

# 1            *Separation in Biorefineries by Liquid Phase*

## 2            *Adsorption: Itaconic Acid as Case Study*

3    *Kai Schute,<sup>a</sup>    Chaline Detoni,<sup>a,b</sup>    Anna Kann,<sup>a</sup>    Oliver Jung,<sup>a</sup>    Regina Palkovits,<sup>a\*</sup>    and*  
4    *Marcus Rose<sup>a\*</sup>*

5    a) Lehrstuhl für Heterogene Katalyse und Technische Chemie, Institut für Technische Chemie  
6    und Makromolekulare Chemie, RWTH Aachen University, Worringerweg 2, D-52074 Aachen,  
7    Germany.

8    b) Chemistry School, Federal University of Rio de Janeiro, RJ 21945-970 Brazil.

9    \* palkovits@itmc.rwth-aachen.de, rose@itmc.rwth-aachen.de

10

### 11    **KEYWORDS**

12    Itaconic acid, selective liquid phase adsorption, fermentation solution, porous hydrophobic  
13    polymer, adsorbent

14

### 15    **ABSTRACT**

16    In biorefinery processes often the downstream processing is the technological bottleneck  
17    for an overall high efficiency. Based on recent developments the selective liquid phase  
18    adsorption applying highly hydrophobic porous materials opened up new opportunities  
19    for process development. In this contribution, the efficiency of selective liquid phase

1 adsorption is demonstrated for the separation and purification of itaconic acid from  
2 aqueous solutions for the first time. A wide range of different adsorbents was screened,  
3 revealing the surface polarity as well as textural properties as critical parameters for their  
4 performance. Adsorption from mixed solutions of itaconic acid and glucose exhibited  
5 extraordinary high selectivities for adsorbents with highly hydrophobic surfaces, especially  
6 certain activated carbons and hypercrosslinked polymers. Evaluation of the pH dependence  
7 showed that the respective molecular species of itaconic acid/itaconate has a major impact on the  
8 adsorption performance. Additionally, experiments on a continuously operated fixed-bed  
9 adsorber were carried out and the desorption behavior was evaluated. Overall, the technical  
10 feasibility of the selective adsorptive removal of itaconic acid from aqueous solutions with  
11 hydrophobic adsorbents is demonstrated as a model system for an alternative technology to  
12 conventional separation strategies in biorefinery concepts.

13

14

## 1 INTRODUCTION

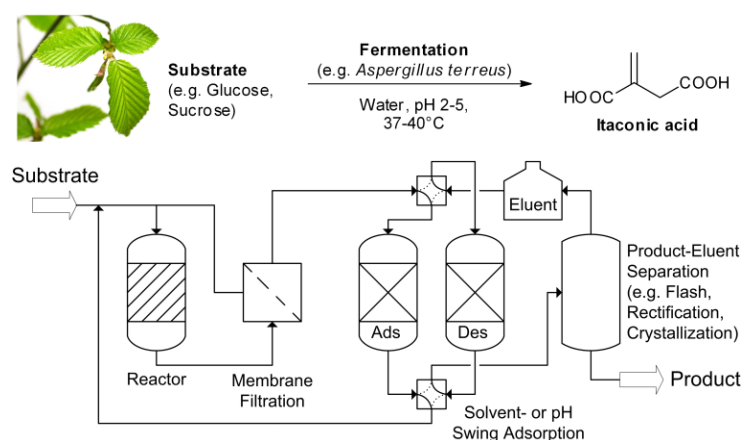
2 The development of efficient separation and purification technologies is one of the major  
3 challenges in the improvement and development of biorefinery processes.<sup>1-2</sup> Mainly  
4 water-based reaction mixtures require separation technologies that are less typical  
5 compared to conventional large-scale industrial processes. Especially for all products with  
6 vapour pressures below and boiling points above that of water conventional thermal  
7 separation processes are not an option. Thus, the main focus is on alternative technologies  
8 such as extraction with organic solvents and crystallization for the final recovery and  
9 purification of solid products. In the last years a lot of effort was put in the development  
10 of efficient membrane filtration processes ranging from micro- and ultrafiltration over  
11 nanofiltration to pervaporation and electrodialysis.<sup>3-4</sup> For carboxylic acids produced by  
12 fermentation ion exchange methods have been reported, mainly applying solid, polymeric  
13 ion exchange resins.<sup>5</sup> Also the physical adsorption from aqueous solutions based on the  
14 weaker van der Waals interactions has been proposed and investigated for various  
15 biogenic platform chemicals such as levulinic acid,<sup>6</sup> hydroxymethyl furfural,<sup>7-8</sup> furfural,<sup>9</sup>  
16 succinic acid<sup>10-11</sup> and many more. As adsorbents mainly commercially available porous  
17 materials such as zeolites and activated carbons have been investigated. Typically the  
18 adsorption capacity depends on the available specific surface area. However, the  
19 selectivity determines the overall process efficiency especially for *in situ* product  
20 recovery with residual substrate and by-products in the aqueous reaction or fermentation  
21 mixture. In the above mentioned reports one trend is obvious: the selectivity for the  
22 physical adsorption of the least polar compound from a polar mixture is increased when  
23 using an adsorbent with a low surface polarity and thus, a high hydrophobicity. This

1 behavior is typically obtained using well-known and commercially available adsorbents  
2 such as activated carbons and highly hydrophobic zeolites. However, the performance of  
3 these adsorbents is by far not sufficient to justify the further development of liquid phase  
4 adsorption as a large scale separation process.

5 In recent years the field of porous materials has made tremendous progress by the  
6 development of novel classes of materials with tunable structures and surface properties.  
7 Especially organic framework compounds and polymer networks with a high permanent  
8 porosity have to be emphasized.<sup>12</sup> These materials exhibit specific surface areas of  
9 typically 1000-2000 m<sup>2</sup>g<sup>-1</sup>, some even exceeding these values. Due to the organic  
10 building blocks which form the pore walls they possess the highest hydrophobicity ever  
11 reported for porous materials and definitely exceeding conventional activated carbons and  
12 all kind of inorganic adsorbents. In recent studies by our group such adsorbents, in  
13 particular hypercrosslinked polymers (HCP),<sup>13</sup> were found to exhibit exceptional  
14 selectivities, e. g. in the adsorption of the biogenic platform compound hydroxymethyl  
15 furfural over fructose from aqueous solutions.<sup>14-15</sup> On this basis the selective liquid phase  
16 adsorption of all kinds of biogenic platform chemicals especially from aqueous  
17 fermentation broths seems feasible. Thus, detailed investigations on the adsorption  
18 performance with a focus on the selectivity for these novel adsorbents are essential to  
19 elucidate its potential as separation process in biorefineries.

