4.1.1- The Biotech Industry in Italy.

Over the last few years Italy has finally witnessed the birth of an industrial biotechnology sector, although much later than other countries of similar economic importance. This sector has shown a rapid and remarkable growth, especially in diagnostics, therapeutics and related fields, by highlighting its remarkable potential, in terms of small or medium-sized R&D dedicated biotech companies. However, it is still "young", small, mainly located in the country's north, and dominated by "red" or healthcare biotech productions.

A survey conducted by Assobiotec in 2006 (after *Assobiotec-Blossom Associati, "Biotechnology in Italy, Strategic and Financial Analysis, 2006"; "www.blossomassociati.com"*) identified 163 Italian companies operating in the sector of biotechnology along with 16 technological parks hosting biotech companies or/and research centres. According to the Report, about 80% of all biotech organizations (companies, biotech-related national science parks and research centres, etc.) are located in northern Italy and mostly active in niche markets in the diagnostics, therapeutics and related fields. Many of the R&D biotech companies have sprung from spin-off processes and buyouts of research laboratories from multinational companies. Furthermore, some well-established pharmaceutical and chemical companies recently turned to biotech. Around 60% of these companies have a project portfolio that is predominantly in the research stage, 26% are at an early development stage (Stage 1 or 2), and 18% of them have projects in Stage 3 or in the approval stage. Lastly, only 46% of the companies obtained patents on their own research products. About 50% of them have fewer than 15 employees; more than 90% of all the Italian biotech companies are classified as SMEs according to the criteria of the European Commission (fewer than 250 employees and a turnover below €27 million euro per year).

The Italian biotech industry grew markedly during the last few years (growth rate: around 10% per annum) with an impact of R&D investment on sales that grew from 34% in 1999 to 46% in 2004.

The geographic distribution of biotech companies shows a high level of territorial fragmentation. In fact, only 21 Italian provinces have a biotechnology presence in their territory and only one third has set up incubators to support the development of start-ups. About 70% of all biotech companies are located in northern Italy, whereas about 14% and 16% of them are located in central and southern Italy, respectively.

The biotech industry in Italy is primarily focused on the production of human health products (about 33% and 25% of the total Italian biotech production consist of therapeutic and diagnostic products, respectively). On the contrary, agro-foods and bioprocesses currently represent minor fields of activity: only 22% of the Italian biotech companies seem to be active in these fields, where they mostly focused their production on specific market niches not covered by the big international companies.

4.2 - Industrial/White Biotechnology and Environmental Biotechnology and Their Impacts on the Future Economy.

4.2.1 - Industrial/White Biotechnology Sector

On the agenda of every responsible government, humanitarian organization, economic entity and analyst there are major issues or concerns regarding all of humanity that can no longer be avoided.

With a fast growing world population, the two major concerns are the supply of energy and water. The demand for energy and water is rising rapidly. New economies with a double-digit growth rate like China and India are absorbing more fossil fuels. It is forecasted, for example that China's energy consumption will be 5 quadribillion BTU higher than the US by 2030[1] (from *http://www.eia.doe.gov*).

The consequences of this situation are multifold (from environment to politics, to economics) and need to be tackled from various standpoints (energy and water saving and equal distribution, lifestyle changes, increased efficiency in industrial processes, and energy production, to name just a few). Locally, the consequences on the environment are already apparent, with high levels of pollution in all industrialized countries: all major Asian cities are good examples of fast economies which are not sustainable from an environmental point of view.

On a global scale, we are experiencing climate changes. As a consequence of the increased concentration of the so-called greenhouse gases in the atmosphere, the earth is expected to become warmer worldwide. Eleven of the latest 12 years are amongst the warmest 12 years on record since 1850[2]. The main contributor of global warming is CO_2, primarily resulting from the burning of fossil resources (coal, oil and natural gas) in addition to other

[1] www.eia.doe.gov

[2] *Intergovernmental Panel on Climate Change (IPCC). Climate Change 2007: The Physical Science Basis. Summary for Policymakers. http://www.ipcc.ch/SPM2feb07.pdf*

factors such as the deforestation of tropical forests. In 1997 the Kyoto protocol was signed by numerous countries with the aim of reducing their emissions of greenhouse gases within a short time; as of today, this protocol has been accepted by most countries, Italy included.

The consequences on our life style may be significant. As already mentioned, there are many strategies for solving these issues. An innovative approach might come from industrial biotechnology (IB).

IB, also known as white biotechnology, like health care (red biotech) and agricultural (green biotech) applications, is the application of biotechnology for the processing and production of chemicals, materials, and energy. White biotech uses enzymes, microorganisms and cultured cells from plants and animals to make products in sectors such as chemistry, food and feed, paper and pulp, textiles and energy. While its application in the production of fine chemicals and pharmaceuticals is already well established (e.g. insulin, interferon, erythropoietin, hepatitis B vaccine, vitamin B12, etc.), now IB is being increasingly applied to produce bulk chemicals such as biofuels (e.g. ethanol) and bio-plastics. Other fields of application include food additives and supplements, colorants, vitamins, nutraceuticals, cosmetics, pesticides, solvents, enzymes, bio-energy, etc. White biotechnology also provides new products and services by producing enzymes and cells at sustainable costs and in protected environment proteins.

IB has the potential to develop clean processes with (i) reduced water consumption, (ii) reduced energy consumption, (iii) less or no waste generation, or recycling thereof and (iv) reduced or neutral CO_2. Thus, industrial biotechnology is a key underpinning technology that may contribute to the transition of our current society, towards a more sustainable one, where renewable resources provide a growing contribution to our energy, chemical and material needs.

To explain the process by which biomasses are transformed into energy and products, the concept of "biorefinery" has been introduced. A biorefinery is a fully integrated manufacturing facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass, with minimal waste and emissions. A biorefinery is analogous to today's oil refineries, which produce multiple fuels and products from oil.

These factories of the future will integrate agriculture and a part of the transformed chemical industry. By providing new markets for crops and crop by-products and residues, the chemical industry may support agriculture and the rural economy which can, in turn, become less dependent on subsidies. Recent reports[3,4] predict annual growth rates of nearly 5% for fermentation products (compared to 2-3% for overall chemical production) in the coming years. McKinsey & Company[5] predict that by 2010 bio-based products will account for 10 percent of sales within the chemical industry, accounting for $125 billion in value. Starting with the chemical industry, white biotechnology will make inroads into a number of other industries. For example, enzymes will transform production processes in the pulp and paper industry, and new polymers will find multiple applications in the automotive and consumer industries. The greatest impact of white biotechnology may be on the EU fine chemicals segment, where up to 60% of products may be produced through biotechnology by 2010. A key driver here is the growth of biological pharmaceuticals such as antibodies for cancer treatment – drugs for which no traditional chemical synthesis exists. The impact on the specialty chemicals segment could vary broadly.

For instance, fermentation and enzymatic processes are commonly used in the fine chemicals sector to produce, for example, vitamins, pharmaceutical intermediates and flavours. They are also making their first inroads into larger volume segments such as polymers, bulk chemicals and bio-fuels, and many more industrial sectors. As of 2005, products made from biobased feedstocks or through fermentation or enzymatic conversion account for 7% of sales and $77 billion in value within the chemical sector, and different studies agree that these products will play an increasingly significant role in the chemical and other manufacturing industries in the future. Therefore, white biotechnology will be the key to the competitiveness of many European industries, including chemicals, textiles and leather, animal feed, pulp and paper, energy, metals and minerals, as well as waste processing. Two factors would contribute to this. One is the low costs for raw materials and processing, combined with small-scale investments in the fermentation of plants. The other is additional revenues from innovative, new, or performance-enhanced products (from *Industrial Biotechnology SRA of ETP SusChem, http://www.suschem.org*).

