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Standardizing Microbiome Research – Culture from Discovery to Production

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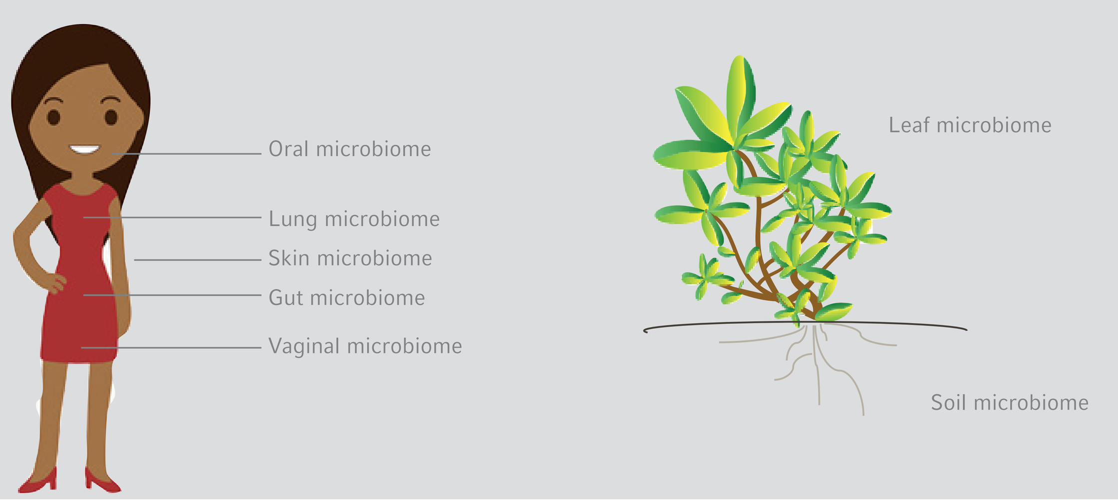
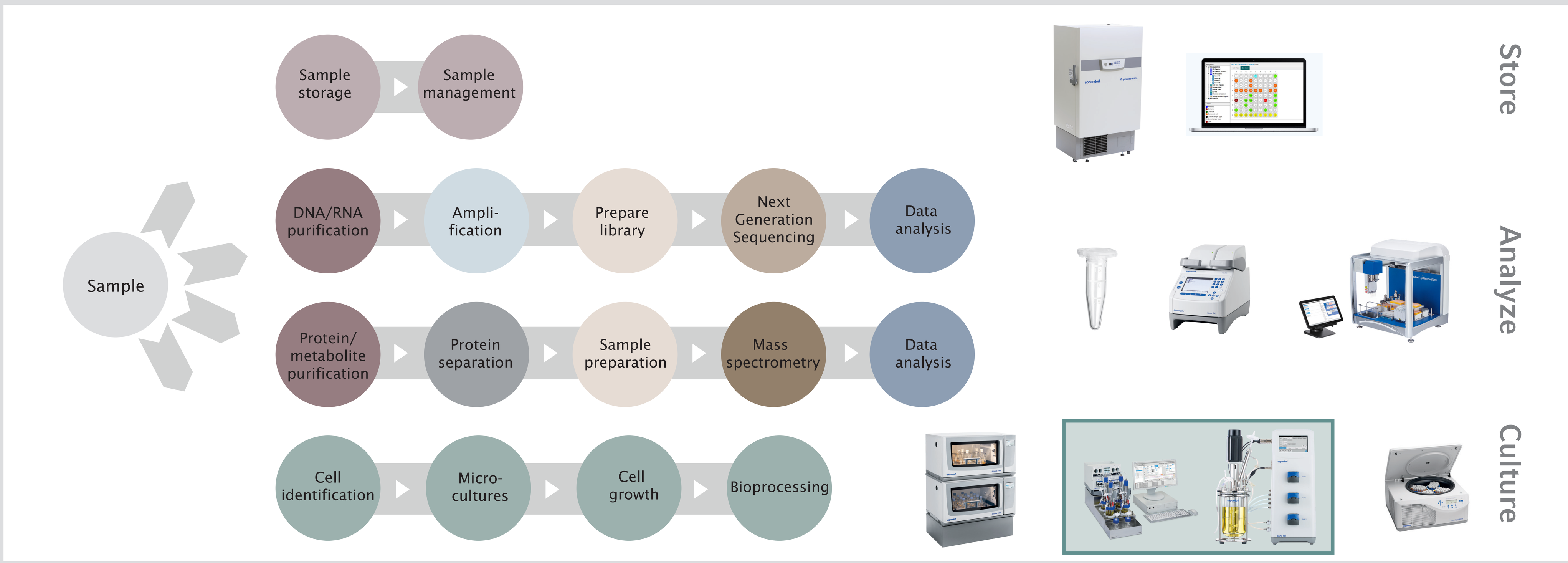
Various microbiomes

