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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.

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

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

In addition to membrane integrity, the biocompatibility of ILs

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should be of concern too, as it may significantly affect longer-term activity of enzyme mixed and immobilized in it [24]. In fact, Martins et al. [16] have also underlined that biocompatible and environmental-friendly ILs can be favored for SILM synthesis. It was noted in previous works that small quantities of CA enzyme (0.1 mg/g IL) [16,23], even in partly-purified form after recovering it from biomass [15] can work and effectively shuttle CO_2 across the SILM membrane. However, to our knowledge, the time-dependent change of CA activity in ILs 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. $CaCl_2 \times 2\,H_2O$ was the product of Sigma-Aldrich, USA.

2.2. Enzyme activity assays

2.2.1. 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 °C) 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 introduced in our previous paper [15]. This was then normalized by the mass of enzyme in the reaction mixture to get the values in W-A unit mg^{-1} enzyme.

2.2.2. Modified procedure I

The *Basic procedure* was adopted with some alterations to check CA activity in the membranes prepared. The membranes were cut to 4 × 4 mm pieces, some of which was placed to the reaction vessel together with 15 mL Tris-HCl buffer (0.02 M, pH = 8.3) and 6 mL substrate solution.

2.2.3. Modified procedure II

The *Basic procedure* was adopted with some changes to reveal the effect of [Bmim][NTf_2] ionic liquid on the CA enzyme activity. During these experiments, 9 mL [Bmim][NTf_2] ionic liquid was mixed with 1 mL SES, giving a mixture referred as IL-SES. Next, the enzyme activity was measured every 5 min for a couple of cycles. To do so, 3 mL of the IL-SES was transferred to 12 mL Tris-HCl buffer (0.02 M, pH = 8.3), supplemented with 6 mL substrate solution and the time required for 1 unit of pH drop (from 8.2 to 7.2) was recorded in order to compute the corresponding W-A unit mg^{-1} enzyme, as mentioned before. Additional test were run under enzyme-less circumstances.

2.3. Membrane preparation

Porous, hydrophilic, cellulose acetate membrane (pore size: 0.2 µm, porosity: 60%, thickness: 120 µm, Sartorius AG) with 5.6 cm diameter was placed to a Petri-plate and then it was moved to a vacuum desiccator for 30 min. This was followed by two consecutive steps: (i) filling 2 mL SES to the membrane surface/pores and (ii) 30 min 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 min 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 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), respectively. Similar driving force (~0.7 bar) was applied by Neves et al. [26], as well.

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

During binary gas experiments with CO_2/N_2 mixtures, feed and permeation chambers were initially flushed with N_2 and then closed. This step ensured that this particular gas had the same, 1 bar(a) pressure everywhere inside the cell. Thereafter, carbon dioxide was loaded to the feed compartment until a total pressure of around 1.7 bar(a) (0.7 bar(a) of CO_2 plus 1 bar(a) of N_2) was observed. At that point, because of the partial pressure difference of CO_2 between the sides (referred as the driving force), this molecule could begin the migration