Claude E. Zobell – his life and contributions to biofilm microbiology

Hilary M. Lappin-Scott

Environmental Microbiology Research Group, Hatherly Laboratories, University of Exeter, Exeter, EX4 4PS, UK

Introduction

It is a little unusual to dedicate an entire symposium to the work of one microbiologist. However, it became apparent to me that it was wholly fitting to give such a tribute to Claude E. ZoBell. I became interested in the publications of ZoBell during my formative years working on biofilms with Dr J.W. Costerton. In looking into the history of biofilms and growth of bacteria on surfaces I found that several of ZoBell's early publications were most informative. I thought no more about the matter until I started researching into petroleum microbiology only to find that ZoBell had been active in that field too! On appointment to a Faculty position I began researching for my lectures within Environmental Microbiology, only to find that ZoBell was present as a founding father in many other fields. Thus began my interest in the research of Claude ZoBell and from this a curiosity about the man behind the research. This chapter briefly covers ZoBell's early years as a scientist, focusing largely on his work on biofilms. The ensuing chapters cover his contributions to petroleum microbiology (C.J. Bass), barobiology (A.A. Yayanos) and the geosciences (H. L. Ehrlich). These four chapters are by no means an exhaustive account of ZoBell's research, that task awaits a biographer to deal with the topic in the detail that it fully deserves.

Claude E. ZoBell - the man

The family of Claude Ephraim ZoBell originated in Denmark, where the family name was Pederson or Hanson. ZoBell's grandfather (and his grandfather's brother) decided that it was appropriate to have a new name for a new country on arrival in the USA and found the name of Zobel amongst their ancestry. The capital B was added for phonetic purposes and some of the ancestors of the two brothers added a second l to the surname too.

ZoBell was born in 1904, the third of seven children. The family lived in Rigby, Idaho (where he attended Rigby High School) and were members of the Church of Jesus Christ of Latter-Day Saints. From 1924 to 1926 ZoBell was a teacher and the Principal at Rigby, then he left to return to his studies. He attended Utah State Agricultural College (now Utah State University) where ZoBell worked part time in the Bacteriology department to help to fund his studies. He graduated with a BSc in Bacteriology in 1927 and received his MSc in 1929.

ZoBell moved to the University of California at Berkeley for his PhD studies and he was awarded the Thompson Scholarship. His PhD was supervised by Dr Karl Meyer on the growth requirements and metabolism of *Brucella*. This period of research was very fruitful; ZoBell and Meyer coauthored nine papers between 1930 and 1932. ZoBell was awarded his PhD in 1931. His research on *Brucella* developed growth media which are still used today.

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At the time there was a general belief that *Brucella* spp. lacked the ability to reduce nitrates. ZoBell and Meyer reported that over 400 strains of *Brucella* could reduce nitrates but that nitrite did not accumulate as it was utilised rapidly [1].

After the award of his PhD ZoBell took up a position as an Instructor in Marine Microbiology (in 1932) at the Scripps Institute of Oceanography (SIO) and remained on the faculty of Scripps throughout his working life. Here he made a break from medical microbiology and remained largely in the subject of environmental microbiology. He was made a full Professor in 1948 and of Emeritus status in 1972.

ZoBell received many acclaimed awards for his research and many positions in other organisations and it is not possible to list them all here. A brief selection includes Research Associate at the University of Wisconsin in 1938, he published a highly regarded textbook entitled 'Marine Microbiology' in 1946, studied in Europe in 1947 after the award of the Rockefeller Foundation Fellowship and worked at the University of Princeton during 1948. Between 1950 and 1952 ZoBell was a member of the *Galathea* deep sea expedition (this expedition is dealt with in detail in the chapter by A.A. Yayanos). In 1957 ZoBell was the ANZAAS lecturer in Australia and New Zealand. He was the founder of the journal Geomicrobiology in 1976.

ZoBell married in 1930 and had two sons (Dean and Karl). The marriage was dissolved in 1945 and he married Jean Switzer in 1946. Jean worked with him throughout his career, frequently accompanying him on his travels. Claude ZoBell died in 1989.

ZoBell - One of the pioneers of biofilm microbiology

The early influences on biofilms and biofouling

What were the factors influencing ZoBell around the mid 1920's to early 1930's ? Some of the other pioneers of the concept that microbes attach to surfaces included Winogradsky [2], Cholodny [3], Conn [4] and Henrici [5]. The work of Winogradsky, Cholodny and Conn all involved submerging glass slides into soil for specific time intervals then observing the attached microbes using microscopy. They noted that the bacteria that grew attached to the slides differed from those that they could culture from the water phase (an important observation in biofilm research stemming from the early 1930's). Henrici used a similar approach to study the attachment of fresh water bacteria and observed that 'for the most part water bacteria are not free floating but grow attached to surfaces'. All reported that bacteria attached to the surfaces and that the populations were diverse and grew to form microbial films. When commencing his research at SIO ZoBell had access to the ocean, he described the pier at SIO as extending 300 metres seawards from the shore, ideal for commencing his research into marine microbiology! ZoBell was well aware of the research into the damaging effects of biological growth on surfaces submerged into seawater. For example, ZoBell had read of the research undertaken at SIO by Wilson in 1925 [6] on marine algal succession on surfaces. Again, in the same year, both Hentschel [7] and Thomasson [8] looked at the attachment of diatoms to glass, but not the attachment of bacteria. ZoBell had read the report by Hilen from 1923 [9], describing the 'slime on ships' and he knew of the biofouling interests of the U.S. Navy.

ZoBell's research on bacterial attachment to surfaces

ZoBell's contributions to biofilm microbiology were from his early work, on first appointment to SIO. These span from the early 1930's to the mid 1940's and many were co-authored by Esther C. Allen.

