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Potential of the green alga *Chlorella vulgaris* for biodegradation of crude oil hydrocarbons

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ABSTRACT

Oil production and/or transportation can cause severe environmental pollution and disrupt the populations of living organisms. In the present study, biodegradation of petroleum hydrocarbons is investigated using *Chlorella vulgaris* as a green algal species. The microalga was treated by 10 and 20 g/l crude oil/water concentrations at two experimental durations (7 and 14 days). Based on the results obtained, *C. vulgaris* owned not only considerable resistance against the pollutants but also high ability in remediation of crude oil hydrocarbons (~94% of the light and ~88% of heavy compounds in 14 days). Intriguingly, dry weight of *C. vulgaris* increased by the rising crude oil concentration indicating the positive effect of crude oil on the growth of the algal species. This biodegradation process is remarkably a continuous progression over a period of time.

1. Introduction

Crude oil, also called black gold, is the most important natural resource of the industrialized countries. It is a fact that oil-related companies cause potentially major hazards for the environment (Jung et al., 2017; Xaaldi Kalhor et al., 2016). Oil spills are one of the main factors with long-term adverse effects on marine life (Demirel et al., 2017). Oil spills may be caused by releasing the crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products and their by-products, heavier fuels used by large ships such as bunker fuel, or the spill of any oily refuse or waste oil (Chen et al., 2015; Farrow et al., 2016). The oil spill in Genoa (1977, Italy) is an example of oil disaster, where approximately 7350 oil barrels entered the sea. The Exxon Valdez oil tanker accident on the Alaskan coast in march 1989 spilled 240,500 oil barrels of crude oil (Curl et al., 1992). Recently, oil spill in the Gulf of Mexico entered approximately 4.9 million barrels of crude oil in April 2010 (Hook and Osborn, 2012; Sammarco et al., 2013). Growing environmental concerns, especially after several dangerous oil spills in the recent decades, renewed the attention for development of cleanup techniques (Xu and Lu, 2010).

Chemically, crude oil hydrocarbons can be divided into the aliphatic and aromatic fractions (Epps, 2006; Harayama et al., 1999). Aliphatics include alkanes, alkenes, alkynes and cycloalkanes, whereas aromatics as the main part encompass monoaromatics and polycyclic aromatic hydrocarbons (PAHs) (Epps, 2006). Aliphatic and aromatic hydrocarbons generally have very different properties and toxicities. PAHs are known to be the most toxic constituents of crude oil and are also associated with potential carcinogenic, teratogenic and mutagenic effects in aquatic animals and humans (Harayama et al., 1999; Kong et al., 2010; Moreira et al., 2011; Paíga et al., 2012).

There are a number of remedial techniques for crude oil contamination, such as combustion, skimming, booms, biosparging and bioremediation (Prince, 2014). Among these methods, bioremediation is an outstanding eco-friendly pollution cleanup technique by living organisms (Milić et al., 2009; Yang et al., 2009). This technique can be divided into *in Situ* (on polluted site) and *ex Situ* (on another location than contaminated area) categories (Xu and Lu, 2010; Yu et al., 2011). Phycoremediation, a type of bioremediation, is referred to the utilization of micro- or macroalgae for the cleanup of pollutants from wastewater, contaminated soil and CO_2 from polluted air (Gomes and Asaeda, 2009; Lavoie and de la Noüe, 1985; Peng et al., 2009; Rawat et al., 2011). Algae culture provides a fascinating biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes (Choudhary et al., 2016; Prajapati et al., 2013a; Rawat et al., 2011). Actually, biomass obtained from these contaminated environments could be conserved to biofuels such as

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biodiesel, biogas and bioethanol (Chapelle, 1999; Park et al., 2011; Prajapati et al., 2014; Prajapati et al., 2013b). Therefore, wastewater treatment in conjunction with the production of biofuel offers a promising approach for environmental cleanup.

The use of different algae for the treatment of wastewater has been the subject of research and development for several decades (Cerniglia and Gibson, 1980; Olguı, 2003; Walker et al., 1975). A number of algal species of different genera such as *Botryococcus*, *Chlamydomonas*, *Chlorella* and *Phormidium* were proven for different phycoremediation purposes. Nevertheless, selecting the most suitable algal species for the removal of certain pollutants requires further research in key areas. The use of microalgae in bioremediation of crude oil is still an exciting research area under discussion. Some algal species can oxidize and degrade many types of hydrocarbons into less harmful components, hinting at their potential to remediate crude oil (El-Sheekh et al., 2013; He et al., 2013). However, more studies investigating the role of different algal systems are needed to provide a better understanding of the impact of algae on crude oil hydrocarbons degradation. Thus, in the present work, we examined the potential of *Chlorella vulgaris* (besides *Skeletonema costatum* as control) for crude oil cleanup. *C. vulgaris* as a green microalgal species was considered as an appropriate biosystem for treatment of crude oil contaminated aquatic environment (El-Sheekh et al., 2013; Xaaldi Kalhor et al., 2016). Here, under experimental conditions, the species were treated with different crude oil concentrations and their cleanup efficiency was calculated. The findings of this work shed more light on phycoremediation of crude oil using algal species and provide a platform for further studies.