20 A suitable model compound and highly promising platform chemical is itaconic acid  
21 (IA).<sup>16-17</sup> It is an unsaturated dicarboxylic acid with a wide range of (potential)  
22 applications and a focus on the use as co-monomer in synthetic rubber or to  
23 replace petrochemistry-based monomers such as acrylic or methacrylic acid.<sup>16-18</sup> Itaconic

1 acid is obtained by the fermentation of glucose in the presence of microorganisms such as  
 2 *Aspergillus terreus* (Scheme 1). The biotechnological production of approximately  
 3 80.000 t/a is mainly concentrated in China, but also USA, Japan and France have  
 4 production facilities.<sup>17</sup> However, the current market of products derived from itaconic  
 5 acid is still rather small and only a few applications are yet established due to the high  
 6 production costs. A major factor of the costs is the product recovery. In a typical process  
 7 the fermentation broth is filtered, the solution is concentrated by evaporation of water  
 8 followed by several crystallization steps to obtain the pure itaconic acid.<sup>17</sup> Recent  
 9 attempts to lower the amount of required energy and the overall costs were based on new  
 10 membrane separation technologies such as electrodialysis<sup>19</sup> and reverse-flow  
 11 diafiltration<sup>20-21</sup> to increase the product concentration for the subsequent crystallization  
 12 step to obtain the pure itaconic acid as product. Very recently, also ion exchange using  
 13 solid ion exchange resins has been described, although this method suffers from rather  
 14 low capacities while neither selectivities for real mixtures nor the desorption behavior  
 15 have been described.<sup>22</sup>



16  
 17 **Scheme 1.** Reaction scheme of the fermentative production of itaconic acid (top) and simplified process  
 18 flow diagram for a solvent- or pH-swing adsorption process for the separation of itaconic acid and similar  
 19 biorefinery products.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17

A further alternative for the separation of itaconic acid is the physical adsorption from the aqueous fermentation broth. In case of adsorbents with sufficient selectivity and the possibility to desorb with an organic solvent or an aqueous solution with a different pH value an *in situ* product recovery based on solvent- or pH-swing adsorption is feasible (Scheme 1). So far, the adsorption of itaconic acid was mentioned only once in literature using a low-surface area alumina with negligible capacity as adsorbent.<sup>23</sup>

In this study we investigate and evaluate the selective separation of itaconic acid from model solutions as well as fermentation broths by liquid phase adsorption on highly hydrophobic adsorbents such as the hypercrosslinked polymers as mentioned above. Batch and continuous experiments are carried out and modelled accordingly to quantify the thermodynamic and kinetic behavior. Also the desorption properties are investigated in detail. Overall, the separation of itaconic acid by liquid phase adsorption using novel adsorbents is evaluated. Its potential as downstream process in biorefineries in general is demonstrated.

# 1 EXPERIMENTAL SECTION

## 2 **Materials**

3 D-glucose (>99 %) was purchased from Merck and itaconic acid (>99 %) from Aldrich  
4 Chemistry. Activated carbons A Supra EUR, Darco G60, SX1G and RX3 Extra were provided  
5 by Cabot, Norit activated carbon. NaY zeolites CBV 500 ( $\text{SiO}_2/\text{Al}_2\text{O}_3 = 5.2$ ) and CBV 712  
6 ( $\text{SiO}_2/\text{Al}_2\text{O}_3 = 12$ ) were provided by Zeolyst International and were calcined at 500 °C for 6 h  
7 ( $10\text{ °C min}^{-1}$ ) prior to the experiments. Zeolite 13X was supplied by UOP<sup>TM</sup> adsorbents and was  
8 calcined at 500 °C for 12 h ( $10\text{ °C min}^{-1}$ ). Zeolites Na-CZF and Na-LSX were supplied by  
9 Clariant and were calcined at 500 °C for 12 h ( $10\text{ °C min}^{-1}$ ). Amberlite® IRA-96 free base was  
10 purchased from Fluka Analytical. 4,4'-bis(chloromethyl)-1,1'-biphenyl (BCMBP, 95 %),  
11 acetone ( $\geq 99.8\%$ ) and 1,2-dichloroethane (DCE, 99.8 %) were provided by Sigma Aldrich®.  
12 Iron trichlorid hexahydrate ( $\text{FeCl}_3$ ,  $\geq 98\%$ ) was supplied by Merck. Methanol (99.8 %) was  
13 provided by ChemSolute from Th. Geyer. Purified water from a Werner Reinstwasser system  
14 ( $>18.2\text{ M}\Omega\text{cm}$ ) was used to prepare the aqueous solutions. All chemicals were used without  
15 further purification.

16

## 17 **Methods**

18 *Synthesis of hypercrosslinked polymer (HCP):* The synthesis of the hypercrosslinked  
19 polymer (HCP) was carried out as described by Detoni et al. 2014.<sup>14</sup>

20 *Adsorption experiments:* Adsorption experiments were carried out in a water  
21 shaking bath equipment with controlled temperature. Prior to all experiments, the  
22 adsorbents (except the resin Amberlite) were dried in oven at 100 °C over night. In  
23 typical adsorption experiments 0.04 g of adsorbent were added to 2 g of solution and kept

1 shaking at isothermal conditions for 1 h. The adsorption time was kept constant to ensure  
2 a state of equilibrium which was confirmed by previous kinetic evaluation. At the end of  
3 each experiment itaconic acid and glucose loaded adsorbents were filtered and resulting  
4 solutions were analyzed by HPLC. A screening of adsorbents was performed to evaluate  
5 which characteristics were relevant for the separation of glucose and itaconic acid by  
6 adsorption. Therefore, an equimolar solution ( $0.25 \text{ mmol g}^{-1}$ ) of glucose and itaconic acid  
7 in millipore water was prepared. The adsorption was performed at  $20 \text{ }^{\circ}\text{C}$ . Adsorption  
8 isotherms for HCP and the activated carbon A Supra EUR were measured for itaconic  
9 acid and glucose in single solutions and in competitive adsorption in a concentration  
10 range of  $0.005\text{-}0.5 \text{ mmol g}^{-1}$ . Additionally, isotherms were measured at  $50 \text{ }^{\circ}\text{C}$  and  $80 \text{ }^{\circ}\text{C}$   
11 for HCP in mixed solutions of glucose and itaconic acid within the same concentration  
12 range. Further adsorption experiments were carried out based on real fermentation  
13 solutions provided by the Chair of Biochemical Process Engineering at RWTH Aachen  
14 University. A summary of the composition of the fermentation solution is given in  
15 Table S1. HCP and A Supra EUR were tested with the real solution by adding  $0.1 \text{ g}$  of  
16 adsorbent to  $5 \text{ g}$  of solution and shaking at  $20 \text{ }^{\circ}\text{C}$  for one hour. The total uptake of  
17 itaconic acid and glucose in the equilibrium on a specific mass of the adsorbent  $q_{eq}$  was  
18 calculated using equation (1). The uptake is calculated based on the change in  
19 concentration of the substance in the liquid phase during adsorption whereas volume  
20 changes were not considered.

21

$$q_{eq} = \frac{(c_0 - c_{eq}) \cdot m}{W} \quad (1)$$



1  $c_0$  and  $c_{eq}$  are the liquid-phase concentrations of the substrates at initial and at  
2 equilibrium, respectively.  $m$  is the total mass of solution and  $W$  the mass of dry adsorbent  
3 used.

4 *pH evaluation:* The solutions of itaconic acid without an additional acid/base or a  
5 buffer typically showed pH values of around 3.7. For the pH-dependent measurements  
6 different pH-values were adjusted by adding 35-37 % HCl or a 4 M solution of NaOH.  
7 The experiments were carried out in 8 mL vials, containing 4 g of solution, to which  
8 0.08 g of pre-dried adsorbent was added. After being sealed, samples were kept in a state  
9 of constant agitation at 20 °C or 40 °C for 1 h in a water shaking bath. After 1 h adsorbent  
10 was removed by filtering. The final concentrations in the solutions were determined via  
11 HPLC analysis. pH values were measured before and after adsorption using a pH meter.

12 *HPLC analysis:* The solid adsorbents were filtered from the resulting solutions.  
13 The filtrate was analyzed using an High Performance Liquid Chromatography (HPLC)  
14 system (LC-20AD, Shimadzu) equipped with a refractive index detector as well as an  
15 organic acid resin column (Polystyrene-divinylbenzene-copolymer (PS-DVB), CS-  
16 Chromatography) maintained at 50 °C. 5  $\mu$ L sample injections were made and a mobile  
17 phase of 2 mM trifluoroacetic acid in water was used with a flow rate of 1 mL min<sup>-1</sup>.

18 *Adsorbents characterization:* N<sub>2</sub> physisorption measurements were carried out for  
19 all adsorbents in order to determine textural properties of the materials. The adsorbents  
20 were pre-treated prior to analysis by evacuation for 12 h at different temperatures  
21 depending on the material (120 °C – HCP, 300 °C – zeolites, 150 °C – activated carbons,  
22 60 °C – Amberlite). Nitrogen physisorption isotherms were measured at -196 °C using a

1 Micromeritics ASAP 2000 (activated carbon, zeolites and Amberlite) and a  
2 Quantachrome Autosorb IQ (HCP) equipment. Surface areas and pore volumes were  
3 calculated from the adsorption branch of collected isotherms.

