ABSTRACT

ASHRAFI, ZAHRA. Superhydrophilic-Superoleophobic Membranes for Oil-Water Separation. (Under the direction of Dr. Dr. Wendy Krause and Dr. Lucian Lucia).

Cellulose is one of the most abundant organic substances on the earth. There are two types of pathways to produce cellulose: first way is from plants which requires a lengthy purification process to remove all the undesired components such as lignin, pectin, and hemicellulose, the second way is from the biosynthetic processes of microorganisms such as bacteria.

Although, the molecular formula of cellulose synthesized by bacteria is identical to that made by plants, but the properties of bacterial cellulose are far better than plant cellulose. Bacterial cellulose possesses a unique three-dimensional nonwoven network of nanofibers which is flexible, biocompatible, and highly hydrophilic. It has high porosity, high wet tensile strength, high crystallinity, and high-water absorption capacity. Therefore, the production of cellulose by bacteria could be an interesting alternative for the plant-derived cellulose.

As such, my research project aims to develop a non-fouling, superhydrophilic-superoleophobic membrane for oil-water separations based upon bacterial cellulose (BC) membranes, improving current fluorinated strategies. Our membranes will remove or adsorb water from an oil water mixture/emulsion and helps to isolate and capture the oil fraction.

In this work, a facile and effective method is described to engineer original bacterial cellulose fibrous networks with tunable porosity. We showed that the pore shape, volume, and size distribution of bacterial nanocellulose membranes can be tailored with appropriate culture conditions specifically carbon sources. Pore characterization techniques such as capillary flow
porometry, bubble point method, gas adsorption-desorption technique as well as visualization techniques such as scanning electron and atomic force microscopy were utilized to investigate the morphology and shapes of the pores within the membranes. Engineering the various shape, size and volume characteristics of the pores available in pristine bacterial nanocellulose membranes lead to fabrication and development of eco-friendly materials with required characteristics for a broad range of applications.

Bacterial cellulose has proven to be a remarkably versatile bio-polymer gaining attention in a wide variety of applied scientific applications such as electronics, biomedical devices, and tissue-engineering. However, difficulties to handle and expand a thin layer of BC membrane limit its applications. To address this issue, BC-NW composite membranes with special superoleophobic/superhydrophilic wetting behavior were fabricated. We have demonstrated that the network of BC-NW composite matrix shows powerful water trapping capacity which makes it an ultralow oil-adhesion material. Taking advantage of the underwater superoleophobicity, a BC-NW membrane was successfully used for oil–water separation.

Our water infused system showed an extremely high separation efficiency and anti-oilfouling properties even for separating surfactant-stabilized micro-/nanoemulsions. The simple strategy of the current method for fabricating three-dimensional oil-repellent structures during biosynthesis makes it possible to design BC-NW membranes with desirable geometrics whose potential applications will emerge in a number of avenues such as droplet-based microreactors, microfluidic devices, and bio-detection.
Superhydrophilic-Superoleophobic Membranes for Oil-Water Separation

by
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North Carolina State University
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Doctor of Philosophy

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DEDICATION

To my parents and Majid
BIOGRAPHY

Zahra Ashrafi was born on January 23, 1989 in Esfahan, Iran. In 2007, she was admitted to the Isfahan University of Technology to study Textile Chemistry and Fiber Science Engineering. In 2012, she graduated with her Bachelor of Science degree and started her Master of Science at Amirkabir University of Technology (Tehran Polytechnic). In 2014, she received a Master of Science in Nanofibrous Structures Engineering.

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ACKNOWLEDGMENTS

Over the past few months, I often spent time reflecting back on this journey that started in August 2015, it has been a period of intense learning for me both personally and professionally. Now that I am writing my PhD final dissertation, I am filled with gratitude and countless fond memories and incredibly proud of what I accomplished and thankful to the people who made this possible.

I would like to express my sincere gratitude to Dr. Lucian Lucia and Dr. Wendy Krause who gave me the opportunity to pursue my doctoral research. I will forever be grateful for the advice, coaching, supporting, and encouragement that they provided me. I wish also to thank Prof. Saad Khan and Dr. Tahira Pirzada who helped me to pursue my minor degree in Chemical and Biomolecular Engineering.

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CHAPTER 1: Superhydrophilic-Superoleophobic Membranes for Oil-Water Separation

1. Introduction

Growth in the global population and economies have led to increased oil extraction, transportation, storage, and processing, which increases the risk of oil spill accidents and oceanic pollution. In the United States of America alone, a massive 10 to 25 million gallons of oil is spilled each year.\(^1\) Furthermore, fires, chemical burns, and explosions can result from oil spills, exacerbating oil spill impact and damage on earth.

Oil-contaminated water threatens human health and safety, causing fire and explosion and severe environmental impacts to ecosystems, and compromises agriculture by killing wildlife and plants, destroying habitats and food, and diminishes air and water quality. Therefore, development of an efficient and sustainable method for the separation of oil from various oil polluted water is crucial to decrease the impact and damage of water contamination by oils and grease.

Existing techniques such as oil skimmers,\(^2\) centrifuges,\(^3\) in-situ burning,\(^4\) air flotation,\(^5\) and electrochemical treatment\(^6\) have limited efficiency especially for emulsified oil/water mixtures which are stabilized by surfactants. Furthermore, these approaches of water remediation suffer from high energy consumption, skilled manual operation and complicated instrumental setup, undesirable secondary pollution, and costliness.\(^7\)

Membrane filtration is one of the most promising methods for separating oil/water mixtures.\(^8-11\) These materials are generally inspired by natural superhydrophobic surfaces of plant leaves or
underwater superoleophobic surfaces of fish scales and boost high-pressure endurance, low energy cost, and high separation efficiency.\textsuperscript{8,10,12}

Membranes are not perfect, however, and tend to suffer from poor mechanical strength, low water flux, and fouling by adsorption and/or clogging of pores.\textsuperscript{11,13} Thus far, researchers have studied fabricating membranes from polymers,\textsuperscript{8,10} ceramics,\textsuperscript{9} or by modifying metallic meshes.\textsuperscript{14-18}

To address current filtration membrane issues of selectivity, permeability, and fouling, recent research focus has shifted more toward studying hydrophilic and oleophobic membranes, particularly membranes that exhibit such properties underwater, or underwater filters.\textsuperscript{19-22} In addition of having oleophobic and hydrophilic properties, membranes must exhibit low oil adhesion to overcome oil fouling problem.

As such, our work has aimed to develop a non-fouling, superhydrophobic-superoleophobic membrane for oil-water separations based upon bacterial cellulose (BC) nanofibers, improving upon current fluorinated strategies. Our membranes will remove water from an oil water mixture and help to isolate and capture the oil fraction.

Bacterial cellulose is a unique, nontoxic nonwoven mat consisting of pristine highly crystalline cellulose nanofibers with good mechanical properties. Its ability to be shaped into three-dimensions structures during biosynthesis, provide the basis for a wide range of applications such as medical,\textsuperscript{23-26} electrical,\textsuperscript{27-29} optical,\textsuperscript{30,31} and magnetic.\textsuperscript{32,33}

The specific targets of the project are as follows:
• To synthesize hydrophilic-oleophobic bacterial cellulose pellicles/membranes;
• To evaluate the influence of culture conditions such as carbon sources in order to tailor the morphology and water passing properties of the prepared BC pellicles;
• To investigate and evaluate the physiochemical properties and the performance level of engineered BC membrane for oil-water filtration;
• To develop and evaluate composite membranes based on bacterial nanocellulose in order to improve filtration performance such as selectivity and permeability.

2. Background

2.1 Special Wetting Membranes

In general, two types of membranes with special wettability properties, having opposite affinities toward water and oil, are appropriate for oil/water filtration: (1) oil-removing membranes which are hydrophobic/oleophilic and separate oil from oil/water mixtures by selective oil permeation, and (2) water-removing membranes which are hydrophilic/oleophobic and separate water by repelling oil while allowing water to penetrate through the membrane.

A material must have a water contact angle above 90° to be considered hydrophobic, while above 150° to be superhydrophobic. Materials with water contact angles less than 90° are hydrophilic. The same logic is applied to materials tested with oil contact angles. Materials with oil contact angles above 90° are oleophobic, oil contact angles above 150° are superoleophobic, and oil contact angles below 90° are oleophilic.
These wettability properties arise from surface chemistry/energetics and/or surface roughness. Unless the material has amphiphilic or amphiphatic wettability, exhibiting both hydrophilic and hydrophobic properties, materials are hydrophobic and oleophilic or hydrophilic and oleophobic. To achieve high oil/water separation, membranes should be at the extreme ends of wettability, namely superhydrophobic and superoleophilic or superhydrophilic and superoleophobic, as demonstrated in Figure 1.1.

![Image of Oil/Water Mixture](oil_and_water_mixture.png)

**Figure 1.1.** Special wetting membranes with extreme opposite affinities towards water and oil.

**Table 1.1.** Oil removing and water removing membranes characteristics.

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Water contact angle (WCA)</th>
<th>Oil contact angle (OCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil removing</td>
<td>higher than 150° (superhydrophobic)</td>
<td>close to 0° (superoleophilic)</td>
</tr>
<tr>
<td>water removing</td>
<td>close to 0° (superhydrophilic)</td>
<td>higher than 150° (superoleophobic)</td>
</tr>
</tbody>
</table>
It is important to note that fabricating superoleophobic surfaces is very challenging. Traditional methods for the creation of superoleophobic surfaces possessing high oil contact angle and low hysteresis rely highly on utilizing fluorinated compounds.\textsuperscript{34-36}

In 2009, for the first time, Liu et al\textsuperscript{37} introduced wetting/antiwetting behavior of oil droplets on the surface of fish skin in water. In fact, underwater superoleophobicity is a result of water penetration into the hydrophilic surface structure of the scales made of calcium phosphate and protein and ensuing formation of a third repulsive liquid phase. Furthermore, the presence of a thin layer of mucus on the skin surface of fish creates a micro/nano hierarchical roughness and thus decreases contact or adhesion of low surface tension liquids such as oil on the surface of the membrane.\textsuperscript{34,37}

Inspired by this finding, they proposed a novel fluoride-free strategy to create a superoleophobic and low oil-adhesive interface.

Herein, we investigated the bacterial nanocellulose membranes as a promising candidate for oil/water separation. Bacterial nanocellulose matrices are hydrophilic and possess unique fibrous structure with large surface area which enables them to hold a large amount of water. These properties make them beneficial porous nonwoven materials for passing through of water, whereas prevent oil-wetting and consequent fouling.

\textbf{2.2 Bacterial Cellulose}

Cellulose is one of the most abundant organic substances on the earth. There are two types of pathways to produce cellulose as shown in Figure 1.2. The first way is from plants. However, to
obtain neat cellulose from plant, a lengthy purification process is required to remove all the undesired components such as lignin, pectin, and hemicellulose. The second way is from the biosynthetic processes of microorganisms such as algae, fungi, and bacteria.\textsuperscript{38}


![Diagram showing two pathways to cellulose, one from plants and another from microorganisms.]

**Figure 1.2.** Two different pathways to the cellulose

Although the molecular formula of cellulose synthesized by bacteria is identical to that made by plants, the properties of bacterial cellulose are far better than plant cellulose. Bacterial cellulose possesses a unique three-dimensional nonwoven network of nanofibers which is flexible, biocompatible, and highly hydrophilic. It displays high porosity, high wet tensile strength, high crystallinity, and water absorption capacity > 99% of its total weight.\textsuperscript{38,39} Therefore, the production of cellulose by bacteria could be an interesting alternative to plant-derived cellulose.
Bacterial cellulose has found applications in fields such as paper products (e.g., audio speaker diaphragms by Sony Corporation, in conjunction with Ajinomoto), flexible electronics, supercapacitors, cosmetics, and soft tissue engineering. Many authors have reviewed fabrication, synthesis, and applications of bacterial cellulose in for example, composite preparations because of their good mechanical properties, biomedical applications due to their non-toxicity, biodegradability and biocompatibility, functionalization, and the biosynthesis of bacterial cellulose.

Bacterial cellulose products are commercially available for biomedical applications, but mostly as topical coverings for severe wounds under the brand names of Bioprocess®, XCell®, and Biofill® whereas for cosmetic use under the name NanoMasque®. Studies have shown BC to be superior to conventional wound dressings in terms of accelerating healing times and alleviating pain because it is non-allergenic and a biomimetic to human skin. It also displays high water content (98-99%), good sorption of liquids, and can be safely sterilized without any change to its characteristics.

2.3 Biosynthesis of Bacterial Cellulose

Several species of bacteria have an ability to extracellularly secrete cellulose nanofibers mat with unique properties. The most important of these is Acetobacter xylinum, now renamed Gluconacetobacter xylinus, discovered in 1886 by A. J. Brown. In nature G. xylinum is found in soil, unpasteurized or unsterilized juice, beer, and wine and is commonly found on the surfaces of rotting fallen fruits that have fixed carbon in the form of sugars or alcohol. Micrographs of the surface of the bacterial cell indicate the presence of tens of cellulose-synthesizing sites arranged along the longitude of the cell. These small pore-like sites produce
10–15 cellulose chains and extrude them into the culture medium to associate with each other forming the so-called sub-fibrils with a lateral width of about 1.5 nm. These sub-fibrils aggregate to form microfibrils 3–6 nm in width, and the resulting microfibrils are further self-assembled to form fibrillar ribbon assembly with a lateral width of 40–60 nm, which are ~ 200 times finer than cotton fibers with a remarkable high surface area.

