PRODUCTION, OPTIMIZATION AND MEMBRANE DIFFUSION STUDIES OF NANOCELLULOSE OBTAINED FROM *GLUCONACETOBACTER* SP. BCZM 1

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DEDICATION

This thesis is dedicated to my mother; Hajiya Maijidda, my late father; Alhaji Abba Abubakar Nakonkomeri and other members of the family whose support and encouragement have led to the success of completion of this thesis. Further, I would like to also dedicate this thesis to my wife; Aishatu Bin-Umar Barde, whose patience and support has also helped in achieving the thesis completion.

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ABSTRACT

Bacterial nanocellulose (BNC) has displayed significant advantages over cellulose obtained from plants due to the absence of lignin, hemicellulose, pectin and other contaminating materials of animal origin. Hence, it possesses a high degree of purity, crystallinity index, and biocompatibility. In this study, nanocellulose-producing bacteria were isolated from the environmental samples and screened for their ability to produce nanocellulose. The best among the selected bacteria were identified and characterised using 16S rRNA gene sequences analysis. Physicochemical factors affecting BNC production were identified using One-Factor-At-a-Time (OFAT). Statistical optimization of the BNC production was carried out using Central Composite Design (CCD). Purification of the BNC was achieved using 0.1 M NaOH at 80 °C. The BNC dried film was characterised to determine its morphological, structural, chemical and thermal properties. Furthermore, biomedical application of BNC for transdermal delivery of crocin as a model drug using vertical Franz cells diffusion was determined and presented. The isolated bacterium was able to produce nanocellulose on the surface of the culture medium under static condition at 30 °C. Maximum concentration of 4 g/L of dried BNC was obtained at the end of the fermentation. The bacterium was identified as *Gluconacetobacter* sp. BCZM 1 by 16S RNA gene sequencing method using universal 27F and 1492R primers. Based on OFAT study, the pH, temperature, incubation time, and inoculum size were the significant factors affecting BNC production by the bacterium. CCD analysis conducted on the significant factors showed optimum condition for BNC production with a maximum BNC concentration of 6.7 g/L. The regression model of the ANOVA was found to be significant with $p<0.0001$ and R^2 value of 0.9963. Characterization of the dried BNC membranes based on Fourier transformed infrared (FTIR) spectrum showed strong absorption peaks at 3335.36 and 2901.40 cm^{-1} representing band signature for pure nanocellulose. Scanning electron microscopy (SEM) revealed its morphological characteristics as an interwoven network structure. Thermogravimetric (TG) analysis had confirmed the BNC produced was thermally stable with degradation temperature above 340 °C. Analysis on BNC-crocin film showed a uniform distribution of the crocin into the BNC membranes. Dissolution studies on the BNC-crocin film displayed significant release of crocin (80-90 %) into the phosphate buffer solution within 40 minutes. Diffusion studies conducted with Franz cells showed that the incorporation of crocin into the BNC membrane provided a slow release pattern with an average flux of 0.53 µg cm^{-2} min^{-1}. Moreover, the production and purification steps adopted had displayed significant influence on BNC unique properties. The high swelling ratio of 33.47 for BNC dry film had indicated a high water absorbing capacity as an important quality for wound dressing materials. The release profile and simple preparation and incorporation of drug – loaded (BNC-crocin) membranes clearly indicated the potentials of using BNC membranes for transdermal application of the active compound. The findings of the current research have revealed the potential of local bacteria for the efficient production of BNC as a value added product with wider biotechnological potential and suitable for biomedical applications. This will further offer a robust platform for future direction in the area of research and innovations

ABSTRACT

Nanoselulosa bakteria (BNC) telah menunjukkan kelebihan yang ketara berbanding nanoselulosa tumbuhan disebabkan oleh ketiadaan lignin, hemiselulosa, pektin dan bahan-bahan tercemar dari sumber haiwan. Oleh itu, ia mempunyai tahap ketulenan yang tinggi, indeks kehabluran, dan biokeserasian. Dalam kajian ini, bakteria penghasil nanoselulosa telah diasingkan dari sampel alam sekitar dan disaring untuk kebolehan penghasilan nanocellulosa. Bakteria terasing yang terbaik yang dipilih telah dikenal pasti dan dicirikan menggunakan analisis urutan gen 16S rRNA. Faktor fizikokimia yang mempengaruhi pengeluaran BNC telah dikenal pasti menggunakan Satu Faktor Pada Satu Masa (OFAT). Pengoptimuman statistik bagi penghasilan BNC telah dijalankan menggunakan Reka Bentuk Komposit Sentral (CCD). Penulenan BNC dicapai menggunakan 0.1 M NaOH pada 80 °C. Filem BNC yang kering dicirikan berdasarkan sifat morfologi, struktur, kimia dan haba. Selain itu, penggunaan BNC dalam bioperubatan untuk penghantaran transdermal krosin sebagai model ubatan yang menggunakan penyebaran sel Franz menegak telah ditentukan dan dipersembahkan. Bakteria yang terpencil itu menghasilkan nanoselulosa sebagai lapisan gelatin putih di permukaan media penapaian pada keadaan statik pada 30 °C. Kepekatan maksimum 4 g/L bagi BNC kering diperolehi diakhir penapaian. Bakteria itu telah dikenal pasti sebagai *Gluconacetobacter* sp. BCZM 1 dengan kaedah penjujukan gen 16S rRNA menggunakan primer umum 27F dan 1492R. Berdasarkan kajian OFAT, pH, suhu, inkubasi dan saiz inokulum adalah faktor yang ketara mempengaruhi penghasilan BNC oleh bakterium.Analisis CCD yang dijalankan ke atas faktor penting menunjukkan keadaan optimum untuk pengeluaran BNC dengan hasil maksimum 6.7 g/L. Model regresi ANOVA didapati ketara dengan nilai p <0.0001 dan R^2 0.9963. Pencirian membran BNC kering berdasarkan spektrum Inframerah Transformasi Fourier (FTIR) menunjukkan puncak penyerapan yang kuat pada 3335.36 dan 2901.40 cm^{-1} mewakili jalur pengenalan untuk nanoselulosa tulen. Pengimbasan mikroskop elektron (SEM) menunjukkan ciri morfologinya sebagai struktur rangkaian yang bercabang. Analisis Termogravimetrik (TG) telah mengesahkan bahawa BNC yang dihasilkan sebagai stabil pada suhu tinggi dengan suhu degradasi melebihi 340 °C. Analisis pada filem BNC-krosin menunjukkan krosin tersebar secara seragam ke dalam membran BNC. Kajian perlarutan terhadap membran BNC-krosin memperlihatkan pembebasan krosin yang ketara (80-90 %) ke dalam larutan penimbal fosfat dalam 40 minit. Kajian penyerapan yang dijalankan dengan sel Franz menunjukkan bahawa penyebatian taburan krosin ke dalam membran BNC telah memberi corak pelepasan yang lambat dengan purata fluks 0.53 µg cm^{-1} min^{-1}. Tambahan pula, langkah-langkah pengeluaran dan penulenan BNC telah menunjukkan pengaruh yang ketara terhadap sifat unik BNC. Nisbah pengembangan yang tinggi iaitu 33.47 untuk filem kering BNC menunjukkan kapasiti penyerapan air yang tinggi sebagai kualiti yang penting untuk bahan pembaut luka. Profil pelepasan penyediaan mudah dan penggabungan dengan ubat (BNC-krosin) dengan jelas menunjukkan potensi menggunakan membran BNC untuk aplikasi transdermal bagi komponen aktif. Dapatan dalam kajian ini telah membuktikan potensi bakteria tempatan untuk pengasilan BNC yang cekap sebagai produk yang mempunyai kelebihan dengan potensi bioteknologi yang luas dan sesuai untuk aplikasi bioperubatan. Ini akan terus menawarkan platform yang kukuh dalam bidang penyelidikan dan inovasi pada masa hadapan.

