1. Publishable summary

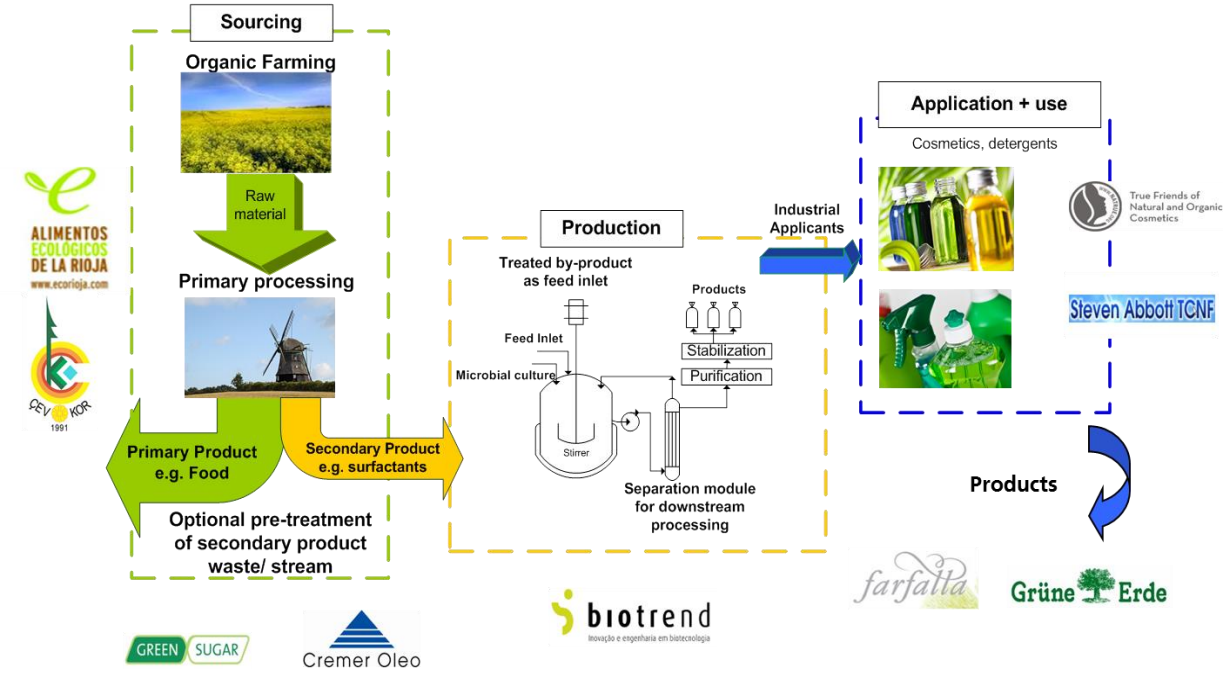
Project acronym:	O4S
Project title:	Sustainable surfactant production from renewable resources through natural fermentation for applications in natural, organically-certified products
Funding Scheme:	FP7-BSG-SME-AG
Period covered - start date:	01/01/2012
Period covered - end date:	31/12/2014

Executive summary

O4S (=Organic for Surfactants) has been primarily designed to address a critical necessity of the European Natural Cosmetic Sector which is lacking a sustainable source of surfactants (Surface Active Agents) that can be considered natural and are of certified organic origin to meet the growing market demands. This development aimed at providing the natural cosmetic sector with an alternative to replace the current use of chemically-derived surfactants from oleo-chemical feedstocks. One of their main applications is in personal hygiene products representing 40% in volume of the cosmetic ingredients used in the EU. This work aimed at optimizing the production of natural bio-surfactants by fermentation to achieve industrial efficiency and economic yields by using organically certified sources (e.g. post-harvest loses by-products from primary process). The work offers an opportunity to achieve the full utilization of organically certified raw materials increasing the efficiency of the European Organic Farming Systems.

The O4S project demonstrated the production, separation, performance and application of organic-certifiable biosurfactants in cosmetics. Suitable organically certified raw materials were identified and evaluated in terms of terms of price, availability, composition and performance as a substrate. Suitable surfactant applications and formulations for the manufacturing of certifiable high-performance Rinse-off products (e.g. pump foam) using mannosylerythritol lipid s (MEL) and further foam boosters were identified. Also suitable applications for cellobiose lipids (CL) were defined and tested. Within the project, selected in vitro-tests did show a very low response and no irritation potential of CL and MEL, which can be categorized as "mild" ingredients. The industrial layout for the production of MEL according to "organic" criteria has been outlined. Economic viability of industrial MEL production still needs to be evaluated in detail by considering the whole supply chain.

