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Carbonic anhydrase production by *Pseudomonas fragi*

Andrzej Tietz, Małgorzata M. Jaworska*

Faulty of Chemical and Process Engineering, Warsaw University of Technology, Waryńskiego 1, 00-645 Warsaw, Poland

*** Corresponding author:**
e-mail:
malgorzata.jaworska@pw.edu.pl

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Abstract
Carbonic anhydrase is an important enzyme that can play a significant role in the processes of lowering carbon dioxide concentration in the atmosphere. The aim of the work was to investigate the extracellular carbonic anhydrase (CA) production by the bacteria *Pseudomonas fragi*. In the research, we focused on the evaluation of the phase of bacterial growth correlated with carbonic anhydrase production and on the evaluation of induction of CA production by calcium carbonate concentration in the nutrient medium. Presented data indicated that calcium carbonate can serve as the only carbon source for *Pseudomonas fragi*, inducing carbonic anhydrase secretion to culture broth. The enzyme was produced mainly in the adaptation growth phase reaching the maximal activity at the end of this phase or at the beginning of the growth phase. The maximal enzyme activity detected in all batches was at a similar level. The enzyme activity was constant but lower in the exponential phase growth. Therefore, the enzyme production is not growth-dependent, but it is correlated with bacteria adaptation to cultivation conditions.

Keywords
carbonic anhydrase, *Pseudomonas fragi*, calcium carbonate, phase of growth

1. INTRODUCTION

Global warming is one of the most important challenges for scientists in the 21st century. Its solving is connected with limitations in carbon dioxide production from fossil fuels and lowering carbon dioxide concentration in the atmosphere. One of the possible technologies is based on the dissolution of CO_2 in a proper medium and its storage in a stable, chemical form. The dissolution process can be sped up using an enzyme, carbonic anhydrase, that will significantly increase the carbon dioxide dissolution rate in the capture medium. The enzymatic method of CO_2 capture is advantageous because of its high specificity and selectivity, as well as the mild reaction conditions required (Liao et al., 2022). Additionally, the immobilized enzyme can be used in continuous flow systems (Iliuta et al., 2023; Nguyen et al., 2023; Zhang et al., 2022).

Carbonic anhydrase (EC 4.2.1.1; *CA*) is a metalloenzyme that catalyzes the reversible reaction of carbon dioxide (CO_2) hydration into bicarbonate (HCO_3^-) in the presence of water (Lindskog and Coleman, 1973). The enzyme does not shift the equilibrium but increases the reaction rate, allowing for a quicker equilibrium reach. Carbonic anhydrase was found in all life domains. Because of the broad existence of carbonic anhydrase among different species, it has been found that the enzyme has evolved at least six times independently, and six families of carbonic anhydrase are known up to date: α, β, γ, δ, ζ and η (Amata et al., 2011; Del Prete et al., 2014; Lindskog, 1997; Supuran and Capasso, 2015). The α-, β- or γ-CAs have been found in bacteria, α- and β-CAs in algae, and γ-CAs in archaea (Supuran, 2016).

The technology based on the enzymatic dissolution of carbon dioxide needs a low-cost and stable enzyme. It seems that extracellular enzymes produced by bacteria should fulfil these requirements. Bacteria usually use cheap, non-complicated nutrients, and they need cultivation conditions that are easy to prepare. Additionally, extracellular enzymes are easier to separate from fermentation broth than intracellular ones, so their production costs are lower. Carbonic anhydrase is produced by bacteria mainly as an intracellular enzyme, and only a few bacteria produce it as an extracellular one: *Pseudomonas fragi* (Prabhu et al., 2011; Sharma and Bhattacharya, 2010; Sharma et al., 2009), *Bacillus cereus* (Liu et al., 2021; Pan et al., 2019; Shen et al., 2017), *Bacillus sp.* (Sundaram and Thakur, 2018), *Bacillus altitudinis* (Nathan and Ammini, 2019), *Chromohalobacter israelensis* (Han et al., 2017), *Lysinibacillus sp* (Lu et al., 2019), *Pseudomonas* spp. (Giri and Pant, 2019), *Staphylococcus epidermidis* (Han et al., 2018), *Corynebacterium flavescens* (Sharma and Kumar, 2021).

