PROJECT FINAL REPORT

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1. EXECUTIVE SUMMARY

The RoboYeast project was designed to consider crucial characteristics of large-scale production *ab initio* on cellular and sub-cellular level for ensuring a successful and commercially sustainable bioprocess realization. Following this guideline, novel-to-market processes using novel-to-nature pathways in recombinant *S. cerevisiae* were in the focus of research. As such, the yeast-based production of the stilbenoid Resveratrol (RES) was investigated. Furthermore, basic findings of the Resveratrol study were planned to be applied for creating and optimizing polyunsaturated fatty acid (PUFA) producing yeasts, to be precise arachidonic acid (ARA) and eicosapentanoic acid (EPA) producers. Noteworthy, RES and PUFA formation share the common need of relatively high ATP demands which, per se, represent a cellular burden that needs to be managed not only in lab- but also in large scale production processes. Consequently the targeted compounds have in common that cellular energetic management should be optimized while carbon economy needs to be considered as well. This challenging scenario served as a model for identifying effectors that tune cellular robustness.

Unfortunately, the participation of the SME partner Fluxome A/S (FS) was terminated, which prohibited the access to the scheduled base PUFA producers, originally provided by FS. As a consequence, the transfer of key findings of the Resveratrol case and the de-bottlenecking of PUFA producers did not happen like primarily planned.

As a key objective, the academic partners at TU Delft and at University of Stuttgart, together with the (original) SME partner Fluxome A/S aimed at qualifying and quantifying the impact of process parameters relevant for full-scale production at the cellular and sub-cellular level, with a focus on metabolism, energy management and stress responses. Resveratrol producing yeasts were in the focus. The ultimate goal was to decipher underlying regulatory regimes such that robust producers could be developed. Consequently objective (1), namely the thorough sub-cellular analysis of RES producers was successfully performed while objectives (2.x) covering in-depth studies of PUFA producing strains could be treated partially only. The transfer of project results to production scale (objective 3) could not happen due to project termination while objective (4), the publication of scientific results is still in progress.

2. SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

The RoboYeast project was designed to consider crucial properties of industrial-scale production *ab initio* **on cellular** and **sub-cellular level** for ensuring a successful **bioprocess implementation**. Following this motivation, **novel-to-market** bioprocesses using **newly implemented** pathways in recombinant *S. cerevisiae* were in the center of research. As such, the yeast-based production of the stilbenoid **Resveratrol** (**RES**) was investigated as well as the biosynthesis of the polyunsaturated fatty acids (**PUFA**) ARA (arachidonic acid) and EPA (eicosapentanoic acid). It is worth noticing that optimized RES and PUFA producers were expected to fulfil the demands of providing sufficient (challenging) ATP for product synthesis while the carbon economy for the said compounds needed to be achieved as well. Considering that additional stress occurs in large-scale production processes, optimized producer strains have to be robust enough to withstand energetic and metabolic burden properly while performing still optimally.

Consequently, the project was planned to cover the following items: **Objective (1),** the '*Qualification and quantification of the impact of process parameters relevant for full-scale production at the cellular and sub-cellular level, with a focus on metabolism, energy management and stress responses in Resveratrol producing yeasts*' was in the center of research at the academic partners at Delft and Stuttgart. According to the original project topics, lessons learned from these studies should be transferred to PUFA strains. Noteworthy RES and PUFA formation have in common that relatively large amounts of energy-equivalents are needed for product synthesis. Consequently, findings of *'…the impact of energy-intensive (product) formation on cellular level w/o applying production-like process conditions…*' (**Objective 2a**), the identification of *'…metabolic and energetic robustness mechanisms…*' (**Objective 2b**) together with *'… their molecular basis…*' (**Objective 2c**) resulting that appropriate *'…process engineering targets…*' should basically be valid for both applications.

