Investigations on Selectivity of Gas-Liquid Reactions in Capillaries

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Biocatalysis offers a broad spectrum of possible ecological and economic advantages over conventional chemical catalysis processes, e.g., lower energy consumption and high enantio selectivity. The focus of this work is on gas-liquid reactions. These are of great importance in the chemical and biochemical industry and subject of current research since they are often limited by mass transfer or show low selectivity. Different suitable biocatalytically gas-liquid reaction systems were tested in capillary reactor designs in order to obtain information about the interaction between reaction and fluid mechanics. Furthermore, an optical measuring method was established. The experiments were performed in batch mode in a glass beaker with a flow cuvette for UV/Vis measurement of product concentration.

Keywords: Coiled flow inverter, Gas-liquid reaction, Michaelis-Menten kinetic, Taylor flow

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1 Introduction

Microreactors are characterized by a large specific surface area and are, therefore, of interest for many applications in research and development. Because of the resulting enhanced heat and mass transfer, microreactors provide a great potential for process intensification and process control [1, 2].

Gas-liquid reactions such as oxidation, hydrogenation or carboxylation/carbonylation represent essential reactions in chemical and biochemical industry. Within this work, different biocatalytic reaction systems are tested for suitability to investigate gas-liquid mass transfer and selectivity in microreactors, i.e., straight capillary (SC), helically coiled capillary (HCC) and coiled flow inverter (CFI) [3, 4] for oxidation.

Depending on velocity of the fluids, channel geometry, fluid properties, and surface wettability, different flow patterns can be observed in microchannels, or particularly in capillaries. In literature, slug flow (Taylor flow, segmented flow) is the most widely used flow pattern for investigations on two-phase flow in capillaries [5–7]. The hydrodynamics and mass transfer phenomena are well understood, and slug flow represents a stable flow regime with good mixing characteristics and a narrow residence time distribution (RTD) [8–10]. In order to investigate selectivity, reactions forming two or more products or detectable intermediates are necessary. Krieger et al. [11] performed experimental studies on the consecutive oxidation of leuco-indigo carmine in SC and HCC. The yellow leuco-indigo carmine oxidizes via a red intermediate to the blue product keto-indigo carmine. The color changes enable local mass transfer and selectivity studies by using an optical evaluation technique.

Within this work, suitable biocatalytic reaction systems have been developed to investigate mass transport and selectivity for parallel reactions in capillary reactor designs. This makes it possible to compare the investigations of the parallel reaction with the consecutive reaction system from Krieger et al. [11]. Specifically, statements about the correlation between reaction and fluid mechanics can be compared.

2 Materials and Methods

In order to find suitable enzymes for biocatalytic reaction systems, the following selection criteria were applied:

- color change: to be able to use an optical measurement technique, a direct proportionality between substrate conversion and color intensity is necessary,
- reaction kinetics: no inhibition through substrate, product or dye,
- availability and costs.

For catalyzing gas-liquid reactions, the class of oxidoreductases is most interesting for further consideration. In this class, particular attention is paid to enzymes that catalyze oxidations with oxygen. For this reason, chromogenic substrates are selected below that show a color change through reaction with oxygen. The criteria taken into account in the selection of the chromogenic substrate are:

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– stability of the dye in solution,
– high intensity of the oxidized dye,
– compatibility with measurement technique, and
– toxicity.

2.1 Enzyme Characterization

As catalytically active proteins, enzymes have an optimum activity [12]. Since enzymes in living cells are involved in almost all metabolic processes and are adapted to the living organism, the optimum activity is in most cases at atmospheric pressure, temperatures between 10–50 °C and a pH value of 5–8 [13]. For the highest possible enzyme activity, the ambient conditions must be optimally adjusted. For this purpose, the influence of temperature, solvent ratio, and buffer was investigated in test series. The basis of all investigations is the extinction measurement of the formed dye in the UV/Vis spectrophotometer. Fig. 1 shows the experimental setup. The educt solution is heated by a thermostat (Huber, polystat cc2). The heated educt is pumped through the cuvette of the UV/Vis spectrophotometer (Thermo Scientific, Evolution 201) and back into the storage vessel.

