

Research Article

Potential of Biosynthesized Silver Nanoparticles as Nanocatalyst for Enhanced Degradation of Cellulose by Cellulase

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Received 9 January 2015; Revised 13 February 2015; Accepted 13 February 2015

Academic Editor: Zhenhui Kang

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Silver nanoparticles (AgNPs) as a result of their excellent optical and electronic properties are promising catalytic materials for various applications. In this study, we demonstrate a novel approach for enhanced degradation of cellulose using biosynthesized AgNPs in an enzyme catalyzed reaction of cellulose hydrolysis by cellulase. AgNPs were synthesized through reduction of silver nitrate by extracts of five medicinal plants (*Mentha arvensis* var. *piperascens*, *Buddleja officinalis* Maximowicz, *Epimedium koreanum* Nakai, *Artemisia messer-schmidtiana* Besser, and *Magnolia kobus*). An increase of around twofold in reducing sugar formation confirmed the catalytic activity of AgNPs as nanocatalyst. The present study suggests that immobilization of the enzyme onto the surface of the AgNPs can be useful strategy for enhanced degradation of cellulose, which can be utilized for diverse industrial applications.

1. Introduction

Silver nanoparticles (AgNPs) have been extensively used in many commercial products. They are important components for research on biomedicine, optics, electronics, magnetism, mechanics, catalysis, energy science, and so on [1, 2]. The processes of biological synthesis of AgNPs are simple, inexpensive, and environmentally benign and do not use high amount of energy and sophisticated instruments in the synthesis protocols unlike physicochemical methods [2]. Use of ecofriendly reducing and capping agents in biological synthesis route makes the synthesized AgNPs as less-toxic, biocompatible, and useful for biomedical applications [2–5]. Plant extracts have been demonstrated to be promising reducing and capping agents for biosynthesis of variety of metal nanoparticles such as silver, gold, and copper nanoparticles [6–8].

Efficiency of electron transfer of AgNPs is better than gold nanoparticles in biosensors. The metal nanoparticle and biomolecule interactions have received increasing attention

in recent years for the development of diagnostics, sensors, and targeted drug delivery. Immobilization of glucose oxidase on AgNPs was demonstrated to increase sensitivity of glucose biosensors by three times [9]. Many organic syntheses of complex molecules utilize nanoparticles for catalysis based on their exceptional catalytic activities and selectivity to materials. The size and shape of nanoparticles, preparation conditions, addition of support materials, and the capping agent play critical role in catalysis [10].

Cellulose is the most abundant organic polymer on Earth [11]. Cellulose gives structural integrity to the primary cell wall of green plants, many forms of algae, and the oomycetes. Cellulose can be broken down through hydrolysis to smaller polysaccharides like cellodextrins or completely into glucose units. However, hydrolysis of cellulose is relatively difficult compared to other polysaccharides because cellulose molecules bind strongly to each other. Enzymes are biological catalysts that speed up reactions in the presence or absence of cofactors without any change in their activity. The enzyme cellulase performs hydrolysis of cellulose. Cellulases

are chiefly produced by fungi, bacteria, and protozoa, which hydrolyze 1,4- β -D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal β -D-glucans. This process has commercial applicability in food processing, textile industries, laundry detergents, pulp and paper industries, and pharmaceutical industries. Cellulases also have promise in the fermentation of cellulosic biomass into biofuels.

Metal nanoparticles have been demonstrated as beneficial catalysts in industry. Nickel nanoparticles were used as a catalyst for the chemoselective oxidative coupling of thiols to disulfides [12]. Polyvinylpyrrolidone- (PVP-) capped silver nanoparticles were used for the oxidative coupling of *n*-dodecanethiol, *n*-butanethiol, and *n*-octanethiol to their corresponding disulfides [13]. High yield and turnover number were reported after the use of silica-supported AgNPs as solid and recyclable catalysts for Diels–Alder cycloadditions of 2'-hydroxychalcones and dienes [14]. Starch hydrolysis using amylase was enhanced after the use of AgNPs as catalysts [15]. The reduction of dyes by sodium borohydride occurred due to the catalytic properties of AgNPs supported on silica spheres [16].