Currently, two types of feedstock can be used in the industrial biotech value chain for the production of fuels, bulk chemicals, materials and specialties. Fossil feedstock is used for the bioproduction of certain compounds by enzymes and/or microorganisms. These types of conversions are typically confined to biospecialties, such as fine chemicals, and are thus conducted on a relatively small scale compared to most industrial biotech processes based on renewable resources. At present, the use of renewable feedstock, such as agricultural by-products, is gaining importance. To that end, agricultural materials containing (ligno)cellulose or starch are first converted into sugars, which are subsequently transformed into a wide range of products via fermentation. In addition, agricultural or even household organic wastes could be valorized in this way.

However, in order to address these challenges, new or improved biocatalysts have to be developed, and new or improved and tailored biotechnological processes have to be designed, developed and assessed. Recombinant

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[3] *World Market for Fermentation Ingredients, Study GA-103R by Business Communications Company Inc.,Norwalk, - March 2005.*

[4] *Fermentation Chemicals, Industry Study 1921 by The Freedonia Group Inc., Cleveland, May 2005.*

[5] *Presentation Jens Riese – World Congress on Industrial Biotechnology – Toronto – July 13, 2006*

DNA technology allows microorganisms to be tailored to give higher yields of particular chemicals, or even to produce new ones. The increased efficiency of the reaction allows more and more scope for replacing established conventional processes by cleaner, low-temperature fermentation, in a safe contained environment. The highly specific nature of individual enzymes means that chemicals can be produced in the purest form, and biological processes not only require fewer chemical inputs, but also result in smaller and more manageable waste streams. In general, most of the industrial biotech processes developed so far use the most effective and convenient biocatalytic form, which is often a whole microorganism. However, this does not exclude the use of higher organisms, in particular plant, animal and human cell cultures, or the use of isolated enzymes combined with chemical catalysts.

In all cases the main driver is the cost- and eco-efficient production of the desired compounds by developing: a) the best biological catalyst for a specific function or process; b) the best possible environment for the catalyst to perform; and c) the most suitable strategy for the recovery, purification and further chemical conversion of the desired products from the fermentation process.

The first aspect deals with the search for the best possible biocatalyst, with improved or new functionalities. Another important aspect of white biotechnology deals with the containment system or bioreactor within which the catalysts must function. Here, the combined knowledge of the scientist and the bioprocess engineer interact, providing the design and instrumentation for the maintenance and control of the physiochemical environment such as temperature, aeration, pH, etc. Chemical engineering plays a crucial role in the research and development of innovative technologies for the design, creation, and conducting of enzyme or whole-cell bioreactors. A detailed fundamental analysis of the thermodynamic and kinetic aspects and the transport phenomena involved in bioreactors is the necessary basis for the scaling-up of laboratory plant processes to pilot or industrial level. The formulation of transport models accounting for the simultaneous transfer of momentum, heat and mass, and the subsequent identification of both the limiting steps and significant process variables, make it possible to properly design the bioreactor in its most suitable configuration (batch vs. continuous, stirred vs. plug flow, free vs. immobilized or confined biocatalyst, etc.), as well as to define the process control strategies and the integration of the bioreactor with other units in a complete process plant. The third aspect, the downstream processing, can be a technically difficult and expensive procedure. Downstream processing is primarily concerned with the initial separation of the bioreactor medium into a liquid phase and a solid phase, and subsequent separation, concentration and purification of the product. In addition, it includes the further chemical conversion of the fermentation product to yield the final desired compound. Improvements in downstream processing will benefit the overall efficiency and process cost and will make the biotechnology-based processes competitive with the conventional chemical ones. Chemical engineering principles also play a vital role here as well in terms of designing and operation of the separation systems.

Based on these observations, the following 7 R&D strategic areas have been identified for an effective future implementation of white biotech in Europe:

- Selection of novel enzymes and micro-organisms
- Improvement of biocatalysts (enzymes and micro-organisms) through microbial genomics, proteomics, metabolomics and bio-informatics
- Metabolic engineering and modelling
- Biocatalyst characterization and optimization
- Innovative biocatalytic process design
- Innovative fermentation science and engineering
- Innovative downstream processing

They should be integrated and applied properly to generate three different product categories: input of the process (biomass defining and usage, recovery of waste, conversion into fermentable sugars, etc.), (new) bioprocesses and bioproducts, and biofuels.

The specific key scientific and technological challenges related to the first type of product category, i.e. biomass to be applied in the processes, are:

- Identification of competitive biomass feedstocks which are best suited for EU needs (availability and competitive price)
- Conducting of LCAs and eco-efficiency studies to identify optimal biomass feedstocks for the EU
- Development and optimization of viable processes for the conversion of biomass materials into fermentable sugars (e.g. enzymatic, physical, chemical, or combinations thereof)
- Creation of added value for the co- and by-products of bioprocesses, to improve the economics
- Development of bioprocesses based on other alternative feedstocks such as lignin or glycerol, for the chemical and energy industry
- Development of a closed-loop fermentation cycle (where the “biowaste” of one process can be recycled as input for another process), e.g. sugar beet pulp as an untapped biomass feedstock for future use.

In the *Bioprocesses and Bioproducts* area, the main challenges to be dealt with are:

- The development of more efficient processes and new properties for bioproducts, in order to make them more competitive versus the existing ones, which are sometimes cheaper than bioproducts.

- The development of new bioproducts with higher performance in existing applications
- The development of innovative bioproducts with new applications and properties.

In the Bioenergy area, the key technological challenges are:

- The development of optimal enzymes and robust fermentation systems (e.g. thermophilic microorganisms and enzymes) capable of converting lignocellulose directly and fermenting it into ethanol or other biofuels.
- Making these technologies cost effective
- The development of new fermentation processes based on biodiesel-deriving glycerol and CO_2 as carbon sources.

Lastly, another key challenge will be to effictively boost the political and economical environment stimulating research and innovation, entrepreneurship, product approval and market development in the EU sector of white biotechnology.

4.2.2 - Environmental Biotechnology for the Contaminated Site Characterization and Remediation, and Groundwater/Wastewater Clean-up

Biotechnologies may also offer special tools and strategies for a sustainable and effective management and reclamation of degraded or/and contaminated sites and wastewaters which make up one of the main environmental issues that the enlarged Europe must deal with over the coming years. Indeed, in many areas of Europe, soil is being irreversibly lost and degraded as a result of increasing and often conflicting demands from nearly all economic sectors. In Western Europe, pressures come from the concentration of population and activities in localized areas, economic activities and changes in climate and land use. Air depositions and cultivation systems are among the most important influences on the quality of soils in agricultural and natural areas. Consumer behaviour and the industrial sector are contributing to ane increase in the number of potential sources of contamination such as municipal waste disposal, energy production and transport, mainly in urban areas. In Central and Eastern Europe, many of the problems stem from past activities and poor management practices.

The combined action of these activities affects quality and limits many soil functions, including the capacity to remove contaminants from the environment by filtration and adsorption, and consequently increase the possible transfer of contamination to groundwater. This capacity and the resilience of soil mean that damage is not perceived until it is well advanced. This partly explains the low priority given to soil protection in Europe until recently. Moreover, since soil is a limited and non-renewable resource, when it is damaged, unlike air and water, it is not easily recoverable.

The geographical distribution of soil degradation depends on several factors. Soil problems are influenced by the diversity, distribution and specific vulnerability of soils across Europe. They also depend on geology, topography and climate and on the distribution of driving forces. Better integration of soil protection into sectoral policies and better harmonization of information across Europe are needed to move to more sustainable uses of soil resources and a promotion of sustainable models of its use. In particular, soil contamination from diffuse inputs and local sources can result in the damage of several soil functions and frequently causes the contamination of connected water bodies (most often groundwater, but also surface water and related sediments).

