Experiments for modelling and BioBrick characterisation

Experiment for measuring ethanol production of IMU051 strain under different glucose concentrations.

The strain IMU051 was inoculated in 25 ml of YPD medium overnight at 220 rpm and 30 ºC. Then the overnight culture was inoculated into CSM medium supplemented with different concentration of glucose (1.0, 2.5, 5.0 and 10.0 g/L) to an initial OD600 of 0.02. One milliliter samples were taken every two hours and the growth was estimated by measuring OD at 600 nm followed by centrifugation at 3000 g for five minutes and ethanol and glucose concentrations in the supernatant were measured by HPLC (sulfuric acid at 5 mM at a flow rate of 0.6 ml/min as mobile phase, HPX-87H was used as stationary phase at 45 ºC). Dry cell biomass (DCW) was calculated based on the calibration curve DCW (g/L)=0.3726*(OD600)-0.0522

Growth and sucrose conversion of population 2 under different ethanol concentrations

The strains EBY 4000, EBY 4000 FCY2 SUC2 tFba1, EBY 4000 TEF2 SUC2 CYC1 were inoculated into 50 ml of CSM medium supplemented with 10 g/L ethanol and incubated overnight at 220 rpm and 30 ºC. The overnight cultures were used as inoculum to 25 ml of CSM medium supplemented with 10 g/L of sucrose and different concentrations of ethanol (2.5, 5.0, and 10.0 g/L). In order to measure growth, ethanol consumption and sucrose conversion into glucose and fructose 1 ml samples were taken at different time points.

Cell growth was estimated by measuring OD at 600 nm followed by centrifugation at 3000 g for five minutes and dry cell biomass (DCW) was calculated based on the calibration curve DCW (g/L)=0.3726*(OD600)-0.0522. Ethanol consumption was measured by HPLC (sulfuric acid at 5 mM at a flow rate of 0.6 ml/min as mobile phase, HPX-87H was used as stationary phase at 45 ºC). Sucrose conversion was measured in terms of reducing sugars by the method of Miller et al. 1959 [2].

References

[1.] Hegemann, J. H., & Heick, S. B. (2011). Delete and repeat: a comprehensive toolkit for sequential gene knockout in the budding yeast Saccharomyces cerevisiae. *Strain engineering: methods and protocols*, 189-206.

[2.] Miller, G. L. (1959). Modified DNS method for reducing sugars. Anal. Chem, 31(3), 426-428.