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O-477 **Comparative metabolomics of xylose-fermenting yeasts by UHPLC-MS/MS: effects of oxygen levels on fermentation performance**

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Xylose fermentation is a bottleneck in second generation ethanol production since few yeast species are able to convert this sugar to ethanol. Efforts to improve yeast fermentation performance through genetic engineering have been done, however ethanol yield and productivity are still low and industrial conditions. In this work, a metabolomics platform based on Ultra High Liquid Chromatography coupled to tandem Mass Spectrometry (UHPLC-MS/MS) was developed to quantify key metabolites from central carbon metabolism (glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle) in yeast. Afterwards, comparative metabolic flux analysis was performed to identify limiting steps on xylose metabolism. *Spathaspora arborariae* and *S. passalidarum* were cultivated in xylose media under aerobic and oxygen-limited conditions to investigate the oxygen influence in the xylose consumption. A total of nineteen metabolites were quantified in each yeast sample. To our knowledge, for the first time, intracellular metabolites from *S. arborariae* and *S. passalidarum* were successfully quantified. Growth under aerobic and oxygen limited conditions, lead to respiratory and fermentative metabolism, respectively. In the last case, cofactor regeneration was hindered and resulted in xylitol secretion significantly, especially for *S. arborariae*. These results indicated that fine control of oxygen levels during fermentation is necessary to optimize ethanol production with naturally xylose-fermenting yeasts. The metabolomics protocol developed in here can also be applied to other species.

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