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Physiological responses of *Fischerella muscicola* to kirkuk crude oil exposure: growth, pigment, and protein dynamics in different concentrations

Ali Muayad Sultan[™] [□]

Biology Department, College of Education for Women, Tikrit University, Iraq Received: July 28, 2023/ Revised: Aug 21, 2023/Accepted: Aug 23, 2023
(☑) Corresponding Author: alimusu82@tu.edu.iq

Abstract

This study was conducted on Fischerella muscicola isolated from the Iraqi Tigris River. To determine the impact of Kirkuk crude oil (KCO) in four concentrations (0.25, 0.5, 1 and 2) % v/v as well as Control culture (CC). The biomass was measured as growth by optical density (OD) and estimated the content of Chlorophyll a (Chl a), Phycocyanin pigments (PC) and protein content as an indicator of Physiological activities of F. muscicola for 30 days. The results shown that the KCO 0.25% and 0.5% was less impact on F. muscicola. The growth rate has slowed down and the content of Chl a, PC and protein was limited during initial incubation. Then those increased regularly after the second week of the incubation period, which is the adaptation period for the presence of KCO. It records with KCO 0.25% treatment 0.39, (8.2, 2.5, 96.5) μ g/ml for growth, Chl a, PC and protein respectively. These values are higher than the values recorded by the CC. that proved that some petroleum hydrocarbon compounds might support the growth of F. muscicola. Even with KCO 0.5% species was tolerant and grown well, were record 0.27, (7.3, 0.8, 78) µg/ml at day 30 for growth, Chl a, PC and protein respectively. When exposing F. muscicola with impact of KCO 1% and 2% treatments, its requires a longer period for adaptation. As the growth and Chl a, PC and protein were low for the first three weeks of the incubation period. Then the growth rate improved after day 24 of incubation, were recorded 0.112 and 0.069 respectively at day 30. Protein content of F. muscicola was affected by the toxicity of high concentrations of KCO, especially initial of incubation period. However, the F. muscicola resisted pollution efficiently. The presence of oil refineries and an electric power station working with KCO near the Tigris River in the Baiji city made some Species to be tolerant to the influence of petroleum compounds.

Keywords: Cyanobacteria, Fischerella muscicola, Crude oil Chlorophyll a, Phycocyanin, Protein

Introduction

Cyanobacteria are common in most diversity of environments on Earth and are major contributors to atmospheric carbon and nitrogen fixation (Galloway et al., 2004; Zwirglmaier et al., 2008; Whitton and Potts, 2002). They are photoautotrophs, some of their species may exist in extreme environments, and when cyanobacteria are away from the light, they remain dormant, but some of their species can metabolize some organic compounds, including sugars (Stewart, 1973). Therefore, one of the reasons for its widespread is its possession of special adaptive mechanisms (Fay, 1983; Dworkin et al., 2006). Foremost among them is its ability to resist the impact of pollutants, including oil compounds containing a wide range of hydrocarbon compounds, and it may play an important role in the process of biodegradation of some petroleum compounds (Cerniglia, 1992). The Iraqi environment is considered one of the most polluted environments with crude oil due to the operations of extracting, transporting and refining oil. Because of the bombing of pipelines transporting crude oil during the ISIS war in Tikrit and Mosul, which led to pollution of the Tigris River during the liberation period 2014-2016 (UNEP, 2017). Oil derivatives are the most complex mixture of hydrocarbon

