PROJECT FINAL REPORT

Grant Agreement number: 286834

Project acronym: Bio-SURFEST

Project title: Development of novel environmentally added-value surfactants and esters by biotechnological processes from fats and oils waste streams.

Funding Scheme: FP7-BSG-SME

Period covered: From 01/10/2011 to 30/09/2013

Name of the scientific representative of the project's co-ordinator[1], Title and Organisation: Mr. Raul Suescun INDUSTRIAS SUESCUN S.A.

Tel: +34 948690067

Fax:

E-mail: administracion@industrias-suescun.es

Project website address: www.biosurfest.com

[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement

INDEX

LIST OF TABLES

LIST OF FIGURES

1. FINAL PUBLISHABLE SUMMARY REPORT

1.1 Executive summary

FP7 Bio-SURFEST project, “Development of novel environmentally added-value surfactants and esters by biotechnological processes from fats and oils waste streams” (Grant Agreement No: 286834) proposes the use of cheaper oils and oil wastes as substrates for bioproducts’ production that might potentially reduce the overall costs of production. This project Bio-SURFEST has developed two biotechnological processes at semi-industrial scale in order to obtain two different classes of bioproducts; biosurfactants and ester oils.

The overall objective of the Bio-SURFEST project is the biological production of high value bio products from oil wastes by developing two techniques; fermentative process and enzymatic catalysis for the production of biosurfactants and esters oils, respectively.

After a characterization of different substances for its use as raw material, it was demonstrated that the most effective materials for the production of biosurfactants are different oil wastes. Hence, the functionality of the techniques was tested with different types of oil wastes, such as waste cooking oil and restaurant greases.

The main activities planned during project were divided in three steps: development of the biotechnological techniques at lab-scale, up-scaling of the developed biotechnological techniques at pilot scale and assessment of the obtained bioproducts since technological, economical and marketable point of view.

In the first period of Bio-SURFEST, raw materials were selected, choosing waste cooking oils as the most interesting feedstock to test within the project. Specifications of the targeted bioproducts were established and the requirements for fermentation and enzymatic processes were defined to tackle the laboratory development in a suitable way. At this point, testing assays at small scale were set up in order to study the behaviour of the bioprocesses and to define a first production procedure. Fermentative and enzymatic procedures were validated and pilot plants for both processes were design for production up-scaling.

In the second period of the project both biotechnological processes were up-scaled, taking as starting point the conclusions of the previous period, to obtain two pilot plants. Fermentation and enzymatic catalysis processes were validated and optimized to increase the bioproducts yields and to ensure consistent product quality. Also, other potential waste streams will be tested and certificated for its use as raw material.

The bioproducts obtained were analysed since physicochemical, microbiological and environmental point of view and its potential market application were studied. An environmental impact assessment (EIA) and a techno-economic environmental analysis of the products and processes were carried out. Finally, an industrial plant for both developed processes was designed and a Life Cycle Assessment (LCA) was performed to assess the environmental impact of the processes involved during the project.

The consortium of the present project, Bio-SURFEST, consists of nine memberships from five different countries (Germany, Netherlands, Lithuania, Greece and Spain). This consortium combines know-how on biotechnological processes; fermentation technologies and enzymatic processes, and on development of new materials applying bio-catalytic methods. Besides, the industries have provided the techno-economic knowledge to promote the transformation of the results obtained into industrial products.

Further information about Bio-SURFEST project can be accessed online at http://www.biosurfest.com.

1.2 Summary description of project context and objectives

Production economy is the major bottleneck in bioproducts manufacturing, as is the case with most biotechnological processes. Often, the amount and type of a raw material can contribute considerably to the production cost; it is estimated that raw materials account for 10 to 30% of the total production costs in most biotechnological processes. Thus, to reduce this cost it is desirable to use low-cost raw materials. This project proposes the use of cheap and agro-based raw materials as substrates for bio-surfactant production.

Several studies with plant-derived oils have shown that they can act as effective and cheap raw materials for bio-surfactant production. Apart from various vegetable oils, oil wastes from vegetable oil refineries and the food industry were also reported as suitable substrates for bio-surfactant production. Furthermore, various waste oils with their origins at the domestic level, in vegetable oil refineries or the soap industries were found to be suitable for microbial growth and bioproducts manufacturing. These oils and oil wastes are readily available in adequate amounts throughout the world. However, the oils used to date for bioprocesses are mostly edible oils and are not cheap.

Concerning disadvantages, one of the problems is related to large scale and cheap production of bioproducts. One of the main risks or barriers which have been face in the present project is the fact that this kind of products used to have higher costs and more complex value and production chains. Demand for biobased products has not been sufficient to realise economies of scale in the manufacturing of these products. In most cases biobased products have to compete against fossil-based products, which are being manufactured and distributed in well established value chains. In this context, the present project has been focused on reducing costs of the potential industrial production of biobased products by developing biotechnological processes coupled to utilization of waste substrates combating at the same time their polluting effect, which balances the overall costs.

Incorporation of cheaper oils and oil wastes in the industrial production media might potentially reduce the overall costs of biosurfactants and ester oils production, making them challenging targets for Bio-SURFEST project.

This project Bio-SURFEST has developed two biotechnological processes at semi-industrial scale in order to produce two different classes of bioproducts, biosurfactants and ester oils (non useful for biodiesel production). Biosurfactants have been produced by fermentative process and ester oils have been produced by enzymatic catalysis. Both biotechnological methods have used residues from waste cooking oils as raw materials or feedstock.

The overall objective of this project has been the biological production of high value bioproducts from oil wastes. The main technological objectives of the project were as follows:

- Development of a fermentation technique for obtaining biosurfactants enabling the valorisation of waste oils.

  Fermentation processes have involved the microbial fermentation of waste oils with specially selected microorganisms for these processes. Parameters in relation with productivity, inhibition capacity and yield have been optimised. Once the fermentation processes were concluded, downstream processing provided the final products, sophorolipids and rhamnolipids depending on the microorganism used. The obtained products were analysed and surfactant properties were determined and compare with the specifications established previously as well as with commercial surfactants available in the market.

- Development of an enzymatic catalysis technique which allows the incorporation of the waste oils to the manufacturing processes of bioplasticizers and biolubricants.

  The biocatalysis processes were consisted on enzymatic esterifications of oil wastes by lipases to facilitate the conversion of raw materials in ester oils. A commercial lipase was tested and optimised to high activity and the resulting products were separated by settling and decantation. The obtained fatty acid derivates (fatty acid ethyl esters and monoacylglycerols) can be used as high-value chemicals for the production of biolubricants and bioplasticizer.

The main activities planned during project were divided in three steps: development of the biotechnological techniques at lab-scale, up-scaling of the developed biotechnological techniques at pilot scale and assessment of the obtained bioproducts since technological, economical and marketable point of view.

