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RESEARCH ARTICLE

Extracellular Biosynthesis of TiO_2 Nanoparticles Using Supernatant Culture of *Lactobacillus crispatus*

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Abstract

In this study, titanium dioxide nanoparticles were synthesized biologically using isolated *L.crispatus* from vagina of healthy women. The formation of TiO_2 nanoparticles was characterized by atomic force microscope (AFM) using supernatant culture of *L.crispatus* .Extracellular biosynthesized TiO_2 nanoparticles occurred with the average size (87.88) nm. For further characterization, the x-ray diffraction (XRD), energy dispersive x –ray spectroscopy (EDX), and scanning electron microscope (SEM) were used. XRD Confirmed the formation of crystallographic plane of anatase of TiO_2 nanoparticles indicating that nanoparticles corresponded to anatase crystalline, while the EDX analysis of TiO_2 nanoparticles indicated that the synthesized nanoparticles were anatase form and not frutile or brookite form. The SEM images of the synthesized TiO_2 nanoparticles were showed spherical or oval in shape. The effect of independent variables, including different incubation times and storage condition on the biosynthesis of TiO_2 nanoparticles was investigated. The average size (70.98)nm of TiO_2 nanoparticle was observed after 72 h .The stability of the synthesized TiO_2 nanoparticles for (1,2,3)months under cooling condition was studied and the result revealed that the synthesized TiO_2 nanoparticle remained stable without change in color even after storage for three months under cooling conditions.

Keywords: *TiO_2 nanoparticles; Biosynthesis; Lactobacillus Crispatus; Incubation time; Storage time.*

Introduction

In the past two decades, the synthesis of metal nanoparticles using microorganisms received great interest due to their optical, chemical, photoelectrical and electronic properties. The microorganisms are used as possible "nanofactories" for development of clean, nontoxic and environmentally friendly methods for producing nanoparticles [1].Metal nanoparticles have various functions that are not observed in bulk phase [2].Extracellular production of metal nanoparticles has more commercial applications in various fields.

Since the polydispersity is the major concern, it is important to optimize the conditions for monodispersity in biological approaches [3].Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the elemental through enzymes generated by the cell activities[4].The microorganisms such as Lactobacillus sp. and Saccharomyces cerevisiae are used for the synthesis of titanium dioxide (TiO_2) nanoparticles [5].Biologically synthesized nanoparticles have wide application viz., biosensors, bio labelling, in cancer therapeutic and in coating of medical appliances [6] TiO_2 nanoparticles are considered to be among the best photocatalytic materials due to their long-term thermodynamic stability, strong oxidizing power, and relative non-toxicity[7].

TiO_2, therefore is a versatile material that has applications in various products such as paint pigments, sunscreen lotion, electrochemical electrodes, capacitors, solar cells, and even as a food coloring agent [8].

So, far only few reports are available on the biosynthesis of TiO_2 nanoparticles using supernatant cultures of *Lactobacillus crispatus* and the effect of different incubation, storage times on TiO_2 nanoparticles size are also compared.

Material and Methods

Lactobacillus crispatus

Lactobacillus crispatus isolated from vagina of Iraqi healthy women have been selected as the best isolate among seventy five isolates of Lactobacillus spp. Isolated and identified during another study (data not show)

Biosynthesis of TiO_2 nanoparticles using Supernatant of bacteria

Flask containing 100 ml sterilized MRS broth were inoculated with 2% of a fresh culture of *L.crispatus*. The flasks were incubated at 37C° for 24 h. After incubation, the cultures were centrifuged at 12,000 rpm for 5min ,and then their supernatants were filtered by Millipore filter paper (0.22 µm), 20 ml of supernatants were added to a flask containing 10 ml of (0.025M) TiO_2 solution and was stirred for half an hour by a magnetic stirrer then incubated at 37C° for (24,48,72) h. The second flask contained supernatant only to be used as blank .Change in color was observed and production of sediment were observed as the primary detection of extracellular produced TiO_2 nanoparticles [9].

Characterization of Biosynthesized TiO_2nanoparticles

Samples of biosynthesized nanoparticles (prepared by drying sediment at room temperature) were characterized after 72 h of incubation. The formation of metal oxide TiO_2 nanoparticles was confirmed by Atomic Force Microscopy (AFM), X-Ray Diffraction (XRD) technique, Energy-dispersive X-ray spectroscopy (EDX) and Scanning Electron Microscopic (SEM).

