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Efficient conversion of CO_2 to formic acid by formate dehydrogenase immobilized in a novel alginate–silica hybrid gel

Yang Lu [a,b], Zhong-yi Jiang [a,b,*], Song-wei Xu [a,b], Hong Wu [a,b]

[a] *Key Laboratory for Green Chemical Technology of Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China*

[b] *State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China*

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Abstract

The efficient utilization of CO_2 will not only help to alleviate greenhouse effect, but also to obtain useful chemicals as well. A novel bio-pathway has been explored to convert CO_2 into formic acid by the aid of formate dehydrogenase (FateDH) as the biocatalyst and reduced nicotinamide adenine dinuncleotide (NADH) as the terminal electron donor. In order to simplify subsequent separation of the enzyme and improve its catalytic stability, the enzyme FateDH was encapsulated in a novel alginate–silica (ALG–SiO_2) hybrid gel. This hybrid gel was prepared by in situ hydrolysis and polycondensation of tetramethoxysilane (TMOS) in alginate solution followed by gelation of alginate with Ca^{2+}. The leakage of the enzyme was significantly reduced by hybridization compared to pure alginate. The optimum reaction condition was found to be at pH 7.0 and 37 °C. Under these conditions, the highest yield of formic acid catalyzed by the immobilized FateDH was up to 95.6%, only a little lower than that of the free form enzymatic reaction (98.8%). The relative activity of immobilized FateDH after 10 cycles could be maintained as high as 69%. Storage stability test showed that the relative activity of FateDH in hybrid gel was about fourfold higher than that in pure alginate gel after being kept at 4 °C for 1 month.

Keywords: Carbon dioxide; Formic acid; Formate dehydrogenase; Alginate; Silica; Hybrid gel

1. Introduction

1.1. Enzymatic conversion of CO_2

The efficient utilization of CO_2 has attracted considerable attention from fundamental research to industrial application in recent years. Heterogeneous catalysis, electrocatalysis and photocatalysis are presently the three predominant chemical methods for converting CO_2 into some useful chemicals, such as methanol, formic acid and formaldehyde, etc. [1–3]. These methods need high temperature and pressure or additional electric or luminous energy. However, both the selectivity and yields are low. In comparison, the enzymatic approach to convert CO_2 has several advantages such as high yields and selectivity under milder reaction conditions (lower temperature and pressure) without pollution [4–6]. For example, a combination of formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH) and alcohol dehydrogenase (ADH) was used in consecutive reduction of CO_2 to methanol [6–8].

Herein, we report a novel and promising approach to convert CO_2 into formic acid through a reduction reaction catalyzed by formate dehydrogenase (EC 1.2.1.2) encapsulated in a novel alginate–silica hybrid gel prepared by in situ hydrolysis and polycondensation of tetramethoxysilane (TMOS) in alginate solution, followed by Ca^{2+}-induced gelation. Reduced nicotinamide adenine dinuncleotide (NADH) acts as a terminal electron donor for enzymatic reaction (1).

$$CO_2 + NADH \xrightarrow{\text{Fate DH}} HCOOH + NAD^+ \quad (1)$$

1.2. Enzyme immobilization

To increase the enzyme stability and reusability, reduce the enzyme cost, and facilitate the subsequential purification, enzyme immobilization is essential for this enzymatic reaction.

* Corresponding author. Tel.: +86 22 27890882; fax: +86 22 27892143.
E-mail address: zhyjiang@tju.edu.cn (Z.-y. Jiang).

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Calcium alginate (Ca-alginate) gel is one of the most commonly used matrices for enzyme immobilization owing to their significant advantages such as good biocompatibility, low cost, easy availability and simplicity of preparation. However, high enzyme leakage due to its open structure and large pore size significantly restrict the lifetime of these biomaterials and limit its application in enzyme immobilization [9–11].