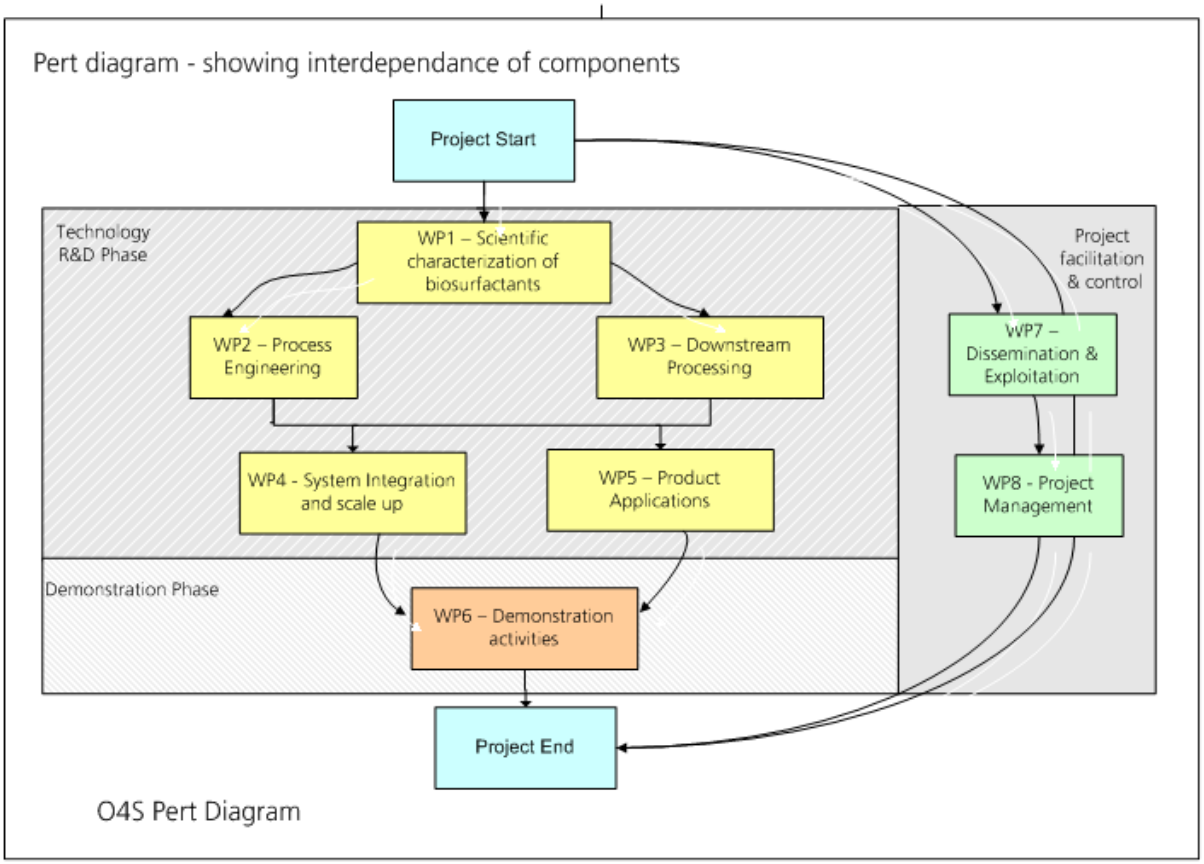
Overall, project results may have a great socio-economic impact for the European Natural Cosmetic Sector (ENCS) especially because it has been proven that high-value biosurfactants can be obtained from organically-certified raw materials and certifiable processes, the results of the O4S project proposes a consistent alternative to valorize organically-certified by-products and agricultural wastes that can positively impact the economic and social sustainability of European organic farming systems.



Project context and objectives (not exceeding 4 pages):

Overall scientific objectives of the O4S project were: 1) To investigate supply of raw materials from waste streams or by-products to lower the initial costs; 2) To develop scientific knowledge on the interdependencies of the various process parameters and substrate properties ; 3.)To establish of a database of compound characteristics, stability and performance for suitable applications. Further, the overall technical objectives were to define the characteristics of the product to meet the organic certification standards and the performance requirements for their application in the natural cosmetic industry. This was followed by the specification, optimization and scale-up of an appropriate industrial bio-process for bio-surfactant production (up to 1 m3 to 10 m3) by developing an integrated fermenting and in-situ removal concept. Further, a technology platform for product information, formulation and applications was be created for the benefit of the SME-AG members.

