“Treasures from the sea”

Inaugurele rede van prof.dr.ir. R.H. (René) Wijffels bij de aanvaarding van het ambt van hoogleraar Bioprocestechnologie aan Wageningen Universiteit, 15 juni 2006

Mr. Rector, colleagues, family, friends, ladies and gentlemen,

In the past 8 years I have been working on a new area: marine biotechnology. It was a new area for me, for our university and for our country. We have been pioneering in this area and it feels great that Wageningen University awarded me with this great position.
The ocean is large and inside the ocean there is an enormous diversity in life. In general a great diversity in biology also results in a diversity in the chemicals produced by the organisms. In addition a lot of the life forms is dependent on chemical war. Many of the animals, like sponges and corals can not swim and the only way to protect themselves is by producing toxic chemicals. This is the reason that many of the natural products discovered nowadays originate from the sea. Marine biotechnology studies the methods to produce these marine natural products. We started to work in this area about 8 years ago. There are a number of leading institutes in the world in this field. Leading institutes are for example located in the USA: the Center of Marine Biotechnology in Baltimore SCRIPPS Institute of Oceanography in California and Harbor Branch Oceanographic Institution in Florida. In Japan there are many activities going on in the Marine Science and Technology Center. In Australia at the Australian Institute of Marine Science and in Europe in Scotland at the European Center of Marine Biotechnology and BiotekMarin in Germany. As the institutes did not work on technology development we could take a unique position. We wanted to bridge the gap between the discovery of new compounds and the applications. With a bioprocess technology approach we thought we could bridge that gap. In that respect we were pioneers in the field and we wanted to position ourselves in this field. We could build up a network via the organization of 2 international conferences; one in 1998, Marine Bioprocess Engineering, and one in 2003, Marine Biotechnology: Basics and Applications.
The reason we wanted to work in this field was because it was possible to produce new products with marine organisms. Products that could contribute to a better health, to new food ingredients and as new biofuels. Our challenge was to produce these products in a sustainable way so that the natural resources would not be over exploited. What we had to develop for this was methods to improve the production so that it could take place in an economical way. If we are able to do that this will result in new economic activities, companies will use the technologies and new companies will develop.
For us it meant in practice we had to develop bioreactors in which we could grow the organisms of interest and let them produce the components of interest. So we wanted to develop the bioreactor and inside that bioreactor control the cellular processes.
I would like to show you some of our activities and for that I will introduce 2 of our research themes: namely the marine pharmacy and photosynthetic cell factories. In marine pharmacy the goal is to produce natural compounds to be used as medicines in a controlled way. This is something we cannot do at present but we would like to develop the processes such that commercial production of medicines will be possible.
With photosynthetic cell factories our goal is to produce products on sun and seawater and increase the yield of production as much as possible. The productivity should be increased and we would like to make it possible that the products are produced at large scale. From there it is a small step to give my view on the relation science and business as well.
I will end this presentation by saying a few words about the title and of course I would like to thank a number of people that have been or are important in my career.

Marine Pharmacy

More and more medicines are developed from natural products. About 15,000 natural products have been described and about 30% of these natural products have been isolated from sponges. In the last few years approximately 75% of the registered antitumor patents are on products from sponges. These figures indicate that sponges have an enormous potential. It is therefore interesting to study how we can develop medicines from sponges.
Before I will say more about sponges I would like to say something in general about the development of medicines. Before a medicine can be brought on the market it needs to go through a lot of procedures where tests will be done to determine whether a medicine is safe, whether it has a curing effect and at which doses it should be applied. For that initially preclinical tests are performed on cell lines and animals. This is followed by clinical tests in 3 phases. Every phase involves more patients, initially the

