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Substrate and water adsorption phenomena in a gas/solid enzymatic reactor

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Keywords:	gas/solid biocatalysis, thermodynamic activity, alcohol dehydrogenase, adsorption, adsorption isotherm



Research Article**Substrate and water adsorption phenomena in a gas/solid enzymatic reactor**

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10

Keywords: gas/solid biocatalysis, thermodynamic activity, alcohol dehydrogenase, adsorption, adsorption isotherm

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20

Abbreviations

2-prop	2-propanol
AcPh	acetophenone
25 BSA	Bovine serum albumin
IU	international unit
LBADH	<i>Lactobacillus brevis</i> alcohol dehydrogenase
RH	relative humidity
SEM	scanning electron microscopy
30 YADH	yeast alcohol dehydrogenase

Abstract

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13 35 The adsorption of water and substrates to dry deposited alcohol
14 dehydrogenase from *Lactobacillus brevis* (LBADH) is studied in a continuous
15 enzymatic gas/solid reactor. This work is aiming at obtaining a deeper and
16 more thorough understanding of the enzyme microenvironment in the
17 gas/solid system of acetophenone reduction with concomitant oxidation of 2-
18 propanol. Extensive water adsorption studies showed that the effect of
19 sucrose in the enzyme preparation on the water adsorption isotherm is
20 significant for water activities exceeding 0.5 and reaches a factor 2 with
21 respect to bead mass at $a_w=0.9$. Significant hysteresis during water
22 desorption is identified, resulting in up to $0.6 \text{ mg}_{\text{water}}/\text{mg}_{\text{protein}}$, for lyophilized
23 enzyme preparation and up to $10 \text{ mg}_{\text{water}}/\text{mg}_{\text{protein}}$ for deposited enzyme
24 preparation. The adsorption of the substrates is quantified here for the first
25 time. Whereas the adsorption of the main substrate, acetophenone, may
26 reach a significant level of up to $6 \text{ mg}/\text{mg}_{\text{protein}}$, at an acetophenone activity of
27 0.32, the secondary substrate, 2-propanol is not adsorbed at a detectable
28 degree. The presence of water leads to a decrease of the adsorbed amount
29 of acetophenone, by approximately 25%, at a water activity of 0.54.
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Introduction

55 Gas/solid biocatalysis has been shown to have various potential applications,
12 including fine chemical synthesis [1] and toxic pollutant degradation [2].
13 Furthermore gas/solid biocatalysis constitutes an efficient tool to understand
14 the effects of the microenvironment of the biocatalyst on its activity and
15 stability [3].
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60 The thermodynamic activity is used to quantify the availability of water and
substrates to the biocatalyst in non-conventional media. In the case of
organic solvents, the calculation of the thermodynamic activities of the
compounds requires the prior knowledge of the activity coefficients. On the
contrary, for gas/solid systems, where the gas phase can be assumed as
ideal, the thermodynamic activity a_i is calculated in a more straightforward
manner. It is given by $a_i = \frac{p_i}{p_i^s}$, where p_i is the partial pressure of a compound
 i and p_i^s is the saturation pressure of this compound at the operating
temperature. The partial pressures of the different compounds of the reaction
mixture can be adjusted separately. Thus, independent control of the
thermodynamic activity of each compound present in the microenvironment of
the biocatalyst is achieved. This constitutes the major advantage of the
gas/solid system, as a fundamental research tool [3].

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6 The water activity (a_w) specifically is a key parameter in non-conventional
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8 biocatalysis. The role of water during biocatalysis is multifaceted. Water is a
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10 75 substrate or product of a hydrolytic or synthetic reaction, respectively. For any
11
12 reaction water can act either directly on the structure of the enzyme or as
13
14 solvent facilitating the diffusion of the reactants. The water adsorption
15
16 isotherm is used to describe the state of the water present in the
17
18 microenvironment of the biocatalyst [4]. The dependency of enzyme activity
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21 80 on the water activity in the gas/solid systems has already been demonstrated
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23 [5-8].
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27 A typical water adsorption isotherm (Fig.1) shows two characteristic break
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29 points, A and B. Point A corresponds to the water activity level below which
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31 the water is highly structured and forms the first hydration layer (water
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33 85 monolayer) of the dried enzyme. The intercept with the y-axis at $a_w=0$
34
35 corresponds to the tightly-bound water amount. Between points A and B, the
36
37 water content changes in a linear fashion with increased a_w , resulting in the
38
39 formation of subsequent hydration layers. Point B denotes the water activity
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41 level above which free water appears [4].
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46 90 Like water, any other compound present will compete for adsorption sites on
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48 the biocatalyst [2,3]. While previous studies have focused exclusively on the
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50 adsorption of water equilibrating with the solid phase and the resulting effect
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52 on the enzyme activity and stability, there are no studies so far dedicated to
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54 the adsorption of substrates to the enzyme. There have been studies
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6 95 addressing the substrate adsorption [9] or the possible product adsorption
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8 [10], without further quantification. In an effort to fill this gap, the present work
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10 focuses on the investigation of the adsorption of substrates on the biocatalyst,
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12 taking place in parallel or in competition to the water adsorption.
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16 In order to be able to interpret the results, a well characterized reaction [11]
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18 100 was chosen: the conversion of acetophenone, catalyzed by the *Lactobacillus*
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20 *brevis* alcohol dehydrogenase (LBADH) deposited onto non-porous glass
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22 carriers together with the cofactor NADPH. In order to overcome the
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24 limitations posed by the batch measuring methods used in the past [12-14]
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26 and to mimic the conditions of the gas/solid system, the adsorption
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29 105 measurements in our study are performed in a continuous gas/solid system,
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31 as proposed previously [2].
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35 Initially, the water adsorption on the enzyme preparation is quantified
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37 indicating the effect of sucrose on the adsorption isotherm. Moreover, the
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39 adsorption of the two reaction substrates, namely acetophenone and 2-
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41 110 propanol, is investigated and quantified. The competitive adsorption, with
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43 respect to the water presence influence on the substrates adsorption, is also
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45 studied.
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53 115 **2 Materials and Methods**

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2.1 Enzyme

Purified and lyophilized wild type LBADH expressed in *E. coli* was obtained from Liliya Kulishova, Institute of Molecular Enzyme Technology (IMET) at Research Center Jülich according to B. Riebel, 1996 (PhD thesis, Heinrich-Heine Universität Düsseldorf, Germany). The resulting lyophilized enzyme had a concentration of 0.4 mg_{protein}/mg_{solid}, the rest being salts resulting from the buffers (10 mM triethanolamine (TEA) adjusted to pH 7 using HCl and 1 mM MgCl₂) used during the purification process, and a specific activity of 88 IU/mg_{protein}.

2.2 Chemicals

Acetophenone and 2-propanol were of >98% purity and purchased from Fluka (Germany). All other chemicals were of analytical grade and obtained from Roth (Germany). Bradford reagent and BSA were obtained from Bio-Rad (USA).

