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Next Generation Technology to Minimize Ecotoxicity and to Develop the Sustainable Environment: White Biotechnology

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Abstract

This review aims to show that industrial sustainable chemistry, minimizing or reducing the ecological impacts by the chemicals, is not an emerging trend, but is already a reality through the application of 'White Biotechnology' such as 'green' chemistry and engineering expertise. A large number of current industrial case studies are presented, as well as new developments from the chemical industry. The case studies cover new chemistry, new process design and new equipment. By articulating the requirements for industrial application of sustainable chemistry, this review also seeks to bridge any existing gap between academia and industry regarding the R & D and engineering challenges needed to ensure green chemistry research enables a more sustainable future chemical industry considering eco-toxicological impacts.

Keywords: White Biotechnolgy, ecotoxicity, sustainable environment

White Biotechnology

Biotechnology has been established during the last decades as one of the most promising future technologies. Today, solutions of future challenges related somehow to biology or technology will not be sought without considering biotechnology. In Germany it was started to name the main application areas of biotechnology by colors. 'Red' stands for medical and pharmaceutical biotechnology, 'green' for agricultural, and 'blue' for marine biotechnology. EU decision making bodies proposed recently to add "white" as name for industrial and environmental biotechnology. White Biotechnology has unquestionably the deepest roots in the history of science and technology. Biochemical and microbiological processes are among the oldest productive activities of mankind. Their dissemination in the chemical industry of the 20th century and their treatment in the framework of chemical technology has promoted the search for a shorter term than "biochemical and microbiological technology and engineering"-just 'biotechnology and bioengineering'-of which 'biotechnology' stood out again. The rapid progress of molecular biology in the second half of the 20th century and the resulting genetic engineering raised the importance of biotechnology for medicine and agriculture far beyond its previous role: The seeds of its subdivision have been sown. The recommendation of BIO (Biotechnology Industry Organization) to speak in future of 'biotechnologies' instead of 'biotechnology' can be regarded as heading right in the same sense. BIO is spreading the experts' opinion that industrial biotechnology is about to change the sector of industrial production in a similar way pharmaceutical biotechnologies have already done in medicine. Indeed, a 'third wave of biotechnology' is expected comprising industrial and environmental applications.

Sustainable Chemical Industry

According to the Organization for Economic Cooperation and Development (OECD), industrial sustainability is defined as the continuous innovation, improvement and use of clean technologies to reduce pollution levels and consumption of resources. In practical terms, industrial sustainability means employing technologies and know-how to use less material and energy, maximizing renewable resources as inputs, minimizing generation of pollutants or harmful waste during product manufacture and use, and producing recyclable or biodegradable products. The chemical and process industries are well placed to embrace and drive the challenge of industrial sustainability as they enable so many other sectors in the value chain, such as construction, building, transportation and health, to provide high quality products and services. The chemical industry creates materials for multiple consumer markets, which need to be produced, used and recycled by manufacturing processes that are clean, safe and economical. These proce-

