From petroleum microbiology to biotechnology: Prospects for Saudi Aramco

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From Petroleum Microbiology to Biotechnology: Prospects for Saudi Aramco

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ABSTRACT

Recent technical advances in the biosciences have driven a resurgence in interest in petroleum microbiology and biotechnology. This century so far has seen a large increase in the number of papers dealing with petroleum microbiology. The reason for this is that our knowledge of the bacteriology of petroleum reservoirs was formerly limited by the inability to culture most of the organisms present. Technically, it is now feasible to infer the presence of bacteria in a reservoir without having to grow them in a laboratory using nucleic acid technology. It has become clear that many more types of bacteria exits in petroleum reservoirs than we could have guessed. This has renewed interest in biotechnology applications such as biocompetitive exclusion, microbially enhanced oil recovery (MEOR) and anaerobic biocracking.

It is much less technically demanding to work in the downstream, and the revolution in molecular biology has also opened up new fields of study. The field of biocatalysis is now benefiting from the ability to modify and enhance the performance of enzymes of interest as a result of targeted genetic engineering. In this category, biodesulfurization of whole crude and refined fuels is being researched worldwide, and there are economic prospects for the biosynthesis of high value chemicals and biochemicals from crude oil.

INTRODUCTION

To traditional oil industry workers the idea of applying biotechnology within oil operations is a strange one. Historically, microbiology has only been an issue concerning oil field problems such as bacterial corrosion, heat transfer resistance and fouling of a variety of equipment[1]. With the increasing ease of handling biological molecules such as DNA and enzymes, a surprising range of biotechnology solutions are beginning to be applied to add value to existing operations. That is not to say that the old problems have disappeared. As Saudi Aramco operations increase during this historic period of expansion, inevitably the problems will increase and vigilance is required of the Biotechnology Unit to prevent material damage caused by bacterial growth. This article, however, will focus on the emerging areas where biotechnology can make positive contributions to the oil industry.

Biotechnology offers new approaches to operations both in the upstream and the downstream. Given the size and nature of oil fields, the range of biotechnology applications in the upstream is relatively limited just now, but that situation may change in the future as we learn more about the bacterial life forms that exist in oil reservoirs. In the downstream, the range of applications is rather more diverse and numerous as it is much easier to apply aerobic systems, and we are not dealing with the vastness of oil reservoirs. Designing a bioreactor, for example, for a downstream application is an easier task than trying to influence all or part of the conditions in a subsurface reservoir, often at extremes of temperature and pressure.

UPSTREAM BIOTECHNOLOGY: HOT AND ANAEROBIC

The challenges for life forms in oil reservoirs are several, but perhaps the greatest challenges arise from the fact that they are anaerobic environments and that the temperature is higher than would normally be expected for life to flourish. This has restricted life in oil reservoirs to the ultimate survivors on our planet – the bacteria.

The bacteria represent perhaps 95% of the biodiversity on the planet, and there is hardly an ecological niche that they have not conquered. Evidence indicates that the earliest versions of bacteria arose on our planet about 3.5 billion years ago[2]. By necessity, the first bacteria must have been anaerobes as they lived in an atmosphere completely devoid of oxygen. Currently there are many very extensive anaerobic ecosystems and these teem with bacterial life. Many bacteria – the facultative bacteria – are able to switch between anaerobic and aerobic lifestyles, and therefore it is no surprise that a great diversity of bacteria is capable of life in the total absence of oxygen.

High temperature need not be a barrier to bacterial life either. The highest temperature at which microbial life has been confirmed is 113 °C for a deep-sea hyperthermophile that grows by gaining energy from hydrogen oxidation[3]. Its optimum growth temperature is 106 °C. The beginning of this century has seen an explosion in interest and discoveries in oil reservoir microbiology[4, 5], mainly due to the emergence of molecular techniques which do not require the culture of bacteria. This will fuel new biotechnology applications in the upstream.

$$C_{16}H_{34} + 19.6\ NO_3^- + 3.6\ H^+ \rightarrow 16\ HCO_3^- + 9.8\ N_2 + 10.8\ H_2O \quad \Delta G' = -983\ kJ\ mol^{-1}$$

$$C_{16}H_{34} + 12.25\ SO_4^{2-} \rightarrow 16\ HCO_3^- + 12.25\ HS^- + 3.75\ H^+ + H_2O \quad \Delta G' = -61\ kJ\ mol^{-1}$$

Fig. 1. Thermodynamics of n-hexadecane biodegradation under nitrate- and sulfate-reducing conditions. ΔG' refers to nonstandard conditions at pH = 7.0.

