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Extraction of phycobiliprotein pigments from *Oscillatoria princeps* by using different methods

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Abstract - In the current study, one isolation was obtained from the Cyanophyta Oscillatoria princeps which was collected from pond water located in Khor Al-Zubair/Basrah during August 2018. The current study aims at extracting and purifying of some effective pigments from the above isolate, test them chemically. Allophycocyanin (APC) pigments were highest among other pigments in sample removal-oil compared to the quantities of concentrations of phycoerythrin (PE) and phycocyanin (PC) pigments. The stationary phase in the industrial media have produced the largest amount of pigments. Phycobiliproteins were observed through the UV-Visible peaks. Freeze-dried samples or removal-oil showed that phycobiliproteins aren't affected by increasing or decreasing the sonication period. The study showed that it's possible to use different media for pigments extraction as buffers and acetate buffer has been mastered. The freezing-thawing method is the best compared with other methods, the concentration of phycoerythrin (PE) pigments began to increase gradually from 6-12 days at PC and PE pigments extract, respectively. According to the statistical analysis, there are significance differences in Phycoerythrin. pigments concentration from 9th day to the 12th day.

استخلاص صبغات Phycobiliproteins المنتجة من Oscillatoria princeps باستخدام طرائق مختلفة

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المستخلص - تم الحصول على عزلة محلية واحدة من الطحالب الخضر-المزرقة، وجرى تنقيتها وتشخيصها الى النوع Oscillatoria princeps. أظهرت النتائج ان كمية صبغة Allophycocyanin (APC) هي أعلى مقارنة بكميات Phycoeryhthrin و Phycocyanin المستخلصة من العينة الحقلية. وقد سجل طور الثبات وفي الوسط الزرعي الصناعي Chu-10d أكبر كمية من الصبغات. وقد تبين من خلال قمم الأشعة فوق البنفسجية-المرئية. ان صبغات Phycobiliproteins لم تتأثر بعملية تجفيد العينة الأصلية أو إزالة الزيوت منها كما لم تتأثر بزيادة فترة الذبذبات فائقة السرعة او نقصانها. وأظهرت الدراسة إمكانية استخدام منظمات مختلفة، لغرض استخلاص الصبغة، وكان أفضلها منظم Acetate. وجاءت طريقة التجميد والذوبان في استخلاص PC و PE على التوالي هي الأفضل مقارنة مع الطرائق الأخرى. لوحظ زيادة تركيز صبغة PE تدريجيا من 6- 12 يوما، ووفقا للتحليل الإحصائي، هنالك اختلافات واضحة من يوم إلى آخر ابتداءً من اليوم التاسع ولغاية اليوم الثاني عشر.

الكلمات المفتاحية: صبغة الفايكوسيانين، خور الزبير-البصرة، Oscillatoria princeps، صبغة الفايكوبيلبروتين، صبغة الفايكوارثرين.

Introduction

Cyanophyta is a morphologically. diverse. and. widely. distributed group of photosynthetic prokaryotes. that. exhibit. oxygenic. (O_2) photosynthesis. Similar to plants (Whitton and Potts, 2000). The genus. *Oscillatoria* is. one of. the. dominant blue-green algae (Cyanophyceae). which.

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grows. in. various. habitats (Galetović *et al.*, 2020). Cyanophyta. have a wide range. of. colored. components, including carotenoids, . chlorophyll, and phycobiliproteins (Fig. 1).

Phycobiliproteins are deep-colored, water soluble, fluorescent proteins that can be found in microalgae, especially Cyanophyta, e.g. *Spirulina platensis* and Rhodophytae ex; *Galdieria*. *sulphurari*. and Cryptophyta. Phycobiliproteins. are constituents. of. the. subcellular. structures called. phycobilisomes, that were attached. to the. outer surface. of the. thylakoid. membranes (Galetović *et al.*, 2020), phycobilisomes are covalently. bound via. cysteine. amino. acid. chromophores. (Mulders *et al.*, 2014).

They. can capture. light. energy and. pass. it to chlorophylls. during. photosynthesis. (Watanabe and Ikeuchi, 2013). Phycobilisomes (PBPs) are beautifully colored as a result of one to three linear tetrapyrrole prosthetic groups, or bilins, connected to their backbone PBP which was made up of two types of polypeptide, one had low molecular weight (MW: 12-19 KD) and the other with a high molecular weight (MW: 14-21 KD) (Mörschel *et al.*, 1980).

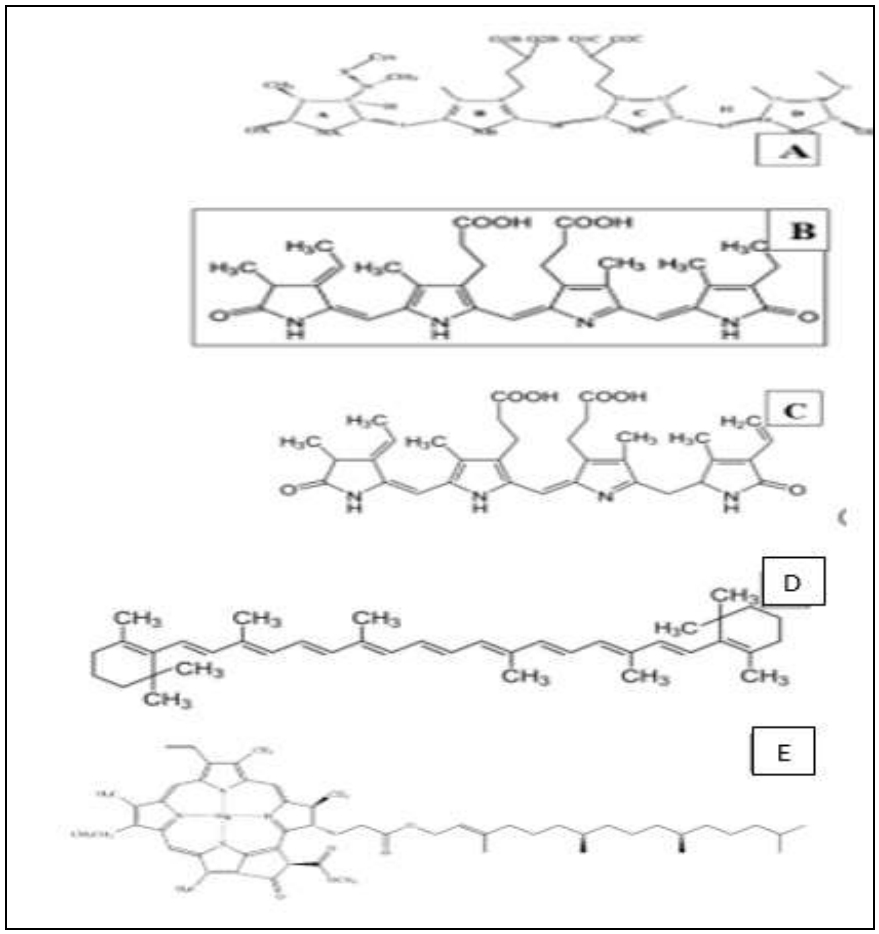

Figure 1. Bio-active structures of *Arthrospira* cells pigments; (A) Allophycocyanin (APC), (B) Phycocyanobilin (PC) and Allophycocyanin, (C) Phycoerythrin, (D) B-carotein, (E) Chlorophyll (Zarzuelo *et al.*, 1991; Al-Mousawi, 2007).

