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Inoculation with bacteria in floating treatment wetlands positively modulates the phytoremediation of oil field wastewater

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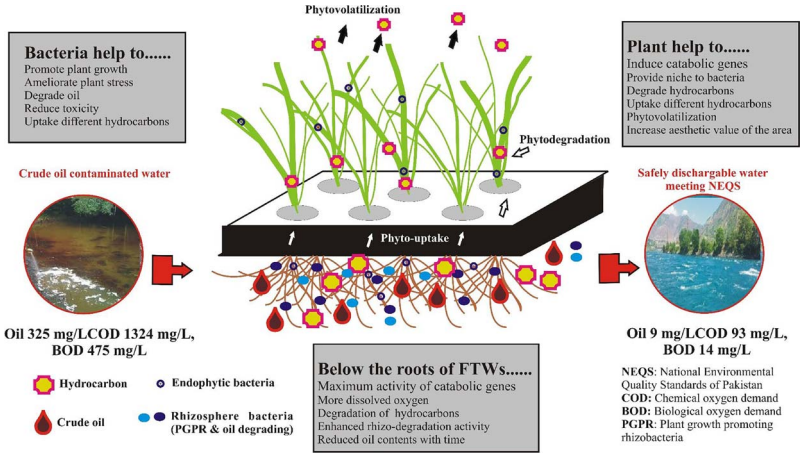
GRAPHICAL ABSTRACT



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ABSTRACT

The aim of the present study was to investigate the potential of plant-bacterial synergism in floating treatment wetlands (FTWs) for efficient remediation of an oil field wastewater. Two plants, *Brachiara mutica* and *Phragmites australis*, were vegetated on floatable mats to develop FTWs, and inoculated with bacterial cons which were then inoculated with a consortium of hydrocarbon-degrading bacteria (*Bacillus subtilis* strain LORI66, *Klebsiella sp.* strain LCRI87, *Acinetobacter Junii* strain TYRH47, *Acinetobacter sp.* strain LCRH81). Both plants successfully removed organic and inorganic pollutants from wastewater, but bioaugmentation of *P. australis* significantly enhanced the plant's efficiency to reduce oil content (97%), COD (93%), and BOD (97%), in wastewater. Analysis of alkane-degrading gene (*alkB*) abundance and its expression profile further validated a higher microbial growth and degradation activity in water around *P. australis* as well as its roots and shoots. This study provides insight into the available phytotechnology for remediation of crude oil-contaminated water and introduces a wetland macrophyte, *P. australis,* with tailor-made bacterial consortium as an effective tool for improved phytoremediation efficiency of FTWs.

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1. Introduction

Crude oil and its constituents are a major source of energy for the rapid urban and industrial growth taking place in the world. During oil exploration and extraction, a large volume of wastewater is produced that has a high content of crude oil, salts, heavy metals, and radionuclides [1–3]. The strategy currently in practice to deal with crude oil-polluted water is to discharge it into running streams and, ultimately, into the seas [3–5]. Furthermore, 0.1–0.25% of petroleum products enter the environment from various other sources [6], which has resulted in a wide-scale degradation of global land and water resources. Crude oil and its associated pollutants cause adverse effects on not only plants and beneficial microbiota [7,8] but also exert deleterious health effects on humans and animals [9,10]. These problems are especially prominent in the areas surrounding oil exploration and production sites [7].

The effective removal of crude oil and other contaminants from oil-contaminated wastewater is one of the major environmental challenges facing mankind today. In comparison to expensive and labor-intensive physicochemical methods, bioremediation is an environment friendly, efficient, and cost-effective approach for the remediation of wastewater. It exploits plants, bacteria, and their synergism for the remediation of contaminated soil, water, and air. Bacteria contain specific catabolic enzymes for degradation of hydrocarbons; this characteristic enables them to support plant growth in contaminated soil and water [11–14]. Plants, being active partners in such a synergistic interaction, provide metabolites, nutrients, and habitat to microbes in their rhizosphere and endosphere [15,16]. Consequently, higher microbial populations and activity can be witnessed in the (endo)rhizosphere, and thus, the abundance and expression of genes, mainly alkane hydroxylase *alkB,* is also higher in this niche, which results in enhanced degradation of contaminants, including hydrocarbons, in the vicinity of plant roots [7].

Floating treatment wetlands (FTWs) carrying different types of plants, provide a sustainable solution for the remediation of wastewater due to their low cost and energy requirements [17,18]. These wetlands can be natural or artificial, but in either case, they combine the properties of natural ponds and hydroponic floating vegetation [19,20]. Plant roots hanging down into the water column not only act as a natural filter for contaminant removal but also provide surface area for enhanced growth of microorganisms and biofilms formation [21,22]. While bacteria growing within or on roots are involved in the breakdown of organic matter, aerial parts of the plant provide habitat for wildlife and are aesthetically pleasant [23,24].

