HYDROCARBON EMULSIFYING ACTIVITY OF BACTERIAL STRAINS: POTENTIAL OF *ARTHROBACTER PARAFFINEUS*

MANJEET SINGH and J. D. DESAI
Applied Biology Division, Research Centre, Indian Petrochemicals Corporations Limited, Baroda 391 346, India.

RECENT years have witnessed a growing interest in the surface active compounds of microbial origin as many of them are potential candidates for commercial application in petroleum, pharmaceuticals, and food processing sectors, and have been a subject of many recent reviews[1–4]. The function of bioemulsifiers/biosurfactants is related to the hydrocarbon uptake and are therefore, synthesized predominantly by hydrocarbon degrading microorganisms[5–7]. We obtained from different sources some bacterial strains capable of growing on alkanes. The production of bioemulsifier during their growth on hexadecane was investigated and the results are presented in this note.

Acinetobacter calcoaceticus, *Arthrobacter paraffineus* (ATCC 15591), *Corynebacterium hydocarboxydans* (ATCC 21769) and *Pseudomonas putida* CAM-OCT were obtained from Dr H. P. Kleber, Karl-Marx University, Leipzig, (GDR), American Type Culture Collection, Maryland, (USA) and Prof. A. M. Chakrabarty, University of Illinois at Chicago, (USA), respectively. Stock cultures were maintained on the nutrient agar slopes by subculturing every fortnight, incubated at 30°C for two days and subsequently stored at 5°C. Inoculum was prepared by growing overnight the organism in nutrient broth at 30°C in shake condition (200 rpm). About 1% inoculum was transferred to a growth medium (0.5% peptone, 0.3% yeast extract, 1% hexadecane, pH 7) and incubated at 30°C on a rotary shaker for 72 h. After the indicated growth period, cells were removed by centrifugation at 10,000 *g* for 20 min at 4°C. From the supernatant obtained, emulsification activity was determined using the method of Reddy *et al*[8] in which 2.5 ml supernatant, 2.5 ml water and 0.1 ml hydrocarbon were vortexed for a minute and allowed to stand for 2 h at room temperature. The turbidity of stabilized emulsion was measured at 610 nm and a unit of emulsifying activity was expressed as optical density under the conditions of measurement.

The results of emulsification of aliphatics, aromatics and mixed hydrocarbons by culture supernatant of bacteria grown on hexadecane are listed in table 1. Straight chain aliphatics (octane and hexadecane) and mixed hydrocarbons (naphtha, kerosine) were not emulsified. However, good emulsification of aromatics was observed using culture supernatants of *A. calcoaceticus* and *A. paraffineus*. Among aromatics, tetraline was easily emulsified in the above condition. Bioemulsifier was isolated from alkane grown *C. tropicalis* which stabilized hexadecane in water emulsions[9,10]. The emulsifier produced by *Acinetobacter* sps. fails to emulsify aliphatic, aromatic or cyclic hydrocarbons[11,12]. However, mixtures of aliphatic, aromatic and cyclic hydrocarbons have been shown to be readily emulsified.

Table 1 *Emulsification activity of culture supernatant of bacteria grown on hexadecane*

	Hydrocarbon tested		
Culture supernatant	Xylene	Toluene	Tetraline
A. calcoaceticus	0.16	0.21	0.51
A. paraffineus	0.36	0.38	1.05
C. hydrocarboxydans	0.11	0.14	ND
P. putida	0.12	0.12	ND

Emulsification activity against octane, hexadecane, naphtha and kerosine was not detectable (ND). No emulsification activity was detected when these cultures were grown on glucose as the carbon source.

