, and the remaining microalgae with eukaryotic cell structure.

Today, microalgae have a vast application in various aspects of human life; they are used as dietary supplements, in medicine, pharmaceutical, in the cosmetics industry, in the search and obtaining new fuels, for the feeding of poultry, pigs, ruminants, and in aquaculture, in fish feeding and zooplankton, numerous researches citing higher production rates with the use of live food.

3.1 Algae and their main areas of application

Algae are a diverse group of industrially important organisms found in every corner of the world. They may be smaller (single-celled) or larger (kelps) inhabiting the marine or freshwater environment. Numerous applications of eukaryotic cyanobacteria and microalgae have been proposed and developed in various technological fields, in mass or continuous cultivation, free or immobilized, live or processed, some of which are in full commercial exploitation, as shown in the following table:

Table 2. Main areas of use of microalgae.

MICROALGAE					
Medicine and pharmacy	**Sources of energy**	**Products of commercial interest**	**Bioremediation**	**Animal nutrition**	**Feeding**
•Antibiotics and antivirals •Antitumor •Diagnostic agents •Other agents •Bioactive •Excipients •Cosmetics	•Biofuel •Bioethanol •Biomethane •Hydrogen	•Carotenoids •Fatty Acid (PUFA) •Polysaccharides •Oligosaccharides	•Heavy metals •sewage water •CO_2	•Feed •Aquaculture	•Foods •functional •Additives •Dyes •Emulsifiers

Microalgae have gained remarkable attention due to their potential to accumulate lipids (70%) carbohydrates (60–65%). In addition, essential amino acids (50% of total biomass) and pigments such as chlorophyll, carotenoids and phycobilin can accumulate in small amounts. Because of their high lipid content, they seem promising to produce biofuels (mainly biodiesel), and some microalgae containing a higher carbohydrate content have also been used to produce bioethanol. Microalgae have been used as an alternative source of food since the 1960s due to its nutritional value. In aquaculture, microalgae have been used for food purposes, where 30% of the world's production microalgae are consumed as animal feed.

The specific composition of microalgae makes them applicable in various branches of the food industry, as well as in the pharmaceutical and beauty industry. Industrial processes can apply both microalgae and multiple cells. They are also researched for their medicinal properties. For example, spirulina has been reported for the prevention of cardiovascular disease, viral infections, and cancer due to its immunogenic properties. Similarly, Chlorella (freshwater microalgae) has the potential to lower cholesterol and blood sugar and is believed

to be hemoglobin and immune enhancers. Microalgae pigments have also been shown to be anti-inflammatory, antibacterial, antifungal, anticancer and antioxidant properties **[8]**.

For example, biopigments obtained from microalgae are commonly used in food, textile and paper and pulp industries due to the presence of carotenoids, flavonoids, chlorophyll, etc. In the food industry, they give the characteristic color for jams, jellies, gum, etc. In addition, marine microalgae are a good source of vitamins (A, B complex, E), antoxanthin, polyunsaturated fatty acid and s-carotene and are commercially produced as nutraceuticals and food additives **[7].** They have been used in the cosmetic industry for eyeliners and lipsticks. Microalgae extracts have UV protection, anti-aging and skin tightening ability and are widely used in the cosmetic industry.

The initial idea of producing lipids from microalgae for transformation into alternative fuels to petroleum derivatives is very old. The current energy situation has given new impetus to these initiatives seeking to reduce dependence on oil and greenhouse gas emissions.

The use of plant crops for energy purposes is not new. The first approach was the use of sugars and plant-derived oils. By digestion and fermentation of starch from cereal grains such as corn, wheat or barley or sugar cane bioethanol and biobutanol are generated. Oil oils such as palm, soybeans or rapeseed are used for biodiesel production. However, these raw materials are the basis of global nutrition, with the disjunction of its use as food or fuels. On the other hand, the large tracts of land and water needed for these crops have driven the search for other more sustainable raw materials **[4].**

In the table below, you can see the type of microalgae that is destined in the different areas of application with their respective products.