BIOCOMPETITIVE EXCLUSION USING NITRATE AS A TERMINAL ELECTRON ACCEPTOR

The injection of nitrate into reservoirs is now an accepted full-scale upstream biotechnology, with successes being claimed worldwide. For example, several oil fields on the Norwegian Continental Shelf have been treated with nitrate since 1999, among them all the Gullfaks platforms[6]. The basis of the technology is combat with the greatest microbial foe in the oil industry, the sulfate-reducing bacteria (SRB). The activities of the SRB result in metal corrosion, crude oil souring and a metabolic end product, hydrogen sulfide (H_2S) is extremely toxic. Sulfate-reducing bacteria are strictly anaerobic bacteria that use sulfate as an electron acceptor instead of oxygen. While this is rather an inefficient mode of growth there is no shortage of sulfate in many environments. In the oil industry the practice of seawater injection into reservoirs is standard for maintaining reservoir pressure. For example, Saudi Aramco operates the largest seawater injection plant in the world at Qurrayah. Unfortunately, seawater contains an abundance of sulfate (2,700 mg l^{-1}, or 28 mM)[7]. The seawater is also stripped of oxygen to prevent aerobic corrosion, and the natural pH of about 8.0 is reduced to 7.2 to reduce scaling[8]. These conditions are near perfect for the proliferation of the SRB, and historically their growth has been controlled with biocides, which by their nature are toxic, environmentally damaging, and as they are specialty chemicals, they are also expensive.

Nitrate, however, is also a terminal electron acceptor, and a considerably more efficient one than sulfate, Fig. 1. The theory is, then, that injecting nitrate into a reservoir will stimulate the growth of bacteria which reduce nitrate, and thereby inhibit the growth of the SRB.

The inhibitory effects of nitrate addition on sulfide production have been known for at least 20 years[9]. Nitrate is nontoxic, and at low concentrations is not environmentally damaging, and is much more cost-effective than using biocides[10]. In addition, there is experimental evidence for enhanced injectivity as a result of nitrate treatment, a phenomenon that is as yet not understood. The technology has run ahead of the science, such has been the rush to implement it to control this severe oil field problem. Much of the microbiology that occurs when the switch from sulfate reduction to nitrate reduction remains to be elucidated. Nitrate is reduced to nitrogen gas via nitrite. The nitrate-reducing sulfide-oxidizing bacteria (NR-SOB) reduce nitrate to nitrite, and it is probable that inhibition of SRB by NR-SOB is caused by nitrite production[11]. This carries the possibility that inhibition of SRB may only be transient. The enzyme nitrite reductase (Nrf) reduces nitrite to ammonia. Nitrite reductase-containing SRB can therefore overcome the inhibition. Nitrite reductase, which is widely distributed in SRB, can thus be regarded as a threat to the technology.

Laboratory tests over the last decade on various waters from both Northern and Southern hemispheres revealed a significant shift in redox potential associated with nitrate dosing and the establishment of a dominant NRB population. The shift of some 300 mV in positive direction of redox potential associated with a viable NRB (compared to an SRB biofilm) could be another important reason for the extended control of NRB over coexisting and detrimental SRB population.

MICROBIAL ENHANCED OIL RECOVERY (MEOR)

Microbial enhanced oil recovery has been discussed and researched for several decades, and has been taken to the field on many occasions, but still remains unpredictable, and therefore is not yet a mainstream method of enhanced oil recovery. There are several companies offering MEOR services, and most of these advocate the addition of microorganisms to the reservoir (known as bioaugmentation). There is good reason to believe that this approach is flawed, and that the addition of nutrients that will stimulate bacterial growth *in-situ* is a more logical approach (biostimulation). Nutrients such as nitrate and a low-cost sugar source such as molasses are likely to travel further into a reservoir than bacteria due to the disposition of bacteria to attach to surfaces and form biofilms[12].

Biosurfactants that are capable of producing very low interfacial tensions are required to overcome capillary pressure. Moreover, biosurfactants have the potential for *in-situ* generation. Biosurfactant technology is the linchpin to success of MEOR. A range of structures of biosurfactants have been isolated from microorganisms, Table 1. Encouragingly, biosurfactants are now known to be produced under anaerobic conditions[13].

Many bacteria produce extracellular polysaccharides, which

Microorganism	Biosurfactant
Arthrobacter RAG-1	heteropolysaccharides
Bacillus licheniformis JF-2	lipopeptides
Bacillus subtilis	surfactin
Clostridium pasteurianum	neutral lipids
Pseudomonas aeruginosa	rhamnolipid
Rhodococcus erythropolis	trehalose dicorynomycolate
Torulopsis bombicola	sophorose lipids

Table 1. Biosurfactants produced by microorganisms[14]

manifests itself by the production of slimy bacterial colonies when grown on solid media, Fig. 2. The biopolymer xanthan already has a successful history of application in the oil industry as it is used as a viscosifying agent for drilling muds. In MEOR *in-situ* or *ex-situ* biopolymers can be used both in selective plugging and to increase the viscosity of injection water, thereby increasing sweep efficiency[15]. Selective plugging is possible using bacteria by targeted injection of microbes and/or nutrients into thief zones to allow the growth of bacteria to plug pores. The Selectively Induced Flow Technique (SIFT) was developed in the UK for this very purpose[16].