The present study aims to investigate potential of Korean traditional medicinal plants for the synthesis of AgNPs and utility of plant synthesized AgNPs in enzyme-catalyzed cellulose hydrolysis. The nanoparticle-bimolecular interaction could be potentially useful in diverse industrial applications.

2. Materials and Methods

2.1. Chemicals and Reagents. Cellulase (Viscozyme, Sigma, USA), carboxymethyl cellulose (CMC, Sigma, USA), silver nitrate (Samchun pure chemicals, Republic of Korea), and all other chemicals were of high purity. Ultra-filtered Millipore water (Millipore, USA) was used for all the experiments.

2.2. Preparation of Extracts from Medicinal Plants. Medicinal plant materials (*Mentha arvensis* var. *piperascens*, *Buddleja officinalis* Maximowicz, *Epimedium koreanum* Nakai, *Artemisia messer-schmidtiana* Besser, and *Magnolia kobus*) were purchased from local medicinal plant market of Cheongju, Korea, and dried for 2 days at room temperature. The plant extracts were prepared by boiling 10 g of each plant material in 200 mL of sterile distilled water for 15 minutes. The extracts were filtered through vacuum filtration assembly using Whatman number 1 filter paper. The filtered plant extracts were immediately used for the synthesis of AgNPs.

2.3. Synthesis and Time Course Studies of Silver Nanoparticles. Typically, 25 mL of leaf extract was added to 475 mL of 2 mM aqueous precursor AgNO₃ solution for reduction of Ag⁺ in an Erlenmeyer flask for a reaction. The reaction was allowed to occur at temperature of 55°C in shaking incubator at 175 rpm for 24 hours. The reduction of pure Ag⁺ was monitored by measuring the UV-visible spectrum between 200 and 800 nm wavelengths of the reaction medium using UV-visible spectrophotometer (UV-1601, Shimadzu, Japan). Time courses of synthesis of AgNPs by plant extracts were monitored by taking absorption spectra at absorption

maxima of AgNPs synthesized by each plant extract in UV-visible spectrophotometer.

2.4. Characterization of Silver Nanoparticles. The AgNPs synthesized by plants were purified by repeated centrifugation at 15,000 rpm for 20 minutes followed by redispersion of the pellet in deionized water. The purified dried powder of silver nanoparticles was further analyzed by electron dispersive X-ray spectroscopy (EDS, Philips XL-30). Morphology of the silver nanoparticles was characterized by scanning electron microscopy (SEM, Philip model CM 200) and X-ray photoelectron spectroscopy (XPS) using an ESCALAB 210 with an Al X-ray source (1486.6 eV) and analyzer. Transmission electron microscopy (TEM) micrographs were obtained using energy filtering transmission electron microscope (JEM-2100 F, HR, Jeol Ltd.) operating at 200 kV.

2.5. Quantification of Reducing Sugars upon Degradation of Cellulose by Cellulase. Tests were performed in tubes containing CMC as a substrate and plant synthesized AgNPs. Free CMC solution without AgNPs was used as control. Fungal β -glucanase units ≥ 100 (~1.2 g/mL at 25°C) of cellulase (Viscozyme, Sigma, USA) were used to hydrolyze cellulose. CMC (10 mg/mL) was added in separate tubes containing 5 mL distilled water and autoclaved at 121°C for 15 minutes. The tubes were cooled to bring temperature of the solutions at 25°C. To these separate tubes, different plant synthesized nanoparticle solutions (5 mL) were added and 500 μ L Viscozyme was added to each tube. The reactions were carried out at 25°C in shaking incubator and formation of sugar (glucose) was monitored in microtiter plates at 500 nm using glucose assay kit (Bio Clinical System Co., Ansan, Republic of Korea). The amount of glucose generated was estimated from standard graph of glucose.

