

Bioremediation of Naphthalene by the Chlorophycean *Coelastrella saipanensis*

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ABSTRACT

The freshwater green alga *Coelastrella saipanensis* was studied to assess its ability to degrade the PAHs (naphthalene). This species was cultured in Chu-10 medium under controlled conditions and treated with different concentrations (1, 3, 5, 10, 15, 25 and 25mg/ l) of naphthalene. The results showed that high removal efficiencies were obtained by the alga *C. saipanensis* for the concentrations: 1, 5, 10 and 15mg/ L, reaching 100% after 12 days of incubation. The increase in naphthalene concentration significantly decreased all the photosynthetic pigment contents studied, such as total chlorophyll, chlorophyll a, chlorophyll b and carotenoids. The results showed that the lowest values of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids were 0.2533,0.1367,0.15,0.2133µg/ml, respectively, recorded at 50ppm naphthalene. Moreover, the results showed a gradual increase in all the antioxidant parameters studied, such as CAT, SOD, MDA and ROS with increasing naphthalene concentration. The highest value was 0.292,0.953,1.74,16.416mg/ g, respectively, at 50ppm of naphthalene. It was concluded that *C. saipanensis* was a promising tool for the bioremediation of PAH compounds, particularly naphthalene, achieving 100% removal efficiency. Furthermore, the microalgae showed reduced levels of photosynthetic pigments and increased antioxidant levels, suggesting that the algae may have tolerance to naphthalene and could potentially degrade this harmful compound.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have emerged as a prominent category of environmental contaminants in numerous ecosystems (Houshani *et al.*, 2019). These compounds have adverse effects on the environment, organisms and human health due to their toxic, mutagenic and carcinogenic properties (Zhang *et al.*, 2013; Kalhor *et al.*, 2017).

Naphthalene, a two-ring PAH, is a common micropollutant found in drinking water, air and soil (Samanta *et al.*, 2002). It is commonly derived from the distillation and fractionation of coal tar, crude oil and petroleum products and is used in manufacturing resins, dyes, and pesticides (Höfer & Bigorra, 2008). Designated by the US Environmental Protection Agency (US-EPA) as a priority pollutant for environmental

monitoring (EPA, 2008), naphthalene forms covalent bonds with molecules in liver, kidney, and lung tissues, increasing its toxicity and leading to conditions such as haemolytic anaemia and eye defects (Srogi, 2007).

Several methods and tactics, including both physical and chemical approaches, have been developed, refined and implemented to mitigate the effects of contaminants and remediate contaminated sites. Some traditional remediation methods have significant drawbacks, such as complicated technology, high costs, and limited public acceptance. In contrast, bioremediation offers a biological solution that is cost effective, environmentally friendly, and widely accepted. Bioremediation is a pollution management strategy that uses biological systems to convert various harmful compounds into harmless forms such as CO₂ and H₂O (Sonune, 2021). Researchers have identified various microbial species, including bacteria, fungi, yeast, and microalgae, as effective agents for degrading hydrocarbons in natural environments (Chan *et al.*, 2006).

In this study, we investigated the ability of the green alga *Coelastrella saipanensis* to bioremediate naphthalene and its effect on photosynthetic pigment levels and antioxidants, such as CAT, SOD, MDA and ROS.

MATERIALS AND METHODS

Algal cultivation and biomass production

A pure culture of *Coelastrella saipanensis* (Fig. 1) was obtained from the Environmental Laboratory at Al-Qadisiyah University. The culture was grown on nutrient agar and incubated for 72 hours at 37°C to ensure the purity and absence of bacteria and fungi (Andersen, 2005). To produce biomass, the algae isolate was then transferred to a sterile 1000ml glass flask containing 500ml of Chu-10 culture medium and incubated at 26°C under a light intensity of 60µmol m⁻² s⁻¹.