Unfortunately, the SME partner Fluxome A/S had to terminate the project participation unexpectedly which implied that access to yeast-based PUFA producers, proprietary strains of the SME partner, was not given any more. During the subsequent amendment process, research activities of the academic partners concentrated on RES producing strains. This focus was scientifically driven by the urgent finding that RES producers revealed robustness problems that obviously deteriorate the production performance. In-depth studies at Delft and Stuttgart identified key effectors that are of general interest not only for RES production but also for all types of products that are triggered by copper induction in *S. cerevisiae*.

After the decision was made to terminate the project, **objective 3**, namely '*the transfer of promising metabolic engineering approaches, strains and process scenarios … in production trails*' could no longer be followed and needed to be cancelled. Nevertheless it is still the interest of the academic partners to follow **objective 4**, namely 'the development of a profound knowledge base', for instance by publishing related results in the scientific community.

3. MAIN S&T RESULTS

This final report corresponds to the RoboYeast Project (FP7-KBBE-2011-5) and is part of the contractual obligations between the European Commission and the project partners.

The collaborative project '*Robustness of Yeast Strains and Bioprocesses for Industrial Production of Novel Compounds*' comprised the cellular, metabolic and genetic engineering for novel compounds for the benefit of Industrial Biotechnology.

The project partners at TU Delft and the University of Stuttgart cooperated in a scientific way to study the efficient production of novel compounds based on robust producer strains. At project start, the SME partner Fluxome A/S joined the project until its participation stopped unexpectedly. As indicated under (1) and (2), no PUFA producers could be investigated further which finally caused the termination of this project.

Short Name	Participant organisation	Country
STU	Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, D 70569 Stuttgart Prof. R. Takors (coordinator)	Germany
TUD	Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft Prof. J. Pronk	The Netherlands
FS	Fluxome Sciences A/S Gymasievej 5, 3660 Stenløse Dr. J.-M. Mourillon Previous project partner before termination of participitation.	Denmark

3.1. Work Package (WP) 2: Dynamic Analysis (STU)

During the project lifetime the tasks **I** ('Set-up of experimental methods'), **II** ('Fermentation studies mimicking production conditions'), **III/V** ('Data analysis for elucidating impacts of process dynamics on subcellular levels'), **IV** ('Fermentation studies mimicking production conditions') and **VIII** ('Identification of targets for engineering strains with improved robustness') were intensively executed focusing on resveratol (RES) producers. Due to early project termination, as a consequence of the stopped participation of the SME partner Fluxome A/S, the tasks **VI** ('Process and strain optimization considering production constraints'), **VII** ('recommendation for industrial fed-batch fermentation') and **IX** ('Large scale tests') could not (yet) be treated with the intensity as originally planned.

At project start, the reference resveratrol (RES) production process of SME FS was transferred to the academic partners. At IBVT, the transfer was successfully done achieving the requested and expected performance criteria disclosed by company.

The process is characterized by an initial biomass formation phase (I) followed by two subsequent phases with limited growth to enable optimum RES formation. Phases (II) and (III) are controlled by limited supply of glucose thus establishing growth rates of 0.1 and 0.01 1/h respectively.

Notably, this low-growth production approach is exactly the type of production scenario which is in the foreground of the RoboYeast project and which demands a well-equilibrated carbon economy and energy management of the yeasts – especially in large-scale production processes when facing environmental heterogeneities.

In addition to phenomenological studies in 2- and 30-L scale aiming at bioprocess optimization, IBVT activities focused on the elucidation of intracellular flux patterns for investigating whether and how targets for metabolic engineering can be identified for further optimizing strain and process performance. Consequently, a stoichiometric model was set up consisting of 88 reactions and 97 metabolites. The model was composed of two compartments representing the cytosol and the mitochondria. 10 transformer fluxes for exchanging metabolites between cytosol and extracellular matrix together with 21 transformer fluxes controlling the mass exchange between mitochondria and cytosol were considered. Details are given in figure (1). Table (1) indicates, that applying the model for the analysis of the production phases (II) and (III), well-closed elemental balances were achieved.

