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Research Article

INVESTIGATION OF PHYSIOLOGIC AND KINETIC EFFECTS OF CHICKEN FERTILIZERS ON MICROALGAE GROWTH AND BIOMASS PRODUCTIVITY

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ABSTRACT. The effects of broiler fertilizer, obtained from Broiler Research Unit of Gazi University, Turkey, on microalgae growth, chlorophyll concentrations, productivity and CO_2 fixation rate were investigated. A series of experiments including pH, temperature, initial biomass concentrations and light intensity were studied. *Chlorella vulgaris* and *Phormidium animale* adapted to broiler fertilizer, as a newly produced culture media, quickly and showed improvement in almost every concentrations. The maximum biomass yield was obtained as 0.78 g/L for *C. vulgaris* at pH 8 and was obtained as 1.34 g/L at pH 7 for *P. animale. C. vulgaris* improved better than *P. animale* in this new broiler culture medium. This study contains the important and rare results of a research that show the transformation of the broiler fertilizer to an aquatic culture media; moreover, the new culture medium has reduced both the disposal of environmental waste and the cost of production of microalgae. Microalgae biomass produced in this culture medium could be used as an organic fertilizer in further studies.

Keywords: *Biomass productivity, broiler fertilizer, cyanobacteria, microalgae, photosynthesis*

INTRODUCTION

One of the best developed livestock sector in Turkey is poultry. Although this sector is important in terms of low cost and fast meat protein production, it brings with it a number of problems. The biggest problem is the “waste” that occurs during the production and causes to environmental pollution. The "fertilizer" occupies the biggest place in poultry wastes. Broiler fertilizers creates a big environment for the development of flies and insects and leading to pollution and contamination problems.

A broiler produces an average of 150 g fertilizer in a day and this is a very important problem for managements. This fertilizer can meet the C/N and NO_3 needs of microalgae for growing [1]. Microalgae have great biomass productivity potential with little area requirements and therefore they have been considered as the most important bio-energy source with their natural oils and organic compounds. Researchers try to increase this great biomass productivity potential by different production methods [2,3]. The important thing in the methodology is that the culture medium is low cost. But a commercial microalgae culture media requires an increased amount of $NaNO_3$ and other chemicals, which increases the production cost. Microalgae media are generally prepared with premixed stock solutions of some nutrients such as $NaNO_3$, $CaCO_3$,

K_2HPO_4, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Na_2CO_3, $NaHCO_3$, KCl [4,5]. Media can be classified as defined or undefined. The components of defined media are all known and can be formulated as chemically. Undefined media contain one or more natural complex ingredients, for example, fertilizer, agar, or lake water, the composition of which is unknown and may vary [5].

Using of broiler fertilizer in microalgae growth has not been reported as an undefined media example. The aim of this study is to evaluate the fertilizer as rich and low cost nutrients to growth microalgae and eliminate an important environmental waste. Based on the some research results microalgae could be used as a natural bio-fertilizer on plant growth [6] and therefore, microalgae biomass produced in this study may be used as organic fertilizer in plant growth. The novelty of this study is producing a low cost and environment friendly microalgae growing media formulated from broiler fertilizer.

MATERIALS AND METHODS

The six microalgae [green algae] and six cyanobacteria [blue-green algae] isolates were used in the experiments. The microalgae strains were; *a.* Isolate 1 [un-defined pure microalgae strain] and *b. Botryococcus* sp. [from culture collection of Gazi University, Health Services Vocational School], *c. Chlorella* sp. [7], *d. Scenedesmus* sp. [8] and *e. C. sorokiniana* [from culture collection of Gazi University, Health Services Vocational School]; the cyanobacteria cultures were; *a. Leptolyngbya* sp. [9], *b. Geitlerinema* sp. [from culture collection of Gazi University, Health Services Vocational School], *c. Oscillatoria amoena* [from culture collection of Gazi University, Health Services Vocational School], *d. O. tenuis* [from culture collection of Gazi University, Health Services Vocational School] and *e.* Isolate 2 [un-defined pure cyanobacteria strain]. The strains were isolated from Central Anatolia and Aegean Region in Turkey and cultivated-stored in our laboratory culture collection. The selected undefined strains were identified as molecularly with PCR and sequencing and presented at below.

