Sequential Co-immobilization of Enzymes in Metal-Organic Frameworks for Efficient Biocatalytic Conversion of Adsorbed CO$_2$ to Formate

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The main challenges in multienzymatic cascade reactions for CO$_2$ reduction are the low CO$_2$ solubility in water, the adjustment of substrate channeling, and the regeneration of co-factor. In this study, metal-organic frameworks (MOFs) were prepared as adsorbents for the storage of CO$_2$ and at the same time as solid supports for the sequential co-immobilization of multienzymes via a layer-by-layer self-assembly approach. Amine-functionalized MIL-101(Cr) was synthesized for the adsorption of CO$_2$. Using amine-MIL-101(Cr) as the core, two HKUST-1 layers were then fabricated for the immobilization of three enzymes chosen for the reduction of CO$_2$ to formate. Carbonic anhydrase was encapsulated in the inner HKUST-1 layer and hydrated the released CO$_2$ to HCO$_3^-$, Bicarbonate ions then migrated directly to the outer HKUST-1 shell containing formate dehydrogenase and were converted to formate. Glutamate dehydrogenase on the outer MOF layer achieved the regeneration of co-factor. Compared with free enzymes in solution using the bubbled CO$_2$ as substrate, the immobilized enzymes using stored CO$_2$ as substrate exhibited 13.1-times higher of formate production due to the enhanced substrate concentration. The sequential immobilization of enzymes also facilitated the channeling of substrate and eventually enabled higher catalytic efficiency with a co-factor-based formate yield of 179.8%. The immobilized enzymes showed good operational stability and reusability with a cofactor cumulative formate yield of 1077.7% after 10 cycles of reusing.

Keywords: metal-organic framework, sequential co-immobilization of enzymes, storage of CO$_2$, CO$_2$ reduction, improved conversion

INTRODUCTION

High emissions of greenhouse gas such as CO$_2$ into the atmosphere have caused global environmental concern. To obtain a sustainable society, it is highly desirable to explore energy-efficient technologies for the conversion of CO$_2$ to useful chemicals or fuels (e.g., methanol). Up to date, considerable efforts have been made to catalyse the hydrogenation of CO$_2$ via chemical
electrochemical (Wang et al., 2016; Coskun et al., 2017; Miller et al., 2019), photochemical (Bachmeier et al., 2014; Tu et al., 2014; Wang et al., 2014; Wang and Wang, 2015; Xu et al., 2015; Sokol et al., 2018; Zhang et al., 2018), enzymatic conversions (Yang et al., 2012; Fixen et al., 2016; Shah and Imae, 2017; Cai et al., 2018; Liu et al., 2018), or photocatalyst/biocatalyst integrated systems (Yadav et al., 2014). Compared with other methods, the transformation of CO\(_2\) to methanol by enzymatic catalysis is preferable due to the significant advantages of high selectivity and specificity, high efficiency, and mild operational conditions (Obert and Dave, 1999; Wang et al., 2014; Ji et al., 2015; Kuk et al., 2017; Nabavi Zadeh et al., 2018; Zhang et al., 2018).

The reduction of CO\(_2\) to methanol by enzymatic cascade reactions mainly involves three enzymes, formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH), and alcohol dehydrogenase (ADH) (Obert and Dave, 1999; Wang et al., 2014; Ji et al., 2015; Kuk et al., 2017; Nabavi Zadeh et al., 2018; Zhang et al., 2018). FateDH converts CO\(_2\) to formic acid, which is subsequently reduced to formaldehyde catalysed by FaldDH. And formaldehyde is further converted to methanol by ADH at the final step. Although this enzymatic cascade reaction features high specificity, it has a relatively low yield with a methanol conversion of merely 43.8% reported by Dave et al. (Obert and Dave, 1999). The possible rate-limiting step is the first reaction in the sequence catalysed by FateDH since the reaction rate of formic acid oxidation is 30 times faster than CO\(_2\) reduction (Rusching et al., 1976). One of the conceivable reasons is the low substrate concentration due to the limited solubility of CO\(_2\) in water. As a result, the increase of CO\(_2\) substrate concentration in the solution may accelerate the forward conversion of CO\(_2\) to formic acid. This assumption was well-demonstrated by Zhang et al. who adopted ionic liquids with high CO\(_2\) solubility to assist the multi-enzymatic conversion of CO\(_2\) to methanol (Zhang et al., 2018). The yield was increased to approximate 3.5-fold compared to the parallel control experiments.