There are different theories why *G. xylinus* species produce nanocellulose networks. The cellulose membrane is believed to protect bacteria from dehydration and helps them to float which assist them to reach the air-liquid interface for access to more oxygen. It is also hypothesized that bacteria produce cellulose for protection from ultraviolet radiation and harsh chemical environments. However, these are all hypothesis and many unanswered questions remain for the reasons behind the synthesis of bacterial nanocellulose.

**Figure 1.** The details arrangement of fibrils extruded from a bacteria cell surface and their self-assembly process.
2.4 Cultivation Conditions

*G. xylinus* is a strict aerobic rod-shaped bacterium which has the ability to produce a mat-like pellicle on the interface of air-liquid mediums in stationary cultures. In shaking cultures, *G. xylinus* produces cellulose in the form of spherical or star-shaped structures.23

A typical culture medium, which is widely used for the fermentation of *G. xylinus* to synthesize cellulose, contains a carbon source (sugars, mostly glucose and sucrose), a nitrogen source, and other compounds required for bacteria growth.

The growth media components have a great impact on bacterial nanocellulose production in terms of cellulose production rate, yield, and network structure. The productivity of the BC membrane can be maximized depending on the type of nitrogen and carbon sources used in the culture medium.48 In recent years, various nitrogen and carbon sources have been used to achieve high bacterial cellulose production at low cost. Researchers demonstrated that among the various nitrogen sources added to the HS medium, peptone is found to be the most effective.50

The type of carbon has also been shown to affect the rate of cellulose synthesis and fibrillar network of BC membranes.
Figure 1.4. Schematic illustration of *Gluconacetobacter* biosynthesis nanocellulose in a static cultivation method.

2.5 Chemical and Physical Properties of BC

Bacterial cellulose nanofiber is composed of linear β-1, 4-glucan chains connected by virtue of hydrogen bonds. It is free of contaminant molecules, such as lignin, hemicelluloses, and pectin, etc., which are normally found in plant-derived cellulose.

By using alkaline solution, non-cellulose materials such as proteins and nucleic acids from bacterial cells and the culture broth can be removed from the BC membrane, allowing the formation of strong inter- and intra-chain hydrogen bonds which also improve the modulus of these membranes.
The degree of polymerization (DP) of BC ranges from 300 to 10,000 depending on the cultivation conditions, various additives, and finally the bacterial strains.\textsuperscript{51} Depending on how the chains are assembled, crystalline and nanocrystalline region exist. The crystallinity of BC is up to 84\textendash89\% due to the arrangement of the pre-microfibril aggregation.\textsuperscript{52}

The crystalline structure is one of the most important factors controlling the mechanical and interfacial properties of the cellulose. Cellulose derived from different resources has two crystal allomorphs: cellulose $I_\alpha$ and cellulose $I_\beta$, that correspond, respectively, to triclinic and monoclinic unit cells. It has been purported that most plant cellulose is $I_\beta$ -rich, while BC is found to be $I_\alpha$ -rich.\textsuperscript{50} Cellulose $I_\alpha$ is characterized as a triclinic unit with single chains that form water-insoluble crystalline microfibrils, whereas cellulose $I_\beta$ has monoclinic unit cells with two parallel chains.

Due to its unique structure, BC membrane shows mechanical anisotropy, with a high tensile strength in the range of 200\textendash300 MPa and Young’s modulus in the range 15\textendash35 GPa along the fiber layer direction, but a low compressive modulus (0.007 MPa) perpendicular to the layered direction.\textsuperscript{48,53}

The network of cellulose nanofibrils shows great ability to be shaped into three-dimensional structures during biosynthesis. Cultivation can be done in the molds of different sizes and shapes, which makes it possible to design membranes with desirable geometries.
Figure 1.5. Illustration of a bacterial nanocellulose membrane from different angles and corresponding microscopic images (scale bar 10 µm); top) FESEM image from top surface; bottom) CRYO-SEM from the cross-section.
3. Outline of the Thesis

The thesis is organized in series of chapter as described below.

Chapter 1 provides a general description of the project. The novelty and importance of application of an environmentally friendly, renewable bacterial cellulose matrices for filtration application is discussed.

Moreover, the superiority of membrane filtrations over other methods is reviewed and different types of membranes that are suitable to be used for oil/water separation along with required interfacial properties are introduced. Finally, a short but complete introduction on BC membranes including bacterial nanocellulose biosynthesis, structure, and cultivation conditions as well as physical and chemical properties are given.

Chapter 2 provides a complete review on nature-inspired liquid infused systems as a superwettable interface. Discussions are applicable to the superior special wettability properties of BC membranes which fulfill fundamental and practical application requirements for oil/water separation. The first section introduces the evolution in the field of wettability that begins in 1804 and is ongoing today. Then, it will discuss how the concept of the oil repelling action of fish scales inspired research on inducing wettability in oil/water/solid tri-phases and how this biomimetic approach inspired the concept of liquid-infused surfaces less than a decade ago. Then, it focuses on principles to design and fabricate liquid infused materials and will discuss wettability of a liquid/liquid/solid tri-phase and air/liquid/liquid/solid tetra-phase systems. It will also explain in detail that how the liquid infused technology can address some of the intrinsic limitations of repellent surfaces inspired by the lotus effect.
In the next section, we review a potentially promising approach for making liquid infused interfaces which involves incorporation of a lubricating liquid into gels (hydrogels, organogels, and organohydrogels) as robust repelling substrates is reported. Then, the super-wettability and super-spreading properties of gels and other liquid infused surfaces are discussed as greatly promising for the fabrication of ultrathin solid films with controlled thicknesses. Liquid infused surfaces with an anisotropic wetting property as a new design in the field of surface engineering is the main point of discussion of this section.

Liquid infused anisotropic surfaces can be achieved through imposing gradients in the chemical and structural features of the surface which account for differences in the energy barrier of wetting on patterned surfaces which can induce a self-running motion of liquids without any other forces applied. Afterward, some successful application examples of liquid-infused materials are discussed such as liquid repellency for a wide range of liquids, immiscible liquid separation, solid repellency such as ice, insects, and dust, anti-bioadhesion, and droplets manipulation and collections. The final section explains several unanswered yet fundamental questions that need to be addressed to carefully design an optimal non-wetting liquid infused interface.

Chapter 3 discusses that among two different types of oil/water filters hydrophobic-oleophilic materials are more straightforward to make however, they face some critical limitations for separating oil-water mixtures. On the other hand, from surface tension stand point, it is very challenging to fabricate hydrophilic-oleophobic membranes although they are more desirable for separation applications. Lastly, it shows that water infused nanocellulosic membranes that bacteria produce in the static cultivation method, have superhydrophilicity and
superoleophobicity with ultralow oil-adhesion properties. The role of hydroxyl groups on the backbone of cellulose polymer chains and the porous microstructure on the surface wettability and trapping capacity of the membrane are discussed comprehensively. Moreover, the oil/water separation performance of the BC membrane along with its nature-inspired design principles is examined.

Chapter 4 tries to address the current issues of BC membranes for passing water faster. Thus, a facile and effective method is described to architecture bacterial cellulose fibrous network with tunable porosity. It demonstrates that the culture conditions, specifically applying different types of carbon sources and post treatment process, can be employed to tune the pore shapes and size distributions, fluid permeability, and overall porosity and morphology of bacterial nanocellulose matrices at the macro and micro scales.

Chapter 5 introduces a facile and cost-effective method to fabricate a filter based on composite of a nonwoven mat and bacterial cellulose (BC) membrane for highly effective separation of micro-/nanoemulsions with strong stability under harsh environment. In this chapter, it is demonstrated that the employment of the reinforcement nonwoven mat plays no role in the separation itself, but as a supporting scaffold to facilitate fabrication and carrying a smooth, uniform, and ultrathin BC membrane which can efficiently serve in separating oil phase even from nanoemulsions.

Chapter 6 revisits the main objectives of the project to summarize all the results and findings from each experiment. In addition, it provides insight into the fruitful field of bacterial nanocellulose matrices as promising liquid impregnated materials for a wide range of applications and offers a fresh perspective for future research.
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CHAPTER 2: Nature-inspired Liquid Infused Systems for Superwettable Surface Energies

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1. Abstract

The development of an innovative interfacial wetting strategy known as liquid infused surfaces offers great promise for the advanced design of super-wetting and super-antiwetting substrates to overcome the drawbacks of textured surfaces classified under the heading of Cassie/Wenzel states. The potential value of nature-inspired surfaces has significant potential to address scientific and technological challenges in the field of interfacial chemistry. The objective of the current review is to provide insight into a fruitful and young field of research, highlight its historical developments, examine its nature-inspired design principles, gauge recent progress in emerging applications, and offer a fresh perspective for future research.

2. Background

Many phenomena dealing with wettability in nature, such as the water-resistance properties of duck feathers,\textsuperscript{1,2} the self-cleaning effects of lotus leaves and cicada wings,\textsuperscript{3,4} the anisotropic surfaces of the rice leaf and filefish skin,\textsuperscript{5,6} and the legs of water strider,\textsuperscript{7} inspire efforts to uncover the mechanisms of the special wettability on these surfaces. Such efforts are of paramount significance because potential solutions have vast implications for resolving a wide variety of real-world challenges.

“Wetting”, as a technical term for the current review, is limited to the wetting of solids by liquids that represents an intrinsic property of a solid surface under the control of intermolecular
adhesive interactions between the two phases. Wetting/wettability as an interdisciplinary area covering numerous fields of science and technology has received significant attention for over two hundred years, but has gained extensive attraction very recently. Young was a pioneer who in 1805 promoted the concept of “contact angle”, the angular displacement equilibrium behavior of a droplet on a flat and perfectly smooth surface.

What is now understood as surface tension was readily apparent to a genius such as Young; however, physico-chemical interactions between atoms or molecules would not come to light until the following century. In the late 1800s, the nascent discipline of thermodynamics saw wetting from the perspective of two-dimensional surfaces and interfacial energetics. In 1932, Langmuir received the Nobel Prize in Chemistry for helping to pioneer the field of “surface chemistry” while his work on the concept of absorbed monolayers describing a surface supported a two-dimensional perspective in which contact angles were controlled by interfacial free energies of solid surfaces.

Discovery of low-surface-energy polymers such as fluoropolymers (e.g., polytetrafluoroethylene; PTFE) and silicones (e.g., polydimethylsiloxane; PDMS) allowed for the development of surfaces with low water wettability. In early work, Coghill and Anderson found that deposition of stearic acid, a waxy solid, on the rough surface of galena, a natural mineral form of lead (II) sulfide, leads to large water contact angles. However, chemical modification has limitations on surface wettability; for example, although fluorine is an effectively small atom that possesses one of the lowest free energies and highest hydrophobicities, Nishino et al. determined that a flat -CF₃-packed surface only achieves a maximum water contact angle of 120°.
Later developments lead to a deeper understanding of the critical factors controlling wettability such as surface topographic structure. In 1936, Wenzel\textsuperscript{15} explained how the roughness of a solid surface enhances apparent water contact angle and hydrophobicity. The paper titled \textit{Wettability of Porous Surfaces} in 1944 by Cassie and Baxter\textsuperscript{16} introduced the “composite wetting model”, a theory of the wettability of porous surfaces, in which drops rest on an interface whose energetics are a composite of the roughness features and trapped air. While water contact angle has commonly been used as a criterion for the evaluation of surface hydrophobicity, this alone is insufficient to assess adhesion of a water droplet.\textsuperscript{17} In fact, on most surfaces, due to the chemical heterogeneities and/or roughness, contact angle hysteresis is observed.\textsuperscript{18-21}

Contact angle hysteresis (CAH), which is defined as the difference between advancing and receding contact angles, can be employed to evaluate liquid-solid adhesion. This difference is a function of the presence of multiple equilibrium positions in which liquid contact takes place on a non-homogenous and rough surface. As a result, a spectrum of possible contact angles are obtained ranging from a minimum value called the receding contact angle (RCA) to a maximum value called the advancing contact angle (ACA).\textsuperscript{24,28} Specifically, contrary to the lotus leaf effect that shows superhydrophobicity properties with very low adhesion forces and low CAH toward water droplets, rose petals exhibit superhydrophobicity and high adhesive forces with water. In the rose petal example, water cannot roll off when the surface is tilted at any angle or even turned upside down because of low contact angle hysteresis.\textsuperscript{23-25}

These theoretical insights into wetting of non-ideal surfaces are very illuminating and still applicable after more than a century. These theories are briefly described in Table 2. 1.
The famous “lotus effect” phenomena familiar to most surface scientists was understood in detail in 1948.³ Approximately twenty years ago, the effect finally was able to be visualized, uncovering the basis for the self-cleaning properties of the lotus leaf.³ This finding prompted a flood of biomimetic approaches for the design of microstructure-based superhydrophobic materials.¹¹,¹⁷,²⁷-³¹ A time-line of several major milestones in the area of repellent surfaces is depicted in Figure 2.1.
Table 2.1. Main theories in the field of wetting.