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LIST OF ABBREVIATIONS

16S rRNA	-	16 Subunit Ribosomal Ribonucleic Acid
3D	-	Three dimensional
ATP	-	Adenosine Tri-phosphate
ATR	-	Attenuated Total Reflectance
BBD	-	Box-Behenken Design
BLAST	-	Basic Local Alignment Search Tools
BNC	-	Bacterial Nanocellulose
CCD	-	Central Composite Design
Cr. I	-	Crystallinity Index
CSL	-	Corn Steep Liquor
DNA	-	Deoxyribonucleic Acid
DNS	-	Dinitrilosalicylic Acid
DOE	-	Design of Experiment
DO	-	Degree of Polymerization
DTG	-	Differential Thermogravimetric
EDTA	-	Ethylene di-aminetetraacetic Acid
EDX	-	Energy Dispersive Electron Microscope
EtBr	-	Ethidium Bromide
FTIR	-	Fourier Transform Infrared
gDNA	-	Genomic DNA
GI	-	Gastrointestinal
GRAS	-	Generally, Recognise as Safe
HCL	-	Hydrochloric acid
HS	-	Hestrin and Schramm
ISO	-	International Standard Organization
MAI	-	Mean Annual Increment
MEGA	-	Molecular Evolutionary Genetics Analysis
min	-	Minutes
mL	-	Millilitre
NCBI	-	National Centre for Bioinformatics Information

NMR	-	Nuclear Magnetic Resonance
OFAT	-	One Factor-at- a-Time
PBS	-	Phosphate Buffer Saline
PCR	-	Polymerase Chain Reaction
PHB	-	Polyhydroxy butyrate
RNA	-	Ribonucleic Acid
RSM	-	Response Surface Methodology
SEM	-	Scanning Electron Microscope
SWR	-	Swelling Ratio
TAE	-	Tris-Acetate EDTA
TC	-	Terminal Complexes
TCA	-	Tricarboxylic Acid
TGA	-	Thermogravimetric Analysis
T_{max}	-	Maximum Temperature
UDPGlu	-	Uridine di-Phosphoglucose

LIST OF SYMBOLS

^oC	-	Degree Celsius
β	-	Beta
α	-	Alpha
ng	-	Nanogram
μ	-	Micron
μL	-	Microlitre
w/v	-	Weight per volume
Θ	-	Theta
%	-	Percentage
±	-	Plus or Minus
h^{-1}	-	Per Hour
g/L	-	Gram Per Litre
g/g	-	Gram per gram
g/L/h	-	Gram per litre per hour
X_{max}	-	Maximum biomass
Kbp	-	Kilo base pair
kV	-	Kilo voltage
cm^{-1}	-	Per centimetre
R^2	-	Regression coefficient
OH	-	Hydroxyl

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Cellulose is the most abundant naturally-occurring biopolymer on earth. They are characterised as an exopolysaccharides glucose polymer consisting of β-1-4–glycosidic linkage with various degrees of polymerizations and a chemical structure (Chawla *et al.,* 2009). It is frequently isolated from plants as the main reinforcing component of their cell walls, which is generally used as raw materials for paper and fibres industries (Moon *et al.,* 2011). Cotton linens and wood pulp are the most common commercial sources of cellulose. However, cellulose obtained from plant sources is still contaminated with non-cellulosic materials/polysaccharides, such as hemicellulose and lignin (Abeer *et al.*, 2014). The removal of such materials can be achieved using chemical and mechanical processes, which may end up weakening the structure and other important properties. This process is therefore, environmentally not suitable for cellulose production (Jozala *et al.*, 2016). Thus, it is necessary to find other alternative sources for obtaining high purity cellulose (Lestari *et al.,* 2014a). With the new development and the emergence of nanotechnology, cellulose has attracted more attention in the new form of ‘‘nanocellulose’’ to be used as a novel and advanced material in modern biotechnology (Islam *et al.,* 2017).

Microorganisms belonging to the genera *Acetobacter, Gluconacetobacter, Achromobacter, Agrobacterium, Athrobacter, Pseudomonas, Rhizobium,* and *Sarcinar* can secrete nanocellulose with the same chemical structure and much higher crystallinity index and chemical purity than plant nanocellulose (Premjet *et al.*, 2007). The nanocellulose obtained from bacteria are generally referred to as bacterial nanocellulose (BNC) and represents the purest form of nanocellulose with remarkable physical and chemical properties suitable for various industrial applications (Islam, *et al.*, 2017). The BNC was first reported as a white gelatinous pellicle, which can grow

up to 25 mm thick on the liquid medium while studying acetic acid fermentation. This membrane is generated by a bacterium, named *Bacterium xylinum,* later renamed as *Acetobacter xylinus,* then *Gluconacetobacter xylinus.* However, a large number of research works still refer this organism *as G. xylinus or A. xylinus* (Yamada *et al.,* 2012).

Biosynthesis of nanocellulose in bacteria has long been documented using a wide variety of bacteria including soil bacteria (*Burkholderia* spp.), nitrogen-fixing plant symbiont bacteria (*Rhizobium leguminosarum*), tumour producing *Agrobacterium tumefaciens* and *A. xylinus / G. xylinus* (Römling and Galperin, 2015; Ross *et al.,* 1991). Bacterial synthesis of nanocellulose is an effective and convenient way of producing nanocellulose from a practical standpoint for the production of pure nanocellulose suitable to be used for biomedical applications and other emerging nanotechnologies (Gatenholm and Klemm, 2010; Römling and Galperin, 2015). The BNC is synthesized in the form of microfibrils, which are arranged in a 3D web shape structure providing high mechanical strength and a porous geometry that is appropriate for a broad range of application (Moniri *et al.,* 2017).

The BNC has attracted academic and commercial interest owing to its great potentials in biotechnology, bio-based packaging, and pharmaceutical as well as biomedical industries (Iguchi *et al.*, 2000). It is one of the most abundant, renewable, natural biosorbents, and biocompatible polymer characterised by high water holding capacity and high surface area allowing the uptake and release of soluble materials leading to wide industrial applications. Consequently, good mechanical properties of BNC are the reason for such interest in producing a fully bio-based cellulosic polymer with high properties, including Young's modulus, and tensile strength. These properties are responsible for the use of BNC as a reinforcing agent in the development of composite materials (Gea *et al.*, 2011). The unique chemical and physical properties of BNC has made it emerge as a new industrial product with a high degree of purity. Hence, BNC does not contain contaminating materials such as lignin, hemicellulose, pectin, wax, and other plants components that are difficult to be removed (Krystynowicz *et al.*, 2002). It is similarly worth revealing that while BNC is the

‘‘gold standard’’ for nanocellulose as it is formed in the nanometer-scale under laboratory controlled conditions by the bacteria (Lee *et al.*, 2014).

Guhados *et al.* (2005) have described BNC as a polymer with a high degree of crystallinity and excellent physicochemical properties than nanocellulose derived from plants. Moreover, these properties have made it an ideal material for a wide range of applications (Young-Jung, 2011). Yet, the high water-holding capacity (WHC), web-like network, and a high degree of polymerization are the added advantages of BNC over plant-derived nanocellulose. The absence of non-cellulosic materials in BNC has led to the concept of extensive utilizations of BNC after mild refinement of the BNC gel produced using aqueous NaOH, for a large number of industrial applications. This includes the use of BNC as a biocompatible polymer for self-constructing protective packaging materials. This finding won the third prize in the Bayer science vision works award in 2007 (Lee, *et al.*, 2014). Most of the studies have investigated cellulose nanofibres as a reinforcing agent in thermoplastics. Favier *et al.* (1995) published the first work on isolating nanocellulose whiskers and demonstrated the reinforcing potential of cellulose whiskers in nanocomposites. Hence, BNC has displayed added advantages as it is more thinner than the plant nanocellulose (Mohite and Patil, 2014).

The BNC can be used in general as a substitute for plant cellulose (Jonas and Farah, 1998). Consequently, practical application in biomedical sciences and other biotechnological fields have been developed and still need further exploration (George *et al.*, 2005). BNC has been used as wound dressing materials and artificial skin. Products include Biofill®, made by a Brazilian company and XCellTM, by an American company. Biofill® has been used for artificial skin to treat trophic ulcerations on limbs. This material shortened the wound healing time, reduced treatment cost and contamination, thereby absorbing wound exudates and eliminating pain symptoms. Other physical properties of BNC were reported by Bielecki *et al.* (2005).

The production of BNC has been receiving great attention in the last decade due to its potential application in the industries and other unique properties over plants nanocellulose. World production of BNC is highly affected by its demand and cost of

production, which is the major concern for industrial-scale production (Bilgi *et al.*, 2016). There are several patented methods and system for BNC production and other published literature in the last decade to understand the major factors affecting BNC production. Such factors and condition need to be optimized using available resources for the production of high quality and cost-effective BNC with the acceptable yield of production to support possible commercialisation (Jozala *et al.*, 2016). By finding new applications for BNC, it is more favourable for the industry to start further exploration of BNC production from different sources. Locally isolated bacteria from food and agricultural wastes that shows nanocellulose producing potentials are expected to be better adapted to the local environments, thereby utilizing the available solid or liquid waste for nanocellulose production. There is increasing demand for nanocellulose and increase in consumption of wood as raw materials for nanocellulose production from plants. This results in deforestation and environmental problems, which could be avoided through the cleaner production of nanocellulose by the bacteria under controlled laboratory conditions.

The BNC has the potential to be employed in topical formulations to overcome and surpass its reported limitations. Such limitations for topical application include lack of drug dose reproducibility due to the loss of material as a result of surface contact with the garment. These are associated with the topical application and formulation of the drugs and other materials. However, the drugs doses for the topical delivery system can be precisely defined by using BNC membranes that can be applied to the skin based on its larger surface area, which can accommodate the required dose and prevent any loss of the drugs applied (Trovatti *et al.*, 2012). Therefore, it is suggested that BNC could be used to represent a biomaterial with an attractive interest in cosmetics to serves as an alternative for improvement of patients compliance based on oily formulation. Other advantages of using BNC film could be extended to transdermal drug delivery systems. Most of transdermal patches that are commercially available for the drug delivery are manufactured by superimposition of different materials (Padula *et al.*, 2003), and since BNC single or fewer layers can be used as the delivery system, this lowers the cost of production and simplifies the preparations.

