René H. Wijffels

Prof.dr.ir. René H. Wijffels is professor in Bioprocess Engineering at Wageningen University.
Since 2007 René is chairman of the Bioprocess Engineering. We work on a variety of applications:

- pharmaceuticals: bacterial and viral vaccines, animal cell proteins, bioactive compounds from sponges, bio-insecticides against the malaria muscito
- food ingredients: anti-oxidants, ω-3 fatty acids, novel food proteins
- bulk chemicals: butanol, biopolymers
- biofuels: bioethanol and biodiesel

Research

Involved in more than 50 projects in the last 10 years; 5 key projects are given below:

1. Photosynthetic Cell Factories. VICI grant, 2005-2010, 3 PhD students and 1 postdoc
    a. Development of high-efficiency photobioreactors
    b. Maximisation of carotenoid production
    c. Strain selection and milking

    VICI is the most prestigious research project funded by the Dutch Science Foundation (NWO) and meant is a stimulus to set up an independent research group
2. Wetsus-algae. Wetsus is a collaboration of the universities of Delft, Groningen, Twente and Wageningen in the field of water technology. The total budget of this program is nearly 100 million €. Funding is based on government support and company support. In this program there is atheme on microalgae with support from 14 companies and presently 8 PhD projects are running under this umbrella:
    a. Carbon dioxide supply in a photobioreactor: a biological approach
    b. Oxygen production in photobioreactors: a biological approach
    c. Harvesting of algae for oil extraction
    d. Biorefinery of algae: make value of protein
    e. An advanced study scenario approach for efficient, robust and flexible algae plants
    f. Maximisation of lipid productivity
    g. Maximisation of photosynthetic efficiency
    h. Post-treatment of wastewater with microalgae
3. AlgaePARC. Project for infrastructure for a pilot facility. Funding from the Ministry of Agriculture and th Province of Gelderland
4. Towards Biosolar Cells. A collaboration between the Universities of Amsterdam, Groningen, Leiden, Delft and Wageningen) with th objective to generate fundamental research on photosynthesis in 3 areas: plants, microalgae and artificial photosynthesis. The total budget is 45 miilion €. Within the algae research program there are 4 area's
    a. Utilization: 5 years support from the project and 15 companies for operation of AlgaePARC
    b. Core program: 3 PhD projects and a postdoc project on Botryococcus
    c. Education: development of an international course for graduate students

Publications

111 publications in peer reviewed journals; a selection of 5:

- Wijffels R.H., Barbosa M.J., Eppink M.H.M. (2010 Microalgae for the production of bulk chemicals and biofuels; *Biofuels, Bioprod. Bioref.* **4:**287-295
- Wijffels R.H. , Barbosa M.J. (2010) An outlook on algal biofuels ; Science **329:**296-299
- Lamers P.P., Janssen M., de Vos R.C.H., Bino R.J., Wijffels R.H. (2008) Exlporing and exploiting carotenoid accumulation in *Dunaliella salina* for cell factory applications. Trends in Biotechnol. 26: 631-638
- Zijffers J.W.F., Janssen M., Tramper J., Wijffels R.H. (2008) Design process of an energy efficiënt photobioreactor. Marine Biotechnol. 10: 404-415
- Norsker N.H., Barbosa, M.J., Vermuë M.H., Wijffels R.H. (2010) Microalalgal production – a close look at the economics.
  dx.doi.org/10.1016/j.biotechadv.2010.08.005

An outlook on microalgal biofuels

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The feasibility of microalgae production for biodiesel and co-products will be discussed. Although algae are not yet produced at large scale for bulk applications there are opportunities to develop this process in a sustainable way. However, it remains unlikely that the process will be developed for biodiesel as the only end product from microalgae. In order to develop a more sustainable and economically feasible process all biomass components (e.g. proteins, lipids, carbohydrates) should be used and therefore biorefinery of microalgae is very important for the selective separation and use of the functional biomass components. If biorefinery of microalgae is applied, lipids should be fractionated into lipids for biodiesel, lipids as a feedstock for the chemical industry and ω-3 fatty acids, proteins and carbohydrates for food, feed and bulk chemicals and the oxygen produced should as well be recovered. If in addition production of algae is done on residual nutrient feedstocks and CO_2, production of microalgae at large scale against low production costs the biorefinery approach for micro algae remains feasible.

In order to obtain that a number of bottlenecks need to be resolved and a multidisciplinary approach in which systems biology, metabolic modelling, strain development, photobioreactor design and operation, scale-up, biorefinery, integrated production chain and the whole system design (including logistics) should be addressed.

