Journal of Environmental Chemical Engineering 5 (2017) 1885–1891

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Biodegradation of petroleum hydrocarbon functional groups in contaminated water using selected protozoan isolates

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ARTICLE INFO

Article history:
Received 8 December 2016
Received in revised form 11 March 2017
Accepted 19 March 2017
Available online 27 March 2017

Keywords:
Protozoan biodegradation
Petroleum
Hydrocarbons
Functional groups
Biodegradation
Thin-layer chromatography
Two-dimensional gas chromatography-flame ionisation detection (GC × GC-FID)

ABSTRACT

Diverse microorganisms are responsible for the biodegradation of various petroleum hydrocarbon chains which are found in both the environment and several existing water sources. Most of these microorganisms are both prokaryotic and eukaryotic in nature. The aim of this study was to investigate the degradation of petroleum hydrocarbon functional groups in contaminated water using individual protozoan species (*Aspidisca* sp., *Trachelophyllum* sp. and *Peranema* sp.) as well as a consortium of these three protozoan species. Chemical oxygen demand (COD) and dissolved oxygen (DO) were analysed using standard methods. The concentration of petroleum oil was determined in triplicate before and after the inoculation of oily wastewaters with the abovementioned protozoan species using the partition-gravimetric method. Various functional groups that had been biodegraded by the protozoan isolates were analysed using thin-layer chromatography (TLC) and GC × GC-FID. Results showed an increase in protozoan biomass from an initial cell count of 1.0×10^3 cells/mL to a final cell count of 2.4×10^3 cells/mL for the consortium of isolates, unlike a minimal biomass increases observed with individual isolates over the study period. The average uptake of DO was found to be low at 0.50 mg/L from an initial concentration of 6.46 mg/L to the final concentration of 6.00 mg/L; however, the concentration of COD released by the isolates was found to be in an average range of ≤2300 mg/L. The consortium of three isolates was able to biodegrade ≥80% of the hydrocarbon fractions, and aliphatic groups such as short-chain alkanes, alcohol/ester compounds and partially aromatic hydrocarbons were biodegraded, while the individual isolates were able to biodegrade an average of 72% of the amount of petroleum hydrocarbon fractions during the study.

1. Introduction

There is a direct relationship between the world's population increase and the demand for petroleum products, which are needed for energy purposes. However, anthropogenic activities such as oil spills and improper discharge of industrial wastes contribute to the pollution of the existing fresh water sources and the environment at large [1–3]. It has been pointed out that most of the groundwater systems in areas that have oil spill might have contained petroleum hydrocarbon-degrading microorganisms [4]. These spills have attracted the attention of researchers in examining the problem of hydrocarbon contamination and bioremediation of water sources.

In recent years, various technologies have emerged in order to manage oil residues and effluents contaminated with various hydrocarbons of petroleum origin. The application of highly efficient and low-cost bioremediation processes represents an extremely important way for the recovery of contaminated areas, among several other clean-up techniques [5]. Bidoia et al. [5] further stated that the success of bioremediation efforts in the clean-up operation of the Exxon Valdez oil spill created tremendous interest in biodegradation potential and bioremediation technology in general.

Bioremediation is one of the most extensively used processes as it is environmentally friendly, cost-effective and highly efficient [6,7]. According to Heitzer and Sayler [8], the biodegradation technology relies on the collective ability of microorganisms to eliminate or reduce the contaminants under prevailing environmental conditions to a non-toxic state. Microbial degradation of organic contaminants such as petroleum-derived hydrocarbons is

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http://dx.doi.org/10.1016/j.jece.2017.03.022
2213-3437/

usually monitored by analysing changes in chemical parameters including the reduction in contaminant oxidants, an increase in dissolved inorganic carbon and reduced species. The use of microorganisms in the biodegradation of hydrocarbons is the main process acting in the depuration of hydrocarbon-polluted environments and various water sources. This process has been extensively studied since 1990 [9–12,7,13]. In many reports, bacteria, filamentous fungi and yeasts have been identified as more efficient crude oil degraders than their protozoan counterparts.

Protozoan isolates have been found to biodegrade crude petroleum oil in polluted water in previous studies done by Kachieng'a and Momba [14,15]. Additionally, a consortium of symbiotic protozoan isolates or supporting materials can be used to enhance the biodegradation process [15]. It is very likely that various hydrocarbons have been ubiquitous throughout Earth's history; this may explain why many types of microorganisms have evolved metabolic capacities to utilise these compounds as electron donors for aerobic or anaerobic respiration, and as carbon sources for cell synthesis [16]. Rahman et al. [17] further stated that protozoa have an important role in wastewater purification systems, such as activated sludge plants, as they prey on and remove bacteria from the bulk solution. According to Madoni et al. [18] they are very useful biological indicators of the condition of an activated sludge and also excellent indicators of aerobic and toxic environments due to their greater sensitivity compared to that of the bacterial/fungal/algal nexus.

