Analysis of ^{14}C modern in coral *Porites lobata* using Liquid Scintillation Counting Method

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Abstract. Analysis of ^{14}C modern in coral aims to determine the activity of ^{14}C modern of marine organism. Samples of living coral *Porites lobata* was taken from inner zone and middle inner zone, Spermonde Archipelago, Indonesia. This research used Liquid Scintillation Counting method. This method used three steps for sample preparation. The first step are physical and chemical washing. In this step, 9 % mass of sample has been removed from impurity. Second steps, CO_2 Absorption. The sample entered in modificated absorption equipment and $^{14}CO_2$ captured with KOH solution. The last steps, 8 mL sample solution mixed with 12 mL scintillator and measured used LSC Hidex 300SL at counting times 5-240 minutes. The activity of ^{14}C modern in coral *Porites lobata* shown the differences activity between marine organism and land organism.

Keywords: Coral, ^{14}C modern, Liquid Scintillation Counting, Radiocarbon.

1 Introduction

Coral-Zooxanthellae is a mutually beneficial symbiotic relationship. Zooxanthellae gets a safe place to stay in the body of coral polyps. The results of food metabolism from coral are taken by zooxanthellae for photosynthesis with the help of sunlight, then the results of photosynthetic algae such as sugar ($(CH_2O)_n$), amino acids, and oxygen are used by coral polyps. In addition, photosynthesis will increase the pH and provide more carbonate ions and accelerate the calcification process to produce calcium carbonate to form deposits into the framework of the coral [1]–[3]. The calcium carbonate calcified coral skeleton contains a useful geochemical proxy arrangement that has been used to enhance our understanding of past climate, ocean circulation, and atmosphere to processes that occur at sea level through ^{14}C (radiocarbon) measurements. Radiocarbon analysis is now also used to understand the sources and biogeochemical cycling of the products of burning in the natural environment. Sixty years ago, the advent of radiocarbon dating rewrote archaeological chronologies around the world [4]–[7].

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Willard Frank Libby received the Nobel Prize in Chemistry in 1960 for his work on the ^{14}C method. Libby was researching about ^{14}C modern in biosfer sample. All of samples are taken from the land organism. The specific activity value of ^{14}C countinue used as a modern reference standard in calculating the age of coral, charcoal, sediment, water, and others [8], [9]. In this case, it is have no report about the ^{14}C activity value in marine organism in general and coral in particular. Therefore it is necessary to develop a new reference standard for the value of ^{14}C modern coral activity specially in the land.

There are three official analytical protocols for ^{14}C determination. The protocols are accelerator mass spectrometry (AMS), benzene-LSC (liquid scintillation counting), and CO2 cocktail-LSC. In the AMS, the CO_2 is converted to graphite and then analyzed by AMS [10]. In benzene-LSC, the CO_2 is converted to benzene and then LSC. AMS and benzene-LSC are used by many laboratories worldwide.The first technique being very accurate but highly expensive, while the second is very demanding though rather popular. In CO_2 cocktail-LSC, CO2 produced from the sample is directly absorbed into a suitable cocktail with high CO_2 affinity and immediately counted by LSC without any further manipulation. This method is simple, safe, and results in significantly reduced analysis time and cost as compared to other methods. [11] [12].

Zagreb Radiocarbon Laboratory since 1968 by proportional counting technique and since 2001 by liquid scintillation counting (LSC) technique by using LSC Quantulus 1220. Zagreb Radiocarbon Laboratory presented the procedures for measurement of ^{14}Cactivity of various samples by LSC.The simple, quick and inexpensive sample preparation technique of direct absorption of CO_2 is proved to be suitable for measurement of environmental samples, when no high precision is required. For precise measurements, and always for archaeological ^{14}C dating, the benzene synthesis is applied and the maximal age that can be determined reaches 50000 years . Liquid scintillation counting techniques are widely used in radionuclide metrology for standardization of pure beta and pure EC radionuclides and also for a growing number of more complex decay scheme radionuclides [13], [14].

2 Material and Methods

This reasearch used liquid scintillation counting method modified. There are several steps of this method including: physical and chemical washing, CO_2 absorption, and measurement of ^{14}C activity with LSC Hidex 300SL.

