Biocatalysis and Agricultural Biotechnology 17 (2019) 203–206

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Microwave irradiation mediated synthesis of needle-shaped hydroxyapatite nanoparticles as a flocculant for *Chlorella vulgaris*

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ARTICLE INFO

Keywords:
Hydroxyapatite
Nanoparticles
Interface
Microwave
Harvesting
Flocculant

ABSTRACT

The hydroxyapatite nanoparticles (HANPs) were prepared by microwave assisted co-precipitation method and assessed their efficacy on flocculation of microalga, *Chlorella vulgaris*. The co-precipitation was initiated by mixing the $Ca(OH)_2$ and H_3PO_4 and allowed them to form the HANPs through exposure of microwave irradiation at 10 min (HA1) and 15 min (HA2). In which the 15 min (HA2) exposure showed the better and stable formation of the HANPs. The results showed the both HANPs was found to be needle-shaped and size from 15 to 50 nm evident by TEM analysis and its crystallinity was confirmed by XRD analysis. The addition of 300 mg mL^{-1} of HA2 enhanced the flocculation of *C. vulgaris* with the efficiency of 90% in 60 min. Hence, this work proposed that use of HANPs in flocculation procedures is a more promising and cost-effective method for microalgal biomass harvesting.

1. Introduction

Very recently, the considerable attention has been paid towards the application of bioceramics in the medicinal field due to their potent rectification resistances, better compressive strengths and lower density (Bafana et al., 2018). Nanosized hydroxyapatite (HA) offers a great interest to search a novel synthetic protocol for accomplishing engineered HA (Bose et al., 2010; Kolanthai et al., 2016). In this study, microwave irradiation has been used to synthesize nanosized HA, which usually consumes only a short time and less energy (Jalota et al., 2006). The literature study indicated that microalgae can be used for various biotechnological applications such as the pigments production, antimicrobials, and nanoparticles and as flocculants in wastewater treatment (Davoodbasha et al., 2017; MubarakAli et al., 2012; Baldev et al., 2015; Sharma et al., 2018; Mathimani et al., 2018; LanChi et al., 2018). Algal harvesting is difficult because of the small size of the algal cells (3–30 μm) and the low biomass concentration (0.02–0.6 g L^{-1} dry weight).

Flocculation is a promising approach to reduce the cost of harvesting microalgae, as it allows separation from the medium by simple gravity sedimentation (Uduman et al., 2010; Ummalyma et al., 2017). Flocculation can be induced by metal coagulants such as alum or ferric chloride or by polymeric flocculants. However, the addition of chemicals results in the inhibition of the microalgal biomass and alters the nature of water (Laamanen and Scott, 2017). Therefore, there is arises the need for alternative flocculants, which overcome the aforementioned limitations. In this context, we generated the HANPs by rapid microwave-assisted co-precipitation method and investigated their effect on flocculate microalgae. To the best of our knowledge, this is first report on use of HANPs towards application of flocculation of *Chlorella vulgaris*.

2. Materials and methods

2.1. Chemicals, microalgal culture, and maintenance

Calcium Hydroxide ($Ca(OH)_2$) and Phosphoric acid (H_3PO_4) were purchased from Himedia, Mumbai, India. A microalgal strain *C. vulgaris* was obtained from National Repository for Microalgae and Cyanobacteria – Freshwater (DBT, Govt of India) and maintained in Chu's 10 medium under white photo fluorescent light at 60 μmol m^{-2} s^{-1} with a photoperiod of 12/12 h of light /dark cycles at 24 °C for 15 days. The growth condition and lipid productivity was reported elsewhere (Baldev et al., 2018).

2.2. Synthesis and characterization of HA nanoparticles

Microwave-assisted synthesis was carried out in a sealed glass vessel located inside a microwave oven (20PG3S, IFB, India) at 280 W. Briefly, 1 M $Ca(OH)_2$ was dissolved in 90% H_3PO_4 and pH was adjusted to 9.0 with ammonia under constant mixing. Microwave treatments were done at 10 and 15 min for the preparation.

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https://doi.org/10.1016/j.bcab.2018.11.025
Received 25 October 2018; Received in revised form 26 November 2018; Accepted 27 November 2018
Available online 28 November 2018
1878-8181/

The functional groups present in the synthesized HA were inferred using a Fourier transform infrared spectrophotometer (FTIR: Spectrum Two, Perkin-Elmer, USA). HANPs were mixed with KBr to make the pellet and resolutions were observed at 4 cm^{-1} in the range of 400–4000 cm^{-1}. The crystalline nature of the synthesized HANPs was ascertained by an X-ray diffractometer (XRD: SmartLAB, Rigaku Co. Japan) operated at a voltage 30 keV with Cu Kα radiation in a θ-2θ configuration. The size and shape of the HANPs were observed by transmission electron microscope (TEM, JSM-JEOL, Japan). For this purpose, the sample was placed on the carbon-coated copper grid then observed at the accelerating voltage of 200 kV at various magnifications.

