

Mauro Doimi

D&D Consulting s.a.s., Department of International and Environmental Research, Mestre-Venice 30171, Italy

Abstract: Trees, generally, are used to remove carbon dioxide from the atmosphere. Other methods need to be considered. NASA (National Aeronautics and Space Administration) uses iron lime to increase the plankton uptake from the ocean bed. We can remove the carbon through the practice of shellfish farming. In French Polynesia, oysters mean pearl oyster farming. An independent assessment of the shellfish industry would be required to determine the carbon sequestration and generate carbon offsets through this method. CO_2 sink in pearl oysters shell, called MOP (Mother Of Pearl) can be allocated into precious button and objects or powder in poultry farming.

Key words: Carbon sink, carbon offset, MOP, pearl farming, French Polynesia.

1. Introduction

Climate change, induced by global warming, has become a reality. It disrupts ecosystems and also human existence. For example, French Polynesia atolls in Pacific Ocean can be underwater in the future. One method for fighting these effects is known as "carbon off-setting", balancing CO₂ emissions and calculating CO₂ reducing actions to create an overall carbon zero growth. There are four categories of offsets: renewable energy, energy efficiency, gas capture, and gas sequestration and reutilization. Generally, afforestation and reforestation are the main known methods to get bio sequestration. Bio sequestration is the general term used to describe activities where organisms, such as trees, are used to "sequester" or sink carbon from the atmosphere. Plants achieve this via photosynthesis, which convert carbon dioxide into oxygen and related material [1]. The amount of carbon sequestered can vary considerably depending on the site, species and other external conditions. Plants that grow in wetland areas can get an important bio sequestration by 210-320 g of carbon per square meter per year

(g/cm³/year) [2]. But this action is correlated to the human life that does, globally, deforestation. To help the reforestation we need alternatives. European wetlands have a great potential but we must explore other solutions. This paper explains as the black lip pearl oyster Pinctada margaritifera can issue CO2 bio sequestration. Carbon is absorbed naturally from the ocean as the shell of the shellfish forms and grows. This practice can generate offsets through CCS (Carbon Capture and Storage) in pearl farming (shellfish farming). A new Italian-European method, PdR UNI 99:2020 can be used to calculate the carbon sink and get carbon credits. (https://www.uni.com/index.php?option=com content &view=article&id=8873&Itemid=2866). Carbon credits (1-ton $CO_2 = 1$ carbon credit), can be used to get money or get a sustainable certification "carbon neutrality or carbon zero" for the product itself. Molluscs, including gastropods and bivalves, have a hard shell around a soft body and, in Pacific Ocean, these are used for pearl aquaculture and this has an economic importance for the local people. The molecular mechanism of calcification in aquatic organisms is well described by Nagasawa [3]. A water-soluble matrix protein, "nacrein", was

Corresponding author: Mauro Doimi, master, Ph.D. biology, research field: marine biology.

characterized in the nacreous layer of the Japanese pearl oyster Pinctada fucata just during 1996 [4]. Nacrein was found to have a similar sequence and similar activity to carbonic anhydrase, which had been thought to be involved in shell calcification. The pearl and the nacre of the shell called MOP (Mother Of Pearl) share a common layered structure showing regular repetition of a unit consisting of an aragonite tablet and an organic sheet, which account for the lustre that they exhibit. Scientist concluded that there is a protein called Pif 80 (gh) that has the ability to bind to aragonite more specifically than to calcite transforming and sink the CO_2 into the shell [5]. Pif-like molecules are found in some species close to the pearl oyster and in the blue mussel, Mytilus galloprovincialis. The shell of Pinctada margaritifera consists of two superimposed calcified layers: the outer layer is made of long calcitic prisms that develop more or less perpendicularly to the outer surface of the shell, while the internal layer is the nacre, made of aragonite thin tablets. There is hardly any overlap between the protein repertoire of each of

the two layers, except for three markers, nacrein, NUSP18 and shematrins [6] (Figs. 1, 2). This means that the mantle epithelial cells responsible for the prisms deposition and that, for the nacre deposition, work in completely different ways.

2. Materials

The aim of this study is to investigate the pearl oyster carbon sink and compare it to others. The area of the study is in the Raroia atoll (Fig. 3), a North Tuamotu French Polynesia atoll, a favourite place for black lip oyster farming.