3. Results and Discussion

The solution of AgNO₃ after addition of plant extracts turned pale yellow and then to brown. This colour change indicated the synthesis of AgNPs (Figure 1(a)). The plasmon peaks for the AgNPs synthesized by *M. arvensis*, *B. officinalis*, *E. koreanum*, *A. messer-schmidtiana*, and *M. kobus* were observed at 450, 434, 438, 421, and 416 nm, respectively. The occurrence of plasmon peaks between 400 and 500 nm confirmed the presence of AgNPs as reported earlier [4–7]. The formation of AgNPs was increased with increase in incubation time. *E. koreanum* showed rapid synthesis of AgNPs among different plant extracts (Figure 1(b)).

EDS measurement showed presence of Ag in all the samples of AgNPs synthesized by plants (Figure 2(a)). The highest Ag content in terms of weight % and atom % was found for *M. kobus* and lowest for *M. arvensis* (Table 1). The Ag 3d_{5/2} and Ag 3d_{3/2} core level binding energies for AgNP samples in XPS appeared at 368 and 374 eV, suggesting presence of metallic silver in the samples. A representative EDS and XPS image for AgNPs is presented in Figure 2(b).

SEM micrographs showed presence of nanoparticles in all the samples (Figure 3). The AgNPs were monodispersed in nature. The size of the particles from TEM image ranged

