# Effects of novel auto-inducible medium on growth, activity and $CO_2$ capture capacity of *Escherichia coli* expressing carbonic anhydrase

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## ABSTRACT

A glucose-based auto inducible medium (glucose-AIM) has been developed to enhance both growth and expression of lac operon-linked carbonic anhydrase (CA) expression in a recombinant strain of *Escherichia coli*. When the *E. coli* expressing CA was grown on various media, the glucose-based auto inducible medium (glucose AIM) resulted in a CA activity of 1022 mU $OD_{600nm}^{-1}$ $mL^{-1}$ at 24 h and a specific growth rate of 0.082 $h^{-1}$. The CA activity was four to fourteen times higher than those by LB-IPTG. The *E. coli* expressing CA grown on the glucose-AIM showed highest activity at pH 8.5 while it kept high stability up to 40 $^{o}$C and an

inlet $CO_2$ concentration of 6%. These findings indicate the glucose-AIM would be a cost-effective medium to support high cell growth, CA activity and stability for effective $CO_2$ capture.

**Keywords:** Carbon capture, carbonic anhydrase, whole cell biocatalyst, auto inducible media, enzyme stability, enzyme characterization

## 1. Introduction

Carbonic anhydrase (CA)-driven carbon capture has been studied as an alternative to conventional absorption and adsorption methods for carbon capture (Kanth et al., 2013; Olajire, 2010). This enzyme catalyzes the hydration of carbon dioxide into bicarbonate and a proton in aqueous solution. Recently *Escherichia coli* expressing CA at its outer membrane has shown high potential to effectively capture and sequester carbon dioxide from flue gas and biogas by its hydration action (Fan et al., 2011).

An economical medium for the *E. coli* expressing CA should be developed in order to make practical the $CO_2$ capture processes that employ them. To date Luria-Bertani medium with isopropyl-β-D-1-thiogalactopyranoside as an inducer (LB-IPTG) has been widely used for expression of enzymes from recombinant bacteria including *E. coli* expressing CA (Fan et al., 2011; Jo et al., 2013; Patel et al., 2013), however, this requires additional chemicals, cost, and careful planning which limited the use of recombinant bacteria in scaled processes (Marbach & Bettenbrock, 2012; Martin et al., 2008).

To circumvent the above drawbacks of LB-IPTG media, the present paper discusses the development of an economical medium for *E. coli* expressing CA to replace LB-IPTG and LB-

AIM. The proposed medium for the *E. coli* expressing CA was a glucose-based auto inducible medium (glucose AIM) that was reformulated from the previous LB-AIM (please seeTable 1). It was specifically designed for lower medium cost, high *E. coli* growth, and high expression of recombinant cell membrane-displayed CA. To the best of our knowledge, there have been no other studies to use glucose-AIM for growth and enzyme (carbonic anhydrase) expression in recombinant *E. coli*. The cell growth and CA activity upon inoculation in three different media (LB-IPTG, LB-AIM, and glucose AIM) were comparatively investigated. Furthermore, the CA stability at various conditions such as pH, temperature and inlet $CO_2$ concentration were investigated.

## 2. Materials and methods

### 2.1. Bacteria transformation and expression of carbonic anhydrase

Chemically competent *Escherichia coli* BL21 STAR$^{TM}$ (DE3) cells were transformed by heat shock with the plasmid pETN-CA for surface display of recombinant carbonic anhydrase (Fan et al., 2011). The pETN-CA plasmid carrying a C-terminal $His_6$-tagged CA from *Helicobacter pylori* fused to the C-terminus of an N-terminal domain of the ice nucleation protein (INP) from *Pseudomonas syringae* for surface-anchoring on the outer membrane of *E. coli* was provided by Professor S. T. Yang (Fan et al., 2011). Transformants were selected on LB agar plate (1% tryptone, 0.5% yeast extract, 1% NaCl, 2% agar) containing 100 μg/mL ampicillin. *E. coli* cells were grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl) with 100 μg/mL of ampicillin. When induced with isopropyl-β-D-1-thiogalactopyranoside (IPTG), *E. coli* strains harboring pETN-CA were grown to $OD_{600}$ = 0.5 in 250 mL of LB medium at 37°C, then cooled to 25°C and induced with 0.2 mM IPTG and 0.5 mM $ZnSO_4$.