2.3 Deposition procedure

Applying a variation of the deposition procedure described in [15], 170 IU of lyophilized enzyme, sucrose (5 times enzyme mass) and NADPH (12 times molar enzyme amount), were dissolved in 1 mL phosphate buffer (I=50 mM and pH 7, resulting from KH₂PO₄ and Na₂HPO₄ solutions) and mixed together

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6 with 500 mg washed with distilled water and dried glass beads 0.25-0.3 mm
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8 (Sartorius, Germany) for 30 minutes in a rotary mixer (RMSW, Welabo,
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10 Germany) at 4 °C. The mixture was dried at 4 °C in a desiccator, containing
11 140 Silica Gel Orange 2-5 mm (Carl Roth GmbH, Germany), with a vacuum pump
12
13 (CVC 200II, Vacuubrand, Germany) at 300 mbar for 4 hours and successively
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15 at 40 mbar until dry, and enzyme was partly deposited to the carriers' surface,
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17 while some amount was lost on the immobilization vessel walls. The enzyme
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22 145 preparations were stored at 4 °C.
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27 **2.4 Protein loading determination**

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29 In order to determine the amount of protein immobilized on the carriers a
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31 Bonde test was carried out [16]. An amount of 10 mg of beads with
32
33 immobilized enzyme were weighted into a cuvette and 800 µL TEA buffer as
34 150 well as 200 µL Bradford reagent were added to the beads and mixed
35
36 thoroughly. After incubation of 5 min and settling of the beads, the
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38 absorbance of the solution was measured at 465 nm by means of a UV-VIS
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40 spectrophotometer (UNIKON 922, Kontron Instruments, Italy). The protein
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42 amount immobilized on the beads was calculated using a BSA calibration
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45 155 curve.
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53 **2.5 Water adsorption measurement unit**

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6 The measurement of the water adsorbed by the deposited enzyme
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8 160 preparation was performed in a water adsorption unit (Fig.2) which has an
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10 operation principle very similar to that of the gas/solid reactor [17]. Prior to
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12 this measurement, the enzyme preparation was dried by means of a pure N₂
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14 stream until a totally dried enzyme preparation was obtained. Two dried, by
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16 means of a humidity trap (MT200-2, Agilent Technologies, Germany),
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19 165 nitrogen streams were defined by mass flow controllers (F201C-FB-11-V,
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21 Bronkhorst Mättig GmbH, Germany) and directed to a thermo stated unit
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23 (WTB, Binder GmbH, Germany). One of the two streams was equilibrated
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25 with water in two successive water flasks. This stream was then mixed in a
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27 mixing chamber with the second stream of pure nitrogen to generate a
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31 170 mixture with total volumetric flow rate of 15 mL/min. This gas mixture was first
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33 directed through the bypass of the packed bed, until a constant humidity level
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35 was measured by a humidity sensor (D07P-EE22, E+E Elektronik GmbH,
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37 Germany). Then, the mixture was directed through the packed bed,
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39 containing a specific amount of immobilized enzyme preparation (19.8 mg
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43 175 and 120 mg lyophilized and immobilized enzyme, respectively, packed
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45 between two glass wool layers), by means of two 2-3-way valves (6604,
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47 Bürkert, GmbH, Germany). The lowering of the water content in the mixture
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49 due to its adsorption by the packed bed was monitored by the humidity
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51 sensor. The adsorption isotherm was generated by repeating the
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55 180 aforementioned procedure in a stepwise manner for humidity levels from
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10%RH to 90%RH. The desorption isotherm was acquired by the reverse procedure, i.e. by decreasing the humidity level stepwise from 90%RH to 10%RH. The operation of the water adsorption unit and data retrieval was achieved using a LabView (National Instruments, Texas, USA) program. The cumulative errors involved in the water adsorption measurement did not exceed 1% for the measurement of the lyophilized enzyme and 15% for the immobilized preparation.

The water adsorption was modeled using the Brunauer-Emmett-Teller (BET) [18] gas adsorption equation:

$$\frac{a_w}{(1-a_w) \cdot M} = \frac{1}{M_0 \cdot C} + \frac{a_w \cdot (C-1)}{M_0 \cdot C} \quad \text{Eq.1}$$

where M is the moisture content (in %w/w dry solid), M_0 is the monolayer water coverage, a_w is the water activity and C a constant. From the linear regression of the plot of $a_w/((1-a_w)M)$ versus a_w , the theoretical monolayer water coverage can be calculated:

$$M_0 = \frac{1}{(S + I)} \quad \text{Eq.2}$$

where S is the slope and I is the y-intercept [19].

2.6 Enzymatic gas/solid reactor

The adsorption measurements of the two reaction substrates, acetophenone and 2-propanol, were conducted in the gas/solid reactor [17]. During the

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6 adsorption experiments no reaction took place. This was achieved due to the
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8 absence of water in the adsorption measurements of individual substrates. In
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10 the case of competitive adsorption studies, with water present, the enzyme
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12 preparation was thermally treated before use (30 min at 105°C).
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15 205 A gas mixture with a volumetric flow rate of 15 mL/min, unless otherwise
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17 stated and a defined substrate thermodynamic activity was generated and
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19 equilibrated through the bypass. Subsequently, the mixture was directed
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21 through the reactor tube containing the deposited enzyme preparation (120
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23 mg and 565 mg for acetophenone and 2-propanol adsorption studies,
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27 210 respectively, unless otherwise stated). The retention of the compound in the
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29 bed due to adsorption was monitored using an online GC and the adsorbed
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31 amount was integrated according to the trapezium rule. The procedure was
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33 repeated for stepwise increased substrate activities in the gas mixture. The
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35 substrates adsorption measurement involved cumulative negative errors of up
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39 215 to 10%.
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43 **2.7 Online gas chromatography**

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46 The outlet of the reactor was directly connected to an online gas
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48 chromatograph (Fisons GC 8000, S+H Analytik, Germany). Sampling was
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51 220 performed via a six-way Valco valve (VICI AG International, Switzerland)
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53 maintained at 150°C. The gas chromatograph was equipped with an FID
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55 detector, maintained at 250°C and a split/split less injector, maintained at
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6 200 °C. The separation was realized through a CP-WAX 52CB (50m x
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8 0.25mm x 0.2µm) GC column (Agilent Technologies, Germany) at the
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10 225 following temperature program: 2 min at 60 °C, 40 °C/min up to 100 °C,
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13 60 °C/min up to 220 °C and 3 min at 220 °C.
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For Peer Review

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3 Results and discussion

3.1 Water adsorption to lyophilized enzyme

Initially, the water adsorption to lyophilized LBADH was studied and the water adsorption isotherm (Fig.3) was obtained. The water adsorption isotherm follows a pattern resembling the BET isotherm. Despite the fact that the assumptions of the BET theory (for non-polar adsorbate and inert surfaces) are not valid for the protein-water system studied, the model is employed, without conferring the physical meaning to the respective hydration parameters calculated.

Using the linear regression of the BET equation, the amount of water adsorbed, corresponding to the monolayer water coverage of the BET theory, was calculated to $0.0865 \text{ mg}_{\text{water}}(\text{mg}_{\text{dry protein}})^{-1}$. This value is in the same order of magnitude as the one reported for pharmaceutical proteins [19] and various other proteins [20].

