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Biosynthesis and characterization of $CaCO_3$ nanoparticles from the leach solution and the aqueous extract of *Myrtus communis* plant

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ABSTRACT

In this study, the biosynthesis and characterization of $CaCO_3$ nanoparticles from the leach solution and the aqueous extract of *Myrtus communis* plant were carried out. The leach solution obtained by leaching from the leaves and branches of *M. communis* growing around Mersin University Çiftlikköy Campus were analyzed by ICP-MS and it was found to be a calcium accumulator plant. Then, $CaCO_3$ nanoparticles were biosynthesized by adding of the leaf extract, as a biological agent, prepared by the extraction with distilled water of the leaves of same plant to the leach solution under favorable conditions. The characterization of $CaCO_3$ nanoparticles was performed by XRD, EDX, and SEM analyses. XRD, EDX and SEM analysis results showed that the biosynthesized nanoparticles were $CaCO_3$ in nano sizes and porous structures. Besides, the availability of the biosynthesized $CaCO_3$ nanoparticles in the color removal from different dyestuff solutions was investigated; the highest color removal yield was determined to be 90% at the end of 5 min for basic Methylene Blue (MB) dyestuff.

1. Introduction

Nanotechnology has a wide range of applications in many scientific and technological areas, such as electric-electronic, catalytic applications, optical applications, biomedicine, automotive, and chemical industry. It could be seen in the literature that various researches in the area of nanotechnology are related to the synthesis, characterization, and applications of nanoparticles due to their outstanding physical and chemical properties. Nanoparticles can be synthesized by various chemical methods such as electrochemical synthesis, reverse micelle/micro-emulsion method, chemical precipitation, hydrothermal synthesis, sono-chemical precipitation, chemical reduction. However, these methods are expensive and there are environmental and biological risks of the used harmful chemicals such as sodium borohydride, sodium citrate, hydrazine hydrate, dimethylformamide, poly- ethylene glycol and ethylene glycol [1, 2]. In recent years, the nanoparticle synthesis, in which microorganisms, enzymes, and plant extracts are used instead of the harmful chemicals, with simple, clean, non-toxic, inexpensive, and environmentally friendly approach (biosynthesis) has attracted much attention as an alternative method to the chemical methods. In the biosynthesis method, nanoparticles are synthesized by reduction or precipitation from a synthetic metal salt solution containing a certain component, $AgNO_3$, $FeSO_4$, $CaCl_2$ etc., with the aid of several biological agents such as microorganism species, enzymes or plant extracts [3]. In the biosynthesis of nanoparticles, plant extracts are more preferred than microorganisms and enzymes due to their some advantages such as easy harvesting, easy and safe processing, and no need for sterilization [4]. Considering the plant diversity of our country, it is possible to transform the unused environmental friendly source into an economic value by using plant leaves as biological agents in the biosynthesis process. There are various studies in the literature about biosynthesis and characterization of different types of nanoparticles by combining various plant extracts and synthetic solutions containing a certain component [5-8]. The biosynthesis of nanoparticles is possible with the aqueous extracts prepared from the forenamed plant leaves as a biological agent and the leach solutions obtained from a plant which accumulates metals in their frame. The metals could be taken up by plants by many pathways such as soil, water, atmosphere and so on. The plants are classified as accumulator, indicator, and excluder according to the

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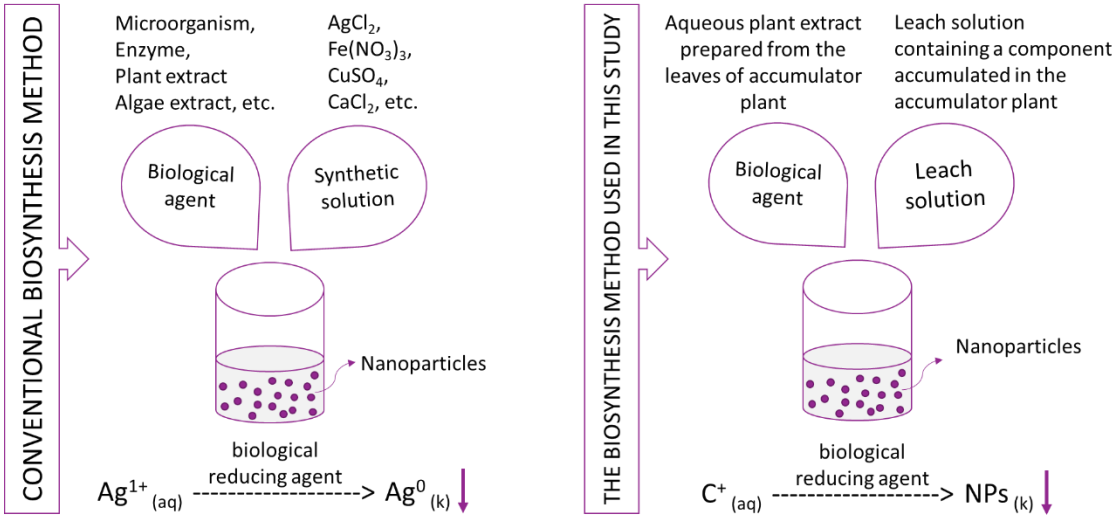
quantities that they can accumulate the metal. On the above basis, the properties the plants are as follows [9]:

- Accumulators store metals mainly in the shoot high and low metal concentration in the soils.
- Indicators grow in some specific environment, allowing an assessment of soil and other conditions in a place by simple observation of vegetation.
- Excluders restrict contaminant uptake into their frame.

