Biotechnological Manufacturing Options for Organic Chemistry

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Abstract: Industrial biotechnology or white biotechnology is an area of growing academic, public and private interest. The global white (or industrial) biotechnology market of ~50 billion US$ is smaller than the red biotechnology (pharmaceutical) market (>70 billion US$). *However*, industrial white biotechnology represents a greater long term business potential than red biotechnology. It is estimated that at least 20% of global chemicals (~2'290 billion US$ today) could be produced by biotechnological means in 2020. The bad news is that this potential is spread over diverse markets and various product/molecule classes. This variety is more difficult to handle on the one hand, but on the other it comes with longer life cycles. Currently there are a number of *in vitro* and *in vivo* methods available for the manufacture chemicals on an industrial scale by biotechnological means. These methods include reactions with enzymes in ordinary stirred tank chemical reactors, microbial fermentation, plant cell culture, manufacturing products with plants (pharming) or other methods. This review summarises and discusses the advantages and limitations of the different *in vivo* and *in vitro* methods which could potentially be used for the biotechnological production of chemicals.

Keywords: Industrial biotechnology, small molecule pharmaceuticals, fermentation, biotransformation, transgenic plants, microorgansms, plant cell culture.

INTRODUCTION

The global chemistry market is estimated at 2'290 billion US$ and is expected to grow to 3'235 US$ by 2015 and 4'000 billion US$ by 2020 [1]. Of the 2'290 billion US$, the global industrial biotechnology market is believed to represent about 50 billion US$ (not including biofuels, see Table **1**). Of these 50 billion US$ biotechnology products, about 25% are fine chemicals and the growth of the share of biotechnologically produced fine chemicals is expected to grow from 8% to 60% between 2001 and 2010 [2].

this market. Global pharmaceutical sales have more than doubled between 1998 and 2006, with the US market in the lead. However, the strong growth of biopharmaceuticals continues to be realised at the cost of small molecules. Since the 1980s with the launch of the first recombinant DNA drug insulin, the 1990s introduction of IFN and interleukins and the first commercial approval of monoclonal antibodies (mAbs) around 2000, the therapeutic protein market has shown a very healthy annual growth of 15-19%. Between 1980 and 2004 about 300 antibodies and 400 other proteins entered clinical

Table 1. The Table Summarises the Different Markets and the Market Volumes in Billion US Dollars, the Compounded Annual Growth Rate (CAGR) and the Approximate Number of Companies Globally Active in the Area. Needless to Say that there is a Lot of Overlap, for Example Between the White or Industrial Biotechnology and the Pharmaceutical or Red Biotechnology

	Industrial Biotechnology	Pharmaceutical Biotechnology	Environmental Biotechnology	Agricultural Biotechnology	Marine Biotechnology
Markets served	Many different markets such as Small molecule pharma & Fine chemicals, Flavour & fragrance, bulk chemicals etc.	Large molecule pharma products such as therapeutic proteins and monoclonal antibodies	Service & solution for bioremediation and waste treatment	Transgenic or genetically modified (GM) seeds and plants	Products and lead substances from the marine environment
Colour code	White	Red	Grey	Green	Blue
Market size US$	>50 bio without biofuels	>70 bio	na	>7 bio	na
CAGR	15%	>20%	na	15-20%	na
Companies	4'000	6'000	na	>50	Na

Including all products (fine chemicals, specialties, polymers etc) it is expected that 20% of the global chemicals in 2020 will be produced using biotechnology. If we take the above mentioned 4 trillion US$ as the basis for calculation, 20% represents 800 billion US$. That is 16 times todays figures of 50 billion US$!

Estimates and definitions may vary, but there is one common denominator and one clear message: the proportion of products manufactured using biotechnology is expected to increase significantly. However, a key question is do we have the all the necessary tools to live up to these expectations of the industry and investors?

