A Tribute to the Pioneering Work of Thomas E. Jensen in the Field of Cyanobacterial Phytoremediation

Richard F.E. Crang*

Department of Plant Biology, University of Illinois at Urbana-Champaign 61801, USA

***Corresponding author:** Prof. Richard F. E. Crang, Department of Plant Biology, University of Illinois at Urbana-Champaign 61801, USA, Phone: +1 708-601-8020, Email: r-crang@life.illinois.edu

Abstract

The impact of the scientific works of Prof. Thomas E. Jensen has often been overlooked. This review paper brings together the focus of his works in a developmental narrative revealing the stages and their practical applications over the course of time. The stages of his research involved the ultrastructural revelations of cyanobacterial organization, the quantification of key components, recognition that sequestering of selected heavy metals and other cations could occur under stress conditions, using that knowledge to formulate applications in waste-water remediation, and developing a model system for both the retention of specific ions and their controlled release. It was found that the release could be predictably controlled by both pH adjustments as well as by regulating the oxygenation of the environmental conditions. Laboratory pilot studies have indicated that the prospects for large-scale applications in municipal and industrial wastewater conditions are possible. This type of phytoremediation has far greater possibilities in commercial circumstances than efforts with higher plants, in as much as the cells can be recycled for continuous ion processing. This review is a partial summary of some of the relevant academic works of Prof. Jensen over a span of nearly 50 years. His research has primarily been directed towards a better understanding of the organization and activities of cyanobacteria, and the special role that they are capable of playing in phytoremediation.

Key words: Cyanobacteria, Polyphosphate bodies, Heavy metals, Phytoremediation.

1. Introduction

In recent years, considerable attention has been given to phytoremediation involving the utilization of seed plants for the uptake of ion pollutants, including that of heavy metals in soils and/or wetlands. Such work was envisioned to encompass phytoextraction, rhizofiltration and phytostabilization (Salt *et al.*, 1995). Recognizing the difficulties in conducting controlled experimental measurements in field studies, efforts have been made to utilize simulated conditions (Ouyang *et al.*, 2005). However, few studies have concentrated on using microorganisms, and most have been focused on immobilization protocols employing the use of anions including oxide, hydroxide, chloride, sulfate, sulfide, phosphates, molybdate and carbonate (Porter *et al.*, 2004) for chemical containment.

Thomas Edgar Jensen (Fig. 1) received his Ph.D. in 1965 from Iowa State University at Ames, Iowa and began his academic career at Wayne State University in Detroit, Michigan that same year. It was a time in which electron microscopy began to play a significant role in the fine structure of organisms, and his work in Botany was centered on elucidating the ultrastructure of cyanobacteria (then referred to as blue-green algae). His work involved finding the right type of preservative/fixative agents needed for reliable and reproducible imaging with transmission electron microscopy. In 1969 Dr. Jensen moved to the City University of New York (CUNY) where he established new laboratory

Fig. 1: Dr. Thomas E. Jensen (1932 – 2014). His work spanned some 50 years with a dedication to the understanding of cyanobacteria, their structure, function and useful applications. Ill health prevented his further leadership role and he died in 2014 in New York State

facilities in conjunction with his research. He remained at CUNY until his retirement in 2010. Through efforts at the time, Dr. Jensen was able to identify various newly discovered structures within cells of cyanobacteria and to trace their movement into dividing cells. From that, Dr. Jensen drew comparisons with other cyanobacteria to determine the extent of common structural organization. At

that time, it was found that most cyanobacteria survived in phosphate-rich waters that were also becoming oxygen depleted due to the abundance of the organisms. Many of these waters were contaminated in natural settings with a variety of heavy metals, and the combined factors prevented most other life forms from inhabiting those environmental conditions. Dr. Jensen's research then took an experimental turn in which he and his colleagues investigated the uptake of inorganic phosphates and discovered that such uptakes often were accompanied with heavy metal uptake as well.

With the early development of energy-dispersive X-ray microanalysis in conjunction with scanning electron microscopy (and later with transmission electron microscopy), the Jensen researchers began to trace and to identify specific heavy metal uptake into inorganic phosphate inclusions in the cells. While qualitative results were first established, the work then progressed into more quantitative studies that were based on controlled laboratory experiments. With further studies it was also found that some conditions existed that would favor the breakdown of the inorganic phosphates along with the concomitant release of contained heavy metals. This opened the concept of employing cyanobacteria for the possible containment of heavy metals from wastewaters, and their controlled release in wastewater management of industrial and municipal contaminated sites.

