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BIOSYNTHESIS OF AMINO ACIDS FROM HYDROCARBONS

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The growth of A-18 (*Bacillus subtilis*), a hydrocarbon utiliser, was studied after supplying different sources of carbon and nitrogen. Kerosene sample supplied by National Oil Refinery (N.O.R.) proved to be the best source of carbon when ammonium nitrate or urea was used as a source of nitrogen. Bacterial growth was maximum when 4% concentration of kerosene (N.O.R.) was used. The growth, however, decreased when kerosene was replaced by other sources of carbon and was minimum in case of n-decane. Natural gas or naphtha did not support the growth. The strain produced the following amino acids: asparagine, serine, glycine, lysine, glutamic acid, threonine, alanine, histidine, arginine, tyrosine, proline, valine, phenyl alanine, isoleucine and leucine.

A rapid growth in the world population and a decrease in the production of good quality protein is posing a serious problem of malnutrition. It has been reported that 26% of the children in Pakistan die before fifth birthday, whereas the figure for the children of the same age group in advanced countries is 2.4%.[1] This lamentable state of affairs can be successfully tackled if an all-out attempt is made to utilize all the available resources for the production of edible proteins.

ZoBell[2] and Beerstecher[3] reported utilization of hydrocarbons by certain microorganisms. Production of proteins by microorganisms utilizing hydrocarbon as the sole source of carbon was reported by various workers. Shah *et al.*[4,5] isolated and identified microorganisms capable of utilizing hydrocarbons, from Pakistani soil. Pilot plants for the production of proteins from hydrocarbons have been set up in U.S.S.R. and France.

This project was undertaken to find out suitability of refinery products available in Pakistan for the production of proteins. The possibility of utilization of natural gas for this purpose was also investigated.

Experimental

The strain employed during these investigations was hydrocarbon utiliser A-18, identified as *Bacillus subtilis*.[5]

The basal medium used was the same as suggested by Yamada *et al.*[6] It contained; HN_4NO_3, 5.0 g; KH_2PO_4, 2.5 g; $MgSO_4.7H_2O$, 1.0 g; Teepol, 0.5 g; distilled water, 1.0 l.

Teepol was added as an emulsifying agent. Tap water was replaced by boiled water, since Shah *et al.*[4] reported that large amounts of salts present in the tap water retarded growth of microorganisms.

Sui gas (a natural gas found in Pakistan), locally available kerosene and naphtha and kerosene supplied by National Oil Refinery were used as sources of carbon.

The unassimilated kerosene present in the cell suspension was extracted with n-hexane and the turbity of the broth was measured at 7500°A, in a Beckman spectrophotometer.

Total nitrogen was estimated by a microkjeldahl method, using $CuSO_4$, K_2SO_4 and SeO_2 (1:9:0.02) as catalyst.

Amino acids present in the hydrolysate of the cell suspension were separated by two dimensional paper chromatography using n-butanol: Glacial acetic acid: water (100:24:100) and phenol: water (80:20), as the solvents; and 0.25% ninhydrin solution was used as a developer.

Discussion

This work has been carried out to study the possibility of production of bacterial protein from hydrocarbons. The influence of different factors like nature of carbon and nitrogenous source and concentration of hydrocarbons have been investigated.

Effect of Sources of Carbon on the Growth of Microorganism.—The microorganism was grown on different hydrocarbons, like n-decane, kerosene from N.O.R. as well as local, naphtha and Sui gas. From Fig. 1 it is clear that this strain showed

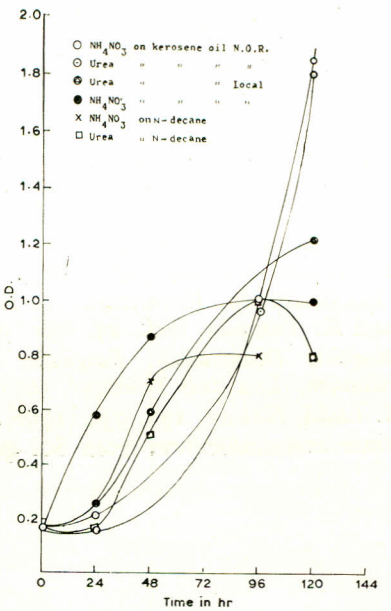

Fig. 1.—Effect of different sources of nitrogen on the growth of A—18.

