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Research Article | **Araştırma Makalesi**

Effect of Microalgae Growth Promoting Bacteria on Biomass Production of *Scenedesmus* Isolates in Co-Culture Systems

Mikroalg Büyümesini Destekleyen Bakterilerin Eş Kültür Sistemlerinde *Scenedesmus* İzolatlarının Biyokütle Üretimi Üzerindeki Etkisi

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Abstract: Microalgae are biotechnologically important microorganisms with the potential to address the challenges posed by rapid population growth and dwindling food resources through their metabolic products. This study focused on the isolation and identification of *Scenedesmus* and microalgae growth-promoting bacteria (MGPB) from wetlands in Erzurum, Türkiye, and investigated the impact of MGPB isolates on the growth performance of *Scenedesmus* species in a co-culture medium. *Scenedesmus* and MGPB isolates were collected from Palandoken Teke Creek Pond and Tortum Lake, respectively, followed by species identification. Co-culture experiments were conducted using 20 MGPB isolates from the same area, with *Scenedesmus flavescens* (from Palandoken Teke Creek Pond) and *Scenedesmus armatus* (from Tortum Lake) serving as test species. The experiment spanned seven days, with daily measurements of cell counts and biomass, along with dry matter weight assessments at the start and end of the study. The results showed that *S. armatus* and *S. flavescens* achieved the highest biomass in co-culture with *Bacillus* sp. and *Pseudomonas* sp., the cell counts of *S. armatus co-culture* with *Pseudomonas* sp. (A5) were 306±16.21 cells/ml, and this group followed by A6 (251±13.37 cells/ml), A7 (260±17.6 cells/ml), and F8 (93±6.01 cells/ml) groups. In conclusion, 6% growth was detected in *S. flavescens* biomass and 7% in *S. armatus* biomass. MGPB bacteria enhanced the growth of microalgae, underscoring their potential for biotechnological applications.

Keywords
- Biomass
- Co-culture
- MGPB
- *S. armatus*
- *S. flavescens*

Özet: Mikroalgler, metabolik ürünleri aracılığıyla hızlı popülasyon artışı ve azalan besin kaynaklarının oluşturduğu zorluklarla başa çıkma potansiyeline sahip biyoteknolojik açıdan önemli mikroorganizmalardır. Bu çalışma, Türkiye, Erzurum'daki sulak alanlardan *Scenedesmus* ve mikroalg büyümesini teşvik eden bakterilerin (MBTB) izolasyonu ve tanımlanmasına odaklanmış ve MBTB izolatlarının eş kültür ortamında *Scenedesmus* türlerinin büyüme performansı üzerindeki etkisini araştırmıştır. *Scenedesmus* ve MBTB izolatları sırasıyla Palandöken Teke Deresi Göleti ve Tortum Gölü'nden toplanmış ve ardından tür tanımlaması yapılmıştır. Eş kültür deneyleri, aynı alandan alınan 20 MBTB izolatı kullanılarak yürütülmüş ve test türleri olarak *Scenedesmus flavescens* (Palandöken Teke Deresi Göleti'nden) ve *Scenedesmus armatus* (Tortum Gölü'nden) kullanılmıştır. Deney, hücre sayısı ve biyokütlenin günlük ölçümleri ile çalışmanın başında ve sonunda kuru madde ağırlığı değerlendirmeleri ile yedi gün sürmüş, ve *S. armatus* ve *S. flavescens*'in *Bacillus* sp. ve *Pseudomonas* sp. ile birlikte eş-kültürde en yüksek biyokütleye ulaştığını, *S. armatus*'un *Pseudomonas* sp. (A5) ile birlikte kültürüne ait hücre sayımlarının 306±16,21 hücre/ml olduğunu ve bu grubu A6 (251±13,37 hücre/ml), A7 (260±17,6 hücre/ml) ve F8 (93±6,01 hücre/ml) gruplarının takip ettiği tespit edilmiştir. Sonuç olarak, Bu çalışmada *S. flavescens* biyokütlesinde %6, *S. armatus* biyokütlesinde ise %7 oranında büyüme tespit edildi. MBTB bakterileri mikroalglerin büyümesini artırarak biyoteknolojik uygulamalar için potansiyellerini vurguladı.

Anahtar kelimeler
- Biyokütle
- Eş-kültür
- MBTB
- *S. armatus*
- *S. flavescens*

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1. INTRODUCTION

Microalgae are simple, single-celled, or colonial microorganisms ranging in size from 1 µm to 1 cm, capable of photosynthesis and thriving under autotrophic or heterotrophic conditions. They contain chlorophyll within the cells (Lee, 2008). Chemically, their structure is expressed as C106H181O45N16P1, whereas their biochemical composition includes carbohydrates, proteins, pigments, and lipids (Sajjadi et al., 2018).

Microalgae production conditions vary depending on the species, although all species require light, appropriate temperature, pH, and medium components (e.g., N, P, and K) to grow effectively. While medium composition varies across species, nitrogen and phosphorus make up 10-20% of the algal biomass and serve as primary nutrients, supplemented by macronutrients (Mg, Na, K, Ca), micronutrients, and vitamins (Andersen, 2005).

Microalgae have found applications in various sectors including biodiesel, food, and pharmaceuticals. However, different microalgae species are suited to specific sectors and their products are influenced by abiotic factors. For instance, Gouveia & Oliveira (2009) identified *Nannochloropsis oleoabundans* (a freshwater species) and *Nannochloropsis* sp. (a marine species) as promising candidates for biofuel production because of their high lipid contents (29.0% and 28.7%, respectively). Modifications in culture conditions, such as adjusting day-night cycles, light intensity, pH levels, and nutrient limitations (nitrogen or phosphorus) can significantly affect lipid and protein production. Ongoing research, for determining ideal culture conditions to optimize biomass yield and metabolic output, continues to evolve (Darki et al., 2017; An et al., 2020).

Although the concept of co-culturing microalgae with bacteria may appear novel in the current literature, its origins have been traced back to the 1930s (Waksman et al., 1937). Interest in beneficial interactions between microalgae and bacteria gained momentum in the 1970s and has continued to expand (Delucca & McCracken, 1977). While these bacteria share mechanisms with "plant growth-promoting bacteria," such as nitrogen fixation, phytohormone production, and phosphate solubilisation, they are now recognized as a distinct category called "microalgae growth-promoting bacteria" (MGPB). This classification reflects their unique capabilities, such as vitamin and CO_2 production, which are essential for microalgae cultivation (Palacios et al., 2022).

