Advances in Biological Research 8 (6): 307-313, 2014
ISSN 1992-006

DOI: 10.5829/idosi.abr.2014.8.6.1215

An Overview of Biocellulose Production Using *Acetobacter xylinum* Culture

Siti Mohainin Mohammad, Norliza Abd Rahman,
Mohd Sahaid Khalil and Siti Rozaimah Sheikh Abdullah

Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment,
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Malaysia

Abstract: Biocellulose, produced by *Acetobacter* species, displays unique properties including high mechanical strength, high water absorption capacity, high crystallinity and an ultra-fine and highly pure fiber network structure. This review article presents the existing information about biocellulose based on previous works that were conducted to improve the production of biocellulose and applications. Biocellulose, a biopolymer, is valuable for production of vital products in food, textile, medicine and agriculture due to its unique properties. Characteristics of biocellulose with respect to its structure and physicochemical properties are discussed. Current and potential applications of biocellulose in textile, nonwoven cloth, paper, films, synthetic fiber coating, food, pharmaceutical and other industries are also presented.

Key words: Acetobacter xylinum · Biocellulose · Coconut water · Fermentation

INTRODUCTION

Biocellulose: Cellulose is a water-insoluble compound which is commonly found in the cell walls of plant, especially in the stalk, stem, branches and woody parts of the plant network. Cellulose fiber is a formed component of plant cell walls. Cellulose is found in plants known as microfibril (2-20 nm in diameter and 100-40000 nm) where its network forms a strong bonding structure in the cell wall. A polysaccharide group is arranged in parallel to form a cellulose microfibril which are tied or packaged together to form a macrofibril. Cellulose molecules make up to 14000 units as chains or microfibril of D- glucose in the form of twisted bundles that are tied together by hydrogen bonds [1].

Cellulose is derived from fermentation process. For example, microbial polysaccharide composed by cellulose fibers are produced by strains xylinum, *Acetobacter aceti* subspecies of a non-pathogenic bacteria and bacteria named as cellulose or bacterial cellulose are derived from fermentation with the help of microbes [2].

Structural: A main component of plant cell walls is cellulose. Biocellulose or bacterial cellulose is produced from some bacteria produce cellulose. Plant cellulose and bacterial cellulose (BC) have the same chemical structure, but different physical and chemical properties [3]. Although bacterial cellulose has the same chemical structure as cellulose from plants, it composes better fibers of cellulose than plant cellulose. Every single fiber of bacterial cellulose has a diameter of over 50 nm. Fiber length cannot be determined because the single cellulose fibers are interconnected with each other forming a network structure [2]. Figure 1 below shows the Scanning Electron Microscopy (SEM) of bacterial cellulose. Figure 2 shows the structure biocellulose with formula $(C_6H_{10}O_5)_n$. Biocellulose are polysaccharides consisting of a few hundred to a thousand chain β (1-4) D- glucose units.

Morphology: New chain biocellulose together and forms subfibril. Subfibril is around 1.5nm wide which belongs to the thinnest natural fibers [5]. Biocellulose subfibril is crystallized as microfibril (bundle) and then turns into ribbons (clot-bound) [6]. Table 1 shows that the resulting

Corresponding Author: Siti Mohainin Mohammad, Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Malaysia.
Tel: +0145093220, E-mail: orzasativa@gmail.com.

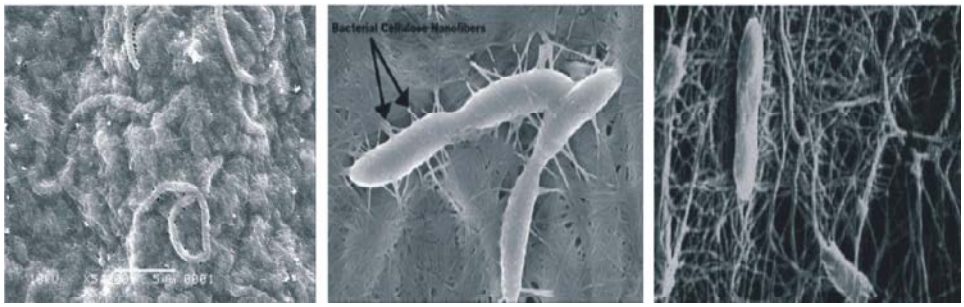

Fig 1: Structure of Bacterial Cellulose (biocellulose)
Source: [2]

