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Advanced Biofuel Development Using Parallel Bioreactor Systems

Christiane Schlottbom[1] and **Sebastian Kleebank**[2]
[1]Eppendorf AG, Bioprocess Center Europe, Juelich, Germany, [2]DASGIP Information and Process Technology GmbH, Juelich, Germany

Executive Summary

To make biofuels such as bioethanol and biodiesel competitive with fossil fuels extensive research in the field of process development is needed. Taking into account the special requirements of biofuel production, such as high temperatures, multi-step procedures and anaerobic conditions, effective tools for streamlining development efforts must be provided.

Process optimization efforts start at the lab scale with industrial standard bioreactors mimicking the manufacturing process and delivering reproducible and scalable results. Parallel bioreactor systems featuring industrial bioreactor design and addressing key requirements have the potential to save time and decrease costs through integrated process development.

Introduction

Scarce oil resources, political instabilities and the increasing global demand for energy have led to skyrocketing oil prices on the world markets, drastically highlighting the urgent need to search for alternative energy sources, particularly for fuels. The European Union's Renewables Directive requires that 20 percent of the energy demand within the EU will come from renewable resources by 2020 [1].
The food vs. fuel dilemma and associated ethical and economical concerns have led to a shift from the so-called 1st generation biofuels (obtained from food crops) to the „advanced" or 2nd generation biofuels from non-edible raw materials. Beyond that, great hopes are attached to fuels generated from algal and microbial biomass (3rd generation), mostly avoiding the critical competition for land and water that even non-food crops are accused of. A variety of fuels and bio-based chemicals are already produced from renewable resources, among them bioethanol, biodiesel, hydrogen, butanol and methane. Only few processes associated with 2nd and 3rd generation biofuels have made it to the commercial stage yet. Optimization of production processes still remains the main challenge in biofuel process development in order to be competitive with fossil fuels. Finding and improving enzymes for the hydrolysis of raw materials, testing and comparing new stocks for biofuel production and overall reduction of technology costs through integrated processes are among the tasks scientists and process engineers are facing [2,3].

Parallel operation of lab scale bioreactors and comprehensive software solutions for data and information management offer an effective means for process optimization reducing time and increasing cost-effectiveness for the manufacturing scale.

Achieving high temperatures for pre-treatment of raw material

Raw materials, especially when talking about second generation biofuels, usually require a so-called pre-treatment as a first step: Enclosed polysaccharides such as cellulose, hemicellulose and pectin cannot be directly processed, but have to be cleaved to their mono- and disaccharide compounds. Although "pre-treatment" comprises a multitude of methods, many of them include chemical or physico-chemical techniques requiring high temperatures.
The Eppendorf DASGIP® Bioblock is a compact tool providing fast and individual temperature control in up to four bioreactors (fig. 1). Combined with the DASGIP TC4SC4 Module for Temperature and Agitation Control, it represents an integrated solution for accurate control of reaction temperatures up to 99 °C.

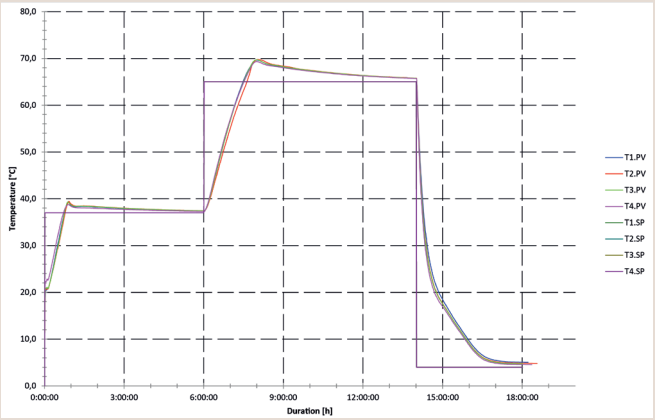

Figure 1: Temperature control with the DASGIP Bioblock. Four parallel DASGIP Bioblock Stirrer Vessels SR1000ODLS (with one cooling finger per vessel, 1.6 L working volume) were exposed to rapid temperature changes using the DASGIP Bioblock (temperature of cooling water: 4 °C; SP: setpoint, PV: process value).

