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Research article

Efficient immobilization of agarase using carboxyl-functionalized magnetic nanoparticles as support

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ABSTRACT

Background: A simple and efficient strategy for agarase immobilization was developed with carboxyl-functionalized magnetic nanoparticles (CMNPs) as support. The CMNPs and immobilized agarase (agarase-CMNPs) were characterized by transmission electron microscopy, dynamic light scattering, vibrating sample magnetometry, scanning electron microscopy, X-ray diffraction, thermogravimetric analysis, and zeta-potential analysis. The hydrolyzed products were separated and detected by ESI-TOF-MS.
Results: The agarase-CMNPs exhibited a regular spherical shape with a mean diameter of 12 nm, whereas their average size in the aqueous solution was 43.7 nm as measured by dynamic light scattering. These results indicated that agarase-CMNPs had water swelling properties. Saturation magnetizations were 44 and 29 emu/g for the carriers and agarase-CMNPs, respectively. Thus, the particles had superparamagnetic characteristics, and agarase was successfully immobilized onto the supports. Agaro-oligosaccharides were prepared with agar as substrate using agarase-CMNPs as biocatalyst. The catalytic activity of agarase-CMNPs was unchanged after six reuses. The ESI-TOF mass spectrogram showed that the major products hydrolyzed by agarase-CMNPs after six recycle uses were neoagarotetraose, neoagarohexaose, and neoagarooctaose. Meanwhile, the end-products after 90 min of enzymatic treatment by agarase-CMNPs were neoagarobiose and neoagarotetraose.
Conclusions: The enhanced agarase properties upon immobilization suggested that CMNPs can be effective carriers for agarase immobilization. Agarase-CMNPs can be remarkably used in developing systems for repeated batch production of agar-derived oligosaccharides.

1. Introduction

Agar is a polysaccharide in the cell walls of some red algae and consists of agarose and agaropectins [1]. Agar or agarose can be enzymatically degraded using two types of agarases on the basis of their hydrolysis patterns. The two agarases, namely, α-agarase and β-agarase, hydrolyze α-1,3 linkages and β-1,4 linkages in agarose, respectively. α-Agarase cleaves the α-L-(1,3) linkages of agarose to produce oligosaccharides of the agarobiose series with 3,6-anhydro-L-galactopyranose at the reducing end. By contrast, β-agarase cleaves the β-D-(1,4) linkages of agarose to produce neoagaro-oligosaccharides with D-galactopyranoside residues at the reducing end [2]. Neoagaro-oligosaccharides from β-agarase have recently received much attention because of their numerous biological functions, such as inhibition of bacterial growth, retardation of starch degradation, improvement of food qualities as low-calorie additives, and even for moisturizing and whitening the skin [3]. Therefore, agar-derived oligosaccharides have broad applications in the food, cosmetic, and medical industries.

Agarase can be directly used to produce agar-derived oligosaccharides. However, these enzymes are often easily devitalized and difficult to segregate from the reaction system during recovery and recycling. Therefore, the enzymatic activity, stability, and recovery of agarase should be improved before its application as an industrial biocatalyst [4]. Magnetic nanoparticles for immobilizing enzymes and cells are recently considered for the commercial application of biocatalyst-processed products [5,6]. The application of biomolecule immobilization mainly depends on the solid-phase magnetic feature which can be rapidly separated and recovered from the reaction medium using an external magnetic field [7,8]. The use of magnetic supports can also reduce the capital and operation costs [9,10].

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To the best of our knowledge, the production of agar-derived oligosaccharides with immobilized agarase on magnetic nanoparticles has not been reported yet. In this study, we described a simple and efficient method to immobilize agarase using carboxyl-functionalized magnetic nanoparticles (CMNPs) as support. The successful immobilization of agarase onto the support was confirmed by characterizing the CMNPs and immobilized agarase (agarase-CMNPs) by transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), vibrating sample magnetometry (VSM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and zeta-potential analysis. In addition, the hydrolyzed agar products were detected by thin-layer chromatography (TLC) and ESI-TOF-mass spectroscopy (MS) to determine the catalytic activity of the agarase-CMNPs. The results provide a foundation for further studies on the production of agar-derived oligosaccharide.

2. Materials and methods

2.1. Materials

Analytical grade $FeCl_3 \times 6H_2O$, $FeCl_2 \times 4H_2O$, $NH_3 \times H_2O$, $KMnO_4$, and oleic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. and used directly without further treatment. Agar was purchased from Guangdong Huankai Microbial Sci. & Tech. Co., Ltd. All other chemicals were of analytical grade.

2.2. Preparation of oleic acid-coated magnetic Fe_3O_4 nanoparticles

The oleic acid-coated Fe_3O_4 nanoparticles were prepared by co-precipitating Fe^{3+} and Fe^{2+} ions with a molar ratio of 2:1 in ammonia and oleic acid solution [11]. $FeCl_3 \times 6H_2O$ (8.10 g) and $FeCl_2 \times 4H_2O$ (2.98 g) were transferred into an Erlenmeyer flask with 142.5 mL of deionized water, and the mixture solution was heated to 70°C. Then, 18 mL of $NH_3 \times H_2O$ (25%, w/v) was quickly added into the flask with vigorous stirring. Oleic acid (4.66 g) was added dropwise into the reaction mixture under constant stirring for 1 h at 70°C. The resulting Fe_3O_4 magnetic fluid was isolated magnetically and repeatedly washed with deionized water and ethanol. Finally, the magnetic fluid was dried by lyophilization for use in the next procedure.