Raroia Pearl Products sca donates the shells of *Pinctada margaritifera* "Pacific Black Pearl Oyster". This specie is farmed and grown by a long line method (Fig. 4).

We consider three main factors in order to calculate the amount of carbon sequestered per year. These are (1) the shell carbon as CO_2 content, (2) the grow-out time and (3) the stocking density. The shell consists of calcium carbonate (CaCO₃) where the CO₂ is sunk. The unit for bio sequestration (CCS) is tonnes of CO₂ per hectare per year (t CO₂/ha/year).



Calcitic prisms:

Alveolin-like, MP10, Shematrins (3, 5, 6, 8, 9), Tyrosinases (1,2), Clp (1, 3), Chitobiase, EGF-like (1, 2), Fibronectins (1-3), PUSP (1-5, 7, 9, 11, 12, 15, 16), Cement-like, KRMP7, Prismalin14, Calmodulin, PSPI (1, 2), CopAmOx, Peroxidase, MPN88, PTIMP (1-3).

Aragonitic nacre:

Pif-177, MSI60, Nacrein, Pearlin, Linkine, MRNP34, NUSP (1-12, 15, 17-20), NSPI (1-5).

Fig. 1 Proteins associated to the CCS in the two different layers of the Pinctada margaritifera black lip pearl oyster [7].



Fig. 2 All these group of enzymes contribute to transform CO₂ into CaCO₃ which is deposited in the different matrix of the shell.

Carbon Capture Storage and Utilization of *Pinctada margaritifera* Black Lip Pearl Oyster in French Polynesia



Fig. 3 Raroia pearl product location in Tuamotu atolls, French Polynesia.



Fig. 4 Long line Raroia pearl product oyster farm: water flux and nutrients.



Fig. 5 Relationship between the atmospheric carbon dioxide and the ocean.

Generally, the method worldwide used for the carbon analysis requires to oxidise the sample by heating in an oxygen atmosphere or reacting it with an acid (Fig. 5). The result is CO_2 and it can be measured or by a volumetric device or by an electronic device by a near-infrared detector. The heating is done in a high frequency induction furnace as this provides both

speed and accuracy. A new method described by Bernard [8] uses the laser to burn the carbon in an oxygen environment to form again CO_2 to be measured with a sensor. Generally, a basic equipment costs \$20-30.000. An economic alternative is to use an Arduino electronic system with a MHZ19 NIR sensor as detector with a cost estimate on \$100 (Fig. 6).



Fig.6 Electronic scheme of the handmade device.



Petri dish with sample area

Fig. 7 Layout of the laser equipment and laser (in red) ray emission.

The laser (Fig. 7) is a 3,500-mW module 450 nm light blue. It must be enabled and powered by varying the 0-5 V voltage from the spindle PWM D9 pin, where 0 V disabled the laser, while 5 V is full power.

The firmware sketch for the Arduino Uno board is: #include <SoftwareSerial.h>

SoftwareSerial SerialCom (A0,A1); // TX = A0 ; RX A1

int myDelay = 10000; byte addArray[] = { 0xFF, 0x01, 0x86, 0x00, 0x00, 0x00, 0x00, 0x79 }; char dataValue[9]; String dataString = ""; const int laser = 8; // the number of the relay pin const int attivazione = 3; // the number of the pwm pin

// constants won't change. They're used here to set pin numbers:

const int buttonPin = 5; // the number of the pushbutton pin

const int buttonpower = 4; // the number of the pushbutton pin

// variables will change:

int buttonState = 0; // variable for reading the pushbutton status

unsigned long tempo;

void setup() {

Serial.begin(9600);

SerialCom.begin(9600);

pinMode(LED_BUILTIN, OUTPUT);

pinMode(laser, OUTPUT);

pinMode(attivazione, OUTPUT);

pinMode(buttonpower,OUTPUT);

digitalWrite(buttonpower, HIGH);

pinMode(buttonPin, INPUT); }

void loop() {

buttonState = digitalRead(buttonPin);

