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Carbon Capture and Storage in the Biofouling Tubeworm *Ficopomatus Enigmaticus*

Doimi Mauro

Department of Electronic Biology, and Manufacturing Engineering, D&D Consulting, via Montenero 90, Venice, Mestre 30171, Italy

Abstract: The study investigates the potential of the serpulid tubeworm Ficopomatus sp., sourced from Jesolo Lido (Venice, Italy), for CCS (Carbon Capture and Storage). Specimens were processed to extract bioactive compounds using a 50% polypropylene glycol-water solvent system. Extracts were analysed and purified using column gel filtration chromatography, with fractions identified by TLC (Thin Layer Chromatography) and further characterized for antioxidant and antibacterial activities. Antioxidant activity was detected via DPPH (2,2-Diphenyl-1-Picrylhydrazyl) spraying on TLC plates, while antibacterial activity was evaluated using the antibiogram paper disk diffusion method. Enzyme activity in the fractions was previously confirmed through a bromothymol blue test followed by spectrophotometric analysis. The primary goal was to explore the CCS potential, using an experimental module involving Arduino Uno embedded microprocessor for the CO_2 measurement and to confirm the conversion into insoluble carbonates (storage). The most active fraction, identified as S1, showed significant CCS action, confirmed by microscopic observation of calcareous deposits on treated sponges. These findings suggest that Ficopomatus sp. can be used in CCS research highlighting its potential for biotechnological applications in mitigating climate change. The paper underscores the importance of marine organisms in CCS and offers insights into innovative strategies for environmental conservation and carbon management.

Key words: CCS, CO_2, marine carbonic anhydrase, Jesolo beach (VE, Italy), biofouling, beach rock.

1. Introduction

Ocean plays a crucial role in CCS (Carbon Capture and Storage) by absorbing large amounts of carbon dioxide from the atmosphere through processes like photosynthesis in marine plants and algae. Phytoplankton, which are microscopic marine plants, absorb carbon dioxide during photosynthesis and serve as primary producers in the oceanic food chain, thereby sequestering carbon. Additionally, the wetland bottom mud like the pet land acts as a vast reservoir for dissolved carbon, storing significant amounts of carbon dioxide over long periods, thus mitigating the impacts of climate change. In addition to photosynthesis, certain marine organisms utilize alternative modes of carbon dioxide utilization, such as chemosynthesis, which occurs in deep-sea hydrothermal vents. These organisms convert carbon dioxide into organic compounds without relying on sunlight, contributing to the ocean's role in CCS. Understanding these diverse mechanisms underscores the complexity of oceanic carbon cycling and emphasizes the importance of preserving marine ecosystems for effective carbon sequestration. Marine biology serves as a valuable way for discovering new active compounds from marine organisms, offering vast biodiversity and unique ecological niches that harbour untapped chemical diversity. Researchers explore marine environments to uncover new bioactive compounds with potential pharmaceutical, agricultural, and industrial applications, for human health and environmental challenges. By studying marine organisms, scientists unlock innovative solutions and expand the repertoire of compounds available for drug discovery, biotechnology, and other scientific endeavours. Carbonic anhydrase in coral [1] and similar marine animals like the sea urchin [2], plays a critical role in facilitating the conversion of carbon

Corresponding author: Doimi Mauro, Ph.D., research fields: marine biology and electronics. E-mail: maurodoimi@ded-consulting.it.

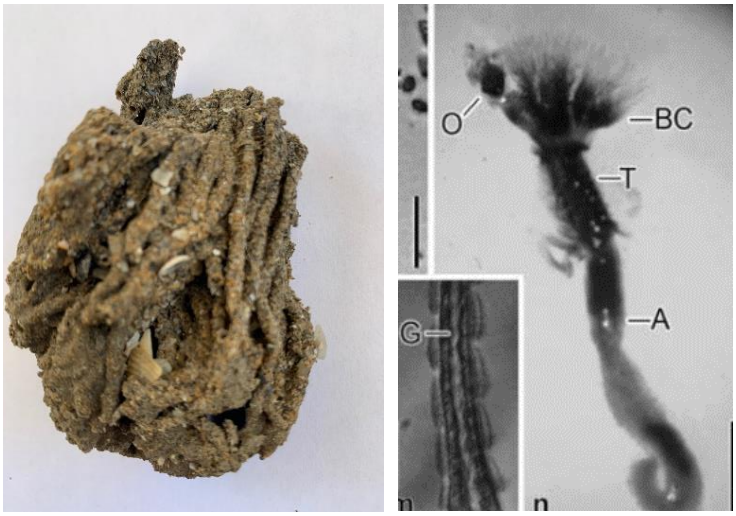

Fig. 1 The beach rock "*Ficopomatus enigmaticus*".
A: abdomen, BC: branchial crown, O: operculum, T: thorax [4].