focus is on safety an lateron more and more on the curing effect. Such a so called registration procedure takes in general 10 years of time and the costs are in the order of 1 billion euro. Apart from the registration itself the pharmaceutical companies should be able to produce the medicines as well. We work on the production of such compounds.
In recent years a number of marine natural products have been in development. According to a recent paper there are at present 44 marine derived natural products. There are 16 of those products in preclinical phase of development, 13 in phase 1, 13 in phase 2, 2 in phase 3 and none of them have been developed into a registered drug so far.
The products are developed to cure certain diseases. Of the 44 drugs 33 have anti cancer activity, 7 of them are used against pain, 3 of them against asthma, 1 of them against psoriasis and another one against Alzheimer. The products originate from a number of organisms. As you see many of them originate from sponges. This is the reason that we are so interested in studying sponges. Apart from sponges medicines originate from mollusks and ascidians as well.
Apart from the complex registration procedure for medicines there is a specific bottleneck in the development of marine drugs. The structure of the components is very complex. This molecule is halichondrin and it originates from a sponge. It can be chemically synthesized, but the number of reaction steps needed is so large that chemical synthesis is not an interesting option for production. An alternative would be to harvest the sponge from nature and isolate the compound. There would be insufficient sponge available to obtain sufficient medicines. At present there are some activities to grow the sponges in nature. The disadvantage of such methods is that production is uncontrolled and therefore it is difficult to guarantee product safety. In my view there are potentially 3 promising methods to produce those drugs. First of all there is the semi-synthetic production. In that case a part of the molecule is produced by a bacterium and the final synthesis is done chemically. In this way the number of synthesis steps is reduced. The second one is transferring the genetic information from the sponge into a microorganism and produce the product in the microorganism. Although this seems simple, the number of biochemical synthesis steps makes also this procedure difficult. The final one is to isolate the cells from the sponge and grow those cells in a bioreactor under optimal conditions. This latter method is the method we focus on in our research. Ideally the procedure would be as such. We go to the sea and collect a sponge, we cut the sponge into pieces and isolate active growing sponge cells, transfer the sponge cells into a bioreactor and supply them with the right medium so that growth of cells and production of bioactive compounds takes place.
In reality, however, the situation is more complex. So far we have not been able to grow isolated cells from sponges. The problem is that for that we still miss a lot of basic information about nutritional requirements of sponges. We do not know what they eat, we do not know how fast they grow and how much bioactive compounds they can produce. So, in order to develop continuous growing sponge cells we need to know how they eat and grow in nature. For that we do research at 2 locations: in the Mediterranean and in the Eastern Scheldt.
The sponges we work on in the Mediterranean are the following. We work on *Dysidea avara*. *Dysidea avara* produces a compound called avarol. Avarol is active against psoriasis. The second one is *Crambe crambe*. *Crambe* produces the drug crambescidine which has an anti cancer activity. The last one is *Corticium candelabrum*. It produces compounds with cytotoxic and antibiotic activity. In the Eastern Scheldt we work on *Haliclona oculata* and *Haliclona xena*. We do not know whether they produce interesting compounds. At present we are testing this. The reason we work on these sponges is that it is nearer by and in addition we believe the diversity in the Eastern Scheldt is very interesting to explore as well.
A sponge pumps water through its body and from the water it filters particles. These particles are bacteria and microalgae.The amount of water that they pump through their body is enormous. What can be seen here is when a fluorescent dye is introduced it is taken up rapidly, pumped through the body and released very quickly. The water contains food and while passing through the sponge particles are taken up and consumed.
Based on the enormous amount of particles eaten by sponges we would expect sponges to grow very fast. This seems, however not to be the case. In the Mediterranean we monitored the growth of Corticium in time. We see here a pictures of a sponge at different stadia and we can not see an enormous increase. We monitored a lot of sponges in this way and it becomes obvious that the animals grow very slowly. We are doing similar kind of experiments in the Eastern Scheldt. We placed a platform on the bottom of the sea and monitor the increase in sponge volume in time. Initial results show that the sponges are able to grow there, although the rate at which they grow is limited.
We would like to understand why sponges eat so much but almost do not grow. Apparently the sponges are very inefficient. For that we feed sponges with labeled food. For this the food is labeled with carbon