"We start to understand that microbes are important to our ecosystem and our health"
"Bacteria, if we change the ones inside you have the potential to cure diseases"

(Dr. Jack Gilbert, Professor in the Department of Pediatrics and the Scripps Institution of Oceanography, UCSD - Center of Mirobiome Innovation)



Microbiome workflow



Eppendorf supports the microbiome workflow from sample management, to sample preparation and analysis, to bacterial culture

Microbiome culturing needs are very diverse

Growth conditions can vastly differ depending on sample origin

> Soil
> Skin
> Gut
> ...
> Vaginal

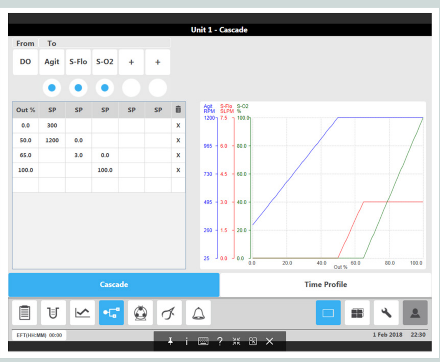
They require ideal match to source the environment

> Presence of oxygen: Aerobic
> pH
> No presence of oxygen: Anaerobic
> Nutrients and metabolites
> Temperature
> ...

Oxygen control

Bioprocess systems allow monitoring and control of the dissolved oxygen (DO) concentration in the culture medium

> DO monitoring using a DO sensor
> Monitoring of redox potential using redox sensors under an-aerobic conditions
> Gassing with air, O_2, N_2, CO_2
> Anaerobic conditions by gassing with anaerobic gas mixtures
> Automated gas mix via thermal mass flow controllers (TMFC)

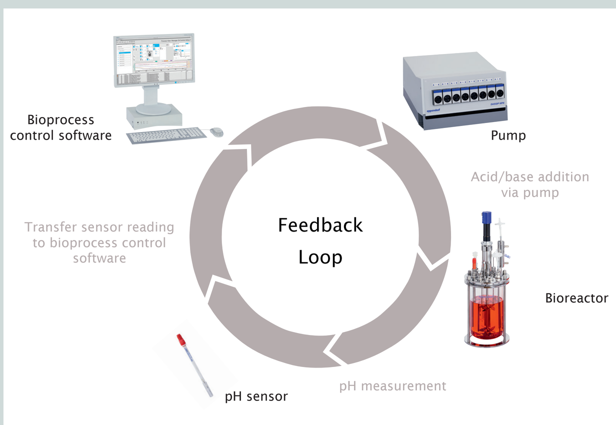


Automated DO control via a cascade. Oxygen transfer is increased by sequentially increasing the agitation speed, the flow rate, and the oxygen concentration in the gas mix.

pH control

The growth of the cells will cause the pH to drift. Bioprocess systems allow online monitoring and control.

> pH monitoring using pH sensor
> pH control by automatic addition of pH agent to the culture using the system's integrated pumps
> One-sided (usually only base) or two-sided (acid and base) pH control is possible.