The pharmaceutical market is still the most important driver for innovation, not only for large but also for small molecule technology innovation, and it is helpful to recognise the burning issues of trials, totalling about 740 products [3]. Of the 252 drugs that entered clinical trials in 2007, 135 were small molecules while 61 were already mAbs, proteins, or other large molecules. While the annual growth of small molecule pharmaceutical was in the double digit range until 1990, it has been steadily decreasing since then to 6%. There is a general trend towards a reduction in the development of small molecule drugs, and an increased focus on protein-based therapies instead. The only small molecule pharma with a high annual growth of ~12% are high potency drugs; if they continue their growth, they will be in the 90-95 bio € range in 2012.

Despite this downturn, the pharmaceutical industry continues to be the largest market for fine chemicals, consuming over 50% of fine chemicals production - and this figure is increasing. The world market for chiral molecules was approximatively 7 billion US$ in 2002 and is expected to grow to ~15 billion US$ in 2010. According to O'Driscoll [4] the chiral portion of approved small molecule drugs increased to 75% in 2006 up from 58% a decade earlier.

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Table 2. Characteristic of the Small Molecule Pharmaceuticals Market and Trends Influencing

▪ 3 segments with above average growth: ❶ Biopharmaceuticals ❷ High potent drugs ❸ Generics.
▪ Reduction of number of small molecule APIs (NCE) in the pipeline
▪ Increased share of chiral small molecule APIs
▪ Need of stable small molecules and affordable novel antiinfectives
▪ Understanding the mechanisms underlying diseases and protein protein interactions
▪ Novel formulations allow switch from parenteral to oral applications
▪ Molecular diversity corresponding to patient diversity (# blockbuster or "one size fits all" mentality)

Table 3. The Sketch Shows a Hypothetic Scenario of Long Term Trends in Pharmaceuticals. One One Hand the Trend Will Direct Away from Today's Proteins and Monoclonal Antibodies (mAb) towards Smaller Entities such as Peptides, Nucleotides and Small Molecules on One Side. But there is also a Second Trend Towards e.g. Gene Therapy and Tissue and Organ Repair

Small molecules		**Gene therapy**
Peptides	**<= Protein & mAb =>**	**Cell therapy**
Nucleotides		**Tissue repair**

Table **2** summarizes in a nutshell the most important characteristics of the small molecules market. Some trends are expected to indirectly influence the small molecules pipeline and products. The understanding of disease mechanism and protein-protein interactions will allow the design of small molecule drugs and inhibitors and it will bring old drugs to a new level of efficacy. It is anticipated that climate change will favour the emergence of new diseases as well as the spread of existing ones for which stable and affordable drugs are required.

Small molecule drugs are positioned in markets that are becoming increasingly generic, thereby adding further pressure. We may observe a decreasing number of small molecule drugs on the one hand, but we can also observe an increasing complexity and functionalisation of these small molecules (Table **3**). But there are numerous arguments for small molecule products as well as we will see later. Also several deals and cooperations have been agreed upon, often by companies typically active in biopharmaceuticals such as GENENTECH (CGI PHARMACEUTICALS on kinase inhibitors**;** INOTEK on polymerase inhibitors**;** PIRAMED on kinase targets), AMGEN (PREDIX (EPIX) for S1P1 modulators**;** PROSTRAKAN on compounds for renal disease), SERONO now SCHERING AG (NEWRON PHARMACEUTICALS for safinamide; RIGEL on kinases;) or BIOGEN IDEC (BIOGEN IDEC with UCB for a small molecule α-integrin inhibitor).

One can conclude that biotechnology continues to play an important role not only in large molecule biopharmaceuticals, but also in small molecule pharmaceticals. However, the pharmaceutical market is going through rapid changes and it seems reasonable to assess some factors which influence the future and where small molecule biotechnology has technical gaps to fill to meet the high expectations. There are a number of questions which arise when considering the great expectations placed upon biotechnology, such as:

(a) What are the drivers of this development?

(b) What products will be produced and with what priority by biotechnological means?

(c) Where are the technology gaps to meet these expectations?

Or as the former German Chancellor and Mayor of Berlin Willy Brandt said "The best way to predict the future is to shape it".

