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

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Introduction

The biocatalytic regioselective *ortho*- and *para*-carboxylation of phenol derivatives using bicarbonate as CO_2 source is a relatively new development.[1,2] Unfortunately, the downstream-processing of (de)carboxylase reactions is still a challenge and, to the best of our knowledge, typically a subsequent column chromatography is required to remove remaining substrate and further impurities. In this study, the downstream-processing of regioselective enzymatic carboxylation reaction using non-oxidative (de)carboxylase has been investigated. 3,5-Dihydroxytoluol (orcinol) **1** was converted to its corresponding carboxylated benzoic acid derivative 2,6-dihydroxy-4-methylbenzoic acid (DHMBA) **2** at larger scale (Fig. 1).[3]

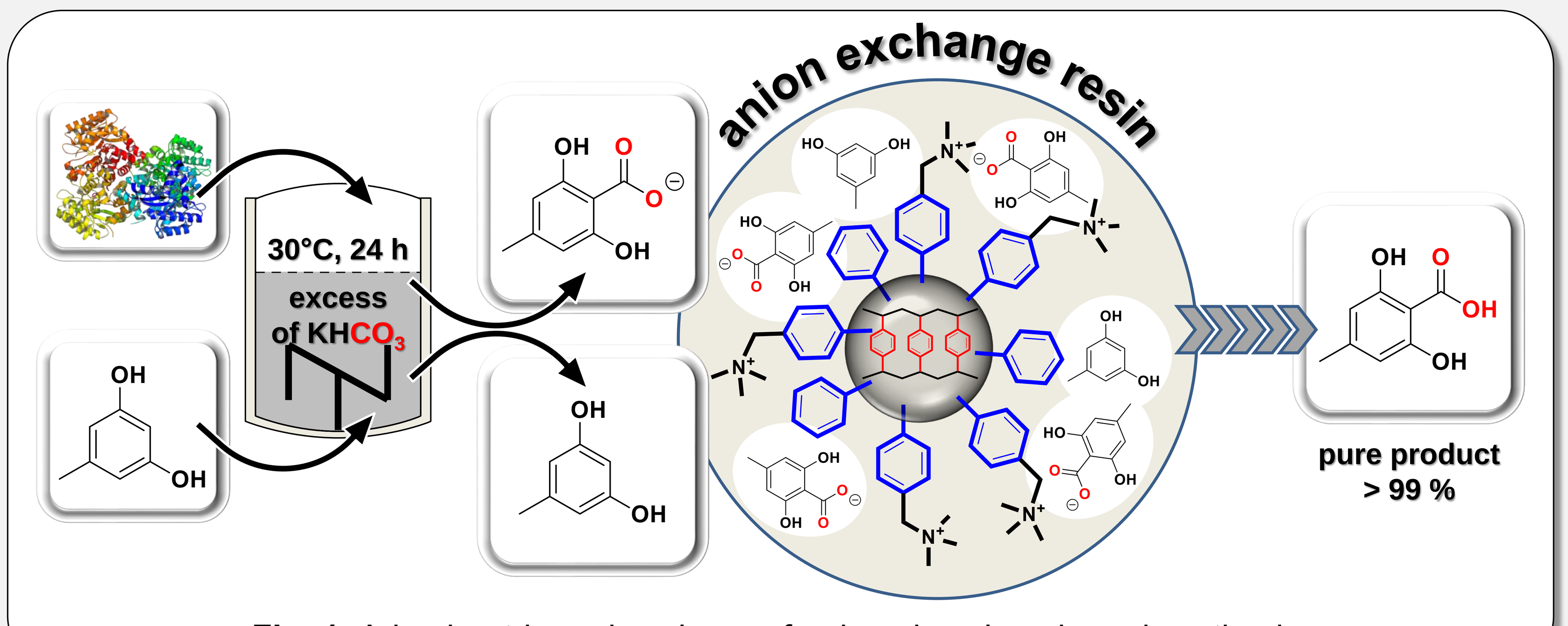


Fig. 1: Adsorbent-based scale-up of a decarboxylase-based synthesis.

