FINAL PUBLISHABLE SUMMARY REPORT

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Project acronym: TRANSBIO

Project title: BioTRANSformation of by-products from fruit and vegetable processing industry into valuable BIOproducts

Funding Scheme: Collaborative Project targeted to a special Group (such as SMEs)

Start Date of Project: **01.12.2011**

Duration of Project: **48 Months**

Website: www.transbio.eu

Dissemination Level:	**PU - Public**

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LIST OF PARTNERS

Nº	NAME	SHORT NAME	COUNTRY	TYPE
1	TECNALIA Research & Innovation	TECNALIA	SPAIN	RTD
2	WETLANDS INCUBATOR SPRL	WETLANDS	BELGIUM	SME
3	TTZ BREMERHAVEN	TTZ	GERMANY	RTD
4	PROMIC S.A.	PROMIC	SPAIN	SME
5	BIOESPLORA SRL	ESP	ITALY	SME
6	Centro Nacional de Tecnología y Seguridad Alimentaria – Laboratorio del Ebro	CNTA	SPAIN	RTD
7	UNIVERSITY OF COSTA RICA	CITA	COSTA RICA	RTD
8	SOLUCIONES VERDES SRL	ECO	ARGENTINA	SME
9	UNIVERSIDADE DO MINHO	UMINHO	PORTUGAL	RTD
10	BIOTREND – INOVACAO E ENGENHARIA EM BIOTECNOLOGIA S.A.	BIOTREND	PORTUGAL	SME
11	BIOZOON GMBH	BIOZOON	GERMANY	SME
12	UNIVERSIDAD AUTONOMA METROPOLITANA	UAM-I	MEXICO	RTD
13	NATUSTOFF-TECHNIK GMBH	NST	GERMANY	SME
14	PROTEOS BIOTECH S.L.U.	PROTEOS	SPAIN	SME
15	TRITECC SRL	TTC	ROMANIA	SME
16	ORGANIC WASTE SYSTEMS NV	OWS	BELGIUM	SME

EXECUTIVE SUMMARY

The European research project TRANSBIO aimed in the implementation of an innovative cascading concept for the betterment of sub-products using environmental friendly biotechnological solutions like fermentation and enzyme-conversion strategies to obtain valuable bio-products like biopolymers (PHB), nutraceuticals / platform chemical (succinic acid) and enzymes for detergent applications.
Thus the overall sustainability of biomass processing industry will be improved and the competitiveness of European biotechnology industry will be increased through new applications.

In order to target and realize different biotechnological approaches for transforming by-products from fruit and vegetable processing industry into value-added bioproducts, the consortium consist of 16 partners coming from nine countries and two continents (Europe / Latin America) combining their knowledge & experiences to reduce production costs for biopolymer PHB, bio-based succinic acid and enzymes for detergent application. Finally, remaining biomass were evaluated for their potential to be used for biogas production. In order to achieve the TRANSBIO strategy, the project had been designed as a cascade of technical work packages (WP1-12) with continuous progress assessment between the individual scientific and methodological approaches. In parallel, economic and environmental evaluation (WP14), testing activities in pilot plant scale (WP13), as well as dissemination activities (WP15) were performed. During the project duration several by-products coming from fruit and vegetable processing industry were characterized and hydrolysis procedures could be established. Beside, promising strains for PHB, succinic acid and enzyme production could be identified and were successfully used in the implementation of fermentation design and up-scaling experiments. In parallel, down-stream processing developments for the different bioproducts were developed.

Furthermore, the partners established dissemination strategies (webpage, leaflet, newsletters) to inform European society and interested stakeholder about the research activities in TRANSBIO and got in contact with other European research projects for knowledge exchange. One keystone of dissemination was the decision of the project partners to participate in the book “Biotransformation of Agricultural Waste and By-Products, 1st Edition, The Food, Feed, Fibre, Fuel (4F) Economy“ to be published by Elsevier in March 2016. http://store.elsevier.com/product.jsp?isbn=9780128036228&pagename=search

The project participated in several congresses, especially to be mentioned the XVIII Latin American Seminar and V National Congress of Food Science and Technology, in March 2014 in San Jose, Costa Rica or the International Conference “Envisioning a Future without Food Waste and Food Poverty: Societal Challenges” in Bilbao (Spain) in November 2015.
http://www.wageningenacademic.com/books/doi/10.3920/978-90-8686-820-9

Additional, four TRANSBIO workshops could be performed, one in Costa Rica, one in Argentina, one in Italy and one in Spain. Finally, a conference was organized in November 2015 in Vitoria-Gasteiz (Spain) to inform interested stakeholders on the TRANSBIO results.

Summary description of the project context and the main objectives

As worldwide demand for petroleum, our main fossil resource to produce energy, chemicals and materials is steadily increasing, petroleum prices are expected to rise. Current price reductions are mainly due to supply has outstripped demand. As the economic growth of China has slowed, also its demand for oil has fallen. In contrast supply has increased, to some extent due to the rise of US shale oil. In addition, to defend market shares of OPEC, the world's largest exporter of oil, Saudi Arabia, has refused to cut production. About one million barrels of oil are being produced above demand every day, analysts estimate. Nevertheless, the crude oil price will reach again a price of approximately 70 US$ / bbl in the next 5-6 years predict the World Bank Commodities Price Forecast (October, 2015).

Whereas this fossil resource will certainly not become exhausted from one day to another, it is clear that its price will follow a long-term upward trend. Its scarcity and high price will not only afflict the chemical industries and energy sectors drastically all around the world, but it will impact on society as a whole (Soetaert and Vandamme 2006). Therefore already in 2002 the Working Group “Renewable Raw Materials” coordinated by ERRMA suggested that by employing physical, chemical and biochemical processes, renewable raw material can be converted into polymers, lubricants, surfactants or specialty chemicals. Besides ensuring security of supply of feedstock for industrial processes, also environmental concerns playing an important role due to policy development and consumer awareness of the need to ensure sustainable production and consumption. Therefore, the European Commission has taken different Actions (e.g. 6th Environment Action Programme, directive 2006/12/EC) to restrict the production of waste particularly by promoting clean technologies and products which can be recycled and re-used. As world demand and competition for finite and often scarce resources will continue to increase, Europe can benefit economically and environmentally from making better use of those resources. Therefore, a **circular economy** system which keeps the added value in products as long as possible and eliminates waste must be implemented (COM(2014) 398 final/2).

In this context ***TRANSBIO aims in the utilisation of by-products from fruit and vegetable transforming industry,*** in order to ***reduce the environmental impact of food waste, reducing landfill*** and ***lessening noxious residues and odours.*** On the other side the ***project*** will ***enhance*** the ***sustainable management of biomass from fruit and vegetable processing industry*** as it can be used as ***feedstock for bio-based products like PHB, succinic acid and enzymes for detergent industry.***

Major factors driving future markets and demand for bio-based materials in the European Union are the limited availability and increased costs of fossil resources, policy development due to climate change as well as employment growth. In this context, by-products from existing fruit and vegetable processing procedures attract notice. Fruit and vegetable biomass is renewable, CO_2 neutral and has the potential to deliver an array of raw materials for industry in place of mainly petrochemical sources. According to the European project AWARENET, approx. 192 million tons of waste and by-products from fruit and vegetable processing industry are produced every year in EU-15. However, currently a number of factors limit the application for bio-based products. Due to demand for bio-based products has not been sufficient, to realize ***economies of scale in the production***, bio-based products have in many cases ***higher manufacturing costs***. However the change from a non-renewable to a renewable feedstock base in the chemical industry is accelerating. This is not only due to the increase in the prices of non-renewable feedstock but also because of the growing ability of certain microorganisms to yield higher productivity of the desired chemicals. Global Industry Analysts predict that the global market for Renewable Chemicals will reach 73.8 billion US$ by 2020, mainly driven by the rising focus on reducing CO_2 emissions and increasing demand for healthier, greener and cleaner products.

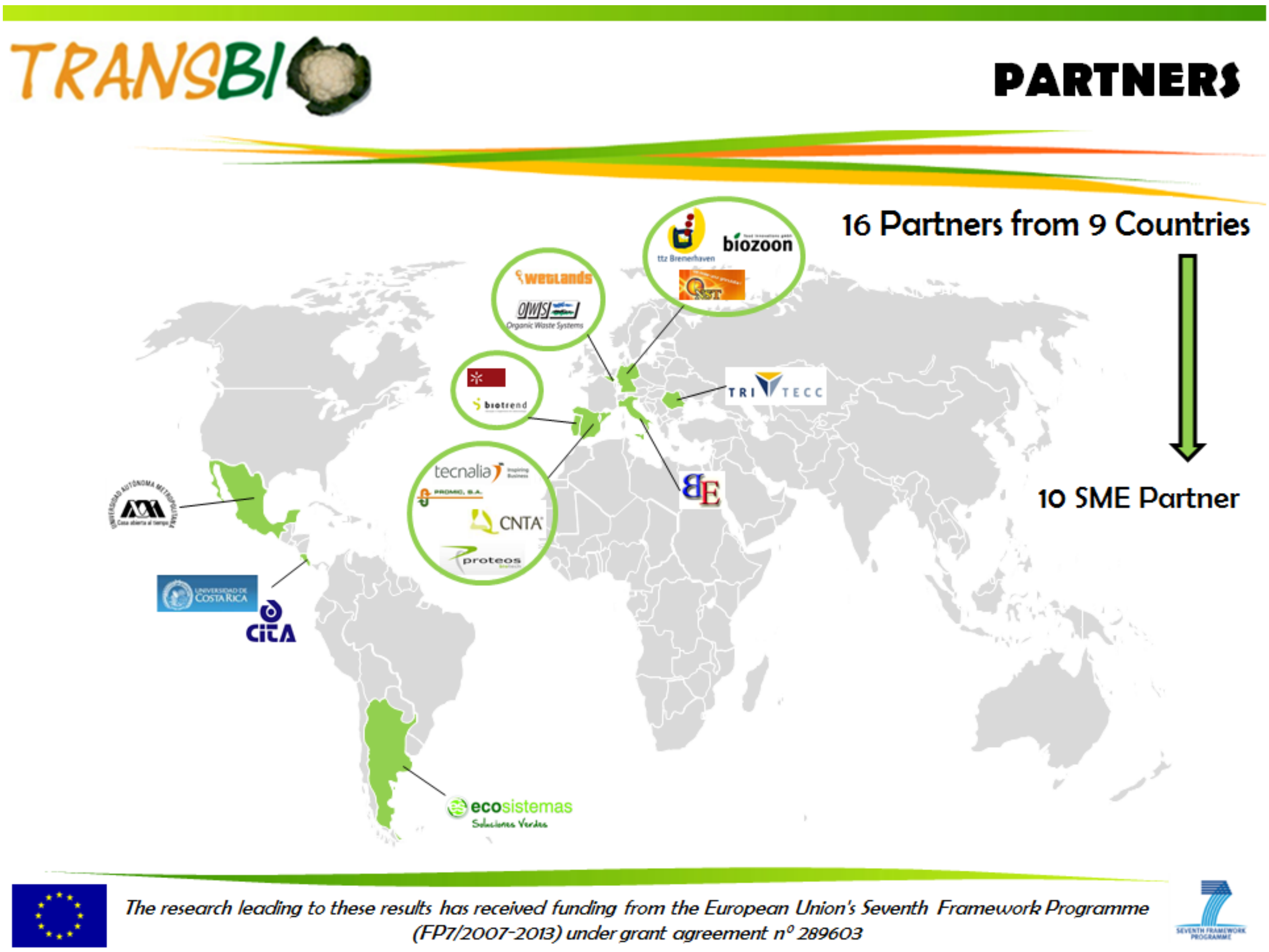
The main aim of the European funded project TRANSBIO (project nº 289603) is the implementation of an innovative cascading concept for the valorisation of sub-products from fruit and vegetable processing industry using environmental friendly biotechnological solutions like fermentation and enzyme-conversion strategies to obtain valuable bio-products like plastics (PHB), nutraceuticals / platform chemical succinic acid and enzymes for detergent applications.
This will improve the overall sustainability of the biomass processing industry and will increase the competitiveness of European biotechnology industry through new applications. Therefore the project is fully in-line with the ***lead market initiative*** for bio-based products (COM(2007)860 final); the ***Strategy on life sciences and biotechnology*** (COM(2002)27 final; SEC(2007)441) by development of cleaner industrial products and processes based on use of enzymes and microorganism (biocatalysis) as well as the ***Initiative for developing a coherent European strategy for key enabling technologies*** (COM(2009)512) supporting applications for industrial biotechnology (fermentation of microorganisms, enzyme application).