2.3. Preparation of CMNPs

Carboxyl-functionalized Fe_3O_4 magnetic nanoparticle was prepared using a modified method described by Lv et al. [12]. The oleic acid-coated magnetic nanoparticles were dispersed in 160 mL of 10 mg/mL $KMnO_4$ solution under continuous sonication for 8 h. The resulting CMNPs were subsequently separated by additional magnetic field, washed thrice with distilled water, and freeze-dried for the subsequent step.

2.4. Preparation of the crude agarase

Agarase-producing strain *Vibrio natriegens* CICC 23820 was used to produce agarase. The optimal culture medium and conditions of agarase production on the *V. natriegens* CICC 23820 were as follows: agar, 0.3%; yeast extract, 0.6%; NaCl, 2%; $MgSO_4 \times 7H_2O$, 0.5%; KCl, 0.1%; $CaCl_2$, 0.02%; K_2HPO_4, 0.01%; $FeSO_4 \times 7H_2O$, 0.002%; initial, pH 6.5; autoclave temperature, 121°C; and treatment time, 20 min. The samples were cooled, inoculated with 2% seed broth, and incubated at 25°C. The samples were cultivated for 24 h, and the supernatant was collected from the culture broth by centrifugation at 12,000 × g for 10 min. The resulting crude agarase solution was directly used for immobilization. The activity of free agarase was determined by measuring the rate of agar hydrolysis [13]. One unit of agarase activity was defined as the amount of enzyme which hydrolyzes agar to liberate 1 μg of the reducing sugar per minute under the assay conditions.

2.5. Preparation of the agarase-CMNPs

The CMNPs (20 mg) were suspended in 5 mL of 4% (v/v) glutaraldehyde (GA) and then ultrasonically treated for 5 min. The mixture was then left to stand at room temperature for 2 h. The CMNPs were then isolated magnetically and washed thrice with PBS (0.05 M, pH 7.0). Subsequently, the crude agarase solution (1 mL, 850 U/mL) was combined with the obtained CMNPs at a constant temperature of 5°C for 1 h under a stationary state. Finally, agarase-CMNPs were collected and rinsed thrice with PBS (0.05 M, pH 7.0).

2.6. Characterization methods

The size and morphology of the CMNPs and agarase-CMNPs were observed by TEM (FEI Tecnai G20, USA) with an acceleration voltage of 200 kV, and hydrodynamic sizes were surveyed by DLS (Malvern Zetasizer NANO ZS, UK). The magnetization curves of the dried CMNPs and agarase-CMNPs were recorded using VSM (Quantum Design MPMS XL7, USA) at room temperature. The crystalline properties of the magnetic microspheres were examined by XRD (Bruker D8 ADVANCE, Germany), and thermal stability was measured by TGA (TA SDT Q600, USA) under nitrogen atmosphere at 20–1000°C at a heating rate of 10°C/min. Zeta potential was measured by dispersing the CMNPs and agarase-CMNPs in water at 25°C in the Nano-Zetasizer (Malvern Zetasizer NANO ZS, UK).

2.7. Reusability of agarase-CMNPs

Hydrolysis reactions were conducted at 40°C in an Erlenmeyer flask containing 20 g of agarase-CMNPs and 10 mL of 0.2% agar. Agarase-CMNPs were isolated by a magnet after each enzymatic reaction, and the supernatant was collected. Then, the agarase-CMNPs were washed thrice with deionized water for the next reaction.

The enzymatic hydrolysate was identified by TLC and ESI-TOF-MS. Then, the samples were first applied to a silica Gel 60 TLC plate and developed with an n-butanol-acetic acid-water solution (2:2:1 by volume). The developed oligosaccharides were detected by submerging the plae with 10% (by volume) H_2SO_4 in ethanol and heating at 90°C. The spots corresponding to the hydrolyzed products were compared with the standard compounds. The total oligosaccharides produced after six reuses were dried in vacuum and extracted with ethanol. The molecular mass distribution was then determined using an ESI-TOF mass spectrometer.

2.8. Preparation and analysis of agaro-oligosaccharides

The different amounts of agarase-CMNPs and agar solution (20 mL, 0.2%) were incubated at 40°C in an Erlenmeyer flask. Samples were collected at different time points to determine the reducing sugar in the reaction liquid. Then, the reaction solutions were separated using an external magnetic field to terminate the reaction. The oligosaccharides were obtained by alcohol precipitation after centrifugation and then analyzed by ESI-TOF-MS after the samples were freeze-dried.

3. Results and analysis

3.1. TEM, SEM, and particle size distribution analysis of the CMNPs and agarase-CMNPs

Transmission electron micrographs of the CMNPs and agarase-CMNPs are shown in Fig. 1a and Fig. 1b, respectively. The CMNPs were well