The solution and prevention of soil and ground water degradation problems has recently become a policy issue in Europe. Soil contamination is considered a priority threat within the issue. The status of site contamination, the state of the art in assessment and remediation, and strategic harmonized solutions have been analyzed over the past 10 years by the EEA and several European networks (CARACAS, CLARINET, NICOLE, Common Forum for Contaminated Land in Europe).

The current activities for the development of the EU Soil Thematic Strategy are clearly oriented toward risk-based solutions of historical contamination problems. The work carried out by European cooperation programmes provides the grounds and the technical and scientific references for the formulation of the strategy. Several Member States have already formulated risk-based oriented policies for the management of contaminated land at their national level, and favoured the adoption of innovative technological approaches to remediation. In addition, many Member States have established criteria for distinguishing new and historical contamination.

According to a recent survey, there are over 1,500,000 sites, often former industrial sites, contaminated by organic pollutants and/or heavy metals in the European Union (EU). EU candidate states also possess a large number of contaminated lands, often located in areas within or near highly populated cities. Such sites constitute an enormous environmental problem, which the recently enlarged EU must take care of over the next few years. To address it, innovative and effective site-monitoring tools and strategies along with remediation technologies capable of combining high decontamination efficiency with low costs and impacts on the site infrastructure and living organisms are necessary. In terms of monitoring, biosensors, along with ecotoxicity tests and molecular microbiology measurement techniques, have become essential tools to reinforce the modern analytical chemistry tools, as they can offer specific information on the actual toxicity and microbial life occurring at the site. In terms of decontamination technologies, the biological ones, i.e. those that typically exploit the activity of pollutantdegrading site-occurring organisms, are greatly preferred over the chemical and physical technologies currently available on the market, as they fully fit the requirements listed above.

The *in situ* application of biological degradation processes of soil and groundwater pollutants on a field scale is approximately 10-15 years old. Numerous laboratory and mesocosm studies have been published to form a sound basis for the application of these techniques. During the last decade, many biological degradation processes of various pollutants in different soil types and groundwater have been developed into *in situ* bioremediation techniques. Among these techniques, we also classify spontaneous biodegradation processes in soil and groundwater (natural attenuation), provided that it is adequately monitored for acceptable prolongation of this process with time. At present, *in situ* biological soil clean-up has evolved into a full-fledged and cost-efficient alternative to other bioremediation techniques.

It is also a priority of the European Union to develop promising wastewater bioremediation technology to encourage SMEs from different industrial sectors to accept the challenge of treating their wastewater (ecological image and money saving) and to benefit from this new technology. This objective supports the "Environmental Technologies for Sustainable Development" (COM 122-2002), a communication from the European Commission approved by the Barcelona European Council in March 2002 stating that "environmental technologies contribute to sustainable development by boosting our economies, protecting our environment and providing new jobs", as well as the "EU Water Framework Directive".

However, today remediation technology development is evolving differently and at different rates in unconnected, isolated pockets of Europe, without a joint sharing of experiences, successes, and lessons learned through technology demonstration. Despite the successful development and demonstration of novel technologies with these features, conventional methods still generally prevail in the market (EURODEMO Newsletter 1. 2006. Available from: http://www.eurodemo.info).

Making national remediation efforts and especially innovative remediation efforts visible and accessible on a European level would support international experience exchange and transnational knowledge transfer. Thus, planned remediation activities and especially innovative applications – often connected with high learning costs – could be optimized with regard to improved technology performance and more sustainability with optimal cost-efficiency. By better connecting European remediation efforts and thus minimizing the duplication of efforts, an overall increased effectiveness of remediation activities and a faster advancement of innovative technologies could be achieved to the benefit of all involved parties. Finally, the competitiveness of European technologies could be strengthened in a global market and a European State-of-the-Art in remediation could be approached.

4.3 - State-of-the-Art on the White Biotech R&D and Industry in Italy and Some Notes on the Environmental Biotech for the Contaminated Sites Reclamation.

A few very small Italian companies are involved in the biotech production of primary metabolites, microbial starters and enzymes and in particular of the main white biotech products, such as fine chemicals, microbial polymers and biofuels. However, a large number of qualified applied research projects focused on the use of enzymes and microorganisms for making new and conventional products such as fine and bulk chemicals, pharmaceuticals, food and feed, energy, and polymers, are in progress in Italy at universities, research centres (belonging to CNR, ENEA, etc.) and/or national science and technology parks.

In particular, according to a recent survey performed by the scientific committee of the Industrial Biotechnology section of IT-SusChem, around 130 large and interdisciplinary projects have been just concluded or are in progress in the country; these are mostly national but also international, and focus on improving the production of conventional or innovative primary and secondary metabolites, enzymes and proteins, new or improved fine chemicals, bioplastics, lubricants, and biosurfactants, and on intensifying the production of biofuels (mostly biogas and, to a lesser extent, bioethanol, biodiesel and biohydrogen (bioH2)) from a variety of biomass sources and agroindustrial wastes and wastewaters. Over 40 different universities, several CNR Institutes and ENEA centres, along with private/public research centres (Stazione Sperimentale Olii e Grassi, Stazione Sperimentrale per la Seta, CRPA, etc.), some spin-offs, and small private companies are involved in such projects. Most of the projects have been supported through public funds provided (on a competitive basis) by the Ministry for the University and Research, the Ministry for Productive Activities, and the Ministry for the Environment (through programmes such as PRIN, PNR, FIRB, FAR, FISR, PON, etc) and, to a minor extent, by some local institutions (i.e. Campania, Emilia-Romagna, Friuli Venezia Giulia, Liguria, Lombardy, Marches, Piedmont, and Sardinia regions, Province of Ravenna, etc.), small biotech or chemical companies (mostly located in the northern part of Italy), or the European Union (through the 6 FP).

The reviewed projects can be grouped into the six clusters reported below.

1. Isolation and characterization (also through bioinformatics, proteomic and synthetic biology approaches) of new enzymes and microorganisms and improvement (through in vivo and in vitro manipulations) of the existing ones specifically for white biotech applications. Among the new or improved enzymes are: laccases, penicillin acylases, nitrilases, mono- and di-oxygenases, cheratinolytic enzymes and enzymes from marine organisms, thermophilic or cold-adapted bacteria, and a variety fungi. Among the new isolates or improved microorganisms there are a large number of probiotic bacteria, thermophilic bacteria, and fungi, all capable of displaying new or improved properties of industrial and/or environmental interest. There are over 30 projects,

mostly carried out through national cooperation among universities, research centres (belonging to ENEA, CNR, etc.), and technological parks, thanks to the economic support of the Ministry of the University and Research (PRIN, FIRB, CNR grants), the universities, or together with some private companies (mostly of the Chemical area) and foundations. A couple of projects were funded by the Friuli Venezia Giulia, Emilia Romagna and Piedmont regions; six of the projects were funded through the 6FP.

