

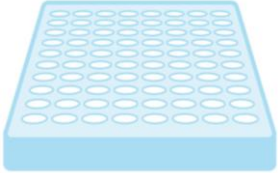
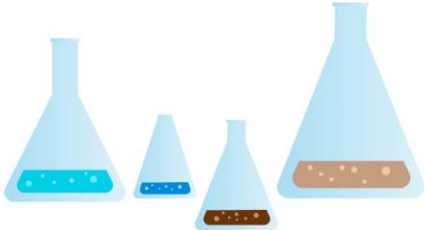



Liquid Injection System (LIS)

AUTOMATED FEEDING IN SHAKE FLASKS

Before, automated feeding experiments could not be performed in shake flasks, limiting their use for bioprocess development.

Problem: Lack of Feeding Technologies for Shake Flask Cultures

	Microtiter Plate	Shake Flask	Bioreactor
			
Automated Feeding Availability	✓	✗	✓
Feeding Applications	Fed-batch, pH control, automated induction	✗	Fed-batch, pH control, addition of antifoam, automated induction

LIS is the first technology allowing for automated feeding of liquids into shake flask cultures.

Liquid Injection System (LIS)



The LIS Drive and the LIS Cartridge are the two key components that allow for feeding of liquids into shake flasks.

Components of the LIS System (1/2)



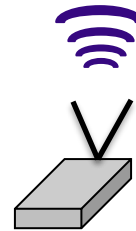
The DOTS Software and LIS hub enable you to set up, control and monitor LIS experiments wirelessly.

Components of the LIS System (2/2)

