IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 11 Ver. III (Nov. 2014), PP 45-48
www.iosrjournals.org

Sequestration of atmospheric Carbon dioxide by microbial carbonic anhydrase

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Abstract: *The increase in atmospheric concentrations of carbon dioxide contributes to global warming to a large extent. In this context biological sequestration of carbon dioxide hold promise as a means for addressing this problem. In the present study atmospheric carbon dioxide sequestration is achieved with the help of carbonic anhydrase produced by Bacillus schlegelii. A maximum activity of 0.0453 µ mol/ml/min was observed at pH 9 and 37^{0}C. The enzyme was active up to 70^0 C.*

Key words: *global warming, greenhouse gas, carbon dioxide, sequestration.*

I. Introduction

Increased atmospheric carbon dioxide (CO_2) levels are known to contribute to global warming. This is a major cause of concern to environmentalists. The concentration of atmospheric CO_2 has increased by 40% from the pre industrial level of 280 ppm. [1,2]. Various methods currently available for reducing the levels of atmospheric CO_2 are to, either reduce emissions or reduce formation. However, it is unlikely to reduce formation in the near future [1]. To reduce CO_2 in emissions, it can be stored in three main ways: geo sequestration, transportation to suitable locations for storage and chemical fixation. In geo sequestration carbon dioxide is converted into 'supercritical CO_2' which can be directly injected into sedimentary rocks. Various locations for CO_2 storage, includes the oceans, deep aquifers, and depleted oil and gas reservoirs.In chemical fixation captured CO_2 is reacted with naturally occurring magnesium- (Mg) and calcium- (Ca) containing minerals. [3,4,5]. These methods have adverse environmental impact [1]. Recently the use of carbnonic anhydrase (CA). to sequester CO_2 has been demonstrated as an eco-friendly and cost-effective approach to this problem. This zinc containing metallo enzyme discovered by Meldrum and Roughton in 1933 catalyzes the reversible hydration of CO_2 to form bicarbonates.[6,7]. The data presented here demonstrates the enzymatic sequestration of CO_2 directly in solution.

II. Materials and methods

Soil samples were collected from a playground and garden in Thane (west) and from a farm in Palghar village and used as a source for carbonic anhydrase producing bacteria [8]. These samples were suspended in sterile saline streaked on Nutrient agar plates containing 10 mM para- Nitro phenyl Acetate (p-NPA) (Hi Media). The plates were incubated at ambient temperature for 48 hrs. [8].

The isolates were identified using standard microbiological methods. Enzyme production was achieved by growing the isolates in 100 ml nutrient broth supplemented with 1 mM $ZnSO_4$ for 72 hr on a rotary shaker at 100 rpm (Scigenics orbitech) [9]. The fermented broth was centrifuged at 12,000 rpm for 20 mins at 4^{0}C (Superspin R-V/FA, Plasto crafts). The supernatant constituted the extracellular crude enzyme. The pellet was washed with distilled water and resuspended in lysis buffer comprising 1ml 250 mM Tris-HCl and 0.5 ml triton X-100, sonicated for 15 mins, centrifuged as above. The supernatant so obtained constituted the intracellular crude enzyme. [9]. Standard graph of p-NPA was prepared ranging from 1µg/ml-10µg/ml. All spectrophotometric readings were taken on UV-vis spectrophotometer (WPA Biowave II) at 348 nm [9]. The protocol depicted in Table I

Table 1: Protocol for carbonic anhydrase assay

Tube	Buffer (ml)	p NPA (ml)	Distilled water (ml)	Crude enzyme (ml)
Test	1.4	1	0.5	0.1
Enzyme blank	1.4	1	0.6	-
Substrate blank	1.4	-	1.5	0.1

Protein concentration was estimated using Follin – Lowry method. The effect of pH and temperature on enzyme activity was determined.

* All media used are from Hi Media and all chemicals are from Loba Chemie unless otherwise stated.
1.1 Assessment of carbon dioxide sequestration by the extracted enzyme.