The *E. coli* displaying CA was harvested by centrifugation (5,000 g for 5 min) at 4°C, resuspended in PBS buffer (8 g of NaCl; 0.2 g of KCl; 1.44 g of $Na_2HPO_4$; 0.24 g of $KH_2PO_4$, pH 7.4) containing a protease inhibitor cocktail, and lysed with a Qsonica sonicator. After centrifugation (20,000 ×g for 15 min), the supernatant was boiled with SDS-PAGE loading buffer directly, and then was loaded to 12% SDS-PAGE. After being separated by electrophoresis, proteins were then transferred to a PVDF membrane. Thereafter, western blotting was performed using mouse monoclonal anti-His tag (Lamda) as primary antibody and antimouse antibody conjugated with HRP (Southern Biotech) as secondary antibody to verify the presence of N-CA in the protein sample.

2.2. Effect of different medium on cell growth and CA activity

The LB-IPTG, LB-AIM and glucose AIM were formulated according to protocol (Table 1), sterilized by autoclave, and supplemented with ampicillin at 50 μg $mL^{-1}$ to prevent plasmid loss and 1 mM $ZnSO_4$ to enhance expression of CA. The glucose AIM was prepared based on the composition in Table 1 by excluding tryptone, keeping only slight amount of yeast extract (0.125 g $L^{-1}$ compared with 5 g $L^{-1}$ in LB-AIM; 2.5% of yeast extract concentration in LB-AIM) and increasing glucose and lactose concentration by factor of 2 compared with LB-AIM. The *E. coli* grown in these three media was cultivated under continuous shaking (200 rpm) while the temperature, pH and dissolved oxygen concentration were controlled to 25 $^{o}$C, pH 7 and 1-1.5 mg $O_2$ $L^{-1}$, respectively. IPTG was introduced to the LB-IPTG culture after 2 h of incubation for a final concentration of 50 mM. The growth and activity of *E. coli* expressing CA on LB-IPTG, LB-AIM and glucose AIM were comparatively investigated by monitoring $OD_{600}$ and CA activity using the Wilbur-Anderson reaction.

2.3. Effect of pH, and temperature on CA activity

The *E. coli* expressing CA was grown on glucose-AIM at pH 7 for 24 h, and was harvested to examine CA stability at various pH and temperature. The cells were incubated at various pH (7-10) or temperature (25-50 $^{o}$C) for 4 h to determine the residual CA activity (%).

2.4. Effect of inlet $CO_2$ concentration on removal of $CO_2$

The effects of inlet $CO_2$ concentration (1 – 15 % v/v) on the removal of $CO_2$ were determined in a bubble column at the selected conditions (1 L $min^{-1}$ gas flow rate, culture density of 2 $g_{dw}$ $L^{-1}$, pH of 8.5 and temperature of 25 $^{o}$C). The bubble column (4 cm ID and 32 cm high, for a volume of 0.4 L) was filled with 0.3 L of the *E. coli* culture grown on glucose AIM. A metered stream of air enriched with $CO_2$ was continuously introduced through a fine gas sparger (VWR, Radnor, PA) to the bubble column where the *E. coli* culture was kept for 1- 2 hours. Batch operation of the liquid without wasting any biomass was warranted as the experiments were relatively short in duration. The pH of the bacterial culture was continuously monitored using the pH sensor and online data acquisition system (Vernier Software, OR). The pH of the bacterial culture was maintained at 8.5, which is the optimum pH for CA activity by frequent addition of acid and base (HCl and NaOH) using a pipette.

2.5. Analytical methods

The CA activity (mU $OD600^{-1}$ $mL^{-1}$) and capacity (mU $mL^{-1}$) using the Wilbur-Anderson reaction were measured by the methods previously reported (Kim et al., 2012; Wilbur & Anderson, 1948). 400 µL of *E. coli* culture and 2 mL of $CO_2$-saturated deionized water (at 0 °C) were added to 3mL of 20 mM Tris-sulfate buffer (pH 8.3) on ice. The time required for the pH

to decrease from 8.3 to 6.3 in the reaction with CA (t) and without CA ($t_o$) was used to determine the CA activity (mU $OD_{600nm}^{-1}$ $mL^{-1}$) and capacity (1022 mU $mL^{-1}$) by using Eq. 1 and 2 (Fan et al., 2011):

$$\text{CA activity} = \frac{\left[\frac{t_0 - t_f}{t_f}\right] \times 1000}{OD_{600} \times \text{culture volume}}$$

(Eq. 1)

$$\text{CA capacity} = \frac{\left[\frac{t_0 - t_f}{t_f}\right] \times 1000}{\text{culture volume}}$$

(Eq. 2)

where $t_o$ is the time in seconds for the sterile media's pH to decrease from 8.3 to 6.3 , t is the time in seconds for the *E. coli* culture to decrease in the same range, and culture volume the volume of *E. coli* culture used in the reaction.

