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Evaluation of the effectiveness of green algae Scenedesmus amatus in bioremediation of petroleum-derived polycyclic aromatic hydrocarbons

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ABSTRACT

Iraq's economy is primarily based on oil exports, making it one of the richest oil-producing nations in the world. However, the transportation, refining, and extraction of oil lead to major environmental issues that impact water, people, and other living things. Pollution rates rise and ecosystems are harmed by oil spills and emissions from oil refineries. As a result, achieving sustainable development in Iraq has become essential. The green algae Scenedesmus amatuswas used in this study to bioremediate acenaphthylene in three different concentrations (100, 200, and 300 ppm/L). The results of the High Performance Liquid Chromatography (HPLC) device demonstrated 100% remediation for both concentrations (100 and 200 mg/L).

KEYWORDS: ***Bioremediation, Petroleum hydrocarbons, Green algae.***

1-INTRODUCTION

Water is an essential element for the existence of human civilization, and life has always been associated with water. The water bodies have been an element of attraction for humans since time immemorial, and man depends on water in various areas of life (1). The increase in population numbers and development in various industries, which increased pressure on water resources and their irregular investment, in addition to water scarcity, led to water pollution. The main cause of water pollution is man. One of the most important industries that cause pollution is the oil industries (2). Crude oil is one of the main pollutants in the world, its extraction and remediation generates water contaminated with hydrocarbons harmful to human health, plants and animals. The pollution with organic hydrocarbon compounds is the most dangerous and widespread type of water pollution around the world due to industrialization and increasing global demand for oil day by day, as well as the growth of oil industries such as refineries and petrochemical industries, which these industries can create a harmful effect on the environment through discharges, oil spills, and waste generation during storage and processing and transport leading to ecosystem damage (3).

Due to its chemical structure, polycyclic aromatic hydrocarbons have carcinogenic and mutation-causing effects, such as those found in oil residues. Acenaphthylene is a polycyclic aromatic hydrocarbon found naturally in fossil fuels like coal tar, petroleum and biomass combustion. Additionally, it is produced commercially and is considered a chemical material that has many main commercial uses becouse it is used in the manufacture of intermediate chemicals (such as phthalic anhydrides), dyes, surfactants, leather tanning agents, dispersants, pesticides, insecticides, resins, solvents, mite repellents, and deodorants are thus a ubiquitous environmental pollutant (4). The International Agency for Research on Cancer (IARC), the US Environmental Protection Agency (US EPA) and the German Research Foundation (DFG) have classified acenaphthylene as a potential human carcinogen, and the European Union is currently preparing a new risk assessment report (5).

Petroleum hydrocarbons affect aquatic organisms, especially algae, which are the base of the food chain (6), they are living organisms, most of which are microscopic and widespread and have recently been introduced in many industries, especially the production of antibiotics and as a good source of energy and have different uses. The most of algae species tolerate severe environmental conditions, especially pollution, because they are used as biological indexes for the presence of pollutants and some of them can also be used in the remediation of pollution (7). Many techniques are used to mediate pollution with hydrocarbons, and the most commonly used techniques for remediating spilled oil include chemical-physical methods that can remove most pollutants and have many damages as well as they cannot be finally disposed of, so the modern trend is bioremediation by the use of microorganisms. Microalgae are involved in the bioremediation of petroleum hydrocarbons effectively, and act directly on pollutants and this is done by activating various mechanisms ranging from the production of enzymes capable of hydrocarbon decomposition, to the attack through the liberation of free radicals, and the advantages of using microalgae are not limited to their ability to remove toxic compounds and release oxygen into the atmosphere, but their biomass can then be used in the circular economy process to produce biofuels (8). In order to provide algae in the Iraqi environment with increased hydrocarbon pollution, the study aims to test the efficiency of algae for the remediation of acenaphthylene, one of the hydrocarbons of high concentration in Iraqi waters (9).

2-MATERIALS AND METHODS

2.1 Preparation of hydrocarbon compounds

Acenaphthylene is one of the most prevalent oil pollutants in the aquatic environment within the sites of oil refineries. In this study, three concentrations (100, 200 and 300) ppm were prepared by dissolving acenaphthylene with distilled water (10).

