Journal of Limnology and Freshwater Fisheries Research
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Growth Kinetics of *Scenedesmus obliquus* Strains in Different Nutrient Media

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ABSTRACT

In this study, the effects of different media and natural mineral waters on the growth dynamics of *Scenedesmus obliquus* (Turpin) Kützing were investigated. *S. obliquus* strains were isolated from various freshwater wells and pools in Ankara, Turkey. In the production of three *S. obliquus* strains, both four culture medium and four natural mineral waters were used. Cell density, dry weight, specific growth rates, duplication time and chlorophyll-a of the cultures were calculated. The results showed that the different culture media and natural mineral waters significantly affected the final cell density, biomass and growth rates of strains. In three isolates, there was a significant difference in ScnGP strain in terms of cell density, biomass weight and specific growth rate compared to others. Cell density ($7.4x10^4 \pm 1.3x10^3$cells/mL), biomass amount (0.212±0.032g/mL), specific growth rate (0.024 h^{-1}) and chlorophyll-a (1.71±0.22µg/mL) of ScnGP grown in Bold Wynne medium were significantly ($P<0.05$) higher than that of grown in all other treatments. It has been determined that the results obtained from natural mineral waters were as good as the results obtained from culture media. It can be thought that various chemical compounds and reproductive factors can be added to increase the production within the natural mineral waters.

Keywords: *Scenedesmus obliquus*, microalgae, cell density, dry weight, natural mineral water

ARTICLE INFO

RESEARCH ARTICLE

Received : 17.01.2019
Revised : 15.02.2019
Accepted : 23.02.2019
Published : 27.08.2019

DOI:10.17216/LimnoFish.514166

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Scenedesmus obliquus Suşlarının Farklı Besin Ortamlarındaki Büyüme Kinetiği

Öz: Bu çalışmada, farklı besi ortamları ve doğal maden sularının *Scenedesmus obliquus* (Turpin) Kützing'in büyüme dinamikleri üzerine etkileri araştırılmıştır. *S. obliquus* suşları, Ankara (Türkiye) ilindeki çeşitli tatlı su kuyuları ve havuzlarından alınan örneklerden izole edilmiştir. İzole edilen üç *S. obliquus* suşunun üretiminde, dört kültür besi ortamı ile dört doğal maden suyu kullanılmıştır. Kültürlerin hücre yoğunluğu, kuru ağırlık miktarı, spesifik büyüme oranları, ikilenme süreleri ve klorofil-a miktarları hesaplanmıştır. Sonuçlar, farklı kültür besi ortamları ve doğal mineralli suların, son hücre yoğunluğunu, biyokütleyi ve suşların büyüme oranlarını önemli ölçüde etkilediğini göstermektedir. Bu üç suş içerisinde, ScnGP suşunda diğerlerine oranla hücre yoğunluğu, biyokütle ağırlığı ve spesifik büyüme hızı açısından anlamlı bir fark bulunmuştur. Hücre yoğunluğu ($7,4x10^4 \pm 1,3x10^3$ hücre/mL), biyokütle miktarı (0,212±0,032 g/mL), spesifik büyüme oranı (0,024 h^{-1}) ve klorofil-a (1,71±0,22 µg/mL) miktarı Bold Wynne besi ortamında yetişen ScnGP suşunda diğer uygulamalara göre önemli ölçüde ($P<0,05$) daha yüksek tespit edilmiştir. Doğal mineralli sulardan elde edilen sonuçların kültür besi ortamlarından elde edilen sonuçlar kadar iyi olduğu gözlenmiştir. Doğal mineralli sulara çeşitli kimyasal bileşiklerin ve üreme faktörlerinin eklenerek üretimin artırılabileceği düşünülmektedir.

Anahtar kelimeler: *Scenedesmus obliquus*, mikroalg, hücre yoğunluğu, kuru ağırlık, doğal mineralli su

How to Cite
Yalçın Duygu D. 2019. Growth Kinetics of *Scenedesmus obliquus* Strains in Different Nutrient Media. LimnoFish. 5(2): 95-103. doi: 10.17216/LimnoFish.514166

Introduction

Microalgae are the main producers of organic matter in aquatic environments. They are mostly simple structured unicellular or multicellular, mostly photosynthetic microscopic organisms like other plants organisms with the chlorophyll in their cells. By the photosynthetic pigments which they contain, they convert solar energy into chemical energy through photosynthesis (Humpry 2004; Murdock and Wetzel 2009; Shelly et al. 2002). Because of this function, they occupy an important place in the inland waters (lakes, lagoons, streams, and dams) and in the feeding of living beings in the seas and are the basis of all production in aquatic environments (Wang et al. 2008). The collection and use of microalgae from the natural environments where it

grows as a food source begins with the earliest written date. However, the first microalgae culture was obtained by Beyerinck as the pure strain of *Chlorella vulgaris* on agar plates (Pringsheim 1946). In the time according to the needs of mankind, various usage areas developed (Brennan and Owende 2010).

The cell content of Scenedesmus consists of the essential amino acids, protein, lipid, mineral matters and pigments (especially chlorophyll) (Toyub et al. 2008). The biomass obtained by the cultivation of *Scenedesmus obliquus* is used in many fields. These include biodiesel and bioethanol production (Hodaifa et al. 2008), nutritional supplements for animals (Fallahi et al. 2014), efficient CO_2 fixation, the ability to grow in wastewaters and accumulate lipids (Sforza et al. 2014).

