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# Degradation of hydrocarbons and alcohols at different temperatures and salinities by *Rhodococcus erythropolis* DCL14

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**Abstract**

*Rhodococcus erythropolis* DCL14 cells were able to metabolise C5–C16 hydrocarbons and C1–C12 alcohols as sole carbon and energy sources, both at 15 and 28 °C. Metabolic activity was also observed at 1.00%, 1.95% and 2.50% sodium chloride. Almost complete degradation of *n*-, *iso*- and cyclo-alkanes and aromatic compounds present in fuel oil was achieved after 9 months, 60% being consumed in the first three months. The results from the conditions tested here suggest that this type of bacterium could be involved in bioremediation processes in marine environments such as the Atlantic, Pacific and Indian Ocean.


*Keywords:* Bioremediation; Motor oil; Degradation; Fuel oil; Saline conditions

## 1. Introduction

Bioremediation of sites contaminated with spillage of motor oils and fuels is needed where at the same time temperature and salinity may exclude the activity of certain microbes. Temperature has a large impact on both the degradation rates and the toxicity of the hydrocarbons present in the contaminants [12]. Tolerance mechanisms to the hydrocarbons may involve altered compositions of the cytoplasm and outer membrane of microorganisms [20], as well as efflux pumps to remove the solvent from the cells [14]. For instance, *Rhodococcus* sp. strain Q15 modulates its membrane fluidity in response to low temperature and hydrocarbon toxicity [25]. The prerequisite access to the hydrocarbons is affected by membrane modifications in the lipid composition altering cell surface hydrophobicity [1,25], and by production of extracellular products such as polysaccharides or surfactants [22].

In this study, the strain *Rhodococcus erythropolis* DCL14, which was isolated from a ditch sediment sample in The Netherlands and has been shown to biodegrade terpenes in organic–aqueous systems [6,7] is used. The rough variant used does not produce extracellular polysaccharides or surfactants, and thus its interactions with the hydrocarbons and the alcohols would be most influenced by membrane properties. The cells are adapted to solvent tolerance, displaying high cell hydrophobicity as shown by migration towards the organic phase in *n*-dodecane–aqueous systems [10] and cell aggregation in stressful conditions [9,11]. *R. erythropolis* DCL14 cells also consumed some of the compounds used as organic phase in biotransformation systems [7,8].

Other hydrocarbon-degrading *R. erythropolis* strains with high hydrophobicity and solvent tolerance were isolated from deep sea [15] and coastal [17] sediments, showing salt tolerance. Consequently, to determine if strain DCL14 would be competent under such natural

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conditions, one aim of this study was to assess the ability of strain DCL14 to degrade hydrocarbons and alcohols at 15 and 28 °C and in saline conditions. The ability of this strain to degrade both C5–C16 hydrocarbons and C1–C12 alcohols was compared to determine which degradation pathways are present. Two- and four-stroke engine oils and a low sulphur fuel oil were used to assess the potential of this strain to degrade contaminant mixtures that resemble motor oil spills in the environment.

## 2. Materials and methods

### 2.1. Microorganism

*Rhodococcus erythropolis* DCL14 was delivered by the Division of Industrial Microbiology of the Wageningen Agricultural University, Wageningen, The Netherlands. The strain was isolated from a sediment sample from a ditch in Reeuwijk, The Netherlands.

### 2.2. Chemicals

The organic solvents used as carbon sources were ethanol (99.8%), butanol (>99.5%), propanol (>99.5%), *n*-dodecanol, cyclohexane (>99.5%) and toluene (>99.5%) from Merck; *n*-octane (>99%) from Merck–Schuchardt; methanol (>99.8%), pentanol (>99%), *n*-hexane (>99%) and *iso*-octane (>99.5%) from Riedel-de Haën (Seelze, Germany); *n*-undecane (99%), *n*-tetradecane (99%) and *n*-hexadecane (99%) from Sigma; cyclohexanol (99%) and *n*-dodecane (>99%) from Aldrich; pentane (99%) from Fluka; *n*-heptane (95%) from Lab-Scan and *n*-nonane (99%) from Acros. The four stroke motor oil tested was Shell Helix Standard 20W-50. The two stoke motor oil used was Mobil Super 2T. Fuel oil was from Petrogal, SA (Portugal).

### 2.3. Growth

Cells were grown in cylindrical 100-ml flasks closed with rubber bungs and containing 20 ml of medium (minimal salts medium, pH 7.0) [26], incubated at 15 or 28 °C and 150 rpm on a rotary shaker with an amplitude of 12.5 mm. The organic compounds used as sole carbon sources were present at an initial concentration of 0.125% or 0.25% (v/v). The extent of growth was monitored by measurement of the optical density (OD) at 600 nm and the dry weight, and, in addition, by microscopy and image analysis techniques. The initial OD at 600 nm in the assays carried out at 15 °C was 0.90, which corresponded to a dry weight of 0.30 g and an average of 311 cells per image, while in the experiments at 28 °C the initial OD was 1.24, the dry weight was 0.40 g and an average of 424 cells was observed per image. The percentages of motor oil tested were 0.13%, 0.25%, 0.50%, 1.00% and 2.00% (v/v). Assays were carried out at least in duplicate.

### 2.4. Carbon source consumption rate

The cells were grown in 10-ml test tubes closed with rubber bungs wrapped in aluminium foil, containing 2 ml of minimal salts medium and 0.125% (v/v) of carbon source. The initial OD was 0.12, the dry weight being 0.04 g. The internal diameter of the test tubes was 12.5 mm and the depression caused on the liquid centre by the round bottom of the tubes was 2 mm. The test tubes were incubated at 28 °C and 200 rpm. During growth, at least five samples were taken at different times in order to follow the carbon source consumption. Each sample corresponded to three test tubes: one for biomass measurement and two for determination of the carbon source present. The content of these latter two tubes was extracted with 0.5 ml of ethyl acetate and, after phase separation, the ethyl acetate layer was analysed by gas chromatography (GC). Blank assays, carried out without cells, were also analysed.

### 2.5. GC analysis

The samples were analysed by gas chromatography on a Hewlett–Packard 5890 gas chromatograph with a FID detector, connected to a HP3394 integrator. The capillary column was a SGE HT5, 25 m in length and with internal and external diameters of 0.22 and 0.33 mm, respectively. The oven temperature was 120 °C and that of the injector corresponded to 200 °C. The detector was set at 250 °C.

### 2.6. Fuel oil consumption

Cells were grown in 100-ml flasks, closed with rubber bungs, containing 20 ml of minimal salts medium, *R. erythropolis* cells and 20, 40, 80, 160 or 320 μl of fuel oil adsorbed on the surface of a plastic tip. Two batches of flasks were used: on one batch, the initial $OD_{600}$ was 0.78 (0.26 g dry weight); on the other, the initial $OD_{600}$ was 0.39 ( 0.13 g dry weight). Fuel oil degradation was followed by extraction of the whole volume of 2 flasks with 2 ml of ethyl acetate and by extraction of 1 ml of the aqueous phase of 2 other flasks with 200 μl of ethyl acetate. The ethyl acetate layer was analysed by GC with the following oven temperature program: 50 °C in the first 4 min, followed by an increase of 10 °C $min^{-1}$ up to 150 and 150 °C in the last 10 min. Several samples were taken in duplicate over a 10-month period, including blank assays without cells.

### 2.7. Cell hydrophobicity test

Cell hydrophobicity tests were carried out according to the ''microbial adhesion to hydrocarbon'' (MATH)