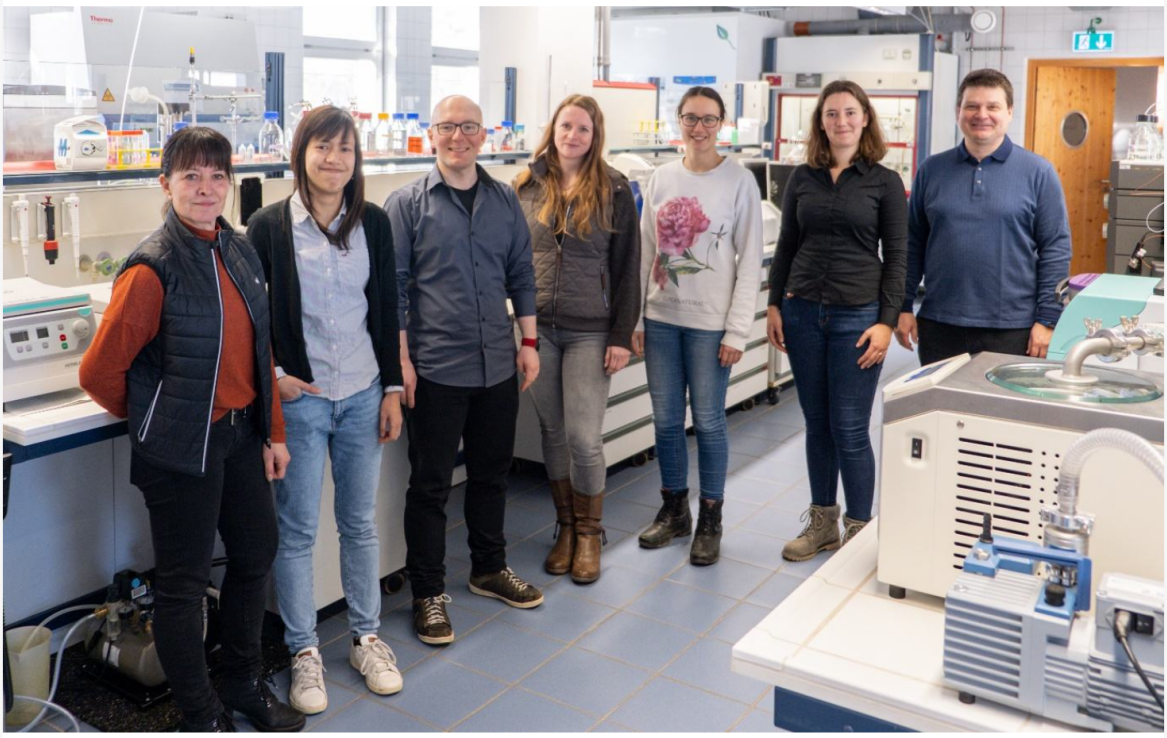
Overcoming Challenges in Filamentous Fungi Research: An Interview with Winda Soerjawinata



Winda Soerjawinata (second from the left) and her working group at the Trier University for Applied Sciences

In this interview, we speak with Winda Soerjawinata, who has extensive experience in fermenting filamentous fungi. Winda discusses her work producing a protease inhibitor using *Penicillium* sp. in bioreactors, as well as the challenges of working with filamentous fungi and the benefits of using non-invasive sensors to monitor biomass.

sbi: Who are you and what is your role?

Winda: My name is Winda, and I am a 4th year PhD Student at the Institute for Biotechnical Process Design, at the Trier University of Applied Sciences.

sbi: Which organism do you work with and what does your bioprocess look like?

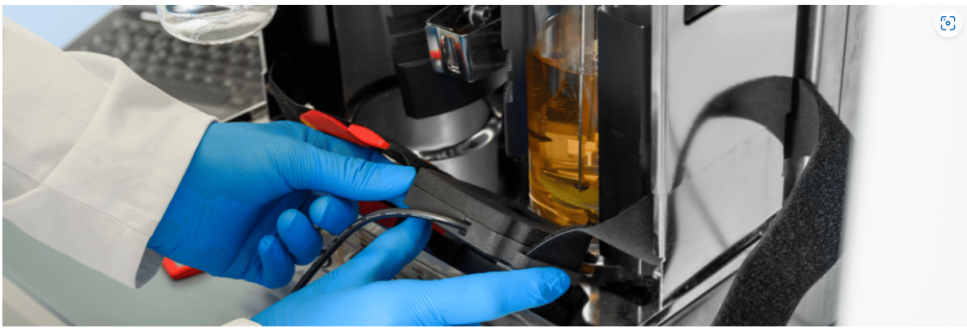
Winda: We are doing submerged cultivations of *Penicillium* sp., a well-known filamentous fungi species, in a 2 L double wall glass bioreactor. The cultivation usually lasts 5-6 days and is started by inoculating medium with fungi spores. During the fermentation, we regulate the dissolved oxygen (DO) in the medium; as soon as the DO-value drops below 30%, the atmospheric air flow is increased and if this is not sufficient, we provide pure oxygen. Before using the CGQ BioR, we could only roughly estimate the fermentation status by monitoring the pH of the cultivation broth.

sbi: How can you infer fermentation status from pH values?

