Development of FTIR method for simultaneous baker's yeast Saccharomyces cerevisiae biomass and trehalose quantifications

Marita Gavare, Janis Liepins, Mara Grube

Institute of Microbiology and Biotechnology, University of Latvia
Kronvalda blvd 4, LV-1010, Latvia

Baker's yeast is among the most favorite eukaryotic model organisms; it is also popular for various biotechnological applications.
Yeast biomass and reserve carbohydrate measurements are of paramount importance since it is a way how to follow the population's growth dynamics and its eventual stress tolerance. There are different conventional methods used for microbial (including yeast) biomass measurements – gravimetrics, spectrophotometry, cytometry [1]. Sometimes the biomass determination might be affected by additional substances present in particular broth (non soluble particles, complex carbohydrates, which attaches to cell wall, etc.). Trehalose has been quantified by enzymatic methods (trehalose assay), but it is also possible to use total glucose moiety measurements after sequential extraction steps from biomass (anthrone assay) [2]. Usually trehalose concentrations are normalized to the biomass dry weight or cell number. So, routinely different methods are used for biomass and trehalose assays. To overcome technical problems of biomass estimation by traditional methods and to make trehalose quantification simple and simultaneous with biomass measurements – FT-IR based method was developed.
Biomass and trehalose analyses were done by microplate reader HTS-XT, Bruker, over the range 4000 - 600 cm^{-1}. For the calibration curves spectra were evaluated by baseline correction (Rubber band correction). The appropriate amount of samples for calibration curve was determined according to the absorption spectra intensity to be 0.35 - 1.25 to fulfill Lambert-Buger-Beer Law.
Absorptions of several typical (amides, nucleotides, proteins, lipids, carbohydrates) markers were estimated and linear range between biomass dry weight and absorbencies determined. Lipids (3002 - 2797 cm^{-1} region) turned out to form the best linear curve over large range of biomass concentrations. Besides, the calibration curve was indifferent to yeast growth mode (fermentative or respiratory), which turned out not to be the case for other biochemical markers investigated. For trehalose calibration curve seven spectra replicates cut in 1164-962 cm^{-1} region and integrated by the mode "R"- peak intensity at a given frequency were averaged and evaluated by baseline correction (Rubber band correction), characteristic peak of trehalose was at 992.09 cm^{-1}.
To confirm applicability of newly developed FT-IR method for trehalose and biomass measurements, it was applied to the *S. cerevisiae* strains of altered trehalose metabolism (trehalose deficient *tps1, tps2* mutants and engineered strain of increased trehalose content). Results obtained were in good accordance with previously published trehalose and biomass measurements. We conclude that newly developed FT-IR method for trehalose and biomass quantification gives results similar to the traditional biochemical and gravimetrical methods and thus can substitute them.

References

[1] R.E. Madrid, C.J. Felice, *Crit Rev Biotechnol.* 25(3), 97-112 (2005).
[2] J.C. Ferreira, V.M.F. Paschoalin, A.D. Panek, L.C. Trugo, *Food Chem.*, 60(2), 251-254 (1997).

Acknowledgement: this work was supported by the project
Nr.2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128, http://www.sysbio.lv