Synthesis of Hydroxyapatite from Biological Sources Prepared by The Microwave Irradiation Method: A Review

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Abstract—**Microwave irradiation has attracted considerable attention as a promising approach for synthesizing hydroxyapatite (HA) from biological sources. This article presents a systematic review of the utilization of microwave irradiation for producing HA from biological sources. Using biological sources for HA synthesis offers several advantages, including sustainability, biocompatibility, and waste utilization. Compared to conventional methods, microwave irradiation presents various notable benefits, such as reduced processing time, enhanced crystallinity, controlled morphology, energy efficiency, and scalability. Furthermore, microwave irradiation is an energy-efficient technique for synthesizing HA from biological sources that align with Sustainable Development Goals (SDGs) numbers 3, 9, 12, 13, 15, and 17. The results of this study indicate that biological sources such as shells and fish bones are generally used for the synthesis of HA using the microwave method. However, biological sources such as mammalian bones and crab shells are still under-explored. The HA synthesis process from biogenic sources utilizing the microwave method is influenced by numerous factors, such as pH, temperature, Microwave Power (MWP), and synthesis time. Therefore, HA with the desired properties, such as crystalline phase, crystallite size, crystalline form, purity, size, and shape of particles, can be produced by determining the appropriate synthesis parameters.**

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I. INTRODUCTION

Hydroxyapatite (HA) is an inorganic component that provides 60-70% of the mineral phase in human bone. HA is classified as a calcium phosphate molecule due to its chemical formula $Ca_{10}(PO_4)_6(OH)$, which is composed of phosphorus (P) and calcium (Ca). The biomedical industry frequently utilizes HA due to its resemblance to the primary constituent minerals of bones and teeth [1]. The diverse applications of HA as biomedical materials due to their biocompatibility and osteoconductivity properties have sparked growing interest. These applications include bone fillers, bone tissue scaffold engineering, bioactive implant coatings, bone tissue repair, and drug delivery systems. Furthermore, due to its adaptability and ease of acceptance, HA has immunogenic qualities and is well-suited for bone tissue. It is non-toxic, non-inflammatory, and has a chemical composition similar to bone, which aids in the repair of injured bone tissue [2]–[10].
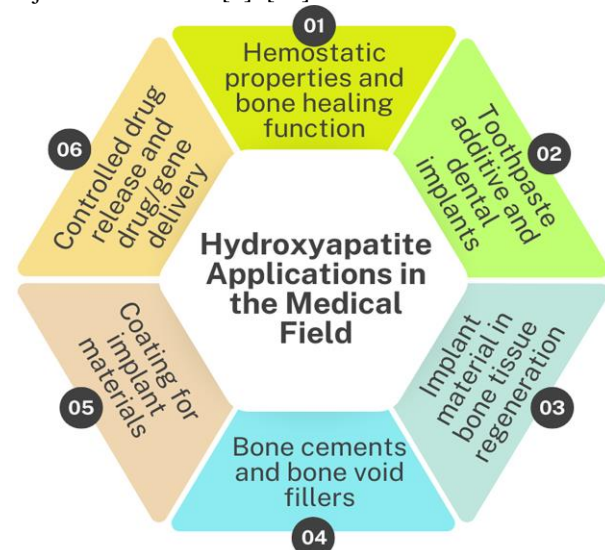

Fig. 1. Hydroxyapatite applications in the medical field

HA possesses distinctive characteristics that render it a crucial substance in numerous medical and related applications. Fig.1 depicts the adaptability of HA in various medical and dental applications. Due to its bioactive properties and biocompatibility, it is highly suitable for implant materials in bone tissue regeneration and as a coating on implant surfaces to improve bioactivity.

Furthermore, bone cement, bone void fillers, and dental implants employ HA to accelerate bone healing and provide a framework for new bone development. Furthermore, its efficacy in promoting regulated medication release and drug or gene delivery systems enhances its significance in medicinal contexts. HA has been used as an ingredient in toothpaste to facilitate the remineralization of dental enamel [11]–[19].

Researchers have long been interested in synthesizing HA due to its biocompatibility and high affinity for biopolymers. In addition, many studies have shown that HA has biocompatibility with human oral tissues, osteoconductive properties, and the ability to stimulate osteoblast growth and bone formation [20], [21]. Synthetic compounds like $Ca_2P_2O_7$, Na_2HPO, and K_2HPO can be used as starting materials to make synthetic HA utilizing various synthesis methods. The use of synthesized compounds facilitated the production of HA with excellent stoichiometric properties (Ca/P 1.67). However, due to the rise in production costs, the synthetic HA created has a high selling price. Additionally, many crucial ions, including iron, potassium, silicon, magnesium, strontium, and sodium, which are crucial in determining the biological characteristics of HA, may not be present in the final product [22].

Therefore, the synthesis of HA from natural resources such as fish bones, eggshells, crab shells, and clam shells is an alternative, as it can yield HA with superior osteoconductive, biocompatibility, and biological activity compared to the synthesis of HA from synthetic compounds. A further advantage of HA derived from natural sources is that it contains small quantities of organic matter, which can positively influence its properties. Additionally, HA, derived from natural resources, is advantageous since it typically contains the ions necessary for quicker bone regeneration with enhanced osseointegration [22]–[24].

Biocompatibility and osteoconductive properties are critical biological characteristics that HA must possess. This allows osteoprogenitor cells to adhere to the newly generated bone and endure partial remodeling by the host's osteoclasts and osteoblasts. Moreover, HA can be used as a scaffold to help bone tissue grow from the stage of osteoblast proliferation to the deposition of Extracellular Matrix (ECM) and early cell-mediated mineralization. Adding a small amount of HA to the scaffold substantially increases Ca deposition and has a minor osteogenic effect [25].

HA can be synthesized using several processes (Fig. 2), including dry methods such as solid-state synthesis and mechanochemistry, wet methods such as conventional chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, and sonochemistry, as well as high-temperature operations such as combustion and pyrolysis [26], [27]. The remaining difficulty in determining the synthesis method for producing HA is the possibility of obtaining a product with improved physicochemical properties. For example, with the capability of performing excellent control of crystallite and particle size and structural homogeneity while avoiding the formation of by-products and impurities [26]–[28].