A large number of artificial media have been developed in the cultivation of small-scale production of monospecific microalgae cultures. Since this type of cultivation is usually carried out in laboratories, the costs of media are not considered. However, the cost of mass production in the large scale of microalgae cultivation is an important issue. Domestic, agricultural and industrial wastewater sources are being investigated for the solution of this problem. During the cultivation, the usage of wastewater which contains pollutants like pathogenic bacteria and viruses, pesticides and heavy metals, might threaten human and animal health. For this reason, cultures exposed to these types of pollutants are not required by the pharmaceutical, cosmetic or food industries. It is very important to carry out all kinds of cultural studies with the developed different artificial cultivation medium. The usage of natural mineral waters which is rich in nutrients, in large-scale microalgae cultivation, is thought to be an alternative solution as a cheap nutrient source for microalgae production.

The aim of this study was to isolate *S. obliquus* from freshwater sources, to investigate the effect of culture conditions on growth kinetics and to determine the effect of different media and natural mineral waters on these dynamics.

Materials and Methods

Isolation and culture conditions

S. obliquus (Turpin) Kützing strains were isolated from different freshwater pools and wells in Ankara, Turkey. The collected freshwater samples were brought to the laboratory and were transferred to the liquid nutrient medium (1) prepared for pre-enrichment of cultures (Table 1). Isolation of strains was carried out by micromanipulation method (Parvin et al. 2007). *S. obliquus* cells observed under the microscope were taken by means of a micropipette and transferred onto a sterile agar plate. This procedure was repeated several times to reduce bacterial contamination. These cells were then transferred to the culture tube and placed under appropriate low temperature conditions. The strains were then inoculated into agar medium (2) for fulfill purification (Table 1). Shortly thereafter, microalgae colonies appeared on the agar plates. These colonies produced into the agar surface were re-inoculated into (1) numbered liquid nutrient medium and control of growth was carried out by microscope. Characteristic cells of *S. obliquus* were identified by using species keys (Prescott 1973; Guiry and Guiry 2018). The coding of the strains was made according to the locations where samples were taken (ScnWW - Well Water), (ScnMP - Medico Social Pool) and (ScnGP - Gazi University Pool).

Morphologies of algal colonies were determined and examined the size and shape of the cells under the microscope. In Figure 1, the taxonomic classification of *S. obliquus* (Guiry and Guiry 2018) and its appearance under the light microscope were given.

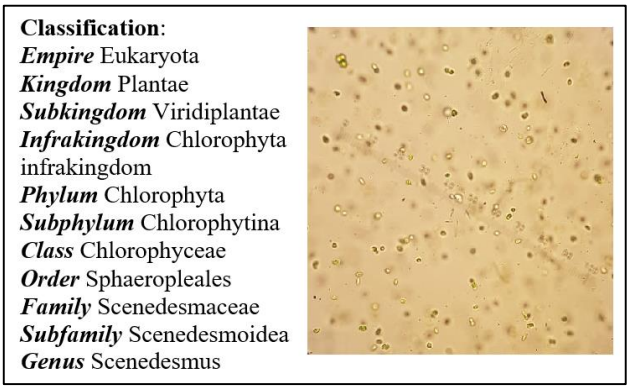

Figure 1. Classification (left) and microscopic images (right) of *S. obliquus*.

In this study, Prat, Yagojinski, Knop, and Bold Wynne Medium were used as culture nutrient media (Andersen et al. 2005). Besides, four different natural mineral water samples from various regions of Turkey were used to grow *S. obliquus*. Compositions of medium and natural mineral waters were given in Table 1 and Table 2 respectively.

The light source (Philips, 50µmol photons $m^{-2}s^{-1}$) was applied at a distance of 22 cm from the cultures, horizontally, with a period of 16L: 8D and cultivated at 22-25°C. *S. obliquus* has been found to have a maximum grow rate of pH at 6.5-7. At the beginning of the study periods, dilutions of 10 ml of medium (10^{-1}, 10^{-2}, 10^{-3}) were inoculated to determine the amount of inoculum. Cultures that were allowed to incubate in the appropriate culture conditions were recorded with and without proliferating tubes, and (10^{-1}) efficient growth was

recorded in dilution tubes. By determining the amount of inoculum, media and natural mineral waters cultures were realized as 9 ml of nutrient+1 ml of suspension culture.

Table 1. Chemical composition of the culture medium tested

Macroelements	Culture medium compositions					
	(1)[1] (g/L)	(2)[2] (g/L)	Prat (g/L)	Yagojinski (g/L)	Knop (g/L)	Bold Wynne (g/L)
$MgSO_4 \cdot 7H_2O$	2.50	1.250	0.01	-	0.01	0.075
K_2HPO_4	-	-	0.01	-	0.02	0.075
KH_2PO_4	1.25	0.62	-	-	-	0.0175
NaCl	-	-	-	-	-	0.025
$CaCl_2$	-	-	-	-	-	0.025
KNO_3	5.0	2.5	0.1	0.5	0.1	-
$NaNO_3$	-	-	-	-	-	0.250
$MgSO_4$	-	-	-	0.1	-	-
Na_2HPO_4	-	-	-	0.05	-	-
$FeSO_4 \cdot 7H_2O$	0.009	0.009	-	-	-	-
$FeSO_4$	-	-	-	0.002	-	-
$FeCl_2 \cdot 6H_2O$	-	-	0.001	-	0.0001	-
$Ca(NO_3)_2$	-	-	-	-	0.01	-
Agar	-	5	-	-	-	-
DW (mL)	1000	500	1000	500	1000	1000