The stoichiometric model of figure (1) was applied for metabolic flux analysis focusing on the phases (II) and (III). For reducing the inner degree of freedom, published information of intracellular fluxes found in *S. cerevisiae* (CEN.PK113–7D) was considered. To be precise, the flux ratio of tyrosine to phenylalanine as important precursor metabolites in resveratrol production was set constant as identified as a rigid property in the wildtype strain. Measured rates of glucose uptake ($q_{glucose}$) (if so glycerol and ethanol uptake or synthesis ($q_{glycerol}$, $q_{ethanol}$)), oxygen uptake (q_{O2}), carbon dioxide emission (q_{CO2}), sulfate uptake (${q_{SO4}}^{2-}$), ammonia uptake (${q_{NH4}}^{+}$), phosphate uptake (${q_{HPO4}}^{2-}$), and resveratrol production (q_{res}) were used as experimental input values. Table (2) gives an excerpt of the cellular energy management. As indicated, measured ATP values of the phases II and III indicate slight decreases. Still, the adenylate energy charge levels [(ATP + 0.5 ADP) $(\text{ATP + ADP + AMP})^{-1}$] stay at physiological levels. Interestingly, the estimated P/O ratios derived from metabolic flux analysis decrease significantly. Considering that the theoretical optimum in *S. cerevisiae* could be about 2[1], this level is not reached by far. Even worse, the longer the process runs, the more the P/O ratios is obviously deteriorated. Consequently, improving strain performance by e.g. enhancing RES formation will result at more and more challenging ATP supply - thus tackling a core objective of the RoboYeast project. This finding is even more pronounced by considering the additional flux balance analysis (fba) results. Only if the theoretical optimum values of about 3 are assumed, maximum RES formation will be achieved at the given production conditions.

Results of mfa simulations were further compared with independent studies applying ^{13}C labelling analytics. As depicted in table (3) ^{13}C labeling studies revealed intracellular NADPH formation in S. cerevisiae wildtype of 1224 µmol/g_{CDW}h at the growth rate µ=0.1 1/h. Notably, very similar values were estimated by own studies lumping NADPH formation via pentose-phosphate pathway (PP) and malic enzyme activity (mae) in stoichiometric models. Interestingly, different growth scenarios (A, B)

[1] Here we assume that only 2 proton efflux pumps are active in the yeast while the theoretical P/O ratio of 3 requires for 3 active proton efflux pumps.

during RES formation support this finding, too. Biomass specific RES formation is proportional to NADPH synthesis which itself is (at least partially) linked to growth. At no-growth conditions, net NADPH formation and demand for RES formation should equilibrate or even allow a surplus of 1 NADPH. Taking into account that anabolic reactions usually require NADPH for biomass formation there may be a NADPH conflict for RES formation in growing cells. However, this putative shortage is not (yet) pronounced in the RES producers investigated.

Besides mfa studies, intracellular metabolome measurements were performed. As an example, figure (2) illustrates intracellular pool sizes measured during the RES production period. This approach was followed in addition to measurements of extracellular metabolomics. As a result, precursor supply as well as by-product formation of RES production could be monitored.

Cinnamic acid accumulated as the product of PAL2 catalyzed conversion of l-phenylalanine. Pinosylvine levels increased as a by-product of 4CL mediated cinnamic acid conversion. Phloretic acid was found, obviously derived from coumaric acid via DH. Additionally phloretin was measured due to enzyme promiscuity of the same enzymatic step. Summarizing, hints were found that RES formation was not fully equilibrated under the production conditions studied. Further studies even enabled the identification of a mechanistic model, being able to describe metabolism dynamics in the aromatic amino acid pathway.

Analyzing metabolomics also gave evidence for another impact that severely affected the robustness of the production system. The heterologous genes of the non-native pathway for RES formation were constructed under the control of a copper inducible promoter. Investigating the performance data of the RES formation with respect to a varying range of copper stimuli, it was clearly found that an optimum copper triggering signal must be implemented. With 40 µmol an optimum induction level was found that allowed relatively high RES formation while preventing too high by-product formation of cinnamic acid at the same time. Additionally, consequences of copper induction were also observed phenomenologically when studying the growth rate. Too high copper stimuli significantly reduced growth rates, obviously forcing the cells to adapt to the new conditions.

