CHAPTER IV
RESULTS AND DISCUSSION

In the presence of water, titanium isopropoxide (TTIP) undergoes hydrolysis reaction to form titanium oxide (TiO_2) and propanol as shown in Figure 4.1 (Agoudjil *et al.*, 2007).

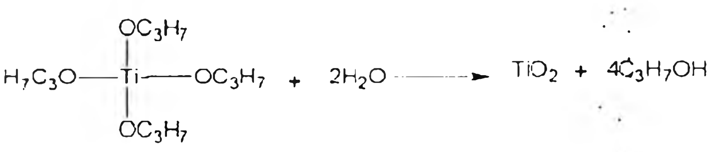


Figure 4.1 Hydrolysis reaction of titanium isopropoxide.

Because the molar ratios of TTIP to water may affect morphology and properties of the synthesized titanium dioxide, in this study molar ratios of TTIP to water in EtOH were varied as shown in table 4.1.

Table 4.1 The molar ratios of TTIP to water for preparation TiO_2 impregnated bacterial cellulose

Number of sample	EtOH (ml)	TTIP (ml)	TTIP (mole)	Molar ratio of TTIP to H_2O in EtOH
1	300	0.29	0.00093	0.031
2	300	0.57	0.00189	0.063
3	300	1.14	0.00375	0.125
4	300	2.29	0.00750	0.250
5	300	4.57	0.01500	0.500
6	300	9.15	0.03000	1.000
7	300	17.16	0.06000	2.000

* EtOH 300 mL contains water 0.03 mole.

* EtOH was included average EtOH in BC pellicle (125.51 % v/w)

4.1. Morphology of Pure Bacterial Cellulose and TiO_2 Impregnated Bacterial Cellulose

From SEM images of surface (see Figures 4.2A and 4.2B) and cross-sectional (see Figure 4.2C) morphology of pure bacterial cellulose, the average fibers diameter of pure bacterial cellulose was found to be 37 ± 12 nm Bacterial cellulose has the fibrous network structure whereas the SEM image of the cross-sectional morphology shows the multilayer and porous structure of bacterial cellulose.

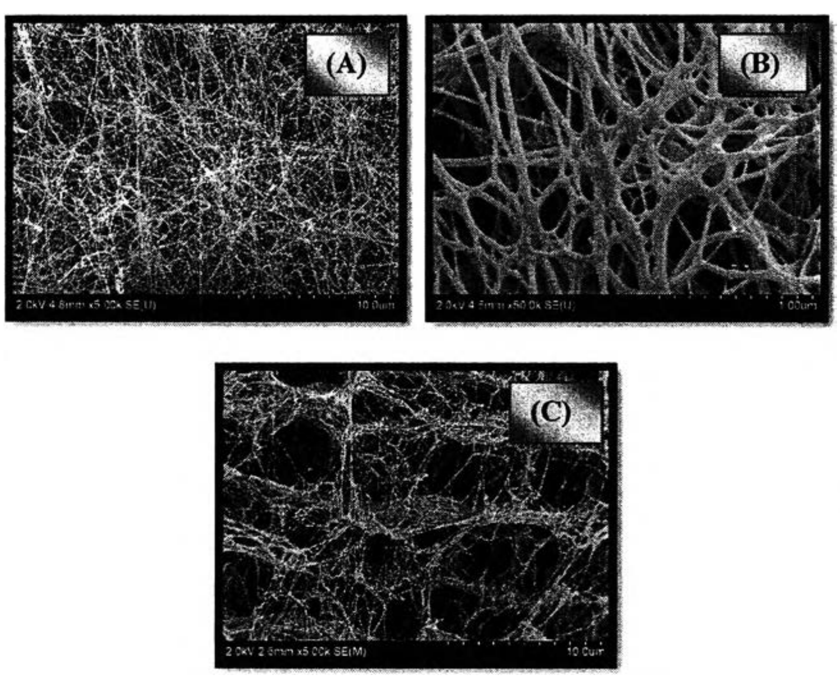


Figure 4.2 SEM images of surface (A, B) and cross sectional (C) morphology of freeze dried pure bacterial cellulose with average fibers diameter of 37 ± 12 nm

Figure 4.3, 4.4, 4.5, 4.6, 4.7 and 4.8 show SEM images of surface morphology of TiO_2 impregnated bacterial cellulose prepared by using various molar ratios of TTIP: H_2O in EtOH. It could be observed that TiO_2 particles covered on ratios of TTIP: H_2O in EtOH. It could be observed that TiO_2 particles covered on nanofibers of bacterial cellulose, resulting in the increasing of the average diameters when ratios of TTIP: H_2O in EtOH were accrued. However, when much excess in the amount of TTIP was used in the hydrolysis reaction at the molar ratio of TTIP: H_2O in EtOH equals to 7.5, TiO_2 was not only deposited on bacterial cellulose fibers but also agglomerated to form spherical particles embedded in porous structure of bacterial cellulose matrix (see Figure 4.8).

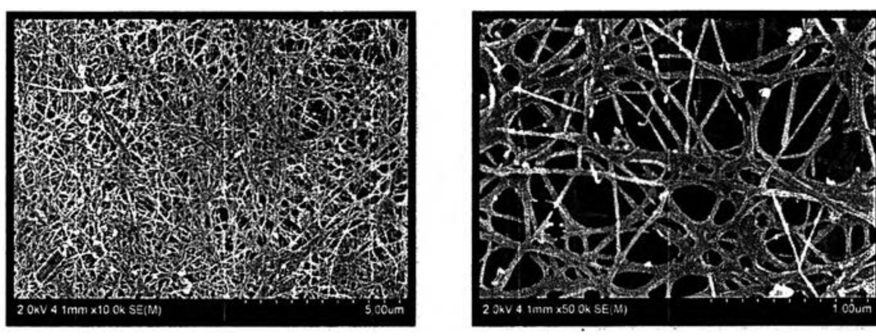


Figure 4.3 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average fibers diameter of 19 ± 6 nm prepared by using molar ratio of TTIP: H_2O in EtOH = 0.031 (water in excess).

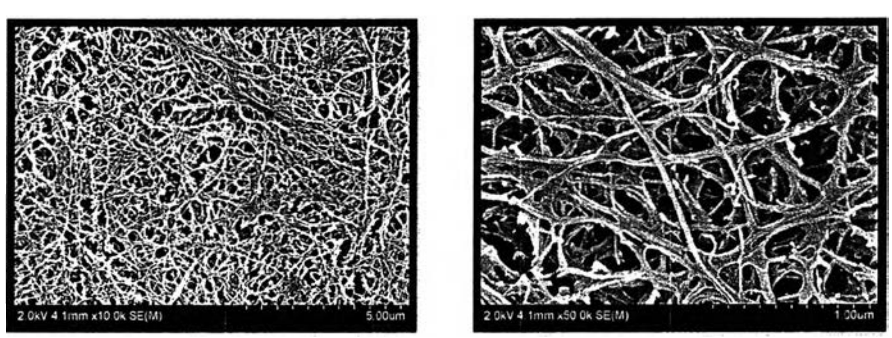


Figure 4.4 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average fibers diameter of 24 ± 6 nm prepared by using molar ratio of TTIP: H_2O in EtOH = 0.063 (water in excess).

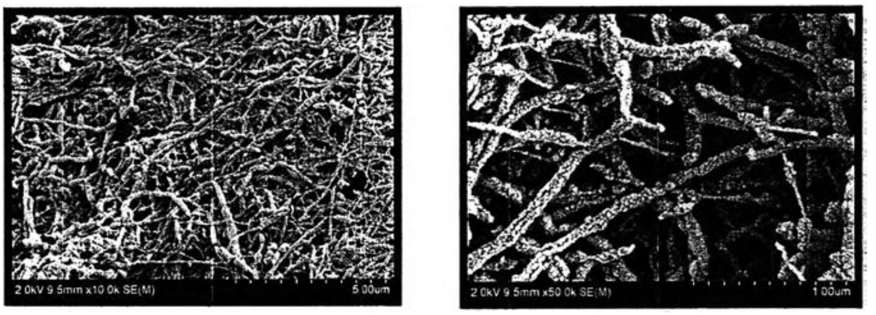


Figure 4.5 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average fibers diameter of 74 ± 21 nm prepared by using molar ratio of TTIP: H_2O in EtOH = 0.125 (water in excess).

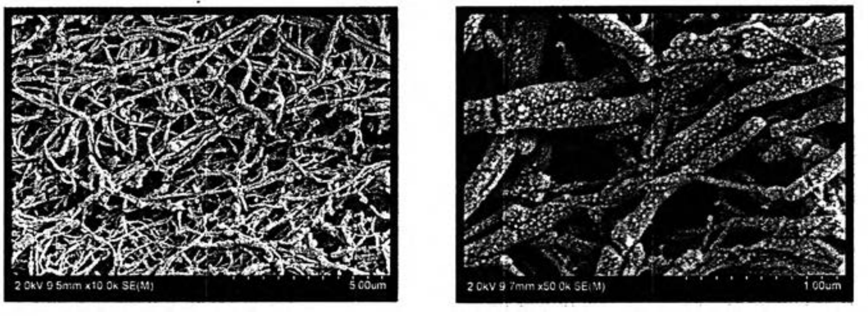


Figure 4.6 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average fibers diameter of 119 ± 41 nm prepared by using molar ratio of TTIP: H_2O in EtOH = 0.5(stoichiometric molar ratio).

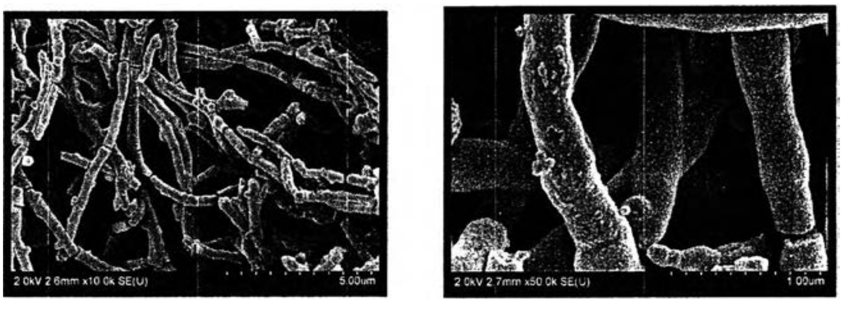


Figure 4.7 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average fibers diameter of 281 ± 76 nm prepared by using molar ratio of TTIP: H_2O in EtOH = 2 (TTIP in excess).

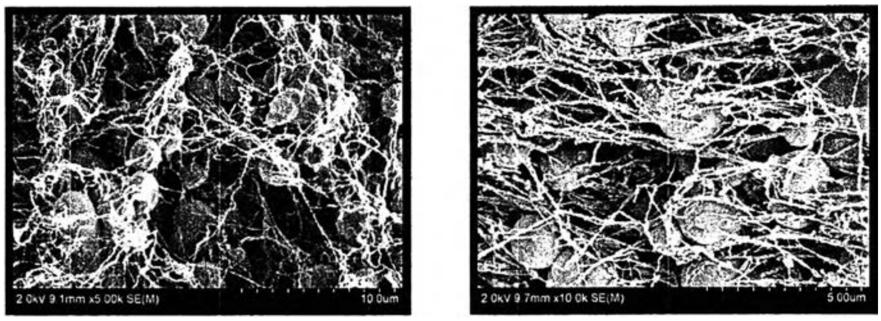
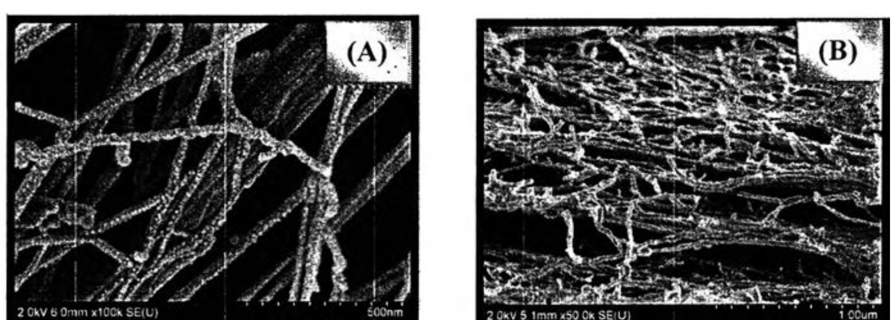


Figure 4.8 SEM images of surface morphology of TiO_2 impregnated bacterial cellulose with average individual spherical particles of TiO_2 diameter of 2 ± 1 μm. prepared by using molar ratio of TTIP: H_2O in EtOH = 7.5 (TTIP in excess).

Figure 4.9A-4.9G show SEM images of cross-sectional morphology of TiO_2 impregnated bacterial cellulose prepared by using various molar ratios of TTIP: H_2O in EtOH. It was found that TiO_2 was synthesized not only on BC surface but also at interior part of BC.



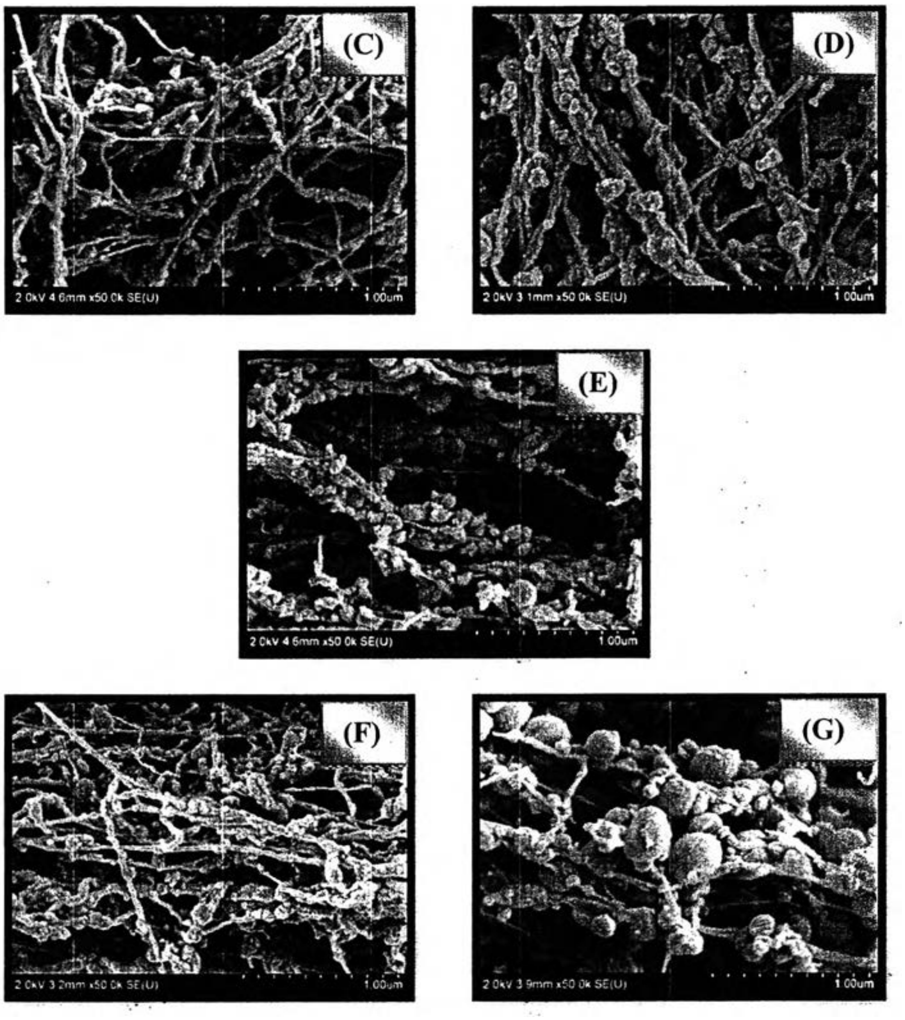


Figure 4.9 SEM images of cross-sectional morphology of TiO_2 impregnated bacterial cellulose prepared at various molar ratios of TTIP:H_2O in EtOH, (A) 0.031, (B) 0.063, (C) 0.125, (D) 0.25, (E) 0.5, (F) 1, (G) 2.

From scanning electron microscopy technique, it was demonstrated that the hydrolysis reaction by using TTIP as titanium precursor and using autoclave at 127 degree Celsius for 7 hours succeed to contrive TiO_2 coating on nanofibrils and existing in interior multilayer of bacterial cellulose as well as It was found that molar ratio of TTIP: H_2O in EtOH effects upon morphology of TiO_2 impregnated bacterial cellulose. The diameter of TiO_2 impregnated in BC increased with the increasing of molar ratios of TTIP to H_2O in EtOH and formation of individual spherical particles of TiO_2 in the porous structure of bacterial cellulose was occurred when using the large excess in the mole of TTIP to H_2O in EtOH.

4.2 XRD Analysis

Figure 4.10 shows the XRD spectra of TiO_2 impregnated bacterial cellulose prepared by using molar ratio of TTIP: H_2O in EtOH equals to 0.5 and heating at 127 degree Celsius in an autoclave for different heating time of 3, 5, and 7 hours. It was found that crystalline structure of TiO_2 impregnated in bacterial cellulose prepared by heating at 127 degree Celsius for 3 and 5 hours was amorphous. However, when the heating time was increased to 7 hours, the anatase form of TiO_2 was obtained as evidence by the occurrence of peaks at 2 theta equal to 25.18, 37.84, 47.76, 54.06, 62.44 corresponding to (1 0 1), (1 0 3, 0 0 4 and 1 1 2), (2 0 0), (1 0 5 and 2 1 1), (2 0 4) crystal planes, respectively (Xie *et al.*, 2007).

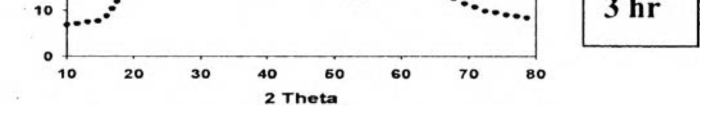


Figure 4.10 XRD spectra of TiO_2 impregnated bacterial cellulose prepared by using molar ratio of TTIP: H_2O in EtOH equals to 0.5 and heating at 127 degree Celsius in an autoclave for different heating time of 3, 5, and 7 hours.

Figure 4.11 shows the XRD spectra of TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH by heating at 127 degree Celsius in an autoclave for heating time of 5 hours. It was found that crystalline structure of TiO_2 impregnated in bacterial cellulose prepared by heating at 127 degree Celsius for 5 hours were affected by the molar ratio of TTIP: H_2O in EtOH, the molar ratios of TTIP: H_2O in EtOH were 1 and 2 that reveal the formation of amorphous. However, when the the molar ratio of TTIP: H_2O in EtOH was increased to 7.5 hours, the anatase form of TiO_2 was obtained as evidence by the occurrence of peaks at 2 theta equal to 25.18, 37.84, 47.76, 54.06, 62.44 corresponding to (1 0 1), (1 0 3, 0 0 4 and 1 1 2), (2 0 0), (1 0 5 and 2 1 1), (2 0 4) crystal planes, respectively (Xie *et al.*, 2007).

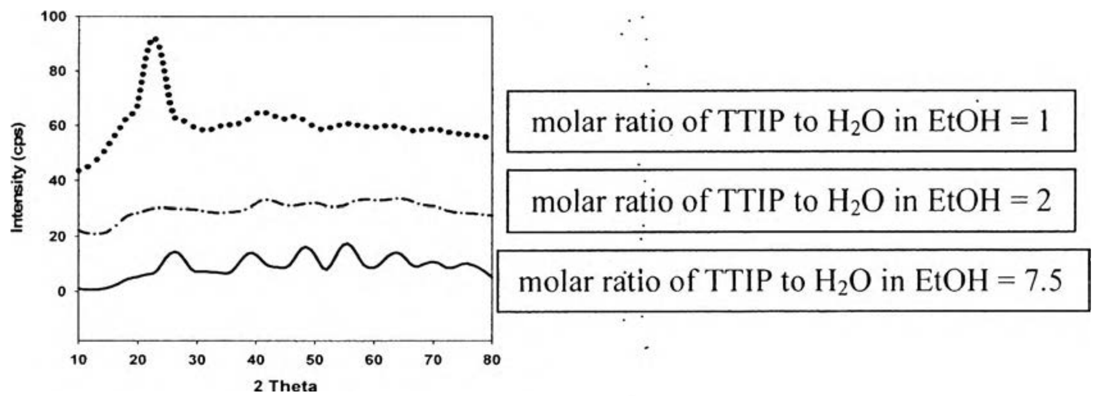


Figure 4.11 XRD spectra of TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH; 1, 2, and 7.5 by heating at 127 degree Celsius in an autoclave for heating time of 5 hours.

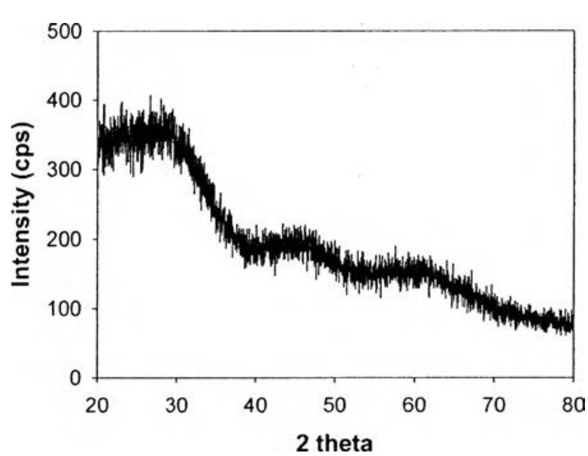


Figure 4.12 XRD spectra of TiO_2 impregnated bacterial cellulose prepared from pure TTIP by heating at 127 degree Celsius in an autoclave for heating time of 7 hours.

Figure 4.12 shows the XRD spectra of TiO_2 impregnated bacterial cellulose prepared from 97% TTIP (without water). It was found that the formation of TiO_2 was not generated seeing that water has none for the hydrolysis reaction so as to convert TTIP to TiO_2.

From XRD analysis, It was suggested that TiO_2 in crystal structure of anatase into bacterial cellulose by using TTIP as titanium source and using autoclave at 127 degree Celsius can invent depended on molar ratio of TTIP: H_2O in EtOH and heating time of autoclaving. What's more, water is importance factor for synthesizing TiO_2 impregnated bacterial cellulose by the hydrolysis reaction pathway.

4.3 Thermogravimetric Analysis of Pure Bacterial Cellulose and TiO_2 Impregnated in Bacterial Cellulose

The result on thermogravimetric analysis of pure bacterial cellulose in air at heating rate of 20 °C / min show the differential thermogram of pure bacterial cellulose and TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH show the loss of water at temperature below 120 °C as well they was completely degraded at about 500 °C. (see figures 4.13A, 4.14A-4.14E) In addition, the total weight loss of 97.5 % on heating at 1000 °C was observed in TGA thermogram of pure bacterial cellulose. (see figure 4.13B) and the total weight loss on heating at 1000 °C of the TiO_2 impregnated bacterial cellulose prepared at molar ratios of TTIP: H_2O equal to 0.031, 0.063,0.125, 0.5, and 2 were 86.0%, 85.8%, 81.7%, 52.1% and 40.7%, respectively (see figures 4.15A-4.15E).

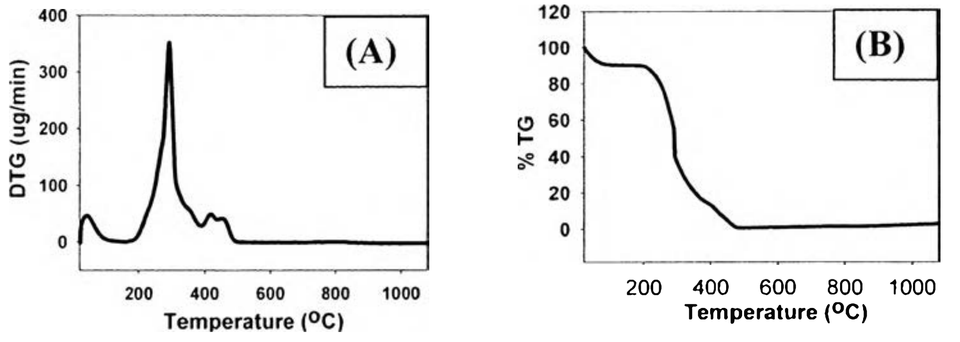
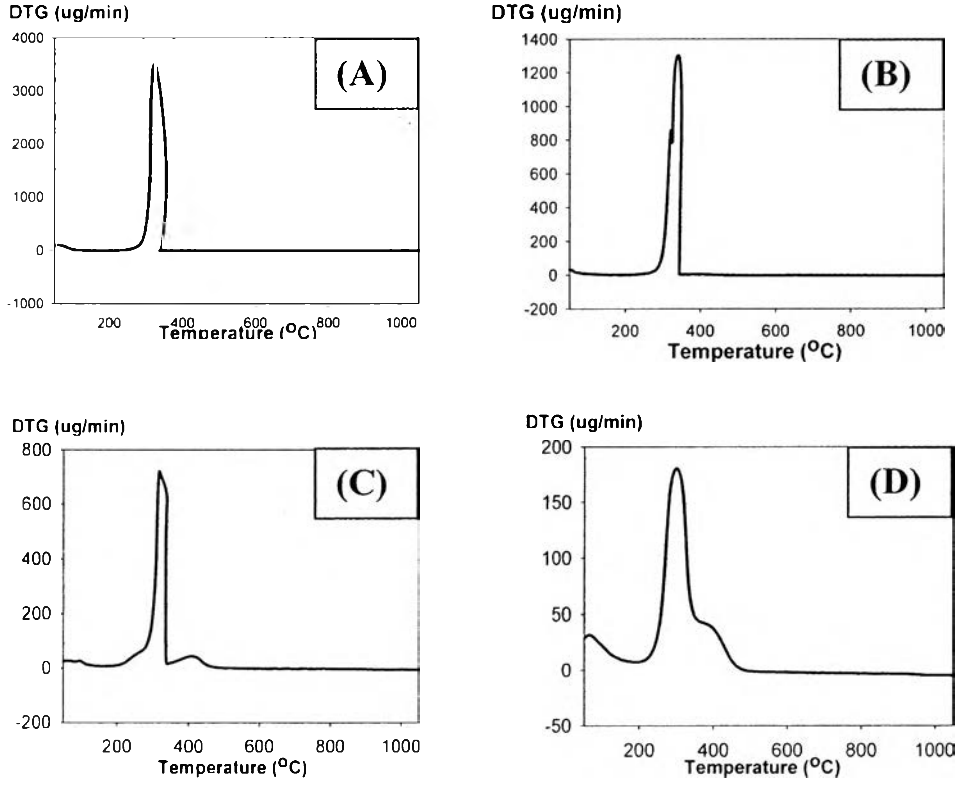


Figure 4.13 Differential thermogram of pure bacterial cellulose (A), TGA thermogram of pure bacterial cellulose (B).



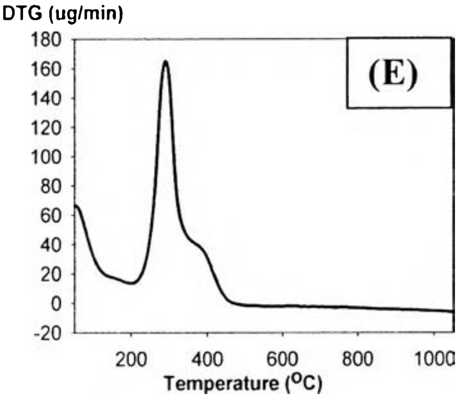
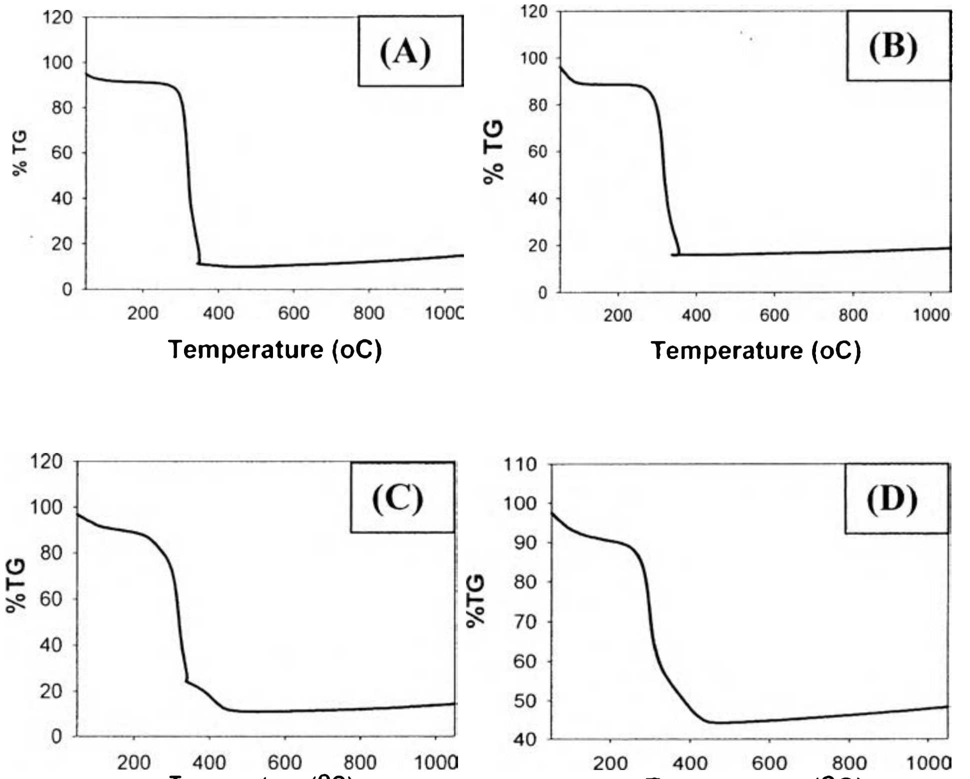


Figure 4.14 Differential thermogram of TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH; 0.031 (A), 0.063 (B), 0.125 (C), 0.5 (D), and 2 (E).



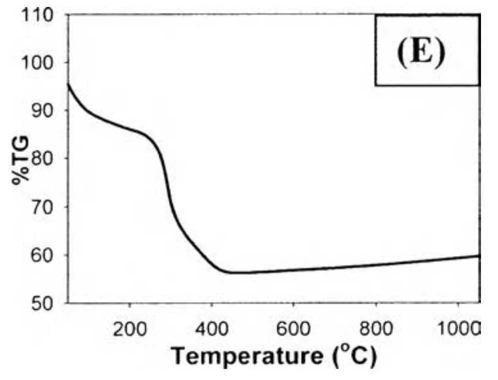


Figure 4.15 TGA thermogram of TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH; 0.031 (A), 0.063 (B), 0.125 (C), 0.5 (D), and 2 (E).

From Differential thermogram of pure bacterial cellulose and TiO_2 impregnated bacterial cellulose, It was found that pure bacterial cellulose was completely degraded at about 500 °C, so the white residual is TiO_2 impregnated bacterial cellulose which can find content of one in BC by using temperature above 500 °C to loss of BC template and weighing white residual (as shown in section 4.4) in addition, molar ratio of TTIP: H_2O in EtOH effect on contaminant in TiO_2 impregnated bacterial cellulose, degrading at around 400 °C, when increasing molar ratio of TTIP: H_2O in EtOH.

From TGA thermogram of TiO_2 impregnated bacterial cellulose, It was observed that molar ratio of TTIP: H_2O in EtOH affect thermal stability of materials. The thermal stability of TiO_2 impregnated in BC increased with the increasing of molar ratios of TTIP to H_2O in EtOH.

4.4 The content of the Synthesized TiO_2 in Bacterial Cellulose

The content of the synthesized TiO_2 in bacterial cellulose was determined by calcination in a furnace at 1000 degree Celcius. It was found that when the molar ratios of TTIP to water in EtOH were increased from 0.031 to 2, the content of TiO_2 increased from 6.45% to 48.44%, therefore molar ratio of TTIP: H_2O in EtOH effects upon content of the Synthesized TiO_2 in Bacterial Cellulose. The formation of TiO_2 impregnated in BC increased with the increasing of molar ratios of TTIP to H_2O in EtOH.

4.5 Study on Titanium tetraisopropoxide Convert to Titanium dioxide into Bacterial Cellulose by Using FTIR

Figure 4.16 shows the FTIR spectrum of Titanium tetraisopropoxide the positions of CH_x stretching modes in the ~3000 cm^{-1} region; C–C stretching, 1132 cm^{-1}; C–O stretching in TTIP, 1008 cm^{-1} ; C–O stretching in isopropanol, 950 cm^{-1}; C–H stretching in propene, 915 cm^{-1}; CH rocking, 850 cm^{-1} ; Ti–O stretching, 626 cm^{-1} (Ahn *et al.*,2003).

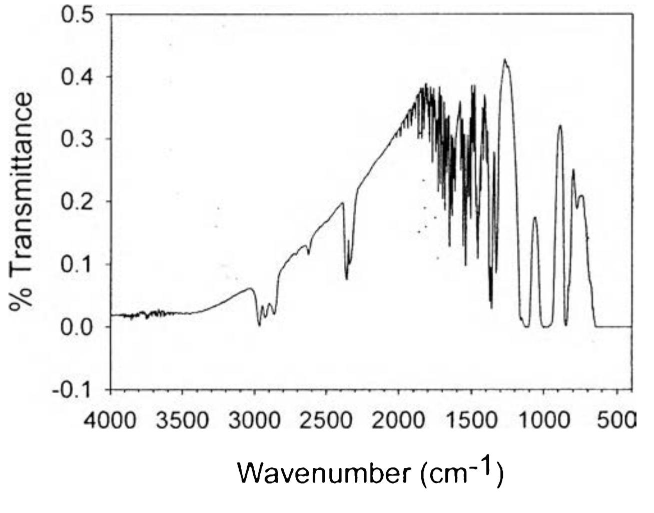


Figure 4.16 FTIR spectrum of titanium tetraisopropoxide.

Figure 4.17 shows the FTIR spectrum of Titanium dioxide. It was found that CH_x stretching modes in the ~3000 cm^{-1} region, C–O stretching, 1008 cm^{-1} and C–H stretching in TTIP were absent. Besides, vibration of the Ti–O bonds was observed at range of 608–620 cm^{-1} and near 400 cm^{-1} (Erkov. *et al.*,2000).

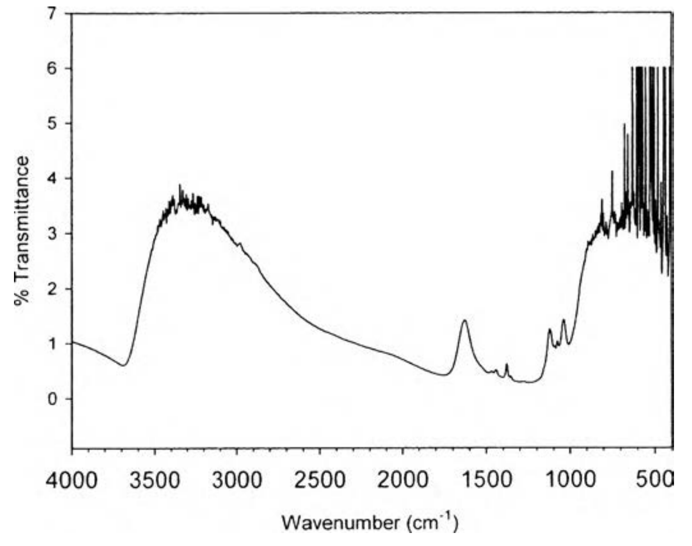


Figure 4.17 FTIR spectrum of Titanium dioxide.

Gladfelter *et al.*, (1994) studied the reaction mechanism for the formation of by using TTIP. They identified the following reaction:

$$Ti(OC_3H_7)_4 \longrightarrow TiO_2+4C_3H_6+2H_2O \quad (\geq 400\ °C) \qquad (1)$$

$$Ti(OC_3H_7)_4 \longrightarrow TiO_2+2C_3H_6+2HOC_3H_7 \quad (\leq 400\ °C) \qquad (2)$$

The FTIR spectrum of pure bacterial cellulose is shown in Figure 4.18. It was observed the OH stretching (3500 cm^{-1}), C–H stretching vibration (2910 cm^{-1}) O=C vibration (1642 cm^{-1}), C–OH stretching (1060 cm^{-1}) and C–O–C bending vibration (1163 cm^{-1}). Resulting from coverage by thin layer of TiO_2, this C–H in-plane stretching was blocked, C–H stretching vibration (2910 cm^{-1}) of FTIR spectrum of TiO_2 impregnated bacterial cellulose is weakened (see figures 4.18 and 4.19) (Sun. *et al.*,2009).

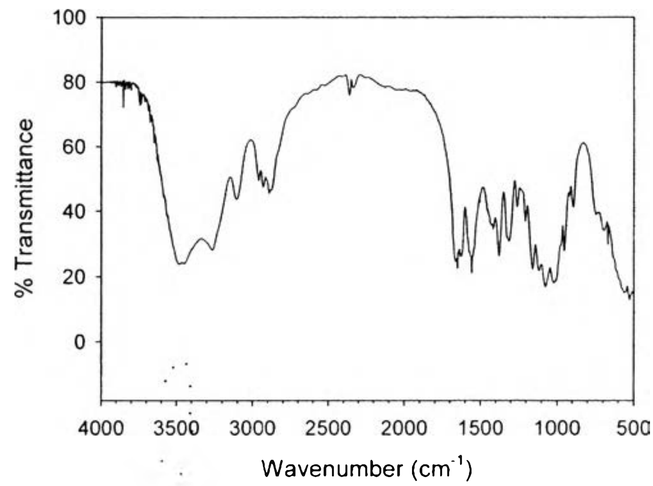


Figure 4.18 FTIR spectrum of pure bacterial cellulose.

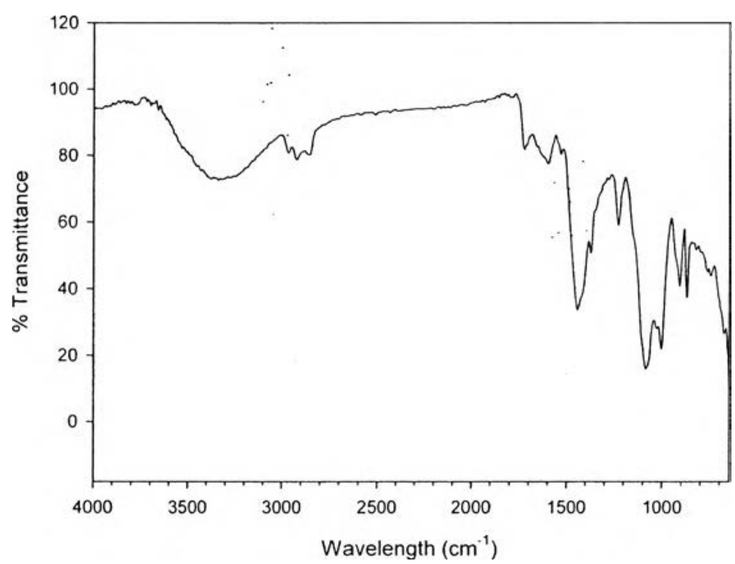


Figure 4.19 FTIR spectrum of TiO_2 impregnated bacterial cellulose prepared at different molar ratio of TTIP: H_2O in EtOH equal to 0.5.

Figure 4.20A-4.20D show the ATR-FTIR spectra of TiO_2 impregnated bacterial cellulose prepared at molar ratio of TTIP: H_2O in EtOH equal to 0.5 after washed by sonication in absolute EtOH for different time. It was found that the patterns of ATR-FTIR spectra are the same hence, sonication time none effect for elimination excess TTIP containing TiO_2 impregnated bacterial cellulose.

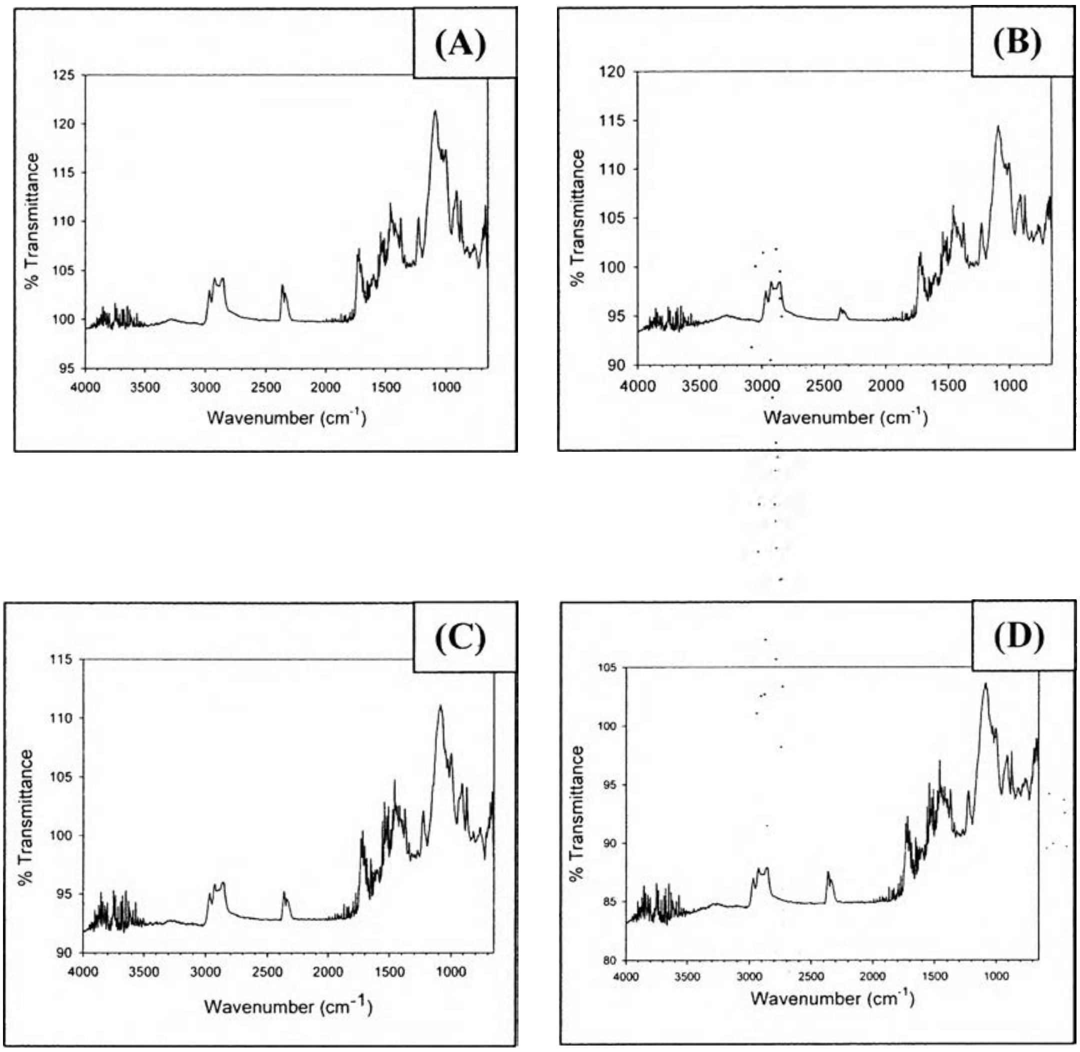


Figure 4.20 ATR-FTIR spectra of TiO_2 impregnated bacterial cellulose prepared at molar ratio of TTIP: H_2O in EtOH equal to 0.5 after washed by sonication in absolute EtOH for for different time of 15 min (A), 30 min (B), 45 min (C) and 60 min (D).

4.6 Photocatalytic Activity of TiO_2 Impregnated Bacterial Cellulose

The photocatalytic activity of TiO_2 impregnated in bacterial cellulose prepared at different molar ratios of TTIP: H_2O in EtOH was investigated by determining photodegradation of methylene blue under UV light (see Figures 4.21 and 4.22).

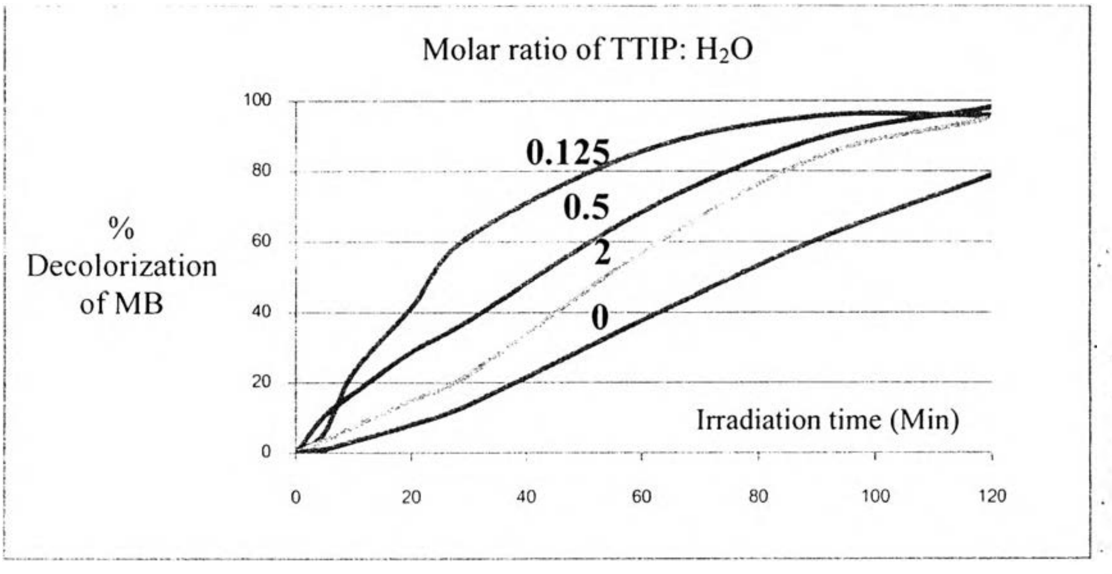


Figure 4.21 Photodegradation of methylene blue under UV light by TiO_2 impregnated in bacterial cellulose prepared at molar ratio of TTIP: H_2O in EtOH equal to 0, 0.125, 0.5 and 2.

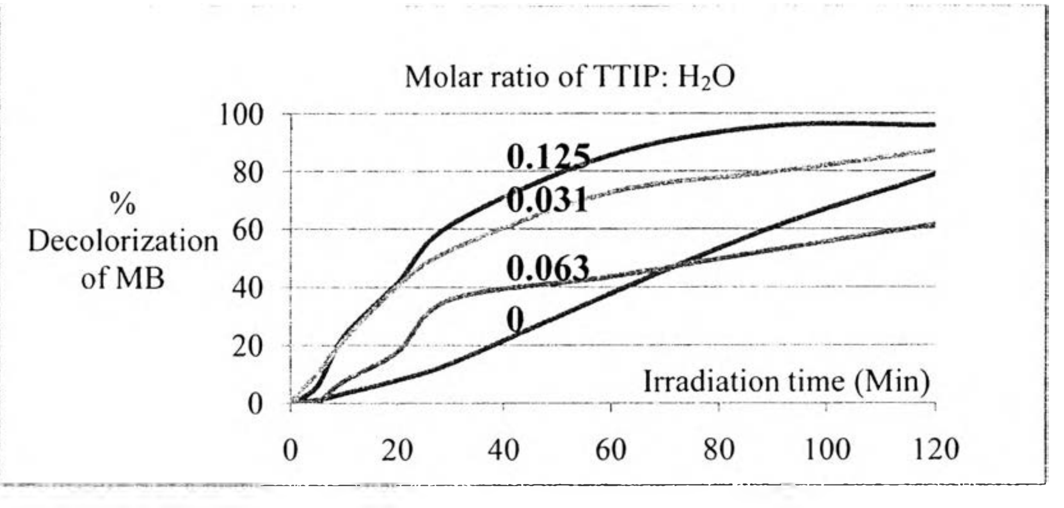


Figure 4.22 Photodegradation of methylene blue under UV light by TiO_2 impregnated in bacterial cellulose prepared at molar ratio of TTIP: H_2O in EtOH equal to 0, 0.031, 0.063 and 0.125.

It was found that the photocatalytic activity of TiO_2 impregnated in bacterial cellulose prepared at the molar ratio of TTIP: H_2O equal to 0.125 had the highest photocatalytic activity. The molar ratio of TTIP: H_2O equal to 0.125 was less than the stoichiometric molar ratio of TTIP: H_2O in the hydrolysis reaction of TTIP or water existed in the reaction in an excess amount compared to TTIP. Accordingly, most of the added TTIP was converted to TiO_2 and deposited on the surface of bacterial cellulose nanofibers. For the molar ratios of TTIP: H_2O equal to 0.5 that is the stoichiometric molar ratio, the synthesized TiO_2 was deposited more on bacterial cellulose as evidence by the increasing of the average fiber diameter and the increasing of TiO_2 content in bacterial cellulose. However, it did not result in the increasing of photocatalytic activity. This might be explained that the dense TiO_2 covered on the surface of bacterial cellulose preventing the penetration of UV light to the interior part of bacterial cellulose. Then only TiO_2 at the surface of bacterial cellulose could expose to the UV light and undergo photocatalytic reactions. In case of the molar ratio of TTIP: H_2O was decreased to 0.031, TTIP was used in a tiny amount in the hydrolysis reaction. The formation of TiO_2 was occurred in some area on the surface of bacterial cellulose nanofibers (see figure 4.9A) as a result, the irradiation of UV light was not promote reactive species from TiO_2 in areas none of TiO_2 and they concealed the direction of UV light resulting in loss of the photocatalytic activity (see figure 4.22). On the other hand, when the molar ratio of TTIP: H_2O was increased up to 2, TTIP was used in a large excess amount in the hydrolysis reaction to the formation of TiO_2 on the surface of bacterial cellulose nanofibers, some TiO_2 was synthesized in a form of spherical particles impregnated in the porous structure throughout the bacterial cellulose matrix. Since the amount of water was fixed for each molar ratio of TTIP: H_2O, the increasing of the added amount of TTIP might result in the increasing of rate of the reaction. Then the crystalline structure of TiO_2 was not gradually formed on the surface of bacterial cellulose fibers but it formed as an individual spherical particle of TiO_2 embedded in the porous structure of bacterial cellulose. The decreasing of the photocatalytic activity might be due to the formation of these large TiO_2 particles, resulting in the decreasing of photocatalytic efficiency of TiO_2.

4.7 Antibacterial Activity of TiO_2 Impregnated Bacterial Cellulose

The antibacterial activity of TiO_2 impregnated bacterial cellulose prepared at different molar ratios of TTIP:H_2O, was determined by colony forming count method. In case of antibacterial activity against *E. coli*, it was found that the TiO_2 impregnated bacterial cellulose prepared at molar ratios of TTIP: H_2O equal to 0.5 showed the effective against *E. coli* (see Figures 4.23A-4.23D).

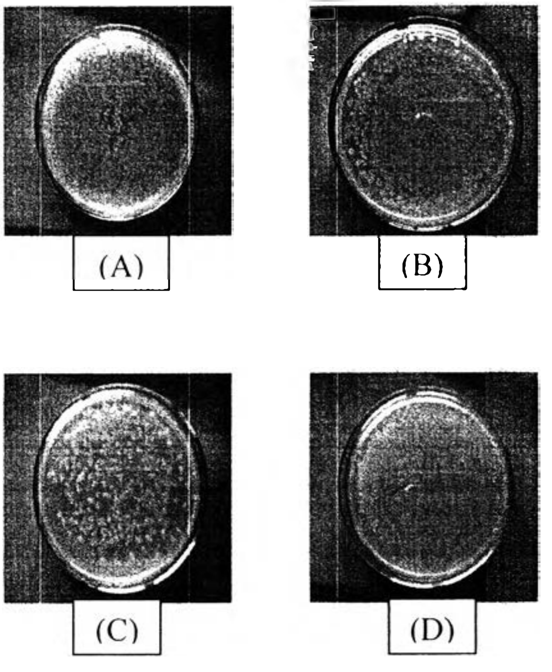


Figure 4.23 The antibacterial activity of TiO_2 impregnated bacterial cellulose prepared at different molar ratios of TTIP:H_2O equal to 0 (A), 0.125 (B), 0.5 (C) and 2 (D) against *E. coli*.

In case of antibacterial activity against *S. aureus*, it was found that the percent reduction of viable cells for the TiO_2 impregnated bacterial cellulose prepared at molar ratios of TTIP: H_2O equal to 0.125, 0.5, and 2, were 38.21%, 84.86%, and 20.35%, respectively.

From antibacterial activity against *E. coli* and *S. aureus*, It was observed that not only content of TiO_2, but also the direction of UV light and position of surface areas of TiO_2 impregnated in BC for supporting UV light affects antibacterial activity.