Adsorbent-based downstream-processing of a decarboxylase-based synthesis of 2,6-dihydroxy-4-methylbenzoic acid

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SC 57202375754

Introduction

The biocatalytic regioselective *ortho*- and *para*-carboxylation of phenol derivatives using bicarbonate as CO_2 source is a relatively new development.[1] Unfortunately, the downstream-processing of (de)carboxylase reactions is still a challenge.[2] In this study, a highly efficient work-up strategy of this enzymatic carboxylation reaction using non-oxidative (de)carboxylase has been investigated. Orcinol **1** was converted to its corresponding carboxylated benzoic acid derivative DHMBA **2** at larger scale (Fig. 1 + 2).[3]

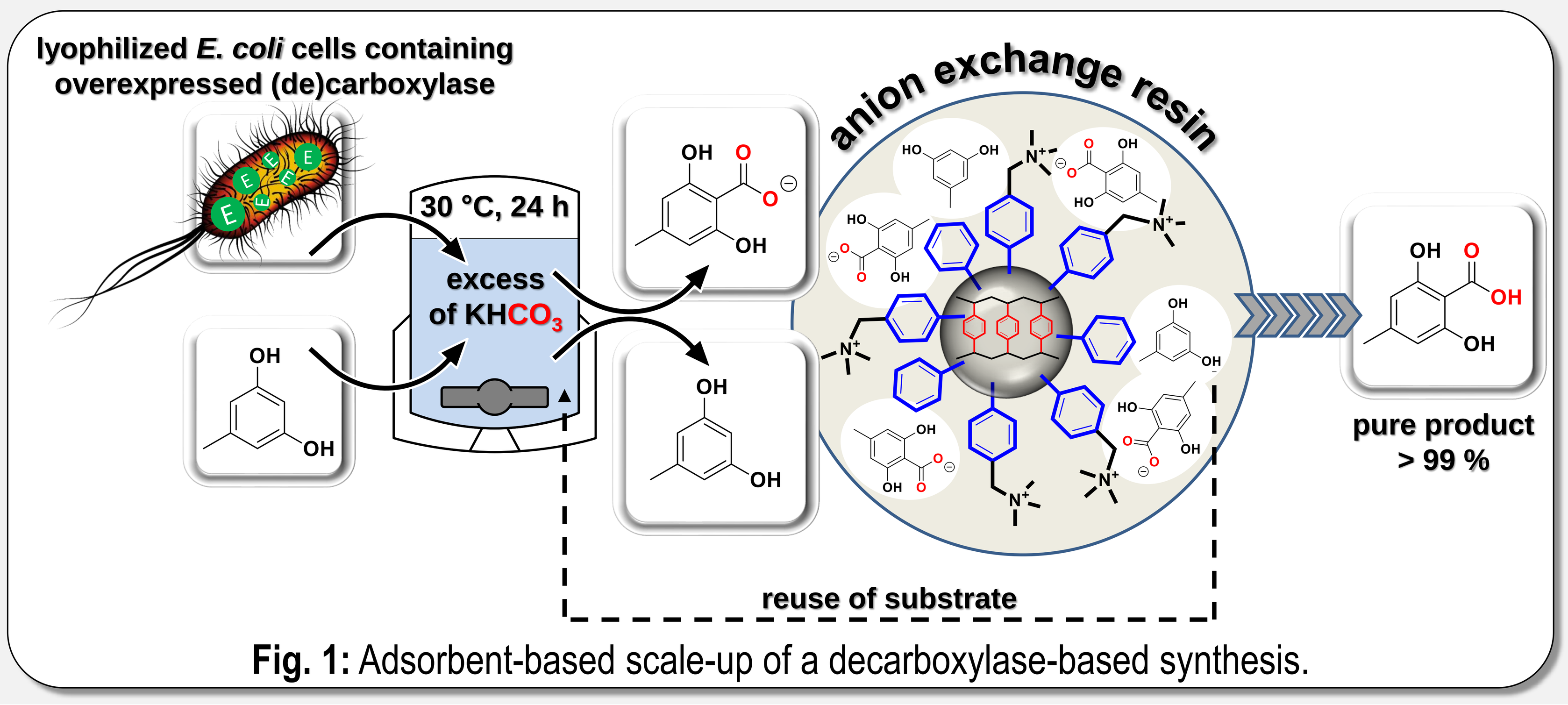


Fig. 1: Adsorbent-based scale-up of a decarboxylase-based synthesis.