To qualify as MGPB, bacteria must exhibit at least two growth-promoting mechanisms for microalgae, beyond basic CO_2 supplementation via natural carbon catabolism. For example, the use of *Azospirillum* spp. in association with higher plant roots has been widely studied. Similarly, co-culture studies involving *Azospirillum* and *Chlorella vulgaris* have demonstrated the potential for advancing sustainable microalgal production technologies (De Bashan et al., 2002; Hernandez et al., 2009; Do Nascimento et al., 2013; Liu et al., 2020; Gonzalez-Gonzalez et al., 2021).

In this study, we investigated the co-cultivation of *Scenedesmus* species and MGPB isolates obtained from wetlands in the Erzurum Province, and aimed to assess the effects of MGPB isolates on the growth performance of *Scenedesmus* species.

2. MATERIAL and METHOD

2.1. Study of Microalgae and MGPB Sampling

In this study, microalgae samples were collected from Palandoken Teke Creek Pond and Lake Tortum within Erzurum using a plankton net with a mesh size of 10 µm. The samples were transferred to the laboratory in polyethylene sampling containers using a cold chain protocol. Bacteria were sampled simultaneously from the same stations as those used for the microalgae sampling. Water samples for bacterial sampling were taken with a Ruttner, and sediment samples were collected with an Ekman grab. Collected samples were transferred to sterile sample containers and transferred to the laboratory using the cold chain protocol, in which aseptic conditions were maintained (Wetzel and Likens, 2000).

2.2. Isolation and Identification of *Scenedesmus* Isolate

First, the collected samples were examined under a binocular microscope and selected for the isolation stage of the *Scenedesmus* isolate. *Scenedesmus* was isolated from selected samples using the micropipette method under an inverted microscope with a camera attachment (Zeiss Binocular Microscope). For the conventional identification of *Scenedesmus* isolates, binocular microscope examinations were matched with literature data (Prescott, 1973; Lind & Brook, 1980; Komarek & Fott 1983; John et al., 2002).

Bacteria used for MGPB research were isolated by preparing dilution series from 10^{-1} to 10^{-6} using phosphate buffered saline from each water and sediment sample and spreading each dilution onto Luria–Bertani (LB) agar and nutrient agar (NA) media. Cultures were grown at 28 °C for 24-48 hours and each of the bacteria showing discrimination in terms of colony morphology was subjected to repeated cultivation until pure colonies were obtained using the 3-phase streak method (Figure 1) (Vaikuntapu et al., 2014; Alaylar et al., 2019; Liu et al., 2020).

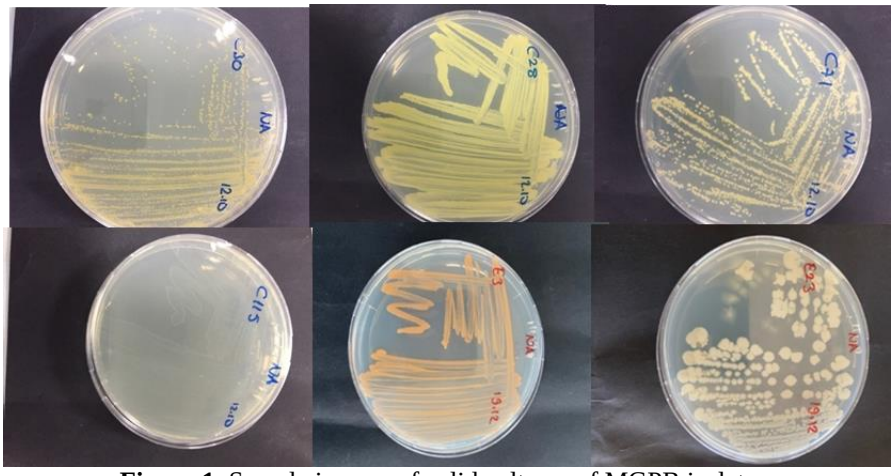


Figure 1. Sample images of solid cultures of MGPB isolates.

2.2.1. Selection of MGPB isolates

Nitrogen fixation, phosphate solubilization, and IAA (Indole-3-Acetic Acid) production criteria suggested in the literature were used for the selection of MGPB isolates for use in co-culture studies with *Scenedesmus* (Liu et al., 2020; Palacios et al., 2022).

2.2.2. Selection of nitrogen-fixing MGPB isolates

The content of selective media, recommended in the literature for the selection of bacterial isolates with nitrogen-fixing ability, used in the present study was as follows: Ashby's [for 1 L: 20 g mannitol, 0.2 g K_2HPO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, 0.1 g K_2SO_4, 5 g $CaCO_3$ and 15 g agar], Jansen's medium [for 1 L: 20 g sucrose, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 0.1 g $FeSO_4$, 0.005 g Na_2MoO_4, 2 g $CaCO_3$ and 15 g agar], Jansen's medium [for 1 L: 10 g glucose, 1 g K_2HPO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, 0.1 g $FeSO_4$, 0.005 g Na_2MoO_4, 1 g $CaCO_3$ and 15 g agar] (Liu et al., 2020).

2.2.3. Selection of phosphate-solubilizing MGPB isolates

Pikovskaya's agar [for 1 L: 10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)2SO_4$, 0.2 g NaCl, 0.1 g $MgSO_4$ $7H_2O$, 0.2 g KCl, 0.002 g $MnSO_4$ $7H_2O$, 0.002 g $FeSO_4$ $7H_2O$, 0.5 g yeast extract, and 15 g agar] medium recommended by the literature was used for the selection of isolates with the potential to solubilize phosphate compounds (Alaylar et al., 2019; Liu et al., 2020).

2.2.4. Selection of indole-3-acetic acid (IAA)-producing MGPB isolates

For the selection of IAA-producing MGPB isolates, each isolate was allowed to grow for 72 h at 28 °C in Luria–Bertani Broth medium supplemented with 1% tryptophan. Then cells were sedimented by centrifugation (12000 rpm for 10 min), 4 ml of Salkowski's reagent was added to 1 ml of supernatant, and the mixture was incubated at 37 °C in the dark for 30 min. At the end of the study period, IAA concentrations of the samples were determined by colorimetric measurement at 530 nm (Liu et al., 2020).

2.3. Cultivation Studies

Scenedesmus isolates were inoculated into a 3N-BBM+V solid medium using the streak method under aseptic conditions (sterile cabinet). After waiting for 10 days in the incubation cabinet, the cells were placed in a 10 ml liquid medium and developed in an incubator for 7-8 days. Then, 10 ml tubes were transferred to liquid 3N-BBM+V medium in 250 ml conical flasks and cultured at 25 ºC, 43.15 µmol/m^2s illumination and 110 rpm in an incubator (JRS Lab 32 brand) with a 16:8 hour day-night photoperiod.