First of all, a fine characterization of different potential waste streams was carried out. Various physicochemical properties were defined in order to establish and valorise the wastes collected for future biotechnological processes. Potential organic residues, enriched in fats, which content in triglycerides (fatty acids), can be used as resources by some microorganisms, were selected for its testing. The waste streams checked and chosen for its use as feedstock in the biotechnological processes, were waste cooking oils, fried oils (olive oil and sunflower oil), sesame oil and restaurant oil.

Different requirements and specification of the potential add-value bio products were defined with the aim of describing the main candidates to be converted into bio products. Among different candidates, rhamnolipid and sophorolipid were selected for its testing by fermentative techniques and ester oils (ethyl esters and monoglycerides) by enzymatic catalysis. The properties of the bioproducts to be developed during Bio-SURFEST project were defined according to the properties and specifications of similar products of the market.

For the development of the fermentative and enzymatic techniques at laboratory scale, the necessary requirements for both processes were identified and the critical parameters were controlled. Once the suitable substrates to operate were defined, and the operative conditions for both biotechnological processes were established, these technologies were developed at lab scale.

The biotechnological techniques mentioned above were validated by means of the modification of different parameters such as: amount of initial glucose in the fermentation broth, amount of waste oil, synthetic air flow, temperature and the ethanol to oil ratio. The optimum conditions of every parameter were studied with the aim to reach high yields of both biotechnological processes.

Once the bio-processes were validated, other potential waste streams such as, olive oil mill wastewater and cheese whey were evaluated. Finally, both biotechnological processes were re-designed at pilot scale.

In the second stage of the project both biotechnological processes were up-scaled to obtain two pilot plants. Fermentation and enzymatic catalysis processes were validated and optimized to increase the bioproducts yields and to ensure consistent product quality. Also, other potential waste streams will be tested and certificated for its use as raw material.

The bioproducts obtained were analysed since physicochemical, microbiological and environmental point of view and its potential market application were studied. An environmental impact assessment (EIA) and a techno-economic environmental analysis of the products and processes were carried out. Finally, an industrial plant for both developed processes was designed and a Life Cycle Assessment (LCA) was performed to assess the environmental impact of the processes involved during the project.

1.3 Description of the main S&T results/foregrounds

The main objectives at the beginning of the project were the definition and establishment of the basis of Bio-SURFEST project in relation with the raw materials for the production of add-value bio-based products, and the establishment of the general operative conditions for each biotechnological process. Once the general operative conditions were established, the final requirements for the production of every bioproduct to be developed during the project were defined at the outmost level of detail.

Different waste streams were characterized to check its potential use as raw material for the development of the biotechnological techniques; fermentation and enzymatic catalysis. The wastes studied and defined during the kick-off meeting were: waste cooking oils, restaurant fats, fried oils and animal fats.

The specifications of the bioproducts synthesised by fermentation and enzymatic processes were defined and the relevant market sectors for surfactants and glycerides were studied to determine the marketable potential of the bioproducts developed by fermentation and enzymatic processes during the present project.

Common characteristics and requirements of similar chemical products to surfactants and ester oils were analysed with the aim of studying the replacement possibilities of the chemical synthesis by fermentation and enzymatic processes.

The requirements and specifications of fermentation process were defined after the target products were determined. The target products selected to develop through fermentation process were rhamnolipids and sophorolipids.

Once the target products were determined, the main parameters that influence the fermentation process were analysed. Among them, type of microorganism, pH, temperature, dissolved oxygen concentration, aerobic or anaerobic conditions, nitrogen and carbon content, etc.

The operation conditions of the fermentation technique were established also. These operation conditions were: type of bioreactor according to the presence or absence of oxygen and according to the feed supplying and downstream procedures. Once the operation conditions were selected, the fermentation process was ready, for starting the lab scale testing.

The parameters of the enzymatic process were defined according to the different bioproducts selected, the selected enzyme and the production route. The target products selected for the enzymatic process were ethyl esters of fatty acids and, mono- and diacylglycerides, obtained through alcoholysis of fats and oils.

Besides the characteristics of the substrates, the reaction system and enzyme properties, other technical parameters of the process were taken into account due to its influence on the yield and cost of alcoholysis and glycerolysis. These parameters were, for both processes (glycerolysis and alcoholysis), the immobilization of the enzyme and the organic solvents.

The following steps were the construction of a laboratory scale biotechnological plant of every process, the development of respectively methods for the production of bioproducts through fermentation and enzymatic processes and the establishment of the analytical methods to analyse the bioproducts obtained.

For the construction of lab-scale fermentation and enzymatic plants, firstly, a bibliographic study of the biotechnological processes was executed with the aim to identify the main parameters that affect fermentation and enzymatic processes. Once the parameters were identified, a range of work of these variables and conditions were established taking into account also the data gathered previously.

A laboratory scale plant was designed to support both processes, taking into account important variables such as, volume of feedstock, desired amount of bioproduct, need of reagents, temperature conditions, automatization or addition of gas for inert atmosphere. The necessary modifications of the plant were detected to adequate the plant for every methodology of synthesis. Finally, a lab-scale biotechnological plant was designed to test it in the following stages of the project.

To develop the fermentation process was necessary to control the critical parameters that affect the biotechnological process. Hence, the main variables of the process were studied and analysed to check its evolution during the process and to obtain the best conditions for the fermentation.

Testing with different volumes of work and the variation of some parameters were executed to identify the best conditions of the fermentation process for the production of biosurfactants. This optimization was developed through the modification of some parameters such as pH, temperature, agitation rate, aeration rate, nutrients concentration, medium, reaction time and amount of raw material.

In the same way, ester oils production were tackled to test and optimize the conditions of the enzymatic process to obtain high yield and productivity and to develop a method of bioproduction of plasticizers by an enzymatic catalysis process, which encompassed the laboratory scale development of the targeted biological processes. The way of work, similar to the fermentation production, was based on the identification and control of the main parameters that affect the enzymatic process. The main parameter to develop the enzymatic biocatalysis process was the selection of the enzyme, in this case, lipases and the variables that affect it. For this selection, an extensive literature research was performed using as criteria:

- Activity: ability to use efficiently the substrate resulting in high product yields
- Robustness and stability: the enzyme should be able to withstand variations in substrate composition, the use of denaturing reagents, variations in temperature and pH, and should be stable over several reaction cycles allowing recycling.
- Reaction rate: to reduce process duration

Several enzyme reactors were taken into account to develop the biotechnological process and for the enzymatic transesterification, the reactions were performed in mixed batch reactors. With the aim to facilitate enzyme recycling, immobilized enzyme was used.

The optimization of the reactions was executed according to the modification and establishment of the ratios of reagents, temperature, water content, alcohol feeding strategy and enzymatic pre-treatment.

