Evaluation of pectin-reinforced supported liquid membranes containing carbonic anhydrase: The role of ionic liquid on enzyme stability and CO_2 separation performance

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Abstract

In this paper, pectin-reinforced, supported liquid membranes (SLMs) prepared with carbonic anhydrase (CA) were investigated for CO_2/N_2 separation. In the first part of the study, the effect of [Bmim][NTf_2] ionic liquid (IL) – as possible solvent to fill the pores of cellulose acetate support during SLM fabrication – on enzyme activity was tested. It turned out that this particular IL caused rapid and severe loss of initial biocatalyst activity, which fact can be seen as a threat in the membrane process design. Afterwards, the stability of pectin-containing SLMs (containing CA but lacking the IL having adverse impact) was addressed and their improved resistance against higher transmembrane pressures (up to 7.2 bar) was found, representing an approx. 3-fold enhancement compared to their control. Thereafter, the performance of the membranes was tested under single and mixed gas conditions with carbon dioxide and nitrogen. Employing single gases, it was demonstrated that CA enzyme could notably increase CO_2 permeability (from 55 to 93 Barrer), while that of N_2 remained unchanged (1.6-1.7 Barrer). Thus, the highest CO_2/N_2 theoretical selectivity was attained as 54 using the pectin-reinforced SLMs enriched with CA biocatalyst. For comparison, the outcomes were plotted on the Robeson upper-bound.

Keywords: gas separation; supported liquid membrane; ionic liquid; carbonic anhydrase; CO_2 separation

1. Introduction

The enhancement of CO_2 separation from various gaseous mixtures (including flue-, bio- as well as natural gas) via the design of novel, facilitated-transport membranes has become a topic of wide interest [1]. Improved CO_2-permeation capability in these types of membranes can be achieved in several different ways [2], where popular methods cover the incorporation of membrane materials such as polymers with specific chemical agents/solvents and in recent year, membrane preparation by using enzymes, in particular carbonic anhydrase (CA) has drawn attention too. This latter, biocatalytic route – that transfers carbon dioxide via a reversible reaction to form bicarbonate as introduced in our previous paper [15] – has been emphasized as a possible way forward in advancing new-generation carbon dioxide capture technologies, which are less energy-intense, show faster reaction kinetics [3] and provides membranes with better permselectivity. The separated CO_2 can be used for the synthesis of valuable components [4] such as organic acids [5], energy carrier e.g. methane [6]. Further utilization path of CO_2 may involve algae cultivation [7], intensification of anaerobic hydrogen fermentation [8], etc.

So far, the CA enzyme has been applied with success in different membranes applications. Relevant examples by Hou et al. [9,10], Yong et al. [11] proved that CA or its mimicking substance i.e. Zn-cyclen [12] can fit to upgrade gas-liquid membrane contactors and membrane reactors [13]. In another research direction, supported liquid membrane (SLM) prepared with

61 the addition of CA was found as a feasible approach in membrane
62 development [14-17]. Conventional SLMs are fabricated by filling various
63 sorption liquids to the pores of polymer membranes.

64 Among SLMs, those made with solvent e.g. ionic liquids (IL) are
65 regarded as supported ionic liquid membranes (SILMs) and represent an
66 emerging class for gas separation purposes [18-21]. Though SILMs are
67 promising from many aspects, issues related to their mechanical stability due
68 to the removal of ILs from the pores at relatively low transmembrane pressure
69 differences may occur. To overcome such liquid washout and consequent
70 membrane degradation, solutions such as membrane gelation (achieved via
71 the blending of ILs with polymers) have been tested [22]. As gelling material,
72 the group of Coelhoso [22,23] applied gelatin, which is a cheap and widely
73 available biopolymer. This example is a good indication of the potential that
74 naturally-occurring components can have in SILM development.

75 In addition to membrane integrity, the biocompatibility of ILs should be of
76 concern too, as it may significantly affect longer-term activity of enzyme mixed
77 and immobilized in it [24]. In fact, Martins et al. [16] have also underlined that
78 biocompatible and environmental-friendly ILs can be favored for SILM
79 synthesis. It was noted in previous works that small quantities of CA enzyme
80 (0.1 mg/g IL) [16,23], even in partly-purified form after recovering it from
81 biomass [15] can work and effectively shuttle CO_2 across the SILM membrane.
82 However, to our knowledge, the time-dependent change of CA activity in ILs
83 has not been monitored so far.

Given that SILM durability can be influenced by the above-referred structural and biological impacts, the aim of this study were two-folded. Firstly, we have assessed the IL-CA interactions as a crucial parameter of membrane lifetime employing [Bmim][NTf_2], which was used for the preparation of enzymatically-boosted SILMs in our previous investigation [15]. Secondly, CA-containing membranes gelated with pectin – a natural biopolymer found in plants [25] – were evaluated against pressure-resistance, followed by gas permeation tests carried out with pure (CO_2, N_2) and mixed (CO_2 – N_2) gases.

As far as we know, this is the first report on the behavior and use of CA-enriched, pectin-containing membranes for CO_2 separation and hence, the information delivered can be novel enough and helpful for the international research community of membraneologists.

2. Materials and methods

2.1. Enzyme and chemicals

Throughout the experiments, the CA enzyme purchased from Sigma-Aldrich, USA – product ID: C2624, purity: >95 %, specific activity: >3500 Wilbur-Anderson (W-A) unit mg^{-1} protein – was used. The ionic liquid, 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([Bmim][NTf_2], purity: >99 %) was obtained from Io-Li-Tec, Germany. Pectin (type: Pectin Amid CU 025; degree of esterification and amidation is 29 % and 23 %, respectively; galacturonic acid content: 89 % according to the certificate of analysis

provided by the manufacturer) was ordered from Herbstreith & Fox KG, Germany. Although a huge variety of pectin is available on the market, this one was specifically chosen for the experiments since it does not contain sugars, which can be considered as an advantageous property from the microbiological stability viewpoint of the gels prepared with it. $CaCl_2$ x 2 H_2O was the product of Sigma-Aldrich, USA.

2.2. Enzyme activity assays

Basic procedure. The activity of CA (EC number: 232-576-6) was determined in W-A unit mg^{-1} enzyme. To conduct the measurements, a stock enzyme solution (SES) (2 mg CA mL^{-1}) had to be first prepared using Tris-HCl buffer (0.02 M, pH = 8.3). Thereafter, 20 µL SES was diluted (D-SES) to 10 mL with Tris-HCl buffer (0.02 M, pH = 8.3). Afterwards, 14 mL Tris-HCl buffer (0.02 M, pH = 8.3) was mixed with 1 mL D-SES in a reaction vessel (thermostated to 0 ^oC) and 6 mL substrate solution (CO_2-saturated distilled water) was added simultaneously. The whole container was continuously stirred at 450 rpm with magnetic bar. Once the reaction mixture was complete, the time needed for 1 unit of pH fall (in the range of 8.2-7.2) was measured by stopwatch. Complementary tests were also performed under enzyme-less circumstances. The W-A unit was delivered from the times elapsed under the two conditions (with and without CA enzyme) according to the formula