The combined studies over the period of more than four decades have revealed the unique properties of cyanobacteria related to ionic uptake, containment over time, and conditions of controlled release, which have helped open a field of study with broad environmental applications. It is therefore the purpose of this paper to document the progressive series of events pursued in a lifetime of studies by Prof. Jensen and his colleagues that have contributed uniquely to the field of phytoremediation.

2. Documentation

2.1. Cyanobacterial ultrastructure

In the early work on descriptive transmission electron microscopy while at Iowa State University, Bowen and Jensen (1965) investigated the ultra-structure of *Aphanizomenon flos-aquae*, which revealed the presence of unique gas vacuoles which could be collapsed by induced pressure, and that appeared to be bound by unit membranes in the collapsed state, but by half-unit membranes when expanded by gas. It was later established to be a mechanism for cell flotation under favorable conditions, and the changes in expansion were due to pressure relationships regulated by a unique vesicle membrane protein designated GvpC (Walsby and Hayes, 1988).

Jensen also carried out further studies on *Nostoc pruniforme* in which he was the first to characterize inorganic polyphosphate bodies at the ultrastructural level (Jensen, 1968, 1969). Staining for the polyphosphates was carried out in a protocol involving 20% $Pb(NO_3)_2$ at a pH value of 3.4 and then a transfer to 1% $(NH_4)_2S$ for 30 min as a modified procedure from that by Ebel *et al*. (1958).

Later, ultrastructural studies encompassed some 60 different collections of blue-green algae from at least 30 species, mostly in the Nostocales, gathered from various field sites in Iowa and from established culture collections (Jensen and Bowen, 1970). They revealed that heterocysts were comprised internally of membranous scrolls, which were not unlike the appearance of myelin sheaths that encompass the axons of neurons (Fig. 2), and that there were a

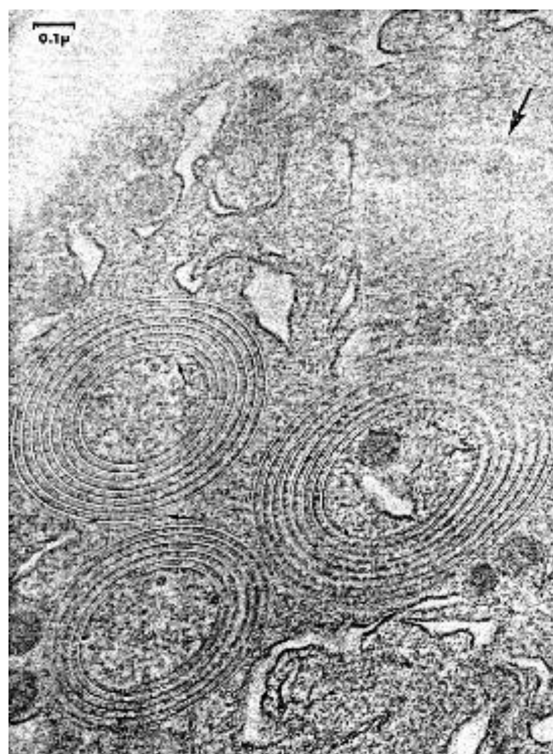

Fig. 2: Scrolls found in heterocysts of *Gloeotrichia pisum*. Large arrow points to a longitudinal view of the structure. The two small arrows point to the beginning (inside) and end (outside) of the spiral. The scrolls are interpreted as being cylindrical

variety of intrathylakoidal granules, polyhedral bodies, aggregates of filament bundles and crystalline inclusions, all of which were of unknown function at that time. In other studies Jensen, and his collaborators, explored the fine structure of a number of cyanobacteria species and reported on unique inclusions including such bodies as the cell wall and coat of akinetes (Jensen and Clark, 1968; Clark and Jensen, 1969; Sicko-Goad and Jensen, 1981), microtubules, microfilaments and polyhedral bodies (Jensen and Ayala, 1976, 1980), paracrystalline inclusions (Jensen, 1978) and tri-lamellar bodies (Jensen, 1979).

While many of these investigations revealed newly discovered organelles, as well as a description of their development and organization for the first time; subsequently the object of primary interest emerged as the unique polyphosphate body. This occurred during laboratory work in which dynamic changes were found in these bodies in respect not only to the phosphate content, but also concomitant changes in accompanying cations—in particular certain heavy metals.