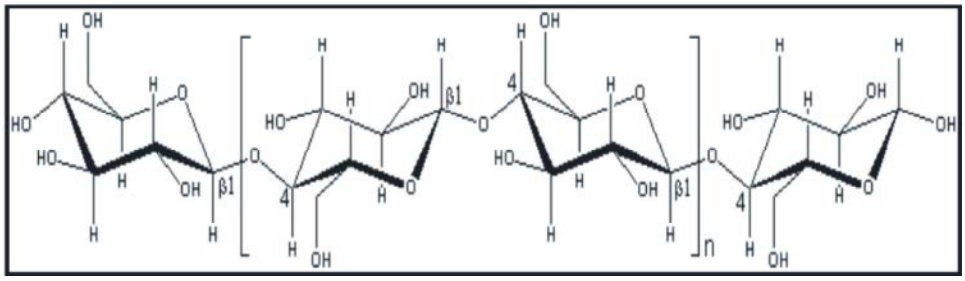

Fig 2: β, 1-4 glucan chain containing cellulose
Source: [3]

Table 1: Dimensions of ribbons of different reference

Dimensions ribbons	Reference Width	Thick
3- 4 nm	70-80 nm	[7]
3.2 nm	133 nm	[8]
4.1 nm	117 nm	[9]

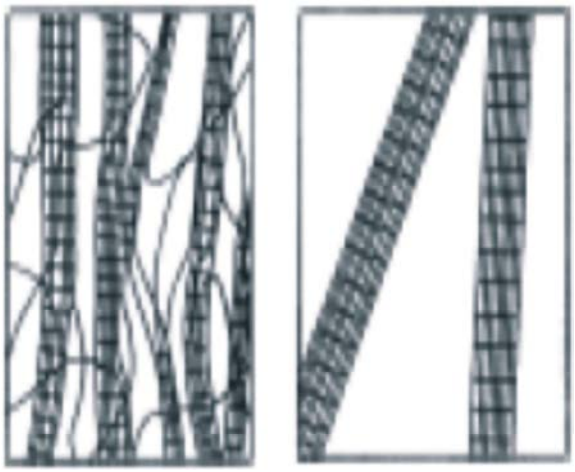

Fig 3: Biocellulose (left), Plant cellulose (right)
Source: [10]

ribbon dimensions vary according to the references. Referring to Brown, Zaar and Yamanaka [7-9], ribbon thickness produced is in the range of 3nm to 4 nm. The greatest width is 133 nm which is referred to Brown *et al*. [8].

The width of cellulose fiber pulp produced by birch or pine is greater, respectively 1.4 - 4.0 × 10-2 and 3.0 - 7.5 × 10-2 mm. The length of very thin ribbons of biocellulose is about 1-9 ìm which forms a dense reticulated structure being stabilized by hydrogen bonds [6] Figure 3 shows different biocellulose structures formed naturally from the extra- cellular bacteria and plants. Ribbons are formed at biocellulose structure to strengthen the structure of the resulting biocellulose. Cellulose structure in natural vegetation is formed linearly without having any ribbon formation.

Characteristics: The transparency feature in Biocellulose was found by Klemm *et al* [11]. This feature can be identified during the process of purification and drying pellicle microbial cellulose in surface culture. After filtering and drying, Pellicle was placed on writing paper to observe that colors on the paper are clearly visible through the thin layer (pellicle) microbial cellulose.