130 introduced in our previous paper [15]. This was then normalized by the mass
131 of enzyme in the reaction mixture to get the values in W-A unit mg^{-1} enzyme.
132 *Modified procedure I.* The *Basic procedure* was adopted with some
133 alterations to check CA activity in the membranes prepared. The membranes
134 were cut to 4 x 4 mm pieces, some of which was placed to the reaction vessel
135 together with 15 mL Tris-HCl buffer (0.02 M, pH = 8.3) and 6 mL substrate
136 solution.
137 *Modified procedure II*. The *Basic procedure* was adopted with some
138 changes to reveal the effect of [Bmim][NTf_2] ionic liquid on the CA enzyme
139 activity. During these experiments, 9 mL [Bmim][NTf_2] ionic liquid was mixed
140 with 1 mL SES, giving a mixture referred as IL-SES. Next, the enzyme activity
141 was measured every 5 minutes for a couple of cycles. To do so, 3 mL of the
142 IL-SES was transferred to 12 mL Tris-HCl buffer (0.02 M, pH = 8.3),
143 supplemented with 6 mL substrate solution and the time required for 1 unit of
144 pH drop (from 8.2 to 7.2) was recorded in order to compute the corresponding
145 W-A unit mg^{-1} enzyme, as mentioned before. Additional test were run under
146 enzyme-less circumstances.
147
148 **2.3. Membrane preparation**
149
150 Porous, hydrophilic, cellulose acetate membrane (pore size: 0.2 μm,
151 porosity: 60 %, thickness: 120 μm, Sartorius AG) with 5.6 cm diameter was
152 placed to a Petri-plate and then it was moved to a vacuum desiccator for 30

minutes. This was followed by two consecutive steps: (i) filling 2 mL SES to the membrane surface/pores and (ii) 30 minutes of vacuum again. As the time expired, a mixture of 4 mL pectin solution (0.25 wt%) and 140 µL $CaCl_2$ solution (1 wt%) was distributed as equally as possible on the surface of the membrane. Another 30 minutes was allowed to achieve partial gelation. In the last stage, the membrane was taken out of the desiccator and forced between 2 glass panes to (i) remove excess pectin that did not strongly bind to the membrane pores and (ii) finish the gelation process.

Afterwards, activity, stability and gas permeation tests on the membranes could be performed. Besides these membranes containing the CA, additional ones lacking the enzyme were made too for comparison. Based on weighing, the reinforcement by pectin resulted in an average gain of of 400-500 mg (on wet basis) for the freshly made membranes. Furthermore, the thickness of the pectin/cellulose acetate membranes was 160 ± 30 µm.

2.4. Gas permeation device

The gas permeation experiments were carried out in a two-chamber permeation apparatus, including a permeation cell that hosts the membrane [19].

In the course of single gas tests, both (the feed and permeate) chambers of the permeation cell were purged with the given gas, followed by setting the pressure on the feed and retentate sides to 1.7 bar(a) and 1 bar(a),

176 respectively. Similar driving force (~0.7 bar) was applied by Neves et al. [26],
177 as well.

178 Under these conditions, once the chambers were closed, the gas started
179 to pass from the higher pressure to the lower pressure compartment. This
180 progress (pressure equalization) was monitored by pressure transducers on
181 both sides as the function of time by in LabVIEW. A typical time profile of the
182 permeation experiments is displayed in **Fig. 1**. The (pressure vs. time) data
183 were first processed by the methodology described in the paper of Neves et al.
184 [17], Afterwards, the permeability (p) of each gas component was converted to
185 Barrer (10^{-10} cm^3 (STP) cm cm^{-2} s^{-1} $cmHg^{-1}$). The theoretical selectivity was
186 calculated as the ratio of gas permeabilities (p_i/p_j, where $p_i>p_j$), similar to our
187 earlier article [19].

188 During binary gas experiments with CO_2/N_2 mixtures, feed and
189 permeation chambers were initially flushed with N_2 and then closed. This step
190 ensured that this particular gas had the same, 1 bar(a) pressure everywhere
191 inside the cell. Thereafter, carbon dioxide was loaded to the feed compartment
192 until a total pressure of around 1.7 bar(a) (0.7 bar(a) of CO_2 plus 1 bar(a) of
193 N_2) was observed. At that point, because of the partial pressure difference of
194 CO_2 between the sides (referred as the driving force), this molecule could
195 begin the migration into the permeate chamber, while no transport of N_2
196 (background gas) had to be considered because of the equal nitrogen partial
197 pressures on both membrane sides [27,28].

198 The CO_2 (commercial grade) and N_2 (>99.9 % purity) were products of
199 Linde, Hungary. The permeation cell was thermostated at 37 ^oC.
200
201 **3. Results and Discussion**
202
203 **3.1. Enzyme activity and its change in the presence of [Bmim][NTf_2] ionic**
204 **liquid**
205
206 The initial activity of the free CA enzyme was determined to be 3580 W-
207 A unit mg^{-1} protein by following the procedure introduced in Section 2.2. This,
208 in the light of the data indicated by the manufacturer (3500 W-A unit mg^{-1}
209 protein), proved that the enzyme assays worked properly and the results
210 obtained could be considered quite reliable, similarly to our previous study with
211 biomass-derived CA enzyme preparation [15].
212 In case of the CA enzyme immobilized in the pectin-reinforced
213 membrane, the initial activity measured was 9 W-A unit according to the
214 modified procedure I in Section 2.2.. This, by taking into account the
215 membrane surface corresponds to 1838 W-A unit m^{-2}, confirming that the CA
216 was efficiently immobilized in the membrane.
217 So far, there has been an agreement in the literature studies that
218 boosting the CO_2-separation in SILMs does not necessarily require great CA
219 enzyme loadings. In recent investigations of Portuguese scientists, SILMs
220 were successfully designed with as low as 0.1 mg CA/g IL enzyme

221 concentration [16,17], while Bednár et al. [15] demonstrated the appropriate
222 performance of SILMs containing partly-purified CA enzyme preparation,
223 obtained after plant biomass processing. Though longer-term experiments
224 revealed the good time-stability of the enzymatically-accelerated membranes
225 [15], no information regarding possible deterioration of CA activity in the
226 presence of IL has been reported.

227 Following the modified procedure II in Section 2.2, we attempted to take
228 a look into the enzyme-IL interactions. It turned out from the results that
229 considerable loss of CA enzyme activity can be induced by the [Bmim][NTf_2]
230 ionic liquid. Even as short contact time as 5 minutes caused an extreme, more
231 than 90-95 % drop of relative enzyme activity. However2, in accordance with
232 measurements carried out after 10 and 15 minutes, stabilization of values
233 could be noticed at around 0.5 % compared to the initial value.

234 From these observations, it would appear that depending on the
235 properties of the ionic liquid, quick and notable inhibition/deactivation of the
236 enzyme may take place and this phenomenon should be taken into
237 consideration for process design. Supportive conclusions were made in our
238 recent paper on the enzymatic hydrolysis of cellulose in the presence of
239 [bmim][Cl] ionic liquid [24]. Nevertheless, even if only a smaller portion of the
240 CA enzyme is preserved in an active form with time, it seems still be capable
241 of doing the job that it needs to and facilitate CO_2-transport across the
242 membrane. This might be attributed to the extremely high turnover number of
243 CA (indicating the number of substrate molecules that is converted to product

through the catalytic site of particular enzyme within a given time period), which is reportedly around the magnitude of 10^6 s^{-1}, making it one of the most efficient enzymes in nature and a plausible candidate for biocatalytic CO_2 capture and sequestration [4]. This characteristic, at least for a certain degree, may compensate for the threat of rate-limitation in CO_2-transfer when the number of active enzyme molecules decreases with time in the membrane. These results and considerations help to speculate why the performance of SILMs used in our previous work [15] demonstrated good time-stability (in terms of CO_2 and N_2 permeations) thorough a 4 week period. In brief, it can be supposed that the spinach-derived CA enzyme preparation initially underwent a remarkable activity loss due to the presence of [Bmim][NTf_2], but despite, the residual number of working enzyme was still satisfactory to assure the enhanced CO_2 permeability and concomitantly higher CO_2/N_2 selectivity compared to the non-biocatalytic (control) membranes.

As the stability of the CA was concerned, in another set of experiments (where 1 mg CA enzyme – dissolved in 0.02 M Tris-HCl buffer, pH=8.3 – was entrapped in pectin beads) it was sought if the immobilization of enzyme in the pectin gel itself causes any notable drop of its beneficial properties (expressed as W-A unit/mL of pectin solution (2.5 wt%) in which CA was mixed and subsequently used for gelation in $CaCl_2$ (2.5 wt%) by allowing 12 h hardening time at slightly acidic pH). As a result, 13.1 W-A unit/mL pectin could be initially noted (according to modified procedure I in Section 2.2.) on the first day. Afterwards, although there was some loss of activity too with the time

elapsed, it was definitely much more less significant compared to that noticed in the presence of [Bmim][NTf_2]. In fact, after 3 weeks (during which beads were stored at 4 ^oC in 0.02 M Tris-HCl buffer, pH=8.3), the residual enzyme activity was still nearly 70-80 % of the initial. This experience that the majority of CA activity could be preserved for a longer time correlates well with our recent findings using free, biomass-derived CA enzyme preparation [15]. Accordingly, the application of pectin was not considered harmful for the CA enzyme.