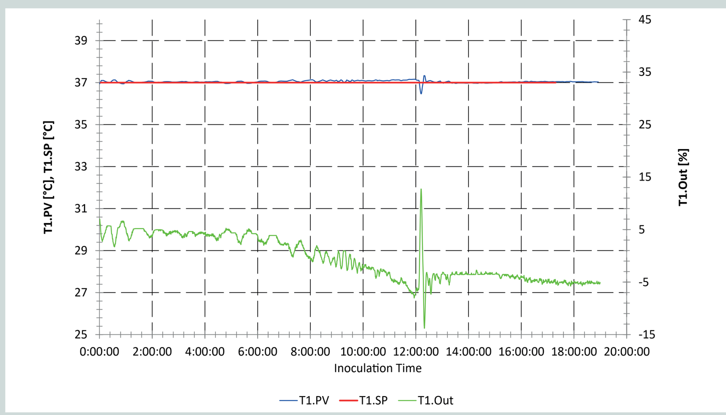


Schematic representation of pH control loop.

Temperature control

Most processes require a mechanism to control temperature at a set point that encourages optimum cell growth.

> Microbial communities need physiological temperature
> Heat-generating processes require cooling within the culture
> Different devices available to control the temperature in a bioprocess: Heat blanket, water jacket or thermowell

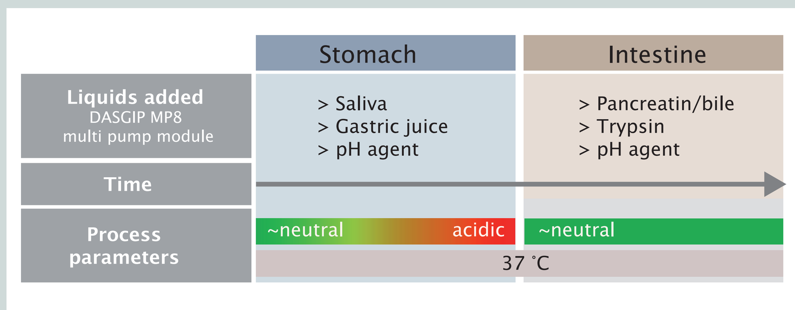


Example for temperature control in a fermentation process in a DASGIP® Parallel Bioreactor System. The temperature setpoint (T1.SP), the process value (T1.PV) and the controller output percentage (T1. Out) are shown.

Liquid addition

Liquids can be added to a bioprocess using the system's integrated pumps.

> pH agent
> Feed solutions
> ...

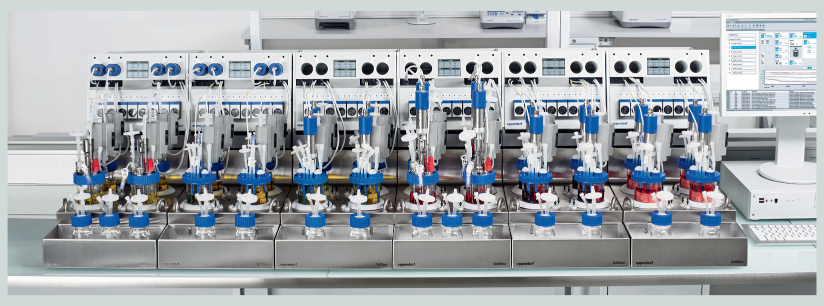


Schematic representation of experimental concept to mimick the gastrointestinal tract in a bioreactor. Liquids (saliver, gastric juice, pH agent, bile, trypsin) werde added to a DASGIP vessel using the DASGIP MP8 multi pump module. Adapted from Luiking et al., Clin. Nutr. 35(1): 48-58. 2016.

Scalable bioprocess systems for microbiome culturing needs from research to production