compounds that cause a global pollution problem affecting soil, air and water. It may penetrate into the groundwater and, reduce large numbers of decomposing turn, in microorganisms, thus destroying and disrupting food chains and impeding the flow of energy. Hydrocarbons exhibit toxic effects in addition to being carcinogenic and genetically mutagenic. The risk of crude oil contamination is due to the non-volatilization and solubility of some (Juhasz et al., 1996; Kridi et al., 2021). While Jia et al. (2017) indicated that crude oil consists of a complex combination of aromatic hydrocarbons, aliphatic hydrocarbons, heterocyclic hydrocarbons, asphaltenes and non-hydrocarbon compounds, about 60-90% of these compounds are biodegradable. Some studies indicated the ability of some species of cyanobacteria to tolerant different concentrations of crude oil and degrade crude oil; Such as Nostoc punctiforme and Spirulina platensis under heterotrophic conditions (El-Sheekh and Hamouda, 2014). The cyanobacterium Fischerella ambigua ISC67 showed the ability to biodegrade crude oil at certain concentrations (Safari et al., 2016; Sultan et al., 2017). Anabaena cicadae isolated from soil contaminated with crude oil showed treatment of crude oil samples according to Agu's study (Agu et al., 2022). The process of biodegradation hydrocarbons occurs in the presence of oxidative enzymes (Plohl and Leskovsek, 2002), which requires the availability of oxygen. As for aromatic compounds, they are often broken down by external enzymes such as Dioxygenase. It is believed that cyanobacteria that are resistant to hydrocarbons possess both Oxygenase and Dioxygenase (Cerniglia et al., 1979).

Materials and Methods

Sample collection sites

Samples were taken from several sites located along the Tigris River passing through Tikrit city and from some swamps and gardens, to ensure obtaining many species of cyanobacteria, and the samples collected for the purpose of the study were placed in sterile plastic bottles that had been previously prepared for this purpose.

Preparation of the culture medium

After experimenting with many culture media, it was found that BG 11 is the best medium for the development of freshwater cyanobacteria and supports the growth of *Fischerella* species perfectly (Stanier et al., 1971; Khamis et al., 2016). The components of BG 11 medium were dissolved in distilled water with continuous stirring Using the Gallen Kamp Magnetic Stirrer Hotplate. The pH was adjusted within 7.6-7.8 using sodium bicarbonate NaHCO3 and dilute hydrochloric acid HCl 0.1 *N*, pH was measured by an Oyster pH meter. Distribute 100 ml of the BG11 medium into conical flasks with size 250 ml. The mouths of the flasks are closed with gauze and medical cotton and then sterilized using Autoclave type Sanshe modl yx280B at 121° C for 20 minutes under a pressure of 15 pounds/inch 2.

Cultivation and isolation of cyanobacteria

Water samples were cultured on Petri dishes containing solid medium (BG11 with Agar 1.5% w/v), the dishes were incubated in a Cooled Incubator made in England by Gallen Kamp. At a temperature of 25 °C with exposure to 2500-lux intensity light for a period of 12 hours/day, after two to three weeks had passed, growth spots of cyanobacteria were observed in the form of green colonies, some of which turned brown. A preliminary identification process is performed under a light microscope for the growing colonies, distinct colonies are marked using a pen from the bottom of the petri dish. Then, it was transferred to Petri dishes containing the solid BG11 culture medium as a preliminary stage of purification to prepare suitable species for the study. Then the dishes are incubated under the influence of the same conditions, the previous process is repeated until pure species are obtained. Then the growing colonies were transferred to 250 ml conical flasks containing 100 ml of liquid BG11, Then the axenic cultures were incubated in the refrigerated shaking incubator at 100 cycles/min at 25°C.

Choosing the *Fischerella* for the study

After isolating and purifying some species, were grown on liquid BG11 media containing KCO residues. It was recultured and transported continuously in several stages while exposing the species to different concentrations of KCO, in order to identify species that are resistant to the toxic effect of petroleum hydrocarbons. The strain was identified as cyanobacterial Fischerella muscicola according to phenotypic properties and taxonomic descriptions available in the literature (Rippka et al., 1979; Desikachary, 1959). It is a filamentous thermophilic species of Cyanobacteria, Some cells in a mature trichome divide in more than one plane, resulting in trichome with multi-seriate and lateral uni-seriate branches. Heterocysts are intercalated or lateral positions. Akinetes can be formed. but according to Martin & Wyatt (1974) Akinetes in Fischerella sp. are not always readily recognizable. F. muscicola was chosen as it exhibits tolerance to the contaminated medium.

