II The Use of Microbes in the Petrochemical Industry

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Only about 5 % of crude oil is used in the manufacture of chemical and agricultural products, the remainder is either burnt to provide power for the internal combustion engine, heat and electricity, or is processed to provide lubricants, asphalts, solvents, *etc.* (Figure 1). At the end of 1976 the world published proved recoverable reserves of oil stood at around 650 billion barrels, which, at our present rate of consumption, is variably estimated to last between 25 and 40 years. Obviously the best way to conserve this dwindling resource is to find alternatives that will satisfy the energy demands currently met by oil. There are already serious attempts to do this using nuclear, solar, and wind power[1] but it is still too early to predict which of these will make any significant contribution to the future energy scene.

Other possibilities for oil conservation do exist. One is to develop efficient means for the recovery of shale oil. The present world reserves of shale oil are estimated to be around 30000 billion barrels of which only about 2 % is available for present day commercial exploitation and even then the best sources yield about six barrels of oil per tonne of shale oil (Table 1). Much of these reserves are left untapped because their recovery, at present, is uneconomic. However, because of the diminishing supply of conventional oil sources increasing pressure to develop economic extraction methods for oil shales is now on. Conventional extraction methods developed by an Irishman, James Young, in 1850 involve crushing and heating the shale to high temperatures to break the bonds between the kerogen and the inorganic matrix and to further crack the kerogen molecule into simpler components. Using this method vast quantities of energy are consumed, only 75 % of the organic material is liberated, and large quantities of expended shale must be disposed of (Figure 2). A biological process has been developed to extract oil that is operated at ambient temperature, gives good yields, and does not result in vast quantities of insoluble residue.[2] The method is based upon the biological production of acids from sulphides by *Thiobacillus* species that dissolve most of the inorganic matrix of the shale oil leaving an organic structure that can be used as a fuel, or can be converted into other fuels or feedstocks such as petroleum or synthetic natural gas (SNG). This bioleaching method can be used either to extract the insoluble material *in situ* by introducing the organisms plus nutrient into cracks and fissures in the oil shale-bearing strata, or the shale oil may be mined, crushed, and placed in a reactor and then bio-

[1] *Int. Herald Tribune*, April 1978.
[2] T. F. Yen, M. D. Appleman, and J. E. Findlay, U.S.P. II 3 982 995.