Multi-step procedures using intelligent software packages

Processes in 2nd generation biofuel research and production typically require multiple steps. The above mentioned pre-treatment is usually followed by a hydrolysis step during which the obtained mixture of different substrates is enzymatically broken down into mono- and disaccharides. After that the actual transformation of the raw material into biofuels, e.g. conversion to bioethanol through microorganisms such as *Saccharomyces cerevisiae*, takes place.
Integrating the different steps into one automated process will save valuable development time, thereby supporting a good business model. Bioprocess control software DASGIP Control enables the use of various process values and time stamps for the activation of pre-defined profiles. DO-activated substrate feed and triggered switch from aerobic to anaerobic fermentation, initiating ethanol production, are among the possible applications. Also, automated transfer of liquid to another vessel, where e.g. enzymes are added, can be implemented.

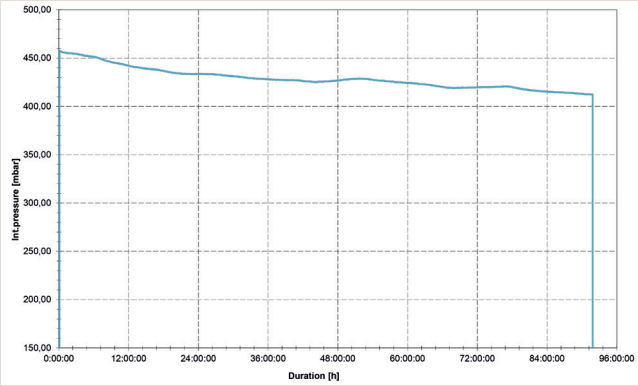

Figure 2: Pressure stability in DASGIP Bioreactors. DASGIP Bioblock Stirrer Vessels SR0700ODLS equipped with Bioprene® tubing were supplied with overpressure (~450 mbar). A pressure sensor was connected to the exhaust filter.

Anaerobic fermentation

Multi-step processes in biofuels research and production often include a switch from aerobic (growth phase) to anaerobic cultivation (production phase).
The DASGIP MX4/4(H) module provides accurate and individual gas mixing from N_2, O_2, CO_2 and air in up to 4 laboratory scale bioreactors. If higher flow rates are required, the DASGIP MX4/1 model provides one vessel with up to 600 sL/h or 1200 sL/h (MX4/1H).

Optional pressure sensors at the outputs allow safe operation of glass bioreactors with all gassing modules.
Vessels preventing uncontrolled gas exchange with the environment are essential for biofuel development, e.g. to maintain strictly anaerobic conditions. DASGIP bioreactors can be equipped with silicon tubing as well as Bioprene tubing. In leakage tests lasting 96 hours gas tightness of vessels with Bioprene tubing was maintained (pressure loss <12 mbar/24 h, fig. 2).

Precisely controlled pH and redox values

In anaerobic metabolism of microorganisms a negative redox potential is essential for specific enzyme activities. As even small changes in pH can influence the redox potential, both parameters must be closely monitored and controlled.
With the Eppendorf DASGIP PH4RD4(L), DASGIP PH8RD8 and DASGIP PH4PO4RD4L users can measure redox potential and pH individually in 4 or 8 parallel operated bioreactors. Addition of acid, base or reducing agents with the DASGIP MP8 and MP4 Multi Pump Modules automatically adjusts pH and redox values to the given set-points.
Users can combine these features with further options such as level/anti foam control („L"), exhaust analysis (DASGIP GA4 Module), and optical density monitoring (DASGIP OD4). All modules are suitable for supporting working volumes from 60 mL up to pilot scale.