Organic–inorganic hybrid gels are now viewed as the next generation of materials for enzyme immobilization. Moreover, incorporating inorganic material into the alginate matrix might be an effective method to overcome its disadvantages. Many researchers have prepared the hybrid silica–polysaccharide composites for enzyme immobilization by physically mixing the silica with alginate or sol–gel process by using a certain precursor [12–14].

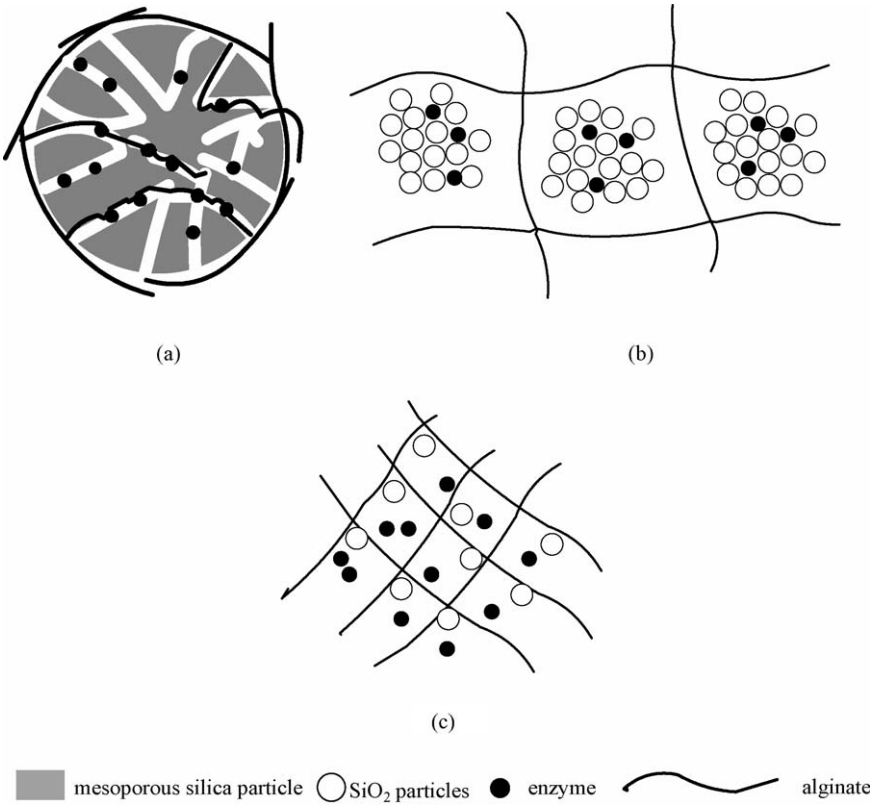
Coradin and Livage prepared a silica–alginate hybrid gel by impregnation of mesoporous silica particles with alginic acid solution and encapsulated β-galactosidase within it [12]. Compared to the pure Ca-alginate gel, the hybrid gel exhibited better stability upon aging and effectively limited enzyme leaching. SEM studies showed that the alginate was located in the interparticle macroporosity of the silica particle, thus, partially coating particle surface (Scheme 1a).

Using a water-soluble precursor tetrakis (2-hydroxyethyl) orthosilicate (THEOS), Shchipunov and coworkers synthesized hybrid silica nanocomposites containing various polysaccharides, which have been successfully applied to immobilize 1 → 3-β-D-glucanase L_{IV} and α-D-galactosidase. The enzyme lifetime was significantly increased after entrapment [13]. The SEM data indicated that the main morphological element of composites was a cross-linked network formed by polysaccharide macromolecules, and aggregated silica nanoparticles which filled the mesh space [14] (Scheme 1b).

However, the most prominent problem for the methods above is that the silica particles tended to aggregate at high concentration due to hydrogen bonding and the homogeneous dispersion was not available. Moreover, the aggregates of silica might break the porous structure of alginate gel and hinder the diffusion of the substrates and products [15]. Coradin et al. prepared a new composite by mixing the gelatine and silicate. SEM data revealed that the composite, which was obtained at low gelatine and high silicate content, was formed of large aggregates of platelets, constituted of closely packed nanoparticles [16].