2.2 High-Performance Liquid Chromatography (HPLC) experiment Design

The results were revealed by HPLC analysis. The SYKAM model was used for analyses to detect acenaphthylene. The mobile phase was an equal flow of a 60/40 mixture (volume/volume) of acetonitrile: buffer phosphate a neutral phosphate flow rate, buffer phosphate of 0.1 molar at 1.0 ml/min, the column was C18-ODS (25 cm * 4.6 mm) and UV detector at 254 nm (14).

2.3 Statistical analysis

The results were analyzed using the Statistical Package for the Social Sciences (SPSS software, version 16) using Dunnett test and One-way ANOVA analysis of variance (ANOVA), and the data were presented as mean±standard error of the mean (SEM). P value at $p \leq 0.05$ was considered as significant difference.

2.4 Design Experiments

The green algae *S. amatus* (Figure 1) was isolated from the bioremediation ponds utilized for the treatment of refinery wastewater and lipid production at the Basra Oil Refinery, Iraq. and was diagnosed and classified microscopically (11). By following the Dilution method, axenic culture of algae was obtained (Kim and Chojnacka, 2015) and cultured *in vitro* under controlled conditions in BG11 cultural media at 25±2°C with a light intensity of 3000 lux and with a light system of 16:8 light: darkness with continuous shaking and growth follow-up by calculating cell numbers and reading absorbance values for the purpose of reaching the sizes required to carry out the study (12). For the purpose of bioremediation and under the same conditions mentioned above, 100 ml of cultural and contaminated medium were placed for each concentration of pre-prepared concentrations in a flask of 250 ml, and then 10 ml of algae was added in a ratio of 1:10 (13). The bioremediation was detected by (High-Performance Liquid Chromatography) HPLC for a period of 12 days by measuring every three days.

Figure 1. Characteristics of the green algae *S. amatus* under the microscope (40x).

3-RESULTS AND DISCUSSION

The highest removal rate of acenaphthylene by the green algae *S. amatus* was recorded during the ninth day at a rate of (39.5%) at a concentration of 100 ppm, and the disappearance of acenaphthylene was observed completely during the twelfth day of the experiment, as well as the highest removal rate was recorded on the ninth day at a rate of (82.45%) at a concentration of 200 ppm, as for the concentration of 300 ppm, the highest removal rate was recorded during the third day at a rate of (88.32%) (Table 1, Figures 2 and 3). The results of the study showed that the highest rate of removal of acenaphthylene mediated by *S. amatus* on the ninth day, which was 39.5 ppm, and the reason is due to the consumption of components in the cultural medium necessary for the growth of algae, and therefore algae do not resort to pulling acenaphthylene as a source of carbon until after the lack of nutrients within the medium (15). The microalgae use hydrocarbons in the process of biodegradation as a source of carbon, converting them into compounds that benefit from them in their vital activity (16). Other studies also indicated that green algae such as *Chlorella vulgaris* and *Selenastrum capricornutum* are good at removing hydrocarbons in the presence of ammonia and that they become more effective for removing pollutants, especially acenaphthylene, with the availability of ammonia, in a longer period and in medium and low concentrations (17). The current results are consistent with (18) although they used lower concentrations of acenaphthylene pollutants (1, 3, 5, 10, 15, 25, 50) mg/L, but they found that the absorption rate of acenaphthylene gradually increases by the green algae *Coelastrella saipanensis* until it reaches a 100% removal rate after 12 days.

The reason for the increased remediation on the third day is due to the increased activity of the dehydrogenase enzyme which was increased after 3 days of culture, and it has been shown that algae tolerate and effectively degrade polycyclic aromatic hydrocarbons which may be toxic in the environment and convert the compounds into a non-toxic form.
Furthermore, (19) showed that polycyclic aromatic hydrocarbons in algae react with the cytochrome P450 monooxygenase enzyme active sites of CYP through hydrogen bonds between molecules, hydrophobic reactions and Van der Waals's reactions, giving algae the ability to remediate polycyclic aromatic hydrocarbons more efficiently than other microorganisms.

Table 1. Total removal rate of Acenaphthylene at a concentration of 100, 200, and 300 ppm by *S. amatus*.

