ZoBell's contribution to petroleum microbiology

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ABSTRACT

Claude ZoBell's recognition of the influence of microorganisms in the ecology of petroleum bearing environments was all encompassing. This translated into a unique contribution over the entire span of the oil industry. Many of his publications in the 1940s and 1950s discussed the key role of microorganisms in the diagenesis of hydrocarbon products. At a time when the industry was treating the supply of crude oil as an almost inexhaustible reserve, ZoBell's thoughts were turning to improved oil recovery using bacterial products such as acids and gases to aid mobilisation and he also recognised the inevitable problems of oil spill pollution and the potential for use of bacteria as control agents. In all areas of the oil industry today there is an increasingly clear recognition of the influence of associated microflora. Perhaps if there had been wider recognition of Claude ZoBell's work throughout the middle decades of this century more microbially mediated improved oil recovery and bioremediation treatments would be in place today.

Introduction

There have been few microbiologists with as wide a knowledge of the petroleum industry as Claude ZoBell, with both a clear understanding of the influence of bacteria in the ecology of oil reservoirs and also a well developed sense of the value of harnessing the activities of those same bacteria to improve oil recovery, or modify the product. He was a member of both the American Petroleum Institute (API) and the American Association of Petroleum Geologists (AAPG) as well as maintaining long term consultative associations with major oil companies such as Texaco Development Corporation (ten years) and Exxon Research (fifteen years).

Petroleum microbiology can be divided into six broad areas, starting with diagenesis of organic components in sediments and subsequent oleogenesis, degradation of hydrocarbons, improved recovery of hydrocarbons from reservoirs, modification of hydrocarbon products either in the formation or post production, mitigation of the effects of 'nuisance organisms' during production and bioremediation of escaped product either crude or processed. Claude ZoBell worked in most of these areas, documented in his output of 66 papers between 1938 and 1978 (of a total 248), and related to one or other of these aspects of petroleum microbiology. His legacy is an unique contribution to the oil industry which is still consulted today, offering source material in many diverse areas of the petroleum industry and also for the growing community of subsurface microbiologists.

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Part played by bacteria in petroleum formation

Following ZoBell's appointment at Scripps Institute of Oceanography (SIO) in 1932 he concentrated on the study of marine bacteria [1, 2] and their role in the transformations of organic material in sediments [3, 4]. The 1938 publication [3] was important in that it described the existence of a rich microflora inhabiting ocean bottom deposits, samples having been taken using a modified Ekman coring device which ensured uncontaminated samples of sediment. ZoBell was able to disprove the assertions of what he termed the 'popular literature', that high hydrostatic pressure and low temperature are inimical to the existence and activity of bacteria. In particular he highlighted the activities of bacteria cultured from the sediments whose 'biochemical activities may geologically modify their environment'.

In 1941 he presented a report on the origins of oil at a meeting of the AAPG in Tulsa [5], which came to the attention of leading US geologists who were setting up a comprehensive research programme into the origins of petroleum. This was a matter of national importance, since the advent of the Second World War had prompted a rigorous inventory of US oil reserves to serve the USA's global military needs. The API commissioned a three centred research study comprising chemists and biochemists at Pennsylvania State University, geophysicists at Massachusetts Institute of Technology and a team of marine biologists and microbiologists at Scripps Institute led by ZoBell. A steady stream of papers appeared under his direction between 1942 and 1954 in a variety of journals and oil industry publications which were collectively submitted in biennial reports 2-6 of the API Research Project 43A entitled 'Fundamental Research on Occurrence and Recovery of Petroleum'.

ZoBell and his co-workers attempted to demonstrate an incontrovertible bacteriological link between the hydrocarbon precursors produced during sediment diagenesis and actual petroleum generation [6]. Although sure that the process was mediated by microorganisms the task of providing absolute proof eluded them, with data suggesting both hydrocarbon production and degradation being attributable to activity of sulfate reducing bacteria in marine sediments [7, 8]. In one of ZoBell's later publications on the topic [9] he stated 'If petroleum is formed from organic matter, it is almost axiomatic that bacteria have contributed to the process'. He continued by citing individual pieces of evidence, including methane production, synthesis of hydrocarbons as cellular components and anaerobic catalysis of hydrogen to a range of substances that may occur in oilfields. This was followed by a strong case for his developing theories on the existence of active indigenous populations of bacteria deep in oilfield reservoirs. In the same paper he discusses new themes relating to degradation of hydrocarbons by microorganisms and also their potential use as tools for improved recovery.

