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Conversion of Calcified Algae (*Halimeda* sp) and Hard Coral (*Porites* sp) to Hydroxyapatite

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Abstract. Calcium phosphate materials can be produced using a number of wet methods that are based on hydrothermal or co-precipitation methods that might use acidic or basic chemical environments. In our previously published works, we have investigated calcium phosphates such as monetite, hydroxyapatite, and whitlockite which were successfully produced by mechano-chemical methods and/or hydrothermal treatments from a range of marine shells and corals which were obtained from the Great Barrier Reef. The aim of the current work was to analyze and compare the mechanisms of conversion of one hard coral species and one calcified algae species from the Great Barrier Reef.

Introduction

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; HAp) is often defined as being the constituent of the bone and teeth mineral phase [1]. As HAp and other calcium phosphate materials are osteoconductive, biocompatible and non-toxic, they make ideal substances to be used for biomedical and dental applications [1, 2].

HAp and other calcium phosphate bioceramics can be synthesized through a variety of wet chemical methods based on hydrothermal or co-precipitations [3, 4]. In these methods, the resulting phase is largely depending on the chemical and physical conditions. Alternatively, HAp can be synthesized through the conversion of biogenically derived minerals [5].

The exoskeletons of marine species, such as coral and calcified algae are composed of calcium carbonate (CaCO_3) primarily in the form of aragonite [5, 6]. In previous research, calcium carbonates from coral and seashells have been successfully converted to hydroxyapatite through mechano-chemical and hydrothermal methods [7, 8]. An advantage of using these starting materials is the preservation of their porous morphologies, which resemble trabecular bone [5, 9, 10]. In hydrothermal treatment, the reagents are sealed in a reaction vessel, and are subjected to elevated temperatures and pressures. This method produces highly crystalline HAp with high phase purity [6].

Extensive research has been conducted on coral as a biomimetic material [5, 8, 9, 11-13]. However, harvesting coral causes extensive damage to coral reefs and consequently, the ecosystem [11, 10]. Alternatively, marine algae can potentially be used instead, as the harvesting procedures are less aggressive and will not destroy the surrounding seabed [10].

In this experiment, the calcareous green algae *Halimeda cylindracea* (Hc) harvested from the Great Barrier Reef was converted to HAp using hydrothermal conversion. *Halimeda* species are

distributed in tropical to sub-tropical environments. Hc consists of bead-like calcified segments linked together through a network of flexible fibers. These form branching chains from a central base to produce a bushy thallus.

The aim of this experiment was to analyze and compare the converted products for both hard coral (*Porites sp*) and Hc calcified algae species. The analysis was achieved by using powder X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) to characterize the materials before and after conversion. Further analysis was done using scanning electron microscopy (SEM) to examine the microarchitecture of both species.

Materials and Method

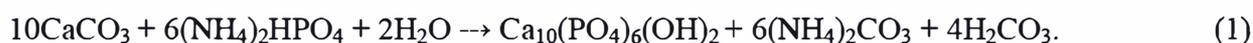
Materials

Hard coral (*Porites sp*) and Calcified Algae (*Halimeda sp*) samples were obtained from the Great Barrier Reef, QLD, Australia. All chemicals used were from Sigma-Aldrich, Australia.

Synthesis and Characterization

Hc calcified algae was first cleaned using 2% (v/v) sodium hypochlorite (NaOCl) in an ultrasonic water bath (UD80 SH-2L). Following this, Hc calcified algae was heat treated at 350°C overnight.

In order to convert Hc calcified algae to HAp, diammonium hydrogen phosphate solution ($(\text{NH}_4)_2\text{HPO}_4$) was prepared in 150 mL ultrapure water according to the Ca/P ratio of hydroxyapatite, 1.67. The clean Hc calcified algae was placed in a Parr reactor (Parr Instrument, USA) with the $(\text{NH}_4)_2\text{HPO}_4$ solution. The reaction vessel was sealed for 24 hours at a pressure of 8.0 MPa with the temperature adjusted between 160 - 220°C. The chemical reaction of the hydrothermal reaction can be described by Eq. 1 below [12]:



After conversion, the converted product was washed with ultrapure water in a vacuum filter in order to remove excess $(\text{NH}_4)_2\text{HPO}_4$ and was dried overnight in an oven at 60°C. The same procedure was applied for hard coral samples.