4 *Continuous adsorption:* The continuous studies were conducted in a fixed bed  
5 column set up filled with the adsorbent A Supra EUR at 20 °C. The adsorptive aqueous  
6 solution of either itaconic acid or itaconic acid/glucose, in general with a concentration of  
7 12.8 mmol g<sup>-1</sup>, was pumped through the fixed bed by a HPLC pump and a feed flow rate  
8 on 0.6 mL min<sup>-1</sup>. For desorption studies ethanol was used with a similar flow rate. A  
9 typical fixed bed contained 0.3 g of adsorbent depending on its powder density. Sample  
10 fraction were taken each minute and analyzed by HPLC analysis. The obtained  
11 breakthrough curves were modelled using the Thomas-model and the Microsoft Excel®  
12 non-linear solver function and maximizing the coefficient of determination. Desorption  
13 degrees were determined using the area under the desorption curve with the integration  
14 function of Origin®.

15

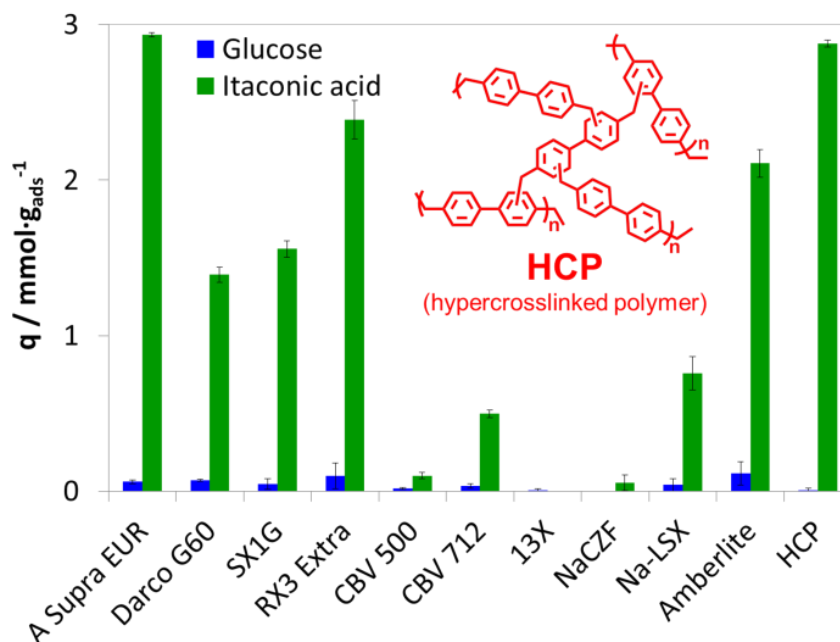
16

# 1 RESULTS AND DISCUSSION

## 2 Adsorbent screening

3 An initial adsorbent screening was carried out by using mixed solute aqueous solutions  
4 containing itaconic acid and glucose in equimolar concentrations. A set of different  
5 activated carbons, zeolites, basic ion exchange resins and a hypercrosslinked polymer  
6 (HCP) were tested.

7



8

9 **Figure 1.** Adsorbed amounts of itaconic acid and glucose in competitive adsorption at 20 °C from  
10 equimolar solutions ( $0.25 \text{ mmol g}^{-1}$ ). Error bars correspond to the standard deviation at each point  
11 determined from three independent reproduction experiments. The inset shows the hydrophobic porous  
12 polymer (HCP).

13

14 The corresponding textural properties are presented in Table S1. Besides the general  
15 textural properties the polarity has shown to be of importance especially regarding the

1 selectivity. Based on the previous work of Detoni et al. a polarity increase can be noted in  
2 the following order: HCP << Activated carbon (AC) < Zeolite.<sup>14</sup> For the herein reported  
3 adsorbent screening at 20 °C a slightly different behavior was observed (Figure 1). The  
4 activated carbons A Supra EUR, RX3 Extra as well as the HCP showed high adsorption  
5 uptakes up to 2.9 mmol g<sup>-1</sup> for itaconic acid whereas the zeolite-based materials only gave  
6 poor adsorption capacities most probably due to their high surface polarity. For the basic  
7 ion exchange resin with a significantly lower specific surface area a significant uptake is  
8 observed that probably corresponds to the affinity due to acid/base interactions. The  
9 glucose uptake appears to be very low for nearly all of the screened materials. However,  
10 ACs and the HCP exhibit the lowest uptakes and thus, the highest selectivity, which  
11 corresponds to the non-polar surface of these materials. This effect can be attributed to the  
12 highly hydrophobic nature of activated carbons with a low degree of surface  
13 functionalization as well as porous polymers that consist of aromatic building blocks with  
14 very low polarity. In summary, the adsorption of itaconic acid in a solution containing  
15 competitive compounds is highly preferred in terms of capacity as well as selectivity for  
16 non-polar adsorbents with high specific surface areas. Thus, in the following the  
17 adsorption/desorption performance is investigated in detail using HCP and the  
18 hydrophobic activated carbon A Supra EUR.

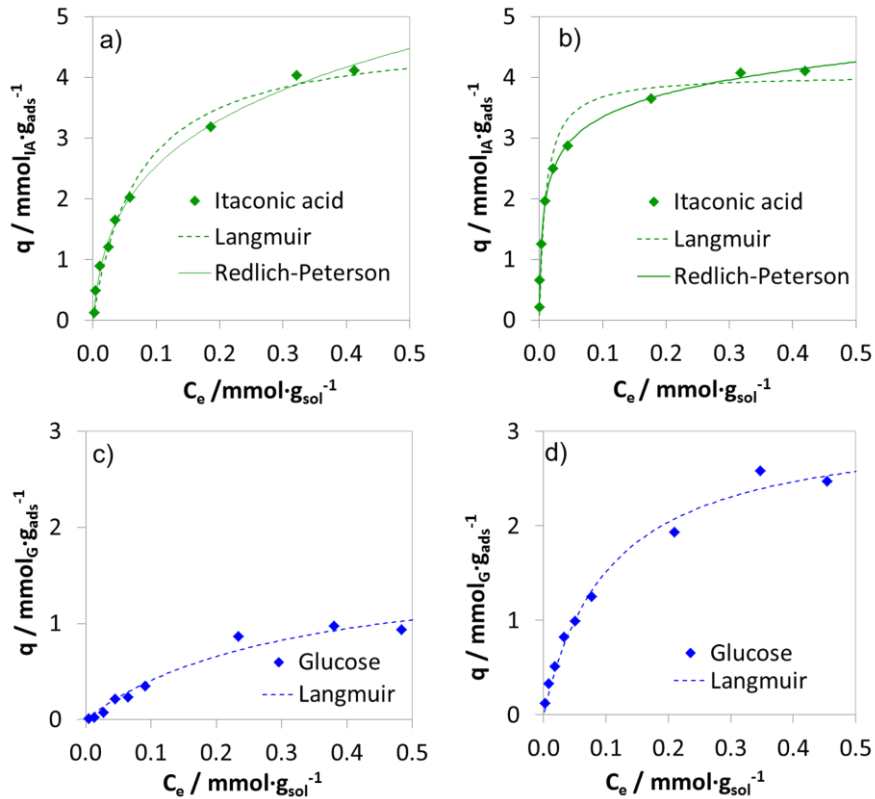
19

### 20 **Single solute isotherms of the non-polar adsorbents**

21 Single solute adsorption isotherms of itaconic acid and glucose were measured for HCP  
22 and A Supra EUR (Figure 2). The isotherms were fitted with three isotherm models:  
23 Langmuir, Freundlich and Redlich-Peterson<sup>24</sup> (see ESI). The adsorption isotherms of

1 itaconic acid (Figure 2a and 2b) correlated better with the Redlich-Peterson model for  
2 both tested adsorbents.