Table 3. Microalgal products and applications

Microalga	Cultivation system and producer countries	Product	Application areas
Chlorella vulgaris	Open pond: China, Japan, Taiwan; tubular photobioreactor: Germany	Biomass	Food supplements, cosmetics
		β-Glucan	Cosmetics
Dunaliella salina	Open pond: Israel, Hawaii, India, China; shallow lagoons: Australia	Biomass	Food supplements, animal feed, aquaculture
		β-Carotene	Food colorant, cosmetics
Haematococcus pluvialis	Open pond: Hawii, India, China, Japan, Taiwan; tubular photobioreactor: Israel, India	Astaxanthin	Feed colorant (salmon), food supplement, cosmetics, pharmaceuticals
Isochrysis galbana	-	Fatty acids	Animal nutrition
Odontella aurita	Open pond	Fatty acids	Pharmaceuticals, cosmetics, baby food
Phaeodactylum tricornutum	Open pond, basin	Lipids, fatty acids	Nutrition, biofuels
Porphyridium sp.	Tubular photobioreactor	Polysaccharides	Pharmaceuticals. Cosmetics, nutrition
		Phycoerythrin	Food colorant

3.2 Characteristics of algae production systems

The development of a microalgae culture method depends on the requirements and quality requirements of the elements mentioned in the various stages in which they must be supplied. The method used in cultivation according to the way of harvesting it is classified as discontinuous, continuous, and semi-continuous culture.

In *discontinuous or batch cultivation,* the population goes through the different phases of growth, generally adjusting to a logistical function, producing physiological changes of the population as the growing time elapses. Discontinued processes operate in closed systems so that the substrate is added at the beginning of the process and the products are removed only at the end of the process. Generally, the use of these crops is for bioassay purposes or for transfer to higher volumes. These types of culture are characterized by being easy to manage and are suitable for studying growth kinetics and parameters that affect cell growth.

Continuous cultivation is one in which the exponential phase is maintained for a long period of time, and the chemical characteristics of the medium, temperature and light are sustained at a constant value. These crops are characterized is that the samples taken at different times are identical. To do this, nutrients must be continuously added to the same extent that they are removed from the medium, to maintain the parameters of growth and cell population at a constant level. To maintain continuous crops, all growth factors must be kept constant and crop density is controlled by keeping it at constant concentration.

Finally, semi-continuous cultivation is the combination of the two methods above. In this type of culture, part of the volume is collected for use, usually at the end of the exponential phase, and the amount that is withdrawn is replaced with fresh culture medium. In efficient light energy systems up to 90% of the culture volume can be collected three times per week at high cell concentrations **[7].**

About crop systems for microalgae, the culture system is designed based on various criteria of the biology of the species to cultivate crop shape, nutritional, light and stress resistance requirements. With regard to the design, the ratio of the illuminated surface/volume of the reactor that determines the speed of growth, orientation and inclination is given importance; the type of gas mixing and dispersion systems, temperature cleaning and regulation systems, material transparency and durability, scaling capacity. Finally, there are also significant low construction and operating costs for commercial purposes

The choice of type of culture system is complex, being important to determine the type and value of the final product developed from biomass, in addition to the availability of water resources and soil type. Depending on the growing systems, two types of designs for microalgae production are observed:

- **Open Crop Systems:** These are the most common systems, encompassing both natural and lagoons and ponds, as well as artificial ones with a variety of designs. The most used design in this type of crop is circular ponds stirred by a rotary paddle used in Japan, Taiwan, and Indonesia for *Chlorella.* Among these, the most used is the High Rate Algal Ponds (HRAP) or Raceway (Illustration*4),* excavation or pond with a depth of between15 to 30 cm, divided by a central wall forming 2 channels. The cultivation circulates through pallets located in one of the channels. This system is one of the most cost-effective, as it can be used for the treatment of wastewater from different sources, which reduces

the costs for nutritional requirements of the crop being able to reach a cell concentration up to 0.7 g L^{-1} and productivity per hectare of up to 50 to-$year^{-1}$.