2.3. Flocculation of microalgae by HANPs

The synthesized HANPs were used for flocculation study using the selected microalga, *C. vulgaris*. Briefly, microalgal suspensions were divided into three replicate tubes of 100 mL. The initial algal biomass concentrations in the tubes were determined by reading the optical density values at 600 nm. Flocculent, HA was added at a concentration of 100–500 mg mL^{-1} under constant stirring (140 rpm) in a magnetic stirrer. All the tubes were allowed to stir for 10 min and then, the stirring was decreased slowly. After 30 min, the optical density of the supernatant at half the height of the cleared phase was measured. The effect of the flocculant was assessed through the value of the flocculation efficiency (FE), calculated using Eq. (1).

$$FE(\%) = ODt0 - ODt/ODt0x\,100 \quad (1)$$

OD_{t0} was the turbidity of the sample at time t_0 and OD_t was the turbidity of the sample at time t.

3. Results and discussion

In this work, we successfully synthesized the needle-shaped HANPs with size < 50 nm and distinct crystalline nature were confirmed by TEM and XRD analyses. Similarly the various shapes including spherical, rod, fiber and flower through the hydrothermal, microwave and precipitation methods of HA synthesis (Bose and Saha, 2003; Chen et al., 2011; García-Pérez et al., 2014; Yuan et al., 2011). The HANPs synthesized in this study revealed the highest peak intensity at 31.72° which is similar reported elsewhere (Vidhya et al., 2015). Moreover, the sharpening of the peaks at 21.35°, 25.87°, 27.81°, 31.72° and 38.11° corresponding to the planes (311), (002), (021), (211) and (111) respectively and it representing the crystallinity of HA (Fig. 3a, b). Evidently, these results were agreement with the standard JCPDS Card No. 09–0432; 35–0495 and 04–0783, consistent with the previous report, this phenomenon might be due to the incomplete reaction at low temperature. The FTIR results of HA powder showed characteristic peaks corresponding to stretching vibrations of PO_4^{3-} ions at 1035.03 cm^{-1}, 1405.84 cm^{-1}, 604.09 cm^{-1} and 565.17 cm^{-1}, respectively. The peaks at 565.17 cm^{-1}, were assigned to the deformation of PO_4^{3-} ions. A peak at 1405.84 cm^{-1} designated the existence of carbonate in trace levels. Moreover, the formation of apatite was confirmed by the appearance of a doublet at around 604.09 cm^{-1}–565.17 cm^{-1}, which denoted the bending mode of P–O bonds in phosphate ions. Furthermore, peaks at 1405.84 cm^{-1}, and 1596.23 cm^{-1} were responsible for the stretching mode of CO_3^{2-} (Fig. 1a). Previous results also revealed weak bonding interactions between carbon and oxygen (Venkatesan et al., 2015).

In the study, HANPs exhibited needle-like structures when being subjected to 10 and 15 min of microwave treatment during synthesis. However, HA synthesized using 15 min of microwave treatment (HA2) showed a definite/stable nature than that using 10 min (HA1) as confirmed by XRD spectra (Fig. 1b,c). TEM morphologies of the as-prepared HA 1 and HA2 are shown in Fig. 2(a–f). The synthesized HANPs showed needle-shaped structures at nanoscale levels ranging from 15 to