3



4

5 **Figure 2.** Single solute adsorption isotherms of itaconic acid and glucose at 20 °C adsorbed on HCP (a+c)  
6 and A Supra EUR (b+d).

7

8 This isotherm model covers a wide range of concentrations combining Langmuir and  
9 Freundlich characteristics.<sup>25-27</sup> It is in good agreement with the Langmuir model for low  
10 solute concentrations, which is expressed by the exponent  $\beta$  in the range of 0.7-0.8  
11 (Table S3). However, at moderate to high solute concentrations the Redlich-Peterson  
12 model fits better to the experimental data than the Langmuir model indicating that the  
13 general assumption of monolayer formation and saturation is less suitable for this

1 complex system. Due to high affinity between adsorbents and adsorbates the values of the  
2 dimensionless equilibrium factor  $R_L$  derived from the Langmuir model as a function of  
3 the initial concentration (Figure S2) are between zero and one indicating a favoured  
4 adsorption of itaconic acid on both adsorbents in the evaluated range of concentrations.  
5 Comparing Langmuir constants for both adsorbents,  $K_L$  is ten times higher for  
6 A Supra EUR than for HCP, indicating a higher affinity of itaconic acid towards the  
7 activated carbon. This assumption is fortified by higher itaconic acid adsorption even at  
8 smaller concentrations. The maximum adsorption of itaconic acid ( $\sim 4 \text{ mmol g}^{-1}$ )  
9 approximates the saturation uptake under equilibrium conditions and is similar for both  
10 adsorbents.

11 The adsorption isotherms of glucose on HCP and A Supra EUR (Figure 2c and 2d) are  
12 well described using the Langmuir model. Adsorption isotherms parameters and  
13 correlation coefficients are shown in Table S4. Overall, glucose is adsorbed by both  
14 adsorbents in the same concentration range although with an overall lower capacity  
15 compared to itaconic acid. In comparison to itaconic acid adsorption, inferior correlation  
16 coefficients were obtained for both adsorbents. These deviations are related to high  
17 concentration of polar groups in the glucose molecule resulting in a higher affinity of  
18 glucose for the aqueous phase presumable by hydrogen bond interactions of the free  
19 hydroxyl groups and the water molecules.<sup>7</sup> Consequently, the adsorption capacity  $Q_0$  and  
20 the equilibrium constant  $K_L$  are shifted towards lower values compared to itaconic acid.  
21 A Supra EUR is able to adsorb higher amounts of glucose than HCP which could be  
22 related to the presence of some polar functional groups on the surface of the activated  
23 carbon that present affinity towards the glucose molecules. Additionally, a maximum

1 uptake was observed for an equilibrium concentration of  $0.4 \text{ mmol g}^{-1}$  followed by  
2 decreasing uptakes with further increasing solution concentrations. This identifies excess  
3 isotherms which describe liquid phase adsorption as a competitive process between solute  
4 and solvent. For the calculation of the glucose uptake, it was assumed that water as  
5 solvent is not adsorbed which is valid for low concentrations and the preferential  
6 adsorption of glucose. For increasing concentrations, this assumption is not applicable  
7 anymore. A maximum uptake followed by decreasing glucose adsorption expresses an  
8 increasing affinity between water molecules and the adsorbent, even though the overall  
9 adsorption is still more favorable for glucose.<sup>7</sup> The coverage of the available surface area  
10 was calculated (Figure S5). Comparing both adsorbents, A Supra EUR shows a higher  
11 coverage with increasing concentration compared to HCP. This observation coincides  
12 with the higher total uptake of glucose on A Supra EUR. In addition, it indicates the  
13 lower polarity of the HCP surface area compared to the activated carbon.

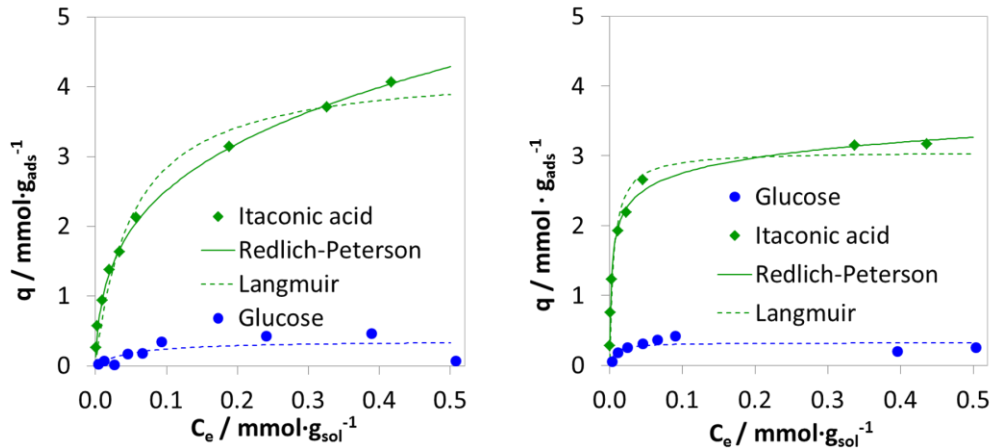
14

### 15 **Competitive adsorption of itaconic acid and glucose**

16 Although knowledge on the single solute equilibrium adsorption is of interest and useful  
17 for the comparison of different adsorbents it is much more important to investigate the co-  
18 adsorption from mixed solute solutions to gain information about the selectivity. Thus,  
19 adsorption isotherms of itaconic acid and glucose in a competitive system and using HCP  
20 as well as A Supra EUR as adsorbents were measured (Figure 3, Table S5). As for the  
21 single solute isotherms the adsorption of itaconic acid is better described by applying the  
22 Redlich-Peterson model. Comparing itaconic acid adsorption in single- and in multi-

1 component solution, the exponent  $\beta$  is between 0.7 and 0.9 for each evaluated system  
2 indicating a convergence to the Langmuir isotherm.

3



4

5 **Figure 3.** Competitive adsorption isotherms of itaconic acid and glucose from mixed solutions on HCP  
6 (left) and A Supra EUR (right) at 20°C.

7

8 The obtained values for  $R_L$  (Figure S6) prove the favourable adsorption of itaconic acid  
9 on HCP and A Supra EUR even in the presence of glucose. Moreover, it is rather  
10 unanticipated that the adsorbed amount of glucose is decreased while the adsorbed  
11 amount of itaconic acid decreases only negligibly when compared to the single solute  
12 isotherms as the single solute isotherm shows a glucose uptake three times higher than for  
13 the multicomponent adsorption. The obtained Langmuir constants  $K_L$  for itaconic acid  
14 and glucose are shifted e.g. from 14.05 to 19.61, when compared to the values obtained  
15 for single solutions. The reason for the higher adsorption amounts could be related to the  
16 competitive interactions in the multicomponent system. Apparently, the main reason  
17 therefor is the significant difference in polarity of the adsorbate molecules, the  
18 hydrophobic surface and water as highly polar solvent. This effect is more pronounced for



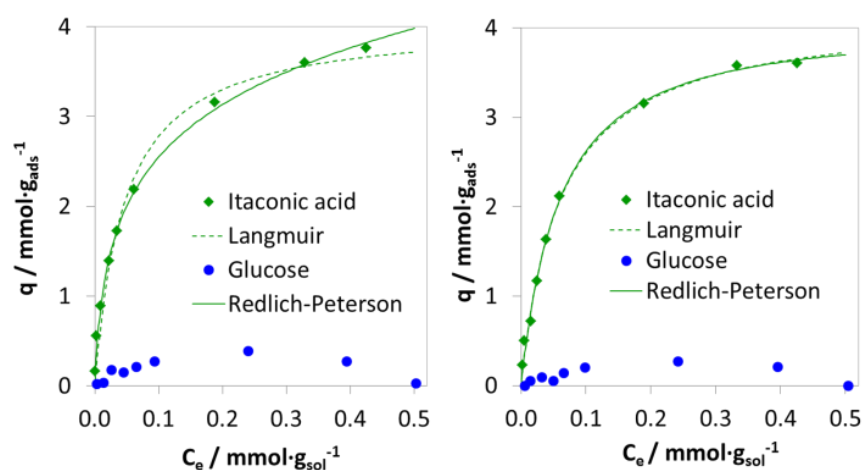
1 the more hydrophobic HCP in comparison to the activated carbon with a slightly more  
2 polar pore surface due to a low degree of surface functionalization, e.g. with oxygenated  
3 groups.

