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Short communication

Enzymatically-boosted ionic liquid gas separation membranes using carbonic anhydrase of biomass origin

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HIGHLIGHTS

- Carbonic anhydrase from spinach was used to fabricate supported ionic liquid membranes.
- The enzyme preparation facilitated CO_2 transport and separation.
- The membranes showed good time-stability for multiple cycles.

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ABSTRACT

Nowadays there is a huge demand for new and sustainable technologies aiming the reduction of the greenhouse gas, in particular carbon dioxide emission. In this work, *enzymatically-boosted supported ionic liquid membrane* (EB-SILM) was developed to permeate carbon dioxide with improved efficiency. Firstly, the selected biocatalyst, *carbonic anhydrase* (CA) was prepared and purified from spinach, a cheap plant biomass containing the enzyme of our interest. Afterwards, the CA enzyme preparation was used for SILM fabrication in order to test the properties towards enhanced carbon dioxide permeation over CH_4, H_2 and N_2. The results indicate basically that EB-SILMs possess an increased ability to permeate CO_2 in comparison with enzymeless controls and therefore, may be viewed as a promising approach e.g. towards enhanced CO_2-capture bioprocesses.

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1. Introduction

Reducing carbon emissions is an urgent task [1], where membranes could play an important role [2]. Among them, those made with ionic liquids (ILs) are potential candidates for the selective removal of CO_2 from gaseous mixtures [3–5]. Recently, the significant CO_2 absorption capacity of ILs – consisting of imidazolium-cathion ($C_n$mim) and [Tf_2N]-anion – was confirmed [6,7]. Additionally, a specific enzyme, called *carbonic anhydrase* (CA) (E.C. 4.2.1.1.) was introduced as a promising option to develop biological CCS method [8]. CA is able to catalyze the reversible hydration of CO_2 [9]:

$$CO_{2(aq)} + 2H_2O \rightleftharpoons HCO_3^- + H_3O^+ \quad (1)$$

Moreover, Neves et al. [10] reported that the performance of supported ionic liquid membranes made of imidazolium-based IL with [Tf_2N]-anion could be improved by CA addition. However, to our knowledge, this *membrane-ionic liquid-enzyme* system was studied only by applying highly-purified, commercially available CA, which is extremely expensive since blood is mainly used as its source in health care applications. Nevertheless, CA can also be found in cheaper resources such as green plants and some works already demonstrated recovery of CA from biomass [11,12]. Therefore, in this research it was aimed to (i) develop a method for CA enzyme preparation from plant origin and (ii) use it in SILM – incorporating [bmim][Tf_2N] as a model IL – to take at least one further step towards CO_2 separation from gaseous effluents and more attractive bioprocesses.

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2. Materials and methods

2.1. Preparation of carbonic anhydrase enzyme

CA enzyme was prepared from fresh spinach leaves (*Spinacia oleracea*) bought from local market (stored at −20 °C until use). The other compounds used were Tris-HCl (Calbiochem) buffer, ethanol, NaOH and ammonium sulfate (Reanal, Hungary) having analytical purity.

Firstly, 400 g spinach leaves were pulled to pieces and put in a kitchen blender. In the device, the biomass was mixed with 96 (m/m)% ethanol (1 mL/g spinach) and chopped (500 W, 5 min). When shredding was done, vacuum filtration (FT-3-104-150 quantitative filter paper, Sartorius AG) was used to remove liquids. Thereafter, the remaining solid fraction (the filtration cake) was transferred to a beaker, fresh alcohol was added (same amount as for chopping) and the mixture was stirred (150 rpm) for 20 min at room temperature (23 ± 2 °C). As the time expired, the mixture was vacuum filtered again. This solid-liquid extraction was repeated for 5 cycles during which alcohol-soluble compounds e.g. pigments, oils, etc. were separated, meanwhile the proteins released after cell disruption (including CA) were aggregated with the cell debris in a denatured form. The alcohol fractions removed after the cycles were collected and regenerated.

In the following stage of downstream, the pulpy fraction was soaked in distilled water (1 mL/g spinach) for 12 h at 4 °C. Subsequently, the liquid phase was taken and centrifuged (12,000 rpm, 20 min). After that, the supernatant (containing our enzyme of interest) was dried at 40 °C under vacuum by a Heidolph VV2000 Rotadest. The obtained solid residue was dissolved in 60 mL Tris-HCl buffer (0.02 M, pH = 7.6) and the solution was then gradually saturated with $(NH_4)_2SO_4$ at 0 °C to cause the fall-out of the proteins. Firstly, at 30% $(NH_4)_2SO_4$ saturation level, undesired (contaminating) proteins were salted out and removed. Then, by further increasing $(NH_4)_2SO_4$ concentration and reaching 50% saturation in the solution, a protein fraction with the highest CA enzyme activity was precipitated. This precipitated substance was centrifuged (12,000 rpm, 10 min), dissolved in 40 mL Tris-HCl buffer (0.02 M, pH = 7.6) and subsequently dialysed to remove salts and other pollutants (e.g. ammonium sulfate residues). Dialysis has been done in diffusion dialysis bag (made of DEAE-cellulose). The sack containing the 40 mL enzyme solution was placed in a bucket filled with 10 L Tris-HCl buffer (0.02 M, pH = 7.6) (continuous stirring, room temperature). The conductivity in the dialysate was followed and the process was considered done once equilibrium was reached. Based on gravimetric analysis, the dialysed enzyme solution could be characterized with a 3.8 mg/mL dry matter concentration. Finally, the dialysed enzyme preparation was dried at 40 °C under vacuum by a Heidolph VV2000 Rotadest and stored in a refrigerator at 4 °C until use.