Therefore, achieving good dispersion of silica particles in the alginate matrix is a challenge. It is imperative to develop a new technique for preparing the hybrid alginate–silica gel, which can achieve the homogeneous distribution of silica in the alginate matrix and retain the porous structure and good diffusion characteristics.

In order to obtain inexpensive and readily available alginate–silica composites, a novel alginate–silica (ALG–SiO_2) hybrid gel was prepared to encapsulate FateDH (Scheme 1c). Such hybrid gels might possess some advantages:



Scheme 1. The schematic representation of enzyme encapsulation in (a) silica–alginate hybrid gel prepared by Livage; (b) hybrid silica nanocomposites containing polysaccharides prepared by Shchipunov; (c) hybrid ALG–SiO_2 gel prepared by this study.

(1) The gelation of TMOS is accelerated by alginate and the cross-linking of silica with alginate matrix leads to a compact and porous composite, which have good diffusion characteristics.
(2) There is a more homogeneous distribution of silica in alginate matrix and combination of high stability of the mineral with better compatibility of alginate.
(3) The enzyme leakage is effectively minimized. The enzyme activity is better retained and enzyme lifetime is increased.

In this work, the preparation of this new hybrid ALG–SiO_2 gel and its application for immobilizing FateDH was presented. The structure of this hybrid gel was characterized by scanning electron microscopy (SEM) and BET surface area. The homogeneity of the silicon distribution within the hybrid ALG–SiO_2 gel beads was determined using energy-dispersive spectroscopy (EDS). The optimum enzymatic condition, the storage activity and the recycling activity of the FateDH encapsulated in the pure and hybrid ALG–SiO_2 gels were investigated and compared.

2. Experimental

2.1. Chemicals

Formate dehydrogenase (50 U/mg, solid) and reduced nicotinamide adenine dinuncleotide (98% purity) were purchased from Sigma Chemical Co. The sodium alginate was obtained from Shanghai Tianlian Chemical Co. Tetramethoxysilane was provided by Makall Brand Co. Tris (hydroxymethyl amnomethane, 99.5%) was used to prepare the pH 7.0, 0.05 mol/L Tris–HCl buffer. All other chemicals were of reagent grade.

2.2. Preparation of pure alginate gel beads containing FateDH

4.5 mg FateDH was dissolved in 1.0 mL of pH 7.0, 0.05 mol/L Tris–HCl buffer to form an enzyme stock solution. Then it was mixed with 4.0 mL of sodium alginate solution to form a sol mixture with the final alginate concentration of 20.0 g/L.

The sol mixture was added dropwise into 20 mL of 0.2 mol/L $CaCl_2$ solution. The hybrid gel beads formed rapidly. After 30 min of aging, the beads were collected by filtration using millipore microfiltration membranes (0.2 μm), rinsed with distilled water several times and stored in the distilled water. All procedures were carried out at room temperature.

2.3. Preparation of hybrid ALG–SiO_2 gel beads containing FateDH

1.47 mL of TMOS was vigorously stirred with 5 mL of 20 g/L sodium alginate solution for 10 min and then mixed with 4.5 mg FateDH to form a sol mixture. The rest of procedure was as the same for the preparation of pure alginate gel beads.

2.4. Characterization of pure alginate and hybrid ALG–SiO_2 gel beads

The cross-sectional morphology of both the pure alginate and the hybrid ALG–SiO_2 gel beads were observed using scanning electron microscopy (SEM) (100-CXII, Philip). The spatial distribution of silica in the alginate matrix was evaluated by silicon mapping using energy-dispersive spectroscopy (EDS) (100-CXII, Philip). Specific surface areas S_{BET}, total pore volume V_p and average pore diameter D_p of the pure and hybrid gel beads were determined by the BET nitrogen adsorption–desorption method (CHEMBET3000, Quanta Chrome).