2. Development of innovative/improved bioreactors, production and downstream procedures and procedures for bioprocess design and modelling. Development of innovative/improved integrated strategies for recycling and valorizing (via recovery and biotech transformation of main constituents) agro-industrial wastes, wastewaters and surplus (biorefinery concept), as well as for closing the carbon cycle in farms (i.e. biomass for producing biogas and hydrogen and successive carbon storing in soils: “carbon sequestering farm”). Around 30 projects are carried out through the cooperation among universities, research centres and companies, and funded through grants provided by the Ministry of University and Research (i.e. PRIN, FIRB, CNR grants), the Ministry of Agriculture, some Regions (Campania, Lombardy), single universities or together with private companies. Five of the reviewed projects have been funded through the EU 6 FP.
3. Development of innovative/improved strategies and/or processes for the direct enzymatic/fermentative production of chiral drugs and synthons, aminoacids, vitamins (ascorbic acid),organic acids (lactic acid), flavours (biovanillin, xilitol), pharmaceuticals (glutathione, polyunsaturated fatty acids), etherologous proteins, fructooligosaccharides (FOS), microbial enzymes, and microbial biomass (probiotics) from conventional media and agroindustrial by-products or wastewaters. Around 15 projects are mostly carried out through national cooperation among universities and research centres (belonging to CNR, ENEA, etc.), thanks to funds provided by the Ministry of University and Research, (via PRIN, FIRB, CNR grants), single universities or together with private companies (mostly of the Food and Feed area).
4. Development of innovative strategies for the recovery of fine chemicals, such as antioxidants, phenols, carbohydrates, FOS, etc., from agroindustrial by-products or wastewaters, and tailored application of new/improved existing biocatalysts for the bioconversion of remaining products/molecules into new fine chemicals, chiral molecules, antibiotics and pharmaceuticals, aminoalcohols, fatty acids, phospholipids and lipopolysaccharides, etc. (biorefinery concept). These are over 20 projects, mostly carried out through cooperation among universities and research centres (belonging to CNR, ENEA, etc.) and grants provided by the Ministry of University and Research (PRIN, FIRB, CNR grants), interuniversity consortia, universities or together with private companies (mostly of the Chemical area). A couple of projects were funded by the Emilia Romagna and Sardinia regions and two others through EU funds (COST and IP programs related to the FP6).
5. Development of innovative/improved strategies and/or processes for the production of microbial polymers (polyhydroxyalkanoates, protein matrices, polylactate, exopolysaccharides, etc) and biodegradable polymers (natural fibers, nanofibrils of chitin, fuel latent bioplastics, biodegradable polyesther blends, etc), as well as lubricants (hydraulic oils) and biosurfactants from biomass and agroindustrial wastewaters and wastes. These are around 20 projects, mostly carried out through by uuniversities and research centres (belonging to CNR, ENEA, etc.), thanks to the economicl support provided by the Ministry of University and Research (PRIN, FIRB, PON, CNR grants), universities or together with private companies (mostly of the Chemical area). A couple of the projects were funded by local institutions (Piedmont and Lombardy regions, Ravenna province). Nine of the reviewed projects are international and mostly funded through EU 6FP money.
6. Development of innovative/improved strategies and/or processes for the production of biofuels (biodiesel, bioethanol, biogas and bioH2) and bioenergy (via biofuel cells) from a variety of agroindustrial and municipal wastes, agroindustrial wastewaters and sludges (also through dry fermentation, in the case of biogas and bioH2). About 15 projects, mostly carried out through the cooperation among universities and research centres (institutes of CNR, ENEA, and CRPA), and within dedicated spin-offs, thanks to the economic support provided by the Ministry of University and Research (via PRIN, FIRB, CNR grants), single universities or together with private companies (mostly of the Food and Feed area), local institutions (Sardinia, Emila Romagna, and Veneto regions). Six of the reviewed projects are international and funded through EU 6FP money.

Taken together, the information provided indicates the existence of solid R&D expertise in all the 7 strategic research priority areas identified in the Industrial Biotechnology section of the ETP SusChem SRA and retained by ETP SusChem IAP, “http://www.suschem.org”). The main Italian IB R&D elements of excellence are in the areas of a) development of improved enzymatic and microbiological tools and technologies (freely suspended and immobilized biocatalytic systems, reactors with tailored configurations) for the production of new fine chemicals of interest for the pharmaceutical, chemical and food industry, as well as of vitamins, proteins and organic acids from simple and complex substrate mixtures, and some agroindustrial by-products, wastes and wastewaters, b) recovery and characterization of biomolecules (antioxidants, vitamins, etc) from agroindustry and food-industry by-products, wastes and wastewaters, and c) biotransformation of main components of agroindustry and food-industry by-products, wastes and wastewaters into flavours, biopolymers, biosurfactants, enzymes and/or biofuels, such as biogas, biomethane and biodiesel. A possible Italian weakness evidenced by the national R&D scenario described above is a widespread lack of knowledge and expertise in the area of biotransformation of lignocellulosic biomass and agroindustrial by-products, and in the production of “second generation bioethanol”.

Some R&D activities on the enzymatic hydrolysis of cellulose, including pretreatment technology, have been and are presently being carried out only at ENEA (Trisaia centre) and by a couple of University research groups.

The Italian R&D scenario described above also indicates that Italian researchers are already accustomed to co-operating and sharing their scientific specialisms within large multi-disciplinary collaborative projects; this feature is essential for successfully performing high-standard industrial biotech R&D.

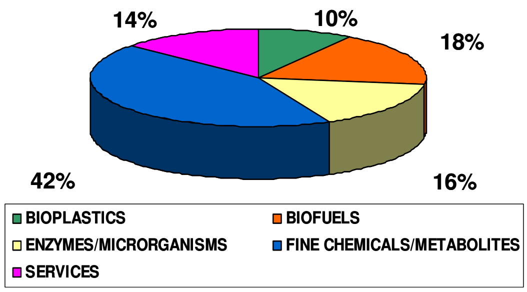
A considerable number of qualified publications (over 700 in the last 3 years, on the basis of the data provided) resulted from the reviewed projects. On the other hand, only a few patents (fewer than 15 during the last 3 years) were produced through the same projects. This evidence, along with the fact that the majority of the reviewed projects resulted or are going to result in laboratory-scale bioreactors or/and processes, indicates a certain lack of aptitude and/or knowledge in the technology transfer phase of new or improved processes and technologies.

According to a recent survey conducted by the Fondazione Rosselli (Turin)[6], Italian projects performed in the fields of production of heterologous proteins/enzymes, primary metabolites, biopolymers and bio-energy have already generated a qualified know-how justifying its transfer to the large scale. Some Italian small, often "single-product", R&D biotech companies, along with some spin-offs, have currently been producing and selling specific IB products. In addition, some Italian chemical or biotech-related SpA companies are gradually orienting some of their R&D activities into the industrial/white biotech area. According to a national survey promoted by *IT SusChem*, around 45 companies (joint-stock or limited-lliability companies) along with 5 spin-offs can be ascribed to the white biotech sector. As shown by the chart below, some of them, including two joint-stock companies (i.e. FAB-Fidia and Novamont), are active in the production of new or advanced biopolymers, others, including joint-stock companies like ENI and Marcopolo Technologies, are active in the area of bioenergy/biofuel production or in the production of microbial starters, probiotics, and enzymes (including CSL Centro Sperimentale del Latte, Biosphere as joint-stock companies), but the majority of the reviewed companies (including several joint-stock companies such as ACS DOBFAR, AGROLABO, DIASORIN, DIASPA, FLAMMA, FATRO, GNOSIS, LONZA, LAMBERTI, NORPHARMA, PRO.BIO.SINT and SEDAMYL) are active in the sector of the production of primary and secondary metabolites, fine chemicals, flavours, prebiotics proteins, hormones and pharmaceuticals. There are also several SMEs providing services to companies and research centres operating in the sector. The majority of the information provided comes from the national survey performed by the scientific committee of the *IT-SusChem* platform, while some of it was collected through a website search.

With regard to the application of biotechnologies for environment protection and remediation, a similar inventory is presently being conducted. Thus only a qualitative picture can be given here, just to highlight the high potential they have in Italy.