In the literature, these accumulators and indicators are usually used for removing or monitoring the metals in environment. The establishment of vegetation on polluted environment helps prevent erosion and metal leaching [10].

In this work, an accumulator plant has been firstly used as both metal source and reducing agent for the metallic nanoparticle synthesis. Accordingly, our study involves the biosynthesis of $CaCO_3$ nanoparticles by combining of the extracts and the leach solutions prepared from leaves and branches of *Myrtus communis*. The comparison of nanoparticle synthesis method by plant extract and metallic salts with the biosynthesis method to be used in this study was illustrated in Scheme 1. In the biosynthesis method to be used in this study, a leach solution prepared from an accumulator plant are used instead of a synthetic metal salt solution (AgCl, $Fe(NO_3)_3$, $CuSO_4$, $CaCl_2$) to combine with the aqueous leaf extract under suitable conditions. It enables to synthesize of the nanoparticle by an environment friendly approach without using harmful chemicals.



Scheme 1. The comparison of conventional biosynthesis method with the biosynthesis method used in this study (C: component accumulated in the plant; NPs: nanoparticles)

2. Materials and Methods

2.1 Chemicals

HCl (37%, Sigma Aldrich), HNO_3 (65%, Riedel-de Haën), ethyl alcohol (≥99.8%, Sigma Aldrich), Procion Red MX-5B (Sigma Aldrich), Acid Red 97 (Sigma Aldrich), and Methylene Blue (Sigma Aldrich) were used in the experiments. All of them were of analytical grade and they were used without further purification.

2.2 Preparation of Leach and Extract Solutions from Myrtus communis

Myrtus communis is from the family of myrtaceae and also is a plant in the form of a bush from the scrub group. The plant, which does not cover its leaves in the winter season and can grow up to 2-5 meters, can be grown in places where Mediterranean climate is experienced, especially in coastal areas. *M. communis* growing around Mersin University Çiftlikköy Campus was used in this study. The leaves of *M. communis* collected for using in the experiments were separated from their branches; after that the leaves and branches were washed separately with firstly tap water and then distilled water to remove impurities. The leaves and branches removed from impurities were dried in the oven at 50 °C for 1.0 day and they were stored in closed vessels in a refrigerator at +4 °C for the further studies.

In the leaching process, the compounds of a solid material are taken into solution by dissolving in the solvents (usually acid or base). In order to determine of the component accumulated in *M. communis*, the leaves and branches, which were purified from impurities and dried, were firstly weighed in certain amounts separately and then they were separately burned in porcelain crucibles in a muffle furnace at 550 °C for 10 h. Ash samples were then cooled and weighed. After that, 5.0 mL of concentrated HNO_3 solution were added to the ash samples and HNO_3 solution was evaporated by using hot-plate until the ash samples dry. The residues were re-dissolved in 5.0 mL of concentrated HCl and diluted to 25 mL by adding distilled water. The contents of the leaching solutions prepared separately from the leaves and branches were analyzed by Agilent 7500ce Octopole Reaction System model Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [11].

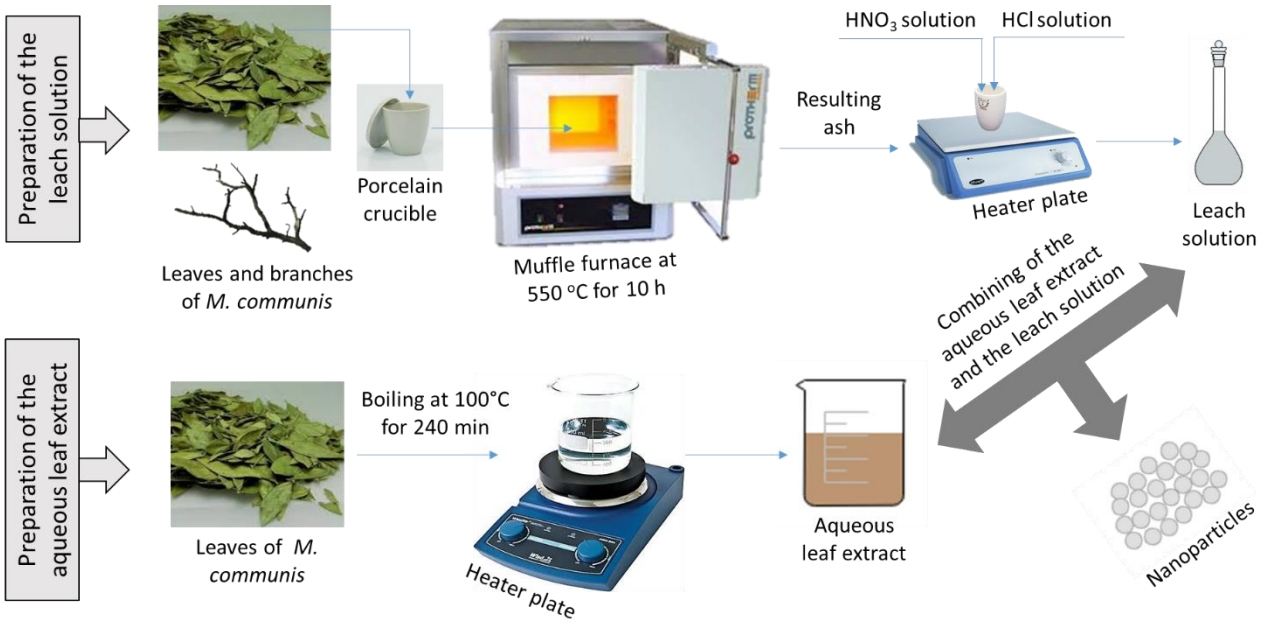
M. communis leaf extract was prepared by boiling 5.0 g of the purified and dried leaves in 100 mL of distilled water at 100°C for 240 min. The extract was centrifuged to remove insoluble fractions and macromolecules. Lastly, the obtained