For the determination of the characteristics of the synthesized bioproducts obtained from fermentation and enzymatic processes, the application of several analytical techniques was developed with the aim to assess the correct operation of both biotechnological processes. Suitable analytical methods were set-up to control the critical values of the biotechnological processes and to measure the parameters that reveal if the products developed fulfil with the specifications established.

Various analytical methods were executed to analyse the main parameters of the biotechnological processes and the bioproducts obtained. The main parameters analysed for the control of the fermentation process were:

- Total carbohydrate content.
- Biomass concentration.

The parameters measured of the bio-products obtained from fermentation process were:

- Surface tension
- Emulsification activity.
- Critical micelle concentration.

On the other hand, in the case of the enzymatic processes, the analytical methods were focused on the measurement of the quality of the products obtained. The main parameters measured were:

- Glycerol content.
- Ester quantification.

Finally, several analytical methods such as test of biodegradability and ecotoxicity were set up to assess the environmental impact of the bio-products developed during Bio-SURFEST project based on the OECD guidelines.

Once the processes were developed at lab scale, optimization of both bioprocesses and the validation of the surfactants' yields obtained from fermentation processes and from the enzymatic biotechnological process were developed. Two different validation were developed; an experimental and technical validation, and an industrial validation of both biotechnological processes.

With regard to the experimental and technical validation, the fermentation process was validated by the measurement and evaluation of the following parameters:

- Optimization of nitrogen content
- Sophorolipid production
- Rhamnolipid production

The result was the obtaining of sophorolipids and rhamnolipids with the following surfactant properties. The values for commercial surfactants are also shown for comparison.

SAMPLE	SURFACE TENSION (mN/m)	CMC (mg/L)	EMULSIFICATION ACTIVITY (E_{600})	DECAY CONSTANT (day^{-1})
Sophorolipids	29-47	50-200	0,876	4,320
Rhamnolipids	40-55	50-200	0,730	5,760
Triton X-100	23-35	50-200	0,782	4,896
Tween 80	40-43	50-200	0,780	5,184
Sopholiance	28-39	50-200	0,617	4,176

Table 1: Surfactant properties of biosurfactants validated at laboratory scale

And in the case of the enzymatic catalysis, the main parameters selected to validate technically the process were:

- Waste substrate
- Ethyl ester production

- Monoacylglycerol production

The result was the obtaining of fatty acid ethyl esters (FAEE) and monoacylglycerols (MAG) with the following properties:

PARAMETERS	FAEE mixture from sesame oil	FAEE mixture from waste oil	MAG mixture from sesame oil
Acid value (mg KOH/g)	2,2	0,9	34
Saponification value (mg KOH/g)	164	138	152
Iodine value (g I/100g oil)	95	70,5	53
Monoglycerides (%)	14,5	7,4	33,2
Ester content (%)	76	83,6	98
Density (g/cm^3)	0,89	0,9	0,94

Table 2: Properties of FAEE and MAG validated at laboratory scale

An important part of the validation of processes is the cost analysis of the production of bioproducts. This cost analysis, named as industrial validation, was executed, evaluating the cost of the processes before and after the optimization. A final comparison of both costs was also developed. Production cost at lab scale was the only cost considered in this previous assessment and as a result of the economic assessment, the cost of the products synthesized at laboratory scale was the following:

PRODUCT	Sophorolipid	Rhamnolipids	FAEE	MAG[1]	MAG[2]
COST (€/Kg)	23,63	25,40	27,28	7,1	8,1

Table 3: Economic assessment of the bioproducts at laboratory scale

To complete the validation at laboratory scale, different waste streams were identified and evaluated to its use as raw materials for the fermentation and enzymatic processes. The waste streams selected for its characterization were olive oil mill wastewater and dairy wastes. Olive oil mill wastewater (OOMW) was chosen to assess its potential as raw material due to two main reasons: the first one, this waste is generated in large amounts from the olive oil industry, and the second one, OOMW was analysed and it was proved that has simple and complex carbohydrates which are a possible carbon resource.

OOMW content of simple and complex sugars, residual oil (lipids), proteins, mineral elements and phenols, turns this effluent into a renewable resource, as its components can be extracted and purified or used for fermentative production processes. Besides, residual oil in OOMW turns this waste into a potential growth medium for lipolytic microorganisms.

To check the viability of OOMW as raw material for the mentioned biotechnological processes, a characterization focused on physic-chemical parameters, fatty acid methyl esters and heavy metal content, was executed.

The other waste stream characterized was dairy wastes. This waste, cheap and viable for its use as substrate for both biotechnological processes, was characterized analysing its physic-chemical parameters. This waste stream contains valuable

[1] In reaction conditions of incomplete ethanolysis.
[2] In reaction conditions of glycerolysis.

nutrients such as proteins, peptides, amino acids, lipids, minerals and vitamins. Hence, its good support for microbial growth and for fermentative production.

The characterization mentioned above of the OOMW showed that this waste can be a suitable substrate in fermentation processes. To test this waste stream, a production assay was carried out, demonstrating its viability as raw material for biosurfactant production.

To finish the technological activities at laboratory scale, the conditions of every system of the biotechnological processes were determined to assure the correct production of the bioproducts at up-scale. The main systems taken into account to achieve successful up-scaling processes were: mixing system, aeration system for aerobic fermentation, sensor system, sampling system, heating or cooling system and aseptic system for pure culture fermentation. Also specifications and details from the laboratory scale testing were taken into account.

Finally, with all data gathered, biotechnological systems designs at pilot scale were executed. The critical areas that were considered as the most important during the process of design were vessel design, process piping, jacket service piping, agitation and mixing, and instrumentation and control.

Starting with the technological activities at pilot scale, conclusions obtained in the laboratory scale activities were used as starting point to tackle the production of biosurfactants, both sophorolipids and rhamnolipids, at pilot scale, taking into account that the bigger dimensions of the processes brought up new challenges.

A pilot plant for fermentative production of biosurfactants was constructed using simple but effective elements that were suitable for both fermentation processes. Specifications and design carried out during the first stage of the project were taken into account for the implementation of the pilot plant.

In this stage of the project the work was focused on testing and optimization of the production processes of sophorolipids and rhamnolipids at pilot scale. In both cases, several fermentation parameters were studied as well as their influence on the reaction yields and the properties of the final products. Preliminary assays were carried out to establish initial conditions and then several tests of fermentation production were completed to synthesize the final products and measure their surfactant properties.

Downstream processing of the fermentation broth for the isolation of the biosurfactants was also an important step of the final production processes. Several strategies were checked during this study and finally elimination of organic solvents and simple techniques of decantation and separation were found as the best option, taking into account efficiency, simplicity, environmental and economic criteria.

Fourier Transform Infrared Spectroscopy (FT-IR) analysis was used as a tool to identify the molecular structures of the obtained biosurfactants by fermentation processes. Obtained spectrums of synthesized products were compared with commercial a biosurfactant and spectrums reported in the literature to confirm sophorolipid and rhamnolipid structures in the experimental production batches.

