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DEVELOPEMENT OF A MILLIFLUIDIC CHIP FOR FRACTIONATION OF A HETEROGENEOUS YEAST CULTURE BY COATED MAGNETIC NANOPARTICLES

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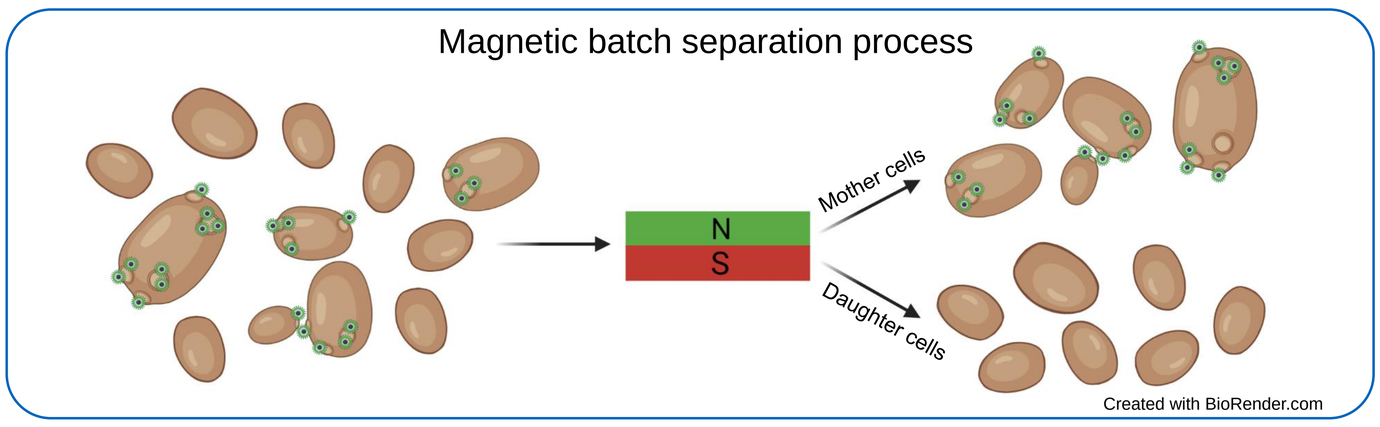
Introduction

Yeast is a widely used microorganism in food and beverage industry as well as in the pharmaceutical industry. This unicellular fungus is characterized by a high stress tolerance against exogenous stressors like temperature, pH or alcohol. In 2017, this market was valued at USD 4,155 million and it is expected to increase to USD 8,940 million by 2026. Here, age related studies become interesting in order to understand viability and vitality of a whole yeast population during cultivation for process optimization. However, the existing analytical methods are either chemically or mechanically invasive and are not representative for a whole yeast population. Therefore, a millifluidic fractionation process is developed via the specific binding of coated magnetic nanoparticles due to a linker-protein. The process is advantageous, as it is non-invasive and low volumes and cell densities are needed. For giving a proof of principle, a magnetic batch separation process of a heterogeneous yeast population is implemented and verifies the age-dependent separation.

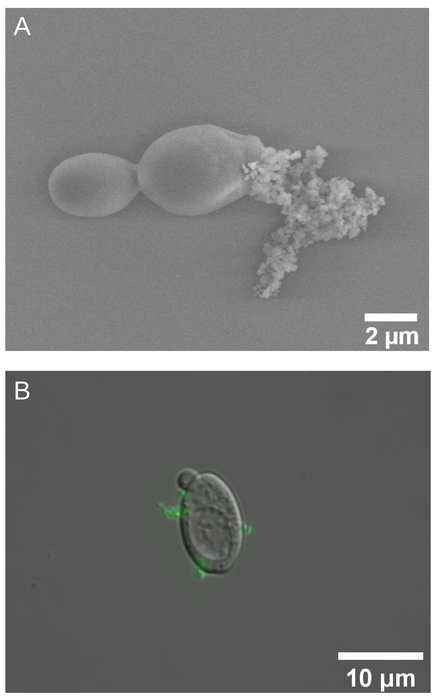
Evaluation of chip prototype via model system



Continuous, millifluidic magnetic fractionation process

Figure 1: Scheme representing adsorption of particles c = 0.08 g L^{-1} to yeast cell OD =1, 1000 rpm, T = 25°C, 3.5 h for magnetic separation. Afterwards, the sample is magnetically separated for t = 30 min and the daughter and mother cells can be analyzed.

Adsorption of nanoparticles to yeast cells



- Synthesis of silica stabilized, magnetic nanoparticles with EDTA functionalization
- High colloidal stability, $d_{hyd} \approx 164$ nm
- Specific adsorption of the MNPs to bud scars of yeast cells via linker-protein → Age dependent magnetic labelling