Generally, phycobiliproteins are categorized into phycoerythrin (PE), a red pigments; phycocyanin (PC), a blue pigments; allophycocyanin (APC), a light blue pigments; and phycoerythrocyanin (A max =575-585 nm; F max =625-635 nm), which would be those

essentially discovered. Phycobiliproteins (PBPs) have various as depicted in Figure (2), following the brief documentation of the application of Phycobiliproteins (PBPs) in various fields.

However, toxicity testing is still required to provide additional justification for the usage of phycobiliprotein in food. In contrast to other pigments, only in the instance of C-Phycocyanin are categorical data on the safety of usage in food accessible; toxicological study is required for the other pigments. Thermostability and aqueous stability must be improved, pH stable, alcohol resistance, light stable, and shelf life of the pigments (Dufossé *et al.*, 2005).

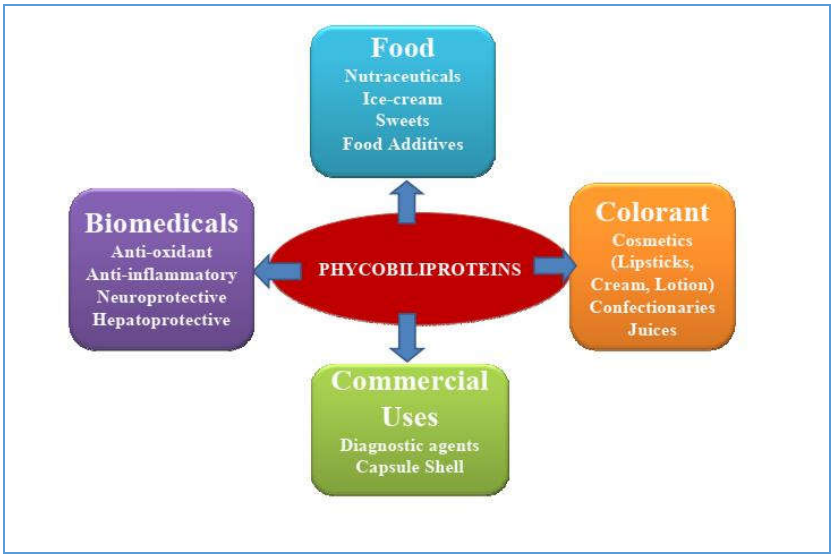

Figure 2. Phycobiliproteins and its potential commercial applications (Al-Mousawi, 2007).

Materials and Methods

Preparation and Sterilization of Media:

Liquid and Solid Chu-10D Media:

The culture media Chu-10D was chosen to isolate, diagnose, and culture *O. princeps* according to Kumar *et al.* (2014); Al-Mousawi and Alwan (2017); Mahmood *et al.* (2017).

Natural Media:

This medium was prepared according to (Al-Mousawi, 2007).

Enrichment Media:

Water samples were distributed in sterile glass flasks by adding 2 liters of pond water per flask, the first flask was treated with the addition of 2ml of $NaNO_3$ to give a final concentration of 50 micromol/l, which represents the treatment of nitrates (+N) alone. The second flask with the addition of 2 ml with addition of potassium phosphate K_2HPO_4 to give a final concentration of 5 micromol/l and this represents the treatment of phosphate (+P) alone, the third flask include the mixing of the two treatments above which then combined to represent the treatment of nitrates and phosphates (+NP) together. The fourth flask (control sample) was left blank for comparison (Al-Mousawi, 2007).

Identification of Algae:

The collected samples were examined according to the following steps; after washing the sample with water and using the sieve to remove the impurities from the sample. A slide was prepared from the collected sample and tested using a composite optical microscope at power of 40X. The following sources were adopted in the diagnosis of the alga (Desikachary, 1959; Prescott, 1978). Algae database (accessed online at http://www.algaebase.org).

The streaking method of the solid Chu-10D medium and the dilution method of the liquid medium were adopted to obtain unialgal axial isolates (Stein *et al.*, 1996). The algae were isolated from the germs depending on the method described by Littler and Littler (1985), the algae were washed with sterile water and then centrifuged at 3000 rpm/min for 50-90 seconds.

The process was repeated at least 12 times, the isolates were incubated on Chu-10D media agar at 25±2 °C, Intensity of illumination 200 Lux, to isolate *O. princeps* from other algae, then *O. princeps* was incubated on nutrient agar (NA) at 37°C for 18 hours, and to examine the absence of bacteria the process was repeated several times until there is no growth of bacteria was found, indicating the purity of the axenic culture.

Estimation of Growth Rate:

The estimation of the growth rate of algae according to dry weight method, algal growth was separated from growth media (log and stationary phase) by centrifugation for 30 min at 3000 rpm/min. After decanting the supernatant, the algal pellets were transferred to known wight vail, dried at 105°C for 48 hr. Then, estimated the difference in weights between the vails (Al-Mousawi *et al.*, 1984).

Preparation of Biomass of Algae:

Harvested algal isolation *O. princeps* in the logarithmic and stationary phases, by centrifugation of the sample and freeze-dried by a dehydrator, the samples were kept in clean, sealed glass containers at 4°C.

Isolation of Oil from Algae:

Gram of (Freeze-drying sample) was weighted then mixed with 250 ml of hexane represented the motile phase. The continuous extraction process was conducted using the Soxhlet at 40°C to obtain a vicious and yellowish liquid (Zarzuelo *et al.*, 1991).

Production of Phycobiliproteins (PBPs):

Optimization of PBPs extraction protocol was done concerning five buffers, cell disruption technique, and protein precipitating agents 0.5 M Tris-HCl in pH 7.0 (Littler and Littler, 1985); 0.5 M acetate buffer containing 0.002 M sodium azide pH 5.1 and 50 mM sodium chloride (Kumar *et al.*, 2014); pond water; double distilled water and Chu-10D media were used.

According to the method described in Abalde *et al.* (1998) and Simeunović *et al.* (2012); once volume of the algae biomass were re-suspended in 5 volumes of each buffer (above), to extract pigment after splitting the cell by mashing the algae with a mortar for at least 5 min, until the algal sample is converted into a watery emulsion. The algal emulsion was exposed to a 10 min Sonication of samples (3 sec. shaking and 3 sec. pause), after Sonication of samples the separation of fragments cell was conducted by centrifuge at 12000 rpm/10 minute, another method was also used for comparison with the aforementioned method.

Another method was also used for comparison with Simeunović *et al.* (2012) where freezing-thawing of samples for 2-12 days using acetate buffer only was conducted. The samples were preserved at a temperature of 4°C until bioactivities were performed.

The concentration of pigments was deterring by using the following formula (Bennett and Bogorad, 1973):

PE (mg/ml) = (A562 - 2.41 * PC-0.849* APC) / 9.62
PC (mg/ml) = (A612 - 0.472 * A652) / 5.34
APC (mg/ml) = (A652 - 0.208 * A615) / 5.09

Where:
PE= (Phycoerythrin)
PC= (Phycocyanin)
APC= (Allophycocyanin)
A= Absorbance

The samples were preserved at a temperature of 4 °C until bioactivities were performed.