Indeed, it is noteworthy that biological processes are well-established in several traditional sectors of environment protection such as end-of-pipe treatment and disposal of wastewater, sludge and wastes (e.g. activated sludge process, biofilm processes, sludge stabilization, anaerobic digestion, composting, etc.). In these sectors, biological processes are much more developed than in traditional sectors of the chemical and process industry, and they also have a leading role with respect to chemical and physical processes. Presently, it can be estimated that in Italy several thousand plants include at least a biological reactor as a key step of the overall process. Most of them are of small-to-medium size, and SMEs are usually in charge of their design, operation and control. On the other hand, in spite of such a large background in traditional bioprocesses, a rather limited use is still made of the modern tools offered by chemical engineering and industrial biotechnology, for both design and control.

Now, as often required by increasingly stringent regulations, there is a growing trend toward combining typical bioprocesses with more advanced approaches in terms of both increasing efficiency of traditional bioprocesses and broadening their application to cope with emerging problems. Among the latter, the bioremediation of contaminated soils and groundwater is particularly important, with particular reference to sustainable remediation of xenobiotic-type organic micropollutants.



In a recent survey by Federambiente (http://www.federambiente.it/) among companies providing environmental services (http://www.federambiente.it/moduli/Rapporto%20Bonifiche%202007/Rapporto%20Bonifiche%202007.htm), about 25% of these companies are active in site remediation, most often in combination with more traditional

6 Report on "National Priorities of Reserach 2002; "http://www.pdf4free.com"

services. Interestingly, most companies are SMEs (less than €30 million/year) but a higher average size is found for companies involved in the remediation of more complex industrial sites. Even though they must be confirmed by a specific analysis, these few figures indicate the potential for the development and application of advanced biotechnologies in the field of site remediation. Moreover, academic and industrial research is quite active at the lab and pilot scale. Several field tests for advanced bioremediation are presently operating in Italy (e.g. in Rho, Naples, Ferrara) and a few projects are under evaluation for full-scale application (e.g. Marghera site).

4.4 - Main R&D Priorities for the Italian White and Environmental Biotech Sectors.

Given the type and the localization of white biotech R&D expertise already available in Italy, the current situation of the industry in the sector and, importantly, the socioeconomic priorities of the country, future national R&D efforts in the white biotech area should be focused on implementing and boosting the development of:

a) new and/or improved biocatalysts and biocatalyzed processes to foster a shift in chemical manufacturing, to enable cleaner, safer and more cost-efficient processes or novel chemo-enzymatic processes with the integrated use of biocatalysts;

b) innovative and/or improved strategies and biotech tools for the integrated valorization of national biomass and agrofood industry by-products, wastes, wastewater and surpluses through the direct recovery of biomolecules and biobased compounds and the biotransformation of the resulting products into biofuels, fine chemicals, biopolymers, biosorbents, biosurfactants and biofuels (biorefinery concept);

c) improved bioprocesses for the production of biofuels (both “second generation Ethanol” and biogas/bioH_2) from Italian biomasses and agro-industrial surplus, by-products and wastes;

d) innovative and/or improved strategies and biotech tools and processes for the integrated monitoring and remediation of Italian contaminated lands and sites.

4.4.1 Priority a).

New and/or improved biocatalysts and biocatalyzed processes to foster a shift in chemical manufacturing to enable cleaner, safer and more cost-efficient processes or novel chemo-enzymatic processes with the integrated use of biocatalysts

Enzymes are increasingly used as efficient biocatalysts to perform a range of chemical reactions. As an example, global sales of single-enantiomer compounds are expected to reach $14.94 billion by the end of 2009, and the share of the market occupied by traditional technology would drop to 41%7. The share of chemocatalysis would rise to 36% and the share of biocatalysis, to 22%, (1.9 billion $). Demand for enantiopure chiral compounds continues to rise, primarily for use in pharmaceuticals but also in three other sectors: agricultural chemicals made up 14.1% of the revenues, and flavours and fragrances accounted for 4.7%. However, natural biocatalysts are often not optimally suited for industrial applications and the major limitation for their commercial use is their price and availability. To develop this sector and the use of enzymes in industrial processes, it is important to expand the range of biocatalysts and improve them for specific uses. Specific types of enzymes such as racemases or oxido-reductases are of particular interest for industrial processes. In this respect, the development of new hosts for the production of endogenous and/or heterologous enzymes is also necessary.

Goals:

a) Optimized enzymatic activity.

Since the major industrial players in the field generally do not sell their own enzymes, it is a major priority to have access to extensive strain and enzyme collections for screening and development. Easy access to protein expression and production, even at a scale that enables preliminary tests on pilot plants, is also a prerequisite.

b) Rapid and efficient screening methods

From an analysis of the European situation[8], it emerges that one of the biggest problems is the still highly empirical nature of catalyst selection.

c) Easy-to-use formulated enzymes

Strategies such as substrate modulation and reaction engineering can often help overcome bottlenecks faster than directed evolution can. The understanding of reactions and catalytic mechanisms is far from complete. Understanding the nature of the catalyst and the optimal process conditions is crucial for successfully identifying and scaling up a biocatalytic process, just as it is for any catalyst. Stronger integration between chemistry and process engineering expertise is crucial on this respect.

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[7] Frost & Sullivan survey; “http://www.frost.com/prod/servlet/svcg.pag/CMCM”

[8] Chemical Engineering News, August 14, 2006 Volume 84, Number 33

d) Improved reaction design and engineering

- Solving reaction and process problems of industrial relevance and accelerating the move to sustainable chemistry by searching for novel biocatalytic functions;
- Overcoming thermodynamic barriers, avoiding high environmental impacts due to the chemical catalysis (both in terms of catalysts applied and reaction conditions required), or reviving natural product biosynthesis;
- Multiphase bioreactors - research projects and network on methodology for high substrate/product concentrations. Methodology for in situ product removal;
- Engineering for complex biocatalytic reactions involving multiple phases – research projects, network;
- Development of cascades of (chemo-)enzymatic methods - R&D projects to make chemo- and biocatalysts compatible with each other.

Actions:

- Multidisciplinary research needed involving molecular biology + enzyme technology + organic chemistry + biochemical engineering;
- Improved and novel protein production systems for efficient and large-quantity enzyme production (including study of protein secretory pathways);
- Fundamental research on understanding of catalytic enzyme/ substrate interactions;
- Integrating evolutionary and computational approaches to rationally design biocatalysts with improved efficiency and (enantio)selectivity, thus shortening the timescale of enzyme discovery;
- Screening and development of new enzymes for specific applications (lyases, racemases, oxidases, oxygenases, peroxidases, and oxido-reductases);
- Development of fast and efficient methodology for screening and activity assay;
- Creation of new functions in existing enzyme scaffolds;
- Search for new biocatalysts and new enzymes from extremophiles and other organisms with the integrated use of bio-informatic approaches;
- Computational tools used in combination with metagenomic and directed evolution to shorten the timescale of enzyme discovery;
- Development of high-performance integrated multiphase bioconversions: enzymatic processes tested under industrial conditions. Research projects and network on methodologies for high substrate/product concentrations;
- Methodology for in situ product removal;
- Biocatalytic reaction engineering - Engineering for complex biocatalytic reactions involving multiple phases – research projects, network;
- Development of cascades of (chemo) enzymatic methods - R&D projects to make chemo- and biocatalysts compatible with each other;
- Formulation and solution of accurate transport models based on the analysis of momentum, heat and mass transfer and capable of predicting the behaviour of enzyme or whole-cell bioreactors.