Generally, only scant information is available regarding the role of protozoan species, especially *Aspidisca* sp., *Trachelophyllum* sp. and *Peranema* sp. in the bioremediation/biodegradation of oil-polluted wastewater; however, the recent study done by Kachieng'a and Momba [15] unearthed their role in the biodegradation of fats and oils. Moreover, their role in the removal of phosphates, nitrates and heavy metals from polluted wastewater systems is well-defined and has been reported in previous studies [19,20]. Therefore, the aim of this study was to use individual protozoan isolates as well as a consortium of these isolates to perform the biodegradation of petroleum hydrocarbon functional groups in contaminated water.

2. Materials and methods

2.1. Protozoan species (Aspidisca sp., Trachelophyllum sp. and Peranema sp) preparation for biodegradation experiment

The targeted three protozoan isolates used in the study were attained from petroleum wastewater sources around Gezina, one of the suburbs of Pretoria, Gauteng, South Africa. These protozoa isolates have been effectively removed heavy metals in wastewater and in addition to nitrate and phosphorus removal in a prior study by Akpor et al. [19] and preceding one by Kamika and Momba [20]. These three isolates had also been used in the biodegradation of fats and oils in a previous study carried out by Kachieng'a and Momba [15]. The inverted microscope (Axiovert S100, Carl Zeiss (Pty) Limited, SA) of 100× and 400× magnification level was used to visualise the individual targeted protozoa species used in the study after they were transferred to microtitre plates from the original culture stock where they were counted. They were aseptically transferred into a sterile Erlenmeyer flasks (250 mL) containing 100 mL fresh sterile proteose peptone glucose (PPG) medium (Sigma Aldrich, Germany) using a handmade capillary glass and a streptomycin of ±30 μg/mL was further added to prevent contamination by bacterial microbes into the media [15]. The individual protozoan isolates were incubated in a dark room (light sensitive species) for a period of 15 days at a normal room temperature and the same procedure was applied for a consortium of three environmental protozoan isolates to enable their growth at satisfactory level for biodegradation study as reported by Kachieng'a and Momba [15].

2.2. Determination of the biomass/growth of the protozoan isolates

The protozoan growth was monitored using an inverted microscope after an incubation period of three days and continued throughout the study period and their biomass was determined according to the method described by Akpor et al. [19] using a spectrophotometer (Spekol® 1300, Analytik, Jena, Germany) at 600 nm. The approximate growth of each isolate (in cells/mL) was calculated with reference to the standard curve previously prepared by Kachieng'a and Momba [15].

2.3. Preparation of the petroleum-contaminated water culture media and sample collection

The study was carried out between September 2015 and March 2016; the petroleum-contaminated water samples were collected during these periods from petroleum-polluted water aquifers in the Gezina suburb of Pretoria, South Africa. The samples of 2.5 L with a known initial petroleum oil concentration of 250 mg/L were subjected to preparation as described by Kachieng'a and Momba [14,15]. The profile of the filtered samples was determined in terms of the psychochemical parameters such chemical oxygen demand (COD), dissolved oxygen (DO) and pH. The COD concentration was determined using a closed reflux method as described in Standard Methods [21]. These parameters were analysed as reported by Kachieng'a and Momba [15]. The carbon source was added as the nutrient supplement to initiate the growth of the microorganism culture during the lag phase was a mixture of sterile anhydrous D-glucose [19,22] and was extremely negligible to COD outcomes in the study. Thereafter, the culture media were sterilised using UV light instead of autoclaving to avoid the loss of volatile fatty acids or other volatile hydrocarbon components. The sterility of the media was determined according to Kachienga and Momba [15]. Each experimental study was run in triplicate and the petroleum hydrocarbon concentrations were determined before and after inoculation with the organisms and this process was also applied to the media before and after UV light sterilisation for a period of 20 days.

2.4. Bioremediation experiment

The experimental protozoan biodegradation study was carried out using five aquarium tanks with a volume of 1.5 L and each containing 50 mL of petroleum-contaminated water at an initial concentration of 250 mg/L of petroleum oil. A cell density of approximately 1.0×10^3 cells/mL was used to inoculate these tanks at a constant of pH of 8 (stable pH for these protozoa isolates obtained in a previous study conducted by Kachieng'a and Momba [15]). In addition, one of the aquarium tanks containing the petroleum-contaminated water but without the specific microorganisms being added served as the control experiment. Each tank was fitted with a thermometer and a constant temperature of 30 °C was maintained using a heater. In previous studies carried out by Kachieng'a and Momba [15] this temperature was found to be favourable for the growth of these protozoan isolates. The increase of biomass of these microorganisms was analysed by taking aliquots after every 5 days until the end of the experiment as reported by Kachieng'a and Momba [15]. Pumps were connected to the tanks to provide a constant oxygen supply for the test