CHAPTER VI
RESULTS AND DISCUSSION

4.1 Production of Pure Bacterial Cellulose

Acetobacter xylinum strain TISTR 975, statically inoculates in a suitable culture medium consisting of D-glucose 4 %w/v and yeast extract 2 % w/v at 30 °C, produced bacterial cellulose and formed a thick gelatinous pellicle at the air-liquid interface of the culture medium within 1-9 days as shown in Figure 4.1

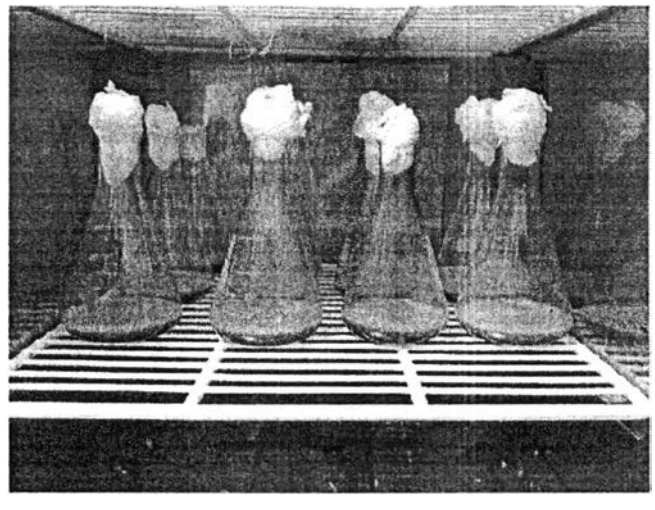


Figure 4.1 Cultivation of *Acetobacter xylinum* strain TISTR 975 : forming of the gelatinous bacterial cellulose pellicles.

The formation of the bacterial cellulose took place on the upper site of the cellulose layer. Bacterial cells increased their population by consumption of oxygen and D-glucose, initially dissolved in the culture medium, as carbon source. During growth and production of bacterial 5cellulose, the bacterial cells were gradually entrapped in the pellicle. As long as the system was kept unshaken, the bacterial cellulose pellicle suspended on the surface of culture medium.

After a certain cultivation time of *Acetobacter xylinum strain* TISTR 975, the gelatinous white pellicle of bacterial cellulose at the surface of culture medium

was harvested. For purification, the bacterial cellulose pellicle was boiled in a sodium hydroxide solution (NaOH) 4.0 %w/v for 24 hrs (3 times) to eliminate *Acetobacter xylinum* cells, protein which is a by-product during the bacterial metabolism and the culture medium that entrapped within the bacterial cellulose pellicle. After that, neutralization with acetic acid solution 3.0 %w/v for 24 hrs, followed by immersing in distilled water until neutral pH was obtained. Figure 4.2 (A) shows the yellow-translucent pellicle of untreated bacterial cellulose became a white-transparent pellicle after purification figure 4.2 (B).

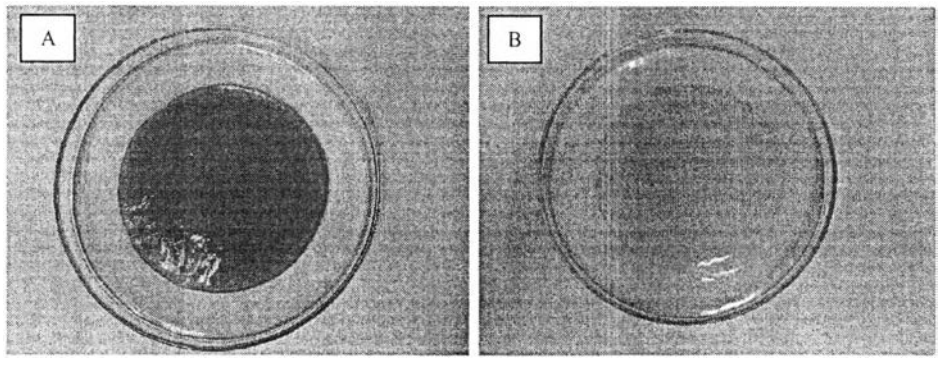


Figure 4.2 Purification of bacterial cellulose, (A) before purification and (B) after purification.

The purification result was also confirmed by using SEM images. Figure 4.3 (A) shows surface morphology of untreated bacterial cellulose compared with the NaOH-treated bacterial cellulose (figure 4.3 (B)). After purification, the random assembly of ultrafine three dimensional non-woven network structure of nanofibrils which constructs high porosity structure was obtained. It can be explained that the bacterial cells, protein, and any component of the culture medium were completely eliminated by NaOH treatment.

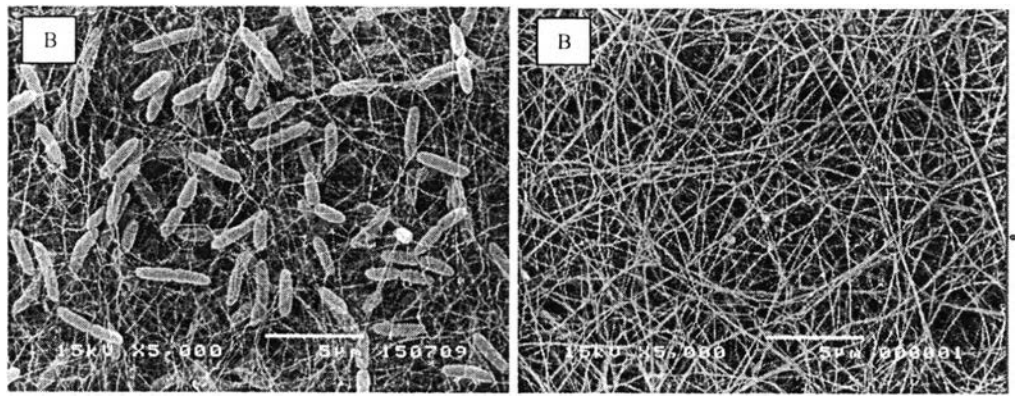
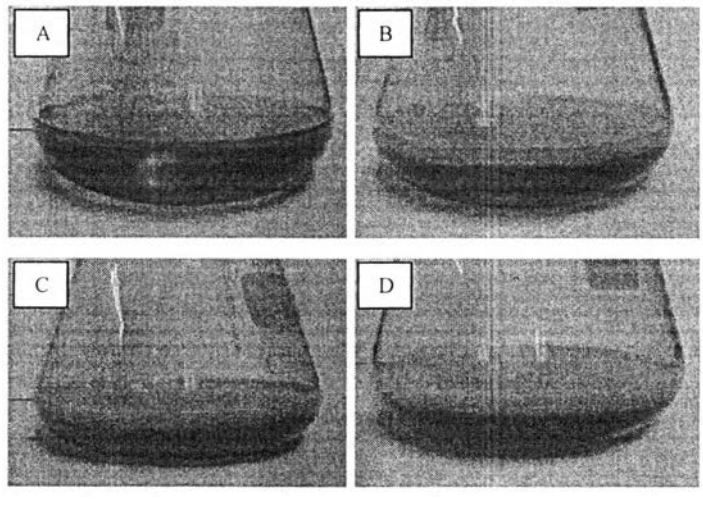


Figure 4.3 SEM images show the surface morphology of pure bacterial cellulose, (A) before purification and (B) after purification.

4.1.1 Effect of Cultivation Time on the weight Bacterial Cellulose Pellicle

Figure 4.4 shows the thickness of bacterial cellulose pellicle under different cultivation time 1-8 days and figure 4.5 shows the relationship between thickness of bacterial cellulose and cultivation time. From the results, after 1 day of cultivation, the thin gelatinous bacterial cellulose was formed at the surface of culture medium. The thickness of bacterial cellulose pellicle increased rapidly for 1-4 days. After 4 days, the thickness of bacterial cellulose pellicle increased slowly. In addition, dry weight of bacterial cellulose also increased rapidly for 1-4 days. After 4 days, dry weigh of bacterial cellulose increased slowly as shown in Figure 4.6



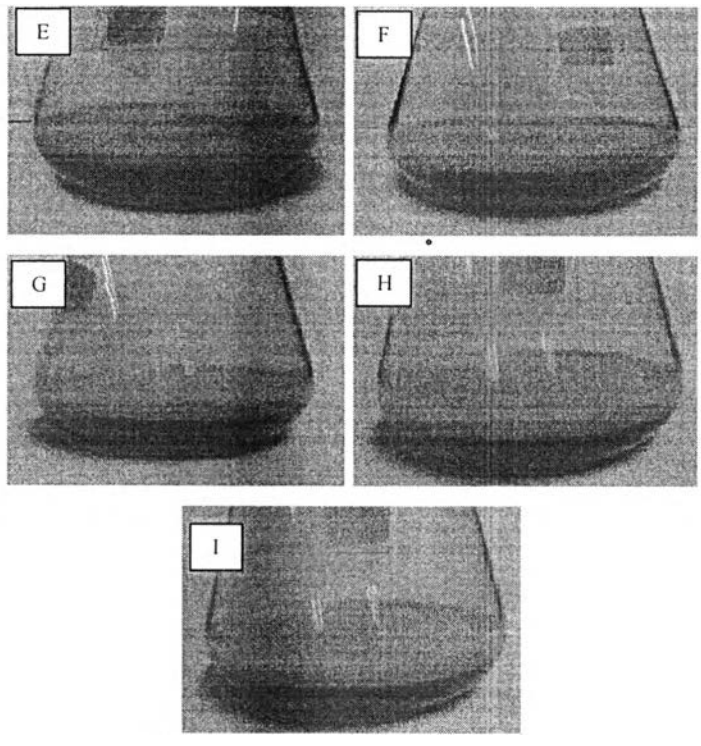


Figure 4.4 Bacterial cellulose pellicle produced by *Acetobacter xylinum* TISTR 975 under different cultivation time (A-I) 1-9 days, respectively.

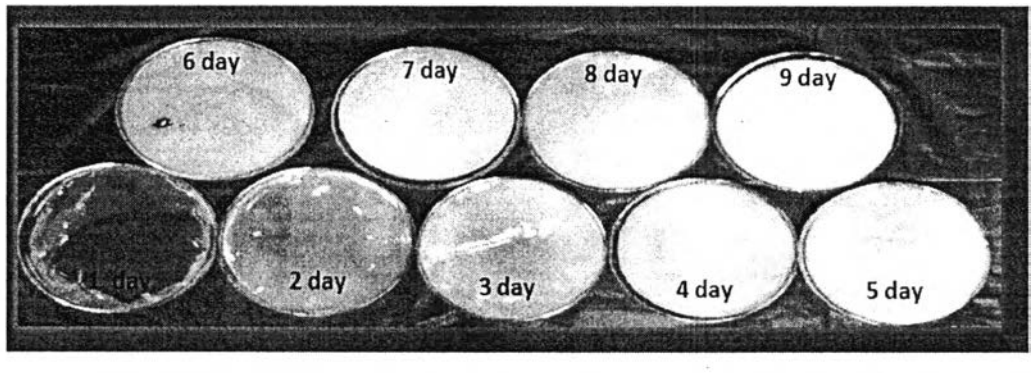


Figure 4.5 Bacterial cellulose pellicle produced by *Acetobacter xylinum* TISTR 975 under different cultivation time 1-9 days.

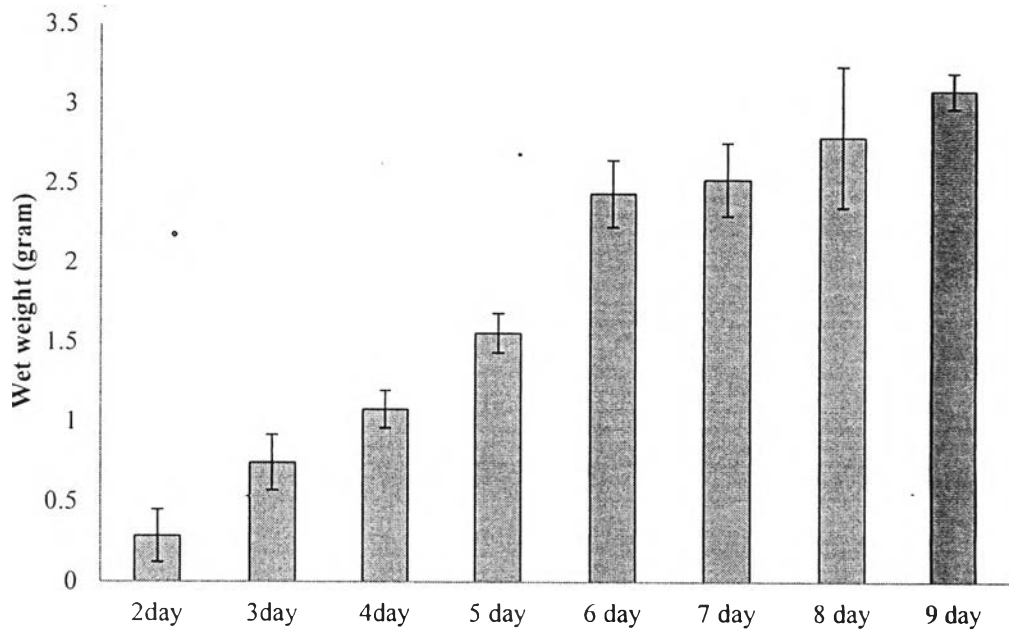


Figure 4.6 Comparison of wet weight of bacterial cellulose pellicle under different cultivation time (2-9 days).

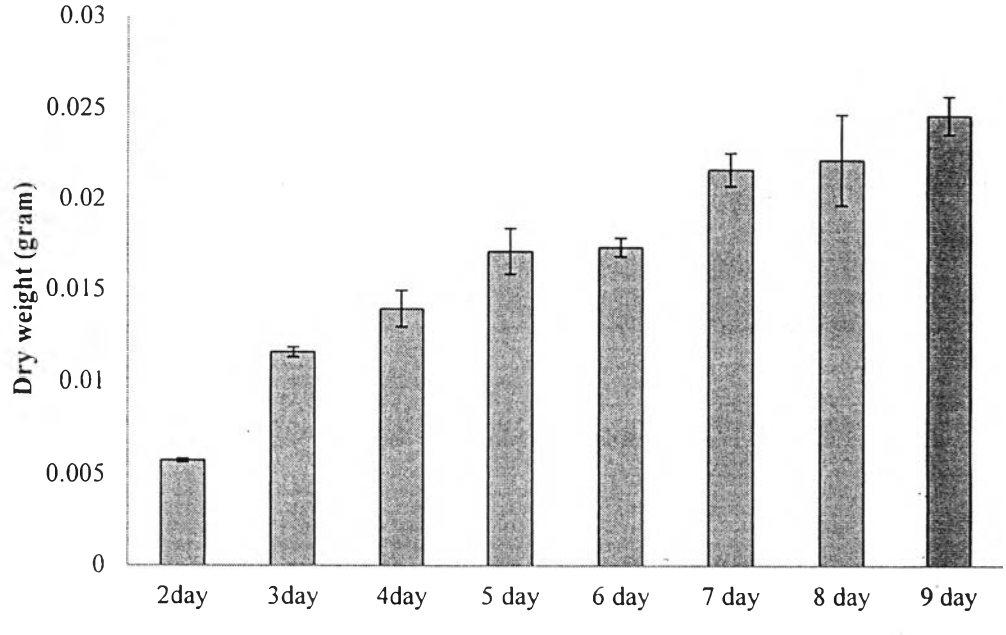


Figure 4.7 Comparison dry weight of bacterial cellulose pellicle under different cultivation time (2-9 days)

From the results, weight of bacterial cellulose increased rapidly in 2-6 days and then slowly until 9 days due to the culture medium not enough for produce cellulose. Suitable cultivation time is 4 days for production of pure bacterial cellulose because it this weight is suitable for wound heal.

4.1.2 Functional Groups of Pure Bacterial

The chemical functional groups of bacterial cellulose (BC) were examined by using fourier transform infrared spectroscopy (FTIR) ATR mode. The FTIR spectra was detected at wavenumber ranging from 4000 to 600 cm^{-1}. Figure 4.7 shown FTIR spectra of pure BC. The peak at wavenumber 3374 cm^{-1} corresponds to the OH stretching, the peak at 2896 cm^{-1} corresponds to the C-H stretching and the peak at 1032 cm^{-1} corresponds to the C-O stretching.

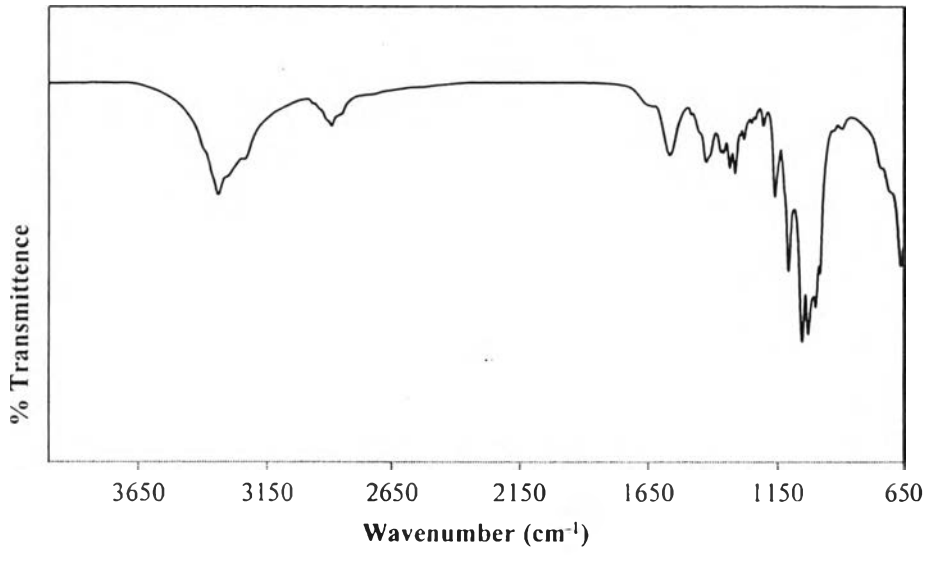


Figure 4.8 FTIR spectra of pure BC.