LIS Drive & Cartridge

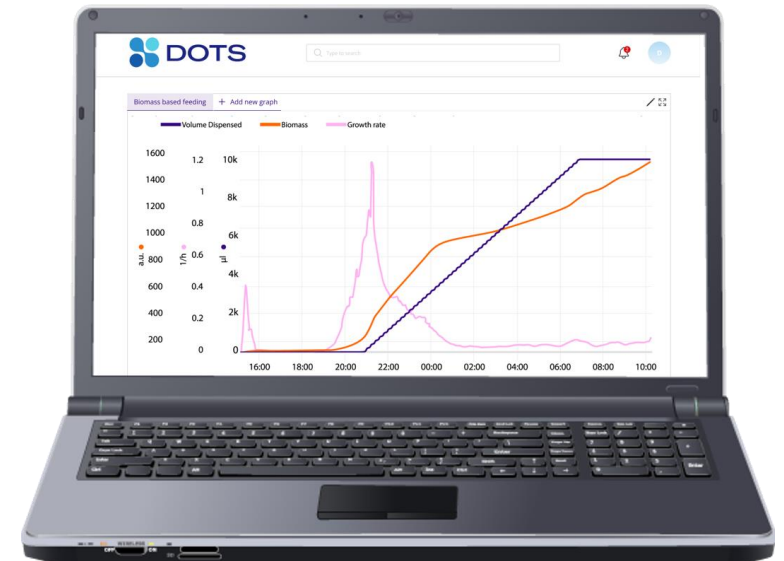


Wireless
Communication



LIS Wireless Hub

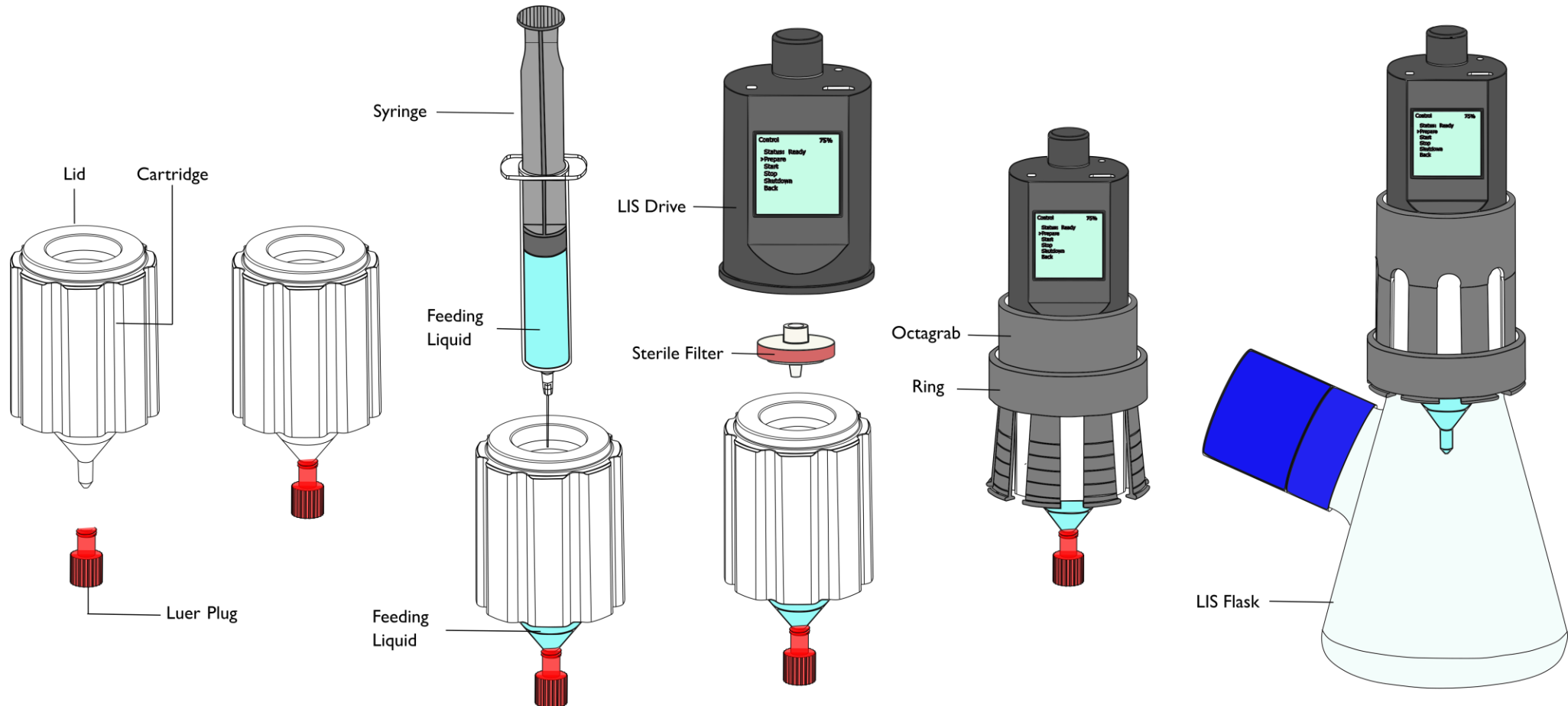
DOTS Software & LIS Coordinator



DOTS Software

LIS is easy to assemble: Fill the cartridge with the feeding liquid, connect the LIS drive to the cartridge and start feeding.

LIS Assembly



LIS creates significant value enabling scientists to perform feeding experiments on a shake flask level.

LIS Key Facts



Key Facts

Wireless control and monitoring

Control and monitor your feeding experiments wirelessly with the DOTS Software

Compatible with various substances

Sugars (e.g., 40% glucose), alcohols (e.g., 50% methanol), glycerol, acids, bases,...

Create any feeding profile you want

Single or multi shot, delay, exponential or continuous feeding

Easy to install and use

Fill the sterile cartridge, program the LIS drive and start feeding your culture

Flexible feeding rates

From 100 μ l droplets to 1 mL/min feeding rate

For a broad range of applications

Fed-batch, gene induction, pH regulation, automated inoculation, toxicity assay,...

The DOTS Software enables easy sensor handling and experiment set up.

Exemplary Screenshots

Create an experiment with pre-defined application templates

The screenshot shows the DOTS software interface with the 'Basic Settings' step selected. The 'Basic information' section is expanded, showing options for 'Quick start application templates' (selected) and 'Custom application templates'. A dropdown menu shows 'Feeding (Shake flask)'. Below this, there are fields for 'Experiment name' (Glucose feed exponential Strain B), 'Number of objects' (1), and 'Project' (Prosugar). An 'Advanced settings' toggle is at the bottom. On the right, a diagram shows a 'Glucose feed exponential Strain B' process with a 'Feeding (Shake flask)' task and a 'Feeding (LIS)' task marked as 'Planned'.