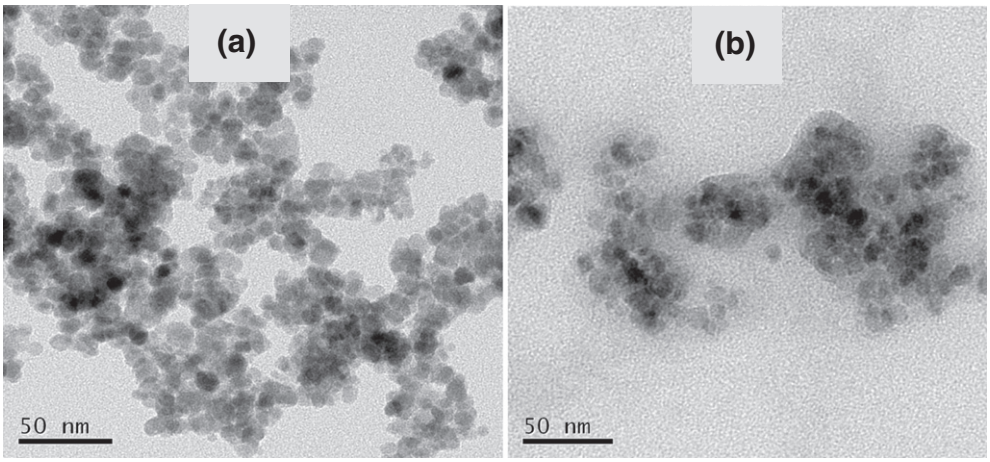

Fig. 1. Transmission electron micrographs of (a) CMNPs and (b) agarase-CMNPs.

scattered, had a narrow size distribution, and with average size of 12 nm. The agarase-CMNPs remained discrete and had a mean diameter of 12 nm after enzyme immobilization, revealing that the agarase-CMNPs and CMNPs were of the same size.

Scanning electron micrographs of the CMNPs and agarase-CMNPs showed that the magnetic nanoparticles exhibited equal size distribution, and the size of nanoparticles were about 12 nm (Fig. 2). No evident difference was found in their sizes before and after immobilization. This type of particulate size greatly increased the surface area, which could increase enzyme loading and benefit agarase immobilization. Additionally, this characteristic could also offer good transmission for the substrate and product during continuous enzymatic reaction.

The DLS is one of the most frequently used methods for determining the particle size and distribution of dispersion. Contrary to the TEM analysis of a dry sample, DLS measures the hydrated radius of nanoparticles, and the size of the magnetic particles also increases with the swelling of the immobilized biomolecules [14,15]. Thus, the sizes of the CMNPs and agarase-CMNPs surveyed by DLS (Fig. 3a and Fig. 3b) were obviously higher than those of the TEM measurements. The DLS measurement showed that the average diameter of the CMNPs was approximately 34.8 nm, whereas the particle size of the agarase-CMNPs increased to 43.7 nm after agarase immobilization. Therefore, agarase was shown to be successfully conjugated onto the surface of CMNPs, and enzyme immobilization caused the increase in particle diameter.

3.2. Magnetic property of CMNPs and agarase-CMNPs

Good magnetic property is highly important for separating and applying the magnetically immobilized enzyme. The magnetization curves for the CMNPs and agarase-CMNPs were measured with a VSM at room temperature. The hysteresis loops of the samples are presented in Fig. 4. The saturation magnetization values were 44 and 29 emu/g for the CMNPs and agarase-CMNPs, respectively. These particles also exhibited superparamagnetism, because no reduced remanence and zero coercivity were detected. This characteristic was beneficial to the separation and reuse of agarase-CMNPs [16,17]. In addition, the decrease in magnetization also suggested that agarase was successfully immobilized onto the CMNPs. The decrease in saturation magnetization was due to the increased amount of agarase immobilized on the CMNPs, because the weights of all particles used to measure the magnetic properties were constant.

3.3. XRD patterns of CMNPs and agarase-CMNPs

XRD is used to analyze the space distribution of the internal atoms of a crystal material. This technique can infer the material structure and particle size of crystals. The XRD patterns of the CMNPs and agarase-CMNPs are compared in Fig. 5. Six characteristic peaks for Fe_3O_4 ($2\theta = 30.08°$, 35.42°, 43.08°, 53.51°, 56.80°, and 69.69°) were observed before and after immobilization, as marked by their corresponding indices (220), (311), (400), (422), (511), and (440).

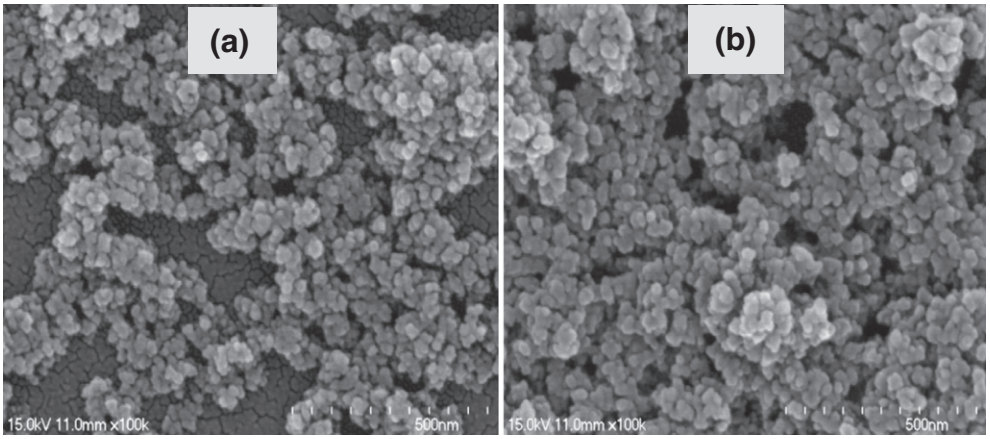

Fig. 2. Scanning electron micrographs of (a) CMNPs and (b) agarase-CMNPs.