13 and we monitor how fast this carbon 13 is taken up and how fast it is introduced in the tissue of the sponge. In this way we can see very quickly, how they metabolize different substrates. In this graph you can see that the sponges indeed have taken up the labeled carbon 13 in a short period of time.
Eventually we would like to use this information to grow cell lines. We and others have done several attempts. For example we isolated cells from sponges and grow them. In that case growth was very slow and we think this is because the sponge cells are differentiated or specialized. In other attempts we tried to isolate cells and reaggregate them to new particles, called primmorphs. Also in those cases sponges grew very slowly.
So we would like the sponges to be a littlebit more opportunistic. There are 2 possible ways to achieve this. First of all we can try to immortalize them. It is possible to turn specialized cells into cancer cells with chemicals and these cancer cells grow a lot faster. Another approach is to work with very young cells. For that we need to isolate cells from sponge embryos or larvae. These young cells are less differentiated and are expected to grow faster.
So far a sum up of the activities we are presently doing. In the future we would like to continue this research line. In this future research physiological studies, studies on nutritional uptake and life cycle will be important. We do that at present with sponges and corals. It would be interesting to do this type of work with other marine invertebrates as well.
We have been successful in developing larvae from sponges and we would like to develop from these larvae continuously proliferating cell lines.
We would like to understand the backgrounds of sponge physiology better as well. Therefore we will not only look to how a sponge develops but also will try to understand the metabolism with of course a special interest in the metabolic routes of the bioactive components.
The sponges produce bioactive compounds to protect themselves and it is expected that they will produce more if they are attacked. We would like to study the effect of stress on the production of bioactive compounds and increase the yield with which they are produced as much as possible.
If we look at the general research line the question will be at the end of course whether all this work will result in new medicines. There are many unknown factors and there will be a huge number of bottlenecks we need to solve. A few of the questions we can address. For example inside a sponge there are symbiotic bacteria and in some cases we do not know whether the sponge or the symbiont produced the bioactive compound. For that reason we work with bioactive compounds from which it is known that they are produced by the sponges.
If we succeed in immortalizing a cell line and growing it we do not know whether the cell line is still able to produce the drugs. We have seen, however, that in the primary cell lines the bioactive compounds were still produced.
Although there are many unknown factors I believe it is interesting to do these studies on sponges. We have seen that sponges have an enormous potential and there are 15,000 sponge species. We would like to develop a technology that can be applied to many systems and for that reason it is worthwhile to invest in this technology.

Photosynthetic Cell Factories

The second research topic I would like to introduce are microalgae. There are 80,000 species of microalgae and we can use them to produce interesting components and just use sunlight and seawater for that. There are already a number of markets for microalgae compounds. Microalgae are sold as biomass and specific microalgae ingredients are sold as well. For example carotenoids and omega-3-fatty acids. These markets are there and these markets are growing. The growth of the markets is, however, limited by a few things. The cost price of microalgae products is at present too high. Often the quality of the product is not good enough and there are serious problems in producing microalgae at larger scale. Before I will concentrate on the technology I would like to say a few more words about some of the products. Interesting products are microalgae used in aquaculture. Microalgae are essential in the developmental stages of mollusks and fish. Microalgae are very interesting for the production of secondary metabolites as they produce those metabolites at high concentrations. Examples of this are carotenoids. Carotenoids are used as pigments, for example in fish feed to give a red color to salmon and as an antioxidant in food. Microalgae contain up to 15% carotenoids. Another example are omega-3 fatty acids. These fatty acids receive a lot of attention nowadays because of their health benefit effects. They are important for the development of brain tissue and as such it is substituted in baby food. Other omega-3 fatty acids result in reduced chances for heart and vain diseases. The omega-3 fatty acids are the reason why it is health beneficial to eat fish and the fish have them from microalgae. Microalgae contain up to 20% omega-3 fatty acids. One last example are C35