sses should use the minimum of resources with the ultimate goal of using only renewable and recyclable feedstock. The global chemical industry represents a significant part of world trade and economic activity with 10 million employees and a combined turnover of some EUR 1300 billion excluding pharmaceuticals, and at EUR 1841 billion including pharmaceuticals, representing 4-5% of world income. It contributes 9% of world trade whilst emitting only 4% of global CO_2 emissions. The OECD publication 'Biotechnology for Clean Industrial Products and Processes: Towards Industrial Sustainability' (1998)[1] and many other publications such as the USA Roadmap for Biomass Technologies[2], note that biotechnology is a powerful tool for achieving industrial sustainability, and is gaining ground for the production of commodity chemicals. Much development has already been done on the possible uses of biomass for production of materials: 1. direct use of specific materials from plants and trees, for construction materials, paper, textiles, etc., 2. specific products based on specific constituents of crops (oil, fibres etc.) by (chemical) conversion e.g. for specialty chemicals, biodegradable polymers, etc., 3. generic conversion of biomass to basic constituents: 'building blocks' such as CO, H_2, CH_4 but also ethanol, acetone etc. through fermentation, 4. potential for production of high added-value products (pharmaceutical products and agrochemicals). Industrial biochemistry seems to be more acceptable to the general public than agricultural biotechnologies, and in the long term, the production of chemicals should be increasingly based on biomass as raw material; including non-food resources, restricted residues and wastes[3]. Chemistry and Chemical Engineering have essential contributions to make in: 1. developing the technologies for conversion of biomass into energy carriers and chemicals, 2. research on which biomass materials are most suited and where and how arable land and water resources can be developed and exploited, 3. determining the metrics and indicators for the assessment of the effectiveness of sustainability projects. There are economic challenges for the development of 'White Biotechnology': feedstocks such as vegetable oils and glucose can be expensive, the enzymes used to convert the material require a high investment in research and long development times. It is also clear that speciality chemicals, with lower volumes of production, are likely to see the most profound early impact from biotechnology. In 'Bio-Refineries', biomass is used for the production of high added-value chemicals, fibres and fine chemicals, together with the production of energy carriers, preferably in liquid phase for higher energy content and easier transportation. Certain technological bottlenecks need to be investigated: a weak point is pyrolysis to obtain 'bio-crude-oil/bio-fuels'. Pyrolysis has not been investigated from the Chemical Engineering point of view, nor considered as a part of the holistic production system : pyrolysis- stabilization of the produced oil-refining-use as fuel or raw material. This could be one of the main production routes in a bio-refinery concept. A parallel approach is the concept of 'Bio-Cascade', using crops in such a way that all constituents of a plant (oils, proteins, fibres, cellulose, secondary metabolites and remaining wastes for energy use) result in a total product mix that offers the highest economic value. Biomass can be used as an energy carrier by gasification and also as a source for base chemicals via the Fischer-Tropsch reaction to 'bio-oil', later refined to liquid fuel. Further biomass utilisation can establish innovative production networks: 1. separation of lignin from cellulose for different downstream product lines, 2. use of solar energy in combination with use of biomass, 3. insertion of biotechnology in normal processes for certain steps, 4. advanced generation of hydrogen by biological processes. Ethical considerations imply that real biomass residues-and not food-are used for chemical feedstocks, and almost exclusively for bulk applications. New processes for commodity chemicals, such as succinic acid and ethylene glycol, are in the pilot stage. Shell has recently invested in enzyme producer Iogen to develop a process making a high octane alcohol by fermentation. DuPont has entered a 6-year alliance with Diversa in a bio-refinery project to produce sugars from corn and biomass (including husks, straws, stovers) and develop fermentation processes to co-produce bioethanol and value-added chemicals (such as 1, 3-propanediol). Vegetable oils (rapeseed, palm) can be used as energy carriers or for production of chemicals. Comprehensive LCA based on ecological indicators (finite energy resources, global warming etc.) conclude that: 1. biobased sources have clear advantages over fossil fuel counterparts, 2. transesterified biofuels are better than pure vegetable oils, 3. vegetable oils are preferred feedstocks to produce surfactants. Biocatalysis is also increasingly important in polymer science.

New Fields of White Biotechnology: Fine and Specialty Chemicals

The examples described till now could arouse the impression White Biotechnology were a means for mass products, only. This is not the case. The rapid development of sophisticated technologies in several branches, especially in physics, but also in chemistry and in biology itself, has created a continuously

rising demand for fine and specialty chemicals with regard to extent and assortment. This branch of chemistry notes a considerable growth. It becomes apparent that the usual chemical methods of their manufacture are often more awkward than biotechnological alternatives, sometimes even do not work. An example is the chiralic compounds. They are molecules, which occur in two forms-like a figure and its mirror-image. They can be recognized in solution by rotating the plane of polarized light into opposite directions. It is spoken of optically active or dextrorotatory or laevorotatory compounds, respectively. In most cases only one of these two forms is able to fulfill the intended purpose. This can already be observed with lactic acid of which only the dextrorotatory form offers the wanted physiological effect. Usual chemical synthesis forms almost equal amounts of both optically active compounds. Their chemical separation is rather complicated. Biological catalysts, enzymes and microorganisms, can differentiate between both forms and attack or produce only one. The demand for such optically active compounds grows mainly for pharmaceutical syntheses. Of late, the question is put how enzymes do perform certain synthesis steps of complicated molecular structures which are much more difficult to realize or inaccessible at all by other means. At BIO 2002 has been shown how a simple detergent's enzyme carried out a determined reaction at a number of differently structured molecules. This opens the view that biological reactions can be valuable means for the production of fine and specialty chemicals when skillfully implemented in chemical synthesis strategies.

New Fields of White Biotechnology: Defense Against Infections

An American research team could show in a mouse model that pathogenic streptococci, causing sore throats in men, could be eliminated within a few hours by application of an enzyme. The enzyme dissolves an often-repeating chemical bond in the cell wall of the bacteria, which is responsible for its strength. As a result the cell wall loses its stability. The bacterial cell is destroyed by its own osmotic pressure, which the so weakened cell wall cannot withstand. This reaction occurs at bonds whose formation is inhibited by penicillin during cell growth. The effect on the bacterial cell is in both cases the same. Thus, enzymes can have the same effect on a bacterial population as antibiotics. But-till now-enzymes cannot be applied in the bloodstream for being proteins and giving rise to defense reactions of the immune system. But is it absolutely necessary for defense against infections to bring the enzymes into the bloodstream? Could it be helpful to place bacteria-destroying enzymes in the free space at the body's outer membranes in order to catch the pathogenic agents before they enter the body? That means to build up an outer biological defense line of the human or animal body, which till present cannot satisfactorily be realized by conventional thermal or chemical sterilization methods. Besides, our organism does already possess such a defense line, e.g. in the mouth, in which enzymes are released continuously showing a certain antimicrobial effect. In contrast to antibiotics enzymes do not act only on bacteria but also on yeasts and fungi. Thus, they have a broader scope of target microorganisms. Use of enzymes for eliminating pathogenic agents in the vicinity of man enables manifold combinations with available techniques of cleaning. Their importance for defense purposes against attacks with bio-weapons has been already recognized and they have been included into the strategies of biodefense[4].