Day	Acenaphthylene		
	100ppm	**200ppm**	**300ppm**
D1	6.3	4.1	6.1
D3	20.7	59.4	92.5
D6	33.5	41	68.61
D9	39.5 udl	60.4	53.56
D12	-	35.1 udl	35.05
Total removal	100	200	255.82
residual	0	0	44.18

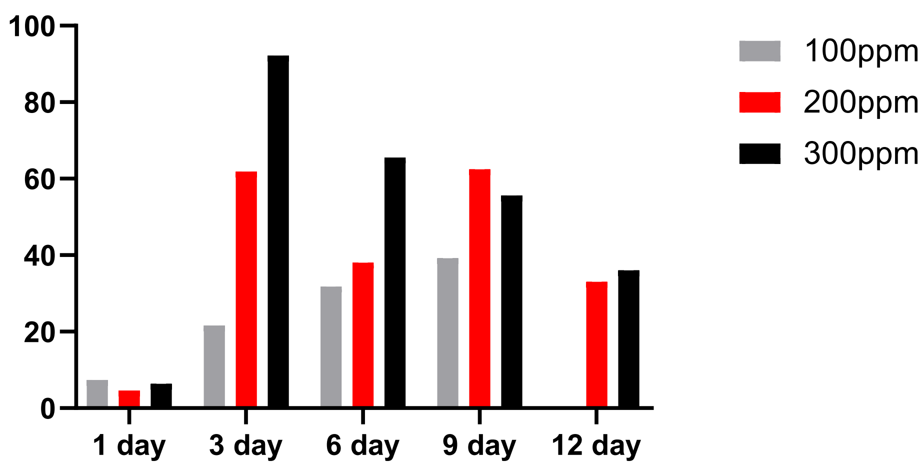
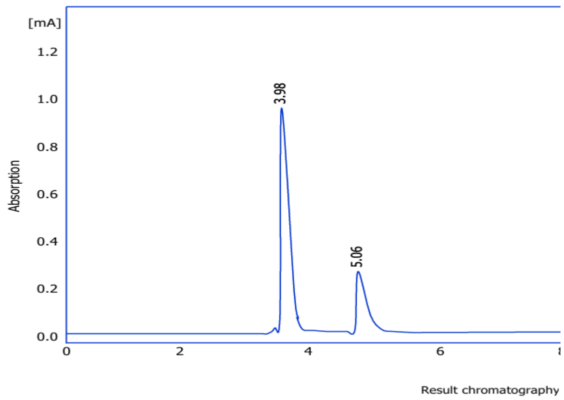
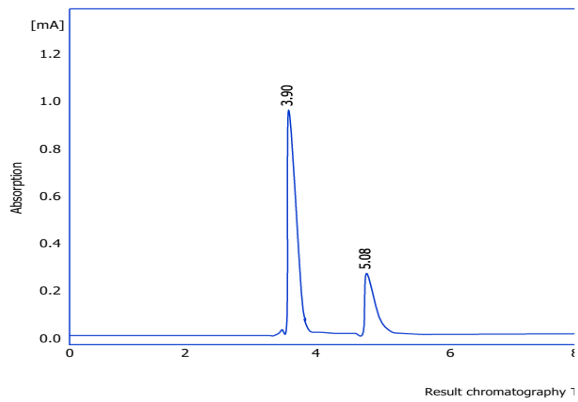


Figure 2. Bioremediation of Acenaphthylene at a concentration of 100, 200, and 300 ppm by *S. amatus*.



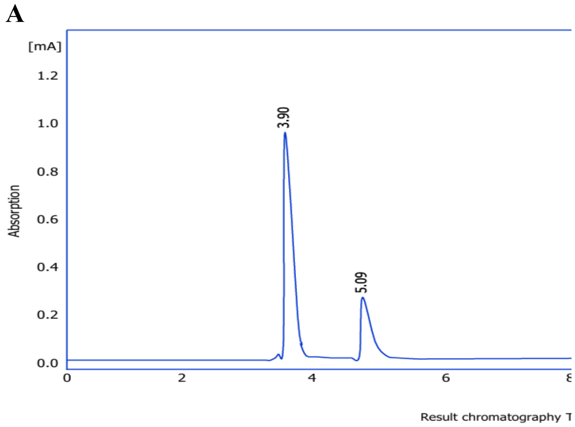
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.98	510452.11	982.65	90.00
2	5.06	65214.44	251.00	10.00
	Total	575666.55	1233.65	100.00