4 For small concentrations of itaconic acid, the Langmuir model is in accordance with  
5 the experimental data for both adsorbents and overlaps with the Redlich-Peterson model.  
6 However, with increasing concentrations, the Redlich-Peterson model exhibits a better fit  
7 for the itaconic acid adsorption. Considering the isotherms of glucose at small  
8 concentrations, isotherms follow the Langmuir model for both adsorbents. Nevertheless,  
9 for raising glucose concentrations the excess isotherms reach a maximum uptake as  
10 already observed in the single solute solution indicating an even decreased affinity for the  
11 adsorption under competitive conditions with itaconic acid in water.

12 As for the single solute isotherms the surface coverage of HCP and A Supra EUR with  
13 itaconic acid was calculated (Figure S7). For A Supra EUR, nearly the same coverage  
14 compared to the single component adsorption was obtained for itaconic acid. For HCP,  
15 the coverage slightly increased in comparison to the single component adsorption. These  
16 results could be associated to an increased affinity of itaconic acid for HCP in the  
17 presence of glucose due to competitive interactions with the surface. Additionally, it  
18 should be noted that the overall uptake for glucose is dramatically lower as for the single  
19 solute adsorption for both adsorbents, while the overall uptake increases. Apparently, the  
20 presence of a less polar solute leads to the suppression of glucose on the surface resulting  
21 in an increased selectivity towards the adsorption of itaconic acid.

22 **Temperature influence on the itaconic acid adsorption**

1 In terms of selectivity combined with outstanding adsorption capacities for itaconic acid,  
 2 HCP appears to be the ideal adsorbent at 20 °C. Since fermentative itaconic acid  
 3 production occurs at a higher temperature, the adsorption behavior was investigated at  
 4 50 °C and 80 °C in order to evaluate the temperature influence on the itaconic acid uptake  
 5 and selectivity under equilibrium conditions (Figure 4, Table S6). Overall, a similar trend  
 6 is observed (Figure S8). The isotherms at elevated temperatures are fitted better by the  
 7 Redlich-Peterson model. However, with increasing temperatures the Redlich-Peterson  
 8 model converges to the Langmuir model indicated by the exponent  $\beta$  close to one.



10  
 11 **Figure 4.** Competitive adsorption isotherms of itaconic acid and glucose on HCP at 50 °C (left) and 80 °C  
 12 (right).

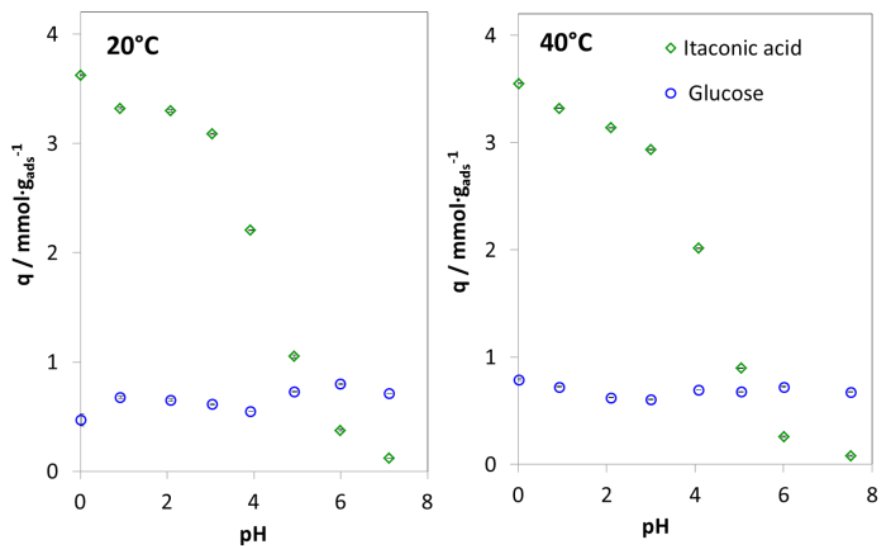
13  
 14 Considering an exothermic adsorption process, the Langmuir constant  $K_L$  and adsorption  
 15 capacity  $Q_0$  are expected to decrease with increasing temperature. This effect could be  
 16 recognised whereas only a slight difference is observed. In general, the influence of  
 17 temperature on the adsorption is negligible which agrees with the previous results of the  
 18 adsorbent screening. The modelled adsorption isotherms confirm the convergence to

1 Langmuir for an increasing temperature since Redlich-Peterson and Langmuir models  
2 overlap at 80 °C (Figure 4). Comparing the maximum uptakes, higher temperatures  
3 resulted in slightly less total itaconic acid and glucose adsorption. The exothermic  
4 behavior of the adsorption is further represented in terms of surface coverage (Figure S9).  
5 Since Langmuir constants and adsorption uptakes decrease with increasing temperatures,  
6 the coverage is shifted towards lower values. However, only slight changes in coverage  
7 and in total uptake were observed. In summary, the influence of temperature is rather  
8 insignificant, and thus, enables operation in a broad temperature range.

9

#### 10 **pH influence on the itaconic acid adsorption**

11 Itaconic acid is a dicarboxylic acid and thus, is able to dissociate twice with pK<sub>a</sub> values of 5.45  
12 and 3.84 (Figure S10).<sup>19</sup> Due to the dissociation behavior of itaconic acid the influence of pH on  
13 its adsorption in the presence of glucose was investigated as the polarity of the adsorptive has a  
14 great influence on the adsorption performance. Furthermore, the pH value needs to be taken into  
15 consideration as fermentative processes may differ in pH. For example, while processes using  
16 *Aspergillus Terreus* work at pH values at ~2, *Ustilago Maydis* requires pH values of ~5 and  
17 thereby mainly produces itaconates. This crucial difference though could have a major impact on  
18 the adsorption performance and needs to be addressed. The influence of the pH was measured in  
19 solutions with the same concentrations used for the screening of the adsorbents (0.25 mmol g<sub>sol</sub><sup>-1</sup>,  
20 Figure 5).



**Figure 5.** Influence of the pH value of the solution in competitive adsorption of itaconic acid and glucose (0.25 mmol g<sub>sol</sub><sup>-1</sup>) on HCP at 20 and 40 °C. Error bars correspond to the standard deviation at each point.