4.1.3 Morphology of Pure Bacterial Cellulose

SEM images of the surface morphology and cross sectional of the pure BC as shown in figure 4.9, SEM image of surface morphology of pure BC shows the random assembly of ultrafine three dimensional non-woven network structure of nanofibrils which constructs high the porosity structure. Figure 4.14 (b), SEM image of the cross sectional morphology of pure BC shows the interconnection

of fibrils linked between the multilayer of cellulose network structure. This result confirms the mechanism of formation of the bacterial cellulose pellicle, only bacterial exist in the vicinity of the surface of culture medium can be associate with oxygen. Production of bacterial cellulose pellicle occurs downwards.

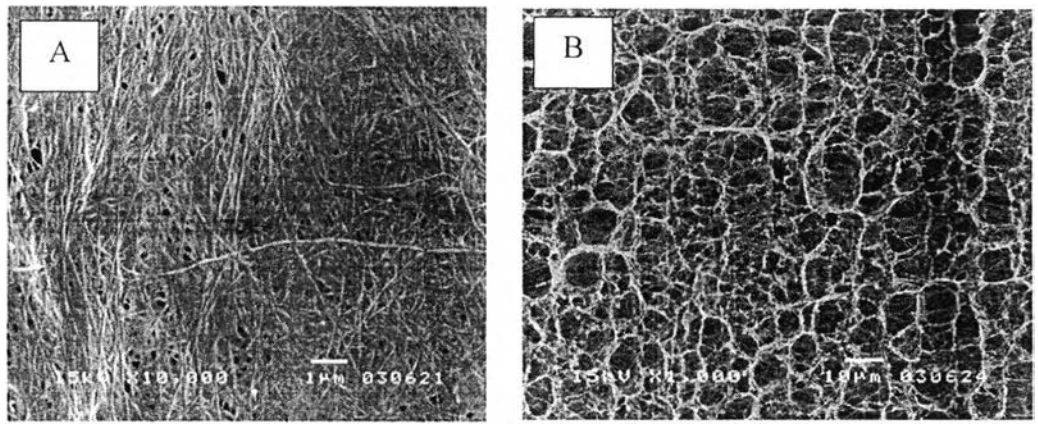


Figure 4.9 SEM images show the surface morphology of pure bacterial cellulose, A) surface, B) cross-section.

4.2 Production of BC/Cotton Composite Using Non-immobilization Technique.

4.2.1 Functional Groups of Bacterial Cellulose Composite

The chemical functional groups of cotton fabrics were evaluate by using fourier transform infrared spectroscopy (FTIR) ATR mode. The FTIR spectra was detected at wavenumber ranging from 4000 to 600 cm^{-1}. Figure 4.10 shown FTIR spectra of cellulose. The peak at wavenumber 3340 cm^{-1} corresponds to the OH stretching, the peak at 2900 cm^{-1} corresponds to the C-H stretching and the peak at 1030 cm^{-1} corresponds to the C-O stretching.

Figure 4.11 shows FTIR spectra of nylon mesh. The spectra shown the peak at wavenumber 3298 cm^{-1} corresponds to the NH stretching, the peak at 2932 cm^{-1} corresponds to the C- H stretching, the peak at 1633 cm^{-1} corresponds to the C=O stretching and the peak at 1532 cm^{-1} correspond to the NH bending. Then, nylon mesh was polyamide.

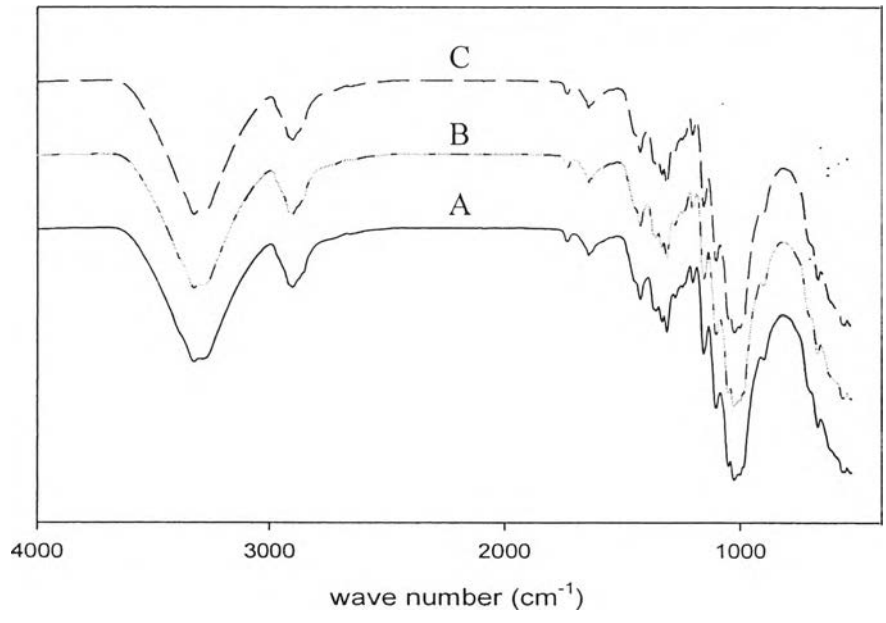


Figure 4.10 FTIR spectra of cotton fabrics A) high density of cotton fiber B) medium density of cotton fiber C) low density of cotton fiber.

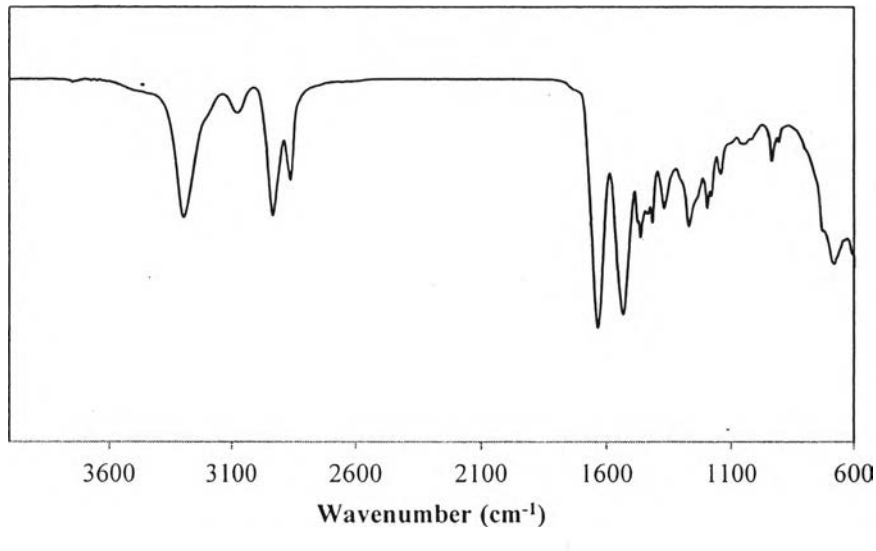


Figure 4.11 FTIR spectra of nylon fabric.

4.2.2 Morphology of BC/Cotton Composite and Cotton Fabric

In this work used 2 types of fabric for perform like supporter of bacterial cellulose fibril, Cotton and nylon fabrics were used as supporter that could increase production yield due to enhance cells attachment.

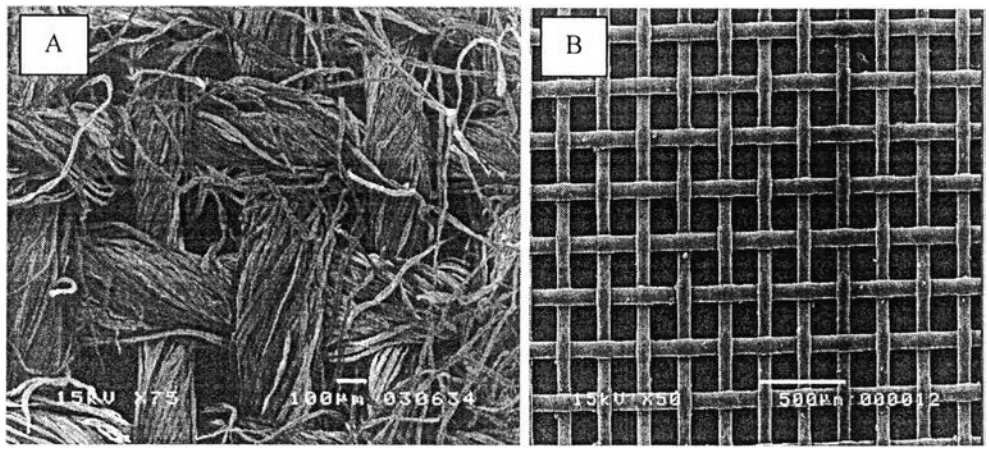


Figure 4.12 Surface morphology of (A) cotton fabric (B) nylon fabric

SEM images of the surface morphology of different types of cotton fabric. That shows fiber density on fabric, high density of cotton fiber consist of 25 horizontal 22 vertical per 1 cm^2, medium density of cotton fiber consist of 15 horizontal 16 vertical per 1 cm^2, low density of cotton fiber consist of 6 horizontal 12 vertical per 1 cm^2. The fabric containing high fibers that means high surface area, Surface area was important parameter for cell attachment.

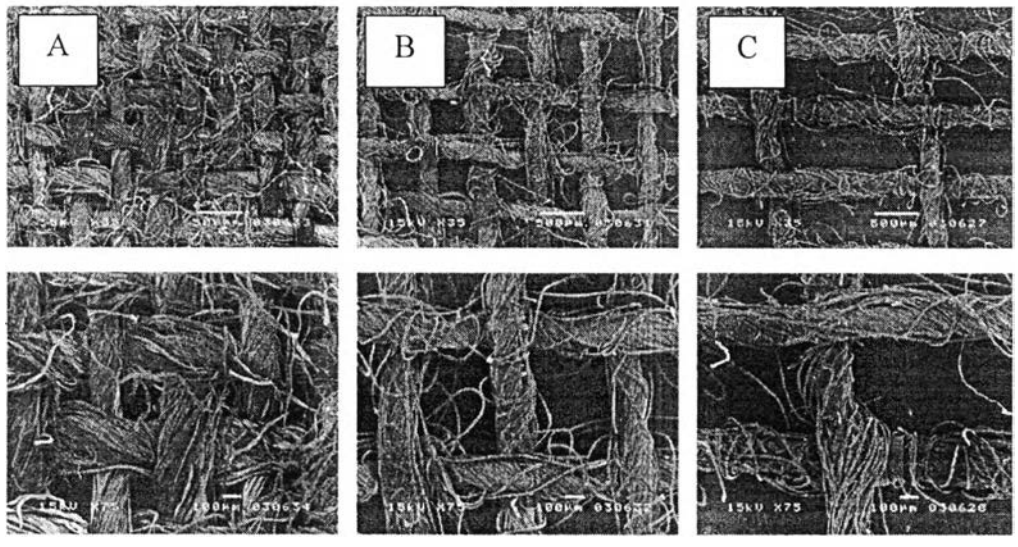
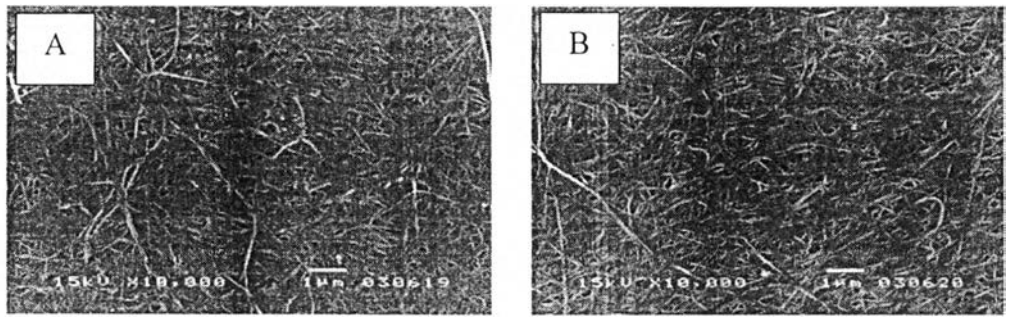


Figure 4.13 SEM images show the surface morphology of different types of cotton fabric, A) high density of cotton fiber B) medium density of cotton fiber C) low density of cotton fiber, respectively.

Morphology of BC/Cotton composites, cross-section morphology (figure 4.13) and surface morphology (figure 4.12), Bacterial cellulose composite with cotton fabrics were same as those of pure BC but the fibrilar structures in the composite were denser than that of pure BC. Used cotton supporting fabric could lead to enhance of the number of cell attachment of bacterial cells on the cotton fabrics especially high density of cotton fiber, resulting could support production yields of bacterial cellulose when compare with pure BC.



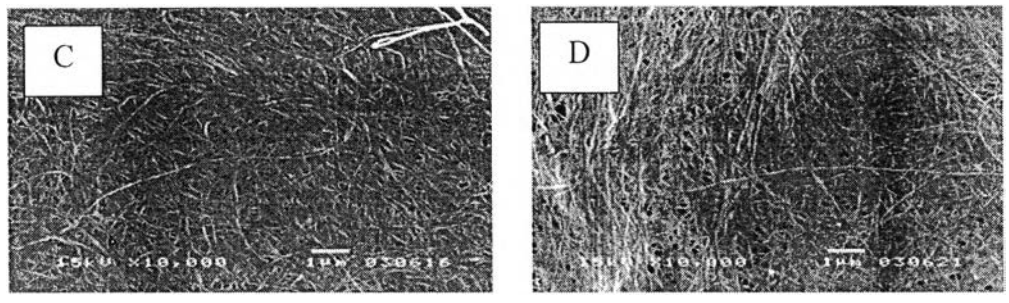


Figure 4.14 SEM images show the surface morphology of BC/cotton composites, A) BC/high density of cotton fiber B) BC/medium density of cotton fiber C) BC/low density of cotton fiber. D) pure BC, respectively.

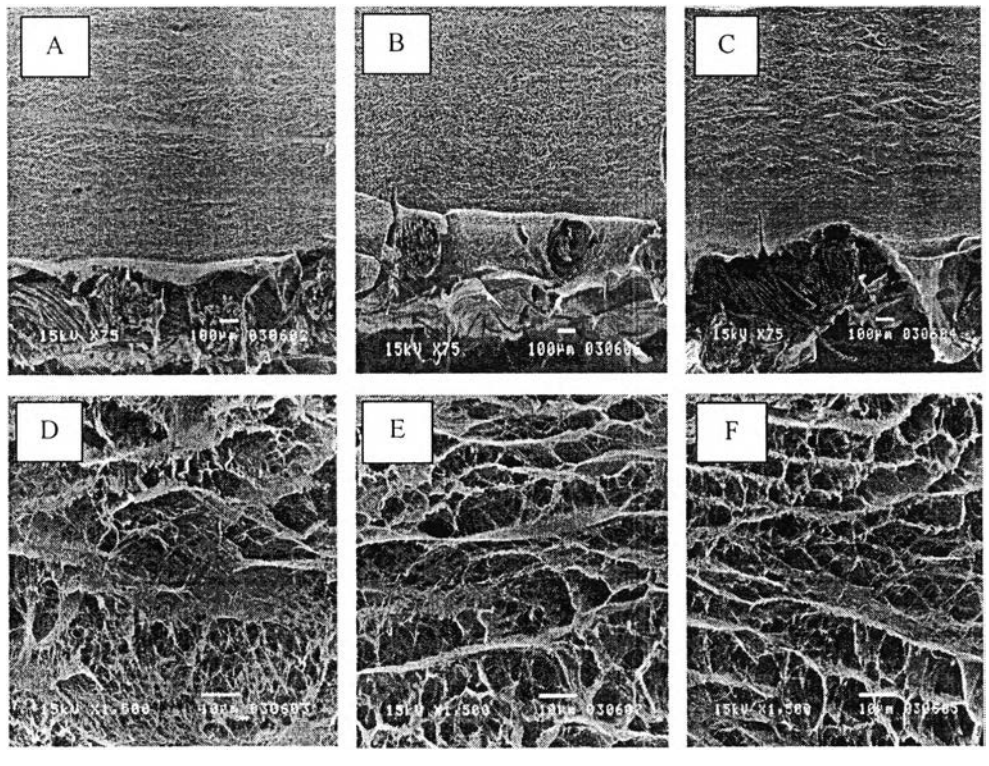


Figure 4.15 SEM images show the cross-section morphology of BC/cotton composites, A,D) BC/high density of cotton fiber B,E) BC/medium density of cotton fiber C,F) BC/low density of cotton fiber, respectively.

SEM images of interaction between BC fibrils and cotton fabric shows in figure 4.16. BC pellicle was removed out of the fabrics, confirmed that the BC fibrils occurred directly interacted on the surface of cotton fabric and held them

together. Although the BC fibrils density was lower than those produces at the surface due to removing, the ultrafine three dimensional non-woven network structure of nanofibrils was formed that connected each fabric fibres. It is suggested that this network structure contributes to the adhesion of BC pellicle to the fabric surface.