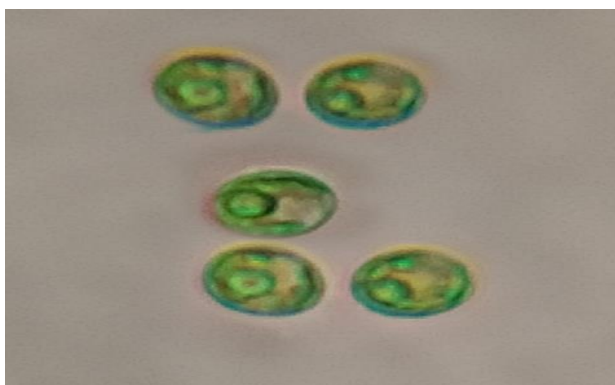


Fig. 1. *Coelastrella saipanensis* algae under a light microscope at 40x

Experimental design

The algae were exposed to different concentrations of naphthalene supplied by Thomas Baker Chemicals/India. Stock solutions of naphthalene were prepared at different concentrations of 1, 5, 10, 15, 25 and 50mg/l by dissolving in a small amount of ethanol. The volume was then adjusted with Chu-0 medium. These solutions were then

exposed to the algae to evaluate their bioremediation capabilities. In addition, the toxicity of naphthalene to green algae at the above concentrations was evaluated. The algae were incubated under conditions of $60\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 26°C temperature, pH 7 and a photoperiod of 16 hours light and 8 hours dark. The antioxidant enzyme activity was determined. Samples (5ml) were filtered through a Millipore filter ($0.45\mu\text{m}$) and analyzed by a high performance liquid chromatography (HPLC) (Fig. 2).

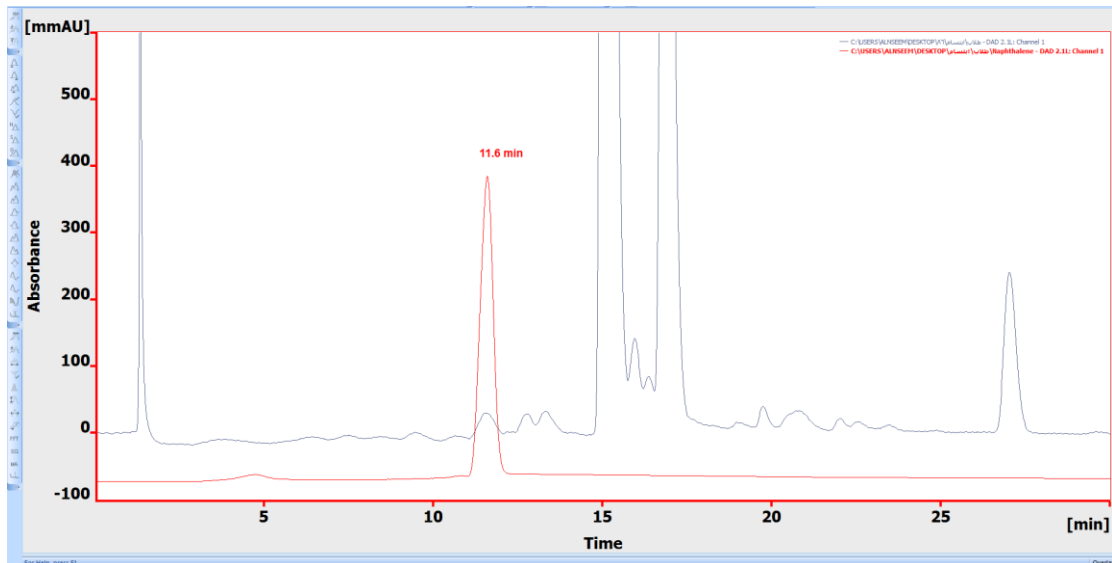


Fig. 2. Detection of naphthalene by HPLC at retention time 11.6, the red color represents naphthalene standard, while the blue color represents the sample treated with *Coelastrella saipanensis*