The water adsorption isotherm of lyophilized LBADH differs significantly from the one of the yeast alcohol dehydrogenase (YADH) reported in previous studies: water adsorption to LBADH follows the pattern of the BET adsorption isotherm, whereas to YADH follows the Huttig isotherm pattern [2]. LBADH also has much higher amounts of water adsorbed at water activity levels

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6 above 0.7, whereas the calculated amount corresponding to the monolayer
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8 water coverage, according to BET theory, is significantly lower than that of
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10 YADH ($0.35 \text{ mg}_{\text{water}}/\text{mg}_{\text{protein}}$) [2]. The water adsorption isotherm of the
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12 lyophilized enzyme powder depicts the capacity of the specific enzyme to
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15 270 adsorb water and depends on the amino acid composition of the protein and
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17 the polar side chains of the amino acid residues [12,18] as well as the salts
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19 used during the lyophilization of the enzyme, in this case in an amount of
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21 60% w/w. However, it provides no further information on the enzyme behavior
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23 in the gas/solid system. For this reason the adsorption behavior of the
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27 275 deposited enzyme preparation was further investigated.

3.2 Water adsorption by the deposited enzyme

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34 The adsorption isotherm of the total deposited LBADH preparation (carriers
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36 with deposited enzyme, containing salts and sucrose) was obtained in
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39 280 replicate (Fig.4). The amount of adsorbed water is referred to as amount of
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41 water per protein amount, for facilitating the representation. Nevertheless the
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43 amounts measured represent the cumulative adsorbed water by the
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45 immobilized preparation, including the enzyme-carriers, sucrose and salts
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47 used during the enzyme purification and immobilization. These components
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51 285 influence the water adsorption very strongly [21]. A subtraction of the
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53 adsorption isotherm of plain carriers, in order to eliminate their effect, was not
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6 considered appropriate due to the unknown distribution of the deposited
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8 enzyme on the carriers' surface.
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11 The close proximity of the two isotherms indicates that the water adsorption
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13 290 measurement is reproducible, with a maximal error of 1%. Similar to the
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15 lyophilized LBADH, the adsorption isotherm also follows the pattern of the
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17 BET model. The water activity level above which water ceases being strongly
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19 adsorbed by the immobilized preparation (point B, Fig.1) was identified to be
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21 slightly higher than 0.5. For LBADH, it has been previously shown that it is
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23 preferable to operate the reaction system at water activities exceeding 0.5 in
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25 295 order to achieve increased conversion in the gas/solid reactor [15]. This
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27 implies that higher conversion in the reaction system is actually achieved at a
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29 water activity level where only weaker adsorptive interactions with water
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31 occur.
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38 **3.3 Effect of sucrose on water adsorption**

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41 The beneficial effect of sucrose on the stability of the LBADH during the
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43 deposition process as well as on the storage and operational stability of the
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45 enzyme preparation has been shown previously [11, 15]. The effect of
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47 sucrose on the water adsorption to the deposited enzyme preparation has
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49 305 been, therefore, investigated here (Fig.5). For water activity levels exceeding
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51 the value of 0.5, the presence of sucrose in the enzyme preparation resulted
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53 in an increased amount of water adsorbed per unit of enzyme preparation
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6 (Fig.5A). The hygroscopic nature of sucrose is responsible for the attraction
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8 310 of more water.

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10 When comparing the two enzyme preparations, with and without sucrose,
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12 with respect to water adsorbed per unit of protein, the two curves were
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14 inverted (Fig.5B). This is due to the lower protein loading of the sucrose-free
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16 material, during the deposition process (protein loading of 0.00074
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18 $\text{mg}_{\text{protein}}/\text{mg}_{\text{beads}}$), compared to the protein loading achieved in the case of
19
20 315 $\text{mg}_{\text{protein}}/\text{mg}_{\text{beads}}$), compared to the protein loading achieved in the case of
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22 sucrose containing material (protein loading of 0.00275 $\text{mg}_{\text{protein}}/\text{mg}_{\text{beads}}$). As a
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24 result in the case of sucrose being present, more protein “sees” less water.
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27 28 29 **3.4 Hysteresis**

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31 320 The fact that the amount of water adsorbed by proteins when the equilibrium
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33 is approached from the “wet” side is higher than the amount of water
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35 adsorbed when the equilibrium is approached by the “dry” side has already
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37 been demonstrated [22]. This hysteresis has already been observed [2,13,22-
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39 24] and was therefore compared in the current study between the lyophilized
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43 325 and the deposited enzyme preparation.

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45 The adsorption and desorption isotherms do not coincide for the lyophilized
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47 enzyme (Fig.6).
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50 The phenomenon of hysteresis is thought to occur due to alterations of the
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52 intra- and inter-molecular interactions of protein molecules during the
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55 330 hydration process [12,25]. Hysteresis has also been attributed to the
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6 formation of hydrates during the protein hydration process. These hydrates
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8 persist at lower humidity levels during the desorption process [22]. In the case
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10 of LBADH it was found that water could not be desorbed entirely, even after
11
12 drying the protein for many hours with anhydrous N₂ (data not shown). An
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14
15 335 unexpectedly high amount of irreversibly bound water of 0.6 mg_{water}/mg_{protein}
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17 was measured. This may be attributed partly to the protein itself and partly to
18
19 the TEA buffer contained in the lyophilized enzyme (60% w/w). Observation
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21 of lyophilized protein particles that have adsorbed water revealed formation of
22
23 agglomerates that appeared rigid and probably able to retain water.
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27 340 Regarding the deposited enzyme preparation, both with and without sucrose,
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29 the hysteresis was even more prominent (Fig.7), amounting in a difference
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31 between adsorption and desorption isotherm of up to 7 mg_{water}/mg_{protein}, for
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33 the enzyme deposited with sucrose and 12.5 mg_{water}/mg_{protein} for the one
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35 without sucrose.
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39 345 The methodology followed for the construction of the adsorption and
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41 desorption isotherms, involving the summation or subtraction of the adsorbed
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43 or desorbed respectively water amounts, on each activity level, caused error
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45 magnification. This was less pronounced for the adsorption isotherm, where
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47 the largest error at highest water activities amounted to less than 2%.
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51 350 However, in desorption, the errors increased predominantly at lower water
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53 activities and that led to an approximately 15% error in the determination of
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6 the irreversibly bound water. This error, though large, does not change the
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8 overall shape of the adsorption and desorption isotherms.
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11 In order to gain insight into the causes of hysteresis, SEM pictures of the
12
13 355 enzyme preparations were taken. The glass carriers with the enzyme coating
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15 were observed before and after measuring the water adsorption (Fig.8). The
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17 enzyme distribution on the carriers' surface was dramatically modified during
18
19 the process. The initially smooth layer of enzyme (Fig.8A and Fig.8C) was
20
21 lost as the enzyme seemed to have been partly washed away from the carrier
22
23 surface. Material bridges appeared between the individual carriers (Fig.8B)
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25 360 and clear areas on the carriers' surface were revealed (Fig.8D). This
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27 suggests that the biocatalyst is leached from the enzyme preparation to the
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29 glass wool layers.
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34 This is supported by the protein loading of the deposited preparation that was
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36 365 reduced to an approximately 30% lower value compared to that of the initial
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38 protein loading of the preparation. In the case of sucrose being absent during
39
40 the immobilization the reduction of the protein loading was even more
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42 pronounced, reaching a level of around 45%. It appears that sucrose acts as
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44 a stabilizer for the attached enzyme on the carriers' surface.
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50 **3.5 Acetophenone adsorption to the packed bed with deposited enzyme**

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52 So far, substrate adsorption in solid/gas biocatalysis has been widely
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54 neglected. Therefore, the effect of the enzyme on the adsorption of
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6 acetophenone by the packed bed was here studied using dynamic
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8 375 experiments (Fig.9). After turning the flow from the bypass to the packed bed,
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10 the partial pressure of acetophenone dropped as a result of its adsorption by
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12 the bed and a negative peak was observed over hours. In a theoretical
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14 prediction the residence and response time of the substrate (acetophenone or
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16 2-propanol) at the outlet of the reactor was estimated to be in the range of a
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20 380 few seconds.