New Fields of White Biotechnology: Safer Food and Commodities

A problem has become visible with the increase of extent and exactness of chemical analyses: What can be defined as safe, what as problematic and what as dangerous? The present uncertainty in the assessment of actual products and systems has been elucidated when dealing with the so-named 'bio-product' from agriculture. On one hand there are a number of chemicals whose real risk potential is not exactly known. On the other hand it is not possible to exclude from the outset possibly dangerous concomitants. It is reminded of a classical case: The toxic m-cresyl phosphate present in the early softeners of PVC. One problem of chemical noxes is that decades can go by between incubation and appearance of damage in the organism. Finding out its cause is then difficult, indeed. Biotechnology cannot solve this analytical or diagnostic problem. But it can provide methods to produce products with another composition, e.g. natural in place of synthetic solvents, colors, fibers, adhesives, thickeners, fillers and other materials.

New Fields of White Biotechnology: Nanotechnology

The upcoming nanotechnology opens new possibilities for biotechnology. Communication is sought between both fields bringing technological solutions to the fore. Transferring the successful principles of application of machines into the molecular range challenges investigations: What are the organizations of systems, drives and controls which can be realized by use of biological methods and principles? In

recent times, terms like bioelectronics or bio-computers haunt literature. This reflects the desire to couple biological and physical systems making use of the advantages of both for the benefit of man. Although all that is still at the very beginning it can be qualified already as a field in which White Biotechnology should cooperate and assume responsibility.

Finding Linkage between White Biotechnology and Eco-Toxicogenomics

It is estimated that approximately 70,000 chemical compounds are used commercially, and an analysis of the toxicological properties of each chemical is estimated to cost US$2-4 million, and require several years to complete[5]. There has been overwhelming pressure to develop cost efficient testing methodologies to screen for the toxicity of these compounds[5]. It is postulated that DNA microarrays may be used as a rapid screening tool to assess the toxicological properties of a chemical, based on the gene expression patterns induced upon exposure to a drug or chemical[6-8].

Toxicogenomics attempts to determine how the regulation and expression of genes control the physiological responses induced by exposure to a drug or chemical compound. The basic premise is that a certain degree of similarity or a generalized pattern of gene expression is expected during exposure to different classes of toxicants[6]. Furthermore, subtle differences in gene expression patterns induced by chemicals belonging to a specific class of toxicants may be sufficiently distinct so as to identify chemical-specific signatures of exposure[6]. Much of the current research in modern toxicogenomics has been aimed at classifying toxicity according to unique gene transcriptional profiles induced by different classes of compounds. Furthermore, in an attempt to expedite the drug screening process, investigators assess the gene transcriptional responses of chemicals of unknown toxicity and compare these responses to those of chemicals that have already been thoroughly characterized[6]. It is suggested that gene expression profiling using DNA microarrays may be used to characterize potential mechanisms of action of environmental contaminants through the identification of gene expression networks[6].

The literature has been bombarded with reports highlighting the considerable scientific merit and potential benefits of using DNA microarrays as tools in toxicological research. However, several assumptions are inherent to the basic concept of using a toxicogenomics based approach in toxicology, and will require validation before conclusive statements about the toxicological properties of a chemical may be drawn. Until these assumptions are validated, it is unlikely that the full potential of DNA microarrays for toxicological research will be realized. In the following sections, we review recent data that supports a toxicogenomic-based approach to assessing biological effects of contaminants.