A



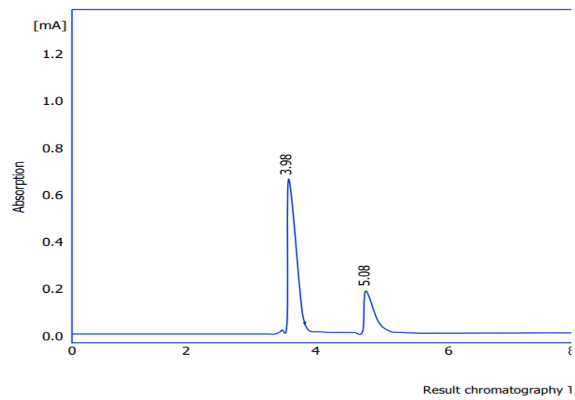
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.90	250652.11	985.44	90.00
2	5.08	55412.65	250.65	10.00
	Total	306064.76	1236.09	100.00

B



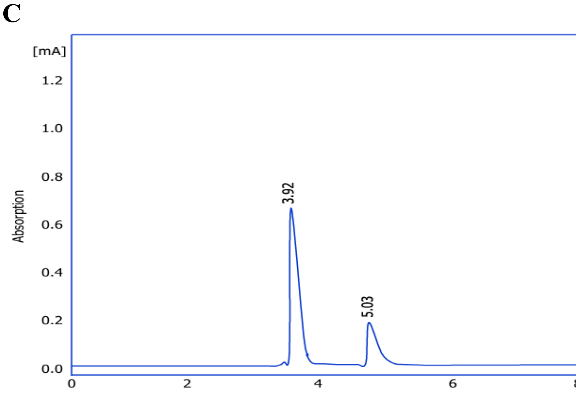
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.90	722654.19	983.11	90.00
2	5.09	71256.98	254.22	10.00
	Total	793911.77	1237.33	100.00

C



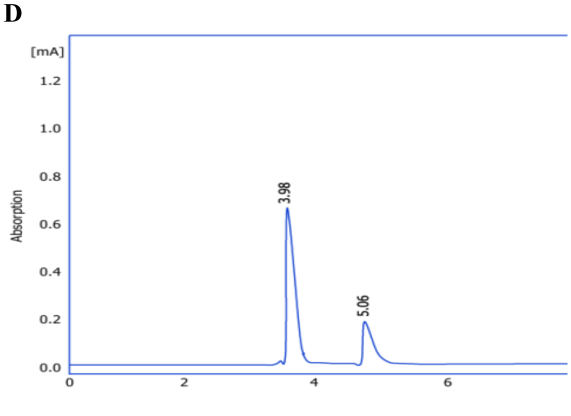
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.98	95624.58	701.25	90.00
2	5.08	21504.55	199.80	10.00
	Total	117123.13	901.05	100.00

D



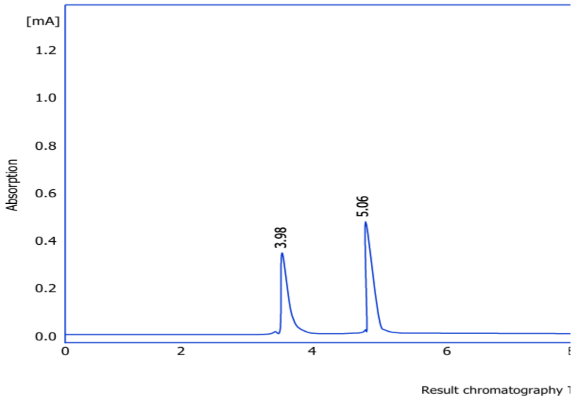
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.92	120652.49	708.95	90.00
2	5.03	25621.44	198.00	10.00
	Total	146273.65	906.95	100.00

E



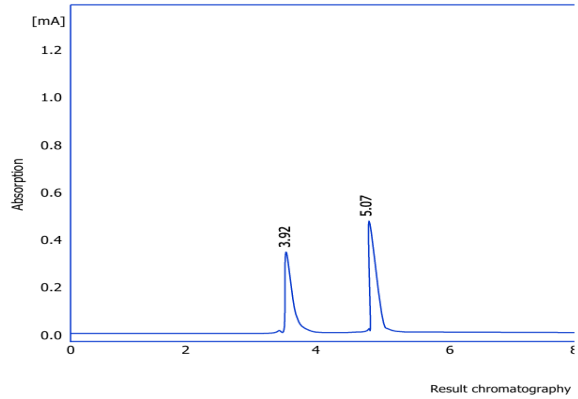
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.98	133952.65	702.14	90.00
2	5.06	29852.44	195.11	10.00
	Total	163805.09	897.25	100.00