[1]Liquid nutrient medium, [2]Agar medium

Table 2. Natural mineral waters and chemical contents

Macroelements (one litter of mg)	Natural Mineral Waters			
	KANMW[1]	KINMW[2]	BENMW[3]	OBNMW[4]
Cations				
Na	735.680	567.847	152.812	51.725
Ca	80.160	76.152	172.344	96.192
Mg	12.946	38.899	138.578	87.516
Fe-Al	1.300	24.691	4.816	0.158
Anions				
SO_4	25.400	110.080	147.300	9.250
Cl	108.800	259.150	19.525	21.837
HCO_3	2.374.120	1.403.000	1.415.200	834.053
H_2SiO_3	35.556	63.700	52.100	45.500

[1]Kızılay Afyonkarahisar Natural Mineral Water®, [2]Kızılcahamam Natural Mineral Water®, [3]Beypazarı Natural Mineral Water®, [4]Ozkaynak Bursa Natural Mineral Water®

Detection of microalgae growth

Thoma slide is the most suitable for cell counts of *S. obliquus* strains. Therefore, cell density (cells/mL) were determined by counting 16 squares in Thoma slide. The cell counts were carried out at the beginning of the showing procedure at ranges of (0), (24), (48), (96), (120) and (140) hours. The cell densities were calculated using Eq. (1) (Guillard and Sierachiki 2005):

$$Cell\ Number = \frac{Tx4000}{16} \quad (1)$$

(T: Total cell number on 16 squares; 4000: volume of one square)

Specific growth rate and duplication time were calculated using the growth kinetics. Specific growth rate and duplication time is calculated shown in Eq. 2 and Eq. 3 (Godoy-Hernández and Vázquez-Flota 2006).

$$\mu = \frac{\ln X2 - \ln X1}{t} \quad (2)$$

(μ: Specific growth rate; X1 and X2: Biomass concentration at $t1$ and $t2$)

$$DT = \frac{ln2}{\mu} \quad (3)$$

(DT: Duplication time)

Determination of yields of cultures was done by measuring the dry weights on the 140th hours of cultivation of the cultures. The Whatman (GF/C) filters were dried at 105°C until reach constant volume. The filters were placed in the desiccator for 20 minutes to allow them to reach room temperature and then weighed. 5 mL algal sample was centrifuged, the supernatant was discarded and

washed with 20 ml of distilled water. The samples were filtered through vacuum filtration. The filters were dried at 105°C for 24 hours, dry weight was determined by a digital balance. The dry weight determination was carried out by taking the difference between these two measurements (Chia et al. 2013). All tests were made in triplicate.

Chlorophyll-a determination

The biomass of *S. obliquus* strains was estimated from their chlorophyll-a content measured through the use of the methanol method (Youngman 1978). The microalgae samples (3 mL) were filtered through Whatman glass-fiber filters (GF/C), taken into a 100 mL beaker, and 14 mL of methanol was added. The beaker was boiled at 70°C in a water bath and rested for 5 minutes in the dark after the boiling. Samples were centrifuged at 5000 rpm and values were determined at 750 and 665 nm wavelengths using Thermo Scientific spectrophotometer (Genesys 10S UV-Vis). The obtained data were calculated by applying the following formula (Eq. 4) (Youngman 1978).

$$Chlorophyll - a\ Concentration = 13.6\ x\ A.v\ /\ d.V \quad (4)$$

(v = volume of the filtrate; d = 1 cm; V = sample to be filtered first (l); A = Absorbance)

Statistical analysis

In this study, all analyses were performed in triplicate (n=3). All data were presented as mean values ± and standard deviation (SD). The data, cell density and biomass weights of strains were analyzed statistically using ANOVA. The statistical analysis was carried out using SPSS software.

Results

S. obliquus was chosen for the study because of high reproductive speed, tolerances to heat, high economic values, resistance to illumination, compliance with high concentration nutrients, resistance to contamination of microorganisms, the chemical structure of biomass, non-toxic substances.

The conditions for establishing an algal growing system in the laboratory were investigated. Prediction of growth through cell density and dry weight of *S. obliquus* in different culture media and natural mineral waters indicated that different growth pattern.

The mean number of cells density and biomass weights in (0) and (140) hours in the nutrient media and natural mineral waters of the ScnWW strain were shown in Table 3. When the mean differences in nutrient media were examined, the number of cells ($5.7x10^4$±$3.3x10^3$ cells/mL) and the biomass weight (0.0673±0.027 g/mL) in the Bold Wynne medium increased the most, while the number of cells ($4.3x10^4$±$1.0x10^4$ cells/mL) and biomass weight (0.0585±0.021 g/mL) in the Yagojinski medium were found to be lower at 140th hour. When the average differences in mineral waters were examined, the number of cells in the OBNMW ($4.1x10^4$±$8.8x10^3$ cells/mL) was highest, while in the KANMW mineral water ($3.5x10^4$±$1.1x10^4$ cells/mL) the least increased (Fig. 2). The results showed that the different culture media significantly (P<0.05) affected the final cell density, biomass and growth rates of ScnWW strain. The final cell density and biomass of Bold Wynne and Knop medium are significantly different from (Prat, Yagojinski, KANMW, KINMW, BENMW, OBNMW) which (P<0.05) but not significantly different between each other (P>0.05).