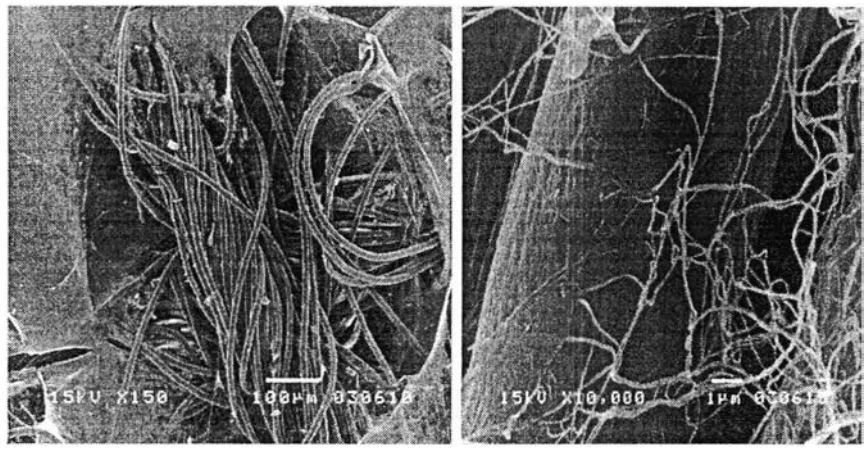


Figure 4.16 The interaction between BC fibrils and cotton fabric

4.2.3 Production Yield of BC/Cotton Composite

Fabric containing high number of cotton yarn density on cotton fabric promoted large surface area that benefit for cell attachment. Cell attachment was important effect on behavior of *Acetobacter xylinum*. Therefore the production yield (figure 4.15) and thickness (figure 4.16) of high number of cotton yarn density on cotton fabric demonstrated highest BC fibril yield follow by medium number of cotton yarn density on cotton fabric and the last on low number of cotton yarn density on cotton fabric.

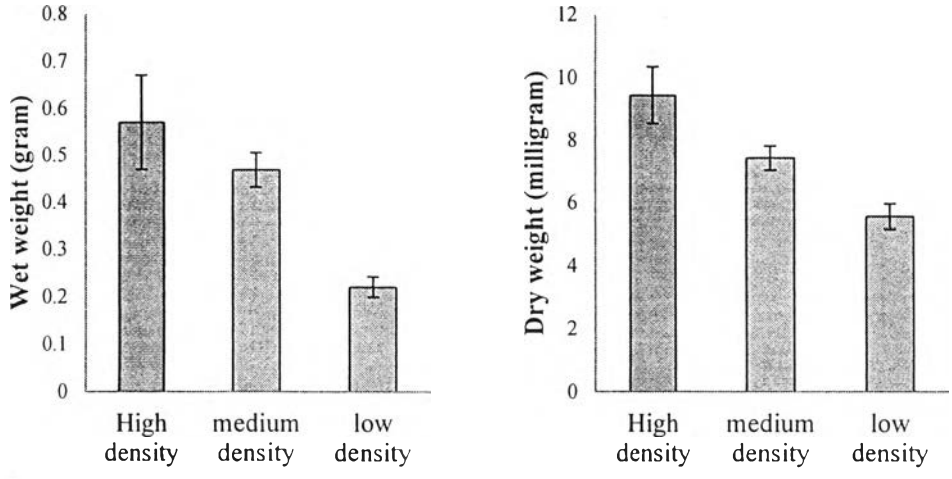


Figure 4.17 Production yield wet and dry weight of BC/Cotton composite by varies different number of yarn density on cotton fabric.

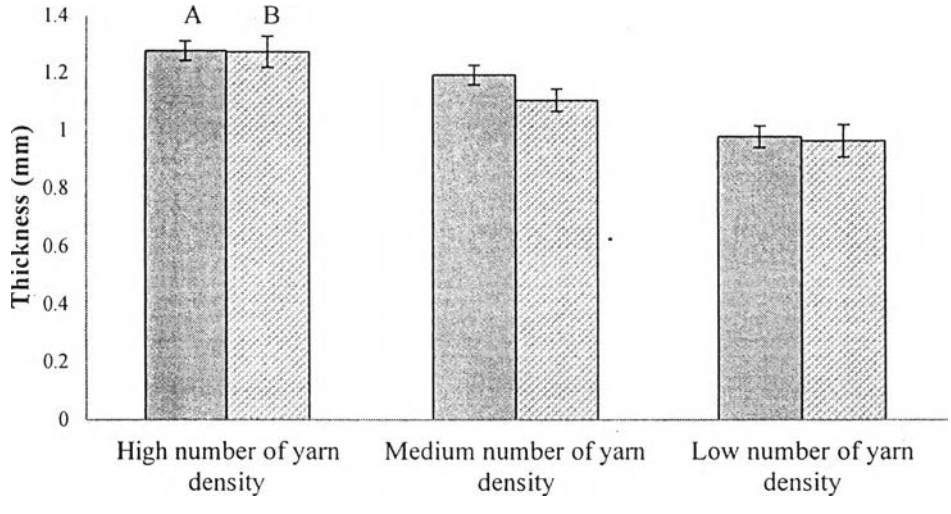


Figure 4.18 Thickness of BC/Cotton composite by varies different number of yarn density on cotton fabric A) wet state, B) dry state.

Comparison between pure BC and BC/Cotton composite (Figure 4.17, 4.18), The weight of both state was similar trends. BC/Cotton shows higher weight than pure BC due to cotton fabric as supporter that bacterial cell could attach everywhere on cotton surface, after that produced BC fibril. In case of pure BC behavior, bacterial cell start to produced BC fibril at edge of beaker and then continue to center. From this behavior used long time and production yield not stable in figure 4.18.

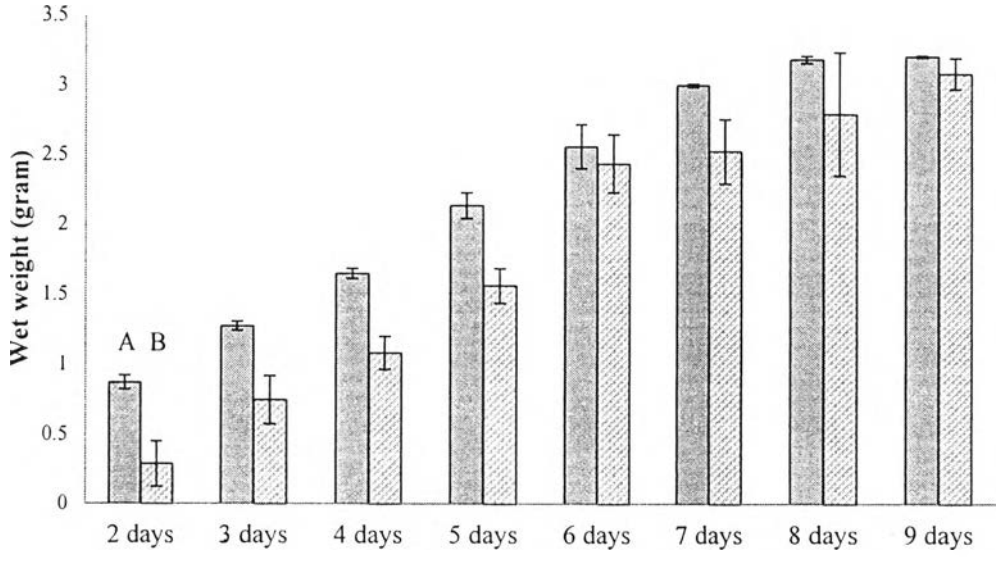


Figure 4.19 Comparison the production yield wet state A) BC/Cotton composite, B) pure BC.

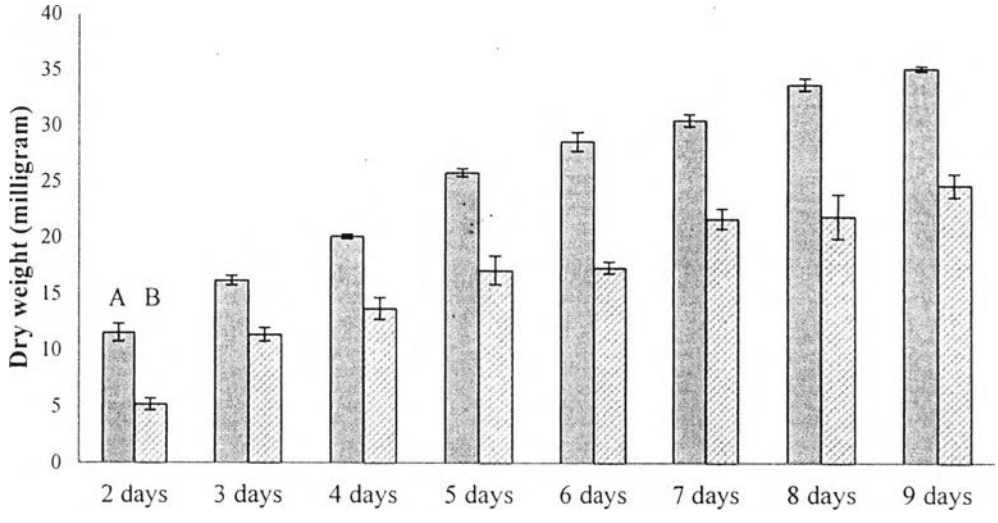


Figure 4.20 Comparison the production yield dry state A) BC/Cotton composite, B) pure BC.

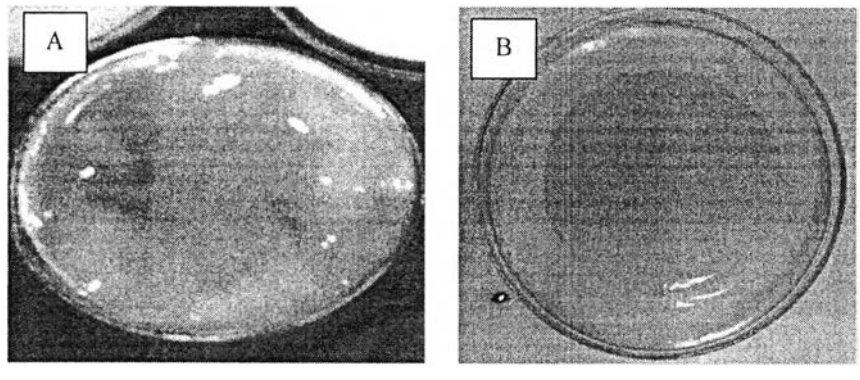


Figure 4.21 Comparison the production yield wet state A) pure BC, B) BC/Cotton composite.

4.3 Production of BC/Cotton Composite Treated Cotton Fabric via DBD Plasma Using Non-immobilization Technique.

4.3.1 Morphology of Cotton Fabric Treated via DBD Plasma

Dielectric barrier discharge (DBD) plasma is widely used to modify the surface properties of polymer in many applications such as improving the adhesion of coating to polymers, printing and biomedical application. The interactions of plasma with polymer surface are physical bombardment and chemical reaction. Surface roughness and surface area were increased by etching polymer surface. In this point, can be improve cell attachment on fabrics, that the advantages of the production of fibrils. Figure (4.19) show the effect of DBD plasma treatment time on the surface roughness of cotton fabric. Surface of cotton were increased at 30 second and start damaged at 240 second. Therefore in this research, DBD plasma treatment time of 180 second was chosen because treatment time too long could lead to damage the structure of fabrics.

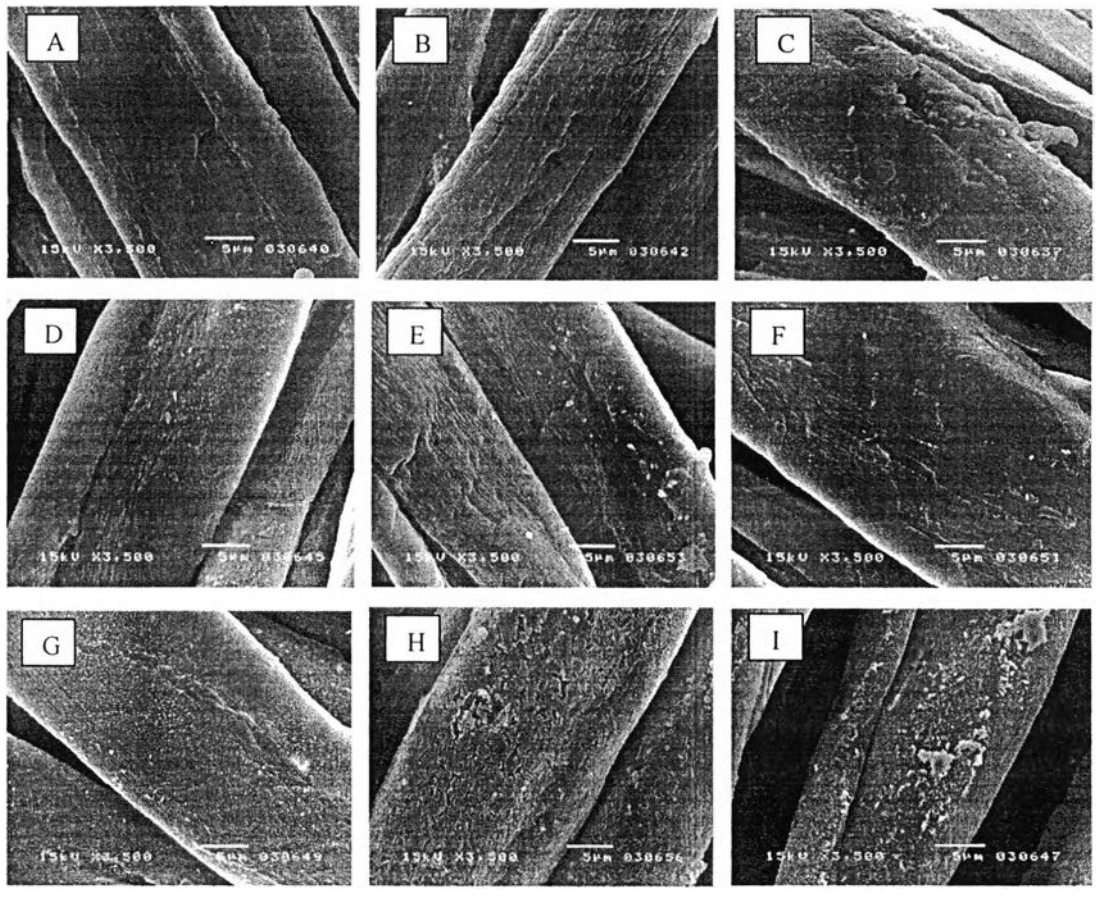


Figure 4.22 morphology the effect of DBD plasma treatment time on the surface roughness of cotton fabric, (a) non DBD plasma treated fabric, (b-i) DBD plasma treated fabric 10 seconds, 20 seconds, 30 seconds, 60 seconds, 2 min, 3 min, 4 min, and 5 min respectively.

4.3.2 ATR-FTIR Functional Groups of Cotton Fabric Treated via DBD Plasma

The chemical functional groups of cotton fabrics (figure 4.20) were evaluate by using fourier transform infrared spectroscopy (FTIR) ATR mode. The FTIR spectra was detected at wavenumber ranging from 4000 to 600 cm^{-1}. Figure 4.10 shown FTIR spectra of cellulose. The peak at wavenumber 3340 cm^{-1} corresponds to the OH stretching, the peak at 2900 cm^{-1} corresponds to the C-H stretching and the peak at 1030 cm^{-1} corresponds to the C-O stretching. The intensity of peak 1690 cm^{-1} corresponds to the C=O and 1610 cm^{-1} corresponds to the C-O-O

were increased because DBD plasma introduced oxygen polar groups in air occur new interaction on cotton fabric.

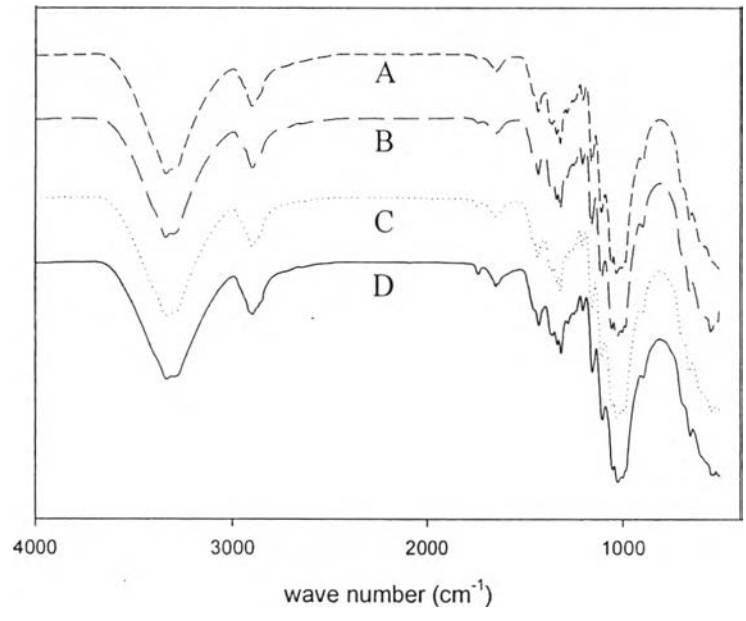


Figure 4.23 ATR-FTIR spectra chemical functional group of cotton fabric after treated via DBD plasma, a) non DBD plasma treated fabric, b) DBD plasma treated cotton fabric 60 seconds, c) DBD plasma treated cotton fabric 180 seconds, b) DBD plasma treated cotton fabric 300 seconds, respectively.