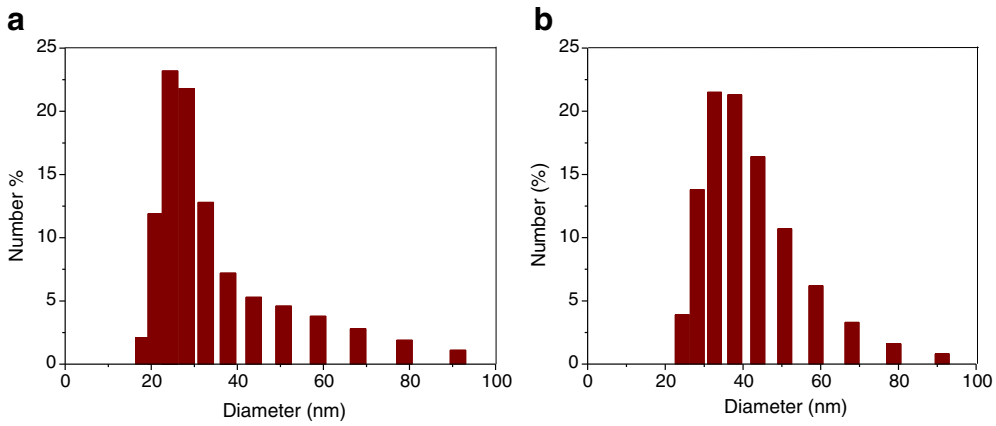

Fig. 3. Size distribution micrographs of (a) CMNPs and (b) agarase-CMNPs using dynamic light scattering.

These peaks conformed to the JCPDS file (PDF No. 65-3107) in the database. This result demonstrated that the modification and the presence of an oleic acid coat on the surface of Fe_3O_4 nanoparticles did not lead to the phase change, because the diffraction peak angles of CMNPs and agarase-CMNPs slightly differed from that of the standard Fe_3O_4.

3.4. TGA and DSC analysis of the CMNPs and agarase-CMNPs

The TGA results of the CMNPs and agarase-CMNPs are shown in Fig. 6a. The weight loss of the CMNPs was mainly attributed to the loss of physically adsorbed water and azelaic acid on the surface of nanoparticles [18]. The weight loss of the agarase-CMNPs, was ascribed to the loss of physically adsorbed water, azelaic acid, and agarase immobilized on the surface. There into, the azelaic acid was produced by oxidizing the oleic acid by $KMnO_4$, which was in the inner layer of MNPs-COOH-agarase supports. One peak was found at 50°C in the DSC curves. This peak could be attributed to the physical and chemical removal of absorbed water. Another obvious peak appeared at 250°C, and the weight loss was related to the degradation of azelaic acid on the surface of CMNPs and agarase-CMNPs. The third peak appeared at 650°C, and this weight loss was due to the degradation of agarase-CMNPs. Thereafter, no dramatic difference in weight was observed over the wide temperature range. This phenomenon reveals the existence of pure Fe_3O_4. The weight losses of the CMNPs and agarase-CMNPs were 8.9% and 11.5%, respectively, as shown by the mass-loss rate of the samples in the TGA curve. Consequently, the amount of agarase immobilized on the surface of CMNPs was 2.6%.

3.5. Zeta-potential and dispersion of CMNPs and agarase-CMNPs in the hydrophilic solvent

The magnitude of the zeta potential indicates the potential stability of the colloidal system. If all particles have large negative or positive zeta potential, they will repel each other and dispersion stability will exist. Stable and unstable aqueous dispersions is generally considered at either +30 or −30 mV [19]. The zeta-potential curve of the aqueous dispersion of magnetic nanoparticles is depicted in Fig. 7a. The prepared CMNPs had a zeta potential of −43.9 mV. The zeta potential decreased to −35.1 mV after immobilization (Fig. 7b). Thus, particles with zeta potentials more negative than −30 mV are normally considered stable.

The CMNPs showed good dispersion in polar solvents, such as water, ethanol, and isopropanol (Fig. 8a), but the particles assembled and quickly settled at the bottom as tested in non-polar solvents toluene and ethyl acetate. The good solubility in polar solvents could be attributed to the high affinity between the carboxyl groups in the magnetic nanoparticles and hydrophilic solvents. In addition, the CMNPs exhibited a superparamagnetic behavior in a magnet field (Fig. 8b). Thus, the CMNPs were well-dispersed in the aqueous solution and quickly aggregated when an external magnetic field

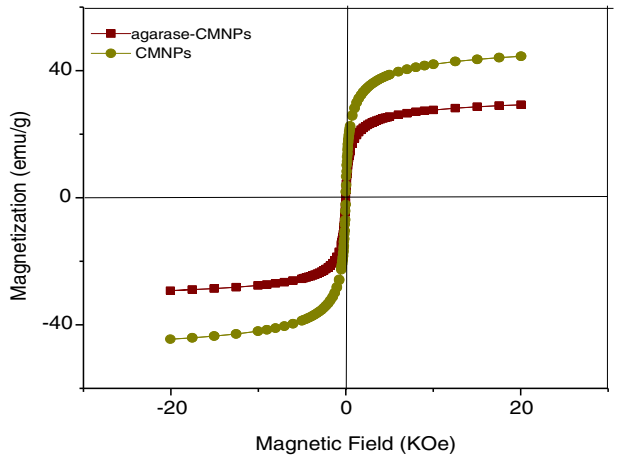

Fig. 4. Magnetic hysteresis loops of the CMNPs and agarase-CMNPs.