2.5. Leakage of pure and hybrid gel containing FateDH

The leakage of FateDH immobilized during the initial gelling period for pure alginate and hybrid ALG–SiO_2 gel was calculated by measuring the absorbance of formic acid in the decanted solution at 280 nm using a UV–visible spectrophotometer (U-2800, Hitachi). The water loss during the bead formation was also determined by the weight change of the beads. The beads were placed in 20 mL of pH 7.0, 0.05 mol/L Tris–HCl buffer solutions at 25 °C, which was used as releasing medium.

2.6. Activity assays of immobilized FateDH

Activity assays were carried out at optimum conditions. The enzymatic activity of immobilized FateDH was evaluated based on the yield of formic acid in the reduction of CO_2 to formic acid. The pure and hybrid alginate gel beads containing 4.5 mg FateDH were mixed with 2.5 mL of 1 mmol/L NADH solution in 0.05 mol/L Tris–HCl buffer. To the mixture, CO_2 was then bubbled for 8 h for production of formic acid. UV–vis spectrophotometer was used for determination of the concentration of NADH in the reaction solution at 340 nm and the yield of formic acid was calculated based on the amount of NADH consumed.

2.7. Determination of optimum pH and temperature of immobilized FateDH

To determine the optimum pH and temperature of immobilized FateDH, the activities of FateDH immobilized in hybrid ALG–SiO_2 gel were examined in the corresponding buffer (0.05 mol/L Tris–HCl buffer in the pH range 6.0–8.0 and temperature range 20–50 °C).

2.8. Operational stability of immobilized FateDH

To evaluate the operational stability of FateDH immobilized in pure and hybrid alginate gel beads, a series of experiments in the batch system were carried out and the retention of the immobilized FateDH activity was tested as described in activity assays. After each reaction run, the gel beads were collected and washed with pH 7.0, 0.05 mol/L Tris–HCl buffer to remove any residual substrate within the beads. They were then reintroduced into fresh reaction medium and enzyme activities were detected in a batch operation mode at optimum conditions.

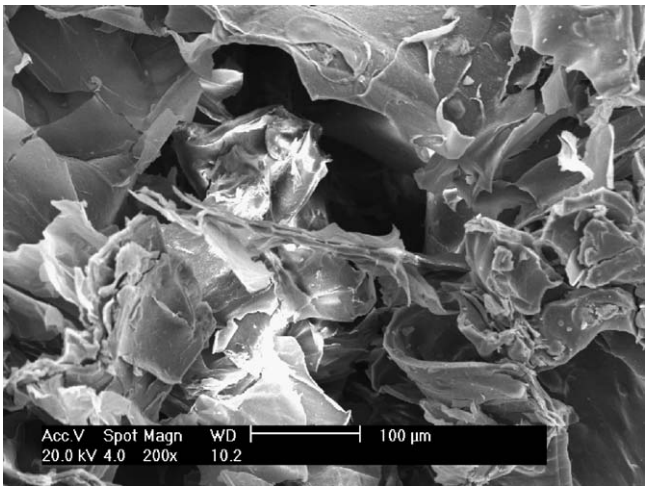


Fig. 1. SEM image of the structure of pure alginate gel.

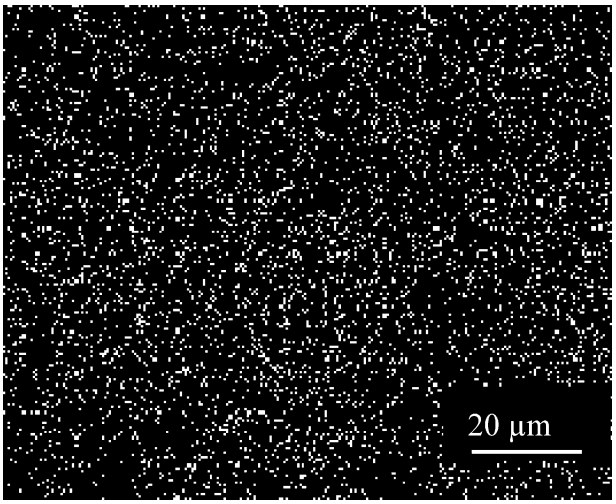


Fig. 3. Silicon dot mapping of hybrid ALG–SiO_2 gel beads taken by EDS.