Acid production by microbes is common, and under anaerobic conditions carbon sources are fermented to fatty acids which lower pH and facilitate an increased permeability of reservoirs. In some MEOR field trials, the pH of produced water has been lowered by 1-2 units. This approach has advantages over the traditional acid treatments in terms of safety and handling requirements, in targeting the acid production to specific regions of the reservoir and in economics.

Gas production, either CH_4 or CO_2 (or both), can be used to stimulate pressure in the reservoir. The most applicable concept is to supply an inexpensive source of carbon, e.g., molasses, and nitrogen and/or phosphorus with the well shut in. This stimulates indigenous or injected bacteria to grow more rapidly and produce large quantities of gases, which serves to push oil from dead spaces and loosen debris that plugs pores.

Fig. 2. On the left is Xanthomonas campestris, *which produces copious extracellular biopolymer. On the right is typical colony morphology of* Serratia marcescens, *not producing extracellular polymers.*

ANAEROBIC HYDROCARBON BIODEGRADATION

Many, if not all, upstream biotechnologies rely on the biodegradation of hydrocarbons in oil reservoirs. The subject has only very recently started to open up. It has only been since the late 1980s that the biodegradation of aromatic, π-bonded hydrocarbons has been observed and described[17]. The alkanes are among the chemically least reactive compounds due to containing exclusively σ-bonds (saturated bonds). But similarly, in the 1990s, evidence for anaerobic biodegradation started to appear under both sulfate-reducing[18] and nitrate-reducing[19] conditions. The biochemistry of anaerobic hydrocarbon biodegradation is poorly understood and appears to be unprecedented in nature.

DOWNSTREAM BIOTECHNOLOGY

The ability to work under aerobic conditions greatly simplifies biotechnology. The well-described bacterial genetic systems are mostly in aerobes, and the critical techniques for genetic manipulation lend themselves most readily to aerobic conditions. Because of the higher efficiency of oxygen as an electron acceptor, aerobic microorganisms tend to grow more quickly and to higher levels of biomass. They are even able to attack solid hydrocarbons such as phenanthrene, Fig. 3. Also, when dealing with hydrocarbons, aerobes have much better systems for catabolism than anaerobes: the well-described oxygenases[20], which are the major enzymes involved in the initial

Fig. 3. Bacterial attack on phenanthrene. The zones of clearing around colonies indicate that the phenanthrene has been catabolized.

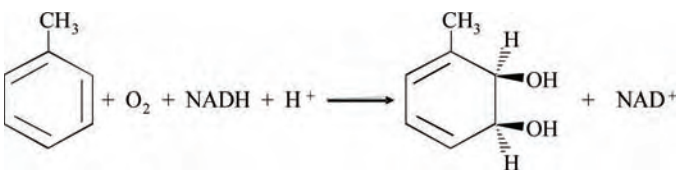

Fig. 4. The action of toluene dioxygenase on toluene to produce toluene cis*-diol.*

biodegradation of aromatic and aliphatic hydrocarbons, and are also involved in economically significant biotransformations[21].

PETROCHEMICAL BIOTRANSFORMATIONS

Bacterial dioxygenases perform the unique function of adding two oxygen atoms to the aromatic nucleus to form enantiopure arene *cis*-diols, e.g., Fig. 4. These cannot be synthesized by conventional organic chemistry, and as bacterial reactions occur at ambient pressure and low temperature, this route to arene *cis*-diols is regarded as a green chemical route to important compounds. The arene *cis*-diols are used as synthons in the development of new industrial and medical products, e.g., indigo, Figs. 5 and 6, polyphenylene and prostaglandin E2α[22].

Additionally, mutant strains have been constructed that prevent catabolism beyond *cis*-toluene dihydrodiol. This dihydrodiol is non-enzymatically dehydrated to *o*-cresol[23]. *o*-Cresol is one of the most important petrochemicals. Dioxygenases are capable of accepting arene substrates with a wide range of functional group substituents, from electron donating groups, e.g., methoxy, to electron-withdrawing groups, e.g., acetyl. By far the largest number of reported substrates has been the mono-substituted aromatic compounds with varying levels of substitution. More than 50 *cis*-dihydrodiols have been formed as the result of dioxygenases activity at the 2- and 3-bond of mono-substituted benzene substrates[21]. Dioxygenases also exist that act on higher aromatics such as naphthalene, anthracene, phenanthrene,

Fig. 5. A bacterium producing indigo. Bio-indigo is a potential replacement for synthetic indigo which requires high temperature and pressure for production[24].