2.2. Discovery and applications of polyphosphate bodies

Jensen early reported polyphosphate bodies imaged with transmission electron microscopy in *Nostoc pruniforme* (Jensen, 1968) were correlated with dense structures that was previously observed with light microscopy (Fig. 3). It was later recognized

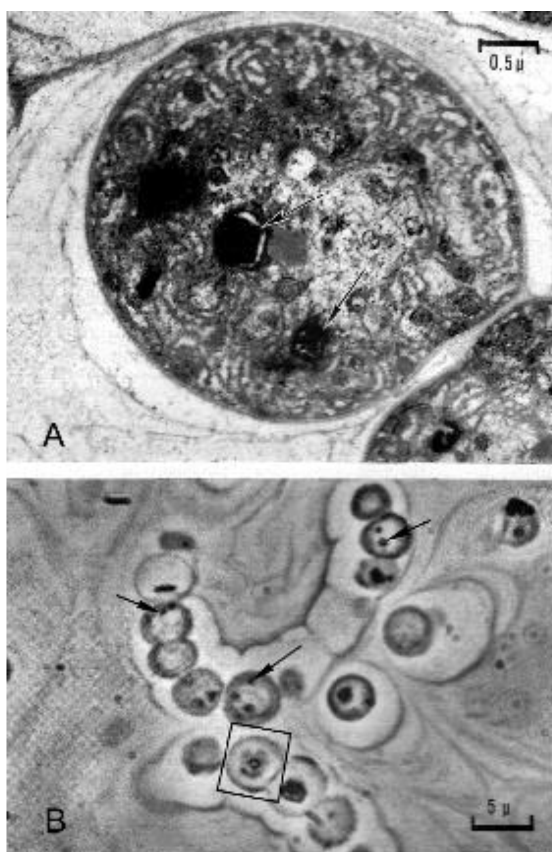

Fig. 3: A. Section through a cell of *Nostoc pruniforme* observed with transmission electron microscopy and showing two polyphosphate bodies (arrows). B. Adjacent thick section observed with light microscopy. The same cell shown in A, is indicated by the box in B. Arrows show the sites of polyphosphate bodies in various cells

by Sicko-Goad and Jensen (1976) that external phosphate concentration influenced the distribution of phosphous-containing compounds in *Plectonema boryanum*. Using TCA extraction techniques, they found that cells, which were phosphate-starved for five days, accumulated large quantities of orthophosphate from the culture medium, most within the first hour after exposure to a phosphate-containing medium. This followed their investigation into what they designated as a "polyphosphate overplus" phenomenon (Jensen and Sicko, 1974). It was found that cells of *Plectonema boryanum* grown in a full nutrient laboratory medium could be transferred for up to five days into a medium with all the same nutrients but lacking phosphates (called a starvation medium). At the end of that time, transferring the cyanobacteria cells back into the complete medium containing phosphates resulted in a rapid uptake of the compound, and its incorporation into the polyphosphate body structures indicated by enlarging dense bodies contained within vacuoles as observed with electron microscopy. It was later shown by Crang *et al*. (1988) that during cell division chromosomes maintained an association with polyphosphate bodies in the imperfect fungus *Aureobasidium pullulans*. Additional studies involving the uptake of cadmium into the microalga *Chlorella saccharophila* and two diatom species, *Navicula incerta* and *Nitzschia closterium*, showed lower levels of intracellular incorporation, especially with the diatoms, but no evidence of retention into polyphosphate bodies was available at that time (Rachlin *et al*., 1982). Thus, while a number of other roles of polyphosphates could be documented, the primary focus of phosphate uptake and retention primarily remained as a study within the cyanobacteria.

2.3. Analytical microscopy

However, while a strong body of facts indicated the incorporation of phosphates and possible accompanying heavy metals into cyanobacteria polyphosphate bodies, the confirming evidence and the potential for quantitative values became possible with the development of energy-dispersive X-ray (EDX) microanalysis in conjunction with both scanning and transmission electron microscopy. This procedure enabled the investigator(s) to specifically localize, and to make qualitative and quantitative analysis of elemental composition comprising specific objects imaged with the electron beam. Early efforts to this experimental direction were reported by Crang and Jensen (1975) and Sicko-Goad *et al*. (1975). Those results showed that polyphosphate bodies may contain the major fraction of total cellular phosphorus which could be disassembled and incorporated into intracellular molecular structures (e.g. nucleic acids, ATP, and other conjugated phosphates) as needed. Due to that important concept, it was speculated that the uptake of heavy metals was really a secondary effect in the functioning of polyphosphate bodies.