dark brown extract was stored in the refrigerator for further the synthesis experiments [12].

2.3 Biosynthesis and Characterization of the Nanoparticles

In the nanoparticle biosynthesis experiments, firstly 50 mL of the leaf extract as a biological reductant at room temperature was dropped by syringe pump to 25 mL of the leach solution prepared from the leaves of *M. communis*. The mixture was magnetically stirred for 1.0 h and then it was kept at room temperature for 2.0 days in order to complete the synthesis. The precipitated nanoparticles were separated from the aqueous mixture by a simple decantation method and then they were washed firstly with pure ethyl alcohol, after that they were washed with distilled water until the pH of wash water was neutral. Lastly, the biosynthesized nanoparticles, named MCI, were dried at 60°C in an oven for 24 h and they were then stored in closed vessels in a refrigerator for the further studies [6].

The preparation of leach solution and aqueous leaf extract as well as the biosynthesis of the nanoparticles were illustrated in Scheme 2.



Scheme 2. The preparation of leach solution and aqueous leaf extract as well as the biosynthesis of the nanoparticles

After that, the biosynthesized nanoparticles were characterized by Philips XPert X-Ray Diffraction (XRD), Zeiss/Supra 55 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Analyzer (EDX) analysis methods.

The same procedure was also performed for the biosynthesis and characterization of $CaCO_3$ nanoparticles from the branches of *M. communis* and it was named as MCII.

2.4 Preparing of the Calcined Nanoparticles

The biosynthesized nanoparticles from the leaves (MCI) and branches (MCII) were calcined in a muffle furnace at 600 ° C for 5.0 h and the characterization studies were also carried out by XRD, SEM, and EDX analysis methods. After calcination process, MCI and MCII were denoted as MCIII and MCIV, respectively.

2.5 Decolorisation studies

1.0 g of the dyestuff was dissolved in 1 L of distilled water in order to prepare 1.0 g/L of the stock solutions of Procion Red MX-5B, Acid Red 97, and Methylene Blue. 50 mg/L of the solutions were prepared with required dilutions from the stock solutions. The color removal studies were carried out with three different types of dyestuff that were reactive (Procion Red MX-5B), acidic (Acid Red 97), and basic (Methylene Blue). These studies were carried out in a 250 mL beakers on a shaker running at constant agitation speed and temperature. For this purpose; the experiments were initiated by mixing the desired amount of nanoparticles with the dyestuff solution at a certain initial dye concentration and initial pH value. After that, the samples were taken at predetermined time intervals during the experiment, then the solid and liquid phase were separated by centrifuging at 3000 rev/min for 5.0 min. The color removal was determined with Specord 210 Plus UV-vis spectrophotometer by recording the absorbance at 538 nm for Procion Red MX-5B, 497 nm for Acid Red 97, and 610 nm for Methylene Blue and also by spectral scanning at 200-800 nm [13].

3. Results and Discussions

The results of the synthesis and characterization of $CaCO_3$ nanoparticles [MCI, MCII, MCIII and MCIV] biosynthesized from the leaves and branches of *Myrtus communis* materials as well as the results of decolorisation studies were also presented in following the subsections:

3.1 ICP Analysis of Leach Solutions Prepared from Leaves and Branches of Myrtus communis

The leach solutions prepared separately from leaves and

branches of *M. communis* were analyzed by ICP-MS; the results were presented in Table 1. From Table 1, it was observed that the leach solutions of leaves and branches contained 1456.4 and 1502.5 mg/L calcium, respectively while it was seen that the other components were in relatively low concentrations compared to the calcium. This suggests that *M. communis* can accumulate calcium element in itself higher than the other components. Moreover, the high content of calcium in *M. communis* may be due to the calcareous nature of the soil in which it grows.