<table>
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<tr>
<th>Theory</th>
<th>Equation</th>
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| Young’s equation            | \[
\cos \theta = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}}
\]                                                                               | The contact angle (θ) of a liquid drop on an ideal (smooth and chemically homogeneous) solid surface, defined by the mechanical equilibrium of the interfacial energies between the solid–liquid (γ_{SL}), solid–vapor (γ_{SV}), and liquid–vapor (γ_{LV}) interfaces can be obtained from Young equation. |
| Wenzel’s equation           | \[
\cos \theta^* = r \cos \theta
\]                                                                                         | Wetting states on rough surfaces are mainly described by two models: first, is the Wenzel model in which liquid penetrates inside the rough surface at the point of contact, and the area of the drop’s solid–liquid interface is enlarged by a factor r; second, is the Cassie-Baxter model for the composite interface, in which liquid sits on top of the roughness features, with trapped air pockets. Here, φ_{s} is the area fractions of the solid on the surface and the liquid–solid contact area is smaller than that in Wenzel. An intermediate state between Wenzel state with high adhesion and Cassie-Baxter state with low adhesion on a same textured surface may also occur when a droplet partially wets a textured surface under external stimuli such as observed by a pushing force. In this case, the drop sinks inside the texture towards a more stable impaled state and displays a strong pinning of the contact line on the surface texture. |
| Cassie-Baxter equation      | \[
\cos \theta^* = -1 + \phi_{s}[\cos \theta + 1]
\]                                                                                       |                                                                                                                                                                                                          |
In 2002, Jiang et al.\textsuperscript{32} pointed out that the cooperation of micro- and nanoscale two-tier structures and the arrangement of the microstructures on the surface are key structural aspects of lotus and rice leaves that both induce a large apparent contact angle and low adhesion. They demonstrated that removing the nanostructures using heat treatment while keeping the microscale parts intact, results in a water droplet pinned onto the microstructure of the surfaces at any sliding angle.

The role of micro-/nanoscale two-tier structures in superhydrophobicity has also been discussed by Robin et al.\textsuperscript{33} and Koch et al.\textsuperscript{34} These findings sparked extensive developmental work that attempted to replicate the surface characteristics of natural examples, which allowed super-antiwetting surfaces fabrication from a wide range of materials (i.e., polymers, metals, and inorganic materials such as ceramic and glass) with more robust repellency, resisting even organic liquids with low-surface-energy that would completely wet typical microstructured surfaces.\textsuperscript{35-37}

One of the most demanding extreme wetting states is superoleophobicity in which the surface repels oil droplets. Due to the low surface tension of most oils, design and fabrication of superoleophobic surfaces is very challenging.\textsuperscript{38,39} In 2009, the concept of the oil repelling action of fish scales inspired research on inducing wettability among oil/water/solid tri-phases. An interesting phenomenon that fish and clamshells can engage in is keeping their body clean in oil-polluted water; this approach inspired researchers to study and innovate strategies for fabricating underwater superoleophobic surfaces.\textsuperscript{40,41}

Liu et al.\textsuperscript{40} measured oil contact angle of fish scales both in air and in water and showed that superoleophilic properties (complete wetting) of scales in the water/air/solid system became
superoleophobic properties (oil contact angle of 156.4±3.0°) in an oil/water/solid system by substituting the air phase with water phase. This oil repellent property results from micro- and nano-structures and high-water content of the hydrogel layers of the fish skin.

In attempts to identify the reasons for such behavior, Waghmare et al. quantified the contact angle of silicon oil on fish scales submerged in water with and without a mucus layer by using a needle-free drop deposition technique coupled with illumination to decipher the drop contact area. This study highlighted the importance of the mucus layer formation on fish scales as part of its defense mechanism.

Unprecedented oil contact angles of ~180° were observed for underwater fish scales containing the mucus layer possessing specific chemical functional groups. However, in the absence of this mucus layer, the angle reduced to 150°, which could be attributed to the hierarchical micro- and nano-structures on the fish scales.

Inspired by this biomimetic approach, the concept of slippery liquid-infused porous surfaces (SLIPS) was introduced in 2011 in which a surface is infiltrated with a lubricating liquid, a process that provides a straightforward and promising solution for exceptional liquid super-wettability and super-antiwettability properties.43

This review therefore focuses on nature-inspired principles used to design liquid infused materials. Furthermore, emerging applications of these materials according to recent advances in this young field of research will be highlighted; finally, it will give perspectives on future research developments.
Figure 2.1. Timeline of evolution in the field of wettability that began in 1804 and ongoing today. The micrographs of a lotus leaf are reproduced with permission from REF 31, Royal Society of Chemistry. The micrographs of fish scales are from REF 40.
3. Liquid Infused Interfacial Materials

Biomimetics deal with revealing the mechanism of the wetting phenomena on the surfaces of plants or animals and has consequently sparked extensive developments of artificial superwettability systems. A total of 64 wetting states have been introduced over the past two decades that have been described in detail by Jiang and colleagues. Among these extreme wetting states, artificial superhydrophobic materials in a water/solid/air tri-phase system (water wettability on a solid surface in air) have been an area of intensive exploration.

Despite several decades of intense research that has resulted in the fabrication of complex liquid-repellent micro-/nanostructured surfaces in the Cassie state, relying on reductions in surface energy and enhancing surface roughness, these surfaces still show limitations to treat real life challenges due to their low durability.

Following a Cassie state strategy, fabricating surfaces that are repellent to low surface tension liquids such as oils are much harder to achieve due to the low surface tension and polarity of the liquids themselves. To address this deficiency, broad attention has been drawn to the wettability of a liquid/liquid/solid tri-phase and air/liquid/liquid/solid tetra-phase systems.

Inspired by the antiwetting behavior of oil droplets on fish skin and Pitcher plants of the genus *Nepenthes*, liquid infused surfaces (LIS) as an interfacial wetting concept was introduced. This strategy is based on infusing a barrier liquid phase into a porous solid surface to make it slippery and repellent toward a liquid of differing polarity. For example, Pitcher plant leaves offer a remarkably simple alternative strategy to design liquid-repellent surfaces. Unlike the lotus strategy that depends on micro- and nanoroughness to exhibit antiwetting properties, *Nepenthes*
Pitcher special leaves use surface microtexture to entrap an immiscible liquid (water) that acts as an effective continuous repellent layer. This wet and slippery surface causes a “hydrophobic” insect to slip and fall into the pitcher plant by repelling the oils on their feet, making escape nearly impossible. The prey-capture effectiveness of the leaf’s surface is further enhanced in moist environments (Figure 2.2).48-50

In the process of designing liquid infused substrates, two critical criteria must be fulfilled: 1) The surface energy of the lubricating liquid and impregnated substrate should be well matched in order to create a durable/stable system. 2) The lubricant and external targeted liquids must be immiscible.

The liquid infused technology can address some of the intrinsic limitations of repellent surfaces inspired by the Cassie state. First, air pockets trapped within the surface texture of the Cassie state can act as vulnerable and fragile spots under external constraints over time. Poetes et al. took a more realistic approach to investigate the lifetime of superhydrophobic surfaces in an underwater application. They observed that these surfaces are prone to a rapid irreversible decay and are therefore not thermodynamically and mechanically stable in underwater experiments.51 However, the lubricating liquid surface is inherently smooth and defect-free.

Second, in general, air is more compressible compared to liquids. Therefore, trapped air cannot withstand high pressures as effectively as the liquid-in-liquid infused materials. In other words, the intensity at which the liquid pressure threshold on the surface can sustain without being impaled by the external liquid is much higher for liquid infused materials. Other than the action of external forces, increases in pressure can occur for several other reasons such as the existence of surface defects due to mechanical damage or fabrication imperfections.
Moreover, the smooth surface properties of the caged liquid layer at the molecular scale shows small CAH even under large external applied forces, which is not the case in the Cassie state where the trapped air acts an ineffective cushion against impalement, resulting in liquid penetration into the texture and a significant accompanying CAH increases.

Finally, most of the liquid-repellent surfaces are effective only for high-surface-tension liquids such as neat water, whereas liquid impregnated materials repel immiscible liquids of virtually any surface tension. There is strong interest in designing surfaces that repel low surface tension liquids, such as organic liquids, and complex fluids, such as crude oil or blood for high performance clothes, screens in electronic devices, optical instruments, microfluid handling, and so forth.52,53

Wong et al., who first used the term “SLIPS” (slippery liquid-infused porous surfaces) discussed that contrary to other surface antiwetting models which show no self-healing ability, a lubricating film in SLIPS serves as a self-healing coating by simply flowing towards any damaged areas due to surface-energy-driven capillary action. Wong and his group also pioneered the designing of impregnating nano-/micro-structured surfaces by incorporating lubricating liquids to repel other immiscible liquids with a broad range of surface tensions.43,54
Figure 2. a) Nepenthes pitcher. b) Waxy inner pitcher wall. c) Schematic of a liquid infused system. d) Filefish N. septentrionalis. e) a side-view and f) a top-view filefish skin with hook-like microspines. Pitcher image is obtained with permission from REF.55 SEM image of the slippery zone of pitcher plant is reproduced from.48 Filefish pictures and corresponding SEM images are obtained with permission from REF.6

4. Porous Templates

To fabricate a liquid infused system, a porous template needs to be designed well in order to confine the impregnating liquid. 2D and 3D porous templates can be constructed through bottom-up or top-down approaches. Bottom up techniques such as electrospinning, self-assembly, layer-by-layer, sol-gel, etc., are based on the arrangement and orientation of 0D and 1D materials as basic building blocks.
The top–down techniques are based on micro-/nano-structured templates constructed by several techniques such as etching,\textsuperscript{57} lithography,\textsuperscript{43,58-60} laser, plasma, direct deposition, chemical treatments, and electrochemical anodizing.\textsuperscript{61}

For example, Huang et al. fabricated tunable underwater superoeophobic films by assembling hydrophilic spherical and non-spherical latex particles, both cauliflower-like and single-cavity. The underwater oleophobicity of the films is mainly attributed to the formation of a water-lubricant layer on the hydrophilic latex particles shell surface after the film is immersed in water. The water contact angle of the film assembled from spherical latex particles was highest followed by the cauliflower-like and single-cavity.\textsuperscript{62}
Figure 2.3. (top row) TEM images of a) spherical, b) cauliflower-like, and c) single-cavity latex particles. The inset pictures are 3D schematic image of the latex particles. (middle and bottom row) schematic drawing of contact states of an oil droplet (L1) on a surface assembled from each correspondent particles in water media (L2). S represents solid particles and the pale blue around each latex particle represents the hydrophilic polyacrylic acid shell that forms after the film is immersed in water. The figures are from REF 62.
In an accompanying effort, Wang et al. prepared anodized alumina surfaces composed of innumerable nanopores which were designed to serve as nano-reservoirs to hold low surface tension liquids (perfluorooctyl acid). After filling up the reservoirs with the assistance of vacuum pumping, substrates displayed superamphiphobicity with contact angles higher than 150° for water, glycerol, CH₂I₂, hexadecane, and rapeseed oil.

When the coating liquid on the top surface is damaged or depleted, low surface energy liquids trapped in the nanoreservoirs move to the outmost surface, a phenomenon that is thermo-dynamically driven. This study provided a proof-of-concept example of designing liquid impregnated surfaces that are capable of intrinsic healing.⁴⁶

Particles can be designed to have micro, nano, or hierarchical micro/nano roughness on their surfaces. Enhancing surface area by introducing hierarchical textures to the surface increases repellency through more efficient hosting of repellent liquids. Three different possible 3D liquid-impregnated surfaces which can be formed through the arrangement and orientation of 0D simple particles are shown in Figure 2.4.
**Figure 2.4.** Possible 3D liquid-impregnated surfaces by arrangement and orientation of 0D simple spherical particles. a) Particles serve to simply hold the infused liquid in place b) Particles partially or totally absorb infused liquid in their structure c) empty half spherical particles act as reservoirs for trapping liquids in their hollow structure.
5. Liquid Dispersed Gels

In contrast with post-impregnation of the porous matrix with lubricants, another potentially promising approach is utilizing liquid dispersed gels as robust repelling substrates.\textsuperscript{63} Gels fundamentally consist of a liquid medium dispersed in a solid 3D cross-linked network assimilated via physical or chemical interactions. Gels are known as a class of soft and flexible materials that can entrap high liquids contents of up to 99\% of their dry weight. Owing to their distinctive liquid sorption and retention capacity, they have attracted extensive attention for the development of super-wetting and super-antiwetting surfaces.

If the dispersion phase is water, it is a hydrogel; similarly, if it is composed of liquid organic phase, it is an organogel. There are also organohydrogels which concurrently employ water and oil as the dispersion medium and quickly adapt their hydrogel or organogel properties in response to the surrounding liquid phase.

For instance, in an underwater situation, the hydrogel component becomes dominant and the surface of the organohydrogel become superoleophobic. However, when submerged in an oil-based environment, the surface of the organohydrogel gradually became more organogel-like and displays superhydrophobic properties. The anisotropic hetero-networks of organohydrogels restrict their volume change considerably contrary to the volume changes typically observed in homogeneous networks of organogels or hydrogels.
Figure 2.5. Representative hydrogel, organogel, and organohydrogel systems formed from hydrophilic, oleophilic, hydrophilic, and oleophilic composite networks, respectively.

