Colonies on oat agar fawn to greyish sepia with abundant aerial mycelium; reverse greyish sepia with black spots. Mycelium composed of septate, branched, smooth, straw yellow to brown hyphae 3–4 μm wide. Conidiomata pycnidial, abundant, solitary or in groups, partially immersed, brown to black, subglobose to globose, 120–300 μm diam., ostiolate. Pycnidial wall composed of 2–3 layers of pseudoparenchymatous cells, the outermost layer brown to dark brown, the inner layers hyaline. Conidiogenous cells globose to doliform, phialidic, arising from the innermost layers of cells lining the pycnidial cavity. Conidia yellowish brown to pale brown, straight to slightly curved, cylindrical, base truncate, apex rounded, 0–3 septate, 10–14 × 2–3 μm, unicellular conidia also present, 6–11 × 2.0–2.5 μm.

Isolated from soil, Mannanur forest, Mahabubnagar District, Andhra Pradesh, India, V. R. Thulasi Reddy and C. Manoharachary, 10-7-1978, OUFS 3 (IMI 232556) holotype.

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UTILIZATION OF HYDROCARBON IN CRUDE OIL BY FUNGI

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PETROLEUM hydrocarbon as substrates for growth of fungi has been a topic of special interest in recent years and reports of studies on the use of purified substrates such as alkanes of specified chain length are available[1–4]. A few studies have demonstrated the ability of fungi to grow and degrade on crude oil[5]. The present study aims at identifying the fungal species potentially useful in degrading the crude oil effluents of the Madras Refineries Limited.

Fungi were isolated from crude oil affluents of the Madras Refineries using serial dilution method[6]. The basal medium consisted of a salt solution of Bushnell and Haas supplemented with 0.1% yeast extract and the pH adjusted to 6. The medium was sterilized by autoclaving for 20 min at 15 Psi. Filter-sterilized crude oil (1%) was added to the medium aseptically. Spore suspensions (1×10^6 spores per ml) of different fungi were prepared separately and 1 ml was pipetted into 100 ml of the medium taken in 250 ml Erlenmeyer flasks. Duplicates were maintained for each fungus. Cultures were incubated at 30 ± 2°C. Oil samples from incubated cultures were recovered periodically by repeated extraction of the mycelium with 10 ml aliquots of petroleum ether (b.p. 40–60°C). The filtrates from duplicate flasks were combined and shaken well in a separating funnel. The layers were allowed to separate after which the lower water phase was drained and the upper oil phase was taken in a beaker and concentrated by evaporation on a steam bath.

Quantitative analysis was done by using a Hewlett–Packard (Model 5730 A) gas chromatograph with dual flame ionization detectors. Stainless steel columns (2% OV stationary phase carbowax with 3% phase loading on chromosorb G-HP 100/200 mesh) were used with column injection of 2 µl of samples. The carrier gas, nitrogen, was run through the columns at a rate of 30 ml per min. Tracings were obtained on a chart recorder and the peaks of the alkanes were identified by comparison with standards of each alkane (C_8–C_{14}). Utilization of fractions of crude oil was thus determined by comparing the peak areas of each alkane from each test corresponding to the control extract.

It is observed that only two species, *Aspergillus flavipes* and *Penicillium* sp registered significant growth and caused a shift in pH towards acidity. Of the two species the shift in pH and the extent of the growth were greater in *A. flavipes* than in *Penicillium* sp.

Gas chromatographic analysis of the residual crude oil of the mycelium of *A. flavipes* and *Penicillium* sp showed that *Penicillium* sp was more effective in degrading crude oil than *A. flavipes*, although dry weight of the mycelium and the decrease in pH of the aqueous phase was comparatively less in *Penicillium* sp than in *A. flavipes*.