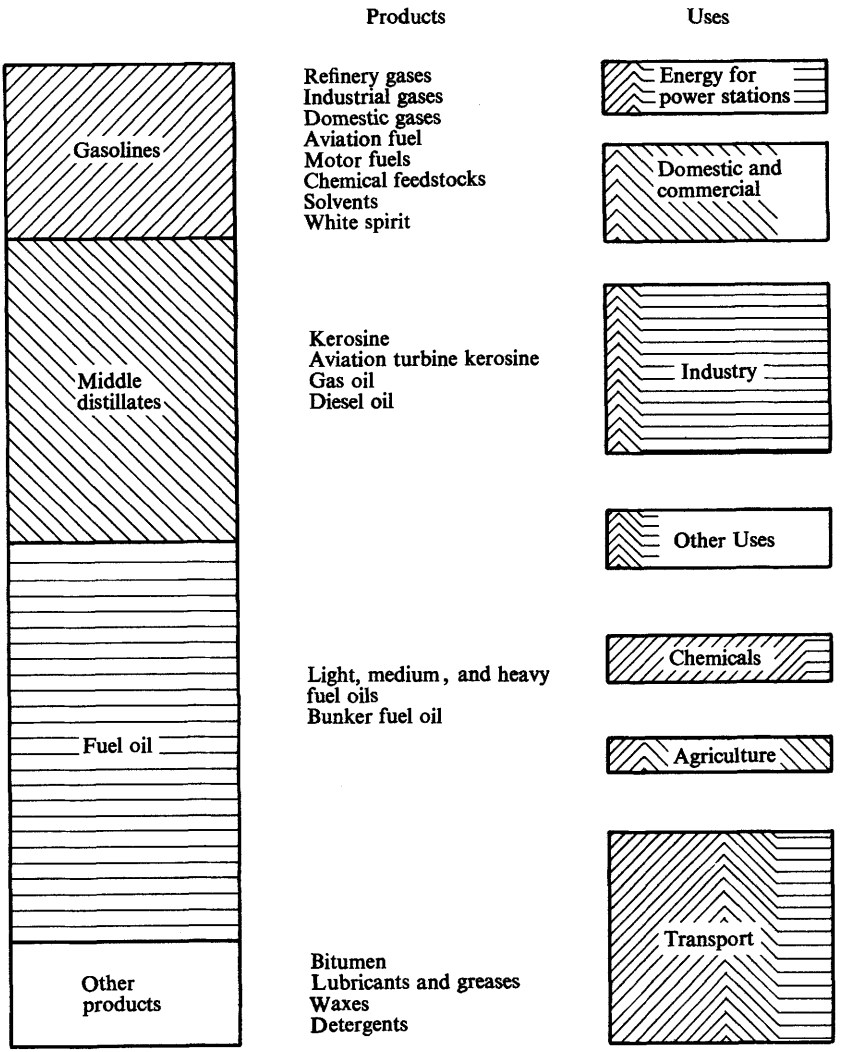

Figure 1 *Products from oil and their uses*
(adapted from 'Our Industry Petroleum', The British Petroleum Company Ltd., London, 1977)

leached in a continuous or batch process. In laboratory batch experiments the weight loss of green river shale was shown to be around 30 % after three days and 40 % after fourteen days, with 97 % of insoluble carbonate being removed. Removal of carbonates prior to refining has its advantages: it eliminates the

Table 1 *Organic carbon content and oil yield of some shale oils*

	Organic carbon (%)	*Oil yield* (gal/ton)
Kiligwa river, Alaska	53.9	139
Piceance Creek, Colorado	12.4	28
Elko, Nevada	8.6	8.4
Dunnit, Scotland	12.3	22
Ione, California	62.9	52
Sao Paulo, Brazil	12.8	18
Puerto Uano, Spain	26.0	47
Shale City, Oregon	25.8	48
Coolaway Mt., Australia	81.4	200
Soldiers Summit, Utah	13.5	17
Ermelo, S. Africa	52.2	100
New Glasgow, Canada	7.9	9.4

<table>
<tr><td rowspan="3">Oil shale</td><td rowspan="2">Mineral matter 86.2 %</td><td>Silicates and iron sulphide 43.06 %</td></tr>
<tr><td>$CaMg(CO_2)_2$ 43.1 %</td></tr>
<tr><td rowspan="2">Organic matter 13.8 %</td><td>Bitumen 2.76 %</td></tr>
<tr><td>Kerogen 11.04 %</td></tr>
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Figure 2 *Average chemical composition of Green River Shale oil*

formation of hydrocarbon polymers in the refining process, a reaction that is catalysed by their presence, and it increases the permeability of the rock and therefore increases the efficiency of recovery of shale oil. Although these experiments were done with *Thiobacillus* species which produce mostly sulphuric acid it is possible to use organisms that produce different acids, *e.g.* rumen bacteria to produce formic, acetic, and propionic acids, or 2-ketogluconic acid by *Pseudomonas* species which have the effect of solubilizing silicates and other components of the insoluble matrix. The major advantage of the biological method is that it operates at low temperatures thus circumventing the problems of loss of organic material through 'burn-off' and the formation of organic chemicals that are difficult to refine into fuel. Furthermore, at high temperatures significant dehydrogenation of hydrocarbons occur which necessitates the use of large amounts of hydrogen in the subsequent upgrading refining process.

Because the organisms oxidize elemental sulphur (*Thiobacillus thio-oxidans*) and pyritic sulphur (*Ferrobacillus ferro-oxidans*) to sulphuric acid they are

probably responsible for removing organically bonded disulphides and polysulphides that are notably absent from bioleached shale oil but present in retorted shale. The dissolution of carbonate from the shale results in the formation of sulphates which can be recovered as sulphides using anaerobic cultures of *Desulfovibrio desulfuricans* which can then be returned to the bioleaching unit.

At present this system has not been proven on an industrial scale and may require modification before it can be operational – one drawback at the moment is the substantial amounts of water that will be required (about 115 gallons per tonne of shale) to effect efficient extraction and its availability will depend on regional water supplies and the meterological conditions of the area. Another problem is the high sulphur requirement (about 400 kg per tonne of shale) for the process, that must be met by the Laseter process for the microbial regeneration of sulphide. If this latter process operates at less than 100 % efficiency then the deficit will have to be made up from imported sulphur which will add significantly to the costs.

