Membrane Aerated Hydrogenation: Enzymatic and Chemical Homogeneous Catalysis

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Dedicated to Roger Sheldon on the occasion of his 60th birthday.

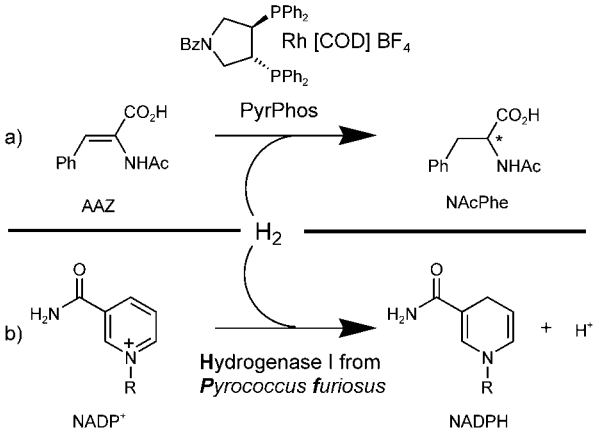
Abstract: Among the most successful systems for homogeneous catalysis, hydrogenation catalysts capable of activating molecular hydrogen, take outstanding roles in research laboratories and in industry. To open up the field of continuous catalytic hydrogenations a novel membrane reactor concept was developed and successfully applied for hydrogenations with dihydrogen both for chemical and for enzymatic catalysis. The hydrogenase I of the archaeon *Pyrococcus furiosus* was utilized for the continuous hydrogenation of $NADP^+$ to NADPH with recycling of the enzyme by means of ultrafiltration. The well known PyrPhos-Rh system was used for the enantioselective synthesis of an amino acid derivative by hydrogenation.

Keywords: asymmetric catalysis; catalyst immobilization; cofactors; enzyme catalysis; homogeneous catalysis; hydrogenation; membranes; reduction

Here we report our most recent efforts to extend the range of feasible reactions in the membrane reactor[1] to hydrogenation with dihydrogen as reducing agent. The direct usage of hydrogen has several advantages over the usage of hydrogen transfer agents like 2-propanol[2] since it constitutes a cheaper and more powerful means of reduction that can be used in large excess and be easily removed, thus not hampering downstream processing.

For this approach a novel reactor concept was developed for the continuous dosage of gaseous reactants *via* a dense polymer membrane. The feasibility of the application of volume-aeration to hydrogenation was investigated for chemical and enzymatic catalysis. We chose the homogeneous hydrogenation catalyst PyrPhos[3] as the chemical representative, whereas the hydrogenase I from the hyperthermophilic archeon *Pyrococcus furiosus* (*Pf*H) illustrates the enzymatic approach.[4] Both catalysts activate hydrogen; the PyrPhos system for the enantioselective reduction of activated double bonds, whereas the *Pf*H is capable of heterolytic cleavage of hydrogen and regioselective 1,4-hydride addition to the oxidized form of the phosphorylated nicotinamide cofactor: $NADP^+$ (Scheme 1). In the case of the enzymatic approach the macromolecular catalyst was recycled by means of ultrafiltration.

Reactor Set-Up: For continuous dosage of dihydrogen in a continuously operated membrane reactor a new set-up was developed. The general scheme is shown in Figure 1. The delivery of gaseous reactants can be achieved by pressure-enhanced diffusion through dense polymer membranes, which has been shown in fluidized bed for animal cell culture.[5] We chose polytetrafluoro-



Scheme 1. Hydrogenation of a) 2-*N*-acetylamidocinnamic acid (AAZ) with PyrPhos and b) $NADP^+$ with the hydrogenase from *Pyrococcus furiosus* (*Pf*H) (for $NADP^+$ and NADPH only the reduced nicotinamide moiety is shown).

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ethylene (PTFE) as the membrane material, combining chemical resistance and relatively high permeability compared with other perfluoro elastomers (data not shown). It was therefore unnecessary to change the aeration membrane for either the enzyme or the chemical catalyst. Furthermore, adsorption phenomena of the enzyme were negligible for PTFE compared to, for example, silicon. The transmembrane pressure drop is the driving force of hydrogen permeation and was controlled by maintaining the inner pressure of the tubular aeration membrane independently from the pressure in the liquid phase with a back pressurizing valve. As a result decoupling of gas and liquid pressure can be obtained within the limits of the burst pressure of the aeration membrane employed. For continuous membrane filtration a closed liquid volume is favorable because the residence time of the reactants can thus be controlled by flow control of the liquid alone, making mass balancing for the gas phase unnecessary. Another advantage is the minimum gas volume needed per liquid volume, which was about 1 to 83. This was achieved by arranging the tubular aeration membrane concentrically inside a stainless steel tube. The module has a surface to volume ratio of 3.8 $cm^2 mL^{-1}$. For the membrane filtration unit a slightly modified version of a previously used membrane reactor[2] was employed as a cross-flow filtration cell (Table 1). As circulation pump a gear pump was applied to establish turbulent flow conditions in the aeration module, thus avoiding concentration gradients over the cross section of the aeration module.

