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RESEARCH ARTICLE

Effect of culture media on biosynthesis of titanium dioxide nanoparticles using *Lactobacillus crispatus*

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Abstract

The present study included the rapid synthesis of Titanium dioxide nanoparticles using *Lactobacillus crispatus* and focus on the effect of different culture media (MRS broth,JKH_1 broth and Whey solution) on TiO_2 nanoparticles synthesis . The synthesized nanoparticles were characterized using X-ray diffraction (XRD) technique, Atomic Force Microscopy(AFM) and Scanning electron microscopic(SEM). The XRD pattern of the sample showed the presence of main peak of θ=25.3 matches the (101) crystallographic plane of anatase of TiO_2 nanoparticles .The morphological characteristics were found to be spherical, oval in shape, have the average size (70.98 ,116.89, 113.67)nm synthesized in MRS broth, JKH_1 broth and whey solution respectively. Adding of 10% glucose to JKH_1 broth and Whey solution lead up to decreasing the average size of synthesized nanoparticles reached to (92.65 and 112.26)nm for JKH_1 broth and whey solution respectively.

INTRODUCTION

Presently,there are chemical, physical and biological(including the use of microorganisms) routes available for the synthesis of metal oxide nanoparticles(Ahmad *et al*.,2013). The chemical and physical synthesis of nanoparticles is expensive and often involves the use of toxic, hazardous chemicals which may pose environmental risks (Malarkodi *et al*.,2013). Biological methods have been put ahead to be advantageous over other synthetic methods as they are cost effective and do not involve the use of toxic chemicals, high pressure, energy and temperatures (Tripathy *et al.*,2010 ;Malarkodi *et al.*,2013).The biological method for nanoparticle production is simple, eco-friendly and allows for getting controlled nanoparticles which can be used as catalysts with specific composition, which cannot be synthesized by classical methods , applications in sensors and medicine are envisaged and the nanoparticles synthesized in the bacteria can be used against the human pathogens(Popecu *et al*.,2010).The microorganisms are used as possible “nanofactories” for development of clean, nontoxic and environmentally friendly methods for producing nanoparticles(Klaus-Joerger *et al*.,2001). Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the elemental metal through enzymes generated by the cell activities (Li *et al.*,2011).The microorganisms such as *Lactobacillus* sp. and *Saccharomyces cerevisae* are used for the synthesis of titanium dioxide (TiO_2) nanoparticles(Jha *et al*.,2009).

Biologically synthesized nanoparticles have wide application viz., biosensors ,biolabelling, in cancer therapeutic and in coating of medical appliances(Prakash *et al*.,2010). The oxide nanoparticles synthesized by several methods appear more and more useful, because these nanoparticles have good electrical, optical and magnetic properties that are different from their bulk counterparts(Sagadevan,2013). The TiO_2 nanoparticles may be one of the most important materials for photocatalysts , cosmetics, and pharmaceuticals(Kirithi *et al*.,2011).TiO_2, therefore is a versatile material that has applications in various products such as paint pigments, sunscreen lotions, electrochemical electrodes, capacitors, solar cells, and even as a food coloring agent and in toothpastes (Byranvand *et al*.,2013). So, far only few reports are available on the biosynthesis of TiO_2 nanoparticles using Lactobacillus sp. Thus in this paper, we report on the synthesis of TiO_2 nanoparticles using *Lactobacillus crispatus* and the effect of different culture media on TiO_2 nanoparticles synthesis 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).

-Synthesis of titanium nanoparticles using *Lactobacillus crispatus*:

A-Using MRS broth

Three flasks were used, each flasks were filled with 40 ml of MRS(De-Man Rogosa Sharpe) broth . Then 20 ml of TiO_2(0.025m) were added to the first and second flask respectively and both were stirred for half hour on a magnetic stirrer while the third flask contain MRS broth only. Final concentration ultimately would be equivalent. *Lactobacillus crispatus* was cultured in first and third flask into CO_2-incubator at $37C^o$ for (24,48,72) hours. Second flask was used as blank for first one , the change in color from light brown to dark brown observed and production of sediment will observed as primary detection of produced TiO2 nanoparticles(Azhar *et al*.,2011).