Compared to dry, wet, and high-temperature methods, the microwave method is more effective and less harmful to the environment for synthesizing HA.
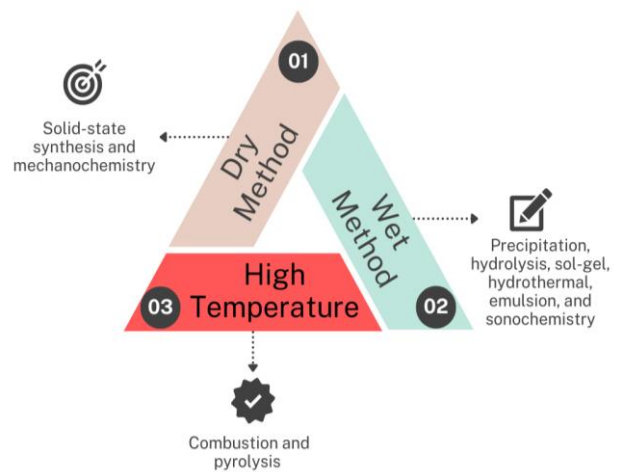

Fig. 2. Method for the synthesis of HA

The microwave method for HA synthesis provides numerous advantages, such as rapid heating to the crystallization temperature, rapid chemical acceleration, high crystallinity, and fewer impurities. Higher and faster microwave heating accelerates the kinetic reaction in HA synthesis, leading to the selective crystallization, formation, and growth of nanostructured HA. Additionally, it reduces energy consumption. Microwave irradiation can heat the entire volume of the vessel uniformly without creating a temperature gradient in the heated substance. Microwaves are crucial in aqueous media reactions and can produce HA in under 45 minutes [26]–[28]. Demirtaş et al. [29] claimed that microwave-assisted methods have several advantages in various processes, including biomimetic processes. The capacity to execute quick, effective, and homogeneous procedures is one of these advantages, as is the decrease in unfavorable reactions. In the context of this experiment, microwave irradiation proved quicker and more effective than alternative approaches for producing bone-like HA [29].

In earlier research, the hydrothermal approach had been employed to create HA from green mussel shells. The findings of this study demonstrated that the hydrothermal process needed to be conducted at 160°C for 18 hours to manufacture high-purity HA [17]. With its high temperature and lengthy synthesis time, the hydrothermal process renders the synthesis ineffective and inefficient. This causes the cost of synthesizing HA by the hydrothermal method to be very expensive.

As a result, this research aims to assess the possible use of microwave irradiation in synthesizing HA from biological resources.
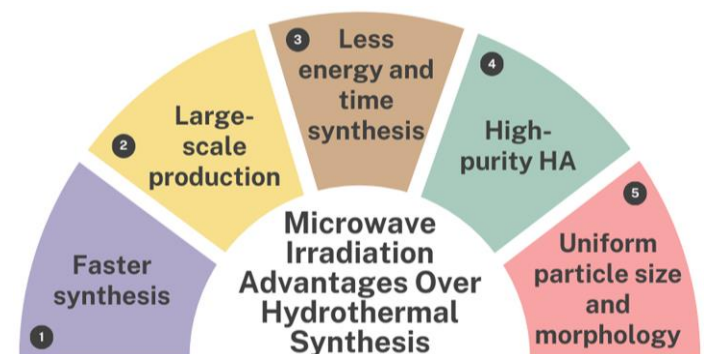

Fig. 3. The advantages of microwave irradiation for hydroxyapatite synthesis

As illustrated in Fig. 3, using microwave irradiation to form HA has various advantages over hydrothermal synthesis. These benefits include faster synthesis, uniform particle size, increased energy efficiency, higher purity levels, and the potential for scalability [27], [30]–[32]. In addition, for the synthesis of HA generated from biological resources, another technique that is more effective and efficient than the hydrothermal technique is required.

Aziz et al. [33] reported that hydrothermal and microwave heating increased the nucleation rate in synthesizing carbonate hydroxyapatite nanoparticles (CHA). Their findings indicated that calculating the total energy for CHA synthesis using hydrothermal heating necessitates more electrical power than microwave heating. The energy required for CHA synthesis using hydrothermal heating ranged from 1.4 to 1.87 kWh. On the other hand, the use of microwaves requires energy between 1.12 to 1.13 kWh. The CHA material produced using hydrothermal heating has a lower crystallinity than that produced through microwave heating, although it consumes more electrical energy. Their findings indicated that microwave heating is more efficient than hydrothermal heating in delivering heat to enhance the nucleation rate, leading to increased CHA crystallinity. Furthermore, their study found that the microwave performs 20 times faster than the hydrothermal method for CHA synthesis. The hydrothermal process requires holding times of 60, 120, and 180 minutes to generate CHA. When employing the microwave method, CHA is created by holding time for 3, 6, or 9 minutes [33].

The study's findings by Aziz et al. [33] exhibited a linear relationship with the research carried out by Baykal et al. [34]. According to research conducted by Baykal et al., microwave methods for material synthesis are two to fifty times faster than conventional methods [34]. Xu et al. [35], Ajani et al. [36], and Kharisov et al. [37] found that material synthesis is faster when utilizing the microwave method compared to conventional heating methods. The microwave approach was 8 [35], 24 [36], 48 [36], and 8 [37] times faster than traditional methods.

II. BIOLOGICAL RESOURCES FOR HYDROXYAPATITE SYNTHESIS USING MICROWAVE IRRADIATION

Natural hydroxyapatite is the term commonly used to describe hydroxyapatite that is obtained from biological sources or waste. Natural hydroxyapatite (HA) typically exhibits non-stoichiometry, meaning it lacks the proper balance of calcium or phosphorus. Trace elements found in certain natural HA closely resemble the apatite formed from human bone. Na^+, Mg^{2+}, Si^{2+}, Sr^{2+}, Zn^{2+}, K^+, Ba^{2+}, F^-, $(CO_3)^{2-}$ and Al^{3+} are crucial for bone regeneration and enhance the process of bone generation [11], [38]. Using natural sources or biological wastes as raw materials for HA synthesis in the future may result in more cost-effective and affordable HA synthesis. Utilizing HA derived from natural sources is an environmentally conscious, sustainable, and cost-effective approach, as these materials are abundant. This can benefit the economy, the environment, and health in general [38]. The synthesis of HA from natural and synthetic sources is feasible; however, natural materials are the most advantageous and cost-effective materials [39].

Natural HA has advantages that are not present in synthetic HA. Akindoyo et al. [40] discovered that prolonged time requirements, complexity, and potential biological hazards characterize most HA synthesis using synthetic sources, leading to safety concerns and high HA synthesis costs. Furthermore, Osuchukwu et al. [41] reported that hydroxyapatite produced from synthetic sources has disadvantages in terms of mechanical and chemical properties.