Isolation of new microalgae

The microalgal culture was isolated from Hıdırlar Thermal Water located in Çanakkale, Turkey. The cyanobacterial culture was isolated from Buharkent Thermal Water located in Denizli, Turkey. The samples were spread on Petri plates containing BG 11 medium [4] and were incubated at 25 ± 2 °C under continuous illumination [cool-white fluorescent, 25 mmol/m^2s [1750 lx]]. The pH of the BG 11 medium was 8. Micromanipulation was used to isolate cells from single colonies on these plates. The microalgal cells were purified under aseptic conditions by streaking the cells repeatedly on the BG 11 medium agar plate. At the final step, the purified microalgal cells were transferred to liquid media. In order to validate the axecinity these liquid cultures were also tested for bacterial contamination by plating on bacteriological media [7]. A series of batch culture experiments in unshaken flasks illuminated by cool-white fluorescent lamps were carried out at 25 mmol/m^2s [1750 lx] light intensity. The microalgal cultures were transferred into 100 mL BG 11 medium in 250 mL Erlenmeyer flasks and incubated at 25 ± 2 °C under continuous illumination for 15 days. The medium was inoculated with 0.1 g/L exponentially growing cultures.

Identification of new isolates with PCR and sequencing

Whole cells from an exponentially growing culture of the *Isolate 1* were used for 18 S rRNA gene amplification in order to identification of the microalgae strain isolated from Hıdırlar Thermal Water. 18 S rRNA region was amplified with primers forward p23SrV_F: 5'- GGACAGAAAGACCCTATGAA-3' and reverse p23SrV_R: 5'-TCAGCCTGTTATCCCTAGAG-3' as described [10]. PCR reaction is carried out in 50 mL reaction mixture. The reaction mixture included in 0.2 mM of each primer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$ and 30 ng of template DNA. 1.5 U Taq DNA polymerase is used in the amplification. Amplification by PCR was carried out by an initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s, and elongation at 72 °C for 45 s, with a final extension of 72 °C for 10 min. The 2 forward primers F371: 5'-AGGGTTCGATTCCGGAG-3' and F1132:5'-GAAACTTAAAKGAATTG-3' and 2 reverse primers R584: 5'-GWATTACCGCGGCKGCTG-3' and R1283: 5'-CGGCCATGCACCACC-3' were used in sequencing. Applied Biosystems ABI3130 genetic analyser [Applied Biosystems Inc., USA] and BigDye 3.1 [Applied BiosystemsInc, USA] are used in sequencing.

Whole cells from an exponentially growing culture of the *Isolate 2* were used for 16 S rRNA gene amplification in order to identification of the cyanobacteria strain isolated from Buharkent Thermal Water. 16 S rRNAgene was amplified with primers forward 106F: 5'-CGGACGGGTGAGTAACGCGTGA-3' and reverse 781R[a]: 5'-GACTACTGGGGTATCTAATCCCATT -3' as described [11]. PCR reaction is carried out in 50 mL reaction mixture in Applied Biosystems Gene AMP PCR System 9700 Thermal Cycler. The reaction mixture included in 0.3 mM of universal primer, 0.2 mM of each dNTP, 5 µL 10 X PCR buffer [150 mMTris_HCl, pH 8.75, T 25 °C, 500 mMKCl, 20 mM $MgCl_2$, % 1 Triton X-100], 100 ng of genomic DNA and 1.5 U Taq DNA polymerase. Amplification by PCR was carried out by an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min, with a final extension of 72 °C for 5 min. The amplified product was analyzed in 1.2% agarose gel with ethidium bromide at 8 V/cm. The PCR products were visualized under UVP gel imaging system. The amplified PCR product was purified using the QIAGEN gel extraction kit.

Transformation of broiler fertilizer to microalgae culture media

Broiler fertilizer [BCF] was obtained from Broiler Research Unit of Gazi University Life Sciences Application and Research Centre with permission to work for animal producer / user / supplier / organizations [Registration number: 109, Republic of Turkey Ministry of Food, Agriculture and Livestock]. It has been provided by veterinarian. On the other hand, different feeded cow fertilizer was used as comparative media. Cow fertilizer was obtained from Cow Farm by veterinarian.