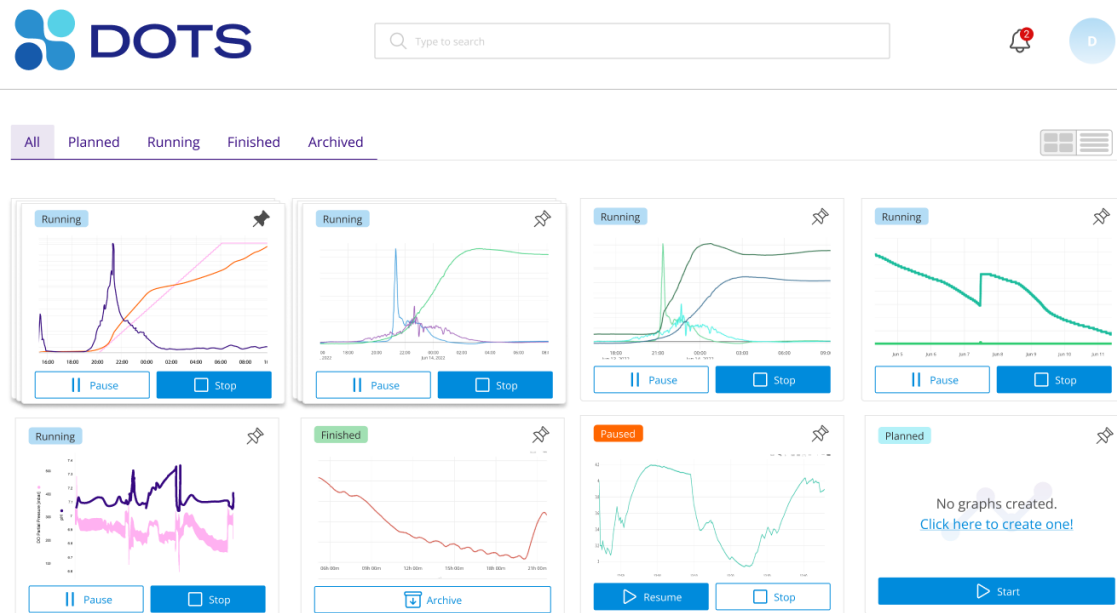
Assign sensors to planned experiments via drag and drop

The screenshot shows the DOTS software interface with the 'Device Assignment' step selected. A progress bar at the top indicates the steps: Basic Settings, General Task Configuration, Replicate configuration, and Device Assignment. A search bar is present. Below, a list of devices is shown: 'LIS-0025478' (80% battery, Not connected), 'CGQ-SP-02548' (CGQ-8-0025 / Port1, Not connected), and 'LIS-00-25478' (Free). A blue arrow points to the 'LIS-00-25478' device, indicating it is being assigned. On the right, a diagram shows a 'MET25 induction strain A' process with tasks: 'Biomass-based feeding (Shake flask)', 'Feeding (LIS)' (Planned), 'Biomass monitoring (CGQ)' (Planned), and 'OD600 (Offline)' (Planned). At the bottom, there are 'Exit wizard', 'Back', and 'Create' buttons.

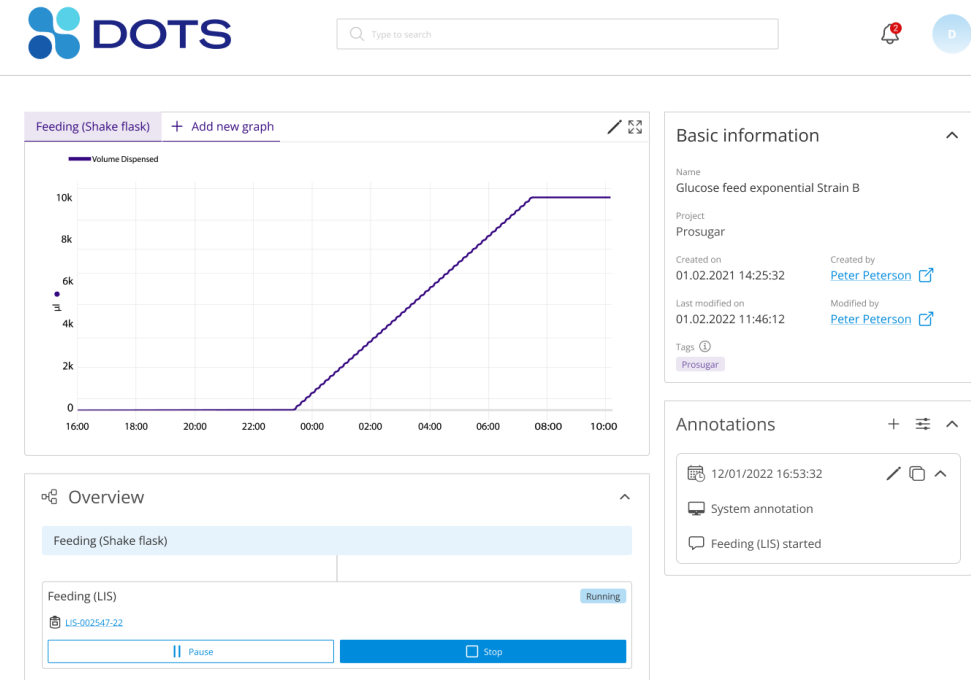
The DOTS Software provides a comprehensive overview of your experiments and visualizes your data in real-time.