Bacterial growth was monitored by measuring the absorbance at 600 nm (OD600) by spectrophotometer (Bio-Rad, Hercules, CA) over time. $CO_2$ concentration was monitored using a Vernier $CO_2$ Gas Sensor with Lab-Pro interface (Beaverton, OR) and $CO_2$ gas analyzer (Servomex, Houston, TX).

Removal efficiency (RE %) is defined by Eq. 1:

$$RE\% = \frac{C_{inlet} - C_{outlet}}{C_{inlet}} \times 100$$

(Eq. 3)

where $C_{inlet}$ and $C_{outlet}$ are the inlet and the outlet $CO_2$ concentrations in $g/m^3$.

## 3. Results and Discussion

### 3.1. Cell growth and CA activity in various media

The *E. coli* successfully grew and CA was induced in various media such as LB-IPTG, LB-based auto-inducible medium (LB AIM) and glucose auto-inducible medium (glucose AIM) as seen in Table 1 in the supplementary information. SDS-PAGE and Western Blot analysis confirmed CA expression in both traditional LB-IPTG and glucose auto-inducible medium (Fig.A.1 and Fig.A.2). The glucose AIM was able to support cell growth and CA induction as well, although it required more time to do so and reached lower cell concentration (Fig. 1A). The specific growth rates of the *E. coli* were estimated to be 0.110, 0.106, and 0.082 $h^{-1}$ for LB-IPTG, LB-AIM, and glucose AIM, respectively. The lower cell growth in glucose AIM (the maximum OD of 2.76 that was 2.2 x $10^9$ cells/mL) was thought to be lack of mineral nutrients compared with the LB-based media. However, the glucose-AIM resulted in highest CA activity at 24 h (1022 mU $OD_{600nm}^{-1}$ $mL^{-1}$) which was four to fourteen times higher than those by LB-AIM and LB-IPTG (Fig. 1B). Besides, the glucose-AIM also showed the maximum CA capacity which was higher than those by LB-IPTG and LB-AIM as depicted in Fig. 1C. The optimal CA activity for glucose-AIM and LB-AIM was found to be after 24 h incubation in the flasks, while it was 36 h for LB-IPTG (Fig. 1B).

In short, the glucose AIM was better than the LB-IPTG and the LB-AIM in terms of cell growth, enzyme activity, and capacity in addition to its low costs. The expected costs for the glucose AIM, the LB AIM and the LB IPTG were calculated in Table A.1. It indicates that the glucose AIM ($2.8/L) was much cheaper than the LB AIM ($6.3/L) and the LB IPTG ($6.7/L).

This substantial increase was likely due to active cell growth and high expression of CA with sequential consumption of glucose and lactose during cultivation of the CA-displaying *E. coli*.

3.2. Characterization of CA displayed by the *E. coli* in glucose AIM

The *E. coli* expressing CA was incubated at various pH and temperature to determine the stability of CA in the *E. coli* when the cells grown in the glucose-AIM were harvested after 24 h. Fig. 2 shows the relative enhancement of CA activity at various pH. The highest CA activity was found at pH 8.5 which was three times higher than that at pH 7. The CA activity at pH 8 and 9 also exhibited almost 1.5 times higher than that at pH 7. Therefore, these results indicate the CA activity in the *E. coli* was significantly enhanced when the pH was adjusted to pH 8.5 followed by pH 8 or 9 compared with that at pH 7. It also supported that CA activity could be stable at broad range of pH (pH 7 – 9).

CA needs its stability at thermal conditions (40 – 50 °C) when it is used for post-combustion carbon capture and sequestration (CCS). The incubation of the *E. coli* expressing CA at various temperature presents that there was slight decrease of CA activity at 40 $^{o}$C (25 – 30% for 4 h), gradual decrease at 45 $^{o}$C and drastic decrease of CA activity (80% for 4 h) at 50 $^{o}$C while negligible decrease of CA activity at 25 $^{o}$C (Fig. 3). Particularly, the *E. coli* kept 65% of the initial CA activity after 24 h incubation at 40 $^{o}$C. The thermal deactivation constants were estimated: 0.02, 0.08, 0.16 and 0.60 $h^{-1}$ at 25, 40, 45 and 50 $^{o}$C, respectively, when the thermal deactivation followed first-order reaction kinetics. Thus, this finding indicated stable $CO_2$ capture by the CA-displaying *E. coli* would be maintained up to 40 $^{o}$C. However, a bioreactor using the *E. coli* would need cooling of flue gases or to be compensated with active *E. coli* cells from an external cell reservoir if the temperature of flue gas is higher than 40 $^{o}$C.