F



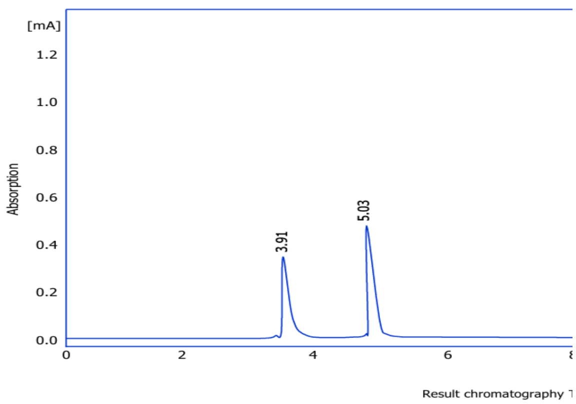
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.98	33652.14	391.00	45.00
2	5.06	22998.80	464.24	55.00
	Total	56650.94	855.24	100.00

G



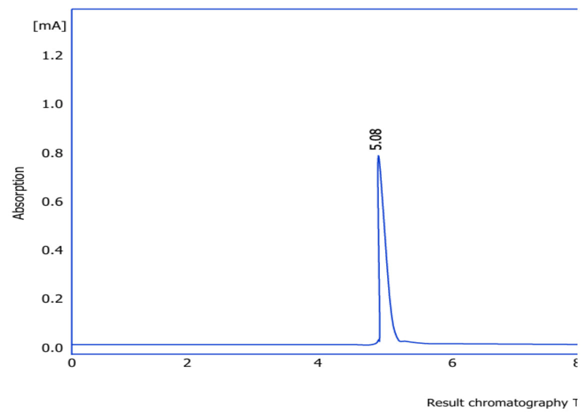
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.92	65214.80	391.31	45.00
2	5.07	23652.11	464.65	55.00
	Total	88866.91	855.96	100.00

H



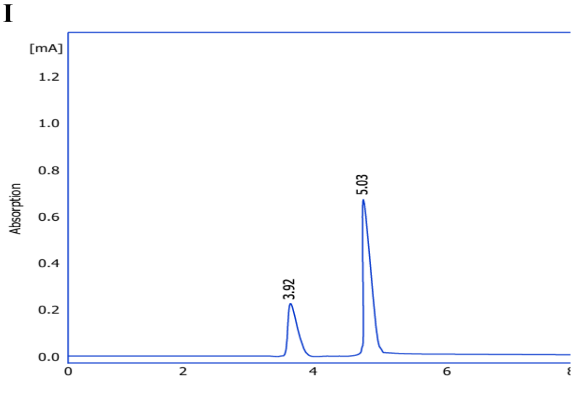
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.91	95124.58	392.65	45.00
2	5.03	22321.45	464.25	55.00
	Total	117452.19	856.90	100.00

I



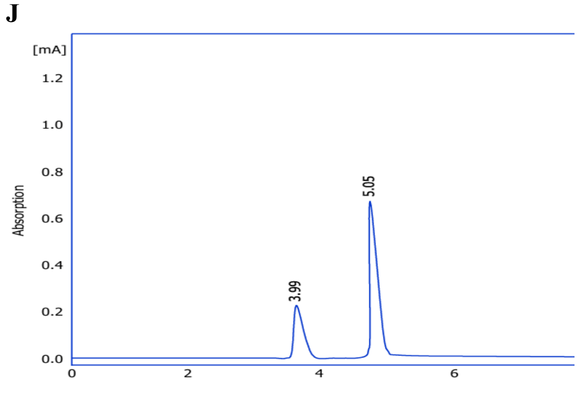
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	5.08	22145.80	800.25	100.00
	Total	22145.80	800.25	100.00

J



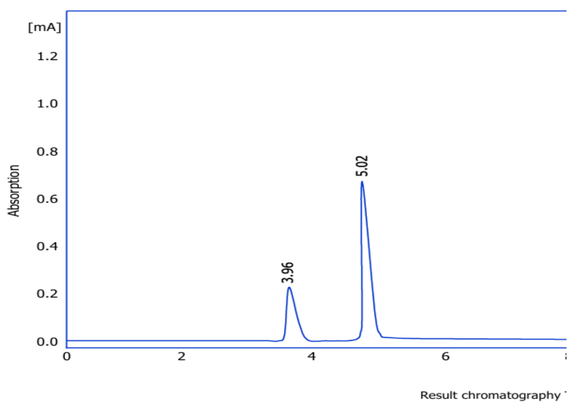
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.92	11250.65	251.00	10.00
2	5.03	22114.58	702.00	90.00
	Total	33365.23	953.00	100.00