dioxide into bicarbonate ions, which is essential for calcification and skeletal formation. This enzyme helps regulate pH levels in coral tissues or sea urchin spine, enabling them to cope with changing environmental conditions, including ocean acidification [3], and supporting the health and growth of coral reefs.

The study on *Ficopomatus enigmaticus* (Fig. 1) is significant because it reveals its dual role: despite being listed as one of Europe's worst invasive species [5], it contributes positively to aquatic ecosystems by acting as a habitat former [6] and a water bioremediation [7]. In this research, the strong enzymatic activity of the carbonic anhydrase of this organism for capturing and transforming CO_2 into insoluble molecules has been verified. Considering the ease of sourcing, the simplicity of enzyme extraction, and the rapid action to reduce the environmental CO_2, *Ficopomatus enigmaticus* can be used in CCS studies and simulations. Additionally, a prototype apparatus for the functional study of the organism in question is described.

2. Materials

A rock of serpulid worm, Ficopomatus sp., measuring 500 g in weight, was gathered in September 2023 from a dock area on the of Jesolo (Venice, IT) lat 45.486234, long. 12.599317 (Fig. 2).

Fig. 2 Reef-like rock by *Ficopomatus enigmaticus* under the pier.

Prior to extraction, the sample was ground and evaporated to dryness under vacuum at 40 ℃ and then filtered through a 100-micron sieve. The extraction was carried out using a solvent made by diluting PGG (Polypropylene Glycol) to 50% in water. This solvent was then used at a ratio of 1:10 (*v*/*w*). After a 12 h sedimentation, the supernatant liquid was collected and centrifuged for 15 min to 10,000 spin speed. The resulting clear yellow liquid extracts were stored at -24 ℃ when not actively utilized. An additional purification step was performed by salting out with 25% saturated ammonium sulphate solution, followed

by a column gel filtration chromatography using E.W.A. (Ethanol-Water-Ammonium) hydroxide as eluent (18 mL-10 mL-10 mL) and a TLC aluminium sheet 10 × 20 silica gel 60 F_{254} using the same eluent. Five microliters (5 μL) of the fraction was applied to 1 cm of the base of the sheet and developed. The separated compounds, using a UV/visible light, were isolated and identified by ninidrine, iodine gas and $FeCl_3$-$K(CN)_6$. The Rf values were measured (and their molecular weight determined against some available standard as Beta-Carotene and pigments).

3. Methods

3.1 Determination of Antioxidant Capacity [8]

The TLC plates were used also to detect antioxidant activity of samples spraying on the plates with oxidizing reagents done with a solution 0.004% DPPH (2,2-Diphenyl-1-Picrylhydrazyl) in acetone. The DPPH radical pale yellow coloured spots were taken as antioxidant positive results.

3.2 Determination of Antibacterial Activity [9]

For the antibacterial evaluation, generic strains obtained by coastal marine water were used. Bacteria were grown on Difco nutrient agar medium. The bacteria strains were inoculated on nutrient broth (Difco) and incubated for 24 h at 30 ± 1 ℃. Adequate amounts of autoclaved Difco Agar medium were dispensed into sterile plates and allowed to solidify under aseptic conditions. Then, bacteria strains were inoculated with a sterile swab on the surface of plates and incubated at 25 ℃ for 12 h. The antibacterial activity was evaluated with the paper disk diffusion method. Briefly, 6 mm paper disks (Whatman No. 1) were soaked with 2 μL of the samples. After incubation, all plates were observed for zones of inhibition growth and the diameters of these zones were measured. All tests were carried out under sterile conditions and repeated three times.

3.3 Determination of Enzyme Activity [10]

A bromothymol blue test was conducted to verify the presence of CO_2 in the water samples. A dilute solution of bromothymol blue indicator was prepared in the CO_2-containing water, showing an initial blue colour. One sample was used as a blank, while the other was injected with 1 μL of the sample to be tested. The colour change of the bromothymol blue indicator from blue to yellow indicated the presence of enzymatic activity.

