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# Controlling the specific growth rate *via* biomass trend regulation in filamentous fungi bioprocesses

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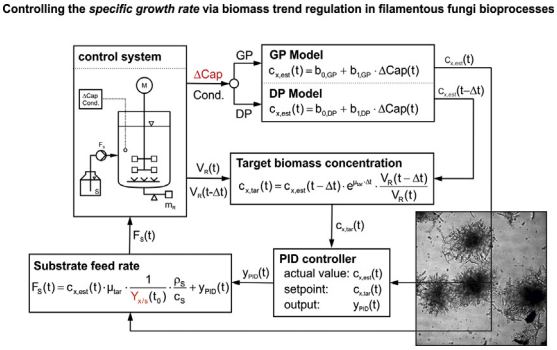
## HIGHLIGHTS

- Viable biomass concentration is predicted online via dielectric spectroscopy.
- The method is applicable for filamentous fungi process in growth and decline phase.
- Holding μ on a constant level is based on controlling biomass trends.
- The control strategy adapts automatically to changing biomass yields.
- The method is robust to changing process parameters, physiology and morphology.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Increasing pressure on product quality and quantity pushes solutions of process control to be a central issue in pharmaceutical bioprocesses. Especially online biomass estimation, and further control of the specific growth rate are of central importance because they describe the catalyst of the reaction. For penicillin producing bioprocesses with filamentous microorganisms, this was underlined by recent findings describing the influence of the specific growth rate on the specific production rate. Hence, the specific growth rate needs to be controlled on a certain level to achieve high productivity.

In this study, we developed a control strategy for the specific growth rate based on online estimation of viable biomass via dielectric spectroscopy. The method was verified using an at-line staining method for viability measurement. The online viable biomass estimation is applicable in the growth and decline phase, coping with physiological and morphological changes of filamentous fungi. Furthermore, the control strategy adapts to changing biomass yields, which is a big issue in the bioprocess for penicillin production applied in this study.Two application runs were conducted, yielding in proper online viable biomass estimation and control of the specific growth rate at a constant level of 0.012 $h^{-1}$. We achieved biomass predictions with an average error of 1.5 g/l over the whole fed-batch process. In the decline phase, the control of specific growth rates was not possible due to physiological constrains. However, in the growth phase, a total specific growth rate of 0.013 $h^{-1}$ was achieved, which met the pre-defined acceptance criterion for this method.

The method is thus ready for viable biomass estimation in the growth and in the decline phase of the penicillin production process. Furthermore, the method is applicable to control the specific growth rate during the growth phase.



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**Nomenclature**

*Symbols*

| | |
|---|---|
| c | concentration [g/l] |
| ΔCap | delta Capacitance [pF/cm] |
| F | feed rate [g/h] |
| μ | specific growth rate [$h^{-1}$] |
| ρ | density [g/l] |
| r | rate [g/h] |
| t | time [h] |
| $V_R$ | reactor volume [l] |
| $Y_{PID}$ | output of PID controller [g/h] |
| $Y_{x/s}$ | biomass yield [g/g] |

*Indices*

| | |
|---|---|
| tar | set-point; target value |
| est | predicted value based on the model |
| s | substrate |
| total | total biomass measured *via* DCW or wet weight |
| viable | viable biomass measurements (vs. total biomass) |
| x | biomass |

## 1. Introduction

Process control is of utmost importance in pharmaceutical bioprocesses to maximize productivity and to ensure product quality. The importance of the latter was underlined by the Quality by Design (QbD) initiative (FDA, 2006). One parameter which is often the focus is biomass growth. Various methods of biomass estimation for different processes and organisms have been applied (Mou and Cooney, 1983b; Riesenberg et al., 1991; Sagmeister et al., 2013; Jenzsch et al., 2006). Studies presenting the influence of the specific growth rate, μ, on penicillin production (Douma et al., 2010; Pirt and Righelato, 1967; van Gulik et al., 2000) underline the importance of μ-control strategies in filamentous fungi bioprocesses.