3.3. Effects of inlet $CO_2$ concentration on $CO_2$ removal in a bubble column

The effects of inlet $CO_2$ concentration (1 – 15 %) on the removal of $CO_2$ were determined in a bubble column at the selected conditions (1 L $min^{-1}$ gas flow rate, culture density of 2 $g_{dw}$ $L^{-1}$, pH of 8.5 and temperature of 25 $^oC$). As seen in Fig. 4, increasing the inlet $CO_2$ concentration from 1% to 6% (thus increasing the $CO_2$ load) resulted in almost no reduction of $CO_2$ removal efficiency. On the contrary, the inlet $CO_2$ concentration above 8% started to decrease the $CO_2$ removal efficiency, with low removal efficiency at an inlet $CO_2$ concentration of 15 %, indicating inhibition with high $CO_2$ concentrations. However, the $CO_2$ removal efficiency at 8 and 10% $CO_2$ increased to almost 90% when the *E. coli* concentration increased from 2 $g_{dw}$ $L^{-1}$ to 4 $g_{dw}$ $L^{-1}$ (data not shown), which indicates potential $CO_2$ removal enhancement with increasing biomass concentration (enzyme loading). These features suggest promising application for carbon capture from flue gas.

## 4. Conclusions

Despite having slightly less cell growth, glucose AIM allows the highest CA activity compared with the conventional LB-IPTG and LB-AIM media. The CA in the *E. coli* showed highest activity at pH 8.5, but remained active between pH 7 and 9. The CA kept high stability up to 40 $^oC$ but drastically decreased at 50 $^oC$. This indicated that *E. coli* expressing CA would support stable $CO_2$ capture up to 40 $^oC$, while cooling of inlet flue gas and compensation of active cells at regular intervals would be required to capture $CO_2$ in flue gases above 50 $^oC$. Additionally, inlet $CO_2$ concentrations up to 6% (v/v) resulted in almost no reduction of $CO_2$ removal efficiency. However, inlet $CO_2$ concentrations above 8% caused decreased $CO_2$ removal

efficiency due to inhibition of $CO_2$ capture activity with high concentration of $CO_2$. Overall, these findings demonstrated that cultivation of CA-displaying *E. coli* in glucose-AIM would be a cost-effective means to support high cell growth, CA activity and stability at various operating conditions for efficient $CO_2$ capture.

**Acknowledgements**

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## Figure captions

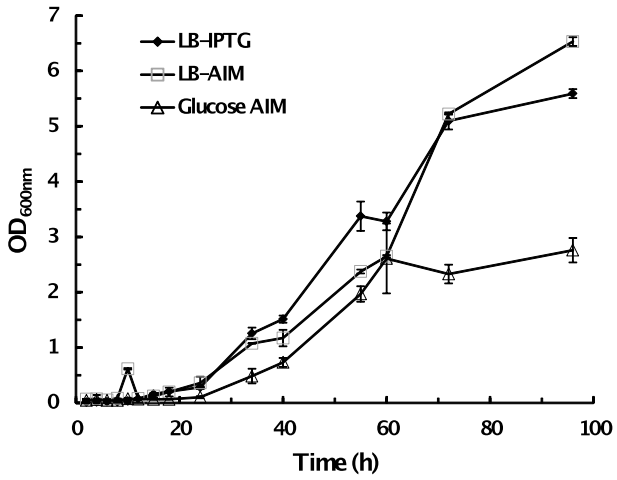
**Fig. 1.** Cell growth (A), CA activity (B), and CA capacity (C) in LB-IPTG, LB-AIM, and glucose AIM. Conditions: culture volume, 0.25 L in 0.5 L flask; culture pH, 7; culture temperature, 25 °C. The error bars were calculated from the triplicate measurement.

**Fig. 2.** Effects of pH on CA stability. Conditions: culture volume, 0.25 L in 0.5 L flask; glucose AIM; temperature, 25 °C. The relative enhancement in CA activity (dimensionless) was calculated by diving the CA activity at any pH by that at pH 7. 100% = 1010 mU $OD_{600}^{-1}$ $mL^{-1}$. The error bars were calculated from the triplicate measurement.