// check if the pushbutton is pressed. If it is, the

buttonState is HIGH: if (*buttonState* == *LOW*) { digitalWrite(LED BUILTIN, HIGH); Serial.println("on"); digitalWrite(laser, HIGH); // turn laser relay on: digitalWrite(attivazione, HIGH); delay(10000); digitalWrite(LED BUILTIN, LOW); Serial.println("off"); digitalWrite(laser, LOW); digitalWrite(attivazione, LOW); } else { // turn LED off: digitalWrite(LED BUILTIN, LOW); // turn laser relay on: digitalWrite(laser, LOW); digitalWrite(attivazione, LOW); SerialCom.write(addArray, 9); SerialCom.readBytes(dataValue, 9); *int resHigh = (int) dataValue[2]; int resLow = (int) dataValue[3]; int pulse* = ((256**resHigh*)+*resLow*); *dataString* = *String(pulse)*; Serial.println(pulse); delay(myDelay); } }

To compare it, we use a chemical analysis using the method described by Van Slyke and Folch [9] and Rader and Grimaldi [10]. A weighed sample is introduced into a vial containing 10 mL of a 1:1 solution in volume of hydrochloric acid and analysed with a Dietrich-Fruehling calcimeter (Fig. 8), equipped with a gas burette graduated up to 200 mL and containing a slightly coloured 0.1 N sulphuric acid solution.

The volume of the gas at normal conditions (i.e., at zero C and 760 mm Hg) shall be reported using Eq. (1):

$$V_0(ml) = V_t \times \frac{273}{(273+t)} \times \frac{(P_t - f)}{760}$$
(1)

 V_t = volume of gas at the test temperature, expressed

in mL; t = temperature at the time of analysis, expressed in °C; $P_t =$ barometric pressure at the time of analysis, expressed in mm Hg; f = H₂O vapour pressure at temperature t, mm Hg. The calculation of the percentage of calcium carbonate follows Eq. (2).

$$CO2 = \frac{V_0 \times 0,0044655 \times 100}{P}$$
(2)

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where: CO_2 is expressed in percentage by weight; $V_0 =$ the volume of gas carried over, brought to 0 °C and 760 mm Hg; P = test sample, expressed in g and 0.0044655 is the gas-volumetric equivalent (g CaCO₃/mL CO₂). We calculate total carbon and its percentage on shell mass. In French Polynesia, the shell grow-out time (GOT) is average 4 years. It is the maximum amount of time that the oyster would spend on the pearl farm until the harvest. The pearl oyster stocking density per hectare depends on the site region and it is nutrient related [11]: high level of nutrients in the water higher stocking density. We consider one kilometer of oyster rail per hectare. The kilometer is made up of 18 m sections, which calculates 55.55 sections per kilometer, or 56 ha⁻¹. Each section contains approximately 3,000 oysters giving a density of 3,000 oysters/section \times 56 sections/hectare = 168,000 oysters/ha [12].



Fig. 8 Dietrich-Fruehling calcimeter layout.

3. Results and Discussion

As described in the following Table 1, the CO_2 analysis done by "maker" laser equipment is suitable for a massive control of farmed shell.

We can estimate the CO₂ content into a shell of 33.5% d.w. Pearl oysters are harvested for two purposes: (1) the pearl production or (2) to get MOP from shell itself. In the warmer areas as French Polynesia, in the final result from the pearl harvest, oysters' shells, are sold for MOP in various sizes like standard (100-120 mm), large (120-150 mm) and jumbo (150+ mm). Generally, the farmer uses a black lip oyster, with a size between 100 and 150 mm, to graft a nucleus inside the pearl sac and obtain a pearl after 1-2 years depending on the environmental condition [13]. The density of the pearl oyster, or carrying capacity, is strictly dependent on the amount of phytoplankton in the water. The OSD/ha (MOP Oyster Stocking Density Per Hectare), in French Polynesia, can be different form different location due to the environment trophic chain [14]. Generally, in French Polynesia a hectare can raise 12,000 black lip oysters and a new pearl farmer can raise only 36,000 individuals. At the other end, just established pearl farmers can farm 150,000 pearl oysters on 20 hectares or 400,000 on 16 hectares. About the weight, the Raroia pearl products sca, gave us the opportunity to check about 15.000 kg of single cleaned and sun dried valve shells. The results are described in the following Figs. 9 and 10.