alkanes. Long chain carbon molecules that could be used as an alternative to petrol as a lubricant or a fuel. Microalgae can contain up to 60% of these compounds.
All these compounds are produced in large amounts under conditions of stress. At high light intensities, high salt concentrations or low nutrient levels microalgae start to do different things than growing.
In order to develop such products industrially a certain number of demands have to be reached. For the production of these compounds we need to be able to produce a lot of biomass and do that preferably in a compact installation. For that reason it is important to develop efficient photobioreactors. Secondly the biomass produced should contain high concentrations of product. We would like to understand the metabolism in such a way that we can control how much product can be produced. I will introduce later one of the processes we developed, 'milking of microalgae' where we specifically concentrate on production and extraction of the metabolite of interest.
Finally the process should be applicable at industrial scale. Scale up to real large scales of 1000s of m^3 is a research issue that should be addressed.
Let me start with the efficient photobioreactors. Most people know microalgae from the green soup in their pond in their garden. The concentration there will be in the order of magnitude of 10 mg/l. We would like to develop systems with a productivity that is at least 1000 times higher than in your pond. An important aspect in this is the photosynthetic efficiency. Photosynthetic efficiency is in this case defined as the percentage of energy in visible light that is converted into biomass. Theoretically it is possible to store 20% of this energy in biomass. In practice it is much lower. In general this efficiency is lower than 2%. Our objective is to develop processes where we reach efficiencies close to 20%. In principle this can be done by reducing the amount of light. At low light intensities the microalgae can convert the light at higher efficiencies. As our light source is the sun this is not a good option. An alternative for this is increasing the biomass concentration. With that the available light per amount of algae is reduced and this results in higher efficiencies if we mix intensively in photobioreactors. Reactors we use for this are flat panel photobioreactors. These reactors are thin and mixing is done by bubbling gas. In these reactors it is possible to obtain very high densities of microalgae. In commercial raceway ponds it is possible to produce algae at concentrations of 0.5 g/l. In the flat panel system concentrations of 15-50 g/l can be reached. This concentration is still lower than normally is obtained in heterotrophic fermentations.
In our work we try to understand the mechanisms that determine the high photosynthetic efficiencies in order to develop processes with that high efficiency. We want to study the primary metabolism in these cells and carefully monitor whether the process is performed optimally and analyze what to do to optimize this. In addition we look at scaling up of the flat panel reactor. One of the technologies we use for this is the so called green solar collector. In that case we do not have a single flat panel but a vessel in which we submerge plates. Between the plates microalgae circulate and the plates illuminate the microalgae. In this way we have a number of flat plates in a single reactor. Light is brought into the plates via Fresnel lenses. The lenses are situated on top of the plates. They focus the light on the top and the light is distributed in the reactor. As the sun moves the Fresnel lenses need to follow the sun. A small scale green solar collector is installed on the roof of the university to grow microalgae.
After producing microalgae at high concentrations we would like to produce metabolites at high concentrations. Preferably the light should not be used to produce biomass but for the production of the metabolite of interest. In general these compounds are produced in large amounts under conditions of stress. In this example we look at the production of carotenoids. In case conditions like nitrogen limitation, high salt concentrations, high light intensities or low temperatures are applied a lot of carotenoids are produced. How this stress should be applied and to which extend is not exactly known. In the picture above we show the results of 9 experiments done by 9 groups of students. They were asked to produce as much as carotenoids as possible within a period of 2 weeks and each group had for that access to the same information and the same equipment. As you see the results were quite different. Sometimes the concentration of biomass was too low and in other occasions the carotenoid concentration was too low. This indicates that small variations in conditions result in very different results. We would like to control these processes better and therefore started to study the metabolism of carotenoid synthesis in more detail. This is the metabolic pathway for carotenoid synthesis. We see here that there are many carotenoids and we would like to control the pathway in such a way that we can produce any specific carotenoid at high concentrations. In the case of carotenoid production not only the synthesis of carotenoids is important but the synthesis of fat globules as well. Fat globules are produced and they form a sink for carotenoids and because of that the carotenoid concentration in the cells can be so high. On the picture we see a stressed cell that is loaded with fat globules full with carotenoids.

