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# Extraction And Determination Of Chlorophyll Content From Microalgae

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**Abstract**: In this experiment, we selected the two microalgae species, Chlorella and Nannochloropsis for their numerous benefits. The microalgae was cultured with Conway medium under laboratory condition to get biomass for the further experiments. The concentration of chlorophyll a and b was evaluated by these procedures; extraction of chlorophyll by solvent, determination of chlorophyll content by spectrophotometric method and calculation by SCOR-UNESCO equation. So, two types of chlorophyll a and b are present in the green microalgae. According to the results, Chlorella has chlorophyll a (8.45 µg/ml) and Chlorophyll b (4.33 µg/ml), Nannochloropsis has chlorophyll a (21.24 µg/ml) and chlorophyll b (9.66 µg/ml) respectively.

**Keywords:** Chlorella, Nannochloropsis, chlorophyll, spectrophotometric method, SCOR-UNESCO equation

## 1. Introduction

Microalgae are photosynthetic microscopic unicellular organisms capable to convert solar energy to chemical energy. These microorganisms exist individually or in chains or groups [1]. They can be grown with simple growing requirement and its biomass can be used to produce human dietary supplements, animal feed and other beneficial subststances for their high levels of protein, vitamins, pigments, essential amino acid composition, which are not synthesized by the human body. Chlorella is single celled green algae found in bodies of fresh water and contains high concentrations of nutrients such as vitamin C, minerals, carotenoids, vitamin B complex and ion. The algae also contain a high amount of protein and can produce healthy oils high in polyunsaturated fats used as a health supplement for a wide range of conditions. The algae also have the activity to treat bacteria, virus and other conditions such as diabetes, cancer and arthritis. Some cultures also believe that the algal can reverse the aging process if consumed in large enough quantities and cleanse the body [2]. Nannochloropsis is highly potential microalgae which has been widely utilized as animal feed in aquaculture [3]-[4]. Moreover, it has been recognized as one of the most potential photoautotrophic producer of pigment such as chlorophylls and carotenoids [5]-[6]. Pigments are regarded as one of the most potential product from microalgae [7]-[8]-[9]. The significant pigment group are found in microalgae, that is chlorophylls, carotenoids, and phycobilins. All these pigments can be applied in food technology, pharmaceuticals and cosmetics industry [10]-[11]. Chlorophyll is one of the useful bioactive compounds that can be extracted from biomass of microalgae. It has been used as a natural food colouring agent and has antioxidant property [6]. Chlorophyll is a photosynthetic pigment present in green plants that absorb light energy and uses it to produce carbohydrates from carbondioxide and water. Chlorophyll is crucial to the process of photosynthesis, which is responsible for sustaining the light process of green plants. There are multiple types chlorophyll in plants, while chlorophyll b and c are present in plants but are not involved in photosynthesis. Chlorophyll is a natural food colouring agent and is more expensive than artificial colourings [12]. There are two main types of chlorophyll, chlorophyll a and chlorophyll b. However, exposure of chlorophyll molecules to weak acids, oxygen or light accelerated their oxidation and result in the formulation of numerous degradation products [13]-[16]. The skeleton of chlorophyll molecule is the porphyrin macrocycle, which comprises of four pyrrole rings [14] [15]. In chlorophyll b, the methyl group in ring II of chlorophyll a is replaced by a formyl group [15]-[16]. The goal of this study was to evaluate the chlorophyll content of microalgae species.

## 2. Materials and Methods

### 2.1 Strains and Culture methods

The strain Chlorella and Nannochloropsis sp. were obtained from Department of Fishery, Tha-kay-ta Township, Yangon, Myanmar. Stocks were cultured under laboratory conditions with the temperature of 25±2°C, 25 ppt of salinity and photoperiod of 18:6 light/dark cycle in Conway medium. Cultures were initially inoculated with about $5x10^6$ agal cells. Cell density was measured by using improved Neubauer hemocytometer. The experiment was carried out in triplicate for 14 days. The medium composition is described in Table (1). Medium solution was previously sterilized before use.