Effect of Incubation Time on the Size of Biosynthesized TiO_2 nanoparticles

Three flasks were used, each flask were filled with 40 ml of MRS broth, then 20 ml of TiO_2 solution(0.025M) were added to the first and second flask respectively, and both were stirred for half hour by a magnetic stirrer while the third flask contained MRS broth only. *L. crispatus* was cultured in a first and third flask and incubated anaerobically at 37C° for periods of 24, 48, 72 h, separately. After incubation, the size of biosynthesized TiO_2 nanoparticles was analyzed and the best incubation time that gave the smallest size was characterized.

Effect of Storage Time on TiO_2nanoparticles Stability

Flask containing 40 ml sterilized MRS broth and 20 ml of TiO_2 solution (0.025M) and stirred for half an hour by a magnetic stirrer then inoculated (2%) with a fresh culture of *L.crispatus*. The culture was incubated at 37C° for 72 h. After incubation the culture flask was kept at 4 C° for 3 months, and then the stability of color was optically observed.

Results and Discussion

Nanotechnology is the prompt emerging discipline in the field of life science.Biologists are highly interested in synthesizing bio-nanoparticles using many of the precious metals . A comprehensive study of the rapid extracellular biosynthesis of TiO_2 nanoparticles from vagina isolate were carried out in this research work. Supernatant of *L. crispatus* was applied and incubated with titanium dioxide solution for 72h.

It exhibited a color change from light to dark brown, which was considered as a primary detection for TiO_2 nanoparticles production. Then the sample was analysed using AFM to determine nanoparticles average size. Results of AFM analysis showed that the average size of synthesized TiO_2 nanoparticles was (87.88) nm (Fig 1).Azhar *et al* [10].

Found that extracellular *Lactobacillus*-mediated biosynthesis of titanium nanoparticles in MRS-broth medium occurred an aerobically after 72h.With nanoparticle size (150) nm. Jayaseelan *et al* [11].Found out that carboxylic acid group which is present in secondary metabolites (broth extract) played an important role for forming TiO_2 nanoparticles. Another study done by Chaudhari *et. al.* [12].

Found the supernatant culture of *Lactobacillus* spp. isolated from VIZYLAC capsule produced extracellular silver nanoparticles. In addition Sarvamangala et al. [13].

Observed that *Lactobacillus bulgaricus* was capable of synthesizing Ag nanoparticles extracellularly using cell free aqueous filtrates, while Saravanan et al [14].Reported biosynthesized Ag nanoparticles extracellular occurred by *Lactobacillus delbrueckii* which secreted hydrolytic enzymes in supernatant. Many hydrolytic enzymes have amino, carboxyl and sulfhydryl group were reduced metal ions to nanoparticles [15]. Extracellular enzyme which acts as electron shuttle or capping agents produced by microorganisms reduced metal ions to nanoparticles [16]. According to Bansal et al [17].Exhibited that extracellular protein mediated hydrolysis of anionic complexes synthesized crystalline titanium nanoparticles.