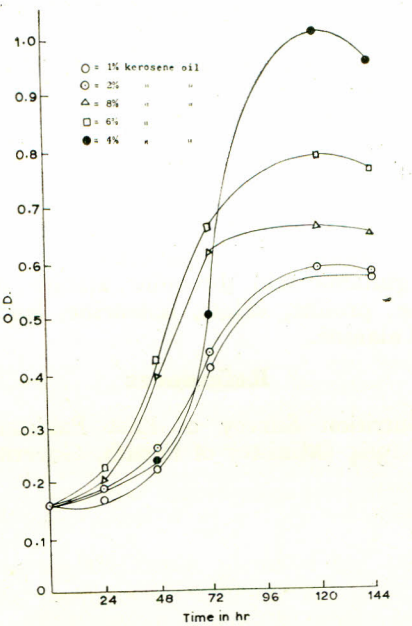

Fig. 2.—Effect of different concentrations of K. oil on the growth of A—18.

maximum optical density, while growing on kerosene from N.O.R. Practically no growth was observed when Sui gas or naphtha were used as the sole carbon sources. The order of preference for the hydrocarbons in the descending order appears to be kerosene (N.O.R.), kerosene (local) and n-decane. These results confirmed the findings of Takahashi *et al.*,[7] who reported a profuse growth on hydrocarbons containing C^{16}–C^{18}.

The growth on local kerosene oil was comparatively less than that on the kerosene from the N.O.R. This inhibition could be due to the coloured material added to the kerosene before sale. These dyes[8] have been reported to have a toxic effect on the microorganisms.

Effect of Nitrogen Source on Growth.—The growth of strain A-18 has also been found to be affected by the nature of the nitrogen source. The bacteria was provided with two different nitrogenous sources, i.e. NH_4NO_3 and urea. The results have been presented in Fig. 1. It is evident that the growth was slightly better when ammonium nitrate was used as a source of nitrogen. However, the difference in the optical density was, in certain cases, so small that both urea and NH_4NO_3 can be recommended as sources of nitrogen. The ammonium salts have been recommended as the best nitrogenous sources for the hydrocarbon utilisers.[9]

Effect of Hydrocarbon Concentrations on Growth.—The effect of change in the concentration of hydrocarbon (1–8% v/v) was also studied. Figure 2 shows the growth curves for the various concentrations used. It is clear from Fig. 2 that the growth of the strain increased with an increase in the concentration of kerosene from 1% to 4%. There was, however, an appreciable decrease in the growth rate at 6–8% concentration of kerosene. This decrease seems to be due to a decrease in the oxidation rate which can be attributed to the lesser availability of oxygen to the microorganisms.

Incapability to Utilise Sui Gas.—The strain could not grow when Sui gas was employed as the sole source of carbon. This could be due to the (i) presence of toxic substances in the gas, (ii) lesser solubility of methane present in the gas or (iii) inability of the microorganism to utilise methane as a sole source of carbon. The analysis of the Sui gas[10] has revealed the presence of the following substances; methane 94.42%; ethane 1.08%; other hydrocarbons 0.40%; nitrogen 3.89%; CO_2 0.02%; mercaptans 0.5–0.7 lb/million ft^3.

The presence of H_2S and mercaptans creates a reducing atmosphere in which the strain, an aerobe, cannot persist. These substances have been reported toxic to microorganisms especially aerobes.

Another factor, for non-assimilation of the gas could be the failure of the strain to oxidise methane gas. It has been reported that majority of hydrocarbon utilisers are capable of assimilating only the medium chain hydrocarbons.

Screening of Amino Acids.—The amino acids produced by the microorganism were detected by the two-dimensional paper chromatography, and were found to be: asparagine, serine, glycine, lysine, glutamic acid, threonine, alanine, histidine, arginine, proline, valine, isoleucine, leucine and phenyl alanine.

References

1. Nutrition Survey of East Pakistan 1962–1964 (Ministry of Health, Government of Pakistan, Islamabad, 1966).
2. C.E. ZoBell, Bact. Rev., **10,** 1 (1954).
3. E. Beerstecher, *Petroleum Microbiology* (Elsevier Press, New York, 1959).
4. F.H. Shah, M.H. Sedi, and T.H. Sheikh, Agr. Biol. Chem., **31,** 645 (1967).
5. F.H. Shah, A.M. Khalid and W. Wahid, *ibid.*, **31,** 1499 (1967).
6. K. Yamada, J. Takahashi, K. Kobayashi and Y. Imada, *ibid.*, **27,** 390 (1963).
7. J. Takahashi, K. Kobayashi, Y. Kawabata and K. Yamada, *ibid.*, **27,** 836 (1963).
8. *Antiseptics, Disinfectants, Fungicides and Sterilisation*, (Lea and Febriger, 1957), p. 71.
9. J.N. Ladd, Nature, **177,** 939 (1956).
10. Private communication from Sui gas fields.