One of the other key features of bacterial cellulose from plant cellulose is a high chemical purity. This refers to the production without lignin and hemicelluloses such plant cellulose. Lignin and hemicelluloses, which form the walls of plant cells, are difficult to remove for purification. Biocellulose bacteria can also be distinguished by a cellulose plant because it has a high crystallization index in the range of 60 % or above. Therefore, different degrees

Table 2: The application of biocellusoe in various industry areas

Industry Areas	Applications
Medical	- Biosensor [12] - Biofill ® [13] - Wound dressing [14]
Production of paper	- Synthetic paper - Paper board as electric insulation material and signboard - Patching the surface of the outer layer of the specific paper [15]
Food processing	- Agent stabilization - Nata De Coco [14] - Kombucha china (the-mold) [16] -Bread
Cosmetic	- Health Tonic - False nails, nail fireplace materials and nail polish [17]
Textile	Product weaving [9]
Tourism and Sport	Sports Equipment [17]
Mining and Refinery Sewage	- Collectors oil spill - Absorbent toxic pesticides - Facilitating recycling of minerals and oil
Others	- Diaphragm microphone - Stereo headphones - Manufacturing of the car body, the body planes and rockets

Table 3: Taxonomy of *Acetobacter xylinum*

Domain	:	*Bacteria*
Phylum	:	*Proteobacteria*
Class	:	*Alphaproteobacteria*
Order	:	*Rhodospirillales*
Family	:	*Acetobacteraceae*
Genus	:	*Acetobacter*
Sub-species	:	*Xylinum*
Scientific name	:	*Acetobacter aceti xylinum*

Source : [18]

of polymerization between 2000 and 6000 [6] can reach up to 16,000 to 20,000 [11] in compared to the degree of polymerization of the cellulose plants in range 13,000 to 14,000 [11].

This suggests that Bacterial cellulose has superior characteristics to cellulose plants. Biocellulose has a high water holding capacity in compared to cellulose plant. This is due to the fact that hydrogen bonding is stronger and more biocellulose cellulose chain (n) long from plant cellulose [12].

Applications: Biocellulose, a polymer synthesized by *Acetobacter xylinum,* has been a high-value biotech product. This is also known as a polymer with high resilience and unique structure which make it a source of biodegradable biopolymers. Production tools and ability to match the plant cellulose have made very important in today's market and are deemed as a future for the mankind. These unique properties have made the bacterial cellulose polymer useful in many applications in various fields. These applications of biocellusoe are involved in medicine, industry production of paper, textiles and so on. Table 2 shows applications of biocellulose in various industry areas.

Acetobacter Xylinum: *Acetobacter xylinum* is a gram negative rod-shaped microorganisms which is also known as *Gluconacetobacter xylinus*. The length of Long *A. xylinum* is in the range of 2 to 10 microns with a width in range 0.5 to 1 micron. *A. xylinum* with molecular formula $CH_2O_{0.52}N_{0.23}$ is a kind of aerobic microorganism that needs oxygen to survive. Table 3 shows the taxonomy of *A. xylinum*.

These bacteria are also known as acetic acid bacteria which are able to form acid from glucose, ethyl alcohol and propyl alcohol rather than oxidizing acids into carbon dioxide and water in the presence of oxygen. The most striking feature of this bacterium is its ability to polymerize glucose into cellulose through only one process of synthesis. These bacteria normally live with short chains formed by the combination of 6 to 8 cells and do not constitute pigment endospore.

A.xylinum has a resistance to sudden changes such as the decrease of water in a medium composition, pH, presence of toxic substances and pathogenic organisms. Although the weather conditions do not permit, but *A. xylinum* bacteria can grow and produce cellulose in an envelope. A total of 23 % of the bacterial cells coated with cellulose survive after treatment with UV radiation. Elimination of protective polysaccharides cellulose from bacteria cells leads to a drastic reduction in the cell where bacterial survival is just 3% [17].