Using biological waste in the microwave process to synthesize HA is a promising option for furthering the United Nations' Sustainable Development Goals (SDGs). This process is relevant to several SDGs, including the following:

- SDG 3: HA is essential to bone and tooth tissue, and its sustainable manufacture can provide medicinal and dental breakthroughs. The discovery of novel procedures for synthesizing HA from biological waste can help improve healthcare solutions such as bone grafts and dental implants, which promote good health and well-being.
- SDG 9: The search for novel technologies to manufacture HA from biological waste can help improve industrial sustainability. Furthermore, this can encourage breakthroughs in manufacturing processes as well as technical innovation and infrastructure, resulting in economic growth and employment creation.
- SDG 12: Using biological waste as a feedstock for HA production promotes responsible consumption, sustainable production, reduces refuse production, and prevents environmental damage and resource depletion.
- SDG 13: Organic waste-based HA production can help reduce climate change effects by promoting eco-friendly practices and reducing greenhouse gas emissions, offering a sustainable alternative to traditional methods.
- SDG 15: The use of biological waste in the manufacture of HA can help to preserve and sustainably use terrestrial ecosystems. This method not only diverts waste from landfills but also reduces pollution, protects biodiversity, and promotes sustainable land management techniques.
- SDG 17: To successfully synthesize HA from biological waste, various stakeholders, including researchers, industry, and policymakers, need to collaborate to achieve sustainable development goals.

In general, natural raw materials or biological refuse such as shells, mammalian bones, fish bones, and scales are readily accessible, abundant, and inexpensive (Fig. 4). Additionally, the extraction of HA from natural resources is considered an environmentally responsible and sustainable process [22]–[25]. The utilization of natural resources may efficiently reduce environmental contamination resulting from inadequate waste disposal methods [42]–[44].

Irfan et al. [45] investigated microwave-assisted nano-Hydroxyapatite (nHA) production from fish bones and mussel shells. Ca-EDTA is formed when Ca from fish bones and mussel shells combines with ethylenediaminetetraacetic acid (EDTA). The Ca-EDTA and disodium hydrogen phosphate (Na_2HPO_4) were microwave-irradiated for 10 minutes at 750 W to generate nHA. HA products were characterized using X-ray diffraction, FTIR, TGA, scanning SEM, TEM, and DSC methods.

The average particle size of high-quality, thermally stable nanocrystals in HA samples from fish bone and mussel shells was 265 nm and 108 nm, respectively [45].
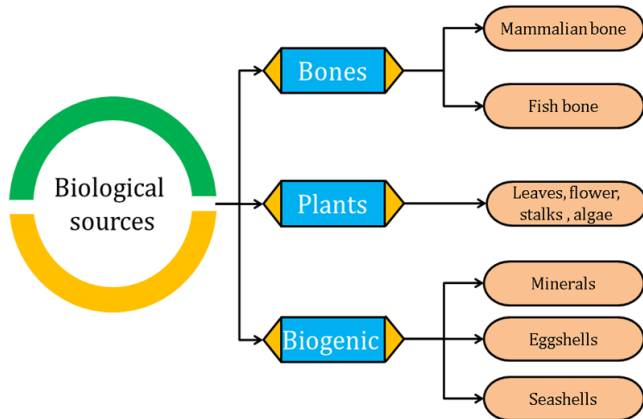

Fig. 4. Biological sources for synthesizing HA

Shavandi et al. [46] used fast microwave irradiation to create nanocrystalline hydroxyapatite from waste mussel shells. Mussel shells were changed into nanocrystalline HA particles of 30-70 nm length using 0.1 M EDTA as a chelating agent for 30 minutes after an acceptable pre-treatment and an irradiation stage in a microwave with a power of 1.1 kW. Furthermore, the mussel shell-derived HA was compared to a commercially pure HA (Sigma-Aldrich) as a benchmark. The thermal analysis discovered that the produced HA had exceptional heat stability at 1000°C, while X-ray Diffraction (XRD) and Fourier transform infrared (FTIR) measurements demonstrated the particles' high purity. When compared to standard hydrothermal therapy, the microwave-assisted approach produces more HA. The extracted HA has engineering applications as a bone tissue materials [46].

Fatimah et al. [47] synthesized HA from the shell of the paddy field snail (*Pila ampullacea*) using microwaves. The calcination process was used to create calcium oxide (CaO) from snail shells, followed by the wet preparation technique using phosphoric acid. Consequently, microwave irradiation and reflux methods were used to create HA. Microwave irradiation was performed at 600 W for 10 minutes, followed by 30 minutes and 3 hours of reflux [47].

In the same experimental procedure used to create HA, pure CaO was employed as a control. The XRD pattern suggests that both the microwave irradiation and the reflux methods created crystalline HA with properties identical to pure CaO. The size of the HA crystals produced by the reflux and microwave techniques with pure CaO was 37.1 nm and 36.7 nm, respectively. Meanwhile, utilizing the reflux and microwave procedures, the crystal size of HA generated with CaO from snail shells was 36.3 nm and 38.9 nm, respectively. Additionally, adsorption experiments revealed that the generated HA possessed a similar capability. For HA generated by the microwave and reflux techniques, the adsorption isotherms are fitted with Langmuir adsorption isotherms with adsorption capacities of 21 and 23 mg g^{-1}, respectively. Based on the minor differences in parameters between XRD and gas sorption tests, the microwave-assisted approach should be the optimum option to produce HA due to its time-saving procedure [47].

Castro et al. [31] used microwave and hydrothermal procedures to investigate the effect of pH and synthesis time on the HA produced from Chicken Eggshells (CES).

Equal volume precursor solutions containing 0.0279 mol of calcium hydroxide (Ca(OH)$_2$) from CES and 0.0167 mol of phosphoric acid (H$_3$PO$_4$) were used to a starting material. Subsequently, Ca/P was kept at a 1.67 molar ratio, and pH was adjusted to 9 or 11 with ammonium hydroxide (NH$_4$OH) solution (1:1). The microwave irradiation synthesis was performed at 800 W for 1 to 36 minutes. To remove impurities, the results of both techniques were filtered and washed three times with ethanol and once with distilled water. Finally, the solid was oven-dried for 24 hours at 100°C. The results revealed that HA generated at pH 9 produced fewer carbonated particles than HA manufactured at pH 11. The results of this study suggest that crystallinity increased with synthesis time, as demonstrated by the increasing intensity, and decreasing width of the peak (211). The crystal expanded in size as the synthesis time passed. The crystal size of three samples with shorter synthesis durations (HT9-6 h, MW9-1 min, and MW11-1 min) was smaller than that of bone HA crystallites, which are in the range of 20-40 nm. The average particle size of hydrothermal samples with a pH of 9 was 30 nm, while samples with a pH of 11 had an average particle size of 50 nm. At pH 9, the average particle size in microwave-irradiated samples was 40 nm, while at pH 11, the average particle size was 50 nm. The material created in this study has the same nanorod form and particle size range of 20 to 80 nm as bone HA, making it suitable for application as a biomaterial in orthopedic implants.