Table 3. Growth performance of ScnWW strain

Medium / MW	Cell Density (cells/mL) Avr.±SD		Biomass (g/mL) Avr.± SD	SGR[1] (μ) (h^{-1})	DT[2] (h^{-1})	Chlorophyll-a (μg/mL)
	(0h)	(140h)				
Bold Wynne	$3.4x10^4$±$3.1x10^3$	$5.7x10^{4*}$±$3.3x10^3$	0.0673*±0.027	0.018	38	1.11±0.43
Knop	$3.1x10^4$±$2.6x10^3$	$5.5x10^{4*}$±$3.1x10^3$	0.0672*±0.011	0.017	40	1.10±0.45
Prat	$3.2x10^4$±$5.6x10^3$	$4.9x10^4$±$6.4x10^3$	0.0594±0.015	0.017	39	1.02±0.39
Yagojinski	$3.2x10^4$±$2.8x10^3$	$4.3x10^4$±$1.0x10^4$	0.0585±0.021	0.016	44	1.01±0.37
KANMW	$3.1x10^4$±$4.9x10^3$	$3.5x10^4$±$1.1x10^4$	0.0407±0.012	0.007	99	0.98±0.10
KINMW	$3.3x10^4$±$4.1x10^3$	$3.8x10^4$±$1.3x10^4$	0.0527±0.017	0.008	92	0.99±0.12
BENMW	$3.2x10^4$±$2.2x10^3$	$3.9x10^4$±$7.6x10^3$	0.0566±0.012	0.011	65	0.99±0.11
OBNMW	$3.3x10^4$±$4.1x10^3$	$4.1x10^4$±$8.8x10^3$	0.0575±0.010	0.012	55	1.01±0.41

*P<0.05 significant difference, P>0.05 there is no significant difference; ANOVA test
[1]Specific Growth Rate, [2]Duplication Time

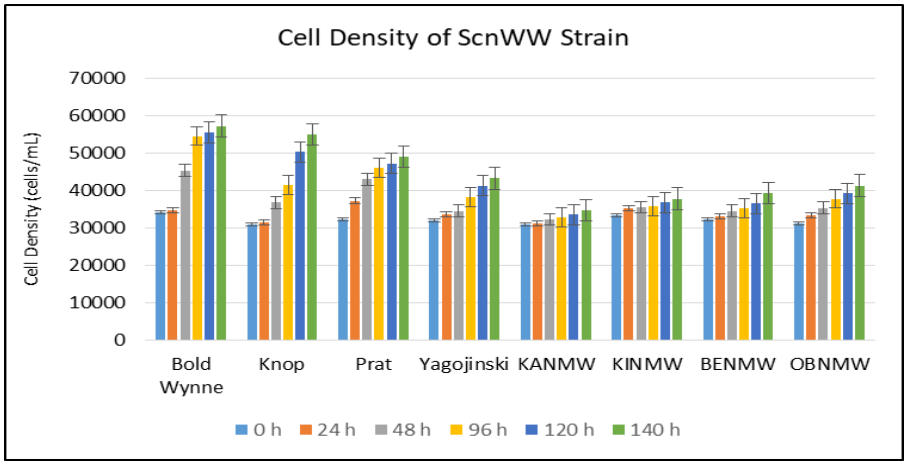

Figure 2. Cell density of ScnWW (cells/mL). Error bars represent the standard deviation for n=3

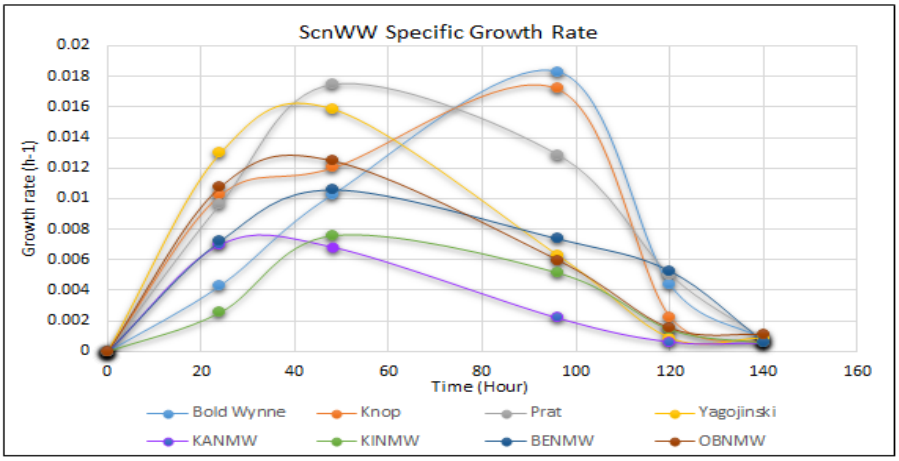

Figure 3. Specific growth rates of ScnWW strain in different nutrient media and mineral waters

The mean number of cells density and biomass weights in the culture media and natural mineral waters of the ScnMP strain were shown in Table 4. The number of cells ($5.4x10^4±3.2x10^3$ cells/mL) and the biomass weight (0.0769±0.029 g/mL) in the Bold Wynne medium increased the most, while the number of cells ($4.4x10^4±9.8x10^3$ cells/mL) and biomass weight (0.0609±0.0219 g/mL) in the Yagojinski medium were found to be lower. When the average differences in mineral waters were examined, the number of cells in the OBNMW ($4.3x10^4±8.1x10^3$ cells/mL) was highest, while in the BENMW mineral water ($3.5x10^4±7.5x10^3$ cells/mL) the least increased (Fig. 4). The final cell density and biomass of Bold Wynne medium is significantly different to the other culture media and mineral waters.