The purpose of this research was to explore and identify new locally isolated bacterium with efficient nanocellulose production potentials that could serve as a model for BNC production for both research and commercialization. This could further reduce the over-dependence on the use of culture isolates from culture collections. Despite the high possibility of identifying newly isolated bacteria with the ability to produce nanocellulose, most of the research reported in the literature depends on culture collections for their isolates used for BNC production. The research further determined the optimum conditions using Design of Experiment (DOE) for maximum BNC production under static culture condition. The BNC produced will be evaluated and characterised to determine its morphological, chemical, structural and other properties under different characterization methods. The application of BNC membranes for the transdermal delivery of crocin (the major active component of Saffron) as a model drug were determined and reported for the first time using Franz cells diffusion method.

1.2 Problem Statement

The increase in utilization of polymers for biotechnological applications has ecological and health implication, especially for environmental and biomedical perspective (Gumel *et al.*, 2013). There is rapid increase in demands for nanocellulose-based materials due to its biotechnological potential. However, the major challenges faced by plant nanotechnologists for the exploration of nanocellulose materials from plants were focusing on how to reduce the number of harsh acids used in nanocrystal preparation and finding an easier recovery method with low energy input for the removal of impurities such as lignin, hemicellulose and pectin. These had limited application of plants nanocellulose for biofabrications and transdermal formulations. The best approach to overcome problems associated with plant's nanocellulose exploration is the production using microorganisms. Thus, bacteria were found to be suitable model for the production nanocellulose with added advantages over plant nanocellulose (Charreau *et al.*, 2013; Jozala, *et al.*, 2016).

Despite of the bacterial ability for nanocellulose production, the producer organisms are yet to be completely exploited, large number of researchers depend on the stock culture isolates for nanocellulose production, and hence, there is high tendency of isolating new local bacteria with high nanocellulose producing efficiency as compared to the existing ones. Fruit wastes are one of the most abundant types of wastes commonly found in Malaysia, which were anticipated to support the growth of different bacterial isolates of biotechnological importance that can support the global production of BNC for scientific and commercial purposes. The current study focused on the development of eco-friendly method to provide better substitutes for plant nanocellulose exploration, through isolation of local bacteria with efficient nanocellulose producing ability in order to supress the over dependence on culture isolates for the production of BNC. Optimization of nanocellulose production by the isolate will further provide a strong platform to scale up BNC production for biomedical and other biotechnological applications. In spite of the potential of BNC membrane for the transdermal delivery of active compounds, to date, transdermal application of crocin as an active compound with clinical significance using BNC membrane has not been studied and reported yet. Hence, this research would therefore contribute substantially to enhancing the body of knowledge.

1.3 Objectives of the Research

Based on the above-stated problems, this study was specifically designed to address the following objectives**:**

(a) To isolate and characterize nanocellulose producing bacterium from fruit waste samples

(b) To characterise and optimize BNC production by the isolated bacterium

(c) To evaluate the potentials of using BNC membranes for the transdermal drug delivery using vertical Franz cells diffusion

1.4 Scope of the Research

The scope of this study involved isolating the potential nanocellulose producing bacteria locally from environmental waste samples and to determine high and efficient nanocellulose producing bacterium using suitable culture medium reported in literature (Hestrin and Schramm, 1954a; Jozala *et al.*, 2016; Premjet *et al.*, 2007; Yamanaka *et al.*, 1989). Nanocellulose production was optimized by varying carbon and nitrogen sources, temperatures, pH, incubation period as well as the inoculum ratio under static culture condition using one-Factor-At-a- Time (OFAT) and Central Composite Design (CCD) methods. The cellular and colony morphologies of potential nanocellulose producing bacteria were analysed. The selected bacterial isolate was identified by molecular tools based on 16S rRNA gene analysis. Chemical and physical properties of BNC were also determined based on SEM, XRD, TGA and FTIR analysis. The potential application of BNC for the transdermal delivery of crocin as the model drugs was also determined by Franz cells diffusion.

1.5 Significance of Research

Research and innovations will provide a strategic and innovative measure for an alternative method of obtaining material from biological entities through sustainable and environmentally friendly methods. The use of bacterial biopolymer will provide an alternative to plants and other synthetic polymers that can endanger an environmentally balanced ecosystem due to deforestation as well as solid waste generation. The production of bacterial nanocellulose will help in solving the ever-increasing demand and pressure imposed on the delicate ecological balance for the exploration of nanocellulose from plants materials. The development of an ecologically clean method of nanocellulose synthesis is of great interest to solve the important problems of biosphere ecology and deforestation. Therefore, this study is in line with the National Green Technology policy of Malaysia where conservation and minimization of environmental impact are highly encouraged.

BNC is one of the most abundant biopolymers produced by several strains of Acetic acid bacteria. This polymer is cost-effective and eco-friendly and its nature makes it an important alternative to synthetic polymers which are costly and causing environmental problems as well as solid waste generation due to its recalcitrant and non-biodegradability. It has also been found to have a multitude of applications in paper, textile, and food industries. Nowadays it is extensively used as biomaterials in cosmetics and medicines. In addition, BNC will serve as suitable raw materials for the production of high acoustic speakers, high-quality papers and dessert foods. In view of the above, it is, therefore, important to isolate and screen for the bacteria able to produce nanocellulose and to further understand the basic, properties, components and structure of BNC and the optimum condition necessary for maximum BNC production.

1.6 Thesis Organization

This thesis is divided into 7 different chapters. Chapter 1 consisted of the background of the research, problem statement, research objectives, scope and significance of the research, which was aimed to highlight the introduction aspect of this research work. Chapter 2 consist of a comprehensive literature review based on the research topic. The literature review covers the general overviews on nanocellulose and major issues related to its application in biotechnology as well as its synthesis by bacteria. The general flow chart of the research and the summary of the materials and methods are provided in chapter 3. Chapter 4 covered the isolation, characterization and molecular identification of nanocellulose producing bacteria using 16S rRNA gene sequence analysis. Chapter 5, consist of production, and purification of BNC produced by the bacterium. The optimization covers conventional and statistical methods. Moreover, different methods for the characterization BNC produced including SEM, FTIR, TGA, and XRD were presented in this chapter. The potential application of BNC membranes for the transdermal application of crocin using Franz diffusion methods was presented in Chapter 6. Finally, the conclusions were presented in Chapter 7 and were based on the results obtained from the experiments conducted and presented in Chapters 4, 5 and 6. In addition, recommendations for further

investigation based on the research vacuums acknowledged during this study were mentioned and highlighted in Chapter 7.

REFERENCES

Abeer, M. M., Mohd Amin, M. C. and Martin, C. (2014). A review of bacterial cellulose-based drug delivery systems: their biochemistry, current approaches and future prospects. *Journal of Pharmacy and Pharmacology*. 66(8), 1047-1061.

Alauzet, C., Teyssier, C., Jumas-Bilak, E., Gouby, A., Chiron, R., Rabaud, C., Counil, F., Lozniewski, A. and Marchandin, H. (2011). Gluconobacter as well as Asaia species, newly emerging opportunistic human pathogens among acetic acid bacteria. *Journal of Clinical Microbiology*. 49(1), 480.

Alavizadeh, S. H. and Hosseinzadeh, H. (2014). Bioactivity assessment and toxicity of crocin: A comprehensive review. *Food and Chemical Toxicology*. 64, 65-80.

Alexander, A., Dwivedi, S., Giri, T. K., Saraf, S., Saraf, S. and Tripathi, D. K. (2012). Approaches for breaking the barriers of drug permeation through transdermal drug delivery. *Journal of Controlled Release*. 164(1), 26-40.

Amin, M., Abadi, A. G., Ahmad, N., Katas, H. and Jamal, J. A. (2012). Bacterial cellulose film coating as drug delivery system: physicochemical, thermal and drug release properties. *Sains Malaysiana*. 41(5), 561-568.

Amnuaikit, T., Chusuit, T., Raknam, P. and Boonme, P. (2011). Effects of a cellulose mask synthesized by a bacterium on facial skin characteristics and user satisfaction. *Medical Devices (Auckland, NZ)*. 4, 77.

Anderson, M. and Whitcomb, P. (2000). DOE Simplified: Practical tools for effective experimentation: Portland, Productivity. Inc.

Aramwit, P. and Bang, N. (2014). The characteristics of bacterial nanocellulose gel releasing silk sericin for facial treatment. *BMC Biotechnology*. 14(1), 104.

Aung, H., Wang, C., Ni, M., Fishbein, A., Mehendale, S., Xie, J., Shoyama, A. and Yuan, C. (2007). Crocin from Crocus sativus possesses significant anti-proliferation effects on human colorectal cancer cells. *Experimental Oncology*. 29(3), 175.

Aydin, Y. A. and Aksoy, N. D. (2009). Isolation of cellulose producing bacteria from wastes of vinegar fermentation. *Proceedings of the 2009 Proceedings of the World Congress on Engineering and Computer Science*, 20-22.

Bae, S. and Shoda, M. (2004). Bacterial Cellulose Production by Fed-Batch Fermentation in Molasses Medium. *Biotechnology Progress*. 20(5), 1366-1371.