In the experiment, 3N-BBM+V nutrient medium was used, which contained: 25 g/l

$NaNO_3$, 2.5 g/l $CaCl_2.2H_2O$, 7.5 g/l K_2HPO_4, 7.5 g/l $MgSO_4.7H_2O$, 0.75 g/l Na_2EDTA, 17.5 g/l $KH_2PO_3.3H_2O$, 2.5 g/l NaCl and supply of essential micronutrients ($FeCl_3.6H_2O$, $MnCl_2.4H_2O$, $ZnCl_2$, $CoCl_2.6H_2O$ and $Na_2MoO_4.2H_2O$) (Andersen, 2005).

2.4. Co-Culture Studies

The experimental co-culture setup for each sample used 20 MGPB and *Scenedesmus* isolates with the highest activity. For each co-culture experiment, the cell count of *Scenedesmus* isolates was taken into the nutrient medium at 2000-4000 cells/mL, and the experiment was established as 50/1 *Scenedesmus*/MGPB by adding the test MGPB isolate at a concentration of $1x10^5$-$2x10^5$ cells/ml. Each experiment was designed to have 3 replications.

The prepared co-cultures were developed for 7 days in the Algae Unit of Atatürk University Faculty of Fisheries at 25 ºC and 43.15 µmol/m^2s lighting conditions with a 16:8-hour day-night photoperiod.

2.5. Microalgae cell counts and biomass calculations

Samples from the homogeneous co-culture experimental medium were taken from a 3 ml sample and Lugol solution was dropped into the counting chamber and kept overnight. Cell counts were performed daily using a Zeiss Primo Vert model inverted microscope (Utermohl, 1958; Anonymous, 2003).

Phytoplankton count (cell/ml) = C × At/As × S × V

Here, C = Number of organisms counted (number), At = Counting cell bottom area (mm^2), As = Field of view (mm^2), S = Number of fields of view counted (number), and V = Precipitated sample volume (mL).

For the measurement of biomass of *Scenedesmus* isolates, 15 ml samples were taken after the trial cultures were mixed homogeneously every day and centrifuged for 5 minutes at 13400 rpm and 4°C and read at 680 nm wavelength in a spectrophotometer. The biomass formula for *Scenedesmus* isolates was calculated using the following equation (Li et al., 2021).

Biomass concentration (g/L) = 0.4818 x (A1-A0)

Here, A1 is the absorbance value of the algal sample and A0 is the absorbance value of the culture medium.

2.5.1. Dry cell weight

In this study, 50 ml culture samples were filtered at the beginning and end of the trial using Whatman GF/C filter papers (0.45 µ mesh size). After the filtration process, they were weighed on a 0.001 g sensitive scale, and their wet weights were determined. After the filtered samples were kept in an oven fixed at 100 °C for 1 h, they were placed in a desiccator, cooled to room temperature, and weighed on a 0.001 g sensitive scale (Vonshak, 1997).

2.6. Research Data Evaluation

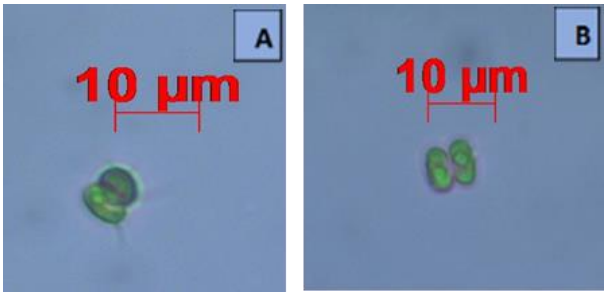
IBM SPSS 20 software was used to evaluate the statistical significance of the findings obtained from all analyses. Cell counting, biomass, and dry matter values were subjected to One-Way ANOVA, and a DUNCAN test was applied to evaluate the differences between them that were significant. In addition, linear correlation among the groups was determined by the Pearson Correlation test.

3. RESULTS

3.1. Identification of *Scenedesmus* and MBTB Isolate

In this study, *Scenedesmus armatus* from Tortum Lake and *Scenedesmus flavescens* from Palandoken Teke Creek Pond were identified (Figure 2).

Group: Chlorophyta
Order: Chlorophyceae
Family: Scenedesmaceae
Genus: *Scenedesmus*
Species: ***Scenedesmus armatus***
(*Desmodesmus armatu*s (Chodat) E.H.Hegewald
Scenedesmus flavescens
(*Desmodesmus flavescens* (Chodat) E.Hegewald)



Figures 2. *S. armatus* (A) ve *S. flavescens* (B)

Information on the bacterial species isolatedfrom Tortum Lake and the properties of MGPBare given in Table 1. The properties of the bacteria isolated from Palandoken Teke Creek Pond are listed in Table 2.

Table 1. Bacteria isolated from Tortum Lake and their MGPB characteristics.

Code	Bacteria Code	Species	Nitrogen Fixation	Phosphate Dissolution	IAA
A1	D22	*Bacillus* sp.	1.2	+	Negatif
A2	D91	*Bacillus* sp.	1.4	+ +	Negatif
A3	D108	*Bacillus* sp.	1.1	+ + + +	Negatif
A4	D144	*Bacillus* sp.	0.9	+ + +	Negatif
A5	D173	*Pseudomonas* sp.	0.9	+	Negatif
A6	D188	*Pseudomonas* sp.	1.3	+ +	Negatif
A7	D243	*Pseudomonas* sp.	0.8	+	Negatif
A8	D275	*Bacillus* sp.	0.4	+ +	Negatif
A9	D290	*Bacillus* sp.	0.6	+	Negatif
A10	D291	*Bacillus* sp.	0.4	+	Negatif

Table 2. Bacteria Isolated from Palandoken Teke Creek Pond and their MGPB characteristics.

Code	Bacteria Code	Species	Nitrogen Fixation	Phosphate Dissolution	IAA
F1	B163	*Pseudomonas* sp.	Negatif	+ + +	0.161
F2	B134	*Bacillus* sp..	Negatif	+ +	0.057
F3	B115	*Bacillus* sp.	Zayıf	+	0.126
F4	B105	*Pseudomonas* sp.	Negatif	+ +	0.602
F5	B98	*Pseudomonas* sp.	Negatif	+ +	0.069
F6	B100	*Pseudomonas* sp.	Negatif	+	0.048
F7	B76	*Pseudomonas* sp.	Negatif	+ +	0.007
F8	B93	*Bacillus* sp.	Negatif	+	0.164
F9	B94	*Pseudomonas* sp.	Negatif	+	0.127
F10	B96	*Pseudomonas* sp.	1.2	+	0.064

3.2. *S. armatus and S. flavescens* Cell Counting and Biomass

In this study, *S. armatus* and *S.flavescens* species isolated from both lakes were prepared as the control group and a co-culture environment inoculated with 20 MGPB each. The experimental groups were designated as control, A1-A10 for *S. armatus*, and F1-F10 for *S. flavescens*. Both the biomass value and cell numbers for both species were found to be statistically significant depending on the day and groups ($p<0.05$). It was determined that there was a correlation between *S. armatus* and *S. flavescens* biomass (Table 3).