Table 4. Growth performance of ScnMP strain

Medium / MW	Cell Density (cells/mL) Avr.± SD		Biomass (g/mL) Avr.± SD	SGR[1] (μ) (h^{-1})	DT[2] (h^{-1})	Chlorophyll-a (μg/mL)
	(0h)	(140h)				
Bold Wynne	$3.2x10^4±3.3x10^3$	$5.4x10^{4*}±3.2x10^3$	$0.0769^{*}±0.029$	0.021	33	1.23±0.52
Knop	$3.3x10^4±2.2x10^3$	$4.7x10^4±2.8x10^3$	0.0684±0.0231	0.015	46	1.10±0.47
Prat	$3.2x10^4±5.5x10^3$	$4.6x10^4±5.9x10^3$	0.0612±0.0165	0.014	48	1.10±0.41
Yagojinski	$3.2x10^4±2.1x10^3$	$4.4x10^4±9.8x10^3$	0.0609±0.0219	0.012	58	1.07±031
KANMW	$3.2x10^4±4.7x10^3$	$4.1x10^4±1.2x10^4$	0.0523±0.0107	0.013	52	1.01±027
KINMW	$3.1x10^4±4.3x10^3$	$3.7x10^4±1.1x10^4$	0.0568±0.0184	0.011	63	0.97±0.09
BENMW	$3.1x10^4±2.7x10^3$	$3.5x10^4±7.5x10^3$	0.0582±0.0121	0.007	95	0.99±.011
OBNMW	$3.1x10^4±4.3x10^3$	$4.3x10^4±8.1x10^3$	0.0602±0.0113	0.013	54	1.04±0.32

*P<0.05 significant difference, P>0.05 there is no significant difference; ANOVA test
[1]Specific Growth Rate, [2]Duplication Time

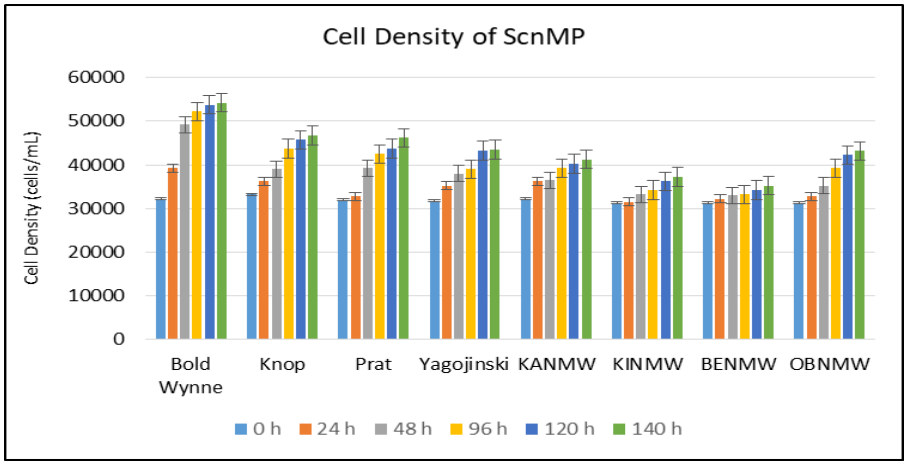

Figure 4. Cell density of ScnMP (cells/mL). Error bars represent the standard deviation for n=3.

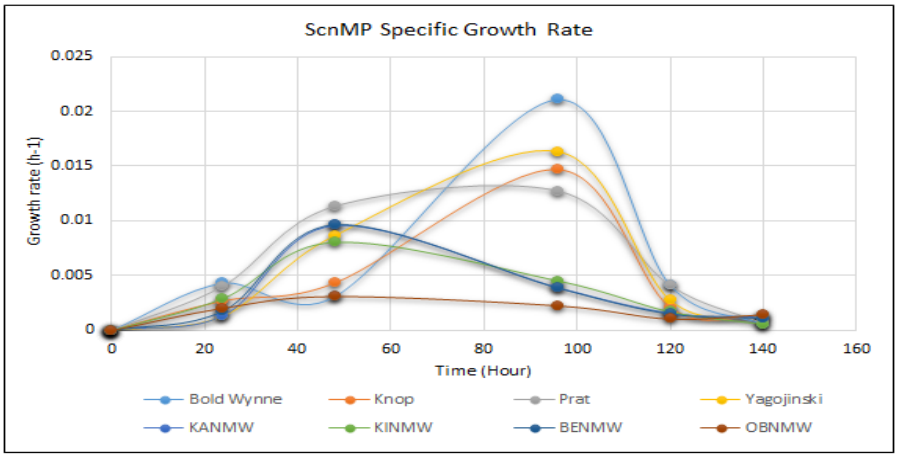

Figure 5. Specific growth rates of ScnMP strain in different nutrient media and mineral water.

Table 5. Growth performance of ScnGP strain

Medium / MW	Cell Density (cells/mL) Avr.± SD		Biomass (g/mL) Avr.± SD	SGR[1] (μ) (h^{-1})	DT[2] (h^{-1})	Chlorophyll-a (μg/mL)
	(0h)	(140h)				
Bold Wynne	$3.2x10^4±2.4x10^3$	$7.4x10^{4*}±1.3x10^3$	$0.212^*±0.032$	0.024	29	1.71±0.22
Knop	$3.2x10^4±2.5x10^3$	$7.1x10^{4*}±3.1x10^3$	$0.207^*±0.013$	0.021	34	1.62±0.43
Prat	$3.3x10^4±3.2x10^3$	$6.7x10^4±3.5x10^3$	0.098±0.017	0.017	42	1.48±0.47
Yagojinski	$3.2x10^4±2.3x10^3$	$6.4x10^4±6.2x10^3$	0.096±0.023	0.016	43	1.23±035
KANMW	$3.1x10^4±3.4x10^3$	$6.1x10^4±2.1x10^4$	0.093±0.012	0.014	48	0.89±0.09
KINMW	$3.2x10^4±3.3x10^3$	$5.8x10^4±2.2x10^4$	0.091±0.019	0.013	52	0.97±0.11
BENMW	$3.2x10^4±1.2x10^3$	$5.7x10^4±8.1x10^3$	0.091±0.014	0.011	66	0.98±0.10
OBNMW	$3.1x10^4±2.2x10^3$	$5.9x10^4±7.2x10^3$	0.089±0.013	0.013	52	1.12±0.42