Crude oil selection

There is no doubt that crude oil is one of the dangerous pollutants that threaten our environment because Iraq is one of the oil-exporting countries. So crude oil from the Kirkuk fields was used because it feeds oil refineries and the electric power station adjacent to the Tigris River in Baiji city near Tikrit city 40 km for the purpose of knowing its effect on *F. muscicola*, with four concentrations of KCO were used 0.25, 0.5, 1, and 2 % v/v.

Growth measurement

F. muscicola were grown on liquid BG11 for a period of 30 days, under cultivation conditions referred to previously, the optical density (OD) of *F. muscicola* biomass growth was measured under the influence of KCO at Four concentrations of 0.25%, 0.5%, 1%, and 2%. The measurements were made at 8, 16, 24, and 30 days of the incubation period, by taking 5 ml from the Cultures then depending on the OD at the wavelength 436 nm using a spectrophotometer type Lapo Med. inc (Gibson and Fay, 1983).

Chlorophyll a Measurement

Chl a was extracted by taking 5 ml of the culture medium, placing it in a centrifuge tube, then centrifuged at 3000 rpm type Hettich for 10 min. acetone 90%v/v is added to the precipitate. extractions and filtrations according to (Kurtis, 2007). The concentration of Chl a was calculated according to (Jeffrey et al., 1997; Oo et al., 2017). The supernatant dissolved Chl a is taken, Acetone was added to the supernatant until the final volume was 10 ml, then the OD was measured by a spectrophotometer with 1.0 cm quartz cell at three wavelengths A630 nm, A645 nm, and A663 nm. Zeroing (blank) by same solvent concentration. The Chl a concentration was calculated in µg/ml through the application flowing equation:

Chl $a \mu g/ml = (11.64A663 - 2.16A645 + 0.10A630) v/ lV$

v = acetone volume used (ml)

l = the spectrophotometric cell length (cuvette) cm

V = the sample volume (ml)

Phycocyanin (PC) Estimation

The absorbance of the PC pigment was measured by taking 5 ml from the growing culture. the cells were isolated from the liquid medium using a Hettich centrifuge at 3500 rpm for 10 minutes. the supernatant was discarded and the cells' sediment was suspended in 5 ml of K2HPO4 buffer 0.1 M phosphate buffer containing 0.15 M NaCl, pH 7.0) (Shayesteh et al., 2023). Then Homogenization in an ice-cooled mortar (Hajnalka et al., 2013) As he tested five methods, he referred to The highest PC concentration obtained when cells were homogenized using a mortar and pestle (B Method). The pellet was re-extracted with buffer. After homogenization, the extract was centrifuged. the PC concentration of the supernatant was measured by the measurement of OD at (652 and 615) nm with UV-Spectrophotometer (Lapo Med. inc). PC concentration was calculated using the equation by (Manikandavelu and Murugan, 2009).

PC (mg/ml) = [A615 - 0.474(A652)] / 5.34

A615 is the absorbance at 615 nm A652 is the absorbance at 652 nm

Protein Estimation

The amount of protein was determined according to Lowry's method (1951) and Modified by Waterborg (1984). the following materials were used: solution (A) 2% NaCO3, (B) 1% CuSO4.5H2O solution, Solution, (C) 2% Sodium Potassium Tartrate and 2 N NaOH, Folin reagent, Standards stock solution by 300 μ g/mL of bovine serum albumin as shown in the Fig. 1.