3.2. Stability of pectin-containing membranes

Bubble-point porosimetry was applied to test stability of the membranes, in terms of their resistance against pressure. This technique enables the user to determine the pressure that exceeds the capillary attraction of a liquid in the biggest pore of a porous material [29]. During the measurements, the pressure of a gas (here N_2) is stepwise increased on the feed side of the membrane until a critical pressure (P_r) is reached, where the bubbles appear on the other side via the largest pore of the wetted material. This means in other words that the flux of the gas below P_r is negligible.

For the membranes reinforced with pectin in accordance with Section 2.3., the value of P_r was obtained as 7.2 bar. This, in comparison with the pectin-free control, presented a nearly 3-fold increase of pressure resistance. Therefore, it can be assumed that the pectin-supported membranes developed

290 in this work can be suitable for higher pressure gas separation task (>0.2 MPa
291 transmembrane pressure difference), where conventional SLMs normally fail
292 due to the instable membrane structure [22]. In our future investigation, such
293 tests will thus be designed to evaluate CO_2-separation under such conditions.

294 In previous works of the literature, various SLMs were manufactured
295 using ionic liquid and natural gelling agent i.e. gelatin [23]. It was found after
296 taking stress-strain curves that membranes prepared only with gelatin (on
297 porous cellulose support) reflected better mechanical properties (stress
298 tolerance) than those containing both gelatin and IL (called Ion-Jelly®
299 membranes). Moreover, gelatin-cellulose membranes could be characterized
300 by an increased stiffness (based on the Young modulus) in comparison with
301 the IL-containing ones [23].

302

303 **3.3. Gas separation performance of pectin-reinforced membranes**
304 **prepared with CA enzyme and lacking ionic liquid**

305

306 As it was inferred in Section 3.1. that [Bmim][NTf_2] can cause the severe
307 deterioration of CA enzyme activity, we aimed to study how the CA-boosted,
308 pectin-supported membranes behave and perform in the absence of this IL
309 during the permeation of pure as well as binary gases.

310 The results of gas permeation experiments are depicted in **Fig. 2**,
311 according to which in case of the non-biocatalytic, pectin-containing cellulose
312 acetate membranes the permeability of CO_2 was an order or magnitude higher

313 than that of N_2 (55 and 1.6 Barrer, respectivetly), which can be ascribed to
314 their distinct solubility and diffusivity traits. Furthermore, it should be also noted
315 that under pure/single gas conditions, no effect was taken on N_2 permeability
316 by the presence of CA enzyme (1.6 vs. 1.7 Barrer). On the other hand, CO_2
317 permeability could increase significantly, from 55 to 93 Barrer. These
318 outcomes match well with those trends communicated by Neves et al. [17],
319 where it was found that both N_2 solubility and diffusivity (the two parameters
320 that determine the permeability) remained unaffected by CA enzyme.
321 Nevertheless, CA does able to positively influence CO_2 solubility coefficient
322 [17], providing an explanation about the mechanism that could play a key-role
323 in the improvement of the theoretical CO_2/N_2 selectivity (from 34 to 54 in the
324 presence of CA enzyme).

325 It is also noteworthy that besides ionic liquid (in more general, solvent)-
326 dependent enzyme inhibition/deactivation that may occur (Section 3.1.), the
327 water activity in the membrane is also a factor that can affect the biocatalyst
328 stability and efficiency [15-17]. Hence, its variation (i) from system to system
329 and (ii) with time is a possible reason leading to altered CO_2-separation
330 performance. Thus, it means that an exact comparison of the already
331 published literature might be done only for results obtained under standardized
332 circumstances, in particular in terms of water activity (a_w). Although in this work
333 a_w was not determined, we can suppose that it was quite high based on the
334 report of Basu et al. [30], where it was deduced that in case of low methoxyl
335 pectin (esterification degree < 50 %, which criteria is satisfied by the pectin

used in our work (29 %), as it can be seen in the Materials and methods) the equilibrium moisture content (g water/g dry matter) and water activity are interdependent. In fact, it was inferred by Basu et al. [30] that higher equilibrium moisture content will be accompanied by higher water activities in a wider range of temperature (30-70 ^{o}C). Since in the current paper the ratio between the mass of water and the mass of dry pectin was most likely above 0.3-0.5 at 30-40 ^{o}C (the interval where the temperature of gas separation tests falls), a_w in the pectin-reinforced membranes may have approached to the vicinity of 1.

Regarding the mixed gas tests conducted, it would appear that CO_2 permeability under these conditions was slightly enhanced from 93 to 102, using nitrogen as background gas. Though N_2 permeation between the cells was not considered (as described in Section 2.4), certain interactions between CO_2 and N_2 may have occurred inside the membrane related to nitrogen dissolved in the membrane material (cellulose acetate support as well as pectin matrix). However, we should also point to the fact that the approx. 10 % difference between pure- and mixed-gas CO_2 permeabilites may arise from experimental uncertainties, as it is more or less the confidence interval of the permeation measurements. Besides, it had been drawn by Scovazzo et al. [31] that mixed-gas selectivities in SILMs can be similar to those obtained with pure gases. These altogether suggest that further experimentation will be required (applying more gases i.e. H_2, CH_4 and their mixtures with CO_2) to unambiguously decide whether the observed differences of CO_2 permeability

359 under single- and binary-gas conditions are remarkable, and should stand in
360 the scope of our next work on pectin-containing, biocatalytic membranes.

361 To demonstrate how the membrane performances fit to the recent
362 trends, the pectin-reinforced gas separation membranes prepared with/without
363 CA enzyme are illustrated against the Robeson upper-bound [32] in **Fig. 3**. As
364 one can observe, this is a double logarithmic relationship, correlating how the
365 CO_2/N_2 selectivity changes as a function of faster compounds (CO_2)
366 permeability. We can see that the enrichment with CA was able to push the
367 separation properties towards the upper-bound line, but further research is still
368 needed for more attractive gas separation behavior of pectin-supported
369 membranes.

370 So far, as it appears in **Fig. 1**, the permeation experiments were
371 performed in rather short-terms (supposing that no significant dry out of the
372 membranes occurred in the closed test cell). However, in longer terms, it is
373 important to note that an issue may arise due to the evaporation of solvent
374 (water) from the aqueous supported membrane when the membrane is
375 coupled to a real gas separation process. In these cases, when the
376 membranes are to be used to separate for example biologically produced gas
377 mixtures (i.e. biohydrogen, biogas), it can be assumed that the humidity
378 content of such gaseous streams (that are generated in a bioreactor via
379 fermentation) would allow the prevention of this undesired phenomena.
380 Therefore, in the continuation of this research, measurements will be
381 dedicated to study this subject.

Conclusions

In this work, pectin-reinforced gas separation membranes containing carbonic anhydrase enzyme were prepared and studied. The results presented that the CA can lose majority of its initial activity in the presence of [Bmim][NTf_2] ionic liquid as a solvent candidate for supported membrane fabrication. Moreover, the pectin-containing membranes (lacking the ionic liquid possessing adverse effect on the biocatalyst) could be characterized with improved resistance towards higher transmembrane pressure conditions. The use of CA enzyme facilitated CO_2 permeation, and as a result, markedly enhanced CO_2/N_2 selectivity was achieved.

