

Biosynthesis of Reduced graphene oxide nanoparticles from Uropathogenic *K. oxytoca*

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ABSTRACT

With advantages over physical and chemical methods from an economic and environmental standpoint, bioproduction possibilities for nanoparticles are becoming a very important topic. The current study's objective is to synthesis of reduced graphene oxide nanoparticles from Klebsiella oxytoca that cause urinary tract infections and characterized the synthesized biogenic nanoparticles by different method for characterization include Fourier transform infrared, energy dispersive spectroscopy, and scanning electron microscopy.

Keywords: Biosynthesis , Reduced graphene oxide , *Klebsiella oxytoca*

Article Information

Received: January 20, 2023; Revised: February 26, 2023; Online: April, 2023

INTRODUCTION

Nanobiotechnology integrates biological concepts with physical and chemical processes to create nanoparticles with specialized functions at a cheaper cost than physical or chemical methods. Based on basic features such as size, distribution, and shape, nanoparticles have new or changed properties. The number of novels uses for NPs and nanomaterials is steadily rising [1]. Nanoparticles can be hollow or solid, and they can be formed of a variety of materials in a variety of layers, each with its function. A core functioning layer, a protective layer, and an outside layer that permits interaction with the biological environment are generally present [2]. The core functional layer usually has some beneficial magnetic or optical activity, with fluorescence being the most frequent. The functional layer is protected from chemical damage caused by water, air, or cell components by the protective layer. as well as any harmful characteristics of the functional layer's chemicals. Nanoparticles can be identified using the outer layer [3].

In the production of NPS, Redox membrane proteins are used by bacteria and fungus for surface synthesis, and extracellular enzymes are used for extracellular synthesis [4]. Synthesizing NPS utilizing natural mechanisms such as carbohydrates, Microorganisms, biodegradable polymers, polysaccharides, vitamins, enzymes from microbes, and biological systems is one approach to achieve this aim. The manufacture of NPs by bacteria is one method with a lot of promise [5]. Recent research has been centered on the creation of a controlled and scalable method for the biosynthesis of monodispersed and extremely stable NPS. As a result, several bacterial species have been exploited in green nanotechnology to research alternate NP manufacturing processes. [6]. GO is a nanomaterial that has been utilized in a variety of applications for more than 150 years [7]. It is the forerunner of graphene, a two-dimensional carbon allotrope that is one of the best in the world. Electrical conductivity [8], thermal properties [9], transparency [10] and mechanical strength [11].

Graphene has been used in medicine in recent years, notably for DNA sequencing [12], biosensor creation, and cell differentiation and proliferation [13]. Its functional derivative, GO, has distinct characteristics that make it better suited for biological applications. It stands out for its capacity to disperse in a variety of solvents, making it simpler to handle. [14]. GO is also utilized to

deliver anti-cancer medications to living things. [15], as well as Aptamers for ATP probing and gene transfer in mouse epithelial cells [16]. The antibacterial activity of GO was investigated by looking for an inhibitory zone around the GO disc, which showed bacterial toxicity against *Staphylococcus aureus* and *Escherichia coli* [17].

MATERIALS AND METHODS

Strain of *K.oxytoca* isolated from UTI and diagnosed by VITEK-2 Compact system was 24 hours of culture at 37 degrees Celsius in brain-heart infusion broth. Following incubation, after 15 minutes of 10,000 rpm centrifugation to separate the precipitate from the supernatant was applied to an industrial Graphene Oxide powder for rGO preparation. The effectiveness of nanoparticles against *E. coli* was assessed using the Muller Hinton agar well diffusion test, which was incubated for 24 hours at 37°C. *Klebsiella oxytoca* was chosen for making Reduced Graphene Oxide nanoparticles because of its bactericidal efficiency, color shift, and spectrum of absorption [18].

LARGE SCALE PREPARATION OF REDUCED GRAPHENE OXIDE NANOPARTICLES USING *K.OXYTOCA*.

Klebsiella oxytoca may both extracellularly and intracellularly biosynthesize nanoparticles. This method was chosen due to the advantages of external synthesizing over intracellular biosynthesis. There are several advantages to extracellular biosynthesis, including a simpler, less artifact-prone approach [19]. *K.oxytoca* was grown in brain heart infusion broth for 24

hours at 37 °C. The precipitate was separated from the colloidal suspension after incubation by centrifuging it for 15 minutes at 10,000 rpm; the supernatant was then used to make nanoparticles. Graphene Oxide was added to the supernatant of *K.oxytoca* at an intensity of 2.4 mg/ml for the preparation of Reduced Graphene Oxide, then the two flasks were incubated , This was repeated at different times [20].

RESULTS AND DISCUSSION

K.oxytoca was employed in the manufacture of rGO nanoparticles, used cell-free supernatant and graphene oxide as a substrate under previously optimized conditions to demonstrate extracellular biosynthesis. Following incubation, the color change and antibacterial behavior of the reaction mixture from light to black served as evidence that *K. oxytoca* had produced the rGO NPs. as appear in Figure 1.

Nanoparticles have distinctive physical and chemical properties as a result of such phenomena as the quantum size effect, the micro size effect, the surface effect, and the macro-quantum tunnel effect. [21]. However, when nanoparticles are swallowed or applied in various ways, there is still considerable concern about human health. Micro-emulsion, electrospray, solvent diffusion during emulsification, and ionic gelation, and biological methods are some of the ways used to make NPs [22].

Because of its repeatability and versatility, the biological technique was chosen. This process is very controlled, allowing for a degree of control over the produced particles' size and surface charge [23]. The utilization of biological systems for manufacturing is still in its early stages, and scientists have been using on microorganisms as prospective eco-friendly nano factories [24]



Figure 1: Biosynthesis of rGO Nanoparticles in BHIB from *K. oxytoca*, the color shift from yellow to black indicate biosynthesis of rGO at optimum conditions (37°C for 24h in shaking incubator with 150rpm).