The Project was divided in 8 Work Packages, which were divided into technology and R&D Phase (WP1 to WP5), Demonstration phase (WP6) and Project facilitation and control (WP7 and WP8).



The main objectives of the work packages are outlined below:

WP1 Scientific characterization of promising biosurfactants

1. To compare biobased and microbial surfactants in terms of eco criteria and their suitability in model formulations as a screening step for further process optimisation.
2. To define the characteristics of the product and the process to meet the organic certification standards and the performance requirements for their application in the natural cosmetic industry.
3. To explore novel processes for supply of raw materials from renewable resources, by-product streams or waste to lower the initial raw material costs involved in fermentative bio-surfactant production.

WP2 Process Engineering

1. To maximize the time-space yield by developing fermentation process with precise adjustment of fermentation parameters and the optimization of feeding strategies to enhance product concentration and production rate.

WP3 Downstream processing strategies

1. To develop an innovative downstream processing solution that can be integrated into the fermentation concept and meets certification criteria.

   This work package focused on the development of new and innovative downstream processing technologies that are compatible with the quality standards for organic

cosmetics. Together with NATRUE an evaluation was made on how membrane technology can fulfil these quality needs. Membranes are perceived as clean technology and therefore this technology was brought to application in this field of cosmetics handling and purification.

WP4 System integration and scale up

1. To define process specifications for industrial scale up, considering the supply of selected by-products or waste streams from organic farming or manufacturing processes.
2. To collect and verify data fermentation and product recovery processes at lab and pilot scale.
3. To develop a concept to integrate all process steps including product purification and stabilization.
4. To specify required units, subunits, peripheral components and process control devices

Within this work package, all results documented from previous work packages wereto be evaluated by methodical procedures and integrated into the design concept of a complete system. Based on the data provided from the WP1, WP2 and WP3, a concept for industrial bio-surfactant production and continuous integrated product removal and purification was to be developed. Further, a concept to integrate waste streams and by-products from organic farming and manufacturing processes was considered

WP5 Product application

1. To establish a database of compound characteristics, stability and performance.
2. To define suitable applications for the natural cosmetic sector and evaluate product performance in model formulations.
3. To explore applications beyond the natural cosmetics

WP6 Demonstration activities

1. To implement organic requirement criteria in core SME and OTH production plants to obtain suitable raw materials defined in WP1. Current processes were revised in WP1 and recommendations to adapt to criteria were given.
2. To review product technology platform developed in WP5. Provide training on handling of product and develop formulation protocols
3. To demonstrate and validate formulations according to recommendations outlined in WP5.
4. To assess results and provide recommendations for end-users.

WP7 Dissemination and Exploitation

1. To raise the level of awareness of the technology, it's benefits, limitations and potential areas of application.
2. To transfer knowledge to the members of the SME-AGs, and hence the larger communities of SMEs, through their respective national organisations in the provision and use of the technology to enable rapid exploitation and commercial gain.

3. To undertake further technology transfer through the networking of the national associations in higher education establishments to enable the students to be fully conversant with the latest technology.

WP8 Consortium Management

1. To manage the consortium of partners and to coordinate the technical activity at a consortium level, in addition to the individual work packages' technical management.
2. To ensure that that the knowledge management processes and innovation related activities are conceived and
3. implemented in a coherent manner.
4. To ensure that all aspects of the EC requirements for communication and reporting are met.
5. To co-ordinate the overall legal, contractual, financial and administrative management of the project

Project outcomes

During the first period and second periods of O4S project, activities have been performed according to 'Description of Work' as agreed per Grant Agreement No: 286859.