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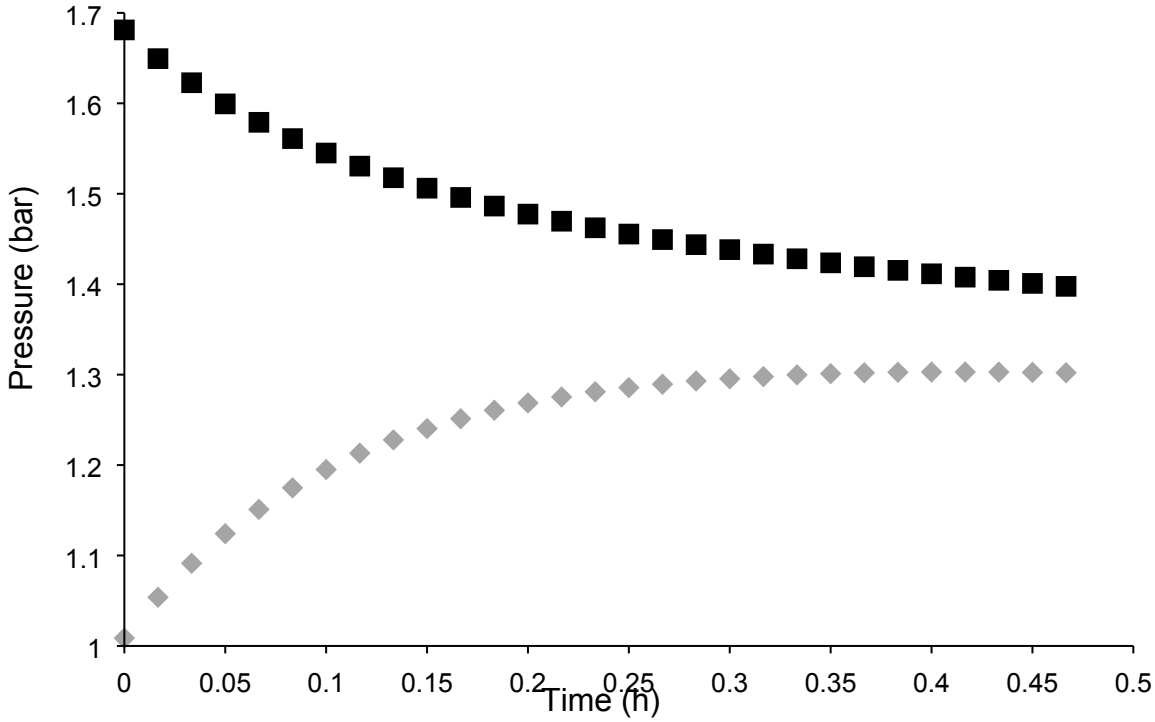
Figure legend

Fig. 1 – Progress curve of a typical gas permeation experiment. Square and diamond symbols represent the pressure in the feed and permeate cells, respectively.

Fig. 2 – Single/mixed gas permeabilites and CO_2/N_2 selectivity in pectin supported membranes with/without CA enzyme

Fig. 3 – The dependence of CO_2/N_2 selectivity on CO_2 permeability. Diamond and star symbols stand for the pectin-supported membranes with and without CA enzyme, respectively. The scattered line represents the Robeson upper-bound for polymeric membranes [32].

519 Fig. 1



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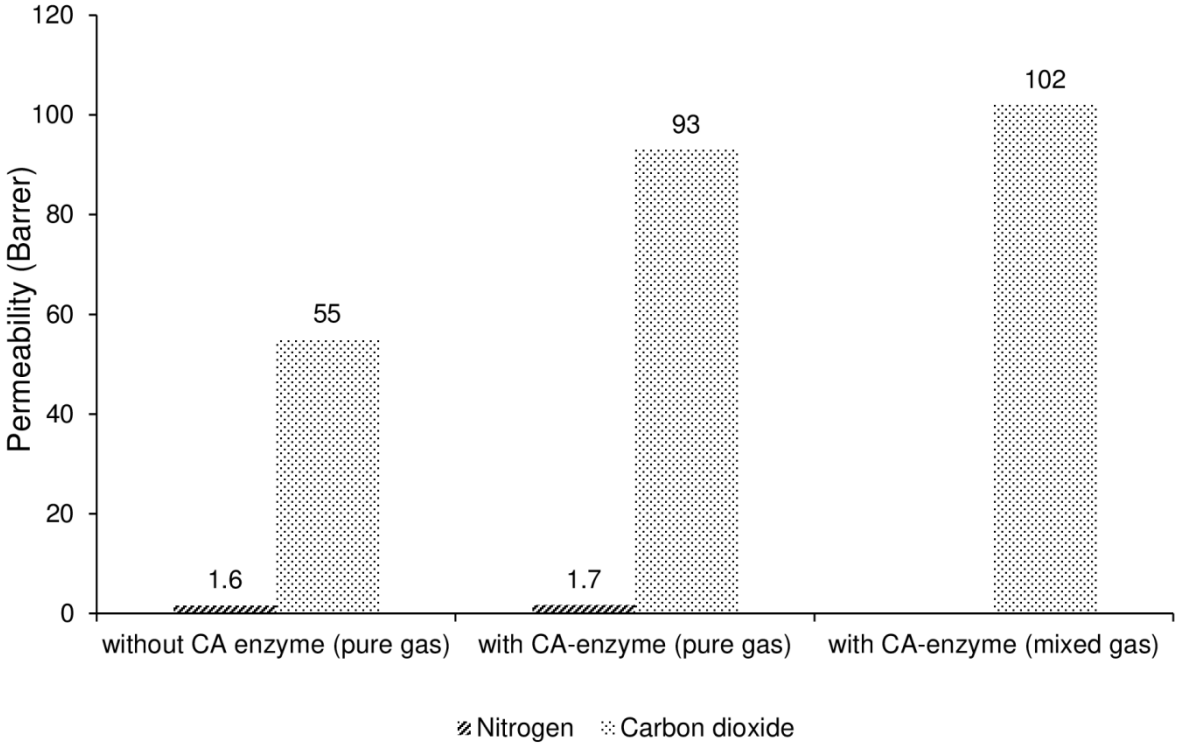
522 Fig. 2



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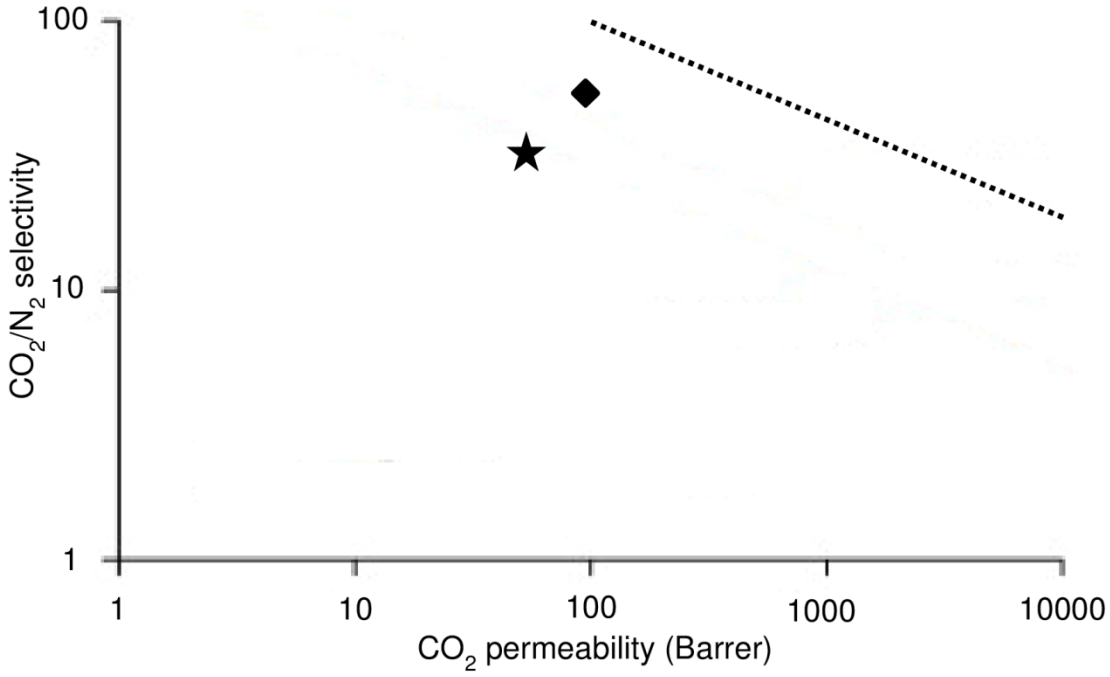
525 Fig. 3



