



Exploring microbiomes in environmental biotechnological processes

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Microbiomes in different processes

Many processes in environmental biotechnology are working due to the presence of a mix of microbes, with each group playing a specific role, like being responsible for one step of a multistage conversion process. Even in industrial fermentations which have the purpose of producing biomass of one specific microorganism, an accompanying flora of other microbes is almost always present.

In all cases, the composition of the microbiome is important for the success of the whole process. A change in environmental conditions (e.g. temperature, pH, nutrient availability) can lead to a shift of the microbiome which affects the result of the process. Therefore, it is important to understand the dynamics of the microbial composition in an environmental biotechnological process.

provide information on biochemical activities that are performed [1].

Shotgun metagenomics are non-targeted and aim to sequence all genomic sequences contained in the sample. However, such an approach is costly. On the contrary, using amplicon metagenomics, short DNA sequences (marker genes) of a group of microbes are sequenced. The targeted sequences must be chosen carefully to capture all relevant organisms and be able to identify them. [1]

Application in environmental biotechnology

The analysis of the microbiome can be useful for all processes involving a mixed or not axenic culture. Some examples of applications in processes in environmental biotechnology that we investigate are given below.

Next Generation Sequencing

Using Next Generation Sequencing methods, the microbial composition of a sample can be analysed.

Depending on the purpose of the analysis, different methods can be used. Analysis of the **DNA** will reveal which organisms are present while analysis of the RNA will

16S/18S rDNA Amplicon metagenomics (Illumina MiSeq[™])



- The sampling method depends on the investigated process/system.
- Pre-treatment might be necessary to homogenize the sample.
- biomass pellet is obtained by • A centrifugation of the culture broth. Long term storage: -20 °C
 - A DNA extraction kit that is suitable for the sample type is used to extract genomic DNA (gDNA).
 - All organisms of interest should be lysed evenly. Defined metagenomic standard may be used.
- Final quality control of extracted gDNA [2].

1st step PCR: A defined 16S/18S rDNA

- fragment with adapter sequences gets amplified. The primer system determines which organisms are detected [1], [3]. • 2nd step PCR: Short sequences are attached to the DNA fragments (barcode sequence for identification and adapter sequence for
- binding to the Illumina-Chip) [3]. The DNA is attached to the Illumina chip and then amplified by Bridge Amplification [4].

Accompanying organisms of microalgae

- Determine the purity of microalgae production strains
- Compare the microbiome of healthy and crashed cultures to identify harmful organisms (Fig. 1)



Fig. 1: Comparison of different samples (β -diversity). The darker the blue circle is, the more similar the samples are.

Microbiomes in gas fermenters, biogas plants, and during anaerobic biohydrogen production from wastewaters

- Detect prevailing microbes
- Determine functional groups of microbes by analyzing their taxonomic relationship (Fig. 2, left)
- Follow the transition of microbial mixed cultures during trials via relative abundance analysis (Fig. 2, right)



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Bioinformatics



The amplified DNA is linearized and sequenced by synthesis [4].

Each of the four bases emits a light signal of a different colour when it is added to the DNA strain. These signals are recorded [4].

- To convert the raw data into DNA sequences, a series of pre-processing steps is done (e.g. demultiplexing, quality filtering, trimming) [4].
- The obtained sequences are compared to databases to identify the organisms [4].
- Depending on the scientific question, parameters (e.g. α - and β -diversity) can be calculated and charts created to allow interpretation of the data [1].

Fig. 2: Left: Taxonomic tree for the analysis of relationships of detected organisms. Right: Change of microbial consortium after adaption to a new substrate.

References

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