The super-wettability and super-spreading properties of gels and other liquid infused surfaces show great promise for the fabrication of ultrathin solid films with controlled thicknesses. The entrapped liquid in these surfaces may act as a precursor film facilitating complete and quick spreading of the liquids over the surface by boosting the advancement of a three-phase contact line, while the defects or impurities on a solid surface can induce local pinning thus restricting infinite spreading of liquids. Following this strategy, a variety of versatile functional multilayer/composite thin films for various potential applications can be advanced including self-cleaning, controllable liquid transport, antifogging, liquid separation, and so forth.\textsuperscript{64-66}

Meanwhile, the extreme liquid repellency and low adhesion properties of liquid-infused gel surfaces can be exploited for the synthesis of supra-particles by evaporation of sessile drops containing nano- or microcolloids. This novel energy efficient approach facilitates fabrication process of supra-particles with various size and compositions which can be used in as self-propelling particles, small-scale magnetic stirrer, storage materials and catalysts.\textsuperscript{67,68}

Such liquid layers can be converted into various functional thin polymer films with adjustable thicknesses (nm- to mm-) through one-step polymerization of the reactants. The current strategy
offers opportunities for large-scale synthesis of versatile functional thin films for wide range of applications such as protection, antifogging and antireflection coating lenses and windows, or in nano-fluidics chip technologies as precursor films for spreading sub-micron scale drops.65,66
Figure 2. 6. Schematic for supra-particle formation on a superlyophobic liquid infused surface. Due to the high liquid repellency, the shape of the evaporating drop remains spherical throughout the process (left). A thin layer/multilayer/composite solid film formation on a liquid infused superlyophilic surface confined in a two-dimensional space. The rapid spreading of the liquids over the surface enables generating an ultrathin uniform film (right).
6. **Anisotropic Liquid Infused Interfacial Materials**

Surfaces with anisotropic wetting properties, widely found in nature, are a new concept in the field of surface engineering. For example, Cai et al. explored anisotropic rolling/pinning oleophobicity of water infused filefish *Navodon septentrionalis* skin that arises from the oriented hook-like spines arranged on the surface of the fish skin.

These asymmetric microtextures impel anisotropic fluid motion in a head-to-tail direction which allow fish to stay clean in oil-contaminated aquatic environments. Anisotropic surfaces can be artificially achieved through imposing gradients in the chemical and structural features of a surface which account for differences in the energy barrier of wetting on patterned surfaces. For example, chemical heterogeneity of the surface induces a self-running motion of water from the hydrophobic region to the hydrophilic region without any other forces applied. In addition, changing periods, width, and spacing of surface structural features affect the transportation of liquid droplets.

For artificial materials, liquid-impregnated surfaces generated from the anisotropic arrangement of micro-/nanostructures are ideal substrates for controlling movement of liquid droplets through high contact angles and low contact angle hysteresis. It has been verified that anisotropic micro-grooved organogel surfaces infused with silicon oil show excellent anisotropic sliding properties using water droplets.
Figure 2.7. Anisotropic sliding of water droplets on the elastomeric micro-grooved organogel surfaces. a) Water droplets can easily slide from the stretched end to the un-stretched end in one direction with a low sliding angle ca. 10°, b) but cannot slide from the un-stretched end to the stretched end at the same sliding angle. The figure is From REF 70.

Moreover, recently, stimulus-responsive anisotropic slippery surfaces were developed by using paraffin, a thermo-responsive phase-transition material, as a lubricating fluid and directional porous polystyrene (PS) films as the substrate. The smart driven anisotropic system offers control of anisotropic sliding motion for several liquid droplets.
Temperature-responsive SLIPS produced by incorporating paraffin, a thermo-responsive phase-transition material, into an anisotropic porous polystyrene (PS) fibrous matrix.

At room temperature, the paraffin solidified on the surface of PS film results in pinning the liquid droplet on the surface in the parallel (∥) and perpendicular direction (⊥) to the fiber arrangements. At higher temperatures, paraffin becomes a slippery liquid, and the droplets easily slide on the composite surface in two directions. Reproduced from REF 71.

7. Emerging Applications

Liquid-infused materials are expected to show superior, special wettability properties to fulfill the fundamental and practical application requirements for self-cleaning, omniphobicity, and separations to increase heat transfer and fluid-flow drag.
Some successful examples are liquid repellency for a wide range of liquids, immiscible liquid separation, solid repellency such as ice, insects, and dust, anti-bioadhesion, and droplet manipulation and collection. To meet the growing requirements in real-world applications, appropriate combinations of surface slipperiness and specific desired physicochemical functionality such as stimuli-responsiveness, self-healing, and optical tunability is highly desired.

### 7.1 Liquid Repellency/Separation

The surface topography not only contributes to anisotropic pinning/rolling properties of the LIS, but also plays a critically important role in maintaining a lubricating liquid in place. It is worth noting that the liquid-lubricated layer is the primary factor that determines the surface free energy and thus has a profound influence on liquid-repellency features of the surface.

Liquid infused systems can be designed by impregnating 3D porous solids with low-surface-tension perfluorinated liquids. The homogeneous and nearly molecularly smooth surfaces not only repel pure liquids such as water, but also complex fluids such as crude oil, blood, and dairy liquids that normally stain most conventional superhydrophobic surfaces.\textsuperscript{43,73,88-91}
Figure 2.9. Superior repellency properties of SLIPS for complex fluids compare to a superhydrophobic Teflon™ porous membrane, a hydrophobic surface, and a flat hydrophilic glass surface. Reprinted by permission from REF 43.

Currently, there is a considerable demand for materials that can efficiently separate immiscible mixtures, especially oil/water either in the form of free mixtures or emulsions. In general, two types of special wetting materials are suitable for oil/water filtration: hydrophobic/oleophilic materials, called “oil-removing” materials that separate oil phase from oil/water mixtures by selective oil permeation, and hydrophilic/oleophobic materials known as “water-removing” materials that separate water phase from oil/water mixture by allowing water to penetrate through them while repelling oil.

Conventional oil-removing membranes which are hydrophobic/oleophilic face limitations for separating oil/water mixtures. First, these materials can be easily fouled and clogged by oil due to their intrinsic oleophilic nature, which greatly restrict their application in filtration due to a
remarkable reduction in permeation flux. Secondly, water normally has a higher density than most of oils, which tends to settle below the oil due to gravity and form a barrier layer above the materials. This water layer directly affects oil permeation by blocking all filtration. Thirdly, these materials show poor recoverability for reuse. In most cases, the adhered oil is hard to remove leading to secondary contamination in the environment. So far, only a few hydro-responsive materials have been reported based on hydrophilic and oleophobic polymeric components.92-94

Although, hydrophilic/oleophobic materials can overcome these issues, due to low surface tension of most oils, the design and fabrication of materials that simultaneously display hydrophilicity and oleophobicity is theoretically very challenging. However, fully wettable water-lubricated anisotropic surfaces is an effective approach to remove only one phase from the oil/water mixture owing to extreme opposing affinities towards water and oil.95 During the separation process, the infused water layer, having no affinity for oil drops, prevents the materials from fouling by oils, while it allows the water phase to pass through. In fact, as shown previously the capillary-stabilized liquid in pore structures can form a reconfigurable doorway which selectively allow liquids to pass through the membrane. This so-called gating mechanism enables SLIPS to coordinate multiphase transport and suppresses fouling, in a highly selective approach without facing pore clogging problem.96,97

Xue et al. constructed a superhydrophilic and underwater superoleophobic hydrogel-coated mesh that showed high separation efficiency for even crude oil and resistance to oil fouling. Stainless steel meshes were used as the substrates and polyacrylamide (PAM) was selected as the hydrogel coating layer because of its excellent water-absorbing and water-retaining capacities.98
Figure 2.10. Scanning electron microscopy (SEM) microimages of a polyacrylamide (PAM) hydrogel-coated mesh. a) uncoated stainless-steel mesh. b) PAM hydrogel-coated stainless-steel mesh. c) magnification of a single pore of the PAM hydrogel-coated stainless-steel mesh. d) the nanostructured papillae on a single wire on hydrogel-coated stainless-steel mesh. e, f) underwater superoleophobic and slippery characteristics of the as-prepared PAM hydrogel-coated mesh. g, h) ultra-low oil-adhesion properties of the coated mesh in oil/water/solid system. i, j) oil/water separation performance of the liquid infused stainless steel mesh; water selectively penetrates through the mesh, while crude oil remained on top. The figure is From REF 98.
7.2 Solid Repellency

Slippery liquid incorporated surfaces have the potential to significantly reduce solid adhesion arising from ice, insects, and dust. The failure of an ant to hold on to SLIPS when the surface is tilted has been captured, though the ant can travel uphill on hydrophobic surfaces such as Teflon.

In another attempt to study the slippery behavior of textured solids filled with silicon oil, the drying patterns left by coffee drops after evaporation, on a non-liquid-impregnated surface (textured or not) and on a slippery pre-impregnated material, were observed. In Figure 2.11, on the non-slippery surface, coffee particles distributed in a ring shape that arises from the pinning of the initial contact line whereas, on the slippery surface, and low hysteresis of these materials stop pinning of the line. Thus, the ring does not form to leave only a round chunk of coffee particles that can be easily removed. The photograph also illustrates the self-cleaning properties of LIS; specifically, a water drop moving down and cleaning the surface (previously sprinkled with silica particles) along its path.
Figure 2.11. Slippery characteristics of liquid-infused materials. a) A water droplet carries away the silica particles on a textured substrate pre-impregnated with a silicon oil. b) Patterns left by coffee drops (initial diameter of 5mm) after evaporation on a non-SLIPS and on a slippery pre-infused substrate. Reproduced from REF 60 with permission from CCC Republication.

Most of the desirable characteristics of anti-icing surfaces, comprising low surface energy and contact angle hysteresis, is conventionally obtained by introducing micro- or nanoscale roughness in superhydrophobic surface to reduce the actual ice-contacting area;\textsuperscript{102-107} however, their practical performance, on impinging droplets, is still largely limited under harsh environmental conditions, particularly high humidity. Alternatively, surfaces with a smooth and stable lubricant interfaces eliminate moisture condensation rapidly because of a reduction in the number of possible nucleation sites.\textsuperscript{108,109}
Further, high mobility of droplets arising from low CAH of the lubricating film suppress frost/ice formation even under humid conditions. Additionally, the lubricant film shows high stability performance under high droplet impact pressures, whereas the voids between surface features of superhydrophobic surfaces encounter physical and mechanical defects under pressure. On this subject, extraordinarily low ice adhesion has been demonstrated using lubricant-infused systems.\(^{61, 56, 110-113}\)

### 7.3 Anti-bioadhesion

The accumulation of biomolecules such as proteins and cells, marine organisms, and bacteria and diatoms on surfaces is a great challenge in a variety of applications ranging from food storage and water purification systems to biomedical devices such as artificial blood vessels and marine and industrial equipment.\(^{114-116}\) Although the fouling species and the type of interaction they make, varies from one kind to another, they nonetheless add to the complexity of the problem, but one approach to develop anti-fouling surfaces, is creating a platform that limits the initial attachment of fouling organisms.\(^{117-120}\)

During the fouling processes of a new surface, several steps take place including adsorption of species, formation of a conditioning layer, primary colonization, and finally formation of biofouling community. Fouling organisms mostly use their superficial biological molecules such as proteins to make the first contact with a synthetic substrate.\(^{121}\) One of the key strategies to combat biofoulants from attaching is use of low-adhesion liquid infused surfaces onto which most organisms have low affinity to settle onto.\(^{89, 122-125}\)
Marine organisms such as sharks, mussels, and crabs have natural antifouling defenses which rely on a combination of surface chemistry, physical attributes, and responses to outside stimuli. Inspired by these criteria, superior anti-biofouling slippery surfaces have been developed.\textsuperscript{121,126-130} For instance, bioinspired biofilms based on porous polytetrafluoroethylene (PTFE) substrate or microstructured fluoro-silanized Si wafers have been reported to inhibit 99.6% of biofilm attachment of \textit{Pseudomonas aeruginosa} over a 7-day period under both static and flow conditions.

These results show 35-time improvement in the total level of bacterial adhesion over PEG-functionalized surfaces. Further experiments confirmed the effectiveness of SLIPS technology for other clinically relevant pathogens, including \textit{Staphylococcus aureus} (97.2%) and \textit{Escherichia coli} (96%). These nontoxic, antibiofilm surfaces demonstrate stability under water and harsh conditions, including extreme pH, salinity, and UV exposure.\textsuperscript{58}

\textbf{7.4 Microfluidic Droplet Handling}

Droplet-based, microfluidic liquid-infused systems are attractive for performing chemical reactions and controlling droplet motion in reactors, collectors, and sensors on a miniature-scale.\textsuperscript{72,90,131-135} The lower surface tension, non-polarity and high viscosity of oil/organic droplets as compared to aqueous droplets make oil droplet manipulation difficult. To perform oil-droplet-based chemical reactions, underwater superoleophobic tweezers are fabricated by attaching frosted glass plates to the tips of tweezers.\textsuperscript{136}

Hydrophilic frosted glass plates trapped water molecules in the gap of their surface microstructures act as a low adhesion oil repulsive slippery layer. Employing these tweezers, oil
droplets motion in water was performed. A series of miniature organic chemical reactions inside oil droplets was explored. As such, a droplet of bromine tetrachloromethane solution in a dark yellow color to come into contact with a transparent droplet of styrene tetrachloromethane. The two droplets merged into a larger one leading to a miniature chemical reaction as shown by a slow change of the color from dark yellow to transparent.