TRANSBIO will characterize and select appropriate by-products from fruit and vegetable processing industry, followed by adapted pre-treatment and enzymatic hydrolysis procedures to obtain fermentable sugars for microbial fermentation. In order to obtain a broad application potential for the by-products selected, the project will investigate three different fermentation strategies – submerged cultivation (SmF) in liquid media (bacteria, yeasts) and solid state fermentation (SSF) (fungi). Beside optimization and up-scaling of fermentation protocols, down-stream processing will be developed keeping in mind economic feasibility, sustainability as well as end-product formation (intracellular, extra cellular).
The procedures will be optimized for extra cellular succinic acid production in SmF using novel non-conventional yeast strains and extracellular enzyme formation in SSF underutilization of fungi as well as intracellular PHB formation in SmF with bacteria. The obtained PHB will be tested for their packaging application, enzymes will be proved for detergent utilization and succinic acid will be purified for food application. Beside, original and pre-treated by-products as well as remaining biomass from fermentation strategies will be tested for their potential to be used as feedstock for biogas production via anaerobic digestion. In order to achieve these aims, the main objectives can be summarized as follow:

- Selection of appropriate by-products from fruit and vegetable processing Industry
- Establishment of environmental friendly enzymatic hydrolysis procedure to liberate sugar components (C5 and C6 sugars) to be used as carbon source
- Selection of fungi’s for enzyme production in Solid State Fermentation
- Screening of bacterial strains for PHB production
- Phenotypic screening for novel nonconventional yeasts strains from natural resources for succinic acid production
- Bacterial Fermentation of intracellular bio-product PHB via SmF
- Solid state fermentation of fungi to obtain extra cellular enzymes (Lipases, Proteases)

- Yeast fermentation of extra cellular bio-product succinic acid
- Development of appropriate down-stream procedures and applications keeping in mind environmental and economic parameters
- Enlargement of raw material base for biogas production by using fruit and vegetable by-products and remaining biomass from fermentation strategies as feedstock for Anaerobic digestion
- Up-scaling to pilot-scale
- Life Cycle Assessment and Economic Feasibility Study

In order to target and realize different biotechnological approaches for transforming by-products from fruit and vegetable processing industry into value-added bio-products, the consortium consist of 16 partners. The consortium has been built as a well-balanced and equal partnership between European (Belgium, Germany, Italy, Portugal, Romania, Spain) and Latin America (Argentina, Costa Rica, Mexico) upon proven expertise in the field of fruit and vegetable by-product valorisation, microbial strain selection, fermentation strategy development and down-stream processing as well as end-product stabilisation and utilization (PHB, Succinic acid, enzymes for detergent application) and biogas production keeping in mind sustainability issues and economic feasibility.



As the TRANSBIO project has not only a high impact on sustainable use of resources and environment but also a significant economic impact, the consortium consists beside 6 RTD partners of 10 SME partners.

Description of the main S & T results / foregrounds

TRANSBIO started its activities with the definition of requirements. Therefore an up-date on state-of-the-art on biowaste valorisation was performed as well as different value-added chains (succinic acid, PHB, enzymes) were analysed. Additional sustainability criteria as well as economic, legal- and end-user requirements were determined.

The general objective of WP2 was to characterize and select appropriate by-products from fruit and vegetable transforming industry, the pre-treatment and enzymatic hydrolysis of said by-products and the providing of cheap fermentation feedstock for the three different fermentation strategies. Based on identified by-products as laid down in deliverable D1.4 Value-Added Chain Analysis Report, the most promising fruit and vegetable by-products from Spain and Costa Rica were analyzed covering a complete physical-chemical characterization (protein, lipid, carbohydrate, fiber, lignocellulose, etc.).The results were summarized in deliverable ***D2.1*** "***Report on By-product Characterization and Selection***". This deliverable is useful to determine the valorization possibilities of these products, not only regarding to fermentation processes, but also provide information about other interesting valorization possibilities (i.e. fiber extraction). Afterwards, the by-products were forwarded for the following pre-treatment and hydrolysis experiments. ***Task 2.2*** investigated several physical disintegration methods (milling, drying, grinding) in industrial scale to obtain useful by-product powders to be used in task 2.3 and 2.4. Based on the results and the necessity of two fermentation strategies (submerged fermentation & solid state fermentation), appropriate pre-treatment procedures were decided and laid down in deliverable ***D2.2*** "***Pre-treatment Protocol.***

In parallel the obtained physical pre-treated by-products were hydrolysed in ***task 2.3*** (chemical hydrolysis) and in ***task* 2.4** (enzymatic hydrolysis). It was decided to use the enzymatic hydrolysis protocol to provide carbon sources for submerged fermentation.

Based on these results **MS5 – Fruit and Vegetable by-products selected, Hydrolysis Procedure established** – could be achieved. Additional economic and environmental performance data were continuously collected and forwarded to WP14.

DELIVERABLE D 2.1

Report on By-product Characterization and Selection

DELIVERABLE D2.2

Pre-treatment Protocol

DELIVERABLE D 2.3

Chemical Hydrolysis Protocol

DELIVERABLE D2.4

Process Parameter for Enzymatic Hydrolysis

Hydrolysates available for Fermentation Strategies

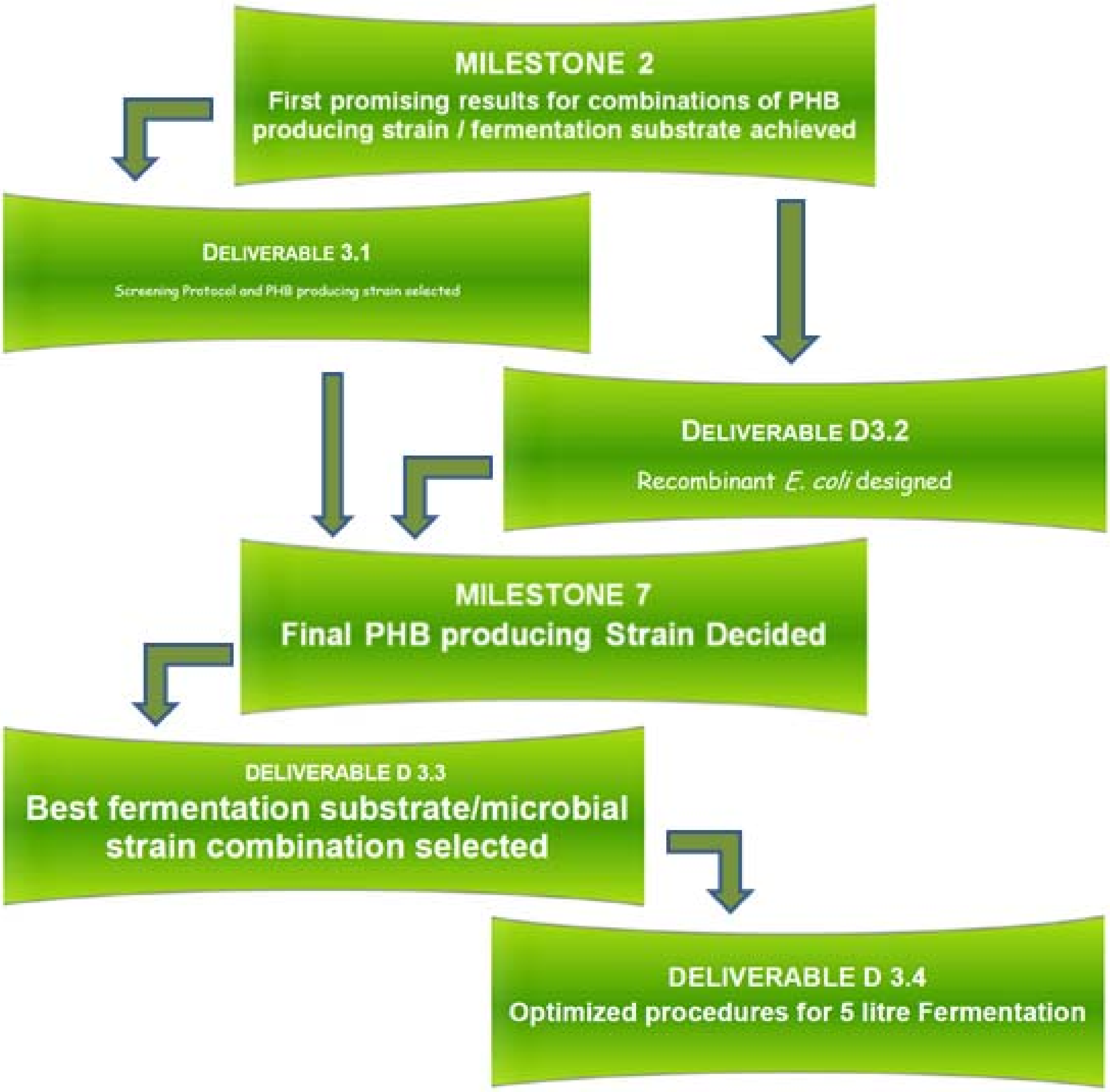
MILESTONE 5

Fruit & Vegetable by-products selected & Hydrolysis Procedures established

Polyhydroxybutyrate (PHB) is a very versatile biopolymer produced directly via fermentation of carbon substrates by a wide range of organisms. In ***work package 3*** the partners established screening procedures for the selection of candidate strains able to produce PHB. The methods were used to evaluate first soil and water samples and 114 natural PHB producing bacteria (wild-type strains of Cupriavidus, Comamonas and Pseudomonas) could be identified, which were further investigated and characterized. The partners found that these bacteria are able to produce PHB when hydrolysates of potato flour, pea pods, turnip waste, banana peels, vegetable waste (grass, chicory waste), frying oil, apple peels are used as carbon source. Based on the work performed milestone ***MS2 First promising results of PHB producing strains*** could be successfully achieved in month 18. Finally a screening protocol for PHB producing strains were agreed and laid down in ***deliverable D3.1***.

Beside natural producers of PHB, the project aimed also in the genetic modification of an *E. coli* to produce PHB. The objective was to clone PHA involved genes (phaCAB, prpE, prpP) from *Cupriavidus necator* to *Escherichia coli*. The vector selected for cloning was pUC19L. This task could be finished successfully with deliverable ***D3.2 – Recombinant E.coli designed***. The partners obtained two recombinant microorganisms: pET24-CAB *E. coli* and pGETS109-CAB *E. coli*. The recombinant microorganisms expresses the three genes, *phaC* (PHA synthase), *pha A* (3-ketothiolase) and *pha B* (NADPH-dependent acetoacetyl-Coa reductase) as demonstrated by the accumulation of PHB in these cells. Nevertheless the **pET24-CAB *E. coli's*** ability to accumulate PHB is around 2 times higher and around 3 times faster in respect to the pGETS109-CAB *E. coli*. Additional, the recombinant pET24-CAB *E. coli* is able to produce PHB when enzymatic hydrolysates are used as broth culture/carbon source and was chosen for further developments. Using the promising strains, the partners selected best fermentation substrate / microbial strain combinations. These results were laid down in ***D3.3***. Finally, the partners optimized fermentation strategy up to 5 Litre scale successfully. The results were laid down in deliverable **D3.4 – Optimized procedures for 5 Litre fermentation.** Therefore work package 3 achieved all necessary developments to be used in WP4 and could be finished successfully.

Fig. Fermentation of wild-type PHB producer using hydrolysate from vegetable by-product at lab-scale



To increase products yields and to ensure consistent product quality, key issues of industrial fermentations, process optimization and scale up are aimed at maintaining optimum an homogenous reaction conditions. For each individual product, process and facility, suitable strategies must be developed by a comprehensive and detailed process characterization, identification of the most relevant process parameters influencing product yield and quality and their establishment as scale-up parameters. Therefore, the main objective of ***work package 4*** was the development of cost-effective fermentation and extraction processes for PHB production up to 50-Liter scale. The work package started its activities in with an intensive study of existing methodologies for PHB determination and extraction procedures in order to give feedback to the strain selection in WP3 and for fermentation design (necessary yields, medium components, etc.) in task 4.1. Afterwards, achievements of WP3 were translated in larger scale and used to produce PHB rich biomass to be forwarded to task 4.2 for PHB extraction and purification. The up-scaling were performed using the most suitable wild strain of

Cupridavidus necator (E9) and the designed recombinant pET24-CAB *E. coli*. The up-scaling protocol was laid down in deliverable ***D4.1*** and submitted to the commission.

Fig. Bioreactors used for up-scaling

The following figure is showing relevant fermentation parameters (temperature, oxygen concentration, pH) during 50 liters fermentation.

