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Simulating Process Limitations in Microbial Cultivation: A Parallel Two-Compartment Scale-Down Approach

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Abstract

In large-scale industrial bioprocesses, the presence of gradients in critical process parameters, such as dissolved oxygen (DO), pH, and substrate concentration, can be observed. They result in inhomogeneous growth conditions within the bioreactor/fermentor and can affect cell yield and/or productivity. Scale-down approaches at the laboratory scale are a tool to analyze the effects of the inhomogeneity.

Scientists at the Forschungszentrum Jülich have been using a cultivation set-up consisting of two connected stirred-tank reactors (STRs) to simulate inhomogeneous cultivation conditions as they can occur in production scale. In this application note, we describe the effects of oscillating DO and substrate concentation.

This study exemplifies the benefits of flexible bench-scale bioreactor solutions and advanced bioprocess control software. Individual control of parallel operated bioreactors allowed different vessel sizes, individual parameter set-points, and multiple cultivation phases to be implemented easily.

Introduction

Corynebacterium glutamicum is an established host used to produce amino acids for the food and feed industries. The research activities of the Bioprocesses and Bioanalytics group at the Forschungszentrum Jülich are focused on the optimization of microbial bioprocesses. As part of this, the scientists perform high-throughput bioprocess development and characterization, including quantitative microbial phenotyping and next-generation omics technologies.

The project focus was to set up a two-compartment scale-down device composed of two interconnected stirred-tank reactors to mimic inhomogeneous cultivation conditions. The cell suspension was supposed to oscillate between an aerobic and a micro-aerobic compartment with different residence times. Such challenging cultivation conditions can occur in the industrial production scale due to insufficient mixing quality [1 – 3]. Inhomogeneous cultivation conditions potentially result in performance losses and the associated increase of production costs, which can imperil the competitiveness of biotechnological production.

For setting up such a scale-down device, the group was looking for a cultivation platform incorporating four or more fermentors to implement two or more parallel STR-STR set-ups. By combining different vessel sizes, fluid levels, and sensor equipment, the volume ratio between the two compartments as well as the online analytics should be adapted to a broad scope of applications. Additionally, the possibility to monitor and control the conditions in each compartment should enable the observation of separated or combined effects of oscillating pH, temperature, DO, and substrate concentration. In the experiments described in this application note, the effects of oscillatory DO and substrate concentrations on a fed-batch cultivation of *C. glutamicum* were analyzed.

Material and Methods

DASGIP® Parallel Bioreactor System

The STR-STR parallel set-up was implemented using an Eppendorf DASGIP Parallel Bioreactor System and consisted of two interconnected fermentation vessels [4]: STR1 (max. working volume = 1.5 L) and STR2 (max. working volume = 300 mL) (Figure 1). The combined working volume of both vessels was 1 L. The volumetric distribution between the compartments was 78 % in the STR1 and 22 % in the additional STR2. Included in the STR2 volume were the connecting tubing (length 1 m; inner diameter 4.8 mm), representing 2 % of the culture volume.

Each vessel was equipped with an optical DO sensor (VisiFerm™ DO 225; Hamilton®, USA) and a pH sensor (405-DPAS-SC-K8S pH probe, sensor length 225 and 120 mm, respectively; Mettler-Toledo®, USA). A DASGIP PH4PO4 monitoring module for pH and DO was used. Titration and substrate feed were realized with a DASGIP MP8 multi pump module. Exhaust was analyzed with a DASGIP GA4. The aerobic compartment (STR1) was aerated by a DASGIP MF4 gassing module and temperature and agitation were controlled by a DASGIP TC4SC4 module. To ensure a constant volumetric distribution between the compartments, a dip tube was implemented in the STR2 at the desired height. Two peristaltic pumps (505U, Watson-Marlow® GmbH, Germany) ensured medium flow between the two vessels: A master pump from STR1 to STR2 was set to a dedicated flow rate and a utility pump enabled flow from

Table 1: Experimental setpoints and configurations in the two compartments. STR = stirred-tank reactor

	STR1	STR2
Vessel (max. working volume)	1.5 L	0.3 L
Working volume	780 mL	220 mL
pH control	7; acid and base	7; acid and base
DO control	30 %; Stirrer cascade	0 %; 0.25 vvm N_2 (non-controlled)
Agitation	Determined through cascade	1,200 rpm
Temperature	30 °C	30 °C
Feeding	none	exponential feed profile

