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μMORE: A microfluidic magnetic oscillation reactor for accelerated parameter optimization in biocatalysis

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ABSTRACT

Enzymatic parameter determination is an essential step in biocatalytic process development. Therefore higher throughput in miniaturized devices is urgently needed. An ideal microfluidic device should combine easy immobilization and retention of a minimal amount of biocatalyst with a well-mixed reaction volume. Together, all criteria are hardly met by current tools. Here we describe a microfluidic reactor (μMORE) which employs magnetic particles for both enzyme immobilization and efficient mixing using two permanent magnets placed in rotating cylinders next to the a glass chip reactor. The chip geometry and agitation speed was optimized by investigation of the mixing and retention characteristics using simulation and dye distribution analysis. Subsequently, the μMORE was successfully applied to determine critical biocatalytic process parameters in a parallelized manner for the carboligation of benzaldehyde and acetaldehyde to (*S*)-2-hydroxy-1-phenylpropan-1-one with less than 5 μg of benzoylformate decarboxylase from *Pseudomonas putida* immobilized on magnetic beads. Here, one run of the device in six parallelized glass reactors took only 2–3 h for an immobilized enzyme with very low activity (~2 U/mg). The optimized parameter set was finally tested in a 10 mL enzyme membrane reactor, demonstrating that the μMORE provides a solid data base for biocatalytic process optimization.

1. Introduction

The efficient application of new enzymes and enzyme variants for application in preparative biocatalysis requires knowledge of appropriate reaction parameters, such as pH, temperature, solvent system, optimal substrate concentration, potential inhibitory effects by substrate and/or product(s), and stability under process conditions. In order to speed up the biocatalytic process development, microfluidic techniques are highly important as was recently excellently reviewed (Bolivar and Nidetzky, 2013; Bolivar et al., 2011; Wohlgemuth et al., 2015). Continuous reaction systems like the continuous stirred tank reactor, e.g. the enzyme membrane reactor, are well suited to obtain all relevant parameters, since the adjustment of constant operating points with respect to substrate concentration and conversion is possible and enzyme inactivation constants can directly be deduced from the conversion curve (Wichmann et al., 1981). This is in contrast to discontinuous batch systems and plug-flow reactors, which do not allow the adjustment of constant operation points as substrate and product conditions change with time or space, respectively.

However, parameter optimization studies on lab scale continuous reaction systems are time-consuming, require high amounts of (purified) enzymes and are difficult to parallelize. In order to rapidly test e.g. improved enzyme variants identified from a respective screening approach (performed at microtiter plate scale) methods are desired that allow in situ immobilization of the target enzyme ideally directly from crude cell extracts, thus enabling parameter optimization with very small amounts of enzyme and without tedious enzyme immobilization procedures (Bolivar et al., 2016).

Abbreviations: BFD, benzoylformate decarboxylase; BSA, bovine serum albumin; 2HPP, 2-hydroxy-1-phenylpropan-1-one = 2-hydroxypropiophenone; μMORE, microfluidic magnetic oscillation reactor for enzymes; EMR, enzyme membrane reactor.

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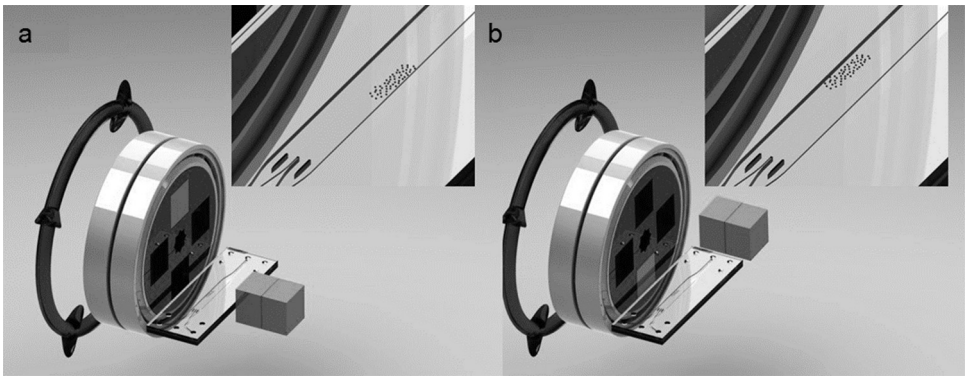

Fig 1. Principle of the magnetic array for the enzyme micro reactor. Two cylinders with magnets staggered by 180° in antiparallel orientation are placed on the left and right side of the chip (cylinder only shown on the left hand side). In position (a) magnetic beads will move to the right hand side and in position (b) they will move to the left hand side (see insets). Switching between the positions is realized by a 180° revolution of the cylinders (see arrows) and will enable mixing of the two influxes into the microfluidic channel. A video demonstrating the μMORE mixing principle can be found in the video supplementary.

