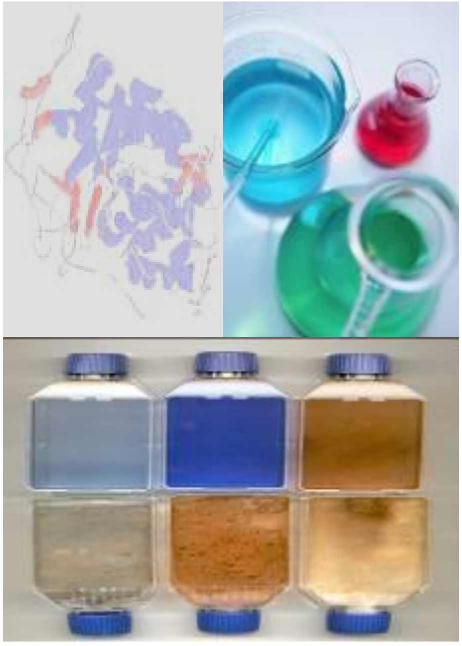
Novel Sustainable Bioprocesses for European Colour Industries

SOPHIED

Integrated Project - FP6-NMP2-CT-2004-505899

Start date: 1st July 2004 Duration: 48 months

www.sophied.net



Publishable Final Activity Report

Coordinator organisation: Université catholique de Louvain, Belgium

Table of contents

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)	
Dissemination Level	
PU	Public

SIXTH FRAMEWORK PROGRAMME

Table of contents

1. Publishable Executive Summary

1.1. Contractors involved

Coordinator and Management Team contacts details:

Function	Responsible Institution	Name and Email	Phone
Coordinator	UCL -Unit of Microbiology -Croix du Sud 3 boîte 6 -1348 Louvain-la-Neuve – Belgium	Sophie Vanhulle coordinator@sophied.net	+32(10) 473737
MT Leader	WET - Wetlands Engineering SPRL - Rue Laid Burniat, 5 - 1348 Louvain-la-Neuve - Belgium	Christian-Marie Bols mtl@sophied.net	+32(10) 861525
Financial & Logistic Manager	WET	Corine Deben finance@sophied.net	+32(10) 861525
Exploitation Manager	WET	Céline Dubois exploitation@sophied.net	+32(10) 861525
IT Manager	WET	Alban Gobin webmaster@sophied.net	+32(10) 861525

The 24 SOPHIED partners are 18 SMEs, 7 Universities and 3 Research Centres from 9 countries.

Participant Number	Participant name	Participant acronym	Country
1	Université catholique de Louvain (SOPHIED Coordinator), 2 labs involved: MBLA (Microbiologie), CHOM (Chimie Organique et médicinale)	UCL	BE
2	Wetlands Engineering SPRL	WET	BE
3	Department of Organic Chemistry and Biochemistry, University of Naples	DCOB	IT
4	University of Westminster	UOW	UK
5	Laboratoire de Bioinorganique Structurale, CNRS UMR 6517	LBS	FR
6	Hydrotox GmbH	HYDRO	DE
7	Labor Grieder	GRIED	CH
8	Setas Kimya San AS	SETAS	TR
10	STAB VIDA, Lda	STAB	PT
11	Instituto de Biologia Experimental e Tecnológica	IBET	PT
12	Maria Curie Sklodowska University	CURIE	PL
13	University of Siena	SIENA	IT
14	Istanbul Technical University	ITU	TR
15	Marwik Informatik	MARW	CH
16	UFZ- Helmholtz Centre for Environmental Research	UFZ	DE
18	The Questor Centre	QUEST	UK
19	Celabor SCRL	CELA	BE
20	BLC Leather Technology Centre Ltd	BLC	UK
21	Ovelacq	OVEL	BE
23	Rayon textile industries and foreign trade co Ltd	RAYON	TR
25	Conceria Antiba S.p.a.	ANTI	IT
26	Tintoria Gori Manifattura Lucchese Lane e Fibre	GORI	IT
27	Eubelius	EUBE	BE
28	Lapière et Libert	LAPLIB	BE

1.2. Website and logo

SOPHIED website is available through the address: www.sophied.eu, www.sophied.net. The website is now also available via other addresses owned by WET (not financed): www.ecocolorant.eu, www.ecocolorant.net, www.eco-colorant.com, www.eco-colorant.eu, www.eco-colorant.net, www.ecocolourant.com, www.ecocolourant.eu, www.ecocolourant.net, www.eco-dyes.com, www.eco-dyes.eu, www.eco-dyes.net.

