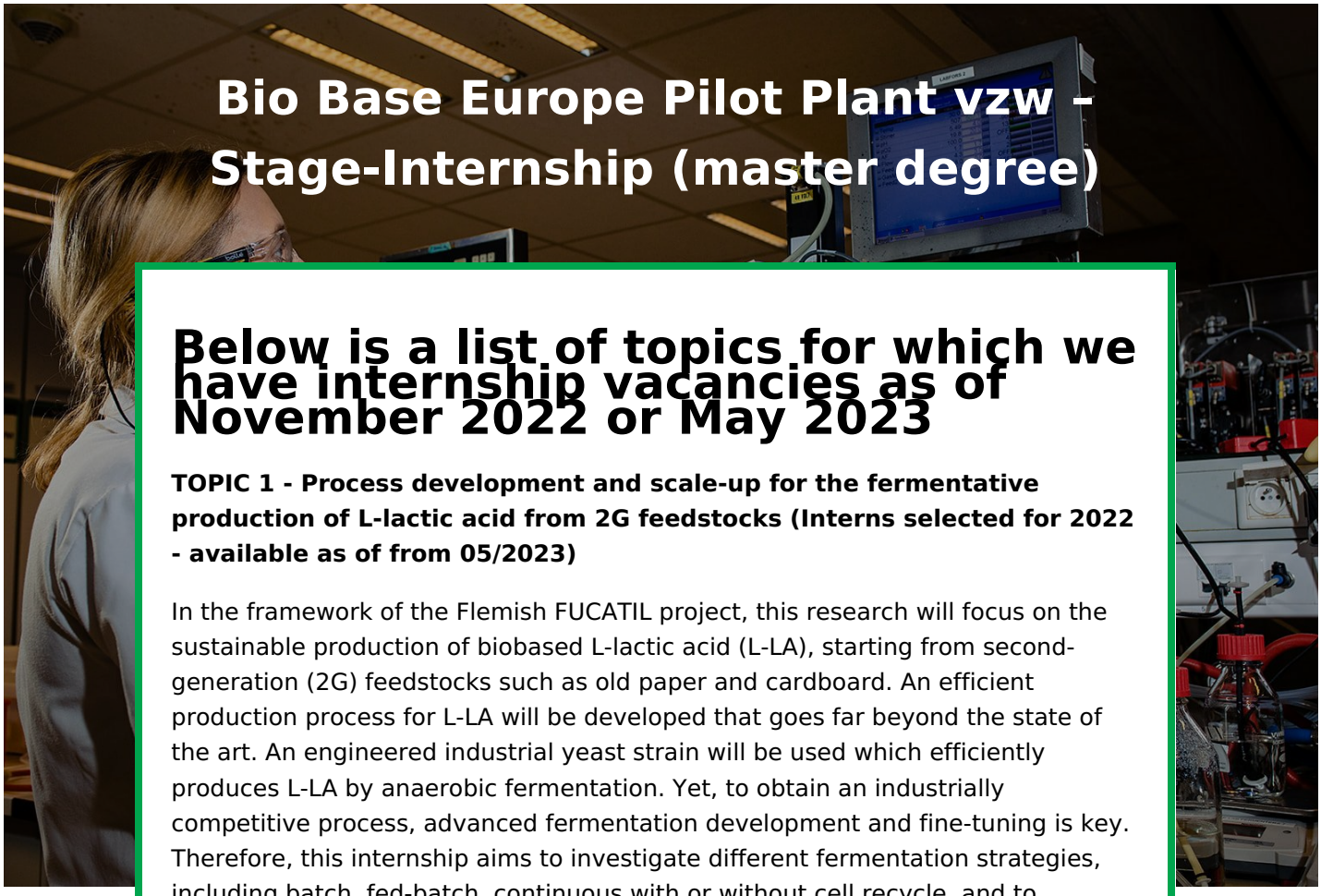

Bio Base Europe Pilot Plant vzw – Stage-Internship (master degree)

Below is a list of topics for which we have internship vacancies as of November 2022 or May 2023

TOPIC 1 - Process development and scale-up for the fermentative production of L-lactic acid from 2G feedstocks (Interns selected for 2022 - available as of from 05/2023)

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 (Interns already selected - not available anymore)

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 (interns already selected - not available anymore)

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 - Process modelling, optimization and techno-economic evaluation for the fermentative production and purification of L-lactic acid (Interns selected for 2022 - available as of from 05/2023)

The industrial production of renewable chemicals and materials through fermentation presents an excellent case of a sustainable biorefinery concept that can boost the transition towards a circular bioeconomy while reducing the dependency on fossil resources. Yet, this relies on the availability of low-cost feedstocks, the efficiency, scalability and sustainability of the various process steps, as well as the final yield and market potential of the obtained target products. This research will focus on the sustainable production of biobased L-lactic acid, using engineered industrial yeast strains in advanced fermentation processes. Here, the efficient and economical recovery of purified LA presents the principal challenge in fermentative LA production today. Various state-of-the-art purification techniques will hence be considered, including (ion exchange) resin adsorption, solvent extraction and membrane filtration.

