Eco. Env. & Cons. 26 (3) : 2020; pp. (1300-1304)
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ISSN 0971–765X

Development of hydroxyapatite from corals obtained from contamination waters of Northern Java by precipitation method

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(Received 15 January 2020; Accepted 1 April, 2020)

ABSTRACT

Research on the development of hydroxyapatite based of coral from the North Coast of Java Island using the precipitation method has been carried out. The research was carried out by identifying the compound content in the coral of the North Coast of Java to find out its potential as a basic ingredient of hydroxyapatite. The preparation of coral powder was carried out using milling for 20 hours with a ratio of samples and ball mill is 1:20. With this treatment, the average powder size of 32.69 nm was obtained. This study was to obtain the highest percentage of hydroxyapatite by varying the concentrations of $Ca(OH)_2$ respectively 0.6 M, 0.85 M, and 1.1 M. Hydroxyapatite synthesized from the basic ingredients of coral, the North Coast of Java, especially in Remen Beach, Tuban, produced the highest percentage of 61.50 %.

Key words : *Hydroxyapatite, Corals, Contamination Waters, Java*

Introduction

Hydroxyapatite ($Ca_{10}PO_4)_6(OH)_2$)is the most stable crystalline compound of calcium phosphate. This compound has better thermal resistance compared to other calcium phosphate compounds such as dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$), dicalcium phosphate anhydrous ($CaHPO_4$), octacalsium phosphate ($Ca_8H_2(PO_4).5H_2O$), tetracalcium dihydrogen phosphate ($Ca_4H_2P_6O_{20}$) and tricalcium phosphate ($Ca_3(PO_4)_2$). Hydroxyapatite is the first material that is processed and synthesized specifically for use as an implant in the human body. This compound has been widely used to repair, filler, and reconstruct damaged bone tissue (Canillas *et al.,* 2017).

Hydroxyapatite (HA) can be synthesized through several methods, including solid state reactions, coprecipitation, hydrothermal, and sol-gel process (Azis *et al.,* 2018). The precipitation method is the most well-known wet chemical method, which is widely used for hydroxyapatite synthesis. This is because the method can synthesize HA in large quantities without using organic solvents and at a relatively low cost. For the formation of HA through the method of precipitation can use various precursors containing calcium and phosphate, for example calcium hydroxide ($Ca(OH)_2$) and phosphoric acid (H_3PO_4). The byproduct of the synthesis using the precipitation method is only water.

Various natural minerals that have high calcium carbonate ($CaCO_3$) content have the potential as a raw material for hydroxyapatite. Mammalian bones, conch shells, corals, and eggshells (Pountos and Giannoudis, 2016) are some examples of sources of hydroxyapatite minerals. Preliminary research on coral as a candidate for hydroxyapatite raw material has been carried out by Siswanto *et al.*, (2019). The XRD (X-Ray Diffraction) test showed that the corals contained 97.69 % of $CaCO_3$ (Aragonite) compounds. The calcium carbonate content in these corals was relatively higher compared to sea shells (87.12%), eggshells (89.98%), snail shells (68.7%) and mammalian bones (95.7%) (Fleet, 2015) This data illustrates that coral is the most potential raw material for hydroxyapatite compounds.

Coral is a marine animal that can secrete calcium carbonate ($CaCO_3$) so that it can make lime deposits on coral reefs. Indonesia is an archipelago that has a large marine area that has a variety of coral species in it. Sea corals have high calcium and phosphate content. The percentage of calcium content in corals is strongly influenced by the content of elements in the sea and species in its ecosystem (Nurmala, 2018).

Coral of the north sea of Java has a different ecosystem with the southern sea coral of Java. Most of the northern seas of Java are estuaries of rivers. This causes a variety of pollutants from the mainland empties into the north sea of Java. Tuban Sea is one of the north seas of Java which is heavily polluted by waste. That is because Tuban is a coastal area so it is a place of industrial waste expansion (Darmawan *et al.*, 2014). Waste generated by domestic is usually liquid waste and contains several chemical parameters such as nitrate, nitrite, ammonia, oil and fat, and detergent. This pollution can cause various impacts such as changes in the structure of the food network, changes in the structure of aquatic communities, physiological effects, behavior, genetics, and resistance. Pollution in the coastal area of Tuban can affect the mineral content of marine biota in it, including coral. Therefore, the content of compounds in coral is not only $CaCO_3$, but also contains other compounds that indicate the occurrence of pollution of the ecosystem.

Materials and Methods

Coral as a hydroxyaptite raw material in this study came from the north sea of Java, namely Sowan and Remen Beach, Tuban , East Java. Also used are 99.8% pure Phosphoric Acid (H_3PO_4) by Aldrich, distilled water and glycerol.