Barbara Surma-Ślusarska, Sebastian Presler and Danielewicz, D. (2008). Characteristics of Bacterial Cellulose Obtained from Acetobacter Xylinum Culture for Application in Papermaking. *Fibres and Tectiles in Eastern Europe*. 16(4), 108-111.

Barry, B. W. (2001). Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences*. 14(2), 101-114.

Bay, H. H., Lim, C. K., Kee, T. C., Ware, I., Chan, G. F., Shahir, S. and Ibrahim, Z. (2014). Decolourisation of Acid Orange 7 recalcitrant auto-oxidation coloured by-products using an acclimatised mixed bacterial culture. *Environmental Science and Pollution Research*. 21(5), 3891-3906.

Bielecki, S., Krystynowicz, A., Turkiewicz, M. and Kalinowska, H. (2005). Bacterial cellulose. *Biopolymers online*.

Bilgi, E., Bayir, E., Sendemir-Urkmez, A. and Hames, E. E. (2016). Optimization of bacterial cellulose production by *Gluconacetobacter* xylinus using carob and haricot bean. *International Journal of Biological Macromolecule*. 10.1016/j.ijbiomac.2016.02.052.

Boesenberg-Smith, K. A., Pessarakli, M. M. and Wolk, D. M. (2012). Assessment of DNA yield and purity: an overlooked detail of PCR troubleshooting. *Clinical Microbiology Newsletter*. 34(1), 1-6.

Borzani, W. and Souza, S. J. (1995). Mechanism of the film thickness increasing during the bacterial production of cellulose on non-agitaded liquid media. *Biotechnology Letters*. 17(11), 1271-1272.

Brain, K. R., Walters, K. A. and Watkinson, A. C. (2002). Methods for studying percutaneous absorption. *Drugs and the Pharmaceutical Sciences*. 119, 197-270.

Brown, R. M. (2004). Cellulose structure and biosynthesis: what is in store for the 21st century? *Journal of Polymer Science Part A: Polymer Chemistry*. 42(3), 487-495.

Cappuccino, J. G. S., Cappuccino, N. J. G. and Sherman, N. (1996). *Microbiology: a laboratory manual*.

Carreira, P., Mendes, J. A., Trovatti, E., Serafim, L. S., Freire, C. S., Silvestre, A. J. and Neto, C. P. (2011). Utilization of residues from agro-forest industries in the production of high value bacterial cellulose. *Bioresource Technology*. 102(15), 7354-7360.

Castro, C., Zuluaga, R., Álvarez, C., Putaux, J.-L., Caro, G., Rojas, O. J., Mondragon, I. and Gañán, P. (2012a). Bacterial cellulose produced by a new acid-resistant strain of *Gluconacetobacter* genus. *Carbohydrate Polymers*. 89(4), 1033-1037.

Castro, C., Zuluaga, R., Alvarez, C., Putaux, J. L., Caro, G., Rojas, O. J., Mondragon, I. and Ganan, P. (2012b). Bacterial cellulose produced by a new acid-resistant strain of *Gluconacetobacter* genus. *Carbohydrate Polymers*. 89(4), 1033-1037.

Castro, C., Zuluaga, R., Putaux, J.-L., Caro, G., Mondragon, I. and Gañán, P. (2011). Structural characterization of bacterial cellulose produced by *Gluconacetobacter* swingsii sp. from Colombian agroindustrial wastes. *Carbohydrate Polymers*. 84(1), 96-102.

Cerrutti, P., Roldán, P., García, R. M., Galvagno, M. A., Vázquez, A. and Foresti, M. L. (2016). Production of bacterial nanocellulose from wine industry residues: Importance of fermentation time on pellicle characteristics. *Journal of Applied Polymer Science*. 133(14), 1-9.

Charreau, H., L Foresti, M. and Vázquez, A. (2013). Nanocellulose patents trends: a comprehensive review on patents on cellulose nanocrystals, microfibrillated and bacterial cellulose. *Recent patents on nanotechnology*. 7(1), 56-80.

Chawla, P. R., Bajaj, I. B., and, S. A. S. and Singhal*, R. S. (2009). Microbial Cellulose: Fermentative Production and Applications. *Food Technol. Biotechnol*. 47(2), 107-124.

Cheng, C., Guo, R., Lan, J., Jiang, S., Du, Z., Zhao, L. and Peng, L. (2017). Effects of sodium hydroxide treatment on microstructure and mechanical properties of lotus fibers. *Fibers and Polymers*. 18(9), 1671-1678.

Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*. 17(4), 840-862.

Çoban, E. P. and Biyik, H. (2011). Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter lovaniensis* HBB5. *African Journal of Biotechnology*. 10(27), 5346-5354.

Czaja, W., Krystynowicz, A., Bielecki, S. and Brown, R. M. (2006). Microbial cellulose—the natural power to heal wounds. *Biomaterials*. 27(2), 145-151.

Czaja, W., Romanovicz, D. and R. Malcolm Brown, J. (2004). Structural investigations of microbial cellulose produced.pdf.

Czaja, W. K., Young, D. J., Kawecki, M. and Brown, R. M. (2007). The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*. 8(1), 1-12.

Dima, S.-O., Panaitescu, D.-M., Orban, C., Ghiurea, M., Doncea, S.-M., Fierascu, R. C., Nistor, C. L., Alexandrescu, E., Nicolae, C.-A. and Trică, B. (2017). Bacterial nanocellulose from side-streams of kombucha beverages production: Preparation and physical-chemical properties. *Polymers*. 9(8), 374.

Donini, Í. A., De Salvi, D. T., Fukumoto, F. K., Lustri, W. R., Barud, H. S., Marchetto, R., Messaddeq, Y. and Ribeiro, S. J. L. (2010). Biosynthesis and Recent Production Advances of bacterial cellulose. *Eclética Química*. 165-178.

dos Santos, F. A., Iulianelli, G. C. and Tavares, M. I. B. (2016). The Use of Cellulose Nanofillers in Obtaining Polymer Nanocomposites: Properties, Processing, and Applications. *Materials Sciences and Applications*. 7(05), 257.

Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. (2006). *The Prokaryotes proteobacteria: Delta and Epsilon subclasses. deeply rooting bacteria*. (3 ed.) New York: Springer Science and Business Media.

E.Embuscado, M., and, J. S. M. and N.BeMiller, J. (1994). Bacterial cellulose. I. Factors affecting the production of cellulose by Acetobacter xylinum. *Food Hydrocolloids* 8(5), 407-418.

El-Saied, H., Basta, A. H. and Gobran, R. H. (2004). Research progress in friendly environmental technology for the production of cellulose products (bacterial cellulose and its application). *Polymer-Plastics Technology and Engineering*. 43(3), 797-820.

El-Saied, H., El-Diwany, A. I., Basta, A. H., Atwa, N. A. and El-Ghwas, D. E. (2008a). Production and characterization of economical bacterial cellulose. *BioResources*. 3(4), 1196-1217.

El-Saied, H., I, A., El-Diwany, Basta, A. H., and, N. A. A. and El-Ghwas, D. E. (2008b). Production and characterization of economical bacterial cellulose. *BioResources* 3(4), 1196-1217.

Fan, M., Dai, D. and Huang, B. (2012). Fourier transform infrared spectroscopy for natural fibres *Fourier transform-materials analysis* InTech.

Favier, V., Chanzy, H. and Cavaille, J. (1995). Polymer nanocomposites reinforced by cellulose whiskers. *Macromolecules*. 28(18), 6365-6367.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39(4), 783-791.

Flink, J. M. and Knudsen, H. (1983). *An introduction to freeze drying*. Strandbergs Forlag.

Foresti, M., Vázquez, A. and Boury, B. (2017). Applications of bacterial cellulose as precursor of carbon and composites with metal oxide, metal sulfide and metal nanoparticles: a review of recent advances. *Carbohydrate polymers*. 157, 447-467.

Fu, L., Zhang, J. and Yang, G. (2013). Present status and applications of bacterial cellulose-based materials for skin tissue repair. *Carbohydrate polymers*. 92(2), 1432-1442.

Garnett, M. J., Edelman, E. J., Heidorn, S. J., Greenman, C. D., Dastur, A., Lau, K. W., Greninger, P., Thompson, I. R., Luo, X. and Soares, J. (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature*. 483(7391), 570-575.

Gatenholm, P. and Klemm, D. (2010). Bacterial nanocellulose as a renewable material for biomedical applications. *MRS bulletin*. 35(3), 208-213.

Gea, S., Reynolds, C. T., Roohpour, N., Wirjosentono, B., Soykeabkaew, N., Bilotti, E. and Peijs, T. (2011). Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process. *Bioresour Technol*. 102(19), 9105-9110.

George, J., Ramana, K., Sabapathy, S. and Bawa, A. (2005). Physico-mechanical properties of chemically treated bacterial (*Acetobacter xylinum*) cellulose membrane. *World Journal of Microbiology and Biotechnology*. 21(8-9), 1323-1327.