At Wageningen UR research on microalgae involves production, metabolic modeling for accumulation of specific compounds and biorefinery. Recently pilot ptpojects started (AlgaePARC). Our research activities as well as an outlook to the future will be discussed.

Wijffels R.H., Barbosa M.J. (2010) An outlook on microalgal biofuels. *Science*. **379**: 796-799

Wijffels R.H., Barbosa M.J., Eppink M.H. (2010) Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts, & Biorefining*, **4**: 287-295.

Norsker N.H., Barbosa M.J., Vermuë M.H., Wijffels R.H. (2010) Microalgal production – a close look at the economics. Biotechnology Advances. In press

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Qualifications:
Bachelor Degree: Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, 1971-1975 (Prof. Minoru Nakajima)
Major: Pesticide Chemistry
Master Degree: Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, 1975-1977 (Prof. Koichi Ogata), Major: Applied Microbiology
Ph.D. Degree: Awarded For Thesis Entitled "Microbial Degradation of Nitrile Compounds". Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, 1977-1980 (Prof. Hideaki Yamada) , Major: Applied Microbiology

Current University Appointments:
Professor of the Department of Biotechnology, Faculty of
Engineering, Toyama Prefectural University, JAPAN

Current External Appointments:
April 1994-Present: 1. Visiting Professor of Toyama College
January 1995- Present: 2. Editor, *Journal of Molecular Catalysis B: Enzymatic* (Elsevier)
2006- Present: 3. Editor, *Frontiers of Chemical Engineering in China*
2009-Present: 4. Chairman of Japanese Society of Enzyme Engineering 2

Awards:
1. Progress Award in Synthetic Organic Chemistry, Japan
(1990)
2. The Japan Bioscience, Biotechnology and Agrochemistry Society Award for the Encouragement of Young Scientists
(1991)
3. Toyama Award (1993)
4. The Chemical Society of Japan Award for Technical Development for 2004 (2005)
5. The Japan Bioscience, Biotechnology and Agrochemistry Society Award (The Best Award of Japan Society of Bioscience, Biotechnology, and Agrochemistry) (2008)
6. The Japan Bioindustry Association Award (2008)

Research interests of Prof. Yasuhisa Asano:
Prof. Asano's group in the Biotechnology Research Center of Toyama Prefectural University carries out an extensive screening for novel enzymes catalyzing new reactions, with their deep knowledge in microbiology, biochemistry, enzymology, synthetic organic chemistry, and molecular genetics, etc. They have been pioneers in the exploitation of novel enzymes, as can be seen in Prof. Asano's discovery in 1977 of nitrile hydratase, which is now utilized in the industrial production of acrylamide (30% of world production by Mitsubishi Rayon, etc.), nicotinamide (Lonza) and 5-cyanovaleramide (Du Pont) in multi-tons scale, and in the first crystallization and characterization of phenylalanine dehydrogenase followed by its successful use in the mass-screening of phenylketonuria in Japan. Not only the exploring studies, but also intensive basic studies are carried out to understand the enzymological characteristics by the use of synthetic chemicals. The structure of the enzymes are analyzed by the techniques in molecular cloning,

nucleotide and protein sequencing. Site-directed mutagenesis of the genes are routinely carried out by random and designed manners.