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Evaluation of pectin-reinforced supported liquid membranes containing carbonic anhydrase: The role of ionic liquid on enzyme stability and CO_2 separation performance

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Abstract

In this paper, pectin-reinforced, supported liquid membranes (SLMs) prepared with carbonic anhydrase (CA) were investigated for CO_2/N_2 separation. In the first part of the study, the effect of [Bmim][NTf_2] ionic liquid (IL) – as possible solvent to fill the pores of cellulose acetate support during SLM fabrication – on enzyme activity was tested. It turned out that this particular IL caused rapid and severe loss of initial biocatalyst activity, which fact can be seen as a threat in the membrane process design. Afterwards, the stability of pectin-containing SLMs (containing CA but lacking the IL having adverse impact) was addressed and their improved resistance against higher transmembrane pressures (up to 7.2 bar) was found, representing an approx. 3-fold enhancement compared to their control. Thereafter, the performance of the membranes was tested under single and mixed gas conditions with carbon dioxide and nitrogen. Employing single gases, it was demonstrated that CA enzyme could notably increase CO_2 permeability (from 55 to 93 Barrer), while that of N_2 remained unchanged (1.6-1.7 Barrer). Thus, the highest CO_2/N_2 theoretical selectivity was attained as 54 using the pectin-reinforced SLMs enriched with CA biocatalyst. For comparison, the outcomes were plotted on the Robeson upper-bound.

Keywords: gas separation; supported liquid membrane; ionic liquid; carbonic anhydrase; CO_2 separation

1. Introduction

The enhancement of CO_2 separation from various gaseous mixtures (including flue-, bio- as well as natural gas) via the design of novel, facilitated-transport membranes has become a topic of wide interest [1]. Improved CO_2-permeation capability in these types of membranes can be achieved in several different ways [2], where popular methods cover the incorporation of membrane materials such as polymers with specific chemical agents/solvents and in recent year, membrane preparation by using enzymes, in particular carbonic anhydrase (CA) has drawn attention too. This latter, biocatalytic route – that transfers carbon dioxide via a reversible reaction to form bicarbonate as introduced in our previous paper [15] – has been emphasized as a possible way forward in advancing new-generation carbon dioxide capture technologies, which are less energy-intense, show faster reaction kinetics [3] and provides membranes with better permselectivity. The separated CO_2 can be used for the synthesis of valuable components [4] such as organic acids [5], energy carrier e.g. methane [6]. Further utilization path of CO_2 may involve algae cultivation [7], intensification of anaerobic hydrogen fermentation [8], etc.

So far, the CA enzyme has been applied with success in different membranes applications. Relevant examples by Hou et al. [9,10], Yong et al. [11] proved that CA or its mimicking substance i.e. Zn-cyclen [12] can fit to upgrade gas-liquid membrane contactors and membrane reactors [13]. In another research direction, supported liquid membrane (SLM) prepared with

61 the addition of CA was found as a feasible approach in membrane
62 development [14-17]. Conventional SLMs are fabricated by filling various
63 sorption liquids to the pores of polymer membranes.

64 Among SLMs, those made with solvent e.g. ionic liquids (IL) are
65 regarded as supported ionic liquid membranes (SILMs) and represent an
66 emerging class for gas separation purposes [18-21]. Though SILMs are
67 promising from many aspects, issues related to their mechanical stability due
68 to the removal of ILs from the pores at relatively low transmembrane pressure
69 differences may occur. To overcome such liquid washout and consequent
70 membrane degradation, solutions such as membrane gelation (achieved via
71 the blending of ILs with polymers) have been tested [22]. As gelling material,
72 the group of Coelhoso [22,23] applied gelatin, which is a cheap and widely
73 available biopolymer. This example is a good indication of the potential that
74 naturally-occurring components can have in SILM development.

75 In addition to membrane integrity, the biocompatibility of ILs should be of
76 concern too, as it may significantly affect longer-term activity of enzyme mixed
77 and immobilized in it [24]. In fact, Martins et al. [16] have also underlined that
78 biocompatible and environmental-friendly ILs can be favored for SILM
79 synthesis. It was noted in previous works that small quantities of CA enzyme
80 (0.1 mg/g IL) [16,23], even in partly-purified form after recovering it from
81 biomass [15] can work and effectively shuttle CO_2 across the SILM membrane.
82 However, to our knowledge, the time-dependent change of CA activity in ILs
83 has not been monitored so far.

Given that SILM durability can be influenced by the above-referred structural and biological impacts, the aim of this study were two-folded. Firstly, we have assessed the IL-CA interactions as a crucial parameter of membrane lifetime employing [Bmim][NTf_2], which was used for the preparation of enzymatically-boosted SILMs in our previous investigation [15]. Secondly, CA-containing membranes gelated with pectin – a natural biopolymer found in plants [25] – were evaluated against pressure-resistance, followed by gas permeation tests carried out with pure (CO_2, N_2) and mixed (CO_2 – N_2) gases.

As far as we know, this is the first report on the behavior and use of CA-enriched, pectin-containing membranes for CO_2 separation and hence, the information delivered can be novel enough and helpful for the international research community of membraneologists.

2. Materials and methods

2.1. Enzyme and chemicals

Throughout the experiments, the CA enzyme purchased from Sigma-Aldrich, USA – product ID: C2624, purity: >95 %, specific activity: >3500 Wilbur-Anderson (W-A) unit mg^{-1} protein – was used. The ionic liquid, 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([Bmim][NTf_2], purity: >99 %) was obtained from Io-Li-Tec, Germany. Pectin (type: Pectin Amid CU 025; degree of esterification and amidation is 29 % and 23 %, respectively; galacturonic acid content: 89 % according to the certificate of analysis

provided by the manufacturer) was ordered from Herbstreith & Fox KG, Germany. Although a huge variety of pectin is available on the market, this one was specifically chosen for the experiments since it does not contain sugars, which can be considered as an advantageous property from the microbiological stability viewpoint of the gels prepared with it. $CaCl_2$ x 2 H_2O was the product of Sigma-Aldrich, USA.

2.2. Enzyme activity assays

Basic procedure. The activity of CA (EC number: 232-576-6) was determined in W-A unit mg^{-1} enzyme. To conduct the measurements, a stock enzyme solution (SES) (2 mg CA mL^{-1}) had to be first prepared using Tris-HCl buffer (0.02 M, pH = 8.3). Thereafter, 20 µL SES was diluted (D-SES) to 10 mL with Tris-HCl buffer (0.02 M, pH = 8.3). Afterwards, 14 mL Tris-HCl buffer (0.02 M, pH = 8.3) was mixed with 1 mL D-SES in a reaction vessel (thermostated to 0 ^oC) and 6 mL substrate solution (CO_2-saturated distilled water) was added simultaneously. The whole container was continuously stirred at 450 rpm with magnetic bar. Once the reaction mixture was complete, the time needed for 1 unit of pH fall (in the range of 8.2-7.2) was measured by stopwatch. Complementary tests were also performed under enzyme-less circumstances. The W-A unit was delivered from the times elapsed under the two conditions (with and without CA enzyme) according to the formula