The samples were also analysed using an AMS AS7265X spectrophotometer at reflectance at 450, 470, 500, 650 nm [11].

To demonstrate the activity of the sample to convert the CO_2, a new test was performed using a solution of 2 mL of 2% sodium bicarbonate+ 0.01 mg Nickel + 1 mL of basic HANNA INS.CO_2 solution + 2 drops of blue thymol + 1 mL sample and CO_2, KH and pH were measured after 10 s.

3.4 Determination of Carbon Capture Activity

To facilitate the CCS observation, we have made a sealed transparent cube. At the bottom, we prepared a layer of calcium carbonate solution. A natural dry sponge, soaked in seawater, served as the CCS substrate. The test began by spraying the enzyme solution onto the sponge surface and then sealing the box. Inside the cube, we placed the following components:

(1) An electrode connected to an oscillator (DollaTek ELA19376, ASIN B09CGXT6CX) generating a 150 kHz square wave signal.

(2) A receiver probe that amplifies the signal using a HALJIA LM358 Weak Signal Amplifier, a two-stage voltage, single power unit operating at 3.5-24 V DC (Direct Current).

(3) An SGP30 CO_2 detector designed for Arduino. This probe uses an innovative metal-oxide semiconductor technology that offers high sensitivity and accuracy in detecting even low concentrations of CO_2. The sensor operates by detecting changes in electrical conductivity when exposed to CO_2. All the changes coming from the oscillator and the CO_2 probe are then converted into digital signals, which can be

interpreted and displayed by plotter mode using an Arduino Uno board (Arduino.cc). The determination of the CCS activity was done analysing the data collected by the system and the electric signal from oscillator over time. We repeated the experiment 10 times to ensure consistency and reliability of results (Figs. 3-5).

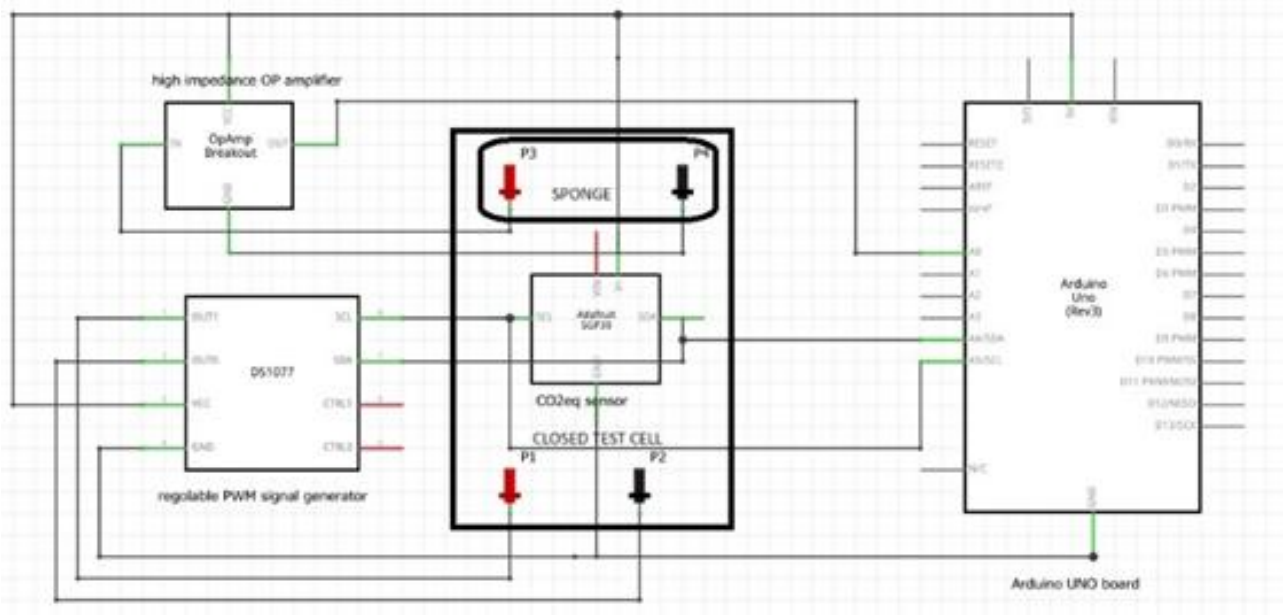

Fig. 3 The lab device scheme used to detect the Ficopomatus sample CCS activity.