B-Using JKH_1 broth

Three flasks were used, each flasks were filled with 40 ml of JKH_1(Jehan-Khawlah 1)broth which prepared from Gourd juice, the clarified juice was collected by coarse filtration (Whatman 40), then the $_pH$ was adjusted to 6.2 , autoclaved for 10 min.(Salman and khalaf , 2014). Then 20 ml of TiO_2(0.025m) were added to the first and second flask respectively and both were stirred for half hour on a magnetic stirrer while the third flask contain JKH_1 broth only. *Lactobacillus crispatus* was cultured in first and third flask into CO_2-incubator at $37C^o$ for (24,48,72) hours. Second flask was used as blank for first one , the change in color observed and production of sediment was observed as primary detection of produced TiO_2 nanoparticles. For another experiment 10% glucose was added to JKH_1 broth and the same procedure for TiO_2 nanoparticles synthesis was done.

C-Using Whey solution

In a typical procedure of nanoparticles synthesis, *Lactobacillus crispatus* inoculated into sterilized 250 ml of whole milk in 500 ml flask and incubated for curdling at 37°C for 24 hours. The whey was collected by coarse filtration (Whatman 40). The filtrate was pale yellow in appearance(Ranganath *et al*.,2012).Three flasks were filled with 40 ml whey solution,then 20 ml of TiO_2(0.025m) were added to the first and second flask respectively and both were stirred for half hour by magnetic stirrer while the third flask contain whey only. *Lactobacillus crispatus* was cultured in first and third flask into CO_2-incubator at $37C^o$ for (24,48,72) hours. Second flask was used as blank for first one , the change in color observed and production of sediment was observed as primary detection of produced TiO_2 nanoparticles. For another experiment 10% glucose was added to whey solution and the same procedure for TiO_2 nanoparticles synthesis was done.

Characterization of TiO_2nanoparticles

Samples of synthesized nanoparticles were characterized after 72 hours of incubation.The formation of metal oxide TiO_2 nanoparticles was confirmed by X-ray diffraction (XRD) technique, Atomic Force Microscopy(AFM) and Scanning electron microscopic(SEM).

Results and Discusion

In the present study *Lactobacillus crispatus* confirmed as positive for biosynthesis of TiO_2 nanoparticles in different media included MRS broth, JKH_1 broth and Whey solution (with and without glucose). For all samples, solution color changed from light to dark and sediment was observed.

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(Fig.1) matches the (101) crystallographic plane of anatase of TiO_2 nanoparticles, indicating that nanoparticles structure dominantly correspond to anatase crystalline (Byranvand *et al*.,2013),which is regarded as an attributive indicator of the biologically synthesized nanoparticles TiO_2 crystallites(Kirithi *et al*.,2011). TiO_2 is preferred in anatase form because of its high photocatalyticactivity, since it has a higher potential energy of photogenerated electrons, high specific area, non-toxic, photochemically stable and relatively inexpensive(Macwan *et al*.,2011).

The Scanning electron microscope (SEM) images of the synthesized TiO_2 nanoparticles have shown spherical, oval in shape (Fig.2 A and B). Similar result of the TiO_2 nanoparticles shape was reported by using *Lactobacillus* sp.(Jha *et al*.,2009.,Azhar *et al*.,2011).