Table 3. Correlation between *S. armatus* and *S. flavescens* biomass.

		Tortum Lake	Palandoken Teke Creek Pond
Tortum Lake	Pearson Correlation	1	1.0**
	Sig. (2-tailed)		0.0
	N	33	33
Palandoken Teke Creek Pond	Pearson Correlation	1.0**	1
	Sig. (2-tailed)	0.0	
	N	33	33

** The correlation significance level is 0.01

In the co-culture experiment with *S. armatus* and MGPB, the difference between the groups and the change depending on the day were found to be statistically significant ($p<0.05$). When the increase in biomass depending on the days throughout the experiment was compared with the control group, the highest biomass was found in the co-culture experiments with *Bacillus* sp. (0.34±0.05 mg/L) followed by *Pseudomonas* sp. (0.32±0.05 mg/L); the lowest biomass was found in the co-culture study with *Pseudomonas* sp. (Table 4). Throughout the study, an increase was detected in all groups except A10 and A9 until the 7th day (Figure 3).

Table 4. Changes in *S. armatus* biomass depending on days and groups (mg L^{-1}, Mean±SD, n=3).

Groups	Days						
	1	2	3	4	5	6	7
Control	0.16 ± 0.0^{Bc}*	0.16 ± 0.0^{Dc}	0.16 ± 0.0^{Ec}	0.18 ± 0.0^{Ec}	0.20 ± 0.0^{Db}	0.25 ± 0.0^{Fa}	0.25 ± 0.01^{Ca}
A1	0.16 ± 0.0^{Be}	0.24 ± 0.0^{Cd}	0.27 ± 0.0^{Cc}	0.27 ± 0.0^{Dc}	0.29 ± 0.0^{Cc}	0.42 ± 0.0^{Aa}	0.37 ± 0.01^{Bb}
A2	0.15 ± 0.0^{Cd}	0.17 ± 0.0^{Dc}	0.18 ± 0.0^{Dc}	0.17 ± 0.01^{Fc}	0.19 ± 0.0^{Dc}	0.28 ± 0.01^{Ea}	0.25 ± 0.0^{Cb}
A3	0.17 ± 0.0^{Bc}	0.18 ± 0.0^{Dc}	0.19 ± 0.0^{Dc}	0.17 ± 0.0^{Fc}	0.18 ± 0.0^{Ec}	0.32 ± 0.1^{Da}	0.22 ± 0.0^{Cb}
A4	0.24 ± 0.01^{Ad}	0.29 ± 0.01^{Ac}	0.37 ± 0.01^{Ab}	0.37 ± 0.01^{Ab}	0.39 ± 0.01^{Aa}	0.36 ± 0.01^{Cb}	0.35 ± 0.0^{Bb}
A5	0.24 ± 0.01^{Ae}	0.30 ± 0.0^{Ac}	0.28 ± 0.0^{Cd}	0.33 ± 0.0^{Bb}	0.34 ± 0.0^{Bb}	0.39 ± 0.0^{Ba}	0.39 ± 0.0^{Aa}
A6	0.25 ± 0.0^{Ad}	0.28 ± 0.0^{Bc}	0.30 ± 0.0^{Bb}	0.30 ± 0.0^{Cb}	0.33 ± 0.01^{Bb}	0.40 ± 0.01^{Ba}	0.39 ± 0.0^{Aa}
A7	0.24 ± 0.0^{Ad}	0.28 ± 0.0^{Bc}	0.30 ± 0.0^{Bb}	0.31 ± 0.0^{Cb}	0.31 ± 0.0^{Bb}	0.40 ± 0.0^{Ba}	0.39 ± 0.0^{Aa}
A8	0.15 ± 0.0^{Cc}	0.28 ± 0.0^{Ba}	0.16 ± 0.0^{Ec}	0.16 ± 0.0^{Fc}	0.22 ± 0.0^{Db}	0.21 ± 0.0^{Gb}	0.16 ± 0.0^{Dc}
A9	0.14 ± 0.0^{Dc}	0.17 ± 0.0^{Db}	0.15 ± 0.0^{Ec}	0.19 ± 0.01^{Ea}	0.13 ± 0.0^{Fc}	0.19 ± 0.0^{Ga}	0.16 ± 0.0^{Db}
A10	0.14 ± 0.01^{Dc}	0.16 ± 0.01^{Db}	0.16 ± 0.01^{Eb}	0.13 ± 0.01^{Gc}	0.14 ± 0.01^{Fc}	0.23 ± 0.01^{Ga}	0.18 ± 0.01^{Db}

*A, B.. Capital letters indicate the difference between groups on the same day, and the difference between groups with different capital letters in the same column was statistically significant ($p<0.05$).
a, b,…: Lower-case letters indicate the difference between days in the same group, and the difference between days with different lower-case letters in the same row was statistically significant ($p<0.05$).