Broiler fertilizer was collected from the healthy broilers. The broilers were grown on floor covered with wood wool in divided special compartments. The 7 broilers were placed in this compartments which divided as 90 x 80 x 80 cm length x width x height. The amount of food that the animals can consume daily was constantly provided in the manger and the ad libitum feeding was provided.

The rate of rations was 23% crude protein and 3000 kcal/kg metabolized energy for male broiler chicks [Ross 308] at 0-21 days of chick period; 20% crude protein and

3200 kcal/kg metabolized energy at 21-35 days of broiler period; 20 % crude protein and 3252 kcal/kg metabolized energy at 36-42 days of broiler period. Rations are based on corn and soy.

A sufficient amount of broiler fertilizer was supplied for using in all experiments. All of them were mixed with tap water and hold for a night. Then filtered with filter paper and autoclaved at 121 °C at 15 min. To standardize the prepared mixture, it was concentrated by dilution. The dilution rates were 5, 10, 15, 20 and 25 g/L. The remaining mixture was kept in the refrigerator for reuse. The C/N ratio was detected for each dilution rate.

A series of batch culture experiments in unshaken flasks illuminated by cool-white fluorescent lamps were carried out at 25 mmol/m2s [1750 lx] light intensity. The microalgal cultures were transferred into 100 mL BCF media with above concentrations in 250 mL Erlenmeyer flasks and incubated at 25 ± 2 °C under continuous illumination for 15 days. The medium was inoculated with 0.1 g/L exponentially growing cultures.

A series of batch culture experiments in unshaken flasks with 100 mL BG11 medium in 250 mL Erlenmeyer flasks were used as control group. All of the experiments were performed as triplicate.

Optimization of culture conditions

The pH values of the culture medium were adjusted to 6, 7, 8 and 9 to determine the effect of pH on growth of microalgae under the influence of BCF media by the addition of 0.1 M H2SO4 and 0.1 M NaOH solutions accordingly.

The effect of temperature was investigated at 15, 25 and 35 °C. The 15 °C was provided in a temperature controlled cooled laboratory, 25 °C was obtained in a room temperature laboratory and the 35 °C was obtained in a heat-controlled orbital shaker [New Brunswick Scientific, Innova 40 R, Eppendorf].

The effect of biomass concentrations was investigated at 0.1, 0.15 and 0.20 g/L biomass concentrations in 100 mL broiler broiler fertilizer media.

The effect of photoperiod was determined at 24:0 light:dark period at 1000, 1500 and 2000 lx light intensity.

The control samples were cultivated at the same conditions with samples. Each of these experiments was performed in triplicates.

Kinetic parameters

The chlorophyll concentrations were determined according the method [12] at 646.6 nm for chlorophyll a and at 663.6 nm chlorophyll b. The chlorophyll concentrations were expressed in μg of chlorophyll per milliliter.

Cell growth of microorganisms was determined by measuring optic density, dried cell mass and specific growth rate parameters for any set of growth conditions. Optic density [OD600] was measured at 600 nm with the spectrophotometer.

The kinetic parameters such as the dried cell weight [X], specific growth rate [μ] and maximum biomass productivity [Pmax] were evaluated asEq 1;

$$Pmax = \frac{X - X_0}{t - t_0} \qquad 1$$

Where X: final and X0: initial biomass concentrations [g/L], t: final and t0: initial time of the culture.

The dried cell weight was saved by the measurement of pellets, which were dried at 80 ºC for overnight [J.R Selecta Digitheat model sterilizator] after centrifugation step [3421x g = 5000 rpm for 10'].

Specific growth rate was calculated according to the Eq 2 [13];

$$\mu = \frac{lnX_2 - lnX_1}{t_2 - t_1} \quad 2$$

where X2 and X1: dry cell weight concentrations [g/L] at time t2 and t1, respectively. Maximum biomass productivity was calculated according to the Eq 3;

$$Pmax = \frac{X - X_0}{t - t_0} \quad 3$$

where X: final and X0: initial biomass concentrations [g/L], t: final and t0: initial time of the culture.