*P<0.05 significant difference, P>0.05 there is no significant difference; ANOVA test
[1]Specific Growth Rate, [2]Duplication Time

The mean number of cells and biomass weights of the ScnGP strain were shown in Table 5. The maximum cell density ($7.4x10^4±1.3x10^3$ cells/mL) and biomass amount (0.212±0.032 g/mL) in ScnGP strain were determined in Bold Wynne culture medium. As in other strains, the lowest cell density ($6.4x10^4±6.2x10^3$ cells/mL) and biomass amount (0.096±0.023 g/mL) were obtained from Yagojinski culture medium. When examined in terms of natural minerals, the highest number of cells and biomass were found in KANMW ($6.1x10^4±2.1x10^4$ cells/mL) and the lowest in BENMW ($5.7x10^4±8.1x10^3$ cells/mL). The final cell density and biomass of

Bold Wynne and Knop medium is significantly different from others (Prat, Yagojinski, KANMW, KINMW, BENMW, OBNMW) which (P<0.05).

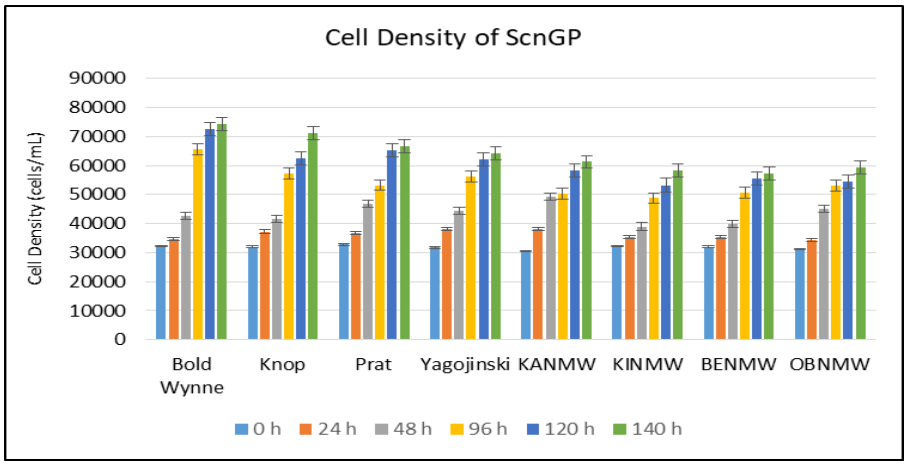

Figure 6. Cell density of ScnGP (cells/mL). Error bars represent standard deviation for n=3.

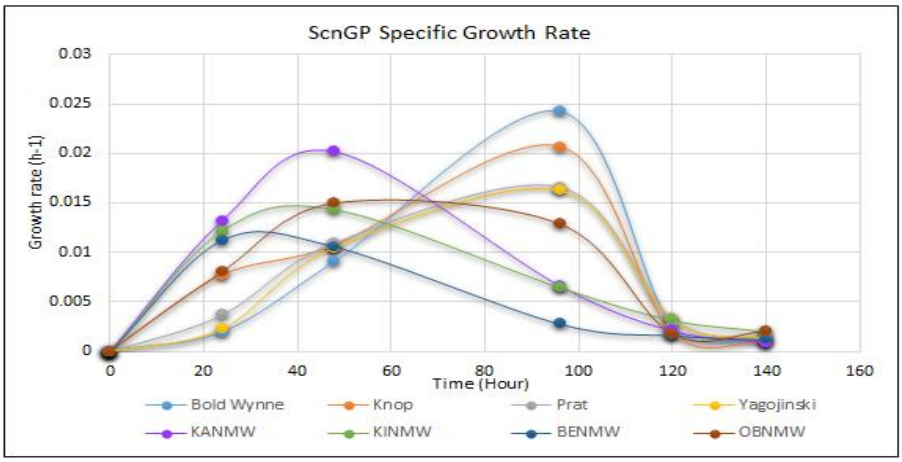

Figure 7. Specific growth rates of ScnGP strain in different nutrient media and mineral waters

The maximum growth rate of ScnWW (0.018 h^{-1}) strain was reached on the 96th hour under Bold Wynne media and its duplication time was found 38 h^{-1} (Table 3 and Figure 3). The maximum growth rate of ScnMP (0.021 h^{-1}) strain occurred on the 96th hour in Bold Wynne media and duplication time was 33 h^{-1} (Table 4 and Figure 5). The algal growth rate was highest under Bold Wynne culture media (0.024 h^{-1}) in ScnGP strain and duplication time was 29 h^{-1} (Table 5 and Figure 7).

Discussion

Nutrient availability, light intensity, temperature, pH and salinity are the main external factors of microalgal growth, reproduction, and morphology. The quantity and quality of nutrients are the most important parameters regulating the growth and reproduction of microalgae. Essential nutrient substances must be supplied for the cultivation of microalgae at the optimum concentration. The presence of nitrogen, phosphorus, sulphur and trace elements is important for the reproduction of microalgae (Blair et al. 2013). Sulphur is a predominant element in proteins and in various coenzymes (Bhamawat 2010). Nitrogen is an indispensable element of all structural and functional proteins in algal cells. The lack of phosphorus in the culture medium affects not only chlorophyll synthesis but also the growth and metabolism of cells (Liang et al. 2013; Chu et al. 2007). In this study, different phosphorus and nitrogen sources were used in the culture media (Table 1). When the media was examined for *S. obliquus* strains, the best reproduction was detected in the Bold Wynne medium and the lowest reproduction was found in the Yagojinski culture medium. The higher biomass productivity of *S. obliquus* grown in Bold Wynne media might be related to concentration of K_2HPO_4, KH_2PO_4, and $NaNO_3$ as a source of phosphorus and nitrogen, respectively.

