

Bio Base Europe Pilot Plant vzw – Internship

Below is a list of topics for which we have internship vacancies as of February 2022, except for topic 1 and 10 for which we still have internship spots open this year as well.

TOPIC 1 - Process development and scale-up for the fermentative production of L-lactic acid from 2G feedstocks

In the framework of the Flemish FUCATIL project, this research will focus on the sustainable production of biobased L-lactic acid (L-LA), starting from second-generation (2G) feedstocks such as old paper and cardboard. An efficient production process for L-LA will be developed that goes far beyond the state of the art. An engineered industrial yeast strain will be used which efficiently produces L-LA by anaerobic fermentation. Yet, to obtain an industrially competitive process, advanced fermentation development and fine-tuning is key. Therefore, this internship aims to investigate different fermentation strategies, including batch, fed-batch, continuous with or without cell recycle, and to optimize the most important process parameters such as pH, temperature, aeration and medium composition.

Besides this, the sustainable and economical recovery of purified LA presents the principal challenge in fermentative LA production today. Therefore, in situ product recovery (ISPR) will be assessed as an innovative strategy to improve the fermentation performance and efficiently obtain purified LA. Different techniques will be investigated, including (reactive) extraction, enzymatic esterification, and adsorption to specific resins. Then, the integrated production and isolation of high-purity L-LA will be fine-tuned and scaled up to industrially relevant scale (150 L). [PDB, EU]

TOPIC 2 - Microbial production of 1,3-butanediol with zero CO₂ emissions

Biotechnology, which makes use of microorganisms and their enzymes to produce chemical compounds plays an important role in the transition towards greener production routes. In the framework of the Flemish GREEN-B2B project, this research will focus on the sustainable conversion of waste paper into 1,3-butanediol (1,3-BDO), an important industrial platform molecule. Furthermore, a cutting-edge novel process will be designed that combines liquid and gas fermentation technology and allows the production of biobased 1,3-BDO while

avoiding any CO₂ emissions. For this, state-of-the-art engineered strains of Knall-gas organisms will be applied that are capable of reincorporating emitted CO₂, using H₂ gas as an energy input, to boost 1,3-BDO production beyond the theoretical limit.

During this internship, pioneering research will be performed that will considerably contribute to the shift towards a true bio-economy. In an initial phase, the lithoautotrophic growth of these bacterial strains on CO₂ and H₂ will be investigated and their production of 1,3-BDO will be evaluated. Then, the production organism will be grown in a state-of-the-art 1L pressure gas fermenter using waste paper as a feedstock. Finally, this leading production process will be optimized and key process parameters including the composition of the fermentation medium, H₂ gas input, pH and temperature will be fine-tuned. [PDB, EU]

TOPIC 3 - Cultivation of industrial high-value biomass with enhanced viability

The commercial interest in functional foods that contain live microorganisms, also named probiotics, has been steadily increasing over the past decade. While lactic acid bacteria have become a fixed value in the dairy industry, the wide use of yeast biomass in foods and nutritional supplements is well-known. This has forced the creation and optimisation of industrial high-value biomass production processes, where innovative technologies are continuously being developed. Indeed, the efficient production of high-value biomass with high cell viability and minimal contamination remains a major challenge up to date.

This research project aims to develop state-of-the-art cultivation strategies to tackle these challenges. Firstly, this involves high cell-density cultivation, where high amounts of biomass are produced by using advanced feeding strategies and close monitoring and control of process parameters such as temperature, pH, oxygen supply and nutrient availability. Secondly, the accumulation of stress metabolites will be induced to prepare the cells for drying later in the process. For this, stress conditions will be imposed in the final stage of fermentation, such as nutrient starvation, osmotic shock or thermal shock. Thirdly, advanced downstream processing (DSP) techniques such as centrifugation, cross-flow filtration and decantation will be evaluated to prepare high-value biomass products while maintaining high quality and cell viability. Finally, the developed global production process will be taken to the next level, where scale-up to 150 L pilot scale will take place alongside experienced engineers. [PDB, EU]

TOPIC 4 - In situ product recovery of muconic acid, a hot industrial platform molecule - Process development and scale up. (Interns already selected- not available anymore]

This research will contribute to the Flemish SPICY project, aiming to provide the chemical industry with new or optimized processes to convert sugars into added value compounds. These include novel biobased chemicals as well as bio-derived equivalents to their current fossil-based industrial counterparts. Muconic acid (MA), a high value-added bio-product with reactive dicarboxylic groups and conjugated double bonds, is such a compound that has garnered increasing interest owing to its high potential for a broad range of industrial applications, among which bioplastics, which are rapidly growing in today's consumer market and greatly contribute to the bio-based economy.