Process optimization with Design of Experiments

Finding the right strain, enzymes and process conditions for optimum product yields can be a time-intensive and costly challenge requiring multiple experiments.
The parallel nature of the DASGIP System provides an effective way of process optimization saving time and resources: 4, 8, and more bioreactors are operated at the same time, including parallel sensor calibration, an intuitive user interface as well as integrated recipe management and report generation. OPC communication enables implementation of solutions facilitating process development in line with industry-standard guidelines such as PAT and QbD.
Design of Experiments (DoE) is a structured approach for development and optimization of processes. Compared to the One-factor-at-a-time method it offers a reliable and reasonable way to determine a proper design space for the production process. As part of the Eppendorf DASware® software suite, DASware design was developed to apply DoE concepts to bioprocesses. It comes with a full factorial DoE Builder and also allows for automated import of DoE designs from most powerful 3rd party DoE statistical tools like JMP® and others. Parallel recipes incorporating the DoE factor variations (e.g. pH, temperature or feedrates) are automatically implemented.

Integration of 3rd party analyzers

Research and process development approaches often include storage and analysis of externally measured data such as HPLC, mass spectrometry (MS) and nutrient analyzers. Calculation of associated fermentation parameters can be time-consuming and labor-intensive.
The Eppendorf software DASware analyze was designed for seamless integration of such laboratory devices into the bioreactor control system. In an isobutanol production process, combination of a parallel bioreactor system with online mass spectrometry allowed for real-time calculation of key process parameters from combined fermentation and exhaust MS data [4]. Charting of process data as well as user-editable recipes streamlined the workflow. Data-driven control decisions enabled automation of the bioprocess.
The integration of nutrient analyzers (e.g. Cedex® Bio, Nova BioProfile®, YSI®) allows real-time monitoring of e.g. glucose consumption or ethanol production including direct feedback control.

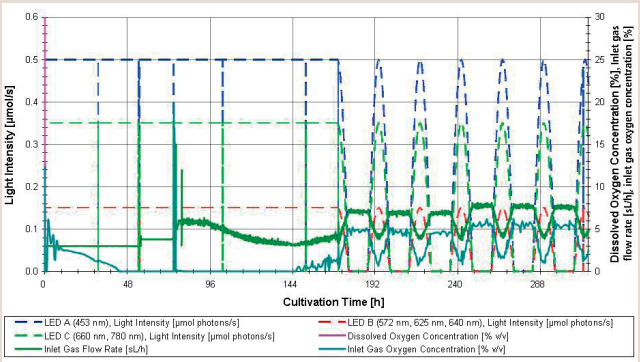

Figure 3: Cultivation of the alga *Dunaliella tertiolecta*. Algae were exposed to constant illumination and day/night cycles, respectively (light intensity [µmol photons/s]). Dissolved Oxygen Concentration [%], Inlet Gas Flow Rate [sL/h] and Inlet Gas Oxygen Concentration [%] were monitored online. [5]

DASware discover enables near real-time retrieval of runtime information from a SQL Server® database by intuitive queries. A Microsoft® Excel® report generator provides recipes, process information and event reporting.

3rd generation biofuels: cultivation of algae

Because of their high lipid content the cultivation of algae for regenerative energy applications arouses great interest and thus algae fuels are expected to contribute a big part when talking about future biofuel production. In order to make algae fuels commercially viable, however, further research must be carried on to increase and optimize production quantities. Evaluating the optimum growth parameters for high product concentrations is both essential and challenging.
DASGIP PhotoBioreactors have been proven effective for the cultivation of various photosynthetic organisms such as the green alga *Dunaliella tertiolecta* [5]. Light emission of integrated LED devices is adjusted to the specific needs of phototrophic organisms, resembling the absorption wavelengths of relevant chlorophyll variants. In combination with the DASGIP PBR4 Module different light spectra and light intensities can be controlled. Day/night cycles can be programmed and optimized to the culture's specific requirements (fig. 3). The DASGIP GA4 Exhaust Analyzer enables precise measurement of CO_2 consumption and O_2 production.

Summary

Eppendorf DASGIP Parallel Bioreactor Systems address the specific demands of biofuel research and development covering various applications such as aerobic and anaerobic fermentation and cultivation of phototrophic organisms. With these systems, processes can be carried out under the absence of oxygen and through independent monitoring of pH and redox potential as well as safe and flexible gassing. Advanced data and information management software, integration of 3rd party analyzers, and DoE support customers in streamlining their process optimization efforts and reducing development costs.

