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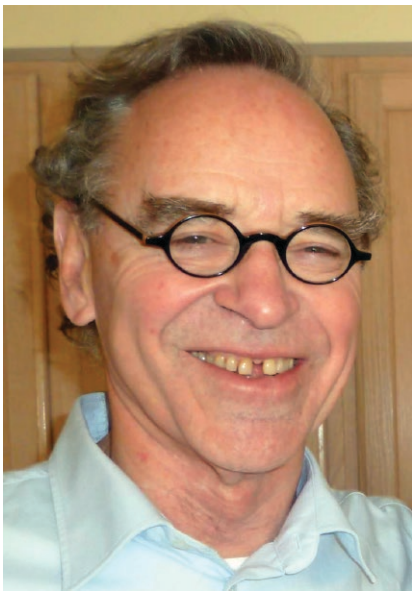
In Memoriam of Prof. Bernard Witholt



Bernard Witholt

With a sad feeling we had to learn that Prof. Bernard Witholt is not with us anymore. He passed away on February 28th in his 75th year after a short illness. Bernard grew up in Brasil as one of six children of Dutch parents. In 1959 they moved on to U.S.A., where he graduated at Amherst College and accomplished his doctoral thesis at Johns Hopkins University in Baltimore, followed up by a postdoctoral appointment at the University of California, San Diego. In 1972 he became Professor in Biochemistry at the University of Groningen, the Netherlands, where he developed novel bioreactors that were typically suitable for two-phase fermentations with organic substrates having low water solubility.

In Groningen, he became one of the driving forces of the Groningen Biotechnology Centre as well as the Science Park Groningen. One of the buildings on this park would later be named after him. He was rewarded with a substantial prize of the local newspaper, half of it to be spent on his research, and half of it for a goal of his own choice. Typical for Bernard Witholt, he chose to throw a great party for family and friends, enabling family members to fly in from the States and celebrate together.

In 2007, a year after his retirement at the ETH, he was rewarded by Queen Beatrix with the title of Knight in the Order of the Netherlands' Lion for his achievements in the field of biotechnology in Groningen and the Netherlands.

It was also in Groningen where he described for the first time a hitherto unknown large class of carbon storage biopolyesters in bacteria, the medium-chain-length polyhydroxyalkanoates (mcl-PHAs). When the strain *Pseudomonas oleovorans* was grown on *n*-octane as a second liquid phase under nitrogen limitation, it accumulated inclusion bodies similar to the ones previously found by other groups. Amazingly, the identification of mcl-PHA was triggered by observations of freeze-fractured *P. oleovorans* using scanning electron micrographs (Fig. 1) [1].

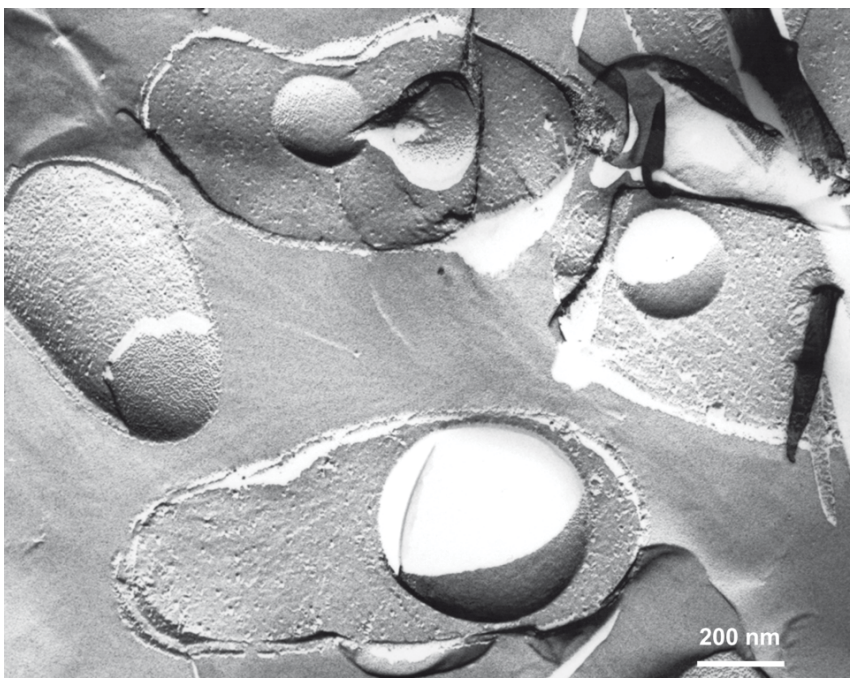


Figure 1. Scanning electron micrograph of freeze-fractured *Pseudomonas oleovorans* GPo1 containing PHA as inclusion bodies.

Fractured inclusion bodies consisting of mcl-PHA resulted in 3D structures like mushrooms and thus clearly differed from the needle type structure that was typical for short-chain-length PHAs such as poly(3-hydroxybutyrate) (PHB). Systematic analysis revealed that the new polymer consisted to a large part of 3-hydroxyoctanoates. Many PhD students in Groningen spent their days and nights with cultivating *P. oleovorans*, studying its growth performance in function of nutrient limitations but also identified and characterized the active enzymes for biosynthesis of mcl-PHA. In 1992, Bernard moved with his wife Renske Heddema and a large group of Dutch PhD students (Marcel Wubbolts, Jan van Beilen, Feike van der Leij, Wil Hazenberg, Marian Kraak, Maarten Nieboer, Qi Qu, Ivo Staijen) to Zurich, where he, together with Prof. Jay Bailey, succeeded Prof. Armin Fiechter in leading the Institute of Biotechnology at

ETH Zurich. With a lot of enthusiasm and contaminating optimism, he established a very productive research team active in the field of selective biocatalysis using oxidative enzymes and mcl-PHA biosynthesis combined with fermentation technology and genetic engineering.

