**Motivation/Introduction**

The objective of the cooperative project FENA is the partial substitution of fish meal and fish oil by algae and oleaginous yeast for a sustainable and high-value fish nutrition. Oleaginous yeasts are characterized by lipid contents of more than 20% (Ratledge and Cohen 2005). They are able to accumulate up to 70% of lipid of their own mass (Agelots et al., 2011). Typical representative yeast genera are Candida, Cryptococcus, Lipomyces, Yarrowia, Rhodotorula and of course Rhodotorula. *Rhodotorula glutinis* is an often used organism with a wide range of industrial usage reaching from biodiesel to carotenoids or as feed for breeding animals as well as potential producer of industrial enzymes for food industry (Kot et al., 2016). For accumulation of high lipid contents, the limitation of minimum one or more essential nutrients for cell proliferation, e.g. nitrogen, phosphor, iron, zinc or magnesium, is required. However, for biotechnology applications nitrogen limitation is preferably used to induce lipid accumulation (Beepoulos et al., 2009; Granger et al., 1993). Therefore, cultivations of oleaginous yeasts are usually carried out in a medium with a high C to N ratio. As long as nitrogen is present, the organism proliferates and produces (mainly lipid-free) biomass. After exhaustion of nitrogen, cell proliferation slows down and the remaining carbon source is channeled into lipid formation.

For the development of a fermentation process, there are a lot of optimization parameters, e.g. medium components, C/N-ratio, pH, DO or temperature. Therefore, process development takes a lot of time and experiments resulting in a large amount of samples. The upcoming samples should be analyzed as fast as possible. Traditional methods like the gravimetric analysis described in detail by Bligh and Dyer, 1959 or the extraction of lipids by organic solvents followed by GC-FID analysis takes several days. We developed three different methods for a rapid detection of intracellular lipid in the oleaginous yeast *R. glutinis*. Thereby, flow cytometry and fluorescence spectroscopy combined with the staining dye Nile Red were applied for fast lipid detection.

**Concept and Results**

- Rapid lipid measurement is possible via photometric or flow cytometric methods
- Online lipid detection is even possible without staining via granularity (side scatter)
- Methods were proven by real HCD production process of *R. glutinis* with 106 g L⁻¹ biomass and 65% intracellular lipid content.

**Conclusions and Outlook**

The course of process development is often dependent on the speed of providing the analytical data. Therefore, we investigated different methods to speed up the process development for maximizing lipid production with *R. glutinis*. In comparison to traditional protocols, the established methods based on flow cytometry and fluorescence spectroscopy could significantly enhance the time of analysis and thus the time of process development from days to minutes. These detection procedures are reliable, valid and easy to handle, indicated by high correlation quotients of up to 0.959. Further, the 96-well-plate spectroscopy assay can also be applied for extended strain screening. As an additional rapid detection method for lipid accumulation, granularity can be measured by side scatter via flow cytometry. Therefore, it is conceivable that an on-line integration of flow cytometry in a production facility for monitoring the lipid formation immediately without staining is of value. However, it has to be considered if the developed methods are suitable for lipid detection in other strains as well.

**Acknowledgements/References**

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