The analyses of materials were achieved using FTIR (Thermo-Scientific Nicolet 6700 instrument using ATR mode, from 2000 to 300 cm^{-1} at a resolution of 4 cm^{-1}), XRD (Bruker D8 Discover diffractometer, radiation Cu-K α 40 kV and 40 mA, from 20 to 80° 2 θ , step size of 0.2° per 0.3 seconds) and SEM (Zeiss Evo LS15 scanning electron microscope with an accelerating voltage of 10 kV).

Results and Discussion

In Fig.1, the differences between unclean-unconverted, clean-unconverted and converted at 220°C Hc calcified algae samples can be seen clearly.



Figure 1: Unclean-unconverted (A), clean-unconverted (B) and converted (C) *H. cylindracea*.

The characterization of all samples was determined by XRD analysis. The XRD patterns for whole samples are illustrated in Fig. 2. Although the major phase of unconverted Hc calcified and hard coral samples refers to aragonite material (JCPDS card No. 00-003-1067) which is a high-pressure stable phase of calcium carbonate (CaCO_3), uncleaned-unconverted Hc calcified algae

contained one minor phase which was identified as NaCl. Additionally, clean-unconverted Hc calcified algae and hard coral samples which were dried at 350C° before conversion show some calcite ((JCPDS card No. 01-072-1650) peaks. Calcite is an atmospheric-pressure stable phase of CaCO₃. Diffraction patterns of Hc calcified algae and hard coral show that the inorganic component of the marine samples have been converted to HAp (JCPDS card No. 00-009-0432) successfully.

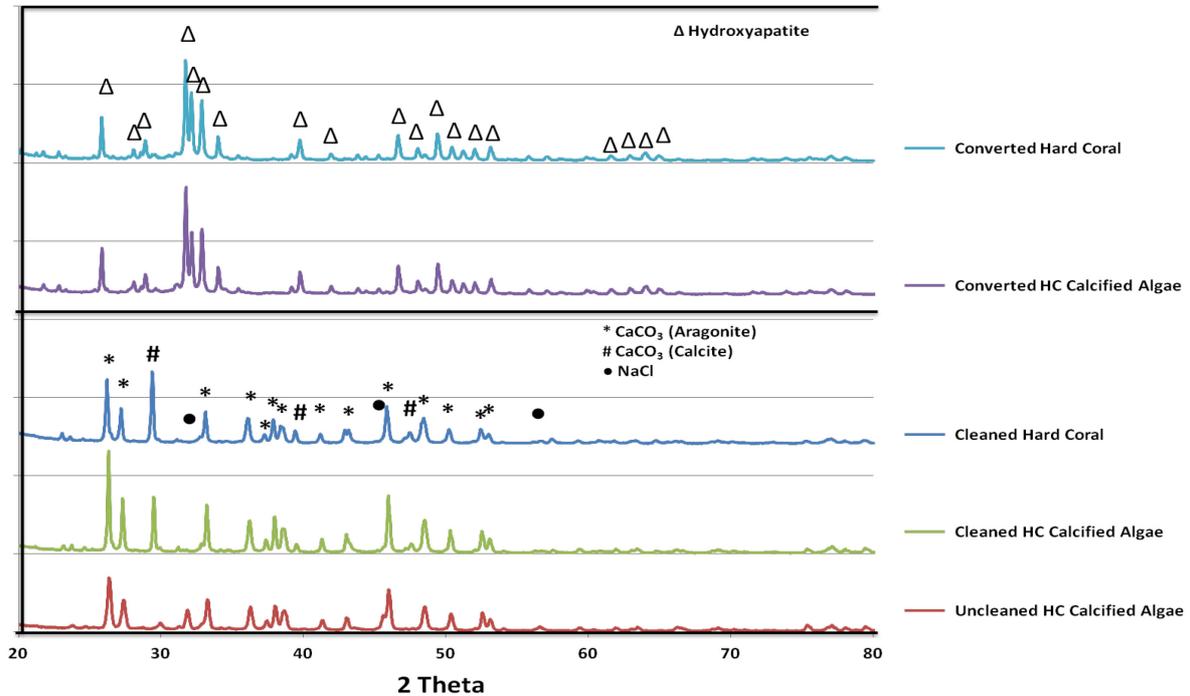


Figure 2: XRD patterns of Unclean-unconverted, clean-unconverted and converted Hc and hard coral samples.

