

DETERMINE THE EFFECT OF SOME CULTIVATION PARAMETERS IN THE DEVELOPMENT OF MICROALGAE FOR BIODIESEL PRODUCTION

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Abstract

The research studies in the past focusing on finding new energy sources, were based on biodiesels which reduce the greenhouse effect by 41% when compared with diesels. Due to this fact, many countries in the world have exempted biodiesel from taxes and encouraged their production and consumption. However, in Turkey, increase in production taxes, and because the producers can only sell their products to distribution firms, the attractiveness of biodiesel has decreased tremendously and could only be developed to the limits provided by the studies of related departments of the universities.

Microalgae carbon dioxide and sunlight through photosynthesis converts it to energy very efficiently, and in this process the oil-producing single-cell biological organisms. Just like any other plant-based oils such as algae oil biodiesel converted directly used as a fuel for diesel engines. Today, experienced negative effects due to oil and oil to eliminate the negative effects to the environment is given a new, clean and cheap energy resources, and these resources have to be used effectively. With a high fat binding properties of micro-organisms, especially microalgae energy crisis on behalf of the resort, be worth studying.

The recent research studies focused on alga cultures which are blue, red, green in color and are transforming CO₂ to O₂ in the ecosystem. The research studies on algs are implemented under two topics which are namely alg production and possibilities of using algs as fuel.

*In this study, species of *Dunaliella salina*, *Palmellopsis muralis* and colored light sources with different wavelengths using the 24-hour period of enlightenment, subjected to constant light intensity of cultivation under the analyzed parameters. The second experiment using white light illumination of different periods of growth parameters, cell number, pH, salinity and conductivity values were measured and analyzed. The third attempt, the temperature in the same way using white light and under constant light intensity, respectively, 21°C, 28°C and 35°C growth parameters were investigated. Finally, using white light source and 12 hours light and dark periods within 12 hours, applying a different light intensity, cell numbers, pH, salinity and conductivity values were analyzed and compared.*

Key words: Renewable energy sources, microalgae, biodiesel, algae.

INTRODUCTION

Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives. In addition, these photosynthetic microorganisms are useful in bioremediation application and as nitrogen fixing biofertilizers (Figure 2). This article focuses on microalgae as a potential source of biodiesel (Chisti, 2007). Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass biodiesel derived from microalgal oil and photobiologically produced biohydrogen

(Demir et al., 2007). The idea of using microalgae as a source of fuel is not new but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels.

Biodiesel is produced currently from plant and animal oils, but not from microalgae. This is likely to change as several companies are attempting to commercialize microalgal biodiesel. Biodiesel is a proven fuel. Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste

cooking oil and jatropha oil (Vonshak, 1997; Gökpinar and Cirik, 1991). Especially, the latest biotechnical and technical studies on microalgae seek to increase their use in food, agriculture, animal feed, environment and cosmetics (Figure 2). Therefore, it is important to base microalgae production upon some biotechnical basis because of its future contributions to the fields mentioned above (Naz ve Gökçek, 2006).



Figure 1. The explosion of microalgae



Figure 2. Microalgae used in food and cosmetics industries

Although there have been many different classifications, algae can be classified simply in two categories as prokaryotic and eukaryotic (Figure 3). They are also separated into two as micro (one-year and unicellular, microscopic) and macro (perennial and cellulosic). Microalgae contain a higher rate of fat.

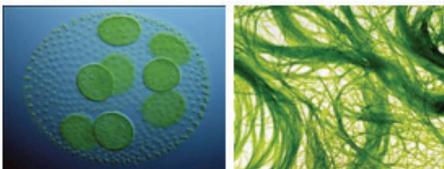


Figure 3. Microalgae cell

Microalgae are known as Cholophyceae (green algae), Rhodophyceae (red algae), Cyanophyceae (blue green algae) and Pheophyceae (brown algae). Important pigments produced are chlorophyll a and b,

Carotene, Astaxanthin, Fitosiyenin, Xanthophyll, fito erythrosine. These pigments are frequently used in food, medicine, textile and cosmetics (Vonshak, 1997).