Among these bacteria, *Pseudomonas fragi* seemed to fulfil all requirements, and it has been chosen as the CA "producer" in our investigations. *Pseudomonas fragi* is a Gram-negative aerobic bacterium present in various environments: soil, freshwater, marine and even the Arctic coast, growing in a broad temperature spectrum (Franzetti and Scarpellini, 2007; Yanzhen et al., 2016). The bacterium is also present in meat (Arnaut-Rollier et al., 1999) and in dairy products (Pereira and Morgan, 1957; Wiedmann et al., 2000), being responsible for their spoiling. It requires a simple nutrient medium and a short time for growth.

The data on the purification and characterization of carbonic anhydrase from *Pseudomonas fragi* has been presented by Sharma et al. (2009). They collected the nutrient medium after 12, 24 and 36 h of cultivation. They reported that CA

activity was observed only in samples taken after 12 and 24 h, while in the sample taken after 36 h, no activity was observed. Other papers focused on CA from *P. fragi* used a commercially available enzyme. Literature data published up to date do not connect the secretion of the enzyme with any of the growth phase. Such information is crucial because it determines which phase of growth should be given the most attention and when the samples should be taken.

Carbonic anhydrase purified from the culture medium of *Pseudomonas fragi* (Sharma et al., 2009) showed the highest pH stability in the range from 7.0 to 8.5 (above 80% of initial value) and thermal stability up to 45 °C (above 80% of initial value). Based on these values, the cultivation conditions of *Pseudomonas fragi* have been chosen as we wanted to keep up high CA activity in the medium.

The aim of the work was to investigate the extracellular carbonic anhydrase production by the bacterium *Pseudomonas fragi*. In the research, we focused on the evaluation of bacterial growth phase correlated with carbonic anhydrase production and on induction of CA production by calcium carbonate concentration in a nutrient medium. It was reported by Sharma et al. (2009) that calcium carbonate present in nutrient induces CA production, but the influence of calcium carbonate on enzyme production has not been reported.

2. MATERIALS AND METHODS

2.1. *Pseudomonas fragi* bacterium

Pseudomonas fragi DSM 3456 was used in experiments. The cultivation nutrient was as follows: [g/L H_2O]: 3.0 K_2HPO_4, 4.0 NH_4Cl, 5.0 NaCl, 5.0 peptone, $2 \cdot 10^{-4}$ $FeSO_4$, $2 \cdot 10^{-4}$ $MgSO_4$ with varying glucose and $CaCO_3$ concentrations depending on experiments.

The same composition of nutrient medium was used for inoculum preparation. The bacterium has been stored in a Microbank (–70 °C). The inoculum was prepared 3–4 days before by immersing one pearl from Microbank in a probe with 10 mL of nutrient medium, cultivated at 30 °C for 12 h, then stored in fridge conditions (5 °C–10 °C).

2.2. Cultivation method

Cultivation of the bacterium was carried out in a BioStat B Plus bioreactor (Sartorius, Germany, total tank volume of 1500 mL) containing 1000 mL of the nutrient medium. The cultivation process was carried out at 30 °C, pH 7.5 (stabilized with NaOH and HCl), with constant mixing (250 rpm) and constant airflow (10 L/min). It was inoculated with 10 mL of inoculum.

The pH and temperature cultivation conditions have been chosen based on enzyme pH stability and temperature stability (Sharma et al., 2009) to keep up the high activity of CA.

2.3. Analytical methods

2.3.1. Determination of biomass concentration

Samples of 3 mL volume were taken in 60 min intervals, and their optical density (OD) was measured at the wavelength of $\lambda = 560$ nm with water as a blind sample (Spectrophotometer UV-VIS 8500, Techcomp Ltd., China). The OD of the sterile nutrient medium was measured, and this value was subtracted from the OD of the measured sample (nutrient medium contained different amounts of insoluble calcium carbonate that could influence the OD measurement of samples taken from the bioreactor during bacterial growth). The concentration (C_{biom}) of the bacterium [mg/L] was calculated using the calibration curve (C_{biom} *vs* OD) prepared with biomass of *Pseudomonas fragi* bacteria.