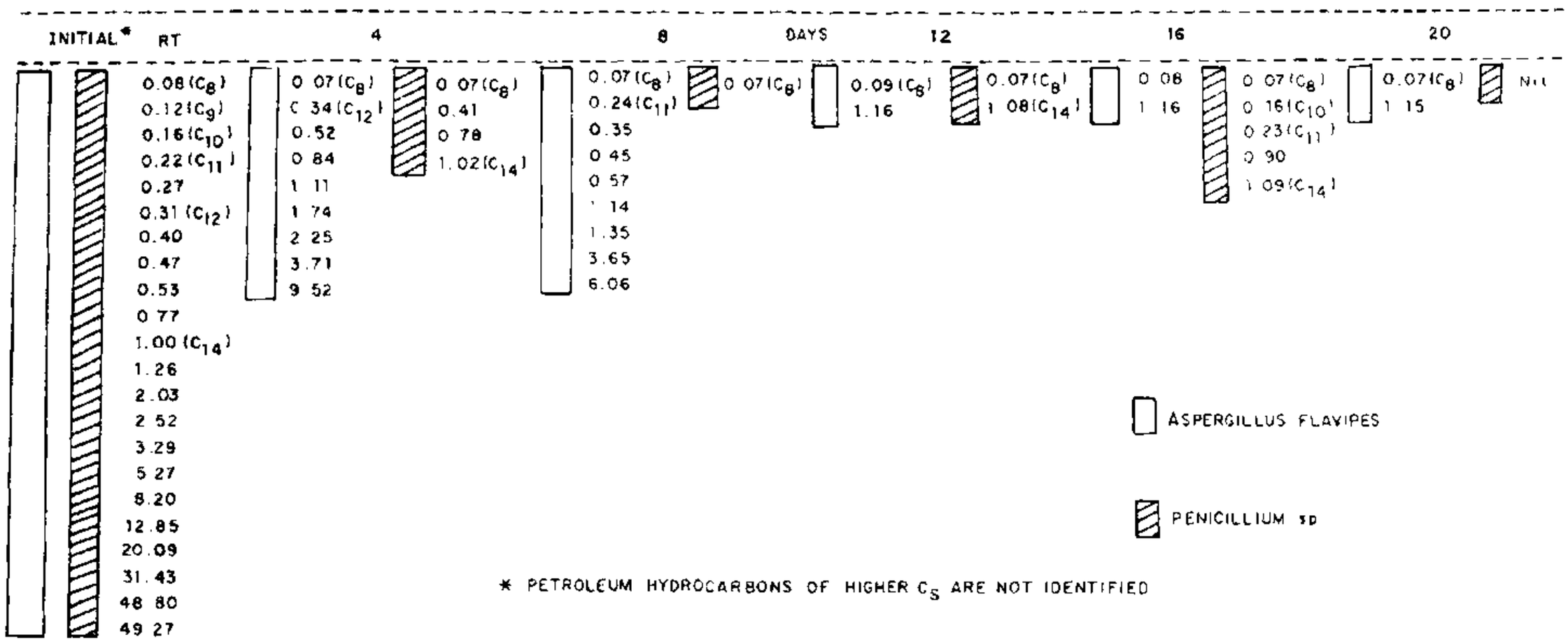

Figure 1. Degradation of petroleum hydrocarbons by *Aspergillus flavipes* and *Penicillium* sp. Results of gas chromatographic analyses (schematic representation).

Penicillium sp utilized both short chain (C_8–C_{14}) and long chain saturates (could not be identified as there was no authentic sample available) within four days of incubation. However, in *A. flavipes* the process of utilization of longer chain saturates was slow but complete within 12 days of incubation (figure 1).

It appears from these data that measurement of growth by dry weight and the shift in pH to acidic levels are not the best index of utilization of hydrocarbons. Gas chromatographic analyses are necessary to evaluate the efficiency of the concerned species in utilizing hydrocarbons.

Other species of fungi grown in crude oil showed only limited growth and dry weight of the mycelium was negligible. Although *Cladosporium resinae*, *Aspergillus fumigatus*, *Cunninghamella* sp, have been frequently isolated from oil-polluted environments and jet fuels[7–9] and are reported to utilize a wide range of hydrocarbons[2,10,12] the isolates of these species tested in the study were not effective in using crude oil.

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