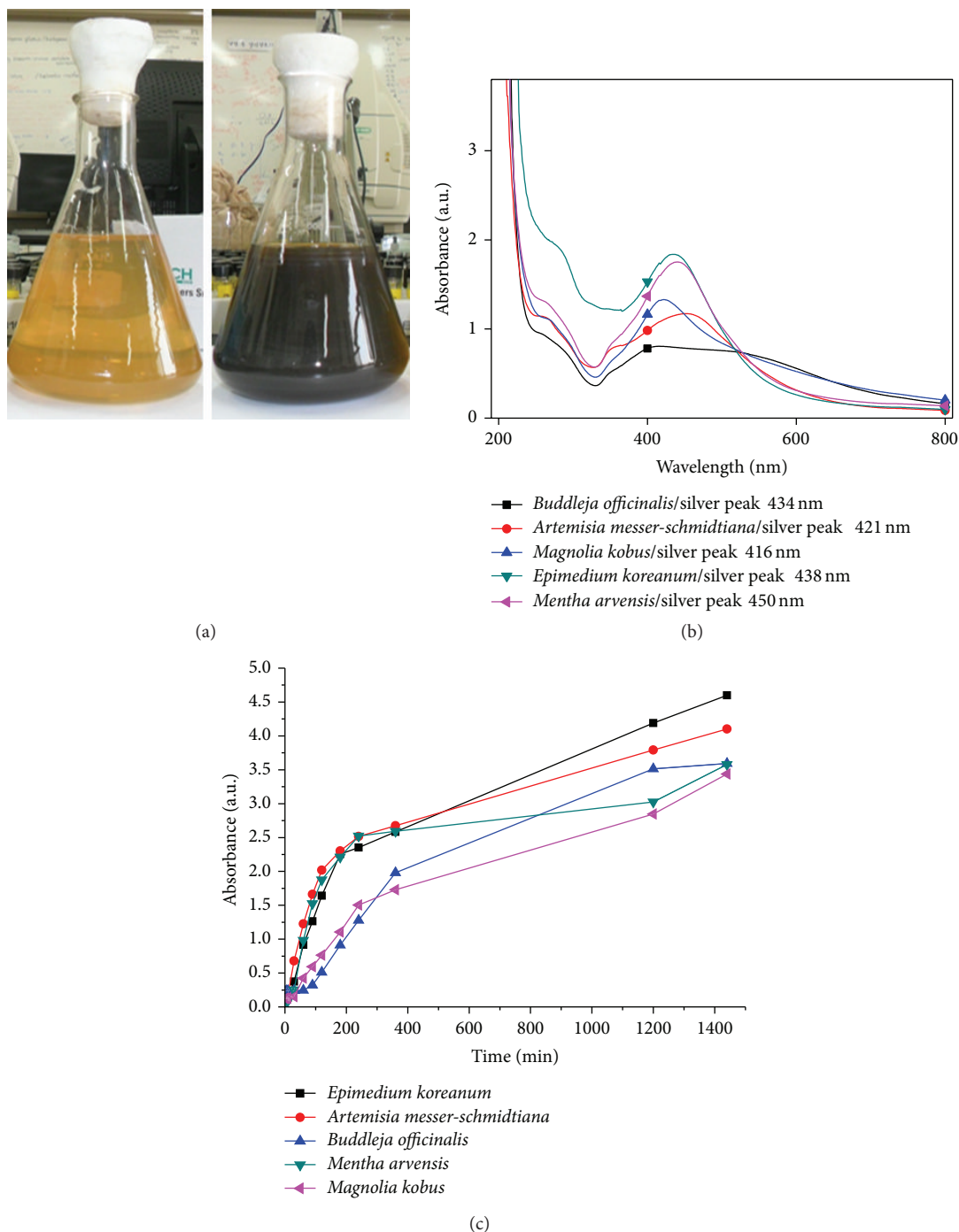


FIGURE 1: Synthesis of AgNPs by medicinal plant extracts by addition in a silver nitrate at 55°C. (a) Flasks at left and right show reaction of 10 minutes and 24 hours after addition of plant extracts in AgNO_3 , (b) UV-visible spectra, and (c) time course of the synthesis of AgNPs by five plant extracts.

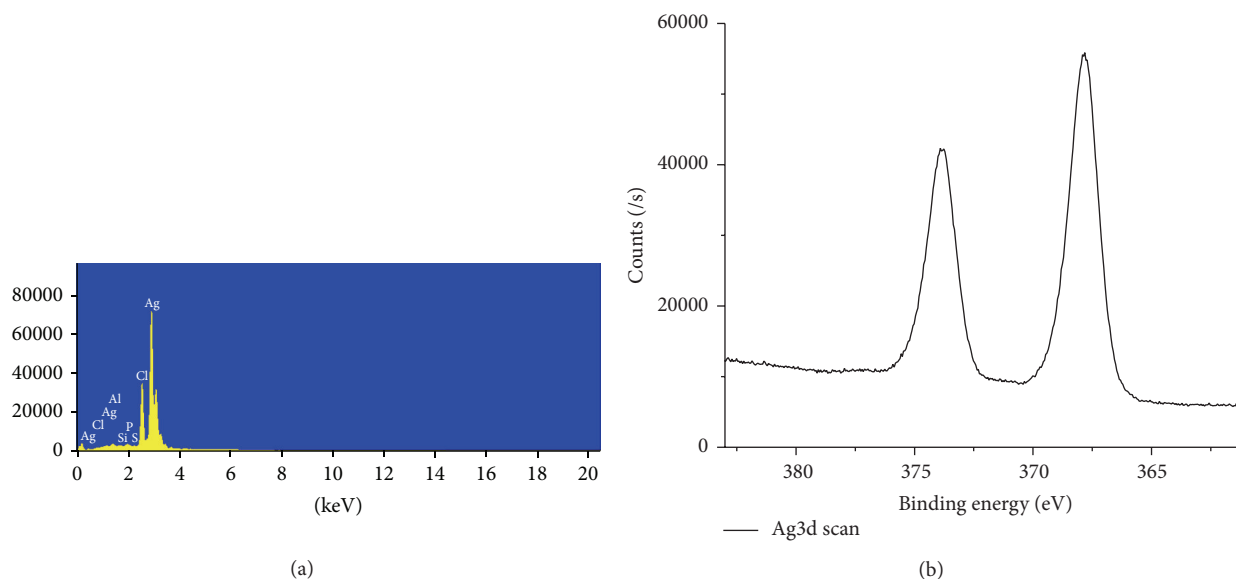
between 5 and 60 nm (Figure 4). The mean diameter of the synthesized AgNPs was found to be different for different plants (Figure 5). The average particle sizes for AgNPs synthesized by *M. arvensis*, *B. officinalis*, *E. koreanum*, *A. messer-schmidtiana*, and *M. kobus* were found to be 40.0, 32.4, 39.7, 29.2, and 24.7 nm, respectively. The largest particle

size was observed for *M. arvensis* and smallest for *M. kobus* (Figures 4 and 5). The resulting nanoparticle solutions were found to be stable for more than two months without agglomeration of particles.