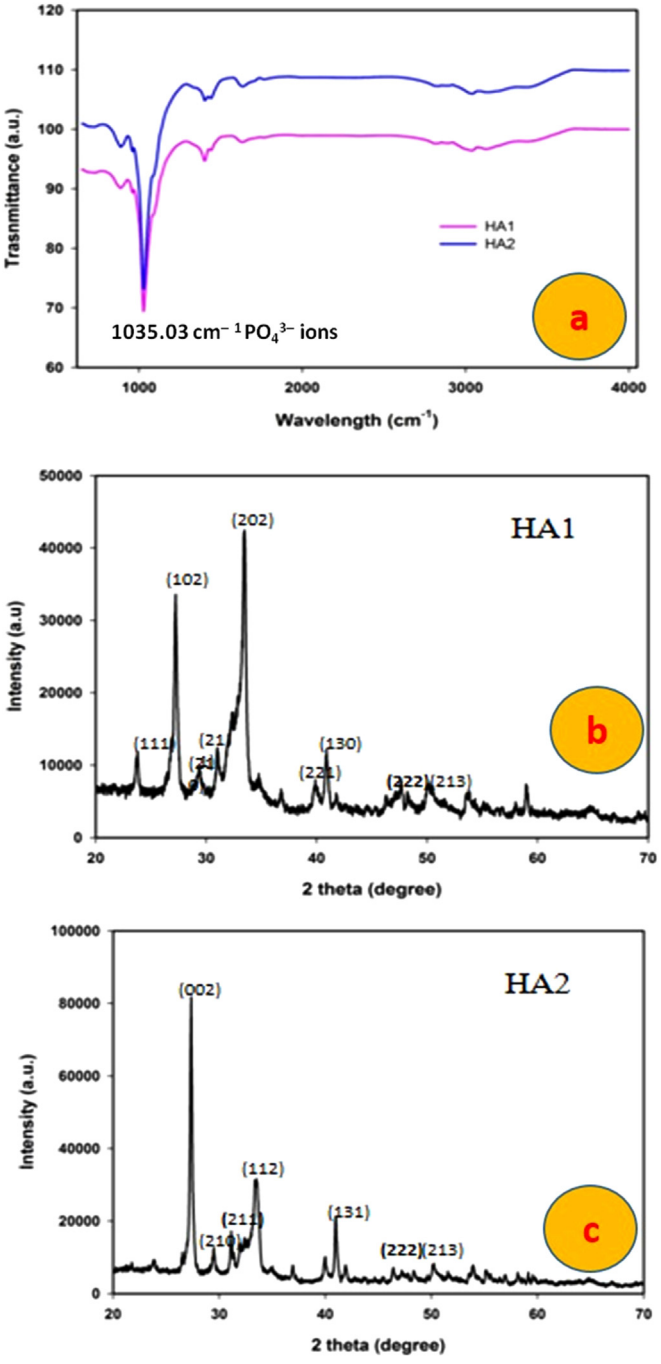

Fig. 1. FTIR results indicating stretching vibrations at 1000 cm^{-1} for HANPs (a); Sharp planes observed at (100), (102), (202) showing the crystallinity of HANPs (b, c).

50 nm. The needles were fine and long with an aspect ratio of 4. HA1 nanoparticles showed more agglomeration than that of HA2. Those results corroborated well with the previous reports on the synthesis of HANPs substituted with silver nanoparticles (Venkateswarlu et al., 2012). A schematic representation of the needle-shaped HANPs is represented in (Fig. 2f).

In the context of algal recovery coupled with wastewater treatment, it is desirable to recover algae at low costs. Chen et al. (2011) suggested that flocculation induced by pH increase is potentially an improvement over other common methods. Most commercial organizations use centrifugation, the traditional method for harvesting microalgae, but it is an energy-intensive process as it consumes a great deal of electric