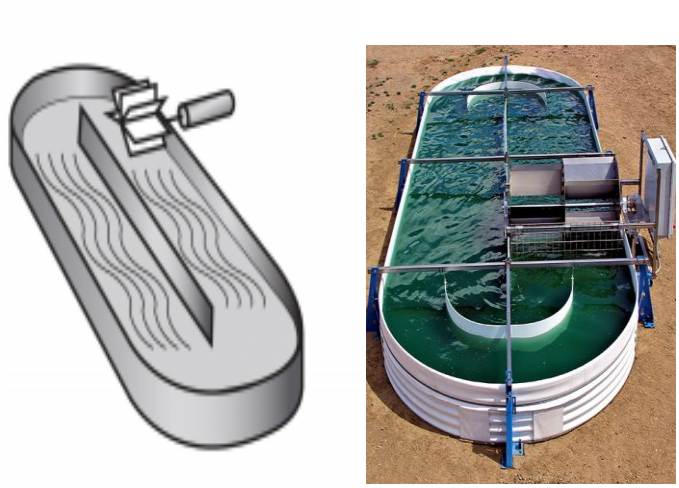


Illustration 4. Open-crop system

The characteristics of open systems is their low cost and ease of construction and operation, as well as in the high durability.

- **Closed Crop Systems:** This growing system is developed from the difficulties of open growing systems. These allow for significant control of parameters, substantially reducing the problems present in open systems. In addition, they allow hyper concentrated crops, whether mixed or monoalgal, with values greater than 1.5g L^{-1} can obtain High Cellular Density (ADC) above 3 g L-1 or Ultra High Cellular Density (UADC) between 15 to 80 g. L^{-1}.

  The designs in photobioreactors are varied flat or plate reactors, stirred by bubbling; tubular, vertical or horizontal reactors with bubbling or other agitation; and annular reactors, a variant of tubular reactors with an internal light source*(Figure 5).*). It is important to consider the speed of the fluid, especially in horizontal tube. At low speeds (< 15 cm s-1) sedimentation, growth to the PBR wall and inhibition by high oxygen concentration is likely. Speeds between 30 and 50cm s^{-1} are sufficient in most cases.