Giménez, J., Martinez, M., de Pablo, J., Rovira, M. and Duro, L. (2007). Arsenic sorption onto natural hematite, magnetite, and goethite. *Journal of Hazardous Materials*. 141(3), 575-580.

Goelzer, F., Faria-Tischer, P., Vitorino, J., Sierakowski, M.-R. and Tischer, C. (2009a). Production and characterization of nanospheres of bacterial cellulose from *Acetobacter xylinum* from processed rice bark. *Materials Science and Engineering: C*. 29(2), 546-551.

Goelzer, F. D. E., Faria-Tischer, P. C. S., Vitorino, J. C., Sierakowski, M. R. and Tischer, C. A. (2009b). Production and characterization of nanospheres of bacterial cellulose from *Acetobacter xylinum* from processed rice bark. *Materials Science and Engineering: C*. 29(2), 546-551.

Goh, W., Rosma, A., Kaur, B., Fazilah, A., Karim, A. and Bhat, R. (2012). Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*. 19(1).

Guhados, G., Wan, W. and Hutter, J. L. (2005). Measurement of the elastic modulus of single bacterial cellulose fibers using atomic force microscopy. *Langmuir*. 21(14), 6642-6646.

Gumel, A. M., Annuar, M. S. M. and Heidelberg, T. (2013). Current application of controlled degradation processes in polymer modification and functionalization. *Journal of Applied Polymer Science*. 129(6), 3079-3088.

Gumel, A. M., Annuar, M. S. M. and Yusuf, H. (2015). Perspectives on the emerging applications of multifaceted biomedical polymeric nanomaterials. *Journal of Nanomaterials*. 16(1), 310.

Guo, L., Chen, Q., Fang, F., Hu, Z., Wu, J., Miao, A., Xiao, L., Chen, X. and Yang, L. (2013). Application potential of a newly isolated indigenous aerobic denitrifier for nitrate and ammonium removal of eutrophic lake water. *Bioresource technology*. 142, 45-51.

Ha, J. H., Shehzad, O., Khan, S., Lee, S. Y., Park, J. W., and, T. K. and Park, J. K. (2008). Production of bacterial cellulose by a static cultivation using the waste from beer culture broth. *Korean Journal of Chemical Engineering*. 25(4), 812-815.

Hall, B. G. (2011). *Phylogenetic trees made easy: A how to manual*. Sinauer Associate, Sunderland, Massachusetts.

Hasan, N., Biak, D. R. A. and Kamarudin, S. (2012). Application of bacterial cellulose (BC) in natural facial scrub. *International Journal on Advanced Science, Engineering and Information Technology*. 2(4), 272-275.

Helenius, G., Bäckdahl, H., Bodin, A., Nannmark, U., Gatenholm, P. and Risberg, B. (2006). *In vivo* biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research Part A*. 76(2), 431-438.

Hemraj, V., Diksha, S. and Avneet, G. (2013). A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Science*. 1(1), 1-7.

Hestrin, S. a. and Schramm, M. (1954a). Factors affecting Production of Cellulose at the Air/ Liquid Interface of a Culture of *Acetobacter xylinum*. *Journal of General Microbiology*. 11, 123-129.

Hestrin, S. a. and Schramm, M. (1954b). Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal*. 58(2), 345.

Hong, L., Wang, Y., Jia, S., Huang, Y., Gao, C. and Wan, Y. (2006). Hydroxyapatite/bacterial cellulose composites synthesized via a biomimetic route. *Materials letters*. 60(13-14), 1710-1713.

Hornung, M., Ludwig, M., Gerrard, A. M. and Schmauder, H. P. (2006a). Optimizing the production of bacterial cellulose in surface culture: Evaluation of product movement influences on the bioreaction (Part 2). *Engineering in Life Sciences*. 6(6), 546-551.

Hornung, M., Ludwig, M., Gerrard, A. M. and Schmauder, H. P. (2006b). Optimizing the production of bacterial cellulose in surface culture: Evaluation of substrate mass transfer influences on the bioreaction (Part 1). *Engineering in Life Sciences*. 6(6), 537-545.

Hornung, M., Ludwig, M. and Schmauder, H. P. (2007). Optimizing the production of bacterial cellulose in surface culture: A novel aerosol bioreactor working on a fed batch principle (Part 3). *Engineering in Life Sciences*. 7(1), 35-41.

Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C. and Sun, D. (2014). Recent advances in bacterial cellulose. *Cellulose*. 21(1), 1-30.

Hungund, B. S. and Gupta, S. (2010). Strain improvement of *Gluconacetobacter xylinus* NCIM 2526 for bacterial cellulose production. *African Journal of Biotechnology*. 9(32), 5170-5172.

Hutchens, S. A., Leon, R., O'Neill, H. M. and Evans, B. R. (2007). Statistical analysis of optimal culture conditions for *Gluconacetobacter hansenii* cellulose production. *Letters in Applied Microbiology*. 44(2), 175-180.

Hwang, J. W., Yang, Y. K., Hwang, J. K., Pyun, Y. R. and Kim, Y. S. (1999). Effects of pH and dissolved oxygen on cellulose production by Acetobacter xylinum BRC5 in agitated culture. *Journal of Bioscience and Bioengineering*. 88(2), 183-188.

Iguchi, M., Yamanaka, S. and Budhiono, A. (2000). Bacterial cellulose—a masterpiece of nature's arts. *Journal of Materials Science*. 35(2), 261-270.

Ishihara, M., Matsunaga, M., Hayashi, N. and Tišler, V. (2002). Utilization of D-xylose as carbon source for production of bacterial cellulose. *Enzyme and Microbial Technology*. 31(7), 986-991.

Islam, M. U., Ullah, M. W., Khan, S., Shah, N. and Park, J. K. (2017). Strategies for cost-effective and enhanced production of bacterial cellulose. *International journal of biological macromolecules*. 102, 1166-1173.

Jahan, F., Kumar, V., Rawat, G. and Saxena, R. (2012a). Production of microbial cellulose by a bacterium isolated from fruit. *Applied Biochemistry and Biotechnology*. 167(5), 1157-1171.

Jahan, F., Kumar, V., Rawat, G. and Saxena, R. K. (2012b). Production of microbial cellulose by a bacterium isolated from fruit. *Applied Biochemistry and Biotechnolology*. 167(5), 1157-1171.

Jia, S., Ou, H., Chen, G., Choi, D., Cho, K., Okabe, M. and Cha, W. S. (2004). Cellulose production from *Gluconobacter oxydans* TQ-B2. *Biotechnology and Bioprocess Engineering*. 9(3), 166.

Jonas, R. and Farah, L. F. (1998). Production and application of microbial cellulose. *Polymer Degradation and Stability*. 59(1), 101-106.

Joseph, G., Rowe, G. E., Margaritis, A. and Wan, W. (2003). Effects of polyacrylamide-co-acrylic acid on cellulose production by Acetobacter xylinum. *Journal of Chemical Technology and Biotechnology*. 78(9), 964-970.

Jozala, A. F., de Lencastre-Novaes, L. C., Lopes, A. M., de Carvalho Santos-Ebinuma, V., Mazzola, P. G., Pessoa-Jr, A., Grotto, D., Gerenutti, M. and Chaud, M. V. (2016). Bacterial nanocellulose production and application: a 10-year overview. *Applied Microbiology and Biotechnology*. 100(5), 2063-2072.

Jozala, A. F., Pertile, R. A., dos Santos, C. A., de Carvalho Santos-Ebinuma, V., Seckler, M. M., Gama, F. M. and Pessoa, A., Jr. (2015). Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. *Applied Microbiology and Biotechnology*. 99(3), 1181-1190.

Jung, H.-I., Jeong, J.-H., Lee, O.-M., Park, G.-T., Kim, K.-K., Park, H.-C., Lee, S.-M., Kim, Y.-G. and Son, H.-J. (2010). Influence of glycerol on production and structural–physical properties of cellulose from Acetobacter sp. V6 cultured in shake flasks. *Bioresource technology*. 101(10), 3602-3608.

Jung, J. Y., Park, J. K. and Chang, H. N. (2005). Bacterial cellulose production by Gluconacetobacter hansenii in an agitated culture without living non-cellulose producing cells. *Enzyme and Microbial Technology*. 37(3), 347-354.

Kačuráková, M., Smith, A. C., Gidley, M. J. and Wilson, R. H. (2002). Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. *Carbohydrate Research*. 337(12), 1145-1153.

Kato, T., Haruki, M., Imanaka, T., Morikawa, M. and Kanaya, S. (2001). Isolation and characterization of psychrotrophic bacteria from oil-reservoir water and oil sands. *Applied Microbiology and Biotechnology*. 55(6), 794-800.

Keshk, S. (2006). Physical properties of bacterial cellulose sheets produced in presence of lignosulfonate. *Enzyme and Microbial Technology*. 40(1), 9-12.

Keshk, S. and Sameshima, K. (2006). Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme and Microbial Technology*. 40(1), 4-8.

Keshk, S. M. (2014a). Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. *Carbohydrate Polymers*. 99, 98-100.

Keshk, S. M., Razek, T. M. and Sameshima, K. (2006). Bacterial cellulose production from beet molasses. *African Journal of Biotechnology*. 5(17), 1519-1523.