4.3.3 XPS Functional Groups of Cotton Fabric Treated via DBD plasma

X-ray photoelectron spectroscopy (XPS) is a surface-sensitive quantitative spectroscopic technique that measures the elemental composition at the parts per thousand range, empirical formula, chemical state and electronic state of the elements that exist within a material. Cotton fabric after treated by DBD plasma were evaluate XPS-Y scan (figure 4.21) and narrow mode (figure 4.22). Y-scan mode displayed after treated by DBD plasma was increased peak intensity both carbon at 284 eV and oxygen at 530 eV due to DBD plasma could incorporate oxygen atom in air on cotton fabric. For narrow mode (figure 4.22) was presented similar peak compare with before and after treated by DBD plasma at 283 eV corresponding to C-C and C-H stretching, 285 eV corresponding to C-O stretching, 287 eV

corresponding to C=O and C-O-C stretching. DBP plasma introduced new peak at 288 eV corresponding to O-C=O stretching from interaction with oxygen in air.

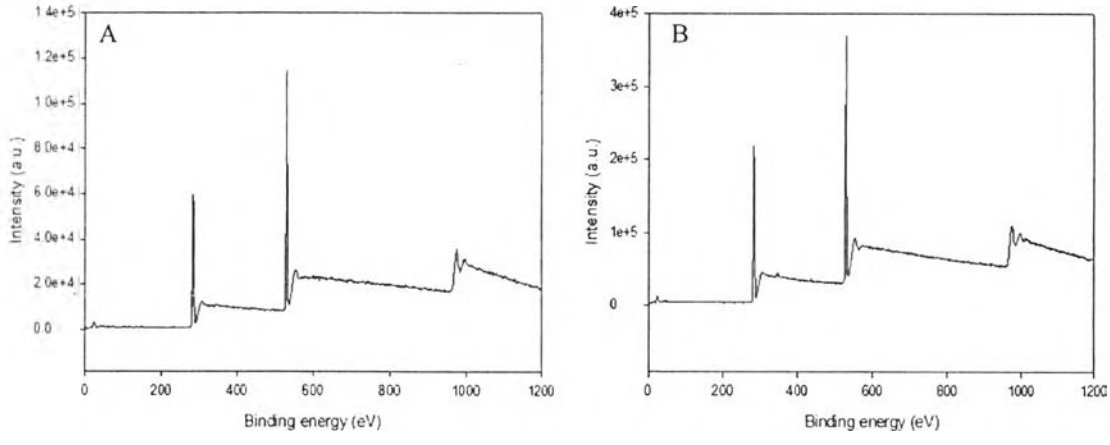


Figure 4.24 XPS-Y scan mode, chemical functional group of cotton fabric, A) non DBD plasma treated fabric, B) DBD plasma treated cotton fabric 180 seconds.

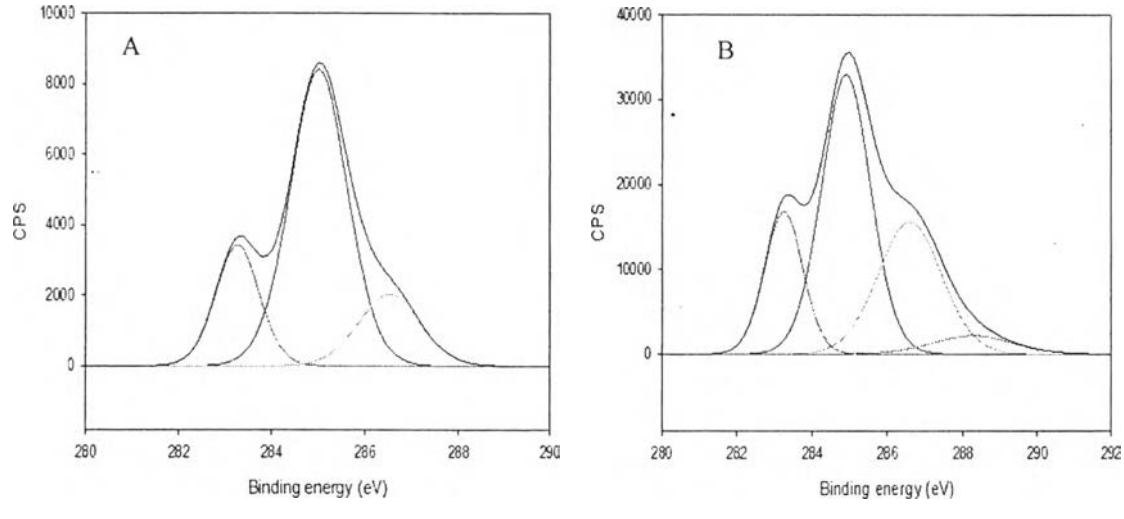
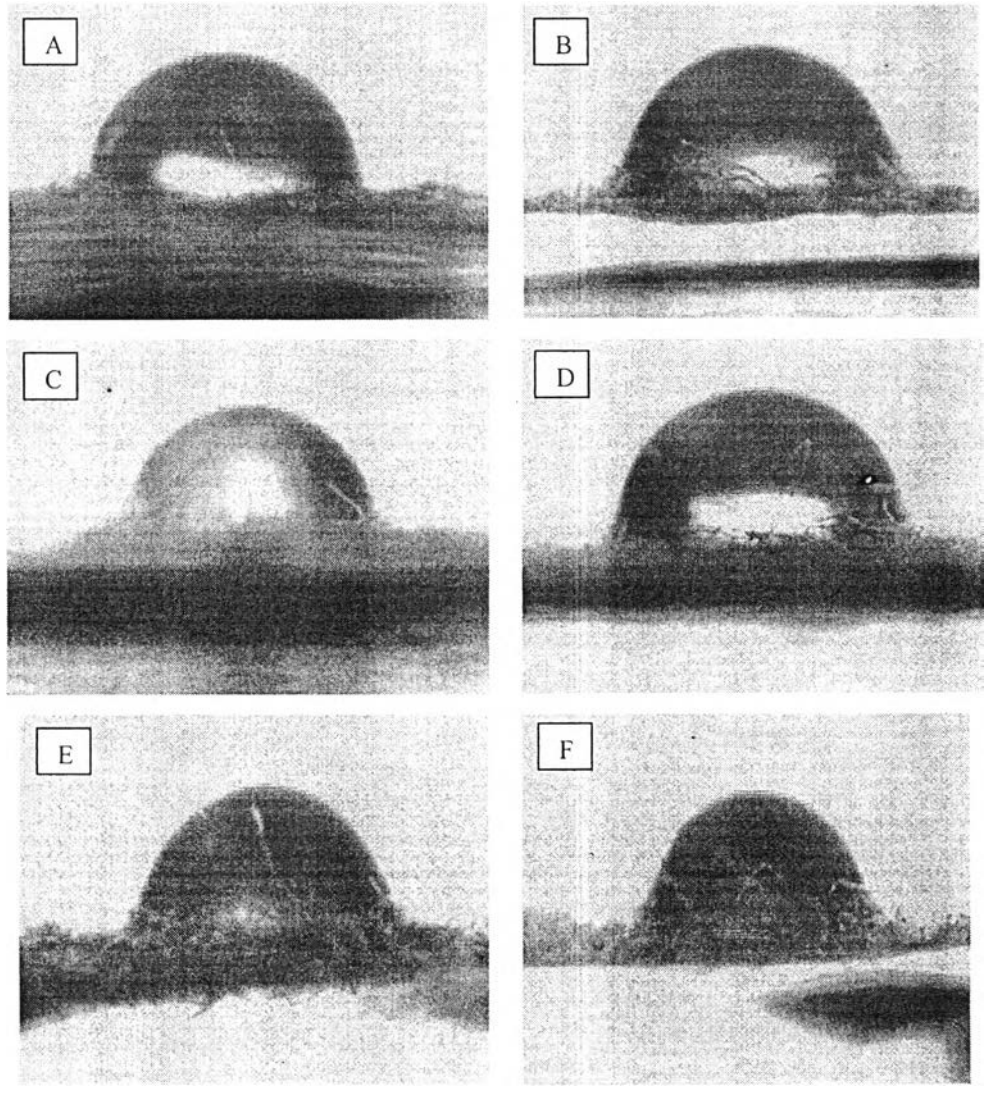


Figure 4.25 XPS-narrow mode, chemical functional group of cotton fabric, A) non DBD plasma treated fabric, B) DBD plasma treated cotton fabric 180 seconds.

4.3.4 The hydrophilic of Cotton Fabric Treated via DBD Plasma

Contact angle was used to evaluate hydrophilicity of cotton fabric. A fabric strip (50 mm x 50 mm) was used and drop distilled water on surface cotton fabric The liquid absorption depend on angle between cotton fabric and distilled water. Figure 4.23 shows the comparison of hydrophilicity abilities between non DBD plasma treated fabric and DBD plasma treated fabrics. The result of contact angle test, hydrophilicity of cotton fabric treated by DBD plasma was increased when treatment time. The behavior could explain, hydrophilicity was increase due to DBD plasma could introduce oxygen polar functional groups on the surface of cotton fabrics that leading to increase hydrophilicity of fabric.



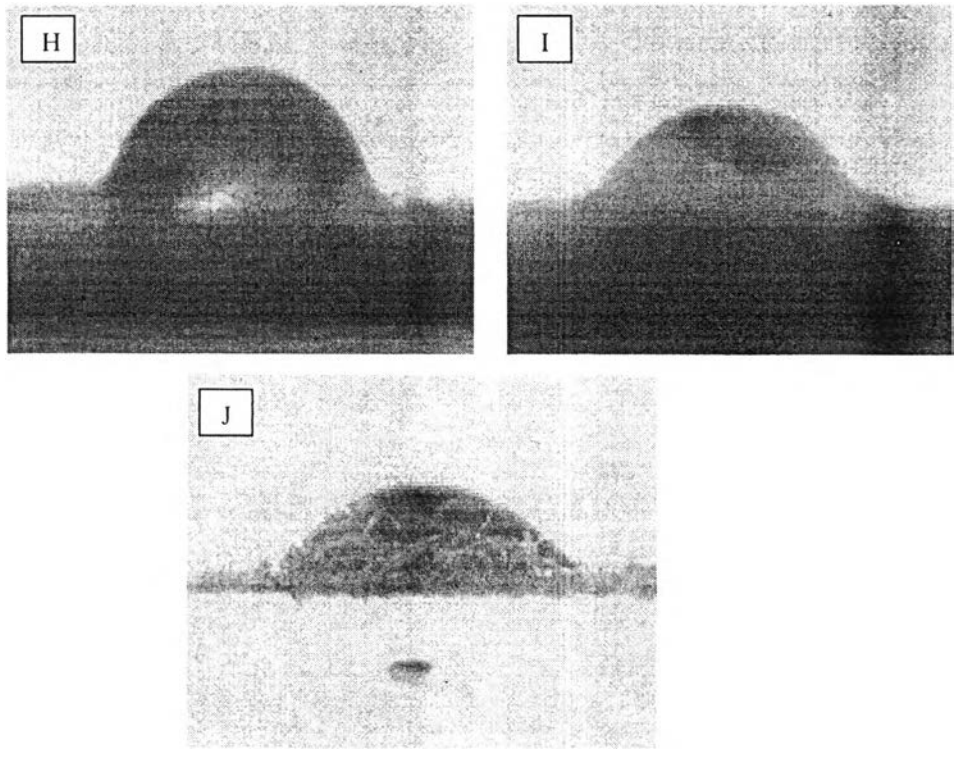


Figure 4.26 hydrophilicity of cotton fabric treated by DBD plasma varies treatment times, (A) non DBD plasma treated fabric, (B-J) DBD plasma treated fabric 10 seconds, 20 seconds, 30 seconds, 60 seconds, 2 min, 3 min, 4 min, and 5 min respectively.

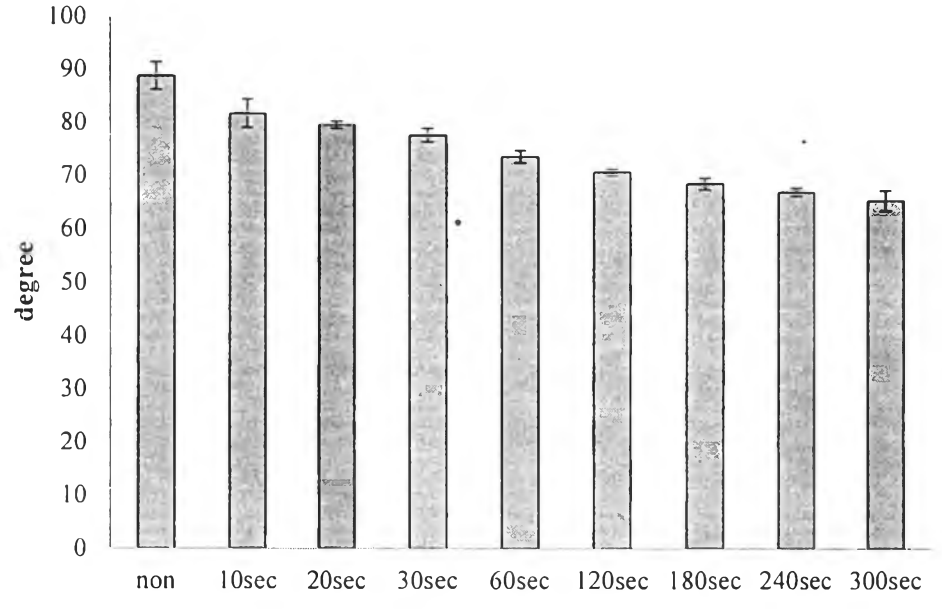


Figure 4.27 Comparison hydrophilicity of cotton treated different treatment times by DBD plasma.

4.2.5 The Water Absorption Capasity of Cotton Fabric Treated via DBD Plasma

Water absorption capacity plays an important role in case of applying in immobilization method. In this work was applied cotton fabric for absorption inoculum. DBD plasma was good candidate for use because could increase hydrophilicity and water absorption. Water absorption capacity presented in figure 4.25, high density of cotton fiber was highest water absorption capacity due to it present largest surface area. After treated 180 seconds water absorption capacity was increased 9.75% compare with non-treat.

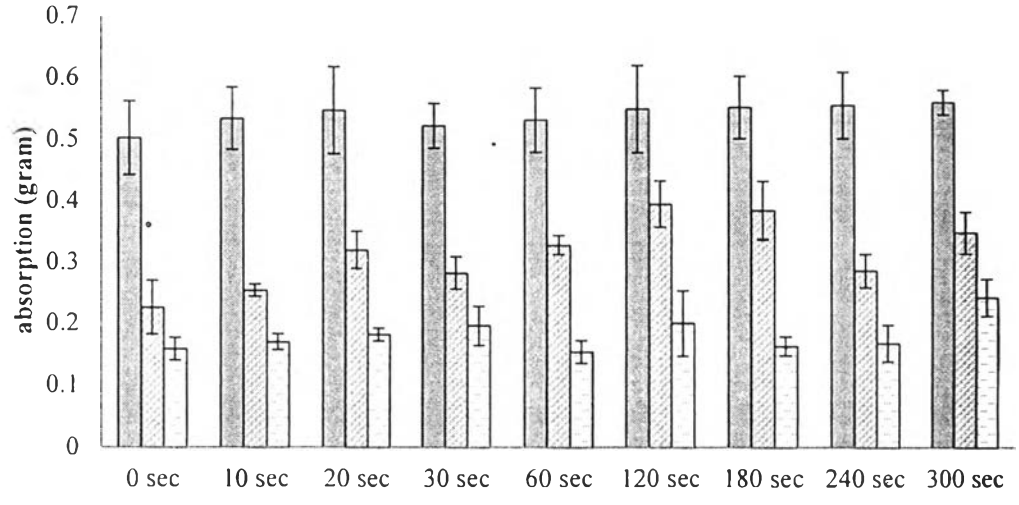


Figure 4.28 Comparison water absorption capasity of cotton treated different treatment times by DBD plasma.

4.3.6 Production Yield of BC/Cotton Composite Treated via DBD Plasma

Surface of cotton fabric after treated by DBD plasma display highest production yield both state because large surface area was introduce via DBD plasma that great for cell attachment and produce BC fibril. The production yield after treated at 180 second was increased 40 % in wet state and 49% in dry state.

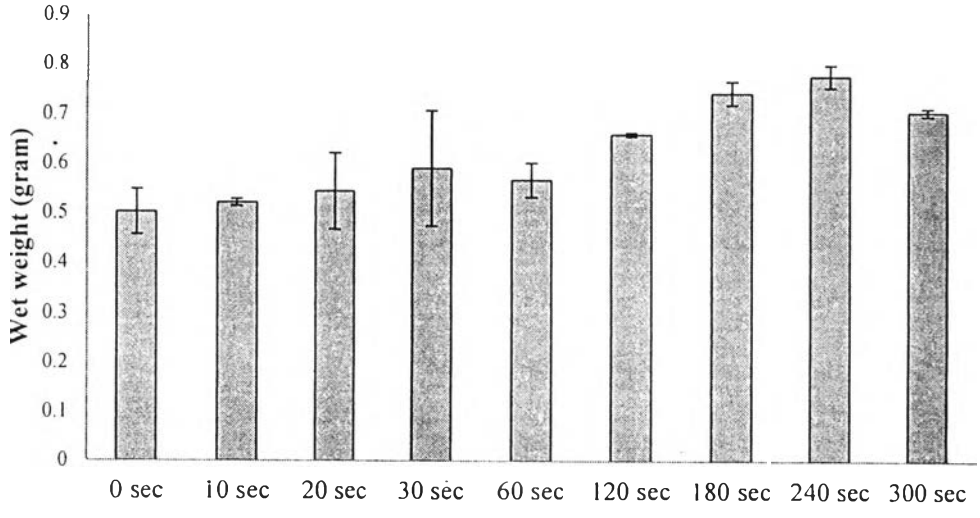


Figure 4.29 Comparison the production yield of BC/Cotton composite treated by DBD plasma in wet state.