Evaluation of properties of the obtained final products was carried out by measurements of surface tension, critical micelle concentration, and emulsification capacity. Suitability of synthesized sophorolipids and rhamnolipids was assessed by comparison of the experimental results obtained in the same tests with commercial surfactants, both chemicals as well as biological one.

For the construction of a pilot scale enzymatic plant, a stirred tank was chosen for the reactor design due to the operational simplicity. It allowed operating the reaction in batch but also continuous mode, but for continuous operation it needs to be adapted to prevent the removal of the catalyst from the reaction mixture together with the product.

Regarding to testing enzymatic parameters, an immobilised lipase selected in the laboratory scale activities was chosen as best alternative for the project. This lipase catalyzed effectively both reactions, ethanolysis and glycerolysis, even at moderate temperature conditions.

To maximize product yield and life time of the catalyst suitable reaction conditions were tested Under the selected ethanolysis conditions, it was shown that the enzyme conserved satisfactory activity even after 5 operation cycles (corresponding to 120 reaction hours). The ethanolysis provided similar yields in the temperature range from 30-40℃, but at higher temperature the loss of acti vity after repeated operation cycles was faster. Up to 83% ethyl ester yields were obtained. The used lipase catalysed glycerolysis, performed at 50℃ for 24h yielded a m ixture of monoacyl- diacyl- triacylglycerols, the highest fraction (around 40%) being represented by MAG. Unfortunately it yielded also a significant amount of free fatty acids as by product.

The quality of the products obtained through lipase-catalysed reactions corresponded mostly to the standards of the plastic industry for additives. Some of these standards depend solely on the available substrate and cannot be influenced by the reaction setup or process parameters. They cannot be optimized. The only standard which could not be achieved was the acidity limit for monoacylglycerides. This limit is hard to keep even when using conventional catalysts, so further downstream processing steps are required.

Fermentation bioproducts   Enzymatic bioproducts

Fig. 1: Final products obtained within Bio-SURFEST project

Once the two biotechnological processes were implemented at pilot scale, technical activities were focused in the validation of the procedures to automatically ensure the standard, quality and consistency of bioproducts developed in pilot scale. Through the qualification and validation procedures, which are applicable to the biotechnological processes for the conversion of waste fats and oils to surfactants (through fermentation) and esters (through enzyme-catalyzed transesterification) can be ensured that their production will be under optimum process control in terms of quality and efficiency. The obtained results regarding to properties of the validated bioproducts were the following:

PROPERTIES	**SOPHOROLIPIDS**	**RHAMNOLIPIDS**
Surface tension (mN/m)	32,98	41-35
CMC (mg/L)	100	100
E24(%)	30,69	39,89

Table 4: Properties of validated biosurfactants.

In case of ester oils, measured parameters were selected in order to check the compliance with the specification addressed at the start of the project with the following results:

SPECIFICATIONS	Value	
	ethanolysis	glycerolysis
Acid value (mg KOH/g)	1,1 (±0,1)	8,1 (±0,2)
Saponification value (mg KOH/g)	356 (±3,1)	362 (±3,7)
Iodine value (g I/100g oil)	231 (±8,6)	226 (±2,9)

Table 5: Characteristics of validated enzymatic products.

The validation process followed included the qualification of the various components of the process like the design, installation, operation and performance. Moreover, a detailed description of the manufacturing processes and a critical analysis were established for the identification of those parameters that could result to product non-conformity. Then, a guide was developed containing all necessary recommendations for industrial validation of the proposed processes as well as a series of experimental and monitoring work for validation purposes in industrial scale.

To conclude with the validation, an evaluation of the potential of other alternative waste streams at pilot scale was carried out. This study is an approach to a more economical and environmentally friendly development of biotechnological processes to obtain high value bioproducts.

In the last stage of the Bio-SURFEST bioproducts developed during the project were analysed to evaluate their physicochemical and microbiological features. In the case of biosurfactants, surfactant properties were measured by direct method and in the case of ester oils, their composition were determined by gas chromatography.

To evaluate final bioproducts from microbiological point of view, total viable microbial count (total viable bacterial plus total fungal counts), presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, bile-tolerant gram-negative bacteria and *Salmonella* were examined. The results of the microbiological examination of all samples of products developed indicated that they are microbiologically safe

An attempt to describe the procedures for the certification of the biotechnological products developed in Bio-SURFEST project was made. In the EU and in International level there is lack of adequate information on the certification processes of bio-based products and an extensive survey was done to tackle this issue. Besides, two fermentation and enzymatic processes products were subjected to the analytical measurement for bio-based determination according to an international standard. that describes the techniques and procedures to be used to any carbon-based content component. The results are shown in the following table.

Biobased material	Mean biobased result
Rhamnolipids	96%
Sophorolipids	100%
Fatty Acid Ethyl Esters (FAEE)	100%
Monoglycerides (MAG)	100%

Table 6: Summary of Results of Biobased Determination

According to the results, all developed products in Bio-SURFEST can be defined as bio-based products.

To assess potentially harmful effect of chemicals, some bioassays are standardised and presented in international (e.g. OECD, ISO) or national (e.g. ASTM) guidelines. In particular, the OECD Guidelines is collection of about 100 the most relevant internationally agreed test methods used by government, industry and independent laboratories to determine the safety of chemicals and chemical preparations. To evaluate environmental safety of bioproducts developed under the project, the following ecotoxicity and biodegradability methods presented in OECD guidelines were used:

- To evaluate effects on biotic systems, terrestrial plant emergence and growth test and nitrogen transformation test were performed;
- To assess environmental fate, ready biodegradability of all final bioproducts was estimated.

Experimental results indicated that all tested bioproducts had no significant inhibitory effect on emergence and dry weight plants tested. Therefore, they were found to be not phytotoxic. It was demonstrated also that all products developed within the project have no long-term effect on nitrogen transformation in soil and can be considered as biodegradable.

An Evaluation Impact Assessment was carried out for the evaluation of the impacts of the project selecting specific alternatives and to minimize potential adverse impacts of the production of biosurfactants and ester oils by processes developed in Bio-SURFEST. The EIA provided information on the environmental consequences for decision-making, and to promote environmentally sound and sustainable development through the identification of appropriate alternatives and mitigation measures. This assessment revealed that fermentation and enzymatic processes developed during Bio-SURFEST project have no negative impact over the environment.

A study of bio-based products market in Europe, a review of potential substrates for processes developed during the project and the potential applications of the biosurfactants and ester oils in the market was made. According to forecasts made by experts in the bio-based products market, the growth of production in Europe by the year 2020 will be 3,6% for biolubricants and bioplasticizers and 3,5% for the biosurfactants. The value of the EU bio-based product market is forecast to grow from 21 to 40 billion EUR between 2008 and 2020. This growth implies significant employment opportunities and reduced dependency on imported fossil fuels. The sector will also facilitate the creation of knowledge driven and attractive jobs and development of new innovations.