References

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[4] Glenn J, Huether CM. Isobutanol from Renewable Feedstock - Process Optimization by Integration of Mass Spectrometry to two 8-fold DASGIP Parallel Bioreactor Systems. Eppendorf Application Note 295; www.eppendorf.com

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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
DASGIP® Bioblock Stirrer Vessels, Rushton-type impeller, L-Sparger, overhead drive, Bioblock	
200 mL – 1 L (SR0700ODLS)	76SR0700ODLS
500 mL – 1.5 L (SR1000ODLS)	76SR1000ODLS
400 mL – 2.0 L (SR1500ODLS)	76SR1500ODLS
DASGIP® PhotoBioreactor, pitched blade impeller, dip tube, overhead drive, 700 mL – 2.7 L (DR03P)	76DR03P
DASGIP® Control System, incl. PC, OS, DASGIP® Control and licenses	
for 4 vessels	76DGCS4
for 8 vessels	76DGCS8
DASware® analyze, OPC client professional incl. 1x tunneller lic. (OPC DA e.g. for ext. analyzer with autosampler)	
for 4 vessels	76DWANA4P
for 8 vessels	76DWANA8P
DASware® design, license for 1 vessel (DoE and local information management)	76DWDOE
DASware® discover	
client-license for 1 vessel (SQL Server® based information management)	76DWDIS
information management server (PC hardware, OS software and server license)	76DWDISPC
server-license (SQL Server® based information management)	76DWDISS
DASGIP® Bioblock, for 4 vessels (4-position heating/cooling block, max. temp. 99°C)	76DGTBLOCK
DASGIP® TC4SC4 Temperature and Agitation Control Module, for 4 vessels, w/o sensors, for Bioblock (TC4SC4B)	76DGTC4SC4B
DASGIP® MX4/4 Gas Mixing Module, for 4 vessels, mass flow controller	
0.1 – 50 sL/h, 0.1 – 40 sL/h CO_2	76DGMX44
0.5 – 250 sL/h, 0.5 – 150 sL/h CO_2 (MX4/4H)	76DGMX44H
DASGIP® MX4/1 Gas Mixing Module, for 4 vessels (4x MX4/1), mass flow controller	
20 – 600 sL/h	76DGMX41
40 – 1200 sL/h (MX4/1H)	76DGMX41H
DASGIP® PH4RD4 Monitoring Module, for 4 vessels w/o sensors, pH and redox	76DGPH4RD4
DASGIP® PH4RD4 Monitoring Module, for 4 vessels, w/o sensors, pH and redox, with level/anti foam option (PH4RD4L)	76DGPH4RD4L
DASGIP® PH8RD8 Monitoring Module, for 8 vessels w/o sensors, pH and redox	76DGPH8RD8
DASGIP® PH4PO4RD4L Monitoring Module, for 4 vessels, w/o sensors, pH, DO and redox with level/anti foam option (PH4PO4RD4L)	76DGPH-4PO4RDL
DASGIP® MP8 Feeding Module, for 8 feeds, w/o feed lines and reservoir bottles	76DGMP8
DASGIP® MP4 Feeding Module, for 4 feeds, w/o feed lines and reservoir bottles	76DGMP4
DASGIP® GA4 Exhaust Analyzing Module, incl. accessories for 4 vessels	
O_2 1 – 50 %, CO_2 0 – 25 %	76DGGA4
O_2 0 – 100 %, CO_2 0 – 25 % (GA4E)	76DGGA4E
DASGIP® Optical Density Measurement, for 4 vessels, incl. transmitter and cables, w/o sensors	76DGOD4
DASGIP® PhotoBioreactor Illumination Module, for 4 vessels, w/o LED Illumination Devices	76DGPBR4
DASGIP® PhotoBioreactor LED Illumination Devices, for 1 vessel, 220 mm, type S (4 sticks w/ 453/572/625/640/660/780 nm)	76DGLED220S

* DASGIP® Parallel Bioreactor Systems are configured to meet individual customer requirements. The systems shown are example configurations. Please contact us for more information.

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