Action of microorganisms on hydrocarbons

ZoBell's work on oleogenesis which began in the late thirties naturally encompassed the biochemical corollary, that of hydrocarbon degradation by microorganisms. His comprehensive review published in 1946 [10] highlighted work carried out during the preceding 50 years in which attack and degradation of a variety of aliphatic, aromatic and naphthenic hydrocarbons were described. He recalled in a later paper [11] that even as late as 1942 the perceived wisdom had been that degradation of hydrocarbons was a 'biological curiosity', in spite of numerous, if scattered, publications which described microbial oxidation of liquid and solid hydrocarbons. Even the classic 1941 paper of Bushnell and Haas [12] which documented their ample evidence for bacterial utilisation of gasoline, kerosene and a variety of mineral oils, was not well accepted.

ZoBell's 1946 publication [10] was acknowledged to be a most thorough review and is still consulted for its source literature and far-reaching appreciation of the implications of hydrocarbon degradation. The ability of hydrocarbon oxidisers (bacterial and fungal) to attack a wide range of aliphatic and aromatic moieties in different conditions of mineral availability, oxygen tension, temperature and dispersion was examined and he collated all available data into a set of criteria to be used as indicators of hydrocarbon degradation. An important outcome was ZoBell's confirmation that the use of paraffin waxes or oils to exclude oxygen from biochemical oxygen demand (BOD) experiments could no longer be supported as a reliable technique due to the widespread occurrence of microbial oxidation of the paraffin substrate. In addition, the passage of oxygen through paraffin oil was found to be relatively easy at the depths often recommended as being exclusive to oxygen for such experiments.

A large section of the review dealt with the issue of dispersion of hydrocarbons and the influence of surface area on reaction rate and extent. ZoBell and his associates set up a series of experiments in which identical weights of paraffin wax dispersed in a variety of ways (from a single block to a thin coating on glass wool) were presented to a population of degradative bacteria in a mineral solution [13]. The unpublished results of this work, which showed the consumption of oxygen being directly proportional to the surface area of the substrate, may seem now to be a forgone conclusion but it represents fundamental knowledge as important to environmental microbiology today as related work which ZoBell had published a little earlier on the effect of solid surfaces on bacterial activity [14].

The overall conclusion was that petroleum hydrocarbons must be expected to be modified or completely destroyed by widespread and diverse hydrocarbon-oxidising microorganisms in soil, water and recent sediments. Accumulation of hydrocarbon deposits was therefore attributed to conditions which were hostile to microbial activity, either through the presence of specifically bacteriostatic components of some crude oils [13] such as certain heavy metals, presence of hydrogen sulphide or low redox [16].

The state of knowledge of deep subsurface microbiology in the 1940s and early 1950s was largely restricted to work on oil formations, and even this was limited at the time. 'Deep' oil formations were considerably shallower than some of the North Sea reservoirs of the 1980s and later [17]. However, it was known that bacteria capable of completely degrading petroleum could be recovered from oil well brines at depths up to 8700 feet [18] and that sulphate reducing bacteria frequently enriched from oilfield waters could attack some hydrocarbons [19, 20]. ZoBell was particularly fortunate to be able to draw on the wealth of data available in the Russian literature, translated by his wife Jean, which was more comprehensive than that available in the English language.

ZoBell's concluding remarks on the effects of microorganisms on petroleum products are in some respects still valid today, "It is an anomalous situation that the petroleum industry, praiseworthy for its many outstanding scientific and technological achievements, has devoted so little attention to the effects of microorganisms on petroleum or its products".