The official project logo, which can be used with or without the project title, is shown below:

1.3. Summary description of the project objectives

Colour… Reflexion of our mood, feelings, society codes, belief or personality… Adding it on textile, plastic, hair, cosmetic or food, man has never stopped inventing new processes to colour his life! But are we really aware of their influence on health and environment? From the dyes and auxiliaries used for the dyeing and finishing yarns and fabrics, to energivorous processes and polluted wastewaters, the colour industry can have a non negligible impact on worker's and consumer's health, as well as to the environment.
The traditional colour industry was an important activity in Europe until the end of 20th century.
It suffers now displacement to the developing world due to increasing production related environmental costs as well as high labour costs in Europe. Azoïc dyes are the largest group of dyes, both in terms of tonnage production as well as the number of different structures. Unfortunately, a survey of oral acute toxicity of 4461 dyes as measured by the 50% lethal dose has revealed that azo and cationic dyes are the most toxic, and there is ample evidence of the mutagenicity of certain dyes, especially azo dyes and amino-substituted dyes such as 4-phenylazoanilin.
Additional problems are that the chemical synthesis pathways, as well as the dyeing of fibres are non environmental-friendly processes, as during dyeing processes, approximately 10 % to 40 % of the dyes are not consumed on the substrate to which they are applied, and find their ways into wastewaters. They are flushed into the environment and constitute a non-negligible risk to living organisms. When raw materials are imported from the far East (India, China, Indonesia, …), their production, made under conditions which are unacceptable in Europe, increases the worldwide sum of global pollution.
To ensure a future sustainable economical development, the colour industry has to rapidly find solutions to face some critical issues:

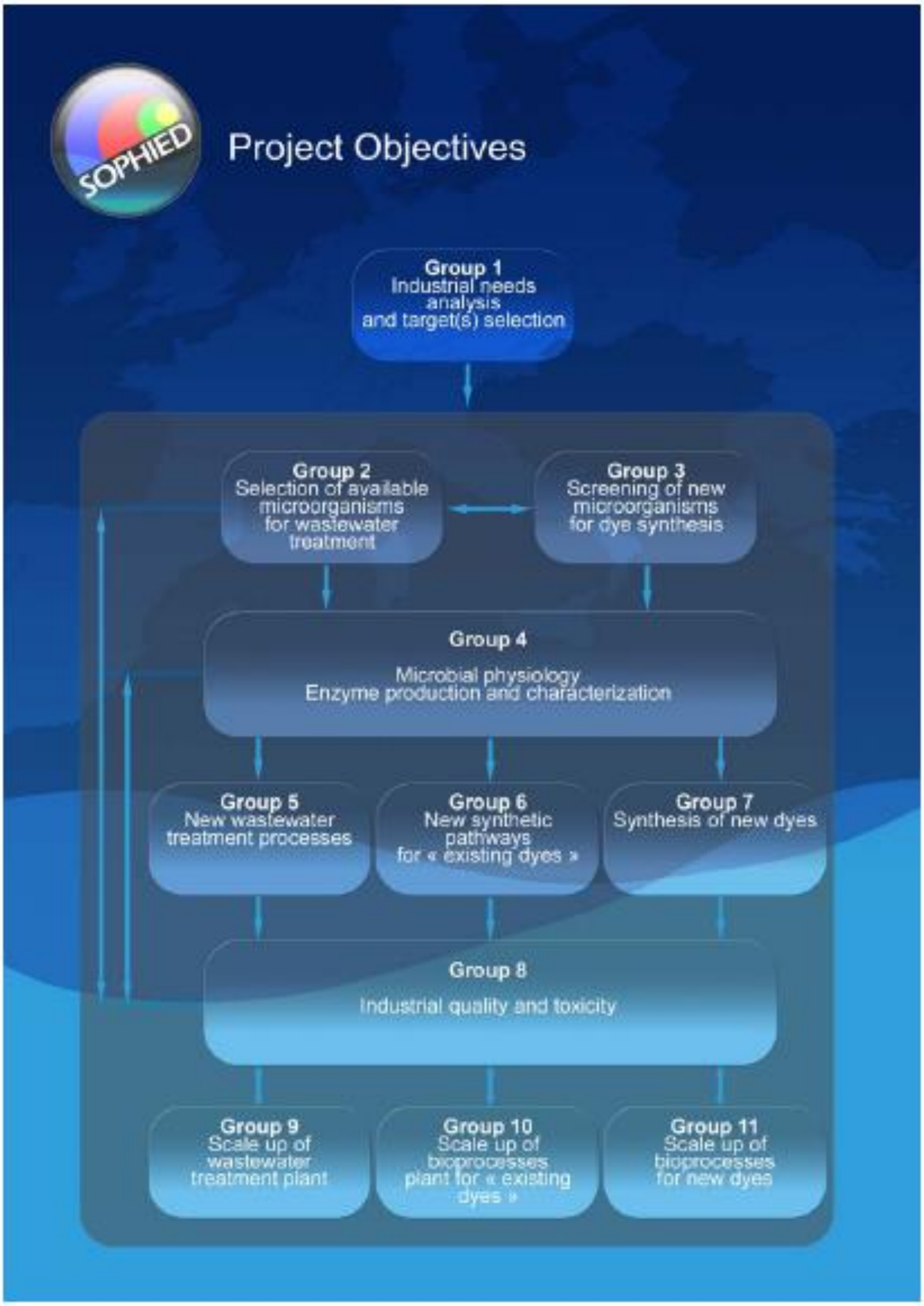
1) how to protect the health of the citizens?
2) How to protect the environment?
3) How to struggle against global climate changes?
4) How to develop alternatives towards fossil feedstock shortage?
5) How to be competitive at a global scale?

Companies need, in addition, innovations which are compatible with their socio-economic and legislative environment, such as 1)REACH – Registration, Evaluation and Authorization of Chemicals; 2) EU directives to protect environment (such as water framework directive), 3) Kyoto protocol (and new protocol planned for 2012), 4) increasing petroleum prices, and 5) the "Lead Market Initiative" (SEC/2007/1729 and SEC/2007/1730) communicated on 21st

December 2007 by the European Commission to the council, the European parliament, the European economic and social and the committee of the regions.
As an answer to the issues of the colour industry, a consortium of 16 SME's and 10 universities amongst 10 countries, have led a research supported by the European Commission. The consortium is named SOPHIED and covered three parallel objectives:

- To develop **new safe processes** for the production of dyes.
- To create **new molecules of EcocolourantsTM** which are **less toxic** and synthesized through "white biotechnology" for high added value markets.
- To develop **new bioremediation** technology **to detoxify coloured wastewaters**.

The repartition of the research within the consortium into "working groups" was as follows:


SOPHIED
Project Objectives
Group 1
Industrial needs analysis
and target(s) selection
Group 2
Selection of available microorganisms for wastewater treatment
Group 3
Screening of new microorganisms for dye synthesis
Group 4
Microbial physiology
Enzyme production and characterization
Group 5
New wastewater treatment processes
Group 6
New synthetic pathways for « existing dyes »
Group 7
Synthesis of new dyes
Group 8
Industrial quality and toxicity
Group 9
Scale up of wastewater treatment plant
Group 10
Scale up of bioprocesses plant for « existing dyes »
Group 11
Scale up of bioprocesses for new dyes

1.4. Work performed : Basics (groups 1, 2, 3, 4)

At first, identification of the bottlenecks of the colour industries was analysed, in order to orient the research and to determine the targets and precursors. As for dye synthesis, acid and natural dyes (with azo, anthraquinonic or new structures) to be used in textile, cosmetic or leather industries were determined as targets. Routes to be explored for their development were proposed. A deep analysis of the industrial issues relating to the wastewater was also undertaken.