130 introduced in our previous paper [15]. This was then normalized by the mass
131 of enzyme in the reaction mixture to get the values in W-A unit mg^{-1} enzyme.
132 *Modified procedure I.* The *Basic procedure* was adopted with some
133 alterations to check CA activity in the membranes prepared. The membranes
134 were cut to 4 x 4 mm pieces, some of which was placed to the reaction vessel
135 together with 15 mL Tris-HCl buffer (0.02 M, pH = 8.3) and 6 mL substrate
136 solution.
137 *Modified procedure II*. The *Basic procedure* was adopted with some
138 changes to reveal the effect of [Bmim][NTf_2] ionic liquid on the CA enzyme
139 activity. During these experiments, 9 mL [Bmim][NTf_2] ionic liquid was mixed
140 with 1 mL SES, giving a mixture referred as IL-SES. Next, the enzyme activity
141 was measured every 5 minutes for a couple of cycles. To do so, 3 mL of the
142 IL-SES was transferred to 12 mL Tris-HCl buffer (0.02 M, pH = 8.3),
143 supplemented with 6 mL substrate solution and the time required for 1 unit of
144 pH drop (from 8.2 to 7.2) was recorded in order to compute the corresponding
145 W-A unit mg^{-1} enzyme, as mentioned before. Additional test were run under
146 enzyme-less circumstances.
147
148 **2.3. Membrane preparation**
149
150 Porous, hydrophilic, cellulose acetate membrane (pore size: 0.2 μm,
151 porosity: 60 %, thickness: 120 μm, Sartorius AG) with 5.6 cm diameter was
152 placed to a Petri-plate and then it was moved to a vacuum desiccator for 30

minutes. This was followed by two consecutive steps: (i) filling 2 mL SES to the membrane surface/pores and (ii) 30 minutes of vacuum again. As the time expired, a mixture of 4 mL pectin solution (0.25 wt%) and 140 µL $CaCl_2$ solution (1 wt%) was distributed as equally as possible on the surface of the membrane. Another 30 minutes was allowed to achieve partial gelation. In the last stage, the membrane was taken out of the desiccator and forced between 2 glass panes to (i) remove excess pectin that did not strongly bind to the membrane pores and (ii) finish the gelation process.

Afterwards, activity, stability and gas permeation tests on the membranes could be performed. Besides these membranes containing the CA, additional ones lacking the enzyme were made too for comparison. Based on weighing, the reinforcement by pectin resulted in an average gain of of 400-500 mg (on wet basis) for the freshly made membranes. Furthermore, the thickness of the pectin/cellulose acetate membranes was 160 ± 30 µm.

2.4. Gas permeation device

The gas permeation experiments were carried out in a two-chamber permeation apparatus, including a permeation cell that hosts the membrane [19].

In the course of single gas tests, both (the feed and permeate) chambers of the permeation cell were purged with the given gas, followed by setting the pressure on the feed and retentate sides to 1.7 bar(a) and 1 bar(a),

176 respectively. Similar driving force (~0.7 bar) was applied by Neves et al. [26],
177 as well.

178 Under these conditions, once the chambers were closed, the gas started
179 to pass from the higher pressure to the lower pressure compartment. This
180 progress (pressure equalization) was monitored by pressure transducers on
181 both sides as the function of time by in LabVIEW. A typical time profile of the
182 permeation experiments is displayed in **Fig. 1**. The (pressure vs. time) data
183 were first processed by the methodology described in the paper of Neves et al.
184 [17], Afterwards, the permeability (p) of each gas component was converted to
185 Barrer (10^{-10} cm^3 (STP) cm cm^{-2} s^{-1} $cmHg^{-1}$). The theoretical selectivity was
186 calculated as the ratio of gas permeabilities (p_i/p_j, where $p_i>p_j$), similar to our
187 earlier article [19].

188 During binary gas experiments with CO_2/N_2 mixtures, feed and
189 permeation chambers were initially flushed with N_2 and then closed. This step
190 ensured that this particular gas had the same, 1 bar(a) pressure everywhere
191 inside the cell. Thereafter, carbon dioxide was loaded to the feed compartment
192 until a total pressure of around 1.7 bar(a) (0.7 bar(a) of CO_2 plus 1 bar(a) of
193 N_2) was observed. At that point, because of the partial pressure difference of
194 CO_2 between the sides (referred as the driving force), this molecule could
195 begin the migration into the permeate chamber, while no transport of N_2
196 (background gas) had to be considered because of the equal nitrogen partial
197 pressures on both membrane sides [27,28].

The CO_2 (commercial grade) and N_2 (>99.9 % purity) were products of Linde, Hungary. The permeation cell was thermostated at 37 ^oC.

3. Results and Discussion

3.1. Enzyme activity and its change in the presence of [Bmim][NTf_2] ionic liquid

The initial activity of the free CA enzyme was determined to be 3580 W-A unit mg^{-1} protein by following the procedure introduced in Section 2.2. This, in the light of the data indicated by the manufacturer (3500 W-A unit mg^{-1} protein), proved that the enzyme assays worked properly and the results obtained could be considered quite reliable, similarly to our previous study with biomass-derived CA enzyme preparation [15].

In case of the CA enzyme immobilized in the pectin-reinforced membrane, the initial activity measured was 9 W-A unit according to the modified procedure I in Section 2.2.. This, by taking into account the membrane surface corresponds to 1838 W-A unit m^{-2}, confirming that the CA was efficiently immobilized in the membrane.

So far, there has been an agreement in the literature studies that boosting the CO_2-separation in SILMs does not necessarily require great CA enzyme loadings. In recent investigations of Portuguese scientists, SILMs were successfully designed with as low as 0.1 mg CA/g IL enzyme

concentration [16,17], while Bednár et al. [15] demonstrated the appropriate performance of SILMs containing partly-purified CA enzyme preparation, obtained after plant biomass processing. Though longer-term experiments revealed the good time-stability of the enzymatically-accelerated membranes [15], no information regarding possible deterioration of CA activity in the presence of IL has been reported.

Following the modified procedure II in Section 2.2, we attempted to take a look into the enzyme-IL interactions. It turned out from the results that considerable loss of CA enzyme activity can be induced by the [Bmim][NTf_2] ionic liquid. Even as short contact time as 5 minutes caused an extreme, more than 90-95 % drop of relative enzyme activity. However, in accordance with measurements carried out after 10 and 15 minutes, stabilization of values could be noticed at around 0.5 % compared to the initial value.

From these observations, it would appear that depending on the properties of the ionic liquid, quick and notable inhibition/deactivation of the enzyme may take place and this phenomenon should be taken into consideration for process design. Supportive conclusions were made in our recent paper on the enzymatic hydrolysis of cellulose in the presence of [bmim][Cl] ionic liquid [24]. Nevertheless, even if only a smaller portion of the CA enzyme is preserved in an active form with time, it seems still be capable of doing the job that it needs to and facilitate CO_2-transport across the membrane. This might be attributed to the extremely high turnover number of CA (indicating the number of substrate molecules that is converted to product

through the catalytic site of particular enzyme within a given time period), which is reportedly around the magnitude of 10^6 s^{-1}, making it one of the most efficient enzymes in nature and a plausible candidate for biocatalytic CO_2 capture and sequestration [4]. This characteristic, at least for a certain degree, may compensate for the threat of rate-limitation in CO_2-transfer when the number of active enzyme molecules decreases with time in the membrane. These results and considerations help to speculate why the performance of SILMs used in our previous work [15] demonstrated good time-stability (in terms of CO_2 and N_2 permeations) thorough a 4 week period. In brief, it can be supposed that the spinach-derived CA enzyme preparation initially underwent a remarkable activity loss due to the presence of [Bmim][NTf_2], but despite, the residual number of working enzyme was still satisfactory to assure the enhanced CO_2 permeability and concomitantly higher CO_2/N_2 selectivity compared to the non-biocatalytic (control) membranes.

As the stability of the CA was concerned, in another set of experiments (where 1 mg CA enzyme – dissolved in 0.02 M Tris-HCl buffer, pH=8.3 – was entrapped in pectin beads) it was sought if the immobilization of enzyme in the pectin gel itself causes any notable drop of its beneficial properties (expressed as W-A unit/mL of pectin solution (2.5 wt%) in which CA was mixed and subsequently used for gelation in $CaCl_2$ (2.5 wt%) by allowing 12 h hardening time at slightly acidic pH). As a result, 13.1 W-A unit/mL pectin could be initially noted (according to modified procedure I in Section 2.2.) on the first day. Afterwards, although there was some loss of activity too with the time

elapsed, it was definitely much more less significant compared to that noticed in the presence of [Bmim][NTf_2]. In fact, after 3 weeks (during which beads were stored at 4 ^oC in 0.02 M Tris-HCl buffer, pH=8.3), the residual enzyme activity was still nearly 70-80 % of the initial. This experience that the majority of CA activity could be preserved for a longer time correlates well with our recent findings using free, biomass-derived CA enzyme preparation [15]. Accordingly, the application of pectin was not considered harmful for the CA enzyme.

