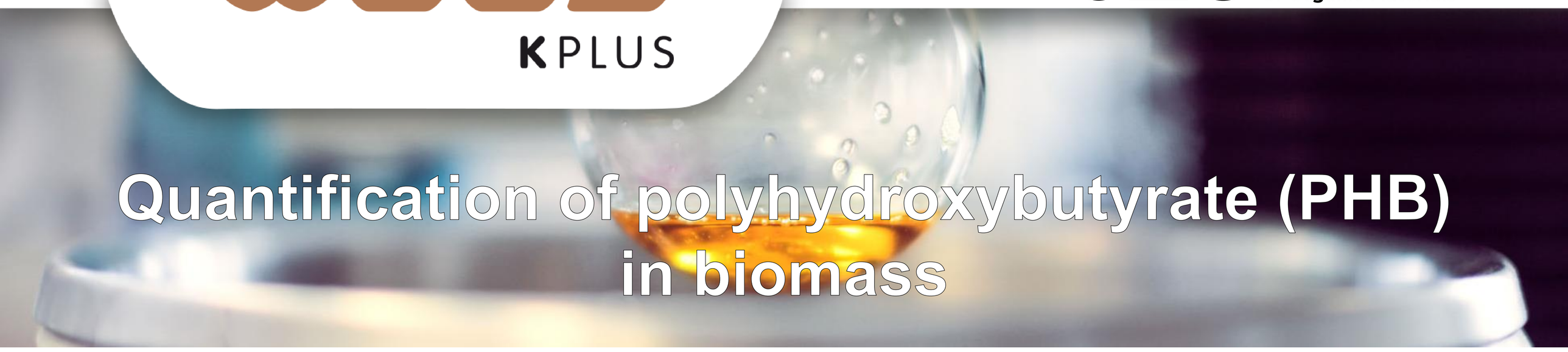

Quantification of polyhydroxybutyrate (PHB) in biomass

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Introduction

Polyhydroxyalkanoates (PHA) are biobased polymers produced by several microogranisms for energy and carbon storage when an essential nutrient terminates the cellular growth. Polyhydroxyalkanoates, produced by bacteria, are biodegradable and biocompatible linear polyesters.[1] The most common member of the PHA family is polyhydroxybutyrate (PHB). Polyhydroxybutyrate is a short-chain-length PHA (sclPHA) with 4 carbon atoms in its monomers.[2] A quick and simple quantification method for PHB in biomass received from *Halomonas halophila* is investigated in this study. Thermogravimetric analysis (TGA), gas chromatography (GC-FID, GC-MS) and high pressure liquid chromatography (HPLC) are used for the quantification of PHB in biomass.

Experimental

For cultivation of *Halomonas halophila* DSMZ 4770 from cryo-stocks the medium DSMZ 434 according to the German Collection of Microorganisms and Cell Cultures are used. For fermentation experiments the cultured bacteria are transferred after 24 h into media with a higher glucose concentration (40 g L^{-1}). For all runs biomass samples are centrifuged to separate the cells from the media and dried at 60 °C for 72 h. These four different methods are used for the quantification of PHB in biomass: the well-established GC-FID method from *Braunegg et al.* [3], GC-MS, HPLC and TGA method. Furthermore, washed and unwashed samples were distinguished to determine the effect of repeated washing with deionized water on halophilic cells. TGA measurements were also performed for wet samples to see if the drying step is necessary.

TGA (Perkin Elmer TGA 4000 Thermogravimetric Analyzer)	**GC-analysis** (GC-FID (HP 5890 Series II) and GC-MS (Shimadzu QP 2020))	**HPLC** (Shimadzu HPLC)
wet & dried samples	dried samples	dried samples
directly measured after centrifugation	dissolved in acidified methanol (5% (v/v) H_2SO_4) and chloroform, benzoic acid as internal standard added, heated (100 °C, 4h). cooled to ambient temperature, mixed with deionized water, shaken well.	H_2SO_4 added, heated (90 °C, 30 min), cooled to ambient temperature, deionized water added, shaken well
Step height = PHB content (%)	measuring the concentration of the resulting methyl ester in the organic phase	measuring the concentration of the resulting crotonic acid