K



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.99	19856.08	252.33	10.00
2	5.05	23665.08	701.33	90.00
	Total	43251.16	953.66	100.00

L



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.96	8956.65	251.10	10.00
2	5.02	23652.05	701.20	90.00
	Total	32608.70	952.30	100.00

M

Figure 3. The results of the bioremediation analysis of acenaphthylene at concentrations of 100, 200 and 300 ppm by green algae *S. amatus* using HPLC device. (A) zero day 100ppm. (B) zero day 200ppm. (C) zero day 300ppm. (D) 3 day 100ppm. (E) 3 day 200ppm. (F) 3 day 300ppm. (G) 6 day 100ppm. (H) 6 day 200ppm. (I) 6 day 300ppm. (J) 9 day 100ppm. (K) 9 day 200ppm. (L) 9 day 300ppm. (M) 12 day 300ppm.

The results of the study showed that the highest rate of removal of acenaphthylene mediated by *S. amatus* on the ninth day, which was 39.5 ppm, and the reason is due to the consumption of components in the cultural medium necessary for the growth of algae, and therefore algae do not resort to pulling acenaphthylene as a source of carbon until after the lack of nutrients within the medium (15). The microalgae use hydrocarbons in the process of biodegradation as a source of carbon, converting them into compounds that benefit from them in their vital activity (16). Other studies also indicated that green algae such as *Chlorella vulgaris* and *Selenastrum capricornutum* are good at removing hydrocarbons in the presence of ammonia and that they become more effective for removing pollutants, especially acenaphthylene, with the availability of ammonia, in a longer period and in medium and low concentrations (17). The current results are consistent with (18) although they used lower concentrations of acenaphthylene pollutants (1, 3, 5, 10, 15, 25, 50) mg/L, but they found that the absorption rate of acenaphthylene gradually increases by the green algae *Coelastrella saipanensis* until it reaches a 100% removal rate after 12 days.

The reason for the increased remediation on the third day is due to the increased activity of the dehydrogenase enzyme which was increased after 3 days of culture, and it has been shown that algae tolerate and effectively degrade polycyclic aromatic hydrocarbons which may be toxic in the environment and convert the compounds into a non-toxic form.

Furthermore, (19) showed that polycyclic aromatic hydrocarbons in algae react with the cytochrome P450 monooxygenase enzyme active sites of CYP through hydrogen bonds between molecules, hydrophobic reactions and Van der Waals's reactions, giving algae the ability to remediate polycyclic aromatic hydrocarbons more efficiently than other microorganisms.

(20) and (21) also reported that although complete degradation of aromatic pollutants s was rare, these organisms were able to bioconvert aromatic pollutants by converting acenaphthylene into hydroxyl intermediates through processes called hydroxyl (Table 1, Figure 1).

The results of the statistical analysis showed that there were significant differences between the days at the level of probability ($P<0.05$). The the differences were recorded between the first day with each of the third day, the sixth day and the ninth day, and the third day was recorded with the sixth day at the level of probability ($P<0.05$).