3.2. Stability of pectin-containing membranes

Bubble-point porosimetry was applied to test stability of the membranes, in terms of their resistance against pressure. This technique enables the user to determine the pressure that exceeds the capillary attraction of a liquid in the biggest pore of a porous material [29]. During the measurements, the pressure of a gas (here N_2) is stepwise increased on the feed side of the membrane until a critical pressure (P_r) is reached, where the bubbles appear on the other side via the largest pore of the wetted material. This means in other words that the flux of the gas below P_r is negligible.

For the membranes reinforced with pectin in accordance with Section 2.3., the value of P_r was obtained as 7.2 bar. This, in comparison with the pectin-free control, presented a nearly 3-fold increase of pressure resistance. Therefore, it can be assumed that the pectin-supported membranes developed

290 in this work can be suitable for higher pressure gas separation task (>0.2 MPa
291 transmembrane pressure difference), where conventional SLMs normally fail
292 due to the instable membrane structure [22]. In our future investigation, such
293 tests will thus be designed to evaluate CO_2-separation under such conditions.

294 In previous works of the literature, various SLMs were manufactured
295 using ionic liquid and natural gelling agent i.e. gelatin [23]. It was found after
296 taking stress-strain curves that membranes prepared only with gelatin (on
297 porous cellulose support) reflected better mechanical properties (stress
298 tolerance) than those containing both gelatin and IL (called Ion-Jelly®
299 membranes). Moreover, gelatin-cellulose membranes could be characterized
300 by an increased stiffness (based on the Young modulus) in comparison with
301 the IL-containing ones [23].

302

303 **3.3. Gas separation performance of pectin-reinforced membranes**
304 **prepared with CA enzyme and lacking ionic liquid**

305

306 As it was inferred in Section 3.1. that [Bmim][NTf_2] can cause the severe
307 deterioration of CA enzyme activity, we aimed to study how the CA-boosted,
308 pectin-supported membranes behave and perform in the absence of this IL
309 during the permeation of pure as well as binary gases.

310 The results of gas permeation experiments are depicted in **Fig. 2**,
311 according to which in case of the non-biocatalytic, pectin-containing cellulose
312 acetate membranes the permeability of CO_2 was an order or magnitude higher

313 than that of N_2 (55 and 1.6 Barrer, respectivetly), which can be ascribed to
314 their distinct solubility and diffusivity traits. Furthermore, it should be also noted
315 that under pure/single gas conditions, no effect was taken on N_2 permeability
316 by the presence of CA enzyme (1.6 vs. 1.7 Barrer). On the other hand, CO_2
317 permeability could increase significantly, from 55 to 93 Barrer. These
318 outcomes match well with those trends communicated by Neves et al. [17],
319 where it was found that both N_2 solubility and diffusivity (the two parameters
320 that determine the permeability) remained unaffected by CA enzyme.
321 Nevertheless, CA does able to positively influence CO_2 solubility coefficient
322 [17], providing an explanation about the mechanism that could play a key-role
323 in the improvement of the theoretical CO_2/N_2 selectivity (from 34 to 54 in the
324 presence of CA enzyme).

325 It is also noteworthy that besides ionic liquid (in more general, solvent)-
326 dependent enzyme inhibition/deactivation that may occur (Section 3.1.), the
327 water activity in the membrane is also a factor that can affect the biocatalyst
328 stability and efficiency [15-17]. Hence, its variation (i) from system to system
329 and (ii) with time is a possible reason leading to altered CO_2-separation
330 performance. Thus, it means that an exact comparison of the already
331 published literature might be done only for results obtained under standardized
332 circumstances, in particular in terms of water activity (a_w). Although in this work
333 a_w was not determined, we can suppose that it was quite high based on the
334 report of Basu et al. [30], where it was deduced that in case of low methoxyl
335 pectin (esterification degree < 50 %, which criteria is satisfied by the pectin

used in our work (29 %), as it can be seen in the Materials and methods) the equilibrium moisture content (g water/g dry matter) and water activity are interdependent. In fact, it was inferred by Basu et al. [30] that higher equilibrium moisture content will be accompanied by higher water activities in a wider range of temperature (30-70 ^{o}C). Since in the current paper the ratio between the mass of water and the mass of dry pectin was most likely above 0.3-0.5 at 30-40 ^{o}C (the interval where the temperature of gas separation tests falls), a_w in the pectin-reinforced membranes may have approached to the vicinity of 1.

Regarding the mixed gas tests conducted, it would appear that CO_2 permeability under these conditions was slightly enhanced from 93 to 102, using nitrogen as background gas. Though N_2 permeation between the cells was not considered (as described in Section 2.4), certain interactions between CO_2 and N_2 may have occurred inside the membrane related to nitrogen dissolved in the membrane material (cellulose acetate support as well as pectin matrix). However, we should also point to the fact that the approx. 10 % difference between pure- and mixed-gas CO_2 permeabilites may arise from experimental uncertainties, as it is more or less the confidence interval of the permeation measurements. Besides, it had been drawn by Scovazzo et al. [31] that mixed-gas selectivities in SILMs can be similar to those obtained with pure gases. These altogether suggest that further experimentation will be required (applying more gases i.e. H_2, CH_4 and their mixtures with CO_2) to unambiguously decide whether the observed differences of CO_2 permeability

359 under single- and binary-gas conditions are remarkable, and should stand in
360 the scope of our next work on pectin-containing, biocatalytic membranes.

361 To demonstrate how the membrane performances fit to the recent
362 trends, the pectin-reinforced gas separation membranes prepared with/without
363 CA enzyme are illustrated against the Robeson upper-bound [32] in **Fig. 3**. As
364 one can observe, this is a double logarithmic relationship, correlating how the
365 CO_2/N_2 selectivity changes as a function of faster compounds (CO_2)
366 permeability. We can see that the enrichment with CA was able to push the
367 separation properties towards the upper-bound line, but further research is still
368 needed for more attractive gas separation behavior of pectin-supported
369 membranes.

370 So far, as it appears in **Fig. 1**, the permeation experiments were
371 performed in rather short-terms (supposing that no significant dry out of the
372 membranes occurred in the closed test cell). However, in longer terms, it is
373 important to note that an issue may arise due to the evaporation of solvent
374 (water) from the aqueous supported membrane when the membrane is
375 coupled to a real gas separation process. In these cases, when the
376 membranes are to be used to separate for example biologically produced gas
377 mixtures (i.e. biohydrogen, biogas), it can be assumed that the humidity
378 content of such gaseous streams (that are generated in a bioreactor via
379 fermentation) would allow the prevention of this undesired phenomena.
380 Therefore, in the continuation of this research, measurements will be
381 dedicated to study this subject.

Conclusions

In this work, pectin-reinforced gas separation membranes containing carbonic anhydrase enzyme were prepared and studied. The results presented that the CA can lose majority of its initial activity in the presence of [Bmim][NTf_2] ionic liquid as a solvent candidate for supported membrane fabrication. Moreover, the pectin-containing membranes (lacking the ionic liquid possessing adverse effect on the biocatalyst) could be characterized with improved resistance towards higher transmembrane pressure conditions. The use of CA enzyme facilitated CO_2 permeation, and as a result, markedly enhanced CO_2/N_2 selectivity was achieved.

