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ALGINATE–SILVER NANOCOMPOSITE MATERIALS: FABRICATION AND ANTIFUNGAL APPLICATIONS

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ABSTRACT

Silver nanoparticles (Ag NPs) were incorporated with a uniform alginate polymer matrix for developing the Alginate-Ag composite. The surface morphology of the nanocomposite was examined by scanning electron microscopy (SEM). The optical characteristics of the nanocomposite were investigated by photoluminescence (PL) and UV-Vis absorption measurement. The study showed that the AgNPs were well-dispersed into the matrix of alginate, the integrated nanoparticles had a size ranging from 7 – 66 nm, and had an average size of about 20.8 nm, and there was aggregation of AgNPs into small clusters. The UV-Visible spectrum showed absorption with a maximum value of 410 nm. The Alginate-Ag nanocomposite demonstrated strong antagonistic properties against the fungi *A. niger* with inhibition rates of 100, 200 ppm at 73.01%, and 87.41%, respectively.

Keywords: Alginate, silver nanoparticles (Ag NPs), nanocomposite, biopolymer, antifungal.

1. INTRODUCTION

Alginates are natural polysaccharides found predominantly in the cell walls of brown macroalgae and certain bacteria, such as Azotobacter and Pseudomonas [1]. Commercially, alginates are derived exclusively from algae and are typically available in the form of sodium alginate, which is the salt form of alginic acid [2]. Alginates have been classified as GRAS (generally recognized as safe) substances by the U.S. Food and Drug Administration (FDA) [3], and their use, along with that of their related salts, has been approved by the European Food Safety Authority (EFSA) under specific dose restrictions [4]. Sodium alginate is frequently used in the culinary sector as a thickening, stabilizing, and gelling ingredient with application [5]. Alginate has a lot of advantages, such as high biocompatibility, non-toxicity, non-immunogenicity, biodegradability, low cost, and easy crosslinking with divalent cations [6]. Due to this, alginate could be very useful in many areas, which include biomedical and tissue engineering, textile printing, water treatment, and drug delivery systems [7,8]. However, Alginate has some problems, such as not being very strong and not having any natural antibacterial properties. To get around these problems, alginate is often mixed with other things like plant extracts, essential oils, chitosan, bacteriocins, enzymes, organic acids, or metallic nanoparticles [3,9].

Silver nanoparticles (AgNPs) have attracted significant interest owing to their unique physicochemical characteristics and extensive biological activity [10–13]. A variety of investigations have shown that AgNPs kill bacteria by entering their cell membranes and damaging their structure, which kills the cells [14,15]. AgNPs release Ag^+ ions, which attach to parts of the cell wall and membrane, making the membrane more permeable and breaking down the integrity of the cells [16,17]. The released Ag^+ can enter cells, prevent respiratory enzymes from working, inhibit ATP production, and cause reactive oxygen species (ROS) to form. This leads to oxidative stress, which makes it more difficult for DNA to replicate itself and cells to divide by connecting to biomolecules that contain sulfur and phosphorus [15]. AgNPs can also get through membranes, which can cause intracellular components to leak out and cells to die. It may also disrupt cellular signal transduction by altering the phosphorylation of proteins [15,18].

The combination of these mechanisms makes AgNPs an outstanding antimicrobial agent. Puttakhun et al. investigate the antibacterial activity of nanocomposite materials based on AgNPs, skim natural rubber (SNR), and bacterial cellulose (BC), reporting that BC–SNR–0.08Ag decreased *Staphylococcus aureus (S. aureus)* by approximately 98% and *Escherichia coli (E. coli)* by 63% [19]. Rozilah et al. integrated AgNPs into sugar palm nanocrystalline cellulose and tested their inhibitory effects against *E. coli, S. aureus, and Salmonella*, finding average inhibition zones of 7.66–7.83 mm, 0.1–0.5 mm, and 7.5–8.0 mm, respectively [20]. The suppression of *S. aureus* using

chitosan membranes combined with AgNPs was investigated by Monica Potara et al., and it was revealed that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the chitosan–Ag bionanocomposite synthesized at 0 °C were lower than those obtained at 35 °C [14].