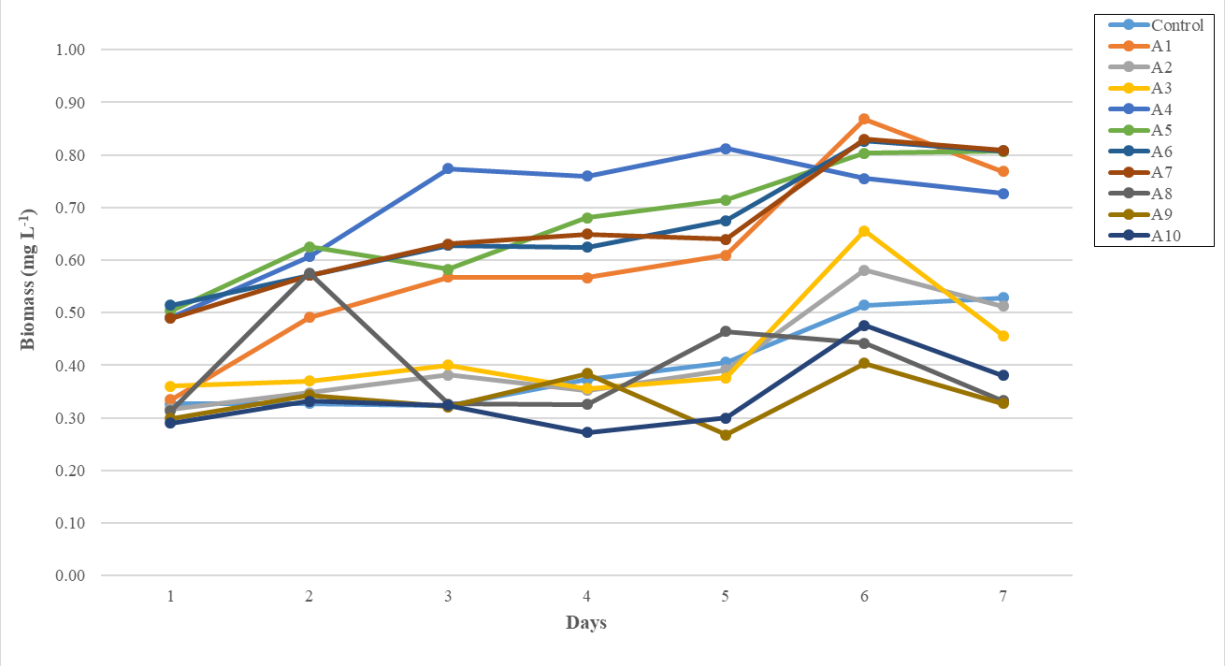


Figure 3. Biomass changes of *S. armatus* and MGPB co-culture groups depending on the day.

In the co-culture experiment with *S. flavescens* and MGPB, the change depending on the experimental groups and days was statistically significant ($p<0.05$). Throughout the experiment, the highest biomass was determined to be *Bacillus* sp. (0.10±0.01 mg L^{-1}) in the co-culture experiment. According to the average biomass value, the effects of *Pseudomonas* sp. were found to be similar to the control group (0.06±0.03 mg/L) (Table 5). In this study, a decrease was detected in all groups, except the F7 group after the 2nd day (Figure 4).

Table 5. Changes in *S. flavescens* biomass depending on days and groups (mg L^{-1}, Mean±SD, n=3).

Groups	Days						
	1	2	3	4	5	6	7
Control	$0.10\pm0.01^{Aa*}$	0.09 ± 0.0^{Aa}	0.10 ± 0.0^{Aa}	0.04 ± 0.01^{Bb}	0.04 ± 0.01^{Bb}	0.03 ± 0.01^{Db}	0.02 ± 0.01^{Cb}
F1	0.07 ± 0.0^{Bb}	0.09 ± 0.01^{Aa}	0.07 ± 0.01^{Cb}	0.05 ± 0.0^{Bc}	0.04 ± 0.0^{Bc}	0.03 ± 0.0^{Dd}	0.02 ± 0.0^{Cd}
F2	0.09 ± 0.01^{Aa}	0.08 ± 0.01^{Aa}	0.09 ± 0.01^{Ba}	0.05 ± 0.01^{Bb}	0.04 ± 0.0^{Bb}	0.04 ± 0.0^{Cb}	0.02 ± 0.0^{Cc}
F3	0.10 ± 0.01^{Aa}	0.09 ± 0.0^{Aa}	0.10 ± 0.0^{Aa}	0.05 ± 0.0^{Bb}	0.04 ± 0.0^{Bb}	0.04 ± 0.0^{Cb}	0.02 ± 0.0^{Cc}
F4	0.09 ± 0.0^{Aa}	0.09 ± 0.0^{Aa}	0.09 ± 0.0^{Ba}	0.05 ± 0.0^{Bb}	0.04 ± 0.0^{Bb}	0.04 ± 0.0^{Cb}	0.04 ± 0.0^{Bb}
F5	0.09 ± 0.01^{Aa}	0.09 ± 0.0^{Aa}	0.09 ± 0.01^{Ba}	0.04 ± 0.0^{Bb}	0.04 ± 0.01^{Bb}	0.05 ± 0.01^{Cb}	0.04 ± 0.01^{Bb}
F6	0.09 ± 0.0^{Aa}	0.09 ± 0.0^{Aa}	0.09 ± 0.01^{Ba}	0.05 ± 0.01^{Bb}	0.05 ± 0.0^{Bb}	0.05 ± 0.01^{Cb}	0.05 ± 0.01^{Bb}
F7	0.08 ± 0.0^{Ab}	0.08 ± 0.0^{Ab}	0.08 ± 0.0^{Bb}	0.04 ± 0.0^{Bc}	0.04 ± 0.01^{Bc}	0.18 ± 0.0^{Aa}	0.03 ± 0.01^{Cc}
F8	0.11 ± 0.0^{Aa}	0.10 ± 0.0^{Ab}	0.11 ± 0.0^{Aa}	0.09 ± 0.0^{A}	0.09 ± 0.01^{Ab}	0.12 ± 0.01^{Ba}	0.11 ± 0.01^{Aa}
F9	0.07 ± 0.0^{Bb}	0.09 ± 0.01^{Aa}	0.07 ± 0.01^{Cb}	0.05 ± 0.01^{Bc}	0.04 ± 0.01^{Bc}	0.05 ± 0.0^{Cc}	0.04 ± 0.01^{Bc}
F10	0.06 ± 0.0^{Bb}	0.13 ± 0.01^{Aa}	0.06 ± 0.0^{Cb}	0.04 ± 0.0^{Bc}	0.03 ± 0.0^{Cc}	0.02 ± 0.0^{Dd}	0.01 ± 0.0^{Cd}

*A, B.. Capital letters indicate the difference between groups on the same day, and the difference between groups with different capital letters in the same column was statistically significant ($p<0.05$).
a, b,…: Lower-case letters indicate the difference between days in the same group, and the difference between days with different lower-case letters in the same row was statistically significant ($p<0.05$).

