BIOINTENSE: CONTEXT

BIOINTENSE was a single stage knowledge based bio economy (KBBE) collaborative project which started on the 1st of August 2012. It is EC-funded through the 7th Framework Cooperation Program that has the strategic objective of supporting research activities to gain or consolidate leadership in key scientific and technology areas and to encourage international competitiveness whilst promoting research that supports EU policies.

The main objectives in **BIOINTENSE** were to **increase biocatalyst productivity and process intensity.** This should result in economically feasible processes by integration and intensification and also shorten the development times by developing optimized tools and protocols that can be widely applicable in industry. More than this, the lack of fundamental understanding on the interactions between reaction, biocatalyst and process characteristics were to be addressed to minimize the uncertainties with respect to the cost of future biocatalytic processes. **BIOINTENSE** was also designed with the objective of increasing understanding about the factors contributing to the total cost and environmental impact of selected processes. The project partners were: Danmarks Tekniske Universitet (Denmark, Prof. John M. Woodley); Technische Universität Graz (Austria, Assoc. Prof. Torsten Mayr); Univerza Ljubljana (Slovenia, Prof. Polona Žnidaršič Plazl and Prof. Igor Plazl); Universiteit Gent (Belgium, Prof. Ingmar Nopens); University of Manchester (UK, Prof Nick Turner); Lunds Universitet (Sweden, Prof. Patrick Adlercreutz); DSM Innovative Systems BV (The Netherlands, Dr. Martin Schürmann); Vlaamse Instelling voor Technologisch Onderzoek N.V. (Belgium, Dr. Winnie Dejongh); iX-Factory GmbH (Germany, CEO Dominique Bouwes); Microfluidic Chipshop GmbH (Germany, Dr. Claudia Gaertner); Luxcel Biosciences Ltd. (Ireland, Dr. Dimitri Papovsky); LentiKat´s a.s. (Czech Republic, Dr. Radek Stloukal); C-Lecta GmbH (Germany, Dr. Sebastian Bartsch); Sigma Aldrich (Switzerland, Dr. Roland Wohlgemuth) and was led by Professor John Woodley at the Department of Chemical and Biochemical Engineering at the Technical University of Denmark.

The BIOINTENSE project has fulfilled all the objectives and produced valuable novel and innovative technical results. The results have contributed to scientific understanding in the field of transaminase development, process chemical engineering applied research and fabrication of miniaturized reactor systems. The collaboration between these fields is presented in Figure 1. The complex interaction between the different scientific and technical fields can clearly be seen. A team of **enzyme developing partners** (UMAN/c-LEcta) had the objective to supply to the consortium the biocatalytic enzymes. The consortium decided to focus on amino transferase (ATA) enzyme catalyzed reactions for the production of chiral amine products (ATAs are sometimes referred to as ω-transaminases in the context of the chemistry presented here). The production of chiral amines is of great importance in the fine chemical and pharmaceutical industry, making this synthesis a very attractive test system, of immediate commercial relevance. Besides the supply of biocatalysts to the partners an important objective of the research partners was to improve the properties of the enzyme such as the stability, solvent tolerance and temperature stability of the enzymes. A second group of partners (ChipShop/iX-factory) have focused on the **development of commercially producible microfluidic systems** that were used in the project for the screening and characterization of enzymes and process options. A close interaction with the partners developing **novel optical sensor technology** (TUG/Luxcel)(e.g. for oxygen, pH and glucose) for advanced on-line measurement was established. This enabled rapid integration of the sensor technology and concepts into the flexible microfluidic systems.

The **process and chemical Engineering** group (DTU, UL, ULUND, VITO, LentiKat`s and UGENT) investigated novel technology by using the new miniaturized platform toolbox for the

evaluation of different *in-situ* product removal (ISPR) strategies for different reactions and reactor configurations. This was done by using minimal amounts of reactants, catalyst and time. In this way it was demonstrated that the developed miniaturized tools can be efficiently used for the intensified characterization of enzymes and processes.