Metal-organic frameworks (MOFs) belong to the category of organic-inorganic hybrid porous materials built from the coordination between organic linkers and metal ions as nodes (James, 2003; Long and Yaghi, 2009; Tranchemontagne et al., 2009). Compared with conventional porous materials, MOFs possess the advantages of ultrahigh surface area and porosity, uniform and controllable pore sizes, structural diversity, as well as diverse chemistry. The superior properties of MOFs enable their wide applications in various research areas. In particular, MOFs are porous materials desired for the adsorption and storage of gases, such as CH\(_4\), H\(_2\), and CO\(_2\) (Li et al., 2009, 2014; Murray et al., 2009; Farha et al., 2010; Liu et al., 2012; Yang et al., 2012; Chaemchuen et al., 2013; He et al., 2014; Tian et al., 2017). In this respect, we envisioned that the transformation of CO\(_2\) to formic acid catalysed by FateDH may also be speeded up if CO\(_2\) is adsorbed in MOFs and used as substrate. On the other hand, MOFs are also ideal solid supports for the immobilization of enzymes as they can maintain the biological activity of enzymes even under denaturing conditions (Lykourinou et al., 2011; Chen et al., 2012; Lyu et al., 2014; Gkaniatsou et al., 2017; Lian et al., 2017; Du et al., 2018; Liang et al., 2019). As a result, we intend to develop a MOF platform aiming at achieving the simultaneous storage of CO\(_2\) and co-immobilization of multienzymes for enhanced cascade reduction of adsorbed CO\(_2\) to formic acid.

Amine-functionalized MOFs are considered as a promising candidate to enhance CO\(_2\) capture capacity as the electronegative N atom has a strong affinity to the positive C atom of CO\(_2\). Tethering amine functionalities in MOFs can be realized by introducing the amine groups on unsaturated metal sites. Chromium(III) terephthalate MIL-101 has a three-dimensional framework consisting of two types of zeotropic mesopores connected by two microporous windows (Férey et al., 2005; Jung et al., 2007). Except for its distinct merits such as large pore volume, high BET surface area, and excellent stability in water, MIL-101 also contains numerous potential open chromium sites (up to 3.0 mmol/g) (Hwang et al., 2008) with an unoccupied orbital that are expected to anchor amine functionalization via a strong binding interaction with the positive nitrogen atoms. It is also demonstrated that amine-functionalized MIL-101 has high CO\(_2\) capture capacities (Lin et al., 2013; Yan et al., 2013; Hu et al., 2014; Lin et al., 2014; Cabello et al., 2015; Darunte et al., 2016; Huang et al., 2016; Emerson et al., 2018; Zhong et al., 2018; Liu et al., 2019). Thus, in our work, MIL-101(Cr) was fabricated and modified with a series of amines to achieve the efficient storage of CO\(_2\) substrate.

Three enzymes were chosen for the transformation of CO\(_2\) to formic acid, carbonic anhydrase (CA), formate dehydrogenase (FateDH), and glutamate dehydrogenase (GDH). The introduction of CA is to accelerate the hydration of CO\(_2\). Moreover, CO\(_2\) is a thermodynamically stable molecule with low reactivity, so the conversion of CO\(_2\) to methanol requires energy which is supplied by co-factor nicotinamide adenine dinucleotide (NADH). GDH was involved into the biocatalysis integrated system to achieve the continuous regeneration of NADH co-factor. To obtain multienzyme systems with enhanced activity, three principles are considered, substrate channeling, kinetics matching, and spatial distribution (Garcia-Galan et al., 2011; Zhang et al., 2015; Walsh and Moore, 2019). The current challenge for the design of multi-enzyme conjugates remains in the development of efficient strategies realizing the accurate control of enzyme positioning and spatial organization (Fu et al., 2012; Schoffelen and van Hest, 2012; Lin et al., 2014). To conquer this limitation, in this work, we adopted a layer-by-layer self-assembly approach to achieve the sequential co-immobilization of multi-enzymes using MOFs in layered structure as the solid scaffold.