The CO2 biofixation rate F [g1/d] was calculated from the Eq 4, as described and used [14-16].

$$F = aP_xV \quad 4$$

where a: 1.833 g CO2, Px: productivity, V: the culture volume.

The harvesting of microorganisms from open pond was performed in the following order; 1. sedimentation, 2. collection of supernatant, 3. centrifugation of pellet. All of the experiments were performed in triplicate. The standard error of data was calculated according to the Eq 5 [17]; where σ represents the square root of the estimated error variance of the quantity.

$$SE = \sqrt{\sigma^2} \quad 5$$

RESULTS AND DISCUSSION

Qualification and standardization of broiler fertilizer

The BCF media were prepared at five different concentrations [Table 1]. The range of C/N ratios can be in a wide range according to the feeding type of broilers. For example, in a study the C/N ratios were 6.7–14.7 [18], while it was 9.6-15 in another study [1]. In present paper the range was 5-7.5.

Table 1. *The Carbon/Nitrogen [C/N] ratios of broiler fertilizers [BCF] at different concentrations*

	BCF [g/L]				
	5	**10**	**15**	**20**	**30**
C/N	7.56 ± 0.09	6.12 ± 0.04	5.44 ± 0.14	5.36 ± 0.06	5.02 ± 0.06

BCF: Broiler fertilizer media; C: Carbon ratio; N: Nitrogen ratio

Microalgae are preferred to produce high biomass in a cheap media. But a commercial microalgae culture media requires an increased amount of $NaNO_3$ and other chemicals, which increases the production cost. Some of the commercial fertilizers should be used for enhancing the microalgal productivity in large-scale culture, by optimizing the well-known commercial media [19]. In the present study the BCF medium was used as a low-cost and high-efficiency growing media without adding any content to a commercial media.

Cultivation of different microalgae species in new designed aquatic media

The microalgae and cyanobacteria species were cultivated in media for selecting the fast grown cultures (Fig. 1).

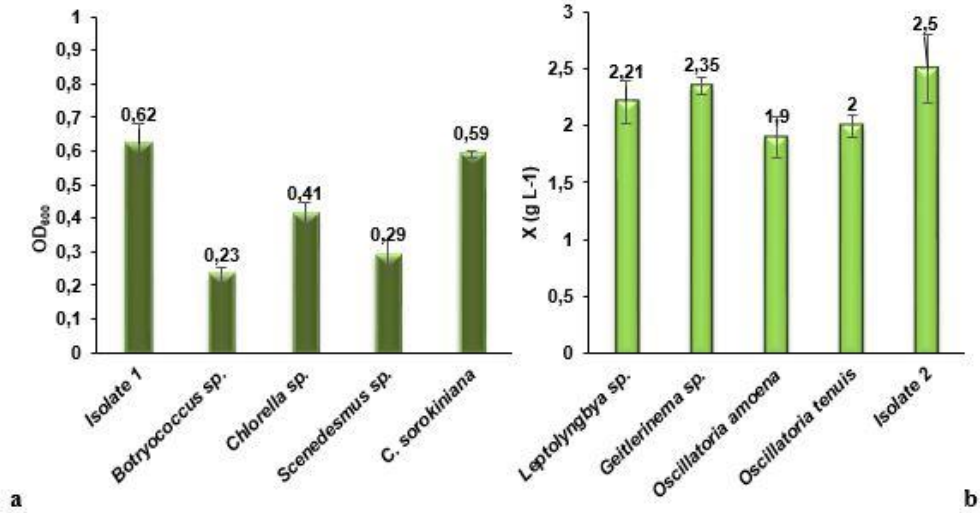


Fig. 1. *Selecting of fast grown microalgae [a] [green algae], [b] cyanobacteria [blue-green algae].*

As seen in Fig.1 after 7 days of incubation period the fastest growing microalgae was selected as Isolate 1 and the fastest growing cyanobacteria was selected as Isolate 2. The species were identified as 18 S and 16 S rRNA sequencing, respectively. According to 18 S rRNA sequencing the Isolate 1 was identified *as Chlorella vulgaris* and according to 16 S rRNA sequencing the Isolate 2 was identified as *Phormidium animale.*

Selecting of new aquatic media concentrations – based on broiler fertilizer

To select of the optimum BCF media concentration *C. vulgaris* and *P. animale* were incubated in BCF media at 5-30 g/L concentrations for 15 days. The BG11 control media was also prepared. It is evident from Fig.2 that the growing yield of microorganisms was really linked to the amount of BCF media concentrations.