Synthesis of hydroxyapatite made from coral using the precipitation method begins by removing the dirt attached to the coral. Furthermore, the coral is dried to remove water content. Dry corals are manually crushed to a smaller form. Small-sized corals are then crushed using a mortar until smooth. After that the sieving was carried out using a 200 mesh sieve to obtain 70 μm of coral. Then it is milled by using HEM (High Energy Milling) with a comparison of coral with a ball mill that is 1:20 for 20 hours.

Coral powder is heated at 900°C for 3 hours to eliminate impurities. At this stage the formation of CaO compounds from $CaCO_3$ occurs. Hydroxyapatite synthesis was carried out by reacting Ca $(OH)_2$ with H_3PO_4. The compound $Ca(OH)_2$ used is the result of calcination of $CaCO_3$ which is carried out in a place that contains water vapor so that it is hydrated and $Ca(OH)_2$ is formed. The reaction was carried out using a stirrer and spin bar at 70°C for 2 hours. Then the solution is allowed to stand for 24 hours at room temperature to form a precipitate. The resulting precipitate is filtered using filter paper and washed with distilled water. The precipitate is removed by dehydration at 110°C for 3 hours. The precipitate resulting from the dehydration process is heated at 900°C for 5 hours to form a hydroxyapatite crystal. After the hydroxyapatite crystal was formed XRD (*X-Ray Diffraction*) characterization was carried out to determine the phase of hydroxyapatite formed.

Results and Discussion

Coral particles that have been millied for 20 hours have a size between (20-50) nm with an average size of 32.69 nm (Fig. 1).

XRD observations were made on corals from Remen and Sowan beaches before milling and calcination treatment. This observation aims to determine the coral content of the North coast of Java and

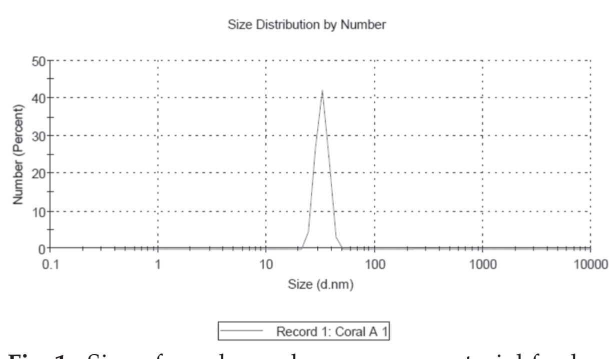

Fig. 1. Size of coral powder as a raw material for hydroxyapatite

the impurity that must be eliminated for subsequent treatment. The results of coral XRD observations before undergoing milling treatment are shown in Figure 2.

Search match analysis results of XRD graphs figure 2 using the Match! ® application showed that the compounds contained in coral on the north coast of Java island, especially in the Tuban area are stated in Table 1. It appears that the Remen Coast coral contains $CaCO_3$ (*calcium carbonate*), SiO_2 (*Dolomite*) and $CaMg(CO_3)_2$ (*Moganite*). While the Sowan coast contains $CaCO_3$ (*calcium carbonate*), SiO_2 (*Moganite*) and $Pb_3(PO_4)_2$ (*Lead phosphate*). In contrast to corals from the South coast of Java, specifically the Banyuwangi coast, the $CaCO_3$ content is very large 94.4% and the remaining Ca_2SiO_5 is 5.6% (Siswanto *et al.,* 2019). The $CaCO_3$ content in corals from the south coast of Java is much greater compared to corals from the north coast. In addition, coral from Sowan beach also contains heavy metal elements in the form of $Pb_3(PO_4)_2$. The existence of these elements is allegedly due to the location of Sowan Beach which is close to the industrial area, so that water and marine biota contained in Sowan Beach are polluted by industrial waste in the form of metal as examined by Darmawan *et al* (2014). The next process used as material hydroxyapatite is coral from Remen Beach, Tuban.

To eliminate Moganite impurity compounds, lead phosphate and dolomite were calcined at 900 ^oC for 3 hours. Besides this heat treatment to decompose $CaCO_3$ into CaO. The heat treatment is carried out in an air (oxygen) furnace so that calcium hydroxide $Ca(OH)_2$ is caused due to condensation, this can be seen in the XRD spectrum in Figure 3 and the results of the search match analysis stated in Table 2

When $CaCO_3$ is heated it will decompose to CaO. The incident occurred because of heating caused the formation of calcium ions which then joined with 1