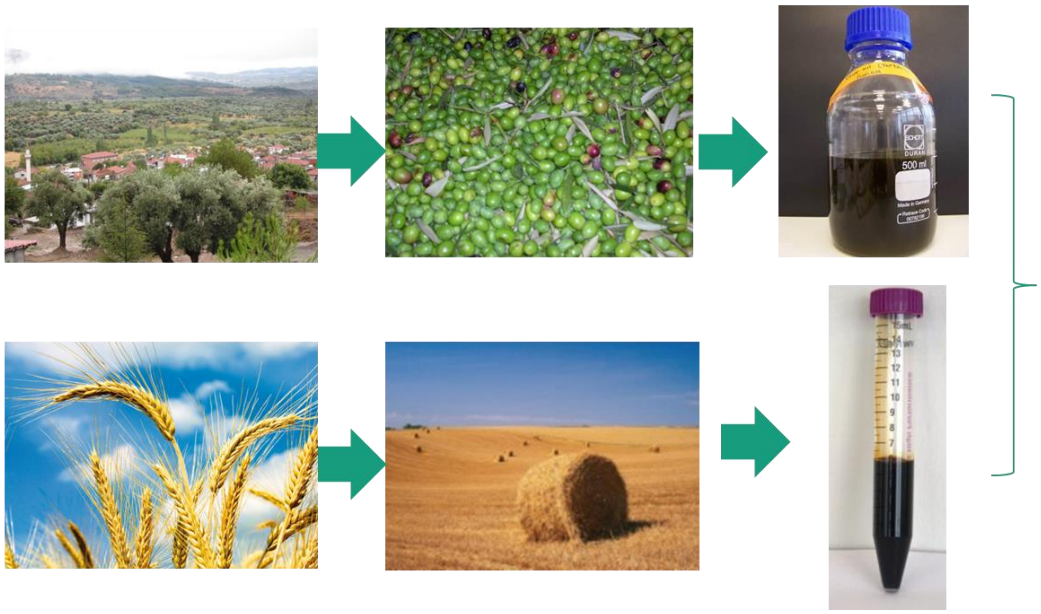
The main results are listed as follows:

WP1 Scientific characterization of promising biosurfactants

- Scientific benchmarking of available surfactants includes industrial, bio-based and bio surfactants. This included physic-chemical and performance evaluation, specially foaming and emulsifying properties. Methods of evaluation have been, collected, tested, standardised and summarized in a Standard Operating Procedure document (SOP) for internal project use. Methods will be constantly expanded, compared and updated till project end, and will be used as a tool for knowledge transfer.
- Definition of criteria for microorganism selection, raw material sources, substrate pre-conditioning process, according to Natrue certification requirements for natural and organic cosmetics. Training workshops and case studies have been conducted. Recommendations have been outlined and communicated to raw material suppliers participating in the O4S consortium. Adjustments on raw material conditioning processes have been conducted to comply with Natrue criteria for natural and organic cosmetics.
- Criteria and approach on suitable separation and purification techniques according to organic certification have been well listed and explained.
- Organically certified renewable resources, by-products and waste streams were selected and systematically evaluated according to eco-criteria, price availability and composition.
- Cultivation performance of pre-selected strains on proposed substrates (sugar and oils) to define suitability of raw material for the production of certifiable biosurfactants was evaluated

- Scientific characterization of promising strain (i.e. *Pseudozyma aphidis*) to produce the most promising biosurfactant (i.e. mannosylerythritol lipids, MEL) have been successfully conducted
- Successful microbial synthesis of the surfactants (cellobiose lipids, CL and MEL) at lab-scale.

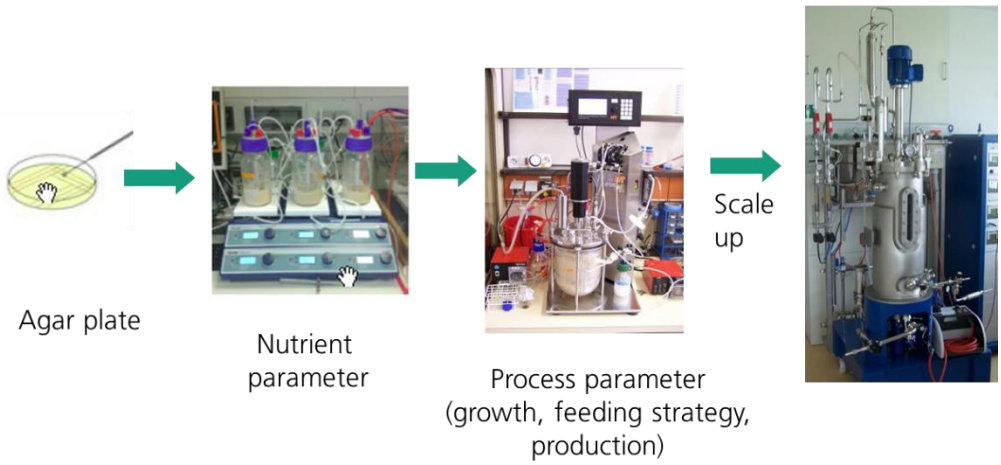
- Raw material selection:
  - Availability, price, conditioning steps, additional costs, performance



