

# Significant enhancement of structural stability of the hyperhalophilic ADH from *Haloferax volcanii* via entrapment on metal organic framework support

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**ABSTRACT:** The use of an *in-situ* immobilization procedure for the immobilization of hyperhalophilic alcohol dehydrogenase in a metal organic framework material is described. The easy and rapid in situ immobilization process enables retention of activity over a broad range of pH and temperature together with a decrease in the halophilicity of the enzyme. The catalytic activity of the immobilized enzyme was studied in non-aqueous solvent mixtures with the highest retention of activity in aqueous solutions of methanol and acetonitrile. The approach demonstrates that this immobilization method can be extended to hyperhalophilic enzymes with enhancements in activity and stability.

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## 1. Introduction

Halophilic enzymes are of interest in biocatalysis due to their resistance to elevated temperatures, high concentrations of salt, and the ability to retain catalytic activity at extreme pH values and in the presence of organic solvents.<sup>1-2</sup> These enzymes are of interest in applications such as synthesis, food processing and bioremediation, among others.<sup>3</sup> Alcohol dehydrogenase (ADH) is used for the production of chiral alcohols that are used as intermediates in pharmaceutical industries.<sup>4</sup> For example ADH is employed in the preparation of (S)-tert-leucine, an intermediate in the production of the antiviral agent, Atazanavir.<sup>5</sup> Enzymes such as *Haloferax volcanii* can possess advantages such as catalytic activity under extreme conditions of temperature and pH.<sup>6</sup> In particular *Archea* microbes live in extreme environments such as high temperature (55-100° C) and salinity up to 6 M KCl.<sup>7</sup> Enzymes that come from this source are active under the same conditions. The alcohol dehydrogenase from *Haloferax volcanii* (HvADH2) utilises NADP(H) (β- nicotinamide adenine dinucleotide phosphate) as a co-factor (with some activity with NADH-nicotinamide adenine dinucleotide)<sup>8</sup> is stable at high temperature over a broad pH range<sup>9</sup> and retains some activity in the presence of organic solvents including DMSO, MeOH and ACN.<sup>1</sup> However, the operational use of this enzyme in its soluble form can be restricted due to its low stability and difficulties in re-use.<sup>10</sup> HvADH2 can undergo rapidly and irreversible denaturation and dissociates in solution at low concentrations of NaCl or KCl (below 1 M). Immobilization of the enzyme can overcome these disadvantages by increasing the reusability of the system, enhancing enzyme and facilitating product separation and biocatalyst recovery. Previous work demonstrated that HvADH2 can be covalently attached to polymeric beads.<sup>11</sup> A wide range of supports can be used for enzyme immobilisation,<sup>12</sup> including natural<sup>13</sup> and synthetic<sup>14</sup> polymers, and inorganic porous materials.<sup>15-19</sup> The use of metal organic framework materials (MOF) as supports for the immobilization of enzymes has been described recently.<sup>20-22</sup> MOF materials have been used for water adsorption<sup>23</sup> and gas storage<sup>24</sup>, for separation,<sup>25</sup> sensing,<sup>26</sup> heterogeneous catalysis,<sup>27</sup> nanoreactors and drug delivery<sup>28</sup> among others.<sup>29-30</sup> The wide range of MOF structures arises from their topological and compositional versatility.<sup>20, 31</sup> While the microporous nature of MOFs makes it difficult to incorporate large macromolecules such as enzymes, recent studies have demonstrated that proteins can be retained within the cavities of some MOFs.<sup>32-34</sup> A number of reports have focused on developing a general immobilization methodology to encapsulate an enzyme in one simple step.<sup>30, 35-36</sup> Many studies focused on the in-situ encapsulation of enzymes in aqueous system in materials such as zeolite-like structures.<sup>37-40</sup> Our recent work demonstrated that it is possible to encapsulate a range of enzymes (including alcohol dehydrogenase from *Saccharomyces cerevisiae*) in a facile manner using a Fe-BTC MOF material that is structurally similar to commercially available Basolite F300.<sup>41-44</sup> With this approach, unlike other immobilisation methods, enzyme immobilization occurs in situ, at mild pH and at room temperature in a rapid (10 minutes) and facile manner. In this work, we describe the in-situ immobilization of the halophilic HvADH2 in the Fe-BTC MOF. Enzyme specificity, stability and tolerance to organic solvents were systematically studied. This *in-situ* immobilization of the enzyme resulted in increased stability over a wider range of pH and temperature with retention of activity in reuse up to 4 cycles. Electrostatic interactions between the halophilic enzyme and the Fe-BTC MOF could explain the enhancement in activity and decreases in halophilicity of the immobilized enzyme.