Table 1. ICP-MS analysis results of the leaching solutions prepared from the leaves and branches *M. communis*

Element	Leaf sample		Branch sample	
	Liquid base concentration (mg/L)	Solid base concentration (mg/g leaf)*	Liquid base concentration (mg/L)	Solid base concentration (mg/g branch)**
Ca	1456.4	7.28	1502.5	3.97
Na	45.46	0.2273	47.97	0.1411
Al	10.50	0.0525	11.68	0.0344
Fe	8.29	0.0415	9.33	0.0274
B	7.25	0.0363	8.43	0.0248
Mn	4.63	0.0232	5.78	0.0170
Si	4.29	0.0215	5.43	0.0160
Ti	4.03	0.0202	5.21	0.0153
Sr	3.10	0.0155	3.98	0.0117
Ba	1.93	0.00965	2.53	0.00744
Zn	1.27	0.00635	1.89	0.00556
Ni	0.967	0.00484	1.244	0.00366
Cr	0.704	0.00352	0.756	0.00222
Cu	0.671	0.00336	0.709	0.00209
As	0.304	0.00152	0.384	0.00113
Ag	0.291	0.00146	0.312	0.000918
Pb	0.100	0.000500	0.123	0.000362
Mo	0.02604	0.000130	0.02731	0.000080
Co	0.01257	0.000063	0.01352	0.000040

* $1456.4 \frac{\text{mg Ca}}{\text{L}} \cdot \frac{1\ \text{L}}{1000\ \text{mL}} \cdot \frac{25\ \text{mL}}{0.5\ \text{g ash}} \cdot \frac{1\ \text{g ash}}{10\ \text{g leaf}} = 7.28 \frac{\text{mg Ca}}{\text{g leaf}}$

** $1502.5 \frac{\text{mg Ca}}{\text{L}} \cdot \frac{1\ \text{L}}{1000\ \text{mL}} \cdot \frac{25\ \text{mL}}{0.5\ \text{g ash}} \cdot \frac{1\ \text{g ash}}{17\ \text{g branch}} = 3.97 \frac{\text{mg Ca}}{\text{g branch}}$

3.2 XRD Analysis of the Biosynthesized Nanoparticles

The phases and crystal structures of MCI, MCII, MCIII and MCIV were determined by using XRD at 40 kV and 30 mA in the 2θ range of 0-90°. XRD analysis showed that MCI and MCII were amorphous (XRD pattern not shown). Furthermore, XRD patterns of MCIII and MCIV were presented in Figure 1. Accordingly, the calcined nanoparticles showed the same phase and crystal structure. Similarly, Mekprasart et. al. (2015) reported that the all calcined samples have identical patterns in single phase without contaminated peaks [14].

In this work, MCIII and MCIV had the characteristic peaks of hexagonal vaterite $CaCO_3$ phase and the diffraction peaks of it were observed in the planes of (002, 100, 101, 102, 110, 104, 202) [15]. It can be concluded that the calcination process enabled the high crystallinity as reported in many studies in the literature [16-18]. It was also seen in Figure 1 that there were some peaks that did not match with the obtained phase due to some impurities in the nanoparticles.

It can be concluded that the calcination process caused the change of the crystal phases of the nanoparticles from amorphous to hexagonal vaterite $CaCO_3$ phase.

3.3 SEM Analysis of the Biosynthesized Nanoparticles

The morphologies of MCI, MCII, MCIII, and MCIV were examined by SEM; SEM images at different magnification ratios were given in Figure 2. Although Figure 2.a-d showed that the biosynthesized particles were in nanoscale and porous structure, it was also noteworthy that the agglomeration occurred resulting in growth of nanoparticles by leaguing together. Furthermore, it was seen that some lamellar structures, which were the triangular forms for leaf samples and the round forms for branch samples, occurred in SEM images of MCIII and MCIV. This may be due to the change in crystal phase by the calcination process as determined in XRD analysis. Moreover, the sizes of nanoparticles did not change after the calcination process. It was seen that the particle sizes were in the range of 20-50 nm for all of the nanoparticles.