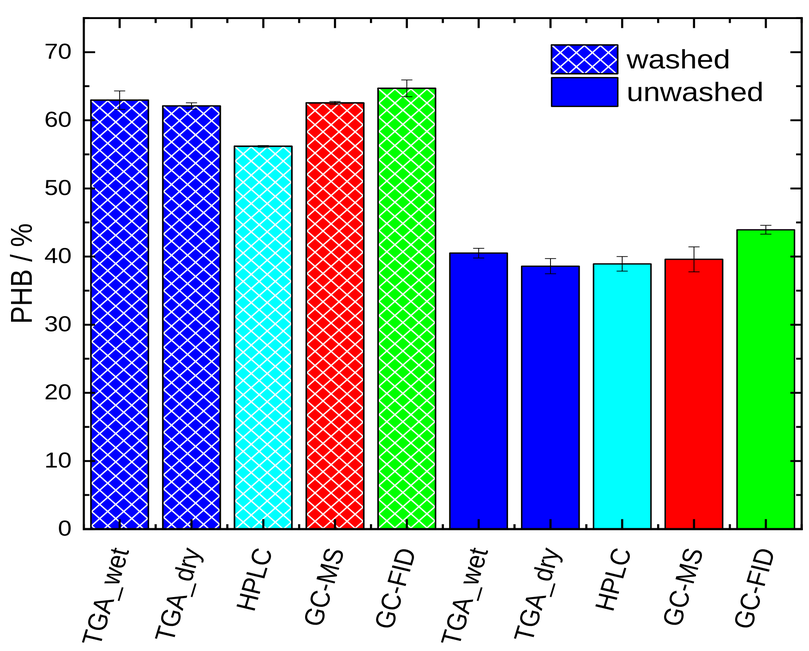

Figure 1 *Comparison of the results of the different quantification methods TGA, HPLC, GC-MS and GC-FID.*

Results

Quantification of PHB in biomass, produced by *Halomonas halophila* was achieved with all tested methods. A comparison of the methods is shown in Figure 1. The determined PHB contents were similar in all the methods applied, also for the wet and dried samples measured with TGA. An example for the quantification of PHB for a wet biomass sample is shown in Figure 2. The mass loss due to water evaporation can clearly be distinguished to the mass loss of PHB. A big difference in total PHB content was shown when the results of washed an unwashed samples were compared. We suggest that this is related to cell burst when halophilic cells are washed with deionized water *(Vreeland* [4]) and due to the additional dry matter content of the media (11%) when unwashed cells are used. Consequently, the PHB content is underestimated for unwashed samples and overestimated when cells are washed.

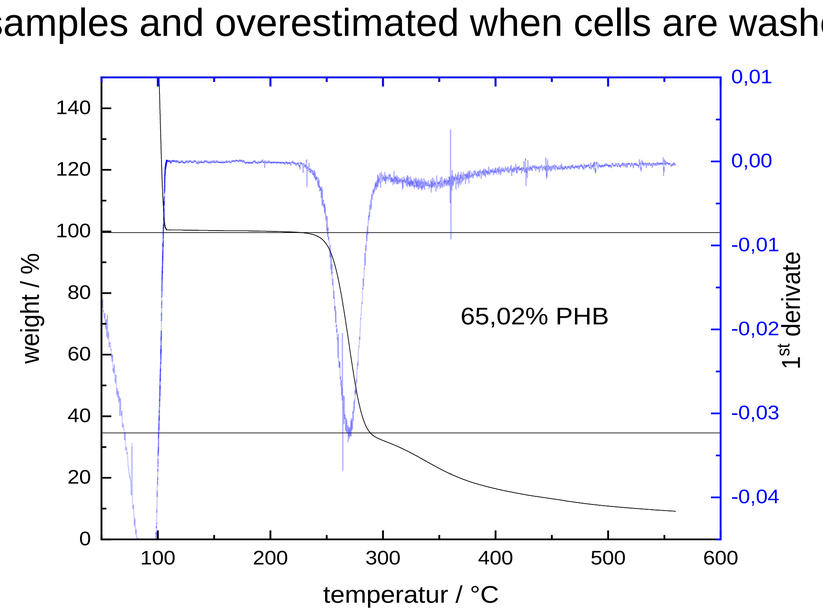

Figure 2 *TGA curve for quantification of PHB in a wet biomass sample.*

Conclusion

HPLC, GC-MS and TGA analysis showed comparable results to the well-established GC-FID Method from *Braunegg et al*. for PHB quantification in halophilic cells. Regarding total analysis time TGA method is superior to the other methods used. It is possible to get results approximately 2 hours after sampling. To reduce the influence of washing on the quantification results further experiments with defined salt solution need to be performed.

Acknowledgement
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