Service Catalogue

The project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement n. 730976
PROTEIN DISCOVERY AND ENGINEERING

Automated library generation 4
Automated protein expression 5
Automated enzyme screening 6
Computational gene sequence search and selection 7
Enzyme/protein engineering 8
Enzyme/protein discovery 9
Functional characterisation of proteins 10
Computational genome annotation and mining 11

PRODUCTION STRAIN DEVELOPMENT

Automated strain construction 13
High throughput phenotypic screening 14
Metabolic reconstruction & Phenotype modelling 15
Exploratory cultivation in bioreactors 16
Rational strain engineering 17

BIOPROCESS DEVELOPMENT/ OPTIMISATION

Computational pathway design 19
Conceptual process flowsheet modelling 20
Integrated bioprocess development and optimisation 21
Downstream processing 22
Development of enzyme-catalysed processes Scale-up with algae 23
Scale-up with bacteria 24
Scale-up with filamentous fungi 25
Scale-up with membrane bioreactors 26
Scale-up with yeasts 27
Scale-up with plant cells 28

OMICS & ANALYTICS

$^{13}$C Fluxomics 31
Proteomics, interactomics and peptidomics 32
Fluorescence correlation spectroscopy for single molecule studies 33
High throughput genome sequencing & transcriptomics 34
Metagenomics and metabarcoding 35
Biomass compositional analysis 36
Metabolomics and metabolite analytics 37

TEA/LCA

Techno-economic assessments 39
Value chain analysis 40
Protein discovery and engineering
Automated library generation

DESCRIPTION
DNA libraries are collections of DNA fragments cloned into vectors, used to identify and isolate the DNA fragments of interest. We generate expression libraries from coding sequences cloned into expression vectors under the control of heterologous regulatory elements. The screening of expression library allows the identification of specific properties, improved or novel properties. Expression libraries in combination with a powerful screening assay provide a useful approach to the systematic study of protein properties, regulation, function, and for protein engineering strategies.

SERVICE
• Expression library for any organism (DNA)
• Expression library and screening: E. coli, Saccharomyces cerevisiae, Yarrowia lipolytica
• Combinatorial libraries for co-expression: E. coli, S. cerevisiae

EQUIPMENT
• Robotic liquid handler systems
• High throughput colony picker
Automated protein expression

DESCRIPTION
The successful engineering of interesting enzymes requires a suitable method to produce the recombinant proteins. Several alternatives can be considered although prokaryotic expression with host *Escherichia coli* remains the most widespread method, benefitting from easy genetic manipulation and simple cultivation strategies. Yeasts such as *Saccharomyces cerevisiae, Pichia pastoris* or *Yarrowia lipolytica* can also be considered, as they offer post-translation glycosylation and extracellular production allowing straightforward downstream purification. Alternative methods can be tested upon discussion with the partner. In all cases, optimisation of gene expression through strain selection and growth condition is a key factor for many projects. Fast results can be obtained thanks to miniaturisation and automatisation of this screening phase via high-throughput gene expression. Thanks to expert technical back-up, growth in microtiter plates (standard/deep-well) coupled to experimental design enables to test an adequate number of strains, medium compositions, temperatures and thus contribute to set up the most efficient cultivation conditions.

SERVICE
- *E. coli / B. subtilis / S. cerevisiae / Y. lipolytica / P. pastoris / cell free*
- Strain selection
- Medium / temperature optimisation
- Experimental design / Response Surface Methodology

EQUIPMENT
- Liquid handling automats
- Colony pickers
- Microplate incubators

| Phase I : cloning | - gene synthesis with optimized codon sequence  
| - cloning into vector of choice |
| Phase II : selection of expression host | - transformation (*E. coli*, *S. cerevisiae, P. pastoris, B. subtilis,..*)  
| - cell free possible |
| Phase III : small scale expression | - microtiter plate format, robotic platform  
| - medium / temperature optimisation,  
| - experimental design / Response Surface Methodology |
| Phase IV : lab scale validation | - flask scale production  
| - protein quantification |

Gene coding enzyme of interest
Recombinant enzyme
Automated enzyme screening

DESCRIPTION

To explore the enzymatic diversity coming from genomes and metagenomes, or generated through directed evolution, it is necessary to create libraries, containing from 105 to 1013 clones, which are subsequently screened to isolate the most interesting clones. As a consequence, to be successful, these projects need to rely on the development of fast and reliable robotic screening strategies. Depending on the enzyme, different strategies can be set up to precisely isolate the most interesting proteins. If cell viability can be linked to enzyme activity, selection methods will be privileged as this usually allows to explore the largest diversity. Otherwise, activity of individual clones will be assayed either in solid or liquid format. Native substrates will always be favored for setting up screening assays, providing cost and method of analysis satisfy the expected throughput. Alternatively, screening can rely on chromogenic or fluorescent substrates, allowing quick and higher throughput assays. As a consequence, enzyme screening service comprises first a thorough setting up of the automated protocols prior to library screening to ensure adequate isolation of interesting catalysts (“you get what you screen for”). Subsequent high-throughput experiments rely on specific equipments such as colony picker/microplate handlers, liquid-handling automats and microplate readers.