As illustrated in Scheme 1, amine-functionalized MIL-101(Cr) was first prepared for the adsorption of CO\(_2\) as substrate. The amine functionalities in MIL-101(Cr) then chelated Cu\(^{2+}\) via the formation of a complex followed by further coordinating with 1,3,5-benzenetricarboxylic acid (H\(_3\)BTC). These reactions provided a high density of Cu\(^{2+}\) and H\(_3\)BTC on the MOF surface, which then functioned as nucleation sites for the direct formation of HKUST-1 (Hong Kong University of Science and Technology) layers. On the surface of H\(_3\)BTC@Cu\(^{2+}\)@MIL-101(Cr), the first HKUST-1 layer encapsulated with CA was
Li et al. MOF Enzymes for CO₂ Reduction

SCHEME 1 | Schematic illustration of the preparation of HKUST-1@amine-MIL-101(Cr)-based multienzymes for the reduction of adsorbed CO₂.

Fabricated using a co-precipitation method via the self-assembly of metal ions, organic linkers, and enzymes. Based on the first HKUST-1 layer, the second HKUST-1 shell immobilizing FateDH and GDH was constructed using the identical approach. In this respect, when CO₂ was gradually released from MIL-101(Cr), it got access to carbonic anhydrase and was hydrated to bicarbonate ion. The second HKUST-1 layer containing FateDH and GDH directly converted bicarbonate ion to formic acid. The presence of GDH in the second MOF layer achieved the continuous regeneration of NADH co-factor. We found that this sequential co-immobilization route significantly accelerated the cascade biocatalysis reaction rate. The increase of concentration of CO₂ substrate by storing in MIL-101(Cr) also remarkably boosted the conversion yield.

EXPERIMENTAL

Materials and Reagents
Terephthalic acid (99%), trimesic acid (99%), cystamine hydrochloride, and 2,3,4,5,6-pentafluorobenzyl bromide (99%) were purchased from J&K Scientific Ltd. (Beijing, China). Chromium nitrate, copper(II) acetate, and L-glutamic acid were bought from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Carbonic anhydrase (CA, bovine red blood cell), formate dehydrogenase (FateDH, lyophilized), and glutamate dehydrogenase (GDH, bovine liver) were provided by Sigma-Aldrich (St. Louis, MO, USA). Nicotinamide adenine dinucleotide (NADH, 98%) was obtained from Aladdin Biotechnology Co., Ltd. (Shanghai, China). CO₂ (>99%) and ^13^CO₂ (>99%) were purchased from Beijing Ruyuan Ruquan Technology Co., Ltd. (Beijing, China). All other chemicals were obtained from Beijing Chemical Factory (Beijing, China). Double-distilled water was used in all experiments.

Instrumentation

The transmission electron microscopy (TEM) images of MOFs were performed using a JEOL 2100F transmission electron microscope (Hitachi, Ltd., Japan). Scanning electron microscopy (SEM) images of MOFs were taken with a JEOL JSM-6700F field emission scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan). Elemental analysis of HKUST-1@amine-MIL-101(Cr) was carried out using an energy dispersive X-ray spectrometer Quantax 200 XF 5010 (Bruker, Germany). Powder X-ray diffractions of MOFs were taken with a JEOL JSM-6700F field emission scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan). Elemental analysis of HKUST-1@amine-MIL-101(Cr) was carried out using an energy dispersive X-ray spectrometer Quantax 200 XF 5010 (Bruker, Germany). Powder X-ray diffractions of MOFs were obtained from a D/max-UltimaIII (Rigaku Corporation, Japan). X-ray photoelectron spectroscopy (XPS) measurements of MIL-101(Cr) and amine-MIL-101(Cr) were performed by a Escalab 250Xi (Thermo Fisher Scientific, America). Nitrogen adsorption/desorption isotherms and pore size distributions of MOF scaffolds were collected at 77 K using V-Sorb2800P surface area and porosimetry analyzer (Gold APP Instruments Corporation, Beijing, China). High-pressure CO₂ sorption measurements were carried out using an H-Sorb2600 high pressure and temperature gas sorption analyser (Gold APP Instruments Corporation, Beijing, China). The ^13^C spectrum of formic acid product was recorded on a 600 MHz Bruker AVANCE III (Bruker Corporation, Germany). A high-performance liquid chromatography (HPLC) 2030 system (Shimadzu, Kyoto, Japan) was applied to determine the concentrations of formate derivatives using a 5020-39001 WondaSil C18 column (15 × 4.6 cm i.d., 5 µm, GL Sciences) with UV detection at 280 nm.
Amine-Functionalized MIL-101(Cr) for the Storage of CO₂

The transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images in Figures 1a,b exhibited the octahedral morphology of MIL-101(Cr) nanocrystals with apparent corners and edges, which were in good agreement with literatures (Férey et al., 2005; Hwang et al., 2008). Amine-functionalized MIL-101(Cr) was obtained by the modification of MIL-101(Cr) with a series of amines including HMD, cystamine, and branched PEI with different loadings (50% and 100%) [here denoted as amine-MIL-101(Cr)]. We found that the original morphology of MIL-101(Cr) was preserved after loading of amines confirming that the amine functionalization

**RESULTS AND DISCUSSION**

**Amine-Functionalized MIL-101(Cr) for the Storage of CO₂**

High pressure CO₂ adsorption experiments were performed at 298.15 K and at pressure of 0–30 bar for 24 h using the Hi-Sorb2006 high pressure and temperature gas adsorption analyser. Before the adsorption of CO₂, the MOFs (500 mg) were dried in a sample tube under vacuum at a temperature of 120°C overnight.

For the enzymatic catalysis of bubbled CO₂ using immobilized enzymes, a mixture solution containing 10 mM L-glutamate and 2 mg/mL NADH in 6 mL of 50 mM phosphate buffer saline solution was purged with nitrogen for 0.5 h to remove the dissolved air. And 30 mg of HKUST-1@amine-MIL-101(Cr)-based multienzymes with stored CO₂ was quickly added to the above solution. The reaction was performed in a sealed flask at 25°C for 6 h.

After reaction, the supernatant was collected by centrifugation. The formic acid product was derivatized by mixing 200 µL of the sample, 100 µL of 100 mM Na₂HPO₄, and 400 µL of 20 mg/mL pentafluorobenzyl bromide in acetone, and reacted at 60°C for 1 h. The derivatized product was detected by HPLC.
step had little damage to the generic MOF (Figures 1c–f). The powder X-ray diffraction (PXRD) patterns of amine-MIL-101(Cr) were also essentially identical with its pristine counterpart indicating that the high crystallinity and purity of MOF were well-maintained (Figure 2A). However, the intensity of diffraction peaks below 7° decreased due to the filling of MOF pores by amines. The similar phenomenon has been observed by other researchers (Lin et al., 2013; Lin Y. et al., 2014). XPS in Figure 3A revealed 6.0 at% Cr, 59.6 at% C, and 34.4 at% O in MIL-101(Cr), which corresponded well
Li et al. MOF Enzymes for CO\textsubscript{2} Reduction

FIGURE 3 | X-ray photoelectron spectroscopy measurements of MIL-101(Cr) (A), HMD-MIL-101(Cr) (B), cystamine-MIL-101(Cr) (C), PEI(50)-MIL-101(Cr) (D), and PEI(100)-MIL-101(Cr) (E).

with its molecular formula C\textsubscript{24}O\textsubscript{16}H\textsubscript{17}Cr\textsubscript{3} (Férey et al., 2005). After functionalization with different amines, the appearances of 13.6, 7.25, 21.6, and 22.4 at% N elements were observed in HMD-MIL-101(Cr), cystamine-MIL-101(Cr), PEI(50)-MIL-101(Cr), and PEI(100)-MIL-101(Cr), respectively, confirming the successful postsynthetic modification (Figures 3B–E). We also measured the nitrogen adsorption/desorption isotherms at 77 K and pore size distributions of pristine and amine-MIL-101(Cr) as depicted in Figure 4. As expected, the postsynthetic modification of MIL-101(Cr) with different amines significantly reduced the specific surface areas and pore volumes as the amine functionalities occupied partial pore space of MOFs. While the surface area of MIL-101(Cr) was 2,477 m\textsuperscript{2}/g, this value was decreased remarkably to 1,922, 1,011, 1,314, and 1,160 m\textsuperscript{2}/g for HMD-MIL-101(Cr), cystamine-MIL-101(Cr), PEI(50)-MIL-101(Cr), and PEI(100)-MIL-101(Cr) (Figure 4A). The pore size distribution in Figure 4B revealed that MIL-101(Cr) had two types of micropores with a pore width of 0.63 and 1.77 nm, respectively. The pore volume was 1.44 cm\textsuperscript{3}/g. Surface modification with amines significantly decreased the number of larger pores. As shown in Figure 4B, the pore volume of bigger pores at around 1.77 nm in amine-MIL-101(Cr) was significantly decreased compared with generic MIL-101(Cr). And the pore volume for HMD-MIL-101(Cr), cystamine-MIL-101(Cr), PEI(50)-MIL-101(Cr), and PEI(100)-MIL-101(Cr) was reduced to 1.08, 0.68, 0.72, and 0.56 cm\textsuperscript{3}/g (Table 1).