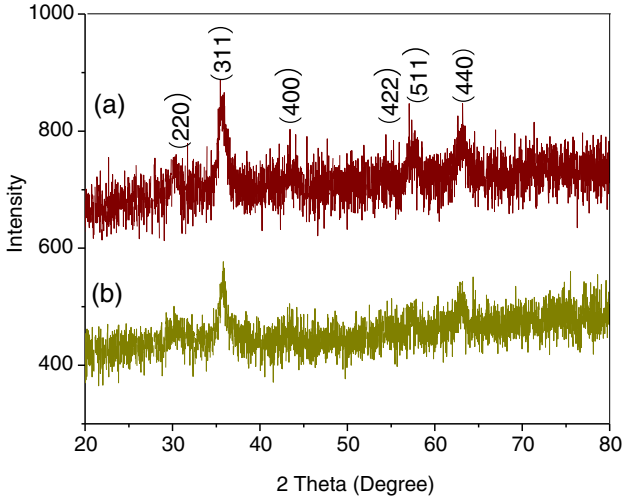

Fig. 5. X-ray diffraction patterns of (a) CMNPs and (b) agarase-CMNPs.

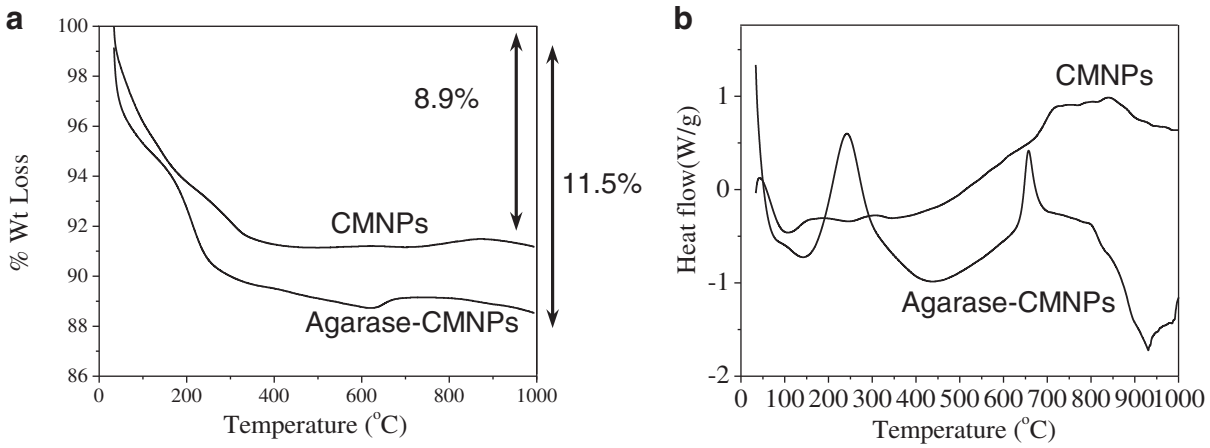

Fig. 6. (a) Thermal gravimetry and (b) differential scanning calorimetry analyses of the CMNPs and agarase-CMNPs.

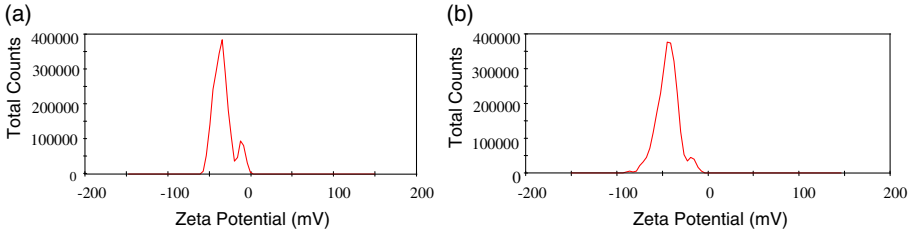

Fig. 7. Zeta potential curve for (a) CMNPs and (b) agarase-CMNPs at aqueous dispersion.

was applied in the test. This phenomenon demonstrated that agarase-CMNPs could be easily separated by additional magnetic field during the using and reusing processes (Fig. 8c). The hydrophilic property of agarase-CMNPs in our study significantly facilitated the recovery of the immobilized enzyme and the purity of the product in the water phase.

3.6. Reusability study of the immobilized agarase

The hydrolyzed products of agar produced by immobilized agarase after six reuses were separated and detected by TLC with the standard compounds neoagarobiose, neoagarotetraose, neoagarohexaose, and neoagarooctaose (Fig. 9a). Three major products were clearly detected. ESI-TOF-MS revealed that the major products have molecular ions at m/z of 629 (2 M–H)$^-$, 935 (3 M–H)$^-$, 981 (3 M + HCOO)$^+$, and 1241 (4 M–H)$^-$, corresponding to neoagarotetraose, neoagarohexaose, and neoagarooctaose (Fig. 9b), which revealed that this separation had the same pattern of mobility as that in TLC. Other peaks, such as 773 (2 M + 2SO_3^-) and 1097 (3 M + 2SO_3^-) m/z, were due to the presence of sulfated group, because the L-galactose-6-sulfate has not been converted into 3,6-anhydro-L-galactose. These results indicated that the agarase-CMNPs still retained activity after six consecutive runs without significant change in the end-products. Thus, the reuse of agarase-CMNPs did not change enzyme specificity. Immobilizing agarase on the surface of CMNPs resulted in good durability and reusability, which may effectively lower the cost of agaro-oligosaccharide production.