Assuming that reasonable alternatives are found that could go at least some way to alleviating the pressure on oil as an energy resource this would spare a larger proportion for the manufacture of chemicals and other products for which there is no obvious substitute. Most of the chemical industry is dependent upon petroleum as its raw material and most technologically advanced countries depend very heavily upon the products of the chemical industry to maintain and even advance that technology. Accepting that there will always be a demand for petrochemicals, then the industry will be forced into either looking for alternative sources of the raw material or trying to conserve the resources that it has. Alternative supplies could be obtained from shale oil as outlined above or from coal in which a Lurgi/Fischer-Tropsch process can be used to produce straight chain hydrocarbons and small amounts of alcohols, ketones, and acids. Unfortunately, only about $1\frac{1}{2}$ barrels of oil can be produced from 1 ton of coal and, like the shale oil, is considered uneconomic at present, but this could change in the future if OPEC drastically increases the price of oil.*

There are two other areas in which micro-organisms could play an important role in conservation. The first is through the efficient utilization of hydrocarbon substrates in the production of protein and allied products. Microbes are capable of growing (with different efficiencies) on a wide range of hydrocarbons. Of those substrates considered over the years the most promising appear to be methanol and alkanes. Already there exist, or are planned, a number of SCP production plants based on these substrates (Table 2). The primary market for these products is as compounded feed for animals and fish, although one eventual aim is for human consumption. At the moment the economics of SCP production from paraffins is an unfavourable one in many countries (apart from those that are agriculturally inefficient and where the need for protein supplemented diet is high). At present this is due to (*a*) the relatively high cost of substrate and (*b*) the relatively low cost of soya and fishmeal protein. To make

*At the time of writing the price of oil was under $13 a barrel. In September 1979 the price was around $20–25 which is close to the 'economic' price for the exploitation of shale oil.

these processes economically viable large tonnages of SCP must be produced or the product has to be upgraded by using it as an immediate source of vitamins, enzymes, oils and fats, polysaccharides, crude protein other than animal feed and even the carcasses.

The development of SCP processes within the EEC has been due to our need to become independent of the vast imports of American soybean and fishmeal. Our compounded animal feed production in 1975 was around 63 million tons and is rising steadily. The protein constitutent of this feed is made up largely from imports, the supply of which can fluctuate tremendously as the 1973 soybean embargo showed. This, coupled with the devastatingly poor anchovy harvest, caused havoc among animal feed compounders who have pressed for domestic processes to produce a similar commodity whose supply will not fluctuate drastically. Hydrocarbon-based SCP can supply this need.

As mentioned above, one of the most important factors in hydrocarbon-based SCP is cost. Since the raw material can make up about 50 % of the production cost the choice of substrate is important. The cheapest hydrocarbon is still methane, but there are problems associated with its use such as the high energy input required to keep both methane and oxygen in solution, its explosive nature, and the high heat production (in the region of 10 kcal gm^{-1} cells) necessitating cooling.

In many countries large quantities of methane gas are flared off at source and this represents a considerable loss of a natural resource. The catalytic conversion of this 'waste' gas to methanol or its direct use as a substrate for methane-oxidizing bacteria in SCP production would constitute a major attempt at resource conservation. The technology for methane-SCP is currently available but has not been used for large scale production because of economic constraints. Large sums have already been spent in testing the product as a protein supplement in the diet of certain farm animals, and as such the bacterial SCP is a reasonable success. Present indications are that the supply of natural gas will outlive other hydrocarbons, and if the technologies to produce methane from waste materials by fermentation are successful one could envisage a useful contribution to the SCP scene based on methane.

Methanol, which can be made quite cheaply from methane, coal, or oil, and paraffins are the most attractive feedstocks at present. Although the cost of the n-paraffins is about twice that of methanol (*ca.* \$250—300/ton for paraffins as compared with \$130/ton for methanol in 1976). The yield of organisms on paraffins is also about twice that on methanol. On a crude protein basis, however, the methanol assimilating bacteria are better (about 80 % on methanol compared with only 50—60 % on paraffins). Both processes have their advantages and disadvantages (Table 3). At present there are problems concerning the n-paraffin processes which have caused a number of them (particularly the BP and Japanese operations) to close down. These problems are not of an operational nature but are more political.

The methanol processes are fairly recent innovations. A large number of companies are actively pursuing the use of methanol as an SCP substrate, the

Table 2 *Existing and planned single-cell production plants*
[after W. Dimmling and R. Seipenbusch, *Process Biochemistry*, 1978, **13** (3), 9]

	Company/location	*Raw materials*	*SCP capacity*
CSSR	Kojetin	Ethanol	4000 t.p.a.
Federal Republic of Germany	Hoechst/Uhde	Methanol (n-Paraffins)	1000 t.p.a. Pilot plant under construction
UK	BP, Grangemouth	n-Paraffins	4000 t.p.a. (closed down)
	ICI, Billingham	Methanol	Pilot plant 1000 t.p.a. 50—70000 t.p.a. under construction
France	BP, Lavera	(Gas oil)	16000 t.p.a. (closed down)
GDR	Schwedt	Gas oil	60000 t.p.a. under construction
Italy	Italproteine SpA (BP, ANIC) Sarroch Sardinia	n-Paraffins	100000 t.p.a. Trial production (closed down)
	Liquichimica Biosintesi SpA (Kanegafuchi process) Reggio Calabria	n-Paraffins	100000 t.p.a. Trial production