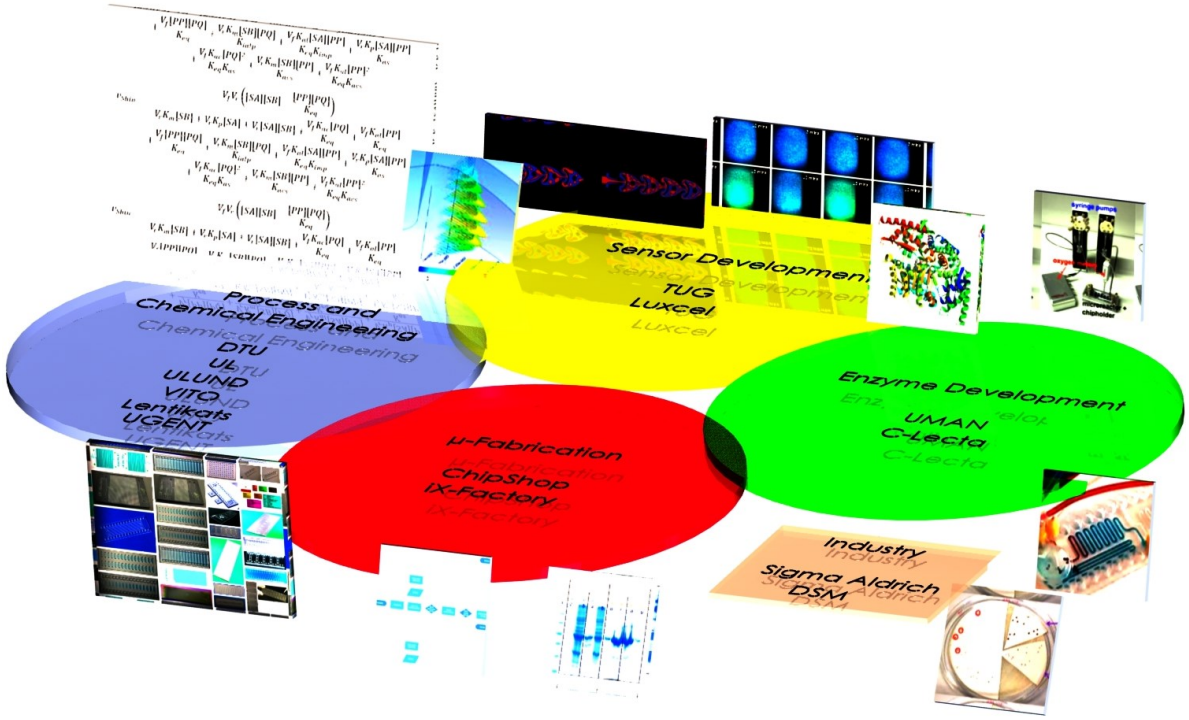

Figure 1: Main interacting elements and partners in the BIOINTENSE project

An essential engineering aspect of the complex interaction was the incorporation and use of **mechanistic kinetic models** and the incorporation into **computational fluid dynamic models** for data analysis and model calibration. With the help of the calibrated models it was possible to successfully study and understand the large amount experimental data acquired in the project. These models serve as the basis for prediction of process performance across different scales and are therefore an integral tool for future development of processes and process technology beyond the ATA catalyzed reaction systems.

The BIOINTENSE project has delivered through this exciting and challenging inter-disciplinary collaboration considerable impact in the field of ***bioprocess intensification by* in-situ *product removal*.** BIOINTENSE delivered a platform-based miniaturized toolbox that facilitates the evaluation of different ISPR strategies for different reaction and reactor configurations, using minimal amounts of reactants, catalyst and time. BIOINTENSE demonstrated the feasibility of the µ-scale and miniature tools developed, integrating the applied sensors and the new developed catalysts, using the results as input for building models that can be applied in further studies. With respect to ***µ-fabrication*** the project has contributed to novel miniaturized reactor systems ready for catalyst and process screening and process characterization. They have been mainly used for studying kinetic data, enzyme stability and activity, solvents use (e.g. extraction and substrate supply), applications of free and immobilized enzymes as well as for operational and configuration studies. The systems have been constructed in various materials (glass, polymers and silicon) and offer a wide range of applications including alternative configurations such as packed bed reactors.

Single stream systems have been produced offering versatility by combining the reactor systems, but a first prototype parallelizing eight reactors has also been fabricated. Those µ-fabrication activities have been accompanied by ***monitoring and sensor development*** focusing on the development of novel optical sensor materials for pH, glucose and oxygen employing indicator dyes that can be excited with red light, emitting in the NIR range. Here the focus has been on the signal stability and minimization of signal drift, the fabrication of calibration-free sensors or at least use of a minimized number of calibration cycles due to higher stability of the sensor material, the enhancement of sensor patches with improved reproducibility between different batches due to defined polymer fractions, the extension of long-term operational and shelf-life stability of sensor layers due to the minimization of the separation of sensing components, the extension of types of processable polymeric support materials because migration of sensor components into other substrate material is suppressed and the better reliability on measurement data will lead to safer decision making stability of the sensor material. The developed sensors have been subsequently integrated into the industrial µ-fabrication processes.