Determination of the photosynthetic pigment content

Total chlorophyll and carotenoids were extracted from 2ml of algal suspension by centrifugation, and the growth medium was decanted. Pigments were extracted in hot methanol for 10min according to the method described by **Jeffrey and Humphrey (1975)**. Cell debris was removed by centrifugation, and the clear supernatant containing the pigments was diluted to a defined volume. Absorbance was measured spectrophotometrically against methanol blank at wavelengths of 663, 644 and 452nm. The concentrations of each pigment fraction (total chlorophyll and carotenoids) were expressed as $\mu\text{g}/\text{ml}$ culture.

Detection of antioxidant enzymes and reactive oxygen species

The antioxidant enzymes CAT, SOD activity, MDA and ROS were investigated for the microalgae *Coelastrella saipanensis*. CAT was determined quantitatively according to the method of **Hadwan & kadhum Ali (2018)**. SOD was determined as described by **Stephenie et al. (2020)**. MDA was estimated by the method of **Hegazy (2011)**, while ROS was measured according to **Erel (2005)**.

Statistical analysis

To assess the ability of microalgae to bioremediate naphthalene and to evaluate the activity of antioxidant enzymes and reactive oxygen species (ROS) in both the control and treatment groups exposed to different concentrations of naphthalene, statistical analysis was performed using one-way analysis of variance (ANOVA) and least significant differences (LSD).

RESULTS AND DISCUSSION

Bioremediation of naphthalene by *C. saipanensis*

The results showed a high efficiency of naphthalene removal by the green alga *C. saipanensis*. Concentrations of 1, 5, 10 and 15mg/l achieved 100% after 12 days of incubation. The results showed that *C. saipanensis* has a high ability to remove naphthalene after 3, 5, 7, 9 and 12 days of incubation for all treatments (Fig. 3).

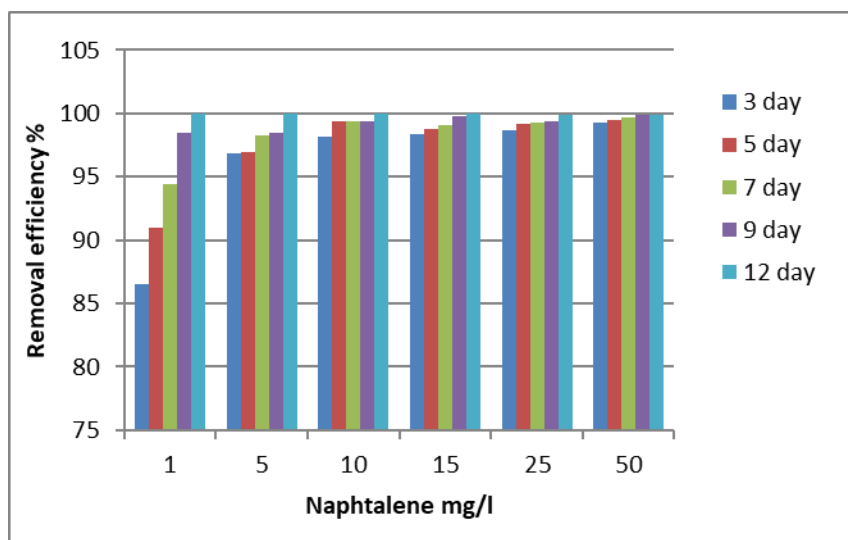


Fig. 3. Removal efficiency of naphthalene by *C. saipanensis* exposed to different concentrations during the experimental period