2.1 Sample

Coral samples are taken at seawater in Spermonde Archipelago. *Porites lobata*$_1$ collected from kayangan island, inner zone. *Porites lobata*$_2$ collected from barrang caddi island, middle inner zone.

2.2 Physical And Chemical Washing

Cleaning methods are designed to remove contaminating carbon sources that accumulate both while the specimen is on the sea floor and while it is stored on land after collection. Water rinses and scrubbing with a brush remove sediment from inside the coral and between the septa. Samples are then immersed in a 1:1 mixture of 30% H_2O_2 and 1N NaOH and ultrasonicated for 15 minutes. However, this process often leaves a brownish/orange organic stain on the $CaCO_3$. Quick dips (30 seconds to 2 minutes) in a 1:1 mixture of 30% H_2O_2 and 1N $HClO_4$ effectively remove this stain. After the dilute perchloric step, samples are rinsed thoroughly with clean distilled water. For the second acid wash, pre-weighed samples are dipped into 6N HCl for 15–

60 seconds followed by rinses in two separate beakers of distilled H_2O. After drying for several minutes in a 60 °C oven, the samples are cooled and reweighed to determine the percent of sample removed. Samples are then crushed in an agate mortar and pestle to facilitate dissolution in the reaction flasks [15].

2.3 CO_2 Absorption

Dried coral were transfered to flask that connected to a separation funnel as hydrochloric acid reservoir. Prior to carbon dioxide absorption, the nitrogen gas was streamed along the system. Solution of 10 % HCl was added by drops to the sample until bubles form end. Gas is channeled into an impinger contains 40 mL potassium hydroxide solution as carbosorb after passed acid trap and water trap. The process was stopped when the gas not formed by adding the hydrochloric acid. Concentration of CO_2 absorbed was quantified from the difference of weight before and after absorption process. The same method is applied to marble as background.

2.4 Measurement

8 mL of sample mixture with 12 mL scintillator in 20 mL vial. The mixture was homogenated by shaking and saved from light exposure, and then lied on 20 mL vial plate tray. Counting the sample as protocol LSC Hidex 300 SL and it was counted at 5-240 minutes in range. The same method is applied to the background.

3 Results and Discussion

3.1 Physical And Chemical Washing

Table 1. Comparison Mass of Samples Before and After Washing

Sample	Mass Before Washing (gram)	Mass After washing (gram)	Lost Mass of Samples (%)
Porites lobata$_1$	155,550	140,082	9,9
Porites lobata$_2$	160,920	145,069	9,8

Washing the sample is done to eliminate all of contaminations contained in the sample. Physical washing is able to remove stains or impurities that are easily lost attached to the surface of the sample. Chemical washing is capable of removing brown / yellow organic stains attached to coral polyps that cannot be lost, and to reduce modern CO_2 adsorbed on the surface of the sample during the washing process. Reducing sample weight as shown in Table 1 shows the loss of natural contamination accumulated during coral in the waters.

3.2 CO_2 Absorption

The third step is CO_2 absorption. Sample entered to absorption equipment until fix weight found. Fig.1 shown the increasing mass of sample when CO_2 absorption process.

3.3 Measurement

Sample counting was carried out in two stages. First, determine the optimum counting time and then determine the average counting value at the optimum time. The fluctuating value of ^{14}C activity was caused by the instability of the interaction between carbonate solution and the scintillator at the beginning of the counting process.