Yet, to get a clear view on the economic and industrial feasibility of these

innovative production routes towards biobased L-lactic acid, and to identify the most promising strategy, the different fermentation and downstream purification processes will be modelled using the SuperPro Designer software, and a comprehensive and in-depth techno-economic assessment (TEA) will be conducted. The latter will comprise technical (performance) and economic (cost) considerations of each process step, as well as a thorough sensitivity analysis, while being applicable from an early TRL level up to full industrial scale. This will allow to compare various production approaches and technologies and to benchmark these against current fossil-based processes to produce L-lactic acid. From a broader point of view, this will also assist the chemical industry in Flanders to implement novel sustainable processes and ultimately, in their transition towards a true bioeconomy.

This internship takes place at Bio Base Europe Pilot Plant (BBEPP), a non-profit SME that assists companies and research groups to bridge the gap between laboratory research and industrial implementation of their innovations. BBEPP is equipped with all process and analytical tools which allow to perform high-quality research in an industrial environment. [EU, ST]

For this internship knowledge and experience in SuperPro Designer is required.

TOPIC 5 - Fermentation and DSP development for the sustainable production of CO2-based bioplastic (available as of April/May 2023)

Plastic has become indispensable in our contemporary society, yet the ever-increasing use of this mostly fossil-based material has a disastrous impact on the environment. In this context, bioplastic derived from renewable resources has attracted attention as promising substitutes. As such, poly-hydroxybutyrate (PHB) is an increasingly investigated biopolymer, known for its excellent biodegradability under various conditions. Its applications are diverse, ranging from compostable food packaging to medical components owing to their biocompatibility.

PHB is obtained by the cultivation of bacteria accumulating the biopolymer as intracellular carbon storage in response of physiological stress. This internship will focus on the development of a biological process to produce PHB using acetate as carbon source. Recently, research at BBEPP showed that acetate can efficiently be obtained from the microbial conversion of CO2, hence, the proposed strategy enables the production of CO2-based bioplastic. In addition, the downstream processing (DSP) to extract the PHB from the microbial cells will be examined. This involves the evaluation of different methods for cell disruption, PHB separation, and purification.

The goal of this internship will be to establish a complete bioprocess including the cultivation of an already modified bacterial strain on CO2-derived acetate, the development of a fermentation in a bioreactor, and the investigation of several DSP technologies. The practical work will be performed in cooperation with experienced engineers at BBEPP by using a wide range of specialized equipment available in house. This research frames within the EU-funded CO2SMOS project for which the obtained results will serve as a starting point for further scale-up (https://co2smos.eu/). [EV, KQ]

TOPIC 6 - 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 labscale and different ISPR methods will be further explored. [CVC]

TOPIC 7 - Meat the future, today! (available as of April 2023)

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]

TOPIC 8 - *In situ* product recovery of fermentatively produced 2,3-butanediol (Interns already selected - not available anymore)

As sustainability becomes increasingly important in the industry, processes more often will become bio based. A biobased process goes hand in hand with the production of biogenic CO2 coming from processes such as fermentation or biomass combustion. It is therefore important that technologies are developed to recycle and convert this CO2 into value-added chemicals, which is naturally occurs in many microorganisms. Because of the low energy content in CO2, energy sources are required in the form of CO and/or H2. The mixture of these three gases results in what is known as syngas.

One such bio-based intermediate, which can be produced via syngas fermentation, is 2,3-butanediol (2,3-BDO), a promising bulk chemical. The

recovery of the product after the fermentation step is a challenging part of the process, as traditional downstream processes such as filtration techniques do not suffice for recovery of the product. An alternative downstream process is *in situ* product recovery via reactive extraction with solvents. This thesis will focus on optimizing the process by screening a variety of solvents on their ability to extract the product and their biocompatibility towards the production organism. Subsequently, the fermentation parameters will be optimized to obtain maximal process efficiency. For the final recovery of the product, a last re-extraction step will be developed.