The study of potential market applications of product developed during the project was carried out and as a result, different applications were found for biosurfactants in several sectors as detergents, cosmetics, in formulation of personal care products, for bioremediation and other emergent applications like bacteriostatic agent, removal of heavy metals, precursor for molecules of interest in perfume and fragrance industry or as active ingredients in pharmaceuticals. Ester oils can be used as precursors of bioplasticizers, as biolubricants, as insulation liquid in power transformer sector, as emulsifier for food, diary, cosmetic and pharmaceutical industry, as precursors for cosmetic surfactants, antibacterial compounds and structured lipids.

The conclusions obtained during the production and validation technical activities were taken as starting point for the design of industrial processes plant for production of biosurfactants, fatty acid ethyl esters and monoacylglycerides. Firstly, processes requirements were established for fermentation processes as well as for enzymatic processes and after that the design of fermentation and enzymatic industrial plants was carried out. In both cases, piping and instrumentation diagrams, detailed reactor designs and 3D solid views of the reactor were developed in order to define the processes and elements in an industrial plant. Safety and techno-economic criteria have been taken into account also with the aim of reducing production costs and energetic consumes, assuring the stability of the elements of the plants to derive in a secured and cost effective production processes.

Little information was found available on the economic viability of the production of bio-based products in general from biotechnology processes because of the sensitivity of this information among competitors and because biotechnology production is still an emerging technology. Designs of industrials plants propose in Bio-SURFEST project were used as model plants for the calculation of economic costs of the production processes.

Taking into account all the production costs for both processes, potential final prices for bioproducts in the market were estimated using the financial parameter ‘Production cost plus profits’ (PCPP) as the best approximation for the estimation of the final potential market price.

Estimated market prices for biosurfactants produce by the fermentative processes developed in Bio-SURFEST project can be competitive compared with estimated market price of biosurfactants presents at commercial level for the users. Nevertheless FAEE and MAG produced by processes design in Bio-SURFEST were around 6 times and 10 times respectively greater that market price for non bio-based plasticizers. These considerable differences in price for biobased alternatives to petrochemical products are an effect of economy of scale and mature of the technology. An expansion in the production scale of enzymes would lead to a decrease in cost du to the high impact of the price of the biocatalyst in the final price of the final products.

Life cycle analysis (LCA) was also carried out at the en of the project to assess environmental impacts associated with all the stages of bioproduct's life. The objective of this study was to generate information on the environmental life cycle of the bioproducts developed within the framework of the project using the existing approaches of LCA methodology. In more detail, the ecological performance and environmental impacts of the production processes of sophorolipids, rhamnolipids, fatty acid ethyl esters and monoglycerides, were estimated in the aim to assess their present environmental status and thus enable future improvements.

LCA was mainly based on data obtained from the manufacturing process of biosurfactants and bioplasticizers produced in Bio-SURFEST at pilot-scale. A series of process assumptions has been made and calculations were carried out based mainly on raw materials use and electrical energy requirement as well as atmospheric emissions during each bioproduct’s process.

1.4 Potential impacts

1.4.1 Economic impact

The value of the EU bio-based product market is forecast to grow from 21 to 40 billion € between 2008 and 2020. This growth implies significant employment opportunities and reduced dependency on imported fossil fuels. The sector will also facilitate the creation of knowledge driven and attractive jobs and development of new innovations.

Annual lubricant and plasticizer consumption in the EU was estimated at 5,2 Mt in 2008 and the share of biolubricants and bioplasticizers at 0,15 Mt (2,9%). It is estimated that biolubricant and bioplasticizer consumption will grow up to 0,23 million tons by 2020 which represents the growth rate potential of 3,6 %/a.

EU	2008	2020
Market value (billion EUR)	21	40
Share of biochemical value of total chemical value	4,0	6,0
Employment in biochemical production	50.200	93.700

Table 7: Estimated EU market value and employment opportunities of bio-based polymers, lubricants and plasticizers, solvents and surfactants

The market penetration of biolubricants and bioplasticizer varies considerably within the EU. It is estimated that their market share is about 15% in Germany and 11% in Scandinavia, but below 1% in France, Spain and the UK.

The consumption of surfactants totalled 2,7 Mt in the EU in 2008. At the same time, the bio-based surfactant market was estimated at 1,52 Mt, representing 56% of the total surfactant market. Annual surfactant growth potential is estimated to be 3,5 %/a, and biosurfactant potential 2,3 Mt in 2020.

EU, millions tons	Total consumption in 2008	Biobased consumption in 2008	Biobased potential in 2020	Growth potential, %/a
Plastics	48	0,13	0,9	16,0
Lubricants and plasticizers	5,2	0,15	0,23	3,6
Solvents	5,0	0,63	1,1	4,8
Surfactants	2,7	1,52	2,3	3,5

Table 8: Estimated EU production volumes of bio-based polymers, lubricants and plasticizers, solvents and surfactants

The economical impact of the project has been calculated taking into account the presented scenario and the following market data:

- Total biosurfactants market in EU in 2014, the first after-project year, could amount to 1,79 billion € (calculating from the year 2008 as the base year with 3,5€ annual growth)
- Total ester oils market in EU in 2014 could amount to 219,6 million €.
- Prices of the products of Bio-SURFEST project were calculated and explained in the techno-economic assessment. Taking average values, the price used for the evaluation of the economic impact of the project were 190,36 €/kg for biosurfactants and 6,42 €/kg for ester oils.
- The target is to achieve 5% market penetration in 10 years.

Sales predictions are difficult as not all target market research has been completed. Furthermore, evolving economic conditions in different markets as well as the development of environmental regulations and standardization rules for bio-base products will have an important impact on market acceptance and uptake. In addition, monitoring competitors will be crucial in determining the correct marketing strategy.

Nevertheless, in order to assess the feasibility of a project, forecast of income and turnover are essential. According to market data and assumptions based on them the consortium will gain 48,06 M€ in turnover during the first five years father the project, being 41,91 M€ from biosurfactants and 6,15 M€ from ester oils. Over a 10 year period, the total turnover will be 95,01 M€, 82,85 M€ from biosurfactants and 12,16 M€ from esters oils. Total production of biosurfactants during a period of 10 years will be 435 Tn and 1.892 Tn of ester oils.

1.4.2 Environmental impact

Environmental performance of bio-based products compared to petroleum-based products, indicates that bioproducts consume less energy and emit less carbon dioxide than products from fossil resources and offer the potential to reduce the generation of toxic wastes. The overall environmental effects of bio-based production are seen as positive.