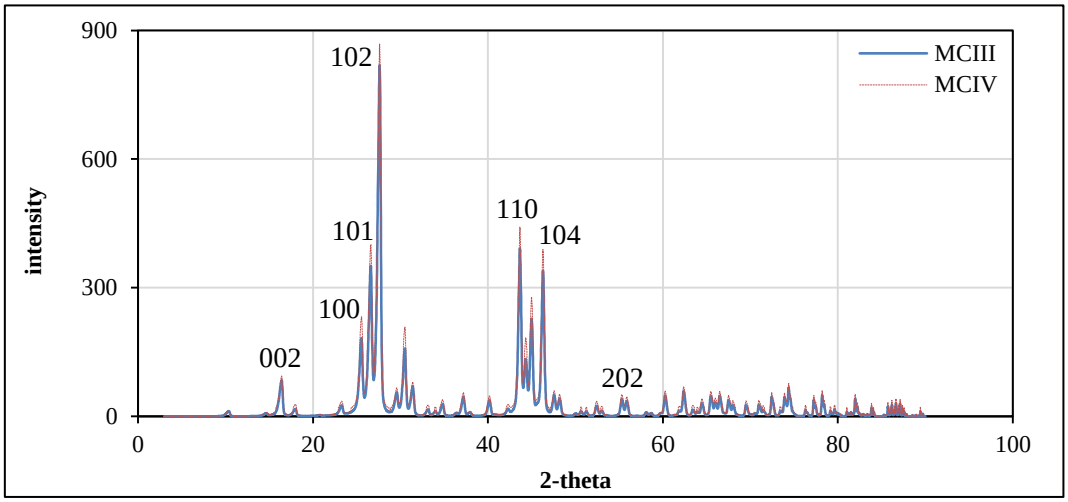


Figure 1. XRD analysis of MCIII and MCIV

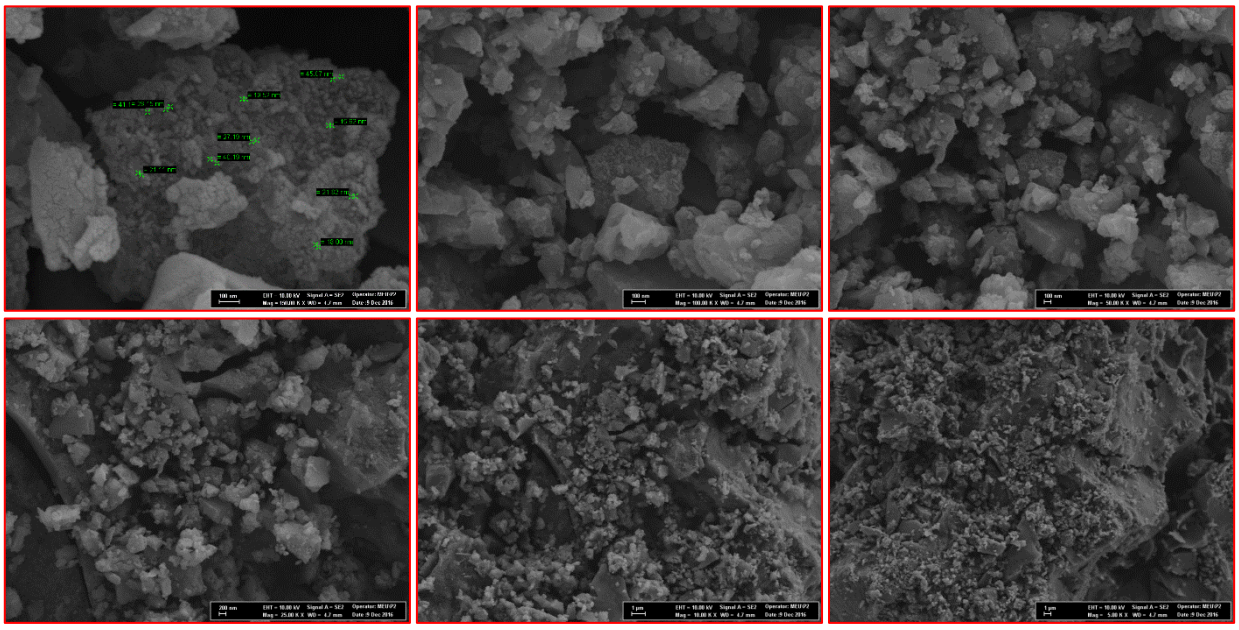


Figure 2.a. SEM images at different magnification ratios of MCI

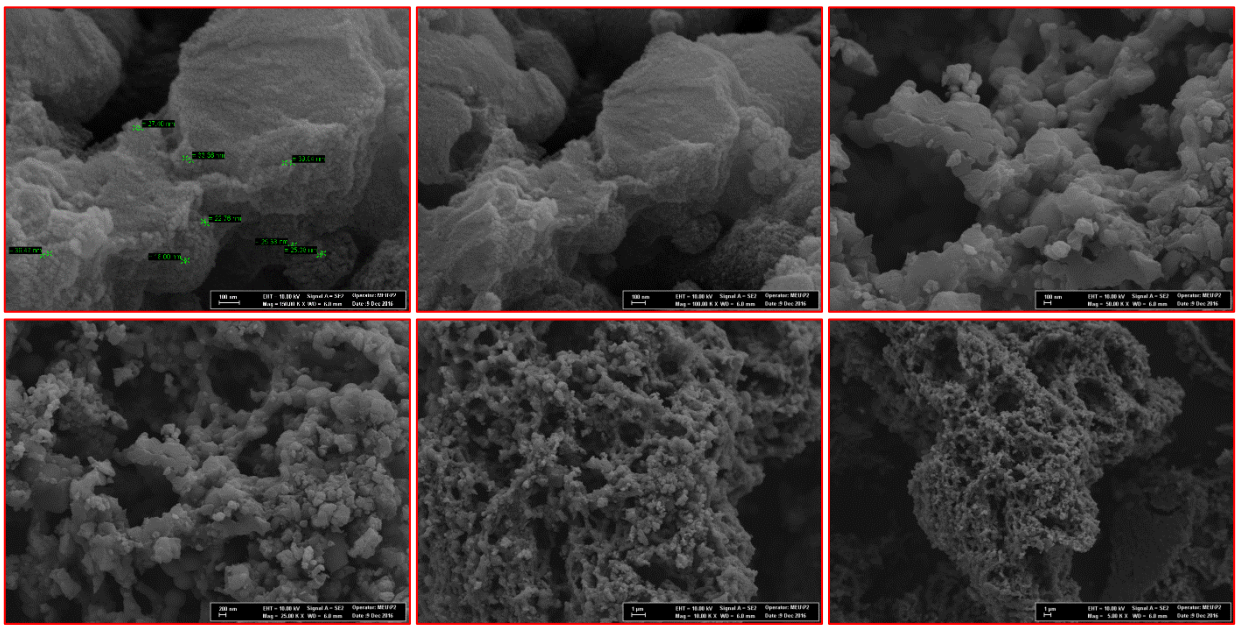


Figure 2.b. SEM images at different magnification ratios of MCII

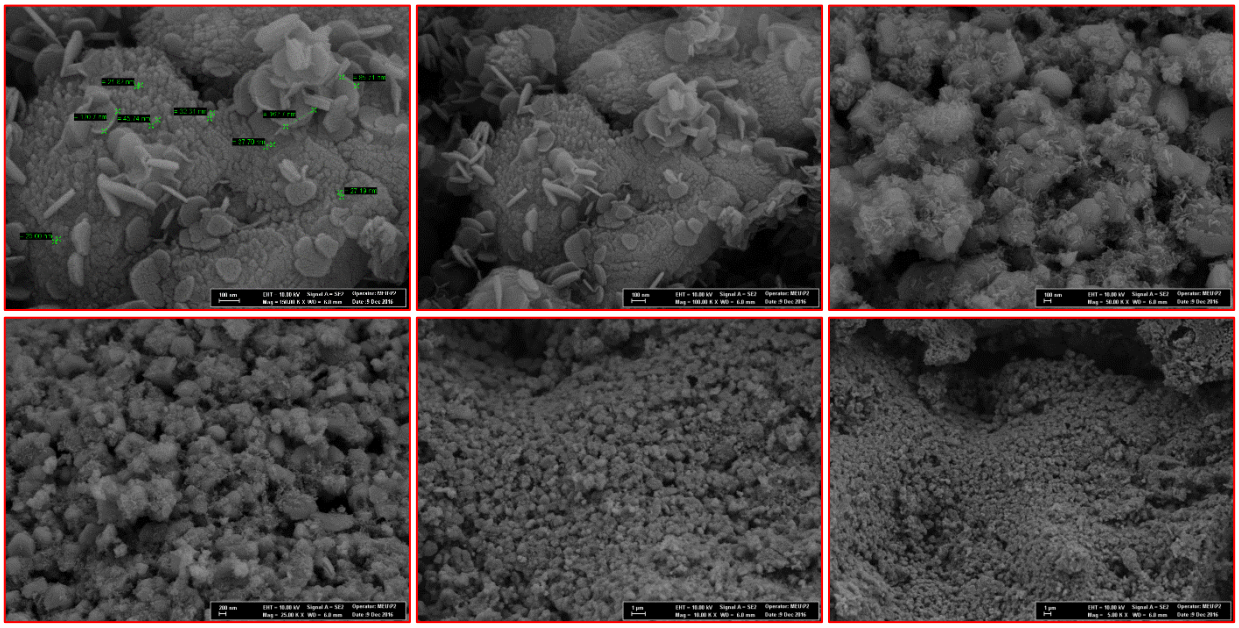


Figure 2.c. SEM images at different magnification ratios of MCIII

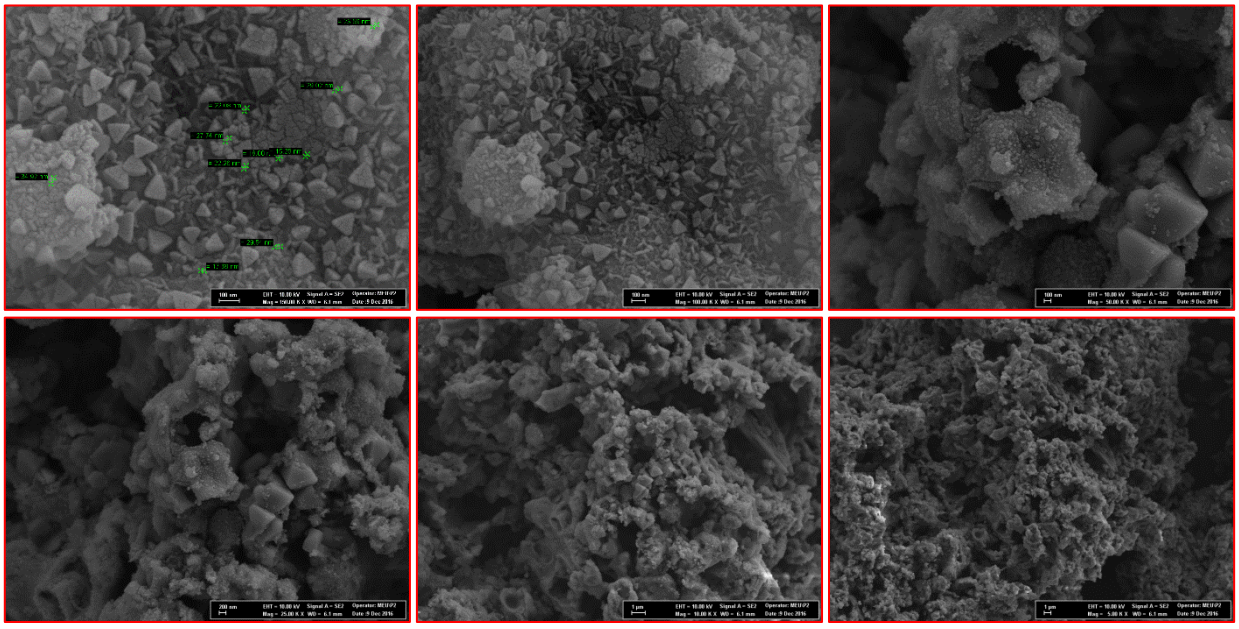


Figure 2d. SEM images at different magnification ratios of MCIV

3.4 EDX Analysis of the Biosynthesized Nanoparticles

The elemental composition of the nanoparticles were determined by EDX. The elemental analysis results of MCI, MCII, MCIII, and MCIV were presented in Table 2. Accordingly, it was obvious that all of the biosynthesized nanoparticles had almost the same elemental composition. Moreover; it could be clearly seen from Table 2 that the theoretical mass and mole percentages of elements in $CaCO_3$ composition and the mass and mole percentages of Ca, C and O elements in the nanoparticles were in agreement with each other, indicating that the biosynthesized nanoparticles were in $CaCO_3$ structure.

Furthermore, the biosynthesized nanoparticles contained trace amounts of Mg, Cl, Fe, Co, and Ni elements compared to Ca, C and O elements due to the some components in the leaves and branches as determined by ICP-MS.