4.4.2 Priority b).

Innovative and/or improved strategies and biotech tools for a more extensive and efficient valorization of national biomasses and agro-food industry surpluses, by-products, wastes, and wastewater

4.4.2.1 New/improved biotech tools and strategies for the valorization of biomass and agroindustry by-products and wastes.

4.4.2.1.1 Improved national biomass use and alternatives to carbohydrates

The diversification of biomass used in industrial processes from the currently available carbohydrates to lignocellulosic biomass, use of agricultural or food industry wastes/by-products, surpluses and wastewater, can provide cheaper biomass, as well as helping to secure the feedstock supply and, in some cases, to reduce the environmental impact associated with their accumulation in the environment. In addition, the use of some national agro-industrial surpluses, such as those associated with sugar beets or cereal cultivation, should be considered. It is also crucial to focus on current Italian biomass, and to develop appropriate biomass sources for industrial processes in conjunction with plant scientists.

Biomass conversion must be adapted to more complex feedstock via improved enzyme cocktails and adapted, robust fermentation microbes which can deal with complex sugar streams. Lipids and glycolipids, with the exploration of specific enzymes for their modification and activation, are a potential source of new bioprocesses and bioproducts, especially for the integration of bioprocesses with traditional oleochemistry.

Activities include:

- Availability and development of biomass for non-food use;
- Conversion of biomass (pretreatment and hydrolysis; robust fermentation systems);
- Alternative feedstock to classic carbohydrates (by-products of pulp factories, biofuel production, food industry (cheese whey, olive mill wastewater, lipids…).

4.4.2.1.2 Development of the next generation of high-efficiency fermentation processes

The main objectives to be achieved are:

- Increase in product yield;
- Facilitation of the scaling-up of processes;
- Development and intensification of novel bioprocesses for bioproducts;
- Reduction of waste production from bioprocesses.

Fermentation processes are commonly used today for the production of numerous products; however, the meeting of growing demands in very competitive markets is still in need of microbiological and technological improvements.

To improve the efficiency of fermentation processes, it is important to better understand the metabolism of microorganisms under extreme environmental conditions and, based on this information, to select better microbes for either axenic or mixed microbial culture processes.

Current technological challenges include low volumetric productivity due to harsh fermentation conditions for microorganisms (pH, temperature, substrate concentration…), the difficulties of scaling-up processes from lab to large-scale fermenters, and the investment needed to develop and implement new or improved bioprocesses which account for a significant part of the whole production costs.

New challenges are also emerging, among them the design and construction of micro-bioreactors and high-throughput screening needed by industry for new and improved processes. Future efforts will also concentrate on the development of new or intensified technologies that can provide a reliable and renewable energy supply (bioethanol, but also bioH_2 and CH_4). These new expectations for fermentation processes require the use of highly advanced technologies and the strong cooperation of experts from various scientific areas.

To improve the product yield, the following issues have been identified:

Microbial genomics and bio-informatics

Availability and usability of genomics information:

a. Functional microbial proteomics, metabolomics and genomics. Analysis of gene knocks out and over-expressions of industrial relevant organisms on a larger scale by chip proteomics and flux analysis to identify unknown functions and regulations;
b. Development of high-performance cell/animal platforms amenable to cost-effective high-throughput technologies for experimental approaches up to the genomic level, since their genomes are entirely sequenced [e.g. *Escherichia coli* (prokaryote), *Saccharomyces cerevisiae* (simple eukaryote), *Caenorhabditis*

elegans (small multicellular animal)]. This set-up can deliver fast answers for a wide range of discovery problems. The assortment ranges from screenings of new therapeutics to the assessment of environmental risks, and can ask questions ranging from the bioavailability of the molecule(s) being studied to the definition of their primary target site and/or their putative systemic effects;

c. Bioinformatics, support for acquiring novel genes with a given function and system biotechnology by tools/strategies for high-quality functional annotation of genes and gene products, and management of databases by integrating in silico and experimental data and applying machine learning / data mining techniques. Consequent improvement, standardization and implementation of ontology-based annotations.

Metabolic engineering and modelling for robust fermentation microorganisms

a) Increasing the understanding of cellular regulation under industrial fermenting conditions (stress, “zero growth”, quorum sensing, fluctuation in nutrient or substrate concentration) to include these parameters in mathematical models: regulatory systems in cells via protein-protein interaction, protein-DNA interactions; product export from cells and metabolic compartmentalization in eukaryotic cell factories, kinetics of membrane transport, product inhibition in relation to product recovery;
b) Studying the physiology of microorganisms under extreme conditions such as: pH and temperature, slow growth rate, dynamic stress and unbalanced growth, high concentration of substrates and products, regulation of gene expression by quorum sensing; screening the most suitable (or improved) variants for a desired process or completely new variants for particular purposes;
c) Studying the population dynamics in mixed microbial cultures for improvement of efficiency and strength of low-cost mixed culture fermentation processes;
d) Developing methodological tools for the mathematical modelling of microbial metabolism in both steady and dynamic models: flux analysis and measurement of intracellular metabolites (industrial conditions).

Process scale-up and intensification:

a) Development of a new generation of reactors: alternative novel reactor concepts that allow for more intensified production, and create optimal environmental conditions for the production of certain metabolites by microorganisms in fermentation processes;
b) More extensive application (for both bioconversion and fermentation processes) of tailored intensified bioreactor systems, such as those relying on specialized bacterial biomass, either passively immobilized on porous carriers or self-granulated, in packed bed or expanded bed or fluidized bed loop reactors, capable of providing higher volumetric productivities, higher stability (genetic and also vs adverse chemical and physical parameters), higher tolerance towards high and variable organic loads, and often an easier product recovery than conventional freely suspended cell bioreactors;
c) Development of engineering tools to design strategies for process intensification, advanced control strategies (non-invasive, highly sensitive, inexpensive), mainly with reference to new biotechnology-based analytical methods, sensors and screening techniques;
d) Development of micro-bioreactors based on realistic large-scale production conditions as a screening tool to shorten the process development time, accompanied by research on scale-dependent differences in microbial behaviour; development of advanced monitoring and controlling strategies of various parameters with the use of advanced simulation techniques (artificial neural networks, hybrid models, etc.) and sophisticated instruments, tools and high-tech equipment;
e) Development of simulation tools for modelling and optimization of fermentation processes on different scales;
f) Improvement of continuous fermentation processes: solving problems of strain instability during long cultivation times by genetic methods (deleting phages in the genomes, creating selective pressure), solving contamination problems by minimal media, selective pressure, harsh conditions like high temperature, solving microbial population shift in mixed microbial systems, increasing process strength in the presence of highly dynamic feeds (organic load and compositions).

Downstream processing:

Development of downstream methods to isolate fermentation products during the fermentation processes and chemical methods to convert fermentation products to interesting chemicals without prior purification of the fermentation product from the broth.

For more advanced combined processes, the following issues have been identified:

a) Development of combined technologies enabling the parallel production of different products;
b) Development of bioprocesses & technologies that make it possible to generate energy from wastes (waste utilization); development of bioprocesses that combine the production of desired products with energy generation (example: integrated valorization of by-products/wastes through the bio-catalytic conversion of some of their specific macromolecules into fine chemicals followed by alcoholic fermentation or anaerobic

digestion - resulting in bioH2, fatty acids, CH_4 generation - of remaining organic material - zero waste processes;

c) Development of bioprocesses based on or with recirculation of waste by-products.

4.4.2.2 Process eco-efficiency and integration: the biorefinery concept

Widespread implementation and use of integrated and diversified biorefineries, in order to use our agricultural/forestry resources in the most efficient (cost and energy) way.