Vidhya et al. [48] compared HA generated from eggshells, calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$.4H$_2$O), and calcium carbonate (CaCO$_3$) synthesized in a microwave using EDTA as the organic modifier. Correspondingly, it was rinsed with distilled water and desiccated for 5 hours at 110°C in a vacuum oven with the eggshell powder. 14 grams of eggshell powder and 0.14 mm of EDTA solution were combined to create the Ca-EDTA compound. After adding 0.08 M of Disodium phosphate (Na$_2$HPO$_4$) solution slowly while maintaining a pH of 13 with sodium hydroxide (NaOH) solution, the mixture was stirred for 30 minutes. The reaction mixture was then deposited in a microwave oven at 600 W and exposed to microwave radiation for 3 hours. The product was washed thrice with distilled water and dried in a hot oven at 110°C for 5 hours. Comparatively, commercial CaCO$_3$ and Ca(NO$_3$)$_2$.4H$_2$O were used as calcium precursors in the production of HA by the same method described previously. The physical and chemical properties of HA synthesized from eggshells were almost identical to those derived from commercial calcium precursors. The crystal sizes of HA synthesized from eggshell, CaCO$_3$, and Ca(NO$_3$)$_2$.4H$_2$O were 59 nm, 62 nm, and 55 nm, respectively. Energy-dispersive X-ray analysis (EDAX) confirmed the presence of magnesium, crucial for bone metabolism, in HA, derived from eggshells. In terms of cytocompatibility, antibacterial activity, and drug release, HA derived from eggshells had slightly superior characteristics [48].

Kumar et al. [49] used microwave irradiation to turn eggshells into flower-like HA nanostructures. EDTA was used as a chelating agent. Consequently, 1 g of dried eggshell powder was mixed with 0.1 M of EDTA solution to make a Ca-EDTA mixture. The Ca-EDTA complex was then obtained by progressively adding 0.06 M of Na$_2$HPO$_4$ solution while stirring for 30 minutes. The pH of the reaction mixture was raised to 13 by adding NaOH solution.

Subsequently, the reaction mixture was placed in a microwave oven (2.45 GHz, 600 W, LG, India) and microwaved for 10 minutes. The white precipitate was cleaned and dried in a 110°C hot air oven for 5 hours. The XRD examination of the produced powders accorded well with the International Centre for Diffraction Data (ICDD) standard data for HA (JCPDS File No. 09-0432), demonstrating the synthesis of phase-pure HA. Furthermore, SEM analysis reveals that the microwave-irradiated product has a typical nanostructured HA that resembles a flower. The flower-like nanostructure comprises leaf-like flakes that stretch radially from the center and measure 100-200 nm in width and 0.5-1 m in length. The resultant material was identified as Mg-containing b-type carbonated HA, and biological applications appear promising [49].

Goh et al. [50] employed the microwave-assisted wet chemical process to investigate the effect of an initial pH value in the range of 8 to 12 on the HA shape, particle size, and Ca/P ratio in a product generated from calcined eggshells as a calcium biosource and phosphoric acid as a precursor of phosphate anions. The reactants' Ca/P molar ratio was held constant at 1.67. After being monitored, the pH of the reacting mixture was adjusted to a value of 8 using an NH_4OH solution. Following the completion of the reaction, the mixture was microwaved in a microwave oven (Panasonic, 2.45 GHz, 700 W) for 15 minutes. The obtained white precipitate was rinsed with distilled water, filtered, and dried in a microwave oven for 10 minutes. Precipitates with different pH levels (9, 10, 11, and 12) were prepared using similar methods. Among the resulting compounds was the pure HA phase. At pH 8, 9, 10, 11, and 12, the average crystallite size of HA powder is 30.4 nm, 29.5 nm, 22.0 nm, 32.3 nm, and 34.4 nm, respectively. The crystallite size was demonstrated to decrease monotonically as pH increased up to 10. This is thought to be owing to an increase in crystal nucleation rate, resulting in a high solute concentration (supersaturation) at pH 10. At pH 10, needle-like particles with a 10-15 nm diameter and a length greater than 80 nm were formed. Correspondingly, when the initial pH of the reaction mixture was increased to between 11 and 12, more rounder crystals were observed [50].

D. Muthu et al. [51] investigated the possible use of CES as HA precursors. HA was generated in the presence of an organic modifier EDTA at various pH levels and MWP intensities. This research aimed to evaluate the effect of MWP on HA yield as a foundation for scaling up HA production from the laboratory to the pilot scale. The results revealed that a 900 W laboratory-scale microwave treatment yielded equivalent results to a 2.2 kW pilot-scale microwave treatment. The duration of the pilot-scale therapy was discovered to be half that of the pilot scale [51].

Sari et al. [52] studied the effect of processing settings on the structural, spectroscopic, hardness, and toxicity of nHA materials. The wet precipitation followed by microwave irradiation was used to create nHA products from Duck Eggshells (DES) (a bio-based calcium source) and phosphoric acid. The microwave trials were carried out with three distinct power inputs of 40, 200, and 400 W for 35, 40, and 45-minute periods. An XRD confirmed the presence of pure nHA, and it was proved that the high energy released by microwave irradiation, together with a long reaction time, changed calcium oxide (CaO) from eggshell into nHA. Moreover, the intensity of microwave irradiation was proportionate to the increase in crystallite size.

The length of microwave irradiation influenced crystallite size. Longer microwave irradiation tends to diminish crystallite size, while shorter microwave irradiation tends to increase hardness. The nHA crystal diameters were 16.72 nm with microwave irradiation and 17.4 - 20.95 nm without. SEM confirmed the shift toward narrower and smaller particles with increased irradiation power and treatment period and increased hardness with prolonged irradiation. Following incubation, a study of the toxicity of nHA on human embryonic cells indicated high cell viability. In summary, this suggests that microwave-irradiated nHA has the potential to be used as a biomaterial [52].