Non-renewable energy consumption and GHGs

Since many bioconversion processes occur at or near room temperature, atmospheric pressures, and neutral conditions, there are often lower combustion energy requirements and fewer associated emissions than in conventional chemical processing. However, energy used for feed preparation, drying and product separations can be a significant source of combustion emissions, which can be partially offset by the use of renewable fuels to meet processing energy demand. Thus substituting plant resources for fossil feedstocks results in lower non-renewable primary energy consumption and mitigates the greenhouse effect.

Bioproducts can create savings in non-renewable energy consumption (that are of the order of magnitude of 30-80%) and in greenhouse gas (GHG) emissions (at least 50% lower CO_2 emissions).

Given the market penetration estimates of bio-based products by 2020 - 4 and 6 percent of sales within the EU chemical industry in 2008 and 2020 - the resulting savings in non-renewable primary energy consumption and GHG emissions will still be modest in the short and medium term. However estimations reaching up to 2050 result in up to a 32% overall reduction of non-renewable energy use in the production of all organic chemicals .The results vary from 3-5% for a low scenario (with rather unfavourable conditions for bio-based chemicals development) to 9-14% in a medium scenario or to 18-32% in a high scenario (with favourable conditions for bio-based chemicals development). The scenarios are based on various assumptions about future production volumes and feedstock price levels. E.g. the total production of organic chemicals in Europe in 2050 reaches 70 MTons, 150 MTons and 300 MTons in the low, medium and high scenarios, respectively.

VOCs and waste

During chemical processing harmful volatile compounds may be released from point sources and as fugitive emissions from valves, vents and pipes in the manufacturing plant. While bioconversion processes are often more benign, they can also release fugitive emissions.

Another attractive aspect of bioproducts is the potential to reduce the generation of hazardous and toxic wastes associated with the manufacture of fossil-based products. Many chemical processes require large quantities of aromatic solvents or strong inorganic acids and bases (e.g., sulphuric acid, sodium hydroxide) that can result in effluent streams that are harmful to the environment. These must be recycled or treated and disposed of. On the contrary, most biological processes require natural catalysts (e.g., enzymes) and solvents (water) and produce few or no toxic or hazardous by-products. In most cases, solid wastes and effluents from these processes are biodegradable or can be recycled or disposed of without excessive treatment processes.

Bio-based production can also contribute to reducing waste by increasing utilisation of current agricultural and other biomass-related waste and by-product streams.

1.4.3 Social impact

Jobs

It is estimated that the production of bio-based products would provide direct employment for 93.700 people by 2020 (an increase of 87% from the estimated 53.200 jobs in 2008).

A major share of these will be in biosurfactants, 47.600 jobs in 2020, and the figures for biolubricants are 4.800. Additional jobs will be created outside the chemical industry, e.g. in agriculture, transportation and other services, equipment manufacturing and R&D. The sector will replace existing jobs based on fossil resources - it can be predicted that bio-based products will account for 4-6 %t of sales within the EU chemical industry in 2008 and 2020.

The production of bio-based products can make a major contribution to agriculture and forestry by creating new markets for biomass. Over time, this could transform the farming and rural economies.

The other transforming factor will be the need to develop bio-refineries in rural areas, to avoid transporting biomass long distances. This would also provide work opportunities in rural areas.

Bio-based production will require new skills, and producing information about emerging needs related to research.

Health and safety

Conventional risks to human health related to the production of bio-based chemicals are comparable to those of fossil-based chemicals. Sometimes, the bio-based chemicals are less harmful to health in production and product use.

1.4.4 Exploitation results and dissemination activities

Exploitation results

The exploitable results of Bio-SURFEST project are defined as knowledge having a potential for industrial or commercial application in research activities or for developing, creating or marketing the bio-products obtained and the bioprocesses.

Although exploitation evolved in parallel with the technical work, it offers a different view of the project results and supports the partners to think about the results more from a market perspective and identify exactly what they have to offer as well as find and reveal the value of the results for the business and scientific world. Exploitation is not the same process as commercialization hence the exploitable results from European RTD projects such as Bio-SURFEST may arise in many forms. As well as technologies which might form the basis for commercial products, such exploitation opportunities could include:

- Developing new services based on the prototypes, methods and tools.
- Creating start-up businesses to commercialize results.

- Protecting results through patents and IPR agreements.
- Inputting to standardization and legislative activities.
- Feeding RTD results and know-how into further research.
- Feeding RTD results and know-how into national or industrial research projects.

The exploitation potential of the project depends mainly on the following critical parameters that are assessed during the project lifetime:

- The project results:
  - The nature of the results (research approaches or concrete prototypes);
  - The degree of innovation (proof of concepts using mature, but risking to be outdated technologies, or forward the state of the art exploiting cutting edge but immature technologies);
  - The quality of the results (results that perform just what they promise, or better than expected (easier and faster for example);
  - Their applicability in the market (they might be the perfect results but not applicable to the market).
- The project partners:
  - The nature of each partner's organisation. RTD and SME partners in general have different exploitation goals.
  - The perspectives and the expectations of each partner from the project.
  - The continuous commitment of each partner.
- The market:
  - The maturity and the trends.
  - The movements and the role of the leaders.
  - The "customers" needs.

Two main business models for exploitation of the main project achievements were proposed by consortium partners:

- The novel technologies for biosurfactants and added products production, which aim is the developing of fermentation and enzymatic catalyst based plants as exploitable products.
- The bio-products obtained from these two novel technologies; biosurfactants and ester oils.

To ensure fluent exploitation of the main business models mentioned above, an exploitation strategy has been prepared, where is stated that SME Partners in cooperation among them and with RTD Performers have developed novel technologies for the production of bio-products (biosurfactants and ester oils). These new technologies consist of fermentation and enzymatic catalyst and the bio-products obtained from them are biosurfactants (rhamnolipids and sophorolipids) and oil ethyl esters and monoacylglycerols respectively.

The use and monitoring of the IPR issues derived from the shared and individual contribution of the partners into the creation of the exploitation assets will ensure that there is a clear understanding and agreement of the ownership of the assets, thus avoiding any potential conflict in the consortium.

Dissemination activities

Every invention has the inevitable problem of being unknown by potentially interested parties. A policy of wide dissemination of project results has been pursued and has been well received by the end users affected by this project.

A policy of broad dissemination of Bio-SURFEST project results has been particularly focused on potential customers and practitioners of various industrial sectors such as cosmetic industry, washing products, plastic industry, construction material industry, fine chemicals, waste water treatment and remediation of soils technology.

The dissemination of information and rising awareness are very important factors to determine the future prospects for the bio-products obtained from both biotechnological techniques; fermentation and enzymatic catalysis.

Pursue of a policy of wide dissemination of project results, which will be supported by all the contractors and end-users of Bio-SURFEST project.

The main objective of the dissemination strategy is to identify and organise the activities to be performed in order to maximise the influence of the project and to promote commercial and other exploitation of the project results.

