APPLICATION NOTE No. 304 I June 2016

High-Density Fermentation of *Corynebacterium glutamicum* for Renewable Chemicals Production

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Abstract

A large variety of chemicals can be efficiently produced by microbial fermentation. They are used in a multitude of applications; for example, as biofuels, building blocks for polymers, food supplements, and ingredients for cosmetics. To be profitable, fermentation processes have to deliver the desired end product at a high yield and a high titer. In this application note we describe bioprocesses which were carried out by the team of Professor Wittmann at the University of Saarbruecken. They used engineered *Corynebacterium glutamicum* strains for the production of the compatible solute ectoine and the polyamide building block 1,5-diaminopentane. The researchers optimized culture conditions in an Eppendorf DASGIP® Parallel Bioreactor System and established high-density, fed-batch processes which delivered high titers of the desired chemicals at high yield.

Introduction

Professor Wittmann's team deals with the systems biology of industrially relevant microorganisms, to develop tailored microbial cell factories for sustainable bioproduction. In this application note, they highlight the bioproduction of two different chemicals which are in great industrial demand. The first is ectoine, a compatible solute with cell protecting properties. Among other applications, it is used as an ingredient of cosmetics and healthcare products. The second is 1,5-diaminopentane, which is a promising building block for the production of polyamide. The global market demands 6.6 million tons of polyamide per year for the manufacturing of fibers for clothing, car parts, materials in medical applications, and many other products [1].

In nature, ectoine is produced by halophilic bacteria. As their cultivation in high-saline media imposes great challenges to the fermentor material, there is a growing interest in the development of bacterial strains which produce and secrete ectoine under more standard cultivation

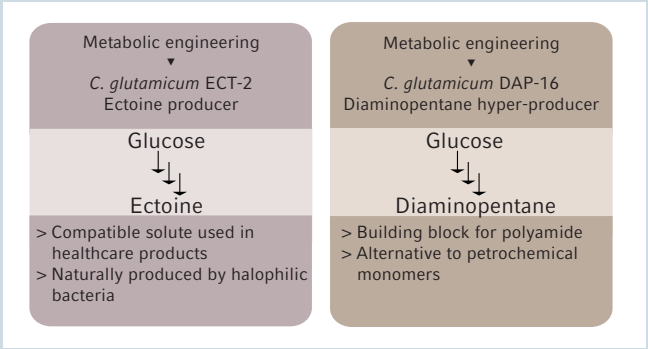

Fig. 1: Ectoine and diaminopentane production.

conditions. In the work presented here, the researchers designed a *C. glutamicum* strain that produces and secretes ectoine without the need for high-saline growth conditions [2], (Fig. 1).

Polyamide is typically synthesized from petrochemical monomers, but its bio-based manufacturing is highly desirable. Producing polymer building blocks through microbial fermentation can save primary energy input and greenhouse gas emissions, and ultimately reduce dependence on fossil fuels. Using systems metabolic engineering, the Wittmann team optimized a *C. glutamicum* strain for diaminopentane production. They achieved this by the overexpression of several enzymes in its biosynthetic pathway, and the shut-down of competing metabolic pathways by gene deletion or attenuation [1], (Fig. 1).

The team used an Eppendorf DASGIP Parallel Bioreactor System to establish fed-batch processes which deliver the desired end products at high yields and high titers. For process optimization, monitoring and control of critical process parameters is key. We will exemplify basic functions of the Eppendorf bioprocess control software, DASware® control 5, in conveniently monitoring, controlling, and displaying process variables.

Material and Methods

Strains

For ectoine production the research team used the *C. glutamicum* strain ECT-2 [2]. Diaminopentane was produced using the *C. glutamicum* strain DAP-16 [1].

Fed-batch production of ectoine and diaminopentane

Table 1 summarizes the fermentation conditions for ectoine and diaminopentane production.

