VALORIZATION OF WHEAT BRAN (WB) BYPRODUCT IN THE PRODUCTION OF *BTK* BASED BIOPESTICIDES

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Bacillus thuringiensis (Bt), is a spore-forming Gram-positive bacterium recognized for its insecticidal activity. This latter is due to its capacity to produce crystals formed by a mix of δ- endotoxins. *Bt* is a key player in the majority of commercially available biopesticides contributing to bio-economy expansion. Wheat bran is a by-product of milling that is treated as industrial waste and is used only in small quantities in human nutrition. However, it is rich in essential nutrients necessary for bacterial development and sporulation. In this context, the European project IPM-4-Citrus (MSCA RISE, No. 734921) aims to optimize δ- endotoxins production by *Bt* in a wheat bran (WB) based culture medium through determination of bio-performances and understanding of nutritional limitations, in order to minimize the cost of biopesticide production at industrial scale. To understand the nutritional limitations of the WB medium during *Bt* production, WB was first sieved.and classified into 4 different sizes: class 1>850 µm, 500 µm<class 2<850 µm, 250 µm<class 3<500 µm and class 4<250 µm corresponding to 1, 19.8, 51.4 and 28.2 %w/w respectively. Two *Bt* serovar *kurstaki* (*Btk*) strains (HD1 and Lip) were cultured in flask and in 2 L bioreactor. The medium was then filtered and the following tests were carried out on permeate and retentate: ashes, starch concentration (colorimetric method), total Nitrogen (Kjeldahl) and elemental composition analysis (statistical test) of the strains and of the medium, before and after inoculation. Bio performances were also examined (CFU, spores, endotoxins). WB properties before inoculation were first determined. On one hand, the moisture (~12% gwater/gdw), ash (~4.4%w/w) and the elemental composition are constant regardless of the class. On the other hand, class 4 is the richest in starch. In suspension, retentate had an initial mass balance of 80-85% that decreased after culture indicating the partial consumption of the WB insoluble fraction while permeate had a mass balance of 14-16%. that increased after culture. Fermentable fraction increased when WB particle size decreased in agreement with starch content. Starch stands as the major carbon source (017-0.34 g/gWB) consumed by *Btk* however hemi-cellulosic fraction was also consumed in a rate of 0.15-0.2 g/gWB. Following, we aimed to determine nutritional limitation during *Btk* culture, based on the analysis of 5 elements (C, H, O, N, S). When compared to WB, the strain's elemental composition shows a higher nitrogen content. After culture, elemental composition was analyzed in both pellet and supernatant. Nitrogen mass balance showed significant differences between permeate and retentate after culture. It decreases in pellet due to WB consumption by Btk and it increases in supernatant due to biomass growth and endotoxins production. These results match with data generated by Kjeldahl technic, and confirmed a threshold for accessible Nitrogen to *Btk* due to a recalcitrant hemi-cellulosic fraction. Nitrogen was identified as a limiting nutrient at flask scale. C/N ratio in permeate and retentate

appears as a reliable indicator to evaluate culture progress in flask and was used to describe biokinetics in bioreactor scale.