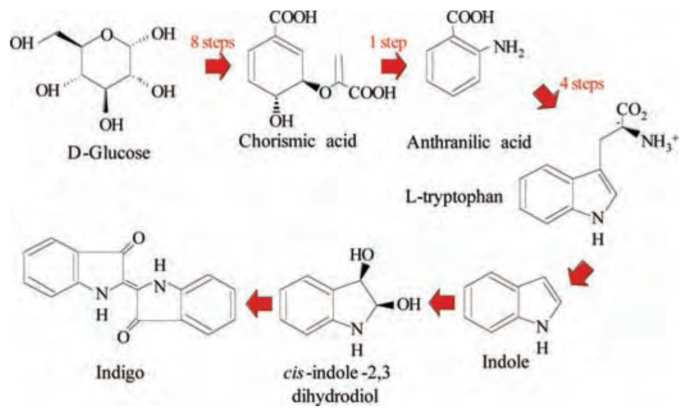

Fig. 6. Biochemical route to indigo.

chrysene, benz[a]anthracene and benzo[a]pyrene. A range of oxygen, nitrogen and sulfur heterocycles also act as substrates. The total number of arene *cis*-diols formed by a small number of dioxygenases exceeds 300.

It has become clear that this enzymatic hydroxylation is not limited to aromatics. Dioxygenase-catalyzed *cis*-dihydroxylation has been reported in several non-aromatic rings. Asymmetric *cis*-hydroxylation of olefins, monooxygenase-catalyzed epoxidation, and *trans*-dihydroxylation of the C-C double bond have also been reported[25].

A rational site-directed mutagenesis approach to improving enzyme function is possible if a detailed characterization of a given enzyme and at best the crystal structure is available. Alternatively, sequence alignments can help in identifying residues critical for enzyme activity (usually highly conserved) or substrate specificity (usually not conserved, so visible as differences in the sequences), and a rational design can lead to improved biocatalysts, Fig. 7.

Besides such site-directed approaches, various DNA-shuffling methods (random fragmentation of a population of mutant genes of a certain family followed by random reassembly) have been developed, which allow the creation of a vast range of chimeric proteins and protein variants[26]. For example, a hybrid toluene/biphenyl dioxygenase has been made by genetic manipulation[27]. It has a substrate range greater than toluene

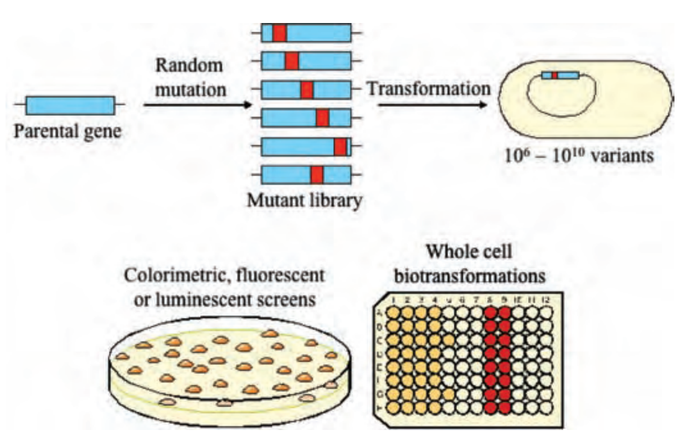

Fig. 7. Schematic of a random mutagenesis approach to improved biocatalysis.

dioxygenase and can generate *cis*-dihydrodiols from toluene, benzene, styrene, *p*-xylene, acetophenone, propiophenone, butyrophenone and trifluoroacetophenone.

BIODESULFURIZATION OF CRUDE OIL AND REFINED FUELS

Biodesulfurization is effectively a petrochemical biotransformation as it involves removal of sulfur from heterocycles, such as dibenzothiophene (DBT) and substituted dibenzothiophenes, in particular the methyl forms, Fig. 8.

It has attained a status in the field of such importance that it deserves to be examined here as an individual biotechnology. It also encompasses the classic reasons for seeking a biological solution to an industrial problem. The vast majority of commercially available desulfurization options involve hydrotreating. But the production of ultra-low sulfur diesel (ULSD) by hydrodesulfurization requires high H_2 consumption, high temperature and high hydrogen partial pressure equipment, which incurs high capital and operating costs. The need to cut these costs in a very competitive, low margin operation offers opportunities to non-hydrogen-consuming processes, operating at low reaction severity in term of temperature and pressure. Aerobic biodesulfurization is consistent with such a strategy. It has attractions from an environmental perspective in its sparing production of greenhouse gases[28].

The principal biochemistry that has been investigated for commercial BDS is the 4S pathway, as present in *Rhodococcus* sp. IGTS8[29]. It is worth noting that this pathway to sulfur removal from DBT involves two monooxygenases, Fig. 9.

Rhodococcus is an exemplar genus for such an operation due to the possession of a hydrophobic cell surface, Fig. 10. As a result, *Rhodococcus* is able to access very hydrophobic substrates such as dibenzothiophenes from the oil phase, where they are present in high concentrations, rather than the aqueous phase (as in the case of *Pseudomonas* in Fig. 10), where these compounds are only available in much lower concentrations[30].

In general much of the work on biodesulfurization has dealt with its integration within an existing refinery treating oil fractions: gasoline or diesel. As much of the research work was driven by oil refiners, this interest is primarily a reflection of the investigators focus. In comparison, much less efforts were devoted to desulfurizing crude oil. Combining BDS and hydrotreating required extremely high activities for the biocatalyst to deliver conversions in very low residence times.