WHAT ARE THE DRIVERS TO USE BIOTECHNOLOGY IN ORGANIC CHEMISTRY?

Whatever source or opinion there is one general element of consensus: biotechnology will play a much greater role in the future. But what are the real drivers for this change? There are four main reasons for these developments summarized in the Table **4** below.

Table 4. Drivers for the Implementation of Biotechnology and the Application of Industrial Biotechnology for the Production of Small Molecule Pharmaceuticals

▪ Increased need for chirality and functionality
▪ Need for sustainability
▪ Technological advances in bio(techno)logy.
▪ Cost and time frame (Increased speed, throughput & selective synthesis. Decreased costs and time)

Sustainability has appeared on the political agenda simultaneously with advances in biology and biotechnology which may provide solutions to manufacturing [5]. The ICAP (International Carbon Action Partnership) initiative [6] and the principles of green chemistry [7] are examples of initiatives bringing momentum to the application of biotechnology in the chemical industry.

One important fact to consider is that the chemical industry uses only about 7% of the annual oil consumption of close to 90 million barrels a day. More than 90% of the oil resources are in fact burned for heating or transportation. If all the oil and the tar sand deposits were hypothetically and exclusively available for synthetic chemistry only, instead of being burned, one would almost speak of an oil glut for chemistry! This aspect is not irrelevant, especially for a discussion of an eventual competition between food crops and crops for chemical feed-stocks. What will the supply chain of tomorrow look like? Will it be a bio-based chemical feedstock or an oil-based feedstock? The likely answer seems to be both, but as the industrial manufacturing methods and tool-boxes in biotechnology are incomplete or partly not existing, decisions are needed regarding where to invest R&D money. The money should be spent specifically and wisely as it can only be spent once. Using food-based feed-stock for e.g. bioplastics increases the pressure on the food prices, but releases pressure on oil prices, as this portion is freed for other consumption such as fuel transport or heating and thus relieving the pressure on the price of oil.

WHAT PRODUCTS SHOULD BE PRODUCED BY BIOTECHNOLOGY AND HOW?

In order to try to find an answer to the question "how to produce", three aspects must be considered. Firstly, as mentioned above, we have two long term options with respect to chemical feedstocks. The two basic options are non-renewable petrochemically derived raw materials and renewable raw materials. The third element interfering with the supply chain is the efficiency and yield of recycling of intermediates and end products.

Sustainability has been defined as one of the key drivers. However, note that sustainability has not only an ecological and economic dimension, but also a social dimension. For example, any use

of renewable feed-stock for chemistry must avoid competition with the food product chain.

In the pharmaceutical area, steroids, antibiotics and a few other classes of compound derived from fermentation and biotransformation are seen as major small molecule biotechnological products. However, there are numerous demands emerging in small molecule pharmaceuticals which will need sustainable methods for their production (Table **5**). Despite the fact that biopharmaceuticals, such as monoclonal antibodies and proteins, clearly dominate the market in terms of growth and potential, there is also a need for small molecule therapeutics. Some of the developments mentioned in Table **2** could even partially reverse the trend back towards small molecules in the longer term.

Some disease areas, such as endocrinology, lend themselves to protein-based drugs while others prefer small molecules applications such as enzyme inhibitors GPCR (or G-protein-coupled receptors) targets. Considering that 50% of the good targets in oncology are actually inside the cell, one can conclude that small molecules have an advantage to reach these intracellular targets. Other aspects are the possibility of oral applications, easier handling and storage. Moreover, small molecule drugs are chemically defined and do not exhibit some of the variations (e.g. during post-translational modification) observed in large molecules.

Whatever the reasons, the common denominator for all of them is that the funtionality required is increasing. Up to now, small molecule fine chemicals and drugs were typically "flat" organic chemicals, sometimes with one or more chiral centers, where –OH, -COOH and $–NH_2$ were the most important functionalities (Table **5**).