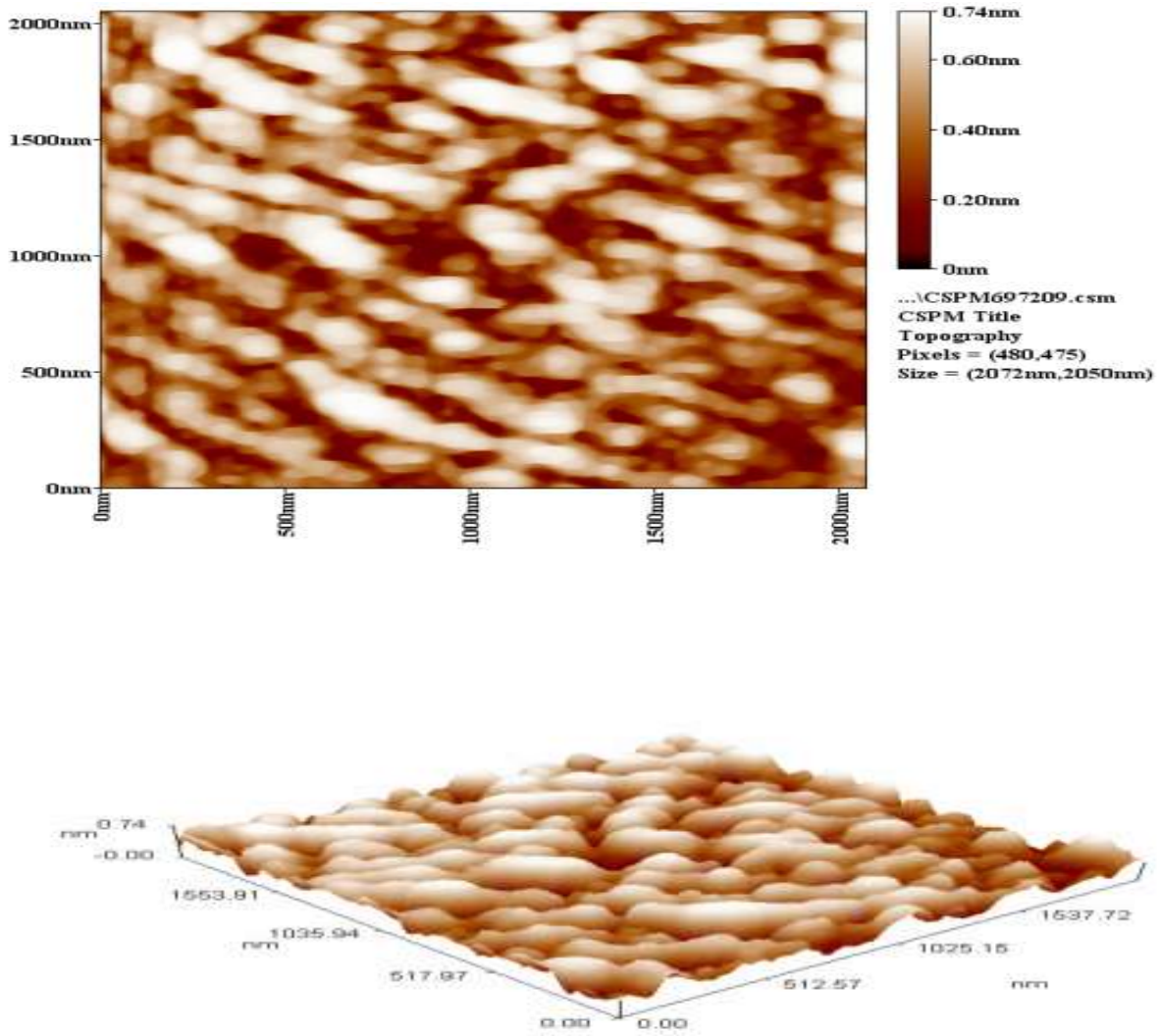

Fig. 1: Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by Lactobacillus crispatus supernatant, Surface and three Dimensional views

For further characterization the X-ray diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDX), and Scanning electron microscope (SEM) were used. The XRD pattern of the sample showed the presence of peaks (2θ=25.3, 37.9, 54 (anatase form)). The main peak of θ=25.3 in (Fig 2) matches the (101) crystallographic plane of anatase of TiO_2 nanoparticles, indicating that nanoparticles structure dominantly correspond to anatase crystalline [8], which is regarded as an attributive indicator of the biologically synthesized nanoparticles TiO_2 crystallites [18].TiO_2 is preferred in anatase form because of its high photo catalytic activity, since it has a higher potential energy of photo generated electrons, high specific area, non-toxic, photo chemically stable and relatively inexpensive [19]. The EDX pattern as shown in (Fig3) is demonstrating that titanium nanoparticles existing in the medium with elemental titanium and oxygen signals as strong peak while weaker signals from other elements like (Ca, Na, O, C, P, Cl, Al, Si, S,k) atoms are also recorded, indicating that TiO_2

nanoparticles produced were anatase form and not rutile or brookite form and expanding of the peak showed the small size of TiO_2 nanoparticles, this result agreed with Ahmad et al [20].