At the concentration of 200 ppm, the highest removal rate was on the ninth day, which it reached (60.4) ppm. It can be seen that the green algae *S. amatus*began to remediate low on the first day of the experiment, due to the adaptation of algae to pollutants, and green algae have a flexible ability to deal with external influences, especially the presence of pollutants in the medium (22). A noticeable increase in absorption occurred after three days until the ninth day of the experiment, which reflects strong vital activity and then the start of decline after the ninth day and until the twelfth day of the experiment, and this is similar to (23) reported, which they indicated that algae differ in their response to hydrocarbon pollution, some of lead to a decrease and slowdown in growth and others stimulate growth, but in both cases the algae passes through a state or period of acclimatization, depending on the species of algae and the pollutant and its concentration (24). When comparing the ability and degradability rate of acenaphthylene, the algae *S. amatus* started from 4.1 ppm on the first day of the experiment and then reached a maximum remediating of 60.4 ppm on the ninth day of the experiment, showing a good absorption rate in the early days, but after the third day, there was a decline in its efficiency, however, this species showed continuity in activity until the twelfth day, achieving a total removal of 200 ppm. The reason for the decline after the third day of the experiment may be the accumulation of acenaphthylene entering the algal cell body, or due to a decrease in photosynthesis. This is consistent with the study of (25) on *Chlamydomonas angulosa* which showed that acenaphthylene accumulates in cells in closed cultures for up to 7 days. Significant differences were found between the days at the level of probability ($P<0.05$) for concentration 200 ppm. The differences were recorded between the first day with each of the third day, the sixth day, the ninth day and the twelfth day of the experiment, and the third day recorded significant differences with both the sixth and twelfth days of the experiment, and also the differences were found between the ninth day with both the sixth and twelfth days of the experiment at the level of probability ($P<0.05$) and at a concentration of 300 ppm. The results indicated that the highest percentage of acenaphthylene removal at a concentration of 300 ppm on the third day, which it reached (92.5) ppm. Through the experiment, it was found that *S. amatus*removal of acenaphthylene increased from 6.4 ppm on the first day of the experiment to 92.5 ppm on the third day of the experiment, and then gradually decreased to 35.05 ppm on the twelfth day. It showed a good absorption rate in the early days but saw a steady decline thereafter. This may be due to the low tolerance or depletion of nutrients it needs to complete biodegradation or because of the surface area, where the smaller the algae cells, the larger the surface area and their exposure to the pollutant more widely. This is consistent with (26) when studying the tolerance comparison between the two species of algae *Microcystis aeruginosa* and *Scenedesmus amatus*when exposed to different concentrations of one of the cyclic hydrocarbons as well as they also mentioned that green algae have a more blue-green algae composition than blue-green algae, and because they are eukaryotic, therefore they have endomembrane systems that enable complex cell structures and an advanced membrane system to better tolerate the pressures of pollutants.

According to ecotoxicity studies, polycyclic aromatic hydrocarbon pollutants interfere with the algae's antioxidant defense system and cause membrane lipid oxidation, and when exposure to high concentrations is persistent, cells produce large numbers of reactive oxides species (ROS), such as O, OH, and H_2O_2, which are removed by the antioxidant defense system. Many species of reactive oxides can also cause or increase the oxidation of membrane lipids, causing severe damage to the membrane system leading to corrosion and damage to membranes (27). Therefore, we observe a gradual decrease opposite to the effectiveness of algae by removing high concentrations of acenaphthylene.

Some studies has suggested that organic pollutants can be degraded by algae to provide a source of carbon and a source of nitrogen, both of which are essential for growth (28). The maximum concentration of pollutant that algae can degrade is defined as the value of the algae's resistance threshold to the organic pollutant. Once this value is exceeded, the organic pollutant harms the algae and inhibits their growth, mainly by affecting photosynthesis, respiration, vital membrane and biochemical construction. The theory of free radical damage is a modern attempt to explain the mechanism of toxicity in cells and is widely used to explain the toxic actions of heavy metals, organic pollutants and pesticides (26).

Significant differences were found between the days of the first day with each of the third day, the sixth day, the ninth day and the twelfth day at the level of probability ($P<0.05$) and the third day recorded significant differences with each of the sixth day, the ninth day and the twelfth day at the level of probability ($P<0.05$) and significant differences were found between the sixth day with both the ninth day and the twelfth day at the level of probability ($P<0.05$). Through the experiment, it was found that the green algae *S. amatus*used had efficient in getting rid of acenaphthylene added to the cultural medium, but at different times and rates of removal depending on the ability of the algae to adapt to the pollutants and its biological activities. The study showed that algae have the ability to form enzymes that work to break down dangerous organic compounds and convert petroleum hydrocarbons into less harmful substances or remove some compounds completely (29). Previous studies have shown that some algae, including green, red and brown algae, have the ability to decompose some hydrocarbons into compounds that are less harmful to the environment, suggesting their ability to deal with crude oil pollution (30).

4-CONCLUSION

The research results proved that the algae *S. amatus* is characterized by resistance to acenaphthylene pollution, as it was able to consume the acenaphthylene added to the medium after 9 days. This indicates that the moss used acenaphthylene as a source of carbon after the main nutrients began to run out of the medium. This was found in concentrations of 100 and 200 parts per million, while the high concentration had a greater impact on the effectiveness of the moss. Despite this, it was able to consume a large part of the pollutant concentration. We conclude that the moss can be used as an effective treatment for acenaphthylene with high efficiency in concentrations of 100 and 200 parts per million .

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