The highest cell density ($7.4x10^4 \pm 1.3x10^3$ cells/mL) of ScnGP strain grown in Bold Wynne was significantly (P<0.05) higher than that of growing in all other treatments. *S. obliquus*'s maximum cell count ($136.3x10^5$/ml) and the specific growth rate (0.32 - 0.42 µg/day) were determined by Toyub et al.

(2008). Al-Shatri et al. (2014) produced the strain of *Scenedesmus dimorphus* in BBM medium and the final cell number was found to be ($2.5x10^4$ cells/mL), the specific growth rate was (0.104 μ/day^{-1}). Latiffi et al. (2017) in their study on *Scenedesmus* sp., the cell number ($1x10^4$ cells/mL), specific growth rate (0.412 μ/day^{-1}) and doubling time (1.680 day^{-1}) was found. In this study, as in cell density, the highest dry weight was obtained from cultivation of ScnGP strain in Bold Wynne medium (0.212±0.032 g/mL). *S. obliquus* was grown at different concentrations of NaCl in Bold Basal Medium (BBM) by Salama et al (2013). The highest dry weight of *S. obliquus* was determined at 25 mM NaCl as 0.65 g/L. In the study conducted by Eida et al. (2018), the highest dry biomass of *S. obliquus* was measured as 0.529 and 0.524 g/L in (0.5% Wastewater and 25% BBM and 100% BBM) mix medium. There are some differences in cell density and dry weight between the studies in the literature and the results of this study. These differences are thought to be the result of the medium used, culture conditions, culture amount, characteristics of the strains and the purpose of the studies.

Different culture medium and mineral waters showed a significantly different effect on chlorophyll-a content of *S. obliquus* strains. The chlorophyll-a content of *S. obliquus* strains grown in Bold Wynne medium was found to be higher than those of other culture media and natural mineral waters. The highest total content of chlorophyll-a found in the ScnGP (1.71±0.22 µg/mL), followed by ScnMP (1.23±0.52 µg/mL) and ScnWW (1.11±0.43 µg/mL) (Table 3, 4 and 5). In other studies on *S. obliquus*, the amounts of chlorophyll-a were found as 0.64 µg/mL, 1.819 µg/mL and 10 µg/mL (Rinanti et al. 2013; Kabir et al.2017; Chen et al. 2011) respectively. It was observed in this study that *S. obliquus* strains were found to have differences in the number of cells ($P<0.05$) and biomass values ($P<0.05$) compared to the culture media and natural mineral waters. Differences were observed between strains in the experiments performed with the media used in the production of *S. obliquus* strains. In 3 isolates, there was a significant difference in ScnGP strain in terms of cell density, biomass weight, chlorophyll-a, and specific growth rate compared to others.

When each culture media and mineral waters are compared, it can be said that the culture medium efficiency better results in terms of both cell density and biomass weight. Also, it was determined that strains which were cultured in mineral waters produced significantly.

When mineral waters were examined in their own form, the strains (ScnWW and ScnMP) values were higher in OBNMW mineral water whereas in KANMW mineral water, ScnGP strain had more reproduction. In aquatic environments, it is thought that Cl, Na and K ions do not directly affect the appearance of algae species in the environment. On the other hand, it was found that the density of the ions mentioned indirectly influenced the osmotic pressure, pH, and single and divalent ion values. These experiments with mineral waters, it was observed that Na and Cl ions were different in four mineral waters (Table 2). In the four mineral waters tested, Ca, Mg, Fe, Al, Si, N elements are available. The amounts of these elements differ in four mineral waters. As a result, natural mineral waters can be used as a cheap source of algae production. In addition, various chemical compounds can be added as factors affecting reproduction to increase production.

In conclusion, the effect of different culture media and natural mineral waters on the growth of freshwater microalgae *S. obliquus* strains were investigated. It was clearly seen that Bold Wynne media has a greater impact on the growth of strains when compared to other media and natural mineral waters. The use of natural mineral water has been considered as a cost-effective and abundant source for the growth of *S. obliquus* strains. The natural mineral water will be beneficial for microalgae production, but the development of microalgae cultivation process should be thought in mineral water. It is thought that in the advanced stages, the effects of the species produced in mineral waters on the amount of intracellular lipid will contribute to the studies to be performed in biodiesel production.

References

Al-Shatri AH, Ali E, Al-Shorgani NK, Kalil MS. 2014. Growth of *Scenedesmus dimorphus* in different algal media and pH profile due to secreted metabolites. Afr J Biotechnol. 13 (16):1714-1720. doi: 10.5897/AJB2013.13455

Andersen RA, Berges JA, Harrison PJ. 2005. Recipes for freshwater and seawater media. In: Andersen RA editor. Algal culturing techniques. London: Elsevier Academic Press. p. 429-538.

Bhamawat PM. 2010. Growth of *Chlamydomonas reinhardtii* under nutrient-limited conditions in steady-state bioreactors [Master's Thesis]. Faculty of the Graduate School of Cornell University. 83 p.