Hence, it is a clear finding, that the copper induction system applied should not be the preferable choice for an industrial approach. Not only the control of optimum induction levels can hardly be established in large scale (with significant heterogeneities) but also the growth disturbance causes severe instabilities counteracting the desire to design robust bioprocesses.

3.2. Work Package (WP) 3: Impact of Growth (TUD)

In industrial fermentation, process parameters and strain performance play a pivotal role in utilizing microorganisms for the production of value-added compounds. For anabolic compounds such as resveratrol and poly-unsaturated fatty acids (PUFAs), aerobic cultivation conditions are the preferred mode of operation because it yields the highest chemical energy (in the form of ATP) yield on substrate glucose, necessary for flux through the metabolic production pathway in of the production host *Saccharomyces cerevisiae*. The drawback is that this extra ATP can also be invested in biomass formation, which is both catalyzer of the reaction, but also a major by product that reduces the product yield on substrate. The optimal cultivation and process condition inherently depends on the physiology of the strain, and more importantly, on the characteristic relationship between growth and production formation. This especially holds for large scale production processes, where slow growth, environmental effects and culture heterogeneity play a role. In work package 3, the impact of specific growth rate on production characteristics is investigated for a resveratrol producing *Saccharomyces cerevisiae* strain. In conjunction with the research from work package 3 and 4, this research delivers a physiological characterization of a strain producing anabolic products such as resveratrol and PUFAs. Furthermore, optimization targets are identified, and properties for improving this producing strain and the process conditions have been investigated; all for an optimally and robust yeast-based resveratrol/PUFA production platform for large scale processes.

During the course of the project the following tasks were undertaken, task I: Setting-up methods for metabolite analysis, Task II: Analysis of the impact of specific growth rate on product formation, Task III: Recommendations for industrial fed-batch fermentation, Task IV: Identification of targets for engineering strains with improved robustness, and Task V: Strain construction and analysis have been executed with focus on resveratrol producers.

Initially, methods were established to measure the compounds of interest produced by the Fluxome *S. cerevisiae* strain, including resveratrol and relevant phenolic metabolites such as coumaric acid, phloretic acid and cinnamic by High Pressure Liquid Chromarography. Furthermore, techniques for the extraction and quantitative analysis of polyunsaturated fatty acids from cell cultures have been optimized and adapted to our laboratory infrastructure. Experiments have been performed to evaluate the method of quantification of neutral fatty acids in isogenic reference strain.

The impact of growth on resveratrol production and yeast physiology has been investigated at cellular and sub-cellular level by mimicking industrial production parameters in lab-scale, under controlled conditions. Aerobic glucose-limited chemostat experiments maintained at different growth rates have been performed in biological duplicate (independent), with both a resveratrol producing *Saccharomyces cerevisiae* and an isogenic laboratory reference strain. Herewith, it was identified that a correlation can be found between the specific growth rate and biomass specific production rate, which is valuable input to define industrial fed-batch parameters.

Based on the results from the chemostat cultures, and more especially the relationship between growth rate and product (by)formation, a stoichiometric model was built to improve resveratrol titers during fed-batch cultivation. The stoichiometric model covers the different phases of the fed-batch and is fully based on mass balances for biomass, product, substrate and change in volume. Based on optimization of resveratrol production, the modelling enabled the prediction of the best feed profile. This *in silico* analysis revealed that choosing between an exponential feed rate with a constant slow growth (modus operandi of choice of SME partner Fluxome A/S), or a constant feed rate with gradual reduction of the growth rate over time, greatly influences the titers of biomass and (more importantly) product over the course of the fermentation (see figure 3). These results could be directly valorised by the industrial partner to improve product formation.