3.7. Hydrolyzed products of agar and analysis of Agaro-oligosaccharides

The time course of hydrolyzed products from agar was examined with agarase-CMNPs incubated at 45°C for up to 90 min (Fig. 10). Then, the hydrolyzed products were separated and detected by ESI-TOF-MS (Fig. 11). Two major products were clearly detected by ESI-TOF-MS which revealed that the major products have molecular ions at m/z of 323 (M–H)$^-$, 629 (2 M–H)$^-$, and 665 (2 M + Cl)$^+$, corresponding to neoagarobiose and neoagarotetraose. Additionally, other peaks, such as 485 and 791 (2 M + 2SO_3^-) m/z, revealed the presence of neoagarobiose and neoagarotetraose because of the incomplete removal of sulfate. This separation did not fully correspond to the pattern of mobility in the TLC in Section 2.7. ESI-TOF-MS analysis of the main spot revealed that neoagarohexaose and neoagarooctaose were hydrolyzed into neoagarobiose and

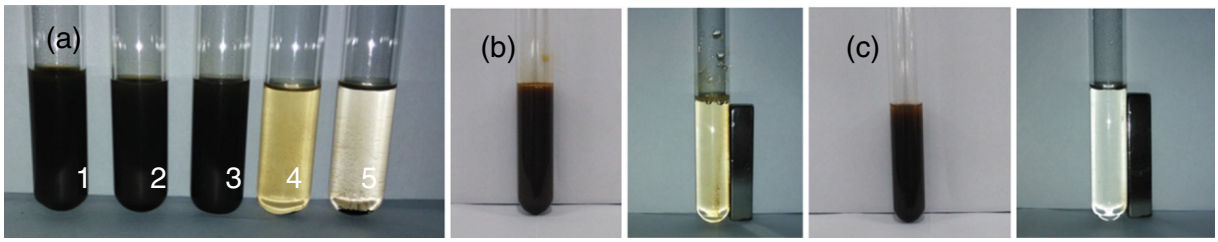

Fig. 8. (a) Dispersion of CMNPs in different solvents(a). (1) Water; (2) isopropanol; (3) ethanol; (4) toluene; (5) ethyl acetate; (b) CMNPs and (c) agarase-CMNPs dispersed in aqueous solution and attracted by magnet.

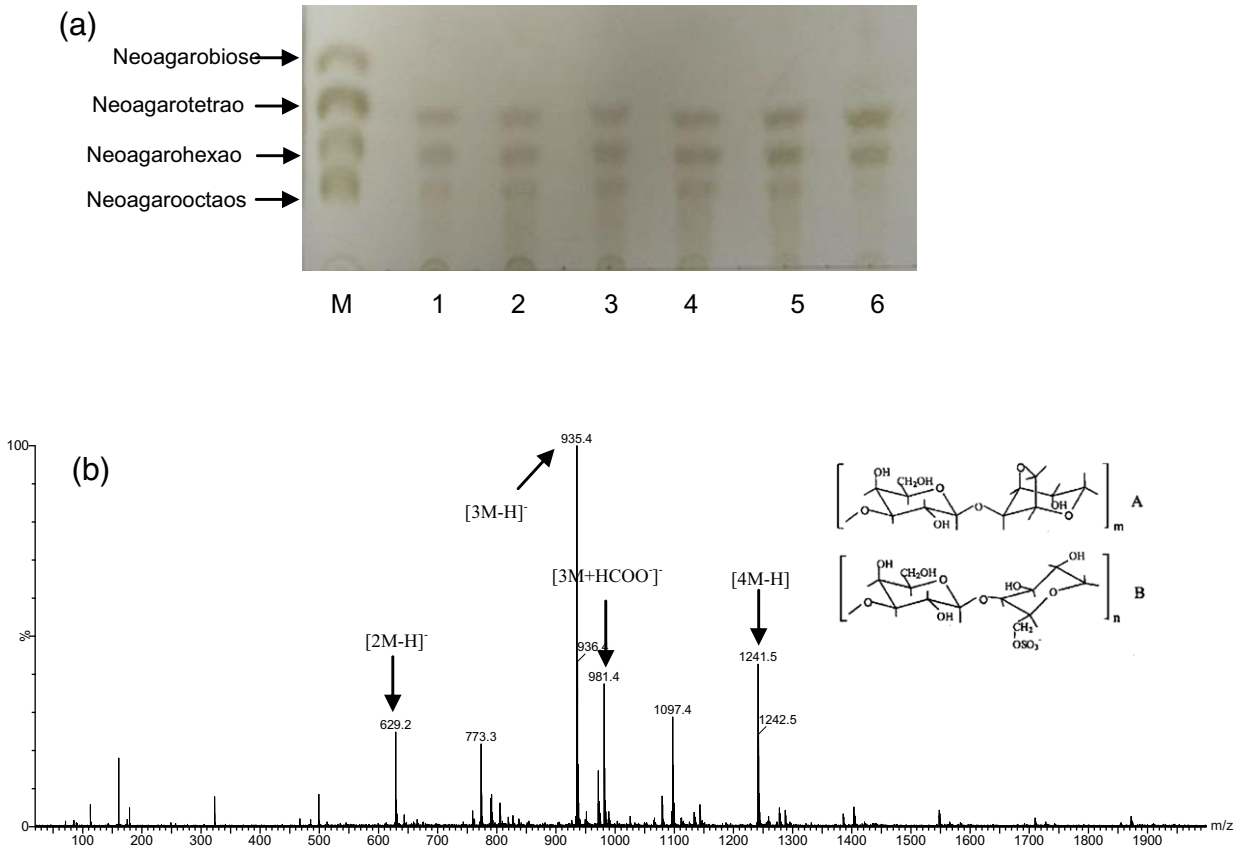

Fig. 9. (a) Thin layer chromatogram and (b) ESI-TOF mass spectrogram of the hydrolyzed product of agar produced by agarase-CMNPs.

neoagarotetraose by agarase-CMNPs for a period of time. Thus, agarase-CMNPs can be a valuable candidate for industrial applications because of their reusability and catalytic efficiency.