Japan	Dainippon Kanegafuchi Kyowa Hakko Kogyo (BP Licence)	n-Paraffins	stopped
	Mitsubishi Gas Chemical	Methanol	Pilot plant 1000 t.p.a.
	Mitsubishi Petrochemical Co.	Ethanol	Pilot plant
Rumania	Boniprot Rumania Dainippon	n-Paraffins	60000 t.p.a. under construction delayed
USSR		n-Paraffins	300000 t.p.a. 500000 t.p.a. projected
		Methanol	Pilot plant projected
USA	Amoco Foods	Ethanol	4500 t.p.a.
Venezuela	Bio Proteinas de Venezuela	n-Paraffins	100000 t.p.a. under construction delayed

Table 3 *Operating parameters for various substrates used in SCP production*
[after W. Dimmling and R. Seipenbusch, *Process Biochemistry*, 1978, **13** (3), 9]

	Methanol	*Ethanol*	*n-Paraffins*
Micro-organisms	Mainly bacteria, few yeasts	Numerous yeasts	Various yeasts
Yield			
kg cell mass/kg substrate	*ca.* 0.50	*ca.* 0.70	*ca.* 1.00
Heat of fermentation			
kcal/kg cell mass	4300	5500	6500
Fermentation temperature	37—40 °C	30—32 °C	30—32 °C
O_2 demand kg O_2/cell mass	1.4	1.65	2.1
Raw protein content	*ca.* 80 %	55—60 %	55—60 %
Nucleic acid content	10—15 %	5—10 %	5—10 %
Advantages	easy to manufacture in pure state easy to separate medium from product completely miscible with water		
Disadvantages	additional treatment necessary for separating the biomass		substrate almost insoluble in water

majority based on bacteria. The most advanced appears to be the ICI process at Billingham using the organism *Methylophilus methylotrophus*. Pilot plant studies over a three year period established the principles of a new design concept for growing the organism in a 'pressure cycle' fermenter. The construction of a 70000 ton/annum plant is well-advanced and should be coming on stream in 1979.

Ethanol, which can be produced catalytically from ethylene is also receiving attention as an SCP source and Amoco in the U.S.A. are already marketing an ethanol grown yeast, 'Torutein', as a nutritional supplement and flavour enhancer in a number of processed foods. They have built a 7000 ton/annum plant in Minnesota, which although not working at full capacity at the moment, may well do so soon. Plans to build much larger plants (around 100000 tons/annum) have been announced in Spain, Germany, and Czechoslovakia.

Whichever SCP process proves to be the most successful in the years to come one thing is clear; the upgrading of simple petrochemical feedstocks into complex protein is probably one of the most efficient ways of making protein that Nature or man has yet devised. It seems rather unfortunate, but a major reality of life, that political considerations have to play such an important role in deciding whether petrochemical based SCP processes ever get off the ground.

I should now like to consider the second way in which biological systems could be used to conserve oil, which is by using microbial systems as efficient catalysts. All commercial catalysts used by the petrochemical industry have been developed by empirical means and in a number of cases tend to be rather inefficient, non-specific, and require high temperatures and pressures to work effectively. As a result, the conversion of a basic feedstock into a certain product can be expensive in terms of energy, and time consuming as far as separation and purification of the product is concerned (Table 4). It is well known, however, that there are microbial enzymes that can effect specifically the transformation of a wide variety of organic compounds. These include oxidative and reductive reactions that are far too numerous so mention specifically here.

Our current understanding of the mechanism of most of these biological reactions is still rather scant but future research could conceivably be very helpful in the design of highly efficient industrial catalysts. This, however, is still rather speculative and futuristic. What is not so esoteric, however, is the immediate possibility of using some of these systems, even if we do not know exactly how they work, to effect desired transformations.

I should like to consider a few specific biological reactions that we have been working on at Warwick which serve as examples of the sort of things that biological systems can do, bearing in mind that there are many other chemical reactions that could be approached in the same way.

Of particular interest to the petrochemical industry are reactions in which oxygen atoms are directly incorporated into hydrocarbon substrates (Table 5). These are generally expensive and difficult to achieve by normal chemical means.