Morphology Properties: *Acetobacter xylinum* is a short rod-shaped bacteria, which is 2 microns long and 0.6 microns wide with a slimy surface. These bacteria can form short chains constituting 6-8 cells. In cell cultures, the young cells are transparent and can be identified individually. Old colonies form a solid layer mask that resembles gelatin and cell colony. Bacteria have a catalytic synthesis of cellulose *A.xylinum* (subunit) organized in a linear fashion on the main axis of the cell as shown in Figure 4. The three subunits are called 'triplet 'and later is referred to as the terminal complex (TC). Figure 5 shows each subunit appears on the outer surface of the bacteria and is gathered into subfibril consisting of cellulose molecules. As shown in the Figure below,

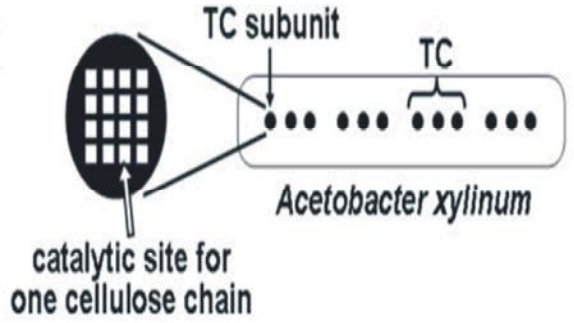

Fig 4: Subunit arranged on a Acetobacter *xylinum*

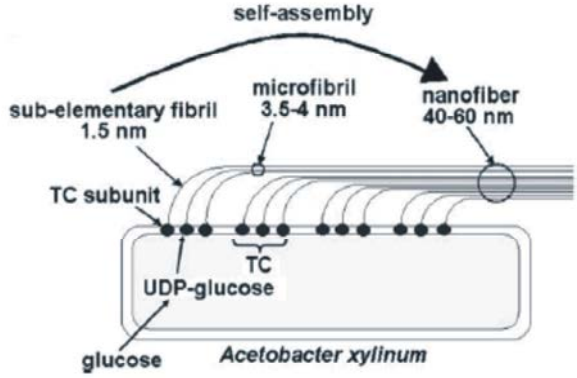

Fig 5: Formation fibril to nanofiber horizontal axis (self-assembly)
Source: [19]

the protruding fibrils gather into microfibril (3.5-4 nm) and subsequent nanofiber (40-60 nm). This spontaneous formation process is called self- assembly.

Physiological Properties: These bacteria can form acid from glucose, ethyl alcohol and alcohol isomer and have the ability to oxidize indole acetic acid into CO_2 and H_2O. The most striking feature of these bacteria is to have the ability to perform the cellulose polymerization of glucose. This cellulose matrix form is known as nata. The dominant factors affecting the physiological properties of nata formation are the availability of nutrients, acidity, temperature and availability of oxygen.

Factors That Affect *Acetobacter Xylinum* Growth

Carbon Source: Carbon source includes carbohydrates which are compounds belonging to the monosaccharide and disaccharides which can be used in the fermentation of biocellulose. Biocellulose formation occurs on media containing compound glucose, sucrose and lactose where sucrose or sugar is most widely used based on economy. The addition of sucrose should refer to the amount needed. Excess addition can be an option considering that the economy will not affect the texture of nata. This can consequently lead to the creation of a new form of residual waste from the sucrose. On the other hand, if the addition is too little, it does result in a normal growing seed nata and cannot yield maximum production.

Nitrogen Source: Nitrogen sources can be used in organic or inorganic compounds. Yeast extract and casein are good products used for the growth and formation of *Acetobacter xylinum* biocellulose. However, ammonium sulfate and ammonium phosphate (in the market known as ZA) are more suitable materials used in terms of economy and quality yield of biocellulose. Many other N inexpensive sources can be used such as urea.

pH: Although *Acetobacter xylinum* can grow at a pH level between 3.5-7.5, pH 4.3 is considered to be the most suitable. Under alkaline conditions, the cell metabolism of these bacteria will be disrupted.