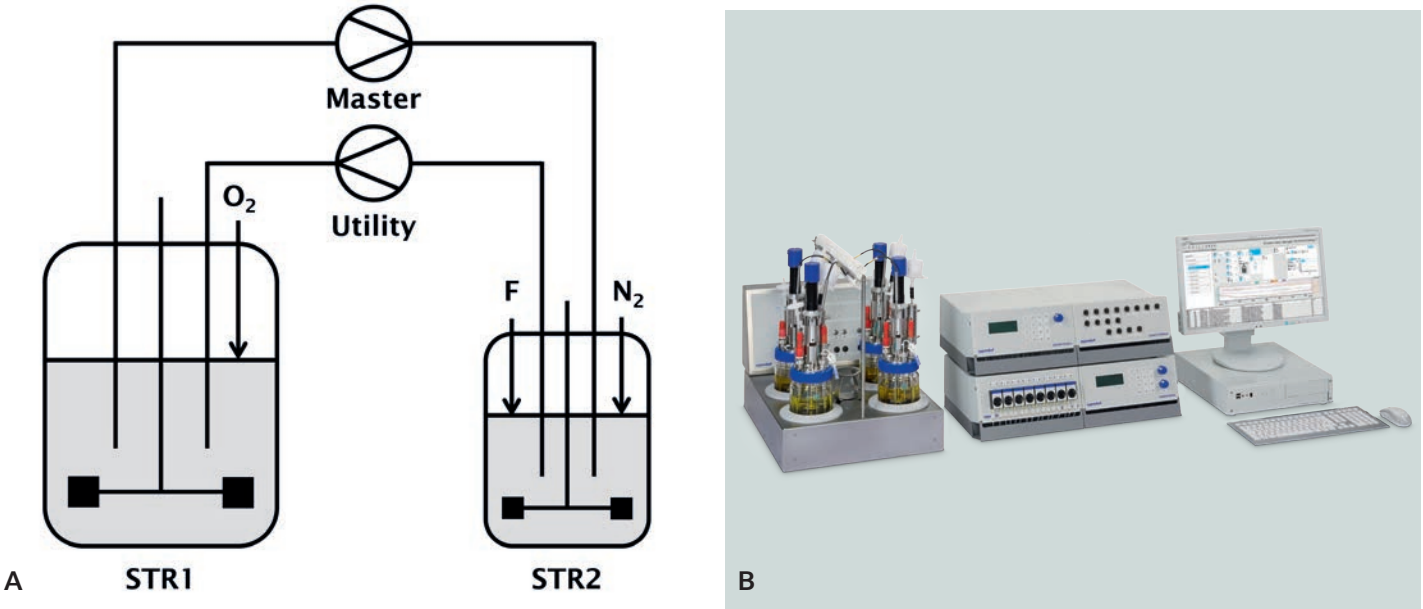

Figure 1: Scale-down setup.
A Illustration of the STR-STR two-compartment cultivation setup. The device is composed of two interconnected stirred-tank reactors. During cultivation the cell suspension circulates between the aerobic STR1 (78 % of the working volume) and the non-aerated STR2 (22 % of the working volume). The master pump controls the flow rate while the utility pump follows with a 30 % higher pump rate. Two sets of the STR-STR devices were implemented in one cultivation platform.
B DASGIP Parallel Bioreactor System. A 4-fold system for microbial applications is shown. From left to right: The DASGIP Bioblock provides independent temperature control of up to four vessels. DASGIP bioprocess analyzer modules deliver accurate monitoring and control of key process parameters. The DASware® control software features parallel process control with individual control of each vessel.

STR2 back to STR1. The latter one was set to rotate with a 30 % higher speed compared to the master pump.

The cultivation runs were performed as a duplicate experiment comprising four vessels in total (2x STR1, 2x STR2). This parallel set-up was controlled by a single PC operating DASware control 5. The software is capable of controlling up to 16 parallel vessels individually and offers process automation features, intelligent recipe management, and integrated reporting.