Chemical Identification of Crude Phycobiliproteins UV-Visible Spectroscopy Identification:

The absorbance spectra for optimizing extraction and freezing-thawing for several days were determined by scanning the sample in the range of 200-700 nm wavelengths. Samples were measured in the laboratory equipment in the Department of Chemistry/College of Science, University of Basrah.

Statistical Analysis:

The statistical SPSS V. 21 program Statistical Package for Social Sciences was used for the statistical analysis. To analyze the data statistically and each according to it's appropriate test, the arithmetic averages using the least significant differences at the level of significance ($P \leq 0.05$), was applied.

Results

Description of Isolated Species:

The results of the present study through the isolation of one alga was done according to Desikachary (1959) and Prescott (1978), and the Algae database accessed online at http://www.algaebase.org.

Empire: Prokaryota
Kingdom: Eubacteria
Subkingdom: Negibacteria
Phylum: Cyanophyta
Class: Cyanophyceae
Subclass: Oscillatoriophycideae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: *Oscillatoria*
Species: *Oscillatoria princeps*

Oscillatoria princeps is simple filamentous, in which the shell is transparent, the alga was dark blue-green, mature trichome straight, cells much broader than long, not constricted cross wall, the optical cell flatly rounded, slightly capitate. The size varies from 25-50 µ in width and 2.5-7 µ in length (Fig. 3).

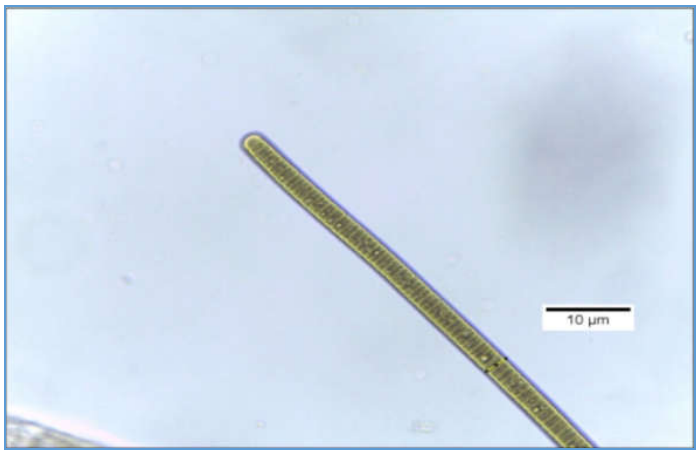

Figure 3. *O. princeps* at (40X) isolated from Basrah city.

Estimation of Growth Rate:

The results of the growth curve of *O. princeps* isolate grown in different media are show in Table (1), which indicated that there was significance growth. The lag phase began in the first four days in terms of dry weight. The phase of exponential growth began from the fourth to the thirtieth-day, where noticeable growth was not observed. The start of the stationary phase lasted until the 40th day, the beginning of the decline phase started from day 40, and later. Based on growth rate (Fig. 4), which showed that the medium (Chu-10D) is the lowest growth medium for isolated algae compared to the other media, natural medium, (+N), (+P), and (+NP) media came after Chu-10D media, respectively.

The results of the statistical analysis showed that there was a significant difference between the treatments at the probability level ($P \leq 0.05$) for growth rate values according to weight, the highest growth of *O. princeps* was 0.119533 ± 0.0049863 mg/dl at (+NP) media, while the growth of *O. princeps* was 0.013933 ± 0.0028290 mg/dl at (Chu-10D media).

Table 1. Growth phases measured in days of *O. princeps.*

Media	Growth Phases by days			
	Lag Phase	Log Phase	Stationary Phase	Decline Phase
Chu-10D media	0-4	4-30	30-40	40-
Natural media (+N)	0-4	4-30	30-35	35-
Natural media (+P)	0-4	4-30	30-35	35-
Natural media (+NP)	0-4	4-20	20-40	40-

Natural media	0-4	4-30	30-35	35-

Production of Phycobiliproteins (PBPs):

Extraction of Phycobiliproteins from Field Sample:

The PBP pigments were extracted from *O. princeps* isolated when the samples were brought directly from the field and called the field sample (control), another sample was freeze-dried called (field sample dehydrate), and the third sample was called (removal oil sample).

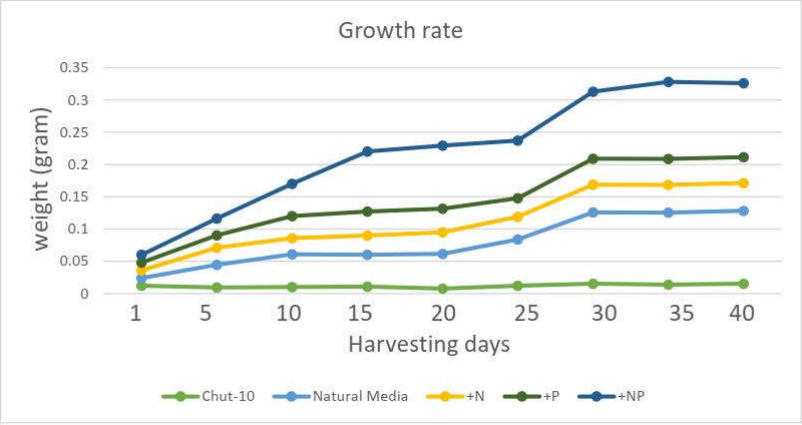

Figure 4. The growth curve of *O. princeps*, grown in several culture media using dry weight method.

Based on the results shown in Figure (5A), the APC pigment was highest among other pigments with concentrations of 0.499 mg/ml in the removal oil sample, followed by the PC pigment at a concentration of 0.296 mg/ml, in the freeze-dried sample.

Figure (5B) showed a comparison of sonication time on the number of pigments extracted from *O. princeps.* It showed that the concentration of APC pigment was highest with sonication of 10 min among other pigments with concentration of 0.493 mg/ml in the field sample.

The results of the statistical analysis showed that there was a significant difference between the treatments at the probability level of $P \leq 0.05$ for Phycobiliprotein values according to treatment quality, the highest value (mean ± SD) was 0.4985 ± 0.00071 mg/ml at Allophycocyanin removal oil, while the lowest value was 0.2063 ± 0.06994 mg/ml at Phycocyanin freeze-dried.

The results of the statistical analysis of sonication period showed that there was a significant difference between the treatments ($P \leq 0.05$) for Phycobiliprotein values according to the treatment quality, the highest value was 0.4920 ± 0.00141 mg/ml at Allophycocyanin field sample at 10 minutes. While the lowest value was 0.0845 ± 0.00071 mg/ml of Phycoerythrin field sample at 3 minutes.

Extraction of Phycobiliproteins (PBPs) Using Different Buffers:

Figure (6) illustrates the use of different buffers on phycobiliprotein extraction from *O. princeps* by the freezing-thawing method. There were clear differences in the number of pigments extracted from *O. princeps* using different buffers, it was found that acetate buffer was the best one in comparison with other extracted buffers.

Moreover, Figure (6) showed that extract of PC pigments with Acetate buffer of using acetate buffer was high at concentration of 0.081 mg/ml, whereas the PE pigment had a concentration of 0.042 mg/ml.

The results of the statistical analysis showed that there were significant differences at the probability level of $P \leq 0.05$, the highest value was 0.0815 ± 0.00071 mg/ml of Phycocyanin in acetate buffer. While the lowest value was 0.0240 ± 0.00001 mg/ml of Phycoerythrin in Tris buffer.