WP2 Process Engineering

- Screening trials using various strains revealed that *Pseudozyma aphidis* had the higher potential for industrial MEL production.
- Fermentation process with *Pseudozyma aphidis* was developed at laboratory scale to obtain optimal and stable production conditions of MEL (Mannosylerythritol Lipid) for maximized time-space yield.
- Experimental trials to parameterize the fermentation, enhance product concentration and minimize the formation of side products have been successfully conducted. The suitable fermentation media for the MEL production has been defined. Other suitable parameters for the fermentation such as airflow and stirring rate have been evaluated and established
- Special aspects, such as the foam control during the fermentation process of MEL and the requirements for connection to downstream processes at a 42 L-scale were integrated into the fermentation concept.
- Evaluation of the performances of produced biosurfactants, include foaming ability, surface tension, purity and water solubility have been conducted.

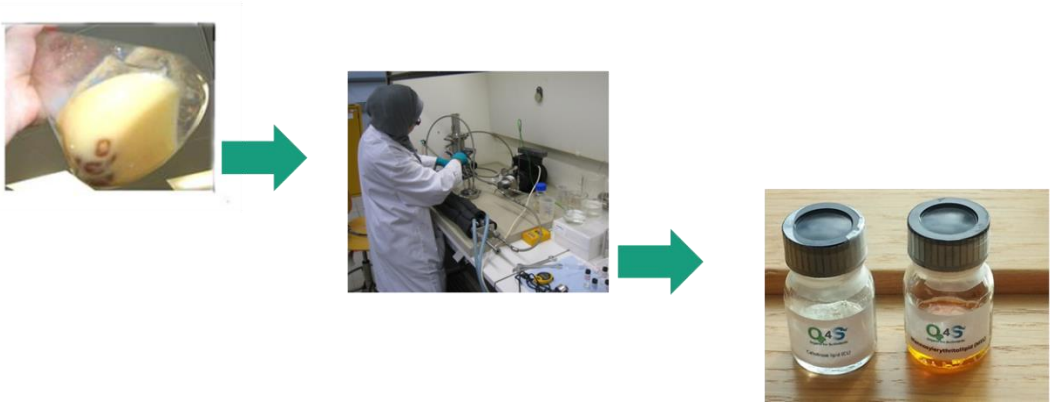
- Process optimisation: Fermentation



WP3 Downstream processing strategies

- Evaluation of membrane technology for the downstream processing of MEL has been conducted.
- Evaluation of bio based solvents to replace petrochemical solvents has been conducted. As a result, bioethanol seems to be the most promising to replace petrochemical solvents in the extraction method to purify the biosurfactants.
- A DSP scheme was developed and proven on lab scale for the purification of MEL based on physico-chemical processes and solvent membrane filtration.
- A strategy for continuous in-situ product removal (ISPR) including options and process layout scenarios towards continuous production of MEL biosurfactant has been proposed based on the lab scale downstream processing (DSP) results.

- Downstream processing:
  - Evaluation of suitable methods against Natrue criteria!
  - →Membrane separation or other techniques approved by Natrue



WP4 System integration and scale up

- Following successful application tests with both MEL and CL biosurfactants, the O4S consortium agreed to evaluate both the process flow of MEL and CL production at

laboratory level. These process flows regarded organic production guidelines and have been further adapted to establish industrial process flows. A mass balance for the production of MEL and CL was calculated and a piping and instrumentation diagram (P&ID) of an integrated system for each biosurfactant was outlined.

- A concept to integrate waste streams and by-products from organic farming and biosurfactant manufacturing process has been outlined. The concept focused on the industrial production of MEL due to the larger availability of data, its higher yield and well established downstream process parameters. The conceptual study evaluated the industrial production of MEL with an annual capacity of 10 MT of MEL, excluding the raw material supply, and presented different theoretical scenarios for industrial layout based on defined raw material. Production costs were further estimated to annual production capacity of 100 MT. However, cost estimations were not within the scope of the O4S project and still require sensitivity studies to support economic validation of industrial MEL production. This activity has been suggested for future post-project activities.

