# FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Workshop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

A. D. McINTYRE
K. J. WHITTLE

Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171: 126–128. 1977.

# STUDIES ON SOME BIOCHEMICAL INDICES OF PHENOL DEGRADATION BY *BACTERIUM ALBUM*

I. A. Divavin, K. K. Ermolaev, and O. G. Mironov
Institute of Biology of South Seas,
2, Nakhimor Prospect, Sevastopol, USSR

Protein, lipids, lipid fractions, nucleic acids and their components, as well as free nucleotides, were investigated during phenol degradation by cells of *Bacterium album* isolated from the Black Sea.

We found a considerable decrease in total cell protein and some increase in total cell lipid when the organism was growing on phenol. The content of nucleic acids and free nucleotides also increased and the RNA level more than doubled.

This study of the biochemical components during phenol degradation indicated a rearrangement in the metabolic processes of the organism. These heterotrophic microorganisms are the main consumers of hydrocarbons in the sea.

## INTRODUCTION

Phenols dissolved in water are used as an energy source by microorganisms which are capable of using them as a sole carbon source.

Phenol degradation by bacteria grown on peptone follows a period of adaptation when the phenol concentration and microbial biomass remain constant (Table 35).

The aim of this study is to determine the changes in cellular proteins, lipids, nucleotides and nucleic acids during phenol degradation by *Bacterium album*.

## MATERIALS AND METHODS

A strain of the phenol-degrading organism *Bacterium album* (Krasilnikov, 1949), isolated from the Black Sea, was used. Pure cultures of this organism were grown on a synthetic medium (Kalabina and Rogovskaya, 1934) with phenol as the sole carbon and energy source. A salt concentration equivalent to the salinity of the Black Sea was achieved by the addition of sodium chloride. Cultures were incubated at 28°C on a rotary shaker at 150 revolutions/min. The same strain, grown in a similar manner, but in a peptone medium was used as a control.

Acid-soluble nucleotides and nucleic acids were determined by the method of Spirin (1958). The concentrations of mononucleotides of RNA and DNA were determined after separation on a DEAE-Sephadex column, A-25 (medium) in the chloride form, using a carbonate-bicarbonate buffer pH 9·7, with a linear concentration gradient of sodium chloride from 0 to 0·4 M. Concentrations of mono-, di- and tri-phosphates of acid-soluble nucleotides were determined after separation on DEAE-Sephadex, A-25, using tris-HCl buffer pH 8·3, with a linear gradient of sodium chloride from 0 to 0·2 M. Separation of acid-soluble nucleotides and nucleotides of DNA was carried out on a column of DOWEX 1×4 (200—400 mesh) in the chloride form, eluting with 0·25 M sodium chloride in 0·2 M ammonium hydroxide. Nucleotide contents were determined using the coefficients of Spirin and Belozersky (1956).

Total lipid content was determined by extracting the bacterial cells three times with chloroform-methanol (2:1), washing with distilled water, evaporating to

Table 35. Rate of phenol destruction by microorganisms

| Time, hour | Phenol conc, mg/litre | Dry wt of bacterial mass, mg/100 ml of suspension |
|---|---|---|
| 0 | 100 | 37[1] |
| 2 | 99 | 37 |
| 4 | 100 | 37 |
| 6 | 101 | 38 |
| 9 | 100 | 37 |
| 12 | 98 | 38·5 |
| 20 | 102 | 37 |
| 28 | 98 | 38 |
| 36 | 99 | 37 |
| 44 | 100 | 38 |
| 52 | 54 | 44 |
| 56 | 41 | 48 |
| 60 | 0·2 | 52 |

[1] introduced bacterial biomass growing on peptone.

Table 36. Content of overall protein, lipids and nucleic acids in cells of *Bacterium album*

| | Protein mg/ 100 mg of dry wt | Lipids mg/ 100 mg of dry wt | RNA mg/ 100 mg of dry wt | DNA mg/ 100 mg of dry wt | RNA/ DNA |
|---|---|---|---|---|---|
| Control..... | 44·36 | 5·64 | 5·21 | 2·41 | 2·16 |
| Experiment.. | 26·84 | 8·29 | 10·95 | 3·42 | 3·19 |

dryness and weighing (Folch et al, 1951). Separation into lipid classes was carried out by thin-layer chromatography on silica gel. The thin layer plates were developed by charring after spraying with concentrated sulphuric acid and the content of the various fractions determined by densitometry. Fluorescence spectra of the lipid fractions were obtained using a microfluorimeter in the range of 400—700 nm.

Total protein content of the cells was obtained spectrophotometrically (protein mg/ml = 1·45 E 280—0·74 E 260).

## RESULTS AND DISCUSSION

The results show large differences in the major components of the bacterial cells (DNA, RNA, lipid and protein) grown on phenol as compared to those grown on peptone. (Table 36). There were increases in the levels of DNA, RNA and lipids and a substantial decrease in the amount of protein present.

The nucleotide composition of the DNA of phenol-grown cells was identical with that of peptone-grown cells. The small variations noted are well within the range of experimental error. Analogous results were obtained on columns with DEAE-Sephadex A-25 and with DOWEX 1×4.