Certain calcium carbonate bands were illustrated in the FT-IR micrographs of uncleaned and cleaned unconverted samples which were completely similar to each other. Besides that, the characteristic absorption bands of HAp peaks in FT-IR spectrum of the converted Hc calcified algae and converted hard coral powders were proved to the hydrothermally conversion from calcium carbonate to HAp, successfully in Fig.3.

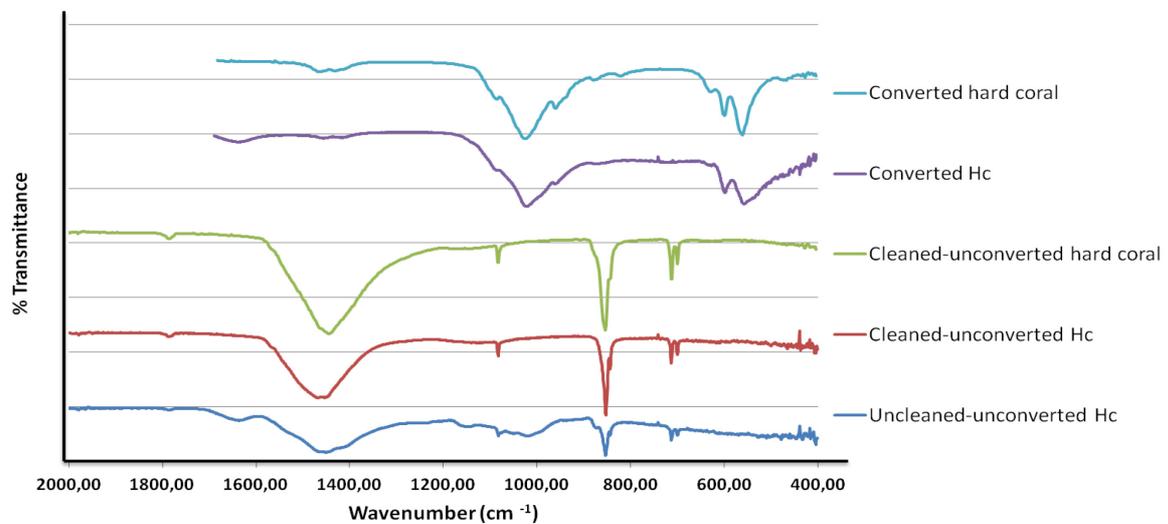


Figure 3: FT-IR spectrum of unclean-unconverted Hc, clean-unconverted Hc, clean-unconverted hard coral, converted Hc and hard coral samples, respectively.