2.9. Storage stability of immobilized FateDH

The activity of FateDH immobilized in pure and hybrid gel beads in pH 7.0, 0.05 mol/L Tris–HCl buffer at 4 °C was measured in a batch operation mode with the experimental conditions given above. Taking the activities of first batch to be 100%, the relative activities of immobilized FateDH were defined as the ratio of the activity assay to the initial activity.

3. Results and discussion

3.1. Characterization of pure and hybrid alginate gel beads

Insight into the structure of the pure and hybrid gel beads was realized by SEM. Figs. 1 and 2 shows the SEM image for the structure of alginate and ALG–SiO_2 gel bead, respectively. As shown in Figs. 1 and 2, there is no evident difference between the structure of alginate and ALG–SiO_2 gel bead. And the hybrid ALG–SiO_2 gel exhibited highly porous structure whereas the alginate matrix was still tightly compact, demonstrating that after hybridization the morphology of the alginate matrix was well retained. In addition, no aggregation of silica in the ALG–SiO_2 gel was observed.

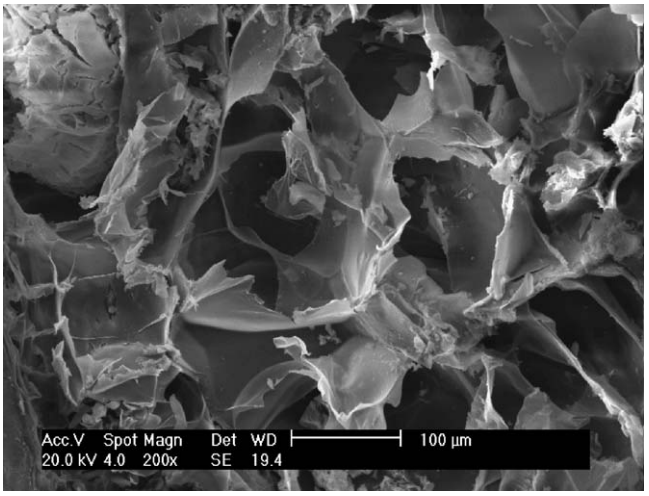


Fig. 2. SEM image of the structure of hybrid ALG–SiO_2 gel.

To investigate the distribution of silica in the alginate matrix, we took silicon dot images using EDS. Fig. 3 showed the silicon dot mapping of hybrid gel beads, clearly indicating a homogeneous distribution of silicon in the alginate matrix.

More information on the porous structure of hybrid gel was obtained from nitrogen adsorption–desorption experiments. Specific surface areas S_{BET}, total pore volume V_p and average pore diameter for the pure and hybrid gel beads are reported in Table 1. It is shown in Table 1 that S_{BET}, V_p and D_p were increased after hybridization, clearly underlining the hybrid gel was sufficiently porous to facilitate the diffusion of substrate and products.

In addition, the effective diffusion coefficients of NADH in pure and hybrid alginate gels were calculated using an unsteady-state model developed by Nguyen and Luong [17]. This method has been used in our previous study [18]. For pure and hybrid alginate gels, the effective diffusion coefficients of NADH were $1.84 \pm 0.02 \times 10^{-10}$ and $1.93 \pm 0.02 \times 10^{-10}$ m^2/s, respectively. It suggested that such porous hybrid ALG–SiO_2 gels with homogeneous silica distribution showed good diffusion characteristics.