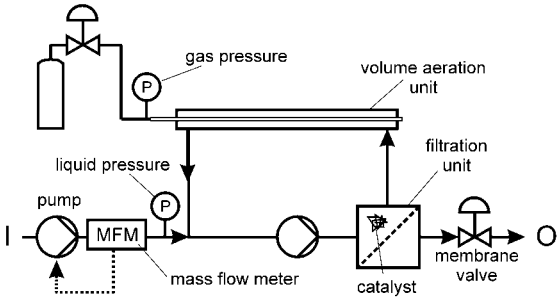

Figure 1. Reactor scheme of the volume-aerated membrane reactor utilized for continuous hydrogenation with the hydrogenase (I = inlet, O = outlet).

Enzymatic Catalysis: The hydrogenase I from *Pyrococcus furiosus Pf*H[4] (E.C. 1.18.99.1) is capable of activating molecular hydrogen for the reduction of the phosphorylated nicotinamide cofactor $NADP^+$ to its reduced 1,4-dihydride form NADPH. The main interest of our investigations is the utilization in cofactor regeneration for biotransformations,[6] since more and more applications with NADPH-dependent enzymes are currently being evolved.[7] Among the general advantages of hydrogen as reducing agent mentioned above, the reduction of the nicotinamide cofactors is an exergonic reaction under physiological conditions, which together with the diminishing product concentration of protons at higher pH values shifts the equilibrium towards quantitative conversion. As in previous investigations[8] we focused on the synthesis of NADPH for the kinetic characterization of the reduction step.

In the volume-aerated membrane reactor, equipped with a commercial hydrophilic ultrafiltration membrane, a continuous experiment was carried out. Figure 2 shows the concentration ratio of NADPH to the sum of $NADP^+$ and NADPH as a function of the number of residence times. A steady-state at a residence time of 2.0 h with almost quantitative conversion was reached. After adjusting the flow rate to the shorter residence time of 1.5 h hydrogen supply proved to be limiting. Raising the differential pressure from 0.9 bar to 1.5 bar compensated for this decrease. The slow decline in concentration ratio to about 0.85 over the period of

Table 1. Technical data of the volume-aerated membrane reactors.

		continuous	batch	
liquid volume				
aeration unit		3.7	110	mL
ultrafiltration unit		10	–	mL
total		25	150	mL
gas volume		0.3	8	mL
liquid/gas volume		83	19	
membrane areas				
ultrafiltration unit		30	–	cm^{-2}
aeration[a]		14	400	cm^{-2}
surface/volume				
aeration	module only	3.8	3.6	$cm^{-2} mL^{-1}$
	total	0.6	2.7	$cm^{-2} mL^{-1}$
ultrafiltration	module only	3.0	–	$cm^{-2} mL^{-1}$
	total	1.2	–	$cm^{-2} mL^{-1}$

[a] Mean logarithmic area

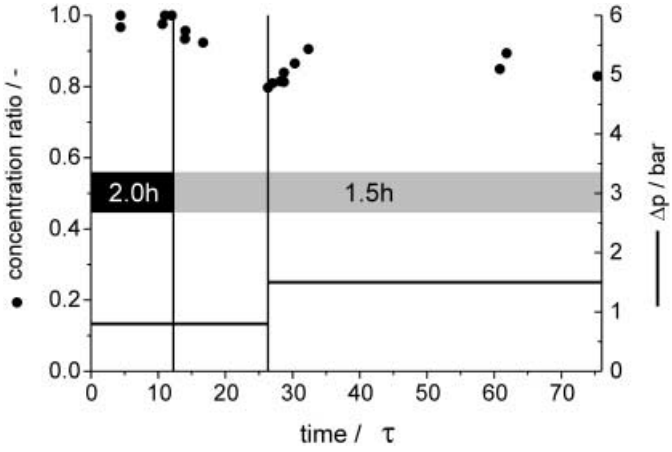

Figure 2. Concentration ratio of NADPH to the sum of $NADP^+$ and NADPH as a function of the number of residence times in the volume-aerated enzyme membrane reactor. Conditions: 1.9 mg mL^{-1} *Pf*H, 12 mmol L^{-1} $NADP^+$, 100 mmol L^{-1} potassium phosphate pH = 8, 40 °C, 5 bar pressure on liquid side, up to 12 residence times $\tau = 2$ h, thereafter $\tau = 1.5$ h.