The stability of hydrocarbon emulsions of bacterial culture supernatant is shown in table 2. The emulsion formed by *A. paraffineus* culture supernatant was quite stable (75–90%) compared to other bacterial culture supernatants tested. The emulsification activity of culture supernatant of *A. paraffineus* was marginally decreased by treating in a boiling water bath for 15 min.; was unaffected between the pH range of 5 to 10, and by NaCl and $CaCl_2$ concentrations up to 10% and 0.3% respectively (table 3). The above results indicate that the emulsifier produced by *A. paraffineus* is thermo-

Table 2 *Stability of hydrocarbon emulsion by bacterial culture supernatant*

	% Stability		
Culture	Xylene	Toluene	Tetraline
A. calcoaceticus	95	5	50
A. paraffineus	95	5	95
C. hydrocarboxydans	10	10	US
P. putida	65	US	US

US, unstable.

Table 3 *Effect of heat, pH, NaCl and $CaCl_2$ on the emulsification activity of culture supernatant of A. paraffineus*

Condition	Emulsification activity
Control	0.36
Heat treatment, 90°C for 15 min	0.32
pH = 5	0.34
pH = 10	0.38
NaCl, 10%	0.35
$CaCl_2$, 0.3%	0.35

stable, effective in saline, acidic and alkaline conditions.

The yield of emulsifier isolated by acetone precipitation was about 1.2 g/l. Although, the emulsifier produced by *A. paraffineus* showed lower yield than reported in the literature, it is quite stable in a wide pH range, at high temperature and salt concentration. The above properties and considerable scope in improvement in the yields of emulsifier, make this organism a potential candidate for the enhanced oil recovery.

19 September 1988

1. Parkinson, M., *Biotech. Adv.*, 1985, **3**, 65.
2. Rosenberg, M., *CRC Crit. Rev. Biotechnol.*, 1986, **3**, 109
3. Desai, J. D., *J. Sci. Ind. Res.*, 1987, **46**, 440.
4. Syldatk, C. and Wagner, F., In: *Biosurfactant and biotechnology*, (eds) W. Kasarie, W. L. Cairns and N. C. C. Gray, Marcel Dekker Inc., New York, 1987, p 89.
5. Singh, M. and Desai, J. D., *J. Sci. Ind. Res.*, 1986, **45**, 413.
6. Zajic, J. E. and Panchal, C. J., *CRC Crit. Rev. Microbiol.*, 1976, **5**, 423.
7. Watkinson, R. J., In: *Hydrocarbons in biotechnology*, (eds) D. E. F. Harrison, I. J. Higgins and R. T. Watkinson, Heyden & Sons Ltd., London, 1980, p. 11.
8. Reddy, P. G., Singh, H. D., Roy, P. K. and Baruah, J. N., *Biotechnol. Bioeng*, 1983, **25**, 387.
9. Kappeli, O. and Fiechter, A., *Curr. Microbiol.*, 1981, **6**, 21.
10. Kappeli, O., Walther, P., Muller, M. and Fiechter, A., *Arch. Microbiol.*, 1984, **138**, 279.
11. Rosenberg, E., Perry, A., Gibson, D. T. and Gutnick, D. L., *Appl. Environ. Microbiol.*, 1979, **37**, 409.
12. Rosenberg, E., Zuckerberg, A., Robinowitz, H. and Gutnick, D. L., *Appl. Environ. Microbiol.*, 1979, **37**, 402.

NEWS

POLYMER CURE FOR MYOPIA

Soviet eye specialists have developed a promising new treatment for myopia involving the injection of polymers mixed with medicinal additives.

Polymers have been used in Soviet ophthalmology for several years now. A foamy liquid synthetic material "penogel" is injected into the eyeball. The material hardens in a minute, enveloping and strengthening the flabby and elastic sclera.

Penogel is itself soon replaced by germinating natural tissues. Over 3,000 patients have already undergone the new treatment.

Professor Eduard Avetisov, Deputy Director of the Helmholtz Institute of Eye Diseases, described the new method as simple and safe and as effective as the traditional surgical treatment. It could be made more reliable by the addition of medicine to penogel. (*Soviet Features*, Science and Technology, Vol. xxvii, No. 130. Published by Information Department, USSR, Embassy in India, P.B. No. 241, New Delhi 110 001).