Then we tested the gas adsorption performance of amine-MIL-101(Cr) for CO\textsubscript{2}. The CO\textsubscript{2} adsorption isotherm at 298 K was illustrated in Figure 4C, and the results of CO\textsubscript{2} sorption data at 5 bar and 298 K for four amine-MIL-101(Cr) were
shown in Table 2. Apparently, amine-MIL-101(Cr) showed much higher adsorption capacity for CO\textsubscript{2} compared with parent MIL-101(Cr). At 5 bar and 273 K, the CO\textsubscript{2} adsorption capacity of PEI(100)-MIL-101(Cr) reached 8.25 mmol/g, which was 4.4-fold higher than that observed in MIL-101(Cr). Similarly, the CO\textsubscript{2} adsorption capacities of HMD-MIL-101(Cr), cystamine-MIL-101(Cr), and PEI(50)-MIL-101(Cr) was 2.57, 3.11, and 4.48 mmol/g, respectively, which was 1.4−2.4 fold higher than that of unmodified MIL-101(Cr). The enhancement of CO\textsubscript{2} storage capacity may be ascribed to the introduction of amine functionalities in the MOF pore environment, which donates electrons and improves the affinity of MOF materials toward CO\textsubscript{2} molecules via dipole−quadrupole interactions (Zheng et al., 2011). Clearly, high loading of branched PEI provided more amine functionalities in MIL-101(Cr) according to the XPS results in Figure 3, which facilitated the enhancement of CO\textsubscript{2} capture capacity. As a result, PEI(100)-MIL-101(Cr) exhibited the highest adsorption capacity for CO\textsubscript{2}. For comparison, we also tested the adsorption capacity of HKUST-1 for CO\textsubscript{2}, which was only 2.17 mmol/g at 5 bar and 298 K.

### Construction of Multienzymatic Cascade System

Three enzymes including CA, FateDH, and GDH were immobilized in HKUST-1 using a layer-by-layer self-assembly
approach. HKUST-1 was selected as the solid support for the immobilization of enzymes because of its good solvent tolerance and mild preparative conditions. To fully utilize the stored CO$_2$ as substrate, the multienzyme system was constructed on the surface of amine-MIL-101(Cr). The enzymes were co-immobilized in HKUST-1 with layered structure to achieve the channeling of substrate. As illustrated in Scheme 1, using amine-MIL-101(Cr) as the core, the first HKUST-1 layer encapsulated with CA was fabricated followed by the second HKUST-1 layer containing FateDH and GDH. In this case, the CO$_2$ substrate released from amine-MIL-101(Cr) first got access to CA and were hydrated to bicarbonate ions. The HCO$_3^-$ intermediate then migrated directly to the FateDH enzyme and was converted to formic acid. GDH in the outer MOF shell was used to achieve the in situ regeneration of NADH co-factor for the continuous production of formic acid. The enzyme immobilization capacity was 267.4 mg/g for CA, and 669.6 mg/g for FateDH and GDH. It is worthy of note that the size of micropores of amine-MIL-101(Cr) does not match the large dimensions of enzymes. As a result, the immobilization of enzymes will not affect the CO$_2$ adsorption capacities of amine-MIL-101(Cr).

