International Biodeterioration & Biodegradation 93 (2014) 18–24

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Crude oil biodegradation activity in potable water

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ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form
4 March 2014
Accepted 4 May 2014
Available online 28 May 2014

Keywords:
Biodegradation
Hydrocarbon utilization
Pollution
Bacterial contamination
Phylogenetic

ABSTRACT

A wide range of aliphatic and aromatic hydrocarbon concentrations were detected in potable water samples obtained from various locations in Kuwait city. Detected aliphatic compounds included hexadecane (2.19 μg l^{-1}), heptadecane (2.49 μg l^{-1}), nonadecane (2.24 μg l^{-1}), eicosane (1.79 μg l^{-1}), docasane (1.4 μg l^{-1}) and pentacosane (1.48 μg l^{-1}), while the aromatic hydrocarbons contaminants included benzene (1.62 μg l^{-1}), phenanthrene (3.19 ng l^{-1}) and several aromatic-degradation intermediates. Culturable microbial loads in the water samples were low, ranging from 3 CFU ml^{-1} to 41 CFU ml^{-1}, but included a high proportion of hydrocarbon degrading bacteria. Hydrocarbon-utilizing bacteria isolated included *Cupriavidus gilardii*, *Pseudomonas* sp., *Bacillus cereus* and *Paenibacillus ehimensis* that constituted >30% of the total number of isolates. Under current experimental conditions, the hydrocarbon-utilizing capacities of individual isolates from the water samples were wide representing self-cleaning activity of hydrocarbons present in water samples. Interestingly, the hydrocarbon-utilizing microbial contaminants also exhibited a wide-range of antibiotic resistance characteristics.

1. Introduction

Much of the concern for water quality is directed towards waterborne microbial diseases usually associated with sewage contamination of water sources (Foster et al., 1998; Du et al., 2014). However, potability of water also takes into account the presence of chemical contaminants (Benotti et al., 2009) such as hydrocarbons that pollute source waters especially in oil exporting countries (McKee et al., 1972). Also, the deteriorated water supply systems and inflow of soil into water distribution networks represent serious sources of water pollution especially polyaromatic hydrocarbons, PAHs (Pensado et al., 2004). The introduction of a diminutive amount of hydrocarbons into ground water or water reservoirs could contaminate a huge supply of potable water, and may persist for long time, representing potential source of health hazards (Hansen et al., 2002; Caylak, 2012). Human exposure to hydrocarbons and chlorinated hydrocarbons that usually form during water chlorination can lead to liver and kidney damage, hematological, gastrointestinal, neurological problems and cancer (Bull, 1985). Thus, contamination of drinking water sources with hydrocarbons is of high priority (Dawson et al., 1993). Unfortunately, inefficient water treatment can result in transferring hydrocarbons to individual users (Baus et al., 2005; Mehta, 2013). However, provision of safe drinking water is achievable through tight control of water supplies and desalination plants, efficient regular monitoring of possible contaminants such as oil, fuels, other chemicals and effective treatment of contamination.

Hydrocarbons in potable water represent sources of utilizable carbon for bacteria that have been documented to thrive for long periods (Byrd et al., 1991) on available nutrients in purified and in potable water (Kulakov et al., 2002). In general, incidence of bacteria in potable water is undesirable due to the potential of bacteria to lower the quality of water, and pose potential health risk for humans (Rusin et al., 1997). Usually, bacteria access potable water due to the inefficient water treatment plants and, in the unique case of Kuwait, can potentially arise sometimes as part of the brackish water supplements. However, the majority of bacterial infiltration into potable water has been documented to arise from old and cracked water distribution networks that usually harbor biofilms of adapted bacteria (Williams et al., 2004) acting as sources of bacterial contamination (Armstrong et al., 1981) surviving on available nutrients that select the bacterial communities in potable water.

The environments of petroleum-producing countries such as Kuwait are constantly and consistently under threat of petroleum contamination that has been widely reported in the marine, soil, and ground water environments of these countries ((Literathy, 1992; Metwally et al., 1997; Bu-Olayan, 1998). Consequently, the use of the potentially hydrocarbon-contaminated sea and ground water as primary sources of domestic water underlay the possibility of petroleum contamination in the supplies of the finished product.

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http://dx.doi.org/10.1016/j.ibiod.2014.05.002
0964-8305/

Therefore, the aim of the current investigation was to assess the occurrence of hydrocarbon contamination in potable water collected from residential areas in Kuwait, and to characterize the hydrocarbon degradation potentials of associated bacterial communities.

2. Material and methods

2.1. Collection of water samples

Three-liter volumes of drinking water samples (in triplicates) supplied to households through the municipal water system were collected from each of 12 residential locations (L1 to L12) of Kuwait city. The collections were carried out in winter (November to February) of 2011. The three representative replicate samples (3 L each) from each location were collected in sterile amber-coloured glass bottles previously cleaned with chromic acid, hot water and rinsed three times with distilled dichloromethane and tap water. Water samples were collected from taps whose nozzles had been cleaned with 70% ethanol and left running for 60 s, to ensure representative samples. Collected samples were kept in ice and transported immediately to the laboratory for immediate analyses and processing.

2.2. Characterization of water samples

2.2.1. Determination of turbidity of water samples

Turbidity (in nephelometric turbidity units) was determined using a model 965-10A turbidimeter following the method of Shelton and Karns (2001).