Applicable to:
E. coli / B. subtilis / S. cerevisiae / Y.lipolytica
Functional metagenomics
Enzyme optimisation via directed evolution
Sample consortia

![Diagram showing the process of automated enzyme screening]

**Diversity**
metagenomic, library, directed evolution libraries...

**SELECTION STRATEGY**
- miniaturisation of enzymatic assay
- optimization of growth conditions
- validation of assay robustness

**SCREENING STRATEGY**
- colony picking, organisation in microtemplates growth
- library screening

**Hits**
- Repetition of assay

**Confirmed hits**
Computational gene sequence search and selection

DESCRIPTION

Selecting the right enzymes to catalyze each reaction step in order to produce a desired target compound is not trivial. Several computational enzyme and gene sequence design tools are available to provide enzyme selection solutions in different scenarios. One such tool is Selenzyme, a flexible online enzyme selection tool for metabolic pathway design that allows the selection of a short list of candidates for a given reaction pathway step. It graphically presents key information about enzymes based on existing databases and tools such as: similarity of sequences and of catalyzed reactions; phylogenetic distance between source organism and intended host species; multiple alignment highlighting conserved regions, predicted catalytic site, and active regions; and relevant properties such as predicted solubility and transmembrane regions. Selenzyme provides bespoke sequence selection for automated workflows in biofoundries. Additional software tools can then be used to design reusable DNA parts with simultaneous optimization of bespoke ribosome binding sites and enzyme coding regions for instance using PartsGenie software. These coding regions and regulatory parts can be combined in silico into large combinatorial libraries of pathway designs, which are statistically reduced using Design of Experiments (DoE) to smaller representative libraries. These libraries allow the efficient exploration of the design space resulting in tractable numbers of samples for laboratory construction and screening.
Enzyme/protein engineering

DESCRIPTION

Protein engineering allows the genetic manipulation of protein structures and the fusion of protein domains, to alter the activity of a given protein or to allow the cost-effective production of proteins or peptides. Since in vivo protein production depends on the balance between protein synthesis, structural maturation, and degradation (proteostasis), protein engineering requires knowledge of several biochemical, genetic and cell biology issues of proteostasis. The service provides scientific and technical support in designing and testing manipulation of amino acid sequences, to improve protein application in pharmaceutical/agroindustrial processes, green chemistry, synthetic biology, and sustainable bioenergy. Bacteria, yeasts, plant cells and whole plants can be used for the production of recombinant proteins. Each of these bioreactors has specific features and issues that need to be taken into consideration. Furthermore, products with unusual useful properties can be produced using extremophilic bioreactors. All cases will be individually studied, choosing the best prokaryotic or eukaryotic platform for engineered protein (EP) expression. Specific tags can be added to the engineered sequences to facilitate detection and purification procedures (if required), and fluorescent tags to allow easy subcellular localization in vivo.

SERVICE

Fully equipped laboratories of protein biochemistry | molecular and cell biology | radioisotope (alpha and beta emitters) | microbiology (class 1) | chemical analysis | plant cell culture and transformation | microscopy | plant growth chambers and artificial green house for germination/growth and maintenance of plants | bacterial bioreactors.

DESIGN

- Customers provide accession# or nucleotide/aminoacid sequence
- Educated in silico protein engineering, codon optimization, cDNA synthesis
- Analysis of the best targeting strategy in the chosen expression host

TEST

- In vivo testing into chosen host

Bacteria  Yeast  Plant protoplasts

- Analysis of protein size, level, stability, post-translational modification
- Permanent expression of the best EP(s) in the most suited host
- Analysis of EP accumulation, intracellular localization and stability in transgenic/transformed chosen host
Enzyme/protein discovery

**Design**

**Build**

**Test**

**DESCRIPTION**

This service is dedicated to broaden the field of industrial applications by mining genomes to discover novel enzymatic activities. To explore the microbial biodiversity of genes coding for enzymes, the service consists of a high-throughput process to clone, express and screen enzymes for the desired activity. Metagenomes (e.g. from wastewater treatment plant) and collections of prokaryote strains (including archaea) from hundreds of different genus are used as biodiversity reservoir to be explored. All the steps can also be carried out in 96-well plates and detection of positive candidates can be done either by spectrophotometry or Mass-spectrometry (LC/MS). The platform has already processed 10,000 CDSs for activity screening on various substrates. The process for searching novel proteins able to carry out a particular enzymatic reaction on substrates of interest includes the following steps: establishing a list of enzymes (or enzyme families) already described experimentally in the literature to catalyse reactions “similar” to the desired one; extending the diversity of this preliminary set of proteins by comparing the known enzymes with the proteome of sequenced bacteria or metagenomes, using low stringency parameters; reducing the number of selected candidates by clustering (based on protein sequence identity) to obtain an optimal set of proteins representative of the diversity of the family; cloning and overexpressing in *E. coli* the corresponding genes; screening on cell free extracts the desired substrates (commercially available and/or provided by the user) using UV spectrophotometry or LC/MS detection. At the end of the process a list of enzymes catalysing the desired reaction will be delivered to the user.

![Diagram of enzyme/protein discovery process](Image)
Functional characterisation of proteins

**DESCRIPTION**
Correct protein function depends on two major issues: 1) Correct folding, oligomerization, co- and post-translational modifications; 2) Correct targeting and sorting to the subcellular compartment of activity. These characteristics can be modified by industrial processing, resulting in increase or reduction of activity, depending on the desired goal. The user will provide the biological material or the industrially processed material containing the protein(s) of interest and, when available, specific antibodies.

**SERVICE**
Folding: resistance to proteolytic digestion, disulfide bond formation, solubility, reaction with conformation-dependent antibodies | oligomerization: velocity gradient centrifugation in different biochemical conditions | glycosylation: analysis with glycan-specific antibodies and deglycosylating enzymes | targeting and sorting: density gradient subcellular fractionation.

**EQUIPMENT**
Fully equipped biochemistry laboratories | protein electrophoresis equipment | equipment for ultracentrifugation | chemical analysis.
Computational genome annotation and mining

DESCRIPTION
Data from completed and ongoing microbial genome projects together with post-genomic experiments (i.e. transcriptomics, re-sequencing of evolved strains, mutant collections) allow users to improve their understanding of gene functions and metabolic pathways. MicroScope (an integrated platform dedicated to prokaryotic genome annotation and analysis in genomic and metabolic contexts) combines tools and graphical interfaces to analyze genomes and to perform manual curation of gene function in a comparative and metabolic context. Expert annotations are continuously gathered in the MicroScope database contributing to the improvement of the quality of microbial genome annotations, and thus to the predicted metabolic networks. Today, the resource contains data for >10K microbial genomes from which about 370K gene functions were manually reviewed.