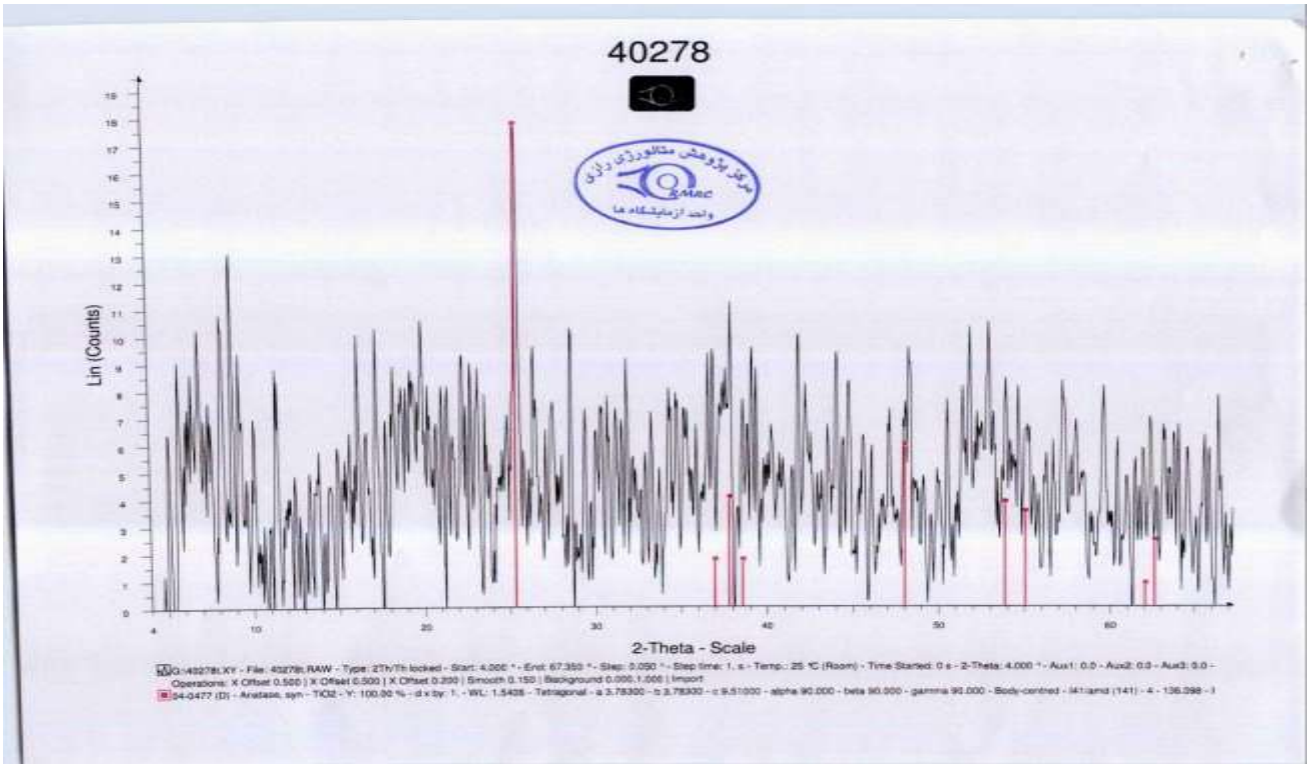

Fig. 2: X-ray diffraction patterns of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus*