2.2. Characterization of the enzyme preparation

To determine CA activity, the modified Wilbur-Anderson method [13] was used. The measurements were validated by commercial CA enzyme (C3934) (Sigma-Aldrich, USA).

To test the activity of the enzyme preparation obtained by the process described in Section 2.1, 600 μL enzyme solution – well-defined amount of powdered CA enzyme preparation dissolved in 600 μL Tris-HCl buffer (0.02 M, pH = 8.3) – was added to 14.4 mL (0.02 M, pH = 8.3) Tris-HCl buffer. The mixture was thermostated at 4 °C and mixed vigorously (450 rpm). Thereafter, 6 mL substrate (distilled water saturated with CO_2) was injected and the decrease of pH was recorded in the range of 8.2–7.2 as a function of time. Control tests without the enzyme were also carried out.

The activity (U) can be calculated from the times corresponding to 1 unit of pH decrease, as follows (Eq. (2)):

$$U = \frac{t_0 - t_m}{t_m} \tag{2}$$

where, t_0 and t_m are the times in seconds measured for the control and the enzyme preparation, respectively.

From the activity (U) measured according to Eq. (2), the unit of U mg^{-1} was derived by taking into account the amount of enzyme preparation (mg dry mass) used during the activity measurement.

To confirm the presence of CA, SDS-PAGE was performed on a Cleaver Scientific Ltd, Nano-PAC – 300 gel apparatus with 4% acrylamide stacking gel and 12.5% acrylamide running gel. The samples were treated with SDS and 2-mercaptoethanol before running. The proteins on the SDS-PAGE gels were stained with Coomassie Blue R-250 and visualised by a GelAnalyzer 2010a image analysis software.

2.3. SILM fabrication and gas permeation tests

Firstly, a 5.6 cm diameter circle was cut from hydrophobic Durapore® PVDF microfiltration membrane (Millipore Corporation, USA), placed in a Petri-dish and put in a vacuum desiccator for 1 h to remove the impurities (traces of water). In the meantime, the enzyme preparation (10 mg dried powder dissolved in 50 μL distilled water) was added to preliminary dried [4] 1950 μL [bmim][Tf_2N] ionic liquid (Sigma-Aldrich, USA). To help dissolution and homogenization, vortexing and ultrasound sonication was applied in several steps. Then, the mixture was loaded by a syringe to the surface of PVDF membrane through a septum on the top of the desiccator, and carefully dispersed. To achieve the saturation of pores by the enzyme-water-IL solution, the vacuum inside the desiccator was allowed to grow up to ambient pressure conditions (the pressure increase aids the penetration of the solution into the pores). Gas permeation experiments were conducted in a device shown in Fig. 2, at a stable 40 ± 0.1 °C with single gases (CO_2, H_2, CH_4 and N_2), all of them having >99.9 vol% purity (Linde, Hungary).

In the beginning of each experimental run, the whole test rig (chambers, pipes) (Fig. 2) was flushed with the actual gas (supplied from cylinders) and the initial pressure in the feed chamber was set to 2 bar(a). At the same time, the permeate chamber contained the same gas at 1 bar(a) pressure. The permeation from the upstream- (high pressure) to the downstream (low pressure) compartment was followed by simultaneously measuring the pressure values in both sides. Data were registered in every 2 min until reaching equalized pressure conditions (loss of driving force).

The permeability values were calculated in accordance with the report of Neves et al. [10]. The theoretical selectivity ($S_{A/B}$) is a product of the permeability ratio of two different gases (A and B). Measurements – for performance comparison purposes – were carried

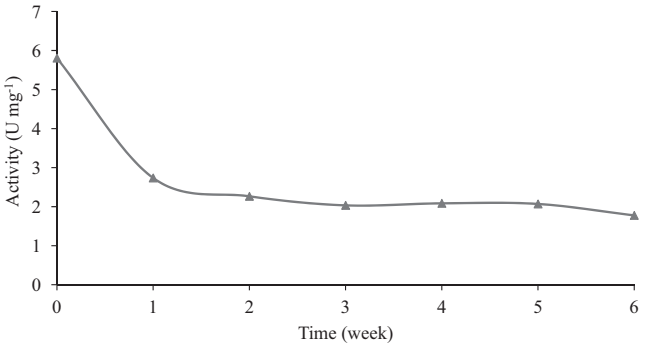

Fig. 1. The stability of the enzyme preparation.

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