## 2. Materials and methods

Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 97%), sodium hydroxide ( $\text{NaOH}$  > 95%),  $\text{KCl}$  (> 99%), trimesic acid ( $\text{H}_3\text{BTC}$  > 95%), alcohol dehydrogenase from *Saccharomyces cerevisiae*, ethanol (96%),  $\beta$ -nicotinamide adenine dinucleotide sodium salt hydrate NADH and phosphate hydrate  $\text{NADP}^+$  (> 99%), acetonitrile (99.8%), dimethyl sulfoxide ACS ( $\geq 99.9\%$ ), methanol anhydrous (99.8%), tetrahydrofuran (> 99%), acetic acid, sodium acetate (anhydrous), citric acid, tri-sodium citrate dehydrate, Tris-base and hydrochloric acid were purchased from Sigma-Aldrich. All reagents were used as received without further purification. De-ionised water (18.2 M $\Omega$  cm) was used for all aqueous solutions.

### 2.1 HvADH2 expression and purification

HvADH2 was expressed as reported in Timpson et al.,<sup>8</sup> single colony of *Haloferax volcanii* containing the pTA1205 vector was inoculated into 5 mL Hv-YPC media and grown at 45 °C over 24 h, and used to inoculate 270 mL Hv-YPC, which was grown a further 24 h. Protein expression was induced by the addition of 5 mM L-tryptophan and the culture shaken overnight. Cells were harvested, lysed by sonication and purified by immobilized metal ion affinity chromatography (IMAC) as reported<sup>8</sup> using a gradient of EDTA to elute. Pure HvADH2 was stored in 100 mM Tris-HCl, pH 8.0, containing 3 M KCl.

### 2.2 Protein concentration determination

The protein concentration of HvADH2 was analysed by Bradford assay<sup>45</sup> using a commercially available Bio-Rad protein assay with bovine serum albumin (BSA) as standard.

### 2.3 Determination of the activity of the Enzyme@MOF

The catalytic activities of ADH from *Saccharomyces cerevisiae* (ScADH) and *Haloferax Volcanii* (HvADH2) were determined spectrophotometrically by measuring the increase in absorbance due to the production of NADH at 340 nm.<sup>46</sup> The activity of HvADH2 and HvADH2@MOF were measured at 50 °C in Tris-HCl buffer (100 mM, pH 10) and KCl (3 M) unless stated otherwise. The reaction mixture was analysed in a quartz cuvette and consisted of 0.25 mL of  $\text{NADP}^+$  (1 mM), 0.01 mL of ethanol (50 mM), and 1.49 mL of buffer Tris-HCl 100 mM (pH 10, 3 M KCl). A 0.1 mL aliquot of the enzyme solution, supernatant or HvADH2@MOF was then added into the reaction mixture and the progress of the reaction was continuously monitored for 10 minutes by measuring the adsorption at 340 nm. The activity of ADH from *S. cerevisiae* was measured at 25 °C, in Tris-HCl buffer 100 mM pH 8 unless stated otherwise. The reaction mixture was analysed in a quartz cuvette and consisted of 0.25 mL of  $\text{NAD}^+$  (1 mM), 0.01 mL of ethanol (50 mM), and 1.49 mL of buffer Tris-HCl (100 mM, pH 8). A 0.1 mL aliquot of the enzyme solution, supernatant or ScADH@MOF was then added to the reaction mixture and the reaction was continuously monitored for 10 minutes by measuring the adsorption at 340 nm and 25 °C. All activity measurements were performed in triplicate and the average value reported.

### 2.4 Temperature effect on the activity of HvADH2@MOF

The activity of HvADH2@MOF was examined over the temperature range from 40 °C to 80 °C, using thermo-bath and Shimadzu spectrophotometer UV-18000 CE 230 equipped with a temperature controller. 10 mg of HvADH2@MOF was resuspended in Tris buffer/solution and an aliquot of 0.1 mL of suspension was used to measure its biocatalyst activity.