Table 1: Process parameters

	Ectoine	Diaminopentane
***C. glutamicum* strain**	ECT-2	DAP-16
Initial volume	300 mL	300 mL
Temperature setpoint	35°C	30°C
DO setpoint	> 30 %	> 20 %
pH setpoint	6.9	7.0

The researchers cultivated *C. glutamicum* in initial working volumes of 300 mL, in an Eppendorf DASGIP Parallel Bioreactor System (Fig. 2) equipped with 1 L DASGIP Bioblock Stirrer Vessels. They monitored and controlled process parameters using DASGIP Control software (now DASware control 5). Temperature was kept constant at 35°C (for ectoine production) and at 30°C (for diaminopentane production), respectively. They controlled the pH by automatic addition of base using an Eppendorf DASGIP MP8 multi pump module. For the control of dissolved oxygen (DO), the researchers implemented a DO cascade which varies the agitation speed and the aeration rate. Such a

Fig. 2: DASGIP Parallel Bioreactor System with Bioblock

Fig. 3: DASware control 5 software facilitates the implementation of customized DO cascades. Agitation, gas flow, and oxygen enrichment can be individually defined, displayed, and edited online.

DO cascade can be easily set up using DASware control 5 software. Agitation, gas flow, and oxygen enrichment can be individually defined, displayed, and edited online (Fig. 3). The researchers carried out fermentations in the media defined in [1] and [2]. To monitor bacterial growth and the production performance, they quantified optical densities, glucose concentrations, and product concentrations offline (Fig. 4). Glucose concentrations were determined enzymatically using a 2300 STAT Plus™ analyzer or a 2700 Select analyzer (YSI® Inc. / Xylem® Inc., USA). Ectoine and diaminopentane concentrations were determined by HPLC. To achieve high product yields, the Wittmann team established fed-batch processes. For ectoine production, feeding was initiated by a DO-based signal, resulting in glucose concentrations below 5 g/L. For diaminopentane production, feeding was initiated when the glucose concentration had dropped to 10 g/L, thus maintaining glucose concentration above 10 g/L.

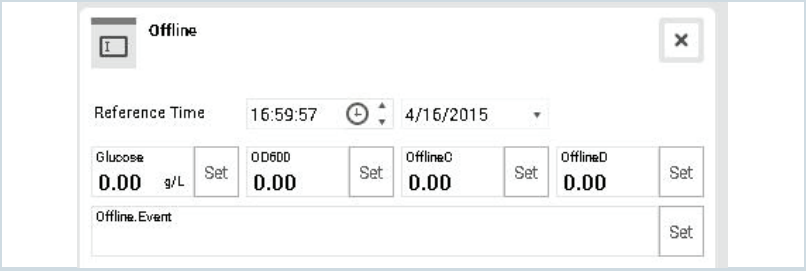

Fig. 4: Using DASware control 5 software, offline measured values, like for example the concentrations of nutrients and metabolites, or the optical density, can be seamlessly integrated. Like online measured values, they can be displayed, automatically exported, and used for calculations. Up to four (DASware control 5) or 26 (DASware control 5 professional) online values can be processed.

Results and Discussion

Ectoine production

Within 8 hours *C. glutamicum* ECT-2 consumed the initially supplied glucose, and the optical density (OD_{600}) of the culture increased to 100 (Fig. 5 A). In the course of this initial batch phase, the ectoine concentration increased to 2 g/L. Growth arrest leads to reduced oxygen consumption and hence an increase in DO. Based on the DO readings feeding was initiated, resulting in glucose concentrations of around 5 g/L. In this fed-batch phase the bacteria did not produce more biomass but shifted the production towards the formation of ectoine. Ectoine concentration reached a final titer of 4.5 g/L after 16 hours (Fig. 5 A). The different cultivation phases gave markedly different product yields. In the initial batch phase, the culture produced 28 mmol ectoine per mol glucose, whereas in the following fed-batch phase the ectoine yield was 298 mmol per mol glucose

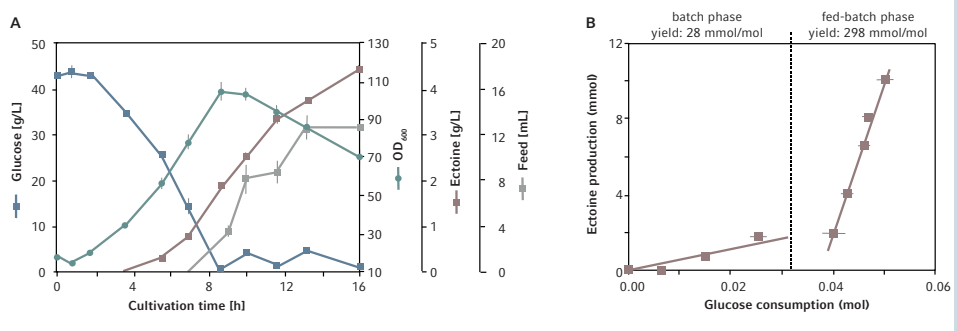

Fig. 5: Fed-batch fermentation of the ectoine producer *C. glutamicum* ECT-2 in a DASGIP Parallel Bioreactor System. **A:** Cultivation profile. **B:** Ectoine yields (mmol per mol glucose) achieved in the batch- and the fed-batch phases. The data represent mean values from two independent fermentation experiments.