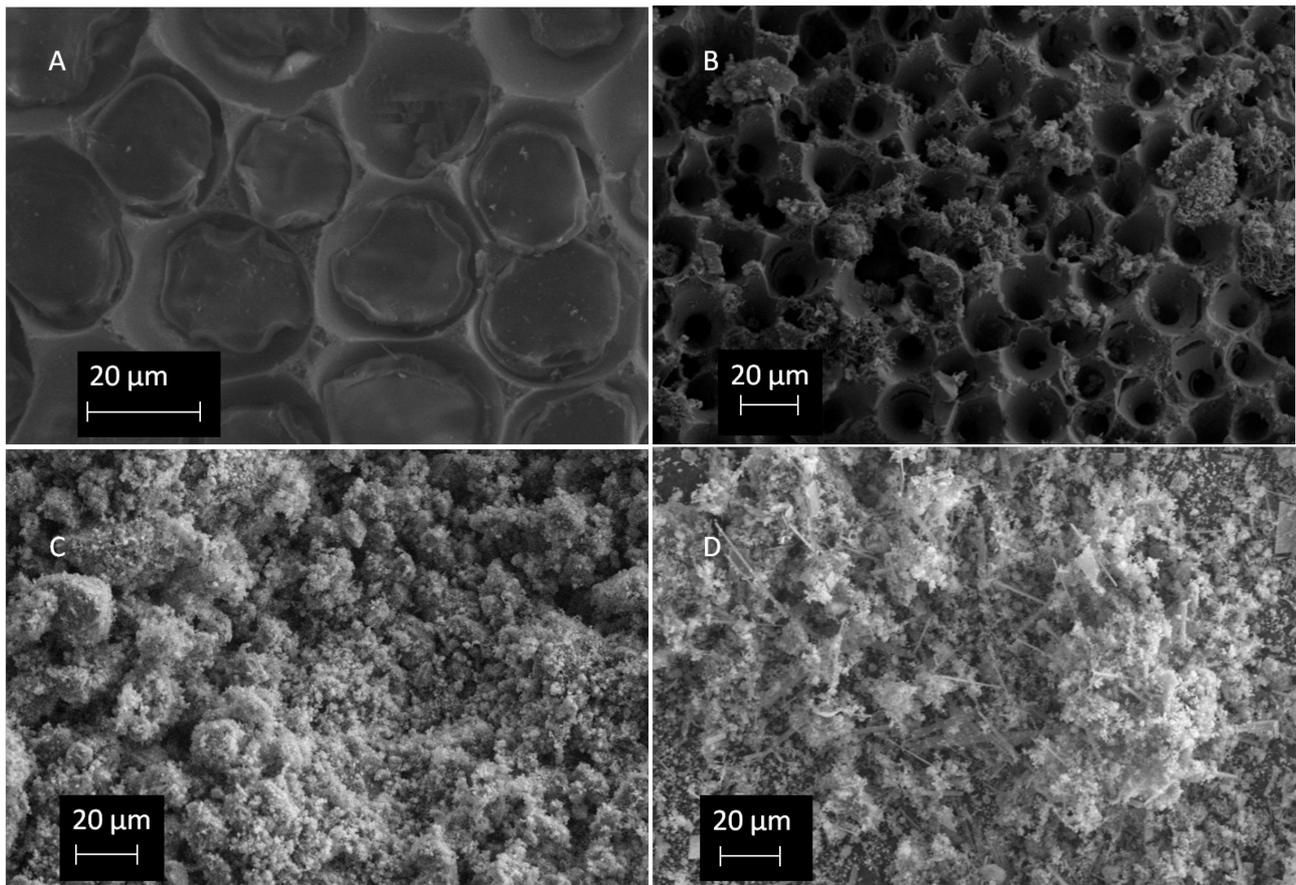


Figure 4: SEM images of clean-unconverted Hc calcified algae (A), converted Hc calcified algae (B), clean-unconverted hard coral powder (C), and converted hard coral powder (D).

The microarchitecture of the samples were determined using SEM analysis. Examination of unconverted calcified algae under SEM revealed the microporous surface. (Fig 4-A, Fig. 4, B). Once converted, the microporous structure and pore distribution of the algae were retained. In addition to this, hydroxyapatite crystals with needle-like morphology were distributed on the algae surface. These observations are similar to those reported by Chou et al., who examined the distribution and architecture of pores in *Foraminifera* hard coral species [14]. Additionally, the SEM micrographs of unconverted and converted hard coral species are given in Fig. 4C and D respectively. In both micrographs, the coral powders are fine in nature. However after conversion, an additional phase of needle-like morphology is formed. As similar observations have been made for both converted calcified algae and hard coral powders, it can be concluded that rod-shaped crystals were formed through hydrothermal conversion. These crystals may be characterized as HAp according to XRD and FT-IR analysis.

Conclusion

Calcified algae *H. cylindracea* and hard coral *Porites* sp. were successfully converted to hydroxyapatite with a Parr hydrothermal reactor. The materials were characterized using XRD and FT-IR analysis, and the microarchitecture was observed using SEM. It was shown that *H. cylindracea* contains a large amount of organic matter that allows flexibility but requires longer cleaning periods and conversion times than hard corals. The unique morphology of these materials may permit drug loading and long term slow and sustained drug delivery.

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