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Model-based analysis of a reactor and control concept for oxidoreductions based on exhaust CO_2-measurement

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ABSTRACT

A novel control concept was developed for a two phase biocatalytic oxidoreduction system. The hydrophobic substrate acetophenone dissolved in *n*-heptane is reduced to (*S*)-1-phenylethanol by *Candida parapsilosis* carbonyl reductase 2, immobilized in a polyvinyl alcohol hydrogel. The cofactor NADH is regenerated via formic acid oxidation using likewise immobilized *Candida boidinii* formate dehydrogenase, increasing the pH-value of the aqueous phase. Therefore, the measured amount of CO_2 leaving the reactor is used to calculate the amount of formic acid to be replaced.

Experiments lead to unexpectedly poor conversions motivating the development of a holistic process model, which was exclusively based on literature data and did not require parameter fitting. Simulation studies identified the CO_2-solubility in *n*-heptane as the root cause for the time-lag in co-substrate feed and the resulting pH-shifts leading to poor conversions. They also indicated enzyme activity and stability as improvement targets, and the choice of an organic phase with low CO_2-solubility. Exemplarily, increased buffer concentration to stabilize the pH within the hydrogel resulted in a predicted 13% productivity improvement, which could be validated experimentally, thus highlighting the potential of process models for complex biocatalytic process evaluation.

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1. Introduction

Enantiopure chiral alcohols constitute diverse building blocks for pharmaceutical applications. Therefore, the stereoselectivity of multiple enzymes renders biocatalysis a viable alternative to organic chemistry [1]. Alcohol dehydrogenases catalyse the oxidation of alcohols or the reduction of aldehydes, and more interestingly, the stereoselective reduction of ketones [2]. Regrettably, many interesting substrates are poorly water soluble, complicating an A promising concept to reduce hydrophobic aldehydes and ketones are two phase systems that are able to dissolve the substrates in reasonable amounts and simultaneously provide an aqueous environment for the enzymes. Depending on the partition equilibria of the reactants, two phase systems provide thermodynamic advantages in driving the overall reaction equilibrium towards the product side by extracting the products [3]. Drawbacks in biocatalysis within non-stabilized two phase systems are hydrodynamic stress and surface inactivation of the dissolved enzymes, which can generally be avoided by immobilization [4,5].

The model reaction system chosen is the *Candida parapsilosis* carbonyl reductase 2 (CPCR2) catalysed reduction of acetophenone (Fig. 1). Since the regeneration of the cofactor NADH is mandatory [6,7], an enzyme coupled cofactor regeneration via formate dehydrogenase from *Candida boidinii* (FDH) is applied. CPCR2 is a highly sensitive enzyme that responds to diverse chemical and physical influences like pH, hydrodynamic stress, temperature, interfaces, and dilution [8–10]. To maximize the residual activity of CPCR2, the enzymes and the cofactor NADH are immobilised in a hydrogel matrix consisting of polyvinyl alcohol (PVA), forming spherical beads in millimetre scale, as suggested by Ansorge Schumacher [11]. These immobilisates are suspended in a 5% w/v ratio in *n*-heptane containing the substrate acetophenone. A scheme of the reaction system is shown in Fig. 1.

In order to keep the pH constant, which is increasing due to formate consumption, a novel reaction pH control concept was developed on a 1 L scale (Fig. 2). As indicator for the consumed formate, the headspace concentration of CO_2 in the N_2 aerated reactor

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Nomenclature

Abreviations

AcPh	Acetophenone
CPCR2	*Candida parapsilosis* carbonyl reductase
DNase1	Deoxy ribonuclease 1
E. coli	*Escherichichia coli*
FCW	Fresh cell weight
FDH	Formate dehydrogenase from *Candida boidinii*
FPLC	Fast protein liquid chromatography
GC	Gas chromatography
IPTG	Isopropyl thiogalactoside
IR	Infrared
NAD+	Nicotinamide adenine dinucleotide (oxidized)
NADH	Nicotinamide adenine dinucleotide (reduced)
OD_{600}	Optical density at 600 nm
PC/DP	Personal computer/data processing
PhEt	1-phenylethanol
PVA	Polyvinylic alcohol
RMSE	Root mean squared errors
RT	Room temperature
TB-medium	Teriffic broth medium
TEA	Triethanolamine