The basis for the control of μ is the availability of real-time biomass measurements. Hereby, two different principles can be distinguished: hard type sensors and soft sensors. The former are *in-situ* measurements of the biomass concentration, while soft sensors estimate the biomass based on mathematical models (Olsson and Nielsen, 1997). Commonly, soft sensors require high process knowledge and historical data for the development of mathematical models. Soft sensors were reportedly applied for biomass concentration and biomass growth estimation of filamentous organisms (Golabgir and Herwig, 2016; Massimo et al., 1992; Thompson and Kramer, 1994; Mou and Cooney, 1983b). The online biomass estimation based on carbon balancing was already applied for the μ-control of penicillin processes (Mou and Cooney, 1983a,b). *In situ* biomass measurements applied for filamentous organisms were fluorescence probes (Haack et al., 2007) and dielectric spectroscopy (Fehrenbach et al., 1992; Mishima et al., 1991; Neves et al., 2000; Sarra et al., 1996). Fluorescence probes were found to be not applicable for penicillin-producing cultures in complex media, as changes in the medium composition influence fluorescence. Furthermore, penicillin is a fluorescent product which interferes with the measurement of fluorescence (Nielsen et al., 1994).

The principle for biomass measurement via dielectric spectroscopy is based on the function of cells as capacitors. The measured signal is a function of the volume fraction of the cells. Only cells with an intact membrane potential are recorded with dielectric spectroscopy. Hence, the method is insensitive to dead cells and only measures viable biomass (Dabros et al., 2009). This fact is of high importance for filamentous organisms who grow pellets, as the inner parts of these hyphal aggregates tend to die due to substrate and oxygen limitation, leading to considerable amounts of dead biomass (Bizukojc and Ledakowicz, 2010; Hille et al., 2005; Manteca et al., 2008). In contrast to this dead biomass, only viable biomass is responsible for biomass growth and productivity (Bizukojc and Ledakowicz, 2010; Paul et al., 1998).

Most contributions, in which dielectric spectroscopy was applied on filamentous fungi for the estimation of biomass concentration, differentiate between a growth phase and a decline phase (at the end of cultivation). For the growth phase, biomass prediction via dielectric spectroscopy is reported to be highly applicable. However, biomass prediction in the decline phase still shows results that are very error-prone (Neves et al., 2000; Rønnest et al., 2011; Sarra et al., 1996).

Within this contribution, an online μ-control strategy in a penicillin production process based on viable biomass measurement via dielectric spectroscopy is established. So far, biomass estimation in the decline phase was reported to not work properly. We aim to predict the viable biomass concentration in the growth and the decline phase to further apply μ-control in both phases. The goal is to predict viable biomass in a similar error range as at-line and offline reference methods (6–12%).

The control of the specific growth rate in filamentous cultures presents a special challenge as growth rate levels are rather low compared to other organisms as *E. coli*. These low specific growth rates make the control and the evaluation of the control strategy difficult as they have a lower signal-to-noise-ratio (Wechselberger et al., 2013). Furthermore, changing biomass yields during the penicillin production process appeared to be a main issue in μ-control. The developed control strategy aims to be applicable in the growth phase as well as in the decline phase and enables an automatic adaption to changing biomass yields. To avoid error propagation in the calculation of the specific growth rate, the control strategy focuses on biomass trends rather than the specific growth itself.

## 2. Materials & methods

### 2.1. Strains and inoculum

One strain of *Penicillium chrysogenum* (code BCB1_V2) was regenerated from an ancestor strain BCB1 and kindly donated by Sandoz GmbH (Kundl, Austria).

### 2.2. Bioreactor cultivation

Cultivations were performed in either of two Techfors S bioreactors (Infors HT, Bottmingen, Switzerland) with a maximum working volume of 10 L and 20 L respectively. The initial batch and fed-batch volumes were 6.5 L for the 10-l-bioreactor and 13 L for the bigger one. The stirrer was equipped with three six-bladed Rushton turbine impellers, of which two were submersed and one was installed above the maximum liquid level for foam destruction.

The fermentation temperature was kept at 25 °C via a double jacket. Aeration was controlled at 1 vvm in batch and initial fed-batch with mass flow controllers (Vögtlin, Aesch, Switzerland). The concentration of dissolved oxygen was measured using a probe of dissolved oxygen (Hamilton, Bonaduz, Switzerland). If not stated otherwise, the $pO_2$ was controlled via an adjustment of the stirrer