SERVICE
Automatic annotation/analysis of the submitted sequences and availability of the data through the MicroScope website | technical support for all users | additional bioanalysis or scientific expertise on a collaborative basis | data conservation and backups (during the whole duration of the service) | computation updates taking into account primary data and sequences.
Training sessions are offered to users with two types of course: “Annotation & Analysis of Prokaryotic Genomes using the MicroScope platform” (1 week, 2-3 sessions/year) and “MicroScope Platform: Advanced course” which aims at presenting the last developments of the MicroScope Platform and tools dedicated to the exploration and the curation of metabolic networks (2 days, 1 session/year). The courses focus on how users can interpret the results of each bioinformatics method and combine them with other evidences to perform biological function expertise.
Production strain development
Automated strain construction

DESCRIPTION
We use genome editing tools to engineer microbial strains and develop strains with new properties for biotechnology applications, namely: Gene knock-in and knock-out, fine tuning of gene expression, multiplex genome engineering.

GMO/non-GMO | Manual/automatic | Homologous recombination (E. coli, yeasts) | CRISPR/Cas9 system (Clustered Regularly Interspaced Short Palindromic Repeats) (yeasts)

SERVICE
Genome editing | Strain selection | Phenotypic screening | Libraries of genes and engineered strains | Combinatorial approach.

EQUIPMENT
Robotic liquid handlers systems | High throughput colony picker.
High throughput phenotypic screening

DESCRIPTION

We use high throughput phenotypic screening tools to screen large micro-organisms libraries to identify specific phenotypes based on the growth or lack of growth on specific media, or linked to the consumption of a specific substrate. We use calibrated high throughput tools (high throughput colony pickers) to efficiently screen libraries of microorganisms and drastically reduce the screening time. Depending on the phenotype and the microorganism background (bacteria, yeasts, fungi, algae), we design custom screening protocols based on growth parameters, size of colonies, halo detection, consumption of a chromogenic or fluorescent substrate, expression of a fluorescent marker. The outcome of this service is the identification of “hits”, i.e. strains with the suitable phenotype(s). Using high throughput devices and automated workflows, we rearrange the libraries in libraries of “positive” strains and we provide biological material in the suitable format for further processes.

High throughput colony picking (up to 3,000 colonies per hour) | Organization of the mutant strains collection in microplates | High throughput screening on different media identification | and selection of hits.

SERVICE

Random mutagenesis libraries screening | Genomic and expression libraries screening | Bacteria, yeasts, fungi, algae.

EQUIPMENT

High throughput colony picker

HT phenotypic screening | HT genetic screening
Metabolic reconstruction & Phenotype modelling

UAB SysBiol | WU ISBE, BIODASH

VTT Bioreactors | CSIC

DESCRIPTION
Advanced Industrial Biotechnology processes require the optimisation of microbial biocatalysts based on the re-design of their metabolic networks synthetic biology technologies. Successful re-designing approaches require a better understanding of genotype-phenotype relationships. Modelling the phenotypic response of the cells to environmental conditions is key in the development of biotechnological processes. It can include different levels of detail, mainly depending on its foreseen application. The most commonly used are: black box, and dynamic steady models based on the elemental or macromolecular composition of the cell.
Algorithms and methodologies, collectively known as COBRA methods (Constraint-Based Reconstruction and Analysis), aim at the reconstruction and analysis of Genome-Scale Models (GEMs), which have also been termed BiGG (Biochemical, Genetic and Genomic knowledge bases). They are structured and species-specific knowledge-bases that contain detailed information on the target microorganism such as the exact reaction stoichiometry and reversibility, the relationships between genes, proteins, and reactions, as well as biomass composition and concurrent bio/chemical reactions together with the biochemical and physiological data available. The result is usually a stoichiometric model to be used in steady state conditions. Thus a mathematically structured knowledge base of a particular strain, species or even a microbial community is obtained.

SERVICE
G Genome sequencing and analysis | high-throughput phenotyping | Manual and omic-data assisted metabolic reconstruction and modeling | Large set of model-based microbial strain designing approaches.
The service allows for a high degree of flexibility, adapted to user requirements, covering from a single step optimisation to a complete optimisation workflow.

Main steps of the metabolic reconstruction & phenotype service
Exploratory cultivation in bioreactors

DESCRIPTION
The power of omics technologies to better understand biological reaction and microbial physiology are more and more used in order to improve bioprocess control or set up. Nevertheless study of impact of environmental conditions, medium culture, culture strategy (batch, fed-batch, continuous process) is limited by the difficulty to combine in an affordable timeline the collection, the analysis and the interpretation of data of significant relevance. Scale-down approaches can solve some of these issues.
The service allows to explore numerous combinations of cultivation process strategies in transposable mini-bioreactors in one shot experiments by combining the speed of our high throughput culture platform and the robustness of statistics tools. Different requirements are necessary: Robust cultivation process | Well known and understood cultivation process | High yield, high productivity, high titer of the desired product | High scalability of the process

SERVICE
Scale-down experiments | Experimental design with DoE strategy | Bioprocess strategy screening | Reproducibility studies Robustness studies.
Rational strain engineering

DESCRIPTION

This service provides a series of GM3 automates that enables long-term strain adaptations to obtain deviated phenotypes potentially interesting for scientific and industrial purposes. Typical strain developments include adaptation to alternative nutritional elements and growth media, temperature and pH optima, etc. Microbial cells can be evolved to degrade chemical wastes or to produce more efficiently useful compounds through foreign synthetic pathways implemented in fermenter strains.

SERVICE

Depending on the complexity and on the purpose of the project (i.e. academic or industrial), the use of the GM3 machines will be subject to a prior agreement to preserve the interests of both the user and the service facility. The strain to be evolved should be non-pathogenic, grow under normal (i.e. non-extreme) aerobic conditions and at reasonable growth rate (†Gen < 8h), and should not have tendency to rapidly form biofilms.