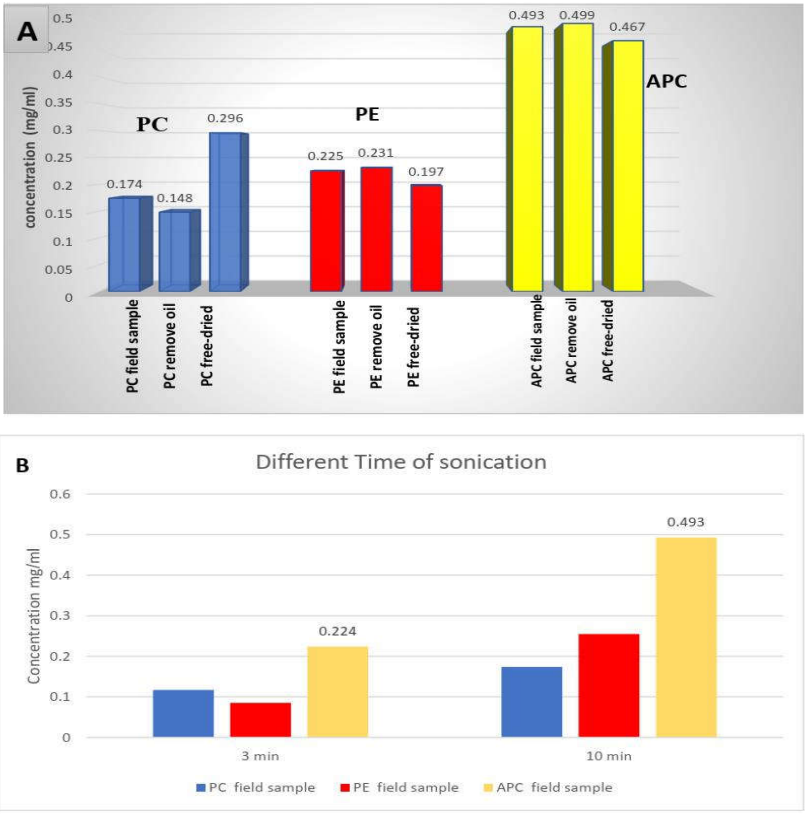

Figure 5. Phycobiliprotein concentrations production from *O. princeps*; (A) Comparison between different treatments of sonication (control) samples; (B) Comparison between different sonication field samples (control).

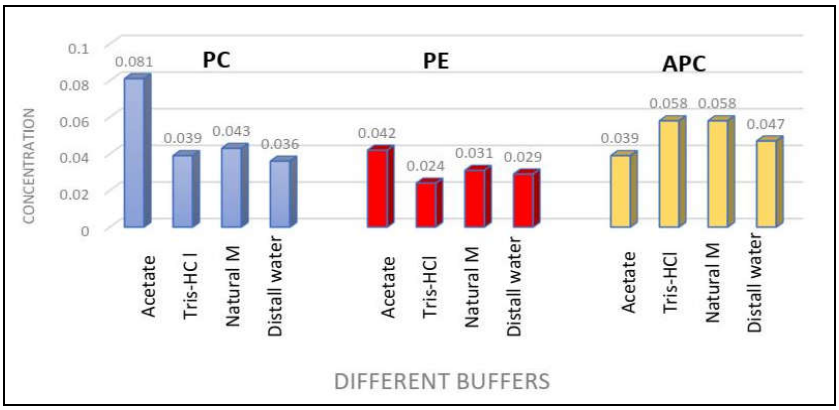

Figure 6. Phycobiliprotein concentrations extracted from *O. princeps* by different buffers. PC-concentrations of phycocyanin, PE-concentrations of phycoerythrin, APC-concentrations of Allophycocyanin.

Effect of Time of Freezing-Thawing on Phycobiliprotein (PBP) Extraction:

The effect of Phycobiliprotein-pigment extraction using the freezing-thawing method was demonstrated, in terms of increased concentration of Phycoerythrin, Phycocyanin, and Allophycocyanin. for several days (Fig. 7), which indicates the use of two methods of extraction using acetate buffer. The freezing-thawing method lasted for 12 days, which is the best time for extracting the PE and the PC pigments compared with the method conducted by Al-Mousawi (2007). The results showed that there were no pigments extraction from *O. princeps* during the first five days. On the sixth day, the extracted pigments were observed and the concentration of PC was 0.013 mg/ml, PE was 0.0095 mg/ml and the APC concentration was 0.0038 mg/ml. On the twelfth day, the PC concentration was 0.0851 mg/ml, while the PE concentration was 0.2711 mg/ml and the APC concentration was 0.0268 mg/ml.

Statistical analysis results showed significant differences at $P \leq 0.05$ in Phycobiliprotein concentrations of different days of freezing-thawing treatment, the highest value was 0.2710 ± 0.00014 mg/dl of Phycoerythrin in twelve-day, while the lowest value was 0.0269 ± 0.00007 mg/dl of Allophycocyanin in twelve days.

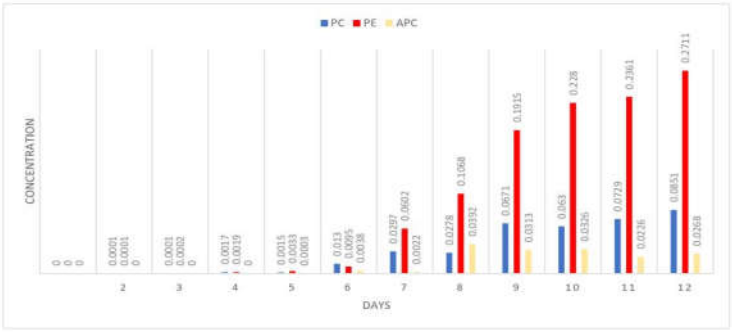

Figure 7. Phycobiliprotein concentrations extracted from isolates of *O. princeps* by acetate buffer, and Freezing-thawing method for 2-12 days.

Effect of Phases of Algal Growth on Concentration of Phycobiliproteins Extract:

To determine the optimal growth phase of *O. princeps* in the extraction of phycobiliprotein pigments (PBP), these pigments were extracted from *O. princeps* in two phases of growth the stationary (Fig. 8A) and logarithmic phase (Fig. 8B).

According to the results of using different media, the stationary phase showed clearly that the concentration of the PC pigment is the highest in the logarithmic phase 0.4652 mg/ml using natural media, while the PE concentration came second with a concentration of 0.155 mg/ml. The logarithmic phase showed the concentration of the three pigments extracted from *O. princeps* in the stationary phase. The PC pigment was also prevalent over the rest of the pigments with a concentration of 0.5324 mg/ml in the Chu-10D, while the concentration of PE pigment came in the second place with a concentration of 0.1728 mg/ml in the same medium. The concentration of APC pigment was the lowest in both the logarithmic (Fig. 8B) and the stationary phase (Fig. 8A) in all the media. The results of the statistical analysis at the probability level $P \leq 0.05$ for significant differences in the extraction of Phycobiliprotein from the growth of the phase of *O.*

princeps has shown that the stationary phase is the best in the extraction of pigments, the highest value was 0.5347±0.00325 mg/dl of Phycocyanin in Chu-10D, while the lowest value was 0.00035±0.00025 mg/dl of Phycoerythrin in (+N) media.

