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Araştırma Makale/ Research article

Antarctic Microalgae Growth in Simulated Wastewater

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ABSTRACT
In recent years, the number of scientific studies in the Antarctic and Arctic regions has increased considerably. While scientific studies allow us to explore the untouched nature of the region and better understand the global climate, they also raise various ecological concerns such as wastewater, air pollution and habitat destruction. This threatens the flora and fauna of the polar regions, negatively affecting biodiversity. It is crucial that we protect the fragile ecosystems of the polar regions through sustainable research practices and international cooperation to prevent pollution, protect habitats and prevent the introduction of invasive species. The Antarctic Treaty and other protocols prohibit the discharge of wastewater into the sea without treatment. For this reason, many research bases in Antarctica have established facilities to treat wastewater. These facilities ensure that wastewater is treated and returned to the sea without harming the environment. However, the wastewater treatment process generates a significant amount of solid waste. As this waste accumulates in the treatment plants, it has to be transported back to the mainland. Antarctica's remoteness and challenging geographical conditions make solid waste transportation logistically difficult and costly. In order to solve these problems, it was aimed to use the algal blooms occurring in Antarctica in the treatment process by cultivating them in wastewater. In the study, it was found that the Antarctic microalgae can be cultivated in domestic wastewater in Antarctic bases and have a high potential for the proposed activities by having approximately 30% of protein content.

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Introduction

Antarctica, the world's fifth largest continent in terms of area, may seem barren and devoid of life at first glance, but it is a real treasure for our planet with its unique ecosystem [1]. It is rich in strategically important resources such as oil, natural gas, minerals and water. The continent is the driest, windiest and coldest region in the world, making life and work very difficult [2]. However, these challenging conditions provide a unique environment for scientific research and an important opportunity to understand the ecological and geological structure of the continent [3].
The Antarctic Treaty and the Madrid Protocol are the principles of peace, science and environmental protection of Antarctica. These treaties aim to protect and sustainably manage Antarctica as a region dedicated to the common good of humanity [4].
The Madrid Protocol includes three important conditions for wastewater management in Antarctica:

- In areas subject to the Antarctic Treaty, there can be no discharge of wastewater that could damage surface ice. This condition aims to protect the continent's fragile ecosystems and ice sheets.
- After certain treatments, wastewater can be discharged to the bottom of deep ice structures. This allows wastewater to be disposed of without harming the environment.
- Wastewater from sparsely populated bases may be discharged from the coast into the sea under specific conditions. However, this discharge must comply with environmental protection standards [5].

Although discharging wastewater into the sea is preferred, especially in low-populated coastal bases, water treatment is necessary in many inland bases. In bases with a permanent population of a few hundred people, water treatment is in many cases inadequate. This is due to technical and infrastructural challenges caused by the isolated nature and extreme conditions of the continent, which render the establishment and operation of

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wastewater management systems difficult. However, the general trend is towards the development and improvement of wastewater treatment systems, with more effort than the legal limitations demand. Some bases are trying to make wastewater reusable through recycling and treatment, aiming to create a completely "closed system" [6].

Different methods are used for wastewater treatment in Antarctica. Certain physical methods are used to remove physical pollutants that cannot be removed by biological methods, and then biotreatment methods are usually used [7]. The materials required for the functioning of treatment plants usually need to be shipped from the mainland. The transportation of these materials to Antarctica is costly and time consuming. In particular, the transportation and procurement of these materials is challenged by the isolated nature of the continent and the extreme climatic conditions. Moreover, the solid waste remaining after treatment is often sent to the countries of origin and treated as organic matter, as no plant cultivation activities are carried out on many bases. This creates an additional challenge and cost for waste management and disposal.

As for the wastewater in Antarctica, it can be divided as black water and gray water. Black water and gray water are wastewater from human waste and sources such as kitchens and showers and contain trace amounts of organic matter and chemical pollutants, respectively [8]. These two types of wastewaters are usually collected through different water systems. This allows different treatment methods to be applied to black water and gray water. While black water contains more intense pollution due to the human waste it contains and requires more extensive treatment, on the other hand, gray water is less contaminated and has a lower organic matter content, so, it is subjected to physical and chemical treatment processes such as filtration and disinfection [9]. Separate treatment of black water and gray water makes wastewater treatment more effective and efficient, leading to more sustainable water management.