WP5 Product application

- Evaluation of the performances of biosurfactants produced at laboratory level (Mannosylerythritol Lipid –MEL- and Cellobiose Lipid), CL), including foaming ability, emulsifying properties, surface tension, purity and water solubility in model solutions and standard cosmetic formulations have been conducted.
- Suitable surfactant applications and formulations for the manufacturing of certifiable high-performance Rinse-off products (e.g. pump foam) using MEL and further foam boosters were identified. Also suitable applications for CL were defined and tested.
- Within the project, selected in vitro-tests did show a very low response and no irritation potential of CL and MEL, which can be categorized as "mild" ingredients.
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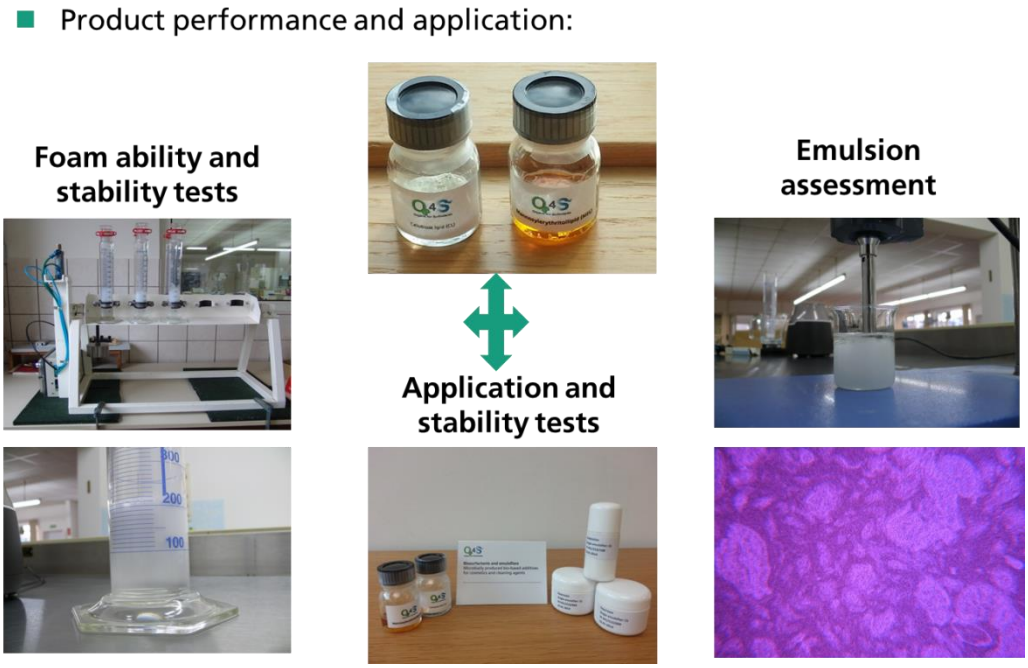

WP6 Demonstration activities

- Demonstration protocols and recommendations for the production of MEL and CL have been developed and described, including product validation in cosmetic formulations and further protocols and recommendations for raw material supply
- During field trials, Farfalla and Grüne Erde tested the application of CL and MEL in their own formulations with the assistance of Inst. Dr. Schrader. Samples were taken home and evaluated internally. Feedback was then provided and recommendations were given by end users.

WP7 Dissemination and Exploitation

- Dissemination activities for transferring the knowledge have been taken place throughout the project time and included various oral presentations at congresses and industrial meetings (e.g. Natrue Annual Members Assembly) , press conference at the BioFach exhibition 2014, as well as the presentation of posters, flyers at various international events

Socio-economic impact

Overall, project results may have a great socio-economic impact for the European Natural Cosmetic Sector (ENCS) especially because it has been proven that high-value biosurfactants can be obtained from organically-certified raw materials and certifiable processes, the results of the O4S project proposes a consistent alternative to valorize organically-certified by-products and agricultural wastes that can positively impact the economic and social sustainability of European organic farming systems.