At the end of each experiment, the total amount of biomass grown was measured. The culture medium containing biomass was transferred into dry centrifuge tubs with known mass and centrifuged for one hour at 4000 rpm (Centrifuge MPW-351e, MPW Med. Instruments, Poland). The supernatants were discarded, and the remaining biomass was dried at 60 °C for 12 hours and weighed. The total amount of bacteria was calculated by knowing the mass of dry centrifuge tubs and the mass of the centrifuge tubs with dry biomass.

2.3.2. Carbonic anhydrase activity determination

The Wilbur–Anderson assay (Wilbur and Anderson, 1948), with modifications proposed by Sharma et al. (2009) has been used for carbonic anhydrase activity determination. Briefly: 6 mL of 20 mM Tris buffer (pH 8.3, 4 °C) was added into a 10 mL beaker. The sample was kept in a water-ice bath and gently mixed. Next, 200 µL of crude enzyme solution taken from the bioreactor was added. The assay was initiated by the addition of 4 mL of ice-cold CO_2-saturated water. The time necessary for pH to drop from 8.3 to 6.3 was measured using a pH-meter (pH-Meter HI 4521, Hanna Instruments, USA with pH electrode LE438, Mettler Toledo, USA) which recorded pH changes in 5-second intervals. The same procedure but without the enzyme addition was applied as the control of the reaction. The time necessary for pH to drop from 8.3 to 6.3 with (t) and without (t_0) enzyme presence was measured. Carbonic anhydrase activity ($CA_{activity}$, U/mL) has been calculated according to the equation:

$$CA_{activity} = \frac{t_0 - t}{t \cdot 0.2} \quad (1)$$

where: t – reaction time for experiment with crude enzyme solution, [min]; t_0 – reaction time without enzyme solution, [min]; 0.2 – the volume of the crude enzyme solution [mL].

2.3.3. Specific growth rate of biomass

The cultures were performed as batch cultures, so the balance of biomass can be presented as follows:

$$\frac{dX}{dt_{exp}} = \mu \cdot X \tag{2}$$

where: X – biomass concentration, [mg/L]; μ – specific growth rate, [1/h]; t_{exp} – time of exponential growth phase, [h].

After integration with the conditions $X = X_0$ for $t_{exp} = 0$ and $X = X$ for $t_{exp} = t_{exp}$, Eq. (2) is transformed to:

$$\ln X = \ln X_0 + \mu \cdot t_{exp} \tag{3}$$

Plotting the experimental data as $\ln X$ vs t_{exp}, a linear relationship is obtained, where $\tan \alpha$ equals μ value.

3. RESULTS

Extracellular carbonic anhydrase produced by *Pseudomonas fragi* can be used in environmental protection for lowering carbon dioxide concentration in the atmosphere. The data published up to date do not show which growth phase of CA bacterial production is correlated with. Additionally, it has been shown in the literature that $CaCO_3$ induces the production of CA, but the influence of calcium carbonate concentration was not shown. In our investigations, we wanted to fulfil these gaps.

3.1. Glucose/calcium carbonate utilisation and carbonic anhydrase production

Glucose is reported as a nutrient component utilized by bacteria as a carbon and energy source. It was used in the cultivation of *Pseudomonas fragi* together with calcium carbonate (Sharma et al., 2009). It has been shown that calcium carbonate is necessary for carbonic anhydrase production. However, it has not been shown whether both components, glucose and calcium carbonate, must be present in the nutrient medium. That is why we compared the culture where glucose or calcium carbonate was the only carbon/energy source, Fig. 1.

Presented data showed that glucose promoted bacterial biomass formation, but no carbonic anhydrase activity was observed. Calcium carbonate present in the culture medium as the only carbon source promoted bacterial biomass and carbonic anhydrase formation. It was interesting to notice that glucose in the culture medium was unnecessary for bacterial growth. *P. fragi* is reported in the literature as a heterotroph, so it is possible that peptone served as the nitrogen and carbon source. Such observation was not reported in the literature to date and needs further investigation. The adaptation growth phase was longer in the culture with glucose