Results and Discussion

biocatalytic carboxylation reaction

- orcinol **1** was directly converted to its carboxylated product 2,6-dihydroxy-4-methylbenzoic acid (DHMBA) **2** (Fig. 2)
- lyophilized *E. coli* cells containing the corresponding overproduced enzyme were used as the catalyst and studied witch respect to the substrate concentration (Fig. 3A)
- potassium bicarbonate at or near atmospheric pressure as a CO_2-source was used
- sufficient conversions were achieved even at low substrate concentrations after 24 hours (Fig. 3B/C))

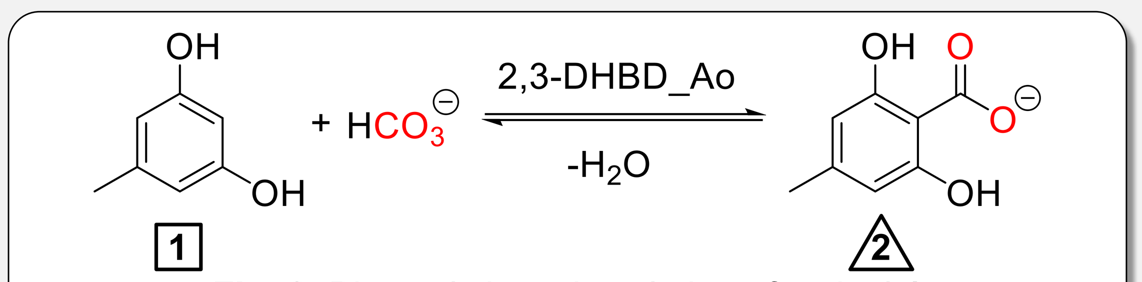
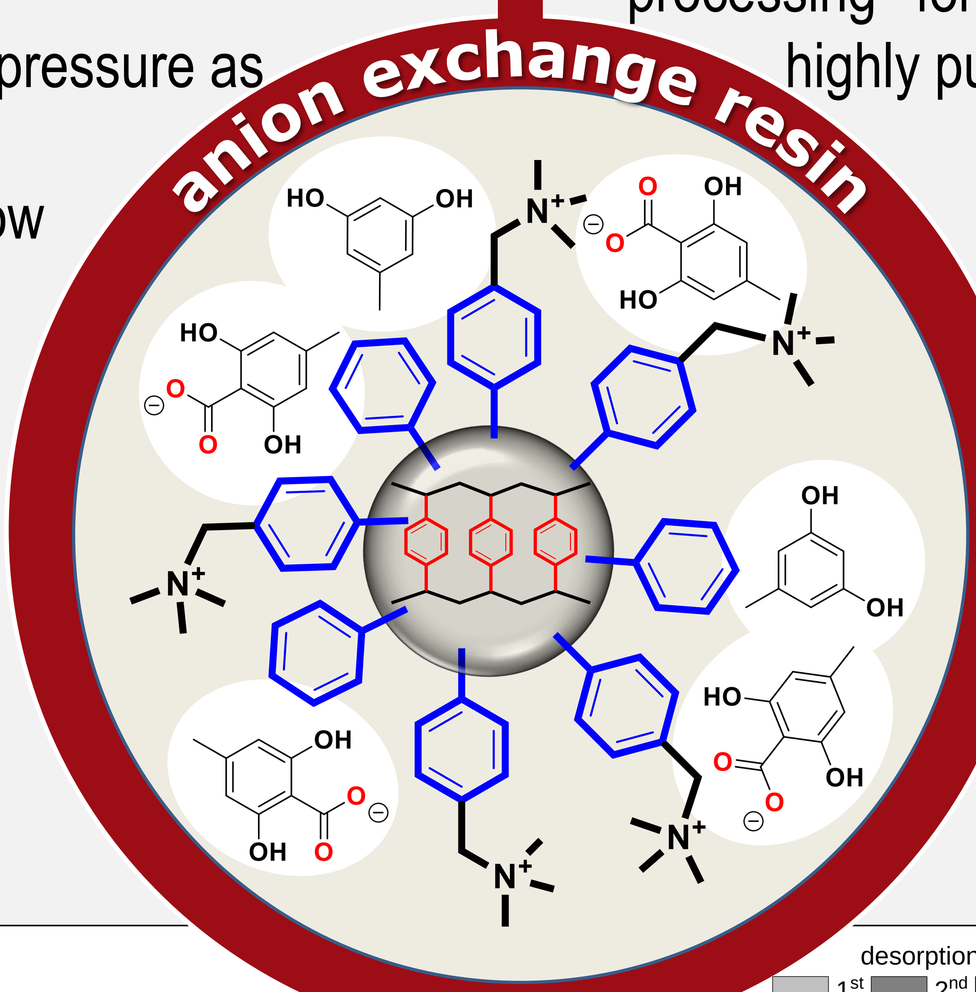