Blair MF, Kokabian B, Gude VG. 2013. Light and growth medium effect on *Chlorella vulgaris*

biomass production. Journal of Environmental Chemical Engineering. 2(1):665-674. doi: 10.1016/j.jece.2013.11.005

Brennan L, Owende P. 2010. Biofuels form microalgae a review of technologies for production, processing and

extractions of biofuels and co-products. Renew Sust Energ Rev. 14(2):557-577. doi: 10.1016/j.rser.2009.10.009

Chen M, Li L, Dai X, Sun Y, Chen F. 2011. Effect of phosphorus and temperature on chlorophyll a contents and cell sizes of *Scenedesmus obliquus* and *Microcystis aeruginosa*. Limnology. 12(2):187-192. doi: 10.1007/s10201-010-0336-y

Chia MA, Lombardi AT, Melao MGG. 2013. Growth and biochemical composition of *Chlorella vulgaris* in different growth media. Annals of the Brazilian Academy of Sciences. 85(4):1427-1438. doi: 10.1590/0001-3765201393312

Chu ZS, Jin XC, Yan F, Zheng SF, Pang Y, Zeng QR. 2007. Effects of EDTA and iron on growth and competition of Microcystis aeruginosa and *Scenedesmus quadricauda*. Huanjing Kexue/Environ Sci. 28(11): 2457-2461.

Eida MF, Darwesh OM, Matter IA. 2018. Cultivation of oleaginous microalgae *Scenedesmus obliquus* on secondary treated municipal wastewater as growth medium for biodiesel production. Journal of Ecological Engineering. 19(5):38-51. doi: 10.12911/22998993/91274

Fallahi M, Rahbary SH, Shamsaii M. 2014. Determination of optimum concentration of diuron for the growth and bloom of the algae (*Scenedesmus obliquus*) in in vitro condition. Iran J Fish Sci. 13(3):739-747.

Godoy-Hernández G, Vázquez-Flota FA. 2006. Growth measurements: estimation of cell division and cell expansion. In: Loyola-Vargas VM, Vázquez-Flota F. editors. Methods in molecular biology. New Jersey: Humana Press Inc. 877:41-48.

Guillard RRL, Sierachiki MS 2005. Counting cells in cultures with the light microscope. In: Andersen RA editor. Algal culturing techniques. London: Elsevier Academic Press. p. 239-252.

Guiry MD, Guiry GM. 2018. AlgaeBase; [cited: 2018 Oct 21]. Available from http://www.algaebase.org.

Hodaifa G, Martínez ME, Sánchez S. 2008. Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. Bioresource Technol. 99(5):1111-1117. doi: 10.1016/j.biortech.2007.02.020

Humpry AM. 2004. Chlorophyll as a color and functional ingredient. J Food Sci. 69(5):422-425. doi: 10.1111/j.1365-2621.2004.tb10710.x

Kabir M, Hoseini SA, Ghorbani R, Kashiri H. 2017. Performance of microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* in wastewater treatment of Gomishan (Golestan-Iran) shrimp farms. Aquaculture, Aquarium, Conservation and Legislation - International Journal of the Bioflux Society. 10(3):622-632.

Latiffi NAA, Mohamed RM, Apandi NM, Tajuddin RM. 2017. Preliminary assessment of growth rates on different concentration of microalgae *Scenedesmus* sp. in industrial meat food processing wastewater. International Symposium On Civil And Environmental Engineering. 103: 1-9. doi: 10.1051/matecconf/20171030

Liang K, Zhang Q, Gu M, Cong W. 2013. Effect of phosphorus on lipid accumulation in freshwater microalgae *Chlorella* sp. J Appl Phycol. 25(1):311-318. doi: 10.1007/s10811-012-9865-6

Murdock JN, Wetzel DL. 2009. FT-IR Microspectroscopy enhances biological and ecological analysis of algae. Appl Spectroscopy Rev. 44(4):335-361. doi: 10.1080/05704920902907440

Parvin M, Zannat MN, Habib MAB. 2007. Two important technique for isolation of microalgae. Asian Fisheries Science. 20:117-124.

Prescott GW. 1973. Algae of the western great lakes area. Michigan: C. Brown Company Publishers 977 p.

Pringsheim E. 1946. Pure cultures of algae. Cambridge: University Press 119 p.

Rasmussen RS, Morrissey T, Steve LT. 2007. Marine biotechnology for production of food ingredients. Advances in Food and Nutrition Research. 237-292. doi: 10.1016/S1043-4526(06)52005-4

Rinanti A, Kardena E, Astuti DI, Dewi K. 2013. Growth response and chlorophyll content of *Scenedesmus obliquus* cultivated in different artificial media. Asian Journal of Environmental Biology. 1(1):1-9. doi: 10.13140/RG.2.1.3370.7926

Salama ES, Kim HC, Abou-Shanab RAI et al. 2013. Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. Bioproc Biosyst Eng. 36(6):827-833. doi: 10.1007/s00449-013-0919-1

Sforza E, Gris B, Silva C, Morosinotto T, Bertucco A. 2014. Effects of light on cultivation *Scenedesmus obliquus* in batch and continuous flat plate photobioreactor. Chemical Engineering Transactions. 38:211-216. doi: 10.3303/CET1438036

Shelly K, Heraud P, Beardall J. 2002. Nitrogen limitation in *Dunaliella tertiolecta* (Chlorophyceae) leads to increased susceptibility to damage by UV-B radiation but also increased repair capacity. J Phycol. 38:1-8. doi: 10.1046/j.1529-8817.2002.01147.x

Toyub MA, Miah MI, Habib MAB, Rahman MM. 2008. Growth performance and nutritional value of *Scenedesmus obliquus* cultured in different concentrations of sweetmeat factory waste media. Bangladesh Journal of Animal Science. 37(1):86-93. doi: 10.3329/bjas.v37i1.9874

Wang B, Li Y, Wu N, Lan CQ. 2008. CO_2 bio-mitigation using microalgae. Appl Microbiol Biot 79(5):707-718. doi: 10.1007/s00253-008-1518-y

Youngman RE. 1978. Measurement of chlorophyll-a. New York: Water Research Centre Technical Report. Report No: TR82.