Toxicogenomics is an emerging discipline that is impacting several areas of toxicology including ecotoxicology. Applying toxicogenomic-based approaches to assess the impacts of a contaminant on the health of an ecosystem has formidable challenges. Two general approaches in ecotoxicology have been used to characterize the impacts that environmental contaminants have on ecosystems; these being exposure and effects assessment. Biomarkers are often used as a measure of exposure to a contaminant, and are physiological markers altered in response to chemical exposure. Biomarkers are not necessarily indicative of an overall toxicological effect of a chemical, but indicate that exposure to a contaminant or class of contaminants has occurred. Under certain circumstances, biomarkers may correlate with adverse health effects (i.e., bioindicators). However, it is often difficult to conclude concrete health effects based on biomarker data collected from environments impacted by sporadic or chronic low dose contamination. Under these conditions, the biological response measured by a biomarker may not necessarily affect long-term health of the organism. In order to extend the use of a specific biomarker to environmental assessment, a biomarker should be predictive of effects at higher levels of biological organization and should precede the ecological effect in time. Biomarkers have the potential to predict contaminant- induced effects on organisms and can therefore be used to minimize ecosystem impacts. Effect monitoring examines the alterations and perturbations in biological fitness of an organism or population. Effect monitoring is limited to the very context of the effect being examined; a lack of effect does not necessarily imply a healthy population. Furthermore, overt manifestations of health indices (e.g., gross morphological effects) need to be violated in order for effects to be concluded. Under these circumstances, there is little doubt that ecosystem health has been compromised. However, these overt effects on biological fitness or population structure pre-dispose remedial action to clean or rehabilitate contaminated areas, and as such, effects monitoring is limited in predicting and preventing environmental impacts by contaminants. Likewise, contaminant-induced effects are difficult to define in areas where sporadic release or low-grade chronic exposures are occurring. In some cases, exposure to low, sub-lethal concentrations of a toxicant

may produce hormetic effects; effects opposite to that elicited by exposure to higher concentrations of a contaminant. The greatest utility for DNA gene expression arrays in the field of ecotoxicology will be to bridge the limitations imposed by either exposure or effects assessment. Gene expression profiling using DNA microarrays may assist in potentially identifying the mechanisms of action of a contaminant on an organism, and help in predicting the overall toxicological effect that contaminant exposure may have on host physiology. Exposure to an environmental contaminant may elicit gene expression patterns indicative of an impaired biochemical pathway, providing DNA microarrays the utility as sophisticated and comprehensive biomarkers of exposure. In turn, traditional function-based biomarkers targeted for these affected systems may be used to validate the toxic mechanisms of the contaminant. Furthermore, gene expression patterns linking exposure to impairment of a biochemical pathway may help in predicting what specific effects to look for in a contaminant-impacted population. Ultimately, applications of DNA microarray technology in the field of ecotoxicology will depend on significant research validating microarray technology with traditional methods of assessing contaminant exposure and effects. To date, most of the work using DNA microarrays have focused on genetically wellcharacterized organisms, including *Saccharomyces cerevisiae, Drosophila melanogaster, Caenorhabditis elegans, Mus musculus*, and *Homo sapiens*. Gene expression arrays are currently being developed for a number of non-model organisms,1 many of which are of interest in the field of ecotoxicology. Gene expression arrays have been developed for a variety of fish species[9,10], frogs[11,12], and birds[13,14], and many more are likely to be developed in the near future. Recent interest in the field of ecotoxicology has focused on the potential of using DNA gene expression microarrays for a toxicogenomics-based approach to assessing the environmental impacts of contaminants. The purpose of the following section is to highlight some of the important considerations in applying DNA microarray technology to the field of ecotoxicology. The considerations presented here are very much dependent on the experimental approaches used (i.e., field applications or laboratory exposures). We also provide a theoretical example of where DNA gene expression arrays may provide important data for incorporating into mathematical models that attempt to explain toxicity based on physiochemical characteristics of the contaminant, gene expression data, and physiological function.

Concluding Remarks

Industry has made great progress in adopting processes and chemistry that have sustainable characteristics: our selection of examples is of necessity limited but we hope reflects the wider picture. Future progress requires technical and political challenges to be addressed. White Biotechnology appears to be a particularly fruitful area for building industrial sustainability and has so far not faced the issues of public acceptance associated with agricultural biotechnology. Radical improvements can also be achieved by new process design including new reactor configurations, integration of operations both within and between enterprises and through a focus on recycling and reusing materials. Many barriers exist to limit the implementation of radical improvements to industrial performance: incremental debottlenecking of plant and expansion is preferred in times of slow growth and a 'first-of-a-kind' process faces a high investment hurdle. The economic and regulatory environment needs to support investment in new technologies that demonstrate sustainable advantage but carry higher technical risk than conventional operations.

As the use and release of chemicals produced by white biotechnology increase, sporadic release or low-grade chronic exposures of the chemicals are occurring. In some cases, exposure to low, sub-lethal concentrations of a toxicant may produce hormetic effects; effects opposite to that elicited by exposure to higher concentrations of a contaminant. Therefore, the greatest utility for DNA gene expression arrays in the field of ecotoxicology, 'Eco-Toxicogenomics', will be to bridge the limitations imposed by either exposure or effects assessment. The impotency of Eco-Toxicogenomics will be considered to assist in potentially identifying the mechanisms of action of low-grade chronic exposure of a contaminant on an organism, and help in predicting the overall toxicological effect that contaminant exposure may have on host physiology.

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