Acid, reactive and direct dyes and dye baths were selected as priority targets to be treated (decolourized and detoxified).

Strains were collected in Solomon Island, French Guyana and Cuba under Rio convention (A), and preserved in internationally recognized Biological Resources Centres, therefore allowing preserving biodiversity (B).


A

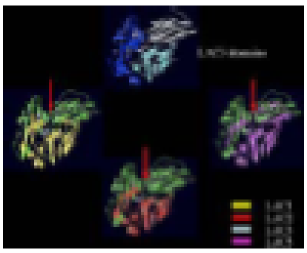

B

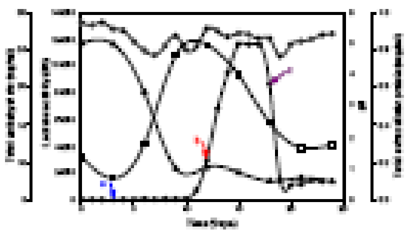
Collection and screening of 280 strains for dye synthesis, and 300 strains for wastewater treatment led to the selection of 15 strains (C) studied further for the conditions for microbial production of selected enzymes (D).


C


D

At a basic level to better understand structure/function relationship and to obtain novel catalytic properties, enhanced stability and higher activity, molecular and genetic tools led to the obtaining of 9 recombinant enzymes, in 5 hosts systems. Evolution engineering and hybrid enzymes were studied (E).


E

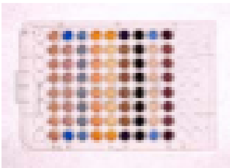

F


G

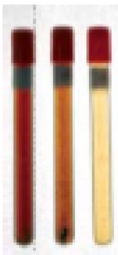
Effective elicitors, production of novel low cost renewable fermentation media (based on valorisation of industrial by-products) and downstream processes were studied. Efficient systems for the production of enzymes (F) with a scale-up potential were developed.
Enzymes production was upscaled up to 150l (G). Enzymes were deeply characterized and a catalogue of enzymes, with a range of industrial specificities was provided.

1.5. Work performed: Wastewater treatment (groups 5, 8, 9)

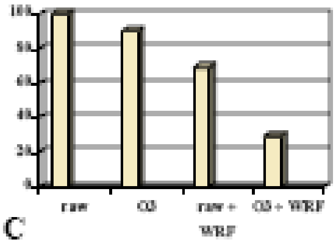
Whole cell process, using free or immobilized mycelium was studied on 11 strains, including bacteria, aquatic fungi and white rot fungi in (WRF) order to compare their efficiency to treat A) model dyes and model wastewaters and B) industrial effluents. Combined treatments with ozonation (O3) were also studied (C). In parallel, the evaluation of efficiency of enzymatic process using free or immobilized enzymes was carried out. Three oxidative enzymes were studied: laccases, manganese peroxidases and versatile peroxidases.



A



B

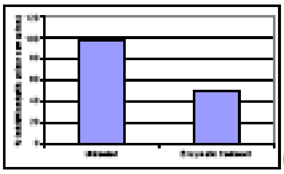


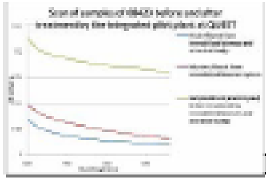
C

Some mechanisms of decolourization, including intermediate metabolites were elucidated for the first time. A benchmarking between those methods was realized and efficiencies of those systems were compared with activated sludge.
Starting at a level of 200µl, the more efficient processes were progressively upscaled to 50ml, 2l, 10l and 20l (100 000 times higher). Whole cells processes revealed to be generally more efficient than enzymatic processes, mainly due to the instability of some enzymes in harsh effluent conditions. There is a need for new enzymes with improved stabilities (starting from microorganisms of extreme environments).
Three pilots were designed, constructed and tested on site within companies. They were based on: D) an enzymatic process (Ovelacq, Belgium), F) fungal bioprocess (Gori, Italy), F) an integrated process comprising electrolysis/fungal biomass/activated sludge (Questor, UK). They were controlled from Wetlands (BE).