### 2.5 pH effect on the activity of HvADH2@MOF

The activity of HvADH2@MOF was examined over the pH range between 2 – 6 (100 mM citrate buffer), pH 7 (100 mM phosphate buffer KCl 3 M), and Tris-HCl (100 mM KCl 3 M) for pH above 7. 10 mg HvADH2@MOF was resuspended in the appropriate buffer and an aliquot of 100  $\mu\text{L}$  of suspension was used for the enzymatic assay. All activity measurements were performed in triplicate and the average value reported.

### 2.6 Ionic strength effect of KCl on the activity of HvADH2@MOF

The activity of HvADH2@MOF was examined in a range of different KCl concentration (0.5/ 1/1.5/3/4 M) after 1 and 24 h in buffer. 10 mg HvADH2@MOF was resuspended in the Tris buffer 100 mM pH 10 at different KCl concentrations and an aliquot of 100  $\mu\text{L}$  of suspension was used for the assay. All activity measurements were performed in triplicate and the average value reported.

### 2.7 Organic solvents effect on the activity of HvADH2@MOF

The activity of HvADH2@MOF was examined in a range of organic solvents. The HvADH2@MOF was resuspended in buffer Tris-HCl (100 mM KCl 3 M) pH 10 with 5% or 10% of organic solvent. The HvADH2@MOF was stored with the buffer/solvent mixture, keeping under stirring and the activity was measured after 24 and 48 hours at room temperature.

### 2.8 In-situ immobilization of HvADH2 in metal organic frameworks

The support material was reported previously<sup>41, 43, 47</sup> and prepared as follows: Solution 1 was prepared by dissolving 0.263 g of trimesic acid ( $\text{H}_3\text{BTC}$ ) in 3.685 mL of  $\text{NaOH}$  1.06 M of pH = 8; solution 2 was made up by dissolving the appropriate amount of enzyme in 6.388 mL of deionized water: solution 3 of pH= 2 was prepared by dissolving 0.508 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 10 g of  $\text{H}_2\text{O}$ .

Solution 2 was added to solution 1, while the pH of the mixture was monitored (pH remained at ca. 7.0). Solution 3 was then added dropwise into the mixture under gentle magnetic stirring. This procedure resulted in the immediate appearance of a reddish brown solid. The resultant suspension was maintained under stirring at room temperature (25 °C) for 10 minutes. The obtained solid was recovered by vacuum filtration and washed with deionised water, and dried at room temperature. Measurements on the effect of temperature, pH, ionic strength and organic solvents were all performed post synthesis, after the formation of the MOF.

## 2.9 Instrumentation

Scanning electron microscopy (SEM) studies were carried out using a HITACHI SU-70. Samples were coated with gold prior the analysis. pH values were measured using a Thermo Scientific Orion 2-star benchtop pH meter. A Shimadzu spectrophotometer UV-18000 CE 230 equipped with a temperature controller was used for activity tests. Dynamic light scattering (DLS) was performed using a Zetasizer Malvern Nano ZSP by dilution (20x) of each sample suspension in de-ionised water.

## 3. Results and discussion

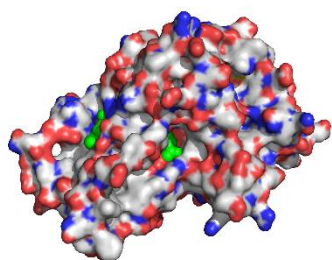


Figure 1. Model of surface residues of HvADH2, with positively charged (blue) and negatively charged side chains (red). The NADP<sup>+</sup> cofactor (green) is buried in the enzyme cleft.