In order to avoid extraction artifacts involved in using conventional fixation and embedding preparation techniques, air-dried fresh samples were employed which gave stronger EDX signals, which

indicated less ionic loss. This procedure was then repeated with a cyanobacterium in semi-quantitative analysis of the polyphosphate bodies (Jensen *et al*., 1982). Also by using EDX techniques, Jensen *et al*. (1986) were able to show that each heavy metal had one or more ions which were accompanying it; for example when Cu was taken up S was also contained, but at the expense of K inclusion in polyphosphate bodies. Thus, it was concluded that heavy metals had a substantial effect on the ion balance in cells. In fact, as heavy metals (such as Cu) became bound to compounds such as proteins, that the biding site may displace the normal elemental S which is then transferred to a polyphosphate body that in turn releases K. For example Fig. 4 shows the concomitant uptake of anions such as S, using EDX procedures (Baxter and Jensen, 1980). The percent volume occupied by polyphosphate bodies at various time intervals following P starvation is illustrated in Fig. 5.

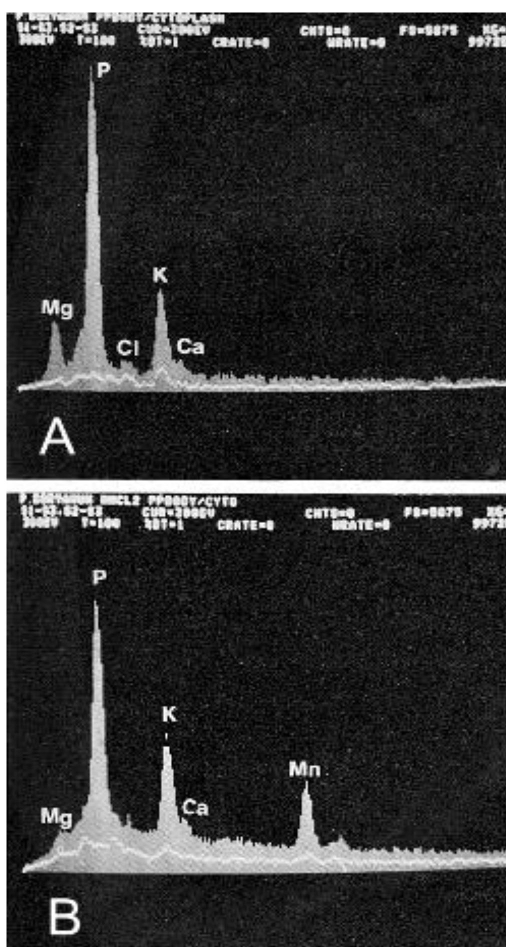

Fig. 4: A. EDX spectrum showing counts from a polyphosphate body of *Plectonema boryanum* after growth in an "overplus" culture. The light dotted line below represents counts from the cell cytoplasm. B. Same scale EDX spectrum from a cell grown for 4 weeks in an overplus culture with the addition of $MnCl_2$. The white dots represent cytoplasmic counts

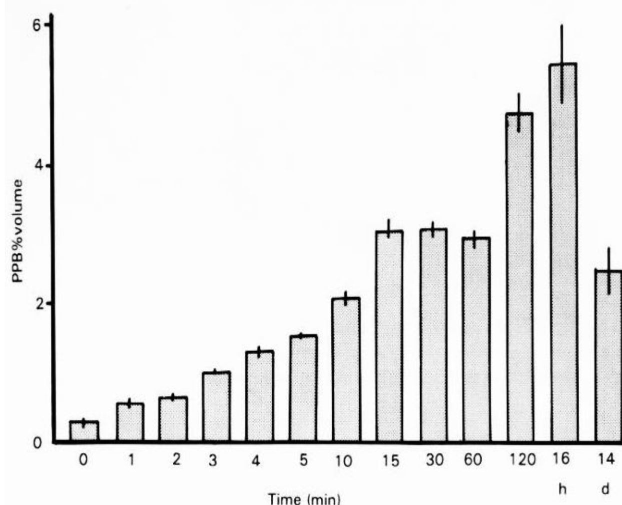

Fig. 5: Bar graph showing the % relative volume from 0.269 to 5.401% of *Plectonema boryanum* cells occupied by polyphosphate bodies at different times (in min) from removal out of starvation medium (0 min) to 120 min. Phosphate level was maximum after 16 hr (next to last bar on right), but dropped by 50% in a stable condition after 14 days (last bar on right)