3.2. Leakage of the immobilized FateDH

To consider the potential reuse of the hybrid ALG–SiO_2 gel beads in the chemical reaction, the leakage of FateDH was measured during the initial gelling period and the loading efficiency was calculated accordingly. In comparison, the

Table 1
Specific surface areas (S_{BET}), total pore volume (V_p) and average pore diameter (D_p) for the pure and hybrid gel beads

Gel	S_{BET} (m^2/g)	V_p (cm^3/g)	D_p (nm)
Pure alginate	319	0.61	3.81
ALG–SiO_2	549	1.27	4.10

Table 2
Leakage of FateDH in pure and hybrid gels (25 °C, pH 7.0 Tris–HCl buffer)

Gel	Water loss (%)	Leakage of FateDH (%)	Loading efficiency (%)
Pure alginate	59.3	42.8	57.2
ALG–SiO_2	36.7	12.5	87.5

leakage of FateDH immobilized in pure alginate gel beads was also studied. The results of the water loss, leakage, and loading efficiency of FateDH measured after 4 h were shown in Table 2.

Both the leakage and water loss in the ALG–SiO_2 gels were found to be significantly smaller than that in the pure alginate gels. After 4 h, 87.5% of the total immobilized FateDH was retained in the ALG–SiO_2 gel beads while only 57.2% was retained in the pure alginate gel beads. This showed that the leakage of FateDH correlates with the water loss. We hypothesized that the leakage of enzyme during the initial gelling period was mainly due to the water loss that could bring the enzyme out. Therefore, the adsorption of water on silica due to its hydrophilicity in ALG–SiO_2 gel beads resulted in the decrease of the water loss and the enzyme leakage accordingly.

3.3. Effect of pH and temperature on the activity of immobilized FateDH

The microenvironment for enzyme molecule may be modified after immobilization because of the interaction between the surface of the matrix and the active sites of the enzyme. The enzyme activity is markedly influenced by the change of microenvironmental conditions, especially pH activity and temperature activity [19]. To understand the interaction between enzyme and matrix, therefore, it is very useful to study the activity of the immobilized enzyme as a function of pH and temperature compared to free enzyme.

To evaluate the optimum temperature of immobilized FateDH, the activity assays were carried out over temperature range 20–50 °C in pH 7.0, 0.05 mol/L Tris–HCl buffer. The pH dependence of the activities of the immobilized FateDH was also studied in 0.05 mol/L Tris–HCl buffer in the pH range 6.0–8.0 at 25 °C. Experimental results were summarized in Table 3.

As shown in Table 3, the highest immobilized enzymatic activity was obtained at pH 7.0, 37 °C, identical to that of free enzyme. Under these conditions, the highest yield of formic acid catalyzed by the hybrid gel containing FateDH was up to 95.6%, while the yield of formic acid catalyzed by FateDH in the free form was 98.8%. It is clearly indicated that the microenvironmental conditions and conformational flexibility of the enzyme were well retained after immobilization.

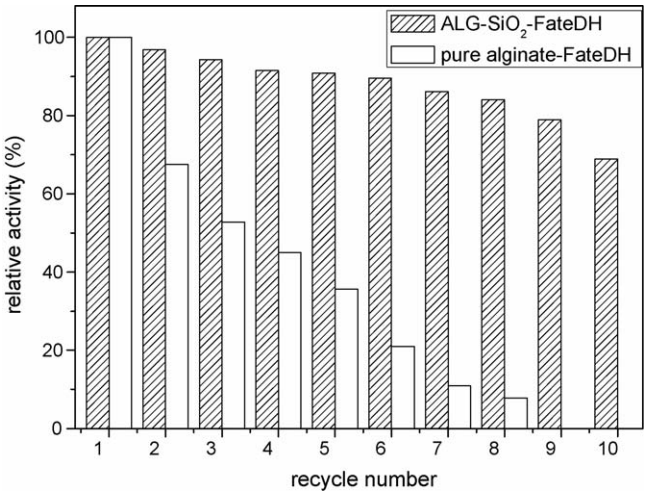


Fig. 4. Recycling stability of FateDH immobilized in pure and hybrid alginate gels.