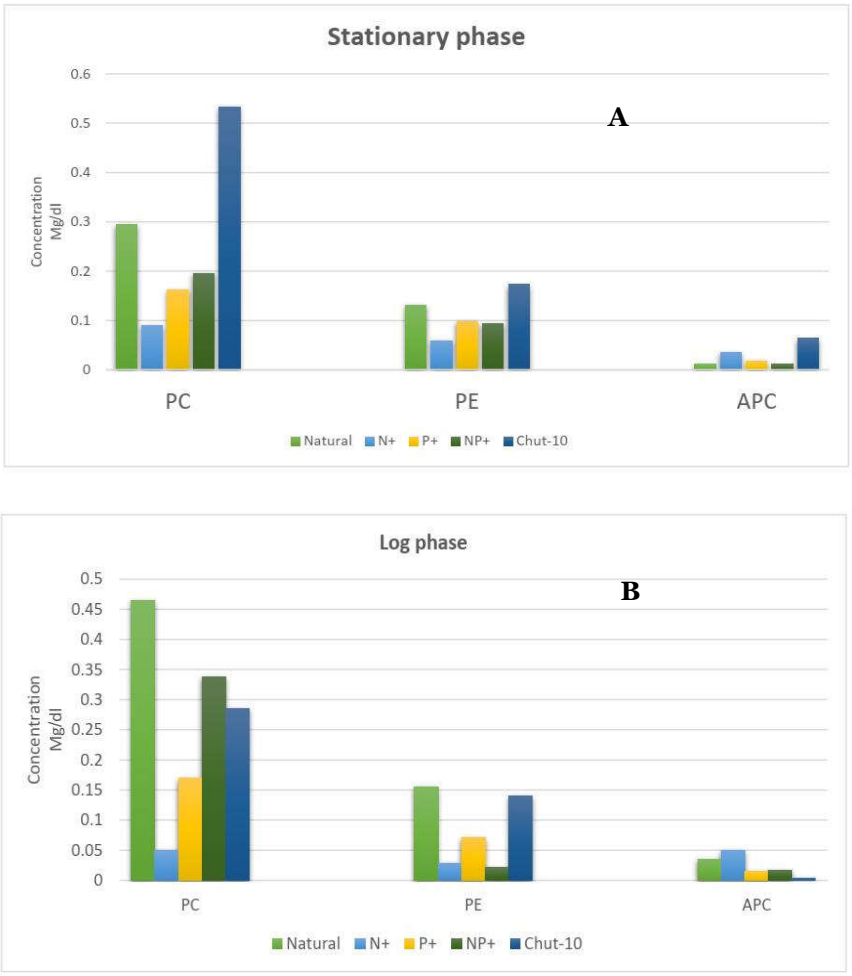

Figure 8. Phycobiliprotein concentrations extracted from *O. princeps* growing in different media during (A) stationary phase and (B) Log phase

Chemical Identification of Phycobiliproteins Extract:

UV-Visible Spectroscopy Identification of Crude Extraction:

Figures (9A, B, C and D) showed that the absorbance spectra for optimizing phycobiliprotein extraction using UV-visible spectrophotometer. The value of phycocyanin was the lowest obtained from the field sample of 0.25 at a wavelength of 630 nm (Fig. 9A), while the pigment value of Phycoerythrin was 0.22 at 579 nm (Fig. 9B). There were similarities of peaks of the two Figures (9C and D), there were no differences in the case of increasing or decreasing sonication.

Figure (10) showed the absorbance spectra of Phycobiliprotein extraction using several buffers. Various buffers were used to extract Phycobiliprotein pigments from *O. princeps*, indicated clearly from Figures (10A, B, C and D), that the use of Tris-HCl buffer and Chu-10D as buffer, had no differences in between Figures (10 A and B). Using distilled water or pond water as buffer extraction compared with acetate buffer, exhibited no differences in between Figures (10C, D and E).

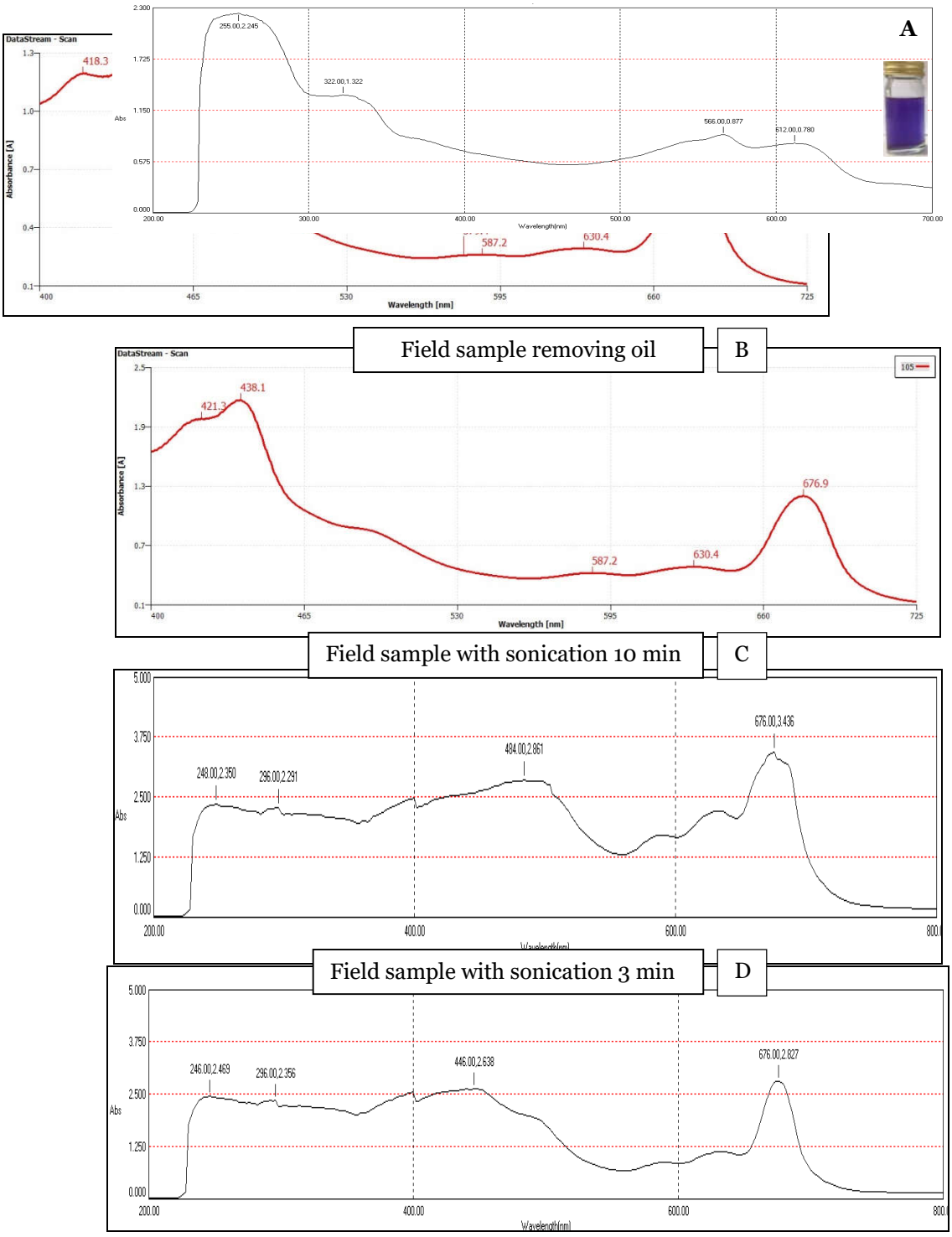

Figure 9. The ultraviolet spectrum of the Phycobiliprotein pigments derived from *O. princeps*; (A) Field sample dehydrate, (B) Field sample removing oil, (C) Field sample with sonication for 10 min, (D) Field sample with sonication for 3 min.