2.4. Quantification methods

A variety of approaches were undertaken to better quantify the ionic composition of polyphosphate bodies by the Jensen team during the late 1980s and through the early 2000s. These included morphometric techniques that began with the utilization of procedures described by Weibel and Bolender (1973) and relative volumes of intrathylakoidal spaces, number and relative volume of polyhedral bodies, lipid bodies, granules, membrane-limited crystalline inclusions and relative volume of the cell wall were determined by Rachlin *et al*. (1985). Relative volumes are the percentage of a structure within the overall cell volume. But soon the primary attention turned to the relative volume of the polyphosphate bodies encountered in their experimental studies. These included research into developing polyphosphate bodies of a cyanobacterium (Baxter and Jensen, 1986), and that in methylotrophic bacteria (Jensen and Corpe, 1988). In *Plectonema* (a cyanobacterium), the percent volume of polyphosphate bodies from 5-day starved cells was at 0.269 but rapidly increased to 5.401 after a 16-hr exposure to a phosphate-rich medium. The question remained, however: "Would heavy metal uptake be at a concomitant rate even when different elements were studied"? This posed an additional problem in as much as the heavy metals could not be defined with electron microscopy alone, but required techniques such as EDX to be somehow coupled.

An elegant study by Goldberg *et al*. (2001) showed that with scanning-transmission electron microscopy (STEM), a fixed electron beam spot mode of energy and time duration on a cell

component could be standardized to determine mass/weight of small latex spheres (1.05 g per cm^3). This study utilized calculation of Heldel (1993) that showed:

$M_{sp} = (W_{sp} \times a)/C_{sp}$

Where M_{sp} is the total mass of a polyphosphate body, W_{sp} = the continuum height of the EDX peak of a particular element minus background noise, a represents the spherical region analyzed in a polyphosphate body, and C_{sp} is the latex sphere correction in the same energy window of the EDX spectrum generated. This led to further calculations that expressed quantitative data under experimental conditions.

2.5. *Controlled uptake and release of cations*

With such determinations as a reference, similar calculations could be made on the mass/weight of polyphosphate bodies under experimental conditions, and was first reported by Goldberg and Jensen (1999). It was further found that the enhanced uptake, sequestering and release of heavy metals from polyphosphate bodies could take place prior to protein denaturation. The control of this process brought about considerable speculation, which in turn led to the concept that aerobic-anaerobic environmental changes could play a significant role. By building on that, attention turned to the use of alternating growth in aerated and non-aerated culture media in order to force the formation and the release of polyphosphates (anions) and their accompanying cations into the surrounding medium. This was first reported by Hagan-Brown and Jensen (2002) at the Microscopy and Microanalysis meeting in Quebec City, Canada. The effort to force the release of inorganic phosphates (anion) and associated cations was also determined to relate to the pH level, there always being a greater release of PO_4^- at more alkaline pH values in the range of pH 7.2 to 9.0. Jensen and coworkers set about to design a pilot project in the laboratory that would aim to incorporate the principles of polyphosphate body accumulation along with that of heavy metal cations, and to precisely release these elements at will (Jensen *et al*., 2008).

2.6. *Pilot studies for phytoremediation*

The procedure involved a radically different approach in which a selected cyanobacterium could be encapsulated into sodium alginate beads that were porous enough for fluid exchange of nutrient media as well as controlled amounts of experimental ions (typically heavy metals). In order to do this, the researchers designed a "bead-maker" (Fig. 6) that produced alginate beads of uniform and reproducible dimensions containing the experimental cyanobacterium in an encapsulated form.

Then, an experimental chamber, termed a "flow-cell", was also designed and built that contained alginate beads with encapsulated cells that could be subjected to varying pH values in the culture media, and also to experimental levels of oxygenation and other factors of air quality (Fig. 7). The flow-cell