2.2.2. Determination of the total dissolved solids of water samples

Values of the total dissolved solids (TDS) in water samples were determined using the method of Karavoltsos et al. (2008) employing a radiometer CDM 230 conductometer (Villeurbanne Cedex, France). Briefly, the conductometer was calibrated using a standard solution at room temperature (20–30 °C). Prior to measurement, water samples were brought to room temperature, then, TDS (ppm) was determined following the manufacturer's instructions for triplicate water samples from each collection point.

2.2.3. Determination of the pH value of water samples

Water sample's pH was measured by transferring 100 ml of sample into 150 ml beaker. pH was measured using a pH meter (Orion 2-Star) after calibration with standard solution (pH 7 and pH 10) according to manufacturer's recommendations. After that, a rinsed electrode was immersed into the sample and pH value was recorded.

2.2.4. Determination of the alkalinity of water samples

Alkalinity was measured by titrating 100 ml water samples with 0.2 N HCl using bromocresol green indicator until an end-point is reached. Samples were titrated in triplicates and the mean value was calculated using standard methods (2320B, APHA, 1995).

2.2.5. Determination of the total organic carbon of water samples

Total organic carbon (TOC) measurements of water samples were carried out using Shimadzu TOC-5000(A) (Shimadzu, Reinach, Switzerland) analyser equipped with a high-sensitive catalyst (High sense TC catalyst; Shimadzu). The analyses were performed according to the thermal catalytic oxidation principle using standard methods (Balci et al., 2009; Bucheli-Witschel et al., 2012). Briefly, water samples were filtered (0.22 mm), acidified by the addition of hydrochloric acid (2 M) and injected (2 ml) into the TOC analyser. Oxygen was used as the carrying gas at a flow rate of 150 ml min^{-1}, and the oven temperature adjusted to 680 °C. The combustion reaction was carried out using platinum catalyst. The TOC analyser was calibrated with potassium hydrogen phthalate standards (0–2 mg C l^{-1}, EN 1484-H3, 1997). The detection limit of the method was 0.1 mg C l^{-1}.

2.2.6. Detection and quantification of hydrocarbons in water samples

Water samples were analysed for monoaromatic, polyaromatic (PAH) and aliphatic hydrocarbons.

2.2.6.1. Detection of monoaromatic hydrocarbons. Monoaromatic hydrocarbons were quantified using gas chromatography coupled to split/splitless injector 1177 and flame ionization detector (Varian GC 3800, Varian, Zug, Switzerland). Samples (10 ml) and internal standard (1 ml) were transferred into headspace vials (20 ml) and placed in the Combi-PAL auto-sampler (CTC, Zwingen, Switzerland). Chromatographic separations of samples were carried out in a GC column CP-Select 624, 30 m × 0.32 mm, df1.8 mm (Chrompack/Varian, Middleburg, Netherlands). Helium was used as the carrier gas (2 ml min^{-1}) and the following oven temperature program was applied: 5 min at 40 °C to 150 °C at 5 °C min^{-1}, to 250 °C at 40 °C min^{-1}, 5 min at 250 °C. The FID detector was adjusted to 300 °C with the make-up gases: nitrogen (25 ml min^{-1}), hydrogen (30 ml min^{-1}), and synthetic air (300 ml min^{-1}). Quantitation of monoaromatic hydrocarbons was performed by means of GC software (Varian SPSS) employing external calibration curves.

2.2.6.2. Detection of PAHs. The whole volume of water samples (3 L) was submitted to liquid–liquid extraction using dichloromethane. Quantities (300 ml) of original water samples were separately transferred to separation funnels (1 L) containing 10 ml dichloromethane (DCM). The inner surfaces of the separation funnels were previously washed with DCM and the bottles in which the water samples were stored. Each separation funnel containing the water/DCM mixture was vigorously shaken for 2 min. Then, the lower organic layer was allowed to separate from the upper aqueous phase, and collected in a 250 ml Erlenmeyer flask previously cleaned with dichloromethane. The same extraction procedure was repeated nine more times using fresh DCM volumes. Extracts were combined (~100 ml), dried with anhydrous sodium sulphate and concentrated to appropriately ml by rotary evaporation. Then, the residual DCM was exchanged to acetonitrile that was evaporated over to 0.5 ml followed by the addition of 0.5 ml of acetonitrile. Extracts were transferred to 2 ml volumetric flasks and the final extracts volumes were made to the mark with ultrapure water. Finally, the extracts were filtered through a 0.22 μm filters (Millipore Co., Bedford, MA, USA) and 20 μl volumes injected in the HPLC coupled with fluorescence and photodiode array detectors (Pensado et al., 2004). The fluorescence detector was used for excitation at 280 nm and emission greater than 389 nm cut-off (Schoeffel FS970 or equivalent).

2.2.6.3. Detection of aliphatic hydrocarbons. The aliphatic hydrocarbons in water samples were extracted as described in Section 2.2.6.2. Following extraction with dichloromethane, equal amount of acetone was added (1:1 acetone/dichloromethane). Then, extracts were analysed by capillary gas chromatography equipped with flame ionisation detector GC-FID as recommended by Adam et al. (2002) and Hojae et al. (2009). The analyses were carried out by means of a gas chromatograph (Agilent, 6890N, Agilent Technologies Co., Ltd., China) equipped with a flame ionization detector and a capillary column (HP-5; 30 m × 0.53 μm I.D. with a stationary-phase film thickness of 0.88 μm). The GC was interfaced