As shown in Supplementary Figure 1, the formation of HKUST-1 on the surface of amine-MIL-101(Cr) turned the MOF aqueous solution from green to blue-green. Energy-dispersive X-ray spectroscopy (EDS) analysis revealed the appearance of 9.98, 2.53, 10.15, and 18.62 at% Cu in HKUST-1@HMD-MIL-101(Cr), HKUST-1@cystamine-MIL-101(Cr), HKUST-1@PEI(50)-MIL-101(Cr), and HKUST-1@PEI(100)-MIL-101(Cr), respectively, implying the formation of HKUST-1 layer. The XRD patterns of HKUST-1@amine-MIL-101(Cr) illustrated in Figure 2B revealed new peaks typical of HKUST-1 nanocrystals. Further characterizations with TEM (Figures 5a–d) and SEM (Figures 5e–h) also confirmed the successful generation of HKUST-1@amine-MIL-101(Cr) nanocomposites.

We next evaluated the gas adsorption capacity of HKUST-1@amine-MIL-101(Cr) for CO$_2$. As shown in Figure 4D, the HKUST-1@amine-MIL-101(Cr) had much lower storage capacity for CO$_2$, presumably as a result of the partial filling of the micropores. But this storage capacity for CO$_2$ is still superior than using bubbled CO$_2$ as its solubility in water is only of 33 mM (Zhang Z. et al., 2018).
Conversion of CO₂ to Formic Acid

The newly constructed HKUST-1@amine-MIL-101(Cr)-based multienzymes containing CA, FateDH, and GDH were employed to reduce CO₂ to formic acid using the stored CO₂ as the starting substrate accompanied by NADH regeneration. The HCOOH synthesis reaction was carried out in batch mode containing 30 mg of MOF-based multienzymes and 2.2 mmol/L NADH in 6 mL reaction system. The preliminary reaction time was 2 h. The formic acid produced from the four multienzyme systems were calculated and compared in Figure 6A and Supplementary Table 1. Clearly, larger CO₂ adsorption capacity of MOFs corresponded to higher HCOOH production yield.

The HKUST-1@PEI(100)-MIL-101(Cr) multienzyme system exhibited the highest HCOOH production amount of 4.0 ± 0.92 mmol/L due to its largest adsorption capacity for CO₂, which was 24 ± 5.5 µmol. This is equal to 71.1% conversion yield taking into consideration that 33.75 µmol of CO₂ was stored in HKUST-1@PEI(100)-MIL-101(Cr) at 5 bar and 298 K.

To further increase the conversion yield of CO₂, we also optimized the reaction time. The production amount of HCOOH from the adsorbed CO₂ catalysed in HKUST-1@PEI(100)-MIL-101(Cr) multienzyme system was depicted as a function of reaction time. As shown in Figure 6B and Supplementary Table 2, the highest HCOOH amount of
5.0 ± 0.22 mmol/L was obtained at a reaction time of 6 h which represented a conversion yield of 88.9%. Obviously, the stored CO$_2$ was not completely transformed to formic acid. One of the possible reason is the partial release of CO$_2$ because the whole enzymatic catalysis process is performed at 1 bar. We also observed that the produced HCOOH amount decreased with the elongation of reaction time. This can be partly explained by the fact that the reaction rate of CO$_2$ to HCOOH catalysed by FateDH is much slower than its reverse reaction (HCOOH to CO$_2$) (Rusching et al., 1976; Zhang Z. et al., 2018). As we know, the production of 1 mol formic acid consumes 1 mol NADH. When the regeneration of NADH catalysed by GDH is not as effective as its consumption, the deficiency of NADH may cause the transformation of formic acid to CO$_2$.