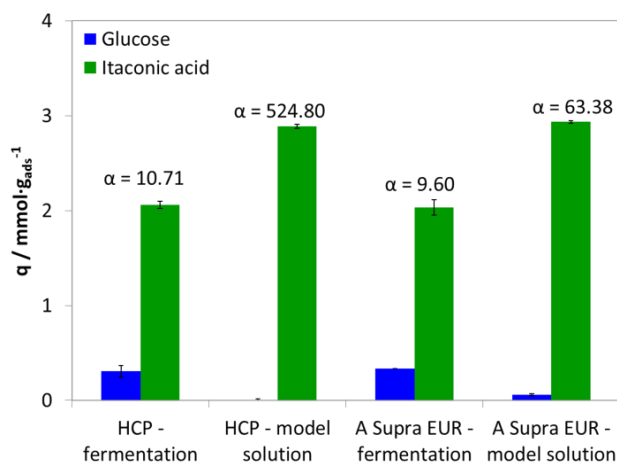
The pH of the solution is able to change the attraction/repulsion forces between adsorbent and itaconic acid/glucose due to changes in the polarity of itaconic acid by shifting the equilibrium towards the mono- or di-itaconate. Under competitive conditions on HCP the itaconic acid uptake was found to decrease significantly when increasing the initial pH of the solution from 0 to 8. The temperature showed only a minor influence. In contrast, the adsorption of glucose remains almost constant upon increase of the pH. Along with an increasing pH-value and the deprotonation, itaconic acid tends to become more polar. It is fully deprotonated at pH=7. Thus, the adsorption behavior correlates directly with the molecules properties, i. e., the polarity that significantly increases upon one- and two-fold deprotonation. With an increasing polarity, itaconic acid is more unlikely to be adsorbed, as the adsorbent is hydrophobic. Moreover, repulsive forces between the molecules increase along with the increasing polarity and therefore decreasing uptakes. This decreasing uptake of itaconic acid is advantageous for the glucose adsorption, as it is no

1 longer competing for active sites as at low pH by the significant difference in polarity.  
 2 This behavior can be observed in the experimental data, indicating that glucose is rather  
 3 unaffected by a changing pH-environment. The influence of temperature is marginal for  
 4 the uptake of itaconic acid as for the glucose over the whole range of concentration. Thus,  
 5 the selectivity decreases significantly upon pH increase. On the other hand, the drastic  
 6 shift of the adsorbed amount by varying the pH value might be considered for a pH-  
 7 dependent desorption of the adsorbed itaconic acid.

8

### 9 Adsorption from real fermentation solutions

10 Itaconic acid is produced by fermentation. Thus, regarding efficient separation  
 11 technologies the performance has to be evaluated not only using model solutions but also  
 12 based on real fermentation solutions to evaluate the influence of other by-products and  
 13 reagents. The performance of HCP as well as of A Supra EUR was investigated  
 14 (Figure 6) based on a real fermentation solution (composition given in Table S2).



15

16 **Figure 6.** Adsorption of itaconic acid and glucose from a fermentation broth and a model solution with the  
 17 corresponding separation factor  $\alpha$  at 20 °C. Error bars correspond to the standard deviation at each point;

18 
$$\alpha = (q_{IA}/q_{Glu}) / (C_{e,IA}/C_{e,Glu}).^{19}$$

1  
2 Overall, the adsorbed amount of itaconic acid is smaller while a slightly higher amount of  
3 glucose is adsorbed compared to the ideal model solutions. The main reason for that is the  
4 presence of some ions that could change the polarity of the solution and thus, increase the  
5 affinity of glucose for the non-polar surface, comparable to the well-known “salting-out”  
6 effect in liquid-liquid extraction. To evaluate the selectivity of the adsorption, the  
7 separation factor  $\alpha$  was calculated as the ratio of itaconic acid to glucose adsorbed  
8 divided by the ratio of itaconic acid and glucose amounts remaining in the solution. The  
9 effect of ions in the adsorption of itaconic acid from a real fermentation solution can be  
10 easier understood considering the separation factor  $\alpha$ . The presence of ions in  
11 fermentation media causes a dramatic decrease in selectivity. The highest selectivity was  
12 found for HCP in the model solution. In the corresponding fermentation solution only 2  
13 and 15 % of the selectivity compared to the model solution are retained for HCP and  
14 A Supra EUR, respectively.

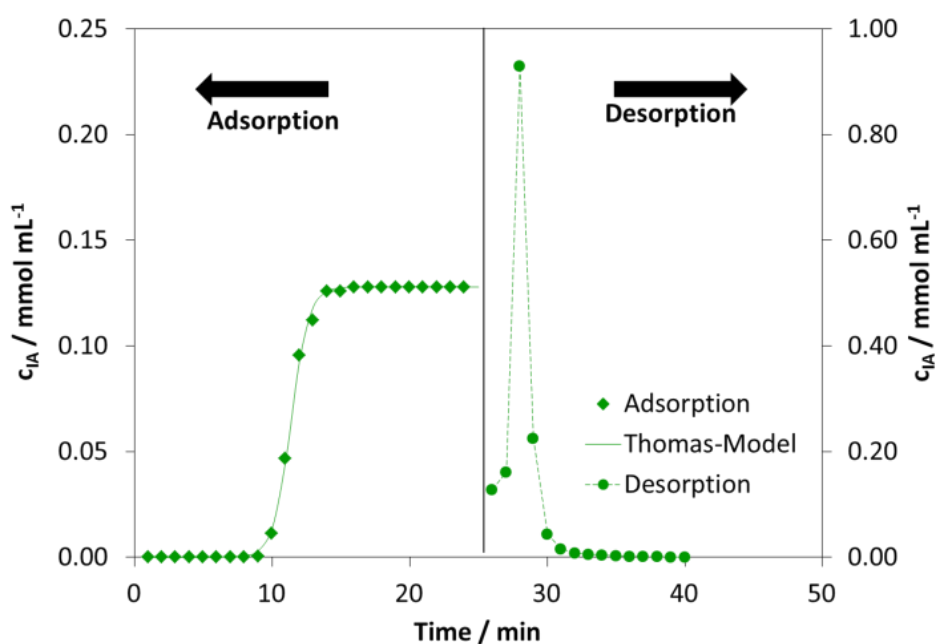
15

#### 16 **Continuous adsorption/desorption on fixed-bed adsorber**

17 Considering an overall adsorption process for purifying itaconic acid from fermentation  
18 broths, continuous process condition need to be evaluated in order to confirm its  
19 applicability. Therefore, breakthrough experiments were carried out in a fixed-bed  
20 adsorber column. The studies primarily focused on the activated carbon A Supra EUR  
21 which performed best in batch adsorption. The HCP could not be evaluated under fixed-  
22 bed conditions, as the material is a fine powder resulting in a high pressure drop.  
23 However, the single solute breakthrough curve of an aqueous itaconic acid solution

1 (Figure 7) exhibits a sharp breakthrough that indicates a comparably small mass transfer  
2 zone in the fixed bed and good mass transfer properties. Nearly 75 % of the solute is  
3 thereby adsorbed before the breakthrough. According to the Thomas model, which is in  
4 good accordance with the experimental data, the overall capacity is  $0.42 \text{ g g}^{-1}$  and  
5 matches with the capacity achieved in the batch study (Table S7). The desorption,  
6 performed with ethanol according to Yang et al.,<sup>28</sup> achieved full recovery of the adsorbed  
7 itaconic acid with a relatively small amount of solvent (here 4.8 mL) resulting in a nearly  
8 two-fold concentrated solution as for the initial aqueous solution.

9



10

11 **Figure 7.** Continuous adsorption and desorption of aqueous itaconic acid solution on A Supra EUR at  
12  $20^\circ\text{C}$ . Ethanol was used as a desorption solvent.