In microalgae production, the purpose of mass-production is to obtain efficient product for a minimal cost (Figure 4). In high scale cultivation systems, effective usage of light, temperature, hydrodynamic balance in cultivation, providing the longevity of culture must be compared.

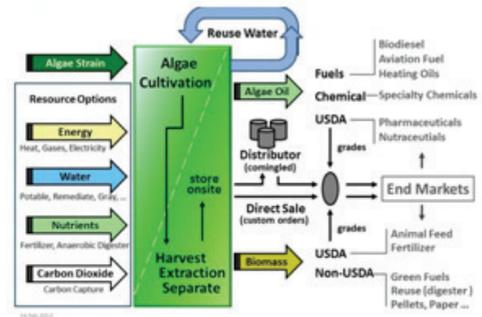


Figure 4. Microalgae production system

The ideal development of any microalgae type occurs in cultivation environments in which distinctive conditions are met. Accordingly, while *Spirulina* achieves most growth in high Ph and bicarbonate density, *Chlorella* seeks highly nutritive environments and *Dunaliella salina* fosters in high salinity. On Table 1, oil content of some microalgae species can be seen.

Open pool systems vary greatly (Figure 5). The main reason of this is that these systems are economical, while indoor production systems require technology which is not cost effective. However fewer kinds of microalgae can be cultivated outdoors (Gökpinar and Cirik, 1991). Outdoor microalgae production systems based on the interior of the significant difference in production systems, the direct environmental impacts of microalgae cultures were exposed. *Palmelopsis muralis*, *Chlorella* and *Spirulina* without any artificial mixture of open-top, shallow, and can be produced by providing a mixture of large circular pools. Microalgae in Table 2 are the average production conditions.

Table 1. Fat content of some microalgae species

Microalgae	Fat Content (dry weight %)
<i>Botryococcus braunii</i>	25-75
<i>Chlorella sp.</i>	28-32
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca sp.</i>	16-37
<i>Dunaliella primolecta</i>	23
<i>Isochrysis sp.</i>	25-33
<i>Monallanthus salina</i>	>20
<i>Nannochloris sp.</i>	20-35
<i>Nannochloropsis sp.</i>	31-68
<i>Neochloris oleoabundans</i>	35-54
<i>Nitzschia sp.</i>	54-47
<i>Phaeodactylum tricornutum</i>	20-30
<i>Schizochytrium sp.</i>	50-77
<i>Tetraselmis sueica</i>	15-23

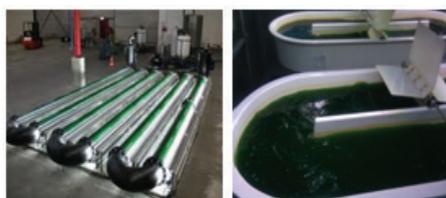


Figure 5. Tubular photobioreactor and growing pool

Table 2. Production conditions of microalgae

Parameters	Limit Values	Optimum Conditions
Temperature (°C)	16-27	18-24
Salinity (g/l)	12-40	20-24
Light Density (lux)	1000-10000	2500-5000
Light Exposure time (Day:Night h)		16:8 minimum 24:0 maximum
pH	7-9	8,2-8,7

Also in outdoor environment, the cultivation is susceptible to contamination. The losses resulting from continuous evaporation, CO₂ emission and contamination risk are the other disadvantages of outdoor systems (Table 3).

Considering indoor systems, it is quite costly to maintain and install. However these systems should be applied for only one kind. Indoor systems have many advantages such as preventing contamination, effective usage of light, high effectiveness, controlling temperature and using the sunlight from reactors installed outdoors. In indoor reactors, it is easy to control the cultivation environment as well as the product is satisfactory in quality and effectiveness.

Table 3. The comparison of open and closed systems

	Outdoor Systems	Indoor Systems
Contamination Risk	Very High	Low
Space Requirement	High	Low
Water Loss	Very High	None
CO ₂ Loss	High	None
Variety of Types	Limited	All kinds
Standardization	Not possible	Possible
Dependence on Weather Conditions	No production in Rain	No dependence
Production Concentration	Low 0.1-0.2 g/l	High 2-8 g/l
Effectiveness	Low	High

To determine the most efficient types of universities in particular, experiments have established direct from production. Finally, Ankara University, Faculty of Agriculture, Department of Agricultural Machinery and University of the Dicle, Faculty of Agriculture the Department of Agricultural Machinery grown *Palmollopsis muralis* and *Dunaliella salina* species. The reason is easy availability of the training of these two species in nature and more influenced by external factors, unless otherwise indicated. (Eliçin et al., 2009) (Figure 6).