Ali et al. [53] investigated the synthesis and characterization of HA and Green Hydroxyapatite (GHA) nanorods from eggshells and Melia Dubia. To synthesize pure HA, 0.1 M EDTA is first dissolved in double-distilled water, followed by the addition of $CaCO_3$ from the eggshell to produce the Ca-EDTA complex. Afterwards, 0.06 M Na_2HPO_4 is added drop by drop to the resultant Ca-EDTA composite solution. After 30 minutes of stirring, a clear, transparent reaction mixture is obtained. The pH of the resulting reaction mixture is adjusted to 13 with NaOH solution. The reaction mixture is then placed in a microwave oven (2.45 GHz, 900 W) and subjected to microwave radiation for 15 minutes. The white precipitate is rinsed with double-distilled water and dried at 110°C for 5 hours. To create GHA nanorods from leaf extract, a solution of Melia Dubia Leaf Extract (MDLE) is added to a Ca-EDTA composite solution and stirred continuously for 1 hour. The primed response combination is then placed in a microwave oven at 2.45 GHz and 900 W and exposed to microwave radiation for 0.5 hours. The greenish impetuous is washed with double-distilled water and dried at 110°C for 5 hours. According to the XRD pattern of the synthesized HA and GHA, the phase formed from the result was a hexagonal crystal of pure HA. HA and GHA crystallite sizes are 50 and 53 nm, respectively. The results demonstrated that GHA had a potent antibacterial action against *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, and *E. coli*. Due to their eco-friendliness, non-toxicity, and compatibility with bio, particularly tribological and electronic applications, GHA nanoparticles can be employed for various purposes [53].

Kumar et al. [54] successfully utilized snail shells to produce hydroxyapatite (HA) nanorods by a rapid microwave irradiation technique, employing ethylenediaminetetraacetic acid (EDTA) as a chelating agent. The outcome of this investigation was determined to be B-type carbonated hydroxyapatite (HA) with a hexagonal crystal system characterized by a specific lattice constant and unit cell volume. The use of microwave irradiation promoted the uneven growth of HA crystallites, resulting in the creation of HA nanorods well-suited for orthopedic purposes. This work emphasizes the possibility of using natural materials, such as snail shells, to manufacture hydroxyapatite (HA). This approach can reduce the requirement for expensive calcium reagents and reduce waste disposal fees.

Wu et al. [55] investigated microwave-induced microrod HA bundles utilizing ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) as a chelating agent and oyster shells as a $CaCO_3$ source. The fabrication of microrod HA bundles was intended to accelerate the material's biodegradability.

The optimal conditions for synthesizing microrod HA bundles were determined to be 700 W MWP and a Na_2EDTA-to-oyster-shell-powder ratio of 2/2. Furthermore, the microrod HA bundles disintegrated more easily than the irregular HA aggregates, indicating that the form of the microrod bundles resulted in a faster HA degradation rate. Bioactivity testing revealed that numerous spherical apatite particles formed on the surface of the microrod HA bundles, indicating good bioactivity. Finally, the microrod HA bundles had a high capacity for protein adsorption, and is beneficial for tissue regeneration [55].

Sajahan et al. [56] investigated the microwave method for producing nHA using Chicken Eggshells (CES) and Duck Eggshells (DES) as $CaCO_3$ sources. To dissolve 25 g of CES powder, a 1:3 mixture of hydrochloric acid and distilled water was used. For 30 minutes, a magnetic stirrer was employed to continually mix the liquid. The eggshell residue was then removed via filtering. The eggshell solution received 1850 mL of $(NH_4)_2HPO_4$ solution, which was vigorously agitated. By adding distilled water and 25% liquid ammonia drop by drop, the pH of the combination was regulated between 10 and 11. The mixture was quickly microwaved for 15 minutes in a home oven set to 2.45 GHz (900 W). The MWP (700 W and 900 W) and irradiation time (MWIT) (15, 30, and 45 minutes) were varied to study their effect on the shape of the HA particle. The precipitate was centrifuged thrice with distilled water at 3000 rpm to eliminate any remaining ammonia. Subsequently, it was dried overnight at 100°C. In its as-dried state, the precipitate was crushed to a fine powder. The experiment was repeated exactly with DES. Under the effect of microwave irradiation, nHA was instantly produced in the solution and implicated in crystallographic change. With only 15 minutes of MWIT and the use of chicken and DES, irregular circular nanosized HA powders were successfully synthesized with no secondary phases or impurities. This study generated nHA with particle sizes ranging from 72.48 nm to 76.17 nm. Increased MWIT had an insignificant effect on phase composition. By varying the MWP, HA particles can be produced in various shapes. It has been demonstrated that combining various biowaste products with microwave heating is the quickest and most cost-effective technique to produce HA [56].

Irfan et al. [45] used microwaves to convert fish bones into HA. The fish bones were cleaned with Deionized (DI) water, baked for 2 hours at 100°C, and pulverized into a powder with a mortar and pestle. 1 g of crushed powders (Ca source) was dissolved in 100 mL of 0.1 M EDTA, followed by 0.06 M Na_2HPO_4. The pH of the solution was kept at 13 by adding NaOH and agitating it for 1 hour at 25°C. In a single process, fish bones and mussel shells were microwaved at 750 W for 10 minutes to produce n-HAp. XRD confirms the existence of HA crystallite phases. Moreover, FTIR and Raman's analysis reveal that HA contains hydroxyl and phosphate groups. SEM and EDS examination discovered sub-micron HA agglomerates with Ca/P ratios smaller than 1.67. According to TEM images, the average size of HA nanocrystals in fish bones was 265 nm. The thermal stability of HA samples was demonstrated at temperatures ranging from 400°C to 800°C [45].

Hamzah et al. [57] investigated the extraction of HA from fish scales using microwaves. CaO extracted from Tilapia fish scales was combined with 0.1 M EDTA to produce a 0.1 M Ca-EDTA complex.

Consequently, the Ca-EDTA complex was drop-by-drop combined with 50 mL of 0.06 M Na_2HPO_4 and stirred for 15 minutes. The pH was maintained at 13 in the liquid mélange. Until the mixtures were completely desiccated, microwave heating at 50% power capacity was applied. The precipitate was washed with deionized water to remove any residual Na and EDTA prior to being dried in a vacuum furnace at 80°C for 6 h. Comparatively, commercial HA was analyzed using XRD and FTIR methods. Examining the phase of the XRD pattern revealed that all the main HA peaks were present. The pattern of HA produced by microwaves is identical to that of commercial HA. Weak bands in the 1478 cm^{-1} range suggested the presence of carbon dioxide (CO_2) in the extracted HA. The HA samples from the atmosphere may have absorbed these groups and substituted them for the P_4^{3-} group during the HA production process. The lack of C–H and C–N peaks indicates that EDTA was absent from the produced HA. Nevertheless, the asymmetries in v1 PO_4^{2-} stretching are eliminated [57].