All of the above publications highlighted the growth of sessile bacteria on surfaces (sceptics of the use of term 'sessile' please note that it has been widely applied to describe bacteria attached to surfaces for at least 65 years!) and the role of biological growth in biofouling of these submerged surfaces. However, ZoBell commented that most research focused on the life histories of the barnacles, molluscs, hydroids, bryozoa and other macrofouling organisms and did not consider the sequence of the initial events of colonisation or the relationship of one group of organisms to another. ZoBell and his colleagues designed a 'slide carrier' which could be fitted with glass slides and lowered over the end of the pier [10, 11]. This apparatus was his first reported design to study bacterial attachment to surfaces.

The carrier was made of lead covered with wood and the whole structure was then coated with paraffin. The lead gave anchorage and stability to the carrier once it was submerged and the wood and paraffin kept the glass slides from having direct contact with the metal. The wood had grooving to hold the glass slides and they were secured into position using string (a schematic of the slide carrier is provided in ZoBell and Allen [10]). The slide carrier could hold 12-16 slides. The carrier was then lowered into the sea and marine microbes allowed to colonise the glass. The slides were removed after between 1 to 7 days [10, 11] and the attached populations were examined. ZoBell and Allen reported that there was a greater diversity of bacteria on the glass slides than they could grow from seawater in culture media. They isolated 73 pure cultures from the films (most were Gram negative rods) and found that of these 24 attached firmly (they defined this as those that were not removed by running water). Some of the bacteria only grew in 'attached films' (the term used by ZoBell for biofilms). Attachment was rapid to clean surfaces, ZoBell observed that it took between 2-4 hours for 'appreciable numbers' to attach. There was evidence that the bacteria were growing on the slides rather than merely attaching to them and ZoBell and Allen described microcolonies of growing bacteria [10, 11]. The highlights from these early studies included: -

1. that it was difficult to ascertain the nature of the attaching mechanism,
2. that some 'material' was present that extended beyond the cells and allowed the cells to be grouped together in islands of the material
3. the tendency to attach was effected by the nutrients present
4. many of the bacteria were capsulated and that some appeared to have a holdfast structure.

The nature of the adhering material has been the subject of much investigation since this report by ZoBell and the nature of the attaching mechanism is still difficult to ascertain [12-16]!

Other publications by ZoBell which are noteworthy for biofilm microbiology were by ZoBell and Anderson [17] and ZoBell [18,19]. These reported that when seawater was collected in sterile glass bottles that there were more bacteria present on the surface of the glass as sessile bacteria than those in the free floating planktonic phase. ZoBell concluded that the nutrients in the seawater were probably concentrated on the solid surfaces and were

thus attracting the bacteria and enhancing their growth and survival. Again, ZoBell found that many marine bacteria were sessile and that they grew preferentially attached to a surface and this area has been further studied since then [20-23]. ZoBell postulated that biofilm holds the nutrients, bacteria and their enzymes close to the surface and therefore they can damage the surface because of this close association [24].

ZoBell developed these concepts of biofilms in one of his later publications on bacterial growth on surfaces [25]. This publication reviewed his earlier work on the attachment of bacteria to glass slides and also made detailed comparisons of the planktonic and sessile populations of seawater and the manner in which these increased in number within stored seawater. Of particular interest to ZoBell was the apparent lack of any relationship between the population size of the planktonic and the sessile fraction, there were millions of attached bacteria but only hundreds of bacteria present in the planktonic phase. He used laboratory studies to test his hypotheses of the survival of bacteria on surfaces. From these he noted that not all of the marine bacteria could attach to surfaces. Of those that did attach some were present as single cells, pairs or occasionally long chains, no thick films were reported. The bacteria were firmly 'glued' to the slide. ZoBell tried to identify them but reported the difficulty of this when working with environmental samples - a phenomenon that many of us can relate to! Many of the marine biofilm bacteria were halophiles and several were new species of *Achromobacter* and *Flavobacterium* , although the latter was renamed as a pseudomonad in his publication of 1943 (ZoBell used the methods of the Committee on the Pure Culture Study of Bacteria, 1930, as his guide for this work). Diatoms were also present within his marine biofilms, but these were less numerous than bacteria yet more abundant than macroscopic organisms.

ZoBell's work during the 1930's and 1940's on biofilms led him to develop a sequence of events at the surface which explained biofouling [10,11, 17-19, 24-25]. Throughout many of his experiments he recorded the numbers of bacteria, other microscopic organisms and the numbers of macroscopic organisms and how these changed during colonisation. From these he considered that the first colonisers were bacteria and that these are rapidly joined by diatoms and actinomycetes. The film that they form then 'favored' subsequent attachment by larger, fouling organisms such as barnacles and hydroids.

Conclusions

I consider that ZoBell made many outstanding contributions to the field of biofilm microbiology including knowledge that: -

* nutrients are concentrated on surfaces.
* there are more bacteria on submerged surfaces than in the seawater.
* bacterial attachment to surfaces is very rapid.
* microcolonies develop on surfaces.
* the attachment is active rather than passive.
* planktonic bacteria are not covered in 'sticky' material but sessile bacteria are and the sessiles secrete a 'cementing' substance.
* the attachment tendency was influenced by the available nutrients.
* it is difficult to ascertain the attachment mechanism.

Present day “biofilmologists” would benefit greatly from studying the work of ZoBell to appreciate many of the origins of the subject. It is sobering to realise that many of the phenomena of biofilm microbiology that are still being discussed today as novel findings were reported by ZoBell over 60 years ago!

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