3.4. Recycling stability of immobilized FateDH

The recycling stability of immobilized FateDH both in pure and hybrid alginate gels were tested for 10 successive batch reactions at 37 °C. The activities of the first batch were taken to be 100%. Fig. 4 shows the relative activity as a function of reuse number. It was observed that FateDH immobilized in hybrid ALG–SiO_2 gels retained about 69% of its activity after 10 cycles, while that in alginate gel decreased almost to zero.

The higher recycling stability of the immobilized FateDH than that in pure alginate was mainly due to the lower leakage of enzyme in early stage of the activity assay and well-retained conformational feasibility of enzyme.

3.5. Storage stability of immobilized FateDH

FateDH immobilized in pure and hybrid alginate gels were stored in pH 7.0, 0.05 mol/L Tris–HCl buffer at 4 °C for

Table 3
Experimental results of conversions of CO_2 to HCOOH catalyzed by FateDH

No.	Temperature (°C)	pH	NADH amount (μmol)	Pressure (MPa)	Yield[a] (%)
0	37	7.0	2.36	0.5	98.8 (free form)
1	37	7.0	2.36	0.5	95.6
2	37	6.0	2.36	0.5	48.1
3	37	7.5	2.36	0.5	38.5
4	37	8.0	2.36	0.5	34.5
5	20	7.0	2.36	0.5	35.1
6	25	7.0	2.36	0.5	48.7
7	50	7.0	2.36	0.5	30.0

[a] The moles of NADH consumed/moles of NADH initial amount × 100%.

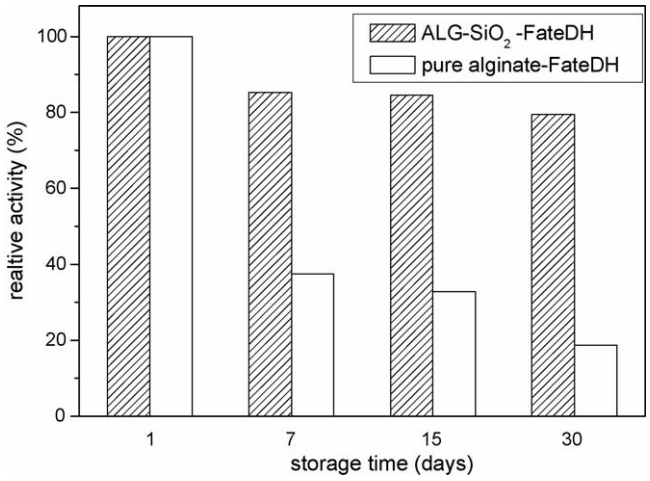


Fig. 5. Storage stability of FateDH immobilized in pure and hybrid alginate gels.

30 days, and the activity assays were carried out during this period (Fig. 5).

As shown in Fig. 5, the activity of the FateDH immobilized in pure alginate gels was reduced by 80% of its initial activity after 30 days whereas FateDH immobilized in hybrid ALG–SiO_2 gels lost only about 20% of its initial activity after storage for the same period. The encouraging result indicated that the hybrid gel effectively retained most of the enzymatic activities.

4. Conclusion

The feasibility of enzymatic conversion of CO_2 to formic acid is tentatively explored. The reduction of CO_2 by FateDH successfully encapsulated in a novel ALG–SiO_2 hybrid gel beads results in high yields for generation of formic acid (95.6%). Compact and porous structure is well retained in hybrid gel. Meanwhile, a homogeneous silica distribution within the alginate matrix is achieved. Enzyme leakage is effectively limited by hybridization, whereas substrates and products diffusion are not hindered. The relative activity of immobilized FateDH after 10 cycles could be maintained as high as 69%. Storage stability test shows that 80% of the relative activity of FateDH in hybrid gel is discerned after being kept at 4 °C for 1 month. Such good operational stability and storage stability of the immobilized FateDH presented in this work may indicate applicability for continuous conversion of CO_2. This will open up a new avenue not only for efficient fixation of the greenhouse CO_2 but also for on-site production of formic acid form cheap raw material.

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