According to the literature review conducted, biological sources such as mussel shells, paddy field mollusk shells, CES, DES, fish bones, fish scales, and oyster shells have been widely used in the microwave irradiation method for producing HA (Fig. 5). However, other biological sources, such as mammalian bones and crab shells, have yet to be thoroughly investigated. Typically, biological sources consist primarily of CaCO3 and trace elements such as Mg, Sr, K, and Na. Biological sources are useful for making HA because they not only make it easy to get rid of biological waste, but they also contain a variety of ingredients that help bone tissue grow and improve HA's overall biological performance. In general, HA synthesized from biological sources exhibits exceptional bioactivity and biocompatibility due to its crystal structure and composition, which are similar to human bone [55].
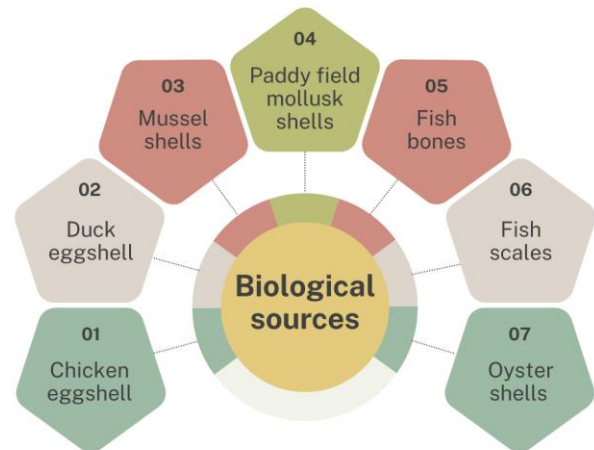

Fig. 5. Biological sources for HA synthesis using microwave irradiation method

Table 1 displays the influence of the type of biological source, the parameters of the microwave setting, and the varying synthesis conditions on the properties of the HA produced. The microwave method for the synthesis of HA from biological sources such as CES, DES, green mussel shells, and paddy field snail shells has application potential in bone tissue engineering [46], [47], [50], [52]. This happens considering that these materials can produce HA with crystal sizes that meet or match bone HA crystallites ranging from 20–40 nm [31].

Table 1 demonstrates that various biological sources can produce high-quality HA under the appropriate microwave setting parameters and synthesis conditions.

TABLE I
THE RELATION OF THE BIOLOGICAL SOURCE, MICROWAVE SETTING PARAMETERS, AND PROPERTIES OF HYDROXYAPATITE

Raw material	The pH of precursor solutions	Power (W)	Duration (minutes)	HA crystallite size[a] (nm) and particle size[b] (nm)	Ref.
Mussel shells	13	750	10	108[b]	[45]
Mussel shells	13	1100	30	30-70[a]	[46]
Paddy field snail shells	-	600	10	38,9[a]	[47]
Chicken eggshells	9	800	1	14.39[a]	[31]
			36	27.56[a]	
	11		1	18.12[a]	
			36	29.20[a]	
Eggshells	13	600	180 (3 hours)	59[a]	[48]
CaCO$_3$				62[a]	
Ca(NO$_3$)$_2$ 4H$_2$O				55[a]	
Eggshells	13	600	10	-	[49]
Eggshells	8	700	15	30.4[a]	[50]
	9			29.5[a]	
	10			22.0[a]	
	11			22.3[a]	
	12			34.4[a]	
Duck eggshells	-	40 200 400	35 40 45	17.4-20.95[a]	[52]
Eggshells				50[a]	[53]
Eggshells with melia dubia	13	900	15	53[a]	
Snail shells	10	900	10	-	[54]
Oyster shells	-	400 700 100	30	43.53[a] 45.09[a] 46.76[a]	[55]
Chicken and Duck eggshell	10-11	700 − 900	15 30 45	72.48 to 76.17[b]	[56]

III. FACTORS AFFECTING THE SYNTHESIS OF HYDROXYAPATITE FROM BIOGENIC RESOURCES USING THE MICROWAVE IRRADIATION

1. The pH of precursor solutions

The synthesis of HA from biogenic sources using the microwave method is affected by a number of variables, including pH, temperature, Microwave irradiation power (MWP), and synthesis time (Fig. 6). Castro et al. [31] synthesized HA from a biogenic calcium source using the Hydrothermal and Microwave Irradiation processes with variable pH values and synthesis times. Other than that, variations in pH and synthesis time affected the morphology of HA, with lower pH values producing more preferable results. HA synthesized at a pH 9 produced fewer carbonated particles than HA synthesized at 11. Moreover, the pH variable is critical in determining the particle size and shape of HA generated from eggshells using a simple wet chemical technique supplemented by microwave irradiation.

Their research produced needle-shaped HA particles with dimensions of 10 to 15 nm in width and 60 to 80 nm in length at a pH of 10. As a result, a change occurred when the pH was raised, resulting in more spherical nanoparticles [50].
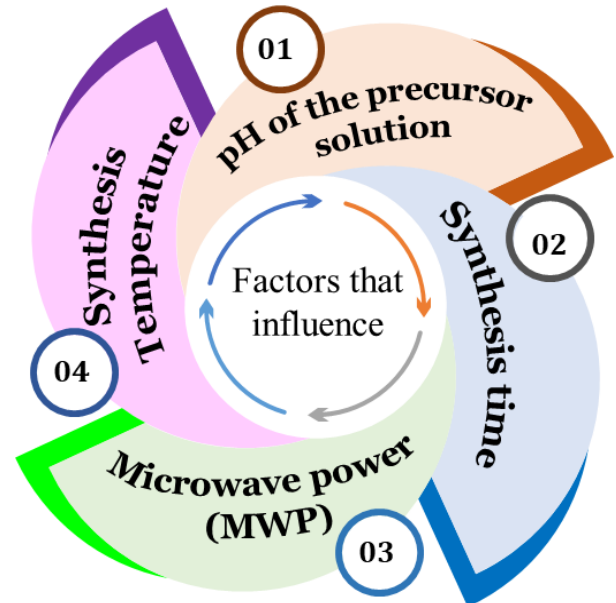

Fig. 6. Factors affecting the synthesis of hydroxyapatite from biogenic resources using the microwave method

Sánchez-Campos et al. [27] studied the effect of pH on HA size, shape, and crystalline structure. The monoclinic phase rose from 85% to 95% as pH increased, while the hexagonal phase declined from 15% to 5%. The pH scale encourages the flow of OH$^-$ and H$^+$ ions, which changes the structure and crystallization of HA particles. The concentration of OH$^-$ ions increases with increasing pH. The higher the concentration of H$^+$ ions, the lower the pH. The size of the crystal decreases as the number of OH- ions increases. Furthermore, as the concentration of H+ ions grows, so does the crystal size [27]. The pH of HA has a substantial effect on the distribution of pore size and total pore volume. The study's findings revealed that reducing the pH from 13 to 8 induced a shift in the pore size distribution. The maximum total pore volume was discovered in samples with a pH of 10.