DOTS Software Modules for Data Visualization

The dashboard schema provides an overview over all running, planned, or finished experiments

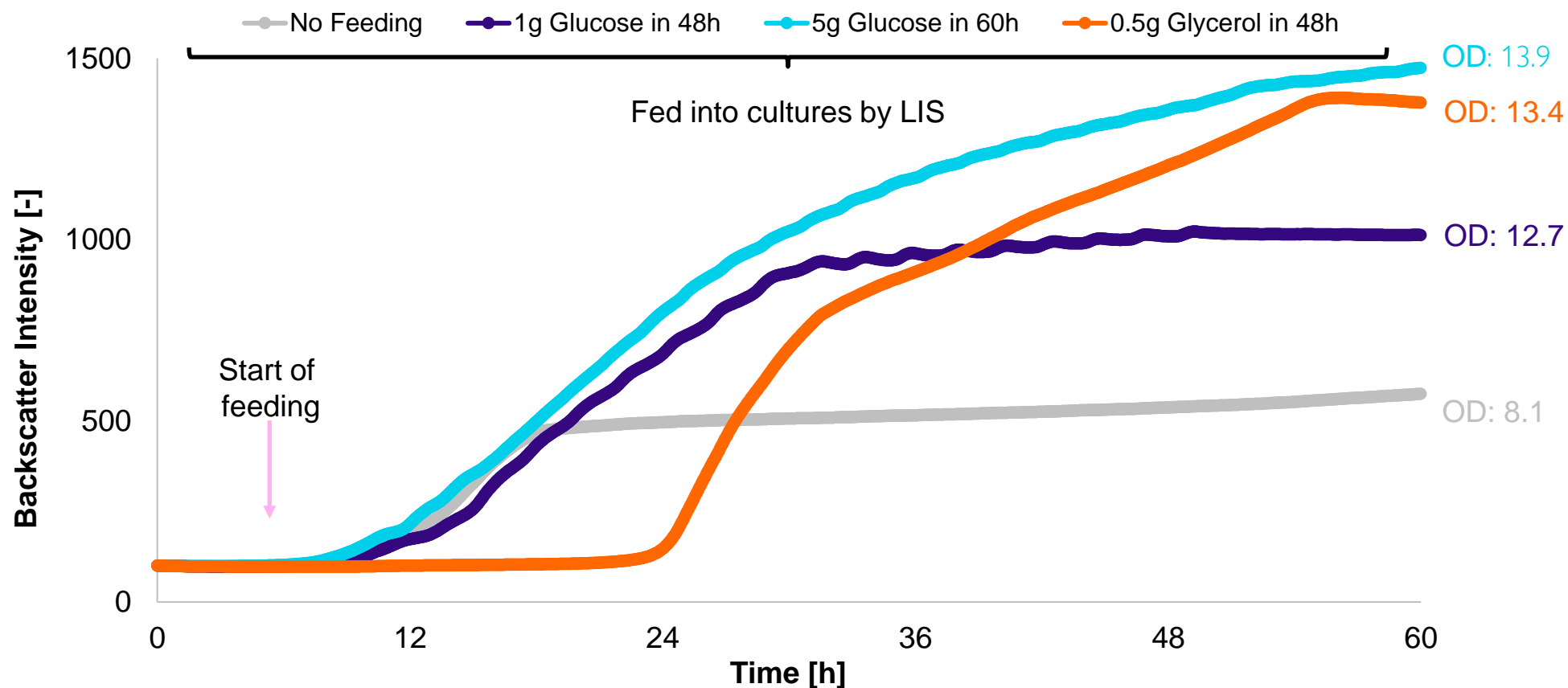


Data visualization tools enable a customized display of data in real-time



LIS can be used to create fed-batch conditions for microbial shake flask cultures and increase biomass and product titers.