Microalgae are third generation biomass feedstock that have been used in various areas such as biofuel production, pharmaceutical and nutraceutical production and environmental applications [10, 11, 12 ,13, 14]. Today, microalgae stand out as an innovative and sustainable solution for wastewater treatment. Microalgae utilize organic matter and nutrients in wastewater for photosynthesis and produce oxygen [15]. This allows wastewater to be treated and oxygenated. Furthermore, the biomass of microalgae can be collected from treated wastewater and converted into valuable products such as biofuels or fertilizers.

In the literature, the high efficiency of microalgae, especially in black water treatment, has been reported in various sources [16, 17, 18]. However, wastewater treatment studies have not yet been carried out by utilizing Antarctic microalgae which has a significant potential for environmental applications [19]. The target of this study is to explore and evaluate the cultivation of Antarctic microalgae in the wastewater that occurred in scientific bases in Antarctica. Given the unique and extreme environmental conditions of the Antarctic region, the microalgae found there possess distinctive physiological and metabolic adaptations that may render them particularly effective in bioremediation processes. This study aims to determine the biochemical content and physiological responses of Antarctic microalgae when exposed to wastewater. Comparing the performance of Antarctic microalgae with that of microalgae from temperate regions will highlight any superior traits or advantages specific to Antarctic species.

By achieving these targets, the study aims to contribute valuable insights into the potential of Antarctic microalgae as a sustainable and efficient source for wastewater treatment, addressing both environmental pollution and resource management challenges.

Material and Methods

Microalgae culture and artificial wastewater media

Chlorella variabilis YTU.ANTARCTIC.001 (MN372092) (Fig 1) was evaluated in this study. Artificial wastewater media with various chemicals (Yeast Peptone Media, Glucose, NH_4Cl, KCl, $NaHCO_3$, $MgSO_4$-$7H_2O$, $FeSO_4$-$7H_2O$, NaCl) were prepared according to Table 1. The pH of the medium was adjusted to 7.8 and then autoclaved at 121°C for 15 min. All the chemicals used in this study were supplied from Merck.

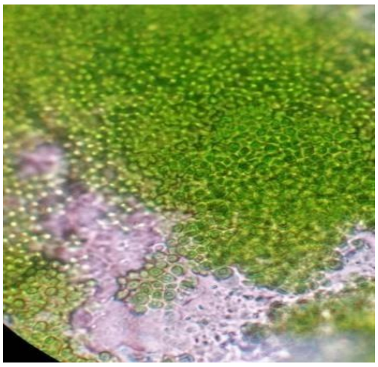

Fig 1 Microscope image of *Chlorella variabilis* YTU.ANTARCTIC.001 cells under (100x)

Antarctic Microalgae Growth in Artificial Wastewater

Microalgae to be grown on solid media and prepared for inoculation were first inoculated into petri dishes from the stock culture. At the end of one week, petri dishes were washed with distilled water and microalgae cells were collected for inoculation and transferred to a modified artificial wastewater medium [20]. A control group was cultivated in BG-11 medium. The experimental study was carried out in 250 mL flasks which were inoculated with 10% inoculum of polar microalgae for 8 days (Fig 2). The cultures were cultivated in a stirred incubator at 24 ± 2 °C and 150 rpm. Continuous illumination was maintained during the growth of the cultures. The pH of the cultures was 7.5- 8.5. The growth of the cultures was monitored by measuring their absorbance using PG 60 Instrument UV-Vis spectrophotometer at 680 nm [21]. The growth rate and doubling time were calculated by Equation 1-2. Cultivation of Antarctic microalgae in artificial wastewater was carried out in two replicates (*C. variabilis* 1 and 2 at growth curves graph). At the end of the cultivation, microalgae culture was centrifuged at 8000 rpm and the media and microalgae culture were separated for characterization studies.

$$\mu = ln\ ln\ \left(\frac{A_t}{A_0}\right)\ /(t - t_0) \qquad (1)$$

$$t_d = ln2/\mu \qquad (2)$$

where; t: Time (day) μ: Specific growth rate (day-1) A_0: Absorbance at t=0, A_t: Absorbance at time t. t_d is the doubling time.

Table 1 Modified artificial wastewater content

Chemical components	g/L
Yeast Peptone Media	5
Glucose	2
NH_4Cl	0.5
KCl	0.2
$NaHCO_3$	0.5
$MgSO_4$-$7H_2O$	0.1
$FeSO_4$-$7H_2O$	0.01
NaCl	2.5

Characterization of microalgae and culture media

The clear water separated from the centrifuged microalgae can be proposed to be used for various purposes such as watering the plants at the Antarctic science base. For this purpose, the pH and dissolved oxygen level of the water were analyzed using a Hach-Lange multimeter [22].