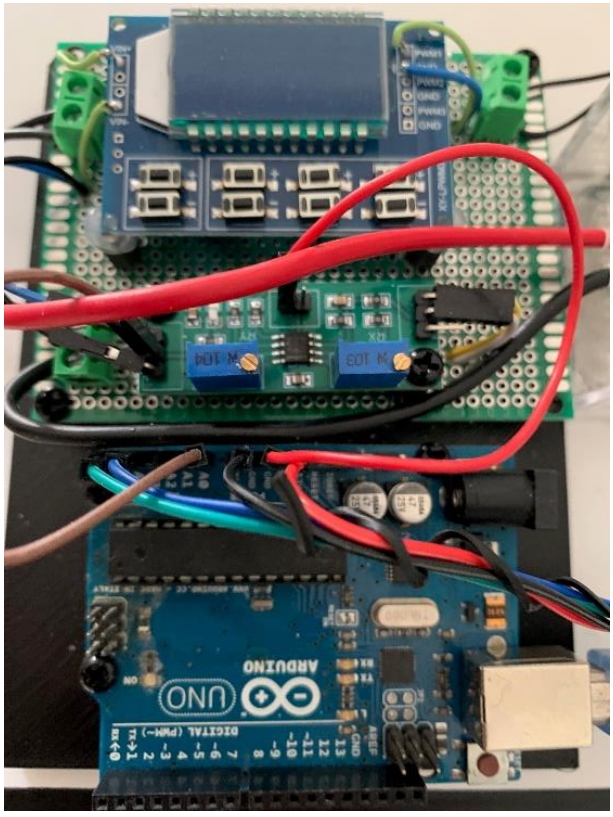

Fig. 4 The Arduino embedded system device.

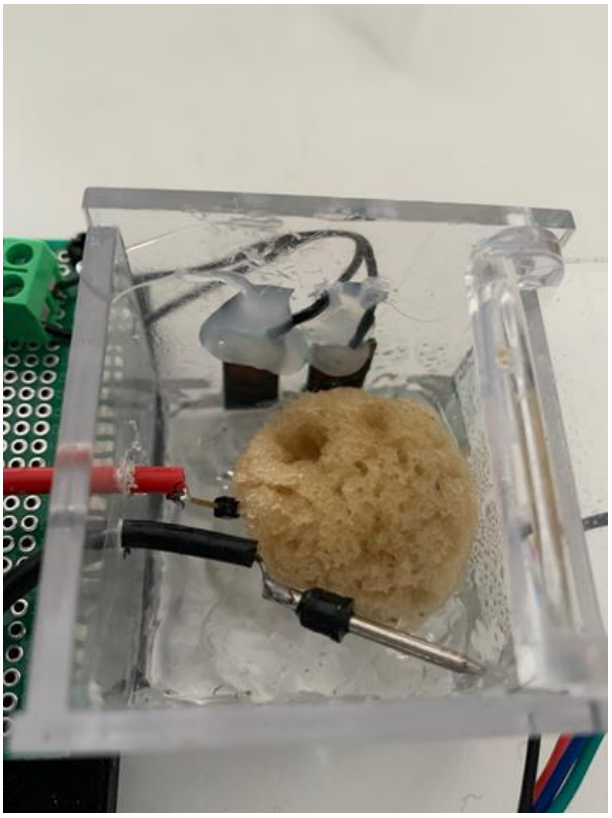

Fig. 5 The test chamber (box) with the sponge support and electrodes connected to the Arduino.

4. Results

Using TLC technique, we separated two different fractions by the liquid extract. The S2 fraction has a 0.22 Rf colorless while the S1 has a 0.78 Rf and is pale yellow in color with a 414 m.w. (Fig. 6).

To obtain 140 mL of extract for bioactivity demonstration from 200 g of dry material containing Ficopotamus, we use 200 mL of solvent in a 1:1 ratio (centrifugation, 12 h sedimentation, then filter using Whatman filter paper).

Using salting-out with ammonium sulfate, we precipitated the proteins and confirmed the two fractions based on TLC test data. From 20 mL of extract, adding 5 mL of ammonium sulfate, we obtained 19 mL of pale yellow supernatant (S1) and 1 mL of colorless precipitate (S2). Both fractions were stabilized in a pH 8 buffer and stored at -20 ℃. By comparison of their absorbance, the two enzymatic fractions differ by a marked absorbance at 585 nm (Fig. 7).