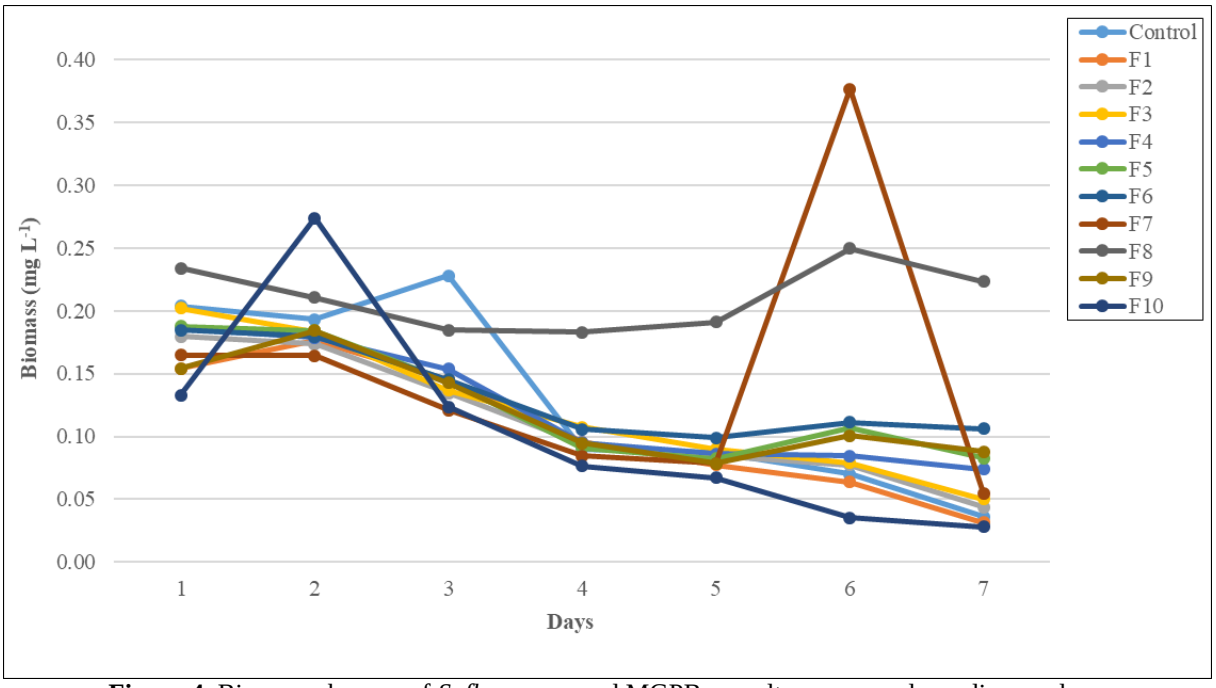


Figure 4. Biomass changes of *S. flavescens* and MGPB co-culture groups depending on days.

In the co-culture study with MGPB isolated from Tortum Lake and *S. armatus*, the change in cell count depending on the day and groups was found to be statistically significant ($p<0.05$). In this study, the highest cell count in the co-culture groups was determined in the experiment with *Bacillus* sp., with an average of 306±16.21 cells/ml. This was followed by the co-culture experiments with *Pseudomonas* sp. with a cell count of 251±13.37 cells/ml and *Pseudomonas* sp. with a cell count of 260±17.6 cells/ml. The cell count in the control group was calculated as an average of 107±45.03 cells/ml (Table 6).

Table 6. Change in *S. armatus* cell number depending on days and groups (cell/ml, Mean±SD, n=3).

Groups	Days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Control	80±22.63^{Ad*}	32±11.31^{Ee}	80±3.30^{Fd}	96±4.50^{Gc}	144±90.05^{Iab}	160±22.67^{Ha}	160±67.88^{Ia}
A1	64±11.3^{Bf}	64±33.06^{Df}	80±3.30^{Fe}	128±56.56^{Fd}	160±33.94^{Hc}	192±0.1^{Fb}	240±67.88^{Da}
A2	16±0.6^{De}	16±10.1^{Fe}	208±56.68^{Cd}	208±56.56^{Cd}	224±56.67^{Fc}	256±0.1^{Db}	288±67.88^{Ca}
A3	16±0.5^{De}	80±9.06^{Cd}	224±22.62^{Ba}	160±13.57^{Ec}	176±33.94^{Gb}	170±4.95^{Gb}	170±67.88^{Gb}
A4	10±0.1^{Ff}	96±11.3^{Be}	160±0.90^{Ed}	208±68.7^{Cc}	304±56.67^{Da}	224±60.78^{Eb}	208±67.88^{Ec}
A5	64±0.1^{Bf}	80±11.3^{Ce}	288±15.23^{Ad}	368±67.8^{Ac}	416±56.67^{Bb}	528±60.78^{Aa}	400±67.88^{Ab}
A6	80±45.25^{Ae}	80±0.1^{Ce}	160±24.36^{Ed}	320±56.56^{Bc}	448±56.67^{Aa}	352±60.78^{Cb}	320±67.88^{Bc}
A7	16±0.1^{Fe}	10±0.5^{Ge}	208±67.8^{Cd}	368±67.8^{Ac}	368±56.67^{Cc}	480±69.8^{Ba}	384±67.88^{Ab}
A8	32±11.31^{Cf}	144±33.9^{Ad}	192±22.62^{Da}	176±12.57^{Db}	160±33.94^{Hc}	80±5.90^{Ke}	32±67.88^{Kf}
A9	16±9.06^{De}	16±0.7^{Fe}	160±11.03^{Ed}	208±56.56^{Cb}	256±56.67^{Ea}	190±5.90^{Ic}	192±22.63^{Fc}
A10	16±9.06^{De}	16±0.7^{Fe}	160±11.03^{Ed}	208±56.56^{Cb}	256±56.67^{Ea}	190±5.90^{Ic}	192±22.63^{Fc}

*A, B.. Capital letters indicate the difference between groups on the same day, and the difference between groups with different capital letters in the same column was statistically significant ($p<0.05$).
a, b,…: Lower-case letters indicate the difference between days in the same group, and the difference between days with different lower-case letters in the same row was statistically significant ($p<0.05$).

In the co-culture study of MGPB with *S. flavescens* isolated from the Palandoken Teke Stream, the change in cell count depending on the groups and days was found to be statistically significant ($p<0.05$). The cell count was calculated to be 93±6.01 cells/ml in the co-culture medium with the highest *Bacillus* sp. added throughout the experiment. This value was determined as an average of 74±6.30 cells/ml and 71±3.01 cells/ml in the co-culture experiments with *Pseudomonas* sp. and *Bacillus* sp. (Table 7).

Table 7. Change in *S. flavescens* cell number depending on days and groups (cell/ml, Mean±SD, n=3).