3.5 The Comparison of MCI, MCII, MCII, and MCIV Physical and Chemical Properties

The comparison of the structures, elemental compositions and morphologies of MCI, MCII, MCIII and MCIV was evaluated according to XRD, EDX and SEM analysis results. Accordingly, the similarities and differences were summarized the following below:

- XRD analysis showed that the crystal phase of MCI and MCII altered from amorphous to hexagonal vaterite $CaCO_3$ phase after the calcination process.
- It was obtained by SEM images that the sizes of the all biosynthesized $CaCO_3$ nanoparticles were in the range of 20-50 nm. Moreover, it was observed by SEM

analysis that the morphologies of MCI and MCII changed depending upon the change of the crystal phase by the calcination process whilst the calcination process did not affect the particle sizes.

- It was determined by EDX analysis that all of the biosynthesized $CaCO_3$ nanoparticles had the same elemental composition notwithstanding the particle type. Thus, it can be concluded that the nanoparticles could be biosynthesized in the same elemental composition without the need to separate branches and leaves. In addition, it just can be indicated that it is possible to obtain the leach solution at higher calcium concentration by using the branches of *M. communis*.

Consequently, it could be said that the calcination process did not cause any change in the elemental composition and particle size while it changed the crystal phase and the morphology of the nanoparticles.