Results and Discussion

biocatalytic carboxylation reaction

- investigated *ortho*-carboxylation reaction (Fig. 2) can be considered as the enzymatic pendant of the well-known Kolbe-Schmitt reaction
- orcinol **1** was directly converted to its corresponding carboxylated product 2,6-dihydroxy-4-methylbenzoic acid (DHMBA) **2**
- lyophilized *E. coli* cells containing the corresponding overexpressed enzyme were used as catalyst and studied in respect of substrate concentration (Fig. 3A)
- in strong contrast to the original industrial applied Kolbe-Schmitt process
  - significantly less harsh reaction conditions are required with a temperature of only 30 °C
  - potassium bicarbonate at or near atmospheric pressure as a CO_2-source was used
- sufficient conversions even at low substrate concentrations were achieved (60% conversion with 50 mmol/L **1** and 3 mol/L bicarbonate ($KHCO_3$) after 24 hours (Fig. 3B/C))

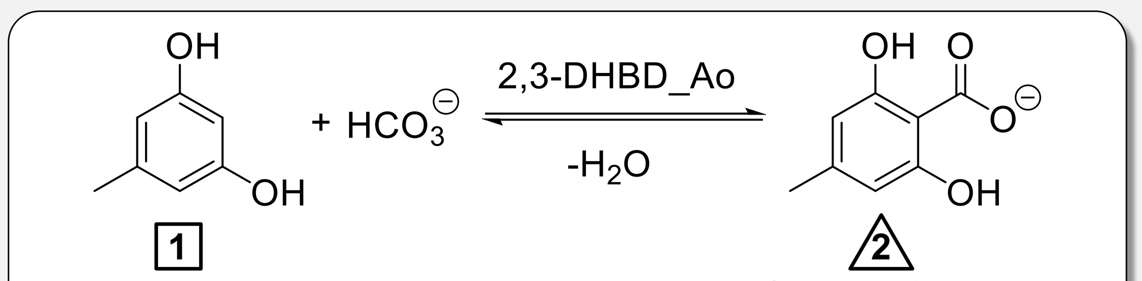
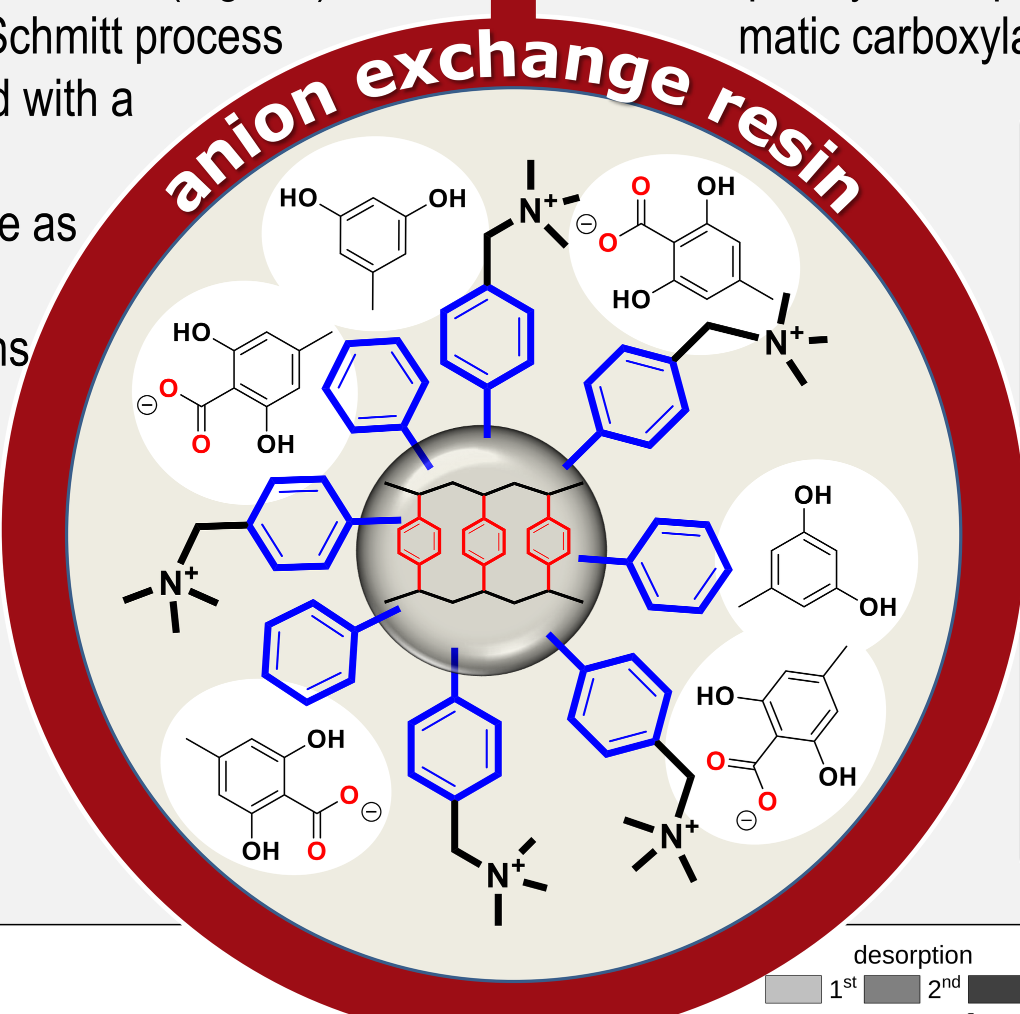


Fig. 2: Biocatalytic carboxylation of orcinol **1** (60 mg lyophilized cells, 50 mmol/L **1**, 3 mol/L $KHCO_3$).