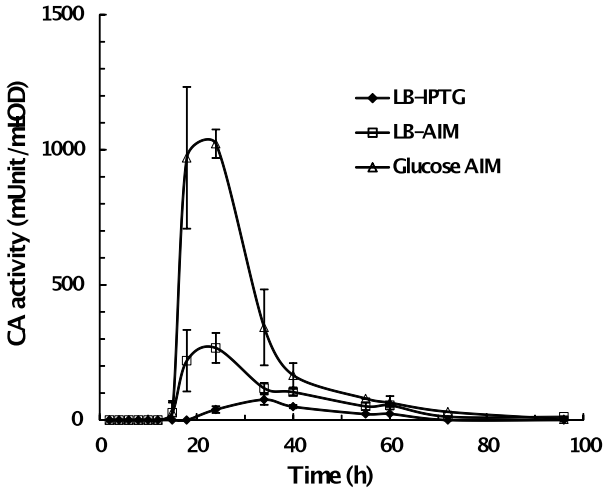
**Fig. 3** Effects of temperature on CA stability. Conditions: culture volume, 0.25 L in 0.5 L flask; glucose AIM; pH, 7. 100% = 985 mU $OD_{600}^{-1}$ $mL^{-1}$. The error bars were calculated from the triplicate measurement.

**Fig. 4** Effects of $CO_2$ inlet concentration on $CO_2$ removal efficiency. Conditions: 0.3 L culture in 0.4 L bubble column, glucose AIM, pH 8.5 (controlled), 25 $^{o}$C, 2 $g_{dw}$ $L^{-1}$, air flow rate of 1 L $min^{-1}$. Please see the detailed information in the methods and material. The error bars were calculated from the triplicate measurement.

(A)




(B)




(C)

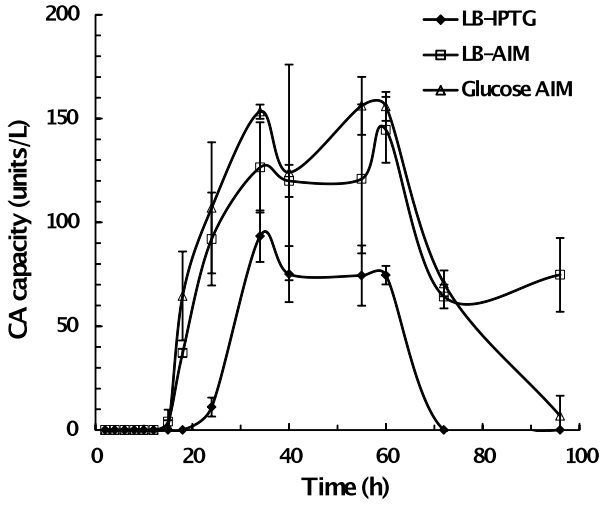
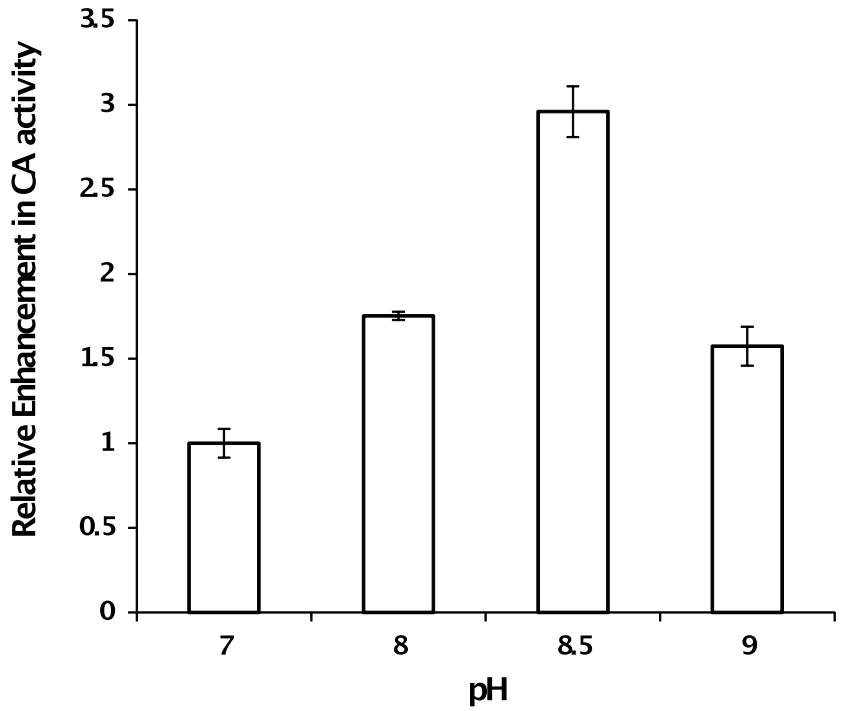