First of all, a microalgae cell count is conducted to determine the number of mature microalgae cells. Microalgae reaching an adequate cell number (which depends on the type of microalgae) are moved into another tank before the "stress" process. A high amount of catalyst is released from nutritive tank in order to expose the algae to stress. Specific parameters are to be modified unless the catalyst is not applied. Stress process forces the microalgae to produce fat quickly before the extraction process. After the stress process, extraction begins. The first phase of fat extraction is the dehydration of microalgae in Microalgae Harvest Unit (Figure 7). With the air inside Microalgae Harvest Unit and a belt rotating continually, water is removed from microalgae.

Microalgae retrieved out of microalgae harvest unit are completely dehydrated. Dehydrated microalgae are also called scales. These scales are sent to pressure machines to extract fat. Custom fat extraction machines are present in many companies producing in small scales. In enterprises without these custom fat extraction

machines, many different fat extractors are used as well (Tawfig et al., 2004).

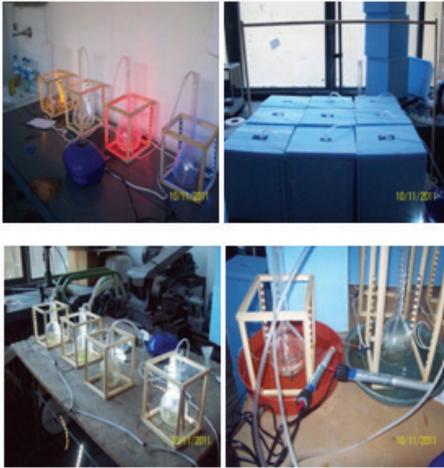


Figure 6. Ankara University Faculty of Agriculture established microalgae cultivation of agricultural machinery in the experimental setup

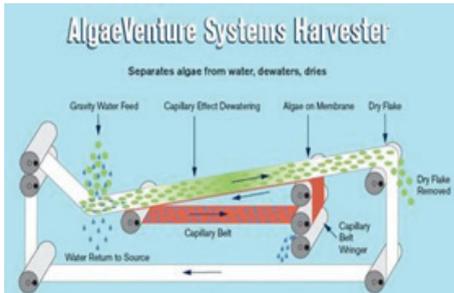


Figure 7. Microalgae harvesting unit operation diagram

Microalgae have high potentials in biodiesel production compared to other oil crops. First, the cultivation of microalgae dose not need much land as compared to that of terraneous plants. Biodiesel produced from microalgae will not compromise the production of food and other products derived from crops. Second, microalgae grows extremely rapidly and many algal species are rich in oils. In order to obtain oil from microalgae culture microalgae and it is expected to reach maturity for the detection of a specific cell count is done. Certain populations as a result of mature cell counts (varies by type) in a separate tank on the stressful applied (Eliçin et al., 2009). Stressful is a catalyst to expose intensive nutrient tank. Some of the parameters given in Table 2 by

changing the applied stressful be executed catalyst. Stressful microalgae oil extraction process prior to application of the oil binding will provide a fast and intense. Stressful oil extraction process begins after the application. Oil extraction process can be accomplished by various methods. Oil extraction process, moving through the air thanks to the free and infinite in the harvest unit with the help of a rotating band enables to water and dried microalgae (Figure 8). Harvest units were extracted from microalgae in the water completely. Microalgae are also known as dehydrated flakes. To be sent to the printing machines have become stamps microalgae oil.



Figure 8. Take the form of flakes harvest microalgae

For the methods of transforming microalgae into biodiesel; Pyrolysis, gasification and transesterification methods can be given as examples (Eliçin et al., 2009). Pyrolysis method; this is the method of transformation in which the biomass is dissolved into liquid (bio-petrol), solid (charcoal) and gas state. Gasification method; “gasification” is the process in which a secondary gas fuel is obtained predominantly during Pyrolysis. Gasification transforms the bio-mass into a flammable gas consisting of carbon monoxide, hydrogen and methane. Transesterification method; in the transesterification method, oils, re-esterification process by reaction with alcohol by means of a catalyst. This method is most effective in reducing viscosity (Demir et al., 2007).