A higher amount of glucose was produced in the presence of AgNPs in comparison with free cellulose (control).

TABLE 1: Elemental composition in energy dispersive X-ray spectroscopy (EDS).

Sample	Element content (weight %)										
	Al	Si	P	S	Cl	Ag	Pt	O	C	Zr	Mo
<i>Mentha arvensis</i>	0.39	0.8	0.21	0.0	12.4	87.28	—	—	—	—	—
<i>Buddleja officinalis</i>	0.61	0.11	—	0.08	4.09	94.15	0.97	—	—	—	—
<i>Epimedium koreanum</i>	0.63	0.06	—	—	7.23	90.21	1.14	0.72	—	—	—
<i>Artemisia messer-schmidtiana</i>	0.48	—	—	—	2.33	94.97	0.91	—	1.30	—	—
<i>Magnolia kobus</i>	0.45	—	—	—	0.50	97.87	—	—	—	088	0.30
Sample	Element content (atom %)										
	Al	Si	P	S	Cl	Ag	Pt	O	C	Zr	Mo
<i>Mentha arvensis</i>	1.24	0.23	0.58	0.01	28.95	68.98	—	—	—	—	—
<i>Buddleja officinalis</i>	2.20	0.38	—	0.23	11.30	85.41	0.49	—	—	—	—
<i>Epimedium koreanum</i>	2.08	0.20	—	—	18.27	74.88	0.52	4.05	—	—	—
<i>Artemisia messer-schmidtiana</i>	1.67	—	—	—	6.10	81.73	0.43	—	10.07	—	—
<i>Magnolia kobus</i>	1.77	—	—	—	1.47	95.42	—	—	—	1.01	0.33

FIGURE 2: A representative (a) energy dispersive X-ray spectroscopy (EDS) and (b) X-ray photoelectron spectroscopy (XPS) image of the AgNPs synthesized by medicinal plant extracts of *Mentha arvensis* var. *piperascens*.

The hydrolysis efficiency of AgNPs showed the following order: *M. kobus* > *A. messer-schmidtiana* > *B. officinalis* > *E. koreanum* > *M. arvensis* (Figure 6). The reducing sugar formation increased by about twofold for *M. kobus* over control, suggesting a significant role of nanoparticles in acting as nanocatalyst for enhanced degradation of cellulose to reducing sugars. The rate of reaction was found to be increased in the presence of AgNPs and is depicted in Figure 6. The results of reducing sugar assay suggested an increased enzyme activity in cellulose hydrolysis.

The exact mechanism behind the enhancement of cellulose hydrolysis by AgNPs is not clear at this juncture but AgNPs may be acting as catalyst by some unknown mechanism, which needs further detailed investigation. Interestingly, plant synthesized AgNPs of smaller size displayed

better catalytic activity than larger sized AgNPs. AgNPs with smaller size have been known to display better bioactivities than larger particles [2]. There may be different possibilities for the faster reaction rate of cellulose hydrolysis in the presence of AgNPs. One of the possible scenarios can be explained as follows. Plant metabolites reduce silver nitrate to AgNPs. The interaction of cellulase with cellulose and plant synthesized AgNPs leads to the immobilization of cellulase on the surface of the AgNPs and degradation of cellulose by the enzyme into reducing sugars. AgNPs generally have the tendency to agglomerate faster in any biological medium and sediment at the bottom. However, such agglomeration was not observed during the reaction, indicating that the AgNPs have the chances of being stabilized by the protein molecule through the thiol linkages, and thus the enzyme