Microalgae separated by centrifugation were dried in an oven at 60°C for one day. For the determination of carbohydrate, lipid and protein contents of microalgae, the phenol-sulfuric acid [23], the Bligh-Dyer [24], and the Lowry [25], methods were carried out, respectively. The change in the main compounds that was used by microalgae was evaluated with phenol-sulfuric acid and modified Lowry method [23], [25].

Results and Discussion

Microalgal growth in artificial wastewater

In the experimental study, it was observed that microalgae cultures grew well within 8 days in the prepared wastewater using the components in the wastewater (Fig 2). It was observed that microalgae grew very rapidly in the first 3 days and starts to slow down after the third day due to the pH level of the culture.

Fig 2 Monitoring the growth of Antarctic microalgae

As can be seen in Fig 3, between days 0 and 8, the absorbance increased from 0.1 to 0.48 for C. *variabilis* cultures. It was determined that the microalgae cultures cultivated in artificial wastewater showed similar growth. When the growth results were analyzed, it was found that the growth rate and doubling time of microalgae was 0.15 day^{-1} and 4.6 days, respectively. Although there is no study on Antarctic microalgae cultivation in wastewater, there are many studies on temperate microalgae cultivation in different wastewater types (Table 2). Liu et al. studied growth of *Chlorella vulgaris* and nutrient removal in domestic wastewater, and reported that, the specific growth rate was found as between 0.3-0.5 day^{-1} when the culture was supplied with air [26]. Reyimu and Özçimen investigated the growth of *Nannochloropsis oculata* and *Tetraselmis* suecica in different concentrations of municipal wastewater. It was stated that, both *N. oculata* and *T. suecica* can tolerate and utilize the wastewater and, the specific growth rate of the cultures can up to 0.5430 d^{-1} (75% of wastewater) for *N. oculata* and 0.4778 d^{-1} (25% of wastewater) for *T. suecica* [27]. Similarly, Gani et al. reported that, highest biomass productivity in domestic wastewater carried out with 0.26 day^{-1} of specific growth rate, and a doubling time of 2.63 days. Meanwhile, the lowest biomass productivity was observed with the lowest specific growth rate of 0.1 day^{-1} and the longest doubling time, which reached up to 7.14 day [28]. Rani et al. studied the growth of *Chlorella sorokiniana* in different tertiary wastewater types (synthetic and real). It was reported that, the highest specific growth rate was found as 0.59 day^{-1} when secondary effluent of domestic wastewater was used. As for the growth in synthetic wastewater, the specific growth rate was calculated as 0.11 day^{-1} [29]. Oliviera et al. analyzed the growth of *C. vulgaris* in the effluent of a Biofloc Technology (BFT) system used in the Nile tilapia fingerlings farming. It was found that specific growth rate was 0.77, 0.63 and 0.53 when the microalgae cultivated in 0, 50, and 100% BFT effluent [30]. It was found out that the specific growth rates of the microalgae species given in the Table 2 were similar to our results, but it should be noted that some of the wastewater types had higher nutrient concentration than the prepared artificial wastewater in this study. Moreover, the increased presence of CO_2 led to higher growth rates in these studies [26].

Characterization of microalgae and culture media

After the growth of Antarctic microalgae in artificial wastewater, carbohydrate, protein, lipid, contents of the microalgae were determined (Table 3). It was seen that, cultures grown in artificial wastewater had partially higher protein content than control sample. By considering the usage areas of microalgae culture after grown in wastewater such as plant growing in Antarctic Scientific Base, it was aimed to focus on protein content. In comparison with the literature studies, it was seen that, protein content of the Antarctic microalgae grown in artificial wastewater is similar [33, 34, 35, 36, 37]. Yet, according to the wastewater type and the nitrate content in the wastewater, the protein content of this sample was determined to be lower than some studies. Higher amounts of nitrate compounds in growth media led to higher protein content in microalgae [27]. The comparison of the protein contents of Antarctic microalgae and the literature studies was given in Table 4.