As a halophilic enzyme, the activity and stability of HvADH2 is strongly affected by the concentration of salt, with concentrations of > 3 M KCl are normally required for detecting catalytic activity. Halophilic enzymes usually possess more acidic residues on the surface that are stabilized by the presence of high concentrations of K<sup>+</sup>.<sup>6</sup> Halophilic enzymes possess less hydrophobic amino acids than other enzymes. They are stable at high salt concentrations, a stability that can arise from increased hydrophobic forces resulting from high concentrations of K<sup>+</sup>.<sup>48</sup> The stability of HvADH2 is extremely sensitive to the electrostatic interactions between the amino acid residues and K<sup>+</sup>. In the absence of salt, the tertiary structure of the enzyme is destabilized, resulting in denaturation.<sup>49</sup>

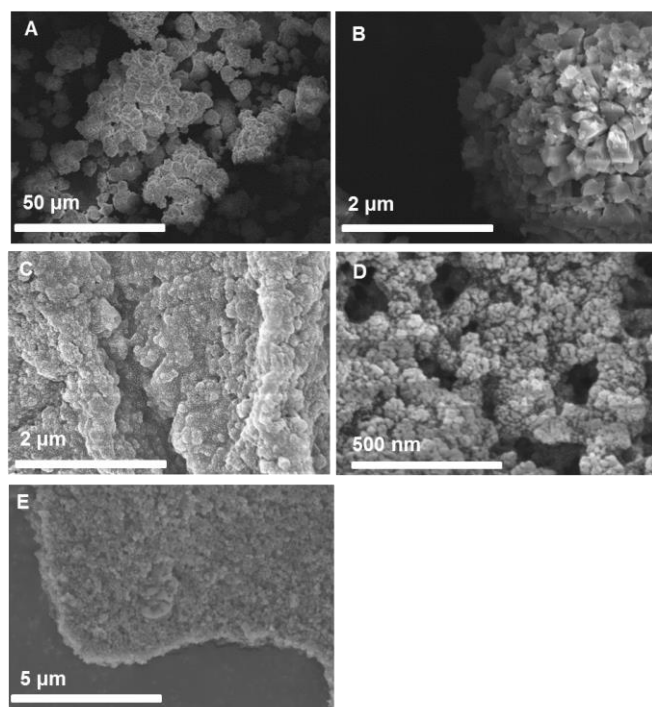


Figure 2. SEM images of HvADH2@MOF (A, B), ScADH@MOF (C, D) and of FE-BTC MOF (E).

ScADH@MOF (*Sc-Saccharomyces cerevisiae*) samples were prepared and characterized for comparison purposes. Scanning electron microscopy images of the ScADH@MOF (Figure 2 C-D) show the presence of uneven agglomerates of particles, in agreement with a previous study.<sup>41</sup> The MOF itself possesses a homogenous particle size and morphology (Figure 2 E). The shape of HvADH2@MOF particles differs from ScADH@MOF. HvADH2@MOF is comprised of semi-cubical sharp particles joined together in neat agglomerates. In comparison with MOF and with ScADH@MOF, the particle size of HvADH2@MOF was significantly larger. DLS measurements show a mean particle size of 150, 500 and 550 nm for MOF, ScADH@MOF and HvADH2@MOF, respectively (Figure S1).<sup>41</sup> It has been previously reported that glucose oxidase and lipase encapsulated in MOF show a particle size of 479 and 483 nm respectively.<sup>41</sup> This change in particle size follows the enzymes size (GOx  $\approx 5 \times 7 \times 9$  nm, LIP  $\approx 3 \times 4 \times 5$  nm, ADH  $\approx 6 \times 7 \times 11$  nm). SEM (Figure 2) and DLS measurements (Figure S1) showed that in comparison to MOF, no significant changes in the structure of HvADH2@MOF were observed. The differences in shape and particle dimensions depend on the enzyme encapsulated in the material and is indicative of the successful encapsulation of the enzyme in the MOF. All of the enzyme is entrapped in the MOF. Confocal measurements demonstrate that, when performing the immobilization on MOF in-situ, the enzyme is present and encapsulated within the material. BET measurements of MOF<sup>41</sup> showed a pore volume of 0.57 cm<sup>3</sup>/g and a total surface area of 753 m<sup>2</sup>/g area which decreased to 0.15 cm<sup>3</sup>/g and 32 m<sup>2</sup>/g, respectively, in the presence of ADH, indicating suggests that the enzyme is encapsulated within the mesopores of the material.