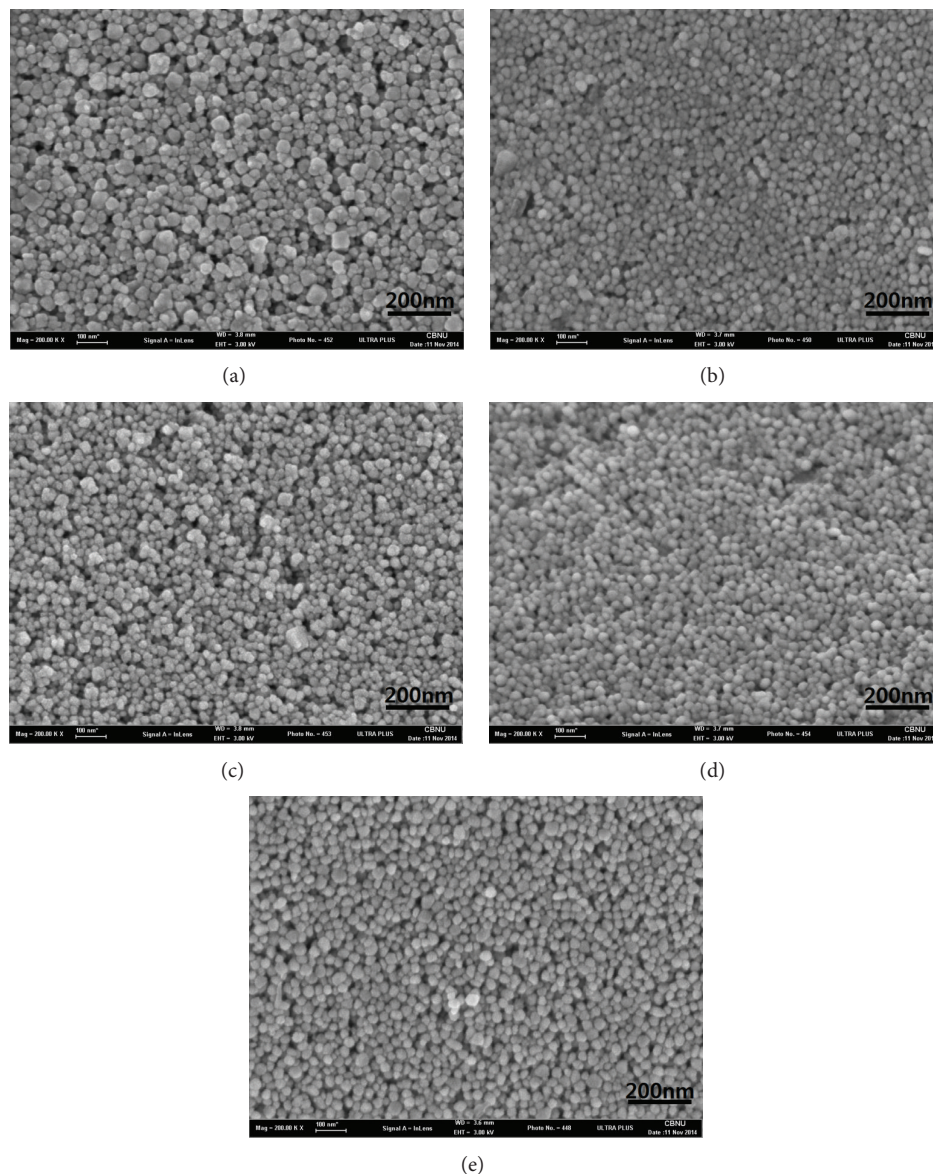


FIGURE 3: Scanning electron microscopy (SEM) of the AgNPs synthesized by five medicinal plant extracts: (a) *Mentha arvensis* var. *piperascens*, (b) *Buddleja officinalis* Maximowicz, (c) *Epimedium koreanum* Nakai, (d) *Artemisia messer-schmidtiana* Besser, and (e) *Magnolia kobus*.

molecule might be immobilized as suggested by Deka et al. [16]. The efficiency of the enzyme increases on solid support as compared to its free form [17]. The degradation in this situation occurs at a higher rate because the collision produced by the system would be less than when compared to free cellulose. AgNPs could possibly be acting as nanocatalyst in the hydrolysis of cellulose catalyzed by cellulase and increase the reaction rate although the exact binding mechanism remains to be explored.