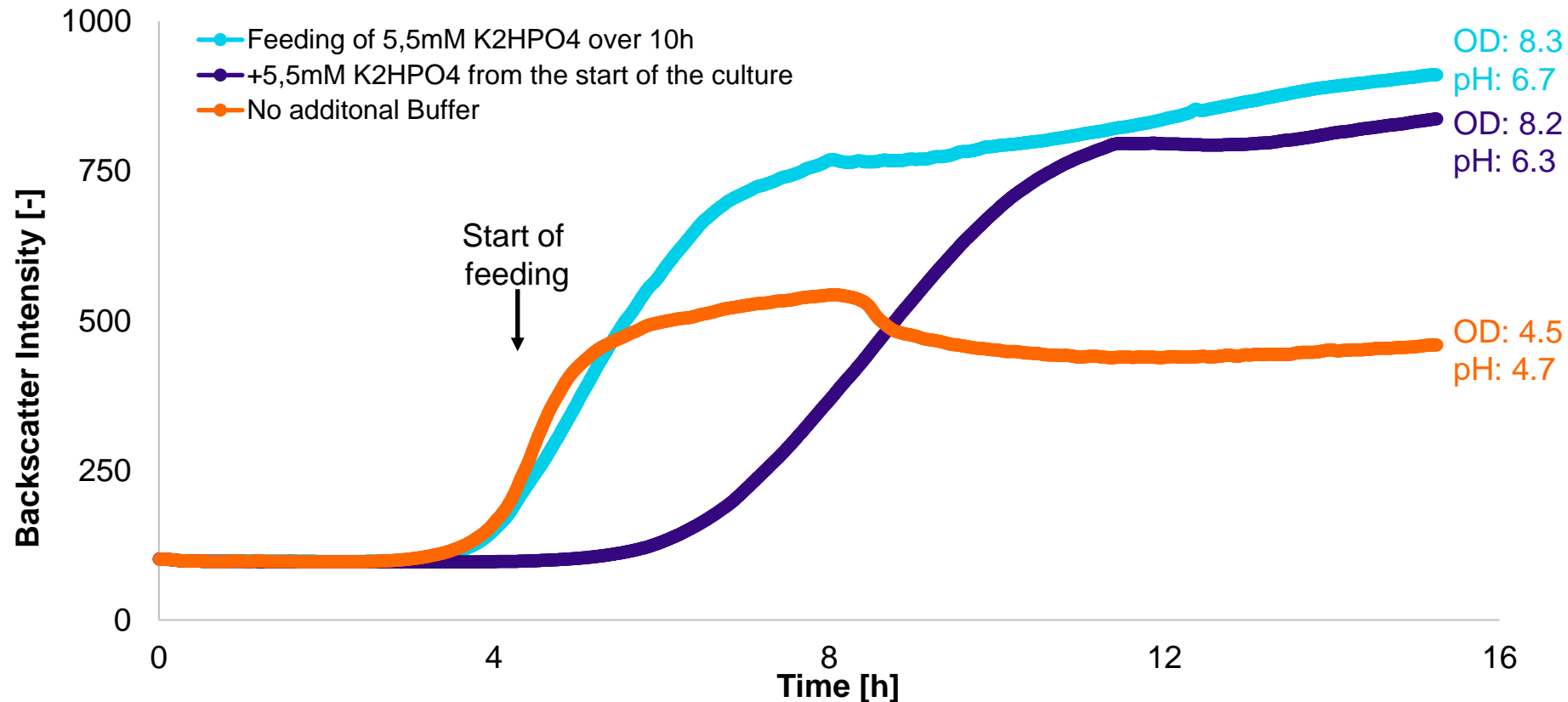
Example Use Case (1/2): Fed-Batch



Saccharomyces cerevisiae, 25 mL YPD Medium, 250 mL Shake Flasks, 30 °C, 250 rpm; growth curves monitored with the CGQ

LIS can be used to control pH drifts of *Escherichia coli* shake flask cultures by automatically feeding buffer to the culture.