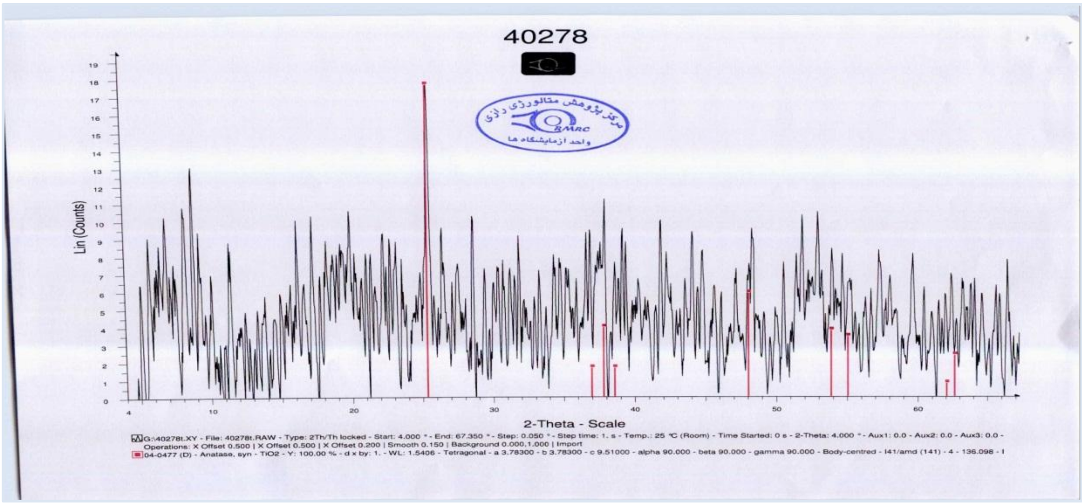
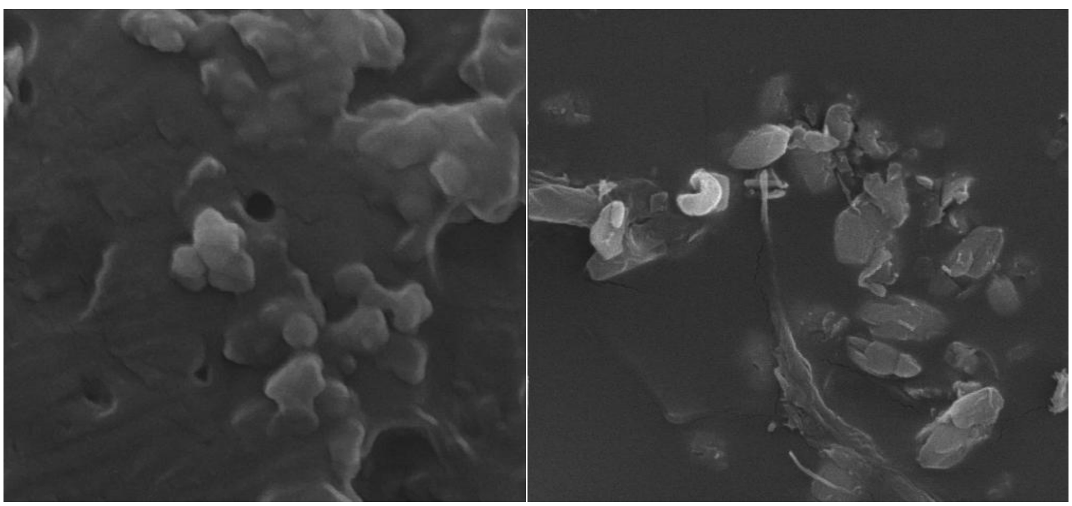


Fig.(1) :X-ray diffraction patterns of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus*

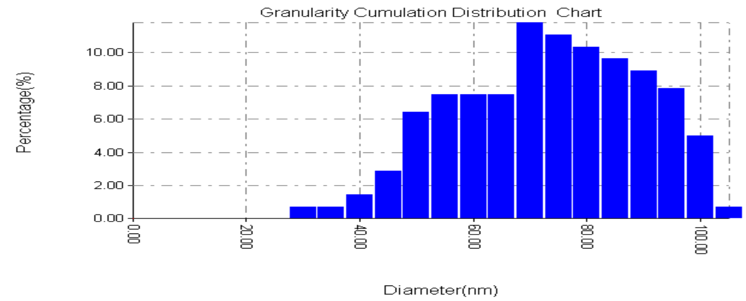


A **B**

Fig.(2): (A and B) Scanning electron microscopic images of the of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus*