Table 5. Functional Groups in Small Molecule Chiral Synthons Used in Pharmaceuticals According to the Authors Experiences

-OH	20%
-COOH	20%
$-NH_2$	15%
$R(NH_2)COOH$	25%
>S=O	5%
Others	15%

However, as we will see later the need for small molecules will grow far beyond these needs in the future representing not only unmet need but also offering new opportunities.

OPPORTUNITIES FOR BIOTECHNOLOGY AND EXAMPLES UNMET NEEDS

Table **6** summarizes selected areas where biotechnology could provide the much needed technical solutions.

Table 6. Examples of Opportunities for Biotechnology and Unmet Needs

Glycosylation of small molecules and peptides
Functionalisation of biopolymers and small molecules
The production of highly active compounds
Peptide biosynthesis
Biotransformation of unusual functional groups and molecules

Glycosylation. Over half of the worlds drug leads derive directly from the natural product pool. Many of them are glycosylated compounds, and glycoside mimetics have attracted interest from the pharmaceutical industry (glycosidase inhibitor Relenza, Tamiflu). Nature changes the chemistry of the N-terminus of peptides and proteins by post-translational modifications: long chain alkylation, acetylation, myristoylation, glycosylation. phosphorylation, acetylation, glycosylation, ethylation and ubiquitinylation. However, chemical glycosylation of small and large molecules remains a challenge due to the need for methods for stereo-and regioselective protection of the sugar hydroxyl groups. Thus enzymatic alternatives have to be developed that use either glycoside hydrolases, glycosyltransferases or glycosynthases.

Biopolymer Functionalisation. New biomaterials are needed for many purposes from regenerative therapies and tissue engineering to industrial applications. Groups with functionality drive the biorecognition of biomaterials, improve fuctions of industrial biomaterials and the applicabilty of biopolymers.

Highly Active Compounds. As mentioned earlier, high potent compounds are the only group within small molecule drugs, which are showing vigorous growth. Highly active compounds and small toxic peptides of microbial, vegetal or even animal origin represent interesting leads for the development of drugs. For example, one *Bothrops jaracara* small peptide toxin (called BPP, bradykinin potentiating peptide) was the lead sequence of captopril, acting as angiotensin converting enzyme inhibitor. About 100'000 venomous animal species are known with an estimated ~10'000'000 different toxins. Companies such as Atheris [8] are using these natural leads which have been optimized by nature over millions of years of evolution.

Peptides. Peptides represent an important class of molecules, which remain difficult to produce sustainably [9]. Peptides such as antimicrobial peptides and small proteins can be naturally derived, produced in *Eschrichia coli* as recombinant oligopeptides, chemically synthesized or use a combination of solid phase synthesis with enzymatic methods for peptide as described by S. Flitsch [10]. However, for most peptides the chemical synthesis remains the prevalent manufacturing method.

Excipients. Excipients are often small molecule binders, disintegrants, fillers or diluants in drugs. The materials accounted for a 3.5 bio US$ global market in 2006. Excipients were considered as simple ingredients with modest roles. In the future they will have more functional roles in drugs, play an important role in new ways formulating drugs and thus allowing for example the switch of selected drugs from e.g. parentheral to oral.

Oligonucleotides. Oligonucleotides, or short segment of RNA or DNA, still have a low profile on markets, despite their therapeutic potential. Aptamers for example are nucleic acid based ligands that can bind to biologically active molecules and receptors [11]. These oligonucleotides fold in well defined 3D structures which are able to recognize various molecules with a high affinity. RNA interference is a natural antisense mechanism and the use of nucleic acid therapies to block RNA functions was first described in the late 1970s. As for peptides, the synhesis of oligonucleotides is tedious and expensive.

THE PRODUCTION OF SMALL MOLECULES – A COMPARISON OF THE CHEMICAL AND THE BIOTECHNOLOGICAL TOOLBOX

This chemical toolbox (Table **7**) has been continuously developed over the 19th and 20th centuries, and it can typically provide the tools necessary for most synthetic routes, albeit at an unfavourable ecological and economic cost.