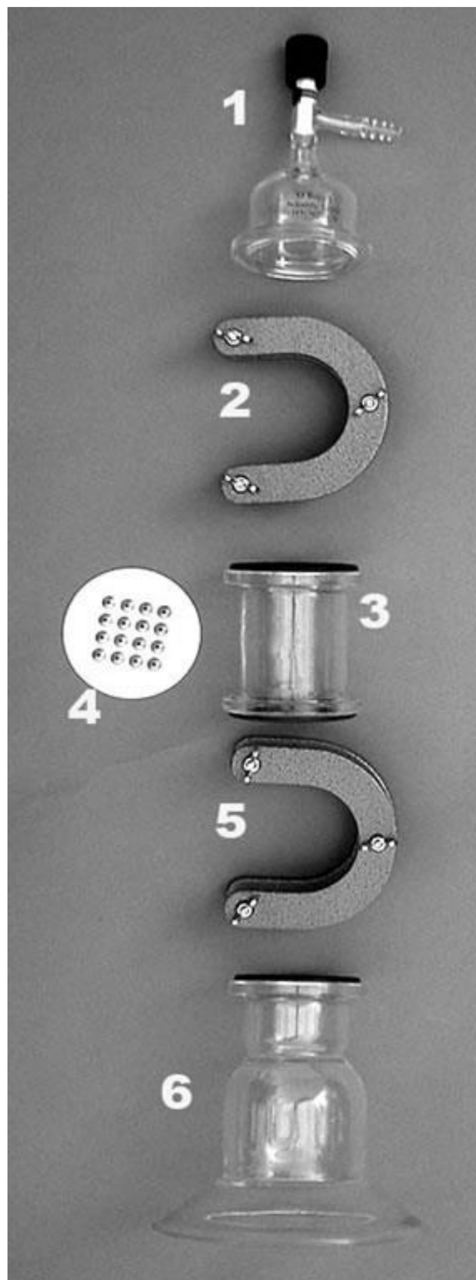

Fig. 6: A disassembled bead-maker showing all the component parts. 1) Top unit with sidearm to which a hand-held bulb-type air pump and gauge can be attached for pressure regulation as needed. Above the side arm is a plastic adjustable valve for backfilling of air or other gas. 2) Horseshoe clamp for holding units 1 and 3 together. 3) Reservoir for fluid sodium alginate with mixed cellular materials. 4) Aluminum separation plate with pores for the flow of the alginate mixture. 5) Lower horseshoe clamp for holding units 4 and 5 together. 6) Bottom unit serving as a receptacle for alginate-specimen droplets that fall into a bath of $CaCl_2$ for hardening

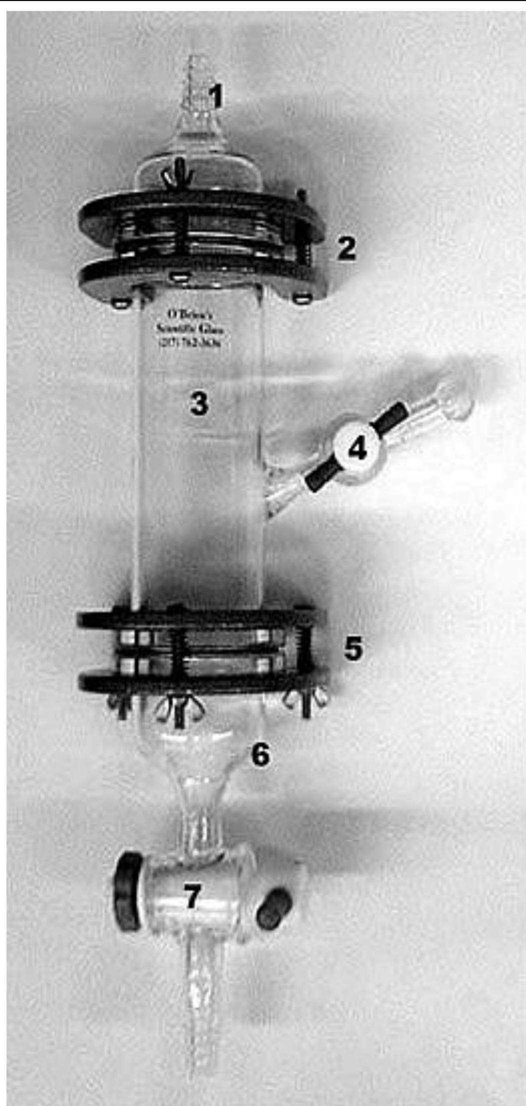

Fig. 7: An assembled flow-cell showing: 1) top fitting for flow-cell. 2) Upper horseshoe clamp for holding top fitting to flow-cell body. 3) Flow-cell central body. 4) Adjustable side arm for the extraction of fluids and beads. 5) Lower horseshoe clamp for holding bottom fitting. 6) Bottom fitting. 7) Adjustable valve for regulating flow and extraction of contents as well as allowing the upward flow of gasses such as N_2 through the complete assembly

assembly enabled encapsulated living cells to be exposed to selected concentrations of phosphates and of heavy metals for experimental purposes. While encapsulation provides a useful means of handling the microorganisms in the laboratory, non-encapsulated cell could also be used with the flow-cell assembly and precluding the use of a bead-maker. In either case, specimen samples could be quickly extracted and injected into fixative solutions or into a cryogenic medium for preservation or for low temperature investigations, and virtually without exposure to air. The Jensen investigators reached a point where pilot studies have paved the way for practical field studies in phytoremediation, especially under circumstances where it has been impossible to work with seed plants.