Delivery of the strain as well as desired medium formulation by the user | Suggestion and implementation of an appropriate continuous culture regime by the staff dedicated to running the platform, depending on the objective of the adaptation | Implementation of the continuous culture protocol (in general, the duration of the evolution experiment cannot be anticipated - it will be limited to six months to provide access opportunities to other users) | Delivery of the evolved strain to the user; upon request, the service can include sequencing of the strain.
Bioprocess development/optimisation
Computational pathway design

DESCRIPTION

The intelligent design of biosynthetic pathways is aided through the use of bioinformatics design tools to identify potential routes to target compounds. This strategy involves:

- Computationally driven strategies using encoded reaction rules
- Use of known metabolic reactions
- The design of pathways that encompass novel reactions not stored in metabolic databases
- Use of retro-synthesis algorithms

A range of algorithms have been developed that can be used to identify possible metabolic pathways and their corresponding enzymatic parts, which can then be ranked according to various properties and modelled in an organism-specific context. To expand access to diverse chemical targets, retrosynthesis approaches have been developed that explore the chemical biosynthetic space. One such tool is RetroPath2.0, which is an automated open source workflow (https://www.myexperiment.org/workflows/4987.html) based on generalized reaction rules that performs the retrosynthesis search from chassis to target through an efficient and well-controlled protocol. Its ease of use and the versatility of its applications make this tool a valuable addition to the biological engineer workbench. Its application has been demonstrated for the identification of alternative biosynthetic routes through enzyme promiscuity, its ability to perform inverse molecular design and to search bioactive molecules over chemical space, providing an automated procedure for chemical target prioritization. RetroPath2.0 plays a major role in reshaping the design, build, test and learn pipeline by driving the process toward optimized bioproduction.
Conceptual process flowsheet modelling

DESCRIPTION

The service converts choices of chemical paths into integrated process flowsheets. The process integration considers three levels of process development, namely process synthesis, process integration and flowsheeting:

Process synthesis addresses the selection of operating units (types of reactors, types and sequencing of separators, etc.) | Process integration builds efficiencies in selecting operating conditions and integration schemes amongst process operations | Process flowsheeting delivers final designs with complete mass and energy balances.

In process synthesis, the methods use a model-based approach that relies on superstructure optimisation to consider all possible types of units and configurations (units in series, parallel etc.; different sequencing and recycle structures). In process integration, the methods combine mathematical methods and thermodynamics using targeting technology, Pinch Analysis and Total Site Analysis. In process flowsheeting, the methods involve a multi-scale modelling approach that combines flowsheeting technology and offer access to commercial simulators (e.g. Aspen Plus, HYSYS, UniSim, SuperPro), modelling suites (e.g. Matlab, gPROMS, GAMS), property suites (e.g. GC, SAFT, Kranium) and services for custom-made models to enable a systematic integration of experimental data from labs, pilots and demos. The latter models may relate to substrate properties, reaction kinetics and/or mass-transfer parameters. Custom-made models may be combined with over-the-shelf models using CAPE-Open protocols to complete the process flowsheet. The service benefits from model libraries and flowsheets already in place from collaborative and industrial projects. It further includes access to Life Cycle Analysis software (SimaPro, Gabi) and/or open source databases to calculate sustainability indicators and impact factors (e.g. based on Ecoinvent).
Integrated bioprocess development and optimisation

DESCRIPTION

Within the area of industrial biotechnology, the aim is the production of bulk or fine chemicals as well as proteins or enzymes from renewable resources. The expectation of chemical industry and their customers are high quality chemicals with well-known functionalities, which leads to high value final products. To reach this processes, pretreatment, bioconversion - fermentation (upstream processing) and purification (downstream processing) need to be well established and optimized. Different requirements are necessary: robust enzymes or microorganisms with high productivity well known and understood metabolism which makes the bioprocesses more and more controllable bio-conversion processes should have a high productivity, high titer of the desired platform chemical and a high yield purification steps should be effective with high yield, eco-friendly and economical and should not require too many process steps scalability of the process itself needs to be considered in an early stage.

SERVICE

Scale down experiments related to industrial processes to investigate specific process parameter (with e.g. dimensionless numbers) | Multifactorial investigations of the influencing factors between renewable raw materials, microbiology, enzymology, technical scale of pretreatment, fermentation and downstream processing (including in-situ recovery) with the aim of economic processes of high social importance.
**Downstream processing**

**DESCRIPTION**

In every industrial biotechnological process, once the biotransformation stage in the bioreactor has been performed, it remains a highly important step to carry out the product recovery and purification: downstream processing. Fermentation broths are in general complex aqueous mixtures containing cells, extracellular or intracellular products, unconverted medium components or substrates. Suitable separation and purification stages must be designed taking into account: Characteristics of the product (size, charge, solubility, chemical nature, extra or intracellular location) | Future application (crystalized product, concentrated liquid, purity, undesired contaminants, bioproduct regulations compliance if any.

Each downstream process requires its own definition and the performance of each operation defined must be characterized determining yields and scale-up parameters.

**SERVICE**

- **Primary isolations**: centrifugation, filtration, disruption (for intracellular products) | Concentration/Purification: impurities removal (e.g. precipitation, chromatography, ultrafiltration, dialysis)

**EQUIPMENT**

- Tubular centrifuges and disk centrifuges at pilot scale | Equipment for tangential membrane filtration (micro- and ultrafiltration, nanofiltration, dialysis processes) | Mechanical cellular disruptor at lab scale and pilot scale ("french-press", homogenizer, sonicator) | Up to 30” filtration housing | Fast protein liquid chromatography (FPLC)

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Typical downstream flowchart
Development of enzyme-catalyzed processes

DESCRIPTION

Enzymatic processes are emerging as a powerful tool for obtaining a huge variety of target products in very different industrial sectors, such as pharma, food, fragrances, fine chemicals, materials, etc. The main bottle-necks for these biocatalytic processes are the biocatalysts availability and stability because enzymes are very unstable under rough reaction conditions (extreme pHs and temperatures, presence of organic solvents). Moreover, a lot of interesting biotransformations, like oxidations, are cofactor dependent (NAD(P)+/NAD(P)H) which should be recycled, in situ, due to its very high costs. On the other hand, most of the interesting biotransformations have to be performed using multi-enzymatic cascade reactions to get the target product. Different knowledge and skills have to be integrated in order to develop a successful and efficient enzymatic process.