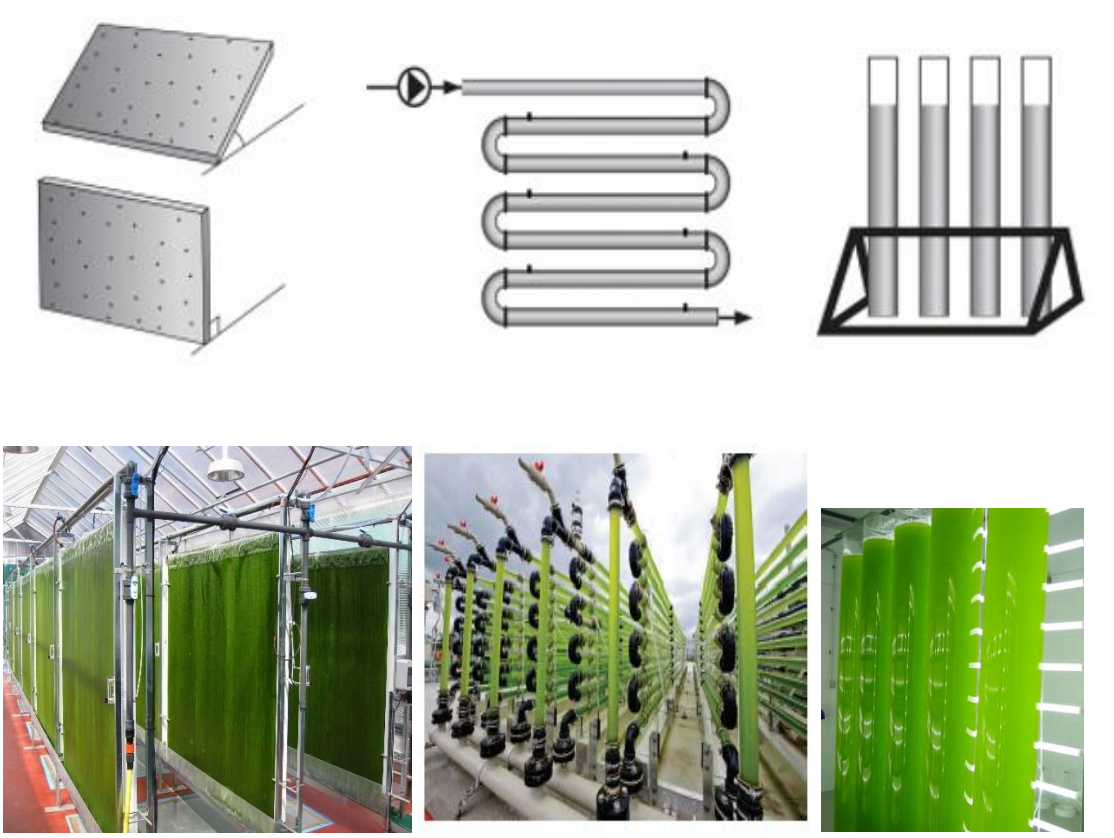


Illustration 5. Closed crop systems

3. Results

3.1. Type of algae

The microalgae used is *Chlorella vulgaris,* is a single-celled alga that develops in freshwater. It is a species that resides in salty aquatic environments and inherent inhabitant of wastewater, has high rates of nitrogen removal and phosphorus.

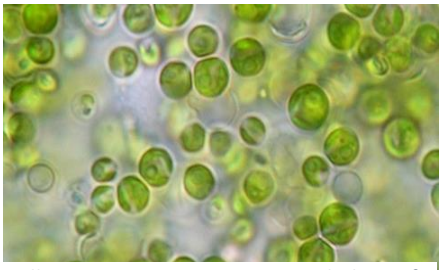


Illustration 6.Microscopic morphology of Chlorella vulgaris

These microalgae are used in crops because its growth rate is high, has a high resistance to environmental changes, mechanical stress and high concentrations of contaminants including nutrients and heavy metals. That is, it is a predominant microalga under extreme adverse conditions, because if wastewater is used as a culture medium without prior sterilization.

4.1 Sedimentation of Chlorella vulgaris algae

From the first study, "Effective harvesting of microalgae: Comparison of different polymeric flocculants"[], it has a culture of *Chlorella vulgaris* in a Bristol medium and maintains a constant low illumination at 24oC in a 1L Erlenmeyer flask with its aeration system.

The study is performed with the concentration of flocculant and the effect of pH in the medium, taking several samples from the discontinuous culture of 5 ml for 4 days in concentrations of 1.5g Wet weight L. The type of flocculants used are Quitosane, PDADMAC and superflock. This type of flocculants is used specifically for this type of microalgae. The result of three trials is obtained.

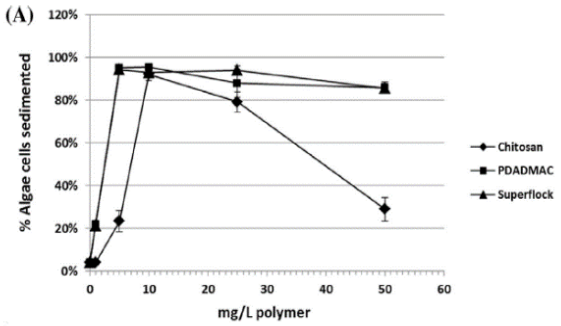


Illustration 7.Sedimentation (A)

In graph (A), a variation occurs in the percentage of sedimented algae cells. All three types of polymers were added to all samples at a pH at 6.5. All polymers have a final concentration of 5 mg/L. After an hour, se produce sedimentation successfully with the flocculants of PDADMAC and the superflock, the other polymer ceases to be effective as the polymer concentration increases.