The synthesized TiO_2 nanoparticles were characterized by AFM to view the nanoparticles both in surface and three Dimensional view , and found the average size of particles (70.98 ,116.89, 113.67)nm (Fig.3,4,5) when MRS broth,JKH_1broth and whey solution were used respectively . Adding of 10% glucose to JKH_1 broth and Whey solution lead up to decreasing the average size of synthesized nanoparticles reached to (92.65 and 112.26)nm for JKH_1 broth and whey solution respectively(Fig.6,7). Jha and Prasad, (2010) showed that the lowered oxidation potential which is formed due to the presence of glucose activates the membrane bound oxidoreductase and makes the requisite ambience for an oxide nanoparticle synthesis. Azhar *et al*.,(2011) found extracellular *Lactobacillus*-mediated biosynthesis of TiO_2 nanoparticles in MRS-broth medium , with nanoparticle size 150nm. Whey solution has been used for synthesis of nanoparticles using *Lactobacillus* sp. by others studies(Prasad *et al*.,2007; Ranganath *et al*.,2012; Salman,2013). To the best of our study this is the first report on synthesis of TiO_2 nanoparticles using locally medium (JKH_1) prepared by Salman and Khalaf (2014) which considered as suitable medium for growth of *Lactobacillus* sp. and production of antibacterial agents. The present study showed that the medium JKH_1 with 10% glucose is the best for TiO_2 nanoparticles synthesis compared with the same medium without glucose.

The explanation of TiO_2 nanoparticles synthesis using *Lactobacillus* sp. is Lactobacilli, like most of the bacteria, have a negative electro-kinetic potential; which readily attracts the cations and this step probably acts as a crux of the procedure of biosynthesis (Ahmad *et al.*,2013).Extra cellular proteins and other biomolecules present in the culture of Lactobacillus mediated the hydrolysis of the anionic complexes and results in the synthesis of titanium nanoparticles (Bansal *et al*., 2005; Jha and Prasad, 2010;Ahmad *et al*.,2013).



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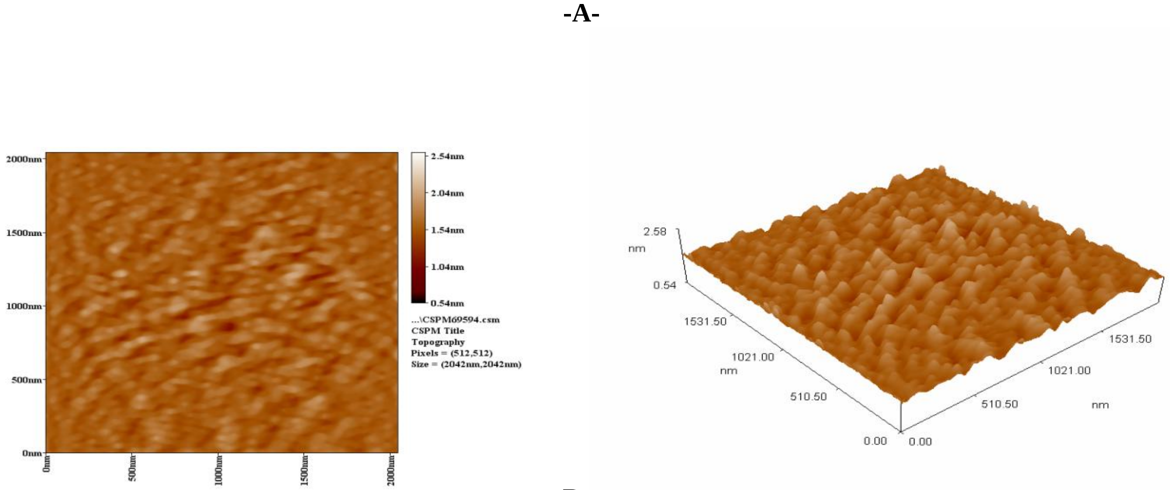