Groups	Days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Control	80±45.25^{Ba*}	32±11.31^{Ec}	16±11.31^{Dd}	10±0.1^{Fe}	64±56.67^{Fa}	16±11.31^{Ed}	16±11.31^{Dd}
F1	64±11.31^{Cb}	64±56.67^{Db}	10±0.1^{Ed}	32±11.31^{Dc}	80±45.25^{Ea}	10±0.1^{Fd}	10±0.1^{Ed}
F2	80±45.25^{Ba}	32±11.31^{Ec}	16±11.31^{Dd}	48±22.62^{Cb}	80±45.25^{Ea}	10±0.1^{Fe}	10±0.1^{Ee}
F3	16±11.31^{Ef}	80±45.25^{Cd}	64±0.1^{Be}	160±67.88b^{B}	176±67.88^{Aa}	16±11.31^{Ef}	16±11.31^{Df}
F4	48±22.62^{Fb}	16±11.31^{Fc}	10±0.1^{Ed}	48±22.62^{Cb}	64±46.67^{Fa}	64±46.67^{Ba}	16±11.31^{Dc}
F5	64±56.67^{Cd}	32±11.31^{Ef}	128±0.1^{Aa}	48±22.62^{Ce}	96±45.25^{Db}	48±22.62^{Ce}	80±45.25^{Ac}
F6	176±67.88^{Aa}	80±45.25^{Cb}	16±11.31^{De}	16±11.31^{E}	48±22.62^{Gc}	48±22.62^{Cc}	32±11.31^{Cd}
F7	80±45.25^{Bb}	128±67.88^{Ba}	16±11.31^{Dd}	16±11.31^{Ed}	128±67.88^{Ca}	10±0.1^{Fe}	64±46.67^{Bc}
F8	32±11.31^{De}	144±67.88^{Ac}	32±11.31^{Ce}	176±67.88^{Aa}	160±67.88^{Bb}	80±45.25^{Ad}	32±11.31^{Ce}
F9	16±11.31^{Ed}	16±11.31^{Fd}	16±11.31^{Dd}	48±22.62^{Cb}	96±45.25^{Da}	32±11.31^{Dc}	32±11.31^{Cc}
F10	16±11.31^{Eb}	16±11.31^{Fb}	10±0.1^{Ec}	16±11.31^{Eb}	80±45.25^{Ea}	10±0.1^{Fc}	10±0.1^{Ec}

*A, B.. Capital letters indicate the difference between groups on the same day, and the difference between groups with different capital letters in the same column was statistically significant ($p<0.05$).
a, b,…: Lower-case letters indicate the difference between days in the same group, and the difference between days with different lower-case letters in the same row was statistically significant ($p<0.05$).

3.3. Dry Matter of *S. armatus* and *S. flavescens*

In this study, dry matter quantity was measured on the first and last days of the co-culture trial conducted with *S. armatus*, *S. flavescens* and MGPB isolates. In the trials conducted with *S. armatus*, the dry matter quantity was obtained from the co-culture trials conducted with *Pseudomonas* sp. compared to the control group (Figure 5). In the co-culture trial conducted with *S. flavescens* and MGPB species, the dry matter quantity was the same in all groups on the 7th day (Figure 6).

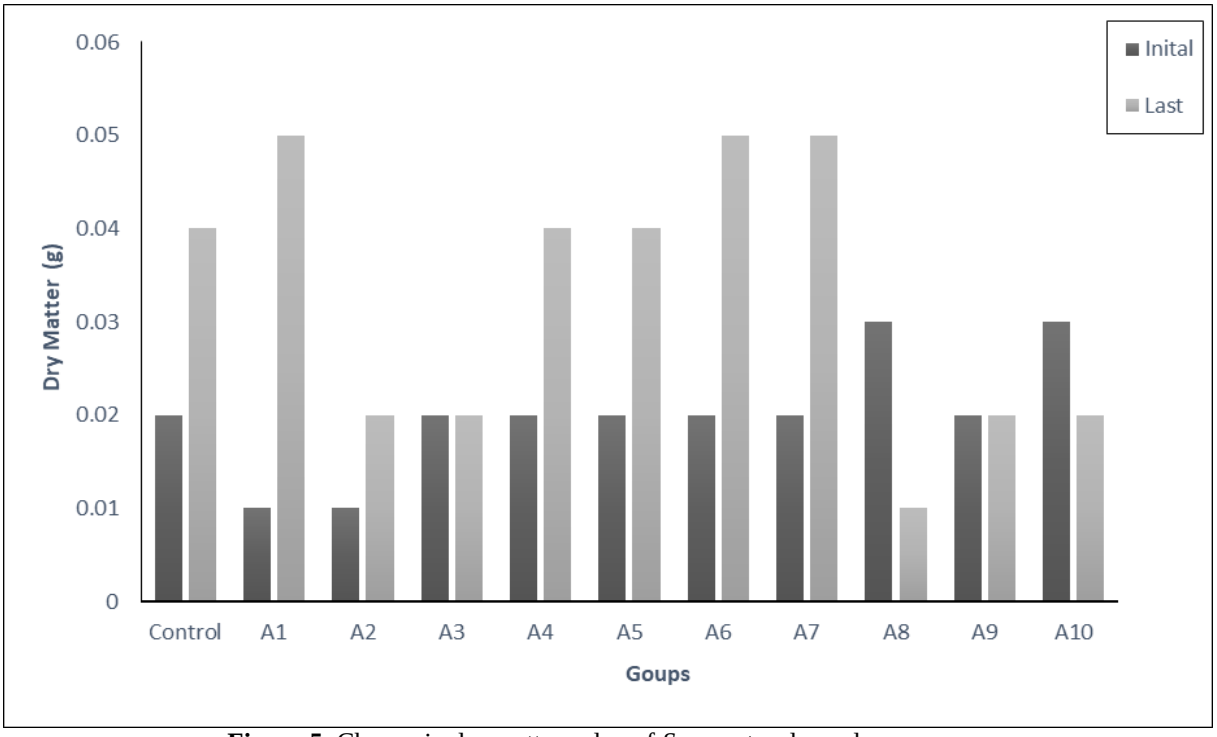


Figure 5. Change in dry matter value of *S. armatus* depends on groups.