After evaluating the chemical and physical properties of the biosynthesized nanoparticles, the usability of the nanoparticles as an adsorbent was tested. It was thought that the color removal capabilities of MCI, MCII, MCIII, and MCIV do not change (it may be possible to change of only removal capacities) since there is not any difference in the elemental compositions and particle sizes of them. For this reason, MCI was chosen as a model adsorbent for the dye decolorisation studies because MCI have some advantages such as being synthesized under mild conditions (no needing of calcination process) and using leaves that are more abundant than branches.

Table 2. EDX analysis results of MCI, MCII, MCIII, and MCIV

Element	**MCI**		**MCII**		**MCII**		**MCIV**		**$CaCO_3$ (theoretical)**	
	Weight %	**Atom %**	**Weight %**	**Atom %**	**Weight %**	**Atom %**	**Weight %**	**Atom %**	**Weight %**	**Atom %**
Ca	39.02	20.03	39.63	19.73	39.89	20.07	40.03	19.98	40	20
C	12.22	19.78	12.41	19.62	12.05	19.66	12.16	19.87	12	20
O	46.93	59.95	46.05	60.03	47.11	59.79	46.87	59.75	48	60
Mg	0.79	0.15	0.88	0.31	0.58	0.29	0.61	0.22	-	-
Cl	0.30	0.05	0.31	0.12	0.19	0.10	0.19	0.09	-	-
Fe	0.28	0.015	0.29	0.09	0.062	0.034	0.048	0.032	-	-
Co	0.26	0.013	0.26	0.06	0.060	0.030	0.047	0.030	-	-
Ni	0.20	0.010	0.24	0.04	0.058	0.026	0.045	0.028	-	-

