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Immobilization of carbonic anhydrase from *Sulfurihydrogenibium azorense* onto Immobead-350 for CO_2 capture.

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Abstract

Carbon emissions increase every year, breaking a record of 35.7 billion tons released into the atmosphere in 2022. In this scenario, the enzyme carbonic anhydrase (CA) has been a promising alternative to carry out the bioprocess of carbon capture and storage. Within biotechnological processes, one can highlight the obtaining of CA from heterologous expression in a simple microorganism such as *Escherichia coli*. Therefore, considering the importance of obtaining the carbonic anhydrase enzyme for use in CO_2 mitigation technologies and for improving CA's industrial applicability, this work aims to produce recombinant thermostable carbonic anhydrase in Escherichia coli and evaluated its immobilization on support Immobead 350. For this, CA from from *Sulfurihydrogenibium azorense* was obtained by heterologous expression in *Escherichia coli* ArcticExpress (DE3) using IPTG as an inducer. The extracts obtained were characterized, purified and immobilized on Immobead 350. The purified extract showed a 416.42 ± 19.83 WAU/mg. The heterogeneous biocatalyst produced (CA-IB350) showed 599.73 ± 45.77 WAU/g of derivative activity, high immobilization yield (≈89 %) and a recovered activity of 52.92 ± 9.49%. This study presented promising and innovative protocols for the effective expression of carbonic anhydrase from *Sulfurihydrogenibium azorense* and its immobilization for CO_2 capture.

Keywords: Decarbonization; Biocatalyst; Carbon capture.

1. Introduction

Over the last centuries, industrialization has been steadily raising the levels of greenhouse gases, leading to notable shifts in the global climate. Carbon dioxide (CO_2) stands out as the most frequently emitted greenhouse gas, and fossil CO2 emissions have surged considerably from 22,683 Mt/yr in 1990 to 38,016 Mt/yr in 2019 [1]. Therefore, the reduction of CO_2 emissions and their conversion into usable materials are crucial for the advancement of sustainable development. A series of technologies that can capture CO_2 have been developed, such as physical absorption [2] and chemical absorption [3]. Although these methods can effectively capture CO_2, they also have problems such as the generation of by-products, high-energy consumption, and secondary pollution [4]. The use of biological processes for carbon capture and storage (CCS) is an eco-friendly means to reduce the concentration of CO_2 in the atmosphere and reduce CO_2 emissions at the source, which can avoid the problem of secondary pollution during CO_2 capture.

Carbonic anhydrase (CA) is an enzyme that catalyzes the conversion of CO_2 into bicarbonate (HCO_3^-), and it is involved in crucial physiological processes [5]. The use of CA to capture CO_2 has attracted increasing attention due to its mild reaction conditions, lack of secondary pollution, and simplicity [6]. Due to the fact that biomimetic carbon sequestration is frequently carried out in challenging environments characterized by elevated temperatures and high levels of organic ions and metals, the use of thermostable carbonic anhydrase emerges as a sensible option5. Among thermostable CA, an α-CA from thermophilic archaea *Sulfurihydrogenibium azorense* (SazCA) stands out because it is one of the fastest CA known with a k*cat* of $4.4 \times 106\ s^{-1}$ [7]. In order to improve the industrial applicability of CA, immobilization has been

investigated as a potential method for large-scale application of CA, because it not only improves the tolerance of CA under extreme conditions but also enables the reuse and recycling of CA, so that the cost of the entire process can be reduced [8].

Thus, this work aims to produce recombinant thermostable carbonic anhydrase in *Escherichia coli* by heterologous expression and evaluated its immobilization onto support Immobead 350 for its use to enzymatic technology for CO_2 capture.

2. Material & Methods

2.1. Bacterial Strain and Construction of the Recombinant Carbonic Anhydrase (rCA)

Escherichia coli ArcticExpress (DE3) were transformed with the vector harboring the recombinant gene of carbonic anhydrase from Sulfurihydrogenibium azorense by the electroporation method and enzyme production was induced through the lac operon. The recombinant protein has 6 histidine residues (His) in the N-terminal portion of its structure.