Table 7. Summary of the Chemical Toolbox for the Production of Optically Pure Compounds

1.	Asymmetric hydrogenation
2.	Asymmetric epoxidation
3.	Jacobsen Hydrolytic Kinetic Resolution (HKR)
4.	Stereoisomer separation by chromatography
5.	Asymmetric sulfide oxidation
6.	Carbonyl group reactivity based transformations
7.	Asymmetric C-C bond formation with Rhodium complexes
8.	Asymmetric Darzens reaction
9.	Organocatalysts (e.g. proline and derivatives)
10.	Organometallic catalysts, Metal-catalysed cross-coupling catalysts

Table 8. An Overview of the Enzyme Classes Presented as Oral or Poster Presentations at the Last Three Biotrans Symposia [12]. An Example of the % Calculation: 34% of all Presentations in 2007 Dealt with Oxidoreductases. The Sum Does Not Add up to 100% Because Some of the Presented Papers did not Deal with Biotransformations

Enzyme class	2007	2005	2003	Reaction Type Carried Out by the Enzyme
1. Oxidoreductases	34 %**	24%	28%	Redox reactions
2. Transferases	8%	6%	3%	Functional group transfer
3. Hydrolases	41 %*	55%	58%	Hydrolysis of functional groups
4. Lyases	12 %	12%	10%	Non-hydrolytic addition/removal of groups
5. Isomerases	2%	2%	1%	Intramolecular rearrangements
6. Ligases	0 %	1%	0%	Formation of C-O, C-S, C-N or C-C bonds

* 64% of all posters on hydrolytic enzymes were concerned with lipases/esterases, or 26% of all papers.
** It seems that the -10% in hydrolytic enzymes are the +10% in oxidoreductases. This increase in availability of some oxidoreductases (e.g. ketoreductases, ene reductases) in combination with protein improvement, e.g. directed evolution, has somewhat improved the situation.

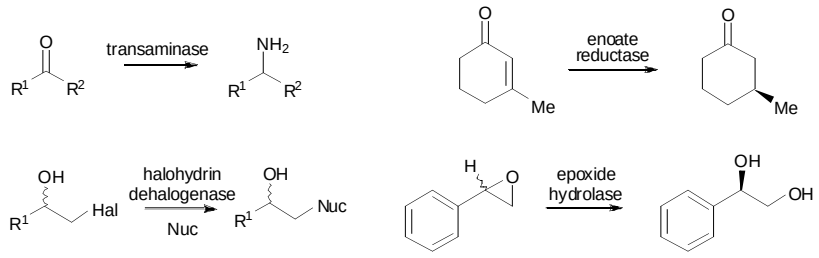

Fig. (1). Four new classes of enzyme currently being developed for applications in biocatalysis.

Small molecule production in industrial biotechnology has been dominated by fermentation and biotransformation for numerous products such as antibiotics, steroids and pharmaceutical intermediates. However, the actual tool box is insufficient to meet current and future needs, especially in the pharmaceutical industry with ever more diversified and functionalised molecules. Natural biodiversity is not fully exploited since most microorganisms have not been cultivated. As a matter of fact, both, whole cells as well as the enzyme toolbox are far from being adequately equipped. Table **8** shows that the most widely studied and used biocatalysts remain the hydrolytic enzymes.

Thus there is a real need to discover and develop new biocatalysts to broaden the range of chemistries available in the biocatalytic toolbox. Examples of enzyme classes that are currently being examined include transaminases, enoate reductases, epoxide hydrolases and halohydrin dehalogenases (Fig. **1**). Transaminases offer a particularly attractive route to enantiomerically chiral amines since they use readily available ketones as starting materials. Chiral amines are highly valued intermediates in a wide range of pharmaceutical products and are currently not easily prepared by alternative methods [13].