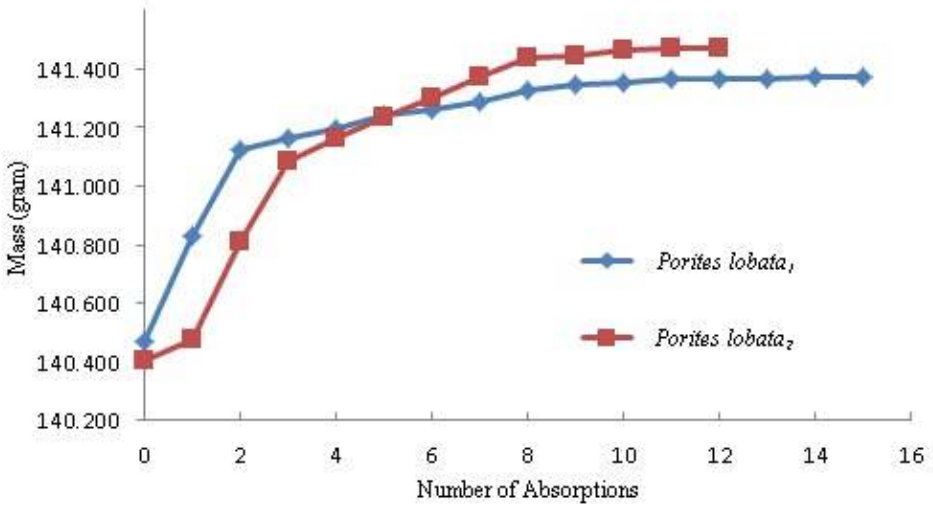

Fig. 1. Improvement Mass of Samples When Co_2 Absorption Process

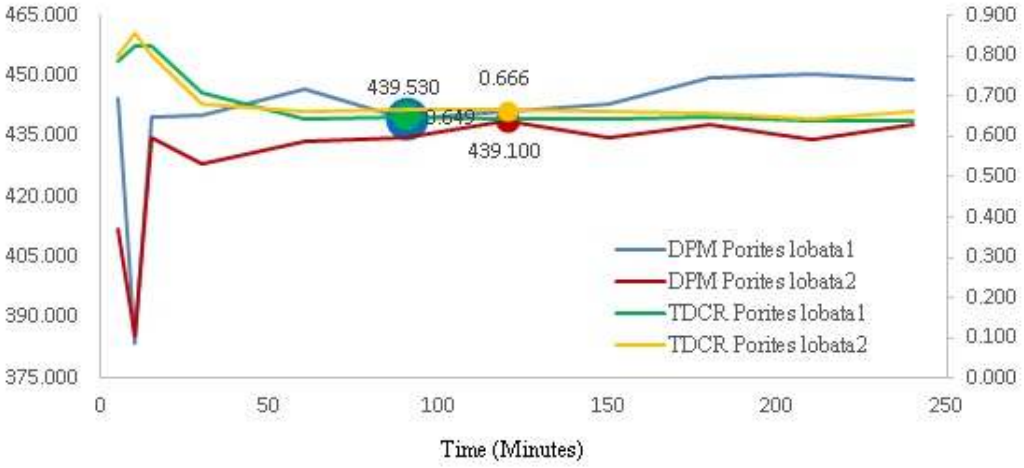

Fig. 2. Counting stability from Samples

Determination of the optimum counting time is done to determine the best time to produce DPM values and have a stable counting efficiency (TDCR) as a sign that the sample counting process runs optimally. ^{14}C activity of *Porites lobata*$_1$ began stable in 90 - 240 minute, as shown in Figure 2. While the activity value of ^{14}C of *Porites lobata*$_2$ began began stable in 120 - 240 minute, as shown in Figure 2. The stability of the ^{14}C activity value is very important to obtain the count chart results which is exponential. *Porites lobata*$_1$ were chopped repeatedly at the optimum time, 90 minutes and *Porites lobata*$_2$ at 120 minutes as shown in table 2.

Table 2. ^{14}C Activity of Samples in Optimum Time

Sample	Optimum Time (minutes)	Activity (DPM)	Efficiency (%)	Background Activity (DPM)
Porites lobata$_1$	90	444.780	65	444.210
	90	443.290	65	442.500
	90	444.410	64	443.740
	90	441.270	65	440.730
	90	442.630	65	442.050
	90	442.020	65	441.360
	90	441.140	65	440.560
	90	442.770	65	442.180
	90	441.030	65	440.440
	90	444.860	64	444.260
Average		442.820	65	442.203
Porites lobata$_2$	120	438.690	66	436.070
	120	438.780	66	436.350
	120	439.090	66	436.520
	120	439.300	66	437.142
	120	439.360	66	437.280
	120	440.040	66	437.580
	120	440.490	66	437.590
	120	440.530	66	437.980
	120	440.610	66	438.840
	120	441.540	66	438.950
Average		439.843	66	437.430

Specific activity of ^{14}C is expressed in units of disintegration per unit mass of carbon (DPM/gramC). Specific activity of *Porites lobata*$_1$ was 15.43 ± 1.81 DPM/gramC. Specific activity of *Porites lobata*$_2$ was 14.62 ± 1.58 DPM/gramC. Specific activity value of ^{14}C modern *Porites lobata*$_2$ is lower than the value of specific activity of modern carbon standards in land organism that are often used. While the value of specific activity of ^{14}C *Porites lobata*$_1$ is higher, shown in table 3. However, the difference in value is not too significant, so further research is needed to be studied thoroughly.