Highlights of current work include; discovery of novel Phenylalanine dehydrogenases (PheDH) from nature, use of PheDHs in the enantioselective synthesis of natural and unnatural L amino acids; microdetermination of L-Phe in blood samples of neonates utilizing PheDH (1/3 of Japanese babies are tested with the enzyme); characterization, gene cloning, structure elucidation, and X-ray structure determination of a new enzyme opine dehydrogenase as one of the first examples of D-stereospecific amino acid dehydrogenase; discovery of 3-methylaspartate ammonia lyase from facultative anaerobes for chiral synthesis and X-ray structure determination; discovery, characterization, structure determination of D-stereospecific peptidases and D-amidases and uses in the syntheses of D-amino acids and D-amino acid containing peptides; discovery and characterization of transphosphorylase producing inosine 5'-phosphate, evolutionary mutagenesis of the enzyme for industrial application (3000-6000 tons/year by Ajinomoto Co. from 2003) and X-ray structure determination, and occurrence of aldoxime dehydratase in nitrile degraders, etc.
An amino acid amide racemase activity was discovered in α-amino-ε-caprolactam racemase and the dynamic kinetic resolution of amino acid amide with an amino acid amidase was realized. From 2003, a new project on protein chips using PheDH was started. Since 2003, A “multi enzyme chip” is being developed for enzyme assay system for diagnosis of phenylketonuria (PKU), galactosemia (GAL), maple syrup urine disease (MSUD) and homocystinuria (HCU), Toyama Medical Bio-Cluster Project, a MEXT project. Since 2008, Hokuriku Innovation Cluster for Health Science was started and his group is working on a new MEXT project “Development of enzyme microchips for amino acid metabolomics for detection of diseases”.
They have been collaborating with Prof. Cooper's group of Biochemistry Department at the Weill Medical College of Cornell University, USA. on the use of PheDH. They are also cooperating with Profs. Engel's and Mayhew's groups of Univ. College Dublin, Ireland, to explore roles of some amino acid residues in PheDH and opine dehydrogenase by the site-directed mutagenesis, and the structural analyses by X-ray crystallography with Prof. Rice's group of Univ. of Sheffield, U.K. On Aug 1, 2006, a scientific exchange agreement was made in between The Faculty of Agro-Industry, Prince of Songkla University, Thailand, and the Graduate School of Engineering, Toyama Prefectural University. They and Drs. H-Kittikun and Hongpattarakere’s groups in Thai side are promoting activities in collaborative research on the development of resources of useful microorganisms and plants, and exchange of scientists and students.
Their laboratory is the first one opened in the Center in 1992. Three laboratories were opened later in 1995, and the expansion of the Center was completed in 1996, when they started enrolling graduate students. Although they have 18 years of history in the Center, some of the projects were started in the Sagami Chemical Research Center, Kanagawa, Japan in 1984. Their laboratory currently consists of 1 Professor, 1 Associate Professor, 1 Assistant Professor, 6 postdoctoral fellows, 4 technicians, 3 postgraduate research students, and 13 undergraduate students. Financial supports have been provided by the Government of Toyama Prefecture, the Ministry of Education of Japan, Japan Society for the Promotion of Sciences, and various private scientific foundations and companies in Japan.

Enzymatic Processes which Replaced Some of Already Established Chemical Processes

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We are developing new enzyme reactions by our screening programs, and improving them by directed evolution techniques to use them in the environmentally benign chemical reactions. Not only the historical works, but also some of our recent results related to the topic will be also introduced.

1. Development of enzymes in the microbial and plant "Aldoxime-Nitrile Pathway"

Nitrile hydratase was discovered in Japan by the author in Kyoto University. Now nitrile hydratase-catalyzed synthesis of acrylamide is recognized as one of the world largest enzyme-catalyzed organic syntheses (it is estimated to be more than 400,000 tons/year). The process is overwhelming the one with Cu catalyst, since most of the new factories established by many companies worldwide seem to be by the enzymatic process.

Aldoxime dehydratase: Aryl- and alkyl-nitriles were synthesized for the first time in high yields from the corresponding aldoximes by *E. coli* cells expressing a new enzyme aldoxime dehydratase, which is located upstream of the "Aldoxime-Nitrile Pathway" distributed widely among microorganisms. The structure has been solved.

α-Amino-ε-caprolactam (ACL) racemase: A combination of amino acid amide hydrolases such as D-aminopeptidase, etc, and ACL racemase enables the dynamic kinetic resolution of amino acid amides to form chiral amino acids. Use of other enzymes in the pathway will be also discussed. The structures of ACL racemase and D-amino acid amidase have been solved.

2. A new enzymatic method of selective phosphorylation of nucleosides:

We have investigated a new enzymatic nucleoside phosphorylation reaction using pyrophosphate (PPi) as a phosphate source to produce 5'-IMP with high regioselectivity. Random and rational mutageneses were successfully carried out. A novel process for producing 5'-nucleotides by a mutant enzyme has been achieved avoiding the use of phosphoryl chloride, a harsh chemical. It was industrialized by Ajinomoto Co. in 2003 and the production is increasing to more than 6,600 tons/year.

Professor Ribo Huang

Professor Ribo HUANG obtained his B.Sc degree on agriculture biology from Guangxi Agriculture University in 1982, and did his M.Sc on microbiology from 1985 to 1986 at Guangxi Agriculture University. He went to Reading University, England, to pursue his Ph.D degree on enzyme engineering and biotechnology from 1986 to 1990 (in the meantime, he worked for then British Biotechnology Limited of Oxford University for nearly two years). Prof. Huang was awarded British ORSA Scholarship and Ph.D scholarship from BBL of Oxford University during his Ph.D study (1988-1990). He worked as a professor at Guangxi University from 1990-1999, and has been working as a professor and a president of Guangxi Academy of Sciences since 1999 and is a highly active scientist involved in molecular enzyme technology, especially in bioenergy-related enzymes. Professor Huang was awarded many national-level titles because his achievements. One important title is National Outstanding Specialists awarded by Chinese Central Government in 2006. Prof. Huang has been authoring over 50 papers and two books. His current interest is on the application of enzyme technology in biotransformation and bioenergy.