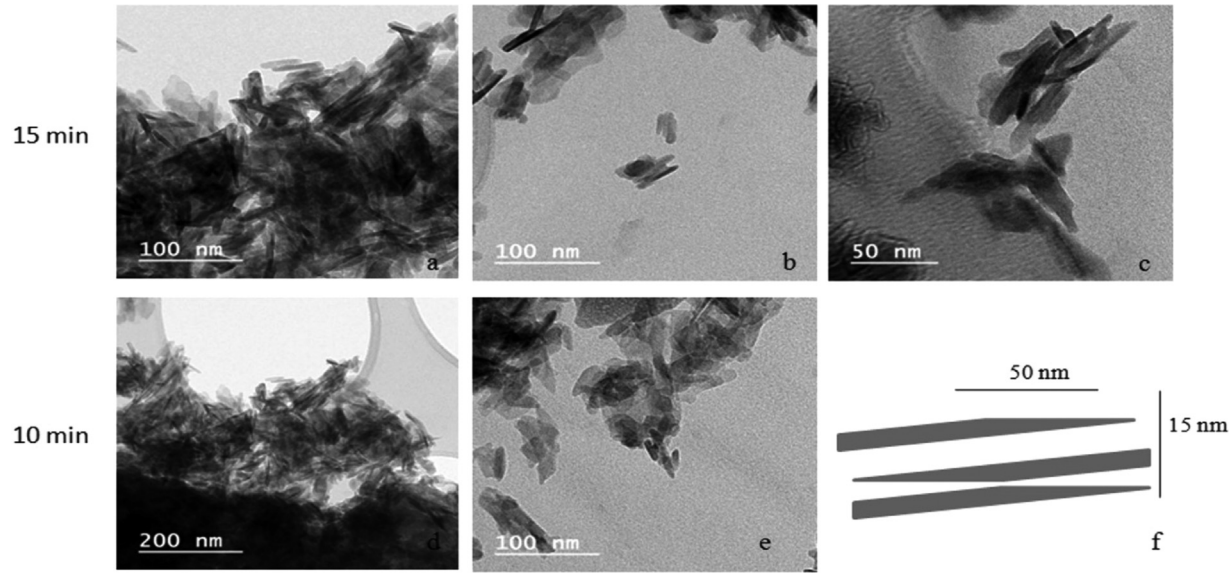

Fig. 2. TEM morphology of HANPs synthesized at 10 and 15 min of exposure to microwave radiation showed needle-shaped nanoparticles of size 15–50 nm (a-e); a schematic representation of HANPs (f).

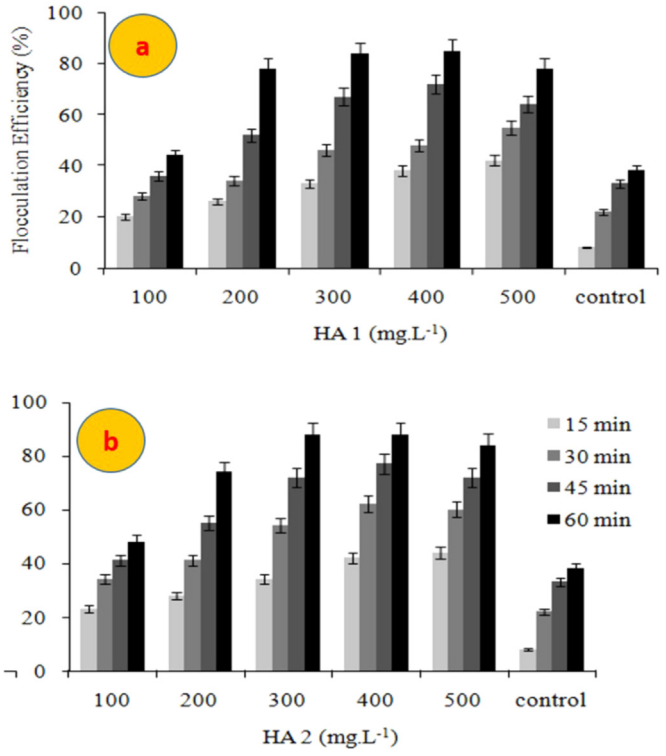

Fig. 3. Flocculation efficacies of HA1 and HA2 nanoparticles (100–500 mg mL^{-1}) for 15, 30, 45 and 60 min showing the increase in flocculation efficiency with the increase in the concentration of the HANPs compared with control (a,b).

power. It was reported that filtration for microalgae harvesting, but membranes are rapid fouled by extracellular organic matter confirmed that microalgae harvested using foam fractionation, but the energy consumption of large-scale harvesting systems is high (Brennan and Owende, 2010). In the present study, it was observed that both HA1 and HA2 could induce flocculation of *Chlorella* sp. at different concentrations. In particular, HA2 at 300 ppm achieved a flocculation efficiency of 90% in 60 min (Fig. 3a,b). It has been reported that the use of cationic polymers has been previously recommended for microalgae flocculation but at doses of 200 ppm, due to the nature of the polymer used cationic polyethylene min (Vandamme et al., 2010).

4. Conclusion

HANPs were successfully synthesized using the microwave-assisted co-precipitation method, which is a fast, cost-effective, and template-free route. The microwave-assisted technique aided in attaining the goal of a greener technology without compromising the efficiency and yield. TEM images revealed different particle sizes in the range from 15 to 50 nm under different reaction parameters. Moreover, HANPs showed promising potentials to flocculate *Chlorella* sp. at different concentrations. However, the flocculation efficiencies decreased considerably with the increase in biomass concentrations. Above all, the flocculated medium could be reused, thereby minimizing the demand for water and reducing the cost of biodiesel production from algae.

Acknowledgement

The author gratefully acknowledged to Dr. N. Thajuddin, National Repository for Microalgae and Cyanobacteria – Freshwater sponsored by Department of Biotechnology (Govt. of India) (BT/PR7005/PBD26/357/2012) for thankfully allowed to perform the experiments.

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