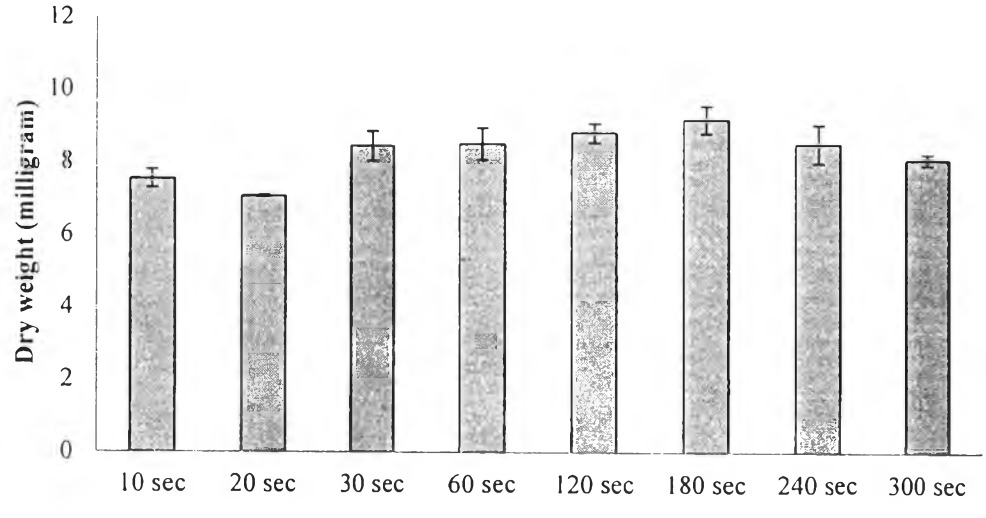


Figure 4.30 Comparison the production yield of BC/Cotton composite treated by DBD plasma in dry state.

4.4 Production of BC/Cotton Composite Using Absorption Immobilization Technique

4.4.1 Morphology of Acetobacter xylinum on Cotton Fabric Prepared by Imnobilization Technique

Figure 4.27 show that *Acetobacter xylinum* cells existing in the cotton fabric had rod shape and smooth cell wall. Although the composite applying the cell absorption immobilization technique did not have higher cell density than the non-cell-immobilized composite, the use of much less amount of inoculum was a great benefit.

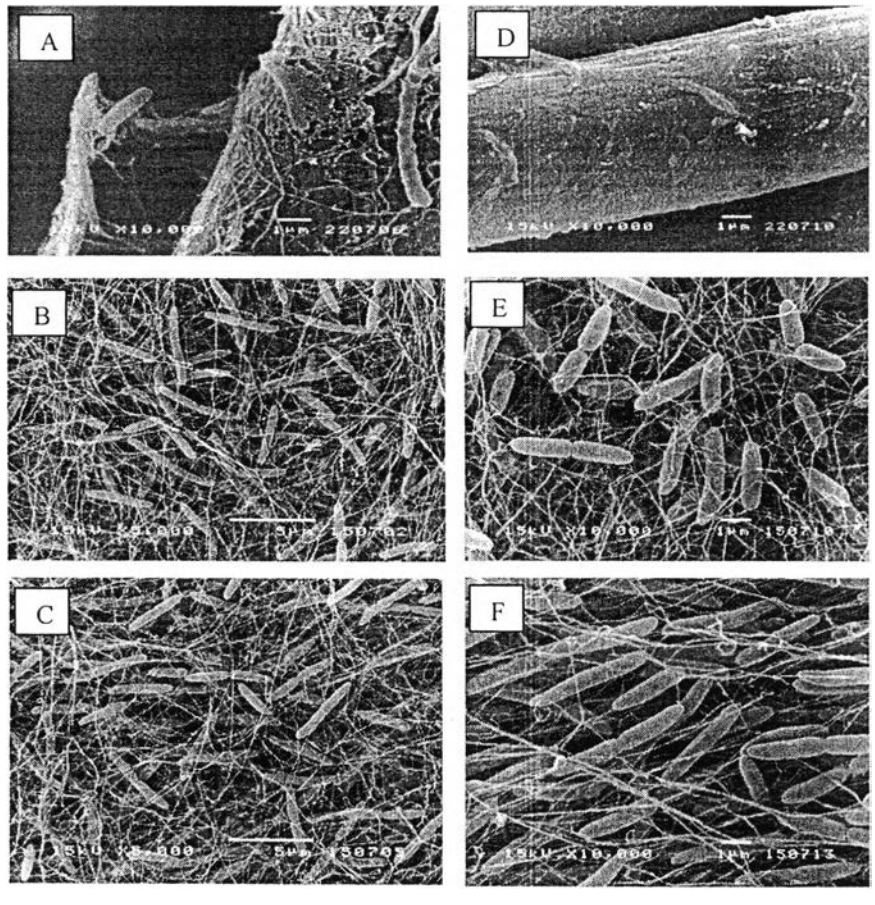
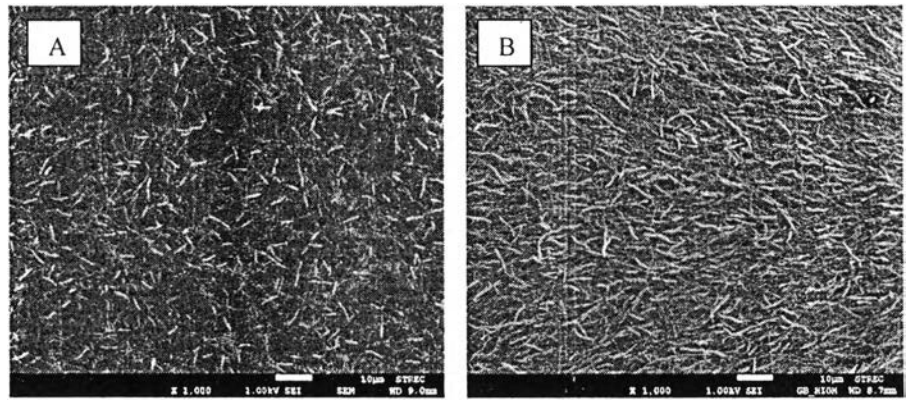


Figure 4.31 morphology *Acetobacter xylinum* on cotton fabric cultivated 0, 24, 48 houses, A-C) preparation by immobilization technique cultivated 0,12,48 horses, D-F) preparation by non-immobilization technique cultivated 0,12,48 horses, respectively

4.5 Production of BC/Cotton Composite Using Absorption and Crosslink Immobilization Techniques

4.5.1 Morphology of Acetobacter xylinum on Cotton Fabric Prepared Different Types of Imnobilization Technique and Surface Modification

SEM images of the *Acetobacter xylinum* attachment of the BC nanofibrils on the surface of cotton fibers after cultivated 48 hours. In case of surface pre-treatment of cotton fabric by citric acid, showed the higest amount of cells density follow by pre-treatment of cotton fabric by acetic acid and pre-treatment of cotton fabric by DBD plasma, respectively. This might be explained that acid condition was suitable for the growth of *Acetobacter xylinum*. Regarding BC/cotton composites applying cell crosslinking immobilization techniques, the cells density of *Acetobacter xylinum* on cotton fabric seemed lowest amount of cells, especially in the BC/cotton fabric composites applying glutaraldehyde solution crosslinking immobilization technique. However, it should be noted that crosslink cell immobilization by using glutaraldehyde might cause cell death due to the toxicity of glutaraldehyde



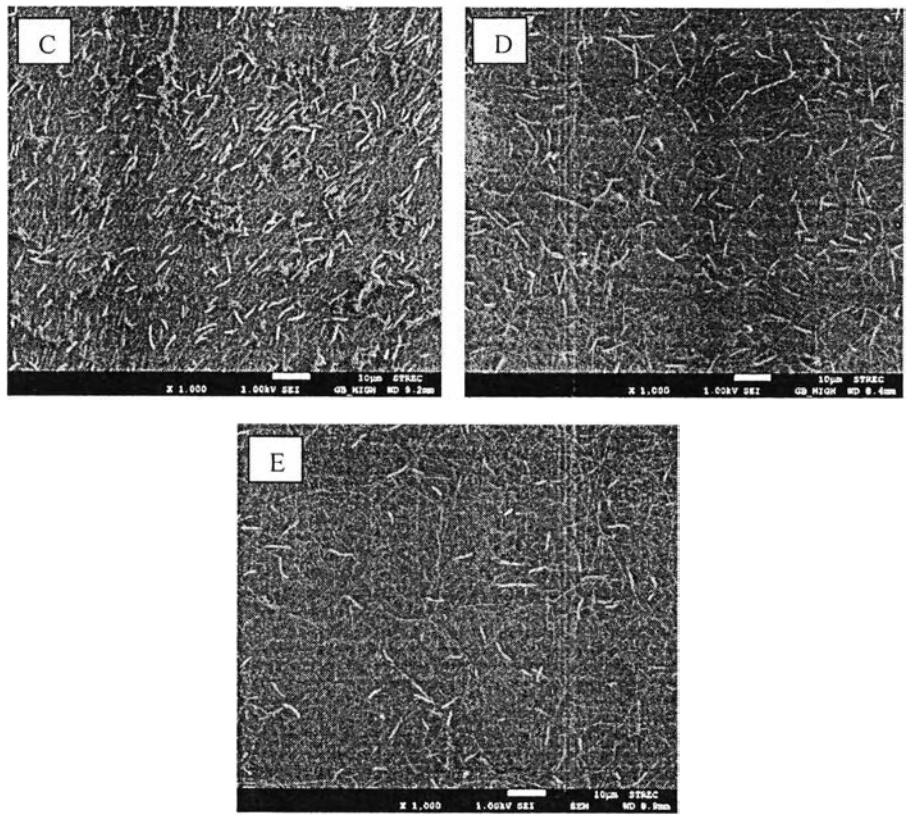


Figure 4.32 morphology *Acetobacter xylinum* on cotton fabric cultivated 48 houses, A) BC/cotton composite applying absorption cell immobilization and DBD plasma pre-treated cotton fabric, B) BC/cotton composite applying absorption cell immobilization and citric acid pre-treated cotton fabric, C) treated surface by acetic acid, D) BC/cotton composite applying absorption cell immobilization and acetic acid pre-treated cotton fabric, D) BC/cotton composite applying absorption and crosslinking (vapor glutaraldehyde), D) BC/cotton composite applying absorption and crosslinking (solution glutaraldehyde), respectively.

Figure 4.28 shows the SEM images of the attachment of the BC nanofibrils on the surface of cotton fibers. For the non-cell-immobilized BC/cotton composite in figure 3a, the attachment of BC fibrils on the cotton fibers was looser than the cell-immobilized composite. In case of surface pre-treatment of cotton fabric by citric acid, the attachment between BC fibrilars and cotton fibers was denser than the control without the acid pre-treatment. This might be explained that acid condition was suitable for the growth of *Acetobacter xylinum*. Regarding BC/cotton

composites applying cell immobilization techniques, the density of BC nanofibrils on cotton fabric seemed to be denser than that of the non-cell-immobilized composite, especially in the BC/cotton fabric composites applying absorption cell immobilization technique. However, it should be noted that crosslink cell immobilization by using glutaraldehyde might cause cell death due to the toxicity of glutaraldehyde. Figure 4.29 compared cells behavior after cultivated 48 hours, *Acetobacter xylinum* not different, It was smooth skin wall and rod shape.

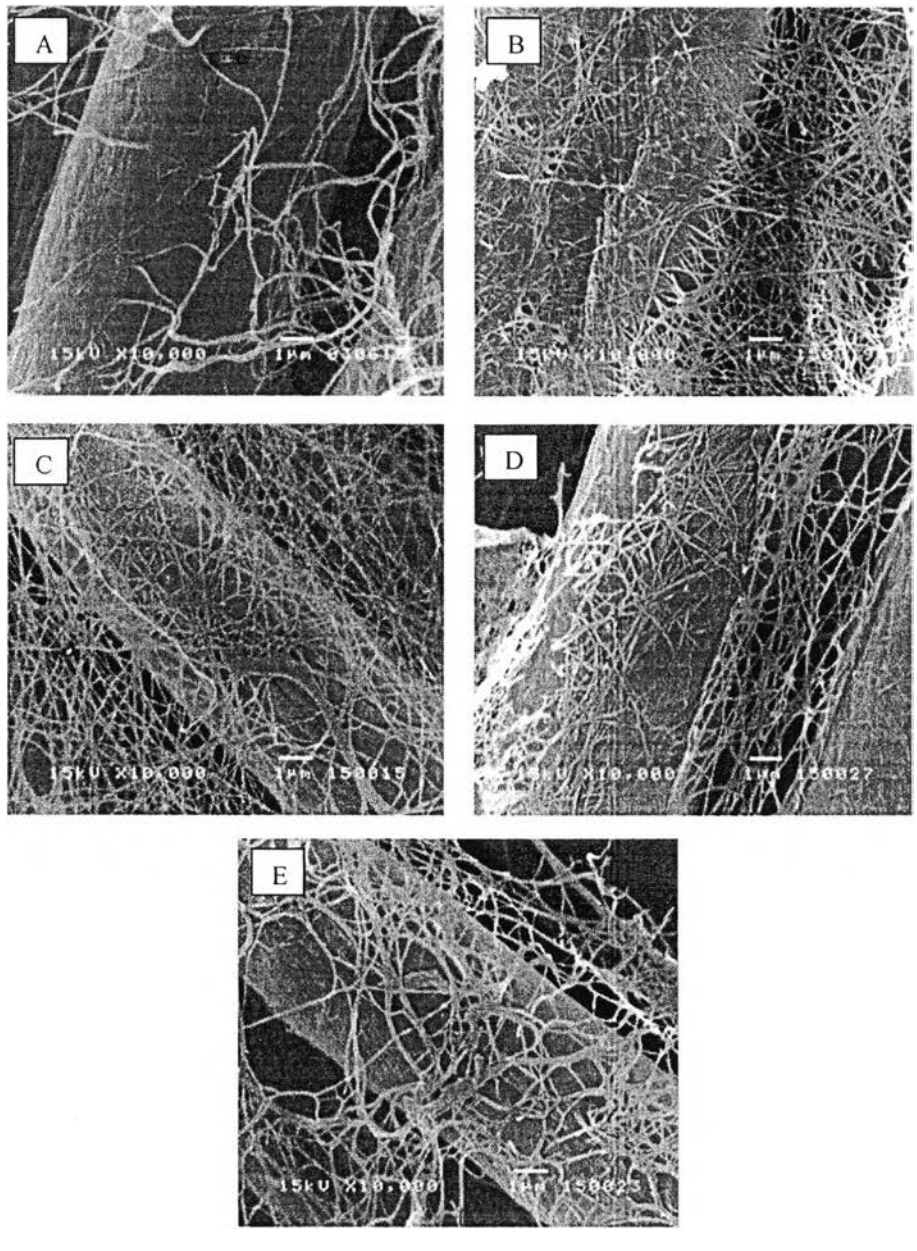
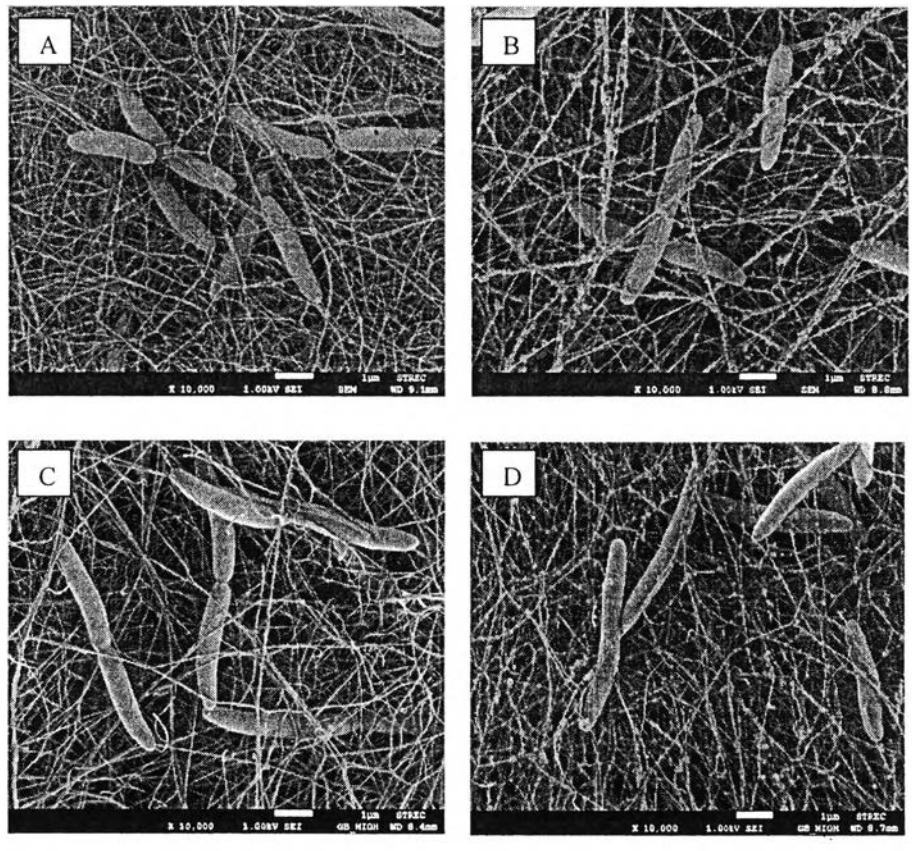


Figure 4.33 Attachment of bacterial cellulose fibers on the surface of cotton fibers for (A) non-cell-immobilized BC/cotton composite, (B) non-cell-immobilized BC/cotton composite using citric acid-pre-treated cotton fabric, (C) BC/cotton composite applying absorption cell immobilization and citric acid pre-treated cotton fabric (D) BC/cotton composite applying absorption and crosslinking (vapor glutaraldehyde) cell immobilization techniques and citric acid pre-treated cotton fabric, (E) BC/cotton composite applying absorption and crosslinking (liquid glutaraldehyde) cell immobilization techniques and citric acid pre-treated cotton fabric.