***Table 1:*** *Chemical Composition of Conway Medium (Walne, 1974) [17]*

| CONWAY MEDIUM (Walne, 1974) | | |
|---|---|---|
| $NaNO_3$ | 100.0 | g |
| $Na_2EDTA$ | 45.0 | g |
| $H_3BO_3$ | 33.6 | g |
| $NaH_2PO_4.2H_2O$ | 20.0 | g |
| $FeCl_3.6H_2O$ | 1.30 | g |
| $MnCl_2.4H_2O$ | 0.36 | g |
| Trace Metal Solution* | 1 | ml |
| Vitamin Mix** | 100 | ml |
| Distilled water (to make) | 1000 | ml |
| *Trace Metal Solution | | |
| $ZnCl_2$ | 2.1 | g |
| $CoCl_2$- $6H_2O$ | 2.1 | g |
| $(NH_4)6\ Mo_7O_{24}\ 4H_2O$ | 2.1 | g |
| $CuSO_4\ 5H_2O$ | 2.0 | g |
| Distilled water | 100 | ml |
| * *Vitamin Mix | | |
| Vitamin B1 | 20 | mg |
| Vitamin B 12 | 10 | mg |
| Distilled Water | 200 | ml |

Utilization: 1 ml Conway medium /litre of seawater

### 2.2 Sample preparation

A volume of 1ml culture sample was withdrawn. Cells were centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and cells were then resuspended in 10 ml of distilled water to remove any salts that could have been retained with biomass and submitted again to centrifugation. This washing process was repeated twice.

### 2.3 Extraction and Determination of chlorophyll

The chlorophyll content was performed by using an extraction with 90% acetone. The obtained algal biomass was added with 90% acetone 1.5ml and homogenized with mortor and pestle. The mixture was incubated in the water bath at 50°C for 30 minutes. The liquid was centrifuged three times in a respective ratio of 1.5 to 8.5ml at 3000 rpm for 10 minutes. The supernatant was then transferred by pipette into a 50 ml centrifuge tube. The solution was allowed to stand for a short period of time prior to an additional 10 minutes of centrifugation. This procedure was completed in subdued lighting. The chlorophyll content of the samples was determined by using the spectrophotometric methods[18]. In order to determine the chlorophyll content of the extract, the sample was measured the absorbance at several wavelengths, between the range of 400 and 700 nm against the solvent (acetone) blank. The concentration of Chlorophyll a and Chlorophyll b were evaluated according to the following SCOR-UNESCO (1966) equations [19]:

Chlorophyll a -

$\mu g_{chlorophyll}/ml_{medium} = (11.64A_{663} - 2.16\ A_{645} + 0.10A_{630})v/\ lV$

Chlorophyll b -

$\mu g_{chlorophyll}/ml_{medium} = (-3.94A_{663} + 20.97\ A_{645} - 3.66A_{630})v/\ lV$

$A_{xxx}$ = the absorbance at xxx nm, after removing the sample absorbance at 750 nm against a blank of the solvent used

v = the volume of acetone used (ml)

l = the spectrophotometric cell length (cuvette) (cm)

V = the sample volume (ml)

## 3. Results and Discussion

In this study, we cultured the two microalgae with initial inoculums of about 5.0 x $10^6$ cells/ml. The growth rate of Nannochloropsis is slightly more than Chlorella. We evaluated the chlorophyll content in the same culture condition. According to the data, the cell counting results are shown in Table (2). Cell density of the two species was gradually increased during the culture period until day 10.

***Table 2:*** *Cell Counting results of Chlorella sp. And Nannochloropsis sp. by using Neubauer Haemocytometer*

| Days | Chlorella sp. | Nannochloropsis sp. |
|---|---|---|
| Day 1 | 4.8 x $10^6$ cells /ml | 6.4 x $10^6$ cells /ml |
| Day 2 | 6.7 x $10^6$ cells /ml | 9.2 x $10^6$ cells /ml |
| Day 3 | 9.6 x $10^6$ cells /ml | 10.0 x $10^6$ cells /ml |
| Day 4 | 9.8 x $10^6$ cells /ml | 10.75 x $10^6$ cells /ml |
| Day 5 | 10.1 x $10^6$ cells /ml | 11.2 x $10^6$ cells /ml |
| Day 6 | 10.95 x $10^6$ cells /ml | 13.40 x $10^6$ cells /ml |
| Day 7 | 11.2 x $10^6$ cells /ml | 13.45 x $10^6$ cells /ml |
| Day 8 | 11.7 x $10^6$ cells /ml | 15.4 x $10^6$ cells /ml |
| Day 9 | 11.8 x $10^6$ cells /ml | 16.8 x $10^6$ cells /ml |
| Day 10 | 16.7 x $10^6$ cells /ml | 20.8 x $10^6$ cells /ml |

The flow diagram for cell counting of Chlorella sp. and Nannochloropsis is shown in Figure (1) and (2). The two species showed similar growth pattern during the culture condition.

