Review

Science-based bioprocess design for filamentous fungi

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Industrial bioprocesses are commonly based on empiricism rather than scientific process understanding. In this review, we summarize current strategies for science-based bioprocess design and control for filamentous fungi aiming at reducing development times and increasing process economics. We discuss recent developments and trends regarding three crucial aspects throughout the bioprocess life cycle of filamentous fungi, namely (i) strain and inoculum characterization, (ii) morphology, and (iii) rheology, as well as their effects on process performance. Complex interconnections between strain, inoculum, morphology, rheology, and process design are outlined and discussed. Only combining different hard type sensors with soft sensor technology and the development of simplified mechanistic models can enable science-based bioprocess design for filamentous fungi.

Industrial application of filamentous fungi

Economic, reliable, and controllable bioprocesses for filamentous organisms, in particular filamentous fungi, are of utmost importance for the large scale production of a wide range of value-added products including organic acids, enzymes, and antibiotics. Due to complex interactions between process technology, filamentous morphology (Figure 1), and overall process performance, traditional bioprocess design is still commonly carried out stepwise by time-consuming, empirical strategies (Figure 2).

In contrast to biopharmaceuticals, the majority of products from filamentous fungi are industrial (white) biotechnological bulk products and thus not subject to tight regulatory demands. Consequently, manufacturers can continuously optimize their production strains and industrial processes to ensure competitiveness. Common empirical approaches, however, lack scientific insight into process technology and key process parameters (KPPs) (see Glossary) inevitably leading to sub-optimally designed bioprocesses and high process failure rates. Therefore, bioprocess engineers pursue two overall goals summarized as 'two-times 50%', which means twice the productivity and reducing bioprocess development time to 50%. In order to meet these economic requirements, it is necessary to understand and control the biological system used.

In this review, we give an overview of recently developed measurement and control strategies for major obstacles throughout the bioprocess life cycle of filamentous fungi that significantly affect the overall process performance. Points that impact the process performance are (i) strain and inoculum, (ii) morphology, and (iii) rheology. Furthermore, we provide future perspectives targeting a holistic understanding of complex interdependencies in science-based process design for filamentous fungi.

Strain and inoculum

Screening for strain characteristics

The first step in bioprocess design is the identification of promising candidate strains (Figure 2). To achieve automation and high-throughput, strains are commonly identified in micro titer plate (MTP) systems. Besides identification of highly productive strains, initial screening should also consider morphological characteristics, such as complex growth morphology or adherent wall growth, which significantly affect reproducibility of results and the subsequent scale-up procedure. Missing these important factors, selected clones may fail at delivering productivities observed at small scale. Reducing the headspace was proven to be a useful strategy for preventing wall growth and consequent heterogeneities [1–3]. Another study compared physiological and morphological process performance in MTPs and 1-liter benchtop reactors. Addition of glass beads promoted mycelial growth

Glossary

Carbon dioxide evolution rate and oxygen uptake rate (CER/OUR): respiratory rates reflecting physiological culture activity.
Critical quality attribute (CQA): a physical, chemical, biological, or microbiological characteristic that should be within an appropriate limit, range, or distribution to ensure desired product quality.
Critical process parameter (CPP): a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure that the process produces the desired quality.
Design of Experiments (DoE): a structured, organized method for determining the relationship between factors affecting a process and the output of that process.
Key process parameter (KPP): an adjustable parameter (variable) of the process that, when maintained within a narrow range, ensures optimum process performance. A key process parameter does not meaningfully affect critical product quality attributes. Ranges for KPPs are established during process development, and changes to operating ranges will be managed within the quality system.
Process analytical technology (PAT): a system for designing, analyzing, and controlling manufacturing through timely measurements of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.
Quality by Design (QbD): a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.

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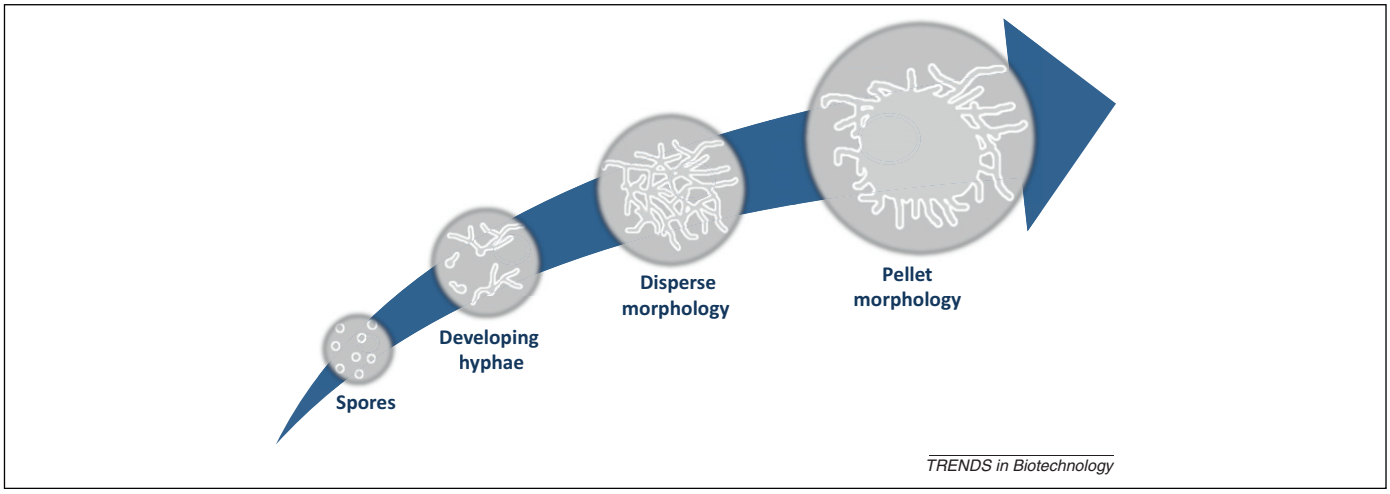