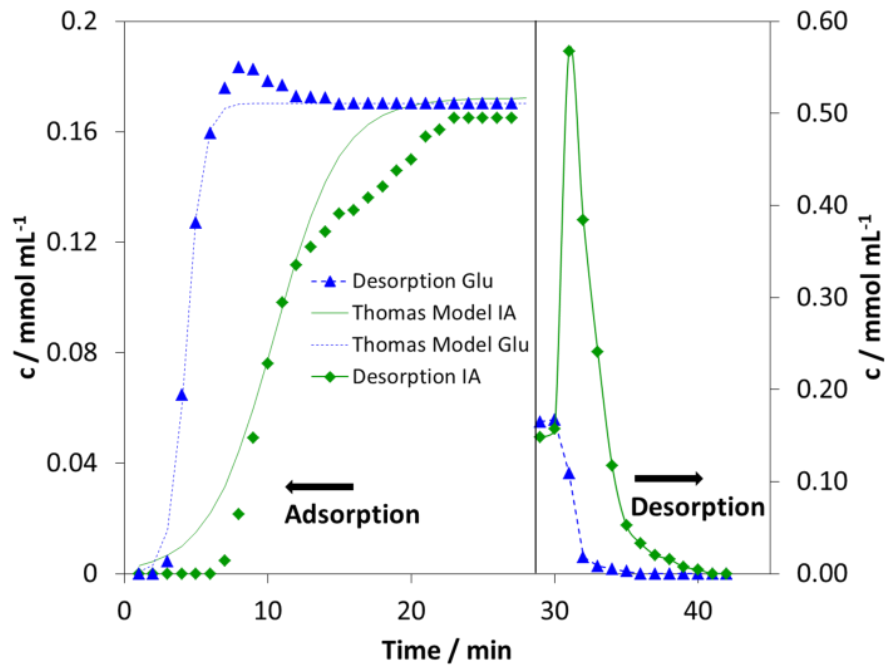
13

14 The competitive adsorption of itaconic acid in the presence of an equimolar amount of  
15 glucose in the fixed-bed is presented in Figure 8. As the glucose capacity is significantly

1 lower for the activated carbon compared to itaconic acid an earlier breakthrough of  
2 glucose is observed compared to the breakthrough of itaconic acid. Thus, adsorption  
3 selectivity towards itaconic acid is present. However, it is noticeable that the Thomas  
4 model is not completely adequate for the competitive adsorption. The experimental data  
5 shows an inflection point in the adsorption curve for itaconic acid. Which results in a  
6 nearly four times lower kinetic constant than for the single solute adsorption (22.5  
7 compared to 93.6; see Table S7). Initially only itaconic acid adsorbs on the adsorbent  
8 surface, leaving a more concentrated glucose solution to pass through the column. This  
9 results in a significantly higher loading of glucose on the surface of the following  
10 unloaded adsorbent due to an increased concentration. For itaconic acid to adsorb, which  
11 is still thermodynamically more favorable, this pre-adsorbed glucose need to desorb first  
12 to expose the surface sites. This leads to an overall lower adsorbent kinetic for itaconic  
13 acid as the desorption needs to be overcome. However, this only occurs after a certain  
14 amount of solution has already passed through, thus, explaining the inflection point. The  
15 glucose adsorption curve interestingly shows a slight over-concentration during a certain  
16 time (7-12 min) additionally proving the desorption of the pre-adsorbed glucose. The  
17 overall adsorbed amounts are comparable the single solute adsorption:  $0.42 \text{ g g}^{-1}$  for  
18 itaconic acid and  $0.19 \text{ g g}^{-1}$  for glucose.

19 If ethanol is used as an eluent, overall a rapid desorption of both components is observed.  
20 Thereby, 100 % of the adsorbed itaconic acid can be recovered with only a minimum  
21 amount of solvent (Figure S7), again leading to a higher concentration compared to the  
22 initial solution. For glucose, 74 % can be recovered in first run, implying a slightly  
23 decreased overall working capacity upon cyclic loading/unloading.





1

2 **Figure 8.** Competitive continuous adsorption and desorption of an equimolar solution (0.17 mmol g<sup>-1</sup>) of

3 itaconic acid (diamonds) and glucose (triangles). Adsorption was carried out from aqueous solutions.

4 Ethanol was used as solvent for the desorption.

5

6

## 1 CONCLUSION

2 This study provides a comprehensive investigation of the separation of itaconic acid and  
3 glucose from aqueous solutions by selective liquid phase adsorption on highly  
4 hydrophobic adsorbents. A broad adsorbent screening proves the necessity of high  
5 specific surface areas in combination with highly hydrophobic surfaces such as in low  
6 functionalized activated carbons or porous polymers. Comparison and modelling of  
7 single- and mixed-solute adsorption isotherms under varying conditions and over a broad  
8 concentration range exhibited synergistic effects of the polarity differences on the  
9 selectivity of the adsorption. It was shown, that a highly selective adsorption of itaconic  
10 acid is only possible at a low pH value with the fully protonated acid. To confirm the  
11 technical applicability, breakthrough curves were measured using a fixed-bed adsorber  
12 column. The experiments revealed a good and selective performance as well as a  
13 quantitative desorption that was achieved using ethanol as eluent. By investigating the  
14 performance in real fermentation solutions in the presence of additional ions it was shown  
15 that the selectivity decreases; an effect that is subjected to future investigations for  
16 improvement of the adsorbents. Regarding the outstanding performance of the porous  
17 polymer HCP, future work needs to deal with shaping of the powdered material for fixed-  
18 bed applications as well as with the investigation and improvement of mass transfer  
19 limitations.

20 In summary, the adsorptive separation of itaconic acid using hydrophobic adsorbents  
21 appears to be a feasible alternative separation technology. Also a good transferability to  
22 other biogenic platform chemicals is expected. Overall, liquid phase adsorption offers  
23 promising opportunities in future process development to overcome the major challenge

1 in biorefineries: the separation from aqueous reaction mixtures and fermentation  
2 solutions.

3

#### 4 **SYMBOLS**

5	$\alpha_{RP}$	Redlich Peterson coefficient [ $\text{g} \cdot \text{mmol}^{-1}$ ]
6	$\beta$	Redlich Peterson coefficient [-]
7	$c_0$	Initial concentration [ $\text{mmol} \cdot \text{g}_{\text{solution}}^{-1}$ ]
8	$c_e$	Equilibrium concentration [ $\text{mmol} \cdot \text{g}_{\text{solution}}^{-1}$ ]
9	$K_F$	Freundlich constant [ $\text{g} \cdot \text{g}^{-1}$ ]
10	$K_L$	Langmuir constant [ $\text{g} \cdot \text{mmol}^{-1}$ ]
11	$K_{RP}$	Redlich Peterson constant [ $\text{g} \cdot \text{g}^{-1}$ ]
12	$K_{Th}$	Kinetic constant in the Thomas model [ $\text{L}_{\text{solution}} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ]
13	$m_{\text{solution}}$	Total mass of solution [g]
14	$q_e$	Adsorbed amount in equilibrium [ $\text{mg} \cdot \text{g}_{\text{adsorbent}}^{-1}$ ]
15	$q_{des}$	Amount desorbed [ $\text{g} \cdot \text{g}_{\text{adsorbent}}^{-1}$ ]
16	$q_{Th}$	Theoretical saturate adsorption capacity in the Thomas model [ $\text{g} \cdot \text{g}_{\text{adsorbent}}^{-1}$ ]
17	$R_L$	Langmuir equilibrium factor [-]
18	$t$	Time [min]
19	$W$	Total mass of adsorbent [g]

20

#### 21 **ACKNOWLEDGEMENT**

22 We thank D. Kreyenschulte and Prof. J. Büchs from Biochemical Process Engineering of  
23 RWTH Aachen University for providing a real fermentation broth for reference

1 experiments. We gratefully acknowledge financial support by the Robert Bosch  
2 Foundation within the Robert Bosch Fellowship for sustainable utilization of renewable  
3 natural resources. This work was also performed as part of the Cluster of Excellence  
4 “Tailor-Made Fuels from Biomass” (TMFB) as well as Seed Funding by the Excellence  
5 Initiative of the German federal and state governments to promote science and research at  
6 German universities. Chaline Detoni thanks the Capes Foundation and Science without  
7 Borders Program (Brazil) for the Postdoctoral scholarship.