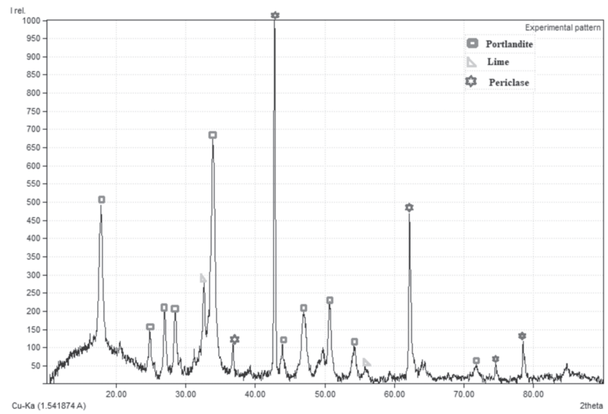

Fig. 3. XRD spectrum of 900 ^oC calcined coral powder for 3 hours

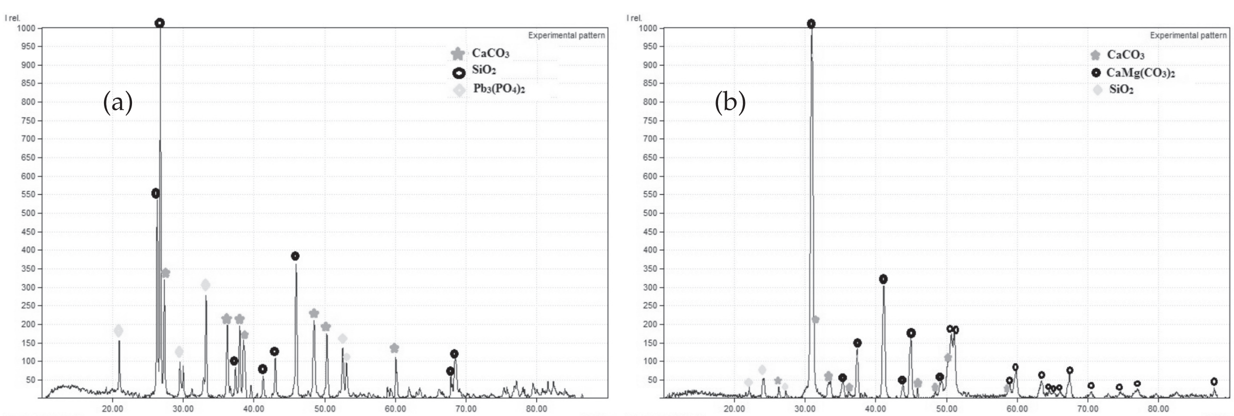

Fig. 2. Coral XRD results before milling treatment from (a) Sowan beach and (b) Remen beach

Table 1. Compounds in corals from Sowan and Remen Beach Coral

Samples	Compound (%)			
	$CaCO_3$ (*Aragonite*)	SiO_2 (*Moganite*)	$Pb_3(PO_4)_2$ (*Lead Phosphate*)	CaMg $(CO_3)_2$ (*Dolomite*)
Sowan Beach Coral	10,1	86,5	3,3	-
Remen Beach Coral	13,7	6,8	-	79,5

Table 2. Compound formed in coral powder which calcined 900 °C, 3 hours

Sample	Compound (%)		
	$Ca(OH)_2$ *(Portlandite)*	CaO *(Lime)*	MgO *(Periclase)*
Coral	30.6	9.3	60.2

oxygen atom. While $Ca(OH)_2$ is formed by the reaction between CaO and water vapor that occurs in the chamber furnace. This calcination also caused the impurity of the Moganite compound not to be found anymore. Whereas the impurity of $CaMg(CO_3)_2$ (Dolomite) which contains Magnesium Carbonate compound changes to form Magnesium Oxide after calcination process. Magnesium Oxide (MgO) compounds that appear after the calcination process are not harmful to the body. In medicine, Magnesium Oxide is used to relieve heartburn and stomach acid, as an antacid, as a magnesium supplement, and as a short-term laxative (Nurmala, 2018). This hydroxyapatite synthesis uses the precipitation method. The process consists of precipitation of $Ca(OH)_2$ powder which is reacted with phosphoric acid $(H_3PO)_4$. Then filtered, removal of water content at 110 °C, and sintering at 900 °C.

Filtration is carried out after the sample on the calcined powder is confirmed to have formed Ca $(OH)_2$. With various concentrations of $Ca(OH)_2$ namely 0.6 M, 0.85 M and 1.1 M reacted with phosphoric acid $(H_3PO)_4$, which was then precipitated for 24 hours and then filtered. The XRD spectrum of the filtration results is shown in Figure 4 and the results of the search match analysis are shown in Table 3.