(Fig. 5 B). Overall the fermentation produced 6.7 g/L ectoine per day, which is among the highest yields reported in the literature so far.

Diaminopentane production

Within 12 hours, the *C. glutamicum* DAP-16 culture reached an optical density of 120, and consumed the initially supplied glucose. Upon glucose depletion, feeding was initiated to maintain glucose concentrations above 10 g/L. *C. glutamicum* continuously secreted diaminopentane, which reached a concentration of 88 g/L within 50 hours. (Fig. 6 A). During the initial batch phase, the culture metabolized 21 % of glucose to diaminopentane. During the subsequent feeding phase, the yield increased to 29 % (Fig. 6 B).

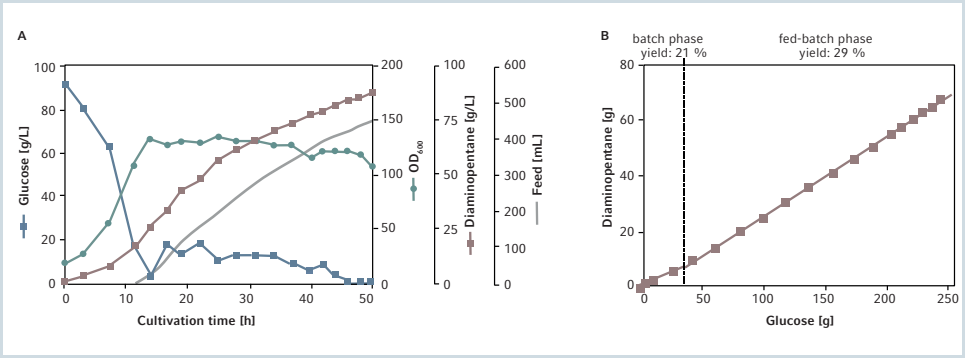

Fig. 6: Fed-batch fermentation of the diaminopentane-producer *C. glutamicum* DAP-16 in a DASGIP Parallel Bioreactor System.
A: Cultivation profile.
B: Diaminopentane yields (grams per gram glucose) achieved in the batch- and the fed-batch phase, respectively. A representative result is shown.

Conclusion

For profitable bio-based production, microbial fermentation processes have to deliver the desired end products at high yields and high titers. The results presented here exemplify the successful high-density fermentation of *C. glutamicum* in a DASGIP Parallel Bioreactor System. Cultivation of metabolically engineered strains in optimized fed-batch processes led to product titers which are among the highest reported so far. Bioprocess control software like DASware control 5 can help the researcher to establish efficient production processes. By allowing intuitive monitoring and control of critical process parameters, and the seamless integration of offline measured values it simplifies process optimization and facilitates fermentation under optimal culture conditions.

Literature

[1] Kind, S. et al. From zero to hero – Production of bio-based nylon from renewable resources using engineered *Corynebacterium glutamicum*. Metabolic Engineering 2014, 25:113

[2] Becker, J. et al. Systems metabolic engineering of *Corynebacterium glutamicum* for production of the chemical chaperone ectoine. Microbial Cell Factories 2013, 12:1

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System for Microbial Applications, max. 250 sL/h gassing	
4-fold system with Bioblock	76DG04MBBB
8-fold system with Bioblock	76DG08MBBB
16-fold system with Bioblock	76DG16MBBB
DASGIP® Bioblock Stirrer Vessels	
2x Rushton-type impeller, L-Sparger, 200 mL – 1.0 L	76SR0700ODLS
2x Rushton-type impeller, L-Sparger, 400 mL – 1.5 L	76SR1000ODLS
3x Rushton-type impeller, L-Sparger, 400 mL – 1.8 L	76SR1500ODLS
DASware® control, incl. PC, OS, and licenses	
for 4-fold DASGIP® system	76DGCS4
for 8-fold DASGIP® system	76DGCS8
for 16-fold DASGIP® system	76DGCS16
DASware® control professional, incl. PC, OS, and licenses	
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for 8-fold DASGIP® system	76DGCSP8
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