A method of transesterification reaction is balanced, complete mixing of the catalyst used in the reaction occurs in particular. The presence of the catalyst is a catalyst of the reaction to equilibrate. Nevertheless, a high proportion of the alcohol to be used to obtain the ester. Triglycerides transesterification process with an alcohol is reacted with a strong

acid or base catalyst, alkyl ester, and glycerin is obtained by reaction of saturated. The entire process is analyzed, the reaction to a chain di- and monoglycerides formed as an intermediate product consecutive two-way and 3-stage appears to be a reaction.

Similarly oil from microalgae, an alcohol (ethanol, methanol), the catalyst (acidic, basic and enzyme) in the presence of methyl esters of fatty acids and glycerol are formed. During the transesterification reaction variables affecting the efficiency of microalgae oil, algae oil, the quality of the alcohol by microalgae oil molar ratio, reaction temperature, reaction time, catalyst type and amount. The amount of conversion occurred as a result of the reaction, the upper phase can be found by the analysis of gas chromatography or thin layer chromatography (Eliçin et al., 2009).

MATERIALS AND METHODS

As plant materials, is more than the amount of fat, easily available, taking into account lifestyle factors such as contamination and temperature resistance, belong to the class *Chlorophyceae*, *Palmellopsis muralis* and still belong to the class *Chlorophyceae*, *Dunaliella salina* sp. types were selected (Figure 9 and Figure 10).

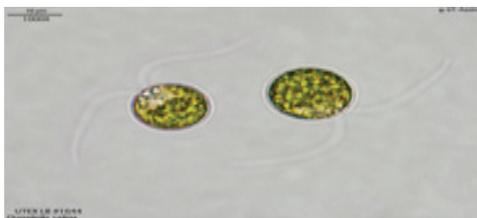


Figure 9. *Dunaliella salina* sp. microscope image

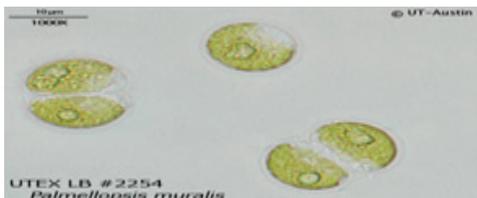


Figure 10. *Palmellopsis muralis* microscope image

Algae are grown right after the planned experiments in 4 different section. Also examined the effects of light intensity and duration of light exposure due to the algae

grown in a light-proof box for each volumetric flask made of styrofoam material (Figure 11).



Figure 11. The protective boxes

Algae at the beginning of the experiment, using the resources of colored light of different wavelengths subjected to a 24-hour light period, the number of cells under constant light intensity, measured were pH and conductivity (Demirbaş, 2010; Tapan, 2006) (Figure 12).

The first experiment, the light source through the 4 different colors with the high intensity white light source that creates a positive impact on the parameters of the cultivation of selected white light source used in the other trials.

Using white light again in the second part algae exposed to constant light intensity, 24 hours light, 18 hours of light - 6 hours darkness, 18 hours darkness - 6 hours of light and 12 h light - 12 h darkness, the same parameters were determined in intervals.

In the third chapter the use of white light and algae under constant light intensity, respectively, 21°C, 28°C and 35°C daki changes in the number of cells with values of were investigated pH and conductivity (Ilgaz 2003; Tapan 2006).

In the last part of the experiment, the white light source is used, and 12 hours of light and 12 hours dark periods algae exposed to applied light in a different light intensity. Respectively, 6V, 9V and 12V adapters 60 cm led light source, light intensity provided by the exchanges were determined on the same parameters.

Nutrients of mixing ratios and algae are given in Table 4. This stock solution was prepared in the Department of Fisheries Engineering, Faculty of Agriculture, University of Ankara, Turkey. Attempts to do such, flasks were produced before it is deployed in a large vase. Here, after performing the production of a sufficient amount of the same volume of volumetric flask (300 ml) were distributed.

