Fine Bubble-based CO_2 Capture Mediated by Triethanolamine Coupled to Whole Cell Biotransformation

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Carbon capture technology can be set up in combination with biocatalysis to utilize the bound CO_2 as substrate in the Kolbe-Schmitt like enzymatic reaction. The exemplary whole cell biotransformation of catechol to 2,3-dihydroxybenzoic acid in a triethanolamine-mediated multiphase system shows increased equilibrium conversion. Apart from the beneficial thermodynamics, the inherent fluid properties of triethanolamine is enabling easy application of CO_2 fine bubbles as highly efficient gassing method to minimize the CO_2 demand and CO_2 emissions.

Keywords: Carbon capture, Fine bubble aeration, Multiphase systems, Triethanolamine-mediated carboxylation, Whole cell biotransformation

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1 Introduction

Greenhouse gases like CO_2 and methane are directly linked to the global temperature. Due to the worldwide efforts to reduce greenhouse gas emission, the optimization of existing and development of new technologies for reduction and recycling of CO_2 is of major interest [1, 2]. The carbon capture technology that uses amines such as triethanolamine (TEA) was shown to be compatible with the enzymatic carboxylation, which is competitive to the Kolbe-Schmitt reaction [1–4]. Here, the bound CO_2, in the form of hydrogen carbonate, is consumed as a sustainable carbon source for the synthesis of carboxylated phenolics (Fig. 1). This type of reaction is limited by an unfavorable thermodynamic reaction equilibrium towards carboxylation [5]. In consequence, there is a need to ensure high concentrations of hydrogen carbonate. The application of the amine-mediated system enables high CO_2 loadings in the molar range, which is shifting successfully the reaction equilibrium towards the carboxylation reaction [6, 7]. Due to the required CO_2 loading of TEA in enzymatic carboxylation, the presaturation of the solution is an important process step, which should be fast and cost efficient. The application of CO_2 fine bubble gassing is a promising solution to increase the mass transfer and shorten the presaturation phase [8, 9]. Fine bubbles are by definition of the ISO/TC 281 less than 100 µm in diameter [10]. This gassing technique provides a high volume-specific surface area and, therefore, quick dissolution rates of gases in the surrounding liquid [9, 11]. In respect to this, there is the potential in reducing the necessary CO_2 gas stream as well as the waste of unreacted CO_2.

This contribution focuses on an application example of bacterial whole cell synthesis of 2,3-dihydroxybenzoic acid (2,3-DHBA) by carboxylation and investigation of the possible application of CO_2 fine bubble gassing as feasible gassing technique. Furthermore, the improvement of the equilibrium yield by variation of the TEA concentration and resulting effects on the bubble size distribution (BSD) were investigated.

2 Experimental Section

2.1 General

All chemicals were purchased from commercial sources with purities of ≥ 99 %. Carbon dioxide 4.5 (≥ 99.995 %) was obtained from Linde AG (Germany). For aeration, a stainless steel PerfectPeak® solvent inlet filter with 0.5 µm

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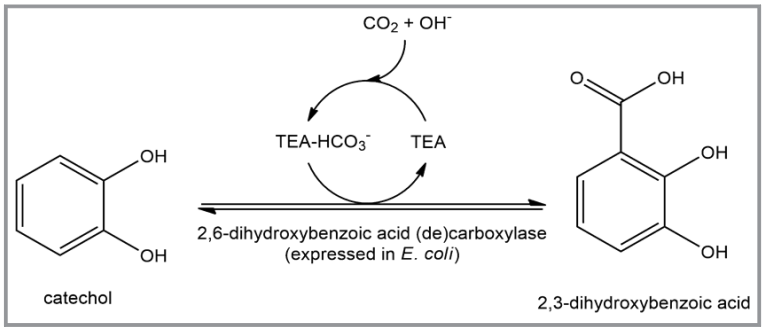

Figure 1. Triethanolamine (TEA)-mediated biotransformation of catechol to 2,3-dihydroxybenzoic acid.

pores was used, with a length of 1 inch and a diameter of 0.5 inch.