Symbols

A	Activity [U = μmol min^{-1}]
c	Concentration [mM]
c^*	Concentration at phase boundary [mM]
d	Diameter [m]
D	Diffusion coefficient [m^2 s^{-1}]
E	Extinction [-]
e	Extinction coefficient [L·$mmol^{-1}$·cm^{-1}]
F	Dilution factor [-]
j	Diffusional flux
k	Mass transfer coefficient [$m.s^{-1}$]
k_{cat}	Turnover number
Ki	Inhibition constant [mM]
K_j	Dissociation constant for reaction j
K_m	Michaelis-Menten constant [mM]
M	Mass [g]
MW	Molecular weight [g mol^{-1}]
N	Molar amount [mol]
$\dot{N}$	Molar flow [mol s^{-1}]
R	Reaction rate [mM s^{-1}]
r	Radius [m]
S	Surface area [m^2]
t	Time [h]
T	Temperature [°C]
V	Volume [L]
$\dot{V}$	Volumetric flow [ml min^{-1}]
y	Mole fraction

Indices

bulk	Bulk phase
hgs	Hydrogel sphere
i	Index for substance
j	Index for reaction
n	Stoichiometric coefficient
b	Backward
f	Forward

is measured via an infrared sensor and used to calculate the formate amount which is then fed to the reactor. As this reactor setup comprises of several chemical and physical phenomena including mass

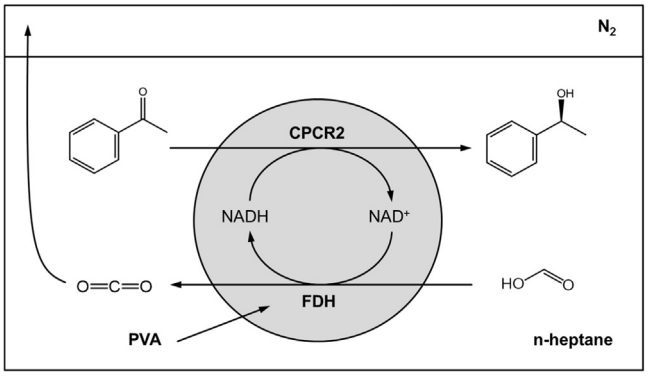

Fig. 1. Scheme of the reduction of acetophenone in a two phase system with enzyme coupled cofactor regeneration.

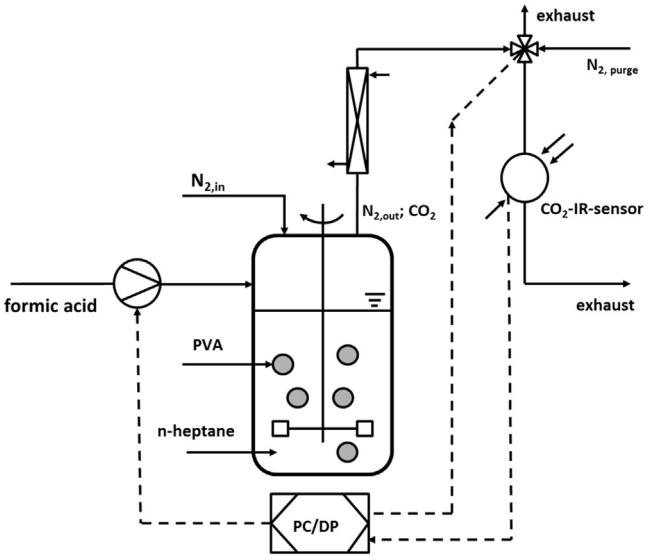

Fig. 2. Scheme of CO_2-measurement controlled process.

transfer, diffusion, partition equilibria, dissociation reactions, and catalysed oxidoreduction, the development of a process model is highly commendable to analyse the potential and limitations for a rational process development.

In biocatalysis, reaction conditions like pH-value or oxygen concentration are mainly measured within liquid aqueous systems. Recently developed optical measurement techniques like dual lifetime referencing enable on-line measurement even in immobilized phases in biphasic reaction systems [11–13]. However, even classical pH-control or in-situ substrate feeding and product removal strategies are rarely found in literature [14,15]. Very few (co)-substrate fed-batch or semi-continuous processes have been reported, many of them focusing on reducing substrate toxicity or inhibition [16–18]. Likewise, only few biocatalytic processes have been modelled in enough detail to allow for model-based process prediction or optimization [19–22].

Therefore, the primary aim of this study is to introduce the novel process control concept. After experimental validation of the CO_2-quantification, the effect of the control concept is shown, comparing a batch and fed-batch experiment. The secondary aim was the analysis of this process, based on a fully literature-based mathematical model. Simulation studies were performed in order to obtain a profound understanding of the process and identify improvement targets, which were experimentally validated for a selected simple case.

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