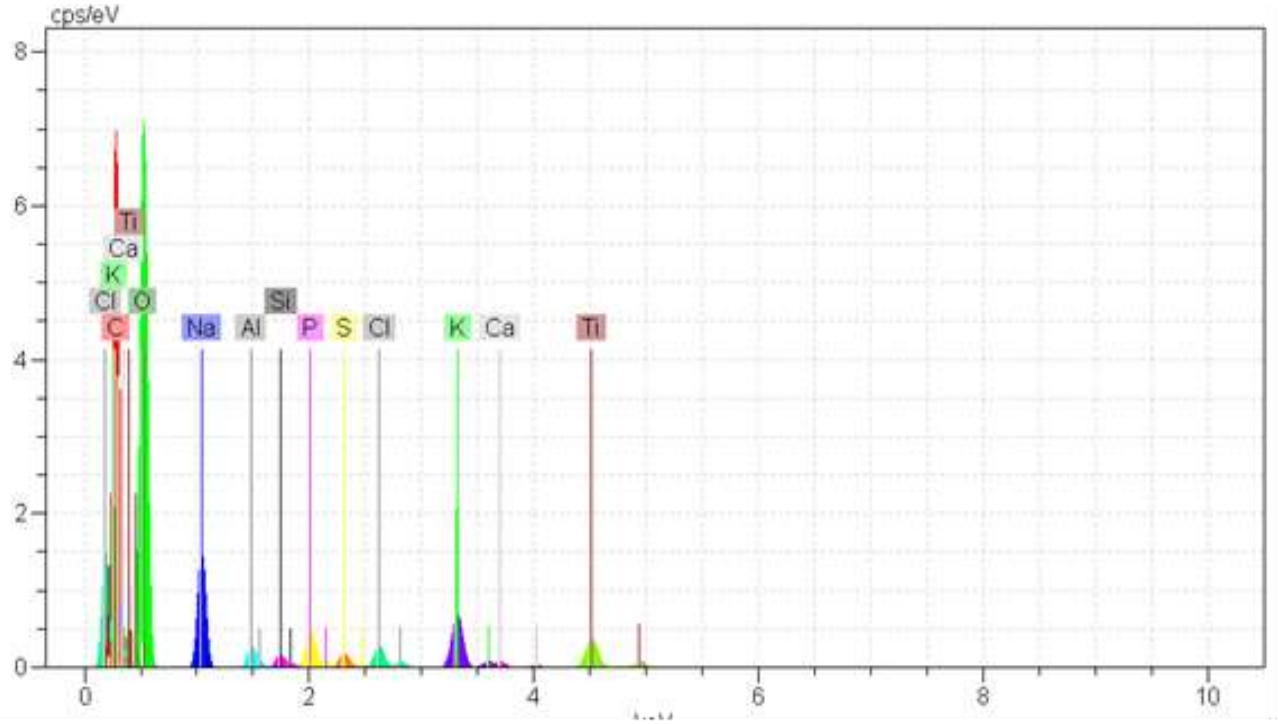

Fig. 3: Energy-dispersive X-ray spectroscopy (EDX) spectrum of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus*

The Scanning electron microscope (SEM) images of the synthesized TiO_2 nanoparticles have shown spherical, oval in shape (Fig 4), this result was corroborated by other studies reported by [21, 10] by using *Lactobacillus* spp. and similar result of TiO_2 nanoparticles shape was reported by using *Planomicrobium* spp. which was isolated from melted ice cream.The nanoparticles synthesized by biological methods have different shape and size, the explanation of the difference in size is due to the fact that TiO_2 nanoparticles are being formed at different times [22].

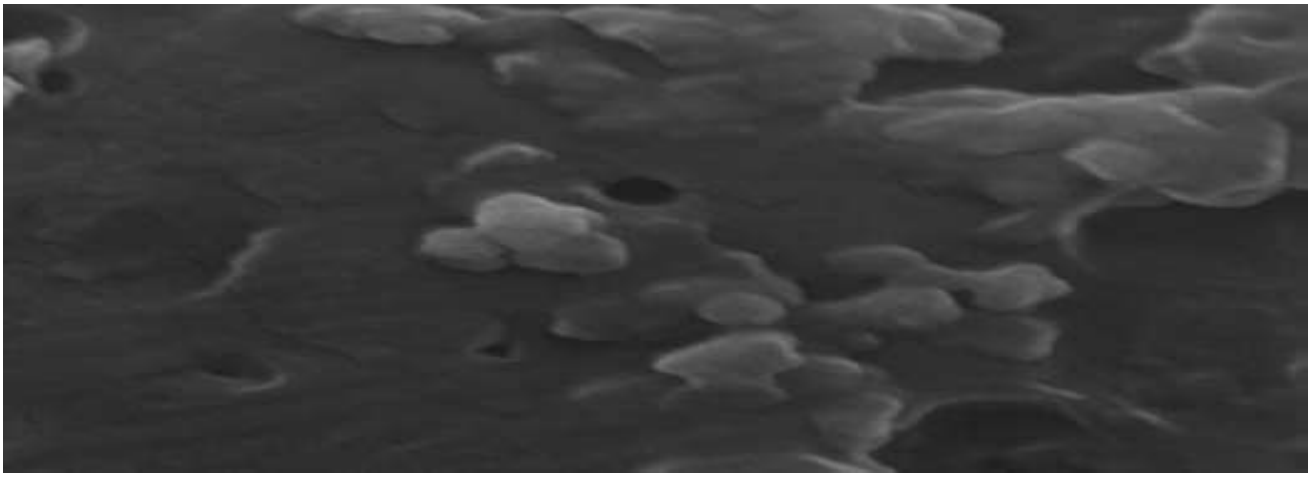

Fig.4: Scanning electron microscopic images of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus*