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22 It was found that the amount of the adsorbed acetophenone to the carriers
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24 when enzyme was deposited on the surface was 35% higher compared to the
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26 adsorbed amount to the plain glass carriers. The acetophenone adsorption
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28 isotherm was measured within the acetophenone activity range relevant to
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31 385 the operation of the gas/solid reactor ($a_{AcPh}=0.05-0.32$) (Fig.10). Following a
32
33 similar practice as in the case of water adsorption by the deposited enzyme
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35 preparation, the amount of adsorbed acetophenone is referred to as amount
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37 of acetophenone per protein amount, for facilitating the representation.

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41 Errors involved in the acetophenone adsorption measurements led mainly to
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43 390 underestimation of the adsorbed acetophenone amount (due to limited tailing)
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45 that summed up to 10%, at the highest acetophenone activities.

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48 The amount of acetophenone adsorbed by the immobilized enzyme
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50 preparation is very low for acetophenone activities below 0.1. Above this
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52 activity level it shows an almost linear increase. By comparing the adsorption
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55 395 isotherms of acetophenone and water (Fig.4) for the same range of activities
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6 it is clear that: (a) the pattern of the two isotherms is different; unlike the water
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8 adsorption isotherm, the acetophenone adsorption isotherm does not follow
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10 the BET pattern and (b) the mass of adsorbed acetophenone is higher than
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12 the mass of adsorbed water at the same levels of thermodynamic activity. At
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15 400 the highest acetophenone activity level tested, namely $a_{\text{AcPh}}=0.32$, the
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17 adsorbed acetophenone amount reached a value of $6 \text{ mg}_{\text{AcPh}}/\text{mg}_{\text{protein}}$. This
18
19 amount of water is adsorbed only at water activity levels above 0.6. In terms
20
21 of mole amounts, the adsorbed amount of water is almost double the
22
23 adsorbed amount of acetophenone ($0.091 \text{ mmol}_{\text{water}}/\text{mg}_{\text{protein}}$ versus 0.05
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27 405 $\text{mmol}_{\text{AcPh}}/\text{mg}_{\text{protein}}$), for the same level of thermodynamic activity of the two
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29 compounds, namely 0.32.
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3.6 2-propanol adsorption to the packed bed with deposited enzyme.

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36 The adsorption of the second substrate, 2-propanol, to the deposited enzyme
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39 410 preparation in the gas/solid reactor was also investigated. In the dynamic
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41 experiment, gas mixtures of nitrogen and 2-propanol, with thermodynamic
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43 activities in the range of $a_{2\text{-prop}}=0.079\text{-}0.38$, were directed through the packed
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45 bed but no negative peaks were detected (Fig.11). The GC sampling of the
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47 mixture took place only 6 min after the flow was switched from the bypass to
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50 415 the reactor. Even if adsorption of this compound occurred, it was not
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52 detectable in the specific set-up.
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3.7 Competitive adsorption of substrates and water

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8 A potential effect of water on the adsorption of acetophenone and 2-propanol
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10 420 to the deposited enzyme preparation for a common water activity level for the
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12 reaction mixture in the gas/solid reactor of 0.54 was also investigated. All
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14 other parameters were kept at the same level as those in the investigation of
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16 the substrate adsorption without water in the mixture. The enzyme
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18 preparation was thermally treated, aiming at the cofactor deactivation
19
20 assuming that this short thermal treatment did not severely alter the enzyme
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22 425 capacity to adsorb water.
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27 By comparing the adsorption isotherms of acetophenone in Fig.10 it is
28
29 obvious that the presence of water in the gas mixture leads to a decrease in
30
31 the amount of acetophenone adsorbed by the enzyme. The substrate and
32
33 430 water molecules compete for adsorption sites of the deposited catalyst and
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35 that leads to a lower acetophenone adsorption. On the other hand, the
36
37 presence of acetophenone molecules may also influence water adsorption.
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39 According to [2] however, due to the low polarity of the acetophenone this
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41 effect is not expected. Due to the incompatibility of the humidity sensors with
42
43 acetophenone, the respective competitive adsorption studies could not be
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45 435 performed.
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50 Under the assumption that the water adsorption isotherm is not strongly
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52 influenced by the adsorption of acetophenone, the quantity of adsorbed
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54 water, at $a_w=0.54$, was obtained from the water adsorption isotherm (Fig.4).
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6 440 Similarly, the quantity of adsorbed acetophenone, at $a_{\text{AcPh}}=0.22$, was also
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8 calculated. The ratio of adsorbed water moles to adsorbed acetophenone
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10 moles was found to be approximately 12.5 ($0.183 \text{ mmol}_{\text{water}}/\text{mg}_{\text{protein}}$ versus
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12 $0.015 \text{ mmol}_{\text{acph}}/\text{mg}_{\text{protein}}$), at the above mentioned water and acetophenone
13
14 activity levels, which are typical values for the reactor operation. Taking into
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16
17 445 account the molecular weight of the LBADH (106 kDa), it is estimated that, at
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19 these conditions, 1 molecule of protein is surrounded by 19400 molecules
20
21 water and 1560 molecules acetophenone.
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24 As it is shown in Fig.11B, the presence of water did not influence the
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26 adsorption behavior of 2-propanol. Once again, 2-propanol adsorption by the
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29 450 immobilized enzyme preparation was not detectable in the dynamic
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31 experiment, exactly as in the case of water being absent.
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4 Concluding remarks

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13 455 In the gas/solid enzymatic reactor, thermodynamic equilibrium is highly
14 controlled by adjusting the carrier gas composition. While hydration of the
15 enzyme has been studied previously, no study had fully covered the
16 adsorption of all compounds to the enzymatic fixed bed.

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18 A simple and efficient experimental set-up, the water adsorption unit, has
19 been used to monitor the water adsorption to the deposited LBADH, at
20 conditions resembling those of the continuous gas/solid reactor. The
21 hydration of the deposited enzyme has been described by a BET-like
22 isotherm. The critical thermodynamic water activity level, above which the
23 system deviates from the initial adsorption behavior, has been found to be
24 approximately 0.5. It has been shown that the presence of sucrose in the
25 enzyme preparation has an important influence on the capacity of the packed
26 bed to adsorb and retain water. It has been also shown that the hysteresis
27 during water desorption from the deposited preparation is very significant.
28 This is accompanied by micro-structural changes of the lyophilized or
29 deposited enzyme preparations, suggesting the choice of covalent
30 immobilization methods in the future. The adsorption of the two substrates of
31 the LBADH, acetophenone and 2-propanol, has been investigated for the first
32 time, at conditions mimicking those of a reacting system, allowing a more
33 complete insight on the microenvironment of the deposited enzyme. It has
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6 been revealed that although 2-propanol does not get adsorbed at a level,
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8 detectable at the specific set-up, acetophenone, on the other hand, is
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10 475 adsorbed to the deposited enzyme preparation at a significant level,
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12 comparable to the amount of adsorbed water. This might lead to mass
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14 transfer limitations due to diffusional transport to the enzyme. Therefore the
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16 overlaying phenomena of adsorption and reaction kinetics will be the topic of
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18 future studies.
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6 480 **Acknowledgements**
7

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Legends

Figure. 1 Water adsorption isotherm [4].