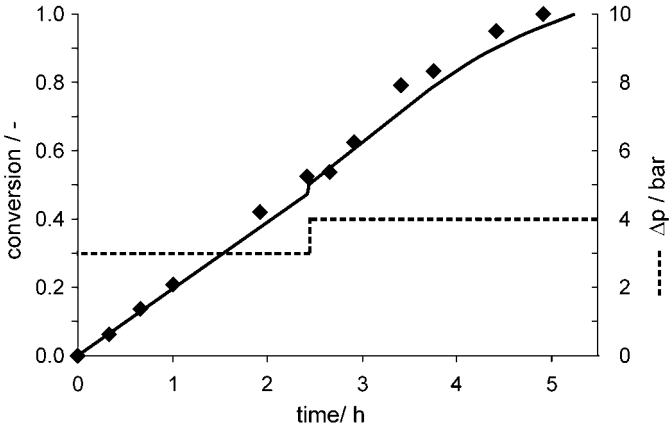

Figure 3. Conversion versus time for the chemical hydrogenation showing the theoretical conversion from mass flow metering of hydrogen (line) and the conversion (diamonds). Conditions: 1.0 mmol L^{-1} PyrPhos, 0.25 mol L^{-1} AAZ, liquid pressure 8 bar; 30 °C.

120 h can be accounted for by deactivation of enzyme. In order to make the degradation effects and the capabilities of the enzyme visible, no stabilizer, as for example the antioxidant sodium dithionite, was added.

In the continuous experiment a space-time-yield of 130 g L^{-1} day^{-1} can be reached. The total turnover number (ttn) of the enzyme was calculated as $66 \cdot 10^3$. This value is the lowest estimate since it was neglected that only approximately one fifth of the protein in the enzyme preparation is active hydrogenase, and that the enzyme is still active after operating the reactor for 70 residence times (120 h).

Chemical Catalysis: A stable macromolecular catalyst was not available for the PyrPhos system, and the retention of the hydrogenation catalyst itself suffers from the low retention of the relatively small molecule.[9] For this reason we used a simplified set-up for the chemical reaction to be performed as a batch, leaving out the ultrafiltration unit. The aeration unit was replaced by a larger one, with a larger membrane area (Table 1). In this set-up a batch reaction, or in respect to hydrogen a fed-batch reaction, was performed. The hydrogen flow necessary to keep the pressure constant on the gaseous side of the membrane was measured. During the reaction the differential pressure was raised from to 3 to 4 bar. The conversion calculated from hydrogen flow matches the conversion estimated by independent analysis from samples (Figure 3). The enantiomeric excess was not altered compared with previous findings (94%)[10] as determined by capillary electrophoresis.[11]

In conclusion, the reactions show the feasibility of the volume aeration concept for chemically and enzymatically catalyzed hydrogenations. In the case of the chemical system a sufficiently retainable and stable catalyst would enable a continuous process, especially as for the PyrPhos system polymer enlarged systems are described.[12] The results obtained with the hydrogenase show the feasibility of the volume-aerated membrane reactor if the catalyst is stable enough for continuous application. The enzyme application is promising, enabling hydrogen to be used as an advantageous reduction equivalent for the reductive generation and regeneration of NADPH.