Moreover, due to the ultra-low oil adhesion property of pedestals in water media, reaction products inside coalesced droplets were attained. Miniature liquid immobilized reaction systems can thus be used for potential lab-on-a-chip applications ranging from microscale components such as biological medicines and toxic chemicals miniature reactors and sensors to high-throughput assays.135-141
**Figure 2.12.** Demonstration of manipulating oil droplets in water to perform a miniature organic reaction. a) A bromine tetrachloromethane droplet was carefully dropped onto a superoleophobic plate, forming a dark yellow spherical shape. b,c) A styrene tetrachloromethane droplet was picked up by a pair of superoleophobic tweezers and placed onto the yellow droplet. d) Two droplets slowly combined. e,f) After 43 seconds, the color of the coalesced droplet slowly changed from dark yellow to transparent, indicating that the reaction was completed. Schematic illustrations of each steps are shown on the right of each image. Reproduced from REF 136.
Inspired by the Namib desert beetle’s back,\textsuperscript{45} which collects microdroplets of water from fog in the desert atmosphere and guides the droplets down to their mouth, Aizenberg and co-workers\textsuperscript{142} designed a surface with asymmetric bumps to optimize fast localized droplet condensation. Later, the slippery surface of pitcher plants inspired them to coat the bumps with a molecularly smooth lubricant layer to render them with higher friction. They achieved a surface with enhanced continuous water condensation and shedding performance superior to conventional superhydrophobic surfaces. Promoting dropwise condensation is critical for water-harvesting applications and many phase-change heat-transfer applications requiring reliable rapid droplet growth and transport performance.\textsuperscript{59,75,143,144}

8. Perspectives

Bio-inspired surfaces infused with a lubricating liquid display a remarkable range of useful properties unlike gas-cushioned surfaces that lose stability and robustness upon damage to the texture and under extreme conditions. These properties originate from the ability of the continuous, fouling-resistance, defect-free, nearly molecularly smooth fluid to flow and configure itself around a variety of surfaces from rigid to elastic.\textsuperscript{134}

Although there is no doubt about the potential of the lubricant-impregnated surfaces, several fundamental questions need to be addressed to carefully design an optimal non-wetting material.\textsuperscript{96} For instance, the infused liquid film may drain due to external shear flow. Although an analytical model showed that manipulating the aspect ratio of the surface grooved pattern-to-wick wetting liquids play a role in this regard,\textsuperscript{131} further investigation on optimization of more complicated topographical length scales is required. Moreover, to satisfy practical demand, lubricant depletion and replenishment need to be considered. As a successful example, a
supramolecular polymer-gel matrix consisting of liquid-storage compartments in which self-regulating and self-reporting secretion materials was introduced. Additionally, self-lubricating hydrogels/organogels are other promising candidates to provide more sustainable replenishing reservoirs.

Although complete immobilization of texture is among the most desirable feature, it restricts the choice of lubricant, and thus gaining a deeper understanding of surface functionality and lubricant chemical/physical properties remains important. For example, the role of lubricating liquid viscosity, texture, and the interactions between components on droplet shedding behavior, whether they slip or roll on the surface, has not been adequately addressed. The technology of liquid infused systems is likely to dominate the field of biomimetic and functional surfaces in practical scenarios for the time being, but more research for understanding the successes and failures of this novel technology is essential.
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CHAPTER 3: Superoleophobic Nanofibrous Materials from Bacterial Bioengineering

1. Abstract

This study demonstrated that water-infused nanocellulosic membranes produced by *Gluconacetobacter hansenii* bacteria under static cultivation conditions displayed superhydrophilicity and superoleophobicity with ultralow oil-adhesion properties. Those properties arose from the following two unique nanofiber features: (1) the three-dimensional porous network of a bacterial membrane made up of cellulose nanofibers yielding an extraordinarily high surface area for trapping water molecules and (2) the native hydrophilicity of the cellulose owing to the large amount of hydroxyl groups on the backbone of the cellulose polymer chains. The bacterial cellulose membrane showed extremely high-water trapping capacity (> 99% of its dry weight), excellent water absorption, and very importantly, superior water retention properties.

2. Introduction

Superwettability of bioinspired nano-/microstructured surfaces in air has been investigated intensively over the past several decades. Inspired by nature, surface energy and roughness are two key parameters for achieving superhydrophobicity in air to display water contact angles larger than 150º. Despite the fact that the trapped air pockets in the structure of superhydrophobic materials in the Cassie state are prone to fail under pressures-induced instabilities and can be dissolved into the surrounding fluid upon temperature and pressure increases a number of superhydrophobic surfaces have been developed for various
applications such as self-cleaning,\(^3\) anti-icing,\(^4,5\) anti-fogging,\(^6\) fluid-flow drag reduction,\(^7\) and etc.\(^8,9\)

Among these applications, oil-water separation is of paramount importance for potable water within water filtration and environmental remediation because of increasing industrial oily wastewater generation and oil spills. Therefore, materials with superhydrophobic-superoleophilic surfaces offering extreme opposite affinity toward water and oil have been extensively studied for oil-water separations.\(^9,10\) However, these “oil-removing” materials easily clog from oil droplets as they pass through. The adhered oil is hard to remove and recycled and therefore leads to a significant decrease in separation flux and materials life.\(^11,12\)

Furthermore, superhydrophobic-superoleophilic materials are not suitable for gravity-driven separation because water settles due to its higher density than most oils and forms a barrier layer which prevents oil from contacting the surface of the materials. Moreover, the oil-sorbent materials are flammable, toxic, and cause environmental contamination.

Superwettable materials with inverse affinity toward water and oil can address these problems. Superleophobic-superhydrophilic materials which are known as “water-removing” have proven to be highly potent in practical applications. They are much less prone to fouling and can have higher oil-water efficiency than oil-removing filters. Also, because of lower viscosity of water compare to organic liquids, water-removing materials are expected to show higher fluid flux.\(^14\)

Despite all these advantages, it is very challenging to obtain a surface that at the same time shows superoleophobicity AND superhydrophilicity. In fact, from a surface tension perspective, if the energy of the surface is low enough to repel organic liquids with lower surface tension than
water, it will repel water molecules too. To overcome this issue, a promising alternative strategy is introducing a repulsive liquid layer into the micro/nano structured surfaces. The concept of water-infused surfaces in an oil/water/solid three-phase system is inspired by the antiwetting and antifouling behavior of oil droplets on fish scales discovered less than a decade ago.

In the case of oil-water separation, water can serve in a simple and fluoride-free way to achieve superoleophobicity while simultaneously behaving as a reconfigurable gate to selectively allow the water phase of the feed stream to pass through. This gating mechanism can be designed to be responsive to environmental stimuli such as pressure and temperature for selective fluid transportation.

In addition to oil-water separation, the study of water-impregnated materials leads to an understanding and development of oil-based micro-reactors, droplet-based manipulation in micro channels, marine antifouling coatings, and bio-adhesion on surfaces and can be extended to wettability by liquid droplets other than oil, submersed in another immiscible liquid rather than water.

A variety of three-dimensional slippery liquid-infused surfaces have been fabricated recently through selectively etching nanoparticle templates or densely packing building blocks such as nanoparticles. However, the lack of interaction between impregnated liquids and these substrates can affect the repellency performance and stability of these materials. In fact, the infused liquid film may drain under external shear flow due to the lack of proper entrapping.
To overcome the weak trapping ability of artificial porous matrix toward intermediary liquid, gels can be promising alternatives because they have shown a potentially more robust approach to prepare lubricated, textured surfaces.25,26

Gels are high-performance three-dimensional crosslinked networks consisting of a dispersed liquid medium. If the dispersion medium is water, it is a hydrogel while if it is composed of a liquid organic phase, it is an organogel. There are also organohydrogels which quickly switch to hydrogel or organogel properties in response to the surrounding phase.17,18

The strong chemical or physical interactions between the dispersed liquid molecules and crosslinked polymeric network of gels make these materials ideal substrates to firmly trap the lubricated medium with high liquid-sorption and retention capacity.28 Gel layers can be utilized to design novel multifunctional soft surfaces with self-lubricating properties.19,29

3. Results and Discussions

This study demonstrated that a water-infused nanocellulosic membrane produced by bacteria in the static cultivation method displayed superhydrophilicity and superoleophobicity with ultralow oil-adhesion properties. Those properties arose from (1) the unique three-dimensional porous network of a bacterial membrane that is made up of cellulose nanofibers yielding an extraordinarily high surface area for trapping water molecules and (2) the native hydrophilicity of the cellulose because of the large amount of hydroxyl groups on the backbone of the cellulose polymer chains. Thus, bacterial cellulose membrane shows extremely high-water trapping capacity (> 99% of its dry weight), excellent water absorption, and very importantly, superior water retention properties.
The bacterial cellulose membrane used in the current set of experiments was produced by *Gluconacetobacter hansenii*. Figure 3.1 (a–c) shows field emission scanning electron microscopy (FESEM) images of the nanocellulosic membrane. The cellulose nanofibers have diameters of $35 \pm 17$ nm among which there are microscale empty spaces which can be filled with liquids. The water trapped in the BC membrane is inherently uniformly distributed and defect-free at the molecular scale for the possibility of allowing rapid self-heal after physical damage. In fact, the lubricant water layer in the porous structure of BC membrane can flow towards any damaged areas due to the surface-energy-driven capillary action and provide immediate self-repairing.$^{1,20,30}$
**Figure 3.1.** (a) Image of water-infused BC membrane. (b, c) FESEM micrographs of the BC membrane with scale bar of 5 µm and 1 µm, respectively. (d) Ultralow oil adhesion of a BC membrane in water. (e) Underwater static contact angle for an oil droplet, n-dodecane, on the water infused BC membrane. (f) Static contact angle of n-dodecane on the dry BC membrane in air. (g) Under-oil static contact angle for deionized water on the oil, n-dodecane, infused BC membrane. (h) Schematic showing superoleophobicity of the membrane occurring because of the presence of the water in the structure and the rearrangement of hydroxyl groups, shown in green, in hydrophilic medias such as water. (i, j) Hydroxyl groups rearrangement in contact with hydrophobic medias such as air or oil, hydroxyl groups point toward the bulk and do not contribute in repulsion any further.
Figure 3.1 d shows an underwater oil-adhesion test which was dynamically measured using an oil droplet (n-dodecane 3 µL, surface tension 24.91 @ 20 °C in mN/m) suspended on a microsyringe needle tip. First, the oil droplet was placed in contact with the membrane surface, then pushed against the surface, and then retracted. However, the oil droplet was repelled from the surface and showed no adhesion force toward the surface. In fact, the oil droplet did not leave the needle during the entire process even after pushing it forcefully toward the surface. When the microsyringe was lifted, the oil droplet did not deform and leave any residue on the surface of the membrane. As shown, the water-impregnated layer of the BC membrane, whose surface is atomically smooth and homogenous, exhibited a very low contact angle hysteresis toward the oil droplet.

Figure 3.1 e shows a droplet of light oil (n-dodecane 3 µL) on a BC membrane immersed in water. The oil contact angle of 174.5 ± 2.5° exceeds by a large margin the established superoleophobicity threshold (> 150°). BC membrane surfaces with a stable, intercalated lubricant water layer show no contact line pinning and hence the oil droplet base is circular in shape and not elongated; this latter result proves that it is extremely difficult for a small underwater oil droplet to adhere and foul the surface of the water-infused BC membrane.

BC membrane shows almost no oil repellency in air when it is dry (Figure 3.1 f). There are void spaces that exist between cellulose nanofibers. When there is no water lubricant layer, oil can infiltrate these spaces quickly. Even the presence of hydroxyl groups on the backbone of cellulose chains do not help very much under these circumstances. In fact, as demonstrated in Figure 3.1 i, hydroxyl groups are more apt to conceal themselves from air as a hydrophobic media and therefore do not repel the oil droplet. In the case of water-impregnated BC membranes
(Figure 3.1 h), hydroxyl groups rearrange themselves to maximize contact with water molecules and intercalate them; therefore, a stable repellent layer at the molecular scale arranges itself to repel oil molecules.

To better understand the role of hydroxyl groups on the oil repellent properties of BC membranes, and oil-impregnated BC membrane was constructed. A piece of BC membrane was freeze-dried in order to maintain pore structures. The obtained dry membrane was soaked in n-dodecane liquid until it was fully wetted. As shown in Figure 3.1 g, once the water droplet touches the surface of oil infused BC membrane immersed in oil media, it infiltrates the membrane in less than two seconds because that hydroxyl groups which are hydrophilic rearrange themselves after the water droplet comes into contact with the surface to absorb it (Figure 3.1 j).

The surface of the membrane possesses a large roughness factor because of the inherent re-entrant curvature of the nanofibers and microstructured pores which can improve the stability of a solid/liquid composite interface. Atomic force microscopy (AFM) equipped with a cantilever with a nanometer-scale tip was used to scan surface features and roughness.

To better investigate the effect of the three-dimensional porous structures of BC membrane on surface roughness, a thin film of pressed BC nanocellulose was prepared and scanned (details explained in experimental section). The corresponding results are shown in Figure 3.2 Three-dimensional porous structures can be clearly seen on BC surface. However, the porous structures disappear from the surface of the pressed sample. Root mean square (RMS) roughness of surfaces was 56.83 nm and 30.28 nm for the original BC membrane and pressed sample, respectively.
A porous microstructure is considered as one of the key factors to influence the surface wettability and trapping capacity and thereby provide different wetting performances.\textsuperscript{31,32}

\textbf{Figure 3. 2.} Surface roughness of BC membrane; (a) original BC. (b) smashed and pressed BC membrane. The measurement results are expressed in 3D based on height information. Possessing re-entrant curvature can lead to a more stable encapsulation of water into the textured substrate to facilitate non oil-wetting properties with high contact angles, low contact angle hysteresis, and no contact line pinning effect. In the oil/water/solid system shown in Figure 3.3, the gap between surface roughness is filled with water; thus, oil drops on a surface of BC membrane underwater are separated by a thin lubricant water film trapped between nanofibers while surrounded and cloaked by an annular wetting ridge.
**Figure 3.** A schematic of the oil-water separation process by a BC membrane (inset) illustrating possible wetting configurations of a slippery water-infused BC membrane; the schematic indicates that the oil droplet is separated from the surface by a thin water layer interlocked between cellulosic nanofibers.

