# Monitoring of *Bacillus thuringiensis* (Bt) growth and sporulation: exploration and comparison of on-line and off-line measurements.

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**Background and aim:** Alternative Integrated Pest Management (IPM) approach based on biological control looks to answer to multiple needs. Production of Bt-biopesticide ready for its use on the field is an identified issue (project IPM-4-Citrus). The present study falls within this project and aims to identify the various physiological states of two *Bt kurstaki* strains during culture, which are critical steps to control and understand cell physiology and δ-endotoxins production.

**Methods:** Two *Bt var. kurstaki* BLB1 and HD1 strains were cultivated in a 3L bioreactor (Biostat B plus) using a semi-synthetic medium (Sarrafzadeh et al, 2005). Batch culture were realized under controlled conditions at 30°C, pH6.8. pO2 was monitored and maintained at different level (25 and 50% pO2sat) with a constant aeration rate (0.18L/min) and variable stirring. On-line (pH, Tp, RPM, $OD_{600nm}$,…) and off-line (HPLC, cell dry matter, OD, cell and spore countings, …) physico-chemical measurements were realized during 48h cultures by sampling.

**Results:** Comparison between on-line measurements, optical density and other observations (off-line measurements) was done and showed the presence of four distinct phases of growth: lag phase (0 to 3 hours), growth itself (3 to 12 hours), transition (12 to 18 hours) and sporulation (starting 18 hours of cultures). pH regulation was found to be a main indicator to detect the exact sporulation phase as acetic acid production slow down. HPLC analysis showed that acetic acid was only present from 3 to 15 hours of culture, confirming the earlier findings. The pO2 level was regulated by stirring at constant aeration rate (0.18L/min). After 21 hours, pO2 level increased, indicating the beginning of sporulation. Meantime, microscopic observations showed the presence of released spores within the culture. For both strains, maximum biomass production was achieved within 12 hours of culture, whereas maximum spore and δ-endotoxin production were achieved after 36 hours of cultures.

**Conclusion:** The complementarity between on-line and off-line measurements was useful to detect the exact phase of sporulation. Future cultures will initiate fed-batch mode considering this critical time, in order to increase the biomass and consequently δ-endotoxins production. The overarching aim will be to transpose biopesticide production with semi-synthetic medium to wheat bran industrial substrate.

**Keywords:** *Bacillus thuringiensis*, BLB1, HD1, sporulation, δ-endotoxin, on-line measurements.