Figure 1. Morphological life cycle of filamentous fungi. Independent of capitalizing *Aspergillus spp.* for the production of enzymes or organic acids, *Trichoderma spp.* for cellulases, or *Penicillium spp.* for antibiotics, these filamentous industrial workhorses all share a unique and complex growth morphology. Fungal bioprocesses are commonly inoculated from spores. After successful germination: branching, extending, and entanglement of single hyphae introduce disperse hyphal networks. Over process duration, pellets may develop via growth from single spores or by agglomeration. High shear forces and nutrient limitation cause pellet disintegration.

and concomitantly decreased wall growth [4]. Using this approach, physiological performance parameters identified during MTP screening were similar to benchtop reactors. The authors also studied the influence of different cultivation regimes on morphological process behavior by measuring particle size distributions. Although physiological screening results were similar at miniaturized and lab-scale, growth morphology was different, probably due to differing shear forces.

A disposable microbioreactor system for high-throughput screening of germination efficiency was introduced recently [5]. Reactor chambers of 100 μl volume enabled parallelized characterization of growth morphology during spore germination as well as quantification of inoculum quality. Although such miniaturized reactors do not allow for complete characterization of growth morphology, it was shown that morphological parameters, such as the hyphal growth unit length, could be correlated to pellet formation kinetics and pellet size [6].

Another crucial factor that has to be considered when analyzing strain characteristics is the growth medium (Box 1).

Characterization of the inoculum

Spore inoculum concentration, quality, and viability severely affect fungal morphology, physiology, and productivity [7]. To date however, the amount of inoculum and the setting of process parameters are still empirically optimized with each inoculum batch. Consequently, initial processes performed with a fresh inoculum batch only gradually reach an empiric optimum after several test runs. This procedure completely disagrees with the overall 'two-times 50%' target in industrial (white) biotechnology.

In this respect, parallel microbioreactor devices and MTP cultivation systems [1,4,5] represent useful tools for the characterization of germination efficiency and spore viability. Moreover, fluorescence microscopy [8] and Fourier transform infrared (FTIR) spectromicroscopy [9,10] provide spatial resolution of the intracellular biochemical composition of filamentous fungi. Although these methodologies are still in their infancy, results show promise for a means to quantify spore inoculum quality in the future.

The correct transfer time point from the seed reactor to the production reactor after successful spore germination is as crucial as the optimal spore inoculum quality.

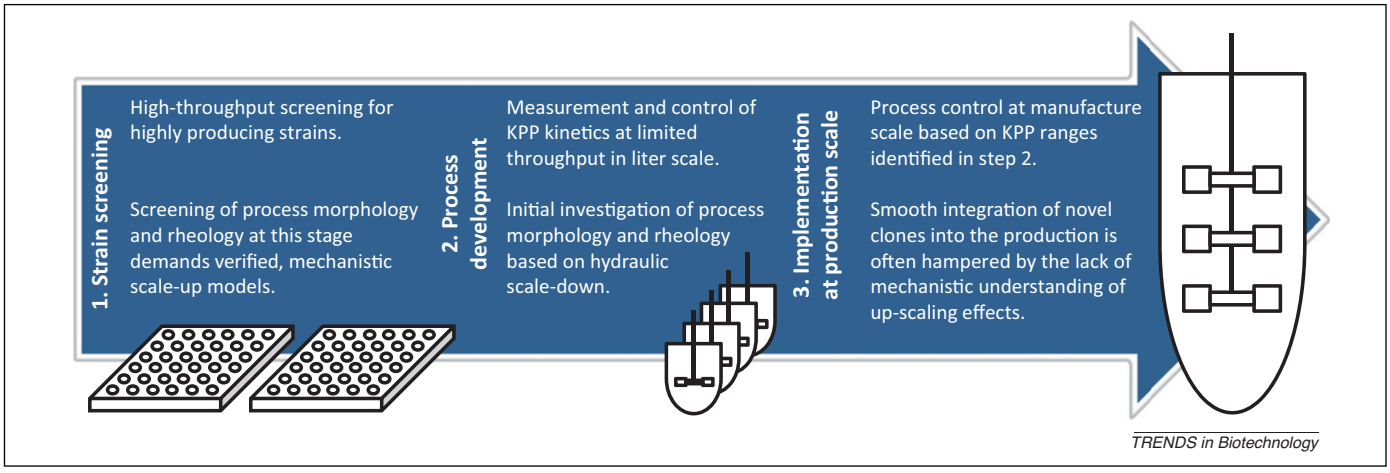

Figure 2. Traditional bioprocess design for filamentous fungi. The development strategy starts with qualitative screening of suitable clones, followed by quantitative bioprocess development in lab-scale bioreactors. Thereby, key process parameters (KPPs) that ensure that the biological system is steered to target product quantity are identified. Subsequent piloting aims at scalability of the process, analyzing adaptation of the process parameters, and scale-up effects [53].