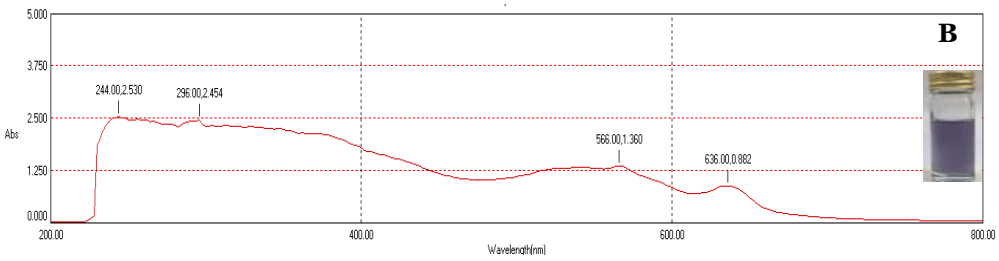

Figure 10. The ultraviolet spectrum of phycobiliprotein pigments derived from *Oscillatoria princeps* using several buffers; A: Chut-10 media, B: Tris-HCl buffer, C: Acetate buffer, D: Pond water, E: Distilled water

Discussion

Cyanophyceae today, usually referred to as Cyanophyta. The term Cyanophyta acknowledges that these prokaryotic bacteria are not eukaryotic algae (Simeunović *et al.*, 2012). *O. princeps* was isolated from one of the farms of Khor Al-Zubair in the surface of rocks (Epilithic algae) (Fig. 3), the cyanobacterium isolate was widely distributed (cosmopolitan) growing in freshwater, sea-water, moist soil, and rock, and this species has been recorded in the Iraqi environment by Lee (2008) and Sharmila Banu *et al.* (2017).

The presence of sheath that surrounds most of Cyanophyta makes it an appropriate environment for the growth of other germs with it, so it must be disposed of and obtain pure isolates in mechanical or physical methods. For this purpose, we have used a re-wash purification and pure isolates have been obtained (Al-Shaheen, 2002). However, indicated that it is not possible to grow many germs on sterile laboratory media. Thus, taking advantage of these pure isolates to obtain phycobiliproteins or active substances (Al-Safi and Al-Mousawi, 2012), and to prevent interference that may occur between algae and bacterial products.

Estimation of Growth Rate:

The growth rate in this study was measured in the dry weight method. Dry weight measurement is a common method for measuring algae (Zarzuelo *et al.*, 1991). Where dry weight was observed over time (Fig. 4 and Table 1), this may be ascribed to a growth in the quantity of cells, an increase in cell size, or an increase in the accumulation of photosynthesis-produced substances including protein, lipids, pigments and stored meals. it causes weight to grow (Bakiyaraj *et al.*, 2014). The response of the blue-green algae *O. princeps* to the natural nutrient-supported medium (+NP) nitrogen and phosphorus were observed together and reached a stabilization phase in 20 days (Fig. 4). This is may be due to the physiological state of the algae understudy as when they transferred from an exposed earth environment to a laboratory, they may suffer from a lack of nutrients (Huang *et al.*, 2019. Also, the natural medium contains trace elements (Co, Fe, Mn and Cd) necessary for algal growth in addition to buffers for growth and enrichment with nutrients (+NP), hence become a good medium for the growth of the blue-green algae *O. princeps* compared with other culture media (Yang *et al.*, 2018).

The high demand for nutrients leads to rapid growth and attaining the stationary phase over a short period (+NP) (Fig. 4), and reaching the decline phase in a period of ten days because lighting is a determining factor for the growth, which leads to death and reaching the decline phase (all transactions) as well as the penetration of nutrients on the culture. The present results

supported those of Wells *et al.* (2017). The growth reached the stability in the mean (+N), (+P) and brackish water after a lapse of thirty days due to the decrease in the quantities of phosphates and nitrates.

Production of Phycobiliproteins (PBPs):

Extraction of Phycobiliproteins from Field Sample:

Figure (5A) indicated that the extraction of the PBPs pigments from the sample (field sample, dehydrated, and de-oiled) using a method described in Simeunović *et al.* (2012) had significant differences in the values of the three extracts. The results showed that the quantities extracted of APC pigments from the field sample are the highest values compared to the quantities of PE and PC, as these pigments may be more adapted to the environmental conditions of temperature, dryness and salinity.

It may indicate abnormal conditions experienced by *O. princeps.* Therefore, it may increase the protection of other photosynthesis pigments such chlorophyll a, the main pigment in photosynthesis. While in laboratory forms (Fig. 8), conditions have become more suitable for the studied algae, so there is no need to increase the pigment (APC).

Also, it was shown through the UV-visible peaks of the extraction methods (Fig. 9), that the peak of phycobiliproteins pigments were not affected by the process of freeze-drying of the original sample or removal of oil from it (Figs. 9 A and B). Also, Figures (9 C and D) showed the same peaks for measuring the UV-light spectrum. This indicates that pigments are not affected by increasing or decreasing the sonication period. The cellular wall of *O. princeps* contains high levels of fat because it is gram-negative bacteria compared to the rest of algae in addition to the presence of Sheath (Praba *et al.*, 2016). It also, indicated that a difference in the number of PBPs varies according to the duration of the sonication (Yang *et al.*, 2018), and therefore, from the above form, it was shown that the amount of PBPs in the sonication period of ten minutes is higher than that of the three minutes' period (Fig. 5B)

Extraction of Phycobiliproteins (PBPs) using Different Buffers:

The quantity and quality of pigments used differed, according to the different buffers used to extract the PBPs pigments, as PBPs pigments can be extracted with a number of buffers (Sarmah and Rout, 2018). It was found in the present study that acetate buffer is the best in extracting pigments PBPs (Fig. 6), and this result agreement with those of Kumar *et al.* (2014) and Mühlsteinová *et al.* (2018). There appear to be similarities in the extraction process (Figures 6 C and D), this is because of the proportions of the salt concentrations in the buffers used for extraction. This is in accordance with the findings of Silva *et al.* (2009) that the local concentration and pH have an effective role in extracting the PBPs pigments.

From the conclusion of the present results, that all buffers have the ability to extract PBPs pigments at different concentrations, which means the possibility of using brackish water and distilled water as buffers and this is very economic as they are inexpensive and available in comparison with other buffers, such as acetate and Tris HCl buffer. So the freezing-thawing method in the PC and the PE extracts are the best compared to the method described in Simeunović *et al.* (2012) (Fig. 7). This approach has various benefits, including speed, simplicity, and independence from biomass quantity (Ruiz-Domínguez *et al.*, 2019). Since the cell is damaged when it freezes, there is no intracellular ice formation, making the extraction of intracellular components is very easy.