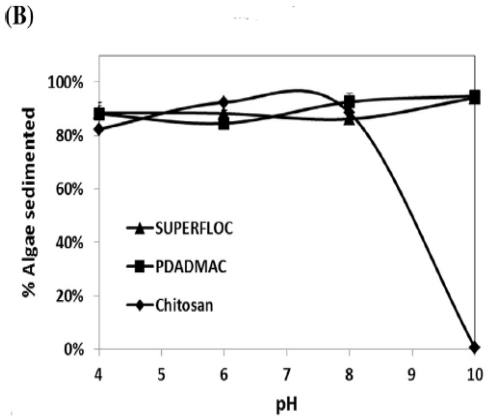


Illustration 8. Sedimentation (B)

In graph (B), the effect of pH on sedimentation with the three flocculants is observed. All polymers have a final concentration of 10 mg/L. As the pH increases in the middle, a flocculant loses its flocculation capacity when the pH of the crop is greater than 8.

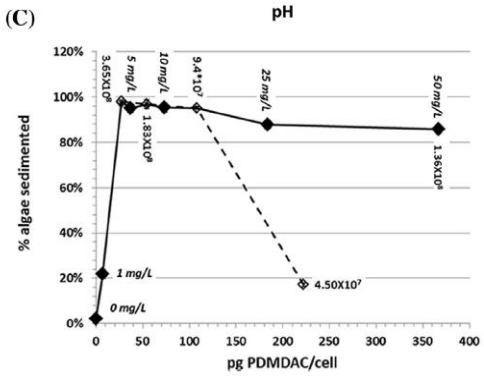


Ilustración 9. Sedimentation (C)

In graph (C), it shows the effect of algae concentration and cell/PDADMAC ratio on sedimentation. The data have a variation in the concentration of algae cells while maintaining constant polymer concentration. A decrease in sedimentation is observed when the portions are about 222 pg PDADMAC /cells. That is, these results fit well with the supposed flocculation mechanism of PDADMAC, i.e. flocculation cannot occur at a low level of polymer concentration or a low concentration of algae.

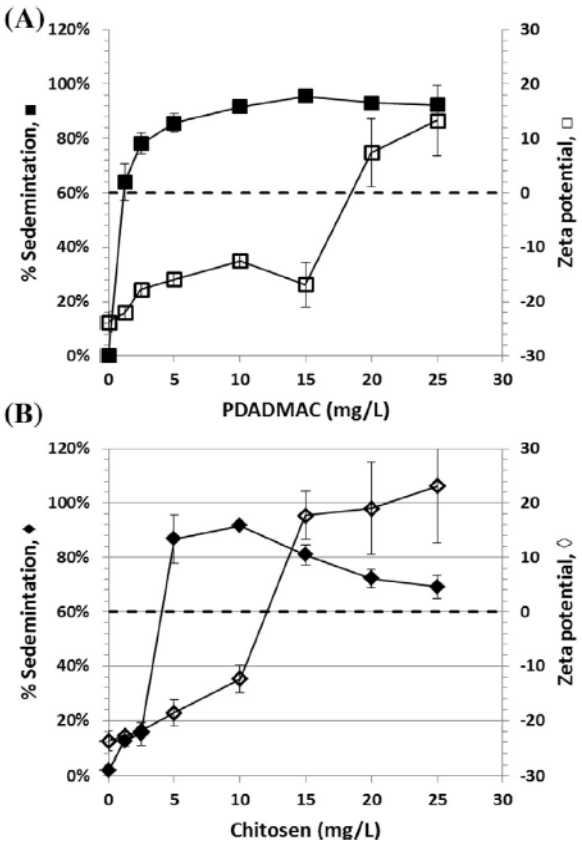


Illustration 10. Zeta potential and sedimentation of C. vulgaris cell suspension as a function

Sedimentation is related to the neutralization of zeta potential; we measure the zeta potential of polymers and the suspension of algae in the presence of different concentrations of PDADMAC and chitosan.

