

Growth of Hydrocarbon Utilizing Microorganisms

N B BHOSLE

National Institute of Oceanography, Dona Paula, Goa 403 004

and

S MAVINKURVE

Centre of Post Graduate Instruction & Research, University of Bombay, Panaji, Goa 403 001

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Two isolates from marine mud having broad spectrum hydrocarbon utilizing profile were identified as *Arthrobacter simplex* and *Candida tropicalis*. Both the organisms grew exponentially on crude oil. The cell yield of the organisms was influenced by incubation period, pH, and different concentrations of crude oil, sodium chloride, phosphate and nitrogen.

Hydrocarbon utilizing microorganisms are ubiquitous in nature and can be isolated from different environments¹. Growth of these organisms and their ability for biodegradation of petroleum products are influenced by environmental parameters such as pH, salinity, temperature, oxygen and nutrients².

Of the 23 microorganisms isolated locally, 2 cultures show broad spectrum hydrocarbon utilizing profile³. The present paper deals with the identification of these two isolates and the effect of some physico-chemical parameters on their growth.

The hydrocarbon utilizing cultures used in the present study were isolated from sediments of Dona Paula Bay (long. 72° 47.5'E, lat. 15° 26.5'N) and maintained routinely³. These isolates were identified using standard methods⁴⁻⁶.

Growth pattern of both the isolates was studied in basal medium containing 1% Saudi Arabian crude³. Incubation was carried out at room temperature (28-30°C) on a rotary shaker. Increase in cell count was observed by surface plating on nutrient agar³.

Effect of substrate Saudi Arabian crude (0-10%), pH (5-10), NaCl (0-10% w/v), phosphate (0-0.15 w/v), nitrogen compounds and nitrogen (0-0.07% w/v) on the growth of these isolates was studied individually.

One of the isolates showed distinct morphological cycle when grown on nutrient agar plate at room temperature (28-30°C). The organisms initially appeared as short rods which turned to elongated rods and then to coccoid forms. They utilized various organic compounds and were able to grow at 37°C and did not require any growth promoting factor. These are some of the salient features of the genus *Arthrobacter*⁴⁻⁶. Morphological and physiological characteristics suggest that the organisms are *Arthrobacter simplex*⁵.

Based on its ability to utilize various organic compounds⁹, to form pseudomycelia and pellicle and to ferment and assimilate glucose, galactose, sucrose, maltose, its inability to assimilate nitrate and to form pigment the 2nd isolate was identified as *Candida tropicalis*^{7,8}.

While growing on crude oil *C. Tropicalis* showed short lag phase (Fig. 1). Thereafter *A. simplex* as well as *C. tropicalis* showed an exponential increase followed by a stationary phase after 6 d. As the cell count remained steady after 6 d for both the cultures, cell count was routinely monitored on 6 d of incubation while studying the effect of various parameters.

Growth pattern and the effect of Saudi Arabian crude oil concentration revealed that increase in cell yield was a function of substrate concentration up to 1 and 0.4% for *A. simplex* and *C. tropicalis* respectively (Fig. 2). At higher concentrations cell yield decreased, the effect being much more pronounced in *C. tropicalis* (Fig. 2). Such harmful effects may be ascribed to the increase in the solubility of some toxic petroleum compound at higher concentration^{10,11}. Lighter fractions of petroleum hydrocarbons are known to exert toxic effect on microorganisms by damaging their cell membrane¹². Further many of the intermediary metabolic products are known to accumulate in the medium and exhibit inhibiting effect. For example, lauric acid produced during the growth of the yeast on hexadecane, inhibits utilization of hexadecane^{2,13}. Similarly biodegradation of crude oil by *Brevibacterium* and *Flavobacterium* sp. was inhibited due to accumulation of fatty acids¹⁴.

While growing on crude oil both the organisms formed black tarry/waxy substances at the end of the exponential phase probably due to accumulation of