2.2 Biocatalytic Reactions

Preparation of *E. coli* whole cells hosting the 2,6-dihydroxybenzoic acid (de)carboxylase (2,6-DHBD) gene from *Rhizobium sp.* was performed according to the protocol by Pesci et al. [12]. Carboxylation reactions were carried out in HPLC screw cap vials (1.5 mL) with 80 mM catechol and 10 mg mL^{-1} whole cells from the same batch at 30 °C and 500 rpm. Reaction mixtures were presaturated with CO_2 prior to biocatalyst addition for the pH neutralization of alkaline aqueous TEA solutions. Further CO_2 gassing during the reaction was applied by a cannula. Samples (100 µL) were withdrawn after 24 h as well as 48 h. No change of analytical yield was observed between 24 and 48 h. Catechol and 2,3-DHBA concentrations were measured by HPLC for equilibrium yield determination.

2.3 Sampling Procedure and RP-HPLC Method

Reaction samples were diluted in 100 µL trifluoroacetic acid (TFA) to stop the reaction and to remove the bound CO_2. The samples were centrifuged for 10 min at 13 000 rpm. The supernatant was then analyzed by an Agilent LC-1100 HPLC system equipped with a diode array detector and a LichroCART 250-4 Lichrospher 100 RP column (5 µm) at 30 °C. The mobile phase consisted of eluent A (TFA/water, 0.1 vol %) and eluent B (TFA/acetonitrile, 0.1 vol %). The following gradient was applied at 0.8 mL min^{-1}: 0–1 min 10 % B; 1–3 min 10–25 % B; 3–10 min 25–60 % B; 10–12 min 60–90 % B; 12–16 min 90 % B; 16–18 min 90–10 % B; 18–20 min 10 % B. Retention times were 7.8 min for catechol and 8.7 min for 2,3-DHBA.

2.4 Bubble Size Distribution

Inline measurements of bubble sizes were performed using the Sopat-VI Sc probe-based microscope and the integrated image analysis software (Sopat GmbH, Berlin, Germany) in a bubble column setup. The column had an inner diameter of 31.9 mm and a working volume of 150 mL. The measuring range of the Sopat-VI Sc is within 9–1200 µm.

3 Results and Discussion

3.1 Biotransformation

The biocatalytic carboxylation of catechol is known to suffer from unfavorable thermodynamics [13]. Up to 12 % conversion is achievable in a typical biotransformation, when applying 10 mM catechol with 1 M $KHCO_3$ at 30 °C [3]. This challenge can be addressed by the application of amines such as TEA. Pesci et al. have already shown that TEA acts as a CO_2 mediator with the concentration of 1 M, which shifted successfully the reaction equilibrium towards the carboxylation [3]. In respect to this, TEA was chosen as a suitable model system in which the effect of higher TEA concentrations was in the focus of this study.

By tripling the TEA concentration as shown in Fig. 2, the equilibrium yield of 2,3-DHBA shifts from 16.1 % to 25.7 %. This improvement is directly related to higher CO_2 loadings at elevated TEA concentrations. However, the binding capacity, which describes the molar ratio of bound CO_2 per amine, does not increase linearly for tertiary amines, because of their reaction mechanism. These compounds exhibit a base-catalytic effect on the hydration of CO_2 forming carbonates, which act as co-substrate [13, 14]. Thus, the resulting concentration of the dissolved inorganic carbon

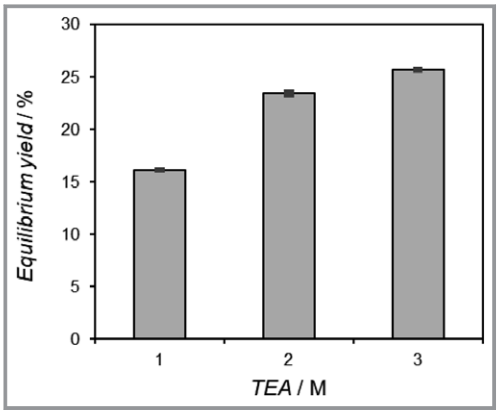

Figure 2. Equilibrium yield for the carboxylation of 80 mM catechol to 2,3-dihydroxybenzoic acid by 10 mg mL^{-1} whole cells containing 2,6-dihydroxybenzoic acid (de)carboxylase in CO_2-saturated triethanolamine at 30 °C with indicated mean deviation (n = 2).