Additionally, the particle size distribution of the produced HA at various pH values was investigated. The particle size decreases when the pH rises from 8 to 13. The pH 10 sample (HA#2) has the smallest particle size with a diameter of 17 nm, while the pH 13 sample (HA#3) has the largest particle size with a diameter of 14 nm. The particle size of the pH 8 sample (HA#1) is 27 nm. The duration of microwave irradiation treatment also affects particle size, with longer treatment durations resulting in lower particle sizes [58].

Amalie et al. [30] discovered that changes in pH levels substantially impact the crystal size, degree of crystallinity and morphological forms of HA. The XRD characterization demonstrates that when only microwaves are used at pH 11, the particle size is approximately 63.43 nm with a Ca/P ratio of 1.59, which is not substantially different from HA.
At pH 7 and 9, however, chlorapatite is produced. Furthermore, the sintering resulted in particles with a particle size of roughly 180.62 nm and a Ca/P ratio of 1.48.

The study discovered that variations in pH levels result in different particle sizes and morphologies. Particles at pH 11 tend to be irregularly spherical and agglomerated, whereas particles at alkaline pH tend to be circular. High pH leads to HA formation, while pH 7 and 9 lead to chlorapatite formation [30].

2. Microwave irradiation power

Microwave irradiation power (MWP) is another component that could influence HA production. An increase in MWP can alter HA particles' crystal structure and morphology, resulting in faster heating and higher temperatures. Particulars include the fact that increasing MWP resulted in a decrease in HA particle size [30], [58], [59]. Rameshbabu et al. determined that 1100 W MWP and 600 minutes of microwave irradiation were the best conditions for synthesizing nanocrystalline HA. They discovered that increasing microwave exposure duration or power enhances HA crystallinity [60].

According to Singh et al. [28], changes in MWP and MWIT alter the bond length and Ca/P molar ratio of Hydroxyapatite Nano powders (HA NPs). The bond length and Ca/P molar ratio of the NPs increased as the MWP and MWIT parameters were changed. This demonstrates that as the MWP and MWIT parameters were changed, the distance between the constituent atoms of the NPs increased, as did the calcium-to-phosphorus atom ratio. In contrast, as the MWP and MWIT settings were changed, the crystal size, crystallinity, and NPS dropped micro strain. This suggests that the NPs' size, crystallinity, and strain decreased inside their crystal structure. Other than that, the structural and physicochemical properties of HA NPs can also be changed during synthesis by varying the microwave intensity and irradiation period [28].

According to Demirtaş et al. [29], microwave irradiation power influences HA production. Their study used microwave irradiation to create a biomimetic process by precipitating a concentrated bodily fluid (SBF) at different microwave powers (90 W, 360 W, 600 W, and 1200 W) and exposure times. These results demonstrated that MWP affected the size and shape of HA crystals, with greater MWPs yielding smaller and more uniform crystals. This is supported by TEM micrographs of HA samples precipitated by microwave irradiation at various strengths. When the MWP is raised, the HA nanoparticles convert into nanorods instead of a needle-like shape with decreasing length [29].

3. Microwave irradiation duration

The duration of microwave irradiation is another element that can influence HA production. Longer microwave MWITs can result in greater temperatures and faster heating, which can change the crystal structure and morphology of HA particles. Moreover, the period of microwave irradiation influence the crystal size, degree of crystallinity, and morphological forms in the synthesis of HA, according to Amalia et al. [30]. The crystal size of samples subjected to microwaves for varied times ranged from 15 to 21 nm. In comparison to the microwave technique, sintering increases crystal size by a factor of three.

In the synthesis of HA, increasing the microwave MWIT causes an increase in crystal size and crystallinity [30].

Yudin et al. [58] discovered a link between the MWIT treatment and the particle size of HA. Nonetheless, it is worth noting that as the MWIT treatment increases, the specific surface area of HA decreases [58]. Nuha et al. [61] studied the effect of microwave exposure time on HA-alginate composite production. For 10 and 15 minutes, the composite solution was exposed to 360 W of microwave radiation. The crystal index and crystallite size of HA in composites rose as MWIT increased. The maximum crystallite size and crystal index of the HA-alginate composite after 15 minutes of irradiation were 13.196 nm and 0.052 nm, respectively. As a result, increasing MWIT can affect the crystal index and crystallite size of HA in composites [61].

The effects of MWP and exposure period on HA NPs' structural and physicochemical properties were investigated by Sing et al. [28]. According to their research, the microwave parameter selections substantially impacted the structural, crystallographic, and physicochemical features of HA NPs. Increasing MWP and adjusting MWIT parameters have increased the bond length and Ca/P molar ratio while decreasing NPs crystal size, crystallinity, and microstrain. As MWP and MWIT were increased, the HA unit cell shrank along the a-axis and grew along the c-axis. The XRD phase analysis of apatite production was supported by the similarity of the NPs' chemical structures. Given the presence of CO_3^{2-} anions, all NPs formed apatite-like to the bone [28].

Asra et al. [62] examined the influence of microwave irradiation time on Carbonated Hydroxyapatite (CHA) formation from CES. Following the precipitation procedure, samples were irradiated in a microwave for 30, 35, and 40 min at microwave powers of 40, 200, and 400 W. The microwave-assisted approach has many advantages, including quick reaction, easy repeatability, high yield, high purity, effective energy transformation, and volumetric heating. Moreover, effective CHA synthesis is a crucial step in constructing scaffolds for bone tissue engineering. As power and duration grow, so does the average CHA mass [62].

Akram et al. [63] evaluated the effect of microwave time on the phase purity, crystal degree, and surface area of HA as the final product of synthesis. Their study employed the Continuous Microwave Flow Synthesis (CMFS) technology for template-free synthesis of mesoporous HA. The particle morphology changed to a mesoporous needle and rod shape when the reaction mixture was exposed to higher MWP and a longer microwave retention time. Hence, increasing the microwave time boosted crystallinity while lowering surface area [63].

Microwave irradiation induces fast vibration of the particles in the solution. This enhanced mobility enables calcium and phosphate ions to have more frequent and forceful collisions, promoting the development and growth of HA crystals with a uniform structure, resulting in increased crystallinity. Microwaves offer a more consistent and even heating effect than conventional methods. The consistent heating facilitates the reaction involving calcium

and phosphate ions, leading to a more thorough transformation to well-defined HA crystals. Furthermore, prolonged exposure to microwave irradiation leads to accelerated atomic vibrations, resulting in elevated temperatures. Elevated temperatures induce atomic diffusion, facilitating the binding and incorporation of atoms into lattice parameters and enhancing the crystallinity level [30].