If one can find biological systems to catalyse these conversions then they would have a number of advantages over the chemical catalysts currently used, *i.e.* they

Table 4 *Energy input and yield of industrial processes for the production of ethylene oxide and cyclohexanol*

	Ethylene → Ethylene Oxide
Power consumption per ton of ethylene oxide	1750 k.w.h. 0.6 tons steam
Reaction	Silver catalyst 200—300 °C 1—20 atm 46—70 % yield 40—60 % selectivity } Depends on catalyst
	Cyclohexane → Cyclohexanol
Reaction	Cobalt naphthenate catalyst 150—160 °C 10—11 atm 10 % conversion per pass ensures modest oxidation rate to reduce amount of over oxidation 63 % selectivity

Table 5 *Substrates and Products of Methane Mono-oxygenase*

Alkanes ($C_1 \rightarrow C_8$)	→	Alcohols
Alkanes ($C_2 \rightarrow C_4$)	→	Epoxides
Cyclohexane	→	Cyclohexanol
Benzene	→	Phenol
Toluene	→	Benzyl alcohol and cresol
Styrene	→	Styrene epoxide
Pyridine	→	Pyridine n-oxide
Methanol	→	Formaldehyde
Carbon monoxide	→	Carbon dioxide

function at low temperatures and pressures, are specific, and very often stereo-selective and efficient.

The mono-oxygenases are a class of enzymes that can effect such transformations. They are found in mammalian tissue where they function in the hydroxylation of drugs, steroids, and fatty acids or in microbial systems where they also catalyse the hydroxylation of hydrocarbons.

Work in our laboratory over the past few years on the methane oxidizing bacteria has revealed that the mono-oxygenase enzyme system from *Methylococcus capsulatus* will insert oxygen into a number of hydrocarbon substrates.

All of the reactions were done at 45 °C and at normal pressures. Using purified enzyme we have achieved rates as high as 6 μmol min^{-1} mg^{-1} protein and the yields for most reactions are close to 100 %. There are, however, several features

about this enzyme which at first sight make it an unattractive one from an industrial point of view. The first is that the enzyme requires NADH as its electron donor which would make its operation on a large scale uneconomical. The second is that the enzyme is not highly specific. To a certain extent these objections may be overcome. For example, it is now possible to supply the reducing power for such a system by electrochemical means, using either surface modified electrodes to supply electrons directly to the system, or liquid crystal membrane electrodes when regeneration of NADH is required. In the latter case, of course, only catalytic rather than stoicheiometric amounts of NAD would be required. Another possibility is to use endogenous formate dehydrogenase activity to regenerate NADH from catalytic levels of NAD and stoicheiometric quantities of formate.

The second objection is specificity. If the feedstock for the enzyme system is relatively pure then this poses no real problems especially if the contaminant(s) is not oxidized by the enzyme. If impure starting material is used then one is forced to look for enzyme systems that are more restricted in their substrate specificities and we already have some evidence to suggest that other methane-oxidizers have a narrower substrate spectrum than *M. capsulatus*.

If the chemical industry is to accept enzyme catalysts in place of traditional ones there are several hurdles that must still be overcome. Stability of the catalyst is paramount. Because of their inherent complexity most enzymes are rather unstable when separated from the cell. We have found that in crude extracts the half-life of methane mono-oxygenase is around 170 hours at 20 °C, which is rather poor when compared with chemical catalysts. When attached to an inert support (immobilization) the half-life of many is considerably extended and may be as long as 1700 hours. If enzyme systems are to be used then careful investigation of ways of enhancing stability will be essential.

One way of possibly avoiding stability problems of enzymes *in vitro* is to rely upon their great stability *in vivo*. In some instances we have observed good whole-cell activity after twenty days in the presence of substrate. Furthermore, we have been able to produce good quantities of several products listed in Table 1 using continuous culture techniques. Here again, conversion efficiences of 100 % can be obtained under suitable conditions but the actual amount of product formed per unit time depends on the number of organisms per unit volume. At the moment we have only managed to produce *ca.* 1 μmole of product min^{-1} mg^{-1} cells. This is still not sufficient to be competitive with industrial catalysts, but with the increasing wealth of information on immobilization techniques one could confidently expect the stability and productivity problems to be resolved in the near future.

If enzymes, either *in vitro* or *in vivo* can be successfully used to replace conventional catalysts in only a few instances then the possibilities of using other microbial catalytic systems may be of great value particularly for reactions that are difficult to achieve conventionally.

At present it is very difficult to determine accurately how much energy is used in chemical processes but with the high temperatures and pressures and product

purification steps involved it must certainly contribute a reasonable proportion. The use of low temperature catalysts will therefore represent a saving in terms of energy and may also be important in preventing a number of unwanted side-reactions that are known to occur only at high temperatures.