4. Discussion and conclusions

Agar-derived oligosaccharides have been considered because of their potentially high economic value as a result of their nontoxic physiological and biological activities. These oligosaccharides exhibit antioxidant activities by scavenging hydroxyl free radicals and superoxide anion radicals as well as by inhibiting lipid peroxidation [20,21]. In addition, studies have shown that the activities of agaro-oligosaccharides are correlated with their degree of polymerization (DP). Neoagarobiose (DP 2) was reported to possess moisturizing and whitening effects on melanoma cells [22]. Sulfated oligosaccharide with DP 4 was reported to be utilized in vitro by intestinal bacteria, stimulating the growth of *Bacteroides*, *Eubacterium*, and *Lactobacillus* [23]. Sulfated oligosaccharide (DP 6) from *Gracilaria* sp., compared with its polysaccharide, has shown a distinctly higher positive effect on survivability in Japanese encephalitis virus-infected C3H/HeN mice [24].

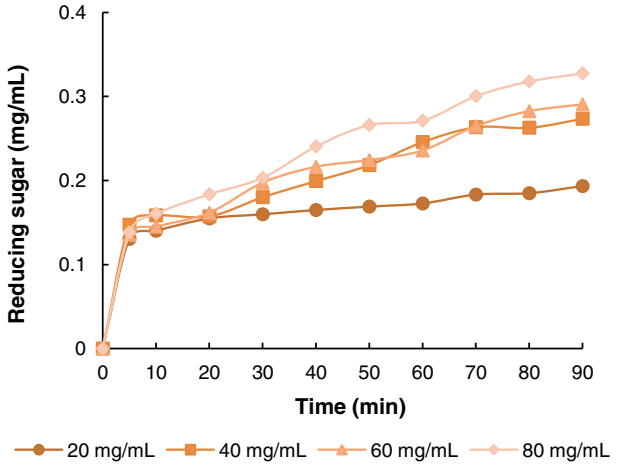

Fig. 10. Time course of enzymatic degradation agar with the agarase-CMNPs.

Agaro-oligosaccharides are prepared by agar hydrolysis using either a chemical or an enzymatic process. The enzymatic process is superior to the former, because a chemical method exhibits an unfavorable effect on the environmental and enzymatic hydrolysate. Agarases are the enzymes that degrade the glycosidic bonds of agar and play an important role in the enzymatic hydrolysis process. Enzyme-catalyzed degradation is generally a low-energy demanding operation and simple downstream operation. Thus, enzymatic hydrolysis is environment-friendly, energy efficient, and potentially cost effective. However, using native enzyme as a biocatalyst shows some limitations in stability, product recovery, and multiple reuses in industrial application. Normal industrial conditions are generally not favorable because of extreme temperature, pH, and the presence of inhibitors. Therefore, enzymes for such processes should be robust enough to withstand such conditions. Numerous studies attempted to overcome these drawbacks by raising the functionality of these enzymes, stabilizing their catalytic properties, and enhancing their reusability by immobilizing them on carriers via some immobilization methods. Some examples of these techniques are covalent binding [25,26], entrapment [27,28], encapsulation in polymers [29], physical adsorption [30,31], and by cross-linking [32]. Several techniques have been applied to immobilize agarase. Lim et al. used Na-alginate as a supporting material for agarase immobilization and showed that