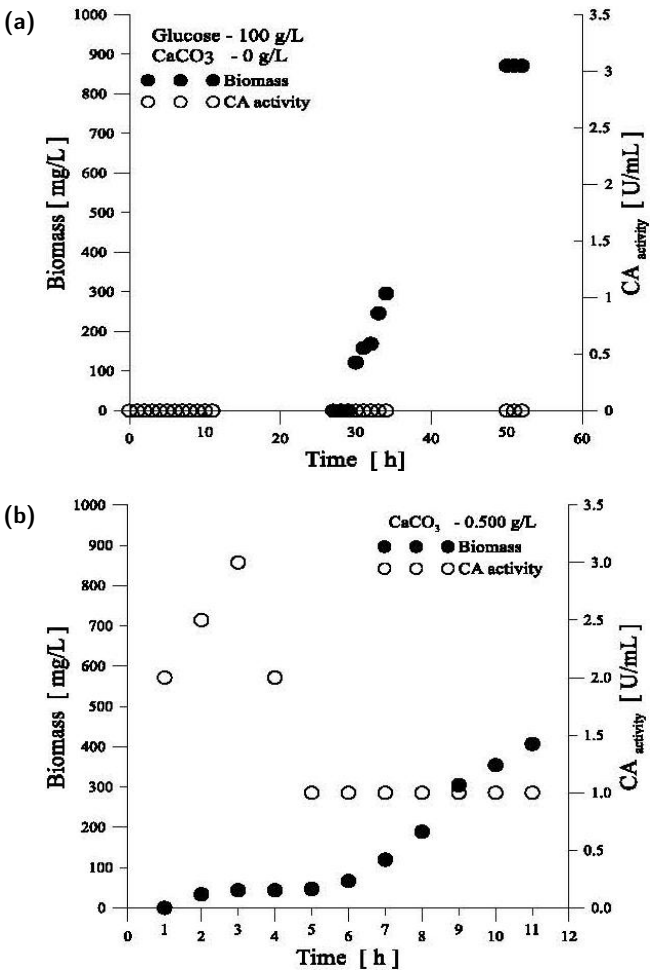

Figure 1. Changes in biomass concentration (•) and activity of extracellular CA (○) in time in a culture with glucose (a) or calcium carbonate (b) as the only carbon source.

than in the culture with $CaCO_3$ (30 h and 2 h, respectively). Biomass concentration was nearly twice larger in the culture containing glucose, but no enzyme was secreted to the culture medium. In the culture where calcium carbonate was used as the carbon source, carbonic anhydrase was secreted nearly just after inoculation (in the adaptation phase) with the maximum at 3rd hour of cultivation, but the activity of the enzyme lowered starting from the 5th hour it was constant till 11th hour. Presented data indicated that calcium carbonate is crucial for extracellular CA production by *Pseudomonas fragi*. This observation is in agreement with data presented by Sharma et al. (2009), but contrary to them, we have shown that glucose is not necessary for bacterial growth.

Based on these preliminary investigations, we decided not to add glucose to the culture medium and to use calcium carbonate as the only carbon source. The influence of the concentration of calcium carbonate in a nutrient medium on carbonic anhydrase production by *Pseudomonas fragi* was investigated.

3.2. Influence of calcium carbonate concentration on CA production

All cultures performed in this step were carried out with calcium carbonate (0.050–0.500 g/L) as the only carbon source (no glucose was added to the nutrient medium), Fig. 2. It must be noticed that calcium carbonate has limited solubility in water with equilibrium at 0.014 g/L (20 °C) (Green and Perry, 1950). It means that in all cultures, the concentration of $CaCO_3$ exceeded the solubility, and some salt re-