All in all, 35 PhD students and many postdocs passed through his lab and profited from his guidance in scientific work. Typically as a PhD student one was asked to elaborate a written report that summarized the results recently achieved in the lab or the most recent manuscripts. One week later the report was discussed in an hour meeting, where he also made sure that the interpretation of the data were correct. He accomplished this by extremely quick calculations and also by considering a cell as a production facility with limited resources steadily looking for optimization. New concepts like the multiple-nutrient-limited growth in continuous cultivation proposed by Prof. Thomas Egli at Eawag, had a hard time in the beginning because this concept contradicted common theory in bioprocess technology. Finally, the experimental proof by Roland Durner and Manfred Zinn could convince him and since then he strongly supported this approach in several follow-up projects that also lead to the tailoring of the monomeric unit composition of PHAs. His ambition for excellence and perfection was also part of his private hobby: rowing in the team of ETH professors, which he continued until recently. In 2006 Bernard Witholt retired from ETH Zurich but kept a lab bench at Prof. Don Hilvert's lab, where he loved to exchange new ideas with postdocs and PhD students. Helping others in management and handling budget numbers was one of his strong competences. In the meantime, he had accepted a position in the Supervisory Board of the University Medical Centre in Groningen, where he monitored the decisions of the board of directors until his death in February 2015.

Bernard has also been such a great friend and mentor to all of us. Two of us (George Guo-Qiang Chen and Sang Yup Lee) have been working on polyhydroxyalkanoates for many years. We remember our many meetings with Bernard at the International Biopolymer Symposium (ISBP). During his fantastic talk on mcl PHA at the first ISBP meeting in Göttingen, Germany, people began to quote his statement that "the monomers of PHA must be limitless". As he correctly predicted, PHAs made by natural and metabolically engineered microorganisms now encompass a family of materials including homopolymers, random copolymers, block copolymers, graft polymers and functional polymers, more importantly, for each class of these polymers there are limitless possibilities to be further modified. No doubt, Bernard was the one who opened this area of vast diversity and limitless potential. Of course, he has made numerous other contributions including his famous biocatalytic conversion works. We also want to share one small less-recognized example of his creativity and enthusiasm. When he pioneered fermentation process using alkane as a carbon source, he became stuck with the oxygen transfer problem. It was amazing to see him actually build a new bioreactor with the highest agitation speed ever; if we remember correctly, he made a bioreactor with an agitation speed of 3000 rpm. Such a challenging engineering strategy Bernard developed is just one of many examples showing his creativity and his perseverance in research. His legacy consisting of numerous scientific high impact publications in the field of biopolymers and biocatalysis and his particular view on science in general will live on for many years to come.

We will all miss our great friend and mentor, Bernard Witholt. Thank you Bernard for everything you have done to us!

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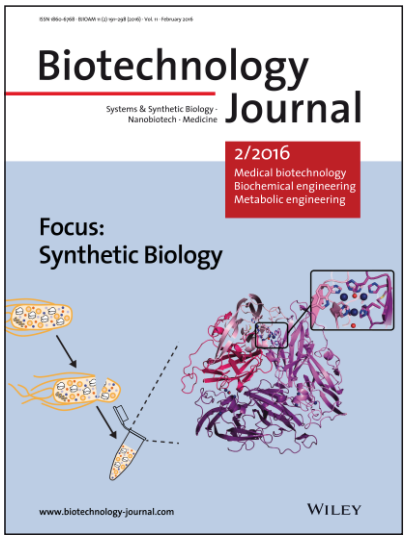
Reference

[1] de Smet, M.J., Eggink, G., Witholt, B., Kingma, J., Wynberg, H., Characterization of intracellular inclusions formed by *Pseudomonas oleovorans* during growth on octane. *J. Bacteriol.* 1983, *154*, 870–878.



Biotechnology Journal – list of articles published in the February 2016 issue.

Research Article

Alphavirus capsid proteins self-assemble into core-like particles in insect cells: A promising platform for nanoparticle vaccine development

Mia C. Hikke, Corinne Geertsema, Vincen Wu, Stefan W. Metz, Jan W. van Lent, Just M. Vlak and Gorben P. Pijlman

http://dx.doi.org/10.1002/biot.201500147

Research Article

Cell-free protein synthesis of a cytotoxic cancer therapeutic: Onconase production and a just-add-water cell-free system

Amin S. M. Salehi, Mark Thomas Smith, Anthony M. Bennett, Jacob B. Williams, William G. Pitt and Bradley C. Bundy

http://dx.doi.org/10.1002/biot.201500237

Research Article

Non-monotonic course of protein solubility in aqueous polymer-salt solutions can be modeled using the sol-mxDLVO model

Marcel Herhut, Christoph Brandenbusch and Gabriele Sadowski

http://dx.doi.org/10.1002/biot.201500123

Biotech Method

Rational plasmid design and bioprocess optimization to enhance recombinant adeno-associated virus (AAV) productivity in mammalian cells

Verena V. Emmerling, Antje Pegel, Ernest G. Milian, Alina Venereo-Sanchez, Marion Kunz, Jessica Wegele, Amine A. Kamen, Stefan Kochanek and Markus Hoerer

http://dx.doi.org/10.1002/biot.201500176