Fig. 2: Biocatalytic carboxylation of orcinol **1** (60 mg lyophilized cells, 50 mmol/L **1**, 3 mol/L $KHCO_3$).



adsorbent based downstream-processing

- different anion–exchange and non ionic adsorbents resins were screened for their ability to remove both the product **2** and the remaining substrate **1** from a test solution (Table 1)
- a subsequent desorption leaded to an efficient downstream-processing for the enzymatic carboxylation reaction obtaining highly pure product **2** (Fig. 4)

Table 1: Adsorption of **1** and **2** onto ten different adsorbers.

applied adsorber	DHMBA (2) adsorption/%			orcinol (1) adsorption/%		
	0.02 g/mL	0.1 g/mL	0.2 g/mL	0.02 g/mL	0.1 g/mL	0.2 g/mL
a) anion exchanger resins						
Dowex 1x2 (Cl)	93	**>99**	**>99**	74	**>99**	**>99**
Diaion PA312 (Cl)	77	99	**>99**	51	96	**>99**
Diaion HPA-25 (Cl)	83	98	**>99**	52	98	**>99**
Diaion SA10A (Cl)	74	99	**>99**	46	96	**>99**
b) non-ionic adsorbens						
Dowex Optipore L493	48	97	**>99**	57	98	**>99**
Lewatit VP OC	20	44	61	21	55	72
Diaion HP-2MG	33	90	97	44	93	**>99**
Amberlite XAD-7HP	42	93	98	47	94	**>99**
Amberlite XAD-4	1	7	16	3	19	29
Amberlite XAD-1180N	2	4	13	1	8	14