So, carotenoid production is induced by stress factors and the final product is stored in oil globules. During this process no growth takes place and the carotenoids are produced to protect the cells against stress. We wanted to develop a process where we could extract the carotenoids from the cells. The cells need in that case to produce carotenoiods again for their protection. In this way we milk the carotenoids from the microalgae. We could do this by bubbling an organic solvent through the stressed cell suspension and in this way carotenoids could be produced continuously.
In this approach we really see the cells as factories and try to understand what is happening inside the cell and how to influence these processes by changing conditions outside the cell and inside the cells. We think we can make larger improvements if we work at the same time outside the cell and inside the cells. So in this research we look at the reactor engineering, we study scale up and the effects of that on the kinetics. At the same time we are looking to the metabolism by analyzing it and by modeling the metabolic fluxes. We expect in the coming years to go levels deeper than that as well and make use of the genome information. All in all these activities should result in a maximization of the productivity in photobioreactors and a maximization of the concentration of secondary metabolites.
So far I illustrated this multidisciplinary approach via the production of carotenoids. I obtained a VICI grant to develop this type of research and to realize breakthroughs in this field. It is, however, not my intention to develop this approach for carotenoids only. I see similarities with microalgae that produce omega-3 fatty acids and C35 alkanes and I would like to apply this approach to these processes as well.
Other interesting components to be developed from microalgae are in my view polysaccharides, recombinant proteins and neuro-toxins.
With this approach and the different opportunities for applications we hope to be an interesting partner in research for industry and fulfill their wishes in respect to productivity, product concentration and scale.

Science and business

From this it is a small step to say something more about science and business. I am a scientist and therefore focus on science in my work. I do believe, however, society should profit from research. Therefore co-operation with industry in our research is important. In other cases science might spin out from university and result into new business. In the Netherlands this has been stimulated via the so called Biopartner program. I obtained a Biopartner first stage grant to develop a business. This business was based on the research of Green Solar Collector. I developed this idea together with Obbo Hazewinkel. The photobioreactor has a high productivity. In the Netherlands our target was to produce up to 100 tonnes of biomass per ha per year and in Spain we thought we could even go higher, up to 200 tonnes per ha per year. With this technology we expected to produce at a lower cost price a product of better quality. This initiative recently resulted in the development of a company: LGem. The company is managed by Eugene Roebroeck, Obbo and Sander Hazewinkel. These people believed in the idea and took an enormous risk by dedicating all their activities to that company. At present they closely co-operate with another start up: Technogrow, headed by Job van der Burg. Together they form a great team that are able to bring the original dreams to business and for WUR a new company was developed with whom we have now our first collaborative research project.

Title

I organized a contest for the most original title for my inaugurational talk. I received an enormous response and I thank everyone for the very original contributions. Nevertheless, I choose in the end for a title nobody of you submitted. 'Treasures from the Sea' covered my own feeling of the contents best. 'Treasures from the Sea' is the title of a DVD, made by Bjørne Vassnes for the European Commission. In "Treasures from the Sea" scientists are followed on their world-wide search for new medical drugs and industrial enzymes. For the film they travelled from tropical reefs to cold Arctic waters in search of new marine organisms and unexplored life forms that might contain interesting compounds, which could lead to new drugs against diseases such as cancer. Research from our lab is also reported in this film. The DVD is used as a documentary at highschools all over Europe. Free copies can be ordered from the web site: http://treats.uib.no/lang4/rep.htm.

The two most original titles that were submitted I would like to share with you and of course authors of the titles will be rewarded. First of all the title submitted by Lisette de Roos: Yellow Submarine. This title is based on a song from the Beatles. The title came very close to my work as in 2001 my son Guido

made a drawing of what he felt that my work was about. He drew a yellow submarine. This drawing was published in the conference book of Marine Biotechnology: Basics and Applications in 2003. The other one I would like to mention is the contribution from Maria Barbosa: Professor: Gone Fishing. I guess this one refers to the rare moments that I am out of office.

