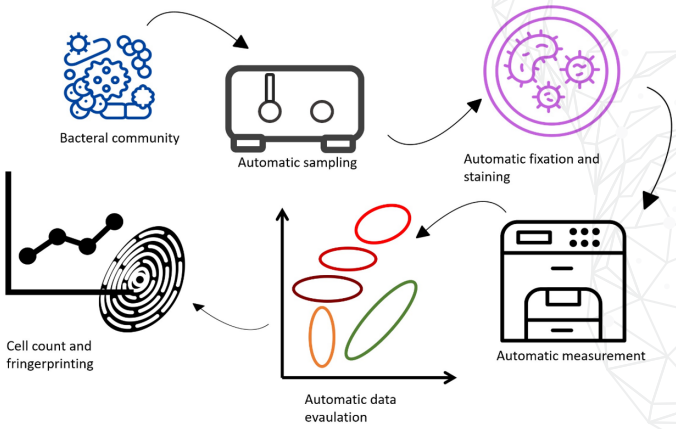
PROMICON

DEVELOPMENT OF AN AUTOMATED ONLINE FLOW CYTOMETRY METHOD TO QUANTIFY CELL DENSITY AND FINGERPRINT BACTERIAL COMMUNITIES

Key message

This method can reproducibly measure both cell density and fingerprint-like patterns of bacterial communities, generating suitable data for powerful automated data analysis and interpretation pipelines. The automated, high-resolution sorting of clustered data into cell subsets allows the identification of operational or abiotic/biotic causes of community disturbances or state changes. Such disturbances or changes can influence the interaction potential of organisms in microbiomes or even affect the performance of individual organisms.



Background

Cell density is among the most commonly used parameters for the operation and control of industrial biotechnological processes. In the past, its determination was often performed offline and manually, resulting in a delay between sampling and immediate data processing, which prevents quick action. While there are now some online methods for rapid and automated cell density determination, they are unable to distinguish between the different cell types in bacterial communities.

Objective

This study proposes an automated monitoring system comprising hardware, software and an automated workflow in order to enable real-time high-resolution analysis of bacterial communities. On the one hand, it allows for the online automated calculation of cell concentrations and, on the other, for the differentiation between different cell subsets of a bacterial community.

Methods

The OC-300 automation device (onCyt Microbiology, Zürich, Switzerland) was coupled with the flow cytometer CytoFLEX (Beckman Coulter, Brea, USA). The OC-300 performs the automatic sampling, dilution, fixation and 4',6-diamidino-2-phenylindole (DAPI) staining of a bacterial sample before sending it to the CytoFLEX for measurement. The resulting data can be analysed in an automated manner using the flowEMMi v2 tool, allowing it to be fed into available bioinformatics tools.

Impact

- Online automated flow cytometry can rapidly provide individual cell data that reflect bacterial community status. This enables highly time-resolved monitoring of a population's dynamics which is essential for controlling biotechnological processes that use bacterial communities. This is of particular relevance for the intended **transition to a circular bioeconomy, which is based on the use of microbial consortia for the revalorisation of waste.**
- The online automated flow cytometry procedure is capable of providing the same high-resolution fingerprints, i.e. profiles of the bacterial community structure, as a more tedious manual procedure and enables their automated analysis and feeding into available bioinformatics tools. This makes the **data available in a very short time, eliminates human error and allows for bioreactors to be controlled online.**

Source

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