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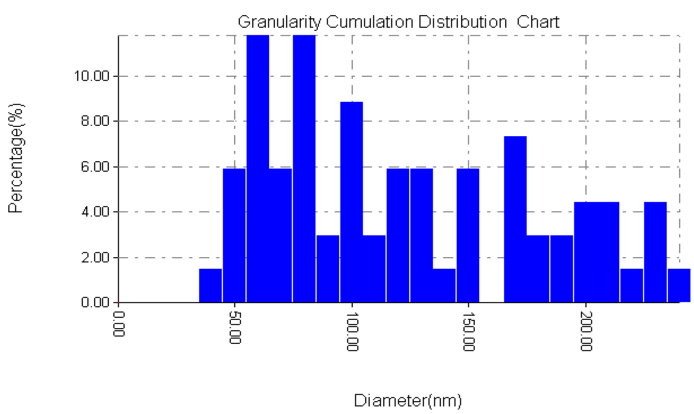
Figure(3):Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in MRS broth.(A-Diameter percentage ,B- Surface and three Dimensional view)



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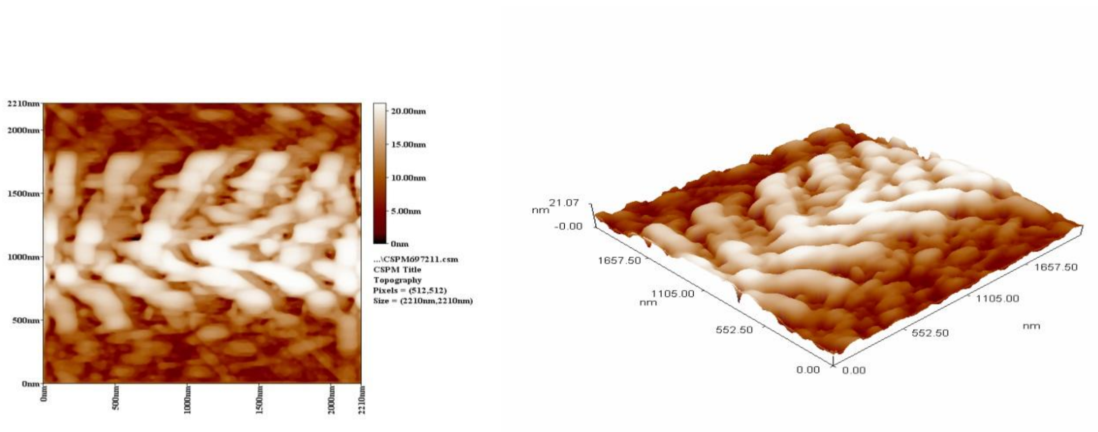

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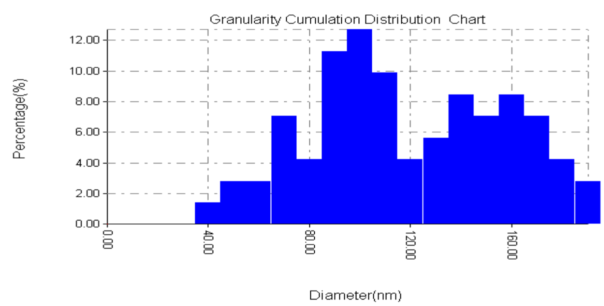
Figure(4):Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in JKH_1 broth.(A-Diameter percentage ,B- Surface and three Dimensional view)



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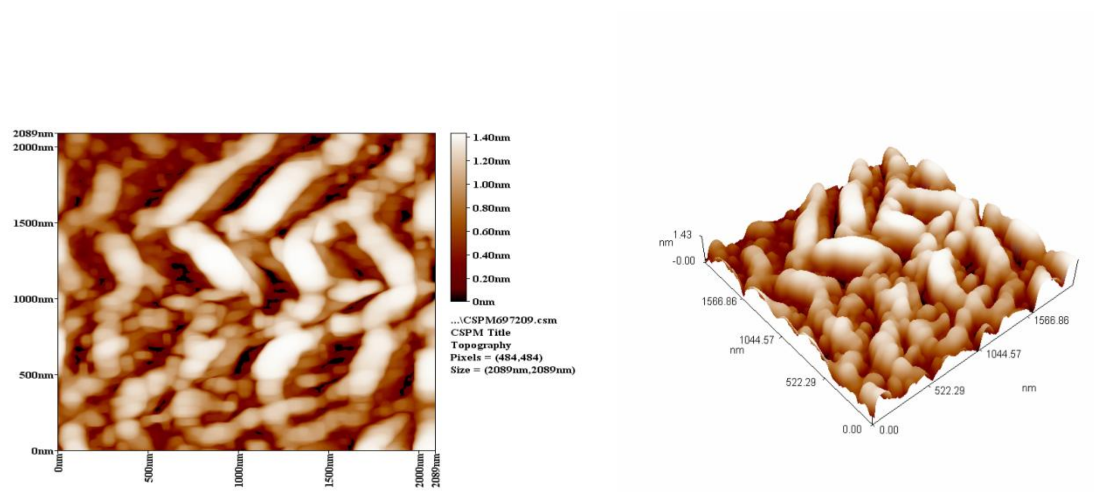