Keshk, S. M. A. S. (2014b). Bacterial Cellulose Production and its Industrial Applications. *Journal of Bioprocessing and Biotechniques*. 04(02).

Kim, S.-Y., Kim, J.-N., Wee, Y.-J., Park, D.-H. and Ryu, H.-W. (2006). Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. *Proceedings of the 2006 Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals*: Springer, 705-715.

Kim, Y.-J., Kim, J.-N., Wee, Y.-J., Park, D.-H. and Ryu, H.-W. (2007). Bacterial cellulose production by *Gluconacetobacter* sp. PKY5 in a rotary biofilm contactor. *Applied biochemistry and biotechnology*. 137(1-12), 529.

Klemm, D., Kramer, F., Moritz, S., Lindstrom, T., Ankerfors, M., Gray, D. and Dorris, A. (2011). Nanocelluloses: a new family of nature-based materials. *Angew Chemie International Edition England*. 50(24), 5438-5466.

Klemm, D., Schumann, D., Kramer, F., Heßler, N., Hornung, M., Schmauder, H.-P. and Marsch, S. (2006). Nanocelluloses as Innovative Polymers in Research and Application. *Advanced Polymer Science*. 205, 49-96.

Klemm, D., Schumann, D., Udhardt, U. and Marsch, S. (2001). Bacterial synthesized cellulose—artificial blood vessels for microsurgery. *Progress in Polymer Science*. 26(9), 1561-1603.

Kojima, Y., Tonouchi, N., Tsuchida, T., Yoshinaga, F. and Yamada, Y. (1998). The characterization of acetic acid bacteria efficiently producing bacterial cellulose from sucrose: The proposal of Acetobacter xylinum subsp. nonacetoxidans subsp. nov. *Bioscience, biotechnology, and biochemistry*. 62(1), 185-187.

Kongruang, S. (2008). Bacterial cellulose production by Acetobacter xylinum strains from agricultural waste products. *Applied Biochemistry and Biotechnology*. 148(1-3), 245-256.

Kramer, M. F. and Coen, D. M. (2001). Enzymatic amplification of DNA by PCR: standard procedures and optimization. *Current protocols in toxicology*. A. 3C. 1-A. 3C. 14.

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Goncalves-Miskiewicz, M., Turkiewicz, M. and Bielecki, S. (2002). Factors affecting the yield and properties of bacterial cellulose. *Journal of Industrial Microbiology and Biotechnology*. 29(4), 189-195.

Kuo, C.-H., Chen, J.-H., Liou, B.-K. and Lee, C.-K. (2016). Utilization of acetate buffer to improve bacterial cellulose production by *Gluconacetobacter* xylinus. *Food Hydrocolloids*. 53, 98-103.

Kurosumi, A., Sasaki, C., Yamashita, Y. and Nakamura, Y. (2009). Utilization of various fruit juices as carbon source for production of bacterial cellulose by Acetobacter xylinum NBRC 13693. *Carbohydrate Polymers*. 76(2), 333-335.

Lee, F. Y., Htar, T. T. and Akowuah, G. A. (2015). ATR-FTIR and spectrometric methods for the assay of crocin in commercial saffron spices (Crocus savitus L.). *International Journal of Food Properties*. 18(8), 1773-1783.

Lee, K. Y., Buldum, G., Mantalaris, A. and Bismarck, A. (2014). More than meets the eye in bacterial cellulose: biosynthesis, bioprocessing, and applications in advanced fiber composites. *Macromolecular Bioscience*. 14(1), 10-32.

Lestari, P., Elfrida, N., Suryani, A. and Suryadi, Y. (2014a). Study on the production of bacterial cellulose from Acetobacter xylinum using agro-waste. *Jordan Journal of Biological Sciences*. 7, 75-80.

Lestari, P., Elfrida, N., Suryani, A. and Suryadi, Y. (2014b). Study on the production of bacterial cellulose from Acetobacter xylinum using agro-waste. *Jordan Journal of Biological Sciences*. 7(1), 75-80.

Li, Y., Cui, F., Liu, Z., Xu, Y. and Zhao, H. (2007). Improvement of xylanase production by Penicillium oxalicum ZH-30 using response surface methodology. *Enzyme and Microbial Technology*. 40(5), 1381-1388.

Li, Y., Tian, C., Tian, H., Zhang, J., He, X., Ping, W. and Lei, H. (2012). Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. *Applied microbiology and Biotechnology*. 96(6), 1479-1487.

Li, Z., Wang, L., Hua, J., Jia, S., Zhang, J. and Liu, H. (2015). Production of nano bacterial cellulose from waste water of candied jujube-processing industry using Acetobacter xylinum. *Carbohydrate Polymers*. 120, 115-119.

Lin, N. and Dufresne, A. (2014). Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*. 59, 302-325.

Lin, S.-P., Calvar, I. L., Catchmark, J. M., Liu, J.-R., Demirci, A. and Cheng, K.-C. (2013). Biosynthesis, production and applications of bacterial cellulose. *Cellulose*. 20(5), 2191-2219.

Lowman, K. K. (2017). Bacterial Cellulose as a Potential Bone Graft. *Journal of Undergraduate Materials Research*. 5(May), 49-53.

Lu, Z., Zhang, Y., Chi, Y., Xu, N., Yao, W. and Sun, B. (2011). Effects of alcohols on bacterial cellulose production by Acetobacter xylinum 186. *World Journal of Microbiology and Biotechnology*. 27(10), 2281-2285.

Lynd, L. R., Weimer, P. J., Van Zyl, W. H. and Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*. 66(3), 506-577.

Maneerung, T., Tokura, S. and Rujiravanit, R. (2008). Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate polymers*. 72(1), 43-51.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J. and Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and environmental microbiology*. 64(2), 795-799.

Martinko, J. M. and Madigan, M. (2005). Brock biology of microorganisms. *ed: Englewood Cliffs, NJ: Prentice Hall. ISBN 0-13-144329-1*.

Masaoka, S., Ohe, T. and Sakota, N. (1993). Production of cellulose from glucose by Acetobacter xylinum. *Journal of fermentation and bioengineering*. 75(1), 18-22.

Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O. and Yoshinaga, F. (2014). A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. sucrofermentans. *Bioscience, Biotechnology and Biochemistry*. 60(4), 575-579.

Meftahi, A., Khajavi, R., Rashidi, A., Rahimi, M. and Bahador, A. (2015). Effect of Purification on Nano Microbial Cellulose Pellicle Properties. *Procedia Materials Science*. 11, 206-211.

Merck (2015). Merck Microbiology Manual 12 th edition. *Merck*.

Mohammadkazemi, F., Faria, M. and Cordeiro, N. (2016). In situ biosynthesis of bacterial nanocellulose-CaCO3 hybrid bionanocomposite: One-step process. *Material Science and Engineering C*. 65, 393-399.

Mohammed, M., Salmiaton, A., Azlina, W. W. and Amran, M. M. (2012). Gasification of oil palm empty fruit bunches: a characterization and kinetic study. *Bioresource technology*. 110, 628-636.

Mohite, B. V. and Patil, S. V. (2014). Bacterial cellulose of Gluconoacetobacter hansenii as a potential bioadsorption agent for its green environment applications. *Journal of Biomaterial Science, Polymer Edition*. 25(18), 2053-2065.

Moniri, M., Boroumand Moghaddam, A., Azizi, S., Abdul Rahim, R., Bin Ariff, A., Zuhainis Saad, W., Navaderi, M. and Mohamad, R. (2017). Production and Status of Bacterial Cellulose in Biomedical Engineering. *Nanomaterials*. 7(9), 257.

Moon, R. J., Martini, A., Nairn, J., Simonsen, J. and Youngblood, J. (2011). Cellulose nanomaterials review: structure, properties and nanocomposites. *Chemical Society Reviews*. 40(7), 3941-3994.

Moonmangmee, S., Kawabata, K., Tanaka, S., Toyama, H., Adachi, O. and Matsushita, K. (2002). A novel polysaccharide involved in the pellicle formation of Acetobacter aceti. *Journal of bioscience and bioengineering*. 93(2), 192-200.

Moritz, S., Wiegand, C., Wesarg, F., Hessler, N., Müller, F. A., Kralisch, D., Hipler, U.-C. and Fischer, D. (2014). Active wound dressings based on bacterial nanocellulose as drug delivery system for octenidine. *International journal of pharmaceutics*. 471(1-2), 45-55.

Movasaghi, Z., Rehman, S. and ur Rehman, D. I. (2008). Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Applied Spectroscopy Reviews*. 43(2), 134-179.

Naritomi, T., Kouda, T., and, H. Y. and Yoshinaga, F. (1998). Effect of Ethanol on Bacterial Cellulose Production From Fructose In Continous Culture. *Journal of Fermentation and Bioengineering*. 85(6), 598-603.

Naz, S., Ahmad, N., Akhtar, J., Ahmad, N. M., Ali, A. and Zia, M. (2016). Management of citrus waste by switching in the production of nanocellulose. *IET nanobiotechnology*. 10(6), 395-399.