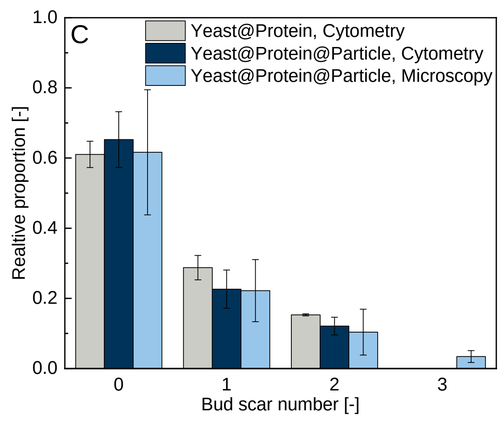
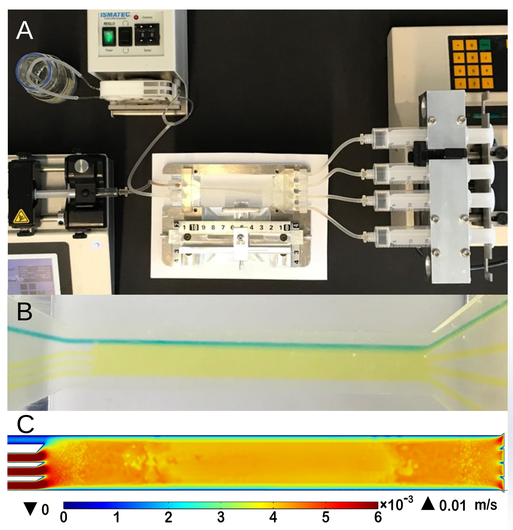


Figure 2: Specific adsorption of particle agglomerates via a fluorescing linker-protein to the bud scars of yeast cells via scanning electron microscopy (A) and light microscopy (B). Quantitative analysis of the adsorption via cytometry and light microscopy (n_{cells} = 120) in triplicates (C).

Millifluidic chip: Design and construction



- Continuous set-up via syringe and peristaltic pumps
- Transparent, 3D-printed chip with channel height of 750 µm
- Laminar flow pattern

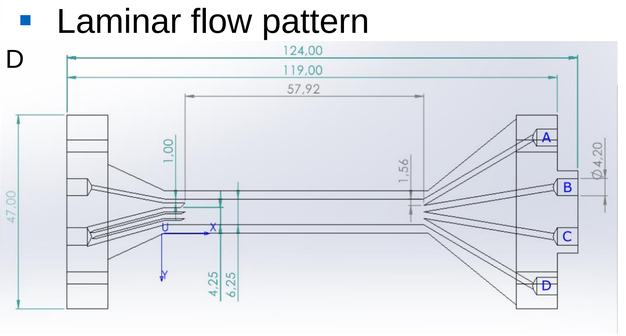


Figure 3: Millifluidic set-up, consisting of a peristaltic and two syringe pumps and the chip placed in a self-designed holder (A). Flow profile of buffer (yellow) and sample (blue) (B) and simulated in COMSOL Multiphysics (C). Dimensions in mm of the rectangular chip, designed with SolidWorks (D).

Determination of influencing parameters on fractionation process:

- Diffusion dependent on concentration and channel height
- Magnetic convection dependent on magnetic field gradient, concentration, viscosity, density and velocity ratio

Magnetic separation of a yeast culture

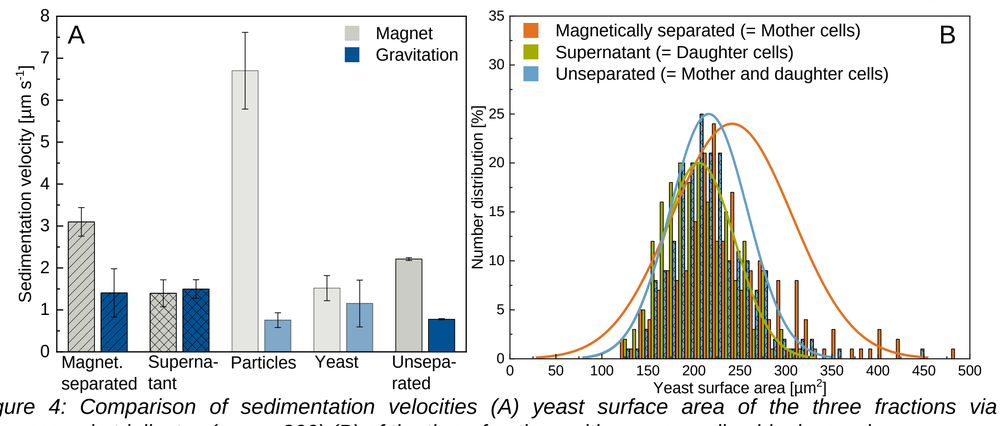


Figure 4: Comparison of sedimentation velocities (A) yeast surface area of the three fractions via light microscopy in triplicates (n_{cells} = 200) (B) of the three fractions with corresponding blank samples.

Batch separation process due to specific adsorption of particles to S. pastorianus

- Age dependent adsorption of particles to yeast cell population

→ Daughter cells: No bud scars → No adsorbed particles

→ Mother cells: Various bud scars → Adsorbed particles

- Magnetic separation process

→ Enrichment of daughter cell content from 60% in magnetically separated fraction to 90% in supernatant fraction via cytometry

→ Verification of separation via optical sedimentation, cytometric analysis and the yeast cell surface area via light microscopy

Summary and Outlook

A heterogeneous yeast cell population (*S. pastorianus*) is magnetically labelled at the bud scars by the specific adsorption of silica-EDTA coated magnetic nanoparticles and a linker-protein agglomerate. The bud scar number is proportional to the yeast cell age, therefore, it is possible to separate old mother cells (bud scar number < 0) from young daughter cells (bud scar number = 0). The daughter cell proportion is enriched from 60% to 90%, which is verified via cytometry, light microscopy and optical sedimentation analysis. Meanwhile, a 3D-printed, millifluidic chip prototype is evaluated regarding influential process parameters (e.g. magnetic convection, diffusion) for the implementation of a fractionation process according different bud scar numbers to increase the selectivity compared to the batch separation.

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DFG Deutsche Forschungsgemeinschaft
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