During the research, you will focus on the fermentative production of MA using state-of-the-art microbial cell factories (MCF), as well as on the in-situ product

recovery (ISPR) of the produced MA. Recently, such a MCF has been custom engineered by the partners within the SPICY project. This promising strain can now be evaluated to produce MA. Following, you will optimize the fermentation conditions such as medium composition, pH, temperature, feeding strategies, oxygen consumption, etc. The fermentation strategy will be developed with the ISPR of MA in mind. For this, you will apply several techniques such as the use of resins, solvents, membranes, and combinations of the above. Finally, you will get the opportunity to bring this process to an industrial level by scaling up the fermentation with ISPR to produce kg quantities of MA, which will allow representative application testing. [PDB, EU]

TOPIC 5 - Process development for the conversion of waste CO₂ into value-added chemicals

This research topic is framed within the BIOCONCO₂ project, aiming to create value from waste CO₂ and therefore assisting in the world's most imminent challenge: global warming. You will be part of the team involved in gasfermentation, a technology which makes use of micro-organisms to convert CO₂ into value-added chemicals. The fixation of CO₂ requires an energy source, most often in the form of hydrogen gas, which possibly results in the formation of explosive gas mixtures. Therefore, BBEPP invested in state-of-the-art gasfermentation vessels, each with a total volume of 1L, which enables the feeding of combinations of CO₂, H₂ and O₂.

The research will enclose a screening of several CO₂-converting microbial strains on the 100 mL scale. After selection of the best performing strain(s), experiments will be conducted in 1L gasfermenters. In this way, process conditions like pH, medium composition, partial gas pressures and gas flow rates will be optimized in order to improve cell growth, productivity, gas uptake rate etc. Some fermentations will also be scaled-up to mid-pilot scale, in a custom-built 10L gasfermenter.

The library of strains consists of two types of micro-organisms, utilizing different pathways for CO₂ incorporation: the acetogenic and the so-called knallgas bacteria. Both require a different approach for cultivation and more importantly have a distinct product portfolio, ranging from e.g. acetic acid and ethanol for the acetogens, to single-cell protein (SCP) and bioplastics (PHB) for the knallgas strains.

You will be a full member of BBEPP's gasfermentation team, who will be guiding you closely through your internship and give you a first impression and broader look into the (biotech) industry. [KQ, EV]

TOPIC 6 - Fermentative conversion of CO₂ into high-value products towards a circular economy

The accumulation of CO₂ in our atmosphere is an omnipresent topic requiring immediate and large-scale solutions. In contrast to considering it as waste, CO₂ could serve as a resource for the synthesis of green alternatives for today's fossil-based products. This concept, known as carbon capture and utilisation (CCU), is gaining industrial attention because of the need for reduction of CO₂-emissions and the opportunity to make useful CO₂-based products.

This internship will focus on the biotechnological utilisation of CO₂. During gas fermentation, CO₂ and H₂ are combined as, respectively, carbon and energy source for microbial cultivation. The main bottleneck of this technology is the uptake of the gaseous feedstock. A strategy to enhance the CO₂ fixation

efficiency is by implementing a two-stage process. CO₂ is first transformed into simple liquid molecules. Thereafter, starting from these CO₂-derived intermediates, the fermentative conversion reaches higher productivities.

The main goal of this research topic is the development of a CO₂/H₂ gas fermentation, which will be intensified by elevated pressure, and a CO₂-based liquid fermentation. In both cases, the product of interest is the bioplastic polyhydroxybutyrate (PHB). The findings resulting from this research topic will be integrated in EU-funded projects and will benefit several companies, going from those who need to lower their emissions, to those who can use the CO₂-based products.

Regarding the gas fermentation experiments, four state-of-the-art parallel fermenters will be used. Liquid fermentation strategies will be developed in the lab and scaled up to 150L fermenters together with experienced engineers. [KQ, EV]

TOPIC 7 - Developing a yeast cultivation and propagation strategy for the production of biofuels from MSW (Interns already selected- not available anymore]

The development of a fermentative production process of isobutanol from paper and cardboard waste comprises three main steps:

1. The saccharification of cardboard to glucose and xylose using a special enzyme cocktail;
2. The propagation of an engineered *S. cerevisiae* strain up to a very high cell density culture;
3. The fermentation of the saccharified cardboard to isobutanol using the freshly propagated yeast culture.