SCANNING ELECTRON MICROSCOPY ANALYSIS (SEM)

The result of SEM shows the biogenic rGO nanoparticles have spherical shape and the size range between (35-85 nm), the average diameter was 49.31 nm as shown in Figure 2. the rGO sheets helped create a special microstructure with microscale roughness on the film surface. Similar very porous microstructures with a dense network of interconnected pores were visible in all of the materials' SEM pictures. rGO was employed to alter the matrix, the exfoliated nanosheets' interaction with one another increased the diameter of the pores and the thickness of the wall. It should be highlighted that this roughness may improve wettability and enable cell adherence, taking into account prospective applications of the material in tissue engineering [25].

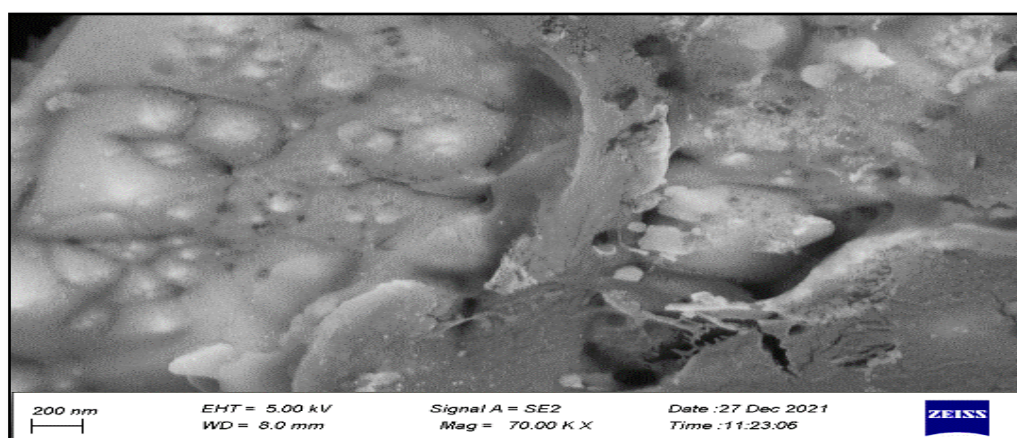


Figure 2: SEM micrograph of Biogenic rGO NPs showed spherical well dispersed with size (35-85nm) and the average diameter was 49.31 nm 200nm.

ENERGY DISPERSIVE SPECTROSCOPY ANALYSIS (EDS)

By examining the optical absorption peaks of elements, Energy Dispersive Spectroscopy (point and mapping analysis) was performed to quantify the existence of rGO. Elemental analysis revealed

that components in reduced graphene oxide nanoparticles have carbon, oxygen and arsenic figure 3. The carbon, oxygen, nitrogen, phosphate, and chloride contents in the formulations created were quantified and qualitatively analyzed using energy dispersive spectroscopy analysis.

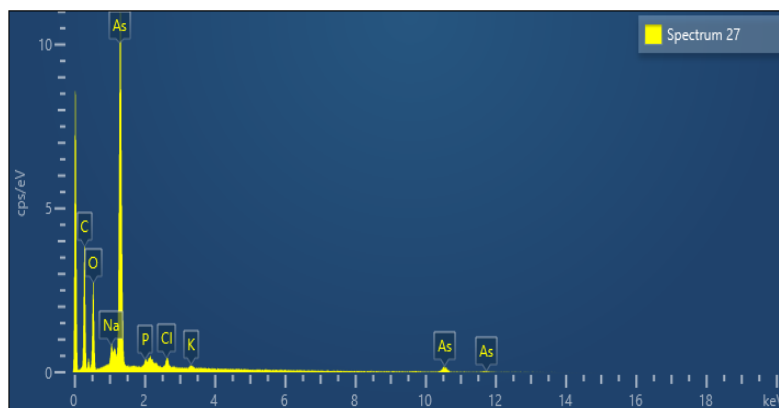


FIGURE 3: EDS Analysis of Biogenic rGO Nanoparticles

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

An FTIR spectrometer was employed. to acquire the Fourier transform infrared (FTIR) spectra of biogenic rGO nanoparticles in order to inspect the complex surface processes of rGO. Table 1 showed the presence of bands at different position, each peak refers to active group in the compound for rGO nanoparticles. The table 1 showed the band's presence at 3421.15 cm⁻¹ due to overlap of (N-H stretching amide binding carbonyl stretch protein), 1643.27 cm⁻¹ overlap (N=H stretching), 1401.82 cm⁻¹ (CH₃ deformation in amide group) 1239.16 cm⁻¹ (C-O-C stretching), 1051.60 cm⁻¹ (C-C stretching), 799.59 cm⁻¹, 620.12 cm⁻¹ (N-H amines, both primary and secondary).

TABLE 1: FTIR CHARACTERIZATION SHOW PEAK NUMBER OF EACH ACTIVE GROUP

Peak NO	X(cm-1)	Y(%T)
1	3421.15	82.70
2	1643.27	84.17
3	1543.91	88.22
4	1400.75	90.12
5	1253.58	92.98
6	1050.25	89.69
7	800.14	41.18
8	603.85	91.17

9	480.16	80.29
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CONCLUSIONS

The isolated *K. oxytoca* from urinary tract infections have the ability to biosynthesized of reduced graphene oxide nanoparticles with size range from 35-85 nm and average diameter was 49.31 , the EDS determine the elemental analysis of rGO that contained carbon, oxygen, nitrogen, phosphate, and chloride and many active group detected by FTIR technique .

ACKNOWLEDGEMENTS

Thank you to every one helped the research team for complete the study research.

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