### 3.1 Thermal stability

For comparison purposes, the activity of ADH (*S. cerevisiae*) was examined and displayed a maximum activity at 25 °C with a specific activity of  $22.7 \pm 1.4$  U/mg. At 50 °C, the enzyme retained approximately 0.4% of its activity ( $0.1 \pm 0.4$  U/mg) demonstrating that the enzyme is not able to function properly at temperatures higher than 25°C. In solution ADH *S. cerevisiae* showed almost no activity at 50°C, the MOF immobilised enzyme was active (0.34 U/mg). In comparison, HvADH2 showed a peak activity at 90°C<sup>8</sup> however, this temperature was not used due to the difficult assay conditions i.e. evaporation of the analyzed solution. On examination of the activity of HvADH2 and HvADH2@MOF as a function of temperature (Figure 3) the optimal activity for the HvADH2@MOF occurred at a temperature 10°C higher than HvADH2 (50 °C versus 60°C). In this range HvADH2 and HvADH2@MOF present the same trend with a decrease in activity of 75% from 60 °C to 80 °C for HvADH and 71.5% for HvADH2@MOF. At 60°C HvADH2@MOF ( $0.30 \pm 0.06$  U/mg) had significantly higher activity than the free enzyme ( $0.08 \pm 0.02$  U/mg).

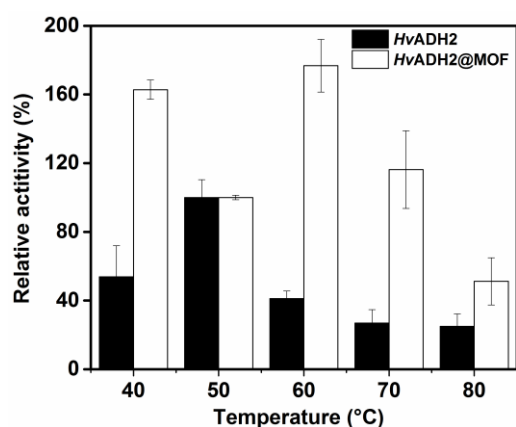


Figure 3. Plot of the activity of HvADH2@MOF and HvADH2 as a function of temperature. The activity was normalized at 50 °C for HvADH2 ( $0.19 \pm 0.02$  U/mg) and HvADH2@MOF ( $0.157 \pm 0.002$  U/mg). Note: the activity of HvADH2@MOF exceeds 100% because its maximum activity occurs at 60°C ( $0.28$  U/mg  $\pm 0.06$ )

### 3.2 pH activity profile

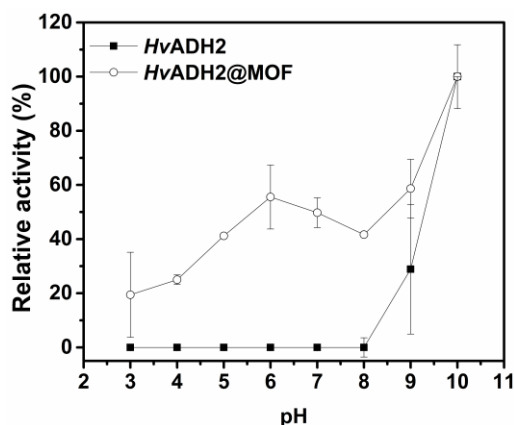


Figure 4. Plot of the specific activity of HvADH2@MOF(○) and HvADH2 (■) as function of pH. The activity was normalized at pH 10 for HvADH2 ( $0.66 \pm 0.03$  U/mg) and for HvADH2 @MOF ( $1.32 \pm 0.15$  U/mg).

HvADH2 in free form and immobilized displayed optimal activity at pH 10 (Figure 4) in agreement with recent studies on the same enzyme.<sup>50</sup> The activity of HvADH2 at different pH values was also examined in the presence of various solvents.<sup>11</sup> As expected HvADH2 shows a decrease in activity in by decreasing the salt concentration. Surprisingly in the presence of DMSO 5% the optimal concentration of salt requirement for HvADH2 decrease from 3 M KCl to 2 M KCl. Moreover at pH 6 free HvADH2 shows almost no activity but it shows up to 40% more activity in the presence of DMSO.<sup>1</sup> They found an explanation in the stabilizing effect of DMSO on amino acidic residue such as cysteines which are involved in the structure of the active site of this halophilic enzyme. A similar reason could be found in the stabilizing effect of the immobilization