SERVICE

Medium reaction engineering (including use of organic solvents) | Reaction optimisation (temperature, pH, substrate concentration, biocatalyst load, etc.) | Enzymatic reactor engineering (DSTR, CSTR, CPBR) | Cofactor recycling Biocatalyst immobilisation (including operational stability of the immobilized derivative).

Aiming to analyze the obtained results during the study, different process metrics are analyzed, such as biocatalyst yield, product concentration, conversion, selectivity, space time yield, etc.

EQUIPMENT

HPLC, GC, FPLC, HPLC-MS | Cell disruptor, centrifuges, lyophilizer | Spectrophotometer, fluorimeter | Enzymatic bioreactor.
Scale-up with algae

Fraunhofer
Fraunhofer CBP

DESCRIPTION

Microalgae belong to a highly diverse group of microorganisms whose components can be utilised both as a material and energy resource. Many algae strains produce high-value substances such as vitamins, carotenoids (pigments) and long-chain omega-3 fatty acids that can be used in the food, feed and cosmetic industries. Once growth is stopped due to limited nutrient availability, and adequate light and CO2 are made available, many algae produce oils (triacylglycerides) or starch as storage products that can be used as a renewable resource for biodiesel, ethanol and biogas production. Further biomass components such as proteins can be used in feed or food products. Algae do not compete for arable land or potable water, hence microalgae species growing in seawater or brackish water can reduce freshwater consumption.

Biomass production in photo-bioreactors for Scale-up. Over longer cultivation periods, high biomass concentrations, which are a prerequisite of high biomass productivities, can only be achieved in closed photo-bioreactors. The volumetric productivity, i.e. the product of biomass concentration and growth rate, is directly related to the amount of light available to the individual algal cells. High light intensities on the reactor surface have to be distributed to all cells by efficient and targeted mixing because of the mutual shading effect in dense algae cultures. Efficient intermixing in flat panel airlift photo-bioreactors allows an optimized specific light availability. As at outdoor conditions the available sunlight varies over the day and with the seasons, affecting the volumetric productivity, the biomass concentration must be adjusted to the available light intensities.

SERVICE

Scale-up of cultivation of microalgae from 6 L to 180 L volume per reactor (photo-bioreactor), reactor could be used single or in a line of 10 reactors (maximal cultivation volume) | Controlled parameter: pH, feed | Batch, fed-batch or continuous process | DSP: different processes available for cell separation, product extraction and drying | Providing samples for technical application tests (kg - t scale depending on the product).

Photobioreactor facility and mechanism of a flat panel airlift photobioreactor
Scale-up with bacteria

**DESCRIPTION**

This service studies the effect of volume increment in bacterial bioprocesses (bio-production, biotransformation, biomass production) and proposes adjustments to maintain the overall performance of the process. The scale-up study can start at different scales (Erlenmeyer flask; 50 mL, 0.5 L, 2 L bioreactors) up to different pre-pilot scales (20 L, 200 L). Scale-up is carried out on already defined processes and determined strains. In order to maintain culture performances from low scale to high scale, different physical parameters (as pH, T°, Np, kLa for example) have to be precisely adjusted. Optimisation of other parameters might also be considered and can be adapted. Medium composition adjusted to industrial practices | Simplification of culture process toward industrial infrastructure | Pre-culture steps (number of steps, inoculation ratio, pre-culture medium) | Stirring mobile device number and design (Rushton, marine blade). As high scale influences mass and energy transfer heterogeneity during the culture process, a robustness study is systematically realised by mapping culture performance according to deviation of the optimum set point for pH, temperature and oxygen pressure.

**SERVICE**

Technology: stirring tank bioreactors | Volumes for scale-up: 500 mL (Minibo, Applikon), 2 to 15 L (Biostat B, Sartorius, Applikon, Infors), 30 L and 300 L (Biostat D, Sartorius) | Measurements (in addition to routine ones): on-line turbidity, capacitance, gas analysis | Type of controls: pH, pO2 | Fermentation modes: Batch, Fed-Batch for all volumes, continuous up to 30 L | Product samples for application testing | Downstream processing available on site: centrifugation, micro-, ultra- and nanofiltration, HHP, freeze-drying, semi-preparative chromatography.
Scale-up with filamentous fungi

**DESCRIPTION**
Fungi are interesting microorganism with a broad range of products and often a high robustness to different substrates. However, cultivation of filamentous fungi is challenging especially submerged in stirred bioreactors because of their morphology. They may be present as mycelium or pellet or are simultaneously in several stages of growth (e.g. spores, germinating, hyphae). The morphology of the fungi usually influences the productivity and depends strongly on several parameters such as stirrer speed, aeration, gas distribution and mixing - which is important to taken into account during scale-up.

**SERVICE**
Process evaluation of the fungi fermentation with regard to scalability (e.g. biomass production during growth and morphology form) | Definition of scale-up parameters (e.g. tip speed, dissolved oxygen control) to obtain stable growth, morphology and production | Providing product sample amounts for technical application tests (kg - t scale depending on the product).

**Fermentation:** Stirred bioreactor in different scales: 10 to 10,000 liter (gross volume) | Batch and fed-batch cultivation (up to 10 m³).