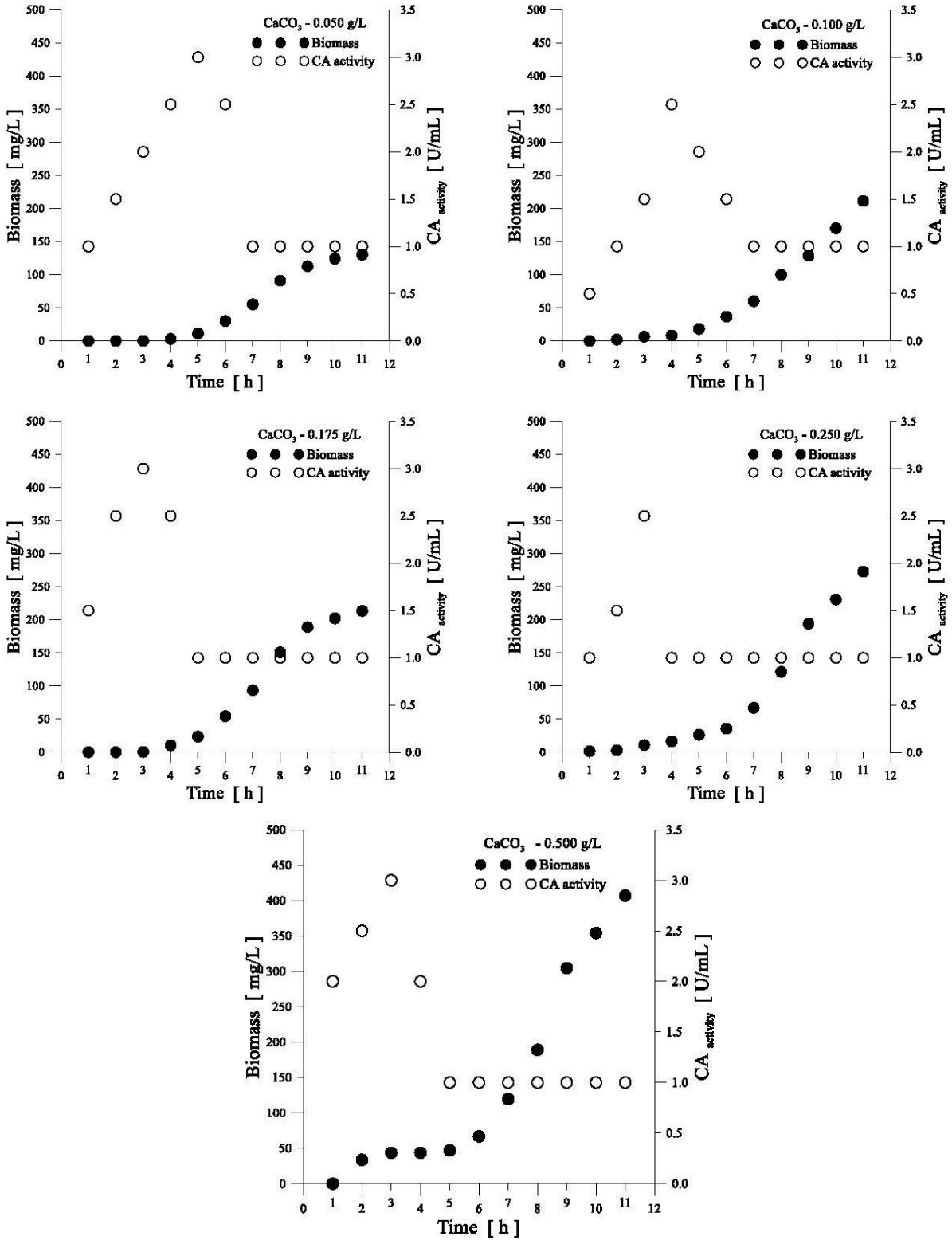

Figure 2. Changes in biomass concentration (•) and activity of extracellular CA (○) in time in cultures with different calcium carbonate concentrations.

mained insoluble. The effect of insoluble calcium carbonate on biomass concentration analysis has been taken into account (see 2.3.1).

The biomass growth and activity of extracellular carbonic anhydrase were observed in all cultures. With increasing concentration of $CaCO_3$, the increase in final biomass concentration was observed: from 130 mg/L to 407 mg/L. Additionally, with increasing $CaCO_3$ concentration, a reduction in the length of the adaptation phase of growth was observed. After 28 hours of cultivation, bacteria reached the stationary growth phase independently of $CaCO_3$ concentration. Surprisingly, the specific growth rate for the exponential phase of growth (calculated based on biomass balance, p. 2.3.3) was similar independently of $CaCO_3$ concentration, Table 1.

Table 1. Specific growth rate at the exponential growth rate

$CaCO_3$	μ	R^2
g/L	1/h	–
0.050	0.553	0.997
0.100	0.502	0.961
0.175	0.511	0.998
0.250	0.503	0.992
0.500	0.478	0.996

R^2 – correlation coefficient

Carbonic anhydrase was secreted to the nutrient just after inoculation independently of $CaCO_3$ concentration. Its activity grew during the adaptation phase of growth, reaching the maximum value after 3–5 h after inoculation. It was interesting to notice that the time when the enzyme reached the maximum activity depended on the concentration of $CaCO_3$: it was shorter for the higher salt concentration. Maximum activity was nearly the same in all cultures (2.5–3.0 U/mL) independent of salt concentration and of the final activity (1 U/mL) of the extracellular enzyme. The decrease in enzyme activity started when bacteria were in the accelerated growth phase and reached the constant value when bacteria were at the beginning of the exponential growth phase.