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over all the studied pH range. Interestingly, while the free form lost its activity at pH below 8, HvADH2@MOF retained up to 60% of its activity at pH 6. In fact specific activity of HvADH2@MOF is higher in all pH range (Figure 4). Even at extremely low pH values as pH 3 HvADH2@MOF shows  $0.3 \pm 0.2$  U/mg. Halophilic enzymes are usually very inactive at acidic pH indicating that the in-situ immobilization strategy in MOF can be used to immobilize extremo-enzymes. The pH profile of HvADH2@MOF is broader than that of the free HvADH2. HvADH2 displayed no activity at pH 3-8, while HvADH2@MOF was active. This may be due to the stabilizing effect of the MOF on the structure of the enzyme. The encapsulation of glucoamylase in a metal organic framework structure stabilized the conformation of the enzyme<sup>51</sup> with FT-IR data showing that a significant change in the secondary structure of the enzyme occurring on immobilization. This change also resulted in an increase in catalytic activity. The increased stability of immobilised HvADH2, even at low pH may arise from the same effect in which the MOF not only protects the enzyme from the external environment but promotes the optimal enzyme configuration even under unfavorable conditions.

### 3.3 Effect of salt concentration

Halophilic organisms adapt to high extracellular salt in one of two ways. Halophilic enzymes inhabit environments at extremely high salt concentrations ( $> 1.5$  M). Most haloarchaea accumulate high intracellular potassium and chloride ion concentrations, usually near saturation levels, in order to maintain osmotic balance with their environment.<sup>52</sup> This "salting in" strategy ensures that the proteins within the cell are capable of functioning at this high salt concentration, and indeed, will likely unfold at low salt concentrations.<sup>3, 53</sup> On the other hand, haloadaptation involves accumulation of organic solutes in the cytoplasm, and exclusion of salt, to maintain osmotic pressure.<sup>1</sup> This "salting out" strategy is much more widespread than the "salting in" method. The organic solutes do not affect the internal proteins and as such, these proteins do not require salt to function.<sup>10</sup> *Haloferax volcanii*, a haloarchaea, utilises the "salting in" strategy and as a result, the proteins within it require high salt concentrations in order to properly fold and function.<sup>54-55</sup> HvADH2 functions optimally in 3-4 M KCl, and will dissociate from a tetramer to a dimer if incubated in concentrations of 1 M salt or less causing the denaturation of the enzyme.<sup>8</sup> Those enzymes possess extensive ion pair networks which prevent precipitation in solution and enable catalytic activity at high salt concentrations. Nevertheless, immobilization of *H. mediterranei* aldolase has been reported to reduce the requirement for high salt concentrations.<sup>56</sup> Since halophilic enzymes have a limited use in industry due to the requirement for high salt concentrations they may become easier to handle when immobilized. For this reason the effect of the salt concentration on the activity of the free and immobilised enzymes was studied in 100 mM Tris-HCl buffer pH 10 over a range of KCl concentrations (0, 1.5 and 3 M). The salt concentration required to have activity with the immobilised form is lower than that one required for the free form. The immobilization of the soluble enzyme was performed in the presence of 3 M KCl. Even at 0 M KCl HvADH2@MOF show 80% retention of activity that remains constant up to 3-4 M KCl. On the other hand HvADH2 presents no activity when no salt is present with an increasing measurable activity just starting from the addition of 0.5 M KCl and with a maximum at 4 M KCl.

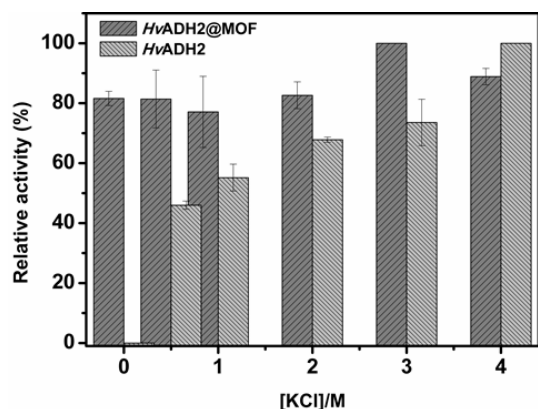


Figure 5. Relative activity of HvADH2 after 1 h incubation at 25 °C in buffer (0.1 M Tris, pH 10) at various concentrations of KCl. The activity was normalized at buffer Tris pH=10 KCl 3M for HvADH2 ( $0.02 \pm 0.01$  U/mg) and for HvADH2 @MOF ( $0.48 \pm 0.16$  U/mg) at enzyme concentration.