Exploitation of subtropical bioresources for bioenergy in Southern China

Ri-Bo Huang [1, 2]

1. China National Engineering Research Center for Non-Food Biorefinery (NERC-NFB); 2. China State Key Laboratory for Bioenergy & Enzyme Technology (SKL-BEET)

Guangxi is a province in the Southern China, and it is full of subtropical bioresources because it accounts for 39% of China's total subtropical area. Guangxi also shares huge similarity in many aspects of subtropical bioresources with most of ASEAN countries. Guangxi is the first province in China in mandatory adoption of E10 (10% ethanol in petrol) derived from non-food feedstock such as sugarcane and cassava. This is regarded as very important event in China because it was implemented by Chinese Central Government, and will be seen as a pattern for the rest of China, and possibly will interest many participants from the ASEAN countries.

In this presentation, following contents will be discussed: 1). The advantages of subtropical bioresources such as bagasse, cassava, rice straw, and microalgae will be presented. 2). The main research areas in my institutes including bagasse bioethanol, lignocellulosic biobutanol, biodiesel and biogas will be introduced. 3). The technological bottlenecks including pretreatment of lignocellulosic feedstock, construction of novel yeast strain capable to use C6 and C5 sugars, yeast strains tolerant to ultra high (over 18% v/v) ethanol concentration, high efficient conversion and utilization of glycerol (the by-product of biodiesel processing) et al., in fulfilling our bioenergy ambition will be discussed and our strategies to overcome these difficulties will be presented.

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Education

Mar., 1995-Dec., 1997 Doctor of Engineering, Department of Fermentation Engineering, Wuxi University of Light Industry, China

Sept., 1987-Dec., 1989 Master of Engineering, Department of Fermentation Engineering, Wuxi University of Light Industry (the former name of Southern Yangtze University), China

Sept., 1983-July, 1987 Bachelor of Food Engineering, Department of Food Engineering, Yangzhou University, Yangzhou, China

Working experience

Dec., 2007-present Professor and Dean, School of Biotechnology, Jiangnan University, China

June, 2003—Dec., 2007, Professor and Vice Dean, School of Biotechnology, Jiangnan University, China

July, 2003- Dec. 2004, Visiting Professor in University of Hawaii, USA

June, 2000-June, 2001 Post-doctoral fellow, Department of Chemical Engineering, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

Jan., 1998-June, 2000 Associate Professor, School of Biotechnology, Southern Yangtze University, China

Mar., 1990-Jan., 1995 Engineer and Vice General Engineer, Zhenjiang Pharmaceutical Factory, Zhenjiang, China

Jan., 1998-June, 2000 Associate Professor, School of Biotechnology, Southern Yangtze University, China

Research Fields

Metabolic engineering, Fermentation Engineering, Enzyme technology

Contributing Papers

More than 120 papers have been published in refereed Journals, the followings are some representative publications.

1. Zhihao Wang, Yun Wang, Dongxu Zhang, Jianghua Li, Zhaozhe Hua, Guocheng Du, Jian Chen. Enhancement of cell viability and alkaline polygalacturonate lyase production by sorbitol co-feeding with methanol in Pichia pastoris fermentation. Bioresource Technology, 2010, 101:1318-1323

2. Jun Lin, Xianyan Liao, Guocheng Du, Jian Chen. Use of Escherichia coli add/ade mutant and Saccharomyces cerevisiae WSH2 to construct a highly efficient coupled system for glutathione production. Enzyme and Microbial Technology, 46 (2010):82–86

3. Jing Zhang, Jie Liu, Zhongping Shi, Liming Liu, Jian Chen. Manipulation of B. megaterium growth for efficient 2-KLG production by K. vulgare. Process Biochemistry 45 (2010) 602–606