2.2 Calibration

To obtain the relationship between substrate conversion and color intensity, calibration curves are recorded. For different substrate concentrations, the absorbance in the UV/Vis spectrophotometer is measured with the experimental setup shown in Fig. 1. The intensity of the radiation in the UV/Vis spectrophotometer is attenuated when passing through an absorbing medium. The correlation between the intensity of the radiation and the concentration of the absorbing medium is given by the Lambert-Beer law, which is illustrated in Fig. 2:

$$A{\sim}E = \log\left(\frac{I_0}{I}\right) = \varepsilon\ c\ e \tag{1}$$

with the absorbance A, which can be correlated with the extinction E [14]. The initial intensity of the radiation is described by I_0 and I is the intensity after radiation of the sample. The parameter ε indicates the extinction coefficient, c is the substrate concentration and e the thickness of the measuring cuvette.

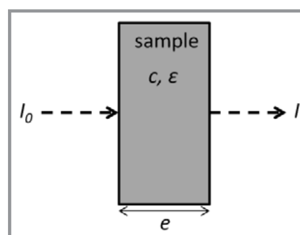

Figure 2. Illustration of Lambert-Beer law [14].

2.3 Enzyme Reaction Kinetics

Compared to chemically catalyzed reactions, biocatalytic reactions offer several advantages, e.g., they run under mild operating conditions in aqueous media [15]. In addition, depending on the enzyme used, different reaction times can be investigated in the range of seconds up to a few hours. Already in 1931 Leonor Michaelis and Maud Menten developed a simple model to explain the kinetics of enzyme-catalyzed reactions called Michaelis-Menten kinetics [16]. The relationship between reaction rate V_0, substrate concentration $[S]$ and K_M value is described by the Michaelis-Menten equation:

$$V_0 = V_{max}\ \frac{[S]}{[S] + K_M} \tag{2}$$

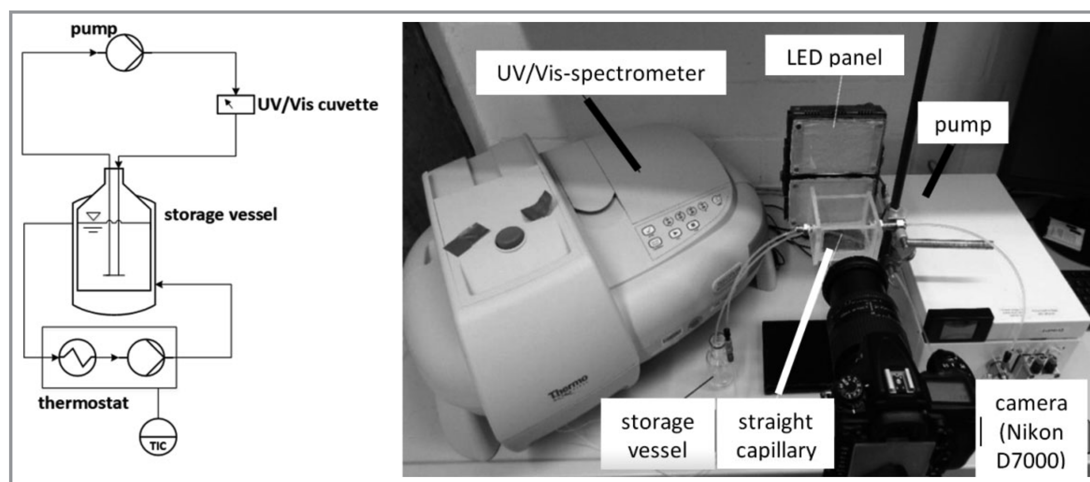

Figure 1. Left: Experimental setup used for enzyme characterization, calibration and determination of enzyme kinetics. Right: Extended setup with camera and LED panel to detect the reaction progress in a straight capillary for optical evaluation.

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Here, V_{max} indicates the maximum reaction rate that is achieved asymptotically when all binding sites of the enzyme are occupied by substrate. The K_M value describes the substrate concentration, when the reaction rate reaches the half maximum value. In Fig. 3, the Michaelis-Menten kinetics and a linearization method according to Lineweaver-Burk are illustrated.