Effect of time Freezing-Thawing on Phycobiliproteins (PBPs) Extraction:

Many researches have indicated that relying on the results of the statistics may show that the freezing-thawing process at a temperature of (-4°C to -20°C) which leads to the process of extraction in one way and without reverse reactions which thus leads to the prevention of any enzymatic reaction, as indicated by extracting at a temperature of 25°C on the same pigments, whether they were PE, PC and APC (Moraes *et al.*, 2011; Yücetepe *et al.*, 2018). The present results matched with the suggestion that the best pavers for PE extraction are acetate buffer for a maximum of three days (Al-Mousawi *et al.*, 1984), while the present method was modified to extract the PE and replaced the acetate buffer with natural water for a period exceeding twelve days (Fig. 7) as the concentration of PE pigment begins to increase gradually from 6 to 12 days. However, the statistical analysis, showed significant differences from day to the other begins from 9^{th} days to 12^{th} days. We noticed the highest of the PE summit increase with increasing in number of freezing-thawing days through the UV-light spectrum, and noticed that no other peak appears, which means that the freezing-thawing method extracts the PE pigments which are pure (Kamble *et al.*, 2018).

Through Figure (8) it is clear that there are significant differences between freezing-thawing and sonication and as Uju *et al.* (2020), indicated that sonication breaks down the cell wall and exits most of the unwanted pigments in addition to the enzymes, namely lysozymes which analyzed pigments and become difficult to control sonication frequencies, but this process varies from one algal species to the another according to the thickness of the cell wall. It is emphasized Kumar *et al.* (2014) and Choi and Lee (2018) that the freezing-thawing process does not have any effect on the active components of the cell and does not affect the degradation of pigments because it is at low-temperature cycles and therefore is the most effective process.

Effect of Phases on Algal on Concentration of Phycobiliproteins (PBPs) Extraction:

Every distinct Cyanophyta strain has a unique composition and quantity of Phycobilin pigments, which is highly dependent on growth conditions and cultivation medium (Simeunović *et al.*, 2012). From Figure (5A), the APC pigments have a high concentration in the field sample, it is very much decreased in the laboratory sample (Fig. 5A). The cyanobacterium may use these pigments (APC) to protect, especially, chlorophyll-a from the effect of high rays such as UV-light or IR, dehydration or heat so that it increases in the field, and when the algae are laboratory cultivated and the normal condition returned, the pigments returned to their normal proportion. The results indicated that the quantities of PC; PE extracted from the stationary phase are more compared to the log phase, this is consistent with the results of Horváth *et al.* (2013). While other studies (Nur *et al.*, 2019); acetate was employed as a buffer and freezing-thawing was utilized to extract PE pigments from *Spirulina platensis*; observed a large quantity of PE pigments during the log phase. However, through the results in Figure (9), it was found that Chu-10D medium is the best producing and extracting of the pigments, compared with enrichment media. The results agree with Simeunović *et al.* (2012), who found that higher contents of phycobiliproteins in most strains grown in nitrogen-free medium (-N), compared to presence of nitrogen (+N), because the synthesis medium (Chu-10D), is not good for the growth (Fig. 4), so increasing the secondary pigments, like PC or PE to protect the chlorophyll-a. While, the natural enrichment medium (+NP) is more active for the growth, of cyanobacterium, so the pigments are in their normal proportion.

Conclusions

- The cyanobacterial strain has an industrial potential to produce phycoerythrin pigment because of its filamentous nature, and it is easier to harvest than the other unicellular cyanobacterium.
- Freezing-thawing method are found to be a better process than other chemical and mechanical processes of extraction of pigment.
- The phycobilin pigment (PBP), can be extracted with a number of buffers, and it was found the acetate buffer is best in extracting pigment.
- It is possible replace to industrial media (Chu-10D) the high cost with natural media (pound water) the low cost, also it is content almost the essential nutritional ingredients needed algae.
- The study explanation, the stationary phase is best to extract phycoerythrin pigment in industrial media (Chu-10D).

References

Abalde, J.B., Liliana, T., Enrique, C.A. and Barwell, C. 1998. Purification and characterization of phycocyanin from the marine cyanobacterium *Synechococcus* sp. Plant Sci., 136: 109-120. https://doi.org/10.1016/S0168-9452(98)00113-7

Al-Mousawi, N.J.M. 2007. Isolation and identification of some active compounds from some blue-green algae (Cyanophyta) and testing their biological activity against bacteria and fungi. Ph.D thesis, College of Science, University of Basrah.

Al-Mousawi, N.J. and Alwan, A.A. 2017. Extraction of carotenoid pigment from halotolerant green algae *Dunaliella* sp., for plant and animals tissues. Al-Kufa Univ. J. Biol., 9: 190-209 (In Arabic).

Al-Mousawi, A.H., Hadi, R.A., Kassim, T.I. and Alaami, A.A. 1984. Biological studies on algae in rice-field soil from the Iraqi marshes. Ph.D. thesis, University of Durham, England, 361p.URL

Al-Safi, A.G. and Al-Mousawi, N.J. 2012. Study of some factors of physical, chemical and species composition of the phytoplankton for waste treatment plants in Hamdan and treatment of tap water processed for the city of Basrah in Al. Brahtyia. Tech. J., 1: 2-7. URL

Al-Shaheen, M.A. 2002. Species composition of algae and its ability to produce toxins in drinking stations in Basra city. M.SC., Thesis, College of Science, University of Basrah, 61p. (In Arabic)

Bakiyaraj, R., Baskaran, L. and Senthilkumar, T. 2014. Original research article isolation and identification of cynobacteria (*Oscillatoria pseudogeminata* G. Schmid) from marine water and its potential on remediation of pesticide. International Journal of Current Microbiol. and Appl. Sci., 3: 256-267. URL

Bennett, A. and Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. Journal of Cell Biology, 58(2): 419-435. https://doi.org/10.1083%2Fjcb.58.2.419

Choi, W.Y. and Lee, H.Y. 2018. Effect of ultrasonic extraction on production and structural changes of C-phycocyanin from marine *Spirulina maxima*. Int. J. Mole. Sci., 19: 220. https://doi.org/10.3390%2Fijms19010220

Desikachary, T.V. 1959. Cyanophyta Indian Council of Agricultural Research, New Delhi, 686p. URL

Dufossé, L., Galaup, P., Yaron, A., Arad, S.M. and Blanc, P. 2005. Microorganisms and microalgae as sources of pigments for food use: A scientific oddity or an industrial reality. Trends in Food Sci. & Tech., 16(9): 389-406. https://doi.org/10.1016/j.tifs.2005.02.006

Galetović, A., Seura, F., Gallardo, V., Graves, R., Cortés, J., Valdivia, C. and Gómez-Silva, B. 2020. Use of Phycobiliproteins from Atacama Cyanobacteria as Food Colorants in a Dairy Beverage Prototype. Foods, 9: 244. https://doi.org/10.3390%2Ffoods9020244

Horváth, H., Kovács, A.W., Riddick, C. and Présing, M. 2013. Extraction methods for phycocyanin determination in freshwater filamentous cyanobacteria and their application in a shallow lake. European J. Phycol., 48: 278-286. https://doi.org/10.1080/09670262.2013.821525