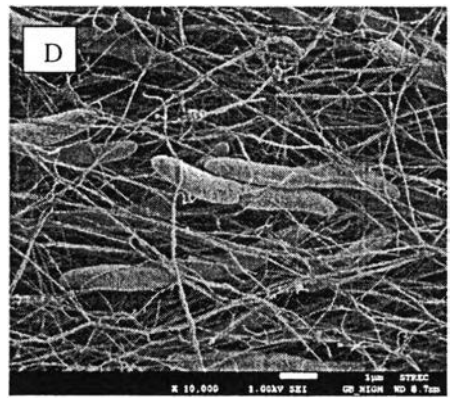


Figure 4.29 morphology *Acetobacter xylinum* on cotton fabric cultivated 48 houses, A) BC/cotton composite applying absorption cell immobilization and DBD plasma pre-treated cotton fabric, B) BC/cotton composite applying absorption cell immobilization and citric acid pre-treated cotton fabric, C) treated surface by acetic acid, D) BC/cotton composite applying absorption cell immobilization and acetic acid pre-treated cotton fabric, D) BC/cotton composite applying absorption and crosslinking (vapor glutaraldehyde), E) BC/cotton composite applying absorption and crosslinking (solution glutaraldehyde), respectively.

4.5.2 Production Yield of BC/Cotton Composite Treated via DBD Plasma

In case of surface pre-treatment of cotton fabric by acid, that help to increase production yield both state wet and dry weight (figure 4.32, 4.33). Especially treated by citric acid increase 33.9% compare with non-treatment. This redult support SEM images, BC fibrilars and cotton fibers was denser than the non-treatment. This might be explained that acid condition was suitable for the growth of *Acetobacter xylinum*. And citric acid consist of 3 carboxylic groups that more than acetic acid.

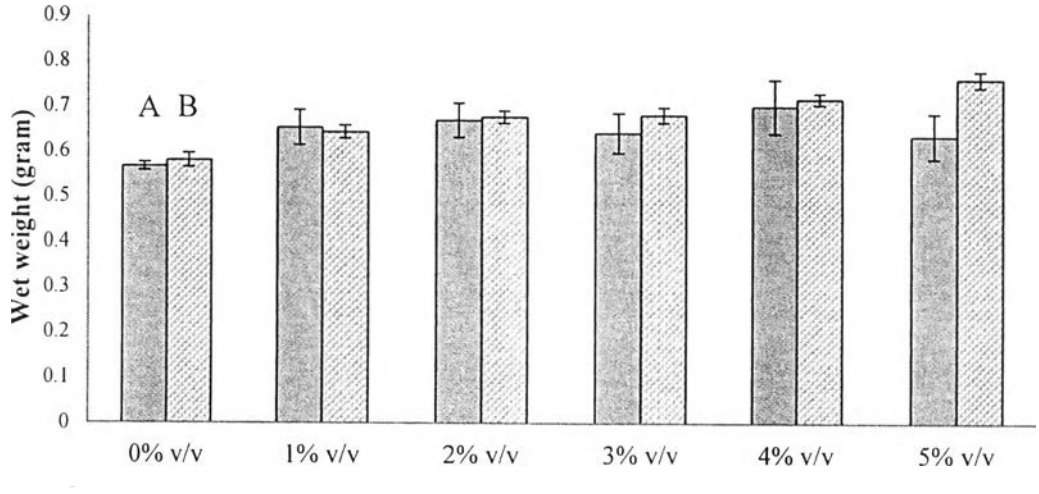


Figure 4.35 Comparison of wet weights of pure BC pellicle and BC/cotton composites obtained by applying (A) acetic acid pre-treated cotton fabric, (B) citric acid pre-treated cotton fabric.

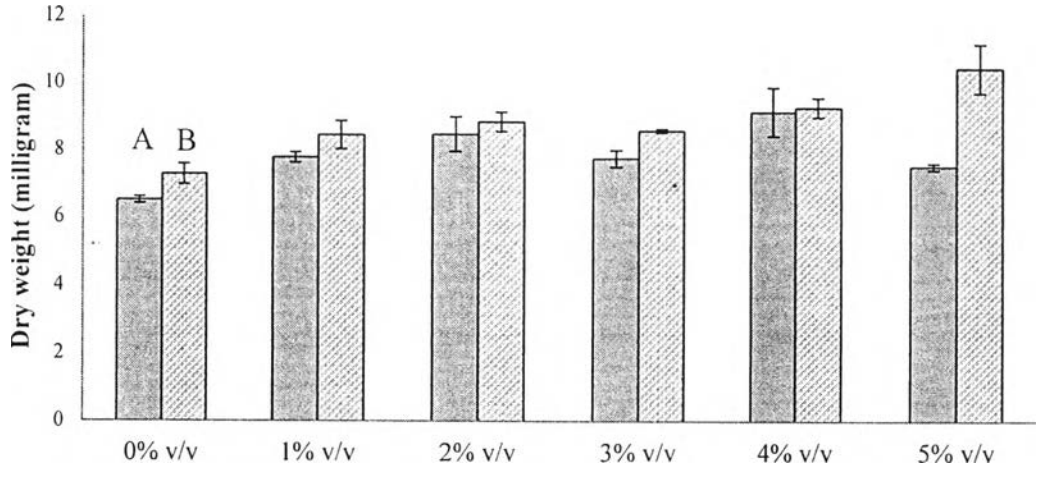


Figure 4.36 Comparison of wet weights of pure BC pellicle and BC/cotton composites obtained by applying (A) acetic acid pre-treated cotton fabric, (B) citric acid pre-treated cotton fabric.

The results of production yields of BC shown in figure 4.33 and 4.34 indicated that the composite obtained without applying cell immobilization technique gave the highest production yield; particularly, when citric acid pre-treated cotton

fabric was used. However, when the cell immobilization techniques were employed, the much less inoculum was used to produce BC. Compared with absorption cell immobilization, crosslinking cell immobilization by using glutaraldehyde resulted in lower yields due to the toxicity of glutaraldehyde.

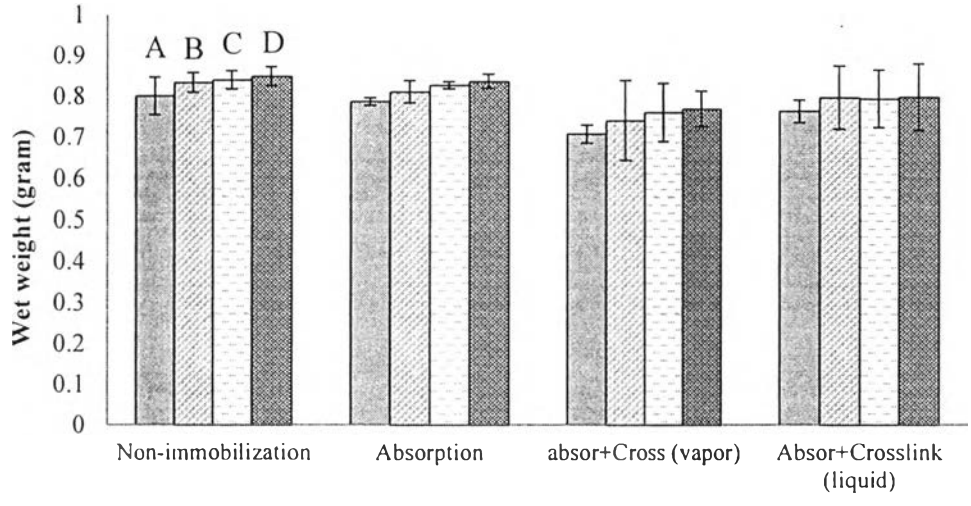


Figure 4.36 Comparison of wet weights of pure BC pellicle and BC/cotton composites obtained by applying (A) no any treatment (B) DBD plasma pre-treated cotton fabric, (C) acetic acid pre-treated cotton fabric and (D) citric acid pre-treated cotton fabric.

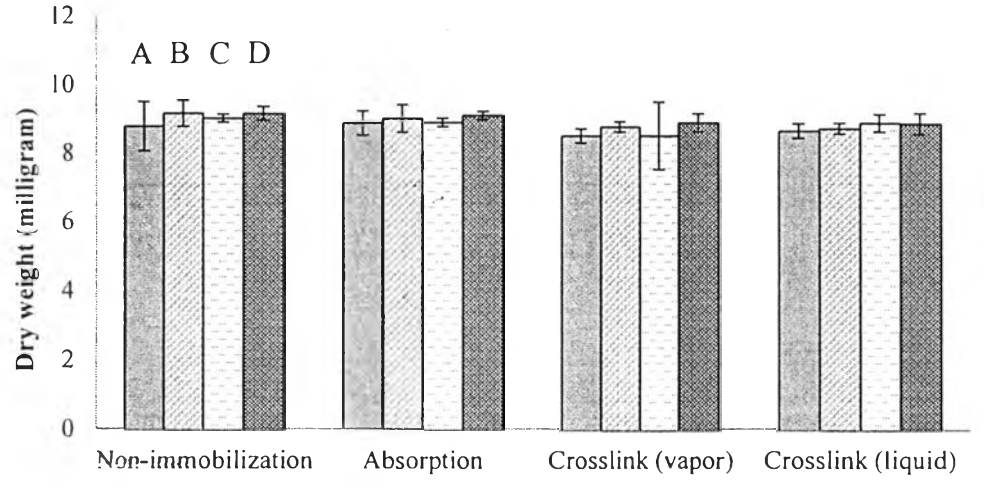


Figure 4.37 Comparison of dry weights of pure BC pellicle and BC/cotton composites obtained by applying (A) no any treatment (B) DBD plasma pre-treated cotton fabric, (C) acetic acid pre-treated cotton fabric and (D) citric acid pre-treated cotton fabric.

From result of production yield in wet and dry state shows figure 4.32 and 4.33 presented the production yield of absorption immobilization technique slightly less than traditional or non-immobilization technique but applied less inoculum. So in figure 4.34 produced BC/Cotton composite by used same amount of inoculum at 3 ml. Result shows that BC/Cotton composite produced by absorption immobilization technique promote higher production yield bolt state because Acetobacter xylinum cells attached on cotton fabric and could start produce BC on cotton fabric after immerse in culture medium.

In case of result in figure 4.32 and 4.33 shows that not similar inoculum that it too difficult to compare between immobilization techniques and non-immobilization or traditional technique. Therefore in figure 4.34 and 4.35 indicate that absorption immobilization technique promote the highest of production yield both wet and dry state, follow by absorption and crosslink immobilization techniques because glutaraldehyde destroy *Acetobacter xylinum* cells. Finally produce by non-immobilization because inoculum too less to produce BC, mostly of *Acetobacyer xylinum* cells distribute in culture medium. For treatment of cotton

fabric display similar figure 4.32 and 4.33 treated with citric acid, acetic acid, DBD plasma, non any treat, respectively.

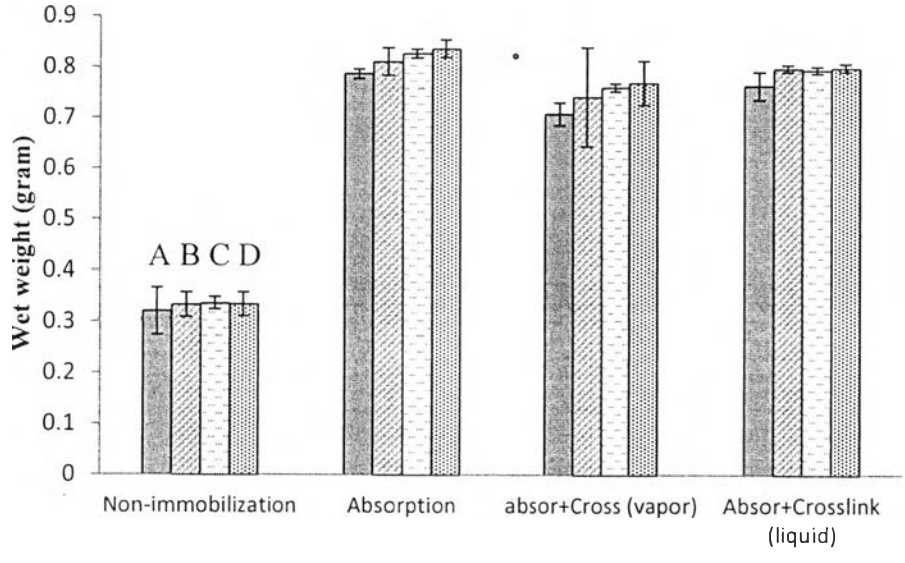


Figure 4.38 Comparison of wet weights of BC/cotton composites obtained by applying similar inoculum and (A) no any treatment (B) DBD plasma pre-treated cotton fabric, (C) acetic acid pre-treated cotton fabric and (D) citric acid pre-treated cotton fabric.

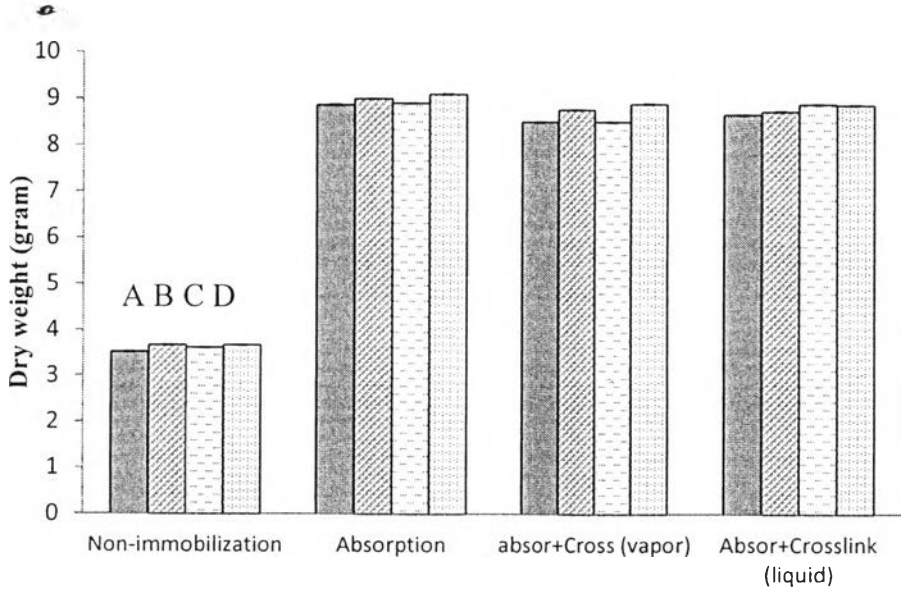


Figure 4.39 Comparison of dry weights of BC/cotton composites obtained by applying similar inoculum and (A) no any treatment (B) DBD plasma pre-treated cotton fabric, (C) acetic acid pre-treated cotton fabric and (D) citric acid pre-treated cotton fabric.

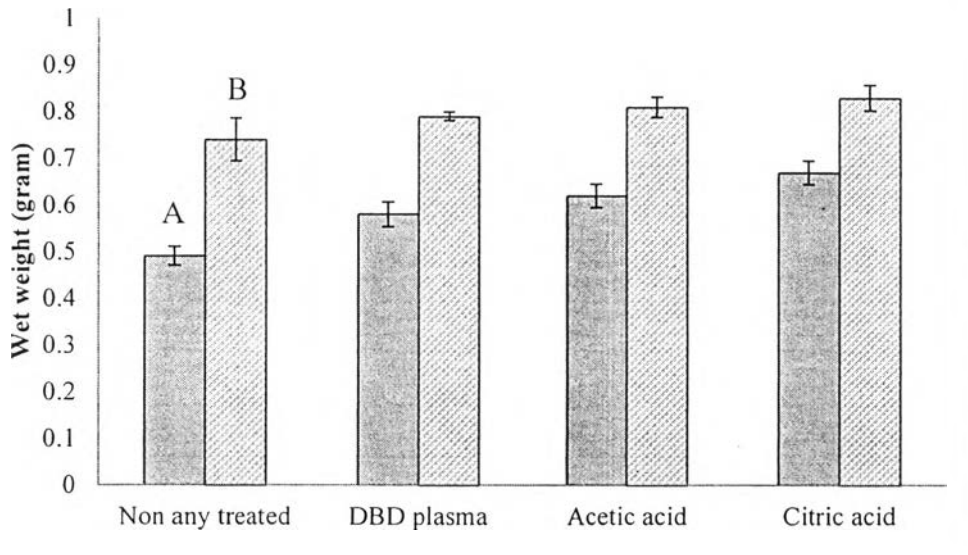


Figure 4.40 Comparison of wet weights of pure BC pellicle and BC/cotton composites obtained by applying (A) Non-immobilization or traditional technique (B) Absorption immobilization technique

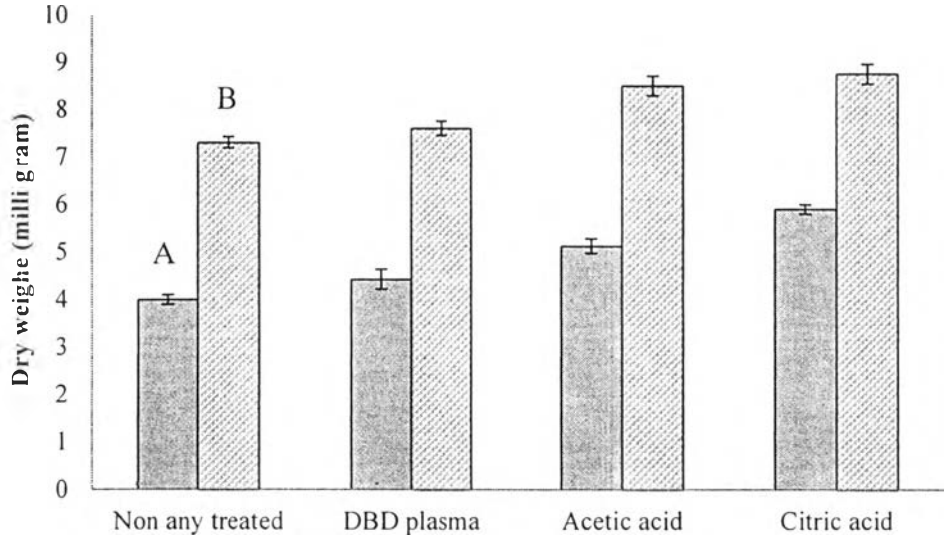


Figure 4.41 Comparison of dry weights of pure BC pellicle and BC/cotton composites obtained by applying (A) Non-immobilization or traditional technique (B) Absorption immobilization technique.