The dissemination actions were aimed to:

– Create awareness about the Bio-SURFEST project on European and national levels,
– Motivate the partners and any interested parties to collaborate,
– Attract researchers and environment and biotechnological sector players to use Bio-SURFEST results and the methodologies to obtain the new bio-products, and promote Bio-SURFEST results through workshops, panels, seminars and etc.

Dissemination was done by means of:

– Project presentation by dissemination the written information
– Project Web site
– Scientific exchange.

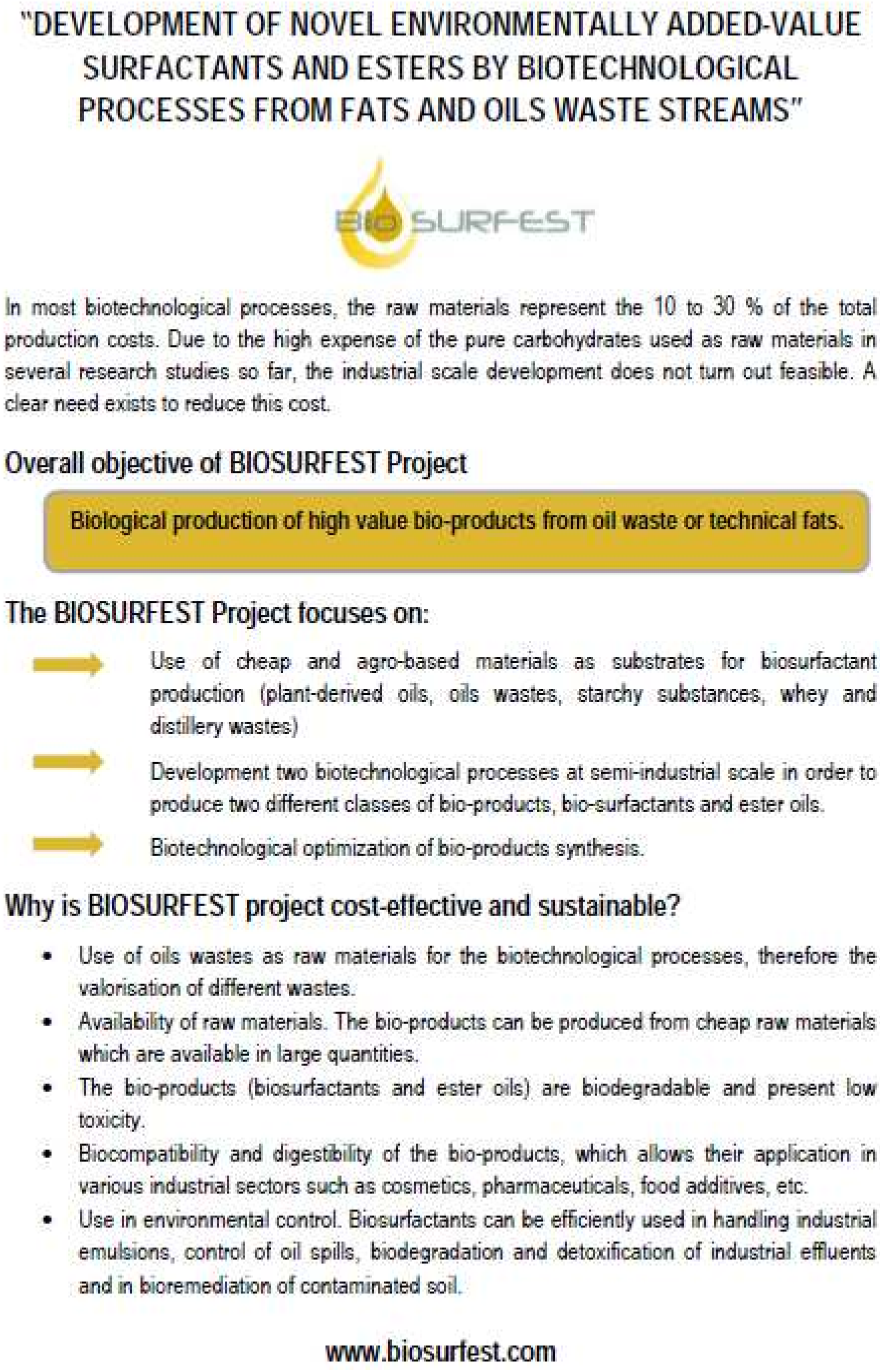
The Bio-SURFEST website represents one of the main tools for dissemination of all project relevant information, such as public downloadable documents (project reports and dissemination papers), presentations, news and events related to the project work scope. Furthermore, the website provides basic information regarding the project partners and links to their websites. Public area is organized in to eight subsections: About the project, Project partners, Documents, Video, Project results and News.

To assist the dissemination effort, a project poster was prepared. This material is distributed at conferences and workshops relevant to Bio-SURFEST. The poster provides general information about the project. The text contained in the poster was designed taking in to account not only experts, but also interested non-specialists. It introduces the main mission and the goals of the Bio-SURFEST.

A leaflet to get a wide public was also developed. The content of the leaflet is similar to the poster’s content where the information is divided in different parts with more pictures and a smaller format being more easily readable.

The last general dissemination tool was a brochure which illustrates the Bio-SURFEST objectives and includes basic information on the project. It is another way of dissemination, in this case without pictures and not structured in little paragraphs as the information is showed in the leaflet.

Fig. 2: Poster of Bio-SURFEST project



"DEVELOPMENT OF NOVEL ENVIRONMENTALLY ADDED-VALUE SURFACTANTS AND ESTERS BY BIOTECHNOLOGICAL PROCESSES FROM FATS AND OILS WASTE STREAMS"

BIO SURFEST

In most biotechnological processes, the raw materials represent the 10 to 30 % of the total production costs. Due to the high expense of the pure carbohydrates used as raw materials in several research studies so far, the industrial scale development does not turn out feasible. A clear need exists to reduce this cost.

Overall objective of BIOSURFEST Project

Biological production of high value bio-products from oil waste or technical fats.

The BIOSURFEST Project focuses on:

- Use of cheap and agro-based materials as substrates for biosurfactant production (plant-derived oils, oils wastes, starchy substances, whey and distillery wastes)
- Development two biotechnological processes at semi-industrial scale in order to produce two different classes of bio-products, bio-surfactants and ester oils.
- Biotechnological optimization of bio-products synthesis.

Why is BIOSURFEST project cost-effective and sustainable?