Alginate–Ag is rarely investigated as a standalone material. Instead, it is commonly combined with additional components such as metal ions, metal oxides, bioceramics, or secondary biopolymers to enhance antimicrobial performance and structural stability. Previous studies have shown that Alginate–Ag-based composite systems effectively inhibit pathogenic microorganisms, including *E.coli, S.aureus, and Botrytis cinerea* [21–24]. Despite these advances, research on the antifungal activity of such alginate–Ag composite systems remains limited, particularly against filamentous fungi, indicating a clear gap in current studies.

This study aims to investigate and fabricate Alginate–Silver (Alg–Ag) nanocomposite materials with improved mechanical characteristics, antibacterial activity, and possible uses in food preservation. The surface morphology of the Alginate–Ag nanocomposite was examined by scanning electron microscopy (SEM). The optical properties of the nanocomposite were further assessed using UV–Vis and photoluminescence spectroscopy (PL). The antimicrobial activity of the materials effectiveness against *Aspergillus niger* **(***A.niger)* was investigated using a dry weight method and disk diffusion

2. MATERIALS AND METHODS

2.1. Materials

Sodium Alginate was purchased from Xilong, and AgNPs solution was prepared by the citrate reduction method [25]. The *Aspergillus niger (A.niger)* strain was provided by the Vietnam Academy of Science and Technology. Other chemical reagents, including peptone (Himedia), agar (Hailong, Vietnam), and dextrose (Xilong), were purchased.

2.2. Preparation of Characterization of Alginate – Ag Nanocomposite

The Alginate – Ag nanocomposite was synthesized by mixing a 1.5% alginate solution with silver nanoparticle (AgNP) solution at concentrations of 25, 50, 100, and 200 ppm. The mixture was stirred continuously until a uniform solution was obtained.

Optical Characterization

The surface morphology of the Alginate and Alginate - Ag nanocomposite was examined by scanning electron microscopy (SEM; Hitachi Regulus 8100). Subsequently, the particle size of the Ag nanoparticles was determined using ImageJ. Photoluminescence (PL) measurements were investigated by exciting samples at a

wavelength of 325 nm using a He-Cd laser (Kimmon), and the PL spectrum was analyzed with a high-resolution spectrometer (SP 2500i, Princeton). UV–Vis absorption measurements were performed using a Shimadzu 2450 spectrophotometer.

2.3. Antifungal Performance of the Alginate - Ag Nanocomposite

A. niger was chosen as the test microorganism to evaluate the antifungal efficacy of the nanocomposite materials. The A. niger culture medium was prepared using Sabouraud Dextrose Agar/Broth (SDA/SDB) media and sterilized in an autoclave at 121°C for 15–20 minutes. After cooling, 10^6 spores were added to 10 mL of medium in conical flasks to make seed cultures. The flasks were then incubated at 30 °C and 180 rpm for 24 hours. The fungal suspensions have been divided into groups according to their concentrations: control, alginate, Alginate–Ag (25, 50, 100, and 200 ppm), and free AgNPs of various concentrations.

We used dry weight and disk diffusion methods to test how well the antifungal worked. For the dry weight test, after 24 hours, treated fungal cultures were filtered through pre-dried filter paper, washed three times with deionized water, dried at 80 °C for three hours, and then weighed to find the dry biomass [26]. We carried out disk diffusion tests using the Kirby–Bauer method. We spread 100 μL of *A.niger* spore suspension on SDA plates, put sterile disks with test samples on top, and incubated the plates at 34 °C for 48 hours [27].