2.2. Culture Medium and Assay Conditions

For the expression of rCA, enzyme production was induced with 0.5 mM of IPTG final concentration as inducer agent. A volume of 1 mL of reactivated culture, 1% (v/v) inoculum, was transferred to 100 mL of Luria Bertani medium containing 50 μg/mL of kanamycin and incubated at 30 °C, 250 rpm until it reached to an OD_{600nm} from 0.6 to 0.8. Immediately, the culture was induced for CA expression by the addition of IPTG. The expression occurred at 12 °C, 250 rpm and 24h. At the end of assay, cultures were centrifuged at 7000×g at 4 °C for 20 min. Supernatant was discarded and cells were subsequently washed with 0.1 M NaCl solution followed by centrifugation at 7000×g at 4 °C for 30 min. Cell pellets were then stored at − 20 °C for further protein expression studies and enzyme activity assays.

2.3. Cell Lysis and Extraction of rCA

Cell pellets were resuspended in 20 mM Tris HCl pH 8.3 and cell lysis were conducted by sonication (Sonicator Qsonic, Q500 Sonicators, USA) at 30% of amplitude, for 4 min (working time and interval of 3 s) at 4 °C. Cell suspension was sonicated until a transparent pale yellow color was obtained. After cell disruption, samples were centrifuged at 5000xg, 4 °C for 30 min and supernatant (crude CA extract) was stored at − 20 °C and further used to determine enzyme activity, protein concentration and SDS-PAGE.

2.4. Protein Quantification

Protein concentration was determined by the Bradford method using bovine serum albumin as standard for calibration curve construction [9].

2.5. Analysis of rCA Expression by SDS–PAGE

Protein expression levels were determined by SDS–PAGE electrophoresis and employing Coomassie Brilliant Blue R-250 as staining agent as described by Laemmli [10].

2.6. Enzyme Purification

Purification of crude enzyme extracts was performed by immobilized metal affinity chromatography (IMAC) using Ni-Sepharose 6 Fast Flow matrix with a degree of activation of 15 μmol of Ni^{2+} ion per ml of wet resin (GE Healthcare). Chromatography column (1.5 cm high per 1.5 cm wide; volume column: 3 mL) was equilibrated with five column volumes of 20 mM Tris HCl pH 8.3 and 150 mM NaCl buffer. Then, 2 mL of enzymatic extract was applied to column. Non-retained proteins were recovered and immediately washed with 20 mM Tris–HCl pH 8.3, 150 mM NaCl, and 6 mM imidazole buffer in order to ensure the removal of the weakly bound proteins. After that, five column volumes of elution buffer (20 mM Tris HCl pH 8.3, 150 mM NaCl and 250 mM imidazole) was applied to the column in order to collect the purified protein. Finally, purified rL-AI was dialyzed for 24 h against 20 mM Tris–HCl pH 8.3 for imidazole remotion and further analyzed by SDS–Page.

2.7. Immobilization on Immobead-350

Preliminary studies of rCA immobilization were conducted a macroporous acrylic polymers support, Immobead-350 (IB-350). Firstly, 1 g of IB-350 was incubated in 12.5 mL of ethanol (95%) for 4 h at 25 °C under gently stirring. Then, the support was filtered and washed with distilled water. After, 1 g of support IB-350 was suspended in 10 mL of

enzyme solutions (protein concentration of 5.0 mg/$g_{support}$) in 20 mM Tris-HCl buffer at pH 10 and 25 °C under gentle stirring for 2 h. The immobilized biocatalysts were incubated in 1 M EDA at pH 10 and 25 °C for 24 h to block the remaining reactive groups in the support. The immobilization parameters was determined as described by Silva and colleagues [12].

2.8. Enzymatic Activity Assay

The rCA activity was assayed by its esterase and hydratase activity. The assay procedures were the same as those reported by others [11]. For hydratase activity, one Wilbur–Anderson unit (WAU) is defined as (T_0 - T) / T, where T_0 and T are the time needed for pH changing from 8.3 to 6.3 in the absence and presence of CA, respectively.