**Downstream processing:** Separation technology (chamber press, vacuum drum filter or disc stack separators) | Purification technology (e.g. membrane filtration, crystallization, decolorization).

Consideration of appropriate fungal morphology during scale-up in stirred bioreactors
Scale-up with membrane bioreactors

DESCRIPTION

Membrane bioreactors are set-ups that couple membranes with bioreactors. The role of the membranes is:

Retention of microbial cells or enzymes: this results in high cell densities or increased biocatalyst concentrations | in situ product recovery: the selective removal of potentially inhibitory or unstable products from the fermentation broth as soon as they are produced, alleviates product inhibition or avoids transformation into undesired products.

The combination with membranes results in increased productivity, and/or improved product quality and/or higher yield.

SERVICE

Fermentor working volumes between 2 and 7 L, equipped with all standard auxiliaries and online measurements (agitation, pH, temperature, gas supply, etc.) | Suited for enzymatic and microbial conversions | Membrane filtration units available as tailor-made mobile skids and equipped with online measurement and control of temperature, pressure, flow, etc. at retentate and permeate side | Coupling possible with micro-/ultra-/nanofiltration for retention of enzymes or microorganisms and for production of tailored oligomer fractions | Coupling possible with pervaporation for selective removal of volatile products | Operation in batch, fed-batch and continuous mode | Certified for operation with GMOs and at Biosafety level 2 | All skids equipped with proper monitoring, automation and (remote) control | Possibility to supply product samples up to kg-scale.

Example of membrane bioreactor: fermentor with coupled pervaporation
Scale-up with yeasts

**DESCRIPTION**

With yeasts the production of a wide range of biobased products is possible, e.g. proteins and enzymes, chemicals such as carboxylic acids or pigments and fatty acids. They often have a broad substrate spectrum and are more tolerant against impurities. Therefore, yeasts are very interesting organisms for fermentation processes and industrial production.

**SERVICE**

**Process evaluation** of the in lab scale developed cultivation with regard to scalability (e.g. growth, induction and production phases, cascade operation) | Adjustment of control parameters and automation procedures within the different bioreactor scales | Definition of **scale-up parameter** (e.g. power input, dissolved oxygen control, feeding rates) to obtain stable growth and production | **Providing product sample amounts** for technical application tests (kg – t scale depending on the product).

**Fermentation:** Bioreactor cascade: 10 / 75 / 100 / 300 liter and 1 / 10 m³ (gross volume) | Automated methanol dosing | Batch and fed-batch cultivation (up to 10 m³) | Continuous cultivation with cell retention (up to 75 liter) | Aerobic and anaerobic process management possible | Designed for microorganisms with biosafety level 1 (BSL1).

**Downstream processing:** Separation technology (Disc stack separators, Chamber press, Vacuum drum filter, Vacuum filter dryer) | Cell disruption (High-pressure homogenizer) | Purification technology (Microfiltration, Ultrafiltration, Low pressure liquid chromatography, Crystallization) | Finishing (Spray dryer, Freeze dryer).

![Fermentation process including downstream processing depending on the product](image-url)
Scale-up with plant cells

**DESCRIPTION**

Plant cell cultures are increasingly applied in various industrial sectors such as pharma, cosmetics and food. The whole biomass can be used or certain fractions or isolated compounds. The service covers the whole process from initiating cell cultures to pilot scale production and various downstream options.

**SERVICE**

Access to biological material according to Nagoya regulation | Initiation of cell cultures from diverse explants | Maintenance of cell cultures in fully controlled growth room | Cryopreservation of cell cultures in liquid nitrogen for long-term storage/deposit | Establishment of suspension cultures | Upscaling of suspension cultures in disposable bioreactors or in stirred steel-tank bioreactors | Harvesting incl. filtering/centrifugation for separation of cells and medium | Downstream processing e.g. freeze- and spray-drying, extraction etc. | Formulation e.g. heat-treatment, encapsulation etc. | Bioactivity testing e.g. antimicrobial activity, digestibility etc. | Chemical analysis.

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Plant cell culture initiation and maintenance on plates; establishment of cell suspension cultures and long term cryopreservation; upscaling in disposable and steel bioreactors followed by downstream processing.
13C Fluxomics

**DESCRIPTION**

Fluxomics is the measurement of the real speeds of metabolic reactions in the integrated biological system (intracellular/intratissular fluxes). There is no direct method to measure intracellular fluxes in a cell or tissues. The most relevant indirect approach is based on **isotope labeling strategies**, here 13C, coupled with fine analysis (measurement of "isotopomers") of isotopic incorporation into metabolites, performed either by mass spectrometry (MS) or by nuclear magnetic resonance (NMR). It leads to obtain **flux maps** that represent the **quantitative distribution of the fluxes** (here carbon) in the metabolic network of the biological system studied.

Prerequisites: **Macrokinetic knowledge**: consumption and production rates, biomass composition | **Metabolic network** knowledge: topology, enzymatic reactions, carbon transitions | **Intracellular pools** from central metabolism | Validation of **sampling protocols** for isotopic profiling by MS or NMR.

**Biological Models**: Bacteria (e.g. *E.coli*) and yeast (e.g. *S. cerevisiae*).

**SERVICE**

HT - Fluxomics: Robotic platform available at INSA-TBI for HT-13C Fluxomics | 48 mini bioreactors fully automatised coupled to automated quenching and extraction modules for cells harvesting.
Proteomics, interactomics and peptidomics

DESCRIPTION
Proteomics and Peptidomics allow the large-scale study of proteomes and peptidomes. A proteome (peptidome) is a set of proteins (peptides) produced in an organism, system, or biological context. We may refer to the proteome or the peptidome of a species, a biological tissue/fluid or a cell compartment. Both are not constant; they can differ from cell to cell and change over time. Proteomics and Peptidomics are used to investigate: when and where proteins (peptides) are present; rates of protein (peptide) production, degradation, and steady-state abundance; how proteins (peptides) are modified (for example, post-translational modifications); the movement of proteins (peptides) between subcellular compartments; the involvement of proteins (peptides) in metabolic pathways/molecular processes; how proteins (peptides) interact with other biological macromolecules.