The Michaelis-Menten kinetics is applicable to reactions with one substrate. In order to apply it on a two-substrate reaction (chromogenic substrate and oxygen), one substrate is assumed to be constant and not limiting the reaction [13]. Here, the buffer solution is gassed with oxygen and the oxygen content can be assumed as constant.

Figure 3. Michaelis-Menten kinetic (left) and linearization method according to Lineweaver-Burk (right) [15].

3 Results and Discussion

Based on the selection criteria, laccase was selected as a suitable enzyme. Laccase is able to catalyze reactions with chromogenic substrates to colored products. Furthermore, there are no inhibitions through substrate, product or dye known. Due to their broad substrate spectrum and the fact that only water is produced as by-product, while no cofactors are required for the reaction, laccases are interesting for many biotechnological applications [16]. Hence, they are readily available and inexpensive. The chromogenic substrates used were ABTS (2,2́-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), SAz (syringaldazine) and OPD (*o*-phenylenediamine). Laccase catalyzes the oxidation of ABTS to a blue product $ABTS_{ox}$ [17]. The oxidation of SAz leads to a pink/violet product SAz_{ox} [18]. DAPN (2,3-diaminophenazine) is the yellow oxidation product resulting from the dimerization of OPD [19]. The corresponding oxidation reactions are illustrated in Fig. 4.

The calibration curves and the enzyme kinetics of the reactions were determined for all three chromogenic substrates via UV/Vis spectrophotometry.

3.1 Characterization of Laccase

The study on optimal temperature was carried out for ABTS and SAz after 90 s and after 3 and 4 min for long-term stability. Considering long-term stability is important when using reaction systems with residence times longer than 3 or 4 min. For fast reactions lasting a few seconds, the long-term stability is irrelevant. With regard to long-term stability, laccase shows an optimum temperature at 35 °C.

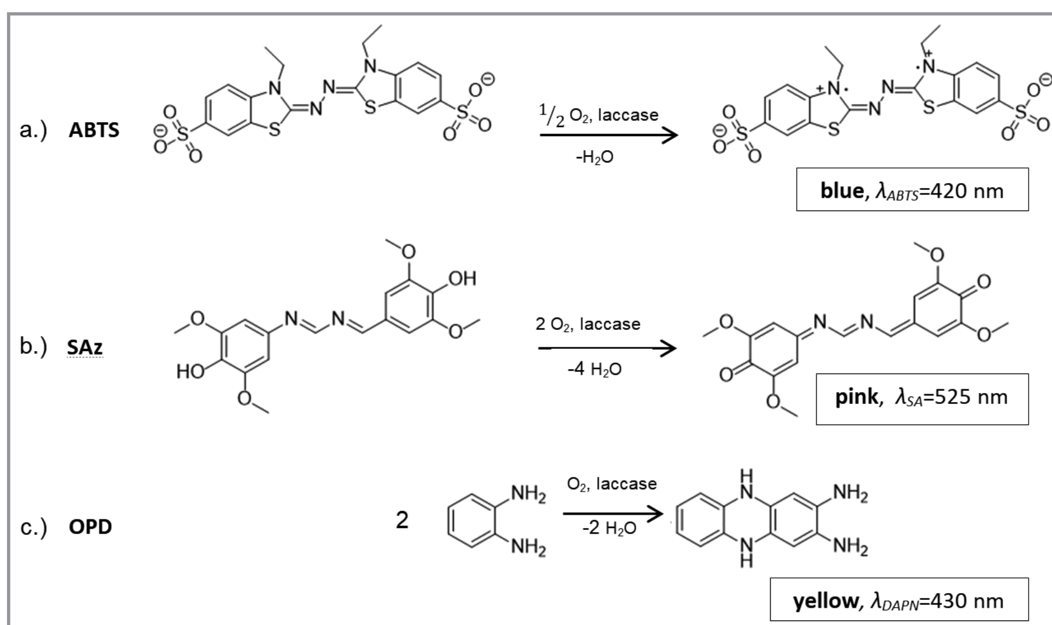

Figure 4. Chemical equation of the oxidation of a) ABTS [17] and b) SAz [18]. The third reaction c) describes the dimerization of OPD [19].