(DIC) is depending on the TEA to water ratio. The highest DIC concentration can be achieved at 50 vol % TEA/water [15], which is additionally dependent on the temperature and pressure within the system [16]. In consequence, the maximum is expected to occur at approx. 3.75 M (50 vol %) aqueous TEA at 30 °C and 1 atm.

Generally, the utilization of gaseous CO_2 as substrate via amine mediation is a promising system that enhances the equilibrium yields compared to the conventional application of carbonate salts [12]. It is shown that TEA is acting as CO_2 mediator. This provides high co-substrate concentrations and shifts the reaction equilibrium towards the carboxylation. Furthermore, it exhibits the advantage of being a reusable system, in which the amine could be reused after product separation. However, equilibrium yields are so far limited under the applied conditions to below 30 % (Fig. 2). Two major approaches can further improve the reaction system: (1) more efficient amines, which achieve higher DIC and, thus, higher co-substrate concentrations, (2) in situ product removal (ISPR) to shift the reaction equilibrium towards the product site. Nevertheless, it is shown for triethylamine in literature, that it causes enzyme deactivation at high amine concentration [6]. This needs to be considered when applying amines.

For an ISPR approach, Ren et al. provided one representative example for the biocatalytic carboxylation of phenols by application of quaternary ammonium ions [17]. They demonstrated that specific quaternary ammonium ions selectively crystallize respective benzoic acid derivatives in situ to remove continuously the formed product reaching nearly full conversion (up to 99 %) [17, 18]. Additionally, they developed a downstream approach in a preparative scale in which the tetrabutylammonium salt of the product was isolated with 90 % yield. The following treatment with hydrochloric acid, filtration and washing procedures resulted in 72 % isolated yield [17]. Simultaneously, an extraction with chloroform recovered the quaternary ammonium compound. This downstream process is a possibility for the amine-mediated system to reach similar results with an optional amine recycling. To get deeper insights into the CO_2 loading efficiency, the influence of TEA concentration and the gassing rate on the BSD is studied in detail.

3.2 Bubble Size Distribution for CO_2 Dissolution

The key aspect of the amine-mediated biocatalytic carboxylation of phenols by decarboxylases is the utilization of CO_2 [3]. In designing an efficient process involving triethanolamine, the gassing plays a major role [2]. Especially, the presaturation of the reaction media to ensure optimal pH and CO_2 loadings is a process step that can be shortened by efficient aeration.

In Fig. 3a, the BSD are displayed using Gaussian fitting with indicated root mean square errors. According to the displayed BSD, fine bubble gassing is accomplished by application of low gassing rates in 1 M TEA. Due to their small size, fine bubbles show elevated residence time coupled with quick dissolution in the surrounding media [8, 9]. The fast shrinking and self-compression are especially occurring based on the enhanced mass transfer performance related to the high volume specific interfacial area and the elevated inner bubble Young-Laplace pressure [8, 11, 19, 20]. The complete dissolution of CO_2 bubbles in the media provides the possibility for the reduction of the applied inlet gas stream as well as CO_2 emission in the offgas [20].

The direct comparison of the measured BSD by the mean diameter d_{50} shows that a shift from 79 µm to 110 µm occurred for $d_{50,20mL/min}$ and $d_{50,100mL/min}$, respectively. In contrast to this, an increase of the gassing rate from 100 mL min^{-1} to 200 mL min^{-1} resulted in no further change of the BSD (Fig. 3a). It needs to be considered that a porous sparger with an inhomogeneous pore size distribution was used affecting the BSD. Neighboring pores can cooperate through coalescence in the initial bubble formation [17, 18]. The increased gassing rate leads to an increase of the pressure drop at the porous sparger, which results in the activation of pores and joint bubble formation between several pores [21]. This effect leads to an increase in bubble diameter until all pores are activated. Furthermore, it is reported in literature that a secondary bubble formation above the pores can occur when the flow rate is increased up to a critical level, resulting in a bubble formation process that is independent of the flow rate [22]. These effects may explain the observed constant BSD for the high gassing rates as shown in Fig. 3a.