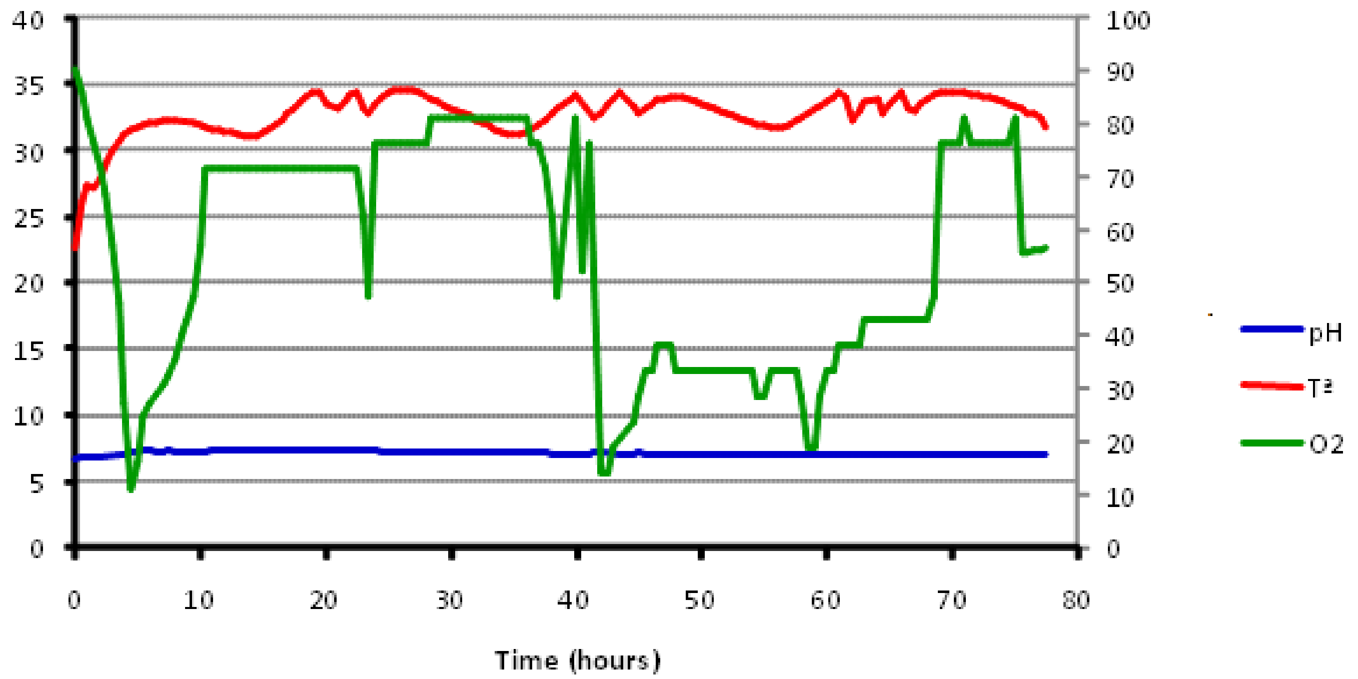


Fig. Evolution of fermentation parameters during PHB production

The obtained biomass was forwarded to task 4.2 to implement biopolymer extraction procedure. In the framework of the TRANSBIO project a combination of mechanical, enzymatic and chemical processes were tested. For polymer characterization, PHB was isolated using conventional chloroform extraction, followed by ethanol precipitation. During process optimization different more environmentally friendly extraction techniques were implemented such as enzymatic extraction or the use of green solvents.

Fig. PHB extraction from engineered *E. coli*

The work started with the selection of appropriate solvents with sufficient solubility characteristics for PHB. For this purpose, the Hansen Solubility Parameters were used. Hansen proposed an empirical equation, which predicts the solubility of a polymer in an organic liquid (Hansen, 1967). The solubility of the product increases approximately as ijR decreases. Furthermore, the respective Hansen parameters for solvents can be found in Hansen (2007).

Tab.: Solubility Index / Hansen radius for different solvents

SOLVENT	HANSEN SOLUBILITY PARAMETERS			
	$^{i}\delta d$	$^{i}\delta p$	$^{i}\delta h$	^{ij}R
Methylene dichloride	18,2	6,3	6,1	2,42
Chloroform	17,8	3,1	5,7	3,77
1,2-Dichloroethane	17,4	5,3	4,1	4,39
Ethylacetate	15,8	5.3	7,2	7,06
2,2,2-Trifluoro ethanol	15,4	8,3	16,4	13,11
Dimethylformamide	17,4	13,7	11,3	10,49
Dimethylsulphoxide	19,0	19,4	12,3	15,34
Isoamyl alcohol	15,8	5,2	13,3	9,90
Cyclohexanone	17,8	6,3	5,1	3,38
n-Butylacetate	15,8	3,7	6,3	7,18
Ethyl lactate	16,0	7,6	12,5	9,34
Propylene carbonate	20,0	18,0	4,1	12,96
Anisole	17,8	4,1	6,7	3,26
Trichloroacetonitrile	16,4	7,4	6,17	6,17

Based on these calculations the eight best solvents were selected for green solvent analysis. The definition of a green solvent is not straightforward and depends on the application. Some criteria may be contradictory and there are no universal metrics set to compare the greenness criteria. Some companies / institutions have edited solvent selection guides in order to help chemists in their choice (Prat, 2015). In this context, the GSK solvent selection Guide can be considered as good solution as it provides clear practical information and guidance for the selection of greener solvents. The guide has a ranking from 1 (worse/red) to 10 (best/green). The scores are based on data or a physical observable property. Considering mainly Waste, Environmental Impact, Health and Life Cycle score versus Hansen radius, the following distribution of the different solvents can be found.

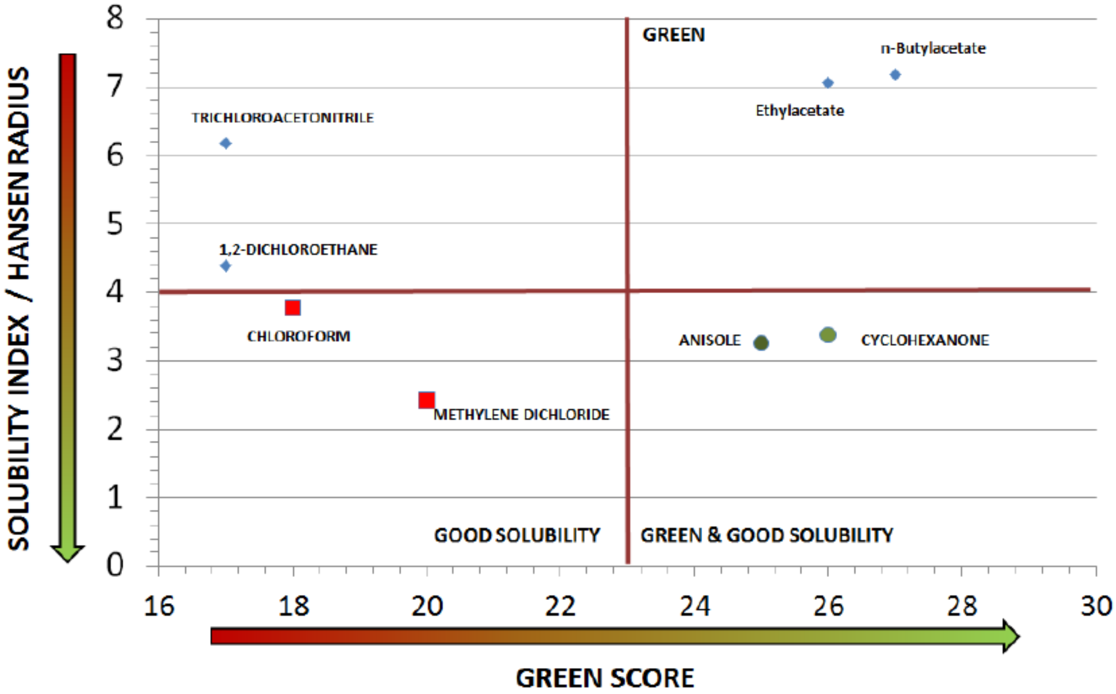


Fig.: Green Score versus Solubility Index (Hansen radius) for the different solvents

Based on this evaluation anisole and cyclohexanone were compared with enzymatic extraction versus chloroform extraction. Taking extract purity and PHB recovery yield as the critical parameters for methodology selection, anisole performed best in terms of extraction capability but also in terms of green solvent characteristics. Therefore, it was decided that anisole would be used as green solvent in the context of TRANSBIO to provide PHB for work package 5. Nevertheless, also enzymatic extraction showed a good performance. Based on these results the final TRANSBIO procedure for PHB extraction and purification could be established and was laid down in deliverable ***D4.2***. The fermentation protocol as well as the extraction procedure was used to provide PHB to work package 5. Therefore, ***milestone MS9*** – PHB fermentation protocol & Down-stream procedure decided could be achieved successfully.

Due to its versatility, plastics are present in almost all human life sectors, generally enhancing the quality of life. However, the durability of most of the petroleum derived plastics has raised much concern for the environment as the large amount of plastic debris dispersed in the biosphere becomes a troublesome inheritance. Therefore, much attention has been focused the last years on the production of biodegradable bacterial polyesters such as PHB. Therefore, work package 5 aimed in the preparation of PHB based biodegradable biomaterials using PHB produced from by-products from fruit and vegetable transforming industry in work package 4. ***Work package 5*** started its activities in month 33 with selecting appropriate ingredients for PHB film development. Additional, PHB polymers obtained from task 4.2 were characterized for molecular weight as well as thermal behavior. Furthermore, final PHB Biofilm formulation was selected and biofilms prepared via hot-pressing. The characterization of the biofilms (e.g. tensile strength, elongation at break) showed that formulation A is comparable with commercial available PHB polymers.

In order to prove biodegradability the TRANSBIO partners used the ISO standard ***ISO 14851:2005*** (Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium). The results show, that the biofilm A, which performed best in technical performance test trials reaches the necessary value of biodegradability - 90% absolute or relative biodegradation – after 96 days. The results were summarized in deliverable ***D5.1*** – Report on Biofilm formation and characterization and submitted to the commission. Therefore, ***work package 5 could be finalized successfully***; developing ***biodegradable PHB based biofilms*** using fruit and vegetable by-products as carbon source for fermentation processes. Finally the developed formulation was used to prepare biofilms for the last workshop as showcase for the participants.

Beside the biopolymer PHB, TRANSBIO focused also in the production of the platform chemical succinic acid. Succinic acid is produced by various microorganisms and is a key building block for a wide range of secondary chemicals and finds use in chemical, pharmaceutical, food and agricultural applications.

A main challenge to produce succinic acid via biological pathways is that the microorganisms that naturally produce succinic acid are often not able to tolerate large concentrations of the acid or its salt and either die or cease growth and active metabolism, making such organisms unsuitable for industrial use. In contrary to bacterial producers it is known that yeasts can release substantial amounts of succinate to the culture medium as end-products. *Saccharomyces cerevisiae* succinate production is mostly studied in the context of wine and liquor manufacturing (Arikawa et al, 1999). Mutants with elevated succinate production profiles are used to modify the taste of rice wine (Arikawa et al. 1999; Camarasa et al., 2003). The emergence of modern molecular genetics continuously extends the industrial importance of *Saccharomyces cerevisiae* beyond traditional fermentation products. Therefore, ***TRANSBIO*** aimed to perform a ***phenotypic screening*** in order to ***identify new robust non-conventional yeasts*** with the potential of producing succinic acid, tolerating large concentration of the acid to be economical feasible. Considering that the microbial flora associated with by-products from fruit and vegetable transforming industry is most adapted to the biotransformation of compounds that prevail in these kinds of residues, the main objective of **WP6** was to explore the genetic and phenotypic variability that nature has created and to select promising yeast strains for succinic acid production. Therefore the partner PROMIC provided several by-products to be used for metagenomic analysis.

From the different by-product fractions 692 yeasts could be obtained, 450 from the fruit by-products and 242 from the vegetables, revealing that the characteristics of the substrate (nutrients, pH, texture) had influence in the abundance and the diversity of yeast species that were recovered. These have been identified by molecular methods: culture-dependent molecular identification and culture-independent molecular identification – metagenomic analysis. In culture-dependent molecular identification, the ITS1-5.8S-ITS2 region was processed by PCR-RFLP, showing a wide variety of species. In term of metagenomic analysis, eight gDNA of microbiome were obtained, sequenced using ITS2 region present in ribosomal operon in fungi. The sequencing data was BLAST against SILVA database. The genera most frequently isolated were *Candida*, *Cryptococcus*, *Wickerhamomyces, Hanseniaspora, Pichia, Rhodotorula* and *Torulaspora.* A higher number of yeast isolates was recovered in fruit samples contrary to the vegetable samples, where more bacterial isolates were isolated. No yeast isolates were recovered from broccoli and cauliflower (edible stems). Considering only the yeast isolates, we observed that the number of yeasts recovered from each biowaste was dependent on the type of biowaste and the sample treatment performed. A total of forty-five different yeast species were identified. Candida tropicalis was isolated from nine different biowaste, followed by Pichia fermentans and P. kudriavzevii associated with eight and seven biowaste, respectively. However, the majority of the species, 28 species, were identified in only one type of biowaste. The metagenomic analysis of microbiome associated with by-products could be finalized successfully and the results were laid down in deliverable D6.1.