Nei, M. and Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford university press.

Newman, M. M., Feminella, J. W. and Liles, M. R. (2010). Purification of genomic DNA extracted from environmental sources for use in a polymerase chain reaction. *Cold Spring Harbor Protocols*. 2010(2), 1-5.

Nguyen, V. T., Flanagan, B., Mikkelsen, D., Ramirez, S., Rivas, L., Gidley, M. J. and Dykes, G. A. (2010). Spontaneous mutation results in lower cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. *Carbohydrate Polymers*. 80(2), 337-343.

Nyakuma, B. B. (2015). Thermogravimetric and Kinetic Analysis of Melon (*Citrullus colocynthis L.*) Seed Husk Using the Distributed Activation Energy Model. *Environmental and Climate Technologies*. 15(1), 77-89.

Oh, S. Y., Yoo, D. I., Shin, Y. and Seo, G. (2005). FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate research*. 340(3), 417-428.

Okpokwasili, G. and Nweke, C. (2006). Microbial growth and substrate utilization kinetics. *African Journal of Biotechnology*. 5(4), 305-317.

Olsson, R. T., Samir, M. A., Salazar-Alvarez, G., Belova, L., Ström, V., Berglund, L. A., Ikkala, O., Nogues, J. and Gedde, U. W. (2010). Making flexible magnetic aerogels and stiff magnetic nanopaper using cellulose nanofibrils as templates. *Nature nanotechnology*. 5(8), 584-588.

Oshima, T., Kondo, K., Ohto, K., Inoue, K. and Baba, Y. (2008). Preparation of phosphorylated bacterial cellulose as an adsorbent for metal ions. *Reactive and Functional Polymers*. 68(1), 376-383.

Österberg, M. and Cranston, E. D. (2014). Special issue on nanocellulose. *Nordic Pulp & Paper Research Journal*. 29(1).

Padmanaban, S., Balaji, N., Muthukumaran, C. and Tamilarasan, K. (2015). Statistical optimization of process parameters for exopolysaccharide production by *Aureobasidium pullulans* using sweet potato based medium. *3 Biotechnology*. 5(6), 1067-1073.

Padula, C., Colombo, G., Nicoli, S., Catellani, P. L., Massimo, G. and Santi, P. (2003). Bioadhesive film for the transdermal delivery of lidocaine: *in vitro* and *in vivo* behavior. *Journal of Controlled Release*. 88(2), 277-285.

Panesar, P., Chavan, Y., Bera, M., Chand, O. and Kumar, H. (2009). Evaluation of *Acetobacter* strain for the production of microbial cellulose. *Asian Journal of Chemistry*. 21(10), S099-102.

Panesar, P. S., Chavan, Y., Chopra, H. K. and Kennedy, J. F. (2012). Production of microbial cellulose: Response surface methodology approach. *Carbohydrate Polymers*. 87(1), 930-934.

Park, J. K., Park, Y. H. and Jung, J. Y. (2003). Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. *Biotechnology and Bioprocess Engineering*. 8(2), 83-88.

Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A. and Johnson, D. K. (2010). Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnology for biofuels*. 3(1), 2-10.

Paul Gatenholm, a. D. K. (2010). Bacterial Nanocellulose as a Renewable Materials for Medical Applications. *MRS BULLETIN* 35, 208-213.

Paximada, P., Dimitrakopoulou, E. A., Tsouko, E., Koutinas, A. A., Fasseas, C. and Mandala, I. G. (2016). Structural modification of Bacterial Cellulose fibrils under ultrasonic irradiation. *Carbohydrate Polymers*. 150, 5-12.

Pecoraro, É., Manzani, D., Messaddeq, Y. and Ribeiro, S. J. (2008). Bacterial cellulose from *Glucanacetobacter xylinus*: Preparation, properties and applications *Monomers, Polymers and Composites from Renewable Resources* (pp. 369-383)Elsevier.

Picheth, G. F., Pirich, C. L., Sierakowski, M. R., Woehl, M. A., Sakakibara, C. N., de Souza, C. F., Martin, A. A., da Silva, R. and de Freitas, R. A. (2017). Bacterial cellulose in biomedical applications: A review. *International Journal of Biological Macromolecules*. 104, 97-106.

Pilz, J. (2011). Statistical Design of Experiments (DOE) *International Encyclopedia of Statistical Science* (pp. 1392-1396)Springer.

Posocco, B., Dreussi, E., De Santa, J., Toffoli, G., Abrami, M., Musiani, F., Grassi, M., Farra, R., Tonon, F. and Grassi, G. (2015). Polysaccharides for the delivery of antitumor drugs. *Materials*. 8(5), 2569-2615.

Pötzinger, Y., Kralisch, D. and Fischer, D. (2017). Bacterial nanocellulose: the future of controlled drug delivery? *Therapeutic Ddelivery*. 8(9), 753-761.

Pourramezan, G., Roayaei, A. and Qezelbash, Q. (2009). Production by Acetobacter sp. 4B—2. *Biotechnology*. 8(1), 150-154.

Premjet, S., Premjet, D. and Ohtani, Y. (2007). The effect of ingredients of sugar cane molasses on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245. *Wei Wei Society*. 63(8), 193-199.

Qi, B., Chen, X., Shen, F., Su, Y. and Wan, Y. (2009). Optimization of enzymatic hydrolysis of wheat straw pretreated by alkaline peroxide using response surface methodology. *Industrial & Engineering Chemistry Research*. 48(15), 7346-7353.

Raghunathan, D. (2013). Production of microbial cellulose from the new bacterial strain isolated from temple wash waters. *International Journal od Current Microbiology and Applied Sciences*. 2(12), 275-290.

Rajendhran, J. and Gunasekaran, P. (2011). Microbial phylogeny and diversity: small subunit ribosomal RNA sequence analysis and beyond. *Microbiological Research*. 166(2), 99-110.

Rajwade, J., Paknikar, K. and Kumbhar, J. (2015). Applications of bacterial cellulose and its composites in biomedicine. *Applied Microbiology and Biotechnology*. 99(6), 2491-2511.

Ramana, K., Tomar, A. and Singh, L. (2000). Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. *World Journal of Microbiology and Biotechnology*. 16(3), 245-248.

Ranade, V. V. (1991). Drug delivery systems. 6. Transdermal drug delivery. *The Journal of Clinical Pharmacology*. 31(5), 401-418.

Ranade, V. V. and Cannon, J. B. (2011). *Drug delivery systems*. (Third Edition ed.)CRC press.

Rangaswamy, B., Vanitha, K. and Hungund, B. S. (2015). Microbial cellulose production from bacteria isolated from rotten fruit. *International Journal of Polymer Science*. 2015.

Rani, M. U., Udayasankar, K. and Appaiah, K. A. (2011). Properties of bacterial cellulose produced in grape medium by native isolate *Gluconacetobacter* sp. *Journal of Applied Polymer Science*. 120(5), 2835-2841.

Razak, S. I. A., Anwar Hamzah, M. S., Yee, F. C., Kadir, M. R. A. and Nayan, N. H. M. (2017). A review on medicinal properties of saffron toward major diseases. *Journal of Herbs, Spices and Medicinal Plants*. 23(2), 98-116.

Reiniati, I., Hrymak, A. N. and Margaritis, A. (2017). Recent developments in the production and applications of bacterial cellulose fibers and nanocrystals. *Critical Reviews in Biotechnology*. 37(4), 510-524.

Rodriguez-Chanfrau, J. E., Dos Santos, M. L., Dos Santos, C., Riccardi, G. M. D. O., Hernandez-Escalona, M., Basmaji, P., Veranes-Pantoja, Y. and Guastaldi, A. C. (2017). Chemical Modification of Bacterial Cellulose for use in Regeneration Medicine. *Cellulose Chemistry and Technology* 51(7-8), 673-680.

Römling, U. and Galperin, M. Y. (2015). Bacterial cellulose biosynthesis: diversity of operons, subunits, products, and functions. *Trends in microbiology*. 23(9), 545-557.

Ross, P., Mayer, R. and Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiological Reviews*. 55(1), 35-58.

Ruiz Espejo, M. (2006). *Design of experiments for engineers and scientists*. (Technometrics 2 ed.) London: Taylor and Francis, CRC Presss.

Ruka, D. R., Simon, G. P. and Dean, K. M. (2012). Altering the growth conditions of *Gluconacetobacter xylinus* to maximize the yield of bacterial cellulose. *Carbohydrate Polymers*. 89(2), 613-622.

Safarinejad, M., Shafiei, N. and Safarinejad, S. (2010). An open label, randomized, fixed-dose, crossover study comparing efficacy and safety of sildenafil citrate and saffron *(Crocus sativus Linn*.) for treating erectile dysfunction in men naïve to treatment. *International Journal of Impotence Research*. 22(4), 240.

Safarinejad, M. R., Shafiei, N. and Safarinejad, S. (2011). A prospective double-blind randomized placebo-controlled study of the effect of saffron (*Crocus sativus Linn.*) on semen parameters and seminal plasma antioxidant capacity in infertile men with idiopathic oligoasthenoteratozoospermia. *Phytotherapy Research*. 25(4), 508-516.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4(4), 406-425.