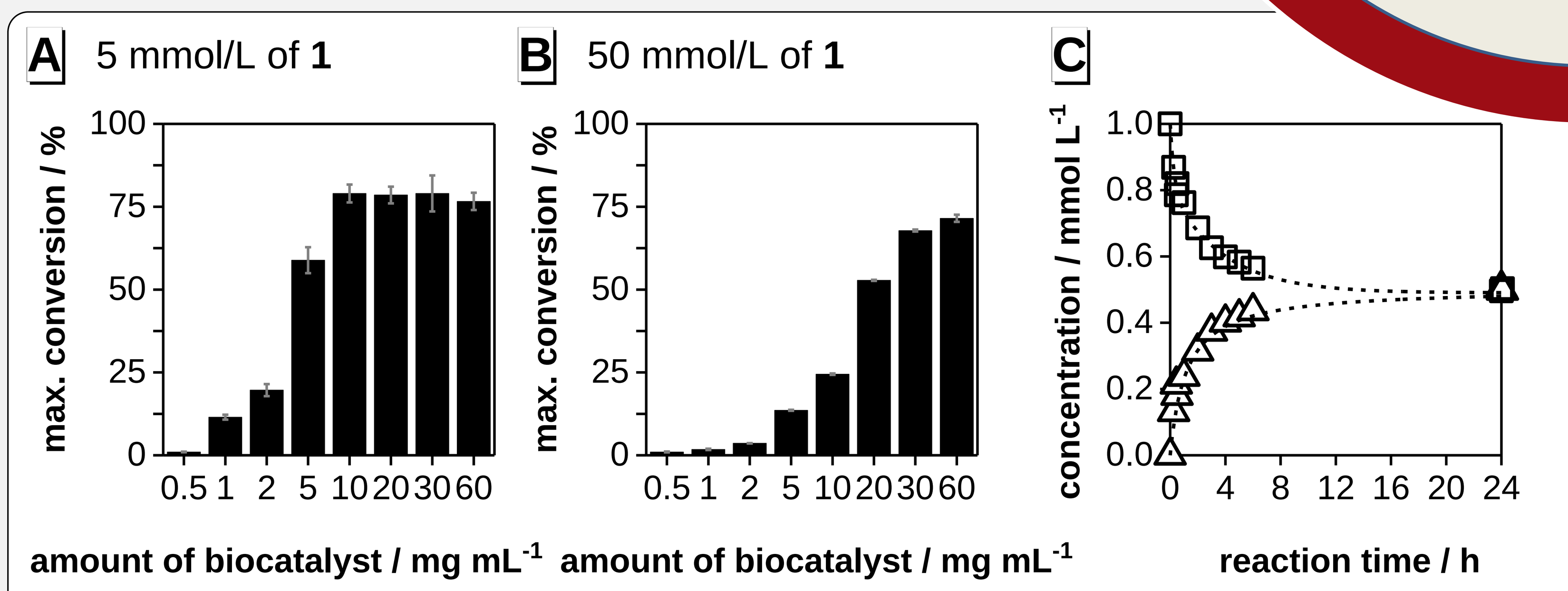


Fig. 3: Biocatalytic carboxylation of **1** (C: 60 mg lyophilized cells, 50 mmol/L **1**, 3 mol/L $KHCO_3$).