3. RESULTS AND DISCUSSION

3.1. Morphological and Structural Characterization of the Nanocomposite

The surface morphology of the Alginate and Alginate–Ag nanocomposite was analyzed by a scanning electron microscope (SEM) in Figure 1. The figure demonstrates that pure alginate has a surface that is rather smooth and uniform. The surface of the Alg–Ag nanocomposite, on the other hand, has several bright spots demonstrating the presence of AgNPs. The size of the AgNPs was calculated using the ImageJ software. Some particles are spread out on their own, and aggregation of AgNPs into a small cluster. The histogram size distribution of AgNPs is shown in Figure 1C, with sizes ranging from 7 - 66 nm, with an average of around 20.8 nm. The clear contrast between Figure 1a and Figure 1b shows that the Ag nanoparticles were successfully added to the alginate matrix, which significantly affected the sample's surface morphology.

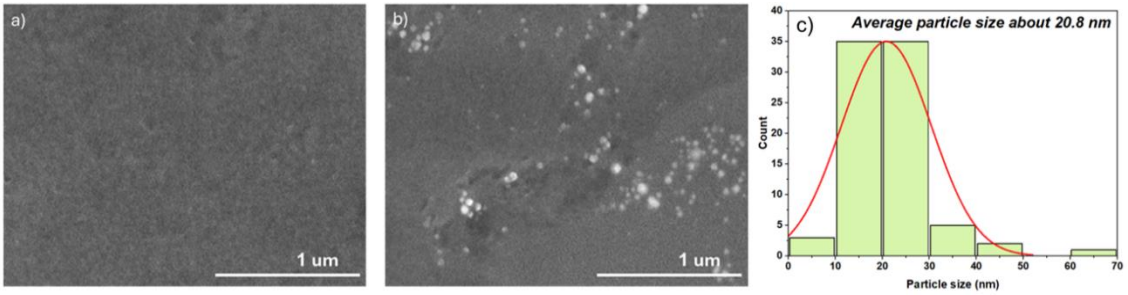

Figure 1. SEM images of a) Alginate, b) Alginate - Ag sample, and c) Particle size distribution histogram of the AgNPs in Alginate

3.2. Photoluminescence and UV-Vis spectrum

The UV-Vis and PL spectra of all samples are presented in Figure 2. It can be seen that the Alginate–Ag nanocomposite exhibits no photoluminescence under 325 nm excitation in Figure 2a. This can be explained by the fact that AgNPs themselves have very weak or even negligible photoluminescence, and alginate is a polysaccharide composed of mannuronic and guluronic acid units and therefore does not exhibit intrinsic fluorescence [28].

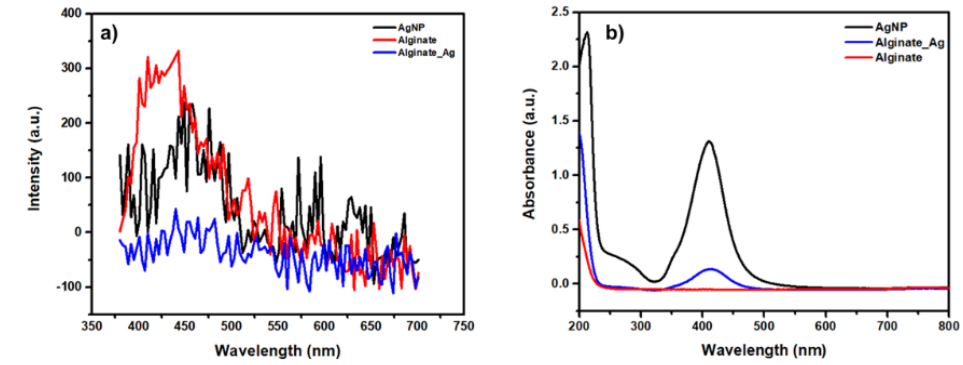

Figure 2. a) PL, and b) UV-Vis spectra of the AgNP (black), Alginate-Ag (blue), Alginate (red)

The absorption properties of the samples were examined by UV–Vis spectroscopy (Figure 2b). Alginate showed no absorption in the visible region, and the Alginate–Ag nanocomposite had a clear SPR peak at 410 nm [29], which indicated that AgNPs were present. At 1000 ppm, free AgNPs showed a strong and sharp absorption peak at the same wavelength. The Alginate–Ag nanocomposite had a lower concentration of AgNPs (100 ppm), but it still had the same SPR peak with a lower intensity and a slightly narrower spectrum (FWHM = 66.13 nm) than free AgNPs (FWHM = 68.14 nm). The results indicate that AgNPs were successfully trapped and interacted with the alginate matrix.