The gassing rate of 20 mL min^{-1} resulted in the smallest measured BSD and was selected for further investigations. At constant gassing rate, the effect of the average sparger pore size and variation of TEA concentration, due to the

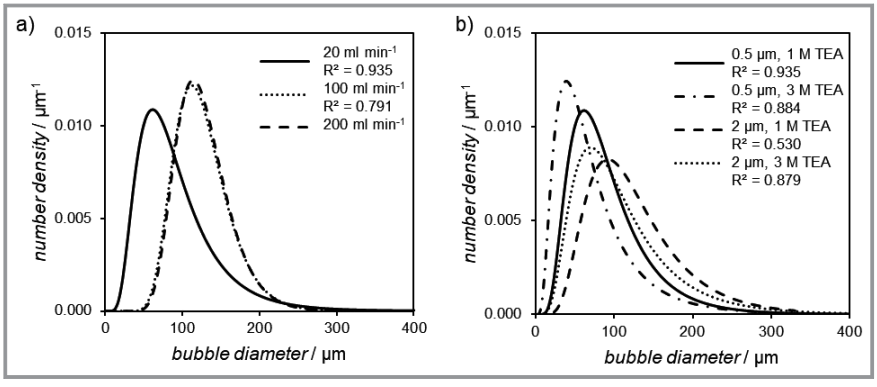

Figure 3. Bubble size distribution for a) variation of gassing rate and b) different sintered stones and variation of TEA concentration at constant gassing rate.

yield enhancing property, was examined (Fig. 3b). In the measurement of both spargers, the increase of TEA concentration led to a shift towards a smaller bubble size. For the 0.5-µm sparger, the d_{50} of 79 µm decreased to 58 µm, while the d_{50} shifted from 109 µm to 98 µm for the 2-µm sparger. This behavior can be explained by the change in the physical properties of the reaction mixture for different TEA concentrations. The bubble formation at single and multiple pores is affected by surface tension and viscosity, which are two of the major influencing liquid properties. In case of TEA, the surface tension decreases from 63.1 mN m^{-1} to 53.4 mN m^{-1} for 1 M and 3 M, respectively [23]. The decrease in surface tension force leads to bubble detachment at smaller bubble diameters and is resulting in a smaller BSD [21]. In consequence, the bubble formation as well as bubble densities are increasing. In contrast to the surface tension, the dynamic viscosity is tripled from 1.56 mPa s (1 M) to 4.47 mPa s (3 M) [23]. In this case, the increase in viscosity is resulting in a higher drag force of the media slowing bubble growth [24]. This causes a pressure increase under the porous surface and activation of smaller pores, when a constant gassing rate is applied [21]. Both physical properties of TEA are beneficial for producing smaller BSD. On the one hand, the generated smaller bubbles are enabling a higher mass transfer, which ensures high co-substrate concentration and higher reactions rates for the biotransformation. On the other hand, the buoyancy force for fine bubbles is decreased, resulting in longer bubble residence times in the aqueous phase.

4 Conclusion

In this study, it was shown that for the whole cell biotransformation of catechol to 2,3-DHBA, an increase of TEA concentration from 1 M to 3 M enhanced the analytical yield from 16.1 % up to 25.7 %. The improved yield can be attributed to elevated CO_2 loadings and hydrogen carbonate content at higher amine concentrations. Simultaneously, the increased TEA concentration enables a more efficient CO_2 aeration, because smaller bubbles are produced. Applying the 0.5-µm sparger at the lowest gassing rate, the major fraction in the BSD was measured in the fine bubble region with d_{50} = 58 µm. The main effects on the decrease of the BSD is based on the lower surface tension and the increase in viscosity when using higher amine concentrations. The enlarged residence time and quick dissolution of fine bubbles makes this gassing technique interesting for applications on industrial scale. Due to this, the overall process time can be shortened. Considering this, the fine bubble technology is an option for reduction of the supplied CO_2 gas stream and CO_2 emissions in the synthesis of valuable products. This was indicated by the exemplary enzymatic synthesis of 2,3-DHBA.

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Abbreviations

2,3-DHBA	2,3-dihydroxybenzoic acid
2,6-DHBD	2,6-dihydroxybenzoic acid (de)carboxylase
BSD	bubble size distribution
DIC	dissolved inorganic carbon
ISPR	in situ product removal
TEA	triethanolamine

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