D E F

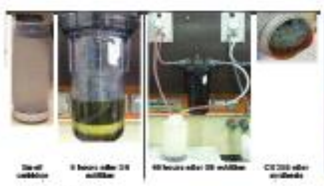
Cytotoxicity was reduced by half through enzymatic treatment (G). Moreover, the integrated system led to much improved results regarding water decolourisation (H), COD and toxicity reduction of dye samples. Results were completed by a SWOT analysis (strengths, weaknesses, opportunities and threats) and a cost analysis, and a LCA (life cycle assessment) showed that global warming could be reduced by 10 with the use of the bioprocess instead of traditional wastewater treatment.

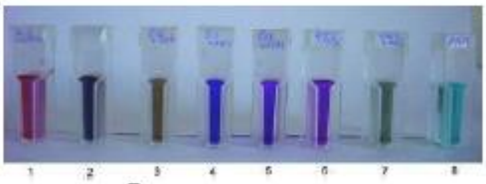

G


H

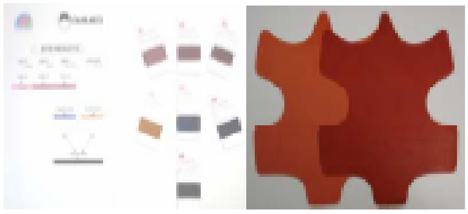

I

1.6. Work performed: Ecocolourants and natural dyes (groups 6, 7, 10, 11)
Concerning dye biosynthesis, a screening of precursors led through “fungal biomass” or “enzymatic” bioconversions to the creation of 500 coloured molecules, amongst which a ranking led to the selection of 10 precursors and 5 enzymes. The mechanisms of Ecocolourants biosynthesis by whole cells (A) or enzymatic (B) bioconversions were deeply studied. Enzymatic processes showed to be generally more efficient and easy to handle. Some processes also led to improved extraction of coloured plants extracts (natural dyes), allowing to remove completely a mutagenic compound.

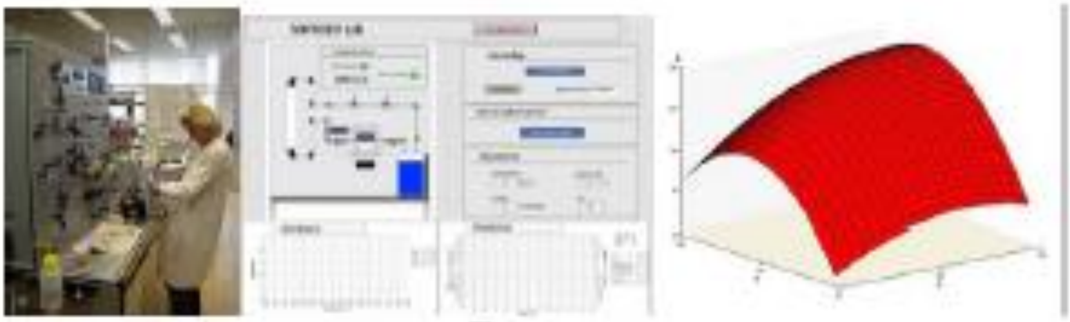

A


B

The 25 more promising dyes were tested for industrial quality (C) (multifibre test, water colour fastness, perspiration fastness, washing fastness…) and toxicity (point 1.7)(bacteria, human cells, fish cells, algae, Ames test…), and 10 non mutagenic dyes with proven low toxicity and improved industrial applicability were selected.


C

Through improved analysis four dyes were selected to be up scaled. Five pilot plants equipments were designed and constructed. A workshop was organised in Belgium in the presence of partners from Turkey, Italy, Poland to perform in three pilots a range of "statistical design of experiments" in order to optimize the enzymatic processes. Based on this, two industrial pilots were performed up to kg.