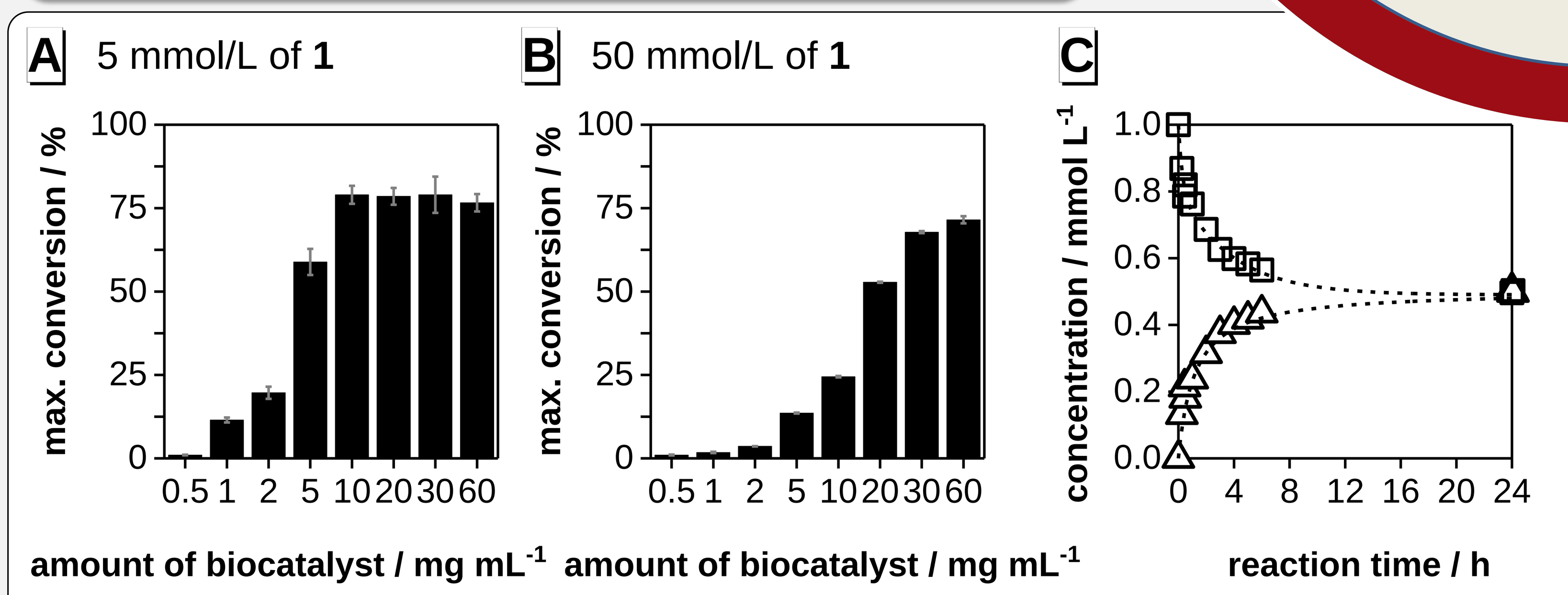


Fig. 3: Biocatalytic carboxylation of **1** (C: 60 mg lyophilized cells, 50 mmol/L **1**, 3 mol/L $KHCO_3$).

adsorbent based downstream-processing

- typically, significant excess of 3 mol/L $KHCO_3$ needs to be removed via acidification (full loss of this reagent and thus lowering atom efficiency)
- different anion–exchange and non ionic adsorbents resins were screened for their ability to remove both product **2** and remaining substrate **1** from a test solution (Table 1)
- subsequently desorption leading to an efficient downstream-processing for enzymatic carboxylation reaction obtaining pure product **2** (Fig 4)

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

applied adsorber	DHMBA adsorption/%			orcinol 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