4.5.3 Water Vapor Transmission Rate (WVTR)

The WVTR of pure BC pellicle was 0.42 g/cm^2/day, as shown in figure 4.38 The WVTR of BC/cotton composites were greater than that of pure BC pellicle due to the water absorption capability of cotton fabric. Although pure BC pellicle had higher thickness than BC/cotton composites, the tightly packing fibrilar structure of BC in BC/cotton composites could reduce the mobility of water molecules throughout the BC pellicle, resulting in the lower values of WVTR. It seemed that the surface pre-treatment of cotton fabrics by citric acid and acetic acid did not cause an obvious difference in WVTR. It is known that the WVTR of wound dressings should be controlled at an appropriate rate. However, there is not an exact ideal value of WVTR for wound dressing materials. If the WVTR is too high, it causes excessive dehydration which will create dry condition around wound area and results in scar formation. In contrast, if the WVTR is too low, this may lead to the delay of the healing process and the increasing of bacterial growth due to the accumulation of exudates. Recently, it has been reported that wound healing takes place faster in moist environment (Bhuvanesh G., 2010).

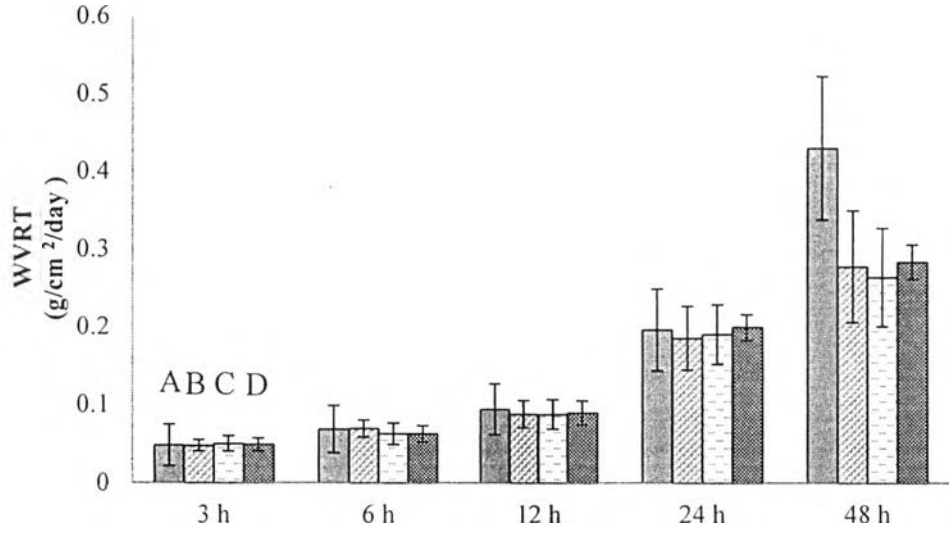


Figure 4.42 Comparison on the water vapor transmission rates of (A) pure BC pellicle (B) non-cell-immobilized BC/cotton composite (C) BC/cotton composite applying absorption cell immobilization technique together with acetic acid pre-treated cotton fabric (D) BC/cotton composite applying absorption cell immobilization technique together with citric acid pre-treated cotton fabric.

4.5.4 Water Absorption Capacity

Water absorption capacity plays an important role in wound dressing application. The capacity of absorbing wound exudates and maintaining moist environment at wound surface are important factors in wound healing process. Bacterial cellulose has high water absorption capacity due to the hydrophilicity and porous structure of nanofibril network. The porous structure of bacterial cellulose generated the high surface areas and capillary force which enhancing the water absorption capacity. Using porous supporting fabrics, water absorption capacity of BC composites were decreased, compared with that of pure BC as shown in figure 4.9 It might be explained that tightly packing fibrilar structure in the BC composites restricted of the swelling behavior of BC.

In addition, the comparison on the water absorption capacity between pure BC and BC composites containing cotton fabrics different types of surface treatment, Bacterial composite shoes similar water absorption capacity and lower value than pure. Because morphology of BC composites containing cotton fabric denser than

pure BC (figure 4.12, 4.13). In case of high density of BC fibril, Water difficult to penetrate in side porous of BC pellicle.

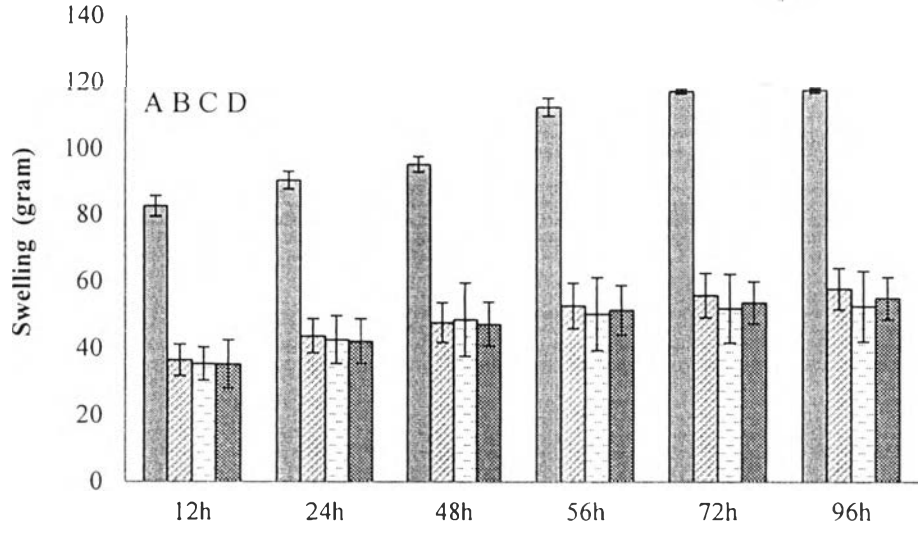


Figure 4.39 Comparison on the water vapor transmission rates of (a) pure BC pellicle (b) non-cell-immobilized BC/cotton composite (c) BC/cotton composite applying absorption cell immobilization technique together with acetic acid pre-treated cotton fabric (d) BC/cotton composite applying absorption cell immobilization technique together with citric acid pre-treated cotton fabric.

4.5.5 Dry Rate

Dry rate of BC plays an important effect that have to concern due to BC compost by over 95% of water. In case of wound healing, if provide moisture environment that important factors in wound healing process. The dry rate of BC/Cotton composite shows similar value due to the thickness and weight were same. BC presents lowest of dry weight because consist of high density of BC fibril from used 4 days for cultivation. After 48 hours, BC/Cotton composite and pure BC were completed day.

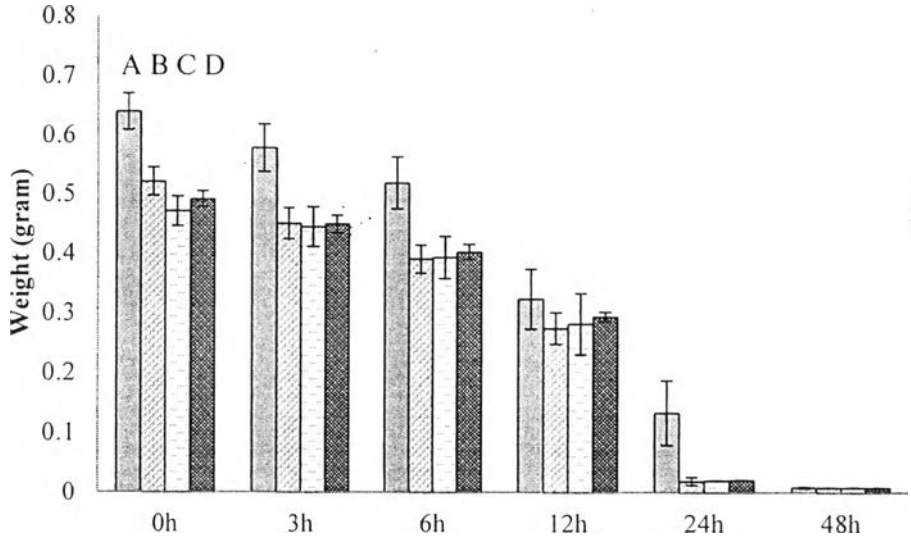


Figure 4.42 Comparison on the water dry rates of (A) pure BC pellicle (B) non-cell-immobilized BC/cotton composite (C) BC/cotton composite applying absorption cell immobilization technique together with acetic acid pre-treated cotton fabric (D) BC/cotton composite applying absorption cell immobilization technique together with citric acid pre-treated cotton fabric.

4.5.6 Cell Acetobacter Xylinum in Culture Medium

Immobilization techniques have to concern about cell fall-off because after cell fall-off out of cotton fabric it distribute in culture medium and cannot control production yield. From result in case of fall-off of *Acetobacter xylinum* cell shows in figure 4.37 and 4.38, BC/cotton composite prepared with immobilization technique displays lower amount of bacterial colony compared with BC/cotton composite prepared by non-immobilization technique due to used lower inoculum and *acetobacter xylinum* cells attach on cotton fabric. For crosslinking immobilization by glutaraldehyde, both solution and vapor exhibited lowest that might explain that glutaraldehyde as crosslinking agent and destroy bacterial cell.

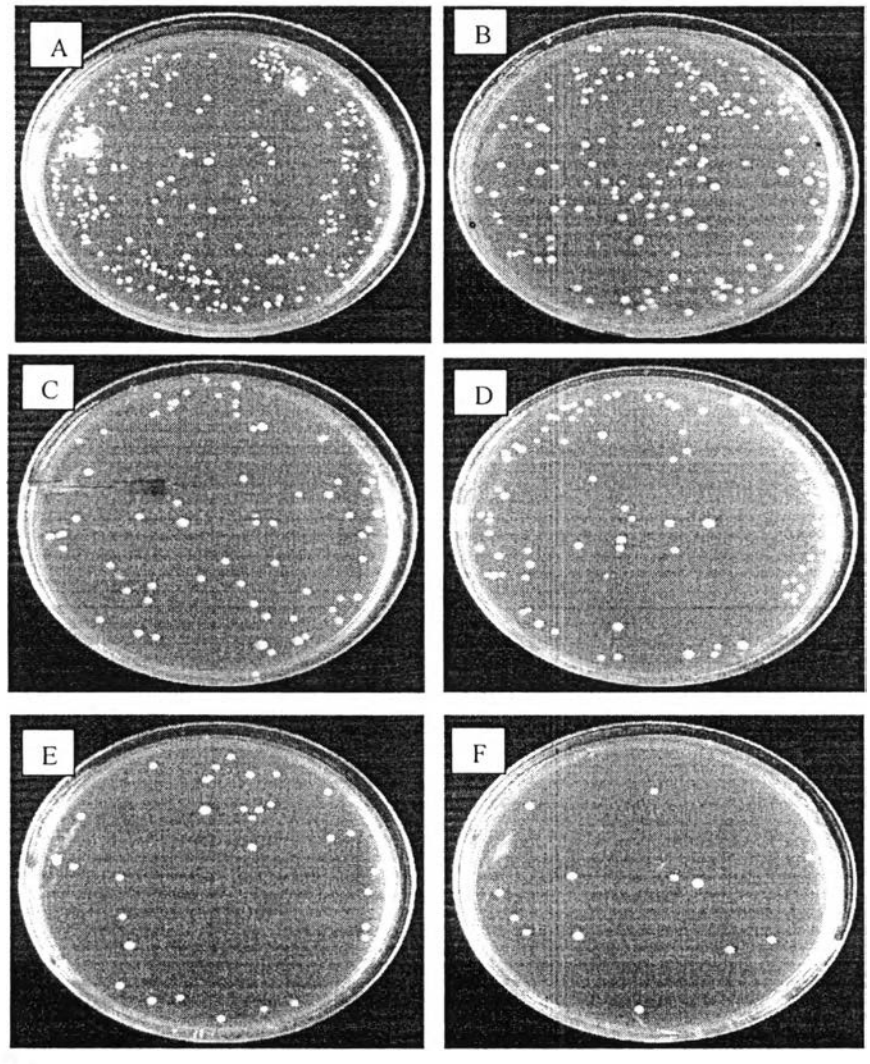


Figure 4.43 Comparison on the colony of bacterial dispersed in culture medium (A) BC/Cotton composite prepared by non-immobilization, (B) BC/Cotton composite prepared by non-immobilization treated with citric acid, (C) BC/Cotton composite prepared by immobilization treated with DBD plasma, (D) BC/Cotton composite prepared by immobilization treated with citric acid, (E) BC/Cotton composite prepared by immobilization treated with citric acid crosslink with glutaraldehyde vapor, (F) BC/Cotton composite prepared by immobilization treated with citric acid crosslink with glutaraldehyde solution, respectively.