The results of this study are consistent with the findings of **Aldaby and Mawad (2019)**, where it was observed that two different algal species, *Oscillatoria* sp. and *Chlorella* sp. showed different abilities to degrade pyrene. Specifically, *Oscillatoria* sp. showed a degradation rate of 95%, whereas *Chlorella* sp. showed a degradation rate of 78.71% when exposed to 50mg/l pyrene over a 30-day incubation period. Furthermore, according to the results of **Asghari *et al.* (2020)**, *Chlorella vulgaris* showed a significant resistance to fluorene and showed promising potential for its degradation. Moreover, **Tomar *et al.* (2022)** postulated that the microalgae *Chlorella vulgaris* was able to remove 92% of naphthalene within 7 days, and added that the activity of dehydrogenase enzyme was increased after 3 days of cultivation. They argued that algae tolerate and effectively degrade PAHs which may be toxic in the environment and convert the compounds into a non-toxic form. Furthermore, in their study, **Kumar *et al.* (2014)** showed that PAHs in algae interacted with cytochrome P450 monooxygenase

CYP active sites through intermolecular hydrogen bonding, hydrophobic π - π interactions and van der Waals interactions, giving algae the ability to remediate PAHs more efficiently than other microorganisms. For the algae, **Semple *et al.* (1999)** stated that, although complete degradation of aromatic pollutants is rare, these organisms are capable of biotransforming aromatic pollutants by converting naphthalene to its hydroxylated intermediates through processes called hydroxylation.

Effect of naphthalene on photosynthetic pigment contents

The results of the current study showed the effect of naphthalene on the chlorophyll content of the algae studied. A decrease in chlorophyll content was observed as the concentration of naphthalene increased. The alga *Coelastrella saipanensis* recorded the lowest concentrations for both total Chl, Chl-a, b and carotenoids reaching (0.2533, 0.136, 0.15 and 4.7933 μ g/ l, respectively, at a concentration of 50mg/ l compared to the control treatment which recorded the highest concentration, as shown in Fig. (4). The results of statistical analysis showed significant differences between treatments and control at different concentrations of naphthalene at $P \leq 0.05$.

Numerous studies have investigated the bioremediation of aromatic hydrocarbons and their effect on chlorophyll and carotenoids in algae. These studies have shown consistent results. For example, **Aldaby and Mawad (2019)** demonstrated the effects of pyrene on chlorophyll and carotenoids in *Oscillatoria* sp. and *Chlorella* sp.; they noted a decrease in chlorophyll and carotenoids with higher pyrene concentrations. Similarly, **Patel *et al.* (2016)** observed a decrease in chlorophyll with higher anthracene and pyrene concentrations in *Anabaena fertilissima*. Furthermore, **Patel and Tiwari (2015)** found that exposure of *Chlorella vulgaris* to fluoranthene and acenaphthene resulted in a decrease in total chlorophyll content. **Kumar *et al.* (2014)** also showed a decrease in carotenoid content in *Anabaena fertilissima*, *Synechocystis* sp. and *Nostoc muscorum* when exposed to pyrene. The current study further supports these findings by showing a reduction in the photosynthetic content of *C. saipanensis* with increasing naphthalene concentration. This reduction can be attributed to the alteration of membrane permeability by PAHs, leading to the production of ROS and subsequent functional changes in PSII, blocking photosynthetic electron flow. This limitation could be caused by increased ROS formation at different points in the photoelectron transport chain, resulting in pigment loss (**Appenroth *et al.*, 2010; García *et al.*, 2011**), or by the accumulation of ROS in PAH-treated plants (**Molina & Segura, 2021**).