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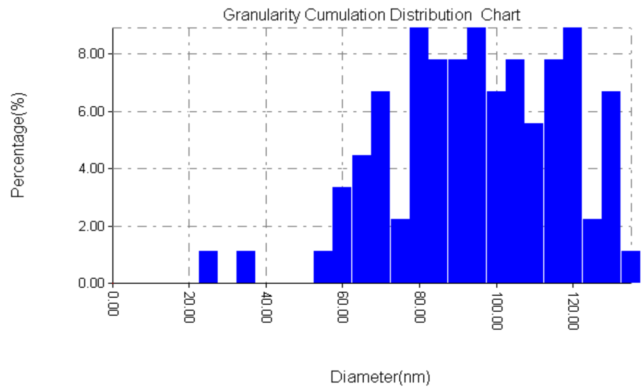
Figure(5):Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in whey.(A-Diameter percentage ,B- Surface and three Dimensional view)



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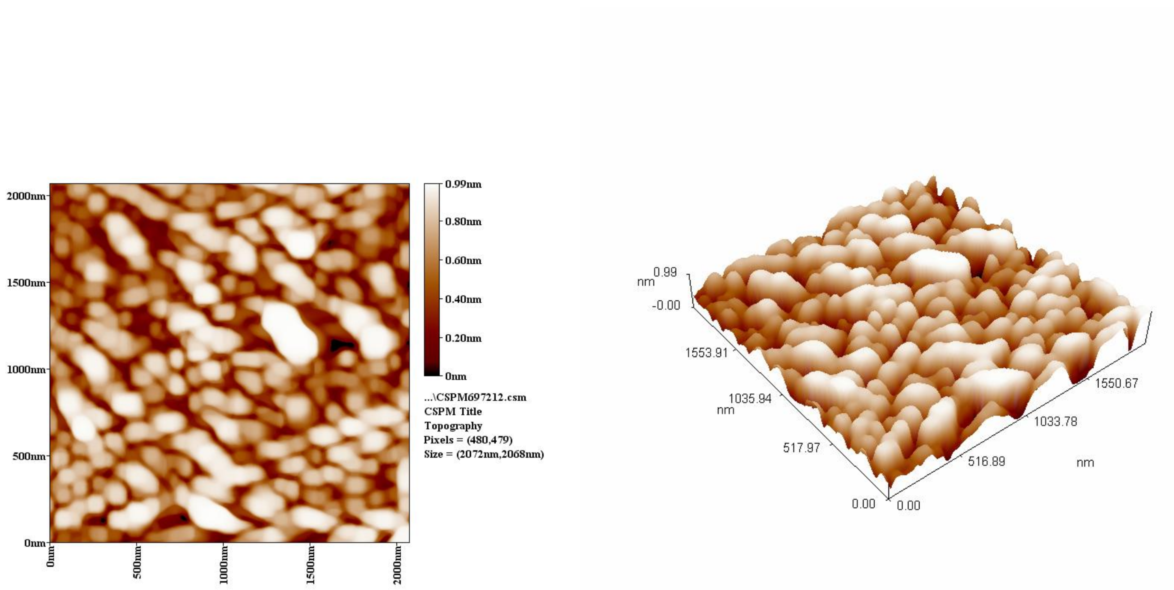