The results are presented that without adding any type of polymer a zeta potential of 25 mV is recorded. That is, electrostatic stabilization occurs is part of the mechanism that prevents agglomeration for both PDADMAC and chitosan.

Maximum sedimentation was achieved at much lower polymer concentrations than those needed to establish total neutralization of zeta potential with sedimentation as soon as 20 mV was achieved.

Regarding the graph (B), they pushed the system beyond the isoelectric point which causes the polymer-algae complex to positively charge and electrostatically destabilize the colloidal dispersion, leading to a reduction in sedimentation efficiency. The different sedimentation behavior of chitosan and PDADMAC at the beginning of the isoelectric point suggests that the flocculation mechanism is different in these two polymers.

In conclusion, the marine microalgae C. vulgaris was not achieved the maximum effect of PDADMAC at 30 mg /L. This effect was probably due to the adsorption of salt anions in the polymer or algae, because of the addition of NaCl at 3% to C. vulgaris in the middle of Bristol in dramatic decrease in the sedimentation effect of PDADMAC.

The second study "An assessment of inexpensive methods for recovery of microalgal biomass and oils", has a culture of Chlorella vulgaris and maintains a constant low fluorescent illumination at 24oC in a 100mL Erlenmeyer flask.

The study on the collection of microalgae by flocculation is carried out. Two types of inexpensive flocculants are aluminum sulfate (Al2(SO4)3 x 18H2O and ferric chloride (FeCl3-6H2O). The solutions of inorganic flocculants were made at a concentration of 20 g L. The mother solution contains 20 g of salt that dissolves in deionized water up to 1 L. The mother solutions were kept at room temperature (24-26oC) and were used within 14 days.

Flocculation-sedimentation tests were performed using a 200 ml suspension of the microalgal cells in each of the six 250 ml beakers.

The flocculation test methodology is distributed samples in 6 beakers for rapid mixing at 80 rpm for 2 minutes to disperse the flocculant, a smooth mixture at 20 rpm for 30 minutes (the flocculation period) to flocculate the cells; and without turmoil for 30 minutes (the settlement period) to allow the floccules to settle. Microalgae broth samples (200mL) are identical but have been dosed with different amounts of the flocculant.

Illustration 11. A jar test unit for flocculation experiments

The sedimentation of the samples is successful. At the end of the 62-minute test, a 5 ml sample of the 100 ml beaker level suspension was extracted to measure the cell concentration suspended by the optical density method. The percentage of microalgae cells removed from the broth was then 100 less than the percentage of the remaining cells in the broth.