Table 3. ^{14}C Spesific Activity of Samples

Sample	Activity* (DPM)	Total Carbon (gram)	Spesific Activity ^{14}C modern (DPM/gram)
Porites lobata$_1$	0.617	0.040	15.43 ± 1.81
Porites lobata$_2$	2.413	0.165	14.62 ± 1.58
	Libby [8]		15.3 ± 0.1

4 Conclusion

The spesific activity of ^{14}C modern from coral *Porites lobata* in the *middle inner zone* and *inner zone* spermonde archipelago shown different values with activities of ^{14}C modern in land organism that have been used so far. But the difference is not significant so further research is needed.

References

[1] G. Muller-Parker, C. F. D'Elia, and C. B. Cook, "Interactions between corals and their symbiotic algae," in *Coral Reefs in the Anthropocene*, 2015.

[2] S. A. Wooldridge, "Is the coral-algae symbiosis really 'mutually beneficial' for the partners?," *BioEssays*. 2010.

[3] S. A. Wooldridge, "Breakdown of the coral-algae symbiosis: Towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae," *Biogeosciences*, 2013.

[4] A. Bolton, N. F. Goodkin, E. R. M. Druffel, S. Griffin, and S. A. Murty, "Upwelling of Pacific Intermediate Water in the South China Sea Revealed by Coral Radiocarbon Record," *Radiocarbon*, 2016.

[5] M. I. Bird, "RADIOCARBON DATING | Charcoal," in *Encyclopedia of Quaternary Science*, 2013.

[6] J. Feathers, "Luminescence dating," in *Encyclopedia of Archaeology*, 2010.

[7] A. Bayliss, "Rolling out revolution: Using radiocarbon dating in archaeology," *Radiocarbon*, 2009.

[8] W. F. Libby, "Radiocarbon dating," *Science (80-.).*, 1961.

[9] K. E. Stenström, G. Skog, E. Georgiadou, J. Genberg, and A. Johansson, "A guide to radiocarbon units and calculations," *Lund Univ. Dep. Physics, Div. Nucl. Phys. Intern. Rep. LUNFD6(NFFR-3111)/1-17/(2011)*, 2011.

[10] A. J. T. Jull, "RADIOCARBON DATING | AMS Method," in *Encyclopedia of Quaternary Science*, 2007.

[11] C. Canducci *et al.*, "Upgrade of the CO2 Direct Absorption Method for Low-Level 14C Liquid Scintillation Counting," *Radiocarbon*, 2017.

[12] C. Varlam, I. Stefanescu, M. Varlam, I. Popescu, and I. Faurescu, "Applying the direct absorption method and lsc for 14C concentration measurement in aqueous samples," *Radiocarbon*, 2007.

[13] I. Krajcar Bronić, N. Horvatinčić, J. Barešić, and B. Obelić, "Measurement of 14C activity by liquid scintillation counting," *Appl. Radiat. Isot.*, 2009.

[14] R. Broda, P. Cassette, and K. Kossert, "Radionuclide metrology using liquid scintillation counting," *Metrologia*, 2007.

[15] J. F. Adkins *et al.*, "Radiocarbon dating of deep-sea corals," *Radiocarbon*, 2002.

[16] C. Varlam, I. Stefanescu, S. Cuna, I. Vagner, I. Faurescu, and D. Faurescu, "Radiocarbon and tritium levels along the Romanian lower Danube River," *Radiocarbon*, 2010.

[17] I. Faurescu *et al.*, "Direct absorption method and liquid scintillation counting for radiocarbon measurements in organic carbon from sediments," *Radiocarbon*, 2010.