4. Microwave irradiation temperature

The temperature of the reaction mixture increased in direct proportion to the increases in MWP, which in turn affected the crystal structure and shape of the HA particles. The synthesis of HA is significantly influenced by microwave irradiation's temperature. A rise in temperature speeds up HA production. The temperature increases boost the production of pure HA, improve crystallinity, and hasten the rate of reaction kinetics, all resulting in the construction of larger and more clearly defined crystalline structures. Additionally, the temperature increase caused the formation of HA particles with a more consistent shape [30], [58], [59].

Siddharthan et al. [59] discovered that forming pure-phase HA would require 24 hours at 25°C but only 5 minutes at 60°C. Therefore, increasing the reaction temperature could significantly reduce the time required to form pure HA. The article also mentions that the crystallite size parallel to the c-axis of the HA structure reaches a maximum at 60°C and that the development of monocrystalline HA along the c-axis is restricted above this temperature, resulting in particles that are more regular and circular [59].

Increased temperatures promote the creation of the most thermodynamically stable phase, which, in this instance, is pure HA. At lower temperatures, calcium phosphate phases and other impurities may form together with hydroxyapatite (HA) [64]. Higher reaction rates, higher product yields, and pure HA as a synthesis product can result from using microwaves, which provide a more efficient heating approach by raising reaction temperatures to levels that can stimulate chemical reactions [65], [66].

Furthermore, increased temperatures can enhance the movement of calcium and phosphate ions in a liquid state. This facilitates more frequent collisions and accelerates the reorganization of ions into the structured crystal lattice of HA. This results in the development of more significant and more clearly defined crystals with enhanced crystallinity. Elevated temperatures generally facilitate crystal growth and pure HA extraction; too high temperatures can have adverse effects. Elevated hydrothermal temperatures can facilitate the formation of undesirable calcium phosphate phases, such as β-TCP and β-DCPA. These contaminants can modify the characteristics of HA and make it inappropriate for specific uses. Furthermore, elevated hydrothermal temperatures can cause high sintering and densification of hydroxyapatite (HA) particles, forming a compact, impermeable material. The reduction in porosity can have a detrimental effect on the bioactivity of hydroxyapatite (HA) and its capacity to integrate with bone [67], [68].

IV. CHALLENGE OF HYDROXYAPATITE SYNTHESIS FROM BIOGENIC RESOURCES BY THE MICROWAVE IRRADIATION

Microwave-assisted extraction is an environmentally beneficial, energy-efficient, and rapid process. Microwave irradiation can heat a small area and speed up the reaction, making hydroxyapatite with better chemical and physical properties, like more surface area, thinner crystals, and better bioactivity [69], [70]. However, the microwave-assisted extraction of hydroxyapatite using biogenic waste sources also presents several challenges that require solutions.

According to Dabrowska et al. [71], microwave reactors are extremely effective for laboratory-scale synthesis; however, scaling up to commercial production presents its own set of challenges. Efficiently optimizing microwave reactors in large-scale synthesis necessitates substantial customization to provide consistent heating and optimize energy use in more significant volumes. Accurate management of reaction conditions, including temperature and pressure, is crucial to prevent uneven heat distribution during synthesis.

Priecel et al.'s [72] research indicated that correctly measuring the temperature within a microwave reactor poses a substantial issue. Microwave reactors can generate localized areas of high temperature, known as hot spots, which are difficult to identify with traditional temperature sensors. These hot spots can result in higher temperatures than those measured, which may impact the reaction's performance and the measurements' reproducibility. Temperature directly influences the rate of chemical reactions. Precise temperature measurement is essential to avoid excessive heat. Excessive heat can cause reactants or products to decompose, releasing toxic gases or causing an explosion.

Hassan et al. showed that the limited ability of microwaves to penetrate large samples might result in uneven heating, with the surface becoming hotter than the core. Insufficient microwave penetration could lead to uneven heating. This can significantly impact the nano-hydroxyapatite particles' dimensions, morphology, and crystal structure [73]. According to Dabrowska et al. [74], an essential challenge in material synthesis through microwave irradiation is the issue of nanoparticles adhering to the reactor's walls. This can lead to erosion and the formation of holes in the reactor layer made of PTFE. Regular cleaning and maintenance can lead to higher production costs. Furthermore, the adhesion and accumulation of materials in the reactor can lead to the formation of hot spots and burns, which may fail the reactor.

As stated by Muthu et al. [51], the production of hydroxyapatite on a large scale is a complex process that presents considerable challenges. They state that to scale up, it is necessary to design larger reactors while still ensuring efficiency. Large-scale experiments necessitate expensive and difficult-to-build specialized microwave reactors. Ensuring consistent heating becomes more challenging as the quantity of material increases, resulting in variations in the quality of the end product. Furthermore, microwave synthesis depends on the material's capacity to efficiently absorb microwave energy.

Fortunately, the material's inability to heat up adequately limits the range of materials it can synthesize. The energy costs of producing larger quantities can be significant, and the need for specialized, high-intensity microwave equipment further escalates the operational costs.

V. CONCLUSIONS

Hydroxyapatite, an important inorganic constituent of bone, provides excellent biocompatibility and bioactivity, rendering it extensively utilized in the biomedical field. Extracting high-quality hydroxyapatite (HA) from biogenic materials such as fish bones, clam shells, chicken eggshells (CES), and oyster shells using microwave irradiation is highly feasible. Microwave irradiation power (MWP), temperature, and irradiation length significantly affect HA nanoparticles' size, shape, crystal structure, and physicochemical properties. The bond length and Ca/P molar ratio positively correlated with the rise in these parameters, while the crystal size, crystallinity, and microstrain showed a negative correlation. The pH level impacts HA's size, shape, and crystal structure. Increasing the pH levels enhances the strength of the monoclinic phase while weakening the hexagonal phase.

Using biological waste as a raw material for microwave-irradiated hydroxyapatite (HA) synthesis aligns with the principles of sustainable development, climate action, and ethical consumption and production. The HA produced is well-suited for biomedical applications because of its exceptional biodegradability, bioactivity, and ability to adsorb proteins. In general, using microwaves to synthesize hydroxyapatite from biogenic materials shows the potential to enhance sustainable manufacturing processes and advance Sustainable Development Goals (SDGs).

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