- Use of oils wastes as raw materials for the biotechnological processes, therefore the valorisation of different wastes.
- Availability of raw materials. The bio-products can be produced from cheap raw materials which are available in large quantities.
- The bio-products (biosurfactants and ester oils) are biodegradable and present low toxicity.
- Biocompatibility and digestibility of the bio-products, which allows their application in various industrial sectors such as cosmetics, pharmaceuticals, food additives, etc.
- Use in environmental control. Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil.

www.biosurfest.com

Fig. 3: Brochure of Bio-SURFEST project

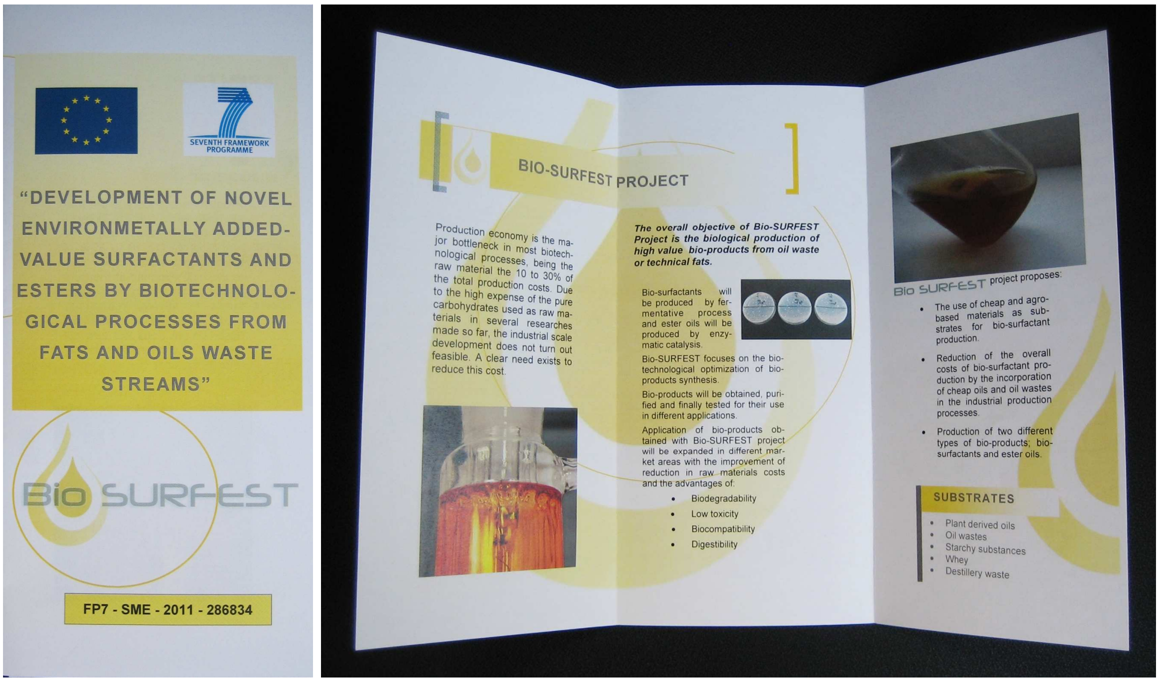


Fig. 4: Leaflet of Bio-SURFEST project

1.5 Project public website

The public part of the Bio-SURFEST website is one of the means for increasing awareness of the Bio-SURFEST project results and the project itself amongst the wider public and for attracting potential users of the Bio-SURFEST project results and its applications. The Bio-SURFEST web page has been created to inform about the project and its events. The Public area navigation bar has been designed to provide general public audience with basic information such as: project goals and structure, presentation of the project partners, description of the objectives, some news and information on upcoming Bio-SURFEST events as well as project partners contact info.

The internal part of the web site is designed for internal use of project members. Its role is to support Project management and coordination – the internal sections are oriented towards aiding in the management and handling of the internal affairs of the Project and comprise essential collaboration information, materials and documents.

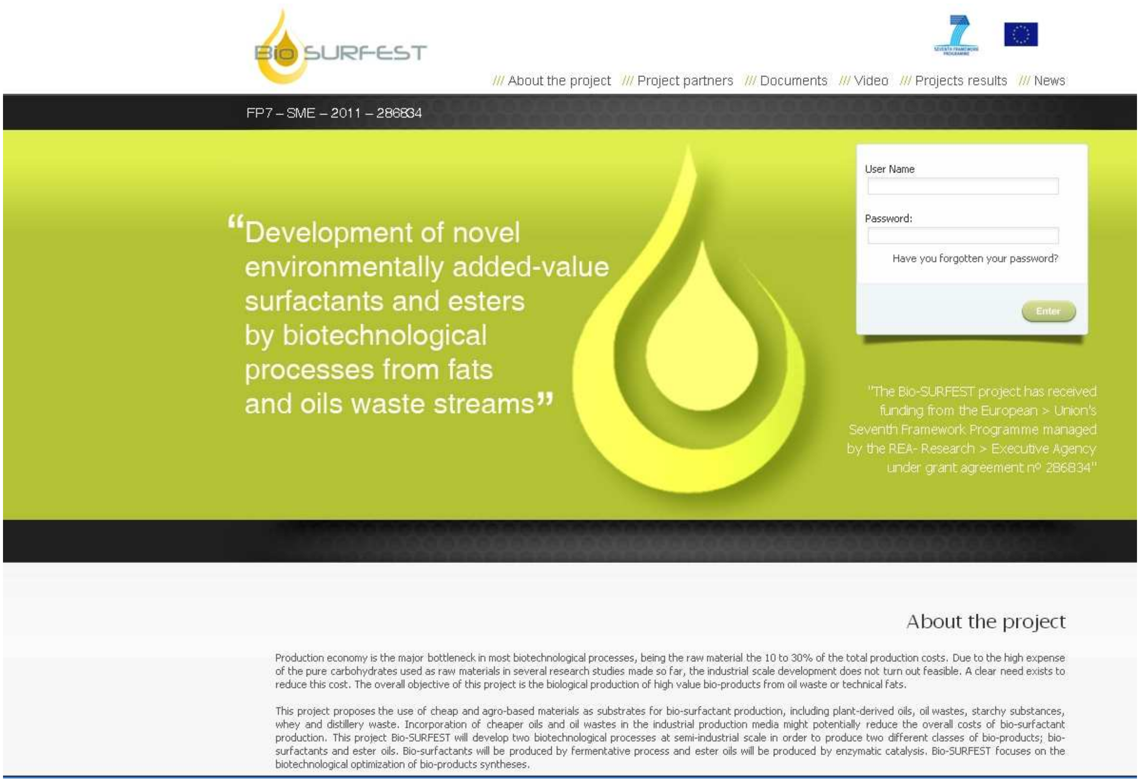


Fig. 5: Website of Bio-SURFEST project

The structure of the Bio-SURFEST website at http://www.biosurfest.com is designed in a clear and consistent way, so that visitors can easily locate all information intended for them. Upon entering the homepage of the Project, users are able to browse the Public area content, while access to Partners area requires entering password in a standard login interface. All subsections of Public area can be accessed by all users. However, all public content within the website is read-only – changes to texts and files therein can be made by administrator only.