3.3. Antifungal activity

The antifungal activities of AgNPs and Alginate–Ag nanocomposites against *A. niger* were evaluated using the disk diffusion method (Figure 3). At low concentrations (25–50 ppm), there were small inhibition zones, which showed weak antifungal activity. However, at 100 ppm and 200 ppm, the inhibition diameter increased significantly to about 10 mm and 13 mm, respectively, showing that the effect depended on the concentration. There was no inhibition zone for alginate without AgNPs, which indicates the polymer matrix itself lacks antifungal properties.

Alginate–Ag nanocomposites showed structures similar to free AgNPs, with distinct inhibition zones at concentrations ≥ 100 ppm, demonstrating that AgNPs preserved their antifungal properties after being incorporated into the alginate matrix. The slightly smaller inhibition zones are due to the fact that Ag^+ ions can't move freely through the alginate network. Penicillin exhibited no antifungal properties, as it is only effective against bacteria [30].

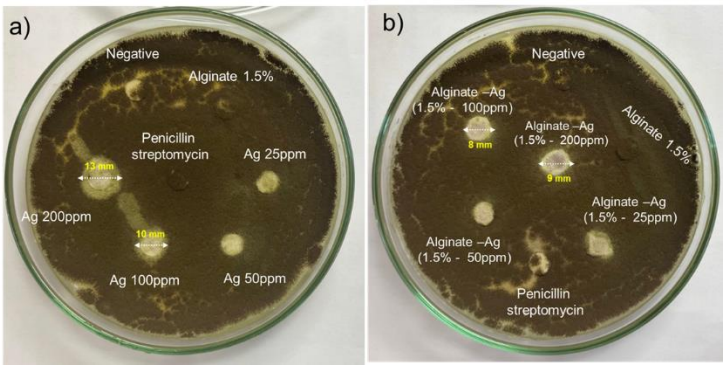

Figure 3. Zone inhibition test for a) AgNPs, and b) Alginate – Ag nanocomposite with different concentrations by using the disk diffusion method after 48 hours.

Figure 4 illustrates the antifungal activity against *A. niger* using the dry-weight method of the AgNPs and Alginate – Ag nanocomposite with different concentrations. As can be seen a clear difference in the amount of fungal biomass between the sample groups. The control sample had the highest dry-weight value, which demonstrated that fungal spores grew normally in Sabouraud Dextrose broth (SDB) after 24 hours. The pure alginate sample had a dry weight that was also rather high, but not quite as high as the control. This little decrease is due to 1.5% alginate is viscous and forms gels, which can inhibit the growth rate of *A. niger* by limiting the spread of nutrients and hyphal extension.

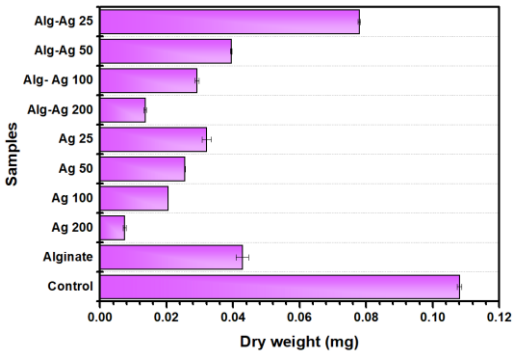

Figure 4. Dry weight value of *A.niger* with different groups after 24 hours of growth