Both fractions have been tested for their bioactive activity in terms of antibiotic activity, anti-oxidation and phenols presence (Table 1).

Both fractions react to ninhydrin indicating the presence of proteins.

The tubeworm extract has no evidence of antioxidant capacity and antibacterial activity, but demonstrates a very interesting activity to use CO_2 and convert it into carbonate. This very interesting function reveals a best anhydrase carbonic action in S1 fraction (Figs. 8 and 9).

Fig. 6 TLC test to separate S1 and S2 protein fractions.

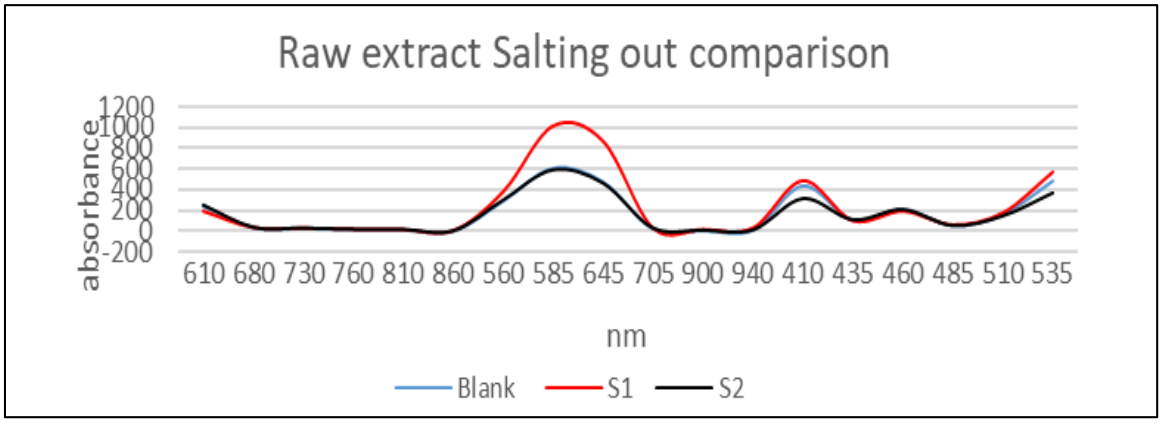

Fig. 7 The salting out protein fraction.

Table 1 Isolated fractions bioactivity.

	Antibiotic	Antioxidant	Phenols
S1	-	-	-
S2	-	-	-

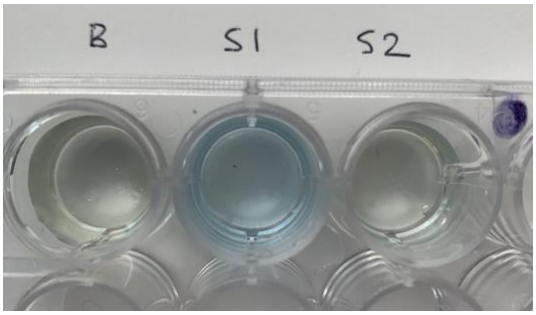

Fig. 8 Test with bluethymol, S1 reveals the best activity to convert CO_2 (B = blank).

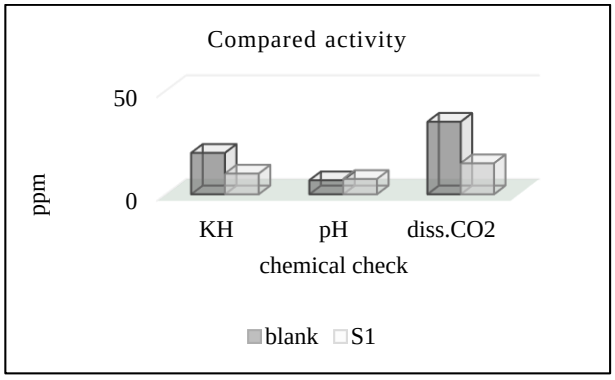

Fig. 9 Enzymatic result by using the S1 fraction against blank.

After spraying the surface of the sponge set on the Arduino device, we noted, a calcareous deposit, after a few seconds (Figs. 10 and 11).

To confirm the result, we saw the reduction of atmospheric CO_2 concentration (Fig. 12) and the electrochemical change due to the calcium deposit (Fig. 13) in the Arduino system closed environment, after spraying the S1 Ficopomatus solution.