Salama, A., Shoueir, K. R. and Aljohani, H. A. (2017). Preparation of sustainable nanocomposite as new adsorbent for dyes removal. *Fibers and Polymers*. 18(9), 1825-1830.

Sani, A. and Dahman, Y. (2009). Improvements in the production of bacterial synthesized biocellulose nanofibres using different culture methods. *Journal of Chemical Technology and Biotechnology*. 85, 151–164.

Segal, L., Creely, J., Martin Jr, A. and Conrad, C. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal*. 29(10), 786-794.

Shah, N., Ul-Islam, M., Khattak, W. A. and Park, J. K. (2013a). Overview of bacterial cellulose composites: a multipurpose advanced material. *Carbohydrate Polymers*. 98(2), 1585-1598.

Shah, N., Ul-Islam, M., Khattak, W. A. and Park, J. K. (2013b). Overview of bacterial cellulose composites: a multipurpose advanced material. *Carbohydrate polymers*. 98(2), 1585-1598.

Shankar, S. and Rhim, J.-W. (2016). Preparation of nanocellulose from micro-crystalline cellulose: the effect on the performance and properties of agar-based composite films. *Carbohydrate polymers*. 135, 18-26.

Sherif, M. (2005). Evaluation of different carbon sources for bacterial cellulose production. *African Journal of Biotechnology*. 4(6), 478.

Shoda, M. and Sugano, Y. (2005). Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering*. 10(1), 1-8.

Silva, N. H., Rodrigues, A. F., Almeida, I. F., Costa, P. C., Rosado, C., Neto, C. P., Silvestre, A. J. and Freire, C. S. (2014). Bacterial cellulose membranes as transdermal delivery systems for diclofenac: in vitro dissolution and permeation studies. *Carbohydrate polymers*. 106, 264-269.

Silvestre, A. J., Freire, C. S. and Neto, C. P. (2014). Do bacterial cellulose membranes have potential in drug-delivery systems? *Expert Opinion on Drug Delivery*. 11(7), 1113-1124.

Singh, O., Panesar, P. S. and Chopra, H. K. (2017). Isolation and Characterization of cellulose Producing Bacterial isolate from Rotten grapes. *Biosciences Biotechnology Research Asia*. 14(1), 373-380.

Sniadecki, N. J., Desai, R. A., Ruiz, S. A. and Chen, C. S. (2006). Nanotechnology for cell–substrate interactions. *Annals of Biomedical Engineering*. 34(1), 59-74.

Solway, D. R., Consalter, M. and Levinson, D. J. (2010). Microbial cellulose wound dressing in the treatment of skin tears in the frail elderly. *Wounds*. 22(1), 17.

Son, H., Lee, O., Kim, Y. and Lee, S. (2000). Isolation and identification of cellulose-producing bacteria. *Korean Journal of Applied Microbiology and Biotechnology*. 28(3), 134-138.

Son, H. J., Heo, M. S., Kim, Y. G. and Lee, S. J. (2001). Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter*. *Biotechnology and Applied Biochemistry*. 33(1), 1-5.

Song, C., Du, J., Zhao, J., Feng, S., Du, G. and Zhu, Z. (2009). Hierarchical porous core− shell carbon nanoparticles. *Chemistry of Materials*. 21(8), 1524-1530.

Stanbury, P. F., Whitaker, A. and Hall, S. J. (2013). *Principles of Fermentation Technology*. (Second edition ed.) United States: Elsevier.

Stanisławska, A. (2016). Bacterial Nanocellulose as a Microbiological Derived Nanomaterial. *Advances in Materials Science*. 16(4), 45-57.

Sugano, M. S. a. Y. (2005). Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering*. 10, 1-8.

Talaei, A., Moghadam, M. H., Tabassi, S. A. S. and Mohajeri, S. A. (2015). Crocin, the main active saffron constituent, as an adjunctive treatment in major depressive disorder: a randomized, double-blind, placebo-controlled, pilot clinical trial. *Journal of Affective Disorders*. 174, 51-56.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*. 30(12), 2725-2729.

Tang, X. C. and Pikal, M. J. (2004). Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharmaceutical research*. 21(2), 191-200.

Thakur, R., Florek, C., Kohn, J. and Michniak, B. (2008). Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing. *International journal of pharmaceutics*. 364(1), 87-93.

Trovatti, E., Freire, C. S., Pinto, P. C., Almeida, I. F., Costa, P., Silvestre, A. J., Neto, C. P. and Rosado, C. (2012). Bacterial cellulose membranes applied in topical and transdermal delivery of lidocaine hydrochloride and ibuprofen: in vitro diffusion studies. *International Journal of Pharmaceutics*. 435(1), 83-87.

Trovatti, E., Serafim, L. S., Freire, C. S., Silvestre, A. J. and Neto, C. P. (2011a). *Gluconacetobacter* sacchari: an efficient bacterial cellulose cell-factory. *Carbohydrate Polymers*. 86(3), 1417-1420.

Trovatti, E., Silva, N. H., Duarte, I. F., Rosado, C. F., Almeida, I. F., Costa, P., Freire, C. S., Silvestre, A. J. and Neto, C. P. (2011b). Biocellulose membranes as supports for dermal release of lidocaine. *Biomacromolecules*. 12(11), 4162-4168.

Tyagi, N. and Suresh, S. (2012). Isolation and Characterization of Cellulose Producing Bacterial Strain from Orange Pulp. *Advanced Materials Research*. 626, 475-479.

Tyagi, N. and Suresh, S. (2016). Production of cellulose from sugarcane molasses using *Gluconacetobacter* intermedius SNT-1: optimization & characterization. *Journal of Cleaner Production*. 112, 71-80.

Vandamme, E., De Baets, S., Vanbaelen, A., Joris, K. and De Wulf, P. (1998). Improved production of bacterial cellulose and its application potential. *Polymer Degradation and Stability*. 59(1), 93-99.

Vaughn, N. and Polnaszek, C. (2007). Design-Expert® software. *Stat-Ease, Inc, Minneapolis, MN*.

Wahid, Z. and Nadir, N. (2013). Improvement of one factor at a time through design of experiments. *World Applied Sciences Journal*. 21, 56-61.

Wasli, A. S., Salleh, M. M., Abd-Aziz, S., Hassan, O. and Mahadi, N. M. (2009). Medium optimization for chitinase production from *Trichoderma virens* using central composite design. *Biotechnology and Bioprocess Engineering*. 14(6), 781-787.

Watanabe, K., Tabuchi, M., and, Y. M. and Yoshinaga, F. (1998). Structural features and properties of bacterial cellulose produced in agitated culture. *Cellulose*. 5, 187-200.

Wei, B., Yang, G. and Hong, F. (2011). Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties. *Carbohydrate Polymers*. 84(1), 533-538.

Whitcomb, P. J. and Anderson, M. J. (2004). *RSM simplified: optimizing processes using response surface methods for design of experiments*. (2nd ed.)CRC Press

Yamada, Y., Yukphan, P., Vu, H. T. L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S. and Nakagawa, Y. (2012). Description of Komagataeibacter gen. nov., with proposals of new combinations (Acetobacteraceae). *Journal of General and Applied Microbiology*. 58(5), 397-404.

Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M., Mitsuhashi, S., Nishi, Y. and Uryu, M. (1989). The structure and mechanical properties of sheets prepared from bacterial cellulose. *Journal of Materials Science*. 24(9), 3141-3145.

Yang, Y., Jia, J., Xing, J., Chen, J. and Lu, S. (2013). Isolation and characteristics analysis of a novel high bacterial cellulose producing strain *Gluconacetobacter intermedius* CIs26. *Carbohydrate Polymers*. 92(2), 2012-2017.

Yoshinaga, F., Tonouchi, N. and Watanabe, K. (2014). Research Progress in Production of Bacterial Cellulose by Aeration and Agitation Culture and Its Application as a New Industrial Material. *Bioscience, Biotechnology and Biochemistry*. 61(2), 219-224.

Young-Jung, W. (2011). Isolation and characterization of a bacterial cellulose-producing bacterium derived from the persimmon vinegar. *African Journal of Biotechnology*. 10(72), 16267-16276,.

Zhang, H., Chen, C., Zhu, C. and Sun, D. (2016). Production of bacterial cellulose by *Acetobacter xylinum*: Effects of carbon/nitrogen-ration on cell growth and metabolite production. *Cellulose Chemistry and Technology*. 50(9-10), 997-1003.

Zhang, J. and Greasham, R. (1999). Chemically defined media for commercial fermentations. *Applied Microbiology and Biotechnology*. 51(4), 407-421.

Zolgharnein, J., Shahmoradi, A. and Ghasemi, J. B. (2013). Comparative study of Box–Behnken, central composite, and Doehlert matrix for multivariate optimization of Pb (II) adsorption onto Robinia tree leaves. *Journal of Chemometrics*. 27(1-2), 12-20.

Zugenmaier, P. (2001). Conformation and packing of various crystalline cellulose fibers. *Progress in polymer science*. 26(9), 1341-1417.