The ability to discover and develop novel biocatalysts has been greatly aided in the past ten years by the emergence of a suite of advanced technologies. Firstly, the sequencing of whole genomes of microorganisms has provided biotechnologists with a vast amount of sequence data that is invaluable when trying to identify novel enzymes as the gene level. Secondly, the cost of obtaining synthetic genes from commercial suppliers has fallen to the point where it is now cost effective to use this approach to rapidly generate starting points for enzyme activity. Thirdly, the ability to improve biocatalysts for their desired application using directed evolution strategies has become a powerful and often used strategy. Provided that suitable screening regimes can be devised, it is now possible to change various properties of enzymes (e.g. solvent stability, enantioselectivity, catalytic activity) so that they are much more robust and suitable for applications on scale in industrial processes [14].

Despite these progresses the enzyme toolbox remains absolutely unsatisfactory for industrial applications. On top of this difficulty, we do not know how to cultivate most microorganisms in the laboratory, although "conventional" microbial fermentation is considered a mature technology. Less than 1% of the microbial world is known, and in the case of the *Archeae* that figure is even as low as 0.01%! How can we speak of conventional microbial fermentations notwithstanding we know basically nothing about how to successfully grow a vast majority of the microbial world. Consequently we are not able to fully exploit natural microbial biodiversity. Although metagenomics represents an approach for searching for genes in uncultivable organism, it nevertheless is based upon existing information and hence excludes radical discovery. Considering that of the oceans a mere 10% are explored, it becomes clear that while the macroscopic world is well known the microscopic world is basically still an unknown world.

BIOTECHNOLOGICAL SMALL MOLECULE MANUFACTURING METHODS OF TOMORROW

Two components will influence development of new manufacturing method tomorrow [9, 15].

1. The broadening of organisms (genetically modified or unmodified) used from the different microrganisms, plants and even animals.

2. Systems biology, which is providing detailed insights into the working of the cells. This will allow the *in silico* design of cells for manufacturing purposes, just as we design automobiles and aircrafts today. The focus is away from investigating single molecules to elucidating whole metabolic networks, in order to assemble the pathway for a desired chemical in an organism of choice. A recent prominent example showing the possibilities of this method is the biosynthesis of the precursor of the antimalaria drug Artemisisine by One World Health supported by the Bill & Melinda Gates foundation [16]. Another example is the expression of 8 taxol biosynthetic genes in *Saccharomyces cerevisiae* [17].

Small molecules can be manufactured by two biotechnological methods or a combination of both: (1) Biotransformation of a

Table 9. This Table Sumarised *in vivo* Biotechnological Manufacturing Options, of which Most are Suitable for the Production of Small Molecules

1	Bacterial fermentation	✓✓✓ excellent fit for small molecules
2	Yeast fermentation	✓✓✓
3	Fungal fermentation	✓✓✓
4	Algae fermentation	✓✓ good fit for the production of small molecules
5	Protozoa	
6	Plant cell culture	✓✓
7	Transgenic plants	✓ fit for selected small molecules
8	Insect cell culture	
9	Mammalian cell culture	
10	Transgenic animals	

chemical precursor. (2) *De novo* biological synthesis by fermentation. Biotransformation can be carried out using whole cells (biotransformation) or enzymes (biocatalysis). Both are principally well established technologies with one serious bottleneck: commercially available enzyme preparations are missing for most reactions. This is regrettable as many competitive and commercial microbial expression systems do exists (e.g. Pfenex of Dowpharma, Aspex of Asahi Glass, XS expression system of Lonza).

Table **9** lists all the bio-based options we do have and those which are suitable for small molecule purposes and for the production of enzymes for biocatalysis. For the assessment which technologies will be used in the future, two aspects are crucial. The first one is that tomorrow's "small molecules" used in the life sciences will considerably differ from today's small molecules, as the required degree of functionality will be much higher, just to mention one example. The second aspect will be that cost at equal or even higher quality aspirations must be contained at an affordable level.

Bacteria, Yeast and Fungi. Of the manufacturing methods mentioned in Table **9**, the first three (bacteria, yeast fungi) have found widespread application, although "burning issues" remain. Also many exciting developments and new tools are being discussed such as grafting of whole pathways as mentioned above, microbial co-production or co-culture and culture of unculturable microorganisms [18, 19] or the development of nanofilms containing active biological principles [20].