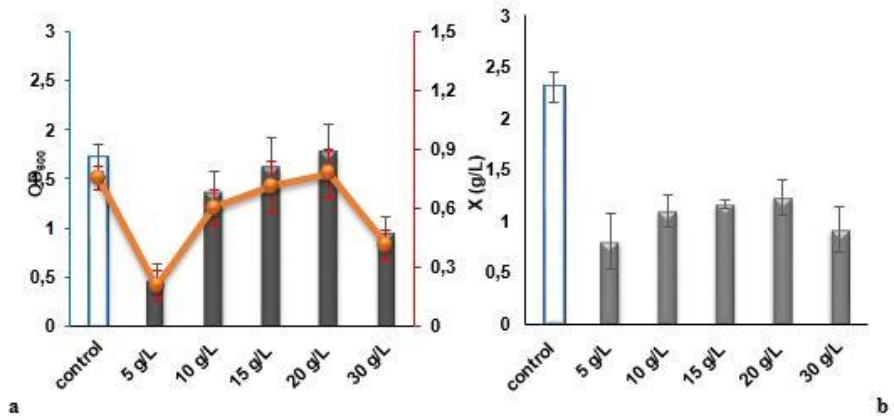


Fig. 2 *Selecting of the optimum broiler chicken fertilizer media concentrations a.C. vulgaris, b.P. animale biomasses [X] [control; BG11 media]*

The highest OD_{600} value of *C. vulgaris* was 1.77 at 15 days of incubation period in 20 g/L BCF media. The highest biomass of *C. vulgaris* was obtained as 0.78 g/L in this media. The OD_{600} value decreased when BCF media concentrations were decreased. The lowest OD_{600} was obtained at 5 g/L BCF media concentration as 0.46 (Fig. 1a). On the other hand, the maximum OD_{600} value that was obtained in 20 g/L BCF media was 30 g/L higher than of control culture [BG11].

On the other hand *P. animale* reached its highest biomass rate as 1.23 g/L at 15 days of incubation period. This was not higher than of control culture. As clearly seen in Fig 1b. the most productive BCF media concentration was 20 g/L for *P. animale*. This concentration was selected as the most productive BCF media concentration for both *C. vulgaris* and *P. animale* for further studies.

In a study five concentrations of an agricultural fertilizer were prepared as 0.1-2 mL/L, in which no other extra nutrients except bicarbonate were used to growth of *Spirulina platensis*. The highest chlorophyll concentration was detected in control group and the chlorophyll concentration at group II [0.1 mL/L fertilizer] was lower than the control group. The paper emphasized that the fertilizer concentration of 0.1 mL/L had a favourable effect on *Spirulina* growth however, the control group had a best effect [20].

In another study the effect of different culture media, water extract of dairy waste, commercial fertilizers mix, carbon sources and Bristol's medium on *Chroococcus turgidus, Temnogyra reflexa, Sirogonium sticticum* and *Uronema elongatum* were investigated. Among the culture media tested, the highest effect was detected in Bristol's medium, followed by dairy waste [21].

Selecting of optimum pH of new designed aquatic media

The effects of pH on biomass yields of *C. vulgaris* and *P. animale* in 20 g/L BCF media are shown in Figure 2 a and b. The maximum biomass yield of *C. vulgaris* was obtained at pH 8 as 0.78 g/L in BCF media. This yield was near to the yield of BG11 media [0.75 g/L]. This means that when *C. vulgaris* was incubated in BCF media, it nearly held the same biomass with a rich media as BG11. The biomass yield of *C. vulgaris* decreased sharply after this pH value (Fig. 3a).