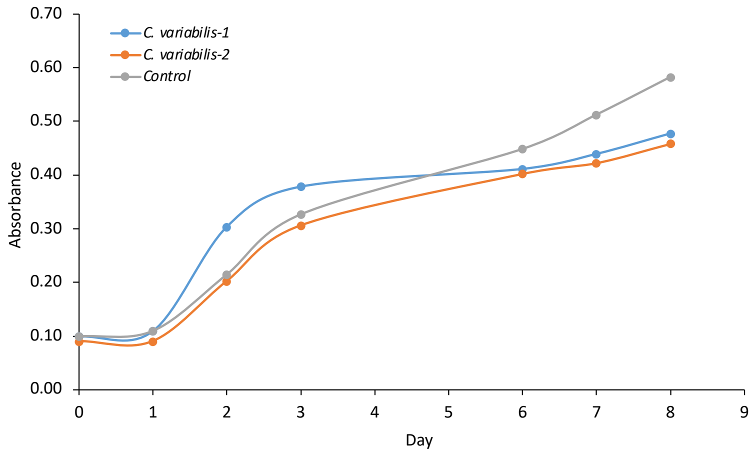

Fig 3 Growth curves of Antarctic microalgae cultivated in artificial wastewater and control samples

After 8 days of cultivation, it was also found that, the wastewater in which the microalgae are cultivated had a pH of 8. The dissolved oxygen level at the end of the day was measured with a multimeter and it was observed that the level increased from 6.2 to 7.6 mg/L. The change in the main compounds that was used by microalgae was evaluated with phenol-sulfuric acid and modified Lowry method. It was seen that, 52% and 48% of the glucose and protein-based compounds in the media were assimilated by microalgae.

Table 2 Specific growth rates of different microalgae species cultivated in various wastewater types

Microalgae	**Wastewater type**	**Specific growth rate (day^{-1})**	**References**
Botryococcus sp.	Domestic	0.10-0.26	[28]
Chlorella sorokiniana	Domestic	0.59	[29]
Chlorella vulgaris	Domestic	0.3-0.5	[26]
Nannochloropsis oculata	Municipal	0.54	[27]
Chlorella vulgaris	Nile Tilapia Farming	1.18-1.73	[30]
Chlorella vulgaris	Nitrate and Ammonium	0.31	[31]
Chlorella zofingiensis	Swine	0.34	[32]
Chlorella variabilis	Artificial	0.15	This study

Table 3 Biochemical contents of Antarctic microalgae species cultivated in artificial wastewater

Cultures	**Carbohydrate (%)**	**Protein (%)**	**Lipid (%)**
C. variabilis 1	25±1.2	33±1.4	39±1.4
C. variabilis 2	26±1.4	28±1.6	44±1.2
Control sample	28±1.3	21±1.2	49±1.1

Table 4 Protein contents of different microalgae species cultivated in various wastewater types

Microalgae	**Wastewater type**	**Protein content (%)**	**References**
Chlorella pyrenoidosa	Artificial	~50%	[33]
Chlorella sorokiniana AK-1	Swine	42.2%	[34]
Chlorella vulgaris	Artificial (25% diluted)	50.7 %	[33]
Chlorella vulgaris	Hydroponic	50.5%	[35]
Chlorella vulgaris	Municipal	43.2%	[36]
Chlorella vulgaris	Municipal	35%	[37]
Oscillatoria sp	Municipal	32.9%	[37]
Chlorella variabilis	Artificial	33%	This study

Conclusion

Microalgae in Antarctica, have not been discovered to a great extent; their potential in possible agricultural areas such as crop production, food enrichment, soil protection has not been put into practice in many ways.

There are many bioproducts fro microalgae such as biofertilizers, biostimulants, biocontrol agents and soil conditioners that are thought to be developed for agriculture. In this study, it was aimed to investigate the utilization of Antarctic microalgae for the treatment of wastewater occurred in Antarctic Scientific Base and evaluation of its potential as a fertilize for plants by determining the protein content. In here, it was found that, Antarctic microalgae can be proposed for the treatment of domestic wastewater in Antarctic bases and has a high potential for the proposed activities by having approximately 30% of protein content. Using Antarctic microalgae for the wastewater treatment in the Antarctica bases will provide various advantages: no transportation of microalgae cultures to continent, utilization of cultures in compatible with continent's environmental conditions, in situ application. In conclusion, although experiments were conducted on a single Antarctic species, there are dozens of microalgae species isolated only from the Antarctic region and each microalgae species has the potential to remove wastewater as biomass.

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Availability of data and material
Please contact the corresponding author for any data request.

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