It is likely that the MOF provides a stable environment for the enzyme, allowing it to retain activity even at lower salt concentration ( $< 3$  M). To ensure a homogeneous diffusion of KCl within the material HvADH2@MOF was also assayed after incubation of 24 h in 100 mM Tris-HCl buffer in a wide range of KCl concentration to ensure a homogeneous diffusion of KCl within the material (Figure 5). On incubating HvADH2@MOF and HvADH in 0 M KCl after 24 h no activity was observed in both cases (Figure 6) in agreement with previous immobilization of HvADH2 on polymeric beads.<sup>11</sup> However, despite previous immobilization strategy on HvADH2, good retention of activity of HvADH2@MOF ( $> 60\%$ ) was observed after incubation in 1.5 M KCl, while the free HvADH2 only show 20 % of its activity under the same conditions. A decrease in halophilicity due to the immobilization has been reported by D'Souza on *Haloferax mediterranei* aldolase<sup>56</sup> and by Koch-Schmidt on the *Halobacterium* malate dehydrogenase.<sup>57</sup> They found a shift in the ionic strength comparing the free malate dehydrogenase and the immobilized one in Sepharose Cl 4B. The free enzyme showed no activity at 0 M KCl while when the same enzyme was immobilized 80% of relative activity was observed. In our study the decrease of KCl requirements may be due to electrostatic interactions between the enzyme (with an excess of negative charge due to the high content of acidic aminoacids) and the ionic nature of the MOF, which may act as a substituent for the stabilizing

K<sup>+</sup> providing a good ion-pair microenvironment for the enzyme. In-situ immobilization in MOF enables good retention of activity at lower salt concentrations that may have applications at lower salt concentrations.

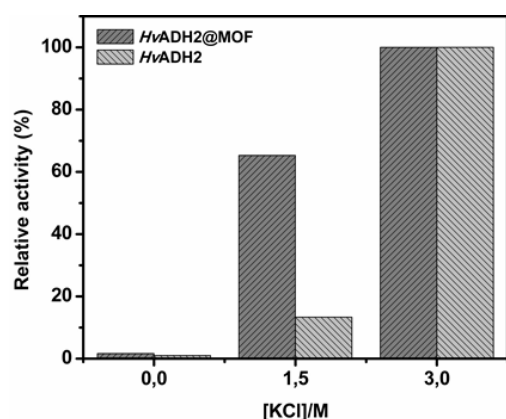


Figure 6. Relative activity of HvADH2 (free and immobilised) after 24 h incubation at 5 °C in (0.1 M Tris, pH 10) at various concentrations of KCl.

### 3.4 Stability

Samples of HvADH2@MOF were kept in the fridge at 5°C. HvADH2@MOF retained 20% activity after at least one week. ScADH@MOF (ADH from *Saccharomyces cerevisiae*) was more stable, with 60 % activity retained after 18 days.<sup>41</sup>

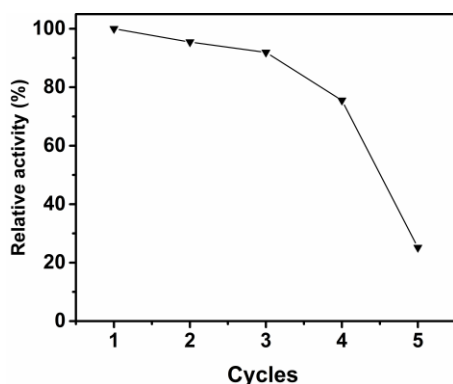


Figure 7. Plot of the activity of HvADH2@MOF as function of number of reaction cycles

One of the main advantages of enzyme immobilization is the ability to recover and reuse the biocatalyst. As shown in Figure 7 the enzyme retained up to 75% of its activity up to 4 cycles losing the most of its activity after 5 cycles (retention of 20%). With ScADH@MOF the activity remained stable (100%) for the first two cycles and then decreased to 55% between the third and fifth cycles. As other MOF immobilized enzymes (glucose oxidase and lipase) retained activity for a number of cycles, the loss in activity for the alcohol dehydrogenases is possibly due to a decrease in the stability of the enzyme on re-use. Furthermore, no leaching and no enzyme activity was detected after each reuse, indicating the immobilized enzyme is stable and catalytically active.