Although FTWs have been successfully applied for the removal of nutrients and organic matter from municipal and industrial effluents [25,26], they have not yet been evaluated to treat oil-contaminated wastewater. The aim of this study was to evaluate the potential of plant-bacterial synergism in FTWs for efficient remediation of crude oil-contaminated wastewater. Two plants, *Phragmites australis* and *Brachiara mutica,* were vegetated in FTWs, which were also augmented with hydrocarbon-degrading and/or plant growth-promoting bacteria. There was enhanced crude oil degradation, toxicity reduction, and expression of metabolic genes in bacterially-augmented FTWs, which shows the great potential of plant-bacterial synergism for remediation of crude oil-contaminated water.

2. Materials and methods

2.1. Analysis of wastewater samples from an oil field

Wastewater produced from an oil field was collected every 3 months for a year (2016). The wastewater was collected from an outlet pipe of an oil well located at FimKassar (32.55°N 72.51°E), Chakwal, Pakistan (Fig. 1). Physicochemical parameters, including pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), etc., of the samples were analyzed (Table 1) using standard methods [27]. Ion content for Na and K was measured using flame photometry. Heavy metal content was determined using atomic absorption spectrophotometry. Crude oil content in the samples was analyzed by a two spectrum hydrocarbon analyzer (Perkin Elmer, USA) as described earlier [28,29].

2.2. Bacterial strains

A set of bacterial strains previously isolated and characterized by our research group was used in this study. The strains included *Bacillus cereus* LCRH93, *Acinetobacter Junii* TYRH47, *Bacillus amyloquefaciens* BRRI53, *Klebsiella* sp. LCRI87, *Acinetobacter* sp. BRSI56, *Bacillus licheniformis* BRSI58, *Acinetobacter* sp. CYRH21, *Acinetobacter* sp. LCRH81, and *Bacillus subtilis* LORI66; all of these were isolated from a crude oil-contaminated site [7]. These strains possessed capabilities of hydrocarbon degradation and/or plant growth promotion as enlisted in Table 2. Moreover, these bacterial strains possessed alkane-degrading genes, *alkB* and/or CYP. The bacteria were cultivated as separate cultures in Luria Bertani (LB) broth for 24 h. Cells were harvested by centrifugation and re-suspended in normal saline solution. Five ml inoculum of each pure culture (10^7 CFU ml^{-1}) was then added in 500 ml of oil-containing wastewater separately. All the flasks were incubated at 37 °C in a shaking incubator.

2.3. FTW structure and experimental setup

Microcosmic floating wetland cells were constructed in 20-liter plastic tanks. Each floating mat (50 cm length, 36 cm width, and 7 cm thickness) was prepared from Diamond Jumbolon-Role with five equidistant holes (Fig. 2). One inch border was left at each side of the mat with the outer sides covered with aluminum foil to protect it from sunlight. Twenty stem cuttings of *Brachiara mutica* and four seedlings of *Phragmites australis* were planted in each hole of the floating mat with coconut shavings as supporting material. Soil, sand, and gravel were added up to 1 inch on the mat to support plant growth and protect the mat from sunlight. The plants used were homogeneous in their weight and height. The plants vegetated on floating mats were initially cultivated in tap water for a month to allow development of extensive root system. After a month, tap water was replaced with oil-contaminated wastewater samples; eight different treatments were designed as below:

Control 1 (un-vegetated): Wastewater without vegetation and bacterial inoculation

Control 2 (vegetated): Tap water with *P. australis* and *B. mutica*

T1 : Wastewater with*P. australis*

T2 : Wastewater with*P. australis*and bacterial consortium

T3 : Wastewater with*B. mutica*

T4 : Wastewater with*B. mutica*and bacterial consortium

T5 : Wastewater with bacterial consortium only (un-vegetated).

One hundred ml of inoculum (10^9 CFU ml^{-1} of each) containing consortium of the four bacterial strains was added in T2, T4, and T5. Three replicates were maintained per treatment.

The experiment was set up at ambient temperature and light (May-June, 2016) at NIBGE, Faisalabad (31°25′0″N73°5′28″E). Water samples were collected after 0, 14, 28, and 42 days of bacterial inoculation followed by analysis for physicochemical parameters, including pH, EC, residual oil content, COD, BOD, Na, K, and heavy metals as described earlier [27,28].

2.4. Determination of bacterial population

To investigate the survival of inoculated bacterial strains, culture-dependent plate count method was used. Briefly, wastewater samples were directly plated onto LB agar plates containing 1% diesel. Root and shoot samples were surface sterilized followed by their grinding; suspensions of ground material were plated onto LB agar plates. The plates