Temperature: The ideal temperature (optimum) for the growth of bacteria *Acetobacter xylinum* is 28°C - 31°C which is typically the room temperature. At temperatures below 28°C, bacterial growth is stunted. Similarly, temperatures above 31°C start to cause damage in *A. xylinum* and can lead to death, although enzymes that have been produced regularly work up biocellulose.

Availability of Air (Oxygen): *Acetobacter xylinum* bacteria are aerobic microbes. This bacterium needs oxygen in its process of growth, development and activity. When deprived of oxygen, these bacteria will experience an interruption in growth which can lead to death. Therefore, the container used for fermentation biocellulose must remain open.

Other Biocellulose Producing Microorganisms: Cellulose can be found in a wide variety of microorganisms including fungi, bacteria and algae. Cellulose is composed of xylem and manna found in green algae, brown (Phaeophuta) and red algae (Rhodophyta) and serves as the structure of the cell wall of polysaccharides in algae. Cellulose such as chitin is also reported to be found in certain type of fungus that forms a layer in the cell wall [14]. However, bacteria have been reported to be superior producers of microbial cellulose from Acetobacter species, Agrobacter and others. Table 4 shows the genus of microorganisms that can produce microbial cellulose. Other cellulose-producing bacteria can also be distinguished by the carbon source

Table 4: The genus of microorganisms producing bacteria biocellulose

Genus	Cellulose structure
Acetobacter	Extra-cellular pellicle
Achromobacter	Fibrils
Aerobacter	Fibrils
Agrobacterium	Short fibrils
Alcaligens	Fibrils
Pseudomonas	No distinct fibrils
Rhibozium	Short fibrils
Sarcina	Amorphous cellulose
Zoogloea	Not well defined

Source: [6]

Table 5: Microorganisms producing cellulose and carbon source

Microorganism	Carbon source	Supplement	Culture time
A. xylinum BRC 5	glucose	Ethanol, oxygen	50 hours
G. hansenii PJK	glucose	Oxygen	48 hours
G. hansenii PJK	glucose	ethanol	72 hours
Acetobacter sp. V6	glucose	ethanol	8 days
Acetobacter sp. A9	glucose	ethanol	8 days
A. xylinum BPR2001	Molasses	none	72 hours
A. xylinum BPR2001	fructose	Agar oxygen	72 hours
A. xylinum BPR2001	fructose	Agar	56 hours
Acetobacter xylinum ssp. *Sucrofermentans* BPR 2001	fructose	Oxygen	52 hours
Acetobacter xylinum ssp. *Sucrofermentans* BPR 2001	fructose	Agar oxygen	44 hours
Acetobacter xylinum E25	glucose	no	7 days
G. xylinum strain K3	Mannitol	green tea	7 days
Gluconacetobacter xylinum IFO 13773	glucose	lignosulfonate	7 days
Acetobacter xylinum NUST4.1	glucose	sodium alginate	5 days
Gluconacetobacter xylinum IFO 13773	Molasses	no	7 days
Gluconacetobacter sp. RKY5	glycerol	no	144 hours
Gluconacetobacter sp. St-60-12 and *Lactobacillus mali* JCM1116	sucrose	no	72 hours

Source: [14]

Table 4: Composition of young and mature coconut water

Components	Young Coconut Water (v/v)	Mature Coconut Water (v/v)
Dissolved solids	6.5	5.4
Reducing sugars	4.4	0.2
Minerals	0.6	0.5
Protein	0.01	0.1
Fat	0.01	0.1
Organic acids	120	60
Potassium	290	247
Sodium	42.0	48.0
Calcium	44.0	40.0
Magnesium	10.0	15.0
Phosphorus	9.2	6.3
Iron	105.0	79.0
Copper	26.0	26.0

Source:[21]

and nutritional supplements used for the production of this product. Table 2.5 below lists different cellulose-producing microorganisms with their necessary supplements of source of carbon and nutrients.