605 **Figure. 2** Water adsorption measurement unit: (1) humidity trap, (2) mass flow controllers, (3) thermo-constant chamber, (4) heating coils, (5) water saturation flasks, (6) mixing chamber, (7) 2-3-way valves, (8) packed bed, (9) humidity sensor.

610 **Figure. 3** Water adsorption isotherm of lyophilized LBADH powder: $m_{enzyme}=19.8$ mg, $V_{tot}=15$ mL/min, $T=40$ °C. The insert shows the linearized BET isotherm according to Eq.1.

615 **Figure. 4** Water adsorption isotherm of deposited enzyme preparation with sucrose. $m=120$ mg, protein loading=0.00275 mg/mg, $V_{tot}=15$ mL/min, $T=40$ °C. Open/close symbols refer to the two replicates. The arrow indicates the water activity level above which deviation from the initial adsorption behavior occurs.

620 **Figure. 5** Sucrose effect on the water adsorption of deposited enzyme preparation: $m=120$ mg, protein loading=0.00275 mg/mg (with sucrose), protein loading=0.00074 mg/mg (without sucrose), $V_{tot}=15$ mL/min, $T=40$ °C. The water adsorbed is calculated here as water amount adsorbed per mg of beads of the catalytic bed (A) and as water amount adsorbed per mg of protein (B). The closed circles correspond to enzyme preparations with sucrose and the open ones without.

625 **Figure. 6** Water adsorption and desorption isotherms of lyophilized enzyme: $m_{enzyme}=19.8$ mg, $V_{tot}=15$ mL/min, $T=50$ °C.