Biorefinery is a concept that has been developed in the food and paper industries and is now applied in biomass-based energy production. It relies on the best use and valorization of feedstock, optimization and integration of processes for a better efficiency, optimization of inputs (water, energy…) and waste recycling/treatments. The production of bioproducts, especially for bulk chemicals, biofuels, sorbent matrices, and polymers, can improve their competitiveness and eco-efficiency by process integration and economy of scale. Many improvements are yet needed to improve the process first: total use of the plant, by-product or waste (better fragmentation and fractionation) and development of processes to add value to all fractions of lignocellulosic material and to valorize by-products, effluents and wastes of other industrial systems (e.g. black liquor in wood/paper industry, glycerol from biodiesel, whey from cheese production, olive mill wastewater …), agro- and food-industry surpluses or products damaged or contaminated and not suitable for food use (e.g. micotoxins in cereals), and downstream processing strategies (low-cost recovery and purification). It is also necessary to study the whole value chain as well as the "biorefinery value chain" for optimization of costs, CO_2 reduction, water and energy minimization.

4.4.2.2.1 Action 1.

To develop new and general strategies to meet the energy- and cost- efficient requirements to be competitive on the market

- Industrial research and development, user groups and forums to define and discuss criteria for sustainability, optimalization of CO_2 reduction potentials, analysis and optimalization of energetic/exergetic use of biomass production/biorefining chains, economic optimalization strategies throughout the whole value chain from the field production to the industrial and/or consumer use use;
- Academic research projects, industrial collaborative research, user groups and forums to develop and validate new business models, criteria for sustainability evaluation, CO_2 reduction potentials, analysis and optimalization of energetic/exergetic use of biomass production/biorefining chains, and economic optimalization for biorefinery value chains.

4.4.2.2.2 Action 2.

Improvement of biorefining technologies

Academic research projects, industrial collaborative research projects, industrial research on:

- efficient harvest and storage technology of various crops directed towards large-scale biorefining;
- whole crop biorefining methods for separating different components such as sugar, starch, lignocellulose, fats, proteins, aminoacids, organic acids from seed, leaf, woody and root parts of various crops;
- the utilization of abundantly produced plant fractions that remain after the use of plant materials for the production of biofuels;
- development of a sustainable closed-loop approach for water saving, through both efficient water use and wastewater reclamation and reuse, in either the fermentation processes or the crop production;
- development of process equipment to open plant/lignocellulose tissues and to fractionate plant components with little energy input;
- Academic research (with industrial support) for the selection of plants that accumulate specific bulk chemicals (precursors);
- development of new processes for bio-ethanol production and valorization of glycerol (resulting from biodiesel production) for incorporation in diesel fuel or base chemicals;
- development of new processes for transforming saccharose to base chemicals.

4.4.3 Priority c),

Improved bioprocesses for the production of biofuels from Italian biomasses and agro-industrial surpluses, by-products, wastes and wastewaters.

Biofuels are the focus of growing interest for transportation. They can provide a reliable and renewable supply of energy as well as reducing greenhouse gas emissions into the atmosphere. The European Union has recently set ambitious new targets[9]: by 2010, 5.75% of both petrol and diesel fuel will comprise biofuels, rising to 20% in 2020.

[9] Directive 2003/30/EC of the European Parliament and the Council of 8 May 2003 on the promotion of the use of bio-fuels and other renewable fuels for transport (OJEU L123 of 17 May 2003)

To be competitive, bioethanol production relies on cheap and reliable sources of renewable raw materials and efficient fermentation processes. At the present time, sugar prices in Italy are too high to allow a competitive production of bioethanol, and only a part of the crops are being used. Therefore new technologies need to be developed to efficiently convert cellulosic, fibre- or wood-based waste biomass into fermentable sugars. However, the availability of such sources of substrates is limited in Italy. Conversely, there is an enormous availability of agrofood industry by-products, wastes and wastewaters, as well as of domestic organic wastes from which ethanol but also biomethane and bioH2 can be generated, with the concomitant reduction of the environmental impacts associated with their accumulation in the environment.

Goal 1

To have "second generation" bioethanol based on Italian specific biomass, such as crop waste (straw, corn cobs), energy crops, wood-industry wastes, agro-food surpluses and wastes**,** available on a commercial scale within 10-15 years.

4.4.3.1.1 Action 1: Improved biomass conversion by hydrolysis based on diversified, cheaper sources of renewable raw materials

Biomass hydrolysis technology involves the breakdown of carbohydrates into its component sugars by a range of chemical and/or biological processes. Biomass is first subjected to pre-treatment to hydrolyze the hemicelluloses and expose the cellulose for subsequent enzymatic degradation. The cellulose then undergoes enzymatic hydrolysis to produce glucose, which can be converted to bio-fuels and chemicals by fermentation.

The following actions are necessary to allow the commercial development of 2^{nd} generation bioethanol:

- Research projects on understanding pre-treatment technologies and designing new more reliable reactors and equipment, minimizing the energy input;
- R&D frontier projects to create a new generation of cheap enzymes for the hydrolysis of cellulose and lignocellulose into fermentable sugars;
- Industry-academia partnerships in developing new catalysts for the selective functionalization of alkanes, gas-to-liquid conversion, use of biomass and waste for energy and chemical applications;
- Industry-academia partnerships in process improvement, including: reduction or economic removal of inhibitory substances in sugar streams for fermentation; introduction of novel feedstocks from biomass fragments, e.g. glycerol in the short term, for chemical production;
- Research, feasibility and impact analysis to develop biotechnological production networks in bio-refineries, including energy management and diversion of by-products to parallel processes.

4.4.3.1.2. Action 2:

Improved biomass fermentation to ethanol

The microorganisms used must be able to fully convert the carbohydrates into ethanol, be robust, and tolerate the toxic compounds formed during the pre-treatment process. They must be able to withstand the stress of high ethanol and substrate concentrations, low pH, etc. At present, no such strains are available and significant challenges still lie ahead in the development of such robust micro-organisms. Developing such strains requires a multidisciplinary approach involving various aspects and research areas through industry-academia partnerships:

- research on microbial metabolic pathways and metabolic engineering to expand the substrate usage spectrum of micro-organisms;
- identification of microbial stress response mechanisms and tolerance to industrial conditions, and subsequent engineering of the system into the production micro-organisms;
- engineering of positive industrial characteristics to increase yield, such as high ethanol tolerance, fast growth, high ethanol yield and productivity, but also production of hydrolytic enzymes to complete the biomass hydrolysis during fermentation.

Goal 2

To improve the qualitative and quantitative production of biogas (biomethane and bioH2) through dark fermentation of agro-food industry by-products and surpluses, domestic organic wastes, the organic fraction of municipal wastes, etc. through the optimization of the already existing medium- and large-scale technology and the development of innovative tailored biotech processes.

4.4.3.2.1 Action 1.

Academic research projects, industrial collaborative research projects, industrial research on the development of innovative single- or double-phase anaerobic digestion processes in conventional or non-conventional reactors under mesophilic and/or thermophilic conditions for an improved biomethane and/or bioH_2 production from organic wastes, residues, sludges and wastewater of national or regional interest, including the development of co-digestion processes based on the optimal formulation of different components such as sugar, starches, fats, proteins, aminoacids, and organic acids.

4.4.3.2.2. Action 2.

Industrial collaborative research projects focusing on identifying strategies for improving the performance and intensifying the use of the medium- and large-scale anaerobic digestors already existing in Italy.

4.3.2.3. Action 3.

Academic research projects and industrial collaborative research projects focusing on developing new integrated systems for producing electricity by means of MCFC (melting carbonate fuel cells) fuelled with biogas and/or biohydrogen.