Effect of Incubation Times on Biosynthesis of TiO_2 nanoparticles using *Lactobacillus crispatus*

Our results showed that after incubation culture of *L. crispatus* with a titanium dioxide solution for (24,48,72)h. at 37C° under anaerobic condition, the color intensity increased with a period of incubation due to the reduction in TiO_2.Color change increased from light brown to dark with white sediments. The three samples were applied to AFM analysis, and the average size of particles for synthesized TiO_2 nanoparticles were (88.75, 81.51, 70.98) nm for (24, 48, 72) h. respectively (Fig5, 6, 7). Afreen and Vandana [23] found that absorption peaks increase at the incubation time (24-120) h. Other studies Kheradmand et al [24].Observed that after 24h. Of incubation with both *L.plantarum* and *L.johnsonii* the concentration of selenium was in the supernatant were considerably reduced and no amount of selenium was present in the culture after 96 h. Extracellular synthesis of highly stable silver nanoparticles occurred within 24, 48, and 72h.Of incubation [12].

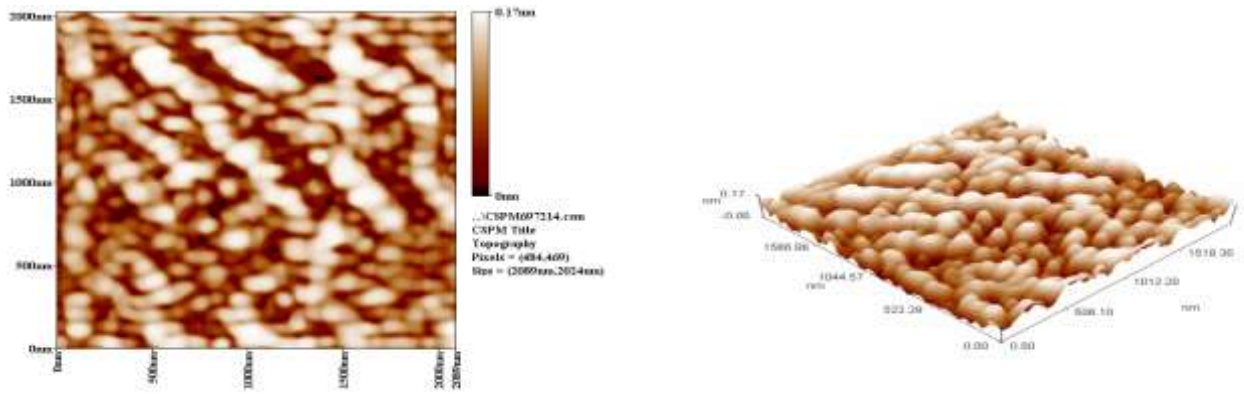

Fig.5: Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in MRS broth for 24 h. Surface and three Dimensional view

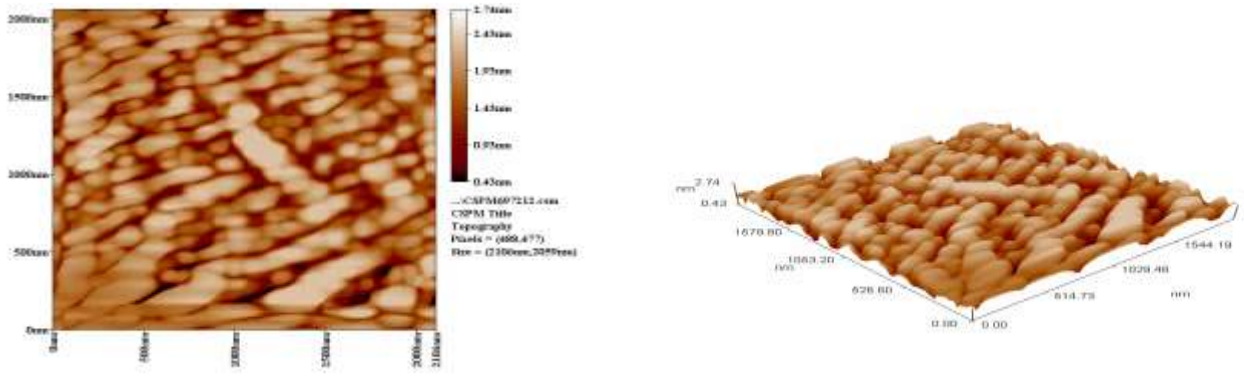

Fig. 6: Atomic Force Microscopy image of TiO_2 nanoparticle synthesized by *Lactobacillus crispatus* in MRS broth for 48 h. Surface and three Dimensional view