8  
9

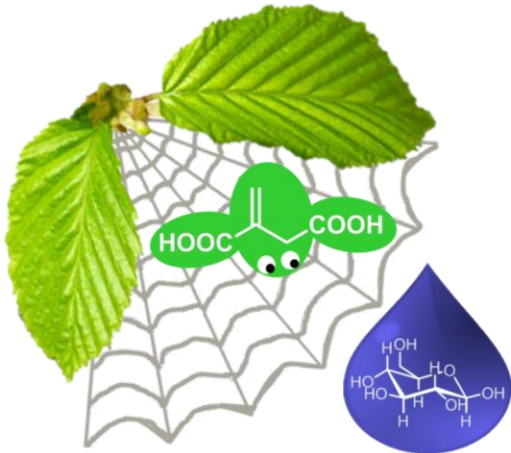
## 10 REFERENCES

- 11 (1) Ramaswamy, S.; Huang, H.-J.; Ramarao, B. V., *Separation and purification technologies*  
12 *in biorefineries*. Wiley-VCH: 2013.
- 13 (2) Huang, H.-J.; Ramaswamy, S.; Tschirner, U. W.; Ramarao, B. V., A review of separation  
14 technologies in current and future biorefineries. *Sep. Purif. Technol.* **2008**, *62*, 1-21.
- 15 (3) Abels, C.; Carstensen, F.; Wessling, M., Membrane processes in biorefinery applications.  
16 *J. Membr. Sci.* **2013**, *444*, 285-317.
- 17 (4) Jiang, L. Y.; Zhu, J. M., Separation technologies for current and future biorefineries -  
18 status and potential of membrane-based separation. *WIREs Energy Environ* **2013**, *2*, 673-690.
- 19 (5) López-Garzón, C. S.; Straathof, A. J. J., Recovery of carboxylic acids produced by  
20 fermentation. *Biotechnol. Adv.* **2014**, *32*, 873-904.
- 21 (6) Liu, B.; Zeng, L.; Ren, Q., Simulation of levulinic acid adsorption in packed beds using  
22 parallel pore/surface diffusion model. *Chem. Eng. Technol.* **2010**, *33* (7), 1146-1152.
- 23 (7) León, M.; Swift, D.; Nikolakis, V.; Vlachos, D. G., Adsorption of the compounds  
24 encountered in monosaccharide dehydration in zeolite beta. *Langmuir* **2013**, *29*, 6597-6605.
- 25 (8) Xiong, R.; León, M.; Nikolakis, V.; Sandler, S. I.; Vlachos, D. G., Adsorption of HMF  
26 from water/DMSO solutions onto hydrophobic zeolites: experiment and simulation.  
27 *ChemSusChem* **2014**, *7*, 236-244.

- 1 (9) Ranjan, R.; Thust, S.; Gounaris, C. E.; Woo, M.; Floudas, C. A.; von Keitz, M.; Valentas,  
2 K. J.; Wei, J.; Tsapatsis, M., Adsorption of fermentation inhibitors from lignocellulosic biomass  
3 hydrolyzates for improved ethanol yield and value-added product recovery. *Microporous*  
4 *Mesoporous Mater.* **2009**, *122*, 143-148.
- 5 (10) Efe, C.; van der Wielen, L. A. M.; Straathof, A. J. J., High silica zeolites as an alternative  
6 to weak base adsorbents in succinic acid recovery. *Ind. Eng. Chem. Res.* **2010**, *49*, 1837-1843.
- 7 (11) Schöwe, N.; Bretz, K.; Hennig, T.; Schlüter, S.; Deerberg, G., Succinic acid removal and  
8 recovery from aqueous solution using hydrotalcite granules: experiments and modeling. *Ind.*  
9 *Eng. Chem. Res.* **2015**, *54*, 1123-1130.
- 10 (12) Slater, A. G.; Cooper, A. I., Function-led design of new porous materials. *Science* **2015**,  
11 *348* (6238), 988-999.
- 12 (13) Xu, S.; Luo, Y.; Tan, B., Recent development of hypercrosslinked microporous organic  
13 polymers. *Macromol. Rapid Commun.* **2013**, *34*, 471-484.
- 14 (14) Detoni, C.; Gierlich, C. H.; Rose, M.; Palkovits, R., Selective liquid phase adsorption of  
15 5-hydroxymethylfurfural on nanoporous hypercrosslinked polymers. *ACS Sustainable Chem.*  
16 *Eng.* **2014**, *2* (10), 2407-2415.
- 17 (15) Schute, K.; Rose, M., Metal-free and scalable synthesis of porous hypercrosslinked  
18 polymers: Towards applications in liquid phase adsorption. *ChemSusChem* **2015**, *8*, 3419-3423.
- 19 (16) Klement, T.; Büchs, J., Itaconic acid - a biotechnological process in change. *Bioresour.*  
20 *Technol.* **2013**, *135*, 422-431.
- 21 (17) Okabe, M.; Lies, D.; Kanamasa, S.; Park, E. Y., Biotechnological production of itaconic  
22 acid and its biosynthesis in *Aspergillus terreus*. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 597-606.
- 23 (18) Willke, D.; Vorlop, K.-D., Biotechnological production of itaconic acid. *Appl. Microbiol.*  
24 *Biotechnol.* **2001**, *56*, 289-295.
- 25 (19) Stodollick, J.; Femmer, R.; Gloede, M.; Melin, T.; Wessling, M., Electrodialysis of  
26 itaconic acid: a short-cut model quantifying the electrical resistance in the over limiting current  
27 density region. *J. Membr. Sci.* **2014**, *453*, 275-281.
- 28 (20) Carstensen, F.; Marx, C.; André, J.; Melin, T.; Wessling, M., Reverse-flow diafiltration  
29 for continuous in situ product recovery. *J. Membr. Sci.* **2012**, *421-422*, 39-50.
- 30 (21) Carstensen, F.; Klement, T.; Büchs, J.; Melin, T.; Wessling, M., Continuous production  
31 and recovery of itaconic acid in a membrane bioreactor. *Bioresour. Technol.* **2013**, *137*, 179-187.

- 1 (22) Magalhaes Jr., A. I.; de Carvalho, J. C.; Ramírez, E. N. M.; Medina, J. D. C.; Soccol, C.  
2 R., Separation of itaconic acid from aqueous solution onto ion-exchange resins. *J. Chem. Eng.*  
3 *Data* **2016**, DOI: 10.1021/acs.jced.5b00620.
- 4 (23) Gulicovski, J. J.; Cerovic, L. S.; Milonjic, S. K.; Popovic, I. G., Adsorption of itaconic  
5 acid from aqueous solutions onto alumina. *J. Serb. Chem. Soc.* **2008**, 73 (8-9), 825-834.
- 6 (24) Redlich, O.; Peterson, D. L., A useful adsorption isotherm. *J. Phys. Chem.* **1959**, 63,  
7 1024-1024.
- 8 (25) Foo, K. Y.; Hameed, B. H., *Chem. Eng. J.* **2010**, 159, 2-10.
- 9 (26) Ng, J. C. Y.; Cheung, W. H.; McKay, G., *J. Colloid Interface Sci.* **2002**, 255, 64-74.
- 10 (27) Yabushita, M.; Kobayashi, H.; Hasegawa, J.; Hara, K.; Fukuoka, A., *ChemSusChem*  
11 **2014**, 7, 1443-1450.
- 12 (28) Yang, W.; Hu, Y.; Chen, Z.; X., J.; Wang, J.; Wang, R., Solubility of itaconic acid in  
13 different organic solvents: Experimental measurement and thermodynamic modeling. *Fluid*  
14 *Phase Equilib.* **2012**, 314, 180-184.
- 15  
16

1 **For Table of Contents Use Only:**



2

3

4 *Title:*

5 Separation in Biorefineries by Liquid Phase Adsorption: Itaconic Acid as Case Study

6

7 *Authors:*

8 Kai Schute, Chaline Detoni, Anna Kann, Oliver Jung, Regina Palkovits, Marcus Rose

9

10 *Synopsis:*

11 Biomass-based chemicals are adsorbed from aqueous mixtures with high selectivity.

12 Thus, efficient separation strategies for future biorefinery processing are enabled.