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Short communication

Effects of illuminance and nutrients on bacterial photo-physiology of hydrocarbon degradation

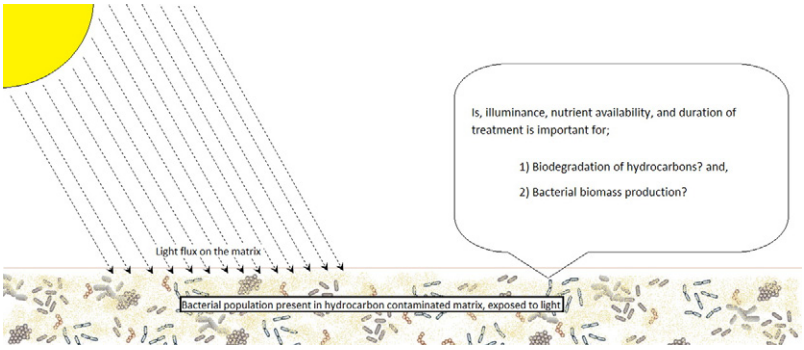
Aqib Hassan Ali Khan [a], Mariam Anees [b], Muhammad Arshad [c], Yousaf Shad Muhammad [d], Mazhar Iqbal [a], Sohail Yousaf [a,*]

[a] Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan
[b] Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan
[c] Institute of Environmental Sciences and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology, Sector H-12, Islamabad 44000, Pakistan
[d] Department of Statistics, Faculty of Natural Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan

HIGHLIGHTS

- First report on bacterial photophysiology of hydrocarbons degradation.
- Nutrients, illuminance and time effect hydrocarbon degradation and biomass production.
- Light exposure and low nutrient levels decreased the rate of biodegradation.
- Biomass was affected negatively in absence of nutrients and presence of light.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacterial photophysiology was previously limited to photoautotrophs. The discovery of bacteriophytochromes in non-photoautotrophs raised a question whether these non-photoautotrophs are affected by the presence or absence of light? In this research work for the first time, bacterial hydrocarbon degradation and biomass production was studied under the influence of nutrients, illuminance (light flux) and time. An experimental model was designed, with six isolated bacterial strains (*Pseudomonas poae* BA1, *Pseudomonas rhizosphaerae* BP3, *Bacillus thuringiensis* BG3, *Acinetobacter bouvetii* BP18, *Pseudomonas proteolytica* BG31 and *Stenotrophomonas rhizophila* BG32) under four different conditions of nutrient media and illuminance at three time intervals of 15, 30, and 45 days without shaking. All strains showed statistically higher hydrocarbon degradation under nutrient rich, dark conditions. Highest biodegradation (80.8, 79.4, and 78.7 mg) was observed in BG31, BG17 and BG3 respectively. Nutrient rich media along with dark conditions improved the biomass production, and when media was nutrient deprived, higher biomass was produced in the presence of light. This work proved that light and nutrients significantly affect bacterial populations and hydrocarbon degradation. The optimal use of these parameters could facilitate to achieve the goal of remediation of hydrocarbon contaminated sites.

* Corresponding author.
E-mail address: rasy_1023@yahoo.com (S. Yousaf).

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1. Introduction

Hydrocarbons discharge, due to accidental or anthropogenic activities, into the environment poses global peril, as they not only ominously threaten human wellbeing, but also have noxious effects on the whole ecosystem (Meng et al., 2007). Trends of developing innovative technologies for the removal of these contaminants have been explored in recent few years. Among these technologies, bioremediation is considered to be the most eco-friendly, economically sustainable, and effective (Madigan et al., 2000; Guo et al., 2014; Lima et al., 2009). The competence of any bioremediation strategy hinges on biotic and abiotic factors. Biotic factors include presence of suitable microbial population and supporting vegetation, and abiotic conditions include type, concentration and bioavailability of contaminants, availability of nutrients (Chaineau et al., 2005; Margesin et al., 2013), pH (Singh et al., 2003), light and temperature (Boopathy, 2000).

Light plays an integral role in the photophysiology of not only plants, but also ferns, algae, fungi and bacteria (Briggs, 2014). Interestingly, light affects photosynthetic as well as non-photosynthetic bacteria (Gomelsky and Hoff, 2011). Discovery of bacteriophytochromes, in nonphototropic bacteria, like *Pseudomonas aeruginosa*, challenged the central dogma that light-response is only found in phototrophs. The presence of LOV (Light, Oxygen, and Voltage), BLUF (Blue Light Sensing using FAD [Flavin Adenine Dinucleotide]), and other photo receptor domains in different proteins of bacteria (Briggs, 2014; Gomelsky and Hoff, 2011) further concreted the concept that light plays an indispensable role in microbial photophysiology, that was previously thought to be restricted to photoautotrophs only. Nutrients are crucial for bacterial degradation. Addition of different nutrients enhanced hydrocarbon degradation potential of degrading bacterial community, as it helps in selective growth of hydrocarbon degrading bacteria (Boopathy, 2000; Röling et al., 2002).

