

Characterization of VFA-producing bacteria with NGS

Characterization of **shift in bacterial communities** with NGS (Next Generation Sequencing) of **Mixed Microbial Cultures (MMC)** of the **16S rRNA** gene in order to find the **optimal conditions** for the final **PHA** polymers. Oxford Nanopore Technologies gave information about the VFA production and ideal conditions for the bacteria.

1. Why PHA?

Because of constant pollution for the past decades, sustainable, green solutions are in serious need. This can be obtained by producing a circular chemistry and reusing materials in order to diminish the waste. PHAs are biodegradable, biobased polymers naturally produced by bacteria and could replace the traditional plastics. However, they are currently expensive due to high costs of substrate (VFA).

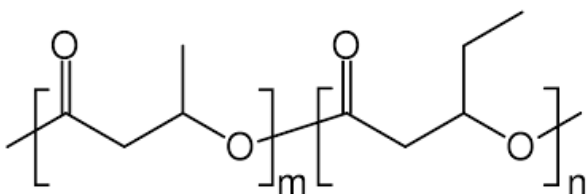


Fig 1: PHBV, part of PHA class.

2. How are VFAs efficiently produced?

Generally, they are produced under anaerobic digestion and can be produced by single-cultured bacteria. However, a cost-efficient manner was done by using MMC where residual streams were repurposed. Finding the optimal conditions was done by analyzing the bacterial growth over time with NGS. The VFAs were produced in bioreactors at different pH conditions: pH 4.5, 5 and 5.5. The VFAs were measured with IC (ion chromatography).

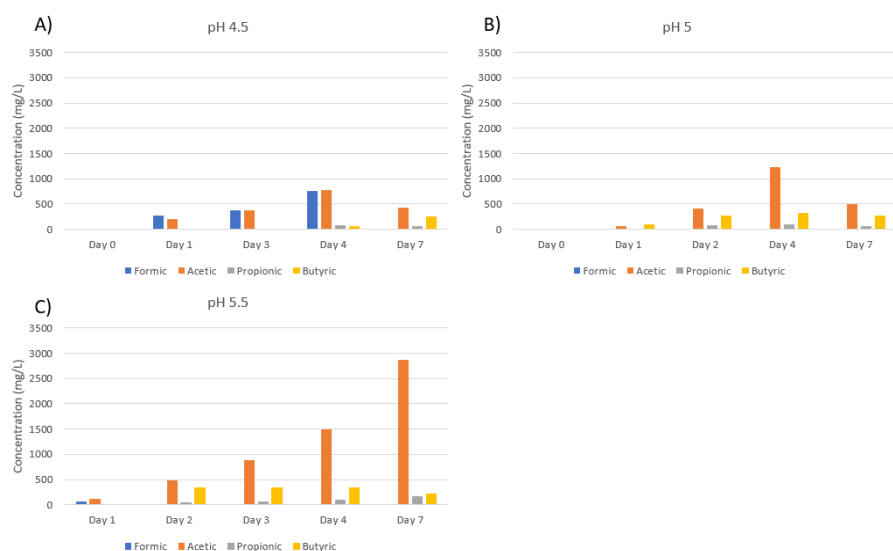


Fig 2: VFA production at different pH 4.5 A), 5 B) and 5.5 C) over time.

3. How to observe shift in VFA-producing bacteria?

16S rRNA is a very conserved gene in bacteria. By DNA collection and isolation of the 16S rRNA gene, a DNA library was prepared with all samples. Analyzed with MinION and with different bioinformatics tools, data was obtained.

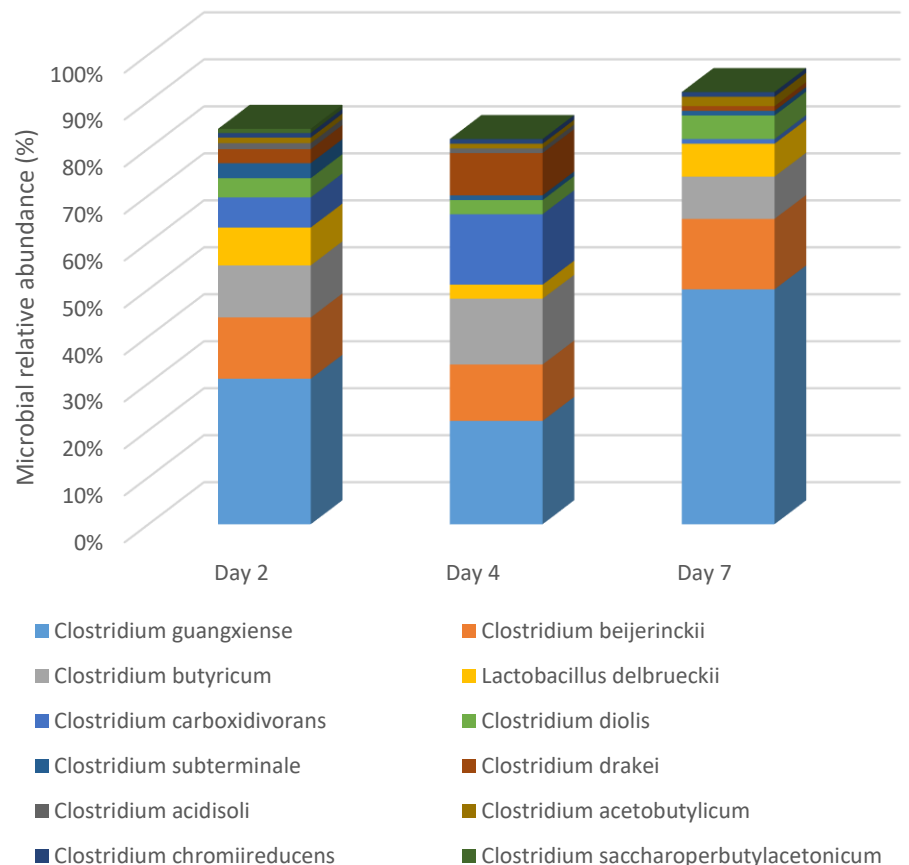


Fig 3: Microbial composition at pH 5.5.

4. Link between pH and bacterial community

There was a clear shift observed in the bacterial community. The main species that were found at pH 5.5 were *Clostridium guangxiense*, *Clostridium beijerinckii*, *Clostridium butyricum*. The dominant species: *Clostridium guangxiense*, *Clostridium beijerinckii* are known to produce acetic, propionic and butyric acids and *Clostridium butyricum* is known to produce butyric and acetic acids. However, it is known that all these species can also produce lactic acid. In order to ensure maximal VFA production, the pathways need to drive towards the end degradation and not stop at lactate, which can be done by maintaining the ideal conditions.

Take-Away

- New way of **repurposing** residual streams;
- MMC are **cheaper** because they do not require specific conditions or aseptic conditions;
- The **same** sludge waste can be used to create their own substrate and create a circular chemistry;
- Generally, the most abundant bacteria was found to be *Clostridium guangxiense* and *Clostridium beijerinckii* which produced VFAs at pH 5.

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