In the present study, cellulose and plant synthesized AgNPs after interaction with cellulase were capable of breaking down the cellulose complex with the attachment

of the enzyme over its surface thereby being immobilized and degrading cellulose much faster than when compared to free cellulose. As the collision frequency between the free enzyme and the substrate molecule and their steric orientations forms the basis of the enzyme activity, the constraint was overcome by the immobilized enzyme with the support of a solid nanoparticle whereas it did not occur in the case of free cellulose similar to reported by Rangnekar et al. [18]. Therefore, the reaction rate was probably high, and the breakdown of cellulose to smaller molecules like monosaccharides and disaccharides was faster. The AgNPs were found to be better nanocatalysts compared

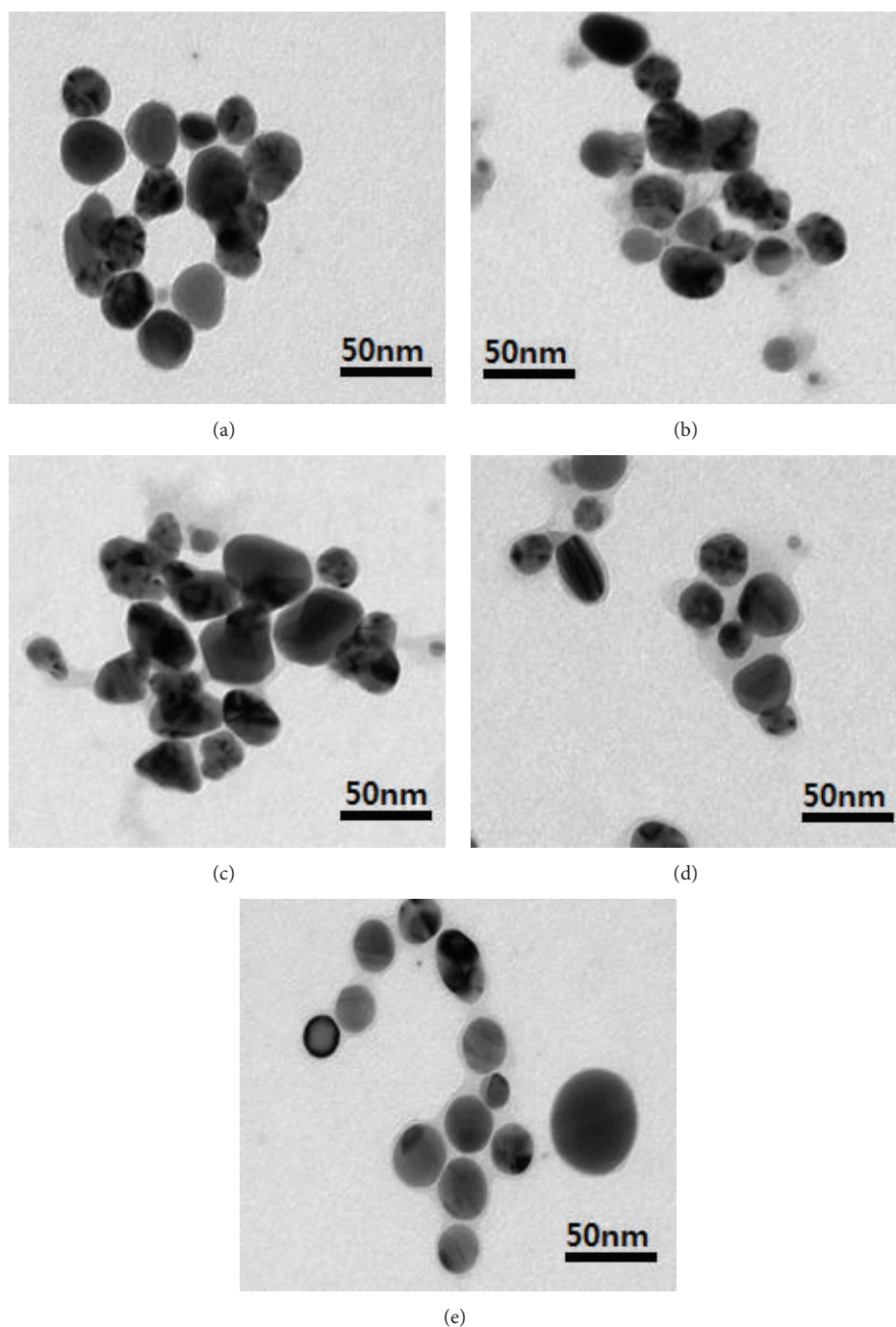


FIGURE 4: Transmission electron microscopy (TEM) images of the AgNPs synthesized by five medicinal plant extracts (a) *Mentha arvensis* var. *piperascens*, (b) *Buddleja officinalis* Maximowicz, (c) *Epimedium koreanum* Nakai, (d) *Artemisia messer-schmidtiana* Besser, and (e) *Magnolia kobus*.