Table 4. The nutrient content

Content	Amount	Amount
NaNO ₃	30 ml / l	10 g / 400ml
CaCl ₂ ·2H ₂ O	10 ml / l	1 g / 400ml
MgSO ₄ ·7H ₂ O	10 ml / l	3 g / 400ml
K ₂ HPO ₄	10 ml / l	3 g / 400ml
KH ₂ PO ₄	10 ml / l	7 g / 400ml
NaCl	10 ml / l	0.4 g / 400ml
P-IV Metal Solution	6 ml / l	0.1 g / 400ml
Soil water: GR + Medium	40 ml / l	1.5 g / 400ml
Vitamin B ₁₂	1 ml / l	2 g / 400ml
Biotin Vitamin Solution	1 ml / l	0.9 g / 400ml
Thiamine Vitamin Solution	1 ml / l	0.1 g / 400ml

RESULTS AND DISCUSSIONS

The first part of experiments investigated the development of algae led of different colors (Figure 12). Boxes made in the measurement of light intensity, the yellow light 117 lux, 194 lux blue light, red light 224 lux and 265 lux have been obtained of white light results (Demir et al., 2007) .



Figure 12. Color experiments

Colorful led trials, *Dunaliella salina* sp. when white light is the best for the type of growth observed in the number of cells. The highest light intensity, blue, red, yellow and white color leds White leds with 265 luxury reserved (Agra et al., 2004) (Figure 13).

Conductivity is directly proportional to the light is increased intensity (Figure 14).

On the contrary, the light intensity increases, ie, the pH value of the conductivity is observed decline in the use of white leds (Figure 15).

Palmellopsis muralis to the color of the cell growth experiments, the best has been reached white leds (Figure 16). Showed the lowest cell count and 13 yellow leds determined at the end of day living cell (Demir et al., 2007; Eliçin et al., 2009).

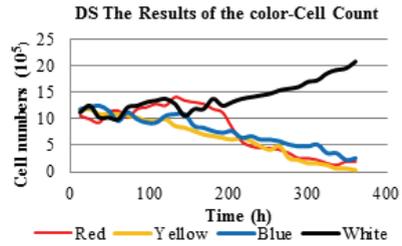


Figure 13. *Dunaliella salina* sp. species, using different colored light sources, which was time-dependent changes in the number of cells

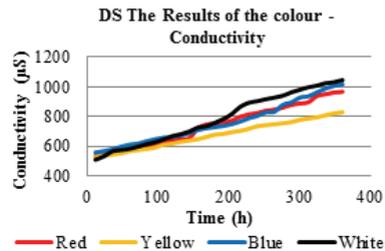


Figure 14. *Dunaliella salina* sp. species, using different colored light sources, which was time-dependent changes in the conductivity values

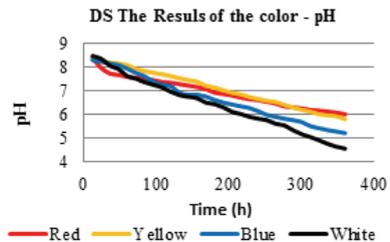


Figure 15. *Dunaliella salina* sp. species, using different colored light sources, which was time-dependent changes in pH values

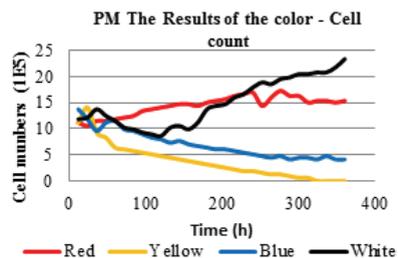


Figure 16. *Palmellopsis muralis* species, using different colored light sources, which was time-dependent changes in the number of cells

Conductivity at the first blue leds *Dunaliella salina* sp. determined to increase up to 2 times (Figure 17).

Evaluation of the data fall in pH in the first 7 days was observed rise in the values of the first day of the next 8 days (Figure 18).

Duration of the light exposure trials, *Dunaliella salina* sp. for the type of environment light of the high number was determined of cells 24 hours (Figure 19). 18 hours dark/6 hours in the light is observed of the low number of cells (Demir et al., 2007; Eliçin et al., 2009).