Description of Fermentation

Coconut Water: Coconut is produced 50-150 ml of water per egg. Coconut water is best used in the production biocellulose, because pregnancy completes amino acid that helps the growth, development and activity of

Acetobacter xylinum. For growth and activity, *Acetobacter xylinum* requires macro and micro elements. Macro elements are made of carbon and nitrogen. In addition to carbohydrates and protein, coconut water also contains many old minerals needed by *Acetobacter xylinum*. Complimentary mineral elements contained in old coconut water are advantageous over other nata making materials. For example, potassium (K), sodium (Na), magnesium (Mg), calcium (Ca) and phosphorus (P) are the main mineral elements contained in old coconut water, which is required by *Acetobacter xylinum*.

The best form of coconut water can be obtained from a matured coconut which is, neither too old nor too young. A very old coconut water contained in coconut oil can inhibit the growth of *Acetobacter xylinum*. On the other hand, young coconut water hardly contains any minerals which are necessary in nata producing materials [20]. Table 4 shows the composition in young and mature coconut water.

Acetobacter xylinum is a source of carbohydrates among the nutrients that play a key role in the fermentation process for the production of cellulose. Acting as the source of carbohydrate, *A.xylinum* makes glucose as the energy source. *A. xylinum* cellulose layer will be formed if the fermentation medium is coconut water fortified with carbon and nitrogen sources in a controlled process. In such cases, bacteria produce enzymes that make up the chain of glucose into millions of cellulose fibers. Millions of chains growing on coconut water will produce millions of pieces of cellulose tapes that finally manifest on the surface of the broth medium.

Static Condition, Agitated and Stirred: A fermentation process can be carried out in three different conditions, such as static, agitated and stirred [22]. Fermentation process in static condition is carried out in the incubation without shaker or rotation. Conditions such as pH, temperature and incubation time remain the same with the other fermentation conditions. Fermentation is performed under static condition in incubator without rotation. Under agitated condition, fermentation can be done by shaking within an incubator (incubator shaker). During agitation, a shock velocity is set to ensure that the shaking in an incubator happens at a constant velocity from the beginning until the end of fermentation.

In the process of fermentation under stirred mode, a large-scale fermentation is usually performed by adulterating in a stirred tank system. On a laboratory scale, for example in the conical fermentation, magnetic stirrer can be used. In the application of *A. xylinum* cellulose fermentation, only the use of laboratory-scale fermentation flasks. The main purpose of shaking or stirring is to ensure the supply and distribution of oxygen homogeneously in a nutrient broth growth.

CONCLUSION

The production of biocellulose can be optimized by controlling all aspects that interfere its production such as pH, temperature and other supplements like nutrients, oxygen and fermentation medium. Plant cellulose and bacterial cellulose (biocellulose) have the same chemical structure, but possess different physical and chemical properties. Biocellulose have superior characteristics over cellulose plants. Therefore, biocellulose has been extensively used in medical applications as well as in industries related to paper, cosmetics and textiles.

ACKNOWLEDGEMENTS

This work is partially supported by FRGS/2/2013/TK05/UKM/02/1. The authors also gratefully acknowledge the helpful comments and suggestions of the reviewers, which have improved the presentation.

REFERENCES

1. Fessenden, J.R., 1986. Kimia Organik, Edisi ke Dua. Jilid II. Jakarta: Penerbit Erlangga.
2. Philips, G.O. and P.A. Williams, 2000. Handbook of Hydrocolloids. Cambridge Woodhead Publishing Limited.
3. Prashant, R.C., B.J. Ishwar, A.S. Shrikant, and S.S. Rekha, 2009. Fermentative Production of Microbial Cellulose, Food Technology. Biotechnology, 47(2): 107-124.
4. Hamonangan, N., G. Saharman, B. Emiliano, P. Ton and D.H. Sabar, 2013. Mechanical and thermal properties of bacterial cellulose fiber reinforced Mater Bi®bionanocomposite. Beilstein Journal Nanotechnology, 4: 325-329.
5. Kudlicka, K., I.M. Saxena, K. Okuda and R.M. Brown, 1989. Characterization of genes in the cellulose-synthesizing operon (acs operon) of Acetobacter xylinum: Implications of cellulose crystallization. Journal of Bacteriology, 176: 5735-5752.