3. Conclusions

In this report, only a portion of the literature produced by Thomas Jensen and his co-workers has been reported in the direction of progressive events towards phytoremediation. Well over 100 peer-reviewed papers were published by Jensen and his research associates primarily on the subject herein addressed, as well as nearly an equal number of presentations at national and international scientific meetings were produced over approximately a 50-year span. A substantial number of these reported studies have given rise to "branch" studies by other investigators. However, despite these widespread publications, many have been overlooked in the recent literature on phytoremediation. The purpose of this review paper is to acknowledge the insightful direction that Prof. Jensen took in his career, and the significant applications that have been made through his research efforts.

References

Baxter, M. and Jensen, T.E. 1980. Uptake of magnesium, strontium, barium and manganese by *Plectonema boryanum* (Cyanophyceae). *Protoplasma* **104**:81-89.

Baxter, M. and Jensen, T.E. 1986. Cell volume occupied by polyphosphate bodies during the polyphosphate overplus phenomenon in *Plectonema boryanum*. *Cytobios* **45**:147-159.

Bowen, C.C. and Jensen, T.E. 1965. Blue-green algae: Fine structure of the gas vacuole. *Science* **147**:1460-1462.

Clark, R.L. and Jensen, T.E. 1969. Ultrastructure of akinete development in a blue-green alga, *Cylindrospermum* sp. *Cytologia* **34**:439-448.

Crang, R.E. and Jensen, T.E. 1975. Incorporation of titanium in polyphosphate bodies of *Anacystis nidulans*. *Journal of Cell Biology* **67**:80A.

Crang, R.E., Jensen, T.E. and Doonan, B.B. 1988. The association of polyphosphate bodies with chomosomes during cell division in *Aureobasidium pullulans*. *Cytologia* **53**:83-90.

Ebel, J.P, Colas, J. and Muller, S. 1958. Recherches cytochimiques sur les polyphosphates inorganiques contenus dans les organisms vivants. II. Mise au point de methods de detection cytochimiques spécifiques des polyphosphates. *Experimental Cell Research* **15**:28-36.

Goldberg, J., Gonzalez, H., Jensen, T.E. and Corpe, W.A. 2001. Quantitative analysis of the elemental composition and the mass of bacterial polyphosphate bodies using STEM EDX. *Microbios* **106**:177-188.

Goldberg, J.J. and Jensen, T.E. 1999. Quantitative elemental analysis of heavy metal exposed cells of *Synechococcus leopoliensis* using regular and overplus cells: An energy-dispersive X-ray spectroscopy study. *Microscopy and Microanalysis* **5**:586-587.

Hagan-Brown, J. and Jensen, T.E. 2002. Uptake and release of phosphorus from polyphosphate bodies by *Synechococcus leopoliensis*. *Microscopy and Microanalysis Annual International Conference*, Quebec City, Canada.

Heldel, M. 1993. Measurement of elemental content and dry weight of single cells: X-ray microanalysis. In: Kemp, B.F. *et al.* (Eds.) *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, FL, pp. 800.

Jensen, T.E. 1968. Electron microscopy of polyphosphate bodies in a blue-green alga, *Nostoc pruniforme*. *Archiv für Mikrobiologie* **62**:144-152.

Jensen, T.E. 1969. Fine structure of developing polyphosphate bodies in a blue-green alga, *Plectonema boryanum*. *Archiv für Mikrobiologie* **67**:328-338.

Jensen, T.E. 1978. Paracrystalline inclusions in various isolates of the blue-green bacteria *Nostoc* and *Anabaena*. *Cytobios* **22**:179-190.

Jensen, T.E. 1979. Occurrence of tri-lamellar bodies in isolates of *Nostoc* (Cyanophyceae). *Journal of Phycology* **15**:112-114.