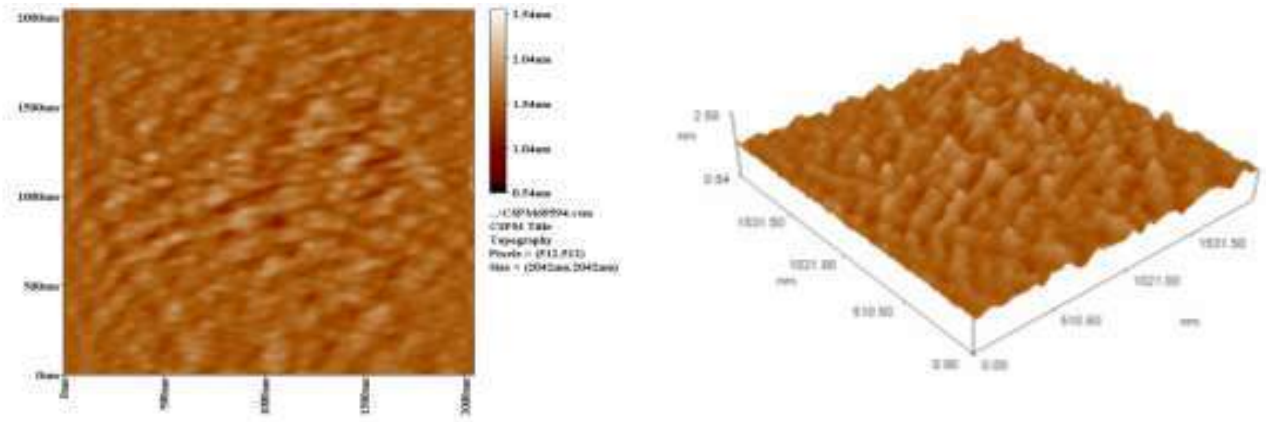

Fig.7: Atomic Force Microscopy image of TiO_2 nanoparticle synthesized by *Lactobacillus crispatus* in MRS broth for 72 h, Surface and three Dimensional views

Effect of Storage Time on TiO_2 Nanoparticles Stability

The TiO_2 nanoparticles suspensions were stored for 1, 2, 3 months under cooling condition, results revealed that the stability of the synthesized TiO_2 nanoparticles produced by *L.crispatus* remained stable without change in color. Raliya and Tarafdar [25] observed that TiO_2 nanoparticles could be stabilized after 105 days and used up for more applications.

Nanoparticles produced by microorganism observed highly stability over a long time (more than 6 months) with no sign of aggregation occurred even at the end of this period due to the presence of proteins which act as capping agents secreted by many microorganisms responsible for stability of nanoparticles, while nanoparticles produced chemically was aggregated together in a liquid media causing surface area reduction of nanoparticles [16]. Zouhir et al [26].Showed that TiO_2 nanoparticles were stable in suspension when the protein is present in culture medium, it has been approved that the amines linkages of proteins have the stronger ability to bind metal ions. The proteins may form a coat covering the metal nanoparticles to avert agglomeration of the particles and stabilizing in the culture media [26, 27].

Conclusion

The suitable conditions to synthesize TiO_2 nanoparticles by *L.crispatus* occurred in MRS broth at 37C° for 72 h. under anaerobic conditions .Extracellular biosynthesized TiO_2 nanoparticles occurred with the average size of (87.88)nm by using a supernatant culture of *L. crispatus* .The synthesized TiO_2 nanoparticles were characterized as anatase form with spherical, oval in shape with average size (88.75,81.51,70.98)nm for (24,48,72)h. respectively. The synthesized TiO_2 nanoparticles by *L.crispatus* remained stable without change in color occurred after storage for three months under cooling condition.

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