All samples containing AgNPs in both free form and bound inside the alginate matrix significantly reduced the fungal dry weight compared to the control. The antifungal effectiveness of the sample groups Alginate, Ag 200, 100, 50, 25 ppm, and Alginate - Ag 200, 100, 50, 25 ppm were 60.32, 93.15, 81.06, 76.39, 70.32, 87.41, 73.01, 63.47, and 27.82%, respectively. This finding aligns with the recognized antifungal activities of AgNPs, which include membrane rupture, enzyme inactivation, and the suppression of cellular metabolic processes [14–18]. These results show that 100 ppm is the lowest amount needed for both free AgNPs and the Alginate–Ag nanocomposite can be used antifungal activity against *A. niger*. The finding points to the prospective utilization of these materials in the fabrication of intelligent biopolymers for the preservation of fruits and food.

4. CONCLUSION

We incorporate AgNPs in the alginate matrix using a simple and effective production method. SEM, PL, and UV-Vis analyses confirmed the presence of AgNPs in the alginate network. Antifungal testing showed that the Alginate - Ag nanocomposite prevented mold growth. AgNPs in all samples, both free and embedded with an alginate matrix, reduced fungal growth more effectively than the control. Along with the eco-friendliness and the biocompatibility of the alginate substrate, the Alginate - Ag showed a strong potential for application in food packaging.

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TỔ HỢP VẬT LIỆU NANOCOMPOSITE ALGINATE – HẠT NANO BẠC: CHẾ TẠO VÀ ỨNG DỤNG KHÁNG NẤM

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TÓM TẮT

Tổ hợp vật liệu nanocomposite Alginate – Bạc được nghiên cứu và chế tạo bằng cách tích hợp các hạt nano bạc vào trong ma trận polymer alginate, từ đó hình thành một cấu trúc vật liệu nanocomposite đồng nhất. Bề mặt cấu trúc hình thái của tổ hợp nanocomposite được khảo sát bằng kính hiển vi điện tử quét (SEM). Đồng thời, các tính chất quang cũng được tiến hành đánh giá bằng phép đo UV-Vis, PL. Từ ảnh SEM cho thấy AgNPs đã được phân tán vào trong ma trận polymer Alginate, kích thước hạt từ 7 – 66 nm và có kích thước hạt trung bình khoảng 20,8 nm, đồng thời có xu hướng tụ thành các cụm nhỏ. Phổ hấp thụ UV-Vis cho thấy đỉnh hấp thụ đặc trưng tại 410 nm. Đáng chú ý, nanocomposite Alginate – Ag thể hiện khả năng kháng nấm mốc *A.niger* tốt với tỉ lệ ức chế đạt 73,01% và 87.41% lần lượt đối với Alginate – Ag 100ppm và 200 ppm.

Từ khóa: Alginate, hạt nano bạc (Ag NPs), nanocomposite, polymer sinh học, kháng nấm.

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Lĩnh vực nghiên cứu: Cảm biến quang học, Vật lý sinh học, Quang học vật liệu...

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Lĩnh vực nghiên cứu: Vật lý sinh học, Quang học vật liệu, Quang học...

Phạm Văn Thành sinh ngày 06/12/2002 tại Hưng Yên. Ông tốt nghiệp Cử nhân ngành Vật lý học tại khoa Vật lý, trường Đại học Khoa học Tự nhiên, ĐH Quốc Gia Hà Nội năm 2024. Hiện nay, ông đang là nghiên cứu sinh chuyên ngành Quang học, Vật lý tại khoa Vật lý, trường Đại học Khoa học Tự nhiên, ĐH Quốc Gia Hà Nội. Ông đang công tác tại Trung Tâm Đổi Mới Sáng Tạo Về Công Nghệ Chất Mềm, Trung tâm Nghiên cứu và Phát triển Công nghệ cao, Viện Hàn lâm Khoa học và Công nghệ Việt Nam.