Fig. 1 Protein standard curve using bovine serum albumin

Results and discussion

The presence of Baiji refineries and the electric power station operating with KCO before the sampling area 40 km away has a role in polluting the waters of the Tigris River and the ecosystem. Perhaps this made algae more resistant and tolerant to the impact of oil pollutants, and some species have become decomposers of oil residues. In this study, the *F. muscicola* was grown under the influence of different concentrations of KCO (0.25, 0.5, 1, and 2) % v/v for a period of thirty days. The

results showed different responses in terms of growth, Chl *a*, PC pigments, and protein concentration, as shown in Fig. 2, 3, 4, and 5, respectively.

As shown in the Fig. 2 the results of the effect of different concentrations of KCO on the F. muscicola biomass growth of as OD. It was observed that the growth rate increased regularly in the control culture (CC) to record 0.354 at 24 days, and then the growth rate slowed down on the 30th day to reach 0.371. When exposing F. muscicola with 25% KCO, the growth is recorded 0.21 at 24 days, that is a half of the value recorded in the control culture, while it jumps to reach 0.39 at day 30, which is the highest value recorded under the KCO influence. This is in agreement with the study of (Khamees et al., 2015) on Oscillatoria splendida they referred Cyanobacteria can metabolize and utilize low concentrations of KCO. When the F. muscicola was treated with a 5% KCO a slowdown in growth is observed in the first two weeks of incubation, to recorded 0.27 at 30 days. F. muscicola resisted pollution efficiently with 0.25% and 0.5% treatment. El-Sheekh and Hamouda, (2014) indicated (or referred) that low concentrations of KCO may promote growth and it is possible to exploit crude oil as a source of carbon. The growth was inhibition under the influence of the two treatments 1% and 2% to reach 0.112 and 0.069 respectively at 30 days. Although the growth slowed down, it was present (Rodas, 2009) indicated that crude oil may hinder the process of photosynthesis. Some species of cyanobacteria endured survival under the influence of 1% treatment of crude oil (Safari et al 2014) while (Adam and Duncan, 1999) indicated that oil and its derivatives are

generally phytotoxic at certain concentrations. But at levels lower than 1%, its growth does not stop, and yet it does not rise to its growth as it was in clean environments. In general, growth depends on the efficiency of photosynthetic pigments such as Chl a, and its results are shown in Fig. 3.

The highest concentration of CC Chl *a* was 8.4 μ g/ml at day 24, to decreased at the end of the incubation period on day 30, and it was 8.2 µg/ml. as shown in Fig. 3. When F. muscicola was treated with KCO 0.25% and 0.5%, the chl a concentration was recorded (0.8, 0.6) µg/ml respectively at 8 days, then it increased during the incubation period to reach (9.4, 7.3) µg/ml respectively at day 30. Although the chl concentration was affected at the beginning of the incubation, but its concentrations increased later. The highest value of chl a concentration was recorded with KCO treatment 0.25% at the end of the incubation period. These results show the ability of F. muscicola to adapt and grow well under the influence of the two KCO treatments 0.25% and 0.5%, which is within its tolerance limits. The chl a content is the most accurate measurement of the toxicity of petroleum hydrocarbon compounds, as Chl a is the most sensitive to all pollutants, and at the moment when Chl a is inhibited, growth and many functions of the living cell are inhibited too (Oberholster et al., 2010). No significant concentrations of Chl a were recorded when F. muscicola was treated with KCO concentrations 1%, 2%. This is similar to the results of El-Sheekh and Hamouda, (2014) on S. platensis, in which the Chl a concentration decreased with the influence of KCO 2%.