E

A "proof of concept award" was organized amongst partners, and some items were made from Ecocolourants TM and natural dyes (F). Results were completed by a SWOT analysis (strengths, weaknesses, opportunities and threats), a cost analysis, and a LCA (life cycle assessment).

F

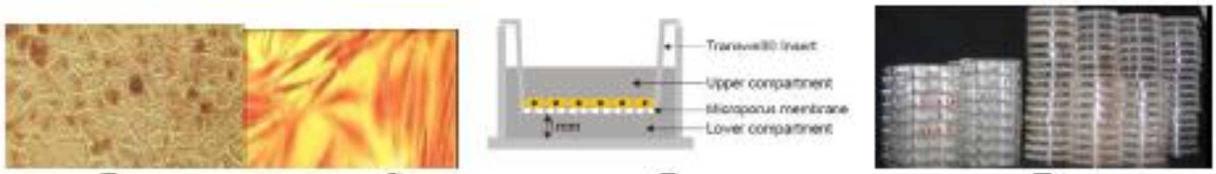
1.7. Work performed: Industrial quality and toxicity (group 8)

The objectives of this group were to assess the industrial quality of the dyes on fibres and leather (shown in point 1.6). Toxicity and ecotoxicity tests were performed to evaluate the potential impact to health and environment of newly developed dyes and waste water treatment methods.

Sophied developed an early toxicity screening battery (A) as part of the risk management in the Research and Development process of new chemicals and new wastewater treatments. The tests were adapted and validated to the specific needs of coloured samples. Non GLP part was used throughout the project.

The European REACH regulation, voted in 2007, by the European Parliament, aims to "to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances." REACH policy would require a lot of testing and hence of labs' animals. Consequently, the European Commission emphasized the need to minimize animal use.

New tests or evaluation methods have been developed which reduce testing time, sample amount and costs: Caco-2 cells, fish cells, fish eggs, and mini Ames test. Those tests were shown as good alternatives to animal testing.



B C D E

New technologies were developed based on optic biosensor reader for measuring oxygen concentrations in vials and microwell plates (G). Pilot experiments with this oxygen biosensor were performed with i) Caco-2 cells in the NRU test (ICCVAM protocol), ii) Ames fluctuation assay for mutagenicity testing of dyed samples (OECD 471) and activated sludge inhibition test for dyed samples (EN ISO 8192).

G

1.8. Work performed: Management and innovation related activities (group 12)

At a total, more than 150 persons from both SME's , research centres and universities amongst 10 countries have contributed to the project. In addition, Sophied increased its impact through interactions with 36 national or regional projects, as well as with other EU projects: Suschem, Euratex, organization of Biotex, Cost Biobio, Cost biotech functionalisation of renewable polymeric materials, COST P15, Bioreffinery Euroview, Quorum, Netzyme...
Therefore a strong coordination structure and management was necessary to conduct this multicultural consortium. This working group carried out activities dealing with management, training, dissemination, and exploitation of the results. The realisation of the project required the use of specific analytical equipments that were shared between partners in an integrated approach to experimentation. Normalized protocols and procedures were shared and international workshops and assays were conducted, bringing together the experiences of partners and leading to an increase in the excellence level of the whole consortium.

Dissemination

Sophied strongly interacted with legislators and policy makers such as DIN group on genotoxicity, DIN group on Bioassay, SETAC, Europabio (e.g. policy day, lead market initiatives, green certificates…) and so on. A website (www.sophied.net) was created with both a public and a private zone. Videos and a documentary were realized, and disseminated through TVs and webTVs. Various dissemination activities were organized such as the participation to the SERI (trade fair related to innovation), DECON: reproduction of a Mondrian painting in presence of dyes and bacteria; participation to the night of researcher (focus to young people);

participation to the Greenweek... Sophied participated to more than 130 conferences and exhibitions to scientific, professional, and whole public, more than 150 papers in scientific, professional and whole public journals, and 20 interventions on TV and radios.