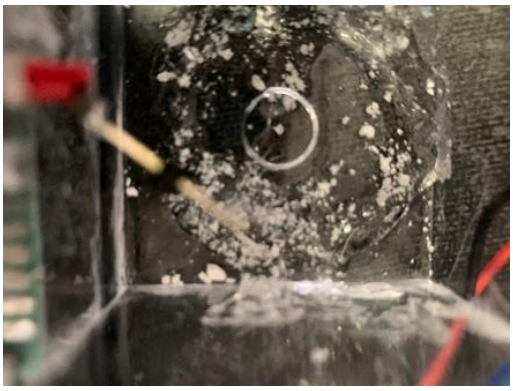

Fig. 10 Calcareous deposit on the bottom of the reaction box.

Fig. 11 Calcareous deposit after S1 spraying on the sponge spike.

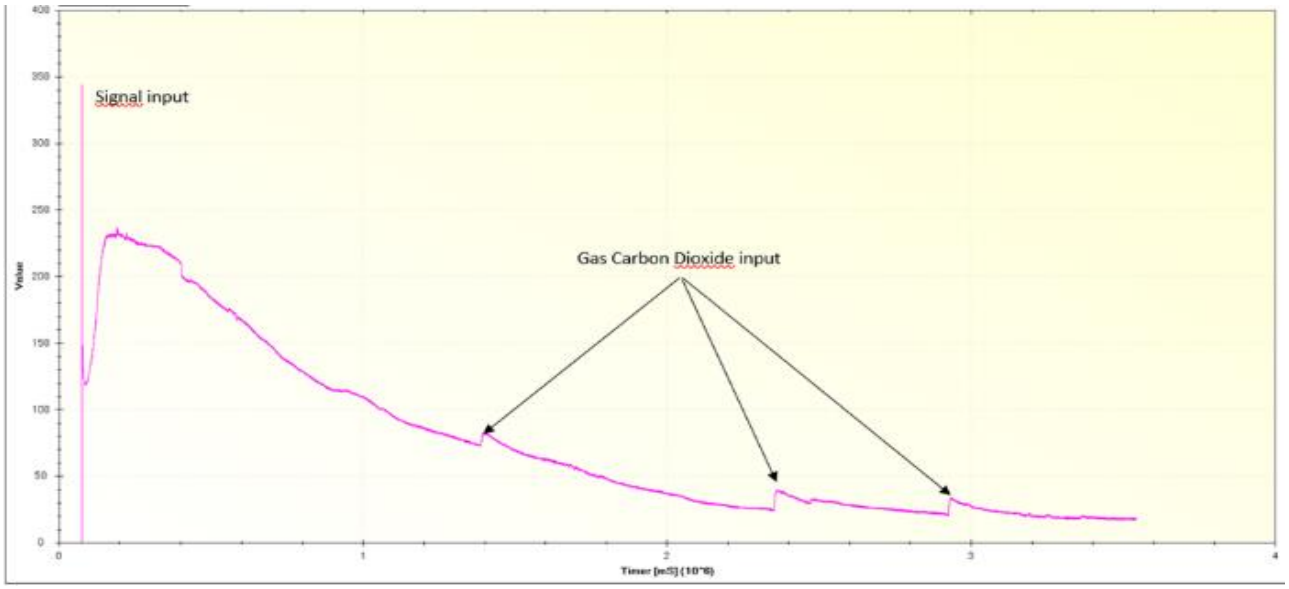

Fig. 12 Atmospheric CO_2 reduction in the Arduino test box after spraying the Ficopomatus S1 enzymatic solution.
The arrows indicate the rapid increase (and decrease) of CO_2 after opening and closing the test box.