Huang, W., Ratkowsky, D.A., Hui, C., Wang, P., Su, J. and Shi, P. 2019. Leaf fresh weight versus dry weight: Which is better for describing the scaling relationship between leaf biomass and leaf area for broad-leaved plants? Forests, 10(3): 256. https://doi.org/10.3390/f10030256

Kamble, S.P., Vikhe, G.P. and Chamle, D.R. 2018. Extraction and purification of phycoerythrin-a natural colouring agent from *Spirulina platensis*. J. Pharm. Chem. Biol. Sci., 6: 78-84. URL

Kumar, S.P., Vikhe, G.P. and Chamle, D.R. 2014. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). Indian J. Plant Physiol., 192: 184-188. https://doi.org/10.1007/s40502-014-0094-7

Lee, R. 2008. Phycology. University of Cambridge Press, 547p. https://doi.org/10.1017/CBO9780511812897

Littler, D. and Littler, M. 1985. Handbook of phycological methods. Vol. 4. Ecological field methods: Macroalgae, Cambridge Univ. Press, Cambridge.URL

Mahmood, H.M. Abed, I.J. and Al-Mashhadani, M.K.H. 2017. Evaluating harvesting of *Chlorella* sp. biomass and chemical composition under the influence of different concentrations of nutrients. Cur. Res. Microbiol. and Biotech., 5(6): 1375-1379.URL

Moraes, C.C., Sala, L., Cerveira, G.P. and Kalil, S.J. 2011. C-Phycocyanin extraction from *Spirulina platensis* wet biomass. Brazilian J. Chem. Eng., 28: 45-49. https://doi.org/10.1590/S0104-66322011000100006

Mörschel, E., Wehrmeyer, W. and Koller, V.K.P. 1980. Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. Electron microscopical and biochemical analysis of B-phycoerythrin and B-phycoerythrin-C-phycocyanin aggregates. Eur. J. Cell Biol., 21: 319-327. https://doi.org/10.1007/BF00403196

Mühlsteinová, R., Hauer, T., De Ley, P. and Pietrasiak, N. 2018. Seeking the true *Oscillatoria*: A quest for a reliable phylogenetic and taxonomic reference point. Preslia, 90: 151-169, https://doi.org/10.23855/preslia.2018.151

Muhlsteinova, R., Mühlsteinova, R., Hauer, T., DeLey, P. and Pietrasiak, N. 2018. Seeking the true *Oscillatoria*: a quest for a reliable phylogenetic and taxonomic reference point. Preslia, 90: 151-169. https://doi.org/10.23855/preslia.2018.151

Mulders, K.J.M., Lamers, P.P., Martens, D.E. and Wijffels, R.H. 2014. Phototrophic pigment production with microalgae: Biological constraints and opportunities. J. Phyco., 50: 229-242. https://doi.org/10.1111/jpy.12173

Nur, M.M., Azimatun, G.G., Boelen, P. and Buma, A.G.J. 2019. Enhancement of C-phycocyanin productivity by *Arthrospira platensis* when growing on palm oil mill effluent in a two-stage semi-continuous cultivation mode. J. App. Phycol., 31: 2855-2867. https://doi.org/10.1007/s10811-019-01806-9

Praba, T., Ajan, C., Citarasu, T., Selvaraj, T., Albin Dhas, S., Gopal, P. and Michael Babu, M. 2016. Effect of different culture media for the growth and oil yield in selected marine microalgae. J. Aqua. Trop., 31: 165-177. URL.

Prescott, G.W. 1978. How to know the freshwater algae. Wm. C.C. Brown Com. Pub., UK., 348p.URL.

Ruiz-Domínguez, M.C., Jáuregui, M., Medina, E., Jaime, C. and Cerezal, P. 2019. Rapid Green Extractions of C-Phycocyanin from Arthrospira maxima for Functional Applications. App. Sci., 9: 1987. https://doi.org/10.3390/app9101987

Sarmah, P. and Rout, J. 2018. Biochemical profile of five species of cyanobacteria isolated from polythene surface in domestic sewage water of Silchar town, Assam (India). Cur. Trends in Biotech. Pharm., 12: 7-15. URL. https://doi.org/10.1007/s11356-018-3079-7

Sharmila Banu, V.M., Santhosh, S., Hemalatha, V., Venkatakrishnan, V. and Dhandapani, R. 2017. Optimization study on extraction and purification of phycoerythrin from red algae *Kappaphycus Alvarezii*. Asian J. Pharm. and Clin. Res., 10: 297-302. URL.

Silva, L.A., Kuhn, K., Moraes, C.C., Burkert, C.A.V. and Kalil, S.J. 2009. Experimental Design as a Tool for Optimization of C-Phycocyanin Purification by Precipitation from *Spirulina platensis*. J. Brazilian Chem. Soc., 20: 5-12. https://doi.org/10.1590/S0103-50532009000100003

Simeunović, J.B., Marković, S.B., Kovač, D.J., Mišan, A.Č., Mandić, A.I. and Svirčev, Z.B. 2012. Filamentous cyanobacteria from Vojvodina region as source of phycobiliprotein pigments as potential natural colorants. Food and Feed Res. 39: 23-32.URL.

Stein, J.L., Marsh, T., Wu, K., Shizuya, H. and Delong, E., 1996. Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase-pair genome fragment from a planktonic marine archaeon. J. Bacter., 178(3): 591-599. https://doi.org/10.1128%2Fjb.178.3.591-599.1996

Uju, D.N., Santoso, J., Setyaningsih, I., Hardingtyas, S.D. and Yopi, A. 2020. Extraction of phycoerythrin from *Kappaphycus alvarezii* seaweed using ultrasonication. IOP Conference Series: Earth and Env. Sci., 414 012028. DOI 10.1088/1755-1315/414/1/012028

Watanabe, M. and Ikeuchi, M. 2013. Phycobilisome: Architecture of a light-harvesting supercomplex. Photosynth. Res., 116: 265-276. DOI: 10.1007/s11120-013-9905-3

Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E, Smith, A.G., Camire, M.E. and Brawley, S.H. 2017. Algae as nutritional and functional food sources: Revisiting our understanding. J. App. Phyco., 29: 949-982. https://doi.org/10.1007/s10811-016-0974-5.

Whitton, B. and Potts, M. 2000. The ecology of cyanobacteria: Their diversity in time and space. Heidelberg, Springer. URL.

Yang, L., Chen, J., Qin, S., Zeng, M., Jiang, Y., Hu, L., Xiao, P., Hao, W., Hu, Z., Lei, A. and Wang, J. 2018. Growth and lipid accumulation by different nutrients in the microalga C*hlamydomonas reinhardtii*. Biotech. Biofu., 11(1): 1-12. https://doi.org/10.1186/s13068-018-1041-z.

Yücetepe, A., Saroğlu, Ö., Duran, A. and Ozcelik, B. 2018. The Effect of NaCl Concentration and pH on Phycocyanin Extraction from Microalgae *Spirulina platensis* as a Traditional Food. International Conference on Agriculture, Forest, Veterinary Sciences and Technologies, 2 -05 April 2018.URL.

Zarzuelo, A., Duarte, J., Jiménez, J., González, M. and Utrilla, M. 1991. Vasodilator effect of olive leaf. Planta Med., 57: 417-419. https://doi.org/10.1055/s-2006-960138