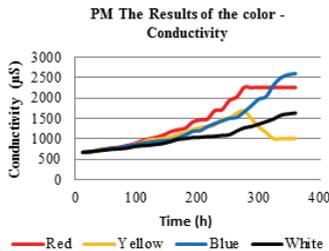


Figure 17. *Palmellopsis muralis* species, using different colored light sources, which was time-dependent changes in the conductivity values

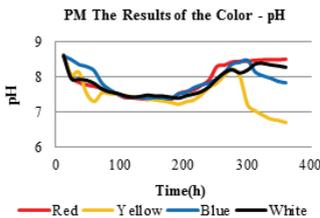


Figure 18. *Palmellopsis muralis* species, using different colored light sources, which was time-dependent changes in pH values

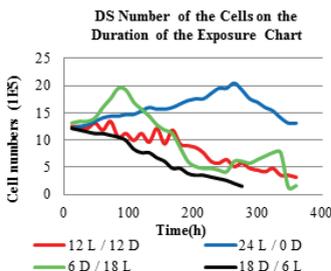


Figure 19. *Dunaliella salina* sp. different times, depending on the type of light can change the number of cells

18-hours darkness/6 hours light environment were increasing conductivity (Figure 20).

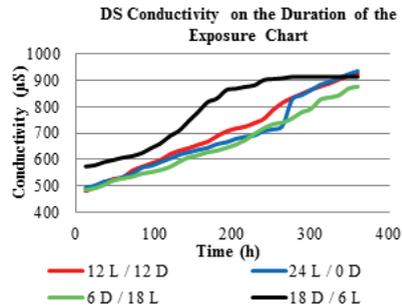


Figure 20. Different light exposure times, depending on the type of *Dunaliella salina* conductivity changes

From a darkness time, the increase in pH values decrease in pH value decreased, but in general there is a decrease in pH values (Figure 21). Looking at the data of the light of life *Palmellopsis muralis* showed the highest number of cells a 24-hours illumination (Figure 22) Lowest showed that the number of cells in the 18 - hours darkness/6 - hours light (Vonshak 1997; Gökpinar and Cirik 1991). Increase in conductivity is observed values in the 24 hours of light (Figure 23).

The best results were obtained from the pH 24-hour periods (Figure 24).

The third section of the white light in the same are used way trials. 268 lux light intensity is below 12 V adapters provided by the algae, respectively, 21 °C, 28 °C and 35 °C changes in cell numbers, pH, salinity and conductivity changes were investigated of the 12 hours light - dark periods of 12 hours based on a measurement in 12 hours the same parameters (Brown et al., 1989; Agra et al., 2004). Temperature experiments, *Dunaliella salina* sp. type the maximum number of cells reaching 35°C showed that a type of heat-loving. Lowest was reached number of cells at 21°C (Figure 25).

Increased in direct proportion to the temperature were evaluated conductivity data (Figure 26).

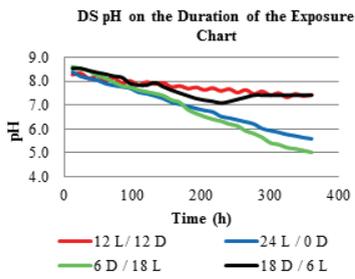


Figure 21. *Dunaliella salina* sp. changes in pH values of different light exposure times, depending on the type

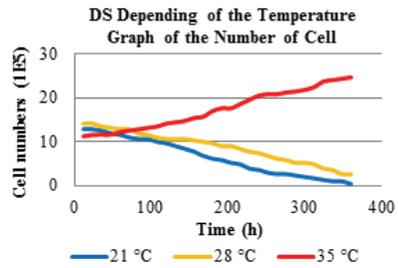


Figure 25. Depending on the temperature variations in the number of species *Dunaliella salina* cells

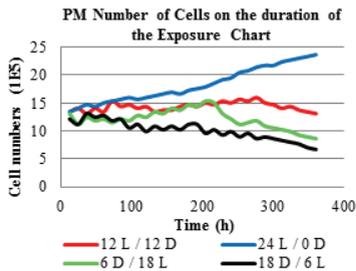


Figure 22. Different times, depending on the type of light can *Palmelopsis muralis* changes in the number of cells