Water-infused BC membranes can repel immiscible liquids of virtually any surface tension. For instance, the oil repellency property of the membrane was herein investigated. A feed of oil-water mixture was passed through a wet BC membrane to yield a visually clear permeate with no oil droplets, highlighting the fact that an oil phase can be separated from the mixture with high separation efficiency.
**Figure 3.4.** Oil–water separation using a water-infused BC membrane. (a) Experimental setup. (b) A mixture of water (yellow color) and hexane (purple color) was poured into the upper glass jar. (c-e) Filtration takes place. (f) The filtrate (left) and the oil phases remained on top of the membrane (right). (g) Membrane after the filtration experiments revealing that it can even concentrate aqueous dyes from the feed solution.
The underlying mechanism behind this separation phenomenon is referred to as gating. Based on this mechanism, the capillary-stabilized water in pore structures form a reconfigurable doorway which selectively allow water to pass through the membrane while prohibiting oil molecules from penetration. Therefore, it enables water-infused BC membranes to effectively suppress fouling and oil clogging.

Meanwhile, the reusability of the membrane was studied numerous times by adding new water to the upper tube to reveal no reduction in separation efficiency and filtration flux. This result proves that a BC membrane has resistance to oil fouling. Therefore, separation can be repeated as long as the BC membrane remains water infused.

The permeability of pure water through BC membranes of different thickness was measured. As shown in Figure 3.5 a, the water flux was inversely proportional to the thickness of the membranes. In contrast, water permeance through the membranes increased linearly with changed in feed pressure over the range of 0–20 psi (Figure 3.5 b).

The membrane of thickness = 312 ± 54 µm exhibited an appreciable gravity-driven pure water flux of 135 ± 26 L.m⁻².h⁻¹ which is in the range of commercial filters. These results showed that a thin BC membrane is able to withstand high pressures up to 20 psi without a concomitant reduction in filtration efficiency (Figure 3.5 b).
4. Conclusion

In conclusion, it has been amply demonstrated that a bacterial cellulose membrane offers underwater superoleophobicity with ultralow oil-adhesion properties due to its powerful water absorbing and retention capacities. Meanwhile, the presence of a micro re-entrant surface texture due to the inherent curvature of nanofibers leads to the establishment of a stable Cassie state composite interface where the water forms a continuous defect-free overlying film. The ability to produce an anti-fouling membrane with different sizes and shapes as well as durability, ease of cycling, and pH-stability match well with the requirements for treating real world problems. It is anticipated that the promising versatility and simplicity of this eco-friendly materials can be employed in designing self-cleaning coatings, stimuli-responsive materials, lab-on-a-chip microfluidic devices, and droplet manipulators systems for mini reactors.
5. Materials and Methods

5.1 Materials

*Gluconacetobacter hansenii* was purchased from American Type Culture Collection ATCC® 23769™. Mannitol, yeast extract, bacto-peptone (Becton, Dickinson and Company), di-sodium phosphate, and citric acid were used for preparing the appropriate culture media. Hydrogen chloride and potassium hydroxide solution 1N (N/10) (Certified), were used to purify membranes. n-dodecane was used to prepare oil-in-water mixtures. All the reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated.

5.2 Preparation of Bacterial Cellulose Membranes

This work utilized ATCC® 23769™ *Gluconacetobacter hansenii* for all cellulose production. Dried bacterial samples from American Type Culture Collection (ATCC) were revived according to the standard ATCC protocol. Inoculation media was prepared using the standard mannitol broth that consists of (w/v): 2.5% mannitol, 5% yeast extract, and 3% peptone in DI water. The mannitol broth media was autoclaved at 121°C for at least 15 minutes before proceeding. This inoculation medium was used as the seed culture medium.

The standard Hestrin & Schramm media (HS media) consists of the following ingredients (w/v): glicerol 2%, peptone 0.3%, yeast extract 0.5%, dibasic sodium phosphate 0.27%, and citric acid 0.114%. Culture vessel was filled with 50 mL of fresh, sterile HS media. To that was added 5% (v/v) of the seed culture medium.
The inoculated vessel was then placed in a static incubator under atmospheric conditions at 30°C for 8 days to develop BC pellicles. Afterwards, the BC membranes were soaked in KOH 1M bath at room temperature for 48 hours to remove non-cellulose materials such as proteins and nucleic acids from bacterial cells and the culture broth. Once the bulk of the media was removed, the BC membranes were transferred to HCl 0.5M bath to neutralize the base. After an hour neutralization, the cellulose was repeatedly rinsed in DI water until neutral pH. Finally, the membrane was stored in fresh DI water at 4 °C for further experiments.

5.3 Morphology Characterization

Surface and bulk morphology of BC membrane were observed by field emission scanning electron microscopy (FEI Verios 460L, FESEM). Samples were gold-palladium (5 0:50) coated (~15m thickness) with Technics Hummer V Sputter Coater to reduce charge interruptions before taking SEM images.

5.4 Contact Angle and Surface Tension Measurements

The oil contact angles in air, underwater oil contact angles and adhesion behavior, and underoil water contact angles were monitored by Data-Physics Contact Angle System OCA 15EC at ambient temperature. The oil (n-dodecane) drops (3μL) were dropped carefully onto the materials, which were in various surrounding media. The average value of three measurements performed at different positions on the same sample was adopted as the contact angle. The pendent drop method was used to measure surface tensions of n-dodecane. It is based on analyzing the shape of a drop hanging from a capillary tube ready to detach.
5.5 Surface Roughness Measurements

Atomic force microscopy (AFM), a very-high-resolution type of scanning probe microscopy, was used to accurately characterize the variation of surface microstructures with resolution on the order of fractions of a nanometer for scanned areas of 1 and 100 µm². In order to keep the pore structures that are available in the wet state within dry samples, the samples were dried in a FreeZone freeze-dryer from LABCONCO at < 50 Pa and -50 °C for at least 48 h without any previous pre-freezing.

For preparation of a thin pressed BC film, a laboratory suction Buchner funnel was used. A slurry of BC nanocellulose was prepared by homogenizing a weighted BC sample in DI water using an IKA T25 dispersing instrument. Filter paper was placed in the Buchner funnel to trap the nanocelluloses during the filtration process and ensure formation of a smooth film. When the slurry was poured in the suction funnel, most of the water was removed by suction, after which a wet gel-like film was formed that was moved to an oven for drying.

5.6 Filtration and Water Flux Experiments

The wet purified BC membrane was tested for oil in water filtration. The surfactant-free oil-in-water mixture were prepared by mixing n-dodecane and water in a volume ratio of 50:50 under stirring for 1h or so. The filtration performances were carried out with a dead-end filtration assembly (Kontes 47mm). The oil contents in the filtrates obtained from filtration experiments were measured by a Total Organic Carbon Analyzer (Shimadzu Scientific Instruments, TOC-VCPN). The filtration efficiency was defined as below.
Filtration efficiency (%) = \( (1 - C_f / C_o) \times 100 \)  \( (1) \)

where \( C_f \) (mg) is oil concentration of filtrate and \( C_o \) (mg) is oil concentration of the original oil-in-water mixture.

In addition, water flow rate through BC samples was measured using a customized filtration assembly. The setup was connected to a controllable external pressure pump enables to apply pressure to the system up to 20 psi and a software capable of measuring the weight of the water that passes the membrane versus time (Figure 3.6).

**Figure 3.6.** Schematic of the filtration set up with external pressure apparatus; (1) filtration cell. (2) air cylinder pump. (3) pressure gauge. (4) digital weighting balance. (5) graduate vessel for permeate collection. (6) computer equipped with appropriate software.
Using this system 200 ml of deionized water passed across the BC membranes with different thicknesses under different pressures to measure the pure water flux (J, L m$^{-2}$ h$^{-1}$), calculated as follows:

\[ J = \frac{V}{A t} \]  

where \( V \) is the volume of the water filtered (L), \( A \) is the effective filtration membrane area (m$^2$), \( t \) is the filtration time (h). The average value was reported from triplicate measurements.

6. Supporting Information

6.1 Purification Step

![Figure 3.7](image)

**Figure 3.7.** Picture of BC membranes and corresponding SEM images: top) before purifying with alkali-treatment, bottom) after purifying with alkali-treatment.
6.2 Diameter Distribution and Thermal Stability of Bacterial Nanocellulose

**Figure 3.8.** Diameter distribution of the cellulose nanofibers shown in the SEM image with average diameter of 35±17 (scale bar 1 µm).

**Figure 3.9.** Thermogravimetric Analysis (TGA) graph of bacterial nanocellulose membrane; weight change as a function of temperature (20 °C/min).
6.3 Intrinsic Wetting Threshold (IWT)

Surface tension is tendency of fluid surfaces to shrink into the minimum surface area possible and the intrinsic wetting threshold (IWT) of the liquid is the contact angle that describes the boundary of the wettability between lyophilic and lyophobic behavior when the liquid is sitting on an ideal, perfectly smooth and chemically homogeneous, solid surface.\textsuperscript{34,35}

In principle, every liquid has its own IWT, which is independent of the solid surface. Recently, the IWTs of several organic liquids were determined by finely modulating the surface tensions of substrates. With the increase of liquid surface tension, the $\theta_{\text{IWT}}$ will also grow gradually.\textsuperscript{23,34,35}

![Image showing contact angles for different liquids]

**Figure 3.10.** The intrinsic wetting thresholds ($\theta_{\text{IWT}}$, shaded blue) for different liquids decrease with decreasing surface tension.\textsuperscript{23}
6.4 Hydrophilic-Oleophobic Membranes

Liquid will spontaneously wet out a surface with higher surface energy. To make a repellent surface, the surface energy of the substrate must be lower than the surface tension of the liquid. Surface energy can be altered by chemical modification, plasma treatment, corona treatment etc.

In order to make an oleophobic membrane the surface energy of the membrane should be lower than the surface tension of the oil (organic liquid). The surface tension of the several organic liquids and water are presented in Table 3.1.

**Table 3. 1. Surface tension of some fluids.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Surface Tension (@ 20 °C in mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>72.80</td>
</tr>
<tr>
<td>Toluene</td>
<td>28.40</td>
</tr>
<tr>
<td>Chloroform</td>
<td>27.50</td>
</tr>
<tr>
<td>n-Dodecane</td>
<td>25.35</td>
</tr>
<tr>
<td>Hexane</td>
<td>18.43</td>
</tr>
</tbody>
</table>
If the surface energy of the membrane is lower than the surface tension of organic liquids, then it will repel water too because surface tension of water is higher than the most of liquids including organic liquids. Therefore, from surface tension stand point, preparing materials that simultaneously display hydrophilicity and oleophobicity is very challenging.

### 6.5 Wetting Behavior of Underwater Surfaces

Consider a liquid sessile drop resting on a perfectly flat, horizontal solid surface. As first described by Young in 1805, the contact angle ($\theta$) of a liquid drop on an ideal solid surface is defined by the mechanical equilibrium of the interfacial energies between the solid–liquid ($\gamma_{SL}$), solid–vapor ($\gamma_{SV}$), and liquid–vapor ($\gamma_{LV}$) interfaces can be obtained from Young equation:

$$
\cos \theta = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \quad (3)
$$

From this equation contact angles are not limited to the liquid-vapor interface on a solid; they are also applicable to the liquid-liquid interface on a solid. And can be modified as follows:

$$
\cos \theta = \frac{\gamma_{L1-V} \cos \theta_1 - \gamma_{L2-V} \cos \theta_2}{\gamma_{L1-L2}} \quad (4)
$$

Where $\gamma_{L1-V}$ is surrender liquid (L1)-vapor interface tension, $\theta_1$ is the contact angle of liquid 1 in air, $\gamma_{L2-V}$ is liquid drop (L2)-vapor interface tension, $\theta_2$ is the contact angle of liquid 2 in air, and $\gamma_{L1-L2}$ is interfacial energy between liquid drop L2 and surrender liquid L1.
Figure 3. 11. Wetting behavior of solid substrates in three-phase systems based on Young’s equation: (left) solid-oil-air, (right) solid-oil-water.

6.6 Nanocellulose Network

The branching of the nanocellulose network is affected by the proliferation rate of *G.xylinus* (Figure 3.12). No breakage of the cellulose ribbon occurs after division, just the creation of a branching point.

Figure 3. 12. The branching of cellulose formed by the cell division of *G.xylinus*. 
6.7 Anti Oil-Fouling Properties

In order to investigate the effect of unique 3D microstructure of the BC membrane in organic liquid repellency properties, a piece of BC membrane and a cellulosic paper were immersed in DI water for a few minutes until fully wetted. Then a droplet of oil (n-dodecane, 1 µm) was slowly released on the surface of each samples. By tilting the plate containing the samples, the oil droplet starts to roll down on the surface of the BC membrane however it completely infused into the paper.