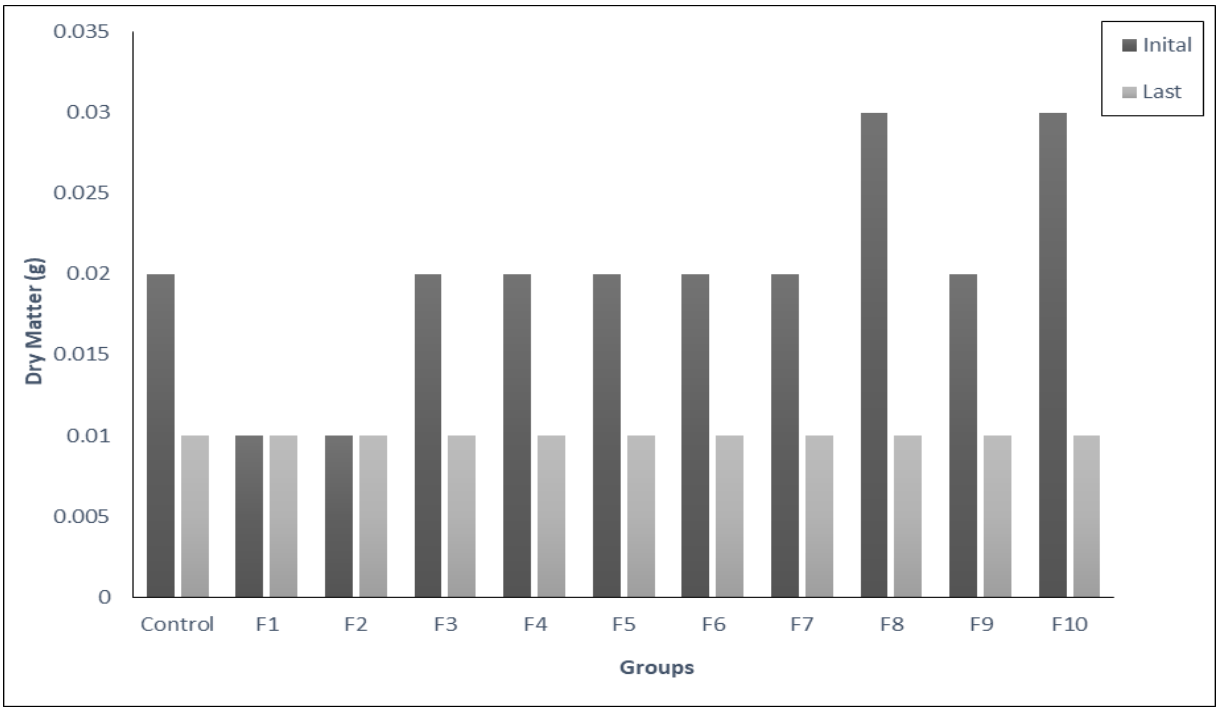


Figure 6. Change in dry matter value of *S. flavescens* depends on groups.

4. DISCUSSION

In this study, the changes in biomass and cell numbers in the co-culture medium of *S. armatus* and *S .flavescens* species isolated from the Palandoken Teke Stream and Tortum Lake, and MBTB species collected from the same locations were examined. MBTB species with nitrogen fixation and phosphorus retention properties were preferred by both species. While an increase in biomass and cell numbers was detected in *S. armatus* and MBTB co-culture experiments, almost the same biomass and cell numbers were calculated in the group with only 3N-BBM+V (control) medium in *S. flavescens* and MBTB co-

culture experiments. However, higher biomass and cell numbers were detected in the co-culture medium of *S. armatus* and *S. flavescens*.

Many studies have investigated the optimal conditions of environmental factors (light, temperature, pH, etc.) to increase cell growth and biomass in microalgae cultures, as well as set up experiments by changing the concentrations of the nutrient medium (N and/or P stress) (Hızarcı, 2004; Şahin, 2010; Uslu et al., 2011; Ağırman, 2015; Bedil, 2015; Öztürk, 2016; Yılmaz, 2016; Akın, 2017; Aygün, 2017; Acar & Fakıoğlu, 2018; Öztürk, 2018; Kaplan Çoban, 2018; Aladağ, 2019; Çoban, 2019; Kurusakız, 2020; Özalın, 2020; Andeden, 2021; Şahin, 2021). In these studies, waste products and glucose galactose were applied as nutrient media for microalgae (Abreu et al., 2012; Aydoğdu and Fakıoğlu, 2022). In this study, the development of MBTB and Scenedesmus species by taking them in the same culture system was tested for the first time in our country. While the biomass of *S. armatus* and *S. flavescens* cultured in the co-culture medium was calculated as 0.25 mg/L and 0.07 mg/L on average, the biomass of *S. armatus* and *S. flavescens* only in the 3N-BBM+V medium was determined as 0.19 mg/L and 0.06 mg/L.

Nitrogen and phosphorus ratios, which are the main sources in the nutrient medium, vary depending on the microalgal species (Richmond, 2004). In this study, cell growth was observed with *S. armatus, which has the highest nitrogen fixation value and lowest phosphorus solubilization value among* MBTB species, while no cell growth was observed in *S. flavescens, which has a* "negative" nitrogen fixation value and high phosphorus retention value. In studies conducted with multiple limiting nutrients, the growth rate varied depending on the intracellular concentration of only the most limiting nutrient in microalgae (Klausmeier et al., 2004).

Pseudomonas asplenii has been reported to secrete heat-stable inorganic substances that have a growth-promoting effect on the microalgae *Chattonella marina* and increase its ability to grow in phosphate-limited environments (Palacios et al., 2022). In this study, *S. armatus* showed good growth in an environment with non-phosphate-limiting bacteria, whereas *S. flavescens* grew in an environment with phosphate-limiting bacteria.

Hernandez et al. (2009) investigated the effect of MBTB *Azospirillum brasilense* on nitrogen uptake by *Chlorella vulgaris* and reported that *Bacillus pumilus* can fix atmospheric nitrogen and produce ammonium to increase the growth of the microalgae *Chlorella vulgaris*. In this study, *Bacillus* sp. promoted the growth of *S. armatus*.

In this study, *Pseudomonas* sp., which can produce IAA, promoted an increase in *S. flavescens* biomass. It has been reported that the interaction between *Euglena gracilis* and five bacterial species capable of producing IAA (Pseudomonas sp. DSM 25356 and *Pseudomonas* sp. DSM25842, *Vibrio natriegens* KCTC 12726, *Paenibacillus yonginensis* KCTC 33428, and *Sphingomonas panaciterrae* KCTC 42646), and IAA-producing *Vibrio natriegens* had the capacity to increase growth (Kim et al., 2019).

Rhizobium sp. isolated from an open pond was cultured in open ponds in a co-culture medium with *Chlorella sorokiniana* and an increase in mass of 13.76% was achieved (Zhou et al., 2021). In this study, 6% growth was detected in *S. flavescens* biomass and 7% in *S. armatus* biomass.

5. CONCLUSION

This study is the first to use both bacteria and *Scenedesmus* isolates obtained from natural sources and monitor their development in a co-culture. MBTB stimulated the growth of *S. armatus* and *S. flavescens*. In this study, *Bacillus* sp. and *Pseudomonas* sp., which have nitrogen fixation, IAA production, and phosphorus retention capabilities, were thought to stimulate the growth of *S. armatus* and *S. flavescens*, respectively. Future co-culture studies are needed.

1. It is recommended to conduct research to determine the intracellular mechanism supporting the development of these species,

2. In addition to biomass increase, it is recommended to investigate the amounts of fat and protein, which are involved in intracellular metabolism, amino acid, and fatty acid compositions.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

AUTHOR CONTRIBUTIONS

Funding: Ö.F.; Literature: Ö.F.; Methodology: Ö. F., M. K.; Data analysis: Ö.F., Z.A.; Manuscript writing: Ö.F., Z. A. ; Performing the experiment: Ö.F. and Z.A. All authors approved the final draft.

ETHICAL APPROVAL STATEMENTS

Approval from the local ethics committee was not obtained because the experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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