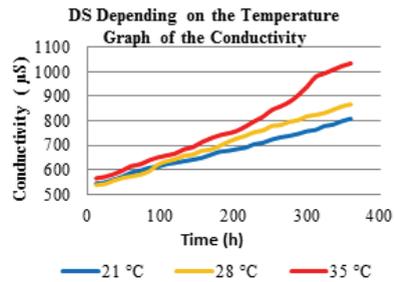


Figure 26. Changes in the value depending on the temperature conductivity type *Dunaliella salina*

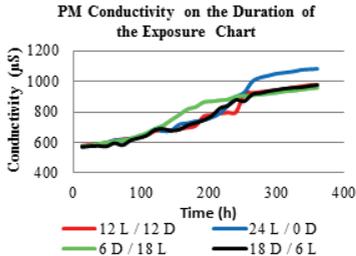


Figure 23. Different times, depending on the type of light can *Palmelopsis muralis* conductivity changes

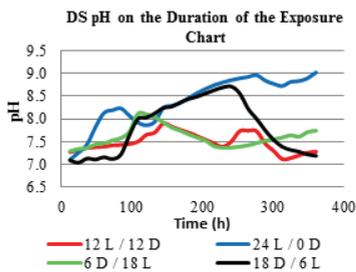


Figure 24. Different times, depending on the type of light *Palmelopsis muralis* changes in pH values

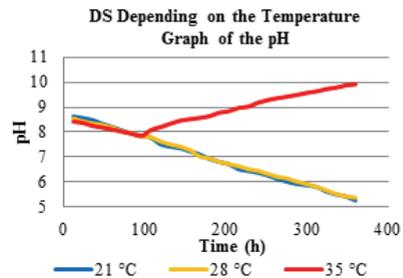


Figure 27. Changes in temperature, pH values, depending on the type of *Dunaliella salina*

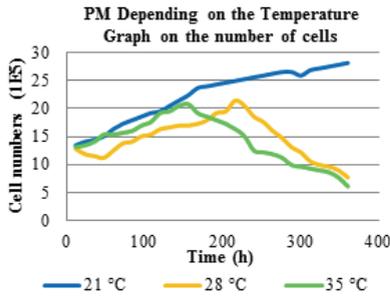


Figure 28. Depending on the temperature variations in the number of cell types *Palmelopsis muralis*

The conductivity is inversely proportional to the pH data (Figure 29). High pH values were measured at low temperatures (Vonshak, 1997; Gökpinar and Cirik, 1991) (Figure 30). Light on the severity of the experiment, the *Dunaliella salina* sp. 265 lux 12 V, the highest has been reached number of cell types (Figure 31) Figure 33 appearance of decline in 12-hours light 12-hours dark period of the reason is intended to trials (Tawfig et al., 2004; Ilgaz, 2003).

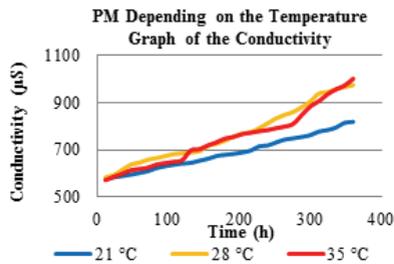


Figure 29. Changes in the value type conductivity depending on the temperature *Palmelopsis muralis*

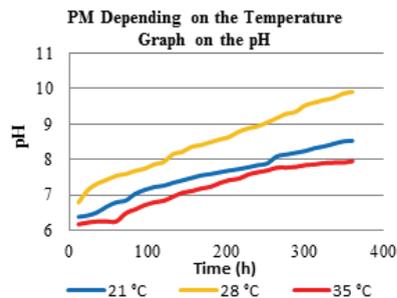


Figure 30. Changes in temperature, pH values, depending on the type of *Palmelopsis muralis*

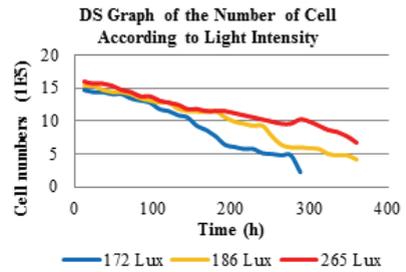


Figure 31. *Dunaliella salina* sp. changes in light intensity, depending on the type of values of the number of cells