Winda: Proton pumps in the plasma membrane of the fungus' cells release protons (H^+) into the medium, when substrate is taken up, thus acidifying the medium. When the substrate has been consumed completely, the pH in the medium rises again, indicating the end of the fermentation, typically after 120-140 hours (5-6 days). Therefore, the pH can be used as an indirect measure to predict the metabolic activity of the fungus and hence the phase of the fermentation.

sbi: You said you are working in bioreactors. Besides batch fermentations, do you also use other processes?

Winda: Besides batch fermentations, we have been developing a repeated-batch fermentation with the implementation of a sporulation support. Principally, during the batch fermentation the sporulation support captures some biomass. After the batch fermentation is ended, the fermenter is cleaned-in-place so that all biomass, except the one from the sporulation support is removed. The captured biomass on the sporulation support will undergo sporulation phase, and the spores will be used to start the next batch fermentation in fresh medium.



sbi: What are you producing with your bioprocess and what is the purpose of this product?

Winda: Our product is a protease-inhibitor protein that can inhibit the protease enzymes of Human African Trypanosomiasis or sleeping disease – an endemic parasitic disease of sub-Saharan Africa, transmitted by tsetse fly. The protease enzymes of this parasite are important for its survival and invasivity. Inhibiting them is one approach in fighting against the spread of the disease.

sbi: Can you tell us a bit more about your work with filamentous fungi? In your opinion, what is especially challenging about working with filamentous fungi?

Winda: Filamentous fungi can grow in several different morphologies, e.g., in free mycelium or pellet form. In our case, the protease-inhibitor is a secondary metabolite that is produced intracellularly and at the end of the fermentation process. So, it is beneficial for us to cultivate the cells in a pellet form, which makes biomass harvesting and product extraction easier.

There are several challenging spots with filamentous fungi. Finding a suitable biomass sensor for pellets, for example, was one of many. Many of the sensors available in the market are mostly not suitable for microorganisms that are not evenly distributed in the fermentation broth. Therefore, we used the gravimetric method of bio dry weight to determine the cell concentration, which is time- and energy-consuming.

sbi: How does a bio dry weight measurement work? What steps are included?

Winda: It is basically done by taking samples from time to time during the fermentation. The samples are then filtered using the pre-dried and pre-weighed filter paper. The filter paper together with its filter cake will be collected and put in an oven until the weight is constant. Biomass concentration is defined as the weight difference between filter with biomass and without biomass, per sample volume. Samples at more time points are for sure beneficial to get more accurate growth curves, for example every 4 or 6 hours, especially at the exponential phase. However, sampling for many time points quickly gets very exhausting.

sbi: Using the CGQ BioR, how did it change your process or your way of work?

Winda: Bio dry weight was our only method to determine the cell concentration, since optical density (OD_{600}) is not suitable for mycel-forming fungi. However, it has several disadvantages. It requires for example a lot of time to process the samples until we can obtain the data we need, and relatively large volumes must be taken from the bioreactor (in laboratory bioreactors this can be up to 10% of the total volume).

By using CGQ BioR, all we need is to attach the sensor on the reactor, start the fermentation, and monitor the whole fungal growth online without the need to take samples for dry weight. In our case, we can trace the fungal growth from the beginning to the end of fermentation very well. Furthermore, since the sensor is attached on the wall of bioreactor, it does not give additional shear stress to the fungi that could eventually affect the fungal growth. Besides the positive effects on the bioprocess, we don't need to be in the lab at random times any more to measure at specific time points, since the measurement automatically proceeds during nights and weekends.

sbi: As a scientist with great experience working with filamentous fungi, what would you recommend others that want to set up successful bioprocesses with this class of organisms?

Winda: It is important to find information about the fungi and the production of the target substance. Each strain has its own tendency to be in either free mycelium or in pellet form. If the fungi are subjected to be cultivated in pellet form, shear stress is one of the most important factors that needs to be kept in mind. On the other hand, with free mycelium, the oxygen mass transfer and the mixing process are crucial. Each fermentation condition must be considered carefully to ensure a good reproducibility of the fermentations. On some things, we must compromise. For example, we cannot use a high stirrer speed and aeration rate with pellets because of the added shear stress, even though that would improve the oxygen availability.