Thank you

At the end of my inaugurational talk I would like to thank a number of people. First of all I would like to thank Wageningen University for giving me the opportunity to develop this research and of course by appointing me as professor in this field.
Secondly I would like to thank Hans Tramper. Hans has been my boss for the last 20 years. This is in my perspective an incredible long time. He was of great influence in my scientific career. He was an example for me in pioneering. He always worked on innovative fields of research and education and in addition gave me all the freedom I needed. Actually, the reason that I stayed so long at Wageningen University was you. I always found new challenges, not a single year was the same. For me it was a perfect seed-bed to develop. I hope I can give as much challenges and freedom to develop to my students as you gave me.

Kees de Gooijer and I started more or less at the same time to work in the group of Hans. We worked together in several papers on diffusion limitation in immobilized cells. The beauty of the experiments was that we used the mathematical models to design the best experiments. I think I learned to do science in that period.

Carlos Vilchez is a very dear friend of mine. We met for the first time in 1988 when he came to Wageningen to learn to work with bioreactors. If I doubt a lot about certain decisions I sometimes go down to Huelva and after a great meal with a lot of the local wine, privilegio, on a terras in the sun it is much easier to take these decisions. I am very happy we recently started a project together where we will study photobioreactors in sunny Spain: the perfect atmosphere to take difficult decisions.

When I started to work in marine biotechnology I found a listening ear and a lot of advises from a lot of people. Tornbjörn Ingemansson from the EU learned me the way of thinking of funding authorities and Oskar Zaborsky from MARBEC in the US to develop large research programs. In respect to the research itself I am grateful to Mario Tredici and Shirley Pomponi. The enthusiasm of Mario for the technology in microalgae was very important to me. Shirley is a pioneer on cell culture and her institute has great facilities. I am very happy we co-operate with this group.

In the Netherlands I would like to thank 2 persons with a warm heart for marine biotechnology. First of all Hans Reith. Hans was the initiator of a large project on microalgae. This project has been very important for us.
Willem Brandenburg is the best stimulator that we can wish for to put Wageningen on the map in the marine world.

In the years after I started to understand micro algae a lot better and sponges a littlebit better our research came into a second phase. At that time Amos Richmond stayed in our lab and I learned a lot from him. Amos is the person that developed the concept of the flat panel photobioreactor, an area where we focus on a lot now.
Sponge research has always been a field that I loved and hated at the same time. I think the love for it appeared already from what I said so far. The hate was determined by the fact that it was incredibly difficult to keep them alive and we had great difficulties having sufficient material for our research. Thanks to Iosune Uriz I stopped hating sponges. Iosune helped me a lot with her solid, quantitative ecological information and helped me to understand how sponges live in the sea. In addition our supply problem for sponges was solved by you. Blanes became my second laboratory and it is always great to go there. I hope we will strengthen this collaboration further in the coming years.

This brings me to my PhD students, both the people that graduated last years and the people that are doing the research now. You are invaluable for me. I endebt you thanks for a lot of things. First of all for the science you are doing. You are a group of talented, smart, enthusiastic, creative people. Secondly, I simply enjoy it a lot to work with you. You are a great team in which a lot of collaboration takes place. You do not only care about your own projects but are interested and supportive to the work of your

colleagues as well. I value the team work higher than the research itself. This does not mean that we do not have to publish papers. I try to give you all the space you need to develop and to have fun in research. I always feel sad in a way when people get their PhD, because that is the moment that the collaboration is ending.

The last words of thanks are for Ida, Guido and Huub. Ida made a great sacrifice. She had a career in environmental hygiene and for that she worked in The Hague. Two careers, travelling and young children are difficult to combine. For a long time we tried to combine these incompatible activities resulting in a continuous feeling of being exhausted. For me she gave up her career and I am aware and grateful for this. You must love me a lot. Guido and Huub are phantastic children. Their enthusiasm, happiness, smartness, creativeness are a greater treasure than we can find in the sea.

Ik heb gezegd.