### 3.5 Stability in organic media

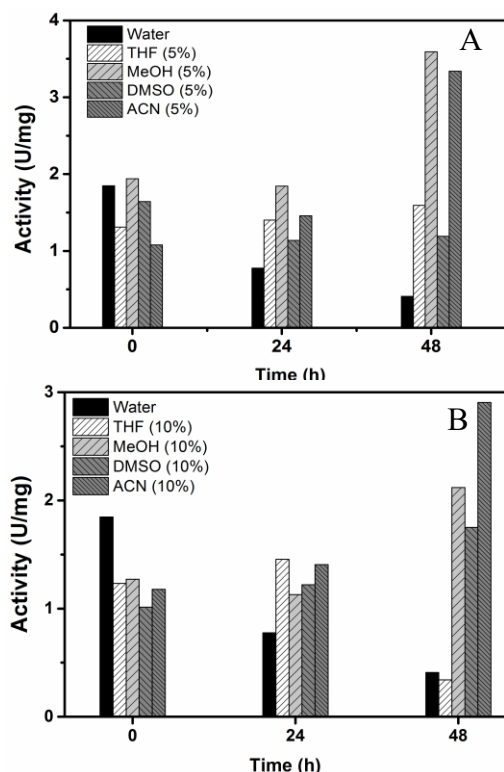


Figure 8. Activity profiles of HvADH2@MOF after storage in the presence of a range of organic media 5% (A) and 10% (B). The immobilised enzymes were maintained at 25 °C and tested after 0, 24 and 48 hours.

Fundamental developments in enzyme engineering and supports allow the introduction of organic media in biocatalysis. Since halo-philic enzymes are active in high salt concentrations with reduced activity in pure water solutions, they are extremely valuable for their use in organic solvents. Many extremoenzymes show improved activity in organic solvents when immobilised.<sup>58</sup> Previous studies on the effect of organic solvents on the activity of HvADH2 demonstrated that the stability of the enzyme, strongly influenced by KCl concentration, was improved on the addition of MeOH and DMSO. In this study the immobilization of HvADH2 in MOF material enhanced the activity in non-aqueous solvent mixtures (5% and 10%) (Figure 8). The activity of HvADH2@MOF was highest in solutions of MeOH and ACN. The activity decreased over time in the absence of nonaqueous solvents, but increased by 50% in the presence of 5% THF and MeOH. The same trend was observed with MeOH (10%) with a smaller increase in activity (43%) while in the presence of THF the activity drops. No clear trend in terms of relating enzymatic activity with properties such as solvent polarity was observed. In contrast the activity of ADH (*H. mediterranei*)<sup>59</sup> increased with increasing solvent polarity. Similar to the results described here, Alsafadi et al.<sup>1</sup> reported that the requirement for high concentrations of KCl was reduced on addition of MeOH and DMSO.

#### 4. Conclusions

This work has focused on the immobilization of a hyperhalophilic alcohol dehydrogenase in a MOF material. The only report to date on the immobilization of the enzyme has been by covalent attachment.<sup>11</sup> The process used to immobilize enzymes in Fe-BTC MOF materials<sup>41</sup> can be extended to a broad range of enzymes improving their properties. The interactions between the material and the enzyme provide a favorable microenvironment broadening the operational conditions. Encapsulation of the enzyme resulted in an increase of optimal work temperature (from 50 °C to 60 °C), a broader range of working pH, a decrease in the requirement for high concentrations of salt, good storage stability and retention of activity in organic media such as DMSO and ACN that is not achievable with the free enzyme.

#### ASSOCIATED CONTENT

Supporting Information, this material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ABBREVIATIONS

ADH, alcohol dehydrogenase; MOF, metal organic framework; HvADH2, alcohol dehydrogenase from *Haloferax volcanii*; ScADH, alcohol dehydrogenase from *Saccharomyces cerevisiae*; MeOH, methanol, THF, tetrahydrofuran; DMSO, Dimethyl sulfoxide; ACN, acetonitrile.

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