Continuous Non-Centrifugal Phase Separation in Biphasic Whole-Cell Biocatalysis

Applied Catastrophic Phase Inversion on a Lab-Scale Prototype.

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Within several chemical and biotechnological processes, the presence of particles (e.g. as catalyst) in a biphasic reaction system (oil/water) often leads to the formation of stable Pickering type emulsions, hindering cost-efficient and effective downstream processing. State-of-the-art-concepts for phase separation fail or include inefficient and costly strategies (centrifugation / de-emulsifiers). Using the phenomenon of catastrophic phase inversion (CPI), efficient phase separation can be achieved by addition of dispersed phase. Based on a patent filed at TU Dortmund, a process concept (termed: Applied Catastrophic Phase Inversion; ACPI) was developed, enabling continuous phase separation of stable emulsion by using the CPI phenomenon. A fully automated pilot-scale prototype was planned and constructed and applicability of the concept to different biocatalysts and aqueous/organic systems confirmed.

Depending on their wettability, cells / particles will either stabilize oil in water, or water in oil emulsions. Wettability is thereby characterized by the three-phase contact angle $_{ow}$ measured through the aqueous phase (see Fig. 1). The par-Θticle stabilized, also termed Pickering-type emulsions, can undergo a phenomenon called catastrophic phase inversion, being the sudden switch of emulsion types from an oil/water to a water/oil emulsion (or vice versa). This inversion is achieved by addition of dispersed phase exceeding a critical volumetric threshold. This catastrophic phase inversion (CPI) is accompanied by a complete destabilization of the emulsion.

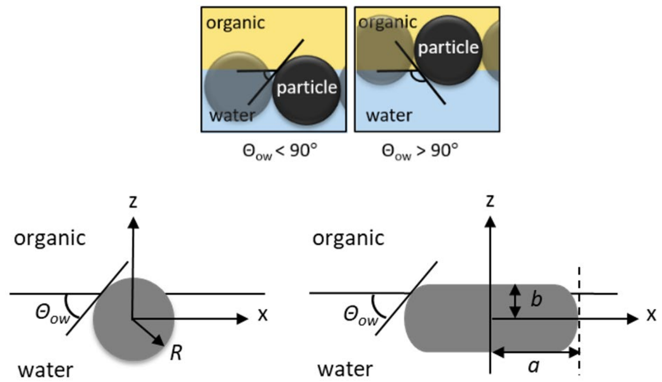

Figure 1: Attachment of particles with three-phase contact angle $_{ow}$ to a planar organic/water interface. (Left) Spherical particles with radius R. (Right) Non-spherical, rod-shape, particles / cells with rod length a, and rod radius b.

Within this work, we designed and constructed a fully automated lab-scale prototype (see Fig. 2) forecontinuous phase separation adhering to the CPI principle. We demonstrate the applicability of the concept for various long-term stable bioprocess-derived Pickering-type emulsions, investigating the influence of both, different organic solvents (n-heptane, ethyl oleate and 1-octanol) as well as biocatalysts (*Escherichia coli* JM101 and *Pseudomonas putida* KT2440). The critical volumetric phase ratio of organic to water phase (V_o:V_w) which has to be applied to achieve phase inversion, was calculated based on the guideline developed in our previous work.

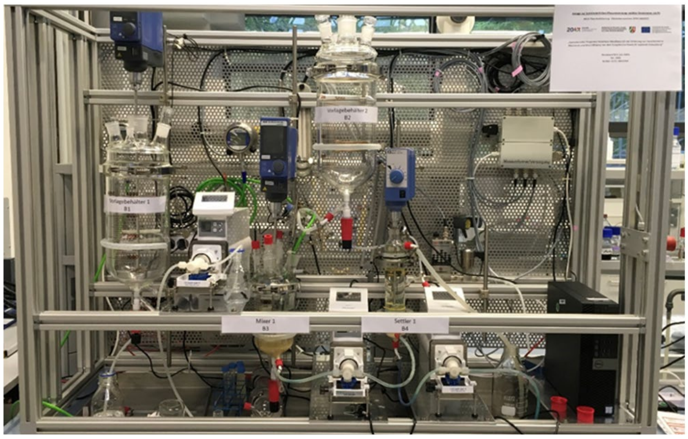

Figure 2: Picture of the lab-scale ACPI prototype as constructed within this work.

A process window that allowed for reliable operating conditions was defined. We investigated the influence of process parameters (e.g., flow rates) on stability and success of the (continuous) phase separation. Furthermore, we investigated the robustness towards perturbations (e.g., fluctuation in water/organic phase ratio of the feed emulsion).
A phase separation efficiency of over 96 % could be achieved for all emulsions considered within this work.
ACPI thus is an innovative and universal tool, overcoming the limitations of the drawbacks in classical downstream processing concepts used in state-of-the-art processing of bioprocess-derived Pickering-type emulsions.

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