On-line monitoring of fermentation of 2G bioethanol

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INTRODUCTION. For many years, cellulosic-ethanol has been considered as an alternative to fossil and non-cellulosic fuels. However, the operational challenges and the lack of understanding of this process often results in poorly operated fermentations that limit their potential to compete with non-cellulosic fuels.

OBJECTIVE. This project aims at implementing spectroscopic-based real-time monitoring methods and to combine them with mechanistic models to predict the dynamics of cellulose-to-ethanol fermentation and to early detect possible contaminations by lactic acid bacteria, achieving an operation closer to the optimal conditions.

FERMENTATION STEPS IN 2G BIOETHANOL

**BATCH PHASE**
- Detoxification phase and cell growth.

**FED-BATCH PHASE**
- Anaerobic phase, to produce of ethanol.
- Anaerobic phase until depletion of substrates.

During the initial batch phase, the cell culture is adapted to the medium and detoxifies the inhibitors. Once it starts growing, a fed-batch phase starts and ethanol is produced. The feeding rate is adjusted so it does not exceed the detoxification capacity of the cell culture. Finally, a batch phase runs until the glucose and xylose are finished.

CURRENT MONITORING APPROACHES

**pH.** Monitoring and controlling pH is used to lower the inhibitory effects of the weak acids. However, alone, pH is difficult to correlate with the dynamics of the fermentation, with the concentration of non-acidic inhibitors (i.e., furfural or HMF), and with the LAB concentration.

**Off-gas.** CO₂, O₂ and ethanol are monitored in the off-gas. CO₂ and ethanol can be correlated with the dynamics of the fermentation but cannot be used to measure the concentration of inhibitors or to detect contaminations.

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CHALLENGES OF 2G BIOETHANOL FERMENTATION

**SUBSTRATE VARIABILITY**
- The substrate changes its composition due to different cultivations and weather conditions.

**INHIBITORS**
- The inhibitors produced during the pretreatment inhibit the yeast and extend the length of the lag phase.

**CONTAMINATIONS**
- The fermentation is non-sterile increasing the risk of contamination by lactic acid bacteria (LAB).

**OXYGEN**
- Early detection of LAB is fundamental to avoid consuming the substrate to produce LAB and lactic acid instead of ethanol.

SPETROSCOPY AS A MONITORING METHOD

The current monitoring methods do not match almost any of the previous monitoring objectives resulting in poorly operated fermentations.

To run the fermentation close to the optimal conditions, it is necessary to add monitoring methods that can deliver information regarding the dynamics of the process in real-time.

Vibrational spectroscopy (NIR, MIR) and Raman spectroscopy can detect several key compounds simultaneously, and combined with the current methods, can deliver a good description of the dynamics of the information.

IMPROVING THE REAL TIME MONITORING OF CELLULOSE TO ETHANOL FERMENTATIONS

**EXPERIMENTAL DESIGN.**

On-line monitoring with a transmittance probe with a slit of 0.5 mm.

Detection in a range from 4000 to 15784 cm⁻¹.

**PLS models** are developed to predict the concentrations of glucose, xylose, and ethanol.

**CONCEPTUALIZATION OF THE MECHANISTIC MODEL.**

Development and validation of a mechanistic model describing the growth of yeast, the interactions with the different inhibitors and a simplified model of the growth of LAB.

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