4. Designing a sedimentator

4.1. Design scheme

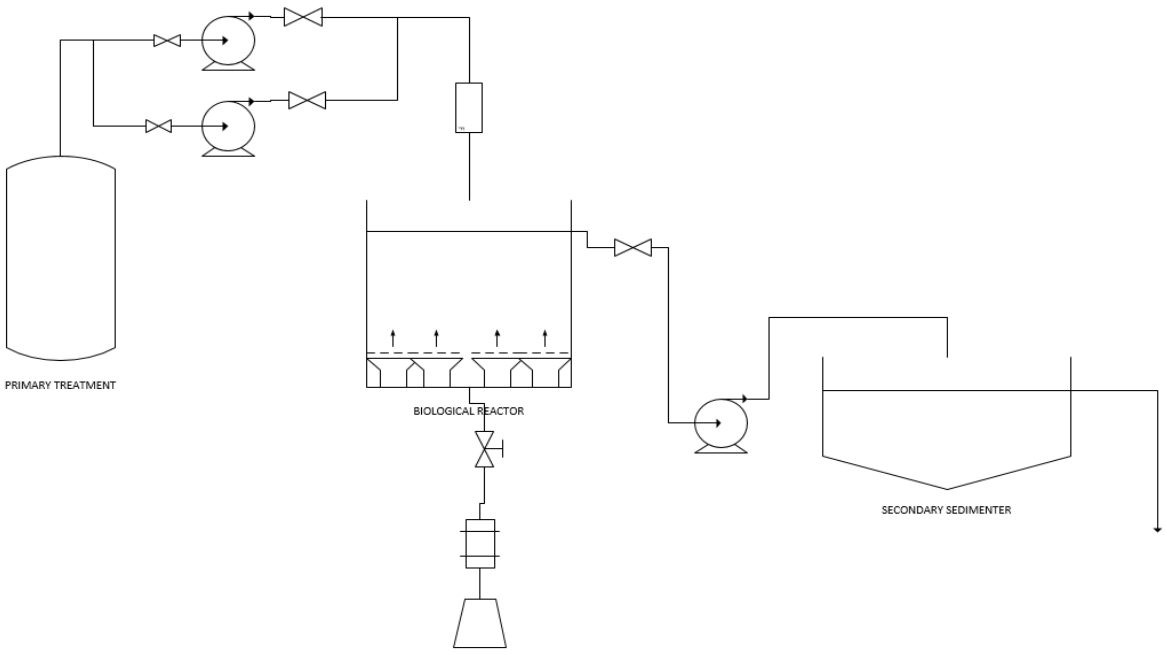
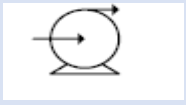
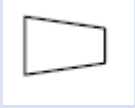


Illustration 11. Assembly of Wastewater Treatment

SYMBOL	DESCRIPTION	UNITS
	Centrifugal pump	3
	Diffuser	185
	Air compressor	1
	Flowmeter	1

	Valve	6

5. Economic Study

The objective of this economic analysis is to estimate the cost of the cubic meter (m^3) of treated water.

5.1. Investment Costs

This is about the expenses that the company will have for the development of the reactor. This section will analyze the cost of equipment and instruments, the cost of installing such equipment and finally the cost of starting the equipment.

Tabla 4. Equipments And Instruments

EQUIPMENT AND INSTRUMENTS			
EQUIPMENT AND INSTRUMENTS	**UNITS**	**PRICE / UNIT (€ / unit)**	**TOTAL PRICE (€)**
Diffuser	185	42	7.770
Centrifugal pump	3	3522.47	10567.41
VAG gate valve	6	1.124,36	6746.16
Flowmeter	1	1.347,52	1.347,52
Air compressor	1	453.32	453.32
InPro 4010 pH and temperature sensor, Mettler Toledo + Analyzed	1	1.598,63	1.598,63
InPRO 6050 Dissolved Oxygen and Temperature Sensor, Mettler Toledo + Analyzer	1	1.675,96	1.675,96
TOTAL COST OF EQUIPMENT AND INSTRUMENTS			
TOTAL (€)			**22396.77**

So, we will have that the total costs of the equipment are **22396,77** euros.

Once the cost of the equipment has been calculated, you would now have to calculate the cost of installing this equipment.

Tabla 5. Installation

INSTALLATION	
Operators	2
Hours of work / day · operator	8
Worked days	20
Hours worked	320
€ / hour · operator	18
Total, cost of operators (€)	5760
TOTAL INSTALLATION COSTS	
TOTAL (€)	**5760**

After performing the calculation of what the installation of the same would cost we can conclude that the total price of the installation of the equipment would be 5760 euros.

Finally, it would be necessary to analyze the start-up costs since prior to the start-up of the reactor, it is necessary to carry out starter tests to ensure that the equipment is functioning properly, and that the reactor operates properly. The cost of this entails (energy, water consumption...), usually corresponds to 10% of the direct and indirect investment costs.

START-UP COSTS	8.453,61 €

Once the costs of each area have been calculated, we can calculate the total cost of the investment.

EQUIPMENT AND INSTRUMENTS	**22396.77**
INSTALLATION	5760
START-UP COSTS	8.453,61 €
TOTAL INVESTMENT COSTS	36610.38

Finally, the total cost of the investment would be about **36610.38** euros

5.2. Annual Operating Costs

About the cost of annual operations, the cost of annual energy, maintenance and finally the cost of workers will be analyzed.