This experiment proves that the anti oil properties of BC membrane is not only the result of cellulose itself, but it roots in microporous structure made of cellulose nanofibers with a lots of hydroxyl groups which provide an ample room for water molecules. Water molecules trap firmly in the structure of BC membrane through physical and chemical bondages.

However, in case of the cellulosic papers, fibers length is much shorter and fibers diameter is much bigger thus the surface area available to water molecules is significantly lower. Moreover, the cellulose fibers are stack on top of each other with limited void volume instead of going around each other and create a bulky entangled fibrous structure with ample room for water molecules to infused to therefore, we do not see the same effect from cellulosic paper.
**Figure 3.13.** Demonstration of anti oil-fouling properties of BC membrane compare to a cellulosic paper.

**Figure 3.14.** Oleophobic characteristic of bacterial nanocellulose membrane.
6.8 Surface Analysis

Surface of BC membrane was observed by confocal laser scanning microscope which combines optical microscopy with laser profilometry.

Figure 3.15. Surface roughness of BC membrane; a) optical microscopy image b) monochrome full-focus laser image c) the measurement result expressed in 3D based on the height information.
REFERENCES


CHAPTER 4: Grown Bacterial Nanocellulose Matrices with Tunable Porosity

1. Abstract

A facile and effective method is described to engineer original bacterial cellulose fibrous networks with tunable porosity. We showed that the pore shape, volume, and size distribution of bacterial nanocellulose membranes can be tailored with appropriate culture conditions specifically carbon sources. Pore characterization techniques such as capillary flow porometry, bubble point method, gas adsorption-desorption technique as well as visualization techniques such as scanning electron and atomic force microscopy were utilized to investigate the morphology and shapes of the pores within the membranes. Engineering the various shape, size and volume characteristics of the pores available in pristine bacterial nanocellulose membranes lead to fabrication and development of eco-friendly materials with required characteristics for a broad range of applications.

2. Introduction

Several species of microbes, most noteworthy among them is *Gluconacetobacter*, have an ability to extracellularly grow a nanoscale cellulosic matrix on the interface of air-liquid medium in stationary cultures. The grown three-dimensional (3D) nanostructured matrices are a pure biopolymer composed of very fine, 40–60 nm diameter, ribbon-shaped cellulose nanofibers which are about 200 times finer than cotton fibers and exhibit a remarkable high surface area.\(^1\)\(^-\)\(^4\) Moreover, BC membrane has exceptional flexibility; meanwhile, it shows high tensile strength (200–300 MPa), higher degrees of polymerization and crystallinity than plant cellulose, high thermal and chemical stability, and high water/oil absorption capacities.\(^3\)\(^-\)\(^6\) Bacterial
nanocellulose has been used for a variety of applications including textiles, food products, flexible electronics and optics, supercapacitors, diagnostic sensors, and cosmetics. 3,7-11

The microstructure of bacterial cellulose mimics the extracellular matrix of human skin; thus, they are promising candidates for medical applications such as wound dressings and soft tissue engineering.12-14 However, the tight network of cellulose nanofibrils can hinder their performance and limit cell infiltration.15,16 Improving porosity of the membrane is critical not only for developing novel functional biomaterials for designing artificial organs and tissue scaffolds, but also as immobilizing various compounds such as enzyme bioreactors, drugs, etc and for separating purposes.17-22

Gatenholm et al.23 have tried to alter the porosity of BC networks by employing particle-leaching techniques. The technique consists of introducing foreign substrates such as solid particles of paraffin and potato starch into the structure of the BC matrices during cultivation, followed by leaching to extract the solid particles residue from the materials. Removing the residue of solid particles trapped into the bulk structure of the BC membrane was challenging and tedious and in some cases resulted in broken pore structures.23

Pore space, which is a portion of the materials volume that is not occupied by solid materials, provides the dominant contribution to improve surface area and control the migration and transportation of cells, solid particles, fluids, and so forth. There are different types of pores in fibrous membranes: one which is known as through-pores is accessible at two external surfaces of the membrane and travel all the way through the membrane, commonly evaluated for pore size measurements. There are also blind or dead-end pores which are open at one end but do not participate in permeability of the membrane and are subject to adsorption and desorption of
particles and living cells. Closed pores are types of the pores that are trapped and encapsulated inside the materials and have no access to external surfaces; thus, they are not associated with any absorption or transportation, but still important in terms of mechanical properties such as strength, pliability, and weight.\textsuperscript{24}

The current work showed that culture conditions, specifically applying different types of carbon sources can be employed to tune overall porosity and morphology of bacterial nanocellulose matrices including pore shapes, size, and volume distributions and fluid permeability behavior. The transparency of the BC pellicles can be altered significantly by growth media carbon components. Capillary flow porometry, bubble point method, gas adsorption-desorption techniques, as well as visualization techniques such as Field Emission Scanning Electron Microscope (FESEM) and Atomic Force Microscopy (AFM) were utilized to characterize the pores as well as air and fluid permeability properties of each membrane.

3. Experimental Section

3.1 Materials

\textit{Gluconacetobacter hansenii} was purchased from American Type Culture Collection ATCC\textsuperscript{®} 23769\textsuperscript{TM}. Mannitol, yeast extract, bacto-peptone (becton, Dickinson and Company), di-sodium phosphate, and citric acid were used for preparing the appropriate culture media. Hydrogen chloride and potassium hydroxide solution 1N (N/10) (Certified), were used to purify membranes. All the reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated.
3.2 Preparation of Bacterial Cellulose Membranes

The medium for inoculation preparation and membrane formation consists of the following (v/v): carbon source (glucose, mannitol, fructose, sucrose, or glycerol) 2%, peptone 0.3%, yeast extract 0.5%, dibasic sodium phosphate 0.27%, citric acid 0.114%. The cultivation medias were autoclaved at 121°C for at least 15 minutes before proceeding.

Fermentation cultures for each carbon sources performed in 6-well plates containing 10 ml of fresh above medium with 5% (v/v) cell suspension media. All five 6-well plates were incubated statically under atmospheric conditions at 30°C for 10 days to BC membranes form. To remove non-cellulose materials, the samples were immersed in 1M KOH solution at room temperature for 48 hours. After this time, BC membranes were transferred to HCl 0.5M bath to neutralize the base. After an hour neutralization, the cellulose was rinsed repeatedly in DI water until shows neutralized pH. Finally, the membrane was stored in fresh DI water at 4 ℃ for further experiments.

3.3 Morphology Characterization

Surface and bulk morphology of BC membrane were observed by field emission scanning electron microscopy (FEI Verios 460L, FESEM). Samples were gold-palladium (50:50) coated (~15m thickness) with Technics Hummer V Sputter Coater to reduce charge interruptions before taking FESEM images.
3.4 Pore Size Characterization

Capillary Flow Porometry and Bubble Point test (Porous Materials Inc, CFP-1100_AEL) were used to measure the flow pore diameter distribution along with the bubble point within the obtained membrane. The samples were dried in a freeze-dryer at < 50 Pa and -50°C for at least 48 h without any pre-freezing to maintain pore structures. Subsequently, a fully wetted sample in Galwick solution with known surface tension of 15.9 Dynes/cm, was placed in the sample chamber and the chamber was sealed.

Nitrogen gas was then allowed to flow into the chamber behind the sample. The bubble point has been found when the pressure reaches a point that can overcome the capillary action of the fluid within the largest pore. The bubble point is defined by the ASTM F-316-03 standard as the pressure at which the first continuous gas bubbles are detected.

After determination of the bubble point, the pressure was continuously increased, and flow was measured until all pores were empty from Gatwick solution. At that point, the sample was considered dry by the instrument and the air flow though the sample was recorded.

To further investigate the through pores within the membrane, 200 ml of deionized water was passed across the membrane under gravity. The pure water flux (J, L.m⁻².h⁻¹) was calculated as follows:

\[ J = \frac{V}{At} \]  

(1)
where $V$ is the volume of the water filtered (L), $A$ is the effective filtration membrane area ($m^2$),
t is the filtration time (h).

Moreover, porosity and surface area of the membranes were determined by the analysis of

4. Results & Discussion

4.1 Morphology Characterization

By playing with the process conditions, altering cellulose mat properties is possible.\textsuperscript{1,25,26} The main nutrient for the bacteria is a form of sugar. A single bacteria cell can polymerize 100 glucose molecules into cellulose within an hour. Because the \textit{Gluconacetobacter} bacteria are obligate aerobes, many synthesized fibrils, suspended in the inoculation medium, gradually form a randomly-assembled fibrous metrics that floats at the air/liquid interface.\textsuperscript{27,28}

Results showed all carbon sources supported growth of the bacteria strain (Figure 4.1). Glycerol and fructose gave the highest cellulose yields of 2.05 and 1.82 g.L$^{-1}$ respectively. The gravity-driven water flux through each sample were carried out with a dead-end filtration assembly as a tool to further study the permeation behavior and membrane morphology.

Results are shown in Table 4.1 and Figure 4.2. Glycerol shows the highest water flux with a significant difference compare to other carbon sources followed by fructose, sucrose, mannitol, and glucose.
Fluid permeation behavior can be attributed to the through pores which are the only form of pores that participate in the transportation of matter. Higher fluid movement through the samples clearly point out either more or larger size distribution transportation pores. Gravity-driven water flux measurements showed that glycerol improves fluid flux by 32% relative to fructose, which was the next best flux system, thus offering the most energy-efficient membrane for permeability and separation applications.

**Figure 4.1.** Bacterial cellulose membranes by applying different types of carbon sources in growth culture with all other conditions constant: (top row) freeze dried samples without pre-freezing which locks the porous structure in place, (bottom row) samples in wet states.
Table 4.1. Effect of different types of carbon sources on yield, water flux, thickness, and density of BC membranes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mannitol</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Glycerol</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC Yield</td>
<td>0.95±0.003</td>
<td>1.82±0.003</td>
<td>0.7±0.001</td>
<td>2.05±0.003</td>
<td>0.42±0.001</td>
</tr>
<tr>
<td>(gL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Flux</td>
<td>12.4±2.5</td>
<td>18.7±1.7</td>
<td>10.9±1.1</td>
<td>27.5±0.67</td>
<td>8.6±1.4</td>
</tr>
<tr>
<td>(L.m⁻²h⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness</td>
<td>0.26±0.078</td>
<td>0.46±0.191</td>
<td>0.70±0.130</td>
<td>0.76±0.137</td>
<td>0.21±0.042</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density¹</td>
<td>0.04±0.013</td>
<td>0.05±0.018</td>
<td>0.01±0.002</td>
<td>0.03±0.005</td>
<td>0.02±0.003</td>
</tr>
<tr>
<td>(g/cm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ The density as calculated from the mass and volume of the membranes.
Figure 4.2. Effect of different types of carbon sources on thickness, water flux and density of BC membranes.

FESEM inspection revealed the microstructure of the bacterial membranes (Figure 4.3). The obvious differences between the fibrous network of the membranes confirm that, applying different types of carbon sources is an important parameter for optimizing porous morphology of the BC pellicles.

As it is apparent from the images, with this bacteria strain, cylindrical pores and micro-channels of varying sizes are present in the structure of all membranes. The size of cylindrical pores is < 1 micron. SEM images verified that among five different types of carbon sources utilized in inoculation medium, glycerol, and fructose-fed samples provide the most open structures.
Figure 4.3. SEM images of BC membranes obtained by applying different types of carbon sources in growth culture with all other conditions constant.

4.2 Pore Size Characterization

Although SEM is a great direct assessment technique to characterize pore structures which exist in a sample, but it is very localized approach and thus, acquiring a reliable representative assessment of the whole material can be quite prolonged especially for BC membranes which are natural products and tend to show more variations in morphology. Therefore, to validate and quantify our findings on the through-pores present in the membranes, both Bubble Point Test and Capillary Flow Porometry techniques were used.

Both of these techniques are based on the fact that there is an inverse proportion between a size of a pore wetted with a fluid with known surface tension and the required pressure to force the wetted fluid to expel from that pore. The Washburn Equation (based on the Young-Laplace
Equation) defines the correlation between this pressure drop and the size of the capillary, or through-pore, as follows:

\[ D = K \frac{4\gamma \cos \theta}{P} \]  

(1)

where \( D \) is diameter of the pore, \( K \) is a shape correction factor since in a practical membrane element are likely to be shaped like capillary tubes, \( \gamma \) is surface tension of the liquid, \( \theta \) is liquid-solid contact angle, and \( P \) is pressure.

While the Bubble Point tests helped us to determine size of the largest through pore within the membranes, in order to understand how many through pores of different sizes are present in the medium, an assessment of the pore size distribution was provided by Capillary Flow Porometry technique.

The results of Bubble Point tests, Figure 4.4, showed that among carbon sources that were fed to the bacteria, sucrose cause formation of the biggest through pore size with a significant difference with other carbon sources, followed by fructose, mannitol, glycerol, and glucose. However, as mentioned earlier, this measurement corresponds to the largest or maximum pore size available in the membranes, yet other through pore characteristics, such as size distribution, shape and tortuous nature determine the overall permeability and release profile associated with the use of the membranes.
The results of Capillary Flow Porometry test, Figure 4.4, illustrated that the presence of glycerol and fructose in growth culture resulted in membranes with higher mean through pore size along with lower mean flow pore pressure.