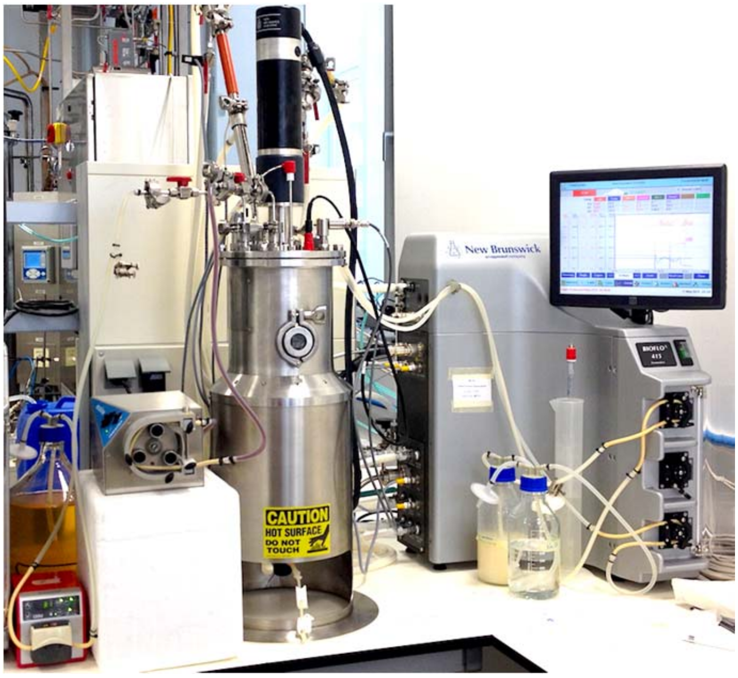
The obtained yeast strains were forwarded to task 6.2 and were used for the constitution of a bio-database of non-conventional yeast strains hosted by UMinho. All yeast isolates were individually stored in cryotubes containing 1.5 ml of glycerol (30%, v/v), at - 80ºC. They constitute a sub-collection that will be included in the Collection of Microorganisms of the Biology Department, University of Minho. The database contains information about the origin and identity of the cultures, as well as all the relevant available phenotypic characterization for each strain. The results were laid down in deliverable D6.2 –

Bio-database for non-conventional yeast strains. The TRANSBIO partners agreed already to further cooperate to exploit the huge potential of this strain collection for industrial biotechnology.

The evaluate the capacity of the isolated yeast strains to produce succinic acid and other value-added compounds, the strains were sent to the partner BIOTREND using its sophisticated high-throughput analysis of succinic acid production. Based on this analysis two non-conventionally yeast strains were selected for succinic acid production and fermentation protocol development in work package 7. The results of the phenotypic screening were summarized in deliverable D6.3.

Beside UMinho gathered within previous research projects, one of the largest bio-database of *Sacharomyces cerevisiae*. These strains were also screened for promising succinic acid producers and interstrain differences among *S. cerevisiae* regarding succinic acid production in different nutritional conditions were evaluated. A promising *S. cerevisiae* strain was identified as laid down in deliverable D6.4. With achieving these deliverables, ***milestone MS8*** could be achieved and therefore work package 6 finalized successfully in month 35. The selected yeast strains were forwarded to work package 7 for fermentation development.

Work package 7 aimed in the development of fermentation protocols for the selected yeast strains in work package 6 and to optimize succinic acid production. The most promising strains identified during WP6 have been challenged against the raw-material hydrolysates produced in WP2 in view of selecting raw-material / strain pairs that resulted in higher productivities and yields. These trials have been performed at micro scale in micro titre plates with enhanced mass transfer efficiency and in shaken flasks and the concentrations of carbon sources and organic acids measured to gather the required information for proper assessment of the most promising conditions to be implemented at higher scale. The results were laid down in deliverable D7.1. Following that, the fermentation processes have been optimized and intensified in fully controlled bioreactors for the implementation of a high-productivity process with high reproducibility and robustness. Fermentation protocol for lab-scale bioreactors has been developed (D7.2) and has been further optimized in a scalable fermentation protocol in pilot scale considering all necessary parameters (D7.3). At this scale it was possible to produce broth samples for the downstream processing activities of WP8. The optimized processes provided also technical data to be included in the feasibility assessment of WP14. By achieving all this results work package 7 could be finalized successfully.



One critical aspect in succinic acid production is the development of cost-effective down-stream procedures. Therefore, WP8 developed appropriate down-stream procedures for succinic acid recovery. The discussion with the fermentation experts of WP6/7 indicated that the formation of several additional metabolites (organic acids) would have a significant effect on down-stream processing and should be considered during fermentation design. Task 8.1 focused on the use of reactive liquid-liquid extraction.

Compared to other systems reactive liquid-liquid extraction is known for high recovery and selectivity as well as low energy. Effective reactive compounds for carboxylic acids as succinic acid are tertiary amines in an organic solvent. TRANSBIO has found trihexylamin in 1-n-Octanol to be more efficient then for example trioctylamin as described in many publications. In the first step the succinic acid forms a reversible amine-acid complex at the interphase. In a second step a back extraction compound is added in order to replace the succinic acid and push into a new water phase. Many trials were performed in order to find the optimal temperature, volume of new water phase, concentration of back extraction compound and time. Oleic acid was used as back extraction compound. After back-extraction succinic acid could be recovered from the new water phase by crystallization and used for further applications.
The final performance analysis of liquid-liquid extraction for succinic acid recovery was laid down in deliverable D8.1. Another interesting cleaning strategy for succinic acid represents the use of electrodialysis, which uses membranes to separate ionic molecules from non-ionic species.

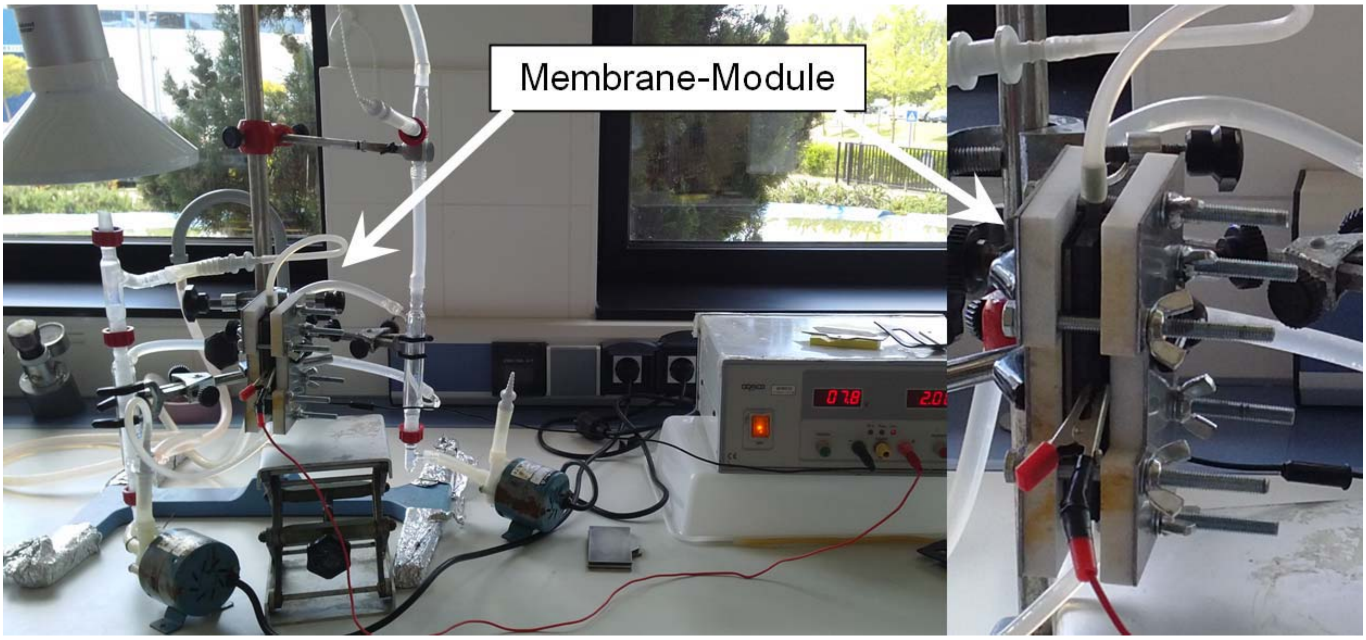


Fig: Lab-scale Electrodialysis experiments in Tecnalia

Electrodialysis showed best results with a combination of bipolar membranes (BPM) with anion exchange membranes (AEM). Based on these results deliverable ***D8.2*** – Evaluation of electrodialysis performance could be prepared. In discussion with WP7 the final down-stream processing was decided and laid down in deliverable ***D8.3***. Using the down-stream processing approach, succinic acid could be recovered. Therefore, also milestone ***MS11*** could be achieved successfully.

The third valorisation chain used filamentous fungi, grown in solid state fermentation to obtain enzymes (lipase/protease). Enzyme production is a growing field of biotechnology. Most enzyme producer use submerged fermentation (SmF). However, Solid State Fermentation offers several advantages over SmF. Therefore the aim of work package 9 was the screening for protease and lipase producing fungi applicable in SSF and the implementation of a lab-scale SSF process. One important aspect was that the enzymes should be applicable in detergent formulations. A first screening procedure provided 175 fungal strains including 65 thermo stable strains. These strains were used for the screening of proteolytic fungal strains. Nine proteolytic strains were isolated and tested for qualitative enzymatic activity. Additional 14 lipolytic could be selected from the thermo stable strains.

The aim was to select at least three protease producing fungis. That could be successfully achieved by identifying ***five GRAS protease producing fungi strains***: *Y. lipolytica R. variabilis SGE39A, alliaceus/lanosus, A. niger, P. polonicus 188Pv.* Additional, ***four pathogenic fungi*** (*A. fumigatus, A. flavus MIAE0129, A. flavus MIAE01259*) could be identified but will not be considered for further implementation. All the strains showed protease enzyme activity under laundry detergent components. The results were laid down in deliverable D9.1 – Selection Protocol for three protease producing fungi. Furthermore, ***two GRAS lipase producing fungis*** *P. commune*, *Y. lipolytic* could be identified as well as one pathogenic strain, *A. fumigates*. Only the two GRAS strains will be considered for further implementation in WP10. All the strains showed lipase enzyme activity under laundry detergent components. The results were summarized in deliverable D9.2 – Selection Protocol for two lipase producing fungi. Additional, the partners implemented the SSF lab-scale fermentation protocol (***D9.3***).
There are different reactors configurations for the SSF process; however the furthermost used is the Tray-Bioreactor, due to the advantages for both operational and cost-effective reasons. Additionally, it offers reliable providing which makes it an ideal to evaluate the geometric design, operational parameters (*e.i.* temperature, pH, substrate, moisture and oxygen); and instrumentation.

Fig: Solid State fermentation – Substrate ready for fermentation

Modeling is a valuable engineering tool for the scaling-up and down processing; it helps in the understanding of the complex interaction between the different transport phenomena and the reaction taking place on the microorganism, allowing the process design and optimization. In WP9 the modeling of the enzyme production in a Tray-Reactor at lab scale was developed. The Tray-Reactor was simulated by using COMSOL Multiphysics 4.4. This study was aimed to understand the interaction of momentum, heat and mass transports with microorganism kinetics. In this document, the analysis of the interaction of transport mechanisms in absence of reaction was carried out. The hydrodynamic local behavior in fluid phase and solid phase were described by the Navier-Stokes and Navier-Stokes-Darcy equations, respectively (Eq 1-6). Besides, heat and mas transfer equations, accounting for convective and dispersive mechanisms, were used to describe the local temperature and concentration in the studied system. These transport equations were the averaged ones and made use of effective transport parameters, dispersion coefficient, interfacial heat and mass transport parameters, wall heat transfer coefficient, and effective thermal conductivity, among others. These transport parameters were obtained from literature. The response variables were temperature, the oxygen, water, and carbon dioxide concentration in and out the reactor. In reaction experiments biomass growth during the reaction was the response variable.

Fig.：Lab-scale tray-bioreactor

Following the simulations, fermentation parameters such as bed height, bed mass, humidity, air flow as well as empry space (bed) were obtained. This information was transferred to reaction system to produce a enzymatic extract by SSF in the Tray -Bioreactor using *Yarrowia lipolytica*. Fruit and vegetable by-products were used as support/substrate for enzymes production. Afterwards, the results were scaled up to 50 kg scale tray reactor

Fig.: Preparation of trays for 50 kg Solid State Fermentation (NST)

The solid state fermentation lab-scale protocol was compiled in deliverable D9.3. As the filamentous fungis are available for WP10 implementation and the lab-scale fermentation protocols are established also milestone ***MS6 could be achieved successfully***.

The main aim of work package 10 was to transfer the results obtained in task 9.2 Development of lab-scale SSF process for selected molds, in technical scale up to 500 kg using a drum solid state bioreactor. Additional, the enzyme recovery procedure from solid state substrate was implemented.
The scale-up up to a 500 kg batch was successfully accomplished including resulting in an even higher product yield and enzyme activity compared to the small scale trial in WP 9. Additionally, some technical hurdles at the large batch size could be identified. The technical problems within the fermentation were solved by additional technical measures (improved temperature & humidity control). The final protocol was concluded in deliverable D10.1 – SSF Technical Scale Performance Report.