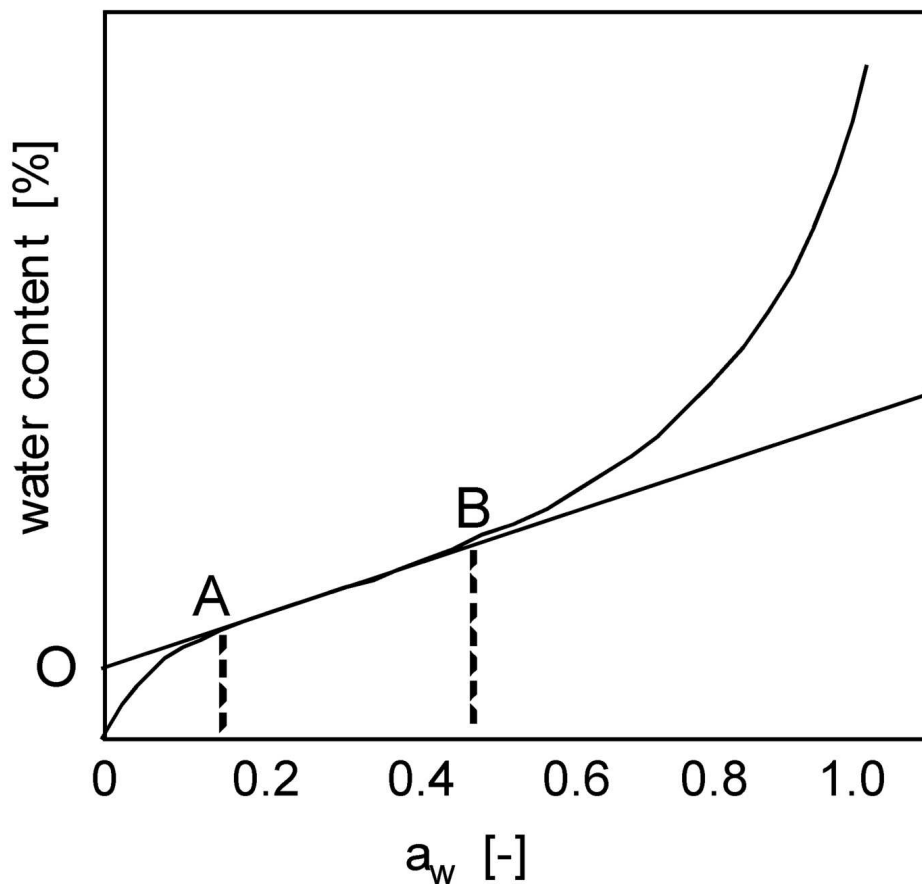
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6 **Figure. 7** Adsorption and desorption isotherms of the deposited enzyme preparation with (A)
7 and without (B) sucrose: $m_{\text{enzyme}}=120$ mg, protein loading= 0.00275 mg/mg (A), protein
8 loading= 0.00075 mg/mg (B), $V_{\text{tot}}=15$ mL/min, $T=40$ °C.
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13 630 **Figure. 8** SEM pictures of the deposited enzyme with sucrose on glass carriers: (A) and (C)
14 carriers before the water adsorption measurement process; (B) and (D) carriers after the
15 water adsorption measurement process. The material bridges created between the individual
16 carriers are indicated in (B) by arrows.
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23 635 **Figure. 9** Effect of enzyme on the acetophenone adsorption capacity of the catalytic bed:
24 $m=565$ mg, protein loading= 0.0021 mg/mg, $V_{\text{tot}}=10$ mL/min, $T=45$ °C. Triangles give the
25 acetophenone partial pressure at the packed bed inlet, the open circles the partial pressure
26 at the outlet of a packed bed with plain glass carriers and the closed circles the partial
27 pressure at the outlet of a packed bed with glass carriers coated with deposited enzyme plus
28 sucrose.
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38 **Figure. 10** Acetophenone adsorption isotherms for the deposited enzyme preparation (with
39 sucrose): with triangles the adsorption isotherm of the gas mixture without water vapors, with
40 circles the adsorption isotherm with water vapors of $a_w=0.54$. For both $m=120$ mg, protein
41 loading= 0.0021 mg/mg, $V_{\text{tot}}=15$ mL/min, $T=40$ °C.
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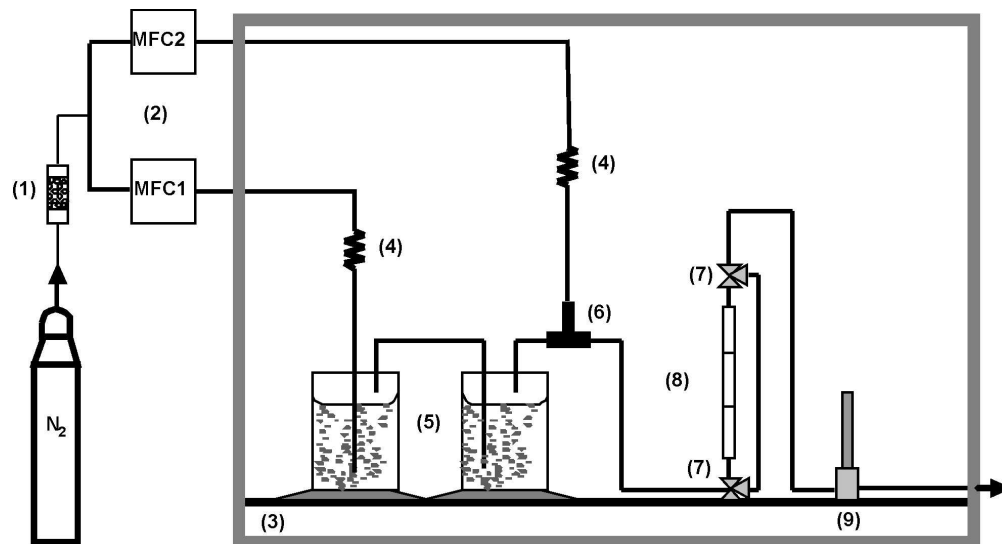
48 **Figure. 11** 2-propanol equilibration without (A) and with (B) water: $m_{\text{bed}}=565$ mg, protein
49 loading= 0.0021 , $V_{\text{tot}}=10$ mL/min, $T=40$ °C, $a_w=54\%$. Open circles correspond to the flow
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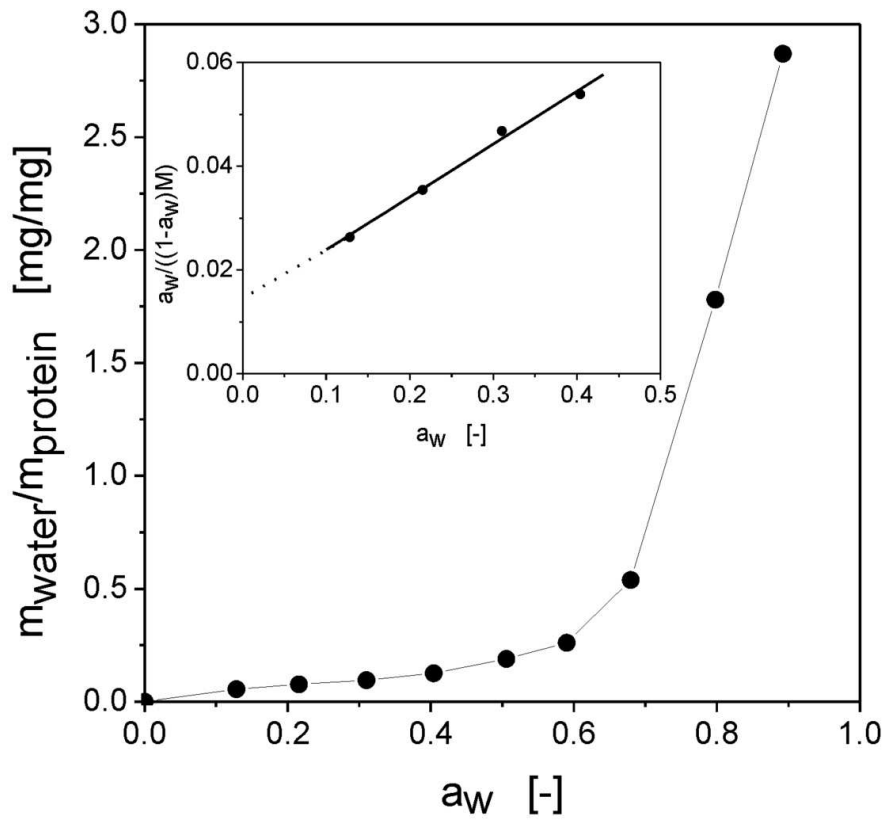
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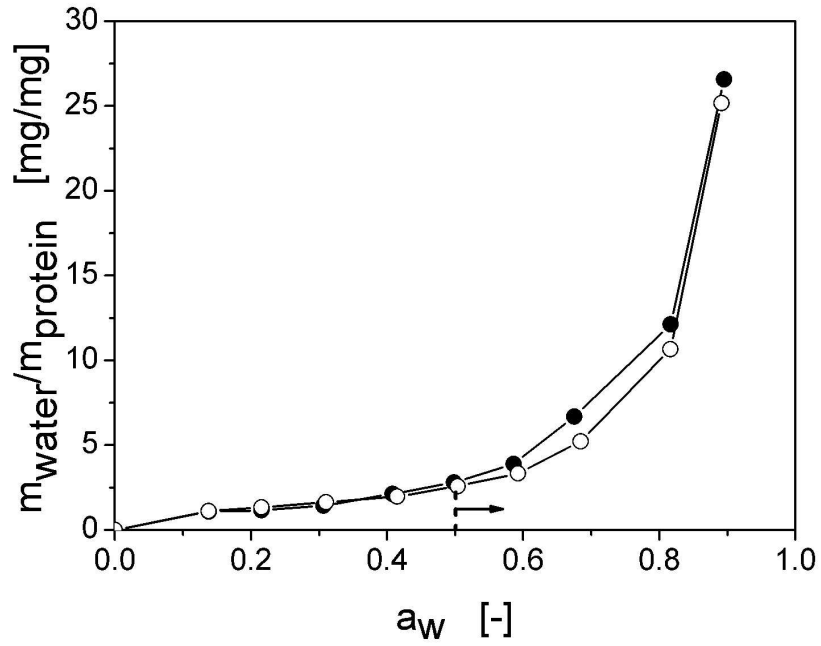


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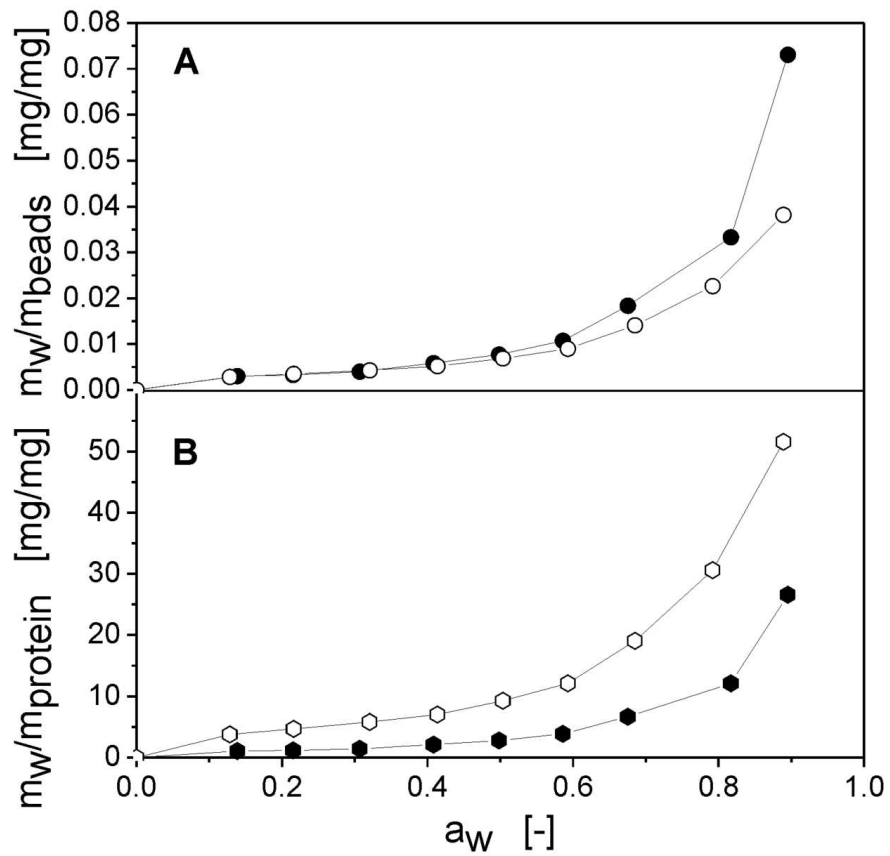


