



Key Words

Technology transfer
Development and Scale-up Process
Implementation of microorganisms
Fermentation - SmF / SSF
Enzyme catalysis
Free / immobilized enzymes
Down Stream Process
Analytical

INSA Biotechnology Platform

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About the platform

The CRITT Bio-Industries was created in 1989. Since 2006, it is a laboratory of the Industrial and Commercial Activities Department (SAIC) of INSA Toulouse.

Its expertise and its 900 m² of technical hall allow the development of processes from proof of concept to pre-industrial scale-up, including the production of qualification batches.

In a partnership approach, it offers services or collaborations in applied research, research and development and experimental development.

The projects carried out at CRITT Bio-Industries generally have TRL levels of between 3 and 5.

Expertise

The CRT/CRITT Bio-Industries has experience and know-how in the development of processes for the use of microorganisms, enzymatic catalysis, purification and analysis of biomolecules.

Liquid state fermentation (LSF) and Solid state fermentation (SSF)

The know-how of CRITT Bio-Industries concerns the implementation of bacteria, yeast, fungi and microalgae with :

- Conservation of microorganism strains (cryoconservation, freeze-drying),
- Definition of optimal culture conditions in terms of physico-chemical parameters (pH, pO₂ via the agitation/aeration couple, impeller, temperature) and nutritional parameters (carbonaceous and nitrogenous sources, trace elements, vitamins) in SmF and SSF
- Implementation of microorganism culture in LSF in batch, fed-batch and continuous mode from laboratory scale (3.6 L bioreactors) to pilot scale (300 L bioreactor),
- Implementation of microorganism's culture in SSF from laboratory scale (2 L bioreactors) to pilot scale (30 L bioreactor).

Enzymatic catalysis:

- Definition of the optimal conditions of implementation of enzymes in terms of physico-chemical parameters (pH, temperature, inhibition, activation...) for realization of enzymatic conversions (hydrolysis, transfer, isomerization, oxidation...),

- Enzyme immobilisation on solid supports,
- Implementation of enzymes in free form in batch mode, or in immobilized form in continuous mode; from laboratory scale (5 mL to 2 L reactors) to pilot scale (300 L reactor).

Down Stream Processing :

- Solid liquid separation (centrifuge frontal and tangential filtration),
- Extraction of intracellular substances (Cell crushing (Beads mill, high pressure homogeniser), chemical and heat lysis),
- Chain of unit operations for the purification of molecules of interest (frontal filtration techniques with or without adjuvant, tangential filtration techniques (MFT, UF, NF, RO), chromatography, activated carbon, ion exchange chromatography, electrodialysis,...)
- Stabilisation (concentration, lyophilisation, fluidized bed),

Analytical tools :

Qualitative and quantitative analysis of many metabolites mainly by HPLC with coupling of different columns and detectors adapted to the searched metabolites (refractometer, fluorimeter, UV, DAD, pulsed amperometry, conductimetry), CPG.

Process book :

Data transfer from laboratory to pilot scale for pre-dimensioning and flow sheet redaction

Equipments

The technical resources available at CRT / CRITT Bio-Industries are of two types: laboratory resources for development assays and pilot-scale implementation resources for batch production and industrial pre-sizing with :

For biotransformation

- An area dedicated to microbiology (microbiological safety stations, scales, pH meters, autoclaves, incubators, microscopes)
- An area dedicated to fermentation/ enzymatic catalysis with bioreactors (from 2 to 300 litres) for submerged process (air lift and mechanically agitated reactors), solid medium fermenters (from 2 to 30 Litres) and centrifuges (laboratory and pilot scale).

For downstream process

- An area dedicated to frontal filtration process from laboratory scale (FPT Lab – Filtration Performance Test Lab) to pilot scale (press filter, centrifuge with filtration bag...)
- An area dedicated to tangential filtration process (micro-, ultra-, nano-filtration, reverse osmosis and electro-dialysis) from laboratory scale (on a few litres, membrane surfaces from 50 cm² to 200 cm²) to pilot scale for batch production and industrial pre-sizing (membrane surfaces from 0.35 m² to 10 m² to treat a few hundred litres), with ceramic or organic membranes
- An area dedicated to chromatographic separation (ion exchange, adsorption, activated carbon) with various columns from 2ml to 15L
- An area dedicated to drying with rotary vacuum evaporators (2L/h of water), freeze dryers with trays (until 6L/batch) and fluidized bed dryer (laboratory scale).

Team

- 1 director
- 5 project Managers : research engineers (master or PhD degrees) with background in chemistry, microbiology, fermentation, enzymology and purification
- 2 administrative staff
- 9 master or engineers degrees : purification Bioprocess, Biochemistry, microbiology, biotechnology (including biopharma)
- 3 technicians : purification bioprocess and fermentation

Continuous projects:

- Fermentation and continuous prepurification of diacid by Membrane Bioreactor with yeast. Production of several kg of purified product by continuous process of 4 weeks
- Production of a protozoa in continuous fermentation mode during 1 month
- Production of volatile fatty acids from fermentation of bacteria and selective extraction by adsorbant chromatography in semi-continuous process

Health projects:

Development and Scale-up projects in *E.coli*: Different development and scale-up projects have been carried :

- i) production, centrifugation and intracellular extraction of recombinant protein from *Escherichia coli* in a 50 L and 200L fermenter;
- ii) Assays for production, extraction, purification and analytical method optimization of different antigen sugars by using genetically modified *E.coli* bacteria strains.
- iii) purification of an active medical enzyme by UF, Chromatography (hydrophobic, molecular sizing, ion exchange)
- iv) Development and optimization of a bioproduction process targeting therapeutic RNA to address animal vaccination applications.

Development and Scale-up of enzymology projects:

- i) Racemic resolution of amino acids by enzymes
- ii) Immobilization of active enzyme on medical device
- iii) Modification of hydrophobicity surface properties of a medical device by enzymatic reaction
- iv) Production of an excipient in an injectable formulation by enzymatic hydrolysis of raw material, followed by purification (UF, NF, chromatography, precipitation)
- v) Enzymatic in vitro characterization of an active cocktail during simulation of digestive system

Others purification projects:

- i) Purification by HPLC of a molecule active on inflammatory phenomenon in order to characterize effect of pure molecule.
- ii) Purification of an antibiotic by separative chromatography