2. Materials and methods

2.1. Water and crude oil source

The fresh water for mesocosms was transported from the Persian Gulf by the assist of the Persian Gulf University of Bushehr. The crude oil of Pazanan oil field in Iran was used for the cleanup experiments.

2.2. Culture condition

Chlorella vulgaris and *Skeletonema costatum* were provided by Culture Collection of Algae, in Bushehr Shrimp Research Institute (Iran). The algal species were grown in the glass aquaria under fluorescent light (2 × 1000 lx) with a 16/8-h (light/dark) photoperiod at a temperature of 25 °C. Konvey medium was used with the following components: KNO_3 (100 g/l), Na_2EDTA (45 g/l), H_3BO_3 (33.6 g/l), $NaH_2PO_4.4H_2O$ (20 g/l), $FeCl_3.6H_2O$ (1.3 g/l), $MnCl_2$ (0.36 g/l), $ZnCl_2$ (21 g/l), $COCl_2.6H_2O$ (20 g/l), $(NH_4)_6Mo_7O_2.4H_2O$ (9 g/l), $CuSO_4.5H_2O$ (20 g/l), Na_2SiO_3 (20 g/l), KNO_3 (100 g/l), vitamin B1 and vitamin B12 (2 and 0.1 g/l, respectively). The treatments were performed in Erlenmeyer flasks. Cell counts on fluids were performed every 24 h (with 3 replications) on a hemocytometer.

2.3. Experimental design and treatments

Aquaria with dimensions of 20 × 20 × 20 cm were used as experimental units. Experimental design was carried out by factorial based on randomized complete block design with 3 replications. Microalgae were exposed to different concentrations of crude oil (10 and 20 g/l crude oil in water) at two experimental durations (7 and 14 days).

2.4. Initial tests

Number of initial cells and initial volume of the algae as well as their resistance against salinity are important factors for a continuous study. Therefore, different initial cells and salinities were selected for the species. Because the salinity of the water of Persian Gulf is about 40 ppt, the different salinity levels used were 20 ppt, 25 ppt, 30 ppt, 35 ppt and 40 ppt. Selected initial values of the cells for *Chlorella vulgaris* were 5×10^3, 6×10^3, 7×10^6, 8×10^6, 9×10^6, 10×10^6 and 15×10^6 (cells per ml) and *Skeletonema costatum* were 5×10^4, 6×10^4, 7×10^4, 8×10^4, 9×10^4 (cells per ml). After preliminary experiments (with 2 to 4-day duration) using different initial volumes of the microalgae, the best volumes for the main cleanup experiments were carefully chosen. Accordingly, the initial volumes of microalgae to enter the polluted environment were 20% of total volume from the 4-day aged suspension culture of *Chlorella vulgaris* (containing 8×10^6 cells per ml) and *Skeletonema costatum* (encompassing 7×10^4 cells per ml).

2.5. Phycoremediation estimation

After 7 and 14 days, the microalgae were removed from the polluted environment (crude oil in Persian Gulf water) by centrifugation at 5000 rpm for 10 min. Then, light compounds (with boiling point < 350 °C) of the contaminated samples were separated by distillation. Consequently, the samples were analyzed by a gas chromatograph with mass detector (Agilent) with HP-5MS elastic silica capillary columns (30 m × 0.25 mm × 0.25 μm). The injection volume was 1 μl. Initial temperature was 50 °C and was heated to a final temperature of 270 °C at a constant rate of 7 °C/min. The quantification of the compounds was performed using GC–MS spectra. The cleanup percentage of heavy compounds was calculated as follows:

$$A = [(C_0 - C_1)/C_0] \times 100 \quad (1)$$

where A is the percentage of cleanup of heavy compounds by microalgae, C_0 and C_1 represent the total mass of the oil in the solution and the mass of the light compounds, respectively.

2.6. Statistical analysis

The data were subjected to analysis of variance (ANOVA) and means were compared by Duncan multiple range test using MSTAT-C and SPSS 20.0. The differences between data were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Phycoremediation of the light compounds (with boiling point < 350 °C)

The cleanup process of the light compounds by *C. vulgaris* was significantly influenced by the different initial oil concentrations ($p < 0.05$). Actually, the oil cleanup percentage at 10 g/l initial oil concentration was higher than that at 20 g/l (Fig. 1). Previously, a negative effect of the initial oil concentration on crude oil bioremoval

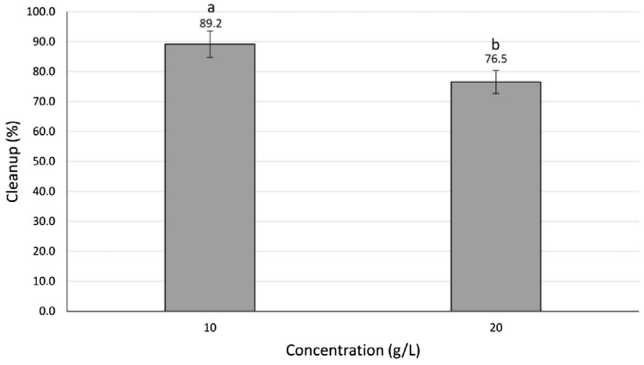

Fig. 1. Effect of initial oil concentration on cleanup of light compounds by *Chlorella vulgaris* ('a' and 'b' show the Duncan's ranking).