Jensen, T.E. and Ayala, R.P. 1976. The fine structure of a microplate-microtubule array, microfilaments and polyhedral body associated microtubules in several species of *Anabaena*. *Archives of Microbiology* **111**:1-6.

Jensen, T.E. and Ayala, R.P. 1980. Microtubule-like inclusions in isolates of the blue-green bacteria *Anabaena* and *Nostoc*. *Cytologia* **45**:315-326.

Jensen, T.E. and Bowen, C.C. 1970. Cytology of blue-green algae. II. Unusual inclusions in the cytoplasm. *Cytologia* **35**:132-152.

Jensen, T.E. and Clark, R.L. 1968. Cell wall and coat of the developing akinete of a *Cylindrospermum* species. *Journal of Bacteriology* **97**:1494-1495.

Jensen, T.E. and Corpe, W.A. 1988. Utilization of morphometric analysis in evaluating the ultrastructure of various methylotrophic bacteria. *Cytobios* **53**:159-171.

Jensen, T.E. and Sicko, L.M. 1974. Phosphate metabolism in blue-green algae. I. Fine structure of the "polyphosphate overplus" phenomenon in *Plectonema boryanum*. *Canadian Journal of Microbiology* **20**:1235-1239.

Jensen, T.E., Baxter, M., Rachlin, J.W. and Jani, V. 1982. Uptake of heavy metals by *Plectonema boryanum* (Cyanophyceae) into cellular components, especially polyphosphate bodies: An X-ray energy dispersive study. *Environmental Pollution (Series A)* **27**: 119-127.

Jensen, T.E., Crang, M.C. and Crang, R.F.E. 2008. Equipment design for biosorption studies with microorganisms. *Electronic Journal of Biotechnology* **11**:1-6.

Jensen, T.E., Rachlin, F.W., Jani, V. and Warkentine, B.E. 1986. Heavy metal uptake in relation to phosphorus nutrition in *Anabaena variabilis* (Cyanophyceae). *Environmental Pollution (Series A)* **42**:261-271.

Ouyang, Y., Shinde, D. and Ma, L.Q. 2005. Simulation of phytoremediation of a TNT-contaminated soil using the CTSPAC model. *Journal of Environmental Quality* **5**:1490-1496.

Porter, S.K., Scheckel, K.G., Impellitteri, C.A. and Ryan, J.A. 2004. Toxic metals in the environment: Thermodynamic considerations for possible immobilization strategies for Pb, Cd, As, and Hg. *Critical Reviews in Environmental Science and Technology* **34**:495-604.

Rachlin, J.W., Jensen, T.E. and Warkentine, B.E. 1985. Morphometric analysis of the response of *Anabaena flos-aquae* and *Anabaena variabilis* (Cyanophyceae) to selected concentrations of zinc. *Archives of Environmental Contamination and Toxicology* **14**:395-402.

Rachlin, J.W., Warkentine, B. and Jensen, T.E. 1982. The growth responses of *Chlorella saccharophila*, *Navicula incerta* and *Nitzschia closterium* to selected concentrations of cadmium. *Bulletin of the Torrey Botanical Club* **109**:129-135.

Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I. and Raskin, I. 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Nature Biotechnology* **13**:468-474.

Sicko-Goad, L. and Jensen, T.E. 1976. Phosphate metabolism in blue-green algae. II. Changes in phosphate distribution during starvation and the "polyphosphate overplus" phenomenon in *Plectonema boryanum*. *American Journal of Botany* **63**:183-188.

Sicko-Goad, L. and Jensen, T.E. 1981. Fine structure of the cell wall of the cyanobacterium *Microcoleus vaginatus* (Vauch.) Gomont. *Cytobios* **30**:19-27.

Sicko-Goad, L.M., Crang, R.E. and Jensen, T.E. 1975. Polyphosphate metabolism in blue-green algae. IV. *In situ* analysis of polyphosphate bodies by X-ray energy dispersive analysis. *Cytobiologie* **11**:430-437.

Walsby, A.E. and Hayes, P.K. 1988. The minor cyanobacterial gas vesicle protein, GvpC, is attached to the outer surface of the gas vesicle. *Journal of General Microbiology* **134**:2647-2657.

Weibel, E.R. and Bolender, R.P. 1973. Stereological techniques for electron microscopic morphometry. In: Hayat, M.A. (Ed.) *Principles and Techniques of Electron Microscopy.* Van Nostrand-Reinhold, New York, pp. 237.