In the case of crude oil desulfurization, where the operation can be done in storage tanks, the constraint of residence time is relaxed. Here, however there is a need for new enzymes that have very broad specificity, thus making attack on a wider range of sulfur heterocycles possible. There is plenty of evidence in the literature of a search for more suitable naturally-occurring enzymes[31, 32], but there has also been a focus on genetic engineering to improve existing enzymes[33, 34]. Genetic manipulation of bacteria will play a major role in delivering activities of commercial interest. The Biotechnology Group in Saudi Aramco's R&DC is embarking on one such collaboration with the Illinois Institute of Technology (ex-GTI) where most of the early work on genetic modification took place.

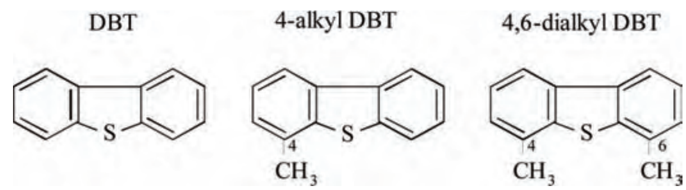

Fig. 8. DBT and related sulfur heterocycles occurring in fossil fuels.

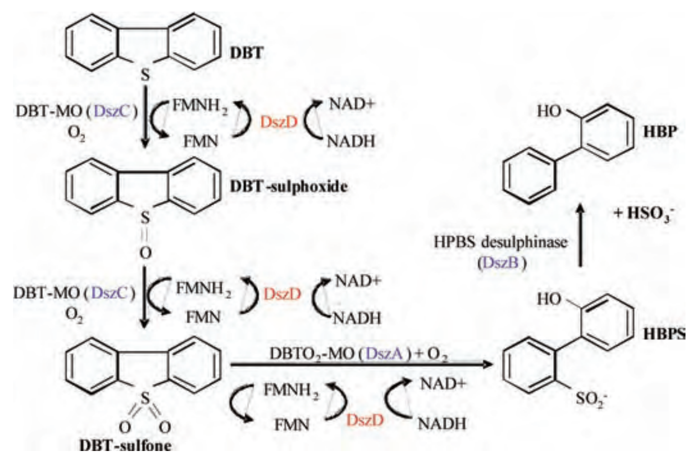

Fig. 9. The 4S pathway of DBT biodesulfurization.

Fig. 10. Phase distribution of Rhodococcus *and* Pseudomonas *cells.*

The engineering problems relate to the need for suitable reactors that can accomplish an intimate mixing of fuel and aqueous phases. All the available evidence suggests that microbial hydrocarbon processing occurs in the interior of cells in what is effectively an aqueous environment. For example, Bouchez et al.[35], unambiguously demonstrated that phenanthrene biodegradation required prior transfer to the aqueous phase. Multiphase reactors that sustain intimate mixing of fuel with water and bacteria at low temperatures and pressure present challenges for biochemical engineering that are as yet unresolved.

BIODENITROGENATION

In a manner similar to the legislation applied to sulfur in fossil fuels, regulation of the nitrogen content will become increasingly stringent. Nitrogen is also present in crude oil and fuels as aromatic heterocycles such as pyrroles, indoles and carbazoles. Pyrrole and indole are readily biodegradable, but carbazole exhibits recalcitrance. Aromatic hydrocarbon heterocycles inhibit hydrotreating, poison catalysts[36], and some are toxic and mutagenic. Like sulfur heterocycles, nitrogen heterocycles are generally removed from fuels by hydrotreating under high temperature and pressure conditions[37].

Once again the bacterial route to denitrogenation is via oxygenases. The initial steps of attack on aromatic nitrogen heterocycles involve the formation of dihydroxylated derivatives by dioxygenases. While recent advances have been made on the microbiology of biodenitrogenation[38, 39], we are not yet close to a working process. In fact, given the similarity in the biocatalysis, to make economic sense denitrogenation needs to be integrated with desulfurization[40]. From Saudi Aramco's perspective, like biodesulfurization, biodenitrogenation has to prove itself on crude oil, rather than oil fractions.

BIOREMEDIATION

Bioremediation refers to the clean up of pollution from soil, groundwater, surface water and air using biological, usually microbiological, processes[41]. It has left the laboratory and is established in some parts of the world as a full-scale, biotechnology-based industry. Bioremediation utilizes the same biochemistry as mentioned several times in this article – the activation of hydrocarbons by insertion of oxygen by oxygenases. Although, here the purpose is different. In bioremediation, complete biodegradation (mineralization) of the compound(s) of interest is sought. That is, the conversion of hydrocarbon to biomass, carbon dioxide and water under aerobic conditions.