The O4S Results will enable SMEs from the organic farming, ingredients, cosmetics and formulation sectors to coordinate their efforts to achieve a common goal: the sustainable production systems, high quality and healthy or non-toxic products.
This will increase the competiveness of organic farmers that could decide between several options a secondary use for the biomass or wastes produced from their primary activities. The main advantage is that biosurfactants can be produced by fermentation of cheap raw materials which are available in large quantities. The carbon source may come from carbohydrates and/or lipids, which may be used separately or in combination with each other. This will help farmers and manufacturers to increase their income by adding value to their agricultural wastes (e.g. postharvest loses or residues from primary processes). This way, they can overcome price pressures from discounter retailers, who are increasingly adopting this market segment and boosted 2009 their sales of organic products in volume by reducing retail prices. According to figures published at the BIOFACH 2015, *"Sales in the whole organic sector have trebled in the last ten years to 7.55 billion EUR, according to figures from the Bund Ökologische Lebensmittelwirtschaft, BÖLW (German Federation of the Organic Food Industry), Berlin (D). The sales barometer of the Bundesverband Naturkost Naturwaren, BNN (German*

Organic Producers and Traders Association), Berlin (D), indicates that organic products worth some 2.5 billion EUR were sold over the shop counters of the organic retail trade in Germany in 2013". The latter unavoidably pushes organic farmers and manufacturers to increase productivity, requiring more efforts in research and innovation.
We are aware of the different opinions on the secondary uses of agricultural wastes from organic production: competition with food production and monocultures should be avoided and development of biodiversity should be strengthened. According to Naturland, prices from organic oil pressed cakes are still very high due to their high protein content and current use as feed for organic meat production. Therefore, sources of raw materials and substrates for the bio-surfactant production were carefully evaluated. According to their organizations experience it can be assumed that the production of bio-surfactants is marginal and will not really compete with food production. On the contrary, secondary uses of certified produce may result in attractive business cases some farmers, helping to increase their turnovers.

In Europe, more than 400 SMEs supply organic cosmetics into this market segment which is growing at approximately 20 percent per annum and with revenues forecast to approach EUR 2 billion during 2010.This growth is driven by the increasing awareness of human environmental impact along with a desire to eliminate the use of products manufactured or incorporating potentially environmentally damaging chemicals.
One of the major problems of the sector is that there is no harmonized regulation associated with natural cosmetics and they are currently not legally protected. As a result, several European and national organizations have developed their own standards and certification labels. Amarjit Sahota, director and founder of Organic Monitor explained that "the major challenge of the natural cosmetics sector is certification, harmonised standards and transparent consumer information".
In Europe a total of 280,000 tonnes of ingredients for cosmetics and toiletries were sold in 2009, being special surfactants the most significant category, with 23% of the value and 40% of the quantity. These include amphoteric, anionic, cationic and non-ionic surfactants18.Thereof the relevance of these compounds in the cosmetic sector. In general, the cosmetic ingredients sector was expected to grow in 2.6% until 2014, with "green" ingredients being the major factor.
Beyond the natural and organic cosmetic sectors there is a huge trend to re-formulate products to align with consumer and regulation trends. In this view TCNF will help to find plausible solutions for other industrial applications.
http://www.organic4surfactants.fraunhofer.de/index.html

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Partners

Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e. V.
(RTD Performer, Germany)

Website: http://www.fraunhofer.de/en.html

Executing Institutes:
Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB

The International Natural and Organic Cosmetics Association
(Natrue SME Association/Grouping, Belgium)
Website: http://www.natrue.org

Naturland - Verband für ökologischen Landbau e. V.
(SME Association/Grouping, Germany)

Website: http://www.naturland.de/welcome.html

Green Sugar GmbH. Produktinnovationen aus Biomasse
(Other enterprises or end-users, Germany)
Website: http://www.green-sugar.eu/index_en.html

Farfalla Essentials AG
(Other enterprises or end-users, Switzerland)
Website: http://www.farfalla-essentials.com

Grüne Erde GmbH
(Other enterprises or end-users, Austria)
Website: http://www.grueneerde.com

Biotrend - Inovação e Engenharia em Biotecnologia, SA
(Other enterprises or end-users, Portugal)
Website: http://www.biotrend.biz

Cremer Oleo GmbH & CO. KG
(Other enterprises or end-users, Germany)
Website: http://www.cremeroleo.de/en/cremer-oleo-gmbh.html

Vlaamse instelling voor technologisch onderzoek
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http://www.vito.be/VITO/EN/HomepageAdmin/Home/Homepage

Insitut Dr. Schrader Creachem GmbH
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Website: http://www.schrader-institute.de/index_engl.htm

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