Three-phased cultivation of *C. glutamicum*

Table 1 summarizes the experimental set-up. The DO concentration in the aerated compartments (STR1) was maintained at 30 % by a stirrer cascade. The DO concentration in the smaller compartments (STR2) was supposed to be near 0 %, however not controlled. To maintain this micro-aerobic environment, a flow of 3 sL/h N_2 (0.25 vvm) was set through the STR2. The stirrer speed in the smaller STR2 was set to remain constantly at 1,200 rpm. pH in the STR1 and STR 2 was maintained at 7 using 30 % (v/v) phosphoric acid and 18 % (v/v) ammonia. The non-aerated compartments were programmed to be fed with an exponential feed rate (Figure 2) while STR1 was not fed. The initial batch medium used was a CGXII minimal medium [5] with 5 g/L glucose. To prevent foaming, 1 mL 50 % (v/v) Antifoam 204 (Sigma-Aldrich®, USA) aqueous emulsion was added. A CGXII minimal medium without protocatechuic acid (PCA) containing 300 g/L glucose was used as feed medium.

The cultivations were divided into three separate phases. The first phase was a batch cultivation in two parallel fermentors (STR1) incorporating a starting volume of 1 L before adding the additional smaller STR2 compartments. This initial phase was characterized by well-supplied cultivation conditions with glucose and oxygen in excess. The second cultivation phase started when the non-aerated compartments were added, initiating the oscillations between well-aerated and oxygen-limited cultivation conditions. This switch in process conditions has been declared as time point t = 0. The third cultivation phase started 1.7 h after adding the STR2 compartments by starting the exponential feed into the non-aerated vessels. A desired growth rate of 0.2 h^{-1} was expected for the whole working volume. This last phase was characterized by glucose excess but O_2 depletion in the STR2 vessels and glucose limitation but sufficient O_2 supply in the larger STR1 compartments.

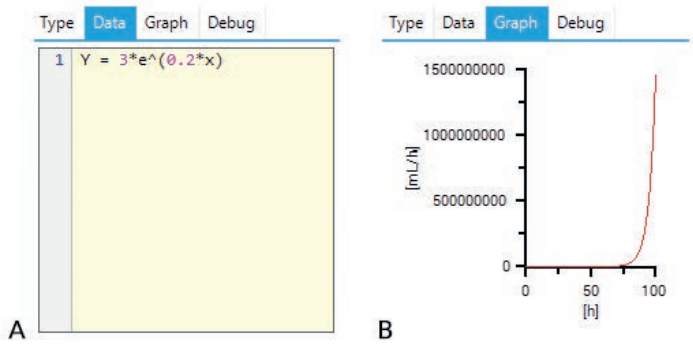

Figure 2: Exponential feed profile in DASware control 5 software. **A:** Formula programmed, **B:** Graphical depiction of the profile.

Results and Discussion

After inoculation, the dissolved oxygen concentration in STR1 compartments decreased in both parallel runs until a saturation level of 30 % was reached (Figure 3). In the time frame of -4.7 h to -4.3 h, the stirrer cascade automatically started in both vessels to maintain a DO of 30 %. At the point of time when the additional compartments were added (t = 0), the DO levels in the STR1 vessels increased due to the volume reduction in the STR1. The DO control adapted quickly to the changed conditions and controlled the DO accurately at 30 % until the process ended. DO levels in the oxygen-limited STR2 dropped immediately to 0 % after oscillation start thanks to aeration with N_2. To ensure that only oscillating O_2 and substrate levels influenced the scale-down cultivation, the pH in both compartment types was controlled at 7.

Offline HPLC analysis revealed that organic acids were produced during oxygen limitation in the STR2 and reassimilated in the aerobic STR1 due to the limited glucose availability. Despite this compartment-specific acid formation, the individual pH control of the vessels was maintained between 7.1 and 6.9 in both compartments, without deregulating the control. The bacterial growth was not significantly affected by pumping the cells back and forth between different cultivation conditions. During the feeding phase, a mean growth rate of 0.19 h^{-1} was observed, which strongly correlates with the expected growth rate of 0.2 h^{-1} given by the exponential feed profile.