4.4.4 Priority d)

Innovative and/or improved strategies and biotech tools and processes for the integrated monitoring and sustainable remediation and reclamation of Italian contaminated environmental resources and habitats (soil, sediments, water and groundwater)

Environmental resources (soil, sediments, air, water and groundwater) and their habitats must be considered of high social, cultural and economic value. All these resources are presently protected by European Directives. For soils there is a Framework Directive draft presently under discussion which defines soil as a non-renewable resource that must be preserved in all its functions (including the conservation of organic matter). The value of these environmental resources can be substantially decreased by the presence of contaminants, which can hinder their present use and/or full exploitation of future and potential uses. In a different perspective, recovery of contaminated sites to obtain new environmental resources (e.g. space for economic redevelopment, clean groundwater for any potential use) is also a "waste" upgrading instead of using fresh (non-contaminated) resources. A big effort is presently focused on restoring the environmental quality of such resources, particularly with reference to soil, sub-soils and groundwater. However, the most currently applied approaches/technologies are not able to fully recover the environmental quality of such resources: the "dig and dump" approach for contaminated soils and the "pump and treat" approach for groundwater soil do not maintain such resources in their original position and functions. Even when dealing with several *in situ* techniques like chemical oxidation or thermal treatment, some secondary damage will occur, such as loss of natural organic matter and soil texture.

Therefore, for a more sustainable approach to the soil and groundwater remediation in Italy it will be necessary:

- to preserve all potential functions of soils as non-renewable resources
- to do so by simultaneously minimizing energy and water consumption as well as waste production
- to do so by avoiding or minimizing any risks for health, also excluding possible secondary contamination (e.g. formation of toxic by-products)
- to do so at a minimum cost, also including full exploitations of economic use at the contaminated areas, even during remediation whenever possible.

For such a sustainable approach, biotechnology-based processes are the obvious candidates but they must be further investigated and implemented at level of both basic knowledge and technical application and performances.

Goal:

To study, develop and implement biotechnology-based processes for sustainable remediation of soils and groundwater

4.4.1.1. Action 1.

To improve knowledge on microorganisms (bacteria and fungi) in natural or modified habitats in order to fully exploit their biodegradation potential on pollutants

- To improve knowledge on the biochemistry and physiology of wild strains involved in the biodegradation of a range of priority contaminants, such as hydrocarbons, MTBE, chlorinated solvents and low-chlorinated and -molecular weight aromatic hydrocarbons;
- To improve knowledge on the behaviour of complex microbial consortia in soils, sediments, groundwater and wastewater, their ecology and relationships with environmental conditions, either natural or enhanced, for faster remediation
- To improve the knowledge of microorganism behaviour in the presence of non-aqueous separate phases (L-NAPL or D-NAPL), including related effects of microbial activity on NAPLs.

4.4.1.2. Action 2.

To improve process engineering

- To create and/or implement new high-performance materials and products specifically targeted to support or enhance bioremediation (e.g. slow-release of oxidizing or reducing compounds, surfactants and pollutant-bioavailability enhancing agents)
- To improve engineering aspects of in situ bioremediation processes, e.g. process configurations more specifically targeted to local constraints like hydrogeology and geochemistry
- To study and develop new processes with a higher efficiency and a more specific focus on selected contaminants, in order to minimize the addition of external compounds, decrease their interaction with natural compounds, and minimize the formation of unwanted by-products.

4.4.1.3 Action 3.

To create or implement new tools for "upstream" and "downstream" of sustainable bioprocess

- To design new biotechnology-based tools to support site characterization, process design, and/or monitoring. These will include both biosensors for the sensitive and specific determination of contaminants and biomolecular tools for microorganism identification and mapping. In situ approach, spatial-sensitive, and continuous signal tools will be preferred whenever possible

- To design sensitive tools for integral evaluation of toxicity and ecotoxicity, including in situ tools

4.4.1.4 Action 4.

To ensure the appropriate scaling-up of biotechnology-based processes and their application to contaminated sites.

- To combine lab- and pilot-scale research
- To promote research directly on real contaminated environmental resources (rather than on model matrices) and research and application directly on the field
- To contribute to removing legal barriers against performing research in the field
- To contribute to removing barriers against the dissemination of results
- To increase public awareness on remediation as a positive action towards economic and social redevelopment.

4.4.5 - Strategies for Boosting Italian R&D and Industry in the Sector of White and Eenvironmental Bbiotechnology.

In conclusion, from the white biotech scenario described, it can be summarized that in the sector of industrial and environmental biotechnology, Italy performs well in science, inputs and vision, but poorly in patenting activity and technological transfer, innovative entrepreneurship (start-up), resources (both public and private) and R&D employees.

Therefore, in Italy, scientific and technological breakthroughs and the aptitude for patenting and developments must be stimulated. Some possible strategies to achieve these results are:

- Translate vision, strategy and input better into roadmaps and action plans;
- Identify selected laboratories (on the basis of their pertinent productivity in terms of qualified papers and international patents produced during the plast 5 years) that have substantial potential to replace fossil-based fuels, power, heat, chemicals, and materials (including water recovery/replacement) with bio-products and/or bio-energy, and support their R&D projects over (5+3) years;
- Selection of a limited number of R&D themes with prospects;
- Selection of tailor-made programs based on marked demands for innovation (> R&D), flexible instrumentation;
- Decrease administrative burden;
- Use targeted demonstration programs to collect data over time and quantify benefits and costs of biobased products and bio-energy use in selected facilities. Demonstration programs must include partnership of national and/or international companies. Adopt six-month evaluation;
- Identify clear indicators to evaluate the state of progress;
- Enhance professional training and development (i.e., attention to critical mass) to support these R&D programs;
- Reserve a portion of the R&D funding for high-risk frontier science opportunities for future innovation;
- Identify in detail the principal barriers against research, development, demonstration programs, and deployment of biobased products and of bio-energy, and systematically develop coordinated policy mechanisms to overcome them. This may include tax incentives, environmental offsets, risk mitigation mechanisms in early employment, buy-down mechanisms, and others;
- Link environmental benefits of biobased products and bio-energy to public policy development;

- Endorsement by national and local istitutions of the integration among different players on specific innovation (R&D) objectives (agriculture, industry, universities, associations);
- Strongly back the use of the most advanced standards for evaluating the innovative products and processes in order to support the competitiveness and the quality level of the territory;
- Use advanced information technologies to collaboratively assemble, analyze, and publicly disseminate information on relevant environmental and ecosystem impacts, informing consumers about the benefits of biobased products and bio-energy so they will support the effort.
- Improve the patenting activity by involving the leading team researchers in any and all stages of the entire process (NOT just the research activities) and actually, not virtually, provide them an opportunity to develop a business activity of their own. Good rules and laws are not sufficient;

Lower the cost of intellectual property protection for SMEs;

- Adopt new ways of communicating to consumers about the technology or production process (biotechnology concept), which will create trust and market pull for such products; Adopt the Life Cycle Assessment tools (such as Environmental Declaration Product) in order to evaluate the overall impact of new products and processes;
- Form consortia of technology providers.

Biocatalysis, and more in general IB, is a strongly multidisciplinary sector and, as a consequence, only major industrial enterprises can afford to integrate the different technologies and translate them into commercial products. Many recent examples indicate that the future expansion of the business will be played on the formation of consortia and alliances of companies sharing synergistic technologies to offer customers broader solutions for solving problems related to the synthesis of compounds. The end-product manufacturers should have access to a wide portfolio of technologies in order to implement that technology which best satisfies the quality and economic requirements of each manufacturing process.The opening of two centres of excellence on biocatalysis in Manchester and Graz is a demonstration of how industries consider that establishing alliances with academia can also represent a route for accessing multidisciplinary expertise and ultimately for generating integrated technologies and products (“from gene to kilo”)