Presented data showed that the concentration of calcium carbonate influenced biomass formation but had limited influence on carbonic anhydrase secretion by *Pseudomonas fragi*.

4. DISCUSSION AND CONCLUSION

Carbonic anhydrase is an important enzyme that can play a significant role in limiting global CO_2 concentration. Implemented in carbon capture technology, it can contribute to the reduction of greenhouse gases' atmospheric concentration. The enzyme plays an important role in living cells as it is responsible for carbon dioxide management. Nearly all organisms produce carbonic anhydrase, but it seems reasonable to find a good "producer" in the world of microorganisms. It seems that *Pseudomonas fragi* can be regarded as one of them. The bacterium is widely present in the environment and can grow in a wide temperature range.

Presented data indicated that calcium carbonate can serve as the only carbon source for *Pseudomonas fragi*, additionally inducing the secretion of carbonic anhydrase to culture broth. The enzyme was produced mainly in the adaptation growth phase reaching the maximum activity at the end of this phase or at the beginning of the acceleration growth phase. The maximum enzyme activity detected in all batches was at a similar level. The enzyme activity was constant but lower in the exponential phase growth. Therefore, enzyme production is not growth-dependent, but it is correlated with bacterial adaptation to cultivation conditions. The reason for the inactivation of the part of the enzyme is unknown and difficult to explain. Such observation was not reported in the literature to date.

One must remember that calcium carbonate has limited solubility in water, which is relatively low (14 mg/L). Therefore, only part of the added salt was dissolved and present in an ionic form of Ca^{2+} and CO_3^{2-}/HCO_3^- while a large part of the salt was in the solid form. Thus, the calcium carbonate concentration in the culture medium was constant and equal to the equilibrium concentration. According to the Monod equation (Monod, 1949) the bacteria's specific growth rate depends on the concentration of the limiting substrate. In our investigations, we observed that the specific growth rate was independent of the concentration of calcium carbonate. This suggests that the specific growth rate is correlated with soluble carbonate ions and not with total carbonate added. This conclusion is in agreement with observations presented by Sharma et al. (2009) and experimentally shown by Zhang et al. (2011) in the study with *Bacillus mucilaginosus*. Therefore, carbonic anhydrase is probably extracellularly produced by the bacteria to accelerate the solubility of the $CaCO_3$ and to reach the equilibrium concentration faster. The faster dissolution allows for faster replenishment of carbonates taken up by bacteria, but only to the level of equilibrium concentration. This faster dissolution of $CaCO_3$ resulted in a larger amount of biomass obtained with increasing concentration of calcium carbonate. As there was a break in analysis between the 11^{th} and 28^{th} hour of cultivation, we cannot judge the length of the exponential growth phase, but the influence of $CaCO_3$ cannot be excluded.

The carbon source was added to the culture medium as $CaCO_3$. In such cases, it is difficult to judge which ion (Ca^{2+} or CO_3^{2-}) is responsible for the induction of carbonic anhydrase production. CO_3^{2-} ion can be used as the carbon source, and CA may accelerate the dissolution of the salt. Therefore, the presence of carbonate ions induces the production of the enzyme. Additionally, carbonic anhydrase is a metal-protein

enzyme with a metal cation in the active center. Usually, Zn^{2+} ions were reported as the metal present in the activity center, but it is possible that Ca^{2+} ions in carbonic anhydrase from *Pseudomonas fragi* could play the same role.