Facilitating the recovery of valuable fluids and improvement of oil

ZoBell was apparently successful at communicating something of this concern to his industry employers, as he was responsible for three patent applications, one on behalf of the API, and two under the patronage of Texaco Development Corporation. At a time when the industry was treating supply of crude oil as an almost inexhaustible reserve, ZoBell's thoughts were turning to improve oil recovery using bacterial products such as acids and gases to aid mobilisation. In US Patent No 2,413,278, entitled Bacteriological Process for Treatment of Fluid-bearing Earth Formations [21] he described a method for recovery requiring the user to deliberately introduce "microorganisms into subterranean formations for the purpose of beneficially influencing the factors which control the quantity of valuable fluids which can be can be recovered…". The invention described the use of bacteria to increase the recovery of oil or gas by increasing porosity of formations (solubilization of calcareous material), releasing fluids from surfaces (acid and gas production), facilitating flow in formations due to surfactant generation, increasing gas pressure (carbon dioxide production) and reducing viscosity of oils (degradation of heavy oils). ZoBell had demonstrated to his own satisfaction that these properties were all to be found in the chosen species, *Desulfovibrio hydrocarbonoclasticus.*

At the time when ZoBell was preparing this work, there was little indication of the severe problems which were to be encountered in later years due to the growth of sulphate reducing bacteria in reservoirs and their contribution to hydrogen sulphide souring [22, 23], corrosion and plugging of formations. This may be attributed to the fact that the majority of wells were still land based at the time, and that where secondary recovery techniques such as water injection were employed, the water injected was low in sulphate, thus avoiding the problems now so prevalent in offshore facilities following sea water injection. Microbially enhanced oil recovery techniques do now have a place as tertiary recovery options, but none is reliant on injecting SRB into the formation. Either a nutrient package is introduced which stimulates the indigenous population into generating by-products which increase formation porosity and aid mobilisation of oil, or organisms may be injected and stimulated into growth in specific zones of the reservoir to promote water flow diversion and increase oil output.

It became clear in the early fifties that the quality of combusted oil products needed to be improved significantly. Refining of high sulfur crudes was expensive because the plant was damaged rapidly due to corrosion, and fuels made from crude oil laden with sulfur-containing heavy oil fractions were releasing sulfur dioxide on combustion, creating acid rain. ZoBell's second patent application in 1953 was therefore timely in its appearance, describing an anaerobic process for removing sulfur from petroleum hydrocarbons (US Patent No. 2,641,564). This relied on a topside treatment plant, operating either as a batch or continuous system in which sulfur was removed by microbial hydrogenation. ZoBell surveyed a wide range of soil and oilfield organisms in order to determine the contribution each might make to the process. The concept of microbial desulfurization still exists today as an improvement technique, although a more rapid aerobic option is favoured and in use as post refining technology in the USA, using *Rhodococcus* spp. which oxygenate dibenzothiophene (DBT) without loss of hydrocarbon. ZoBell's third, and even more farsighted patent, related to a similar sulfur reduction process which was designed to take place in the formation by injection of organisms capable of *in situ* desulfurization, mobilization, and improved recovery; surely an admirable target, but one which still eludes the industry today.

The occurrence, effects, and fate of oil polluting the sea

Zobell was responsible for writing another key review article, of this title, published in 1963 [25]. Like the 1946 publication on the effects of microorganisms on hydrocarbons, this has proven to be another invaluable first resource to researchers and draws together the work he did twenty years earlier with the necessity of predicting the fate of oil spills in a variety of natural environments. His paper charts the progress of legislation which appeared just after the First World War, the first time in history that large quantities of fuel oil were being transported, in preference to coal, to power the war effort. Merchant marine traffic soon followed the naval example, precipitating the need for primary legislation in 1922 and 1924 on each side of the Atlantic. The damage and sinking of hundreds of ships and tankers in the Second World War did little to improve matters, with seepages of oil sometimes occurring years after the event due to corrosion of fuel holds in unrecovered vessels. Since then the traffic in fuel and petrochemicals by sea has increased out of all proportion, and in some cases out of control where governments have been unable or unwilling to regulate shipping. A second paper [26] written as the preface to a workshop at the Center for Wetlands Resources in 1973 was almost the last contribution ZoBell made to the literature in the field of petroleum microbiology.

Conclusion

The contribution of Claude ZoBell to the advancement of knowledge and application of that knowledge to petroleum microbiology has still to be fully realised. In many respects while the petroleum industry was forging ahead on the technology of oil production, ZoBell's foresight was setting the scene for new microbial technology which will be of increasing importance in the 21st century. Perhaps the only area in which his work has been less prominent is that of the control of souring in water-flooded offshore facilities, yet that can only have been an accident of time. The opportunities for improved oil recovery, modification of petroleum products, knowledge of subsurface microbiology and remediation of oil polluted environments have all been greatly enriched by his thorough approach and ability to write informatively.

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