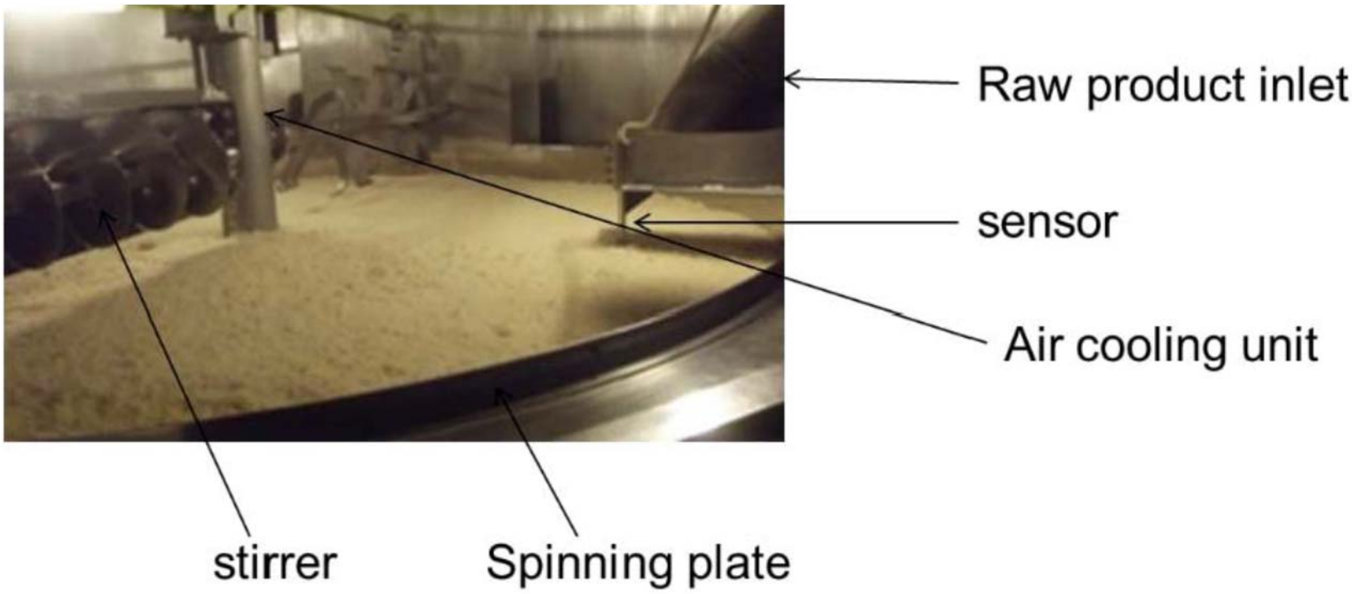


Fig.: Drum SSF Reactor - inside

The obtained ferment was forwarded to task 10.2. The aim of task 10.2 was the development of cost-effective recovery and purification strategies for proteases and lipases produced in solid state fermentation for further detergent application.

The obtained enzymatic extracts were forwarded to work package 11 for detergent development. The adaption of the recovery methodology could be finalized successfully and were laid down in deliverable ***D10.2***. Further purification methodology is described in deliverable ***D10.3***. Based on the results obtained, and the possible up-scaling potential, it was concluded that already the recovery strategy with low water content (hydration of SSF ferment) developed in sub-task 10.2.1 and laid down in D10.2 would provide an adequate enzyme product in sufficient quantity consisting of lipase and protease to be used for detergent development in WP11.

By achieving these results/deliverables, work package 10 could be finalized successfully and ***milestone 10*** could be achieved – SSF established in technical Scale.

The main aim of ***work package 11*** was the development of detergent formulations containing lipases and proteases developed and produced in work package 9 and 10.**The work package started its activities in month 22** with task 11.1 developments of enzyme-based detergents. The partners started their activities with test design as well as performance indicator were agreed. Based on the results obtained in WP9 about enzyme activity in detergents the partners screened literature for detergent components to perform component specific test trials. These test trials could be finalized successfully and resulted in deliverable ***D11.1*** – Enzyme-based Detergent Development. Additional, enzymes are not only degraded by chemicals in the detergent but also by proteases, which attack protein structure of lipases. Therefore, the partners started with the identification of possible protection procedures such as microencapsulation. Task 11.2 aimed in the further protection of enzymes using microencapsulation, analyzed several possibilities. The final results were laid down in deliverable ***D11.2*** – Enzyme stabilization Protocol. Using enzymes provided by WP10 and further processed using results of WP11, were tested in detergent applications for household laundry. It could be shown that the TRANSBIO formulations using enzymes obtained via Solid State Fermentation in WP10 provided similar cleaning properties than commercial detergents. Therefore, also deliverable ***D11.3*** – Performance Evaluation of Household Laundry as well as milestone ***MS13*** – Enzymes recovered and tested in household laundry could be achieved successfully.

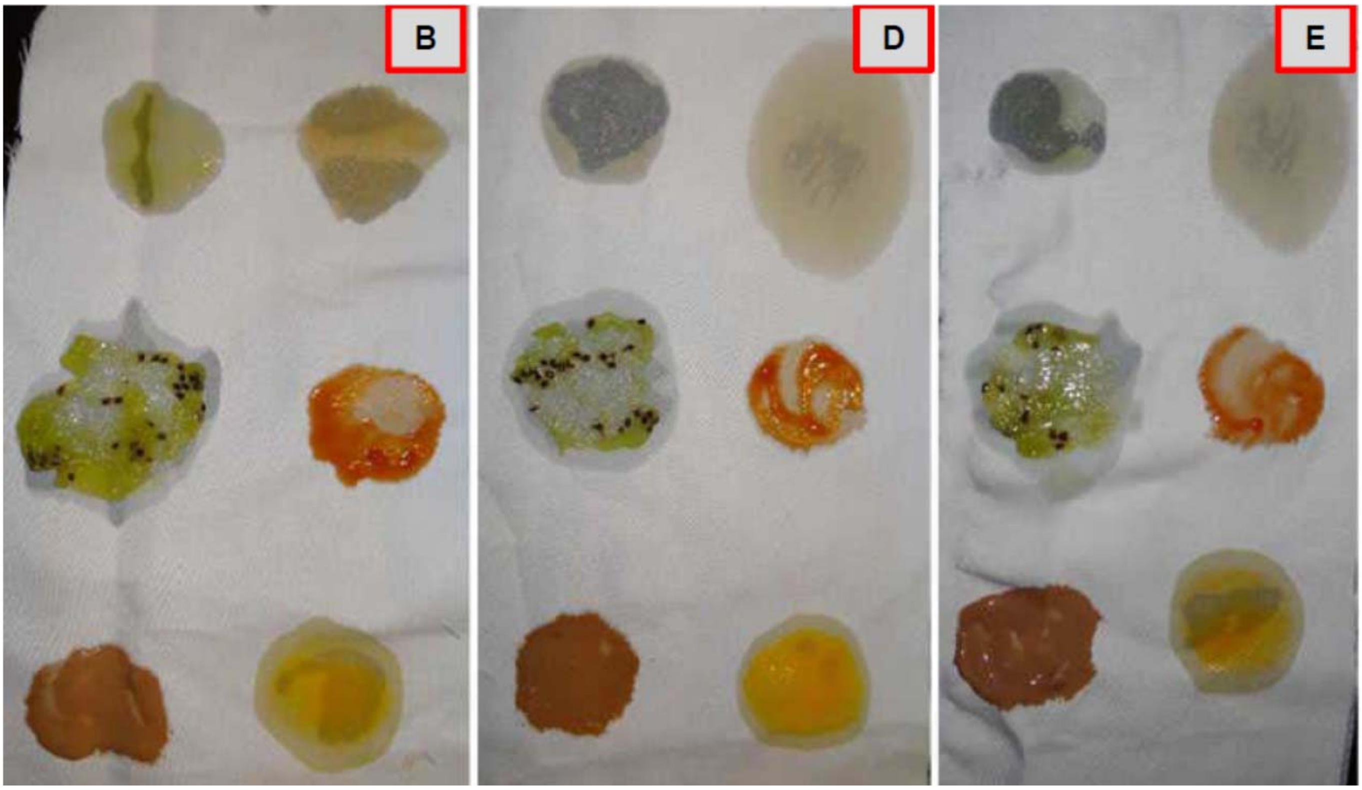


Fig: Stained cloths for 40ºC test (B: without detergent, D: Commercial detergent, E: TRANSBIO)

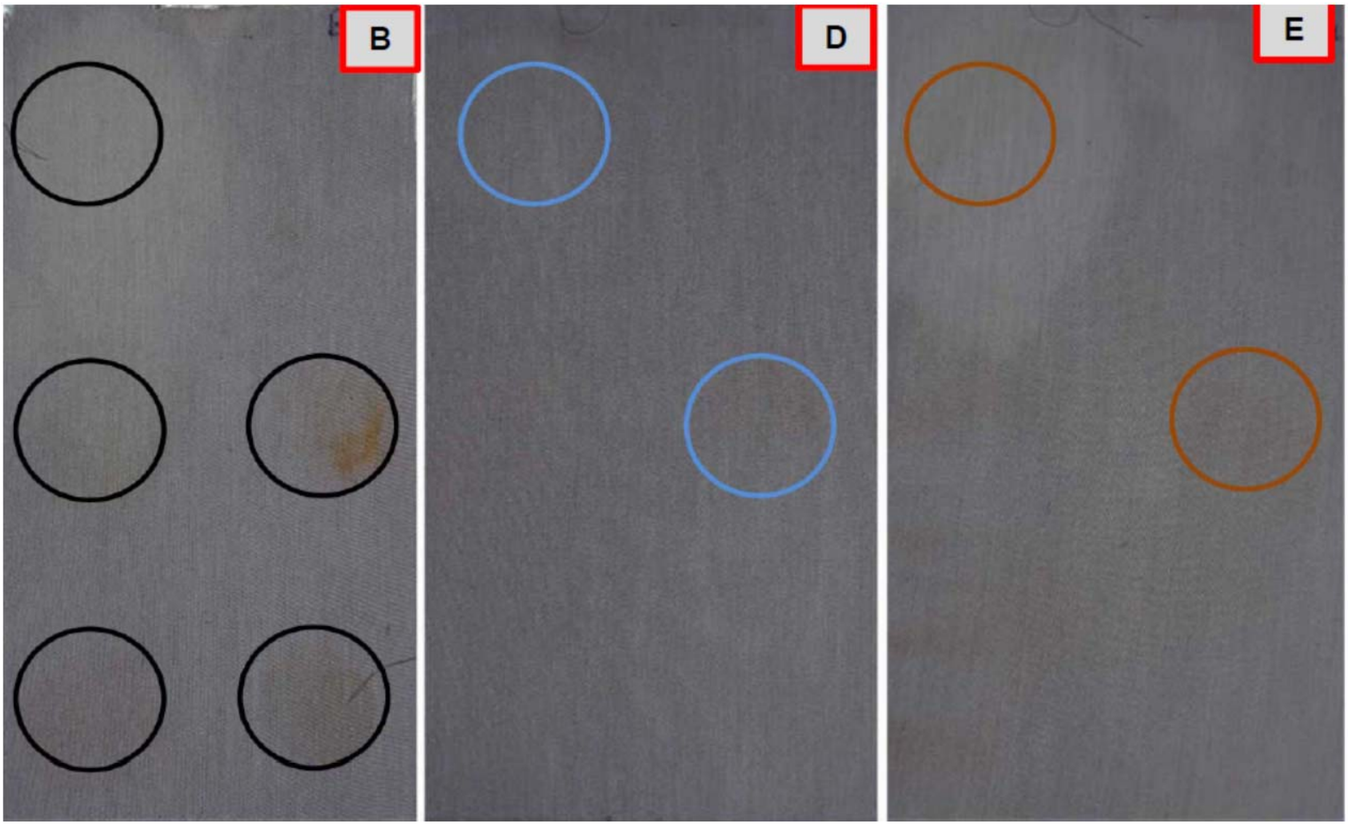


Fig.: Test cloths after washing at 40ºC (B: without detergent, D: Commercial detergent, E: TRANSBIO)

In order to close the cycle and to follow the approach of a circular economy remaining biomass was analyzed for its potential to be used for biogas production. The obtained digestate were characterized for its potential to be used as fertilizer. Work package 12 tested by-products remaining after bio-product extraction to be used as feedstock for biogas production and examining their effect on process stability and effectivity. In order to set up the base line all selected by-products were used for biogas production and methane yield obtained was compared. All tested by-products (broccoli, cardoon, green bean, lettuce, potato, sweet corn, and banana) showed a good potential for use as (co-)substrate in anaerobic digestion. To evaluate the effect of hydrolysis, fermentation and bio-product extraction, the biogas potential was again determined on the solid fraction that remained after bio-product extraction.
In a second phase, full-scale anaerobic digestion of sweet corn and potato pulp after hydrolysis and bio-product extraction was simulated in a lab-scale environment. The goal of these tests is to determine the maximum loading rate and the boundary conditions within which a stable process can be maintained.