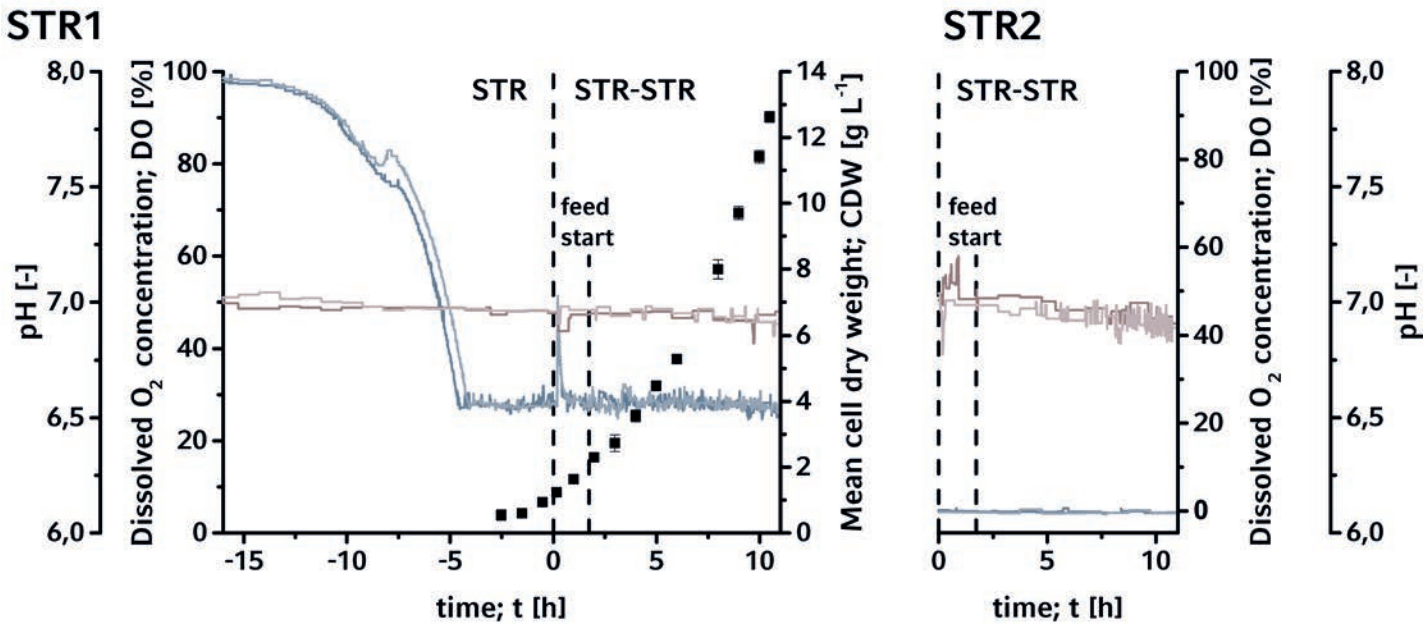

Figure 3: Fed-batch cultivation of *C. glutamicum* under oscillation oxygen and glucose supply. The dissolved oxygen (DO) concentration (blue) and the pH (red) courses over the process time are plotted for the aerated compartment (STR1; left) and the non-aerated STR2 (right) individually. Each line represents one out of two parallel biological replicates. Cell dry weight (CDW; black squares) was determined offline indicating bacterial growth.

Conclusion

Using the described scale-down device, the researchers were able to simulate parameter gradients that can occur in the production scale. Metabolic effects of the oscillating DO and substrate concentrations were evaluated by offline analysis. Two identical scale-down processes could be realized at the same time with highly reproducible results.

The independent control of parameters in each vessel was essential to ensure the aerobic conditions in STR1 and micro-aerobic conditions in STR2. An accurate pH control, despite different vessel sizes and environments, made it possible to separate the O_2-limited conditions from a potential effect of acidic by-product-induced pH drop.

The scripting function of the software enabled the implementation of an exponential feed profile that resulted in the desired growth rate. Once the set-up was defined, the comprehensive recipe management allowed the adaptation and applications of the stored templates to any future cultivations. Like this, the researchers will be able to further investigate the relation between the cellular metabolism and inhomogeneities in the fermentor.

DASGIP Parallel Bioreactor Systems and DASware control have proven to be a powerful platform for scale-down experiments. A flexible design of both hardware and software allow even uncommon process set-ups and enable process automation.

Literature

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Ordering information

Description	Order no.
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4-fold system with Bioblock	76DG04MBBB
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