Training

Mobility of the researchers was promoted, leading to 30 short term scientific missions, but also to the hiring (even through international exchanges) of 6 persons (initially from universities) by the SME's of the project. Thanks to this, Sophied has strongly contributed to improve the RTD level in traditional SME's and to reduce fragmentation of the research, therefore reinforcing the European Research Area. More than 100 training activities were followed by partners, 30 trainings from partners to partners were organized and more than 40 trainings to young students and young researchers were carried out.

Exploitation

Sophied has developed a procedure to determine as objectively as possible, the repartition of the intellectual property amongst partners, based on reports and deliverables (preexisting know how, idea, material, experiments, results…). The introduction of patents were coordinated as well as the sharing of IP between inventors and the writing of contracts (NDA, co-ownership, licence, …). Continuous analysis of patents, legislations and markets were carried out in order to help in decision making. SWOT, PESTEL and costs analyses on the marketable products and services developed through the project, were built in order to prepare the entrance on the market with dedicated marketing plans. A Japanese ministerial delegation came to Belgium and asked to get a meeting with Sophied managers to get advised about good practices in IP management between academics and enterprises.

1.9. Conclusions

As a conclusion, this research led to 1) new safe Ecocolourants TM, 2) enzymes to be used in bioprocesses, 3) new toxicity tests to replace animal testing, and 4) engineering equipment to be used in bioprocesses.

1) New safe Ecocolourants TM produced through bioprocesses. The Ecocolourants TM produced were screened for their safety, following the stages of toxicity assessment (before GLP) in agreement with the REACH (Registration Evaluation and Authorization of Chemicals) legislation. Some safe Natural dyes (starting from plants) were also developed. Industrial quality has been continuously evaluated. As a whole, the Ecocolourants TM and Natural dyes presented here are the result of a strong selection among more than five hundred coloured molecules.

2) Enzymes to be used in less energivorous and sustainable bioprocesses. For example, bioprocesses to produce Ecocolourants TM thanks to enzymes have been developed as an alternative to traditional chemical synthesis. Chemical azo dyes synthesis requires phases of temperature up to 70-90°C and phases at 4°C, in harsh conditions requiring the presence of dangerous chemicals. On the contrary, enzymatic synthesis of Ecocolourants TM can be obtained at ambient temperature, under mild conditions. Those enzymes are renewable raw material produced by microorganisms grown on industrial by-products (therefore leading to the valorization of a waste). A selection of enzymes with a range of industrial characteristics is available.

3) New toxicity tests to replace animal testing (European Council Directive 86/609/EEC). Due to animal protection reasons many attempts were made recently to replace animal tests by more animal friendly procedures. Cytotoxicity tests with cell lines are one alternative which is presented here on mammalian and fish cells, as well as with fish eggs. During the

registration process of chemicals, mutagenicity tests are an important but often quite expensive step.
Therefore, to save samples, time and money, the basic assay, the Bacterial Reverse Mutation Test also known as Ames test (OECD 471, 1997 and 92/69/EEC, B.13/14, 1992) has been miniaturized. The resulting mini Ames test is also presented here.

4) New equipments for bioprocesses. The validation, optimization and scaling up of enzymatic bioprocesses, in particular with immobilized enzymes or biomass, require the development and use of specific engineering equipment. The equipments developed for the synthesis of Ecocolourants TM are presented here. They may be used for other enzymatic processes with free or immobilized enzymes. Another issue from the colour industry relates to their wastewater. In SOPHIED, more than 600 microorganisms and enzymes were tested in order to reduce colour toxicity and mutagenicity. This catalogue presents also the pilots used to validate the process.

Additionally, Sophied led to:i) increased biodiversity preserved in Biological Resources Centres,
ii) efficient training and mobility that led to a strong improvement of RTD level of traditional SME’s, iii) submission of patents, iv) activities with decision makers, and v) a range of dissemination including participation to exhibitions, conferences, papers in scientific and general journals, radio and TV, video…