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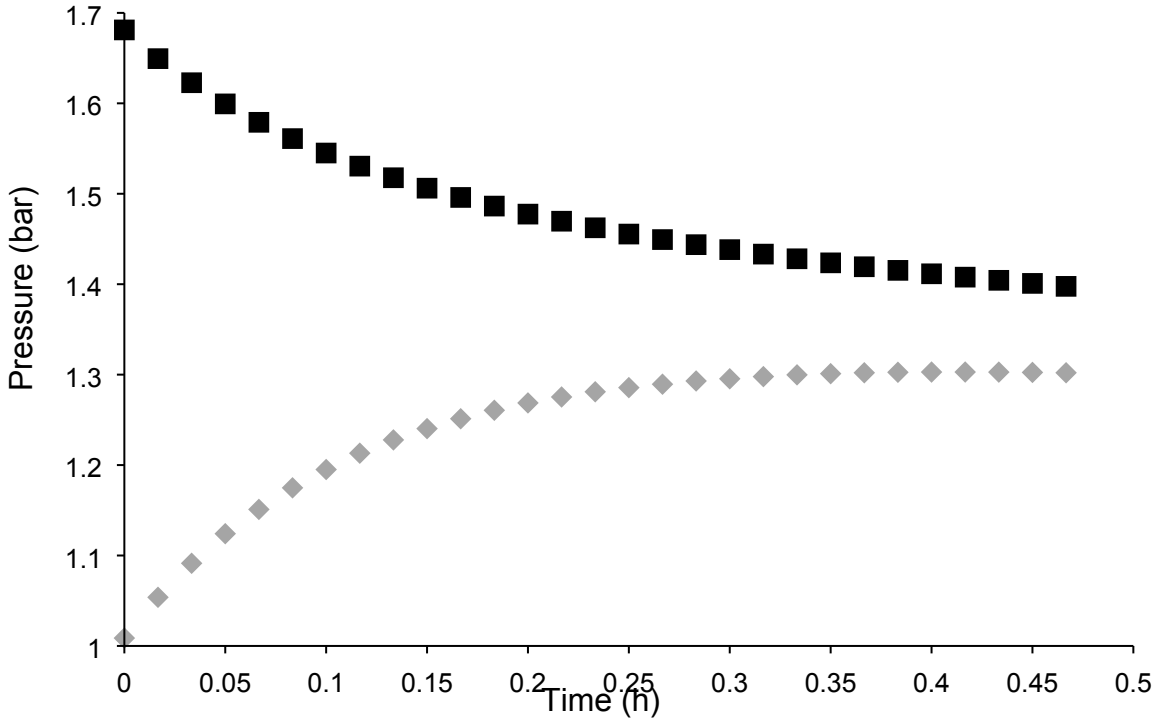
Figure legend

Fig. 1 – Progress curve of a typical gas permeation experiment. Square and diamond symbols represent the pressure in the feed and permeate cells, respectively.

Fig. 2 – Single/mixed gas permeabilites and CO_2/N_2 selectivity in pectin supported membranes with/without CA enzyme

Fig. 3 – The dependence of CO_2/N_2 selectivity on CO_2 permeability. Diamond and star symbols stand for the pectin-supported membranes with and without CA enzyme, respectively. The scattered line represents the Robeson upper-bound for polymeric membranes [32].

519 Fig. 1



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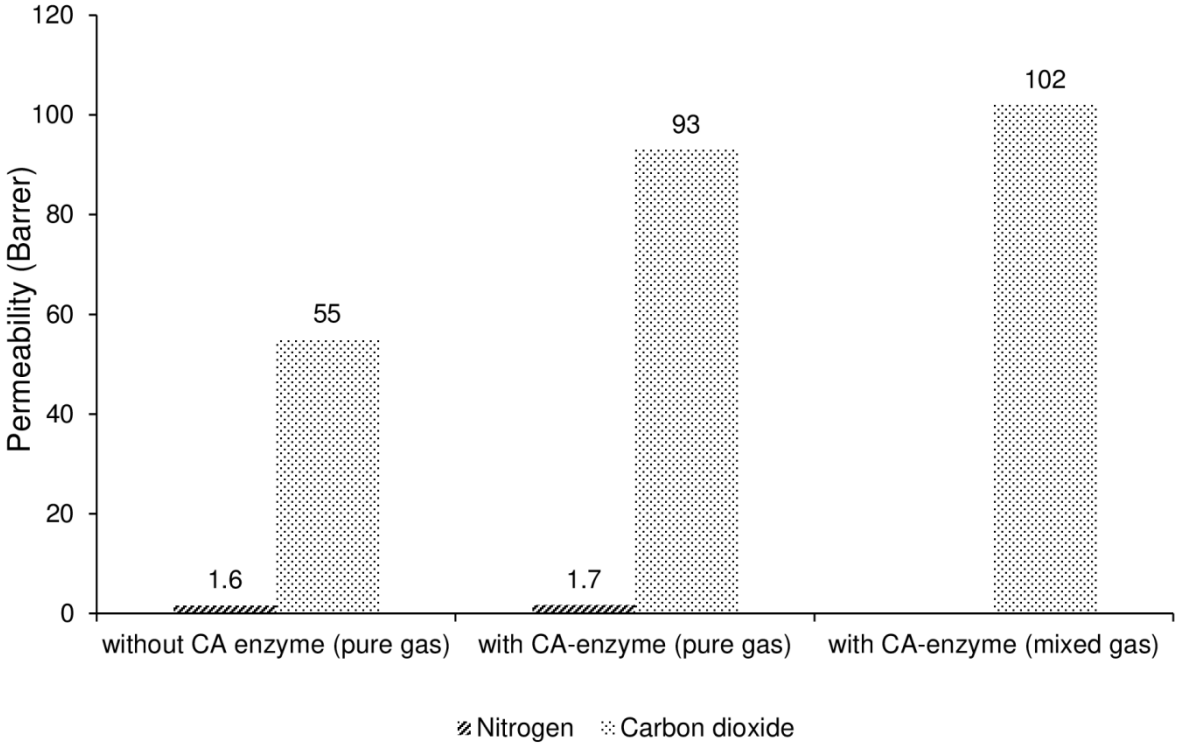
522 Fig. 2



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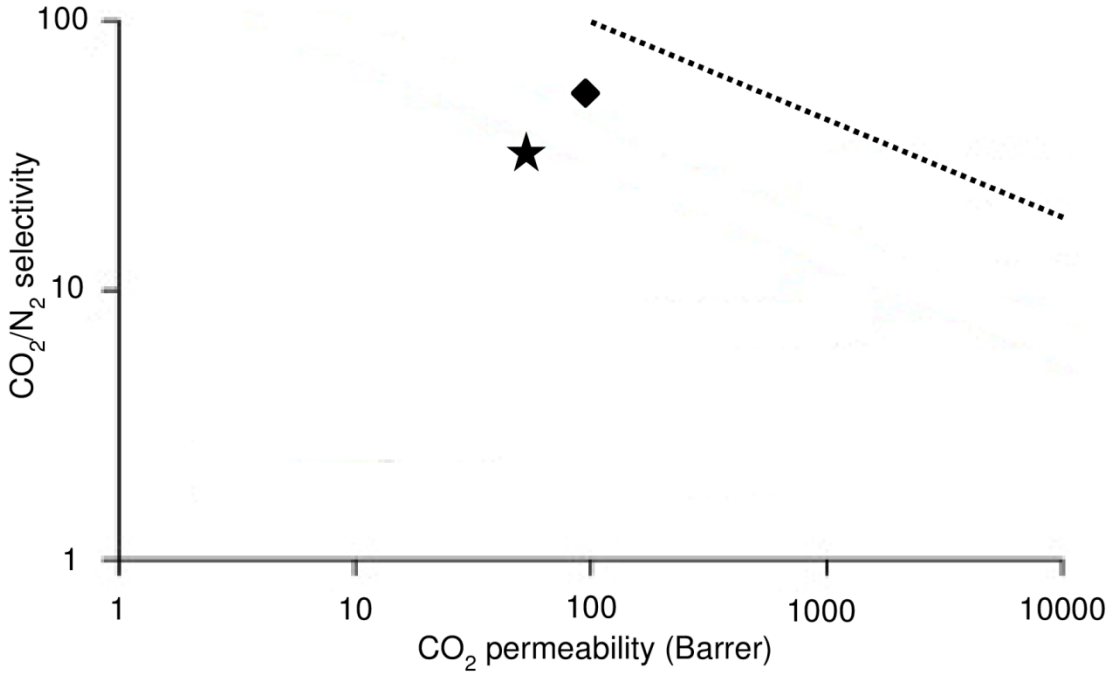
525 Fig. 3



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