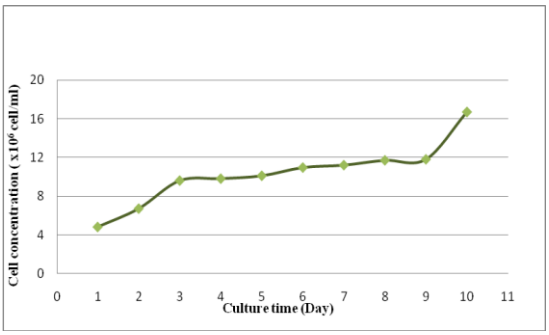


***Figure 1:*** *The Growth Curve for Cell Density of Chlorella sp.*

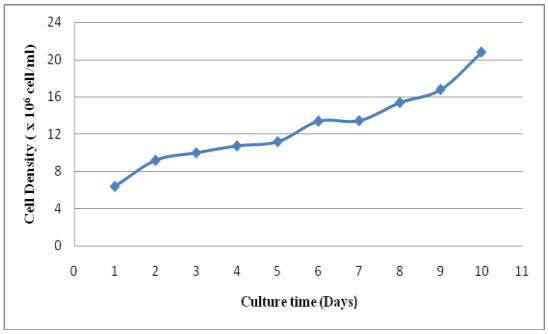


***Figure 2****: The Growth Curve for Cell Density of Nannochloropsis species*

The method for quantifying chlorophyll was performed with biomass from the nearly same cell growth phase. According to Table (3), Nannochloropsis showed a higher content of chlorophyll a and chlorophyll b than Chlorella. Chlorophyll a is the highest levels of pigments found in both species since it

is the main photosynthetic pigment and the other chlorophyll pigments are accessories which may or may not be in combined with chlorophyll a [20]. The concentration of chlorophyll a and chlorophyll b of Chlorella sp. and Nannochloropsis sp. was described in Table (3).

***Table 3:*** *The concentration of Chlorophyll a and b of Chlorella and Nannochloropsis sp. during culture period*

| Chlorella sp. (µg/ml) | | Nannochloropsis sp. (µg/ml) | |
|---|---|---|---|
| Chlorophyll a | 8.45 | Chlorophyll a | 21.24 |
| Chlorophyll b | 4.33 | Chlorophyll b | 9.66 |

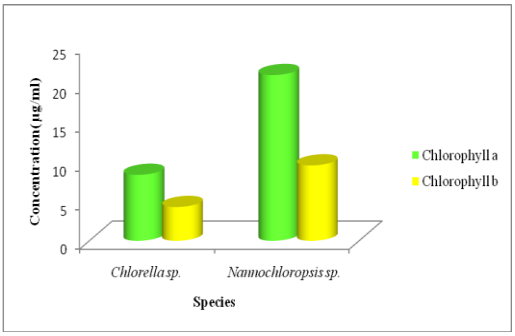


***Figure 3:*** *Chlorophyll a and b Content of Chlorella and Nannochloropsis sp.*

There are many parameters that influence the chlorophyll concentration. The microalgae culture medium may contain various amounts of non-conservative nutrients such as phosphate and nitrate. So, the nutrient composition of the culture medium may also influence the content of chlorophyll. Moreover the selection of the solvent is always important issue because more aggressive solvents can increase the extraction yield in cell and chlorophyll content.

## 4. CONCLUSION

In pigment quantification and their correlation to the amount of biomass present in the sample, it is important to take into account the structure of cell wall, use of additional rupture techniques to be beneficial in chlorophyll extraction. The concentration of chlorophyll may vary in species to species. In addition, to get more chlorophyll content, we need to investigate the significant factors that influence the growth, because high cell concentration resulted in high chlorophyll value [21]. In the literature, Chlorella has one of the highest chlorophyll contents found in nature [22]. However, this work confirmed Nannochloropsis sp. is pointed out as good source for chlorophyll quantification. Moreover, Microalgae seem to be a promising alterative source for chlorophyll.

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