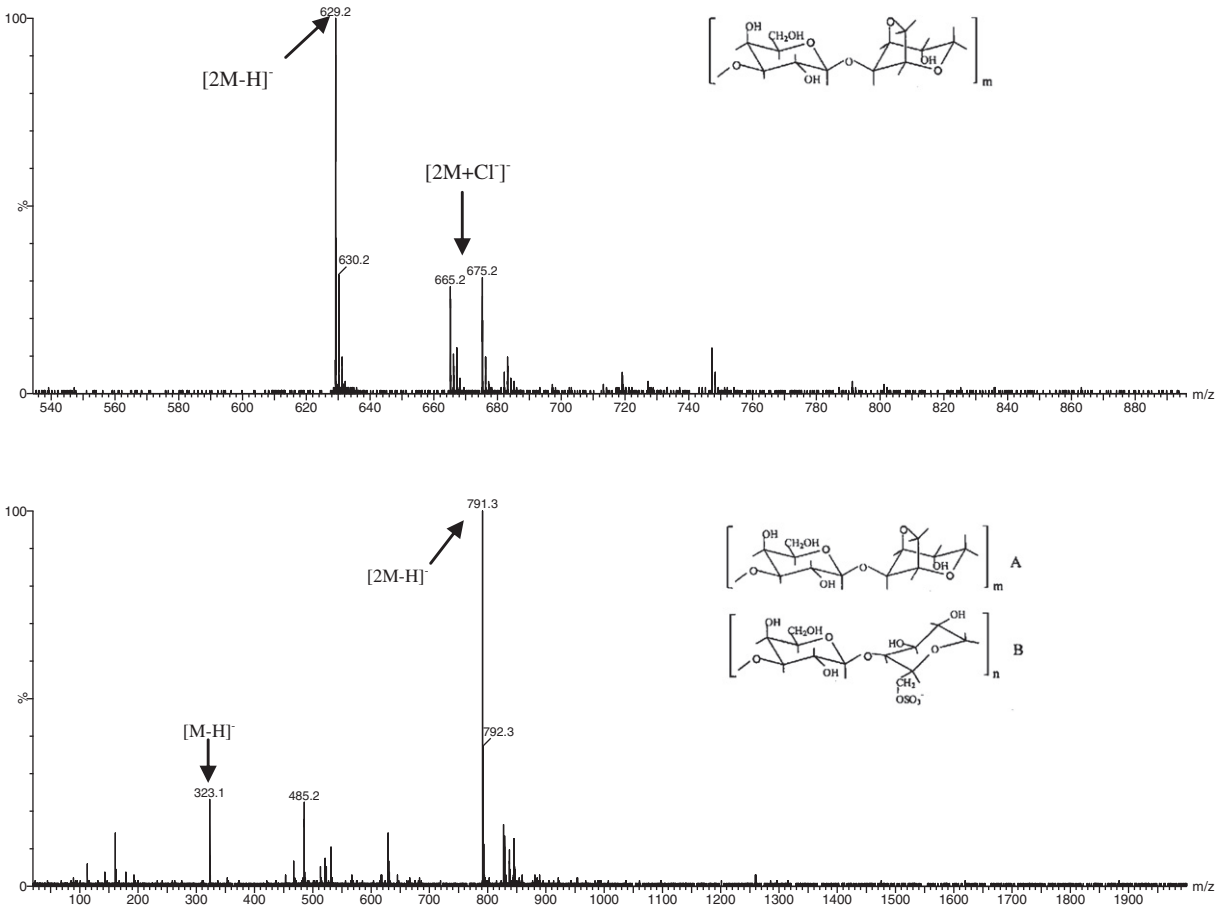

Fig. 11. Analysis of agar hydrolyzed products based upon ESI-TOF mass spectrometry.

the immobilized agarase could degrade agar into agarobiose and agarotetraose [33]. Their total conversion ratio was 89% under the optimal condition. Koti et al. [34] used Amberlite IRA-900 as a matrix to immobilize agarase and found that the agar-hydrolyzed products of immobilized agarase were neoagarobiose, neoagarotetraose, and neoagarohexaose, similar to the hydrolysates of free agarase [34]. Moreover, the immobilized agarase was recycled efficiently eight times and stored for 3 months at 4°C as wet beads and for more than 6 months as dry beads. Therefore, immobilized agarase may be preferred over its free counterpart in the production of agar-derived oligosaccharides.

The choice of the immobilization matrix determines the physical and chemical properties of the final material. Therefore, the selection of an appropriate matrix is an important step in developing an adequate sorption procedure. Magnetic Fe_3O_4 nanoparticles have vast potential for immobilizing enzyme on their surfaces, because they perform better than conventional bulk supports used in enzyme immobilization. Moreover, these particles can be quickly separated by an external magnetic field, resulting in the feasibility of recycling immobilized enzyme [35]. However, pure magnetic Fe_3O_4 nanoparticles tend to aggregate in liquid because of Van der Waals forces. Only a few hydroxyl groups could be found on the surface of Fe_3O_4 nanoparticles [36,37]. Furthermore, pure magnetic nanoparticles are extremely sensitive to air oxidation and easily integrate in an acid medium. Therefore, the surface of pure magnetic nanoparticles should be suitably coated by a surfactant (e.g., oleic acid) or a polymer (e.g., chitosan) to avoid these limitations [38,39]. Additionally, preparation of surface-functionalized magnetic particles with water soluble, biocompatible, and reactive groups is also much desired for the commercial use of an enzyme to increase the loading amount of the biomolecules on the magnetic particles and improve the stability of immobilized biomolecules [40].

The production of agar-derived oligosaccharides with immobilized agarase on magnetic nanoparticles has not been reported yet. In the present study, CMNPs were prepared from primary Fe_3O_4 nanoparticles by co-precipitation. The average size of the CMNPs was approximately 12 nm, with a narrow size distribution, and approximately spherical morphology. The CMNPs showed superparamagnetic behavior and good magnetic properties at room temperature. The agarase-CMNPs did not show significant changes in size and shape after agarase was immobilized on the CMNPs via cross-linking with GA. However, the saturation magnetization was 29 emu/g, which was lower than that prior to immobilization (44 emu/g). Agaro-oligosaccharides were prepared with agar as substrate using agarose-CMNPs as biocatalyst, and the catalytic activity of the agarase-CMNPs did not change after six recycle uses. The ESI-TOF mass spectrogram identified the predominant reaction products hydrolyzed by immobilized agarase as neoagarobiose and neoagarotetraose. Therefore, CMNPs may be promising carriers for agarase immobilization. Agarase-CMNPs can be remarkably applied in the development of systems for repeated batch production of agar-derived oligosaccharides. Further study on systems with agarase-CMNPs is currently undertaken by our laboratory.

Conflict of interest

The authors declare no conflict of interest.

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