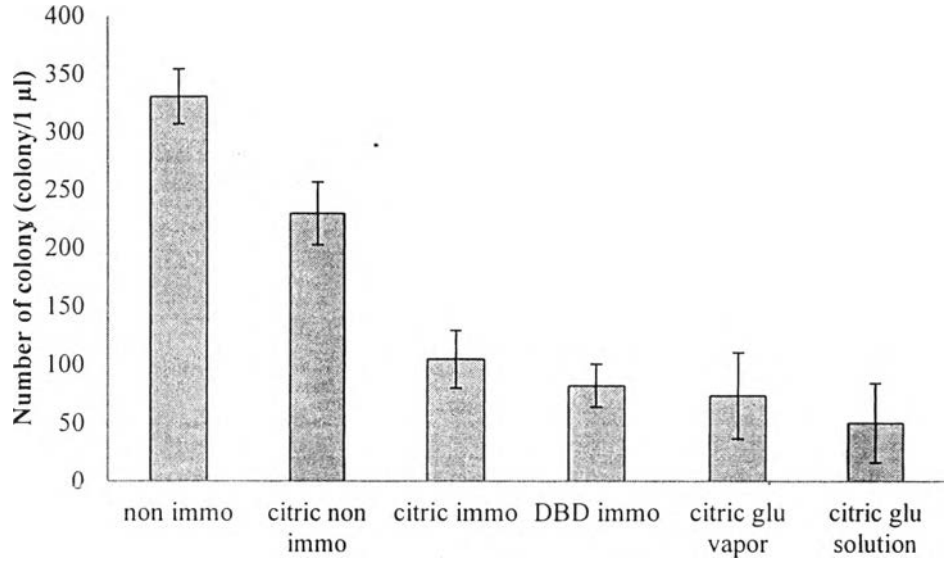


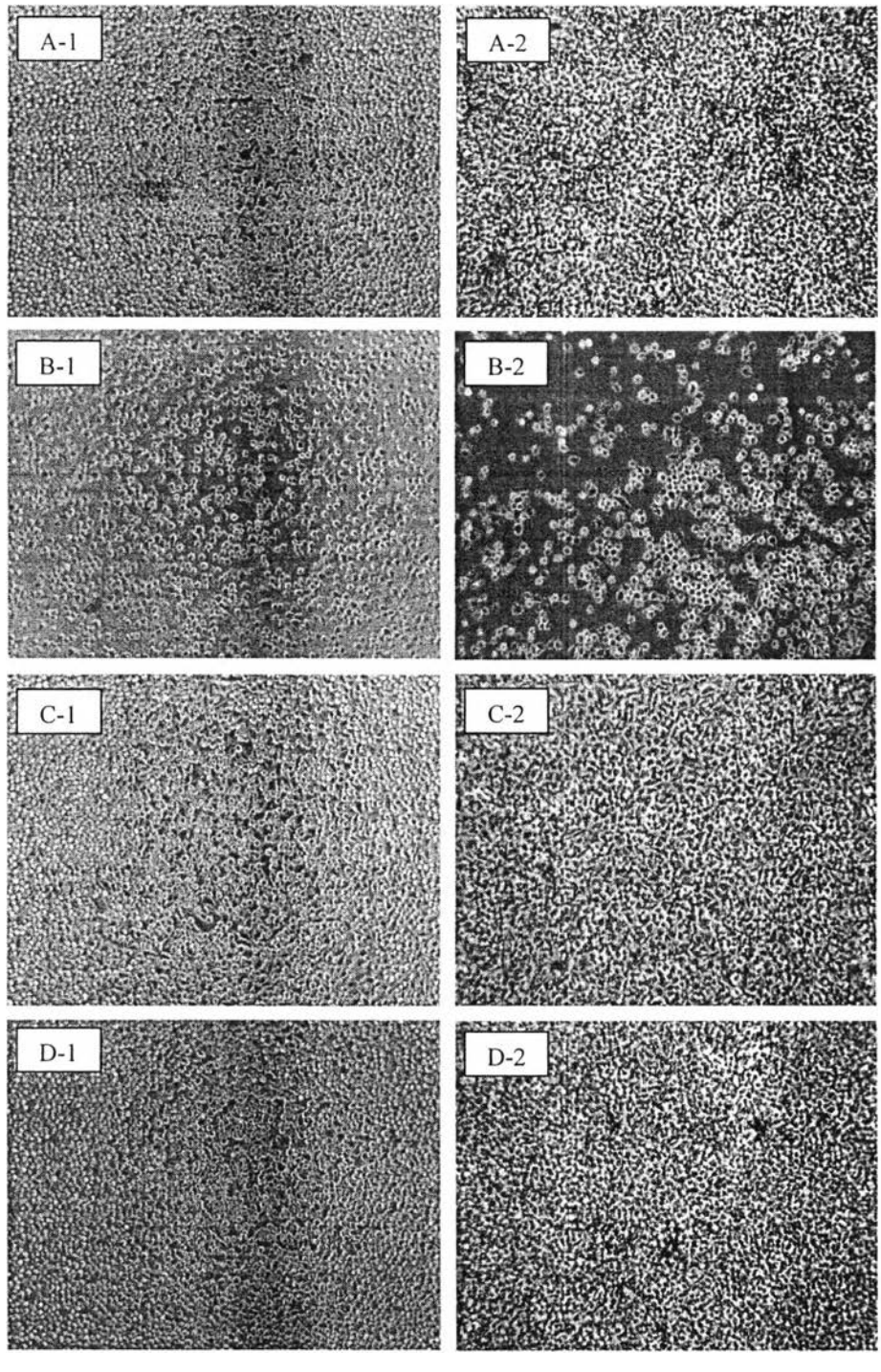
Figure 4.44 Comparison on the colony of bacterial dispersed in culture medium (A) BC/Cotton composite prepared by non-immobilization, (B) BC/Cotton composite prepared by non-immobilization treated with citric acid, (C) BC/Cotton composite prepared by immobilization treated with DBD plasma, (D) BC/Cotton composite prepared by immobilization treated with citric acid, (E) BC/Cotton composite prepared by immobilization treated with citric acid crosslink with glutaraldehyde vapor, (F) BC/Cotton composite prepared by immobilization treated with citric acid crosslink with glutaraldehyde solution, respectively

4.5.7 The MTT Cytotoxicity Assay

This experiment was approved by National Metal and Materials Technology Center (MTEC, NSTDA). The biocompatibility is one of the important factors during wound treatment. MTT assay is colorimetric assay for measuring the activity of enzymes from metabolism of cells. This assay was a modified version of conventional direct and indirect contact tests conformed to the published standard methods (BS-EN30993-5 and ISO10993-5). The MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolism-competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are

metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability.

The percentage survival of the human dermal skin fibroblast cells cultured with samples (compared to control) shown in figure 4.38 and table 4.1.



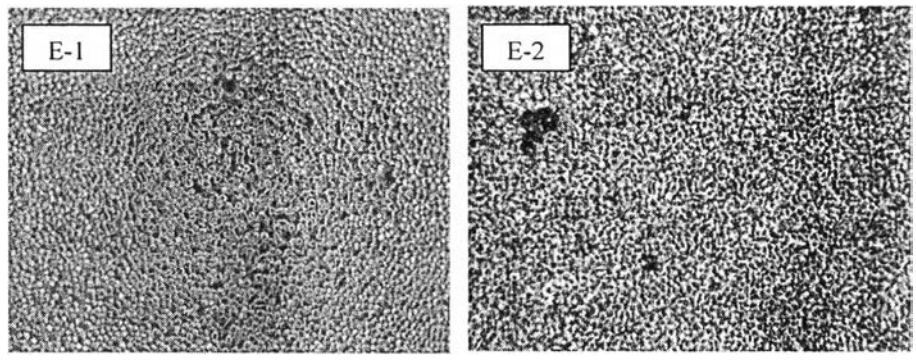


Figure 4.45 optical microscope image of survival of rat dermal skin fibroblast cell L929, (1) before dye cell with MTT color, (2) after dyed cell with MTT color, (A) control non-toxic sample, (B) control toxic sample, (C) pure BC, (D) BC/Cotton composite, (E) BC/Nylon composite.

	Average	
Sample	OD 570	% Viability
Ideal sample	1.470	100
Control sample	1.440	98
Toxic sample	0.008	0
Pure bacterial	1.145	78
Bacterial/cotton composite	1.368	95
Bacterial/nylon composite	1.374	95

Table 4.1 The percentage survival of rat dermal skin fibroblast cell L929 after test by MTT assey.

The percent survival of human dermal skin fibroblast cells cultured with BC composites; BC/Cotton, BC/Nylon, more than pure BC. However, based on the percent survival of each test concentration, the toxicity of sample can be indicated as; a) "non-cytotoxic effect" if cell survived over70% and b) "cytotoxic

effect" if cell survived less than 70%. Then, Pure BC, BC/Cotton, BC/Nylon were cytotoxic to human dermal skin fibroblast cell lines showed by the percent survival 78%, 95% and 95% it more than 70% that mean bacterial cellulose and bacterial cellulose composite not toxic due to not much effect on the percent survival of cells.

4.5.8 The Cytocomplatibility

SEM images of the cell attachment of rat dermal skin fibroblast cells on Pure BC, BC/Cotton and BC/Nylon as shown in figure 4.44 - 4.48. In generally, the cells increase density in figure 4.44 and spread figure 4.45 on the surface and stretched their morphology indicated that the materials show the better biocompatibility. In this experiment, there were many cells attached on surface, increase cells density and change shape from sphere to spread it mean can apply bacterial cellulose and bacterial cellulose composite in wound dressing material.

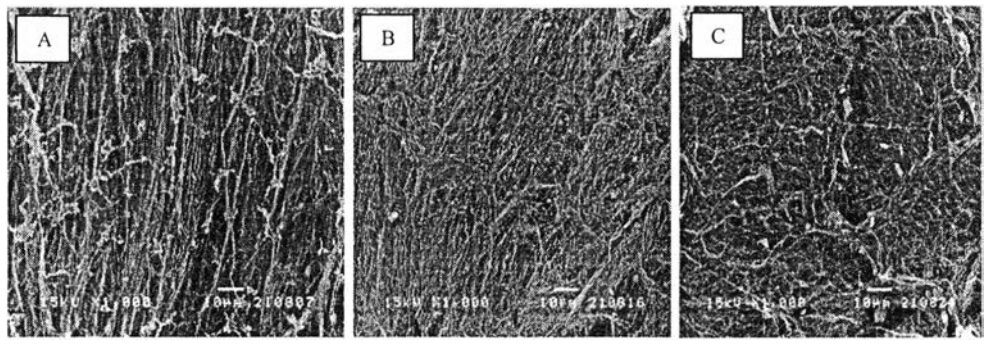


Figure 4.46 No cell rat dermal skin fibroblast on (A) pure BC, (B) BC/Cotton and (C) BC/Nylon respectively.

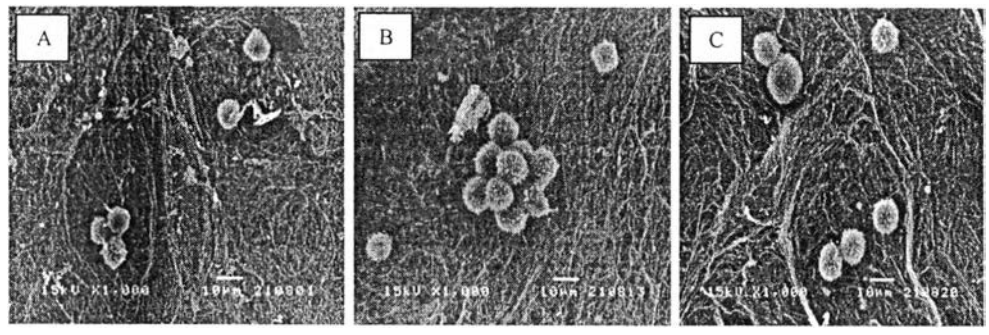


Figure 4.47 Attachment of cell rat dermal skin fibroblast on (A) pure BC, (B) BC/Cotton and (C) BC/Nylon respectively at 24 hours.

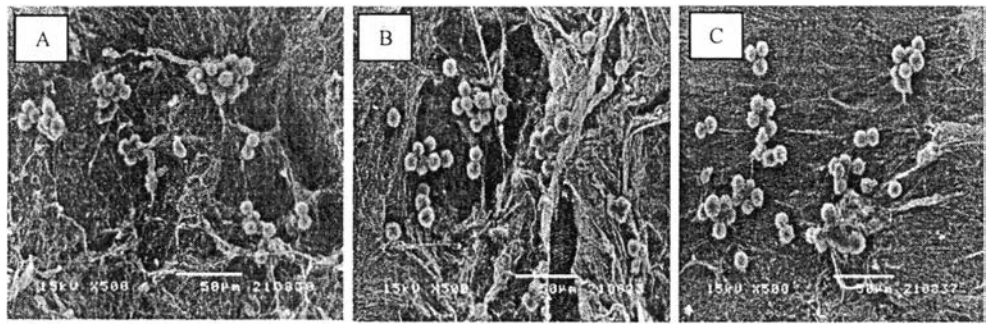


Figure 4.48 Attachment of cell rat dermal skin fibroblast on (A) pure BC, (B) BC/Cotton and (C) BC/Nylon respectively at 48 hours.

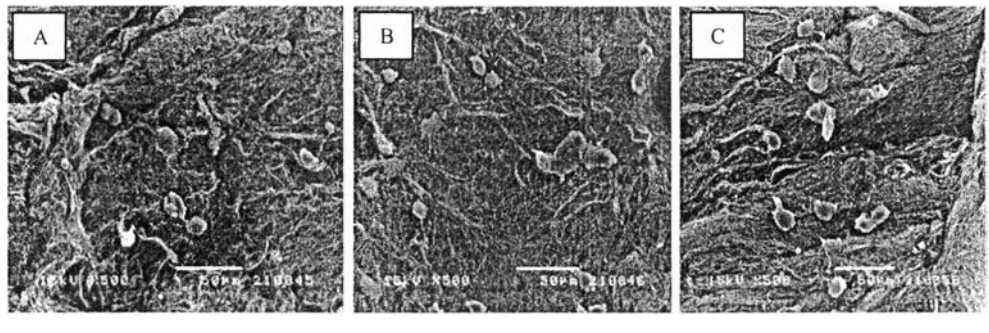


Figure 4.49 Attachment of cell rat dermal skin fibroblast on (A) pure BC, (B) BC/Cotton and (C) BC/Nylon respectively at 96 hours.

4.5.9 Antibacterial Property

Antimicrobrial property is important property wound dressing should be promoted due to can protect bacterial that infect in wound. Form figure 4.48 BC and BC/Cotton composite did not exhibit antibacterial because not occur clear zone both gram positive and gram negative.

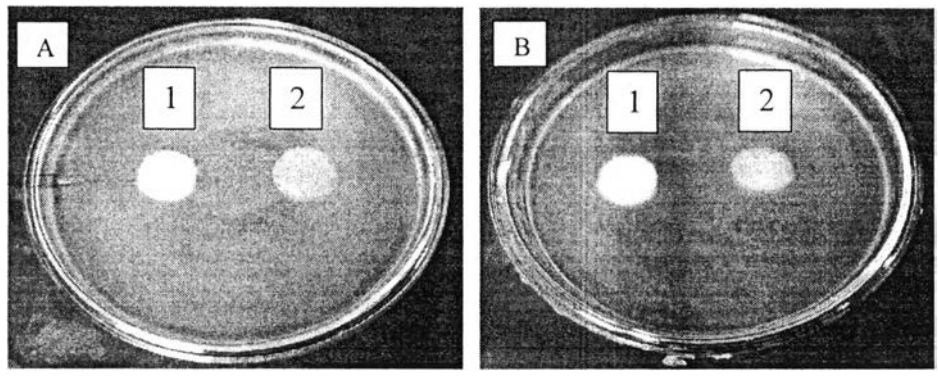


Figure 4.50 antimicrobrial property (A) gram negative, (B) gram positive and (1) pure BC, (2)BC/Cotton composite.

4.5.10 Suitable thickness of BC/Cotton composite for using in wound dressing

Thickness of BC/Cotton composite is important effect for apply to use in wound dressing because it relate with water vapor transmission, extruae absorption capacity and pressure that give on wound. In case of contour on skin is first thing for concern, thickness of BC/Cotton composite approximately 1, 2, 3, 4 mm were suit for skin contour 5, 6, 10 mm show over thickness because BC pellicles not contour skin area. Power of wound healing is correspond with thickness, from this reason could suggest that thickness of BC/Cotton composite around 2, 3, 4 were the best condition because could provide moisture in long period and also great to absorp extruded from wound.

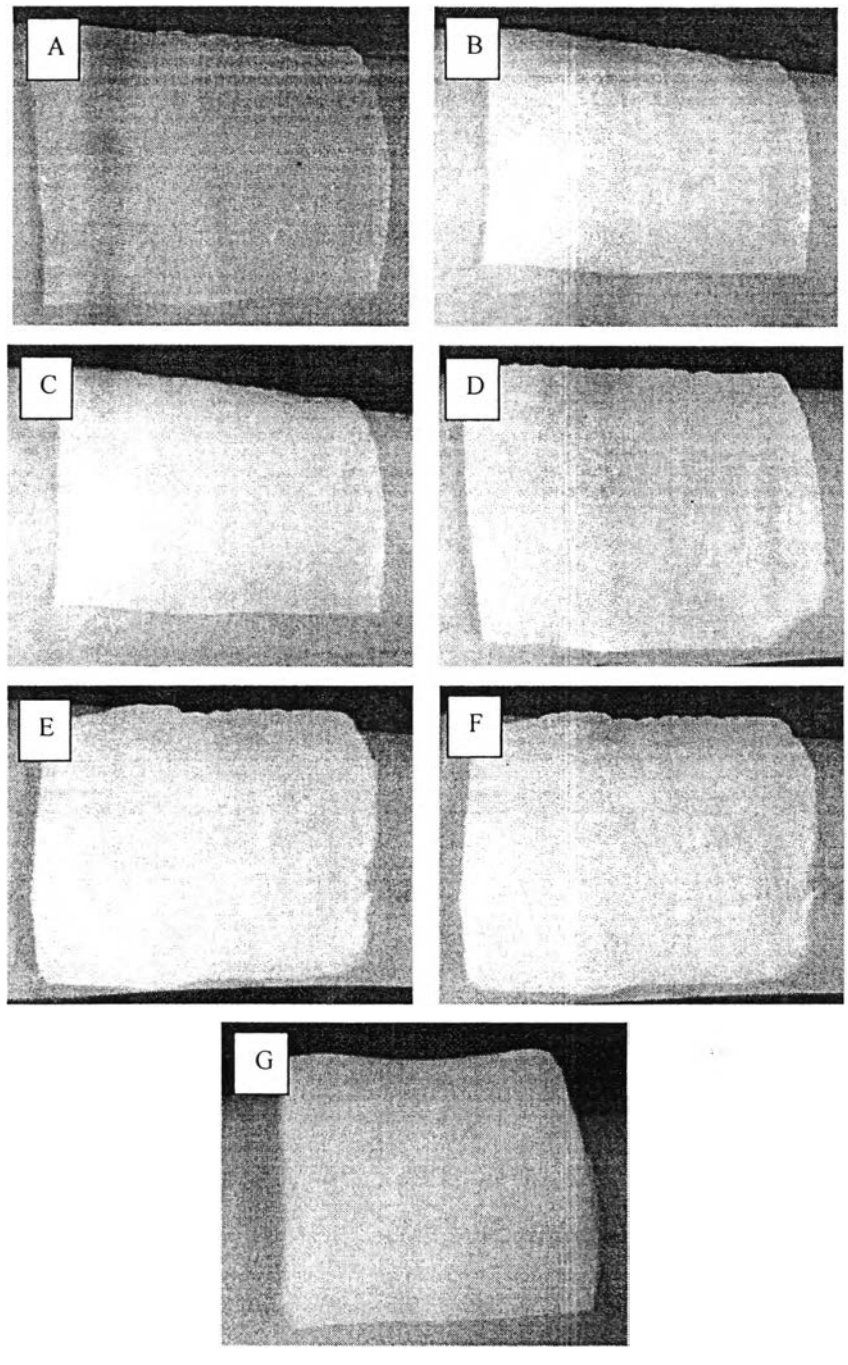


Figure 4.49 Different thickness BC/Cotton composite applying on skin (A) 1 mm, (B) 2 mm,. (C) 3 mm. (D) 4 mm, (E) 5 mm, (F) 6 mm,. (G) 10 mm, respectively.