Small-scale parallel bioreactor systems

> Ensure maximum reproducibility between runs
> Save time

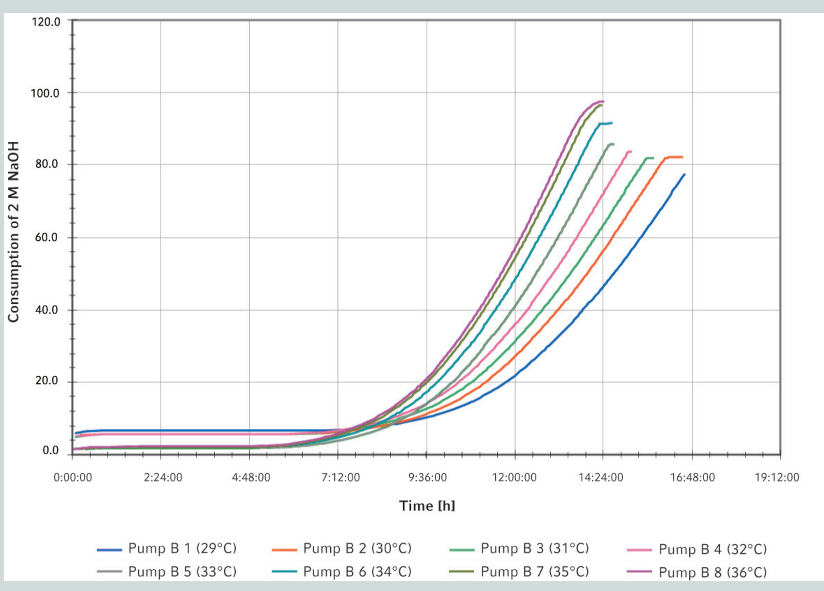


DASbox Mini Bioreactor System

> Parallel operation of up to 24 vessels
> Working volume: 65 mL – 250 mL
> Small working volumes save on the amount of cell material, media and supplements required

DASGIP Parallel Bioreactor System

> Parallel operation of up to 16 vessel
> Working volume: 0.2 L – 3.8 L
> Modular design of control units allows for flexible system configurations that meet the demands of specific applications



Parallel experimentation to compare different culture conditions. Cultivation of *Lactobacillus sp.* in an 8-fold DASGIP Parallel Bioreactor System. Bacterial growth at various temperatures was compared. NaOH consumption was used as a proportional indicator for growth-dependent acidification (*Eppendorf Application Note 299*).

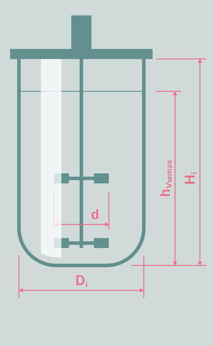
Usually, process development is carried out at small volumes and the process is subsequently scaled up to larger production volumes.

Bench-scale systems

> BioFlo® 120 and BioFlo 320 bioprocess control station
> Working volume (fermentation): 0.4 L – 10.5 L
> Universal connections for digital and analog sensors

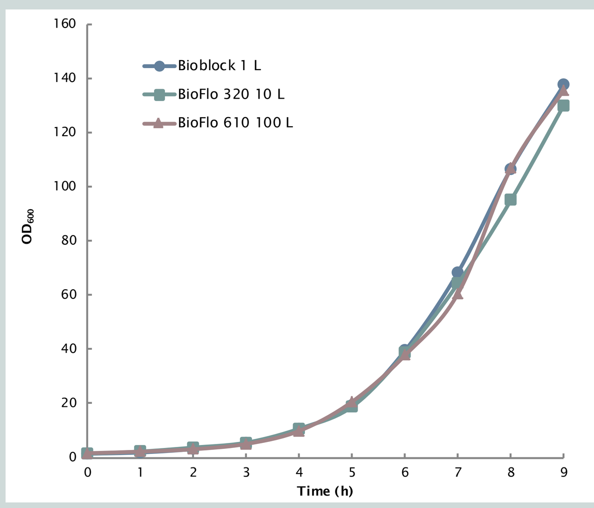
Large-scale systems

> BioFlo 510, BioFlo 610, BioFlo Pro
> Working volume (fermentation): 10.5 L – 1,200 L
> Sterilize-in-place systems
> Modular desing for system flexibility



Stirred-tank bioreactors

> Comparable vessel geometries across scales support scale-up
> Vessel characterization to identify OTR capabilities, tip speed range, and impeller power numbers
> The vessel capabilities need to be taken into account to make sure that process conditions at small scale can be reproduced at larger scales.



Scale-up of *E. coli* fermentation from 1 L to 10 L to 100 L. Biomass growth curves were comparable at all scales. Fermentations were carried out using a constant power input per volume (P/V) value.

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