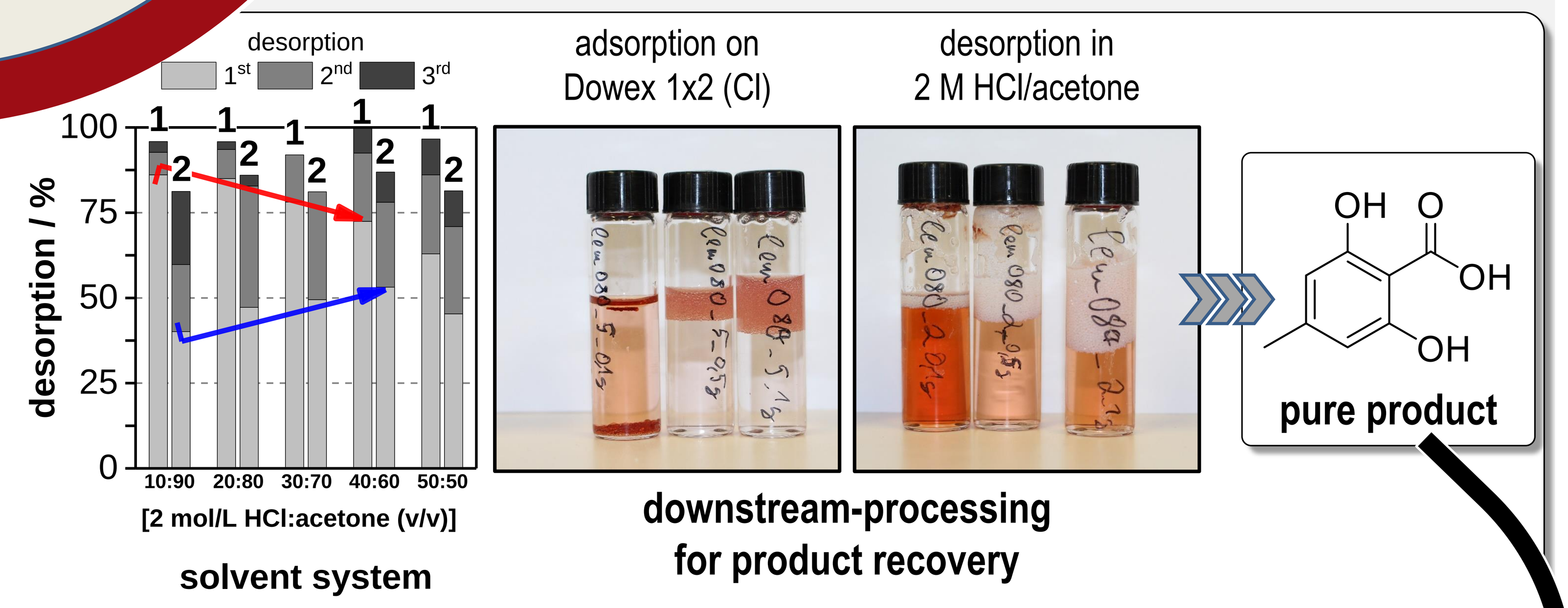
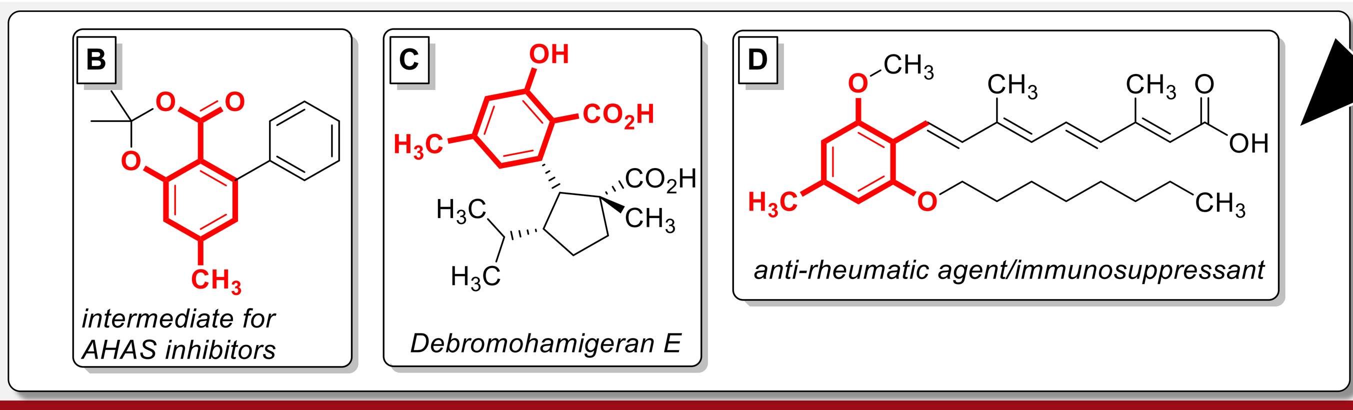


Fig. 4: Desorption and efficient downstream-processing for enzymatic carboxylation reaction.

Summary and Outlook

- classical downstream concepts for carboxylic acid from fermentation processes include mainly the use of adsorption, extraction or crystallization
- to the best of our knowledge, first time that an effective recovery method of aromatic hydroxy acids for enzymatic carboxylation reaction is reported in scientific literature
- product is intermediate for important pharmaceuticals (see right hand side)



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