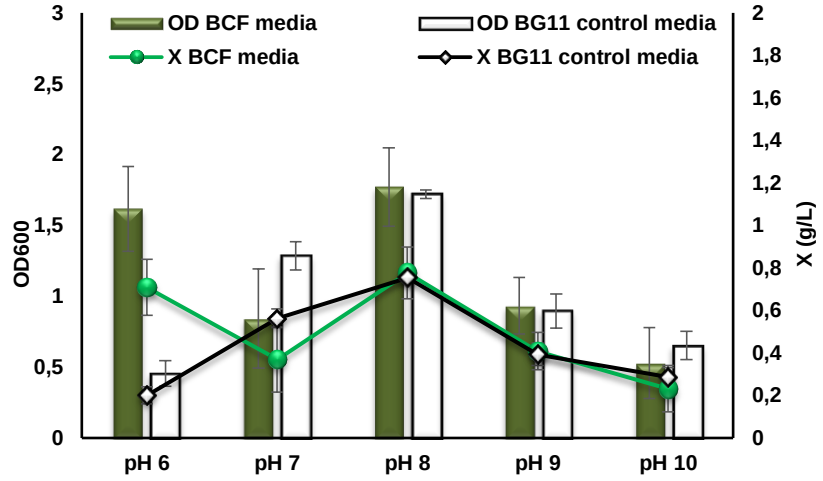
As seen in Fig. 3b *P. animale* reached its maximum biomass value at pH 7 as 1.34 g/L in BCF media. The biomass value was also high at pH 7 in BG11 control media. It seemed to suitable to select the pH 7 as optimum pH for *P. animale* (Fig. 3b).

Selecting of optimum temperature of new designed aquatic media

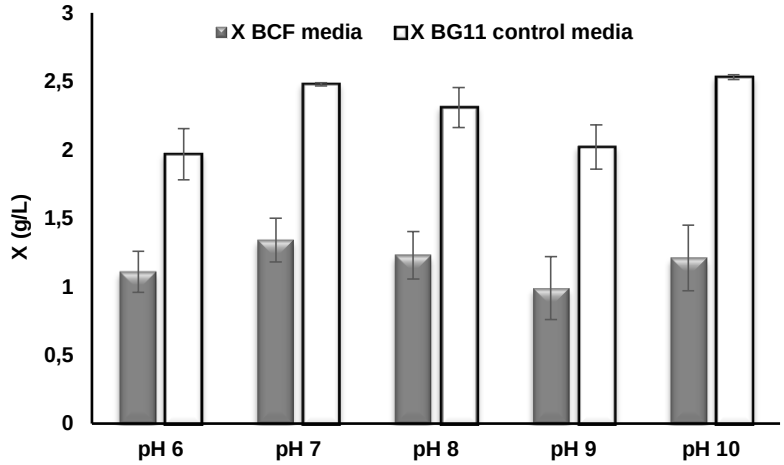
The studies were performed at pH 8 for *C. vulgaris* and pH 7 for *P. animale* with 0.15 g/L initial biomass concentration at 15 °C to 35 °C. As seen in Fig. 4*C. vulgaris* reached its maximum OD_{600} value at 15 °C as 1.86. This means 0.82 g/L biomass yield. This yield decreased sharply to 0.61 g/L when temperature was increased to 35 °C. Therefore, optimum temperature was selected as 15 °C for *C. vulgaris* (Fig. 4a).

As seen in Fig. 4b the changes in biomass yield of *P. animale* was more remarkable at different temperature values. When temperature was 15°C *P. animale* reached its maximum biomass yield as 1.45 g/L however this yield was very low at 35 °C [0.9 g/L].