Algae. Algae (Fig. **2**) have been used for a long time in human nutrition, especially in the subtropical and tropical zones. Today algae are used to produce for example polyunsaturated fatty acids (PUFAs), health foods cosmetics, feed products, thickening agents, amino acids, pigments or pharmacologically active products. The productivity seems to be 2 - 5 fold higher as compared with traditional agricultural crops [21]. Algae became popular again as they are able to harness CO_2 from the atmosphere, from exhaust fumes or sequestrated CO_2 to produce chemicals. The development of photobioreactor technology for microalgae cultivation has become of great interest because of the opportunities for biofuel production with microalgae.

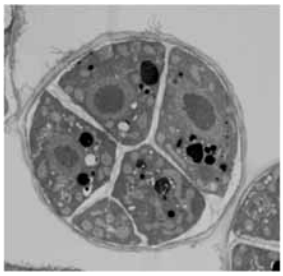

Fig. (2). Polyunsaturated fatty acids (PUFAs) such as docosohexanoic (DHA) acid are important building blocks of human brain tissue or the retina of the eye. The molecule can be produced by fermentation of marine algae. The picture shows the algae *Ulkenia sp*. for the industrial production of DHA. They grow to form "footballs" cosnsiting of single cells – 5 in this case with the product accumulated in the cells as oil drops.

The manufacturing with phototrophic organisms (algae, plant cell culture, transgenic plants) has potential for the production of small molecules (and large molecules) which is presently rarely exploited.

Transgenic Plants. About 25% of all pharmaceuticals contain plant based lead structures, or are obtained directly from plant material, resulting in a market size for plant-derived drugs of close to 20 billion US$ in 2006. Plants were and continue to be an important source of drugs, food additives, cosmetic ingredients, flavour and fragrance compounds, pesticides and other molecules. New candidates and leads can be expected from the numerous ethnobotanical programmes. Unlike primary plant metabolites, most secondary metabolites, by definition biologically/physiologically active compounds, are nothing less than very high value fine chemicals. Approximatively 100'000 plant compounds have been reported [22] but the majority of plant species remains unexplored. Especially the *Dicotyledoneae*, which are particularly rich in secondary metabolites, may contain yet unknown beneficial products and molecules. Thus we can conclude from empirical considerations and also from systematic screening that plants produce many unknown natural compounds which could be used as starting materials, building blocks or final products. Plants can also be a source of enzymes for biocatalysis for example hydroxynitrile lyases of *Hevea brasiliensis* used for enantioselective C-C coupling, soy bean peroxidases or P450 monoxygneases from plants. These enzymes however will almost exclusively be produced in different hosts, preferably microorganisms after cloning the corresponding gene of the plant into a suitable host. Besides, there are a number of issues which negatively affect the use of transgenic plants. The biochemical potential of plants and reliable supplies must be realized without threatening wild populations, independent of seasonal fluctuations and crop failure, under defined culture conditions and assuring quality and activity year round. The use of transgenic plants or genetically modified plants is hampered by regulatory uncertainties and technical challenges in down stream processing [23, 24]. This uncertainty triggered the development of plant made pharmaceuticals (PMPs) in contained systems by culturing plant cells in suspension culture in closed fermentors for large and small molecule pharmaceuticals. Another issue is the general reluctance to accept genetically modified plants in an agricultural environment. A change in opinion could be triggered by large molecules as about 16 plant made large pharmaceuticals are reported in clinical trial, with the main argument that manufacturing costs can be cut using transgenic plants.