While using chemostat cultivation has proven very useful to investigate the relationship between product formation and growth, this cultivation tool is technically limited to growth rates above $0.01h^{-1}$. However, a situation in which cells do not or hardly grow, but remain metabolically active and invest all the carbon and energy in product formation is highly desired. We have previously demonstrated that this situation can be reached using retentostat cultivation. A retentostat can be compared with a chemostat setup, in which there is a constant inflow of carbon-limited medium and a constant outflow of broth to maintain a certain dilution rate. However, in the retentostat the outflow is filtered, thereby retaining the biomass inside the reactor but still keeping the volume in the reactor constant. In response, the inflow of limiting carbon source has to be distributed over an increasing biomass fraction, resulting in very slow growth rates up to the point where the limiting carbon and energy source is solely used for maintenance energy requirements of the cells rather than growth. Although validated for anaerobic processes and catabolic products, retentostat cultivation had to be tailored for high energy-demanding anabolic products such as resveratrol and PUFAs. In this project, a novel bioreactor setup adapted for aerobic conditions has been designed and experimentally validated*.* The design was inspired by the physiological characteristics and especially the energy economics and maintenance requirements of *Saccharomyces cerevisiae*, elucidated from the growth rate studies in chemostat (see Figure 4). This system has allowed us for the first time to explore the physiological response of *Saccharomyces cerevisiae* cultivated at near-zero growth rates under aerobic, fully respiratory conditions.

The resveratrol producing *Saccharomyces cerevisiae* requires copper for induction of the first gene in the heterologous pathway, the *PAL2* gene. The growth studies in chemostat have been performed with an optimized concentration of copper in the medium that facilitates optimal induction of the pathway. However, from the growth studies at different dilution rates, an estimation of the maintenance requirements of *Saccharomyces cerevisiae* were higher than obtained from the studies in retentostat, where culture medium and conditions where the same, except for the lack of extra copper in the medium. This suggested that copper alters the energy economics of the cell, and creates an undesired energetic burden to the cells. Our results corroborates that of WP2 and demonstrate that copper-inducible promoters should be replaced in the resveratrol producing strain.

Whole genome transcriptome analysis allows to uncover genetic regulation of cellular metabolism based on differential transcript levels between strains cultivated under specifically set conditions. To elucidate targets to improve robustness of a resveratrol producing strain, transcriptome samples from individual chemostat cultures of resveratrol producing strain and the isogenic reference strain have been sampled, processed and analysed using the Affymetrix whole genome transcriptome analysis platform. Statistical analysis of the transcriptome data was used to identify the genes that significantly differed (q value cutoff of 0.005) in their growth rate dependent expression profiles between the two strains. This resulted in a substantial number of differentially expressed genes between producer and non-producer. Genes were clustered according to their expression profiles, resulting in four distinct clusters showing both positive and negative correlation between gene expression and growth rate. Our analysis confirmed that genes encoding proteins involved in the *de* *novo* pathway for resveratrol production were significantly differentially expressed between the resveratrol producing and non-producing strain. Several genes belonging to pathways involved in the supply of precursors for resveratrol synthesis were also differentially expressed. Based on these results, overexpression of the genes involved in precursor supply have been identified as a primary target for engineering in the resveratrol producing strain to boost precursor supply. To improve robustness of the yeast-based resveratrol production platform, the first steps for metabolic engineering of precursor supply have been undertaken in *Saccharomyces cerevisiae*, utilizing the latest synthetic biology techniques.

3.3 Work package (WP) 4: Strain Construction and Production Trials (FS)

Because of the early termination of project participation by Fluxome A/S the corresponding tasks were affected significantly leading to the cancellation of all tasks that were originally scheduled after the termination date. However, the task **VI** ('Analysis of the impact of specific growth on product formation in chemostat') was executed for wildtype strains and task **VII** ('Engineering novel recombinant strains for EPA and ARA producers') could only be executed during the participation period of the SME.