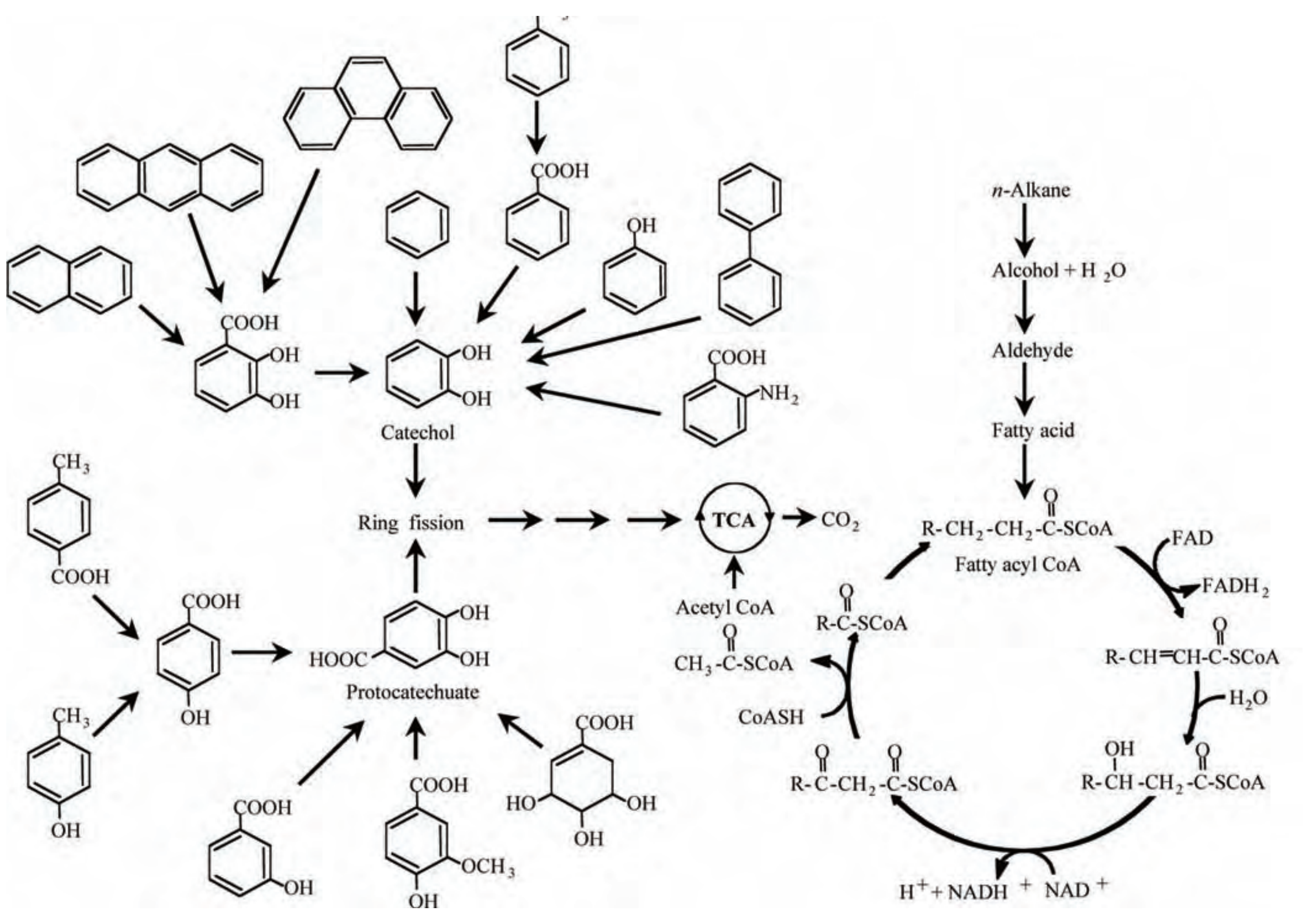

Fig. 11. Bacterial catabolism of hydrocarbons to central metabolites.

How this is achieved is by converging biochemical pathways. The remarkable economy of bacteria can be illustrated, Fig. 11. A vast number of hydrocarbons are converted to just two key intermediates, catechol and protocatechuate. From this point, ring fission occurs, and by a relatively few steps the ring fission products are converted to central metabolites. This economy has great significance to bacteria. It means that for the biodegradation of a new compound, there is no need to develop a complete new biochemistry. For example, the biodegradation of phenylacetic acids appears to have a common aerobic pathway in different bacteria, as suggested by metabolic products and gene sequences[42]. Second, by possession of such capabilities, the bacterium that does so extends its substrate range, thereby putting it at an ecological advantage in the survival struggle.

The technologies for bioremediation have now been developed that allow the treatment of tens of thousands of tons or more of contaminated soil or water in one operation. The technologies are divided between those which are done on the surface or at a dedicated treatment facility (*ex-situ*) and those where the soil or water is not moved (*in-situ*). *Ex-situ* technologies offer a greater degree of process control and are generally faster than *in-situ* treatment. *In-situ* technologies are becoming more popular as they are more cost-effective when the contamination is deep, thus making excavation uncompetitive. The most common *ex-situ* technologies are biopiles and windrows. The soil is graded and heaped with various materials such as bulking agents, and nutrients are applied to stimulate hydrocarbon oxidation by microorganisms. In biopiles forced aeration is practiced, whereas turning of windrows achieves aeration using a variety of equipment. These bioremediation technologies and others are described in greater detail by Philp & Atlas[43].