The internship is framed within the EU-project CO2SMOS, which focusses on the transformation of biogenic CO2 into biobased chemicals. Experimental work will be performed with advanced equipment present at Bio Base Europe Pilot Plant, while being supported by experienced engineers. (KQ) (EVH)

TOPIC 9 - Capturing biogenic CO2 into biobased intermediates through gas fermentation (Interns already selected - not available anymore)

Due to its adverse effects on our climate, the accumulation of CO2 in our atmosphere is and remains a serious challenge. Yet, where CO2 was previously considered as a burden, it is now seen as a possible green resource for the synthesis of chemical intermediates and added-value compounds. This concept is known as carbon capture and utilisation (CCU) and can help to reduce CO2 emissions across industries. Industrial CO2 can be abiogenic, when it comes from fossil carbon, or biogenic, in case it originates from a natural resource. As CO2 has a low energy content, energy sources are required, which come in the form of CO and/or H2. The resulting gas mixture of CO2, CO, and H2 is called syngas.

This internship will focus on producing acetic acid via gas fermentation on syngas produced from biogenic CO2. The optimal process set-up will be evaluated in both batch, and continuous fermentation mode on scales ranging from up to 10 L. The process will be further intensified by means of cell recycle and the use of elevated pressures, aiming to improve the acetic acid productivity. The resulting acetic acid stream will be processed downstream and used for further conversion into high-value compounds in a second fermentation step.

The internship is framed within the EU-funded project CO2SMOS, which focusses on the transformation of biogenic CO2 into biobased chemicals in a truly renewable manner. With the support of experienced engineers, you will get the opportunity to perform work with state-of-the-art gas fermentation equipment, with the final aim of developing an industrially relevant gas fermentation process. (KQ) (EVH)

TOPIC 10 - Development of the fermentative production and purification of new-to-nature innovative biosurfactants, (Immediate available).

Surfactants produced by microorganisms (microbial biosurfactants) are gaining more and more industrial relevance as a more sustainable alternative to conventional – often petrochemically derived - surfactants. For example, sophorolipids produced by the yeast *Starmerella bombicola* are used in Ecover's ecological cleaning products. Through genetic modification of this yeast, several new *S. bombicola* strains have already been developed that produce new-to-

nature biosurfactants. These molecules have potential new applications in the agro, pharma, food, medical and/or cosmetic industries. Despite promising properties and strong interest from industry, productivity is still too low, resulting in an excessive production cost of these microbial surfactants.

In this internship, the bioprocess and purification for these innovative molecules will be further investigated at bioreactor scale in different advanced fermentation set-ups (i.e. fed-batch, high cell density and ex-situ cell retention) to further increase the productivity and yield. Through a combination of medium optimisation, process set-up and bioprocess parameters starting from erlenmeyer flask to 10L bioreactors you will improve the currently low productivities to industrially relevant standards and tackle the lack in uniformity. Finally, the optimized bioprocess and down-stream processing will be scaled-up to pilot scale (150L). In the long term, the results from this internship will lead to commercialization of these innovative and promising microbial biosurfactants.

This internship is framed within long ongoing research on biosurfactants at BBEPP and follows multiple EU-funded and international research projects over the past 10 years (Waste2Func, Applisurf, Carbosurf, Stabosurf, IB2M, Biosufing). You work in a well-equipped laboratory with state-of-the-art equipment and are guided by an experienced team of R&D and process engineers.

TOPIC 11 - **Development of fermentation process to convert 2G feedstocks into acetic acid with 100% carbon conversion efficiency. (available as of April/May 2023)**

In the framework of Flemish AC2GEN project, this research focusses on the production of biobased acetic acid (AA) using anaerobic bacteria (acetogens), starting from second generation feedstocks. Acetic acid serves as a versatile commodity chemical with a variety of applications in both chemical and biotech industries. Acetogens have a diverse metabolism, with advantages such as pathways which allow conversion of all saccharides, including hexoses, pentoses, di-,tri-, oligo- and polysaccharides, into AA, 100% carbon efficiency of sugars to AA,

hydrolytic capabilities, resistance to and even degradation of typical inhibitors, and more.

These characteristics have the potential to significantly increase the carbon efficiency on 2G feedstocks, beyond that of other biochemical pathways.

The first goal of this internship is to screen a variety of commercially available acetogenic strains and evaluate their performance to convert 2G sugars into AA. The best performing strains will be cultivated on real 2G hydrolysates, containing mixtures of 2G sugars, as well as typical inhibitors such as hydroxymethylfurfural. Analytical procedures such as HPLC and HPAEC will be used to follow up the consumptions of the substrates, as well as the accumulation of the target product and other metabolites. Then, the acetogenic fermentation process will be thoroughly studied in bioreactors, and optimized in terms of carbon efficiency and productivity.

The screening and identification of promising strains will be executed using serum bottles, to increase the experimental throughput, while the in-depth optimization of the fermentation process will be executed in bioreactors. To this

end, batch fermentations will be performed in BBEPP’s state-of-the-art 4x1L bioreactors, and the process will be scaled-up to 10L scale. The practical work will be executed at BBEPP, in collaboration with a team of experienced engineers. (NP)

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/