The heterologous production of ω-3 polyunsaturated fatty acids (PUFA) in baker's yeast asks for multiple metabolic engineering measures including the implementation and amplification of heterologous genes from algae, mosses, higher plants, nematodes and mammals. Following this approach it should be possible to convert *S. cerevisiae* native compounds such as mono-unsaturated fatty acids (like oleic acid, C18:1ω9) to the target compounds like arachidonic acid (ARA, C20:4ω6) or – even more attractive – eicosapentanoic acid (EPA, C20:5ω3).

The PUFA synthesis of ARA and EPA starts from oleic acid and requires the heterologous, typically membrane-bound desaturases (D9D, D12D, D6D, D5D and D17D) for introducing double bonds. Additionally, two carbon atoms are added to the 18 carbon atoms skeleton by the elongase (D6E). Noteworthy elongation consists of 4 steps represented by condensation, reduction, dehydration and reduction. Furthermore any metabolic engineering approach should consider that most of the enzymatic reactions are taking place at the Endoplasmic Reticulum which obviously may cause additional problems of appropriate in- and export of substrate and products requested. Consequently the identification of potentially reaction limiting steps is a multiple problem not only consisting of the individual reactions but also by considering the transport steps involved.

A set of yeast transformants was constructed applying the infusion cloning technique of Stratagene e.g. to amplify the D9D gene on plasmid. Strains were tested in shaking flask batch cultures. Fermentation tests were performed in shaken flask cultures applying the liquid Delft medium with 20 g/L glucose. After overnight cultures at 30°C and 150 rpm, these pre-cultures were transferred into 250 mL shake flasks with 25 mL Delft medium containing 0.2 g/L glucose and supplemented with glucose feed-beads to simulate fed-batch processes. In total, these production batches lasted for about 96 hours at the said process conditions.

The single amplification of D9D yielded at relatively low amounts of ARA and EPA mixtures measured inside the cells. Therefore it was included that single D9D overexpression is no suitable, single overexpression target.

Next, the desaturases D12D, D6D, D5D, D17D and the elongase D6E were amplified individually and tested in shaking flask cultures. Again, only relatives low ARA and EPA titers were found. Consequently, the combined amplification of the said genes was scheduled. However, this could not be pursued because of the termination of related activities at the SME partner.

4. THE POTENTIAL IMPACT; DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS

To achieve successful and sustainable bioprocesses it is of crucial importance to investigate and to understand impacts of large-scale production in-depth, already at lab-scale studies. Production strains and processes obviously need to be robust enough to withstand any impacts of large-scale production such that lab-scale performance will be equally achieved under industrial production conditions. As examples of novel-to-nature compounds resulting at new-to-market processes, the challenging products Resveratrol and polyunsaturated fatty acids (PUFA) were addressed. For such type of products the need of robust production strains and processes is even more challenging because cellular energy management needs to fulfill the dual demand for optimum product formation and (increasing) maintenance due to scale-up requirements. RoboYeast exactly tackled this complex problem by elucidating basic robustness mechanisms on the regulatory and molecular level. Once known, findings can be further exploited to allow highest yeast performance at industrial scale.

The teams of the RoboYeast project successfully managed to set up and apply basic metabolic and biochemical engineering tools for investigating metabolic activities on cellular and sub-cellular level. Their application provided insights into intracellular controlling regimes for precursor and energy supply. Besides ATP availability, maintenance demands were quantified, evidences for not well equilibrated enzyme activities in the heterologous biosynthesis from phenylalanine to resveratrol were found and the high sensitivity of the promoter system used outlined.

These findings are not only valid for the RES producer but are of superior significance for other cell-based bioprocesses too, thus strengthening European bioeconomy.

Among others, RoBoYeast had the ambition to accelerate market introduction time of the novel-to-nature production of the PUFAs ARA and EPA by *S. cerevisiae*. Unfortunately, due to the unforeseen termination of participation of the former SME partner Fluxome A/S, this product introduction did not happen. Notably, Fluxome A/S once owned fundamental yeast patents for ARA and EPA production exclusively. However, neither strains nor patent licenses were accessible after the company's breakdown. Finally, this caused the early termination of the RoboYeast project.