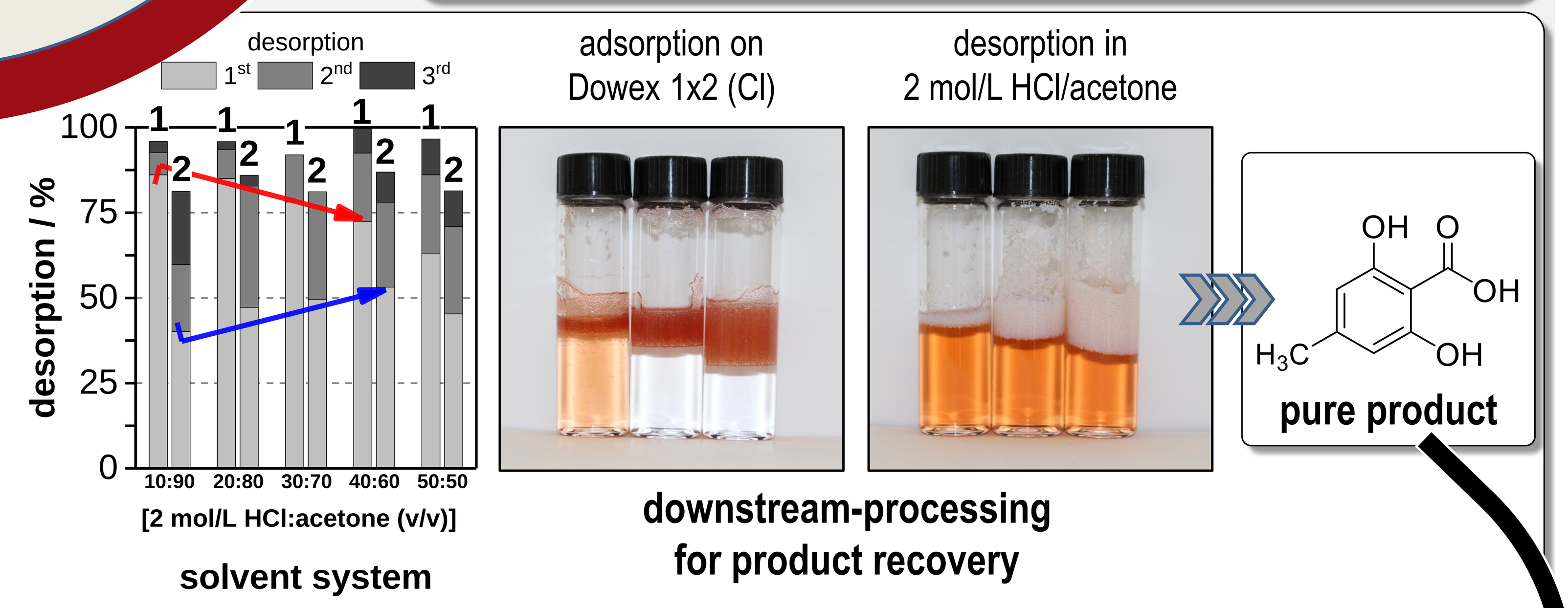
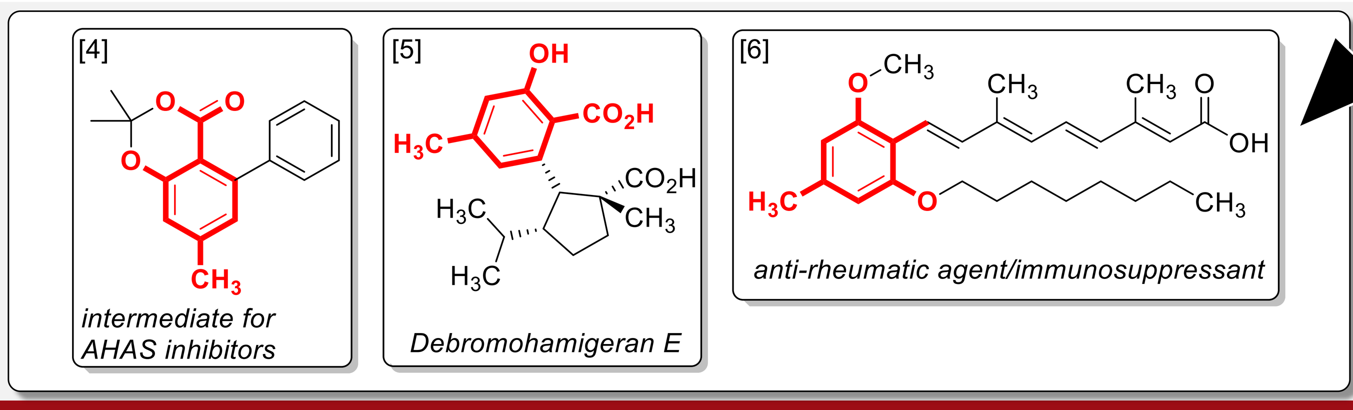


Fig. 4: Desorption and efficient downstream-processing for enzymatic carboxylation reaction.

Summary and Outlook

- product **2** was purified by an adsorption−desorption cycle and subsequently obtained with purities >99% without a full elimination of the excess bicarbonate from its reaction solution
- product is an important intermediate for Active Pharmaceutical Ingredients (APIs) (see right hand side)



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Acknowledgements

Financial support by Deutsche Forschungsgemeinschaft (DFG, Germany) and Fonds zur Förderung der wissenschaftlichen Forschung (FWF, Austria) is gratefully acknowledged.

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