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SYMBOLS

CA	carbonic anhydrase
CA_{active}	activity of carbonic anhydrase, U/mL
CAs	carbonic anhydrases
C_{biom}	biomass concentration, mg/L
OD	optical density
R^2	correlation coefficient
t	reaction time for experiment with crude enzyme solution, min,
t_0	reaction time without enzyme solution, min
t_{exp}	time of exponential growth phase, h
U	enzyme activity
X	biomass concentration, mg/L
μ	specific growth rate, 1/h

REFERENCES

Amata O., Marino T., Russo N., Toscano M., 2011. Catalytic activity of a ζ-class zinc and cadmium containing carbonic anhydrase. Compared work mechanisms. *Phys. Chem. Chem. Phys.*, 13, 3468–3477. DOI: 10.1039/c0cp 01053g.

Arnaut-Rollier I., Vauterin L., de Vos P., Massart D.L., Devriese L.A., de Zutter L., Van Hoof J., 1999. A numerical taxonomic study of the *Pseudomonas flora* isolated from poultry meat. *J. Appl. Microbiol.*, 87, 15–28. DOI: 10.1046/j.1365-2672.1999.00785.x.

Del Prete S., Vullo D., De Luca V., Supuran C.T., Capasso C., 2014. Biochemical characterization of the δ-carbonic anhydrase from the marine diatom *Thalassiosira weissflogii*, TweCA. *J. Enzyme Inhib. Med. Chem.*, 29, 906–911. DOI: 10.3109/14756366.2013.868599.

Franzetti L., Scarpellini M., 2007. Characterisation of *Pseudomonas* spp. isolated from foods. *Ann. Microbiol.*, 57, 39–47. DOI: 10.1007/BF03175048.

Giri A., Pant D., 2019. CO_2 management using carbonic anhydrase producing microbes from western Indian Himalaya. *Bioresour. Technol. Rep.*, 8, 100320. DOI: 10.1016/j.biteb.2019.100320.

Green D.W., Perry R.H. (Eds.), 1950. *Perry's Chemical Engineers' Handbook.* McGraw-Hill Book Company Ing.

Han Z., Li D., Zhao H., Yan H., Li P., 2017. Precipitation of carbonate minerals induced by the halophilic *Chromohalobacter Israelensis* under high salt concentrations: Implications for natural environments. *Minerals*, 7, 95. DOI: 10.3390/min7060095.

Iliuta I., Rasouli H., Iliuta M.C., 2023. Intensified CO_2 capture in wall-coated microreactors with immobilized carbonic anhydrase: Experimental and modelling. *Sep. Purif. Technol.*, 307, 122590. DOI: 10.1016/j.seppur.2022.122590.

Liao Q., Liu W., Meng Z., 2022. Strategies for overcoming the limitations of enzymatic carbon dioxide reduction. *Biotechnology Advances*, 60, 108024.

Lindskog S., 1997. Structure and mechanism of carbonic anhydrase. *Pharmacol. Ther.*, 74, 1–20. DOI: 10.1016/S0163-7258(96)00198-2.

Lindskog S., Coleman J.E., 1973. The catalytic mechanism of carbonic anhydrase. *PNAS*, 70, 2505–2508. DOI: 10.1073/pnas.70.9.2505.

Liu R., Huang S., Zhang X., Song Y., He G., Wang Z., Lian B., 2021. Bio-mineralization, characterization, and stability of calcium carbonate containing organic matter. *RSC Adv.*, 11, 14415–14425. DOI: 10.1039/D1RA00615K.

Lu X., He Q., Wang Z, Cao M., Zhao J., Jiang J., Zhao R., Zhang H., 2019. Calcium carbonate precipitation mediated by bacterial carbonic anhydrase in a karst cave: Crystal morphology and stable isotopic fractionation. *Chem. Geol.*, 530, 119331. DOI: 10.1016/j.chemgeo.2019.119331.

Monod J., 1949. The growth of bacterial cultures. *Annu. Rev. Microbiol.*, 3, 371–394. DOI: 10.1146/annurev.mi.03.100149.002103.

Nathan V.K., Ammini P., 2019. Carbon dioxide sequestering ability of bacterial carbonic anhydrase in a mangrove soil microcosm and its bio-mineralization properties. *Water Air Soil Pollut.*, 230, 192. DOI: 10.1007/s11270-019-4229-3.

Nguyen K., Iliuta I., Bougie F., Pasquier L-C., Iliuta M.C., 2023. Techno-economic assessment of enzymatic CO_2 capture in hollow fibre membrane contactors with immobilized carbonic anhydrase. *Sep. Purif. Technol.*, 307, 122702. DOI: 10.1016/j.seppur.2022.122702.