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For reaction times up to 90 s a higher extinction is measured at higher temperatures up to 42 °C and, thus, more substrate is converted. All further investigations were carried out at the optimal temperature at 35 °C because the experimental handling is much easier when laccase can remain in the storage tank for a few minutes.

Since only ABTS is water-soluble, different methanol/water ratios were investigated for the reaction systems laccase/ABTS and laccase/SAz. The results show that lower extinction values are achieved with increasing methanol content, i.e., less substrate is converted. According to Harkin et al. [18], high concentrations of ethanol have an inhibitory effect, so that this correlation is also suspected for methanol. With a methanol/water ratio of 1:15 the water content required for the enzyme activity seems to be sufficient.

In literature different buffer solutions with various pH values are mentioned depending on the substrate used [20, 21]. The experimental investigations led to the conclusion that the use of sodium acetate buffer (pH 5.2) led to a higher extinction and, thus, to a higher substrate conversion than potassium phosphate buffer (pH 6). This result is consistent with literature data, which also suggest the highest enzyme activity in the acidic range [20].

3.2 Calibration Curves

Information about concentration of the formed product (and thus about conversion and selectivity) can be obtained by means of an optical evaluation method via formation of the dye. The experimental studies for investigating the correlation between color intensity of the formed dye and substrate conversion have shown linear correlations with: $E_{ABTS} = 0.99[S] + 0.002$; $E_{SAz} = 0.21[S] + 0.004$ and $E_{OPD} = 3.5[S] - 0.0004$.

3.3 Enzyme Kinetics

The investigation of the kinetics showed that all three biocatalytic reaction systems follow the Michaelis-Menten kinetics. The values for K_M and V_{max} were determined according to Lineweaver-Burk method. The values can be taken from Tab. 1.

Table 1. Kinetic data K_M and V_{max} for laccase with different substrates ABTS, SAz and OPD.

	K_M [µM]	V_{max} [µmol min^{-1}]
ABTS	5.26	7.20
SAz	0.99	0.44
OPD	2301.58	79.34

The results in Tab. 1 show clear differences in the values both for K_M and for V_{max}. Nevertheless, all are typical values for enzymatically reactions [13]. K_M is an indicator of the strength of the interaction between enzyme and substrate. Based on the results, it was concluded that laccase has the greatest affinity to SAz (lowest K_M value) and the lowest affinity to OPD (highest K_M value). Although the laccase-OPD system can achieve a high reaction rate, the stability of the enzyme-substrate complex is not very high. The residence time for ABTS and SAz was about 3–4 min until the substrate was completely converted. In the case of OPD, the residence time was about 30 min for complete conversion.

4 Conclusion

This work describes the investigations on biocatalytic reactions with laccase. Three chromogenic substrates were examined for their suitability for parallel reactions in capillary reactors. The results show that although all reactions follow Michaelis-Menten kinetics, they are not all suitable for parallel reactions among each other. The reaction times of the single reactions indicate that ABTS and SAz are compatible reaction systems to perform a parallel reaction since the reaction starts within 10 s after addition of oxygen to the reaction mixture in both cases. The dimerization of OPD is too slow (approx. 20 min until start of reaction) for a parallel reaction with ABTS or SAz.

The subject of future work is to distinguish between ABTS and SAz with the optical evaluation method to obtain accurate information about local mass transfer phenomena. This will aid to describe the relationship between fluid dynamics (depending on the reactor design used) and chemical selectivity of parallel reactions.

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Symbols used

A	[–]	absorbance
c	[mol L^{-1}]	concentration
E	[–]	extinction
e	[m]	thickness of measuring cuvette
I	[W m^{-2}]	intensity
K_M	[µM]	Michaelis-Menten constant
$[S]$	[mol L^{-1}]	substrate concentration
V	[µmol min^{-1}]	reaction rate

Greek symbols

ε	[m^{2}mol^{-1}]	extinction coefficient
λ	[nm]	wavelength

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Sub- and Superscripts

0 initial
M Michaelis-Menten
max maximum value
ox oxidized

Abbreviations

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
CFI coiled flow inverter
DAPN 2,3-diaminophenazine
HCC helically coiled capillary
OPD *o*-phenylenediamine
RTD residence time distribution
SAz syringaldazine
SC straight capillary

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