Tabla 6. Energy Cost

ENERGY COST						
EQUIPMENT	**UNITS**	**POWER (kW)**	**Hours of activity at year**	**kW·h/año**	**€/Kw·h**	**€**
Centrifugal pump	1	300	700	210 000	0.13	27300
ANNUAL ENERGY COST					27 300	

The annual energy cost would be 27,300 euros

Then, regarding the cost of maintaining equipment

Tabla 7. Equipment Maintenance Costs

EQUIPMENT MAINTENANCE COSTS	
COST	1200
Repairs	600
Conservation	500
Oil and pump greasing	400
Cleaning material	600
ANNUAL MAINTENANCE COST	3300

The annual maintenance cost would be 3300 euros.

Finally, the cost of staff

Tabla 8. Personal Cost

PERSONAL COST				
	Quantity	€/month·operator	€/year·operator	€/year
Operator	2	1.300	15.600	31.200
Engineer	1	2.200	26.400	26.400
ANNUAL STAFF COST			57.600	

El annual staff cost would be **57.600** euros

With this analysis we can calculate the total operating costs

ENERGY COST	27 300
EQUIPMENT MAINTENANCE COSTS	3300
PERSONAL COST	57.600
ANNUAL OPERATING COSTS	88.200

The total costs per operation would be 88,200 euros

5.3. Cost of the m^3 of treated water

To obtain the cost of the m3 of water, the annual amortized amount of the total investment costs and annual operating costs are added up and divided by the flow of treated water per year. The total annual costs are therefore as follows:

ANNUAL AMOUNT AMORTIZED	13.230
ANNUAL OPERATING COSTS	88.200
TOTAL ANNUAL COSTS	101.430

In the event that 3500 m^3 of water per day were treated

3500 m^3/day · 365 day/year = **1.277.500 m^3/year**

Therefore, the price of m^3 of water will be:

101.430 euro/year /1.277.500 m^3/year= **0.079 Euro/ m^3 water**

According to literature consulted through AEAS (Spanish Association of Water Supplies and Sanitation), the cost of water purification is about 0.53 euros/m3. From this data and according to the cost we have obtained, we can know what percentage belongs exclusively to the biological treatment within a plant:

0,079/0,53 = **15 %**

Therefore, approximately 15% of the total cost of treating one m3 of our wastewater in an entire plant, is equivalent to the cost of biological treatment.

6. Conclusions

From the results obtained it can be concluded that the microalgae and bacteria consortia are efficient for the treatment of wastewater, allowing to eliminate from these effluents not only the organic matter present (COD), but also recover the nitrogen and phosphorus present in such effluents in the form of biomass that can be recovered. The technology and operating conditions should be adequately selected according to the type of wastewater to be purified, and adjusted according to the time of year for optimal operation

Microalgae-based systems can be used for the tertiary treatment of wastewater, eliminating nitrogen and residual phosphorus from conventional treatment from these effluents. To produce microalgae this effluent presents a nutrient limitation as evidenced by the fact that the productivity of biomass achieved with the culture medium with fertilizers is greater than that achieved with secondary wastewater. In any case the secondary water used is adequately purified since the content of organic matter, nitrogen and phosphorus decreases to the limits of discharge. The quality of the treated water is independent of the dilution rate used and the reactor used.

Flocculation is the first stage of the microalgae harvesting process for obtaining biomass. This process involves the binding or grouping of substances that are dispersed in a liquid medium. Generally, it is the second stage in a complete coagulation-flocculation and sedimentation process, which seeks to separate the solid substances from the liquid medium that contains them. The types of flocculants used in the studies facilitate sedimentation to the cultivation of algae.

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[11] Continuous flocculation-sedimentation for harvesting Nannochloropsis salina biomass Tawan Chatsungnoen, Yusuf Chisti ∗ School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand