Synthesis of titanium oxide nanoparticles using Aloe barbadensis mill and evaluation of its antibiofilm potential against Pseudomonas aeruginosa PAO1


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ABSTRACT

Titanium dioxide nanoparticles (TiO2-NPs) were synthesized using the aqueous leaf extract of Aloe barbadensis as a reducing and fabricating agent. The biosynthesis of the TiO2-NPs was initially confirmed by UV–vis spectroscopy. Based on the HRTEM and FESEM analysis, the biosynthesized NPs were found to be polydispersed and predominantly spherical in shape, with an average size of ~20 nm. A sharp and strong characteristic peaks of titanium (Ti) and oxygen (O) observed in the EDS pattern confirmed the synthesis of the TiO2-NPs. The FTIR spectroscopy suggested the presence of terpenoids, flavonoids and proteins which might be responsible for the biosynthesis and fabrication of the TiO2-NPs. The crystalline nature of the synthesized TiO2-NPs constituting of a mixture of brookite, anatase, and rutile phases was indicated by the XRD pattern. The spectral window around 180–1000 cm−1 covered the high-frequency Raman spectra of the TiO2-NPs. The Raman vibrational spectrum showed four Eg modes centered at 197.84, 399.24, 514.50, and 641.58 cm−1 representing the anatase phase of TiO2-NPs. The strongest and broadened peak of anatase was observed at the frequency of 641.58 cm−1. The metabolic activity of P. aeruginosa exposed to the MIC of TiO2-NPs was measured based on the reduction of tetrazolium salt by the dehydrogenase enzyme, produced by the metabolically active bacterial cells. The reduction in TTC was evident from the appearance of a red colored formazan in the solution. A noticeable suppression in the cell viability by 30.76 ± 3.96% of P. aeruginosa in the biofilm mode was found in presence of TiO2-NPs. Furthermore, the Minimum Inhibitory Concentration (MIC) of TiO2-NPs exhibited profound antibiofilm activity against P. aeruginosa by effectively preventing the adherence of the planktonic cells to the substratum. Thus, these NPs may be employed in controlling bacterial infections associated with biofilm.

1. Introduction

The ability of bacterial pathogens to form biofilm on medical implants and in-dwelling is a major challenge faced by global healthcare system today. Biofilm constitute an organized community of bacteria adhered onto a living or inert surface. Encased inside the self-produced exopoly saccharide (EPS) matrix, the biofilm confers resistance to the sessile cells against various antimicrobial stresses and host defenses [16]. Therefore, the eradication of these biofilm is challenging due to their resistance against conventional antibiotics [19].

P. aeruginosa, an opportunistic, gram-negative, aerobic pathogen is related to lower respiratory tract and burn wound. In individuals with cystic fibrosis (CF), this aerobic bacterium is the primary causative agent of chronic pulmonary infections [15]. The exopolymatrix of P. aeruginosa, composed of proteins, extracellular DNA and nucleic acids represents an indispensable part in the biofilm development and

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also contributes to its multidrug resistance [23].

In the past few decades, we have witnessed an outstanding advancement in the field of nanotechnology and its applications in the area of diagnostics and therapeutics [5]. The unusual physicochemical characteristics of the nanomaterials such as its minute size, high thermal conductivity, and reactivity make them remarkably different from their bulk counterparts. Because of its unique features, nanoparticles (NPs) are of great interest in medical diagnostic imaging, targeted drug delivery and production of biocompatible materials [31]. Nanomedicine enhanced the efficacy of the existing anti-infective and anti-tumour drug. The ease of employing these NPs in the delivery of drugs may be attributed to their stability, high loading capacity and targeted delivery [30].

Metallic NPs have acquired much interest in clinical medicine due to their exciting properties which include the possibility of bio-functionalization, ease of synthesis and biocompatibility [2]. Various forms of NPs have been developed to carry imaging probes or drug of choice efficiently the target site [6]. Titanium and its alloys are employed as key materials in dental implants, due to their biocompatibility and efficiency the target site [6]. Titanium and its alloys are employed as key materials in dental implants, due to their biocompatibility and efficiency the target site [6].

With the development of NPs and nanostructure materials, nanotechnology seems to offer an efficient alternative to conventional antimicrobial agents in combating biofilm-associated infections [9]. Traditionally, NPs were synthesized and fabricated to increase its stability via various chemical and physical techniques. However, the toxic non-polar solvent and chemicals employed in various stages of the chemical synthesis greatly limits their applicability in therapeutics [11].

In recent years, plant-mediated biosynthesis of NPs has gained a significant interest as a safe, eco-friendly and less energy-intensive process [13]. The anti-biofilm and anti-adhesive activity of the plant extracts against mono and multispecies biofilms have been reported earlier [12]. The medicinal plant, Aloe vera (Aloe barbadensis mill) is known to possess numerous therapeutic properties including, anti-cancer, anti-ulcer, hepatoprotective, antioxidant, immunomodulatory, anti-inflammatory, anti-microbial, and anti-diabetic [24]. The present work deals with the biosynthesis of TiO2-NPs using Aloe vera extract. Further, the antibiofilm efficacy of the synthesized NPs at its Minimum Inhibitory Concentration (MIC) was investigated against P. aeruginosa PA01.

2. Materials and Methods

2.1. Synthesis of TiO2 Nanoparticles

The chemical and reagents employed in the present study were procured from E-Merck Life sciences Pvt. Ltd., Mumbai. The Aloe vera plants were collected from Salamanatham Village, Vellore District, Tamil Nadu, India. About 100 g of the fresh leaves was washed and boiled for 3 h and distilled water (100 ml) at 70 °C. The leaf extract was filtered using a Whatman filter paper.

A solution of Titanium Chloride (1 M, TiCl4) was prepared in 100 ml of Millipore water and the leaf extract was added dropwise under constant stirring (pH~7). The NPs formed during the process were centrifuged (10,000 rpm) and repeatedly washed with ethanol (absolute). The NPs were washed again with distilled water and dried at 100 °C for 7 h for further characterization.

2.2. Characterization of TiO2 Nanoparticles

The biosynthesized TiO2-NPs were subjected to UV–Vis spectrophotometer and the spectrum was recorded in the range of 200 to 800 nm. The High-resolution transmission electron microscopy (HR-TEM) micrographs were captured at an accelerating voltage of 200 kV to estimate the average size of the TiO2-NPs formed [15]. The field emission scanning electron microscope (FE-SEM) was recorded at an acceleration voltage of 20 kV to examine the microstructure and morphology of the NPs. The elemental composition of the TiO2-NPs was further analyzed using energy dispersive spectroscopy (EDS). The functional groups associated with the TiO2-NPs were graphed from Fourier transform infrared (FTIR) spectrum. The X-ray diffraction (XRD) measurement was recorded with CuKα radiation (λ=1.5406 Å) under 40 kV, 30 mA and scanning between 10° to 80° (2θ). The resonant and non-resonant Raman spectra of the TiO2-NPs were measured using IR (632.8 nm) and UV (325 nm) excitation lasers.

2.3. Bacterial Strains and Culture Conditions

P. aeruginosa PA01 (MTCC 2453) was cultured freshly at 37 °C in LB broth.

2.4. Antimicrobial Susceptibility Test

Agar well diffusion method was used to check the potentials of TiO2-NPs. An overnight culture of the test bacteria, P. aeruginosa (0.1%) was supplemented in sterile Mueller Hinton Agar (MHA). The molten agar media was poured into sterile petriplates and left to solidify. On solidification, wells were made on MHA plates using a borer of 6 mm in diameter. A stock solution of TiO2-NPs was prepared with 0.1% DMSO and 100 μL of the NPs (100 and 150 μg/ml) were added in each well, with one well containing DMSO (0.1%) [23].

2.5. Determination of Minimum Inhibitory Concentration (MIC) and Evaluation of Growth Curve

Broth micro dilution technique was followed to analyze the MIC of the particles [23]. The growth curve of P. aeruginosa treated with the MIC concentration of TiO2-NPs was further analyzed relative to the untreated control. An overnight culture of P. aeruginosa was diluted with LB broth to attain an absorbance (OD600) of 0.05 and further incubated at 37 °C under continuous agitation (200 rpm) in presence and absence of TiO2-NPs (MIC). The absorbance was recorded at 590 nm at every 1 h interval over a period of 24 h [4].

2.6. Antibiofilm Activity of TiO2 Nanoparticles

The anti-biofilm assays were executed with the MIC of TiO2-NPs and DMSO (0.1%) as a negative control.

2.6.1. Microtiter Plate (MTP) Assay for Biofilm Inhibition

The test bacteria (1%) was inoculated into 100 μl of LB broth containing MIC of TiO2-NPs and incubated at 37 °C for 24 h. The free-floating cells were withdrawn by gently rinsing the MTP with phosphate saline buffer (PBS, 0.1%). The wells were air dried and loaded with 200 μl of crystal violet (CV, 0.1%) to stain the biofilm adhered to the wells. The excess dye was discarded and MTP were further rinsed twice with sterile distilled water. The CV attached to the biofilm was eluted using 1 ml ethanol (95%) and the absorbance was recorded at 570 nm [3]. Biofilm inhibition (%) = OD (control) - OD (treated) / OD (control) x 100.

2.6.2. Biofilm Disruption Assay

P. aeruginosa (1%) was inoculated into LB broth (100 μl) to obtain an initial absorbance of 0.4 (OD600) and incubated overnight at 37 °C.
The planktonic cells present in the MTP were gently rinsed with 0.9% NaCl (w/v) and the biofilm formed was further incubated at 37°C for 24 h in presence of TiO$_2$-NPs (MIC), without agitation. The free-floating cells were washed out from the wells using phosphate saline buffer (PBS, 0.1%). As described in the MTP assay, the wells were stained with 0.1% CV solution and the absorbance was measured at 570 nm [23]. Biofilm disruption (%) = OD (control) – OD (test) / OD (control) × 100.

2.6.3. Rhamnolipid Quantification

P. aeruginosa was cultivated in presence of the MIC of TiO$_2$-NPs at 37°C for 24 h. The culture broth (2 ml) was subjected to centrifugation 17,500 × g for 15 min to separate the cell biomass. The resulting cell-free supernatant collected was treated twice with ethyl acetate and the solvent phase collected was air dried. About 500 μl of MilliQ water was added to the dried extract and acidified with 53% H$_2$SO$_4$ containing 900 μl of 0.19% orcinol [17].

2.6.6. Microbial Adhesion to Hydrocarbon (MATH) Assay

P. aeruginosa treated and untreated with MIC of TiO$_2$-NPs was subjected to centrifugation at 10,000 rpm for 15 min. The cell pellet collected was washed with 0.85% NaCl and re-suspended in 3 ml of PBS along with 0.25 ml of toluene. The mixture was vortexed vigorously and the upper toluene phase was retrieved. The absorbance of organic phase was recorded at 600 nm to calculate the hydrophobicity index [32].

Hydrophobicity Index = (Initial OD$_{600}$–Final OD$_{600}$/Initial OD$_{600}$) × 100

2.6.7. TTC (2,3,5- Triphenyl-Tetrazoliumchloride) Reduction Assay

P. aeruginosa cultured with and without MIC of TiO$_2$-NPs in MTP were rinsed with sterile PBS. Freshly prepared TTC (100 μl, 0.5%) was dispensed to each well including control. The MTP was kept for shaking at 400 rpm for 6 h, in dark and the absorbance was measured at 530 nm to determine the cell viability [14].

2.6.9. Microscopic Observation of Biofilm

Light Microscopy and Confocal Laser Scanning Microscopy (CLSM) was used to analyze the antibiofilm efficacy of TiO$_2$-NPs against P. aeruginosa [29]. P. aeruginosa was cultivated in TSB medium until anabsorance of 0.05 was attained at 600 nm. About 1 ml of culture aliquots was added to each well of 24-MTP containing cover glass along with MIC of TiO$_2$-NPs and incubated at 37°C for 16 h, without agitation. After incubation, the un-adhered planktonic cells were rinsed with PBS solution (0.01 M) and the coverslips were stained with 0.1% CV dye. Excess dye from the coverslips were rinsed and observed undera light microscope at 40× [29].

Likewise, the biofilm adhered coverslips were removed from the MTP and stained with acridine orange (0.01% w/v) for 1 min. The coverslips were gently rinsed with PBS and observed using CLSM equipped with an excitation filter (515–560 nm) at a magnification of 20×. The 3D-images of the biofilm matrix were captured and the thickness of biofilm was evaluated from the z-stack analysis [23].

2.7. Statistical Analysis

All the assays were conducted in triplicates (n = 3) and the results represented as mean ± S.D.

3. Results and Discussion

3.1. Synthesis of TiO$_2$ Nanoparticles

The Alovera leaf extract mediated bio-synthesis of TiO$_2$-NPs was successfully achieved by reduction of the 1 M solution of TiCl$_4$ to TiO$_2$. The change in the colour of the reaction mixture to brown was observed after 3 h of continuous stirring indicating the formation of TiO$_2$-NPs. Similarly, in earlier reports, the biosynthesis of TiO$_2$-NPs was achieved using plant extract of Azadirachta indica [13], Psidium guajava [26] and Curcuma longa [22].

3.2. Characterization of TiO$_2$ Nanoparticles

The UV–visible spectroscopy provided preliminary confirmation on the reduction of TiCl$_4$ and synthesis of TiO$_2$-NPs [25]. As shown in Fig. 1, a strong SPR excitation spectrum of the TiO$_2$-NPs was observed between 217 nm to 350 nm indicating the formation of TiO$_2$-NPs. Similarly, Sankar et al. [25] documented that the synthesized TiO$_2$-NPs showed an excitation between 270 nm to 320 nm.

The HR-TEM micrographs of the biosynthesized TiO$_2$-NPs revealed its polydisperse nature. The TiO$_2$-NPs were found to be mostly spherical in shape with an average particle size of ~20 nm as depicted in Fig. 2(a-h). The crystalline nature of the TiO$_2$-NPs was supported by the bright spots observed in the SAED pattern as shown in Fig. 2(i). In a similar report, Krishnasamy et al., (2015) reported the preparation of smooth, spherical shape TiO$_2$-NPs with size ranging from 15 nm to 45 nm, using A. indica leaf extract [13].

The TiO$_2$-NPs was analyzed using FE-SEM to further examine its size and surface morphology. The SEM images as shown in Fig. 3(a-e) revealed poly-dispersed, spherical shaped TiO$_2$-NPs with size ranging from 20 to 50 nm. Hence, the findings were found to be in consistent
with the data obtained from the TEM analysis.

Elemental composition and purity of the TiO$_2$-NPs were determined using EDS. The EDS pattern depicted in Fig. 3(f) showed sharp and strong characteristic peaks of titanium (Ti) and oxygen (O), indicating a high percentage of elemental titanium and oxide peak confirming the purity of the synthesized TiO$_2$-NPs. Similar finding was documented by Santhoshkumar et al. [26] where in the SEM analysis of the TiO$_2$-NPs synthesized using Psidium guajava revealed the smooth and spherical shaped NPs. The EDS proved that the particles formed were metallic TiO$_2$-NPs [26]. In another report, Krishnasamy and Velan [13] reported the synthesis TiO$_2$-NPs using A. indica leaf. The FE-SEM images revealed 25 nm to 87 nm in size. The EDS analysis showed the strong titanium (Ti) peaks at 4.4 to 5 keV, silica (Si) at 1.8 keV, carbon (C) at 0.2 keV and oxygen (O) at 0.6 keV confirming the synthesis of TiO$_2$-NPs [13].

The FTIR spectrum of the TiO$_2$-NPs synthesized using Alovera leaf extract shown in Fig. 4(a) enabled the identification of the functional groups associated with the bio-synthesized TiO$_2$-NPs. A broad peak formed at 3186.2 cm$^{-1}$ represented the broad O–H stretch and corresponds to the carboxylic acids of the major phytocompound present in the Alovera extract. The prominent peak at 3414.69 cm$^{-1}$ was contributed by the O–H stretch of alcohols and phenols from the plant extract. The peak at 1633.73 cm$^{-1}$ can be assigned to the N–H bend of amide I, and the band at 677.92 cm$^{-1}$ to the C–H bending. The absorption band below 1000 cm$^{-1}$ indicated the oxide lattice vibrations of the TiO$_2$. The peak at 677.71 cm$^{-1}$ apparently associated to the Ti-O-Ti stretching vibration of TiO$_2$-NPs. The strong band observed between 899.41 cm$^{-1}$ and 511.80 cm$^{-1}$ corresponds to the characteristic vibrational modes of TiO$_2$. The presence of O–H stretching and C–C group are indicative of the terpenoids from the Alovera leaf extract [18]. Hence, the presence of terpenoids, flavonoids and proteins in Alovera leaf extract were responsible in the formation and fabrication of the TiO$_2$-NPs. The findings were consistent with that of Sankar et al. (2014) wherein the FTIR spectra of Alovera indica leaf extract synthesized TiO$_2$-NPs indicated that the presence of terpenoid, flavonoid and protein which might have acted as reducing and capping agent in the synthesis process. The peaks observed at 758, 593 and 447 cm$^{-1}$ also indicated the stretching vibrations of Ti–O [25].

TiO$_2$ exist in main crystal structures: rutile, brookite and anatase.

![Fig. 2. (a–h). TEM images of TiO$_2$-NPs with different scale bar (ranging from 200 nm–2 nm), (i) SAED diffraction pattern of the synthesized TiO$_2$-NPs.](image-url)
analysis was used to determine the crystallinity of the TiO$_2$-NPs. As depicted in Fig. 4(b), the XRD pattern of the biosynthesized TiO$_2$-NPs exhibited seven distinct diffraction peaks. The characteristic diffraction pattern with 2θ values lying at (101), (200) and (215) corresponds to anatase phase of TiO$_2$-NPs while the peaks at (121) and (123) belong to brookite phase. The observed pattern at (221) and (301) corresponding to the rutile phase was confirmed [JCPDS Card no. 21-1272]. Hence, the synthesized TiO$_2$-NPs constituted of a mixture of anatase, brookite and rutile phases. Further, the presence of sharp diffraction peaks in the XRD pattern also indicated the nanosized and crystalline nature of the biosynthesized TiO$_2$-NPs. Jalill, and R.S.N., A.N. Abd [22] reported a similar observation wherein the XRD pattern of the TiO$_2$ nanopowder synthesized using Curcuma longa plant extract showed the presence of seven peaks indicating the presence of pure anatase in the biosynthesized TiO$_2$-NPs. Strong diffraction peaks were observed at 25.32° (101), 48.05° (200) and 37.81° (004) indicating the crystallinity of the biosynthesized anatase nanoparticles [22].

Further, the Raman spectroscopy (RS) characterization of TiO$_2$-NPs was used to study the vibrational behavior and structural properties. The spectral window around 180–1000 cm$^{-1}$ covered the high-frequency Raman spectra of the TiO$_2$-NPs. The Raman vibrational spectrum showed four $E_g$ modes centered at 197.84, 399.24, 514.50, and 641.58 cm$^{-1}$ representing the anatase phase of TiO$_2$-NPs as in Fig. 5(a). The strongest and broadened peak of anatase was observed at the frequency of 641.58 cm$^{-1}$. The small particle size of the TiO$_2$-NPs might have contributed to the broad Raman scattering peaks. The present findings were in reasonable agreement with the data obtained from the XRD spectrum suggesting that most of the crystalline phase was anatase, with a small amount of brookite and rutile phases.

3.3. Antimicrobial Susceptibility Test

P. aeruginosa is one of the most common and multi drug resistant pathogen associated with nosocomial infections especially in immune compromised individuals [15]. The inhibitory effect of the synthesized TiO$_2$-NPs towards the growth of the test pathogen, P. aeruginosa was indicated by appearance of visible zone of clearance around the wells of the agar medium in Fig. 5(b). The zone of inhibition (ZOI) on addition of...
100 and 150μg/ml of TiO2-NPs was recorded as 11 and 14mm, respectively. The increase in the diameter of ZOI was indicative of the dose-dependent bactericidal effect of the TiO2-NPs. In an earlier report, TiO2-NPs synthesized using the leaves extract of *Morindacitrifolia* showed dose-dependent bactericidal effect towards *P. aeruginosa* with a ZOI of 8 and 9mm on exposure to 100 and 150μg/ml of TiO2-NPs, respectively [27].

### 3.4. MIC and Growth Curve Inhibition Study

Based on the broth dilution assays, the test bacteria showed a significant decrease in the growth of *P. aeruginosa* with increasing concentrations of TiO2-NPs. However, at the concentrations lower than 31.25μg/ml, the NPs fail to exhibit bactericidal effect towards the test bacteria as compared to untreated control in Fig. 6(a). Hence, the MIC was determined to be 31.25μg/ml and the same dosage was used for all the subsequent anti-biofilm assays. According to a report of Jayaseelan et al. [11], the MIC of TiO2-NPs synthesized using *Aeromonas hydrophila* was determined to be 30μg/ml for *P. aeruginosa* [11].

### 3.5. Anti-biofilm Activities of TiO2 Nanoparticles

Biofilm constitutes of aggregated colonies of bacterial cells attached to a substratum and encased inside a polymeric matrix majorly constituting of exopolysaccharides (EPS). Bacteria within the biofilm display profound tolerance and resistance to conventional anti-infective and host immune response. Biofilm-related infections are responsible for the spread of numerous diseases, especially those associated with medical implants. Hence, the control of biofilm formation using NPs is a promising approach to combat the multidrug resistant (MDR) strains [10]. The efficacy of TiO2-NPs in inhibiting the biofilm formation by the multi drug resistant opportunistic pathogen, *P. aeruginosa* was explored.

The biofilm forming ability of *P. aeruginosa* was significantly reduced in the presence of the TiO2-NPs (31.25μg/ml). The inhibition in biofilm formation was found to be 30.69 ± 3.78% as shown in Fig. 6(b). The TiO2-NPs were also able to disrupt already established biofilm in the MTP. Upon treatment with MIC of TiO2-NPs, a considerable disruption in the preformed biofilm of *P. aeruginosa* by 47.04 ± 3.71% was observed as depicted Fig. 6(b).

Rhamnolipids have been demonstrated to regulate swarming motility and aids in biofilm establishment of *P. aeruginosa*. The production of rhamnolipid was considerably suppressed on treatment with TiO2-NPs. As observed in Fig. 6(b), the reduction in rhamnolipid production by 41.77 ± 2.96% was achieved in presence of MIC of TiO2-NPs, both in the context of planktonic and biofilm growth. The synthesis of viscous exopolysaccharide, alginate was significantly reduced with MIC of TiO2-NPs. The MIC of TiO2-NPs exhibited 24.23 ± 4.35% decrease in alginate production, as depicted in Fig. 6(b).
Extracellular polysaccharides (EPS) play a vital role in the pathogenicity of P. aeruginosa. The EPS matrix of the biofilm gives protection to the sessile cells present inside the biofilm against antibiotics and other external stresses. It also provides structural stability to the biofilm and facilitates the initial colonization of the planktonic cells on the substratum [10]. A profound reduction in EPS production was found in presence of TiO2-NPs (31.25μg/ml). The MIC of TiO2-NPs profoundly inhibited the EPS production in P. aeruginosa PA01 by 75.56 ± 3.2% as shown in Fig. 6(b). Rajkumari et al. [23] reported a significant attenuation in EPS production of P. aeruginosa by 81.29% when exposed to the sub-MIC level of AuNPs, synthesized using phytocompound, Baicalein [23].

Cell surface hydrophobicity (CSH) plays a vital role in the initial colonization and establishment of planktonic bacteria to the substratum [28]. MATH assay was employed to assess the efficacy of the bacterial cells to attach to the hydrophobic surface and form a biofilm. The ability of P. aeruginosa to attach to the hydrophobic substrate was greatly reduced to 47.88 ± 5.86% with the MIC of TiO2-NPs as represented in Fig. 6(b). In case of untreated control, the cells however, showed a high degree of cell surface hydrophobicity. The findings from the EPS quantification and MATH assay revealed that the TiO2-NPs interfere with adherence of the planktonic cells to the substrate thereby hampering the establishment of bacterial biofilm. Similar observations were documented by Viszwapriya et al. [28] using 2,4-Di-tert-butylphenol against the biofilm forming ability of group A streptococcus[28]. Hence, the decrease in EPS production along with the reduction the CSH contributed to the anti-biofilm activity.

The metabolic activity of P. aeruginosa exposed to the MIC of TiO2-NPs was measured based on the reduction of tetrazolium salt by the dehydrogenase enzyme, produced by the metabolically active bacterial cells. The reduction in TTC was evident from the appearance of a red colored formazan in the solution. A noticeable suppression in the cell viability by 30.76 ± 3.96% of P. aeruginosa in the biofilm mode was found in presence of TiO2-NPs (31.25μg/ml), as shown in Fig. 6(b).

The notable arrest in EPS production on treatment with the TiO2-NPs was further substantiated by the congo red agar assay. As shown in Fig. 7(b), the test organism treated with TiO2-NPs produced pink colored colonies indicating reduced production of EPS whereas, the control plates devoid of TiO2-NPs showed dry black colonies with crystalline consistency representing significant EPS production in Fig. 7(a). A similar finding was reported by Rajkumari et al. [23] where in P. aeruginosa produced pink colored colonies on CRA plates when grown in presence of Baicalein coated AuNPs, suggesting a decrease in EPS production [23].

The light microscopy and CLSM micrographs gave clear visual evidence on the inhibition of biofilm development in P. aeruginosa when treated with the TiO2-NPs. A remarkable difference in the morphology and thickness in the biofilm of P. aeruginosa on treatment with TiO2-NPs was seen when examined under light microscopy (40×). A compact and dense biofilm structure was evident on coverslides of the control as shown in Fig. 7(c). On the contrary, a considerable reduction in the biofilm development with more scattered bacterial cells was evident in presence of TiO2-NPs as shown in Fig. 7(d). Further, the CLSM images of untreated control displayed denser and thicker biofilm matrix with an average Z-axis value of 6 μm as evident in Fig. 7(e). The 3D images of P. aeruginosa treated with TiO2-NPs (31.25μg/ml) showed less dense biofilm with a calculated thickness of 2 μm in Fig. 7(f). Hence, the biosynthesized TiO2-NPs provided a great potential for the control of biofilm-associated infections. The current study, the green synthesized TiO2 nanoparticles were found to exhibit high antibiofilm efficacy against P. aeruginosa.

4. Conclusion

With the growing resistance of pathogenic microbial strains to the presently available conventional antibiotics, NPs has emerged as an efficient alternative to the conventional antimicrobial agents [11]. This study is the first report on the biosynthesis of TiO2-NPs using aqueous leaf extract of Alovera. The NPs were mostly spherical and polydispersed in nature with an average size of ~20 nm. The biosynthesized TiO2-NPs constituted of a mixture of anatase, rutile and brookite phases. Further, the synthesized TiO2-NPs showed a profound anti-biofilm activity towards P. aeruginosa. This approach provides an eco friendly and economical way for large-scale production of TiO2-NPs.