Fig. 2 Effects of different concentrations of KCO on *F. muscicola* Growth, by OD (436 nm)

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Fig. 3 Effects of different concentrations of KCO on *F. muscicola* Chl *a* for 30 days

The results of PC concentrations are shown in Fig. 4. PC is a secondary pigment in Fischerella sp. and forms the major part of biliprotein in. it occupies half of the Phycobilibroteins composition of the Phycobilisomes (Soltani et al., 2007). In general, the PC concentrations were low compared to the Chl a concentrations. Where the highest value of PC was recorded by control culture 2.8 µg/ml at day 24, and it decreased at the end of the incubation period to record 2.5 μ g/ml. This may be due to the depletion of nutrients with the accumulation of some residues that cause a change in the pH of the medium. When F. muscicola cultures were treated with KCO 0.25%, a gradual and slow increase was observed in the PC content after 8 day of incubation, to record the highest value at 30 days, which was 1.6 µg/ml. This is equivalent to the PC CC value that recorded at 8 days. While the PC content was significantly inhibited with the three KCO treatments 0.5%, 1%, 2% and they were 0.8, 0.35, 0.15 µg/ml, respectively at end of the incubation period. Sundaram and Soumya, (2011) indicate that organic stress affects chlorophyll, PC, and carotene in cyanobacteria. Zhang et al. (2020) also confirmed that petroleum has a negative impact that reduces photosynthetic pigments and slows light absorption. PC content was very sensitive to KCO treatment.

Proteins make up the largest fraction of the cyanobacteria biomass (López et al., 2010). The protein content of F. muscicola was estimated according to Lowry's method, which was described as the best for sensing low protein concentrations (Peterson, 1979). As shown in Fig. 5. results of the protein content with KCO treatments, where the protein content increased in the CC that incubated under day 24, then the protein content decreased on the 30th day, as it recorded 90.3 µg/ml, this is because some nutrients are depleted. When F. muscicola was treated with KCO 0.25% and 0.5%, it was recorded that the amount of protein was affected at the beginning of the incubation period to record 27.5 and 15.5 µg/ml at day 16 respectively, to reach its concentration on the 30th day 96.5 and 78 µg/ml on respectively. El-Sheekh and Hamouda, (2014) indicated the ability of some cyanobacteria species to tolerate different concentrations of crude oil and degrade it, such as Nostoc punctiforme and Spirulina platensis under heterotrophic conditions. These results indicated that some petroleum hydrocarbon compounds may support the growth of cyanobacteria, as happened with the KCO 0.25% treatment. although this differed with the results of the Khamis et al. 2016 study on Oscillatoria chlorina, which indicated that the protein concentration was reduced by half when treated with crude oil 0.25%, in the same study, they referred that the protein content decreased by 80% under the influence (or effects) of the 1% of KCO treatment. While the results showed a decrease in the protein content of the two treatments 1% and 2% at day 16 of the incubation period, a relative increase was recorded 22.6 and 13.4 µg/ml at day 30. This indicates that the protein content of F. muscicola is affected by the toxicity of high concentrations of KCO, especially in the early days of the incubation period. In a study conducted by Soto, (1977) on Chlamydomonas angulosa, a decrease in total protein was observed after its growth under the influence of oil derivatives in the aqueous medium. This may be due to inhibition of DNA or inhibition

standard conditions to record the highest value 94.2 µg/ml at

of RNA replication, followed by a decrease in the formation of starch. This results is also agreements with what happens in Scenedesmus armatus (Zachleder and Šetlik, 1982).



Fig. 4 Effects of different concentrations of KCO on *F. muscicola* Phycocyanin for 30 days



Fig. 5 Effects of different concentrations of KCO on *F. muscicola* Protein for 30 days

Conclusions

The study demonstrated that *Fischerella muscicola* exhibited varying levels of tolerance and adaptation to Kirkuk crude oil (KCO) concentrations. Lower KCO levels 0.25% and 0.5% initially slowed growth and pigment production, but adaptation occurred by the second week, leading to enhanced growth and pigment content. KCO 0.25% even supported higher growth and pigment levels compared to the control

culture. At 1% and 2% KCO, longer adaptation periods were required, with delayed growth and pigment increase. Protein content was adversely affected by higher KCO concentrations initially. Overall, *F. muscicola* displayed efficient resilience against KCO pollution, possibly due to nearby petroleum-related activities.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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