The amount of carbon per MOP oyster and the number of MOP oysters are used to determine the carbon sequestration. This result (Table 2) is divided by the grow-out time to determine sequestration per year using Eq. (3):

(CO₂ per MOP oyster
$$\times$$
 number of MOP oysters) /

Grow-out time = t C/ha/year (3)

Using the Eq. (3), the result is:

MOP CO₂ sink = t CO₂/ha/year = (47 g CO₂ × 12.000) / 2 = 0.3 t CO₂/ha/year

 Table 1
 Compared results obtained with different methods.

	Calcimeter	Laser	
CO ₂			
s.d.	8	16.5	% d.w
Average	32.6	33.5	% d.w
Moda	30	28	% d.w



Fig. 9 Weight/size black lip oyster single valve analysis. The total shell weight is double.



Fig. 10 Age vs. length, French Polynesian official data.

 Table 2
 Amount of carbon per MOP oyster OSD/Ha.

Total Shell weight 10 cm size	g	140
$OSD \times 1$ ha	N°	12.000
CO ₂ sink per oyster	g	47
Grow-out time	Year	2

This approach using the stocking density is realistic. French Polynesian pearl farms are classified into three categories (Table 3).

Small farms, under 5 hectares. Their holders are 284, or 49% of pearl producers;

	,	-		
	Small farm	Average farm	Large farm	
На	5	5-30	> 30	-
MOP farmed	36,000	150,000	400,000	Number
Time to end	4	4	4	Year
End life biomass	12.9	54	144	Tons
Total CO ₂ sink	4.25	17.8	47.5	Tons
Value in carbon offset market	127	534	1,425	Euro's (× 30 €/CO ₂ eq. carbon credit)
Market value of MOP	38.700	162.000	432.000	Euro's (× 3 €/kg)

 Table 3
 CO₂ uptake, and value [15], of MOP oyster jumbo size (> 13 cm) at different pearl farm.



Fig. 11 Compared CCS efficiency expressed as gr. of CO₂ uptake by square meter at year [17].

Average farms, 5 to 30 hectares. Owned by 258 people, or 44%;

Large farms, over 30 hectares. In the hands of 39 people, or 7%, they are the ones who, with 5,831 ha, or 60%, have the most land used.

Despite thorough research, concerning bio sequestration there is still no true understanding of the carbon value of vegetation and numerous interpretations of research exist [16]. Other species such as the salt wetland plant *Tamarix* sp, is sequestering four or five times the amount of carbon per year in front of other land plant. Human controlled wetland gives a good CO_2 sink rate per ha as 500 g/m²/year (Fig. 11).

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We analyze the results and explain that, for MOP oysters, the carbon-offset value is irrelevant in front of the value of the shell itself (or black pearl). However, other market parameters such as "carbon neutral" or "carbon zero" label, can give an additional opportunity. Using a certified protocol, the costs of the sea concession and labor can be sustainable for the farmer and create a new way for green MOP product. MOP could give a positively impact on the amount of carbon present in a water body using the end life carbon allocation into the final products like, for example MOP buttons or objects.

4. Conclusion

Oceans occupy about 71% of the earth; their handling and management can increase carbon sequestering. The coral reef atoll with low phytoplankton primary productivity and with low primary biomass have a limitation. A good water circulation from outside to inside the coral atoll can increase the photosynthetic productivity, reduce turbidity and increase the oyster farming and, at least, the carbon storage and sink. A long-time project is crucial for carbon credit generation and its price. The pearl farming in developing countries, with 4-5 years farm project, offers an opportunity for carbon sequestration. Worldwide, MOP related products are request. Jewel like black pearl or MOP buttons are sold in the most luxury markets around the world. Otherwise, it now is requested a sustainable approach on the market. "Carbon Emission Neutral" can fit the people action to defend our earth. The "VCS protocol" is generally used to verify, calculate and certificate the forestall land project. Now, the new PdR UNI 2020 gives us a practical guide to the calculation and a roadmap to get a certificate by other ecosystem. The coral reef people can benefit also from a generation of "VER" (voluntary carbon credits) [18]. A collaboration with an entirely different concept of pearl farm can be

useful to increase the carbon offset and can give some money back to sustain the life in that particular place. A further improvement can be to produce seaweed and aquatic plants. The importance of these ecosystems for the global budget of carbon dioxide, can be better investigated.

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