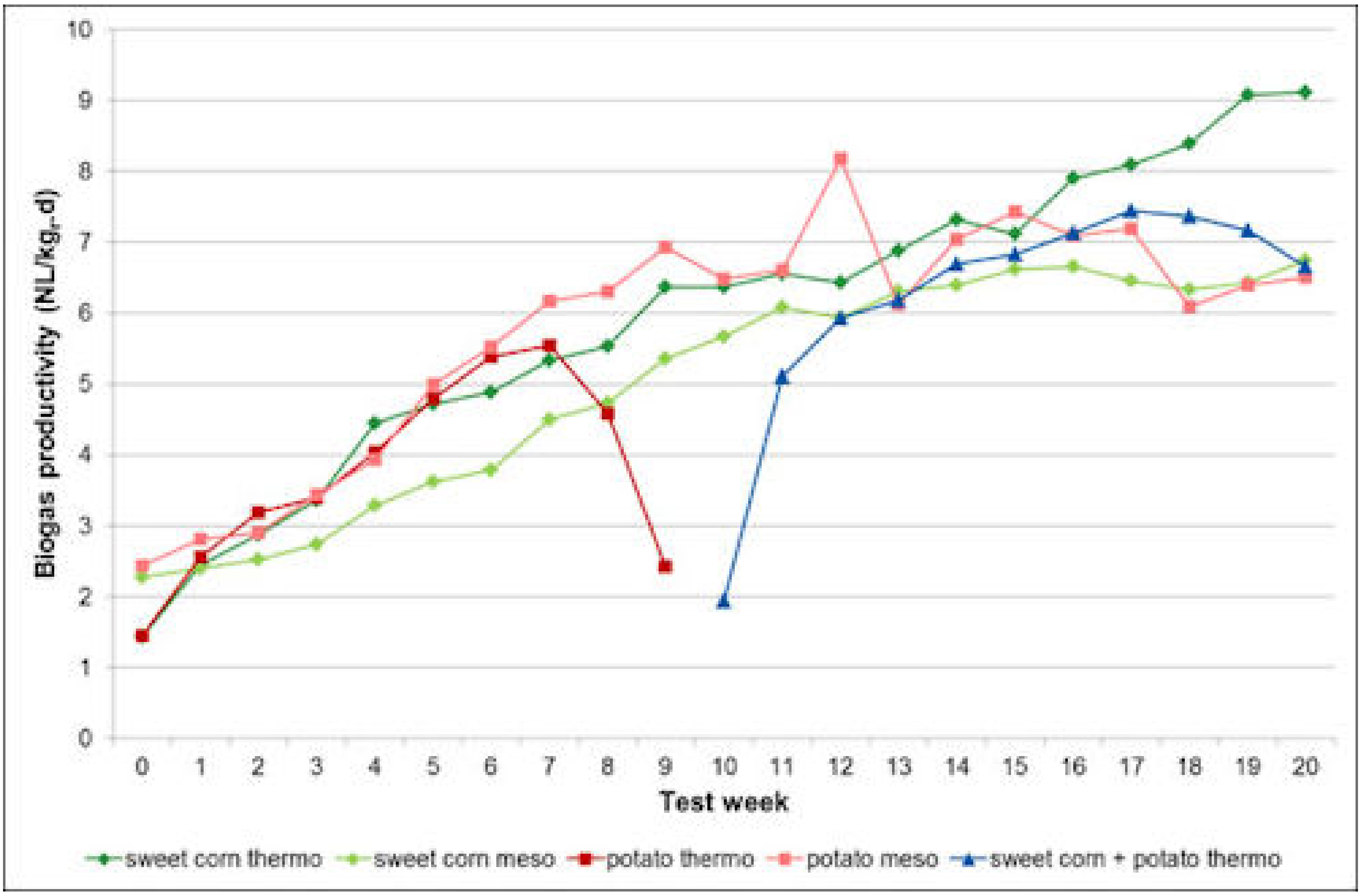


Fig: Evolution of the biogas productivity of the anaerobic digestion test reactors

Anaerobic mono-digestion of sweet corn residuals after bio-product extraction resulted in a stable process, both at mesophilic (37°C) and thermophilic (52°C) temperature. The maximum loading rate was higher in the thermophilic reactor (9 NL/kgr/d) than in the mesophilic reactor (6.5 NL/kgr/d) because of the higher biochemical reaction speed at elevated temperatures.
Anaerobic mono-digestion of potato pulp residuals after bio-product extraction resulted quickly in reactor failure at thermophilic temperature, whereas a stable process could be obtained at mesophilic temperature with a maximum loading rate of 6,5 NL/kgr/d (comparable to sweet corn digestion). The instability at higher temperatures was caused by elevated levels of K in the potato pulp after bio-product extraction, whereas mesophilic bacteria have a higher resistance to K-toxicity.

Thermophilic co-digestion of sweet corn and potato pulp (50/50) after bio-product extraction was tested in a 5th test reactor to evaluate if similar loading rates could be obtained as with thermophilic mono-digestion of sweet corn after bio-product extraction. After 10 test weeks, it seems that a stable fermentation can be maintained, although the slightly elevated K-levels prevent from obtaining a loading rate higher than 6,5 NL/kgr/d. It can therefore be concluded that when treating potato pulp (alone or in combination with sweet corn) after bio-product extraction, mesophilic digestion is the best option (more stable process), whereas with sweet corn after bio-product extraction, thermophilic digestion yields the best results. The methane content in the produced biogas varied around 55%, which is high and indicates a good energetic potential.

To help evaluate the suitability of anaerobic digestion for residual organics of vegetable waste after bio-product extraction, a theoretical model was built using the test results obtained within the TRANSBIO project (D12.1 – Anaerobic Digestion Performance Model). This model can predict the expected biogas potential of vegetable waste using the carbohydrates, fat and protein content of the product in question. The model was able to predict the biogas potential with a maximum error of 10%. This model can help in screening the possibilities to apply anaerobic digestion on vegetable waste that was not tested in the TRANSBIO project, although it is advisable to still do some lab tests on the most promising substrates, as the model does not give information about possible inhibitors (e.g. the K-content in potato pulp).
Furthermore, the biogas obtained during the different test trials were analyzed for major components to CH_4, CO_2, H_2S, O_2, N_2, H_2 and NH_4. The analysis of micro-components was carried out by gas chromatography-mass spectrometry system using a Varian technology 3800 GC equipment with a selective Ion trap detector, Saturn A ZB -5MS (30x0.25 I.D), 0.25µm film thickness (Zebron Phenomenex, USA), chromatography column was used. The gas chromatography conditions cover whole m/z range with a final oven temperature of 300ºC. Ion source and transfer line was set at 200ºC and 280ºC respectively. The mass spectrometer was operated in the full scan mode from 40 to 650 m/z and a special gas srynge was used for the gas sample inyection into the system.

Fig: GC/MS/MS equipment

The analysis results were reported in deliverable ***D12.2*** – Biogas evaluation. Finally, digestate obtained during biogas test trials were forwarded to task 12.3 to be analyzed. The residual specification (***D12.3***) showed good potential of the digestate to be used as fertilizer. As an overall conclusion for **WP12**, it can be stated that anaerobic digestion is a promising technology to valorize vegetable waste after bio-product extraction. The energy (biogas) that is produced during anaerobic digestion can be used in the processes for PHB, succinic acid and/or enzymes production, improving the overall sustainability of this novel process chain. The remaining, stabilized organic matter that remains after anaerobic digestion (digestate) can be applied in agriculture as an alternative for chemical fertilizers, thus recycling valuable nutrients back to the soil. Therefore, also milestone ***MS12*** – Suitability of TRANSBIO biomass for biogas production evaluated could be achieved.

Work package 13 aimed in the implementation of the TRANSBIO approaches in pilot scale. The WP started in month 39. The partner PROMIC provided necessary by-products to be used as carbon source for fermentation. Afterwards the partner Bioesplora with the support of CNTA and Biotrend performed pilot fermentations and provided PHB for biofilm processing. The obtained biofilms were used in the showcase of the fourth TRANSBIO workshop in Vitoria (Spain).

Beside partner Biotrend supported by UMinho and Biozoon established succinic acid production based on potato hydrolysate.

The partner NST, scaled-up the enzyme production in solid-state fermentation up to 1.600 kg scale. The results are very promising, therefore the partner is already implementing further research & exploitation activities.

Fig.: PHB biofilms & encapsulated enzymes at 4th TRANSBIO workshop – show case

Fig: Picture of NST's two-stage SSF plant front and backside

In order to develop sustainable processes and products, TRANSBIO is performing, in parallel to the process developments, environmental and economic impact analysis in ***WP14***. Based on results obtained in the first period, the partners could finalise an Initial Situation Analysis which were laid down in ***deliverable D14.1***. Afterwards, the partners under the lead of OWS performed an Impact Assessment of the novel TRANSBIO approaches. It could be shown that enzyme production in SSF performs ***environmentally competitive***. Furthermore, the PHB production pathway ***could be made environmentally competitive if further considerations were implemented***, especially avoiding the drying of the potato by-products and establishing a just in time processing and fermentation as well as use of other heat sources throughout the chain. This could be achieved e.g. by using waste heat or solar heat. Due to low yield of the natural yeast strain the succinic acid production pathway ***was unable to compete with the reference products*** in terms of greenhouse gas emissions. Nevertheless, the partner BIOTREND, an expert in fermentation approaches concluded already that the natural yeast strain has high potential if further genetically modified. Therefore, already further research opportunities especially with the partner UMinho are analysed to perform the necessary genetic modifications. The LCA analysis was laid down in deliverable ***D14.2***. Additional, an economic feasibility study was performed, showing huge potential for enzyme production as well as PHB formation. Also, succinic acid formation using a genetic modified strain obtained in TRANSBIO forecasts economic feasibility. The economic Feasibility study was laid down in deliverable D14.3. By analysing the environmental and economic feasibility of the three biotechnological approaches also milestone ***MS14*** could be achieved successfully.

Currently, respective SME partners develop their further exploitation strategies for yeast strains for succinic acid production, genetic modified *E. coli* for PHB formation as well as enzyme production using Solid State Fermentation.

Description of the potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results

In a world where demand and competition for finite and often scarce resources will continue to increase, and pressure on resources is causing greater environmental degradation and fragility, Europe can benefit economically and environmentally from making better use of those resources. Therefore, a **circular economy** system which keeps the added value in products as long as possible and eliminates waste must be implemented (COM(2014) 398 final/2). In this context TRANSBIO aims at the utilization of by-products from fruit and vegetable transforming industry, in order to reduce the environmental impact of food waste, reducing landfill and lessening noxious residues and odours. Further, the project targets to enhance the sustainable management of biomass from fruit and vegetable processing industry, demonstrating its potential to be used as feedstock for bio-based products like PHB, succinic acid and enzymes for the detergent industry. This will improve the overall sustainability of the biomass processing industry and will increase the competitiveness of European biotechnology industry through new applications. Therefore the project is fully in-line with the lead market initiative for bio-based products (COM(2007)860 final); the Strategy on life sciences and biotechnology (COM(2002)27 final; SEC(2007)441) by development of cleaner industrial products and processes based on use of enzymes and microorganism (biocatalysis) as well as the Initiative for developing a coherent European strategy for key enabling technologies (COM(2009)512) supporting applications for industrial biotechnology (fermentation of microorganisms, enzyme application).

Currently a number of factors limit the application for bio-based products. Due to demand for bio-based products has not been sufficient to realize economies of scale in the production, biobased products have in many cases higher manufacturing costs. Therefore economic feasibility was one key question investigated during the technical development. The results have been up-scaled up-to pilot scale to obtain meaningful information about the technical and economic performance. Therefore TRANSBIO will increase competitiveness of fermentation industry by providing low costs substrates for fermentation procedures using new, cost-effective enzymatic hydrolysis strategies. The biopolymer production sector will take advantage due to improved bacterial fermentation strategy to obtain PHB and therefore be more competitive regarding petroleum-derived polymers.

Impact on overall sustainability of the biomass processing industry

The continuous use of petroleum sourced raw materials as industrial feedstock is nowadays widely recognized as a major barrier towards sustainable development for reasons concerning the environment, the security of supply and as well as of depleting exhaustible raw materials. By contrast, the production of chemicals and industrial products from renewable raw materials has recently received attention.

Major factors driving future markets and demand for bio-based materials in the European Union are the limited availability and increased costs of fossil resources, policy development due to climate change as well as employment growth. Another important issue represents the consumer demand based on the awareness of the need to ensure sustainable production and consumption.

In principle, any carbon source can be used as substrate, including lignocelluloses from agriculture and forests. However sustainability criteria must be applied by selection of suitable biomass sources. Especially as current strategies consider engineering crops for biofuels, the combustion of crops, the modification of lignocellulosic materials, as well as the exploitation, development and adaptation of novel energy crops and other plant organisms with particular prospects in energetic and industrial applications in the sense of a holistic biorefinery concept. But for bio-cropping systems and the use of lignocellulosic materials, the ***increasing competition for land use between food, industrial products and fuel production*** and the additional pressure on forest use and reforestation has to be taken into account

(WWF, 2006, FAO, 2000). If the demand for industrial products and energy from biomass continues to grow, this will inevitably lead to an expansion of global arable land at the expense of natural ecosystems. All over the planet land is converted to human use, primarily to agricultural land. Currently around 12% of the global land surface is under crop cultivation (Foley et al., 2005, Ramankutty et al., 2008). In order to stay in the planetary boundaries, Rockström et al (2009) propose that no more than 15% of the global ice-free land surface should be converted to cropland. According to the land cover statistics of Eurostat in 2012 more than 24.5% of the total area was already used as cropland in EU-27. This transformation of forests, grasslands, wetlands and other vegetation types to agricultural land is the driving force behind the serious reductions in biodiversity, impacts in water flows as well as biogeochemical cycling of carbon, nitrogen, phosphorous and other important elements. Therefore, ***feedstocks*** must be produced in an ***environmental sound manner***. Greater use of lower-cost feedstocks (i.e. agricultural and ***processing residues***) must be pursued (Biomass Research and Development Technical Advisory Committee, 2007).