Sequestration of carbon dioxide was assessed as per table 2. This enzyme system was incubated in carbon dioxide atmosphere (5% and 10%) in a CO_2 incubator for 72 hrs. A qualitative test for captured $CaCO_3$ was also performed using concentrated HCl.

Table 2: Protocol for assay of carbonic anhydrase activity

Enzyme (ml)	0.1 M calcium chloride (ml)	Tris buffer pH 8.3 (ml)	Distilled water (ml)
2	4.0	4.0	10.0
0 (blank)	4.0	4.0	12

III. Results and discussion

After screening for the CA producers, Colonies showing dark yellow zones around it, indicating formation of para- Nitro phenol, were selected [8].

Fig 1 Carbonic anhydrase producing colonies.

Table 3: Morphological and biochemical characteristics of isolated carbonic anhydrase producer.

Test	Standard #	Observed results
Spore position	terminal	terminal
Catalase	+	+
oxidase	+	+
Anaerobic growth	-	-
Voges Proskauer test	-	-
Acid from D-Glucose	-	-
Acid from D-Mannitol	-	-
Gas from glucose	-	-
Hydrolysis of casein	-	-
Hydrolysis of starch	-	-
Utilization of citrate	-	-
Degradation of tyrosine	-	-
Nitrate reduced to nitrite	+	+
Formation of indole	-	-
Growth at		
$5\,^0C$	-	-
$10\,^0C$	-	-
$30\,^0C$	-	+
$40\,^0C$	-	+
$50\,^0C$	+	+
$65\,^0C$	+	+
Growth in presence of lysozyme	+	+

Key: + : positive test, **-** : negative test

As per the Bergy's manual

Enzyme activity of the crude enzyme was calculated as per the following formula:

$$\text{Enzyme activity / ml:} \quad \frac{\mu \text{ moles of pNPA}}{6 \times 0.1} \quad \ldots\ldots\ldots\ldots (1)$$

The activity of crude enzyme was found to be 0.00615 U/ml as per p-NPA assay (pH8.3) .Protein concentration of the crude enzyme was determined as 0.156 mg/ml. The specific activity was found to be 0.0394.

CA activities were determined in the pH range of 4.0–10.9 using buffers of different pH values [10]. The experiment was performed in duplicates and average values are taken (Table 4). The pH activity profile of crude CA is presented in graph.

Table 4: Effect of pH on enzyme activity

Optimum pH	Buffer	Absorbance (348 nm)	Enzyme activity (µ mol/ml/min)
pH 4	Acetate	0.5055	0.0408
pH 5	Acetate	0.4890	0.0395
pH 6	Phosphate	0.4610	0.0372
pH 7	Phosphate	0.4845	0.0392
pH 8	Phosphate	0.5230	0.0423
pH 9	Borate	0.7705	0.0625
pH 10	Ammonia	0.4775	0.0386
pH 10.9	Ammonia	0.4080	0.0329

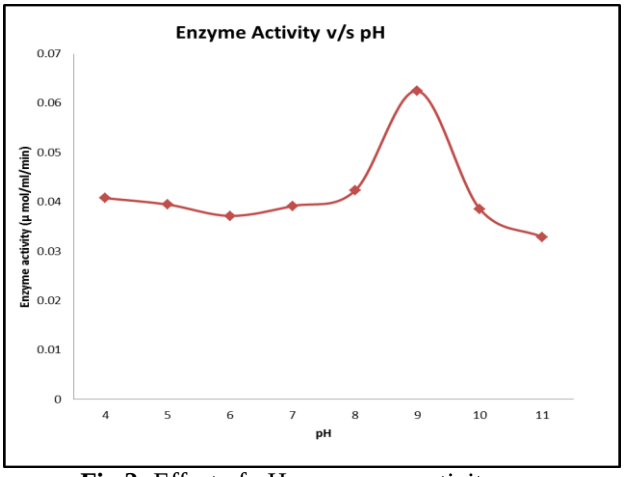

Fig 2: Effect of pH on enzyme activity

The optimum enzyme activity of our enzyme was found to be at pH 9 which was in contrary to the findings by Sib Sankar Giri et al who discovered that pH 8.3 to be optimum for carbonic anhydrase obtained from Bacillus subtilis VSG-4 .The enzyme activity showed decline with the increasing pH.