Plant Cell Culture. The obstacles of transgenic plants mentioned above can be overcome by producing with plant cell culture in closed fermentors instead of whole plants in fields or greenhouses. Plant cell culture technology was developed in the 1930s when the first reports of successful culture of undifferentiated plant cells in flasks were published [25, 26]. However, although valuable chemicals could be produced by plant cell culture, the penetration of the technology so far was very poor and only very few products of plant cell culture processes are reported, such as the antixancer product taxol of Bristol-Myers Squibb [27]. The main factors which must be improved are

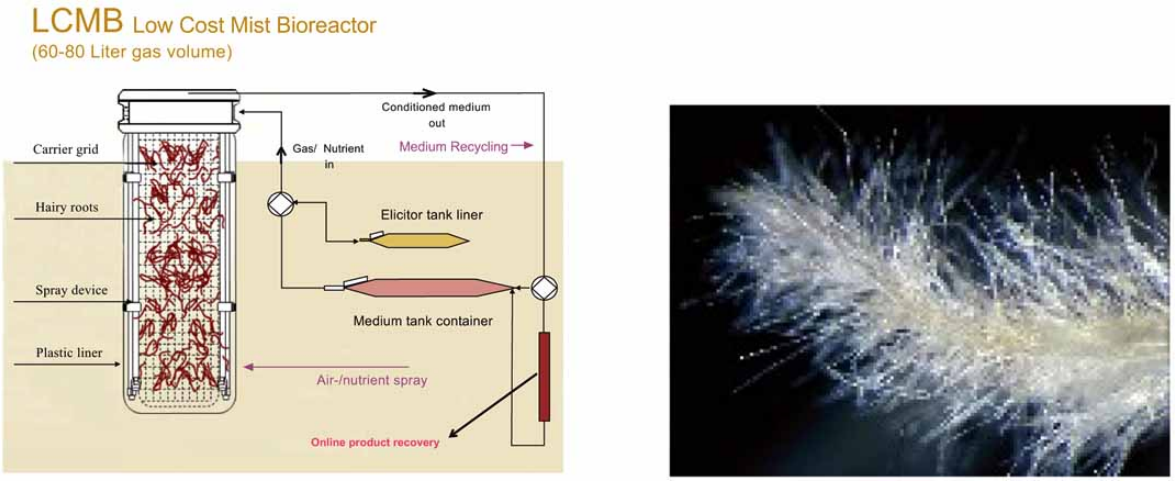

Fig. (3). This scheme shows the basic construction of the mist bioreactor patented and operated by Rootec [28], and hairy root cells on the photograph, which were grown in the reactor for the production of a small molecule anticancer molecule. Instead of growing the cells suspended in a culture broth, the hairy cell mass is growing on a support is sprayed with growth medium, resulting in a much higher cell density and product concentration.

(1) Slow growth and low titers when compared to bacteria and yeast

(2) Overcome the empirism of the (secondary) metabolism

(3) Difficult induction of production using stress factors and elicitors

(4) Tendency to cell aggregation and shear sensititivy

The high value products are typically produced in differentiated and specialized cells. Thus the cells must be grown and maintained in a fermentor in a non differentiated state, until sufficient plant cell biomass is produced. This biomass can then be induced by signal factors or elicitors to differentiate and to produce the desired product or enzyme. This procedure is a standard method used for example in microbial fermentation, but proves to be much more difficult in plant suspension culture. Another issue is that products are often not stable due their extremely complex molecular structure. This is also observed when producing for example labile secondary metabolites in microbial culture, and the solutions (for example in situ product recovery using ion exchangers) can be applied for plant cells as well. Hairy roots (Fig. **3**) are a promising technology with the problem that they do not grow well in suspension culture.

CONCLUSION

Biotechnology holds a lot of promise for the manufacturing of organic chemicals, but the potential unfortunately remains largely untapped. The pharmaceutical and fine chemical markets are the important drivers for innovation, but we have to expect that the degree of sophistication of the molecules and the cost pressure will increase simultaneously at unchanged or even higher quality requirements. Moreover, sustainability is now a "conditio sine qua non". On the other side, we can expect that several important new biological tools such as systems biology, cloning of whole pathways will be implemented for industrial manufacturing. Algae and plant cell culture, whether grown phototrophically or heterotrophically, represent an interesting additional manufacturing method. What is most important, however, is the joint agreement between chemists and biologists concerning a roadmap and priorities, where to focus developments, where to reduce prioririres and where to chose the right feedstocks for the different value chains.

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