In this context, ***by-products*** from fruit and vegetable processing industry must be considered as substrate for fermentative production of biobased products like biopolymer PHB, succinic acid or biocatalysts (enzymes). Fruit and vegetable biomass is renewable, CO2 neutral and has the potential to deliver an array of raw materials for industry in place of mainly petrochemical sources. By employing physical, chemical and biochemical processes, fruit and vegetable biomass can be converted into different bio-based products like chemical intermediates, polymers and catalysts. The principle components of biomass are cellulose (30-50%), hemicellulose (20-30%) and lignin (20-30%); with starch, protein and oils as minor components. Exact compositions of each biomass vary depending both on the plant and on the byproduct collected. The composition, in turn, determines the ease with which the biomass can be converted to useful products and/or intermediates and affects the functionality of the final product. A key barrier is the lack of knowledge on how to deal with natural differences in fruit and vegetable by-products components and characteristics from one plant to the next within the same species for feedstock considerations. Therefore, TRANSBIO evaluated and characterized extensive by-product samples from fruit and vegetable processing industry provided by PROMIC, to identify a select the most appropriate by-products versus valorization strategy.

According to the European project AWARENET, approx. 192 million tonnes of waste and by-products from fruit and vegetable processing industry are produced every year in EU-15. The ***improved exploitation of*** **such** ***by-products*** will have a number of benefits, as it will ***reduce the environmental impact of food waste***, ***reducing landfill***, ***lessening noxious residues*** and ***odors***. It will ***enhance*** the ***sustainable management*** of ***organic matter*** from food production and processing as it can be used as feedstock for bio-based materials. Additional TRANSBIO will contribute to reduction of pressure on arable land by enlarging the biomass base for bio-based products.

Impact on Competitiveness of European Biotechnology Industry

Biotechnology is generally considered one of the key technologies of the 21st century, with a potentially wide range of applications for several kinds of biobased products like biopolymers (PHB), platform chemicals for chemical industry (succinic acid) or new biocatalysts (enzymes). Of growing importance within the broad area of biotechnology are industrial / white biotechnology applications. Industrial biotechnology as a horizontal enabling technology is used in several manufacturing sub-sectors. TRANSBIO mainly supported the NACE DG 24 subsector (Manufacture of chemicals, chemical products and man-made fibers) by developing novel biotechnological approaches for transforming by-products from fruit and vegetable processing industry into value-added products (biopolymer PHB, platform chemical succinic acid, biocatalysts enzymes for detergent application). According to “The Bio4EU Analysis report” (2008) this category not only contains the enzyme and chemical producers, but also

some of the most important users of enzymes (detergents, polymers). Currently a number of factors limit the application for bio-based products. Due to demand for bio-based products has not been sufficient to realize economies of scale in the production, biobased products have in many cases higher manufacturing costs. Therefore economic feasibility was one key question investigated during the technical development in TRANSBIO. The implementation of TRANSBIO results will increase competitiveness of fermentation industry by providing low costs substrates for fermentation procedures using new, cost-effective enzymatic hydrolysis strategies for by-products from fruit and vegetable transforming industry. The biopolymer production sector will take advantage due to improved bacterial fermentation strategy to obtain PHB and therefore be more competitive regarding petroleum-derived polymers. Currently two patent applications are planned in this context.
According to van den Tweel (2010) the current global market for succinic acid is around 35,000 tons and will reach in 2015 around 144,700 tons (Global Industry Experts). In 2020, the global bio based succinic acid market is expected to reach a market volume of 710,000 tons (AlliedMarketResearch, 2014).
By developing a new cost-effective fermentation strategy based on low cost substrates using new non-conventional yeast strains, TRANSBIO will help the European Biotechnology Industry to participate on this growing market.
The market for detergent enzymes grew in the EU and Japan by approximately 4,5% per year (ETEPS, 2006). This exceeds the growth of the EU economy, which was between 1% to 2% in recent years. The world's leading detergent enzyme producing companies are European Nowadays the production of enzymes takes mainly places in submerged culture. By developing a novel strategy using Solid State Fermentation, the current market lead of European enzyme producing companies can be increased. Additional, the development of the detergent enzyme market depends on the market for cleaning products. Looking more detailed in the growth rates, shows, that the use of enzymes as detergents has a high and growing potential. According to the analysis report related to modern biotechnology (IPTS, 2007) the growth rate of the soap, detergents, cleaning and polishing sector was only moderate approx. 1,17% between 2001 and 2003. The growth rate of detergent enzymes of 4,5% per year in the same period indicates a growing share on detergents containing enzymes. These growth rates increased significantly in the last years. Maximum growth is estimated to be recorded in the detergent enzyme segment with a CAGR of 11.3% during 2013-2018 (BCCResearch, 2014). Therefore, the SME partner NST is currently implementing based on the TRANSBIO results, its own strategy for enzyme production using solid state fermentation.

SME-relevant research

The participation in TRANSBIO raised the knowledge and training level of the participating ***10 SME partners***, as they had the opportunity to co-operate with some of the European and Latin American leading research organizations in the concerned areas. Additional they got used to work at an international level (cooperation between Europe & Latin America). These effects support the ***European Commission's horizontal SME policy***, as laid down in the ***White Paper on Growth, Competitiveness and Employment.*** The results of the project will improve the competitiveness of the participating SMEs primarily through the exploitation of new products/knowledge. There are several exploitable developments in the TRANSBIO project in terms of novel products and technologies. Applying the developed products in model systems will validate the process technology and technological functionality of the integrated biorefinery approach of TRANSBIO.
For the SMEs within the consortium, the project improved their general competitiveness as well as provided new business opportunities through process and product development. Furthermore, the SMEs are well established and have a European and global customer base through which they intend to exploit the results of this project. The dissemination work addressed potential end users (biopolymer

processors, chemical industry using succinic acid as platform chemical, detergent industry and users of detergents, biogas producer) outside the consortium, but also the public throughout the European Union. The consortium is convinced that the new technologies and novel renewable industrial products will be successful on the market.

Cross Thematic approaches

In order to implement sustainable fermentation strategies based on biomass from fruit and vegetable transforming industry, a cross sectorial approach is necessary to achieve the breakthroughs needed for the longer term. Therefore TRANSBIO pooled together competences and resources in the domain of biotechnologies, chemistry, enzymology and materials like biologists for strain selection, bio process engineers for optimization of biomass production, chemists and chemical engineers for extraction and valorization of biomaterial PHB, platform chemical succinic acid an enzymes, sustainability experts as well as economists for Life Cycle Assessment and marketing experts in order to reduce the time to market.

Social impact

The use of modern biotechnologies is poised to create “better jobs”. More highly qualified employment like engineers, biologists, molecular biologists, biochemists etc. also potentially contributes to higher labour productivity via efficiency gains. Additional, biomass must be used regionally, as transport costs are affecting final product price, therefore creating new job opportunities in rural areas will be one more of the benefits of TRANSBIO.

Environmental impact

The overall impact of modern biotechnology on environmental sustainability is difficult to quantify in absolute terms. It differs widely between the individual application areas. However, the fact, that biotechnology approaches (fermentation or enzyme application) lead, in general, to improvements in the eco-efficiency of industrial applications, while being themselves a new source of economic activity, strengthen its role in assisting in the decoupling between economic growth and environmental pressures. For the SMEs within the consortium, the project has the potential to improve their general competitiveness as well as providing new business opportunities through process and product development. Furthermore, the SMEs are well established and have a European and global customer base through which they intend to exploit the results of this project.

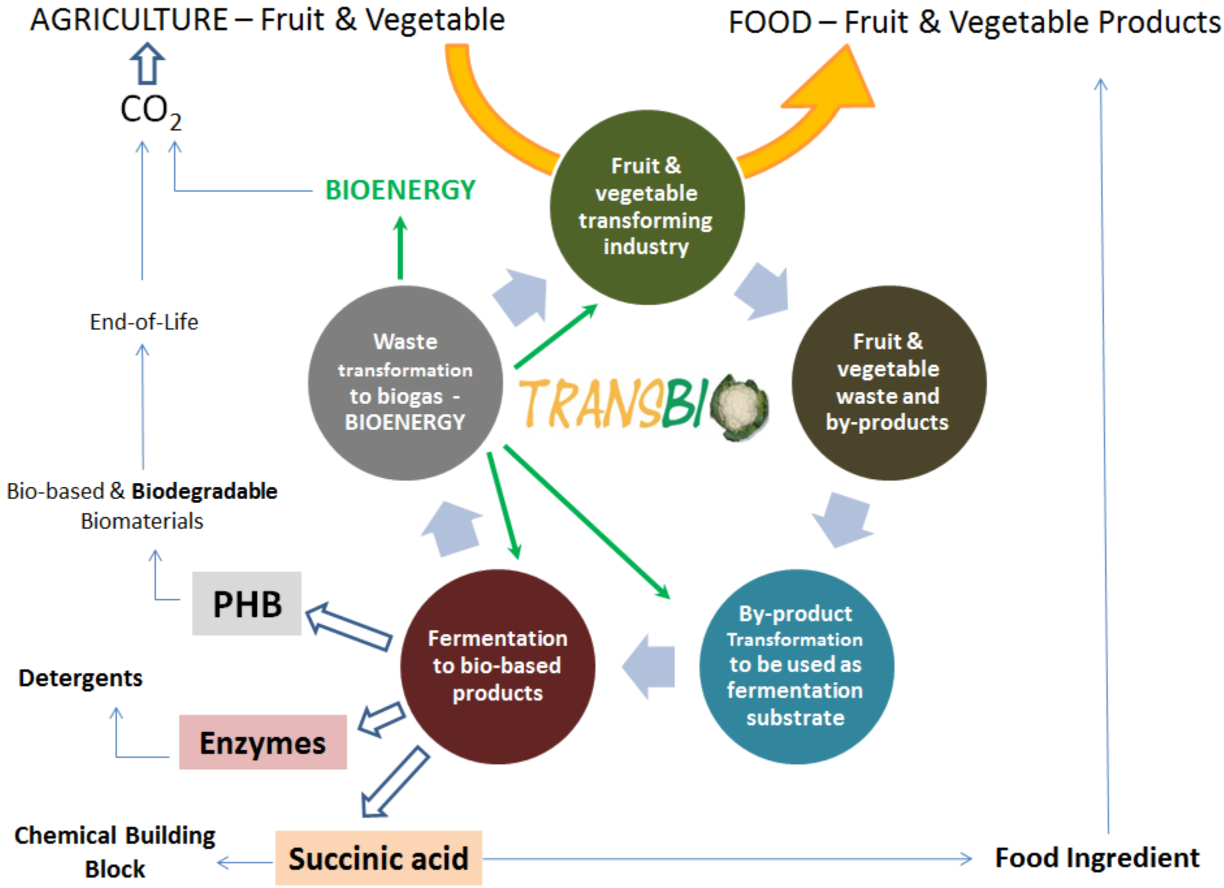
Contribution to European policies and initiatives

The successful implementation of the research objectives foreseen in TRANSBIO will support several European policies and initiatives like:

- the ***Lisbon Strategy*** by creating more and better jobs. In its communication COM(2005)24, the Commission advocated refocusing the Lisbon agenda on actions that promote jobs and growth that is fully consistent with the objective of sustainable development. One objective represents competitiveness through productivity growth.
- the ***Biomass Action Plan*** through cutting Europe’s dependence on fossil fuel (providing new feedstock for biomaterials, succinic acid and enzymes for detergent application), cutting greenhouse gas emissions (saving energy in industrial processing) and through stimulation of new economic activities (novel and robust biocatalyst for several industrial applications).
- the ***Common Agricultural Policy*** by contributing to sustainable development with the need to preserve the natural environment (enzymes for hard-to-degrade waste materials which would be

land filled, reduced input of chemicals used in industrial processes in the natural environment) and to Protect and to improve natural resource (enzymes for efficient use of available resources – efficient polysaccharide degradation).

- the programme "***Towards a circular economy: A zero waste programme for Europe***" by re-directing and reuse of by-products from fruit and vegetable transforming industry into biotechnological production of biodegradable biopolymers, platform chemical and enzymes, closing cycle between primary production of fruit and vegetables and industrial products.



- the ***Energy & Climate Package*** by reduction of greenhouse gas emissions (energy savings in industrial processes by enzyme and fermentative applications) and the efficient production of renewable energy / biogas.
- the ***SET plan*** "...Make second generation biofuels competitive alternatives to fossil fuels, while respecting the sustainability of their production..." due to a holistic biorefinery concept, using by-products from fruit and vegetable processing industry as feedstock for bio-based value added products.
- the ***Lead Market Initiative*** in the field of bio-based products and renewable energy taking into account a demand driven approach.
- the ***Directive 1999/31/EC*** on the landfill of waste requiring the reduction of biodegradable waste disposed to landfill, in order to reduce methane emissions, which are estimated to have more than 20-times the climate change effect of carbon dioxide.
- the ***Directive 96/61/EC*** (IPPC-Directive) concerning integrated pollution prevention and control set out measures to prevent, reduce and eliminate pollution at the source of the pollutant (e.g. biowaste from fruit and vegetable processing industry) and to ensure sensible management of natural resources (biogas production from available biomass resources).