Conductivity values, the number of cells as in the case of an increase in conductivity, with the increase was determined of light intensity (Figure 32)

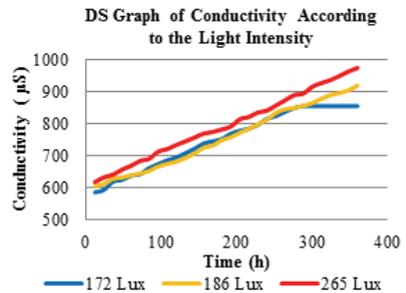


Figure 32. *Dunaliella salina* sp. changes in conductivity, depending on the type of light intensity values

In general sense, there is a decrease in pH data (Figure 33) Light intensity decreases with the increase of pH value decreases (Tawfig et al., 2004; Brown et al., 1989).

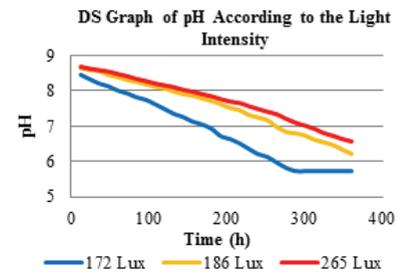


Figure 33. *Dunaliella salina* sp. changes in light intensity, depending on the type of pH values

Light intensity values for the type of *Palmelopsis muralis*, the highest was reached cell number at 265 lux. Figure 34 is the reason

for the decline is the growing number of period due to of 12 hours darkness /12 bright.

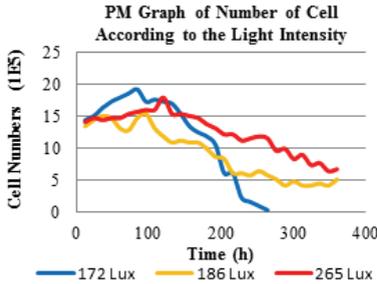


Figure 34. Depending on the type of intensity values *Palmellopsis muralis* changes in the number of cells

Conductivity analysis of the data of the light intensity, as well as the influence on the number of cells is the effect on conductivity and conductivity increased with increasing light intensity (Figure 35) (Demirbaş, 2010; Demir et al., 2007; Eliçin et al., 2009).

Palmellopsis muralis light intensity for the type of experiment, the effects of light intensity were not detected significant in pH changes value (Gökpınar 1983; Scragg et al., 2002) (Figure 36).

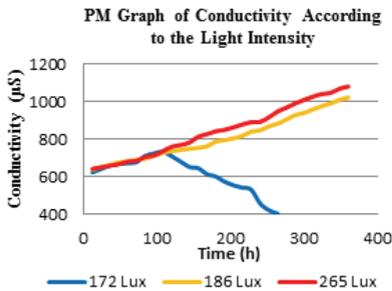


Figure 35. *Palmellopsis muralis* changes in conductivity, depending of the light intensity

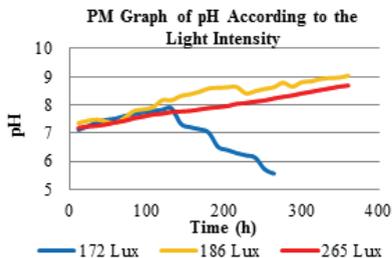


Figure 36. *Palmellopsis muralis* changes in light intensity, depending on the type of pH values

CONCLUSIONS

To obtain the highest cell density culture of microalgae in per unit is objective of the operation. Culture technique used for production process of determining in addition to many factors limiting the process, the culture economy should be noted that an important factor as well. Different production techniques are applied depending on ecologic and economic conditions. For his reason, production enterprises must choose the best methods according to culture purposes.

As a result, *Dunaliella salina* sp. species at relatively high temperatures, salinity, loving and very susceptible to ambient conditions and the external environment, rather than rapidly in closed environments need to be raised because it is contaminated. Show the best growth in the low tempered *Palmellopsis muralis* type and ambient conditions *Dunaliella salina* sp. To be less affected by the contaminated due to the low risk were grown outdoors more comfortable.

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