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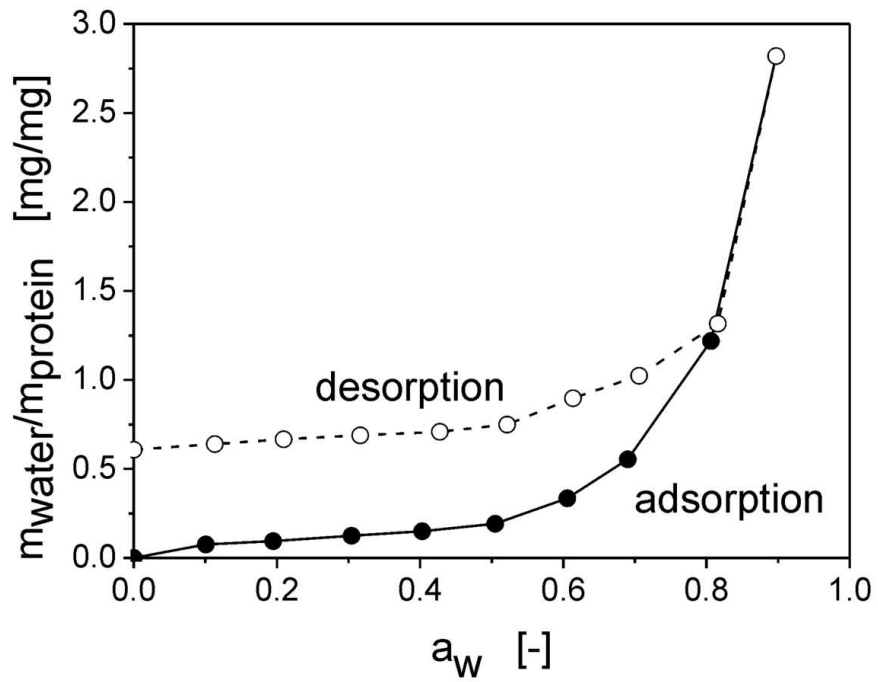


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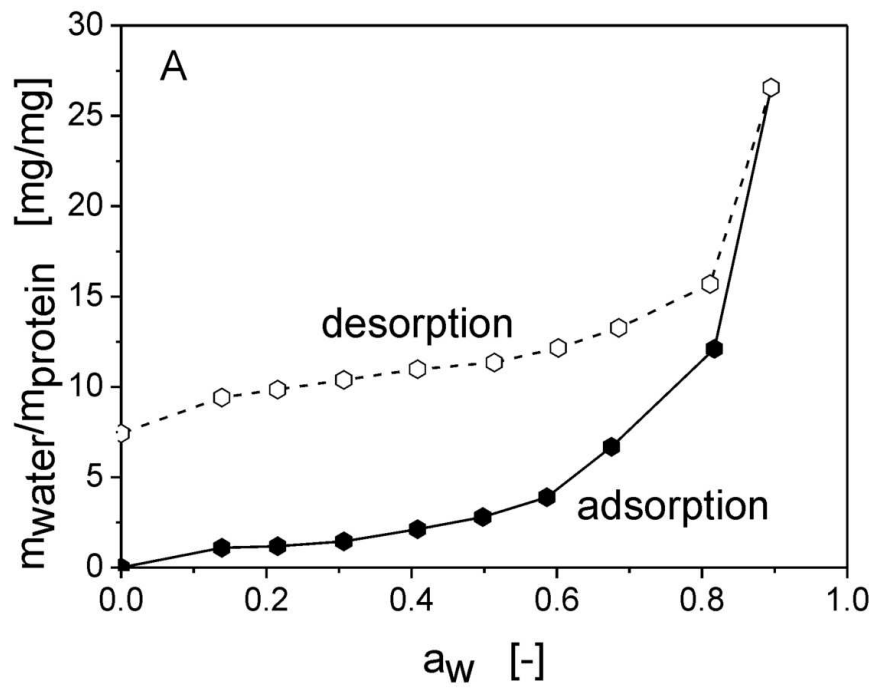


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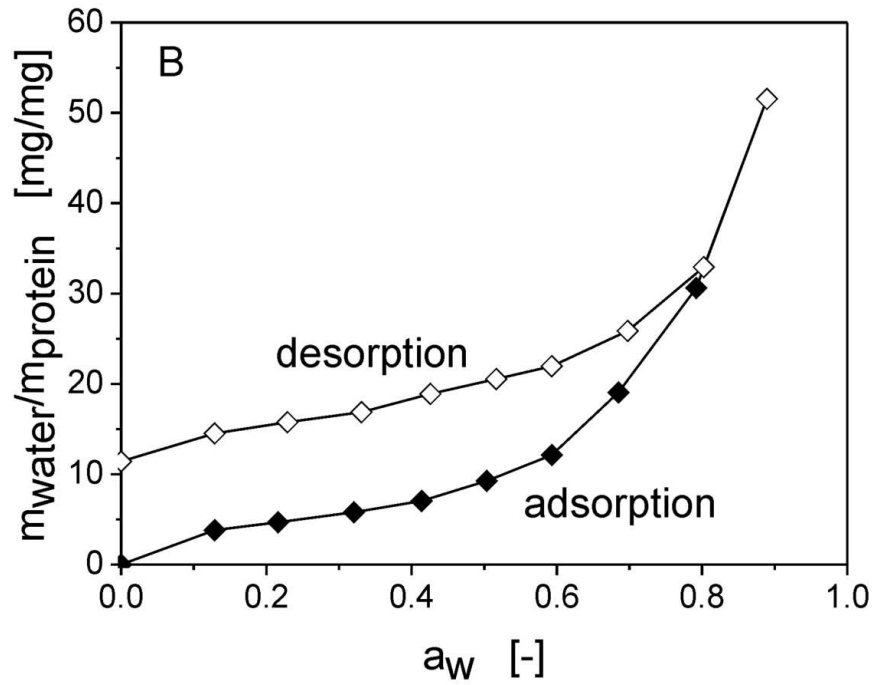