- the ***Strategy on life sciences and biotechnology*** (COM(2002)27 final; SEC(2007)441) by development of cleaner industrial products and processes based on use of enzymes and fermentative processes (biocatalysis).
- the ***Initiative for developing a coherent European strategy for key enabling technologies*** (COM(2009)512) supporting applications for industrial biotechnology. Key enabling technologies (KETs) are knowledge intensive and associated with high R&D intensity, rapid innovation cycles, high capital expenditures and highly-skilled employment. They are multidisciplinary, cutting across many technology areas with a trend towards convergence and integration (Communication from the Commission, COM(2009)512/3; SEC(2009)1257). Industrial biotechnology was identified by several member states and the EC which will be relevant for future competitiveness and prosperity especially in relation to "greening" of the chemical industry by replacing conventional chemical processes by enzyme-based approaches.

Main dissemination activities

The success of the TRANSBIO project relied in the development and implementation of widespread dissemination of objectives and results, also facilitating the later exploitation of project developments. All project partners were well aware of the importance of the widespread of the gained knowledge of the holistic Biorefinery and Cascading Concept based on by-products from fruit and vegetable processing industry to interested stakeholders (research and expert institutes, technology developers, industry managers) as well as policy makers, as such all of them supported the partner TTC, leader of the Dissemination work package in achieving this goal.

Along the 4 years of the project, the partners have been carrying out different dissemination activities for the products obtained in the development of novel biotechnological approaches such as:

- Establishment of TRANSBIO dissemination and communication objectives and development and implementation of TRANSBIO brand image;
- Development of several versions of "TRANSBIO at a glance" brochure;
- Set up of an Internet platform / web page were the valorization strategy for by-products from fruit and vegetable processing industry based on biotechnological approaches and project results was made available and potential users had the chance to get familiar with the concept. The website is available in several languages English, Spanish, German, French and Romanian; www.transbio.eu
- Set up of a regular, biannual newsletter (TTC supported by the other partners) to inform the identified key players on:
  - developments of the project (e.g. to give short abstract on strategies and products);
  - to inform about upcoming events (e.g. stakeholder workshop, conferences, fairs)
  - to distribute common findings out of the workshop;
  - to communicate and make aware of progress in the sustainable utilization of by-products from fruit and vegetable processing industry using novel biotechnological approaches for valorisation.

3. Newsletter: http://tritecc.ro/transbio/
4. Newsletter: http://tritecc.ro/transbio/newsletter%204/index.html
5. Newsletter: http://tritecc.ro/transbio/newsletter%205/index.html
6. Newsletter: http://tritecc.ro/transbio/newsletter%206/index.html
7. Newsletter: http://tritecc.ro/transbio/newsletter%207/
8. Newsletter: http://tritecc.ro/transbio/newsletter%208/index.html

- Participation in several fairs and exhibitions, beside others,
  - Project flyers were distributed by partner TTZ at several editions of the **Hannover Messe**, Germany: International Industrial technology trade fair, during 08.-12.04.2013, 07.-11.04.2014. Also during **waste to energy**: Internationale Fachmesse und Konferenz für Energie und Rohstoffe aus Abfällen und Biomasse, 19.-20.02.2013, In Bremen, Germany, partner TTZ was active in talks with stakeholders and distributed several TRANSBIO dissemination materials.
  - TRANSBIO was widely disseminated at **SIAL**, in Paris, during 19-23 October 2014, as suggested in the 24th Month Meeting in Brussels. The dissemination was done by TTC and Biozoon who presented the project and distributed flyers to all exhibitors in the Green Area of Fruits & Vegetables.
  - Also, partner Ecosistemas, disseminated TRANSBIO at the **Chinaplas exhibition** during April 23 – 26, 2014 and also 20 to 23rd of May 2015 in Guangzhou, China. At present, CHINAPLAS is not only the largest plastics and rubber trade fair in Asia; it is also widely recognized by the industry as the 2nd most influential exhibition in the world, and as such the best channel to get an insight on the future of PHB.
- Organization of 4 Training Workshops (2 in Latin America & 2 in Europe)
  During TRANSBIO duration, 4 successful training workshops for the academia and professionals of the bio-based industry have been organized.
  1st Latin American Workshop, 14-15 October 2014, San Jose (Costa Rica)
  2nd Latin America Workshop, 13th of March 2015, Buenos Aires (Argentina)
  1st European Workshop, 22nd of April 2015, Bari (Italy)
  2nd European Workshop, 11th of November 2015, Vitoria (Spain)
- Organization of a Special Workshop for School kids
  Beside the four workshops organized for interested stakeholders from industry and research, the partners of TRANSBIO also understand the necessity to motivate teenager in Science careers. Therefore the partner Tecnalia modified the 4th workshop to be held for a younger audience and participated in the 1st Lego League organized by InnoBasque (www.innobasque.com/home.aspx?tabid=891). The aim is to motivate children between 10- 16 years in science and technology. The workshop is available in youtube: https://www.youtube.com/watch?v=28bNUqY-mKA
- Organization of a Final Conference, 11th of November, 2015, Vitoria-Gasteiz (Spain)
  Starting from the following questions: Are sub-products your next income opportunity? What are we doing today with sub- and by-products? How can sub-products be transformed in an income opportunity? The Final TRANSBIO Conference took the form of a promising technology transfer activity for around 50 academic and professionals of biotechnological sector.
- Several **press releases, interviews; articles** etc. mentioning the research objectives of TRANSBIO are available in different online and/or printed media beside others for example:
  - http://www.elcorreo.com/alava/v/20120107/economia/tecnalia-lidera-proyecto-mundial-20120107.html
  - Dietrich, T. (2013). Valorisation of by-products: Biotransformation of by-products from fruit and vegetable processing industry into valuable Bioproducts. Bioplastics Magazine, Vol. 8: 12.
- TRANSBIO was presented in several events organized by the **Commnet network** along the **2nd Bioeconomy Forum in Brussels** between 30 Sep - 1 Oct 2013. Therefore the project was also included in the CommNet's first ***Innovation Catalogue.*** The catalogue features selected bioeconomy research projects and discusses their positions in the innovation cycle, both in the agrifood chain and in the field of bioplastics. The catalogue is available digitally and in print.

2013 Innovation Catalogue – page 26-27
http://commnet.eu/01_About_CommNet/Commnet_Community/Business/Innovation-Catalogues.kl

- TRANSBIO was also presented on several events organized by the European Commission such as the workshop “**Maximising the impact of KET Biotechnology”** with a focus on the Project exploitable results, on the 22nd of September 2015, Brussels, Belgium as well as the **Bioeconomy Investment Summit** organized during 9th and 10th of November 2015 in Brussels (Belgium). During the Summit more than 400 participants gathered to discuss how investment can bring speed and scale to the European Bioeconomy. TRANSBIO project had a dedicated stand in the exhibition of projects (“Bioeconomy village”) showcasing the tangible results of some bio-economy projects in the marine, agri-food, forestry and waste themes. At the stand several relevant discussions related to the promising project results took place and contacts were made; also on the second day of the event the project results were presented to key audience during the **Bio-waste, residues and the circular economy Workshop.**
- Presentation at several conferences, beside others e.g.
  - Presentation by Lilia A. Prado-Barragán, Exploitation of agro-industrial waste in the production of biomolecules with high added value, XVIII Latin American Seminar and V National Congress of Food Science and Technology, March 2014. San Jose, Costa Rica organized by the TRANSBIO partner CITA. Therefore TRANSBIO was also mentioned as collaboration partner.
  - Presentation by Thomas Dietrich, Improving sustainability of fruit and vegetable processing industry by sub-product transformation at the International Conference: Envisioning a Future without Food Waste and Food Poverty: Societal Challenges, 17-18 of November 2015, Bilbao (Spain).
- Publications, the project TRANSBIO published several scientific publications, beside others, e.g.
  - J. Drumonde-Neves, E. Vieira, M.T. Lima, I. Araujo, M. Casal, D. Schuller (2013). An easy, quick and cheap high-throughput method for yeast DNA extraction from microwell plates. Journal of Microbiological Methods 93: 206–208.
  - T. Dietrich, J. Wildner, F. D’Urso, R. Virto, C. Velazquez, C. Sacramento Santos Pais, B. Sommer Ferreira, A. Carolas, L. Prado-Barragan, M.P. De Castro, S. Verstichel (2015). Improving sustainability of fruit and vegetable processing industry by sub-product transformation. IN: Leire Escajedo San-Epifanio & Mertxe De Renobales Scheifler (Eds.) Envisioning a future without food waste and food poverty - Societal challenges, 95-102. http://www.wageningenacademic.com/doi/book/10.3920/978-90-8686-820-9
  - Several chapters of the book: Biotransformation of Agricultural Waste and By-Products, 1st Edition The Food, Feed, Fibre, Fuel (4F) Economy. (2016). Edited by Palmiro Poltronieri & Fernando D'Urso, http://store.elsevier.com/Biotransformation-of-Agricultural-Waste-and-By-Products/isbn-9780128036228/

Exploitation of results

Each TRANSBIO partner has specific exploitation interests covering products and services. Therefore the partners will benefit in different ways from knowledge gained. For several of the project results, different exploitation possibilities have been identified and will be followed in order to ensure full benefits from the research results for all involved partners:

- The yeast communities that colonize fruit & vegetable by-products are a most valuable resource for novel biotechnological products, presently poorly studied. The collection of **non-conventional**

yeasts isolated from TRANSBIO by-products set up by partner **UMinho** stands up as a valuable biobank offering a great potential for screening yeasts for several biotechnological applications. Its exploitation is foreseen in future projects with other partners of the TRANSBIO consortium.

- TRANSBIO isolated several natural yeast strains able to accumulate succinic acid at higher levels than yeasts that are currently used as host for the construction of high succinic acid producers. Thus, the TRANSBIO yeast have a genetic background particularly suited to the production of succinic acid and have the potential to be an optimal chassis for building a high performing cell factory used in the **production of succinic acid** and other (di)carboxylic acids. This will be further exploited by the partners UMinho and Biotrend.
- The partner BIOESPLORA is currently analysing the patentability for genetic modification of a bacteria to produce high levels of PHB as well as the connected fermentation strategy.
- Solid-state fermentation (SSF) is ideally suited for the production of enzyme formulations for detergent application. In the case of the **enzymes for detergent application by SSF** isolated within TRANSBIO project, the SME partner **NST** concluded that this result is very promising for their business and as such the path towards exploitation will lead to the development of a specific business plan.
- Through the active participation in TRANSBIO, the SME **Biozoon** is aiming to further **exploit the knowledge gained on succinic acid** and its incorporation in food commodities and as such they are currently performing several tests for its application as food ingredient and exploit properties such as antimicrobial/preservative, flavour enhancer, emulsifier etc.
- **Soluciones Verdes** entered the TRANSBIO consortium with the challenge of expanding their knowledge regarding bioplastics in the final aim of being able in the near future to develop **new packaging products based on PHB polymer** such as bags and other plastic products. Soluciones Verdes will widen thus the merchandising products offer they already have, and also extend the commercialization in other countries. They will try to start selling this kind of products in Argentina, as there are no such items in the local market right now.
- **Proteos biotech,** as a primary manufacturer of enzymes for biomedical use, cell cultures and cosmetics, improved their knowledge regarding **new substrates and processing strategies for the obtainment of enzymes**, in bio-catalysis optimization and bio-hydrolysis, novel enzymes and are thus looking forward towards new exploitation routes.
- **OWS** believes strongly in a circular economy in which organic waste is first used for material recovery and only secondly for energy/nutrient recovery. As a supplier of **anaerobic digestion technology**, the TRANSBIO project yielded very valuable information on the biological parameters when digesting waste after material recovery. Compared to the state of the art for AD, in which the organic waste is immediately digested, the 'spent waste' (= waste after material extraction) has less favorable characteristics. The lab and pilot tests in the TRANSBIO project yielded information on how to overcome these less favorable conditions, making the combination of material recovery and energy and nutrient recovery a feasible process chain for future full scale applications.
- The results achieved during the **Life Cycle Assessment study** for the TRANSBIO project have improved the technical understanding of **OWS** in the case of bio-based production processes and associated environmental challenges. Products and product intermediates which were not available in LCA databases have been modelled during the study and are applied for modelling purposes in other projects, increasing their precision and our understanding of the environmental impacts of novel production pathways. Furthermore, the experience acquired during the project will allow OWS to more effectively evaluate other bio-based production processes in the future.

- The valorization of by-products from fruit and vegetable transforming industry into value-added compounds is one of the main objectives of the Bioprocesses group of the project coordinator **TECNALIA**. Besides TRANSBIO, several research projects such as VALBIO, VALSOST, and NATURDEV could be implemented. Therefore, the **knowledge obtained in the area of extraction technologies for succinic acid, PHB and enzymes** will be quite helpful for further project developments and offers the possibility for the group to be more competitive on the market for contract research for companies.