**Fig. 1.** Cell growth (A), CA activity (B), and CA capacity (C) in LB-IPTG, LB-AIM, and glucose AIM. Conditions: culture volume, 0.25 L in 0.5 L flask; culture pH, 7; culture temperature, 25 °C. The error bars were calculated from the triplicate measurement.

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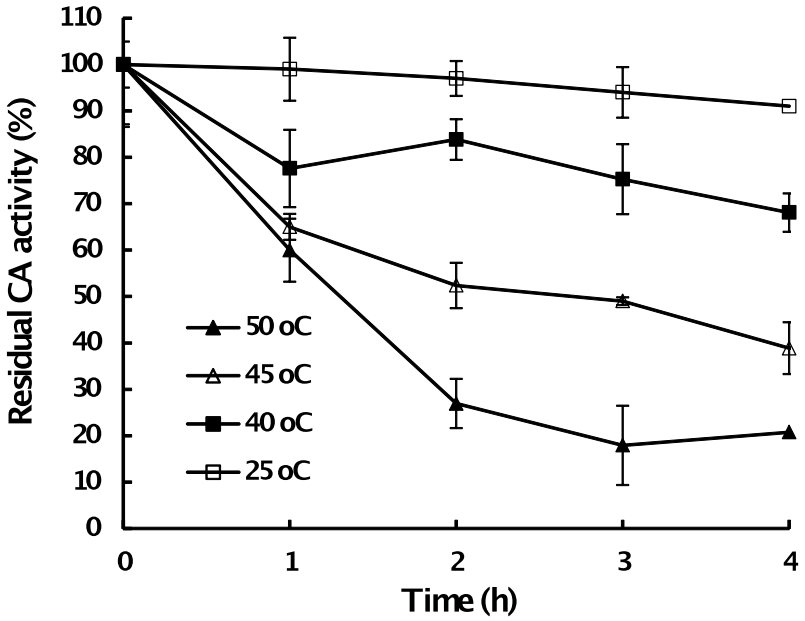


**Fig. 3** Effects of temperature on CA stability. Conditions: culture volume, 0.25 L in 0.5 L flask; glucose AIM; pH, 7. 100% = 985 mU $OD_{600}^{-1}$ $mL^{-1}$. The error bars were calculated from the triplicate measurement.