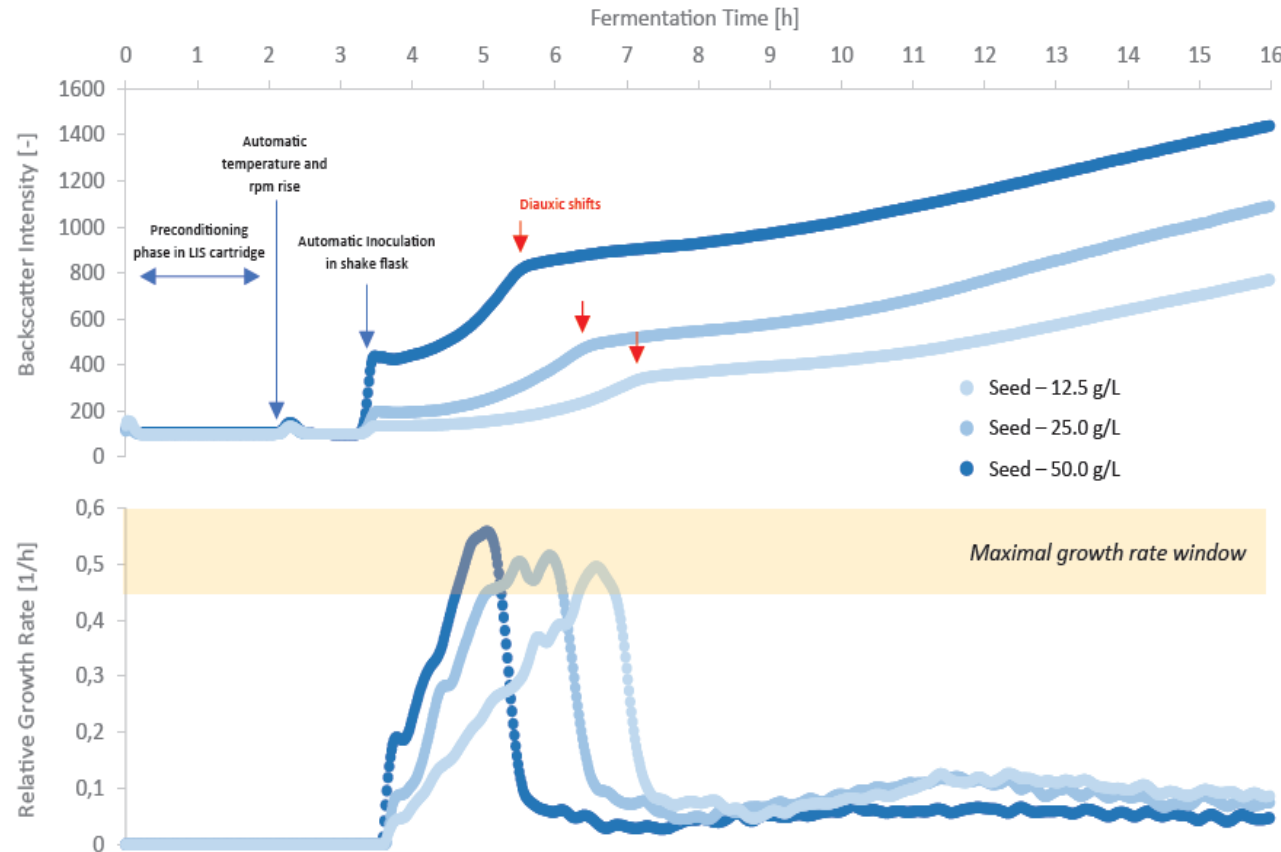
Example Use Case (2/2): pH Regulation



Escherichia coli, 25 mL LB Medium + 2% Glucose, 250 mL Shake Flask, 37°C, 250 rpm growth curves monitored with the CGQ

LIS and CGQ (biomass monitoring) allow for automated seed inoculation for the perfect bioreactor pre-culture in shake flasks.