The U.S. EPA[44] has published a compendium of costs for six common remediation technologies: bioremediation; thermal desorption; soil vapor extraction (SVE); on-site incineration; groundwater pump-and-treat systems; and permeable reactive barriers (PRBs). A unit cost/quantity treated correlation was evident for four of the technologies - bioventing, thermal desorption, SVE and pump-and-treat systems, and economies of scale were observed with all four, i.e., the unit cost decreased as larger quantities were treated. Of these four, bioventing showed the best correlation.

INFORMATION SOURCES

The primary information required for a new oil biotechnology project is the existence of the necessary biochemistry for a biotransformation to occur. There are a number of sources of microbial metabolism information, and the most convenient are those with a dedicated Web site. These have been outlined recently by Wackett[45]. The most comprehensive of these of value to oil biotechnology is the University of Minnesota Biocatalysis/Biodegradation Database, which has been available for more than a decade[46]. It contains information on over 900 compounds, over 600 enzymes, nearly 1,000 reactions and about 350 microorganisms. It has a useful feature that a prediction can be made of the pathway for biodegradation of a particular compound[47]. The database and prediction system develop under the direction of the scientific community.

FUTURE PERSPECTIVES

Legislation has systematically driven down the level of sulfur in diesel, and this is set to continue in the future. Legislation is often a driver of innovation, and biotechnology has an increasing role to play. On the horizon, for example, will be the removal of metals, in particular nickel and vanadium, from low grade marine diesels and therefore possibly from crude oil. These metals exist in porphyrin-like organic compounds in crude oil, and therefore may be a target for biodegradation[48]. Apart from environmental concerns, vanadium is of concern during combustion, as vanadium pentoxide is formed, which is very corrosive to heat-resisting, high alloy steels. This can be a particular problem in turbines[49].

It has previously been noted that the arene *cis*-diols are used as synthons in the development of new industrial and medical products, and that these are easier to produce through a biotechnology approach than through synthetic chemistry. It is now possible to produce arene *cis*-diols by whole cell biotransformation processes in the multi-ton scale. But the world market for these novel chemicals currently remains in the tens of kilograms. There has been plenty of patenting in the area, and it may be that the global development awaits the first "big" product, as often happens with technology-driven manufacturing. If the significant bioprocessing problems can be overcome, there will be an increasing demand for high-value, low-volume chemicals by oil biotransformations. There is a double stimulus if this biotransformation can be done from unwanted components of oil. For example, the near future will see a reduction in the amount of benzene allowed in gasoline. Toluene would also be a good target for bioconversion.

It is also worth mentioning that there must be many potential bio-products that can be made from oil that are as yet not thought of. The highly chemically diverse nature of oil; and the enormous biodiversity of the bacteria are good partners for each other in a generation of new chemicals. And the future may even see the revival of old technologies that can be brought back to production due to the revolution in genetic engineering. For example, *n*-butanol has been manufactured in the past by fermentation by *Clostridium saccharobutylicum*, but the process became uncompetitive in cost. Due to recent technological advances, biobutanol will be used in the future as a gasoline additive[50].

CONCLUSIONS

A lot has changed since Atlas published the seminal book Petroleum Microbiology in 1984[51]. That book concentrated to a large extent on academic issues of microbiology. It also pre-dated the revolution in genetic manipulation that was heralded by polymerase chain reaction (PCR) technology which has made possible many of the genetic techniques mentioned in this article. Paradoxically, however, at that time a lot of attention was being given to bioreactor design because that was the era of single cell protein (SCP) from hydrocarbon feedstocks. Even then, hydrocarbon utilization by microorganisms was being described as "the major topic of microbiology that it is today[52]." A significant area of omission in the 1984 book was upstream applications. Even today, we are only beginning to understand the anaerobic microbiology of oil reservoirs, with its greater technical demands.

An interesting comparison can be made with a relatively new book of the same title[53]. While much of the subject matter is similar but brought up-to-date through huge changes in genetic engineering, other topics covered in this book are: biodegradation of petroleum in subsurface geological reservoirs; microbial enhancement of oil recovery; and petroleum upgrade through the use of biotechnology. Clearly the upstream has begun to take its place in petroleum biotechnology.

These two books and the spatial separation between them are a reminder that, despite the unusual nature of petroleum as a substrate for microbial growth, it is a subject of both massive academic interest and biotechnological potential that has endured for a longtime and has a secure and fascinating future. The Biotechnology Unit of Saudi Aramco is involved both in combating the negative aspects of microbes and in harnessing their positive uses for the benefit of the company.