As a result, optimum temperature value was determined as 15 °C for both of the microorganisms. This is an important result for producing a microbial fertilizer in terms of enabling it to be used in difficult and cold conditions when commercialized in the future.



a



b

Fig. 3. *Effect of pH on biomass yields [X] of [a] C. vulgaris and [b] P. animale in BCF and BG11 control media.*

Selecting of initial biomass concentrations of microalgae in new designed aquatic media

The studies about selecting of optimum initial microalgal biomass concentration was performed at selected optimum pH and temperature for both of the microorganisms at 0.1, 0.15 and 0.2 g/L initial biomass concentrations. As seen in Fig.4b and e when initial biomass concentration increased, microalgal biomass yields increased. *C. vulgaris* reached its maximum OD_{600} value as 1.99 at 0.2 g/L initial biomass concentration. This means 0.88 g/L biomass yield. The biomass yield of *P. animale* was 1.53 g/L at the same concentration. On the other hands control groups had the same high biomass yields with the yields of BCF media at the same initial biomass concentration of 0.2 g/L. For example, the maximum biomass yield of *C. vulgaris* in BG11 control media was 1.03 g/L at 0.2 g/L initial biomass concentration. This yield was 2.27 g/L for *P. animale* at the same biomass concentration. This means that both of the microalgae caught the biomass yields in BCF media as in rich BG11 media. As seen in Fig. 4 b *C.*

vulgaris was seem to show more success to grow in BCF media than *P. animale* (Fig. 4e) when compared with controls.

Selecting of optimum light intensity in new designed aquatic media

The light intensity studies were performed at selected optimum pH, temperature and initial biomass concentrations at 1000, 1500 and 2000 lx light intensities. All of the above studies were performed at 1500 lx light intensity and therefore the effect of lower [1000 lx] and higher [2000 lx] light intensities on microalgal growth was carried out.

As shown in Fig. 4 c *C. vulgaris* reached 0.32 g/L biomass yield [OD_{600} was 0.74] at 1000 lx light intensity. On the other hand, *P. animale* had 1.09 g/L biomass yield at the same light intensity (Fig. 4f). These yields were quite lower than control groups. When the light intensity was increased to 2000 lx, *C. vulgaris* increased its biomass yield to 2.14 [OD_{600}]. This was 40 % lower than of control culture. *P. animale* had 1.64 g/L biomass yield at the same light intensity and this was 45.5 % lower than control culture.

As seen in Table 2 the effect of light intensity parameter under the influence of all above optimized parameters on biomass, chlorophyll concentrations, maximum biomass productivity, specific growth rate and CO_2fixation rate of *C. vulgaris* and *P. animale* were summarized. Between the experimental groups, the highest chlorophyll [a+b] concentration of *C. vulgaris* [1.15 µg/mL] at BCF media was lower than the control group. The maximum µ and Pmax recorded in BCF media by *P. animale* were 0.15 [1/d] and 0.09 [g/Ld], respectively, when compared with 0.174 [1/d] and 0.15 [g/Ld] in control group. Overall the biomass was produced cheaply and besides this, an environmental waste was disposed. The biomass produced in BCF media may be used in agriculture as an effective microbial fertilizer. As stated before the cost of the commercial f/2 media was more expensive than fertilizer. It was concluded that fertilizer had the same amount of biomass when compared with the f/2 media, and it was much cheaper [22].

As the result of optimization studies *C. vulgaris* increased its biomass yield as 21 % when compared to the initial studies. This yield was 33.3 % for *P. animale*. These microalgae should be used as important bio-fertilizers in agriculture. Especially, when compared with their own control groups, the biomass yields of *C. vulgaris* were higher than those of *P. animale*. It has also been identified in previous studies that *Chlorella* sp., have stimulating effects on seed germination and plant growth [23]. However, the subject is rather new in the literature and the studies are very limited.

In general, the researchers try to solve the high cost culture media problem by optimizing culture conditions with adding some commercial fertilizers [19] or using culture media up to several times [24]. In this work, the microalgae culture was directly performed at different BCF media concentration, pH, biomass, temperature and light intensity availability using the best growth medium to identify the optimum growth conditions. The work aimed to discover a low cost microalgae growth media to replace the commercial media. Consequently, a novel media was developed to cultivate both microalgae and cyanobacteria to decrease the production costs and eliminate an environmental waste.