Table 3. The compound formed in the sample after filtration

Molar of $Ca(OH)_2$	Compound (%)		
	$Ca_5OH(PO_4)_3$ *(Hydroxylapatite)*	$CaHPO_4 \cdot 2(H_2O)$ *(Brushite)*	$Ca(OH)_2$ *(Portlandite)*
0.60 M	24.9	63.9	11.2
0.85 M	23.5	70.0	6.5
1.10 M	11.1	86.3	2.6

The compounds formed after the deposition process are hydroxylapatite. brushite, and portlandite. Hydroxyapatite ($Ca_5OH(PO4)_3$) phase is formed because of the processes that occur under acidic conditions. Other phases formed by the acidic environment in the deposition process are *brushite* ($CaHPO_4.2(H_2O)$) or monocalcium phosphate. Brushite is a phosphate mineral that has a structure like calcium diphosphate ($CaHPO_4$) but contains water. Dehydration and sintering treatment at 900 °C for 5 hours formed crystal structure hydroxyapatite, which is a change from amorphous. The formation of hydroxyapatite crystals was observed using XRD, as shown in Figure 5 and table 4. XRD results of figure 5 and table 4 show that the sintering treatment not only produces hydroxyapatite, but also other compounds such as brushite, and Calcium Trihydrogen Phosphate. The compound calcium dihydrogen phosphate or known as monocalcium phosphate is formed because of the reaction between calcium hydroxide ($Ca(OH)_2$ with phosphoric acid (H_3PO_4).

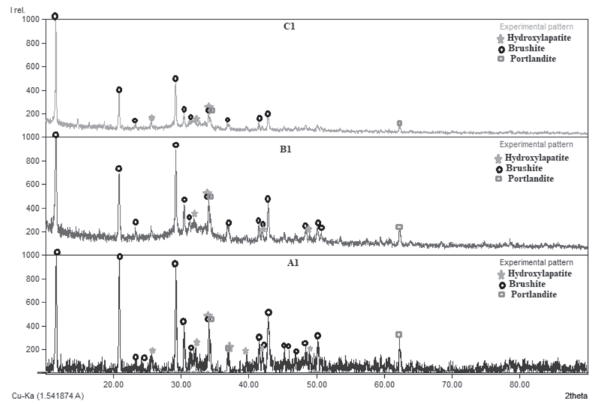

Fig. 4. XRD spectrum of filtration results for the concentration of $Ca(OH)_2$ A_1 (0.6 M), B_1 (0.85 M), and C_1 (1.1 M)

Based on the XRD results of Figure 5 and the results of table 4 analysis it can be seen that the highest percentage of hydroxyapatite produced was

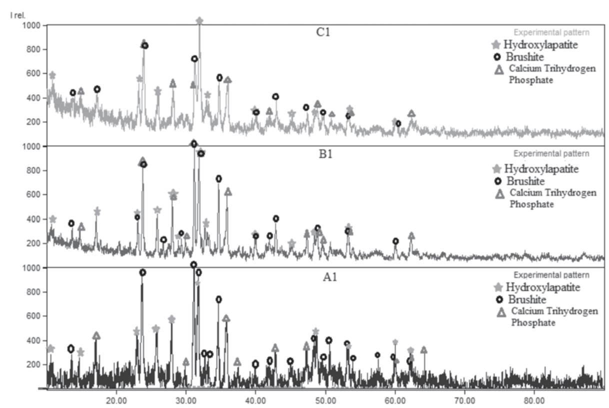

Fig. 5. HA XRD spectrum for $Ca(OH)_2$ for A_1 (0.6 M), B_1 (0.85 M), and C_1 (1.1 M) concentrations

Table 4. The compound formed in the sample after sintering

Molar of Ca(OH)2	Compound (%) $Ca_5OH(PO_4)_3$ *(Hydroxylapatite)*	$CaHPO_4 \cdot 2(H_2O)$ *(Brushite)*	CaH_3PO_4 *(Calcium Trihydrogen Phosphate)*
0.60 M	27.9	55.0	17.0
0.85 M	61.5	6.0	32.5
1.10 M	41.4	44.6	14.0

61.5%, which occurred in samples with a molar concentration of $Ca(OH)_2$ of 0.85 M. Compared to the research conducted by Naldo (2017) using the same $Ca(OH)_2$ method and concentration using corals from the south coast of Java (Tulungagung Beach), 95.3% hydroxyapatite was obtained and the remainder was β-TCP (*β-Tricalcium phosphate*. Likewise, when compared with research conducted by Zamani *et al* (2012). This difference occurs because the concentration of $CaCO_3$ coral in the North Coast of Java is less in percentage compared to the southern coast of Java. Therefore, it can be suggested that the formation of hydroxyapatite made from marine corals should use those originating from the southern coast of Java.

Conclusion

The north coast of Java has relatively little calcium carbonate content compared to coral from the south coast of Java. In addition, coral from the north coast of Java contains elements that contain heavy metals Pb. Therefore, hydroxyapatite formed from the coral of the north coast of Java is also less than the corals originating from the south coast of Java. Pb heavy metals are also relatively difficult to separate from their compounds. Because hydroxyapatite is applied to medical materials, it is better not to use corals that originate from the north of Java.

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