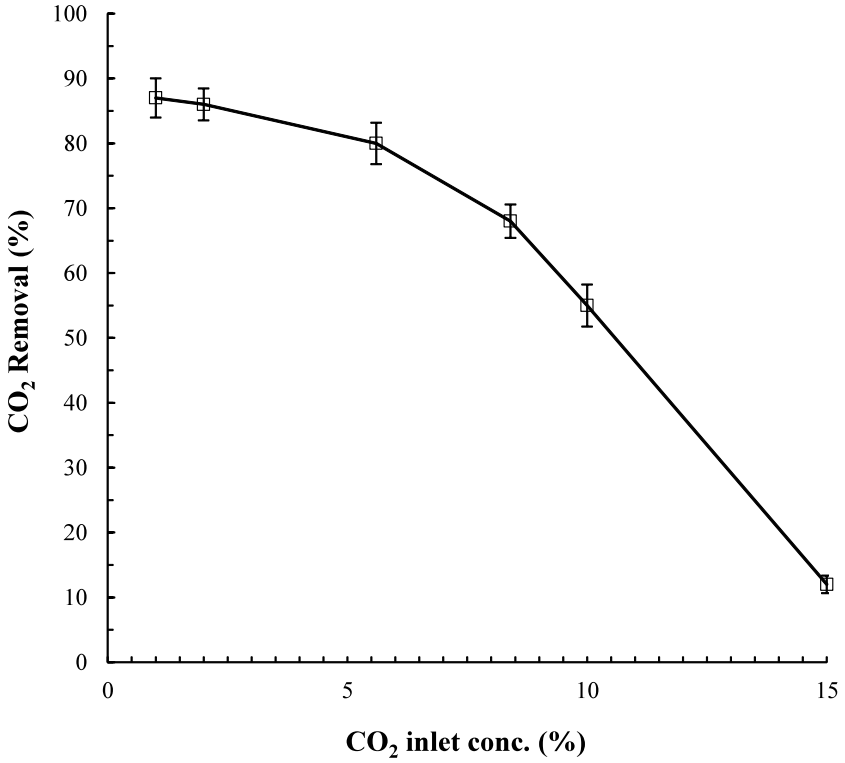


**Fig. 4** Effects of $CO_2$ inlet concentration on $CO_2$ removal efficiency. Conditions: 0.3 L culture in 0.4 L bubble column, glucose AIM, pH 8.5 (controlled), 25 $^{o}$C, 2 $g_{dw}$ $L^{-1}$, air flow rate of 1 L $min^{-1}$. Please see the detailed information in the methods and material. The error bars were calculated from the triplicate measurement.

**Table 1**

Composition of three media used for carbonic anhydrase expression in the *E. coil*

| $g\ L^{-1}$ | LB with IPTG | LB AIM | Glucose AIM |
|---|---|---|---|
| NaCl | 10 | 10 | 0.5 |
| Tryptone | 10 | 10 | - |
| Yeast Extract | 5 | 5 | 0.125 |
| Ammonium Sulfate | - | 3.3 | 1.65 |
| Potassium Phosphate, Monobasic | - | 6.8 | 6.8 |
| Sodium Phosphate, Dibasic | - | 7.1 | 7.1 |
| Glucose | - | 0.5 | 1.0 |
| Lactose | - | 2.0 | 4.0 |
| Magnesium Sulfate | - | 0.15 | 0.15 |