CGQ and LIS for Seed Train Applications



Key Facts

- CGQ (biomass monitoring) and LIS (feeding) work together to prepare the perfect culture in the shortest time
- No interaction required
- No risk of contamination, no interruption of the process, better results

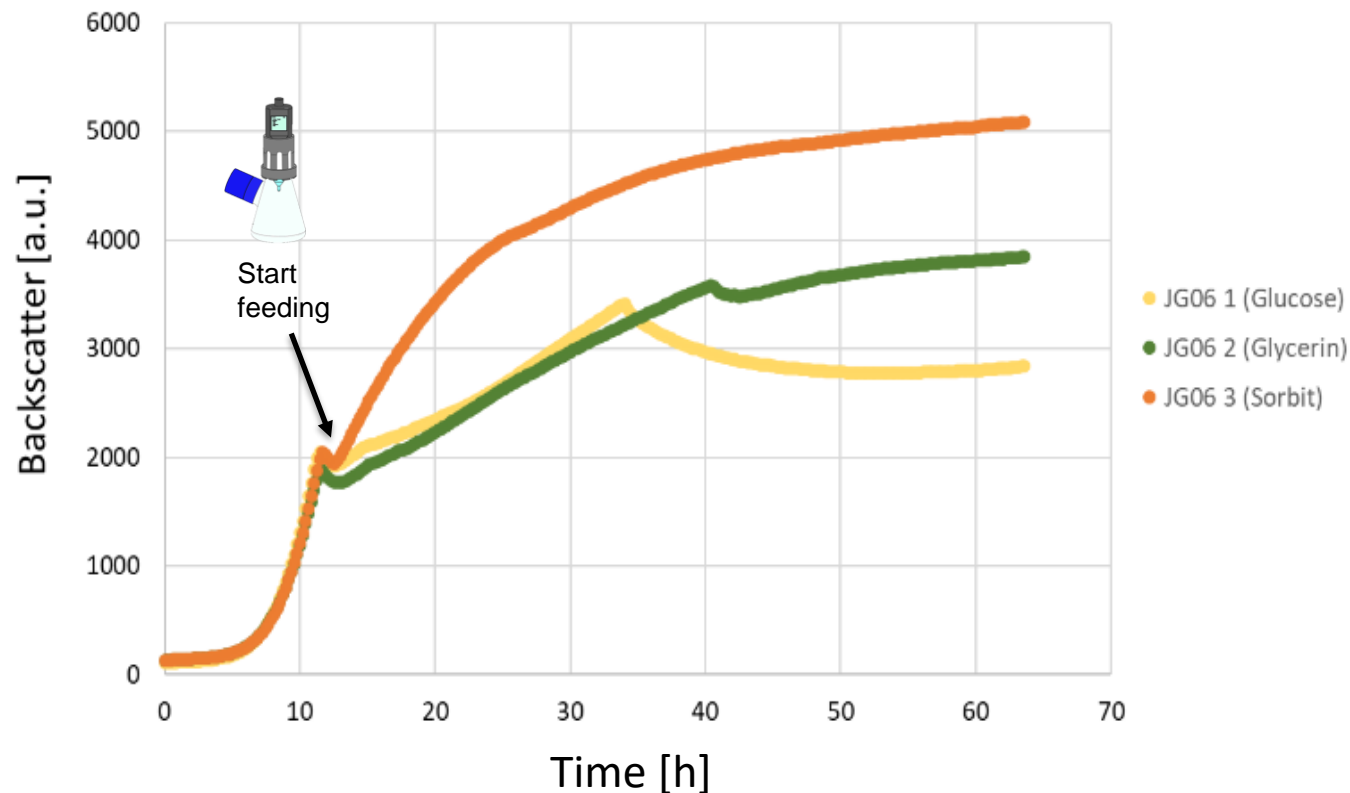
(Data from Eppendorf Application Note)

eppendorf

S. cerevisiae grown on YPD + 20 g/L Glc. Shaking speed: 100 – 210 rpm, 50 mm throw, temperature 10-30°C. Feeding: Yeast with 12.5 g/L – 50 g/L Glc

LIS and CGQ (biomass monitoring) enable bioreactor-like automated experiments in shake flasks.