In situ product recovery (ISPR) of isobutanol during the fermentation will be required to keep the isobutanol below its toxicity concentration.

This internship will mainly focus on the development of a yeast propagation on 7L bioreactor scale, aiming to improve yields and to minimize the overall cost prize. However, the saccharification will also be further improved at lab scale and different ISPR methods will be further explored. [CVC]

TOPIC 8 - Development of a downstream process for the production of a main polymer building block used for the production of eco-polymers

Glaukos is a European project which aims at developing ecologically friendly polymers for usage in the marine environment. The scope of this internship is to develop a downstream process for the production of this building block from a fermentation broth. A literature study aims at mapping the state-of-the-art of this building block's purification from fermentation broth, both at industrial scale and at research level. Practical work will be performed at lab scale to evaluate existing techniques as well as testing new approaches to the downstream process. As the goal is to design an industrially relevant process, the focus is on scalability of the tested techniques. Depending on the progress of the project, some lab-scale fermentations can also be included in this work. [LDB]

TOPIC 9 - Developing an industrial process for the biocatalytic production of vanillin from 4-n-propylguaiacol in the frame of the European SMARTBOX project

An important strategy to combat climate change is the use of biomass as carbon-neutral feedstock in the chemical industry. KU Leuven has developed a new technology for hydrolyzing lignin sources into relatively pure monomer fractions.

This groundbreaking research opens the door to using these monomers for high value components. One of these test cases is the production of vanillin from the monomer 4-propylguaiacol. In the SMARTBOX project, new enzymes have been developed which catalyze this two-step conversion.

In this internship, this enzyme will be used for the development of an industrial process, applying bioprocess engineering principles as most important tool. First, the enzyme production and purification will be developed and optimized at 10 L bioreactor scale. This enzyme will then be used to convert 4-n-propylguaiacol into vanillin. The reaction conditions (substrate concentrations, pH, etc.) during the bioconversion will be optimized through a Design of Experiments using 4 x 1.5 L bioreactors. Finally, the entire developed process will be demonstrated at small pilot scale (150L), and the resulting product medium used to develop an efficient downstream purification of vanillin.

Check the website of the smartbox project for more information: <https://www.smartbox-project.eu/> [TD]

TOPIC 10 - Meat the future, today!

Global food consumption keeps rising and to keep up with the demands of an est. 10 billion people by 2050, especially the conventional meat industry is far from sustainable. Also, a rising consumer concern for animal welfare and the burning issue of climate change, we must urgently revolutionize the foundations of this industry.

Many meat processing companies envision cultured meat as promising alternative to (partly) replace traditional animal-derived raw materials. With the arrival of cutting edge cultured meat technologies, Flanders has everything what it takes to be a pioneer in this field - a leading role in biotechnology on a scientific level, a strong food industry, and a history of expertise in fermentation (cell growth) technologies, both in research and industry.

Animal cell cultures have been used for over a century in research and medical settings providing the basic principles of cell culturing. However, the culturing of these cells for food production comes with its own set of challenges, as this for one requires a low media cost and high cell densities to become economically relevant. During this internship, you will be involved in tackling the challenging task of developing an industrially scalable cell proliferation and differentiation process for an embryonic cell line. This will be achieved by medium engineering, optimizing the feeding strategy, agitation, temperature etc. An optimized process will subsequently be scaled-up to enable the development of a downstream processing method that meets the demands for application in food products.

[MVB]

Which student profile do we require?

Requirements are:

- You are studying for a degree in bio-engineering or related (bioprocessing, chemistry, biotechnology, cell- and gene technology)
- some experience with bacterial, yeast or fungal hosts is an asset
- some relevant experience with biocatalytic processes for the production of

chemicals, food ingredients or cosmetics is an asset

- The duration of your internship is at least 6 months.

What do we offer?

We offer a dynamic, international and young working environment and a full learning experience. You are based at the Bio Base Europe Pilot Plant, situated in the port of Ghent in Belgium.

The internship runs for a minimum of 6 months fulltime to preferably a whole academy year fulltime.

<https://www.bbeu.org/>