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BIOGRAPHIES

Dr. James C. Philp holds a B.Sc. degree from the University of Edinburgh, a M.Sc. and Ph.D. degree from Napier University, Edinburgh, all in Microbiology or related disciplines. His Ph.D. is in Microbial Corrosion of Mild Steel used in canisters for the deep disposal of high level nuclear waste. Jim first worked for Saudi Aramco from 1987-1991 in the Laboratories Department performing projects relating to the microbiology of oil field and water facilities within the company. He returned to his native Scotland to take up an academic post in 1992, and worked for nearly 15 years in applied microbiology and biotechnology as a lecturer, researcher and consultant. Jim served as coordinator of the UK LINK Bioremediation Programme from 2001-2006, a national program of scientific projects dedicated to the clean up of contaminated land and water using microorganisms. He returned to Saudi Aramco in November 2006 to join Research & Development Center (R&DC) as a Science Specialist. Jim is currently on a temporary assignment to King Abdullah University of Science and Technology (KAUST). He has authored over 250 scientific articles, and is co-author of a book on bioremediation.

Dr. Tony Y. Rizk holds a B.Sc. in Industrial Engineering and graduated with a Ph.D. in Corrosion Science from Manchester University in 1992. After graduation, he worked for Capcis Ltd., in Manchester, UK for over 14 years. Tony has generated and led a number of reservoir souring and corrosion projects and worked on investigating the various effects of nitrate biotechnology for some 10 years. He also generated numerous confidential reports on the oil field microbiology and corrosion control for a number of oil majors, chemical suppliers and other industries. Tony has authored and co-authored a number of papers and numerous technical reports mainly on reservoir souring, nitrate treatment and corrosion inhibitors. In 2006 he authored the IP research report to the Energy Institute entitled "The Effect of Nitrate Anti-Souring Treatment on Corrosion of Mild Steel" ISBN: 978 0 85293 458 6. He joined Saudi Aramco in July 2006 as a Senior Scientist in the Research & Development Center (R&DC) Biotechnology Group in Dhahran, Saudi Arabia. Tony is currently the Project Leader for the nitrate biotechnology project (one of the biggest projects in R&DC). Tony is an active member of the Microbiology Committee of the Energy Institute, and currently is the chair of the committee for organizing the Reservoir Microbiology Forum (RMF) planned for November 2008 and the chair person of the conference.

Dr. Kouider Guerinik works in the Biotechnology Group at Saudi Aramco's Research & Development Center (R&DC). He is the Project Leader for the crude oil biodesulfurization work. Kouider graduated with a Ph.D. in Biochemical Engineering, and joined Unilever Research, UK to work on the biotechnology application for chemicals and food. He has worked on a number of bioconversion/biocatalytic processes as alternatives to the chemical processes. Kouider subsequently joined the Biotechnology Department in KISR, Kuwait, where he worked on the biotechnology application for petroleum and environmental problems. In particular, he worked on the oil biodesulfurization in collaboration with Japan Petroleum & Energy Center (JCCP), on waste sulfur utilization through sulfur-oxidizing bacteria with Idemitsu Kozan, Japan. For the last 2½ years, Kouider has been leading the Research & Development Division (R&DD) work on biodesulfurization for crude oil, as well as expanding biotech work in the R&DD. Overall, he has over 20 years experience in Biotech R&D spanning diverse fields: petroleum, chemicals, environment and food, and is a member of Who's Who in Science & Engineering for his work in Biotechnology.

Mazen A. Al-Saleh joined Saudi Aramco's Research and Development Center (R&DC) in November of 2001 as a Lab Scientist. He is currently working in the Biotechnology Group of the Research & Development Division (R&DD) and he joined the Engineering Service's Specialist Development Program (SDP) in June 2005, targeted to become one of the company's specialists in petroleum microbiology. Mazen acquired his B.S. degree in Chemistry at Toledo University, OH. Prior to his employment at Saudi Aramco; he served as a Chemist at the National Methanol Company (IBN-SINA) an affiliate of SABIC. His professional interest lies in biotechnology. Mazen's research focus has been in the development of a biotechnology process to upgrade Saudi Aramco crude oil for improvement of oil properties.

Dr. Peter F. Sanders is a Research Science Consultant and Water Systems Project Leader in Saudi Aramco's Research & Development Center (R&DC) in Dhahran, Saudi Arabia. He holds a B.Sc., M.Sc. and Ph.D. degree in Microbiology from Exeter University in the UK. Peter worked for 12 years as a Senior Microbiologist and Research Manager of Oil Plus Ltd., an oil field consultancy company in the United Kingdom, working on solving microbiological problems for most of the major oil field operators all over the world. Prior to that, he was a Research Fellow at Aberdeen University, Scotland, and ran a small oil field microbiology company for 8 years, servicing the developing North Sea oil industry. Peter joined Saudi Aramco in 2001, and has been working on developing new chemical solutions to combat microbial corrosion, biofouling and contamination problems in oil production and utilities systems. He has recently been studying downhole microbial growth, developing novel biotechnology processes for upgrading crude oil quality *in-situ* and to enhance oil production efficiencies, as well as developing resources and competencies in the Biotechnology Group of the Research and Development Division (R&DD), R&DC.