to gold nanoparticles in degrading starch at a faster rate [16, 18].

4. Conclusion

The plant synthesized AgNPs showed an increased rate of reaction with cellulase compared with free cellulose. The degradation of cellulose in the presence of AgNPs rapidly

produced larger amounts of reducing sugars. AgNPs obtained with *M. kobus* showed highest reducing sugar formation. This study demonstrates that the nanoparticles may be useful in the field of nanocatalysis such as rapid degradation of the complex molecules to simpler ones by immobilizing the enzymes onto the surface of nanoparticles and different industrial applications. They can also be useful in developing assay kits for sensing and biomedical applications.

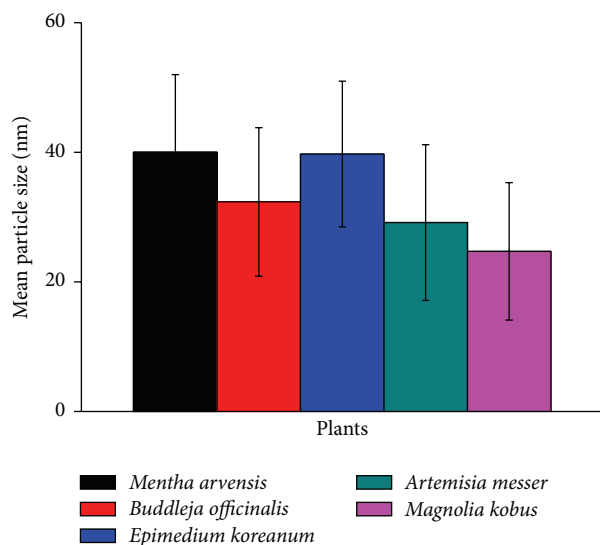


FIGURE 5: Mean particle size histogram of the AgNPs synthesized by five medicinal plant extracts.

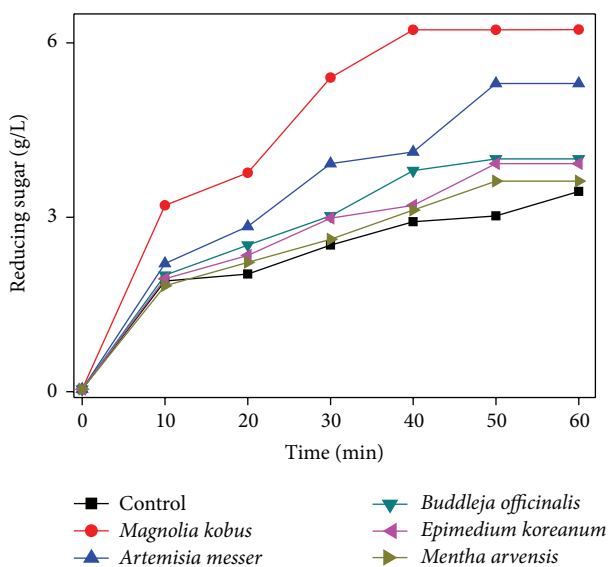


FIGURE 6: Comparative study of cellulose degradation at 25°C in the presence of AgNPs and free cellulose (control).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This research was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2006375 and NRF-2013R1A2A2A01067117).

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