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Transfer of an Itaconate Production Process in *Ustilago maydis* to the BioFlo® 120 Bioprocess Control Station

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Abstract

The unsaturated dicarboxylic acid itaconate is used as a building block for the production of pharmaceuticals and adhesives, as a copolymer for synthetic resins, and is a promising starting material for biofuel production. Researchers at RWTH Aachen University have previously optimized a production process for itaconate using a genetically engineered strain of the fungus *Ustilago maydis*. The following application note highlights the simplicity and ease of use of the new BioFlo 120 bioprocess control station, through a successful process transfer from it's predecessor, the New Brunswick™ BioFlo/CelliGen® 115.

Introduction

For the bio-based production of itaconate (Fig. 1) natural producer as well as heterologous hosts have been used. Itaconate is naturally synthesized by the fungi A*spergillus itaconicus* and *Aspergillus terreus*, and *Ustilago maydis*, among others. *Aspergillus* produces itaconate via enzymatic decarboxylation of the tricarboxylic acid cycle intermediate cis-aconitate. This biosynthetic route has been identified many years back. More recently, an alternative biosynthesis pathway starting from trans-acontiate has been described in *U. maydis* [1]. For large-scale itacontate production *U. maydis* can be better, because unlike *Aspergillus spec.* it has a single-cell, yeast-like morphology that avoids problems typical of filamentous fungi, like high viscosity and clogging, hindered oxygen transfer, and sensitivity to mechanical stress.

Researchers at the RWTH Aachen and Marburg University had previously optimized fermentation of a metabolically engineered *U. maydis* strain and produced itaconate in a high-density fed-batch process controlled by a BioFlo/CelliGen 115 bioprocess control station [1]. Now they transferred process control to the BioFlo 120 bioprocess controller (Fig. 2). To compare the results obtained with the two different bioprocess systems, they analyzed cell growth and the concentrations of glucose and itaconate in the culture medium over time.

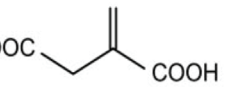

Fig. 1: Chemical structure of itacontate

Fig. 2: BioFlo 120 bioprocess control station

Material and Methods

Itaconate producer strain
The researchers had previously identified and characterized the gene cluster responsible for itaconate biosynthesis in *U. maydis*. This laid the foundation for the design of the itaconate hyper-producer strain MB215 Δ*cyp3* P_{etef}ria1. Overexpression of the gene cluster regulator ria1 and deletion of the *cyp3* gene, encoding for a P450 monooxygenase, increased itaconate production by a factor of 4.5 compared to the wildtype and abolished the formation of the byproduct 2-hydroxyparaconate [1].

Process parameters and control
Using a BioFlo/CelliGen 115 bioprocess control station, the researchers had previously optimized the process conditions for itaconate production in *U. maydis*. For cultivations with the BioFlo 120 bioprocess controller they used the same process parameters and medium composition. *U. maydis* was cultivated in a working volume of 0.5 L in medium containing 200 g/L glucose, 4 g/L NH_4Cl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.5 g/L KH_2PO_4, 1 g/L yeast extract, 1 mL/L vitamin solution, and 1 mL/L trace element solution. The temperature was set to 30°C. The pH was maintained at 6.0 to 6.5 by automatic addition of 10 M NaOH. Dissolved oxygen was controlled at 80 % by aeration with a rate of 1 L/min (2 vvm) and automatic adjustment of the agitation speed between 700 and 1,200 rpm. Process parameters were controlled with the Eppendorf bioprocess control software (Fig. 3) built into the controller. The researchers inoculated the cultures to an optical density (OD_{600}) of 0.75 and cultivated them for 168 hours. After 96 hours they fed the cultures with a single feed pulse of glucose.

Analytics
DO, temperature, and pH were measured online using analog sensors. The cell density was quantified offline by measuring the OD_{600} of the culture. The researchers quantified the concentrations of glucose and itaconate in the culture supernatant by HPLC.

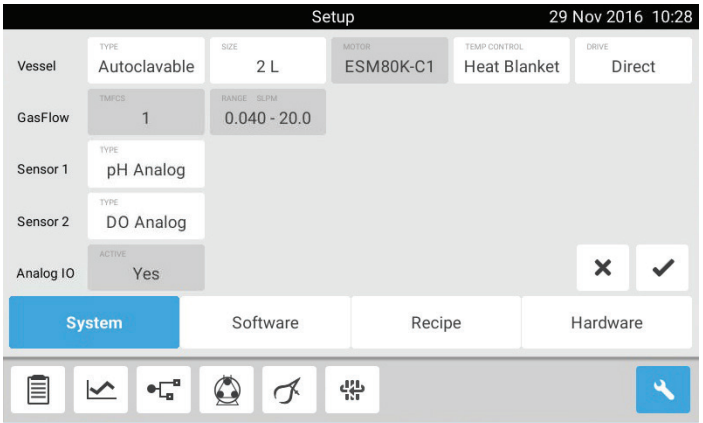

Fig. 3: Eppendorf bioprocess control software. The setup screen allows to select the vessel and sensors intended to use. All fields in the gas flow indicator and the analog input/output indicator section automatically populate based on the controller configuration.

Results

To compare the performance of the processes controlled by the BioFlo/CelliGen 115 and BioFlo 120 bioprocess control stations, the researchers analyzed the cell density of the culture and the concentrations of glucose and itaconate in the culture supernatant over the duration of the processes. Figures 4A and B show previously published results obtained with the BioFlo/CelliGen 115 controller [1]. Results obtained with the BioFlo 120 bioprocess controller were very similar (Figure 4C and D). Within 96 hours the glucose in the culture medium was completely consumed, but temporarily raised again by a single feed pulse at that timepoint. Glucose consumption was accompanied by an increase in cell density to an OD_{600} of around 120 and then stayed almost constant until the end of the process. The itaconate concentration in the culture supernatant increased almost linearly to approximately 60 g/L after 168 hours.