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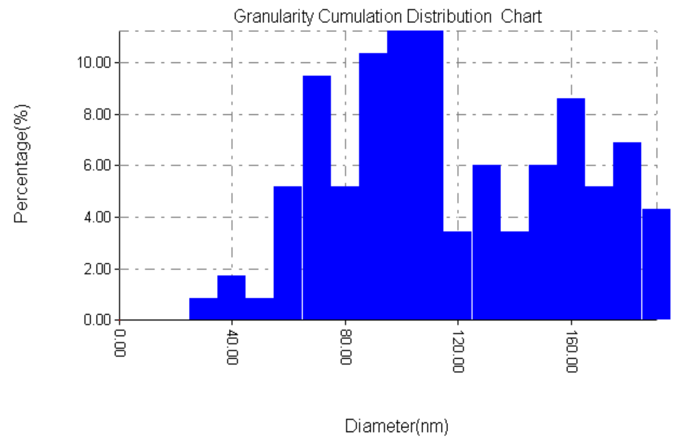
Figure(6):Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in JKH_1 broth with glucose.(A-Diameter percentage ,B- Surface and three Dimensional view)



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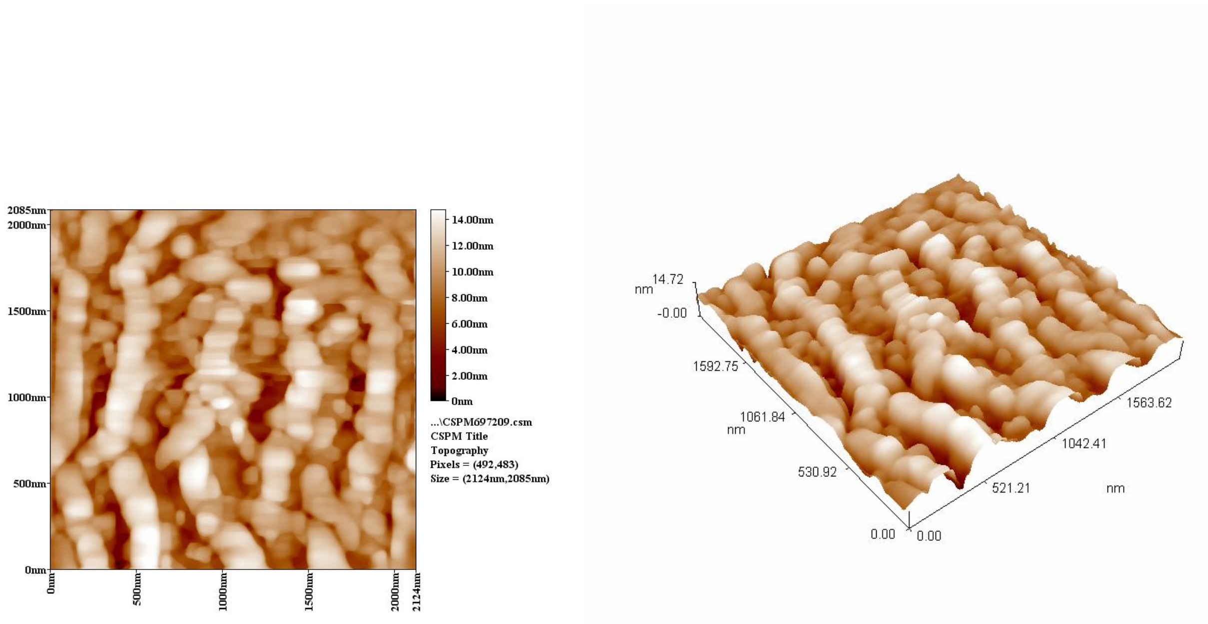

-B-

Figure(7):Atomic Force Microscopy image of TiO_2 nanoparticles synthesized by *Lactobacillus crispatus* in whey with glucose .(A-Diameter percentage ,B- Surface and three Dimensional view)

Conclusion:

The present study reported that the best culture media for TiO_2 nanoparticles biosynthesis using *Lactobacillus crispatus* is MRS broth followed by JKH_1 with 10% glucose .

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