Besides, the partners at TU Delft and University of Stuttgart are still fully convinced that RoboYeast did contribute to industrial and societal benefit as follows:

Industrial benefits:

- The proposal was - and still is - fully aligned with the objectives of KBBE to establish a competitive knowledge bio-based economy in Europe.

- Recommendation and methods for generally improving robustness in industrial bioprocesses are formulated thus serving as a blueprint for similar problems. This argument holds true e.g. for the proper choice of an industrial induction system. Obviously, the copper induction used should not be the first choice at all.

- Recommendations and methods for optimal feed profiles in industrial fed-batch fermentation are created. Based on the transferred process data, improvements were made.

- Education of Ph.D. students at two universities was performed. Skilled fermentation personnel is currently hardly available on the labor market. This need, once formulated at project start, is still valid. RoboYeast succeeded to educate newly skilled fermentation personnel well - until it was decided to terminate the project early. Unfortunately, the cut of project continuation forced to dismiss people.

Societal benefits:

- The RoboYeast team started with the motivation to increase employment in SME by 20 – 40 %. However, independent commercial interests – obviously linked to the financial crisis in the EU market – prevented this development.

- Because, PUFA production could not be studied, related (planned) benefits (such as the access to nutraceuticals that can be used in preventive health care or the replacement of fish oil derived PUFAs) could not be achieved. However, focusing on resveratrol (RES), the remaining core project still managed to install environmentally friendlier and commercially attractive production systems. The attractiveness of the RES business is shown by its acquirement by another SME shortly after the bankruptcy of Fluxome A/S.

Expected impacts listed in the work program

1) Integration of European research actors and activities

This project brought together three highly complementary European research groups around (orignally) two (later: one) high-profile, novel-to-nature biotechnological processes. The information exchange was (and still is) very vivid, mirrored by several project meetings and intensive discussions about the future developments. Furthermore, a fourth, new SME party was planned to be incorporated. However, this was prevented by EU decision to terminate the project.

2) Enlarging the application of industrial biotechnology for the production of novel industrial compounds

Roboyeast improved the Resveratrol project by unravelling key elements for further metabolic engineering. So, the blueprint for next steps of strain and process optimization is given.

3) Increasing the competitiveness of the European biotechnology industry

Obviously, commercial interests of a third party caused the termination of Fluxome A/S. This shows that SMEs need to be financially independent for developing a sustainable position in e.g. biotech markets. Consequently, they need research support by e.g. EU institutions to develop the same in close cooperation with academic partners. So, this case should be taken as an example to support related research activities further in future programs to create a strong SME biotech basis.

4) Developing new and robust microbial industrial production systems

The blueprint for Resveratrol strains was developed in RoboYeast.

5) Development of platform technologies to enhance industry's capabilities for biotechnological applications

Unfortunately, RoboYeast could not show this property within the given project duration. Having no access to PUFA strains developed by Fluxome A/S, the platform character is still limited to resveratrol formation.

6) Explanation why an international and not a national approach is followed

The academic partners offered and applied individual expertise on yeast engineering and cultivation at TU Delft and systems engineering at University of Stuttgart. This ideal matching of individual knowhow was not possible by national cooperations. Furthermore, activities for finding a new SME partner after the Fluxome A/S bankruptcy clearly revealed that pure national searches were not helpful. According to the academic partners, a new, highly attractive SME partner was found – again in Europe and outside The Netherlands and Germany.

7) Indication how account is taken to other national or international research activities.

Both, the academic partners at TU Delft and at the university of Stuttgart are closely networked with other European research activities as project partners or as participants of related conferences. As such, they are well aware of ongoing research activities in this field.

Dissemination activities:

At project start a press release has been published informing the public about RoboYeast. Additionally, a project homepage was activated. Besides, the printing of flyers was scheduled – however not executed due to the changing project status.

Furthermore, project plans had considered to install a summer school for experts of the expanded EU countries in the last year of RoboYeast. Unfortunately, this issue was cancelled because of the project termination.