Lĩnh vực nghiên cứu: Cảm biến quang học, Vật lý sinh học, Quang học vật liệu,…

Nguyễn Thị Ngọc Hân sinh ngày 19/04/2003 tại Quảng Ninh. Bà tốt nghiệp Cử nhân ngành Vật lý học tại khoa Vật lý, trường Đại học Khoa học Tự Nhiên, ĐH Quốc Gia Hà Nội năm 2025. Hiện nay, bà đang là học viên cao học chuyên ngành Vật lý Chất rắn tại khoa Vật lý, trường Đại học Sư phạm Hà Nội 1.

Lĩnh vực nghiên cứu: Cảm biến quang học, Vi laser, Quang học vật liệu.

Nguyễn Duy Thiện sinh ngày 30/03/1986 tại Bắc Ninh. Ông tốt nghiệp cử nhân ngành Vật lý học năm 2008, thạc sĩ chuyên ngành Vật lý chất rắn năm 2012, Tiến sĩ ngành Vật lý học năm 2021 tại Trường Đại học Đại học Khoa học Tư nhiên, Đại học Quốc gia Hà Nội. Ông công tác tại Khoa Vật lý, Đại học Khoa học Tư nhiên, Đại học Quốc gia Hà Nội từ năm 2008.

Lĩnh vực nghiên cứu: Vật lý, Vật liệu nano, cảm biến.

Bùi Thị Trang sinh ngày 15/6/1992 tại Thanh Hoá. Bà tốt nghiệp kĩ sư chuyên nghành Công Nghệ Sinh Học, Đại học mở Hà Nội năm 2014, tốt nghiệp Thạc sĩ hệ Khoa Học, chuyên ngành Công Nghệ Sinh Học năm 2017 tại trường Đại học Bách Khoa Hà Nội. Hiện nay, bà đang công tác tại Trung Tâm Đổi Mới Sáng Tạo Về Công Nghệ Chất Mềm, Trung tâm Nghiên cứu và Phát triển Công nghệ cao, Viện Hàn lâm Khoa học và Công nghệ Việt Nam (VAST)

Lĩnh vực nghiên cứu: Công nghệ sinh học, An toàn vệ sinh thực phẩm,...

Nguyễn Hoàng Dương sinh ngày 16/02/1980 tại Hà Nội. Ông tốt nghiệp cử nhân ngành Vật lý quang năm 2003 tại Đại học Arizona, Hoa Kỳ. Ông nhận học vị Tiến sĩ tại Trường Đại học Colorado, Hoa Kỳ với chuyên ngành Vật lý chất mềm vào năm 2011 và Tiến sĩ chuyên ngành Thương mại hóa sản phẩm nghiên cứu khoa học tại Tổ chức Nghiên cứu Khoa học và Công nghiệp Liên Bang Úc (CSIRO). Hiện tại, ông là giám đốc Trung Tâm Đổi Mới Sáng Tạo Về Công Nghệ Chất Mềm, Trung tâm Nghiên cứu và Phát triển Công nghệ cao, Viện Hàn lâm Khoa học và Công nghệ Việt Nam (VAST) và Phó trưởng ban Ứng dụng và Triển khai công nghệ, VAST.

Lĩnh vực nghiên cứu: Vật lý chất mềm, Vật lý quang,...

Mai Hồng Hạnh sinh ngày 27/11/1984 tại Hà Nội. Bà tốt nghiệp Cử nhân năm 2006 và Thạc sĩ năm 2008 chuyên ngành Quang học, Vật lý tại Khoa Vật lý, trường Đại học Khoa học Tự nhiên, ĐH Quốc Gia Hà Nội. Bà nhận học vị Tiến sĩ chuyên ngành Quang học, Công nghệ Nano năm 2012 tại ĐH Kassel, CHLB Đức và được công nhận chức danh Phó giáo sư năm 2020. Hiện nay, bà đang công tác tại Khoa Điện tử viễn thông, ĐH Công nghệ, ĐH Quốc Gia Hà Nội.

Lĩnh vực nghiên cứu: Cảm biến quang học, Vi laser, Quang học vật liệu…