Biomass-Based Automated Fed-Batch Feeding



H. polymorpha grown on mineral medium, 180 rpm, 30°C. Feeding rate: 2.5 g/L*h

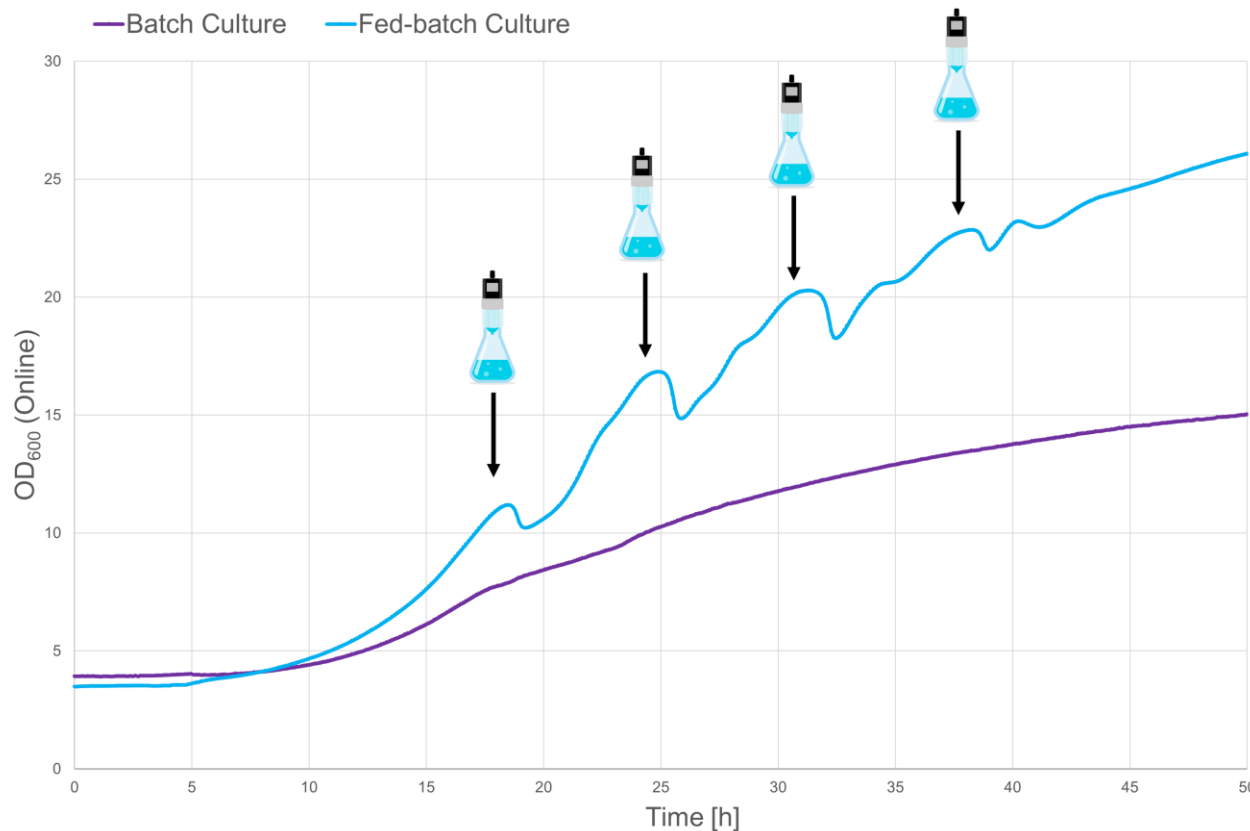
Key Facts

- LIS started feeding when the CGQ (biomass monitoring) detected the end of the batch phase
- Different sugars fed
- No risk of contamination, no interruption of the process, perfect time to feed without the need to be in the lab

(Preliminary data from Application Note)

With LIS, fed-batch cultivations can be created in shake flasks, supporting the modeling of larger scale bioprocesses.

Fed-batch Feeding of Potato Waste with LIS Increases Biomass Production



S. cerevisiae grown on potato waste. Feeding: Multi shot (fed-batch) feeding of 4x5 mL potato waste hydrolysate.

Key Facts

- High biomass yields were required but could only be achieved in fed-batch processes, due to growth inhibitors in the concentrated substrate (potato waste)
- LIS enabled fed-batch in shake flasks which increased biomass yields
- Data was used to model large scale bioreactor runs



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