4. Zhaofeng Li, Zhengbiao Gu, Miao Wang, Guocheng Du, Jing Wu and Jian Chen. Delayed supplementation of glycine enhances extracellular secretion of the recombinant α-

cyclodextrin glycosyltransferase in Escherichia coli. Appl Microbiol Biotechnol. 2010, 85(3):553-561
5. Bo Tang, Xianyan Liao, Dongxu Zhang, Min Li, Rong Li, Kelu Yan, Guocheng Du , Jian Chen. Enhanced production of poly(vinyl alcohol)-degrading enzymes by mixed microbial culture using 1,4-butanediol and designed fermentation strategies. Polymer Degradation and Stability 95 (2010) 557-563
6. Muhammad Salman Qureshi, Dongxu Zhang, Guocheng Du, Jian Chen. Improved production of polygalacturonate lyase by combining a pH and online methanol control strategy in a two-stage induction phase with a shift in the transition phase. J Ind Microbiol Biotechnol (2010) 37:323–333
7. Song Liu, Dongxu Zhang, Long Liu, Miao Wang, Guocheng Du, Jian Chen. Enhanced water absorption of wheat gluten by hydrothermal treatment followed by microbial transglutaminase reaction. Journal of the Science of Food and Agriculture, 2010, 90(4): 658-663.
8. Zhiyu Liu, Dongxu Zhang, Guocheng Du, Jian Chen. Improvement of laccase production and its properties by low-energy ion implantation. Bioprocess and Biosystems Engineering. 2010, 33(5): 639.
9. Hejing Yan, Zhaozhe Hua, Guoshi Qian, Miao Wang. Guocheng Du, Jian Chen. Analysis of the chemical composition of Cotton Seed Coat by Fourier Transform Infrared (FT-IR) Microspectroscopy. Cellulose, 2009, 16:1099-1107
10. Jun Lin, Xianyan Liao, Guocheng Du, Jian Chen. Enhancement of glutathione production in a coupled system of adenosine deaminase-deficient recombinant Escherichia coli and Saccharomyces cerevisiae. Enzyme and Microbial Technology, 2009, 44: 269–273.
11. Long Liu, Jun Sun, Dongxu Zhang, Guocheng Du, Jian Chen and Wenbo Xu. Culture conditions optimization of hyaluronic acid production by Streptococcus zooepidemicus based on radial basis function neural network and quantum-behaved particle swarm optimization algorithm. Enzyme and Microbial Technology, 2009, 44(1): 24-32
12. Yun Wang, Zhihao Wang, Guocheng Du, Zhaozhe Hua, Liming Liu, Jianghua. Lowering induction temperature for enhanced production of polygalacturonate lyase in recombinant Pichia pastoris. Process Biochemistry, 2009, 44 (9): 949-954
13. Long Liu, Guocheng Du, Jian Chen, Miao Wang, Jun Sun. Microbial production of low molecular weight hyaluronic acid by adding hydrogen peroxide and ascorbate in batch culture of Streptococcus zooepidemicus. Bioresource Technology, 2009, 100 (1): 362
14. Guobin Liang, Xianyan Liao, Guocheng Du, Jian Chen. A new strategy to enhance glutathione production by multiple.H2O2-induced oxidative stresses in Candida utilis. Bioresource Technology, 2009, 100 (1): 350-355
15. Jingwen Zhou, Liming Liu, Zhongping Shi, Guocheng Du, Jian Chen. ATP in Current Biotechnology: Regulation, Application and Perspectives.

Teaching activities

Courses for postgraduate student include:
1. “Application and Production of Environmentally Friendly Materials”.
2. “Advances in Biotechnology”
3. “Biochemical Engineering”

Process engineering for the biotechnological production of exopolysaccharides

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Abstract: Microbial exopolysaccharides like hyaluronic acid (HA), Kefiran and xanthan gum are rapidly emerging as a new and important source of polymeric materials. These biopolymers have novel and unique properties and already have found a wide range of applications in the food, pharmaceutical and other industries. The biotechnological production of exopolysaccharides has gained considerable attention in last decades. Various process optimization strategies have been developed for the enhancement of microbial exopolysaccharides production, which is usually challenged by the low oxygen mass transfer, low energy cell status of the bacterium and the inhibition of some end-products like lactic acid. Therefore, besides the mixing performance, the cellular physiology also should be considered for the efficient microbial exopolysaccharides production.

In this work, we present the process optimization approaches for the biotechnological production of HA by *Streptococcus zooepidemicus* and Kefiran by *Lactobacillus kefiranofaciens*. The mixing performance of HA production was improved via the optimization of agitation and aeration, the addition of oxygen vector n-dodecane and the reduction of broth viscosity via the HA degradation. The energy status of *S. zooepidemicus* was improved via the oxidative stress via the feeding of hydrogen peroxide, and the HA productivity was improved by a two-stage model based fed-batch and batch culture mode. The Kefiran productivity was improved via a process kinetics-based two-stage temperature control approach, and the inhibition of lactic acid on Kefiran production was alleviated via the co-culture of *L. kefiranofaciens* and *Saccharomy cerevisiae*.

Keywords: Process optimization, Exopolysaccharide, Mixing performance, Model, Hyaluronic acid, Kefiran