## Appendix

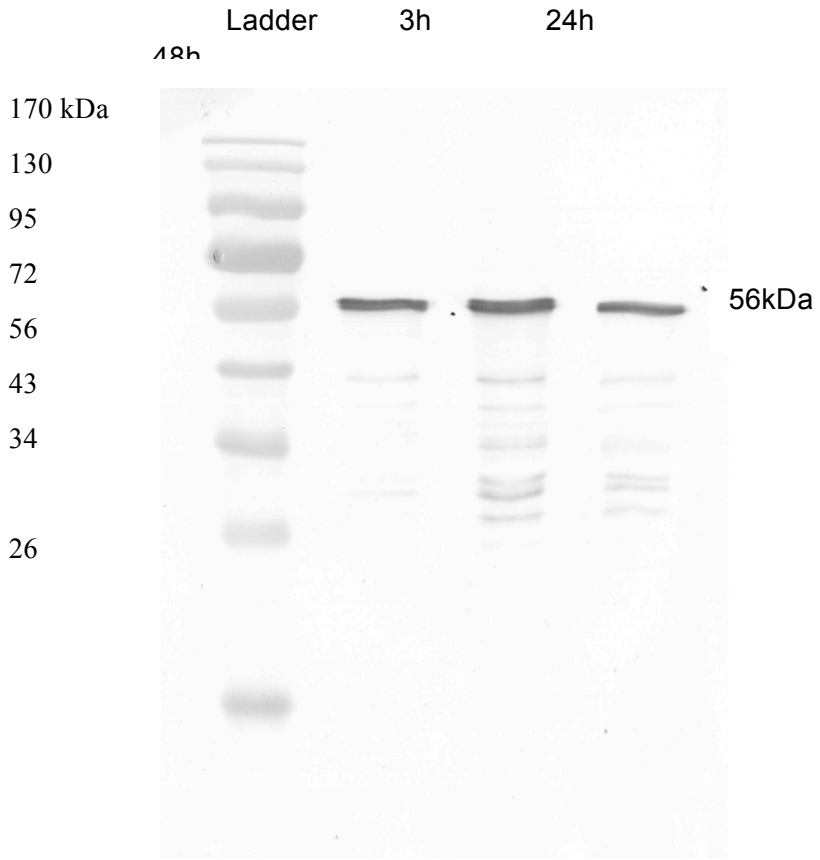


**Fig. A.1.** Western Blot for CA detection at 56 kDa in *E. coli* grown in LB and induced with IPTG.

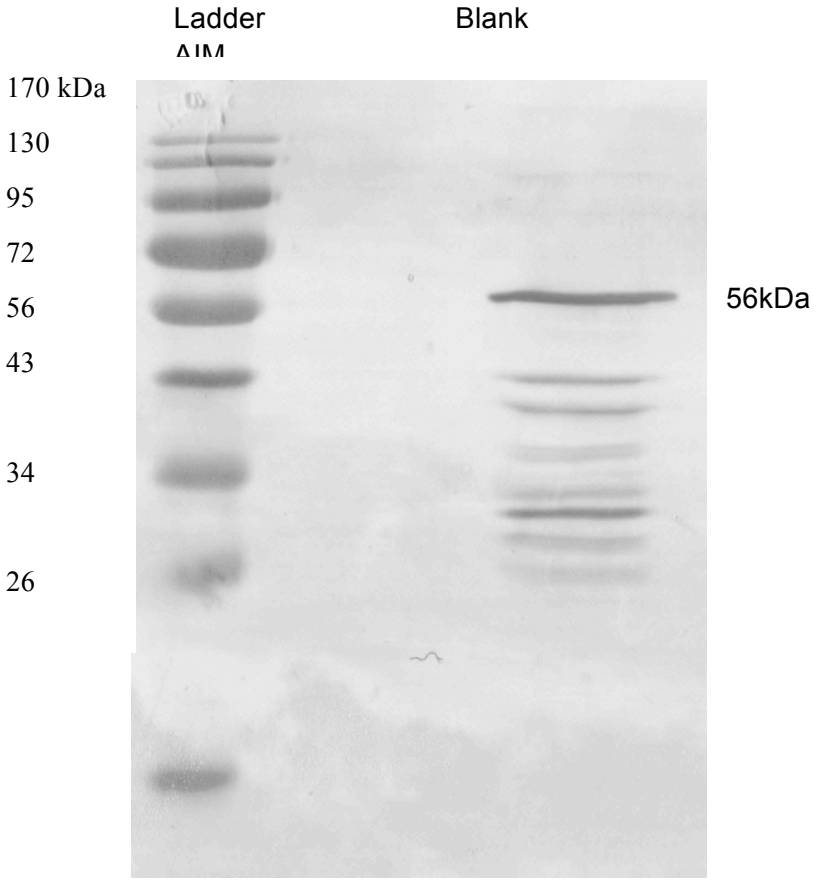


**Fig. A.2.** Western Blot for CA detection at 56 kDA in *E. coli* grown in the glucose-AIM.

**Table A.1.**
Costs of three media used for carbonic anhydrase expression in the *E. coil*

| | Medium composition | | | Costs for each medium ($ per 1 L medium) | | | | |
|---|---|---|---|---|---|---|---|---|
| g $L^{-1}$ | LB with IPTG | LB AIM | Glucose AIM | unit cost ($)* | Unit | LB with IPTG | LB AIM | Glucose AIM |
| NaCl | 10 | 10 | 0.5 | 53 | kg | 0.53 | 0.53 | 0.027 |
| Tryptone | 10 | 10 | 0 | 211 | kg | 2.11 | 2.11 | 0 |
| Yeast Extract | 5 | 5 | 0.125 | 190 | kg | 0.95 | 0.95 | 0.024 |
| $(NH_4)_2SO_4$ | 0 | 3.3 | 1.65 | 90 | kg | 0 | 0.30 | 0.15 |
| $KH_2PO_4$ | 0 | 6.8 | 6.8 | 125 | kg | 0 | 0.85 | 0.85 |
| $K_2HPO_4$ | 0 | 7.1 | 7.1 | 168 | kg | 0 | 1.19 | 1.19 |
| Glucose | 0 | 0.5 | 1 | 135 | kg | 0 | 0.068 | 0.14 |
| Lactose | 0 | 2 | 4 | 44 | kg | 0 | 0.088 | 0.18 |
| $MgSO_4$ | 0 | 0.15 | 0.15 | 108 | kg | 0 | 0.016 | 0.016 |
| IPTG | 0.048 | 0 | 0 | 60 | g | 2.88 | 0 | 0 |
| $ZnSO_4$ | 0.16 | 0.16 | 0.16 | 116 | kg | 0.019 | 0.019 | 0.019 |
| Ampicillin | 0.05 | 0.05 | 0.05 | 103 | 25 g | 0.21 | 0.21 | 0.21 |
| | | | | **Total cost ($/L)** | | **6.7** | **6.3** | **2.8** |

* The unit costs for all chemicals were obtained from Sigma-Aldrich (Saint Louis, MO) except those for lactose and ampicillin which were from Fisher Scientific (Pittsburgh, PA).