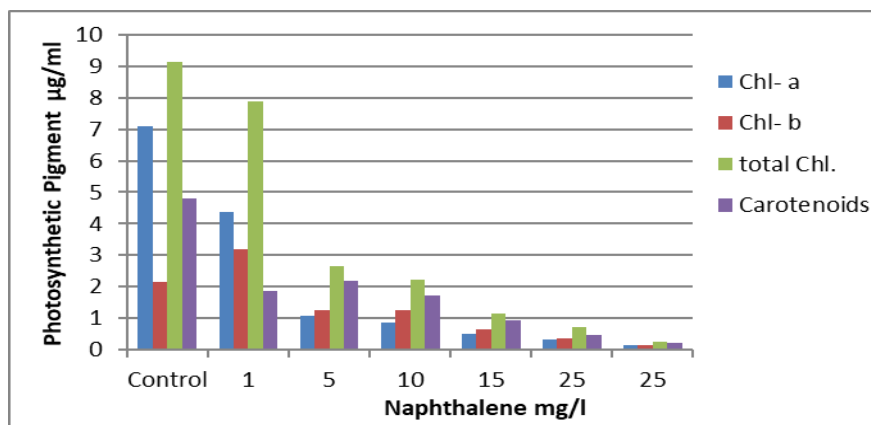


Fig. 4. Mean values of total Chl, Chl-a, b and carotenoids contents of *C. saipanensis* after 12 days of exposure to different concentrations of naphthalene with control

Effect of naphthalene on antioxidant enzymes, MDA and ROS contents

The current study showed a gradual increase in CAT and SOD values of the alga *Coelastrrella saipanensis* exposed to the different concentrations of naphthalene compared to the control treatment, with the highest values being 0.292 and 0.953mg/ g at 50mg/ l for CAT and SOD, respectively. Likewise, the study concluded that a gradual increase in ROS and MDA values with increasing concentrations of naphthalene, the highest value was 1.74, and 16.146mg/ g for ROS and MDA, respectively, at 50ppm of naphthalene (Fig. 5). The results of this study agree with the findings of **González *et al.* (2020)**, since they showed that *Ulva lactuca* displayed an oxidative stress condition reflected in the increase of reactive oxygen species (ROS), mainly superoxide anions, and an increase in the activities of antioxidant enzymes such as CAT and The level of transcripts encoding these antioxidant and metabolizing enzymes were increased in response to PAHs, suggesting that the increase in activities is due to the enhanced expression of genes encoding these enzymes. Alternatively, PAHs may cause changes in the enzymatic reactions of algal cells, as the dehydrogenase enzyme is considered to be one of the major oxidoreductase enzymes that metabolize PAHs in addition to being an important part of the electron transfer system in the cell (**Tomar *et al.*, 2022**).

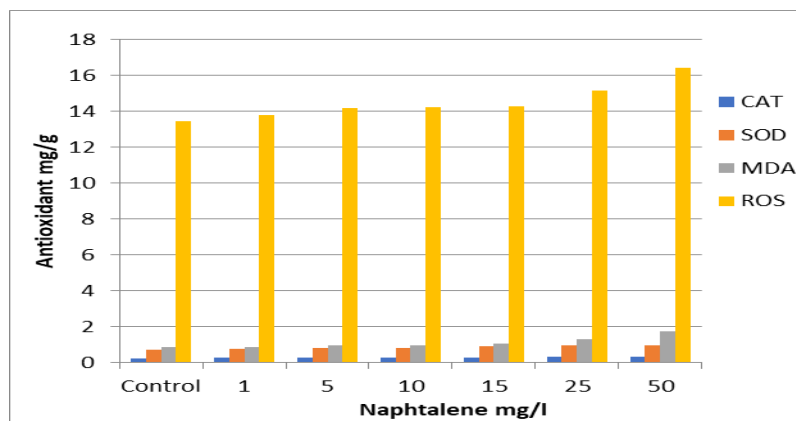


Fig. 5. Mean values of CAT, SOD, MDA and ROS of *C. saipanensis* after 12 days of exposure to different concentrations of naphthalene with control

CONCLUSION

C. saipanensis represented good tools for the bioremediation of PAHs compounds such as naphthalene and had an effective removal of it reaching 100%. Furthermore, the microalgae gave lower photosynthetic pigment and higher antioxidant values when exposed to high concentrations of naphthalene via chlorophyll content, CAT, SOD, MDA and ROS, indicating that the algae have a tolerance level for this toxic compound and can resist and degrade it.

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