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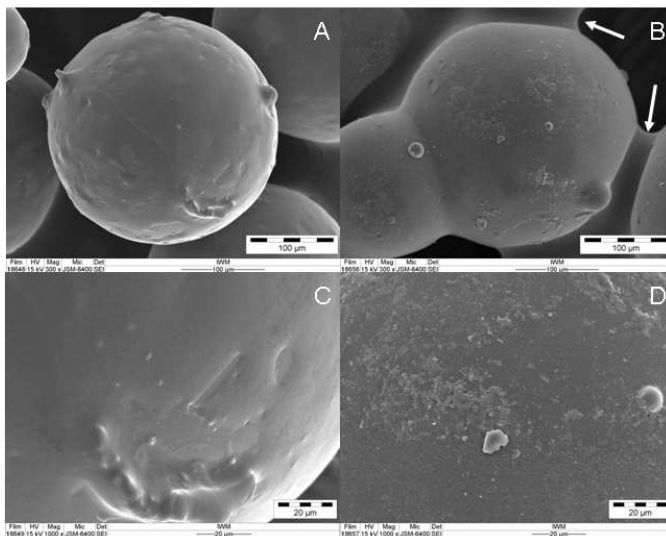


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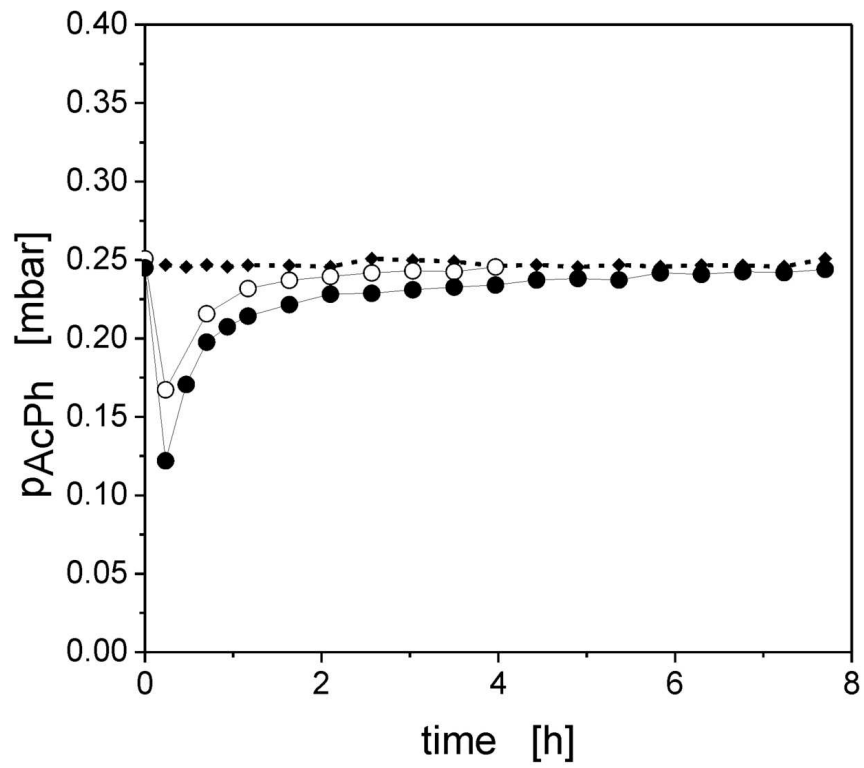
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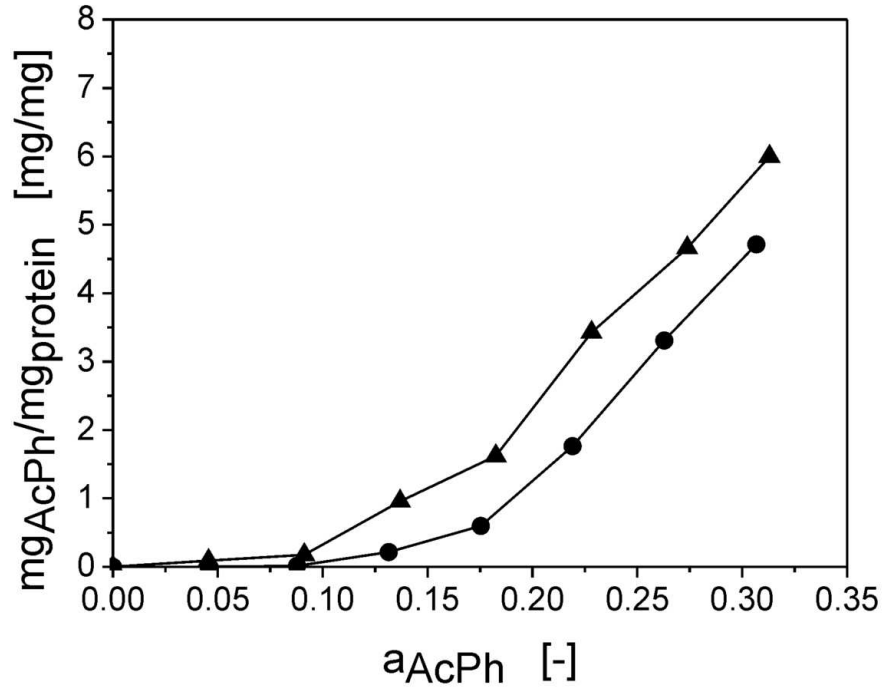
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Review



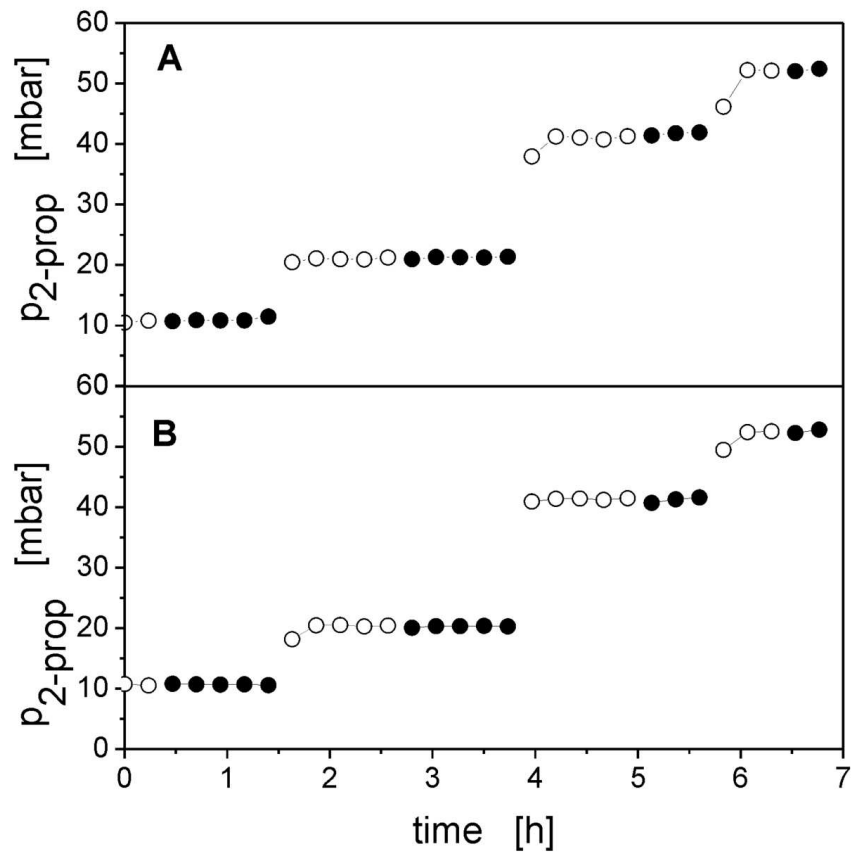
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