SERVICE
1D/2D-E or 2D-DIGE instruments for protein separation/quantification | Chromatographic instruments for peptide separation/quantification | MALDI-TOF-MS instruments for protein profiling | Robotics for automatic protein enzymatic digestion | Robotics for automatic molecular extraction from protein digests/biological samples | MALDI-TOF-MS instruments for peptide mass fingerprinting | MALDI-TOF-TOF-MS instruments for sequencing of peptides | nanoLC-ESI-LIT-MS/MS instruments for sequencing of peptides | nanoLC-ESI-Q-Orbitrap-MS/MS instruments for quantitative profiling and sequencing of peptides/proteins | Dedicated bioinformatics.
Fluorescence correlation spectroscopy for single molecule studies

**DESCRIPTION**
Fluorescence correlation spectroscopy (FCS) measures fluctuations in the small number of molecules in a focused laser beam. FCS is based on the analysis of time-dependent intensity fluctuations that are the result of some dynamic process, typically translation diffusion into and out of a small volume defined by a focused laser beam and a confocal aperture. When the fluorophore diffuses into a focused light beam, there is a burst of emitted photons due to multiple excitation-emission cycles from the same fluorophore. If the fluorophore diffuses rapidly out of the volume, the photon burst is short-lived. If the fluorophore diffuses more slowly, the photon burst displays a longer duration. By correlation analysis of the time-dependent emission, one can determine the diffusion coefficient of the fluorophore. In the case of proteins, these macromolecules are able to display a large set of conformational fluctuations even at thermodynamic equilibrium. The timescale range of these fluctuations is from picoseconds to seconds. Different spectroscopic techniques and in particular fluorescence spectroscopy such as the fluorescence steady state and fluorescence dynamics (time and frequency domain lifetime measurements) that provide data in a millisecond and nanosecond timescale, respectively, give information about the dynamics of proteins. Many dynamic processes in proteins, however, occur on microsecond to millisecond timescales and with these techniques they are difficult to measure. FCS is a very powerful technique to explore dynamics in the microsecond time region.

**SERVICE**
Characterize the dynamics fluctuations of molecules, with biochemical and biophysical interest, through the measure of their diffusion-associated properties | Characterize fluctuations in fluorescence intensity caused by chemical kinetics and/or photophysics events of biomolecules | Study the folding events of a single molecule at a time, through the measurement of intra molecular diffusion coefficients of denatured and partially folded states | Characterize the protein-protein and protein-ligand interaction following the conformational fluctuations associated to the binding event

**Single protein stability studies as function of pH values**
Metagenomics and metabarcoding

**DESCRIPTION**
This service provides scientific and technical support for the metagenomic and/or metabarcoding analyses of different environmental samples (soil, water, gut, etc). A fully operational genome facility with two Illumina Next generation Integrated systems, Droplet Digital PCR, QuantStudio Real-Time PCRs, computer farm equipment for data analysis and storage is available. Metabarcoding analyses consists of advanced microbial community analysis through next generation sequencing/massively parallel approaches directly from environmental samples. Metagenomics, i.e. shotgun whole genome sequencing of the microbiome through next generation sequencing, is the approach used to analyze all of the genes present in a microbial community, providing not just phylogenetic analysis but also insight into the functional capabilities of each species within the community.

**SERVICE**
Extraction of genomic DNAs directly from the environmental samples | Preparation of metagenomic libraries | Shotgun metagenomic sequencing by Next Generation Sequencing using Illumina technology (Metagenomics) | Bioinformatics analyses (meta-assembly, gene findings and annotation, metabolic reconstruction) and data delivery | 16S rRNA sequencing by Next generation sequencing using Illumina technology (Metabarcoding) | Bioinformatics analyses: micro-organisms (bacteria, yeast, fungi) identification.

All cases will be individually studied, choosing the best technical approach for the analyses. Full service plan, including full coverage for parts and reagent replacement upon failures, will be provided. The data are generated in FASTQ format, .txt format and FASTA file and are both stored or sent to the customers.
High throughput genome sequencing & transcriptomics

DESCRIPTION

We offer a wide range of sequencing services, relying on a diversity of state-of-the-art equipment, protocols, methodologies and bioinformatics tools, allowing to apply the right sequencing and analysis strategy and expertise to each project. A typical project includes the following steps: Decide on the project strategy in close interaction with the user | Sample reception: cell culture, lysates, extracted nucleic acids, etc. | Sample Quality Control (QC) & recording in the Laboratory Information Management System (LIMS, tracking system) | Construction of sequencing libraries | Sequencing & primary data treatment (QC) | Bioinformatics analyses as pre-decided on a case-by-case basis.

More specifically, the available sequencing equipment includes massively parallel (NGS) Illumina sequencers (NovaSeq 6000, HiSeq 4000, HiSeq 2500, MiSeq) for ultra-high-throughput genome, metagenome of metatranscriptome sequencing or for more tailored needs. Also, Oxford Nanopore Technologies machines (MinION, PromethION) are now routinely used to produce long reads for efficient genome assembly applications as well as for transcriptomics, allowing full-transcript sequencing and/or alternative splicing analysis. The associated IT infrastructure ensures efficient project tracking and control of the sequencing operations from sample reception to data treatment, analysis and delivery, and guarantees the appropriate level of confidentiality.
Biomass compositional analysis

DESCRIPTION
For the development of a Genome scale model and the analysis of its performance, it is crucial to determine the elemental and macromolecular biomass composition of the organism of interest. To this purpose, the following analyses are performed on freeze-dry biomass as detailed below. Elemental composition analysis In this analysis, the major elemental components of the biomass are determined. Namely: C, H, O, N, S and P content is evaluated. C, H, N, S and P are analyzed by combustion at 1200 °C and subsequent gas chromatography. Oxygen is determined through an oxygen-specific pyrolysis at 1060°C.