Temperature profiles were determined by assaying CA activity at different temperatures between 0^0 C to 70^0 C. The experiment was performed in duplicates and average values are taken (Table 5).

Table 5: Effect of temperature on enzyme activity

Temperature	Absorbance (348 nm)	Enzyme activity (µ mol/ml/min)
0^0C	0.377	0.0304
4^0C	0.486	0.0318
15^0C	0.479	0.0387
30^0C	0.394	0.0393
37^0C	0.560	0.0453
50^0C	0.449	0.0362
70^0C	0.419	0.0338

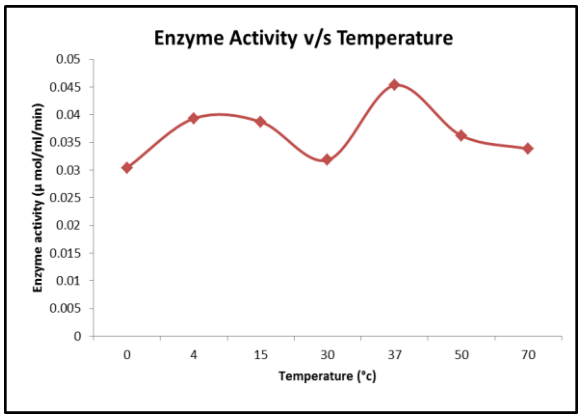

Fig 3: Effect of temperature on enzyme activity

The optimum temperature for crude carbonic anhydrase enzyme activity was determined by varying the reaction temperature from 0^0 C to 70^0C at pH 9 as this pH was found to be optimum for the enzyme. The optimum temperature for activity of crude carbonic anhydrase enzyme was found to be 37^0 C. The enzyme activity steadily declined at 50^0C and 70^0C.

The amount of carbon dioxide sequestered in the form of calcium chloride in 72 hrs at pH 9 was 45 mg/ml of enzyme, when the percentage of carbon dioxide was increased from 5% to 10% more calcium carbonate was found to be precipitated. Thus these results suggests that percentage of carbon dioxide, amount of calcium chloride and buffer plays an important role in precipitation of calcium carbonate along with the enzyme.

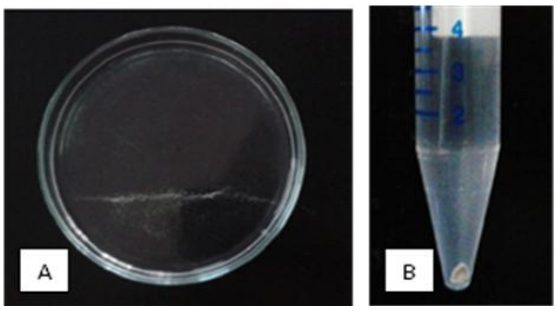

Fig 4 Calcium carbonate A: as line of precipitation
B: as pellet

IV. Conclusion

Carbonic anhydrase producing bacteria was isolated from soil and was identified as a variant of Bacillus schlegelii. There are reports suggesting that Carbonic anhydrase enzyme can precipitate carbon dioxide dissolved in water (CO_2 saturated water). But idea behind this project was to check whether carbonic anhydrase enzyme is able to sequester atmospheric carbon dioxide or carbon dioxide which is in gaseous phase. It was demonstrated that the enzyme carbonic anhydrase was able to precipitate carbon dioxide gas (5%) present in carbon dioxide incubator. The amount of calcium carbonate precipitated increased when level of carbon dioxide in the carbon dioxide incubator was increased to 10%.The major advantage of using enzyme to sequester carbon dioxide is that it does not pollute the environment, it is cheap, and the process is not tedious. This provides us a cheap, effective and novel method for sequestration of carbon dioxide which will help in combating the problem of global warming.

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