Another important working field was the ***fermentation development and production.*** Here the fermentation and recovery of ATA was efficiently optimized in a parallel fermentation unit at c-LEcta investigating also the monitoring sensors developed by TUG. The enzyme was produced in sufficient quantities for immobilization and free volume investigations. The enzyme was distributed within the consortium and implemented in the process optimization in µ-scale. The use of the same biocatalysts quality from micro to industrial scale allowed the minimization of disturbing effects from side products in the enzyme formulation. In a parallel activity UMAN and c-LEcta were performing ***multi-objective biocatalyst screening***. UMAN and c-LEcta designed multi-objective (temperature stability, activity, selectivity) screening protocols which targeted process conditions and used these to screen libraries of ATA mutants to find enzyme variants that are better suited to process conditions and show better process performance. After distribution of the biocatalysts to the partners it was of considerable interest to investigate the ***immobilization and stability*** of the enzymes under realistic conditions. ULUND has investigated methods for the study of inactivation mechanisms of transaminases under process conditions. The established knowledge has been used in order to facilitate the choice of stabilization strategies in a rational way. The most efficient strategy for the improvement of ATA was found to be protein engineering carried out by c-LEcta. With respect to the immobilization of enzymes the surface immobilization and entrapment was successfully implemented. Here the polyvinyl alcohol lentil shaped carrier particles from LentiKat`s showed good performance in the miniaturized screening methods.

An important contribution of the project was the development of methods for the early stage assessment of the ***economic and environmental*** process candidates. The industrial partners Sigma Aldrich and DSM interacted dynamically with DTU in order to evaluate the biocatalytic process potential, including the allowable costs. The study of process design, the process costing and the process potential were investigated by using a set of metric-based development targets. An integral component of the work was the ***modeling*** efforts connecting the different expertise with each other. Integrated models were developed combining different model types (e.g. kinetic process models and computational fluid dynamics for detailed mass transfer studies). Such models were used for the design of experiments as well as for the analysis of experiments or design of new µ-fabricated fluid systems. Here, especially the modeling of spatial heterogeneities was of importance. Using the models for topology optimization resulted in the development of new, wisely placed inlet conditions to the microfluidic reactors, increasing the productivity up to 30% compared with traditional configurations. Finally the ***scale-up and numbering-up of bioprocesses*** have been of interest for

the overall project. It was investigated if the microfluidic enzyme reactors are easily used in parallel configurations. At the same time VITO has been working with larger scale reactors (at 300 mL scale) in order to test the concepts of intensified processes at larger scale.
In summary, the most important successes were as follows:

- Development of miniaturized devices for evaluating ISPR options
- Development of intensified bioprocesses with in-situ product recovery and substrate supply
- Development of new miniaturized sensors for monitoring and control
- Understanding and benchmarking Scale up & Numbering up approaches for biocatalytic processes
- Development of a cost effective fermentation and DSP protocol for transaminase preparations
- Using multi-objective screening protocols to develop new transaminases fit for process conditions
- Understanding stability and inactivation of transaminases
- Development of cost effective immobilization procedures for increased biocatalyst productivity
- Economic and environmental evaluation to quantify process improvements
- Process modelling & design of experiments to optimize process performance and experimental protocols through optimal experimental design

Impact of the project

This project has brought together engineers and scientists from biotechnology as well as microfluidic and modeling experts. This interdisciplinary interaction was at the beginning not straightforward but it resulted in a highly valuable rapid development and progress in the investigated field. This can be observed for instance by:

- The development of new microfluidic products (i.e. the design of 17 new microreactors resulting in 24 different micro reactor prototypes).
- 24 mass fabrication compatible micro reactors for further commercial application.
- Standardization of device formats, making the systems applicable with standard laboratory equipment (microscopic slide and microtiter plate formats were used for internal standards)
- The development of various new substrates and products, previously worldwide not available, by new synthetic methods and by using screening of ω-transaminases against a library of potential substrates.
- Novel LentiKats® particles with immobilized ω-transaminase for the production of fine chemicals.

This short extract of some of the highlights shows the potential of a systematic collaboration across fields and disciplines. Furthermore, it shows the potential for application of miniaturized systems to contribute to the accelerated development of biotechnological-based processes far beyond ATA-based reactions. It is therefore expected that micro-technology will especially in the field of process development gain increased importance, especially when the reaction material is scarce or very expensive. It is expected that the insight gained in this project will be used in the future for ***future collaborations***. Here it is anticipated to use the advantages of micro-technology for studying the synthesis of unstable intermediates in biocatalytic and organic synthesis, unstable and potentially dangerous reactions (e.g. Grignard reactions), as well as reactions using toxic materials.