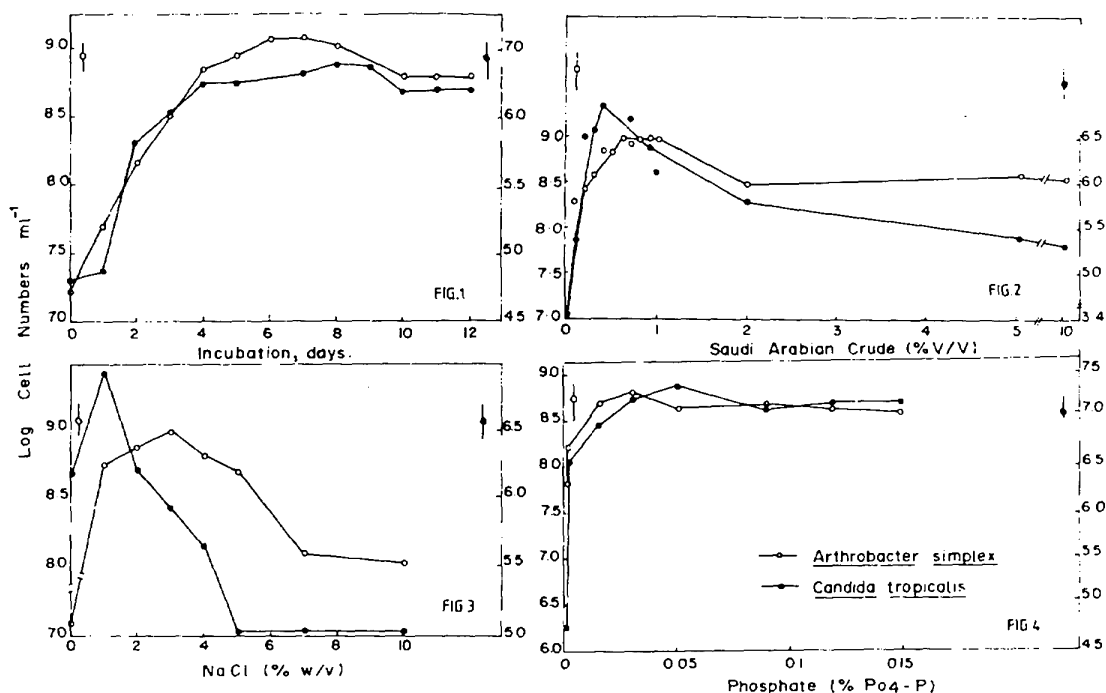


Fig. 1—Growth pattern of organisms in basal medium with Saudi Arabian Crude (1%).

Figs 2 to 4—Effect of Saudi Arabian crude (2), chloride (3) and phosphate (4) on the cell yield of organisms in basal medium.

degradation products formed during the microbial degradation of petroleum hydrocarbons^{2,15}.

In addition to the concentration and composition of crude oil, the growth of hydrocarbon utilizing microorganisms was influenced by variety of environmental factors such as oxygen, temperature, salinity, pH and nutrients².

A. simplex showed wide range of pH tolerance (5 to 10) as compared to *C. tropicalis*, nevertheless, pH optima observed for these organisms (6.5 and 5 respectively) was in agreement with the reported values for bacteria and yeast¹⁶. During the growth of these organisms decrease in pH was observed similar to that reported in the degradation of hexadecane by a mixed microbial culture and *Nocardia* sp^{7,18}.

Cell yield increased with NaCl concentration up to 3% for *A. simplex* and 1% for *C. tropicalis* (Fig. 3). Further increase in NaCl reduced the cell yield of both the isolates. These data are similar to those reported by others^{6,18,19}.

Phosphate at 0.03 and 0.05% concentration was optimum for *A. simplex* and *C. tropicalis* respectively (Fig. 4). Among different nitrogen compound tested, ammonium molybdate and ammonium sulphate were preferentially utilized as nitrogen source by *A. simplex* and *C. tropicalis* respectively (Table 1). When ammonium molybdate and sulphate was used at different

Table 1—Effect of Nitrogen Compounds (0.2% w/v) on the Growth of *A. simplex* and *C. tropicalis*

(Values are expressed as cells. ml⁻¹)

	<i>A. simplex</i> (× 10 ⁸)	<i>C. tropicalis</i> (× 10 ⁶)
Control	1.85	0.06
NH ₄ NO ₃	3.65	4.55
(NH ₄) ₂ SO ₄	4.75	4.80
NH ₄ Cl	5.85	4.25
(NH ₄) ₆ MO ₇ O ₂₄	9.40	0.86
KNO ₃	5.55	0.06

concentrations 0.007 and 0.003% of nitrogen supported maximum cell yield of *A. simplex* and *C. tropicalis* respectively. The growth stimulating effect of phosphate and nitrogen on *A. simplex* and *C. tropicalis* confirms the importance of these two nutrients on the growth of hydrocarbon utilizing microorganisms and biodegradation of petroleum compounds².

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