Experimental Section

General

All reactions and manipulations were performed under an atmosphere of dry argon using standard Schlenk-type techniques. Solutions were degassed by simultaneously passing helium and argon through the solution. *Pyrococcus furiosus* hydrogenase was prepared by an established protocol.[13] PyrPhos was a gift, prepared following a procedure described in literature.[3] $NADP^+$ was obtained from Jülich Fine Chemicals. All other chemicals were obtained from Sigma-Aldrich and were of the best quality available and used without further purification. Conversion and enantiomeric excess were determined by a Beckman Pace/MDQ capillary electrophoresis as described elsewhere,[11] equipped with an uncoated fused silica capillary (Supelco CElectFS25, Sigma-Aldrich, Taufkirchen, Germany) (length to detection window 50 cm, total length 57 cm) in a 125 mmol L^{-1} potassium phosphate buffer at pH = 10.2 containing 25 mmol L^{-1} dimethyl-β-cyclodextrin, 16 °C, 30 kV, migration times: 2-N-acetylaminocinnamic acid (AAZ) 13.4 min, 2-N-acetylaminophenylalanine: *S* 14.1 min, *R* 14.3 min. Nucleotides were analyzed for conversion also by capillary electrophoresis as described elsewhere,[11] equipped

with an uncoated fused silica capillary (CElectFS50, SUPELCO, length to detection window 43 cm, total length 50 cm in a 40 mmol L^{-1} potassium phosphate and 10 mmol L^{-1} borate buffer at pH = 8.5, 40 °C, 30 kV, migration times: uridine (internal standard) 2.9 min, $NADP^+$ 9.1 min, NADPH 12.1 min. Protein content was determined by the method described by Sedmark et al.,[14] by measuring the absorption of the Coomassie Brilliant Blue G250 complex ($\lambda = 620$ nm) against samples of known concentration of bovine serum albumin. Hydrogenase was stored in liquid nitrogen and thawed directly before use under an argon atmosphere, which was replaced with hydrogen before heating to 80 °C for 10 min.

Volume-Aerated Membrane Reactor

The reactor set-up is depicted in Figure 1. All connections were made using metal fittings (Swagelok, Neufahrn, Germany) or if not possible polymer fittings (Upchurch Scientific, Oak Harbor, USA). The dosage pump was an alternating piston pump (P500, Pharmacia, Erlangen, Germany) controlled *via* a personal computer using the signal of the mass flow meter (Bronkhorst, Ruurlo, The Netherlands), as described elsewhere.[2] Circulation flow in the aeration unit was established by a magnet coupled gear pump (Verder, Haan, Germany). The membrane filtration unit was a modified set-up used before[2] equipped with an ultrafiltration membrane (Amicon YM10, Millipore, Eschborn, Germany) and it was at the same time used as heat exchanger. The volume aeration unit was a 6 mm metal tube of 35 cm length. In this tube the PTFE membrane (CS-Chromatographie Service, Langerwehe, Germany) of 1.6 mm outer and 1.0 mm inner diameter was concentrically positioned. Pressure was measured by pressure transmitters (Wika, Klingenberg, Germany). Both liquid and gas pressure were controlled via membrane valves (gas: AGA, Hamburg, Germany; liquid: Jasco, Gross-Umstadt, Germany). The technical data of the set-up is given in Table 1.

Continuous Operation of the Volume-Aerated Membrane Reactor: Enzyme Catalysis

Prior to addition of the catalyst, the reactor was flushed with degassed buffer for at least 5 residence times (reactor volume 25 mL), subsequently with 50 mL of substrate solution. During this time the volume aeration unit was flushed with hydrogen at 2 mL min^{-1}. The reactor temperature was maintained at 40 °C by a thermostatic water bath (Lauda, Lauda-Königshofen, Germany). Prior to addition of the enzyme, 100 mg of bovine serum albumin was added to the reactor *via* the dosing pump, and the enzyme preparation was heated under hydrogen atmosphere for 10 min before adding it to the reactor.[13] The course of the reaction and the other conditions are given in Figure 2. Samples of the outflow were analyzed.

Batch Operation of the Volume-Aerated Reactor: Chemical Catalysis

The aeration unit was replaced by another one with a larger surface area of 400 cm^2. The 10 m of PTFE tubing of the same dimensions used above were coiled onto a hexagonal inner metal block. The total volume was increased to 150 mL. For batch operation the ultrafiltration unit was omitted from the set-up. Prior to reaction, the reactor was flushed with a solution of 25 mol L^{-1} AAZ in methanol (1 L) for ten hours. During this time the volume aeration unit was flushed with hydrogen at 2 mL min^{-1}. The PyrPhos catalyst was dissolved in 5 mL methanol and added *via* the dosing pump (resulting concentration 1 mmol L^{-1}). Throughout the reaction a flow of 1 mL h^{-1} was maintained, and the outflow was analyzed by capillary electrophoresis. The hydrogen flow to maintain constant pressure on the gaseous side was measured by mass flow metering (Bronkhorst, Ruurlo, The Netherlands), and recorded on a personal computer.

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