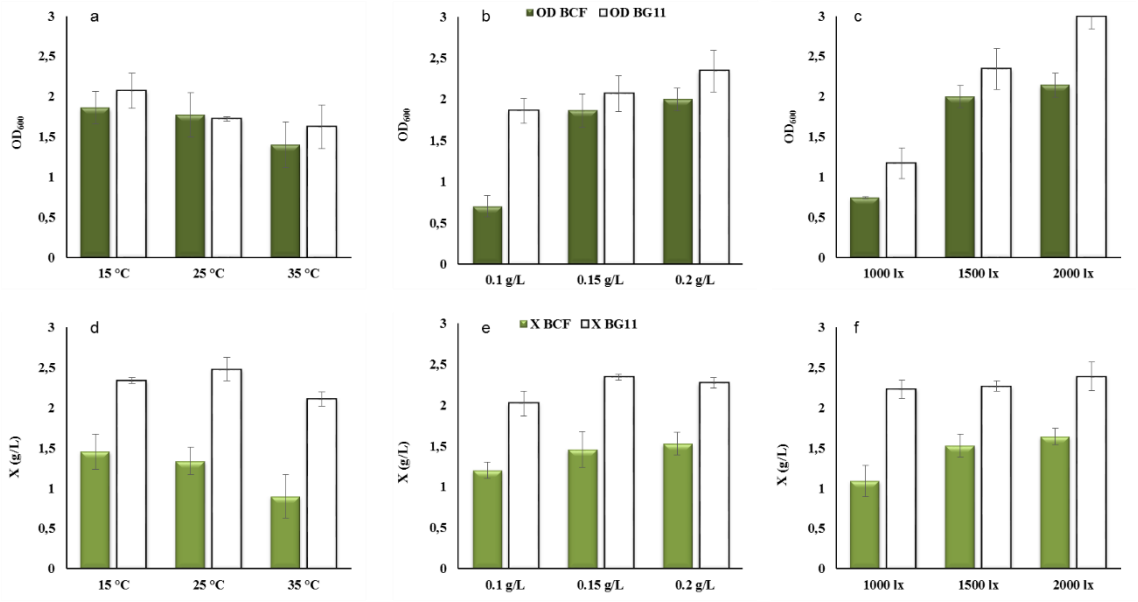


Fig. 4 *Effect of temperature [a, d], initial biomass concentration [b, e] and light intensity [c, f] on biomass yields [X] of C. vulgaris [a, b, c] and P. animale [d, e, f] in BCF and BG11 control media.*

Table 2. The effect of light intensity parameter under the influence of all optimized parameters on biomass, chlorophyll concentrations, maximum biomass productivity, specific growth rate and CO_2 fixation rate of *C. vulgaris* and *P. animale.*

Microorganism	Growth Media	X [g/L]	Chl* [µg/mL]	μ [1/d]	*P max* [g/Ld]	*CO_2 fixation rate [mg/CO_2d]***
C. vulgaris	**BCF**	0.94±0.006	1.15±0.008	0.11±0.001	0.05±0.001	9.30±0.058
	BG11	1.31±0.04	1.61±0.46	0.13±0.002	0.08±0.071	14.0±0.577
P. animale	**BCF**	1.64±0.042	3.15±0.080	0.15±0.002	0.09±0.003	18.0±0.577
	BG11	2.39±0.069	4.59±0.127	0.17±0.002	0.15±0.004	27.0±0.577

X [g/L], biomass concentration at 15thday,
Chl*, chlorophyll [a+b] for*C. vulgaris* and chlorophyll a for*P. animale*,
μ [1/d], specificgrowth rate,
P [g/Ld], maximum biomass concentration,
T, 15 ± 2 ºC; illumination, 2000 lx
** for 0.1 L working volume of culture.

CONCLUSION

A commercial microalgae culture media requires an increased amount of chemicals, which increases the production cost. Therefore, it needs to be produce a low cost growth media. It was concluded from the present paper that BCF media had positive effect on the biomass productivity of *C. vulgaris* and *P. animale*. The microorganisms produced

high biomass in BG11 media and the biomass resulted produced in BCF media followed it. The maximum biomass yield of *C. vulgaris* was obtained at pH 8 as 0.777 g/L and at pH 7 as 1.34 g/L for *P. animale* in BCF media. When take into consideration of the cost effectiveness, BCF media should be recommended or trying large scale cultivation of microalgae. Furthermore, microalgae biomass produced with BCF may be used as organic fertilizer and its effect may be tested in further studies.

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