For comparison, we also performed the enzymatic reactions catalyzed by immobilized enzymes and free enzymes using bubbled CO$_2$ as the substrate. As shown in Figure 6C, the production amount of HCOOH catalysed by free enzymes using bubbled CO$_2$ as substrate was only 0.38 ± 0.03 mmol/L. By using the immobilized enzymes to catalyse the bubbled CO$_2$, the produced HCOOH increased to 3.52 ± 0.13 mmol/L. The conversion using bubbled CO$_2$ as substrate was also calculated based on the CO$_2$ solubility of 33 mM in water (Zhang Z. et al., 2018), which was only 10.67% for immobilized enzymes and 1.15% for free enzymes, far <100%. Clearly, the produced HCOOH catalysed by the immobilized multienzyme system using stored CO$_2$ as substrate was more than 13.1-times higher than that of the corresponding free enzyme systems. These results clearly demonstrated the superiority of our new strategy. The immobilization of enzymes in HKUST-1 layered structure is kinetically advantageous over free enzymes. The adsorbed CO$_2$ was gradually released from amine-MIL-101(Cr) and was directly converted to bicarbonate ions by CA which was encapsulated in the inner layer. The intermediate bicarbonate ions were then in situ consumed by FateDH immobilized on the outer MOF layer without diffusion through long distance. The porous structure of MOF allowed efficient diffusions of substrate and products. This synthetic route facilitated the channeling of substrate and eventually enabled higher rate of the cascade reaction. Moreover, the use of adsorbed CO$_2$ as substrate provided CA and FateDH with a high CO$_2$ concentration stored in a slow-releasing MOF system as required by CA and FateDH, which allowed much more production of formic acid.

To further demonstrate that the formic acid was produced from catalysing the CO$_2$ adsorbed in MOFs instead of free CO$_2$ in the air. $^{13}$CO$_2$ was stored in MOFs and used as substrate catalysed by HKUST-1@PEI(100)-MIL-101(Cr)-based multienzymes. The final product was analyzed by $^{13}$C NMR. Figure 6D displayed the prominent peak of $^{13}$C at 174.6 ppm which belonged to H$_3$COOH. The surface morphology of HKUST-1@PEI(100)-MIL-101(Cr) immobilized enzymes was also characterized by SEM. As shown in Figure 5i, the immobilization of enzymes did not change the shape and morphology of MOF scaffolds.

### NADH Regeneration With Glutamate Dehydrogenase (GDH)

NADH is the co-factor functioning as a terminal electron donor and hydrogen donor in the cascade enzymatic reaction. The production of 1 mol formic acid from CO$_2$ consumes 1 mol costly NADH generating NAD$^+$. As the presence of NAD$^+$ suppresses the reduction of CO$_2$ to formic acid and accelerates its reverse oxidation reaction, the efficient regeneration of NADH is highly desirable. Enzymes such as glucose dehydrogenase (Obón et al., 1998; Marpani et al., 2017; Zhang Z. et al., 2018), xyllose dehydrogenase (Marpani et al., 2017) and GDH (Ji et al., 2015) have been successfully used for the regeneration of NADH. In our

### TABLE 3 | HCOOH production at different NADH concentration.

| NADH (mM) | HCOOH (mM) | $Y_{\text{HCOOH}}$ (%) |
|-----------|------------|----------------------|
| 0.5       | 1.77       | 353.9                |
| 1         | 2.83       | 283.3                |
| 2         | 3.21       | 160.6                |
| 2.8       | 5.04       | 179.8                |

### TABLE 4 | Comparison of the NADH-based methanol or HCOOH yield produced using HKUST-1@PEI(100)-MIL-101(Cr) immobilized enzymes and other immobilized systems reported in published literatures.

| Immobilization matrix | Enzyme$^a$ | Initial concentration of NADH | $Y_{\text{Product, f}}$ (%) | References       |
|-----------------------|------------|-------------------------------|----------------------------|------------------|
| Polystyrene particles | FateDH, FaldDH, ADH, GDH | 50 $\mu$M | 52.6$^a$ | El-Zahab et al., 2008 |
| GelCSi hybrid microcapsules | FateDH, FaldDH, YADH | 50 mM | 71.6$^b$ | Wang D. et al., 2014 |
| Porous silica sol-gel | FateDH, FaldDH, ADH | 50 $\mu$M | 91.2$^d$ | Obert and Dave, 1999 |
| Alginate-silica hybrid gel | FateDH, FaldDH, ADH | 940 $\mu$M | 98.1$^b$ | Xu et al., 2006 |
| Hollow nanofiber membrane | FateDH, FaldDH, ADH | 1 mM | 103.2$^b$ | Ji et al., 2015 |
| ZIF-8 | FateDH, GDH, FaldDH, ADH | 10 mM | 40.2$^b$ | Zhu et al., 2019 |
| Titania nanoparticle | FateDH, FaldDH | 50 $\mu$M | 92.7$^c$ | Shi et al., 2012 |
| Millimeter-scale gel bead | FateDH, FaldDH, ADH | 0.1 mM | 22.5$^b$ | Jiang et al., 2009 |
| HKUST-1@PEI(100)-MIL-101(Cr) | CA, FateDH, GDH | 0.1 mM | 353.9$^f$ | This work |

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$^a$Formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH), alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH), and yeast alcohol dehydrogenase (YADH).