Pan L., Li Q., Zhou Y., Song N., Yu L., Wang X., Xiong K., Yapa LS., Huo J., 2019. Effects of different calcium sources on the mineralization and sand curing of $CaCO_3$ by carbonic anhydrase-producing bacteria. *RSC Adv.*, 9, 40827–40834. DOI: 10.1039/C9RA09025H.

Pereira J.N., Morgan M.E., 1957. Nutrition and physiology of *Pseudomonas fragi*. *J. Bacteriol.*, 74, 710–713. DOI: 10.1128/jb.74.6.710-713.1957.

Prabhu Ch., Wanjari S., Puri A., Bhattacharya A., Pujari R., Yadav R., Das S., Labhsetwar N., Sharma A., Satyanarayanan T., Rayalu S., 2011. Region-specific bacterial carbonic anhydrase for biomimetic sequestration of carbon dioxide. *Energy Fuels*, 25, 1327–1332. DOI: 10.1021/ef101608r.

Sharma A., Bhattacharya A., 2010. Enhanced biomimetic sequestration of CO_2 into $CaCO_3$ using purified carbonic anhydrase from indigenous bacterial strains. *J. Mol. Catal. B: Enzym.*, 67, 122–128. DOI: 10.1016/j.molcatb.2010.07.016.

Sharma A., Bhattacharya A., Singh S., 2009. Purification and characterization of an extracellular carbonic anhydrase from

Pseudomonas fragi. *Process Biochem.*, 44, 1293–1297. DOI: 10.1016/j.procbio.2009.07.022.

Sharma T., Kumar A., 2021. Efficient reduction of CO_2 using a novel carbonic anhydrase producing. *Corynebacterium flavescens*. *Environ. Eng. Res.*, 26, 200191. DOI: 10.4491/eer.2020.191.

Shen T., Li W., Pan W., Lin S., Zhu M., Yu L., 2017. Role of bacterial carbonic anhydrase during CO_2 capture in the CO_2–H_2O–carbonate system. *Biochem. Eng. J.*, 123, 66–74. DOI: 10.1016/j.bej.2017.04.003.

Sundaram S., Thakur I.S., 2018. Induction of calcite precipitation through heightened production of extracellular carbonic anhydrase by CO_2 sequestering bacteria. *Bioresour. Technol.*, 253, 368–371. DOI: 10.1016/j.biortech.2018.01.081.

Supuran C.T., 2016. Structure and function of carbonic anhydrases. *Biochem. J.*, 473, 2023–2032. DOI: 10.1042/BCJ20160115.

Supuran C.T., Capasso C., 2015. The η-class carbonic anhydrases as drug targets for antimalarial agents. *Expert Opin. Ther. Targets*, 19, 551–563. DOI: 10.1517/14728222.2014.991312.

Wiedmann M., Weilmeier D., Dineen S.S., Ralyea R., Boor K.J., 2000. Molecular and phenotypic characterization of *Pseudomonas* spp. isolated from milk. *Appl. Environ. Microbiol.*, 66, 2085–2095. DOI: 10.1128/AEM.66.5.2085-2095.2000.

Wilbur K.M., Anderson N.G., 1948. Electrometric and colourimetric determination of carbonic anhydrase. *J. Biol. Chem.*, 176, 147–154. DOI: 10.1016/s0021-9258(18)51011-5.

Yanzhen M., Yang L., Xiangting X., Wei H., 2016. Complete genome sequence of a bacterium *Pseudomonas fragi* P121, a strain with degradation of toxic compounds. *J. Biotechnol.*, 224, 68–69. DOI: 10.1016/j.jbiotec.2016.03.019.

Zhang Y., Zhu J., Hou J., Yi S., Van der Bruggen B., Zhang Y., 2022. Carbonic anhydrase membranes for carbon capture and storage. *J. Membr. Sci. Lett.*, 2, 100031. DOI: 10.1016/j.memlet.2022.100031.

Zhang Z., Lian B., Hou W., Chen M., Li X., Li Y., 2011. *Bacillus mucilaginosus* can capture atmospheric CO_2 by carbonic anhydrase. *African J. Microbiol. Res.*, 5, 106–112, 001A48612981. DOI: 10.5897/AJMR10.690.