3. Results & Discussion

3.1. Purification and Characterization of recombinant Carbonic Anhydrase

Purification of recombinant carbonic anhydrase was performed by immobilized nickel affinity chromatography and the results obtained can be seen in Table 1. Purified CA extracts showed an enzyme specific activity of 416.42 WAU/mg, which turned out to be 3.03-fold higher than the specific activity of the crude extract (137.56 WAU/mg).

Table 1. Purification parameters of carbonic anhydrase obtained from heterologous expression.

Parameters	Enzymatic Extract	
	Crude	Purified
Enzymatic activity (WAU/mL)	483.36 ± 32.61	437.24 ± 14.72
Protein concentration (mg/mL)	3.51 ± 0.40	1.05 ± 0.04
Specific activity (WAU/mg)	137.56 ± 13.43	416.42 ± 19.83
Purification factor	1.0	3.03

The Fig.1 displays the SDS–PAGE profile of the different samples collected during purification. By comparing lanes one (crude extract) and four (purified enzyme), it can be observed a removal of other contaminant proteins and presence of higher concentrations of target carbonic anhydrase (around 30 kDa).

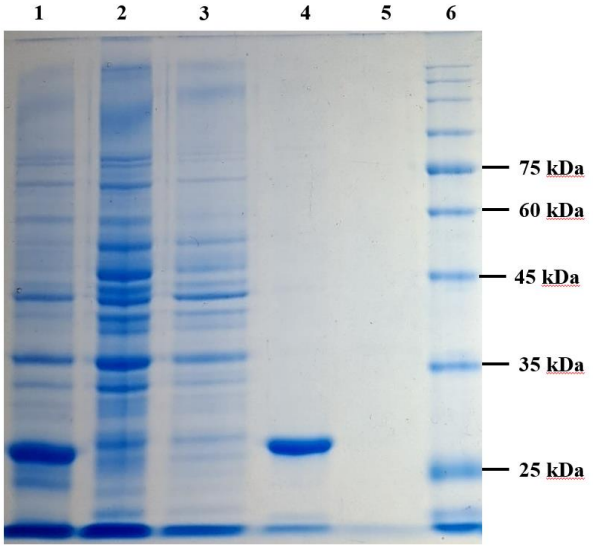

Fig. 1. SDS–PAGE profile of the protein fractions from carbonic anhydrase extracts produced by heterologous expression after each purification step. 1. Crude extract. 2. Fraction not retained in the Ni^{2+}-sepharose column (auto-induction). 3, 5. Washings. 4. Purified enzyme. 6. Molecular weight standards.

3.2. Immobilization of carbonic anhydrase onto Immobead 350

The immobilization of carbonic anhydrase on IB-30 was evaluated and the results obtained are presented in Table 2. The immobilization time was pre-defined at 2h and load of enzyme used was 5 $mg_{enzyme}/g_{support}$. The heterogeneous biocatalyst produced (CA-IB350) showed 599.73 ± 45.77 WAU/g of derivative activity, high immobilization yield (≈89 %) and a recovered activity of 52.92 ± 9.49%.

Table 2. Immobilization parameters of carbonic anhydrase on Immobead-350. Yield Immobilization (IY), Immobilized enzyme activity (At_d) and activity recovered (At_R).

Biocatalyst	IY (%)	At_d (WAU/g)	At_R (%)
CA-IB350	88.99 ± 1.29	599.73 ± 45.77	52.92 ± 9.49

4. Conclusion

The studies presented show that it was possible obtain an enzymatic extract of carbonic anhydrase from *Sulfurihydrogenibium azorense* by heterologous expression in *E. coli* ArcticExpress (DE3) presenting a high value of hydratase activity (483.36 ± 32.61 WAU/mL). The preliminary studies of CA immobilization have shown that the assayed on Immobead-350 immobilization strategy was efficient, producing a biocatalyst with high activity and recovered activity. Finally, this study presented promising and innovative protocols for the effective expression of carbonic anhydrase from *Sulfurihydrogenibium azorense* and its immobilization for its use to enzymatic technology for CO_2 capture.

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