$^b$The final product was methanol.

$^c$The final product was formic acid.
work, GDH was adopted to attain the continuous conversion of NAD\(^+\) to NADH.

We investigated the effects of NADH concentration on the overall reaction efficiency by varying the added NADH amount in the reaction solution at a final concentration between 0.5 and 2.8 mM while keeping the immobilized enzyme amount constant. The NADH-based HCOOH yield (\(Y_{HCOOH}\)) was calculated according to the following equation.

\[
Y_{HCOOH} = \frac{C_{HCOOH}}{C_{NADH, initial}} \times 100
\]

where \(C_{HCOOH}\) is the HCOOH concentration (mM) at a reaction time of 6 h, and \(C_{NADH, initial}\) is the initial NADH concentration (mM).

As shown in Table 3, the production of HCOOH raised up to 5.04 mM when the NADH concentration increased from 0.5 to 2.8 mM, while \(Y_{HCOOH}\) decreased from 353.88 to 179.82%.

This trend was similar to the work reported by Zhang et al. in which the sequential co-immobilization of five enzymes in hollow nanofiber was achieved and used for the synthesis of methanol from CO\(_2\) (Ji et al., 2015). As reported by Pinelo et al. (Zhang Z. et al., 2018), the reaction rate for reducing NAD\(^+\) to NADH is much higher than its reverse oxidation reaction catalysed by FadE DH. The same finding was also observed in our work. NADH was efficiently regenerated by GDH encapsulated in the outer MOF shell. The catalytic performance of our newly designed HKUST-1@PEI(100)-MIL-101(Cr) immobilized systems compares well with the values obtained from other immobilized enzymes published by other groups shown in Table 4.

**Operational Stability and Reusability**

The operational stability and reusability of enzymes immobilized in HKUST-1@PEI(100)-MIL-101(Cr) were evaluated by testing the HCOOH production amount after repeated catalysis of adsorbed CO\(_2\) for 10 cycles. After one batch of reaction for 6 h, the MOF scaffold containing enzymes were dried using freeze drying and used for the adsorption of CO\(_2\) at 5 bar and 298 K before the next batch of catalysis. As shown in Figure 6E, the NADH-based HCOOH yield (\(Y_{HCOOH}\)) was still 86% even after 10 cycles of reuse. A cumulative HCOOH yield of 1077.7% was obtained from the 10 reusing cycles of this reaction system indicating the good operational stability and reusability of the immobilized enzymes.

We also tested the chemical tolerance of the MOF scaffold. The HKUST-1@PEI(100)-MIL-101(Cr) nanocomposite obtained from 10 cycles of reusing was subjected to SEM measurement. As shown in Figure 5j, there was no change in the morphology of MOF support indicating its high chemical stability. The gas storage capacity of HKUST-1@PEI(100)-MIL-101(Cr) nanocomposite after 10 cycles of reusing was also evaluated. As illustrated in Figure 4D, the repeated reaction did not lead to any decrease in the CO\(_2\) uptake capacity thus confirming the reusability of the MOFs as absorbent for the storage of CO\(_2\).

**CONCLUSIONS**

We have developed a new MOF scaffold that functions as absorbent for the storage of CO\(_2\) as well as solid support for the sequential co-immobilization of multi-enzymes via a layer-by-layer self-assembly approach. This new strategy used the adsorbed CO\(_2\) as substrate, facilitated the channeling of substrate, and eventually enabled high catalytic efficiency with a continuous regeneration of NADH co-factor. Improved operational stability and reusability were also observed in immobilized enzymes implying the great potential of our new strategy for the biotransformation of CO\(_2\) used in industrial applications.

**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

YLi and LW carried out the experiments. YLv and TT conceived and designed the experiments. YLv and YLi analyzed the data and wrote the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2019.00394/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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