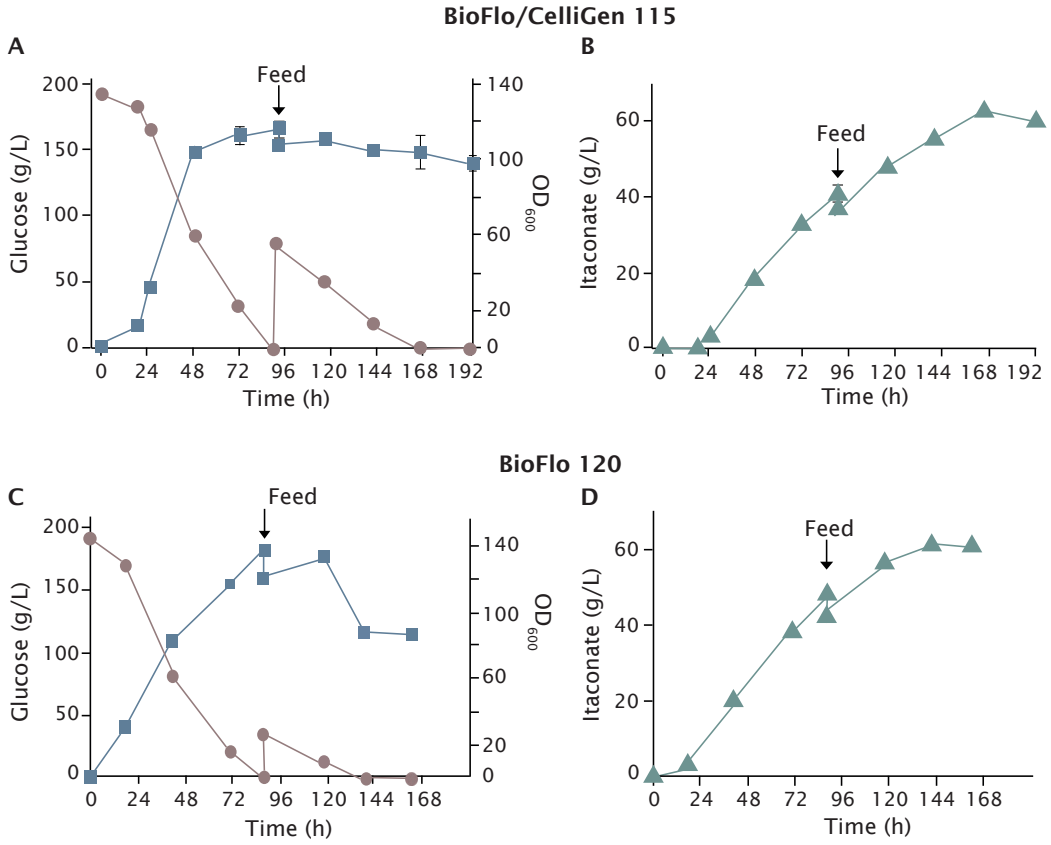

Fig. 4: High-density fermentation of *U. maydis* MB215 Δ*cyp3* P_{eter}ria1 controlled with a BioFlo/CelliGen 115 **(A, B)** and BioFlo 120 **(C, D)** bioprocess control station. The glucose concentration in the culture supernatant, the optical density of the culture **(A, C)**, and the itaconate concentration in the supernatant **(B, D)** were quantified offline.

Conclusion

By metabolic strain engineering and optimization of bioprocess parameters and medium composition, researchers at the RWTH Aachen increased the itaconate production by *U. maydis* to an industrially relevant level. In just a short period of time working with the new BioFlo 120, the researchers were able to reproduce the results from an already optimized production process on the new system. Impressed with its ease of use, Hamed Tehrani, lead researcher on the project concluded "The results were the same when compared to the BioFlo/CelliGen 115, but the BioFlo 120 proved much easier to use."

Literature

[1] Geiser E, Przybilla SK, Engel M, Kleineberg W, Büttner L, Sarikaya E, den Hartog T, Klankermayer J, Leitner W, BölkerM, Blank LM, Wierckx N (2016) Genetic and biochemical insights into the itaconate pathway of *Ustilago maydis* enable enhanced production. *Metabolic engineering*. 38:427-435

Ordering information

Description	Order no.
BioFlo® 120, Advanced	
Plug type B (USA, Canada, Mexico, Japan)	B120ACS000
Plug type CEE 7/7 (EU (except UK, Ireland, Switzerland), Russia, Korea	B120ACS001
Plug type I (Australia, New Zealand, China, Argentina)	B120ACS002
Plug type J (Switzerland)	B120ACS003
Plug type G (UK, Ireland)	B120ACS004
Plug type N (Brazil)	B120ACS005
Plug type D (India)	B120ACS006
BioFlo® 120 Fermentation Vessel Bundle	
1 L, heat blanket	B120AVB000
2 L, heat blanket	B120AVB001
5 L, heat blanket	B120AVB002
10 L, heat blanket	B120AVB003
1 L, water jacket	B120AVB004
2 L, water jacket	B120AVB005
5 L, water jacket	B120AVB006
10 L, water jacket	B120AVB007

For more information on these and other configurations visit www.eppendorf.com/BioFlo120

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