3.6 Decolorization with MCI

The usability of MCI was investigated for color removal from aqueous solutions containing three different types of dyestuffs, which were reactive (Procion Red MX-5B), acidic (Acid Red 97), and basic (Methylene Blue). The color removal yields for Procion Red MX-5B, Acid Red 97, and Methylene Blue (MB) were observed as 3.35%, 2.70%, and 90%, respectively. According to these decolorisation yields, the decolorisation results of MB dyestuff by MCI was evaluated in this section. The time-varying UV-vis spectra of MB dyestuff solution were presented in Figure 3. Accordingly, the characteristic peaks of MB were observed at 665, 610, and 292 nm; and the intensities of these characteristic peaks decreased over time in the presence of MCI. In addition, as a result of the overlapped peaks belonging to 5.0 and 60 min, the optimum contact time was determined to be 5.0 min. Consequently, the color removal efficiency for 50 mg/L of initial MB concentration was determined to be 90% in 5 min.

The application areas of $CaCO_3$ nanoparticles in the literature were presented in Table 3. Accordingly; it can be seen that there are many studies in the literature where $CaCO_3$ nanoparticles synthesized by different methods have been used as additives and antibacterial agents; however it is the first time to evaluate biosynthesized $CaCO_3$ nanoparticles for color removal from aqueous solution.

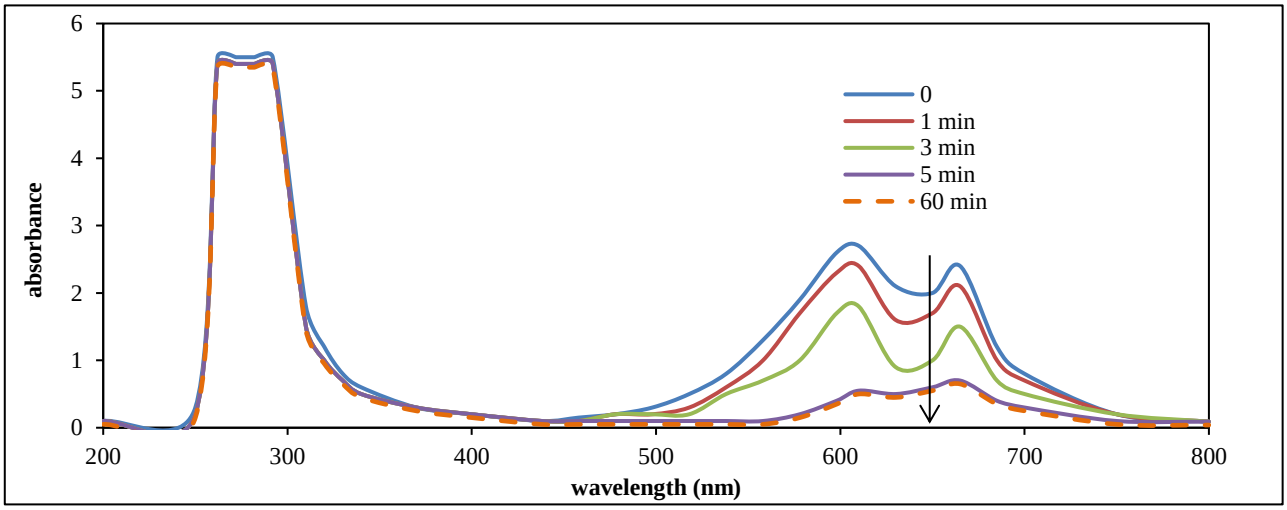


Figure 3. UV-vis spectra of MB dyestuff solution (experimental conditions: initial pH: 9.0, initial MB concentration: 50 mg/L, temperature: 30 °C, MCI concentration: 0.5 g/L)

Table 3. The studies with $CaCO_3$ nanoparticles in the literature

Nanoparticle	Synthesis method	Particle size	Application area	Reference
$CaCO_3$/Ag nanocomposite	Reduction with PPG	5-20 nm	Antibacterial agent against *Escherichia coli*	[19]
$CaCO_3$ nanoparticles	Reverse micro-emulsion method	90 nm	Antibacterial agent against *Staphylococcus aureus*	[20]
$CaCO_3$ nanoparticles	Wet carbonation	40 nm	Additive for epoxy resin	[21]
$CaCO_3$ nanoparticles	Commercial	50-120 nm	Additive in accelerating the hydration of Portland cement	[22]
$CaCO_3$ nanoparticles	Wet carbonation	< 100 nm	Paper coating for the hydrophobic character of the paper surface	[23]
$CaCO_3$ nanoparticles	Biosynthesis	< 100 nm	Color removal from aqueous Methylene Blue dyestuff solution	This work

4. Conclusion

This study has demonstrated that $CaCO_3$ nanoparticles, which are frequently used in nanotechnology applications, could be successfully synthesized with a completely environmental-friendly approach (without using harmful chemicals to the environment and human health) by using only the solutions, which are the leach and aqueous extract solutions, prepared from a bioaccumulator plant. Furthermore, this study has also showed that the biosynthesized $CaCO_3$ nanoparticles could be effectively used for color removal from aqueous solutions containing basic dyestuffs.

Acknowledgment

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