Magnetic beads for particle-based enzyme immobilization offer easy immobilization of enzymes through appropriate fusion tags (e.g. multi-histidine tags) (Hübner et al., 2015). However, high backpressure (Dräger et al., 2007; Srinivasan et al., 2004) or even plugging of the reactor (Li et al., 2007; Nomura et al., 2004) pose additional challenges when using magnets to retain magnetic beads.

Different approaches such as magnetic bead movement, micro-stirrers, and passive mixers have been used to implement mixing in such reactor systems (Kuo and Chiu, 2011; Verbarg et al., 2012). However, while the construction of micro-stirrers is challenging (Zhang et al., 2006), passive mixers cannot be used with carrier-based immobilization as the channel geometry providing the mixing would be clogged by the carrier material. Magnetic bead-based mixing provides an interesting alternative, and different magnetic field configurations for magnetic bead movement, employing a multitude of both electric- and permanent magnet arrays have been published (Lee et al., 2009; Liu et al., 2011; Lund-Olesen et al., 2008; Seong and Crooks, 2002; van Pelt et al., 2011; Wang et al., 2007; Yu et al., 2011). While showing easy adjustability of field direction, strength, and frequency, electromagnets generate heat (Liu et al., 2011), which is unfavourable for enzymatic reaction systems as a defined temperature is crucial for these processes. This problem can be avoided by the use of permanent magnets. However, the permanent magnet systems described so far show complicated magnetic arrays underneath the microfluidic chip (Verbarg et al., 2012), which has numerous drawbacks. First, leakage at the macro-to-micro interface may result in damage of electric or mechanic components of the magnetic field device. Further, integration of a heating/cooling system underneath the chip is impaired by the magnetic agitation setup. The constant application of a magnetic force to the bottom of a microfluidic channel may also damage the enzyme immobilized on the magnetic beads and the particles themselves by shear stress. Furthermore, microscopic observation to study e.g. the mixing properties of the microfluidic system is impaired due to obstruction of the optical path (Werts et al., 2012).

In order to establish a user-friendly magnetic micro-bioreactor system with a combination of magnetic mixing and retention, we developed a novel microfluidic magnetic oscillation reactor for enzymes (μMORE), which is based on permanent magnets placed on both sides of the microfluidic chip (DiB Fig. 1). First, the mixing and retention properties of the new device using dye distribution experiments were studied. Subsequently, we demonstrate the potential of the μMORE system for process development using the carboligation of benzaldehyde and acetaldehyde to (*S*)-2-hydroxy-1-phenylpropan-1-one ((*S*)-HPP) catalyzed by benzoylformate decarboxylase from *Pseudomonas putida* (BFD) as a non-trivial example. This reaction requires the handling of volatile acetaldehyde and strict temperature control. BFD provides a valuable model for stereoselective biocatalytic synthesis of valuable chiral 2-hydroxy ketones and has been used as a test enzyme for microfluidic and small scale reaction systems, like laminar flow microreactors (Valinger et al., 2014), a 200 μL membrane reactor as well as capillary microreactors (Fagaschewski et al., 2012), and a 10 mL continuous stirred tank reactor enzyme membrane reactor (Iding et al., 2000; Valinger et al., 2014). Besides, hexahistidine-tag based immobilization of BFD on magnetic beads was already earlier reported to maintain the enzymatic activity (Tural et al., 2013).

2. Material and methods

2.1. Chemicals and magnetic particles

Rhodamine B was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All further chemicals were obtained from Fluka/Sigma Aldrich Chemie GmbH (Steinheim Germany), Merck KGaA (Darmstadt, Germany, Carl Roth GmbH + Co. KG Karlsruhe, Germany). PureProteome™ Nickel magnetic beads were from Millipore®.

2.2. Microfluidic reactor chips

For details see Data in Brief (DiB Fig. 1).

2.3. Magnetic modelling

AMPERES® software was used for simulation of the magnetic flux density within the microfluidic reaction chambers. The geometries were exported from Solidworks as STEP files and then imported to the AMPERES® program by Integrated Engineering Software (Winnipeg, Canada) for magnetic field simulation. For magnetic modelling the magnetic properties of the used materials as described in the ESI (Table S1) in a vacuum were used.

2.4. Micro-bioreactor setup

A prototype adapted to fit under a Nikon Diaphot microscope was constructed first. For details see Data in Brief (DiB Fig. 2). Microscopy was carried out with a Nikon Diaphot microscope. Images were taken by a Nikon D3100 Camera connected to the microscope's F-mount. For time lapse photography the DIYPhotobits camera control software was used. Microscopic images of dye distribution analyses (see chapter 2.6) were analyzed using