6. Jonas, R. and L. Farah, 1998. Production and application of microbial cellulose. Polymer Degradation and Stability, 59(1998): 101-106.
7. Zaar, K., 1977. The biogenesis of cellulose by Acetobacter xylinum. Cryobiology, 16: 1-15.
8. Brown, R.M., J.H.M. Willison and C.L. Richardson, 1976. Cellulose biosynthesis in Acetobacter xylinum: Visualization of the site of synthesis and direct measurement of the in vivo process. Proceedings of the National Academy of Sciences of the United States of America, 73: 4565-4569.
9. Yamanaka, S., M. Iguchi and A. Budhiono, 2000. Bacterial cellulose-A masterpiece of nature's arts. J. of Material Science, 35: 261-270.
10. Iguchi, M., S. Yamanaka and A. Budhiono, 2000. Bacterial cellulose - a masterpiece of nature's arts Journal of Materials Science, 35(2): 261-270.
11. Klemm, D., D. Schumann, U. Udhart and S. Marsch, 2001. Bacterial Synthesis Cellulose-Artificial Blood Vessel for Microsurgery. Program Polymer Science, 26(1): 1561-1603.
12. Watanabe, K., M. Tabuchi, Y. Morinaga and F. Yoshinaga, 1998. Structural features and Properties of bacterial cellulose produced in agitated culture. Cellulose, 5: 187-200.
13. Fontana, J.D., A.M. deSouza, C.K. Fontana, *et al*., 1990. Acetobacter cellulose pellicle as a temporary skin substitute. Appl. Biochem. and Biotechnology, 24(25): 253-264.
14. Prashant, R.C., B.J. Ishwar, A.S. Shrikant and S.S. Rekha, 2008. Microbial Cellulose: Fermentative Productions and Applications. Food Technol. Biotechnology, 47(2): 107-124.
15. Johnson, D.C. and A.N. Neogi, 1989. Sheeted products formed from reticulated microbial cellulose. US patent, pp: 4863565.
16. Iguchi, M., Y. Nishi, M. Uryu, S. Yamanaka, K. Watanabe, N. Kitamura and S. Mitsuhashi, 1990. The structure and mechanical properties of sheets prepared form bacterial cellulose. J of Material Science, 25: 2997-3001.
17. Vandamme, E.J., S.D. Baets, V. Vanbaelen, K. Joris and P.D. Wulf, 1998. Improved production of bacterial cellulose and its application potential. Polymer Degradation Stabilization, 59: 93-99.
18. Sinta Erythrina, 2011. Kajian Terhadap Selulosa Mikrobial Sebagai Pensubstitusi Selulosa Kayu dalam Pembuatan Kertas. Institut Pertanian Bogor.
19. Ross, P., R. Mayer and M. Benziman, 1991. Cellulose biosynthesis and Function in bacteria. Microbiological Reviews, 55(1): 35-58.
20. Tomita Yoko and Tetsuo Kondo, 2008. Influential factors to enhance the moving rate of Acetobacter xylinum due to its nanofiber secretion on oriented templates.
21. Pambayun, R., 2002. Teknologi Pengolahan Nata De Coco. Teknologi Tepat Guna Kanisius: Yogyakarta.
22. Muhamad Taufiq Munawar. Bacteria Nata De Coco. http://muhtaufiqmunawar.blogspot.com. [12 Februari 2009]
23. Shuler, M.L. and F. Kargi, 2002. Bioprocess Engineering Basic Concepts. United States: Pearson Prentice Hall.