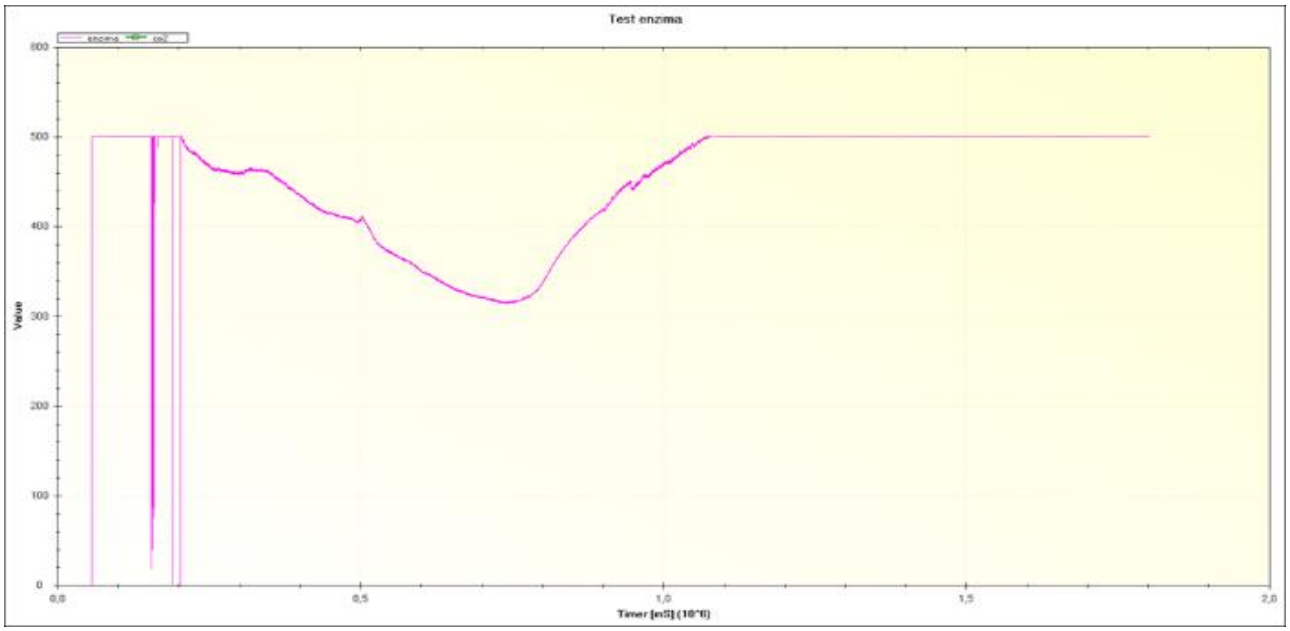

Fig. 13 Electrochemical change for deposition of calcium ore after spraying of S1.

5. Conclusion

The biological synthesis of materials using enzymes [12] with higher compatibility and human safety, has attracted the attention of researchers in the last decade [13]. Other advantages of the enzymatic synthesis are CCS activity [14]. The study of the CCS in marine organisms [15], holds immense significance for our planet. The oceans absorb approximately 31% of human-generated CO_2 emissions, acting as a vital buffer that helps reduce greenhouse gases in the atmosphere. [16] Understanding how marine organisms sequester carbon is crucial for modelling future climate change and mitigating its impact. Beyond natural processes, our research explores a CCS technology that directly removes CO_2 from industrial emissions and stores it in natural or engineered reservoirs. By harnessing *Ficopomatus enigmaticus*'s ability to capture carbon, we can develop innovative strategies to fight climate change [17]. *Ficopomatus enigmaticus* is a troublesome fouling serpulid tubeworm that has spread across temperate waters in both the Northern and Southern hemispheres [18-22]. This species prefers habitats with brackish waters of varying salinity, such as estuaries, where it faces little competition from other serpulids. Adults are capable of reproducing in salinities ranging from 6 to 35 ppt. Recently, its exceptional ability to reprocess has led to its establishment beneath the piers constructed to protect the beach from storms in the Lido of Jesolo (Venice, Italy). Since 2000, these piers have been completely colonized, resulting in the formation of "beach rocks", with structures comparable to coral reefs.

The rapid colonization with the formation of tubing in calcareous material reveals the presence of a carbon-dioxide enzyme which can be easily extracted.

Based on these observations, we confirmed that the serpulid can be used in studies of CCS in calcium carbonate. Recent studies indicate that many marine animals have similar activities such as sea urchin. Carbonic anhydrase is easily extracted from the organic matrix by using a solvent like polypropylene glycol and separating the protein part by salting out with ammonium sulphate. The activity has been verified both chemically and electronically through a special device whose heart is a specially programmed Arduino embedded microprocessor. In this device we observe "in vitro" the rapid making of calcium deposit on the sponge spike and the decrease of the carbon dioxide by the surrounding atmosphere.

Ficopomatus enigmaticus, for its rapid growth and ease of breeding [4], is to be considered as a useful organism for the development of methods of reducing carbon dioxide from the atmosphere.

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