In general, the nucleotide composition of RNA is predominantly G+C but in the present experiments

Table 37. Nucleotide composition of nucleic acids (mol. %) in *Bacterium album*

| | C | U (T) | G | A | (G+C) % | G+C / A+U (T) |
|---|---|---|---|---|---|---|
| | | | RNA | | | |
| Control. | 9·56 | 41·63 | 25·30 | 23·51 | 34·86 | 0·54 |
| Experiment... | 8·82 | 43·61 | 24·06 | 23·51 | 32·88 | 0·49 |
| | | | DNA | | | |
| Control . | 25·92 | 23·30 | 27·50 | 23·28 | 53·42 | 1·14 |
| Experiment ... | 24·10 | 23·10 | 27·95 | 24·85 | 52·05 | 1·08 |

C – cytosine; U – uridine; T – thymine; G – guanine; A – adenine

Table 38. Free nucleotide content in *Bacterium album* (% – A, mg/100 mg of dry wt – B)

| | | C | U | G | A | Total |
|---|---|---|---|---|---|---|
| A | Control..... | 14·05 | 51·35 | 16·55 | 18·05 | 100 |
| | Experiment... | 14·45 | 51·65 | 17·50 | 16·40 | 100 |
| B | Control..... | 0·134 | 0·490 | 0·158 | 0·172 | 0·954 |
| | Experiment... | 0·178 | 0·638 | 0·216 | 0·202 | 1·234 |

C – cytosine; U – uracil; G – guanine; A – adenine

(Table 37) RNA, which was predominantly A+U, was obtained from both phenol- and peptone-grown organisms (65·14 % in peptone; 67·12 % in phenol). The high uridine content could possibly be explained by the deamination of cytidine during hydrolysis and this would then be eluted from the column with the peak corresponding to UMP. Different conditions of hydrolysis (0·5N KOH, 100°C, 5 min; 0·5N KOH, 70°C, 20 min; 0·5N KOH, 37°C, 16 hours), however, gave similar results. In addition, hydrolysis of commercial yeast RNA (0·5N KOH, 100°C, 5 min) did not result in deamination of the cytidine.

No differences were found in the nucleotide composition of RNA or DNA between phenol- and peptone-grown cells. This probably reflects the stability of the genetic material in microorganisms.

The levels of acid-soluble nucleotides and nucleotide phosphates are presented in Tables 38 and 39. The degradation of phenol by this organism is accompanied by an increase in the content of cytidilic, uridylic, guanilic and adenylic nucleotides although the ratio of these components in the nucleotide pool does not change substantially. Some increase in the levels of the three phosphates were found after growth on phenol. Increased enzyme activity resulting in higher levels of RNA and DNA probably can be attributed to an accumulation of precursors of nucleic acids and an increase in phosphorylation. The increase of nucleic acid content could in turn be a result of an increased demand for synthesis of adaptive enzymes required for phenol degradation.

Characterization of the lipid classes showed that phenol-grown cells had a higher lipid content and there were differences in the proportions of the various classes identified (Table 40). The proportion of phospho-

Table 39. Content of mono-, di- and triphosphates of free nucleotides in *Bacterium album*

| | % | | | mg/100 mg | | |
|---|---|---|---|---|---|---|
| | mono- | di- | tri- | mono- | di- | tri- |
| Control..... | 25·39 | 33·50 | 41·07 | 0·242 | 0·319 | 0·392 |
| Experiment.... | 23·60 | 42·96 | 33·43 | 0·291 | 0·531 | 0·412 |

Table 40. Content of lipid fractions in cells of *Bacterium album* (% – A, mg/100 mg of dry wt – B)

| | | Phospholipids | Sterol | Free fatty acids | Triglyceride | Sterol esters + hydrocarbons | Total |
|---|---|---|---|---|---|---|---|
| A | Control ... | 21·31 | 6·55 | 20·76 | 22·95 | 28·41 | 100 |
| | Experiment | 16·00 | 6·85 | 14·95 | 30·28 | 32·00 | 100 |
| B | Control ... | 1·20 | 0·37 | 1·16 | 1·30 | 1·61 | 5·64 |
| | Experiment | 1·33 | 0·57 | 1·23 | 2·50 | 2·66 | 8·29 |

lipids and free fatty acids decreased (21·31 to 16·00 and 20·76 to 14·85 % respectively) whilst the proportion of sterol esters and hydrocarbons increased (28·41 to 32·00 %). The triglyceride fraction increased most from 1·30 to 2·50 mg/100 mg dry wt (22·90 to 30·28 %). This represents an increase in energy resources which may be required during the change to phenol degradation. Sterol, a minor component, showed only small variations in both culture conditions.

The change in intensity and character of UV fluorescence of the phospholipid fraction is of interest. These differences were observed at different stages of microbial growth in more than a hundred observations. It is possible that phospholipid, which is a structural element of the cell membrane, is changed both qualitatively and quantitatively in phenol-grown cells.

This study of certain biochemical components of microbial cells during growth on phenol as a sole carbon and energy source has shown that under such circumstances rearrangement of the metabolism of the cells occurs. Similar organisms are mainly responsible for the degradation of hydrocarbons in the marine environment.

## REFERENCES

Kalabina, M. M. & Rogovskaya, T. I. 1934. Phenol degradation influenced by microorganisms. Gosstroyizdat, Moscow-Leningrad, p. 140.

Krasilnikov, N. A 1949. Krasilnikov's determinative scheme. Academy of Sciences, USSR, Moscow-Leningrad p. 830.

Spirin, A. S. 1958. Spektrofotometricheskoe opredelenie summarnogo kolichestva mukleinovyh kislot. Biohimija, 23 (5): 656.

Spirin, A. S. & Belozersky, A. N. 1956. Sostav nukleinovyh kislot pri eksperimentalnoi izmenchivosti u bakterij kischechnoj gruppy. Biohimija, 21: 768.

Folch, J., Ascoli, I., Lees, M., Meath, J. A. & Le Baron, F. N. 1951. Preparation of lipid extracts from brain tissue. J. biol. Chem., 191: 833.