SERVICE
Macromolecular composition analysis In this analysis different macromolecular components are determined: Protein content: Includes both total protein content as well as fractional amino acid composition | Carbohydrates content: Includes total carbohydrates content as well as trehalose and glycogen content | Lipid content: Includes total lipid content as well as the fractional composition of its major components (Ergosterol, triacylglycerides, etc.) | Total RNA content | Total DNA content | Other relevant compounds (trehalose, glycogen, ...) as proposed by the client can also be added. The obtained data allows for the calculation of stoichiometric and/or mass fraction equations that can also be provided upon request. Additionally data reconciliation analysis between elemental and macromolecular composition analysis can also be provided upon request.

Main biomass compositional analysis service steps
Metabolomics and metabolite analytics

**Description**

**Exometabolomics**, also known as ‘metabolic footprinting’ is the study of extracellular metabolites and is a sub-field of metabolomics. The analytical approaches used for the analysis of exometabolome are: LC-MS | NMR | GC-MS. The analysis of exometabolites 1) is most commonly focused on investigation of the transformations of exogenous metabolite pools by biological systems 2) is performed by comparing metabolites content at different time points 3) can differentiate different physiological states of cells. In many cases, the exometabolite (extracellular) pool is less dynamic than endometabolite (intracellular) pools which are often perturbed during sample processing.

In **Endometabolomics** intracellular metabolites are analyzed by mass spectrometry using: **Untargeted metabolomic approach** which offers broad analysis of a wide range of metabolites including chemically unidentified ones and the data is analyzed using multivariate statistics. The untargeted metabolomics service utilizes either ultra-high performance chromatography (UPLC) with a high-resolution mass spectrometer or comprehensive, two-dimensional gas chromatography coupled to a time-of- flight mass spectrometry (GCxGC-TOFMS) | **targeted metabolomics approach** which provides an optimised analysis of predefined metabolites of interest. The advantages of targeted metabolomics are a higher throughput and accurate quantitation using internal standards. The targeted metabolomics analysis are performed using UPLC-MS/MS or GC-MS techniques.

Rapid sampling and immediate intracellular metabolite extraction are key factors governing the usefulness of endometabolomics since intracellular metabolite concentrations can change within minutes. **Endo-** and **Exo-** metabolomics are also used as a complementary tool with genomic, transcriptomic and proteomic data, to gain insight into the function of genes and pathways.
**Optimal portfolio**

Biomass to:
1. Xylitol
2. Itaconic acid
3. Poly-Urethanes

<table>
<thead>
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**Lignocellulosic Biorefinery**
**Techno-economic assessments**

**DESCRIPTION**

The service provides scale-up and techno-economic analysis of process flowsheets. The assessment involves different levels of scale-up and economic analysis that include: (a) sizing of process equipment and the selection of construction materials; (b) investment analysis based on time-invariant and/or time-dependent economic criteria: payback times, Net Present Values (NPV), Internal Rates of Return (IRR) etc, and (c) economic and environmental trade-offs between capital and operating costs to determine preferred levels of operation. The analysis extends with the integration of process supply chains (upstream costs for logistics and storage; downstream costs for storage and product deliveries) and explains benefits in sharing resources from available infrastructures and nearby chemical plants. Costing and sizing may relate to continuous, semi-batch and batch operations; batch production considers planning and scheduling solutions for the plant.

Scale-up and sizing methods involve commercial (APEA/Aspen Plus) and in-house models that relate to individual pieces of equipment, functional units, processing blocks or the entire manufacturing plant. The range of costing covers problems in Class 5 (technology conception and lab validation) that is appropriate for TRLs 1-4, Class 4 (technology validation and demonstration) for TRLs 5-6, and/or Class 3 (operational system demonstration) for TRL 7. In collaboration with end-users, the service extends to model and cost CAPEX of non-conventional equipment (mainly reactors and separators). Similar collaboration is useful to assess the potential re-use and regeneration of utilities (water, solvents, acids, etc.) and the reduction of waste treatment costs and OPEX. The analysis of trade-offs makes use of mathematical and optimization models formulated at different scales and levels of investment.
Value chain analysis

DESCRIPTION

The service selects chemical paths and chemistries delivering product portfolios to best valorise available feedstocks. Reversely, the service determines the (combination of) feedstocks to produce selected products delivering co-product and by-products to better match the selection. The search is intended to systematically screen chemistries with respect to efficiencies, common intermediates, or underlying complementary patterns with capabilities to better share energy, water and solvents. The analysis assumes a global perspective to intensify processing flows, reduce processing costs, or trade-off economic benefits with environmental performance. Value chain analysis addresses multiple feedstocks, multi-period production further evaluating the plant flexibility and resilience against market and technology uncertainties or regional constraints. The methods deployed combine mathematical optimisation, short-cut models and thermodynamics. Mathematical optimisation analyses degrees of freedom in relation to reaction paths, processing units and stages, engineering and market parameters, access to feedstocks, and integration patterns. Services are benefitted by ontology engineering and semantically-enabled developments that automate the formulation of optimisation models, systematise and parallelise the optimisation search, also monitor and analyse the optimisation results. Results involve favourable patterns of integration that balance economic and environmental objectives under a variety of problem and market constraints. The analysis is flexible to involve multiple objectives, the impact of uncertainties on economic and technological parameters, regional supply constraints, and the impact of different business models.

Conceptual and short-cut cost models enable high-throughput capacity in the calculations whereas thermodynamics are useful to target performance ahead of detailed calculations. Environmental analysis involves the majority of environmental impact factors in LCA; the analysis is applied either directly on selected product portfolios or, alternatively, through in-house surrogate models to pro-actively sSearch for eco-friendly solutions ahead of design.

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