The studies have reported that addition of nutrients improve bacterial hydrocarbon degradation (Boopathy, 2000; Röling et al., 2002; Xu and Obbard, 2004); but no such work exists in the domain of microbial photophysiology, more specifically in hydrocarbon biodegradation. The objective of present study was to investigate the effect of illuminance (light flux), nutrients and time duration on hydrocarbon degradation and biomass production by variant bacterial species. Diesel oil due to multifaceted composition, containing cycloparaffins, paraffins, aromatic and olefinic hydrocarbons with carbon numbers predominantly in the range of C9 to C25, was used for the degradation study. To the best of authors' knowledge this is the first report of bacterial photophysiology and its effect on hydrocarbon degradation.

2. Materials and methods

2.1. Bacterial isolation

Soil samples were collected from historically petroleum-contaminated site of Hattar industrial estate, Haripur, Pakistan. Bacterial strains from 2 mm sieved soil samples were isolated on nutrient agar (NA) plates amended with 1% filter sterilized diesel oil and fluconazole (100 μg ml^{-1}). Morphologically different isolates were pure cultured, inoculated in Bushnell Haas agar (BHA) and incubated for 15 days at 30 °C. Composition of BHA was (g l^{-1}) NH_4NO_3, 1; $FeCl_3$, 0.05; KH_2PO_4, 1; K_2HPO_4, 1; $MgSO_4$, 0.2; $CaCl_2$, 0.02; Agar, 12; pH 7 (Ugochukwu et al., 2013), amended with 5 ml l^{-1} filter sterilized diesel oil as a carbon source.

2.2. Substrate utilization rate experiment

Bacterial strains growing on BHA were subjected to a 15 days preliminary screening experiment. The strains were inoculated in Bushnell Haas broth (BHB) in static conditions at 30 °C. Composition of BHB was (g l^{-1}) NH_4NO_3, 1; $FeCl_3$, 0.05; KH_2PO_4, 1; K_2HPO_4, 1; $MgSO_4$, 0.2; $CaCl_2$, 0.02; pH 7. Diesel at 10 ml l^{-1} was added as the carbon source in BHB. The experiment was conducted in triplicates. After incubation period the optical density of strains was monitored, the strains showing maximum growth were selected for biodegradation experiments.

2.3. Identification of bacterial strains

Six bacterial strains (BA1, BP18, BG3, BG17, BG31, and BG32) were selected for biodegradation experiment after preliminary screening. The bacterial strains were preserved in glycerol and stored at −20 °C. The isolates were sequenced from Genome Analysis Department Macrogen Inc. Korea. Sequences obtained were analyzed using BLAST search from National Center for Biotechnology Information (NCBI) databases revealing up to 99 or 100% similarity to different bacterial species. Multiple sequence alignments were carried out using CLUSTALW after complete deletion of the mismatch sequences. 16S rRNA sequences of organisms related to hydrocarbon degradation were submitted to GenBank and were assigned accession numbers KT758715–KT758720.

2.4. Biodegradation experiment

Six bacterial strains (*Pseudomonas poae* BA1, *Pseudomonas rhizosphaerae* BP3, *Bacillus thuringiensis* BG3, *Acinetobacter bouvetii* BP18, *Pseudomonas proteolytica* BG31 and *Stenotrophomonas rhizophila* BG32) were grown in nutrient broth (NB) supplied with 1% diesel at 30 °C for 24 h. Bacterial cells were washed thrice with isotonic 0.9% NaCl, centrifuged at 8000 g for 15 min. After final washing, bacterial cells were resuspended in 0.9% NaCl. Each strain was incubated in four different conditions 1) nutrient rich (NB) in illuminance, 2) nutrient rich and devoid of illuminance (dark condition), 3) nutrient deprived (BHB) with illuminance, and 4) nutrient deprived and devoid of illuminance. A negative control (NC), containing all ingredients without inoculum, was made for each of the conditions, to check the effect of abiotic factors alone on degradation. For each treatment 10 ml broth was dispensed in test tubes and 100 mg diesel (equivalent to 135 μl) was added. Each test tube was inoculated with 100 μl cell suspension containing 10^8 cells per ml (Wiegand et al., 2008), excluding negative controls. In each of the four conditions, 7 treatments (6 strains + 1 negative control) were used in triplicate for intervals 15, 30, and 45 days. For the purpose of biodegradation experiment under light and dark conditions, we constructed a chamber, in which light flux was maintained at 1000 ± 38 on the surface of test tubes and monitored with the help of Extech light meter 401025, as was done by Czerwik-Marcinkowska et al. (2015) and Olguín et al. (2015). The chamber contained two light sources (100 watt incandescent bulb each). Test tubes for dark environment were packed and sealed, with two layers of paper and one layer of aluminum foil, to prevent light exposure; while test tubes to be exposed to light were left uncovered. All the 84 tubes were placed in the same light chamber maintained at 30 °C.

2.5. Residual hydrocarbon extraction and biomass production

Dichloromethane (DCM) at 1:1 ratio was used for the washing of incubated media to separate the residual diesel oil and bacterial biomass. Bacterial biomass was calculated by filtering the solution from separatory funnel through pre-weighed Whatman membrane filters nylon, having a pore size 0.45 μm. After filtration, wet filter papers were dried at 80 °C, until the attainment of constant weight. DCM washed extracts were taken in pre-weighed screw bottles and placed under fume hood till constant weight attainment and degraded diesel oil was calculated after the complete evaporation of DCM (Elshafie et al., 2007).