**Figure 4.4.** Results of Bubble Point and Capillary Flow Porometry tests on BC membranes obtained from different carbon sources.
Table 4.2. Results of Bubble Point and Capillary Flow Porometry tests on BC membranes obtained from different carbon source.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mannitol</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Glycerol</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bubble Point Pressure (psi)</td>
<td>9.3280</td>
<td>7.863</td>
<td>1.506</td>
<td>10.477</td>
<td>20.183</td>
</tr>
<tr>
<td>Bubble Point Diameter (µ)</td>
<td>0.7074</td>
<td>0.8392</td>
<td>4.3807</td>
<td>0.6298</td>
<td>0.3269</td>
</tr>
<tr>
<td>Mean Flow Pore Pressure (psi)</td>
<td>29.402</td>
<td>11.206</td>
<td>72.818</td>
<td>10.646</td>
<td>73.752</td>
</tr>
<tr>
<td>Mean Flow Pore Diameter (µ)</td>
<td>0.22±0.1608</td>
<td>0.59±0.4661</td>
<td>0.09±0.6794</td>
<td>0.62±0.5067</td>
<td>0.09±0.0609</td>
</tr>
<tr>
<td>Smallest Detected Pore Diameter (µ)</td>
<td>0.0659</td>
<td>0.1992</td>
<td>----</td>
<td>0.1974</td>
<td>----</td>
</tr>
</tbody>
</table>

Although both glycerol and fructose-fed samples show around the same mean diameter for flow pores, but glycerol-fed samples showed around 32% greater water flux over fructose-fed samples (Figure 4.2 and Figure 4.4). To better understand the underlying reasons behind this, we need to consider pore size distribution for each sample which provides a quantitative description of the range of pore sizes present in a given membrane, another information that can be obtained from Capillary Flow Porometry based on the following equation:
\[ f = -d \left[ \frac{f_w}{f_d} \times 100 \right] / dD \]  

(2)

where \( f \) is pore distribution, \( f_w \) is flow rate through wet sample, \( f_d \) is flow rate through dry sample, and \( D \) is pore diameter.

The data obtained from Capillary Flow Porometry experiments clearly indicates that the glycerol-fed sample has a larger pore sizes compare to the fructose-fed sample while the mean pore size for both is around the same. There is a wider range of pore sizes in the fructose-fed sample. In fact, a lot of pores in this samples fall within the range of 0.2-0.22 microns (Figure 4.5 a).

Moreover, if we study the flow rate behavior of each sample under pressure, before 50 psi both samples approximately show the same behavior however, the flow rate of air/liquid through glycerol-fed samples soar up (Figure 4.5 b).
We also measured the density of glycerol and fructose-fed samples and realized that fructose-fed samples are denser than glycerol-fed samples; thus, by deduction, because both samples have about the same average size of through pores, the overall pore volume must be higher in glycerol-fed sample (Figure 4.6). It is worth noting, while the pore diameter varies along a single pore, Capillary Flow Porometry measures the most restricted pore diameter within a pore. Therefore, it is incapable of assessing pore volume data.

Figure 4.5. a) Pore size distribution for glycerol and fructose fed BC membranes obtained from Capillary Flow Porometry test. b) Wet/dry flow rate behavior of glycerol and fructose fed BC membranes under pressure.
Figure 4. 6. Results of water flux, mean flow pore diameter, and density for glycerol and fructose-fed BC membranes.

Figure 4. 7. Schematic illustration of BC membrane structure cultivated with two carbon sources glycerol (left) and fructose (right).
4.3 Pore Volume Characterization

It is important to note that although Capillary Flow Porometry and Bubble Point techniques are quick and convenient, but they are not proficient in terms of providing direct data regarding the pores volume in solid materials. In fact, both of these methods can describe the most constricted pore size of a through pore while the pore shapes are irregular in most cases.

Other techniques, such as, gas adsorption/desorption, are capable to determine pore volume data from the adsorption and desorption isotherms of a gas subjected to condensation in the pores. However, it is important to remember that gas adsorption/desorption techniques are incapable of discriminating between through pores and blind pores present.

Figure 4.9 shows the nitrogen adsorption-desorption isotherms for various BC membrane samples cultivated with applying different carbon sources in the growth media. The obtained isotherms illustrate that the quantity of adsorbed nitrogen is different for each sample. The fructose and glycerol-fed samples with higher surface area found to have higher nitrogen adsorption than the other samples. It is worth noting from the isotherms that higher surface area results in higher adsorption. The nitrogen adsorption property and surface area of the samples showed a deceasing trend in the following order: fructose > glycerol > mannitol > sucrose > and glucose.

The data on BET surface area, pore volume, and average pore size of the BC membranes are presented in Table 4.3 and Figure 4.8 a in which the surface area and pore volume reveal the same trend. The highest surface area attributed to fructose sample with 66% higher value.
compared to the glucose sample with the least surface area. The pore volume results found to show a declining trend from highest, fructose, to lowest, glucose, by 56%.

Contrary to surface area and pore volume data, pore size results show an opposite trend with highest amount associated with glycerol followed by glucose and sucrose, and then mannitol and fructose samples. Considering Capillary Flow Porometry data, the glycerol sample also has the highest average through-pore size, whereas glucose and sucrose had the smallest one. However, it is important to point out that gas adsorption-desorption assess not only through-pores, but also blind pores and channels between pores which do not participate in permeability properties of BC pellicles and thus are not recognizable by flow porometry techniques.
Table 4.3. Brunauer, Emmett, Teller (BET) surface area, pore volume, and pore size of the BC membrane structures cultivated with five different carbon sources.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET surface area (m²/g)</th>
<th>Pore vol.² (cm³/g)</th>
<th>Average pore diameter ³ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>32±0.35</td>
<td>0.12</td>
<td>82</td>
</tr>
<tr>
<td>Fructose</td>
<td>65±0.45</td>
<td>0.25</td>
<td>82</td>
</tr>
<tr>
<td>Sucrose</td>
<td>31±0.16</td>
<td>0.15</td>
<td>85</td>
</tr>
<tr>
<td>Glycerol</td>
<td>52±0.52</td>
<td>0.23</td>
<td>88</td>
</tr>
<tr>
<td>Glucose</td>
<td>22±0.48</td>
<td>0.11</td>
<td>85</td>
</tr>
</tbody>
</table>

² Barrett, Joyner, Halenda (BJH) adsorption cumulative volume of pores between 17.000 Å and 3000.000 Å width
³ Adsorption average pore width (4V/A by BET)
Figure 4.8. a) Brunauer, Emmett, Teller (BET) surface area, pore volume, and pore size of the BC membrane structures cultivated with five different carbon sources. b) BJH pore size distribution of the BC membrane structures cultivated with five different carbon sources.
Figure 4.9. Nitrogen adsorption-desorption isotherm of BC membranes cultivated with different types of carbon sources.
5. Conclusions

Bacterial nanocellulose membranes are promising three-dimensional nanostructured matrices and have been used for innumerable applications because of their unique green structures along with sustainable and cost-efficient production methods. However, tight pore structures present in the native membrane restricts its practical application. Therefore, in order to tailor the pore size, shape and volume of original nanocellulose membranes, different types of carbon sources in growth culture were applied with all other conditions constant.

Our experiments showed that feeding the bacteria different types of carbon sources under static culture conditions alter the morphology of the bacterial nanocellulose membrane at both the macro- and micro-scales.

Among five different types of applied carbon sources, glycerol- and fructose- fed samples result in the most porous structure with highest pore surface area. The higher inner surface area is a critical feature of the bacterial nanocellulose membranes which adjusts many fundamental properties such as mechanical and thermal stability, fluid permeability, pliability and compactability, sorption properties and even the lifecycle of materials. This work offers a simple effective method to induce porosity in native BC membrane without needing to chemically or mechanically destroying the original unique structure and crystallinity of these nature’s masterwork.
6. Supporting Information

6.1 Thermogravimetric Analysis (TGA) Graphs

**Figure 4.10.** Thermogravimetric Analysis (TGA) graph of bacterial nanocellulose membranes obtained by applying different types of carbon sources in growth culture; weight change as a function of temperature (20 °C/min).

6.2 Effect of Alkali Treatment on Permeability Properties

There are protein, medium ingredients, and bacterial cells in native bacterial cellulose membranes. In general, alkali treatment was used to process the biochemically complex pellicles into clean cellulose membranes. However, the influence of alkali-treatment on filtration rate was studied by preparing two samples soaked in 1M solutions of KOH and NaOH for two days. Then
it was neutralized by soaking in acetic acid 0.5 M solution for an hour and washed several times with deionized water until showing neutral pH.

Our results confirmed that treatment with KOH increases surface porosity, which affects the rate of permeation of water through the membrane perhaps due to higher swelling. NaOH solution causes the cellulose fibrils swell thus, reducing the effective pore size available in the membranes, resulting in reduction in the transmission of water through the membrane.

![Figure 4. 11. Effect of alkali treatment on permeation properties of the BC membranes.](image)

**6.3 Cultivation pH During Nanocellulose Network Formation**

It has been shown that the pH outside the bacteria affects the structure and permeability of the bacteria cell membrane, which can have an effect on cellulose production and network formation. The pH of the bacterial nanocellulose growth medias with different carbon substrates (glycerol, fructose, and glucose) is shown in Figure 4. 12.²⁸
There might be no gluconic acid production during glycerol metabolism, thus the pH remains stable which provided a favorable condition for bacteria growth. Whether this is the principal reason for the great difference in nanocellulose network morphology remains unclear.

Moreover, a cultivation pH below 3 results in no production of cellulose and the proliferation of the bacteria are negatively affected therefore glycerol and fructose are better options in this regard.\textsuperscript{28}
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CHAPTER 5: Water-infused Superoleophobic Bacterial Cellulose Composite Membranes for Effective Separation of Oil-in-Water Micro-/Nanoemulsions

1. Abstract

Oil-water separation has become one of the most urgent global environmental problems because the pollution caused by adventitious leakage and frequent oil spills on land and water precipitated by catastrophic material fatigue and rupture or human errors. In this study, we developed a green superhydrophilic-superoleophobic membrane for oil-water separations based on bacterial cellulose (BC) nanofibers. Our study shows that this antifouling membrane can remove/adsorb water from oil/water nano-/microemulsions with a very high filtration efficiency and helps to isolate and capture the oil fraction. The nanocellulose-based membrane we developed can improve current technologies by increasing efficiency and reducing clogging through the judicious application of an environmentally friendly, renewable nanofibrous cellulose materials.

2. Introduction

Oil–water separation is widely recognized as a sustainable strategy to resolve industrial oily wastewater and oil spill problems. New understanding of the mechanisms of special wetting phenomena in nature during past decades, leads to a biomimetic approach to design and fabricate interfacial materials with superwettability. Development of extreme wetting states, that simultaneously show a combination of superhydrophilicity-superoleophobicity or superhydrophobicity-superoleophilicity for potential use in industrial applications, like oil-water separation and surface cleaning have been reported.
Although fabricating superhydrophobic-superoleophilic surfaces is an easier task due to the higher surface tension of water than oils, these materials face critical limitations for separating oil-water mixtures. First, these materials can be easily fouled and clogged by oil, because of their intrinsic oleophilic nature, which results in a remarkable reduction in separation efficiency and permeation flux and greatly restrict their application in filtration process.

In most cases, the adhered oil is hard to remove and can cause secondary contamination to the environment when discarded. Second, water normally tends to settle below most of the oils due to higher density and forms a barrier layer above the materials. This water layer directly affects oil permeation and filtration performance. Moreover, the oil-sorbent wastes are flammable, toxic and, extremely dangerous. Therefore, the safe handling and recycling of oil removing filters is highly crucial.\textsuperscript{9-11}

Superwetting materials that combine superhydrophilicity and superoleophobicity are one of the most promising approaches to address these problems. However, fabricating materials that simultaneously display superhydrophilicity and superoleophobicity is much more challenging as a result of intrinsic difference in surface tension between water and oil. In fact, most of superoleophobic substrates are inherently superhydrophobic too, because generally oils have lower surface tension than water.\textsuperscript{12}

There are a few number of reports on surfaces designed to possess both oleophobic and hydrophilic chemical compositions to show superoleophobic-superhydrophilic properties in air.\textsuperscript{13-15} For example, Pan et al.\textsuperscript{15} developed superoleophobic and superhydrophilic cotton fabric surfaces through fluorosilanization treatment. The low surface tension of fluorosilanization coating (~11.5 mN m\textsuperscript{-1}) yields oil-repellent properties when in contact with polar liquids such as
water; it was found that surface reconfiguration facilitates the migration of cellulose chains to the surface. Abundant ether bonding (-C-O-C-) and hydroxyl groups (-OH) on the cotton cellulosic polymer chains lead to quick wicking of water droplets over the surface. These surfaces still suffer from poor durability under extreme conditions such as acidic/alkaline environments, physical abrasion, oxidation in air and so forth, which significantly limit their practical applications.

Introducing water into the surface offers an alternative strategy to design superhydrophilic-superoleophobic materials and overcome the above drawbacks. This approach is based on slippery liquid-infused porous surfaces (SLIPS), in which the surface is impregnated with a lubricating liquid, a concept that was broached in 2011.16-18 This nature-inspired young field of research offers an effective and straightforward solution for creating excellent superwetting surfaces.19-21

Herein, we fabricated a filter based on composite of a nonwoven mat and bacterial cellulose (BC) membrane for highly effective separation of nanoemulsions through a facile and cost-effective method. Bacterial cellulose membrane is a green fibrous hydrogel consisting of pristine highly crystalline cellulose nanofibers which shows good mechanical strength, chemical stability in a wide range of pH, and environmental compatibility.22

With respect to oil-water filtration applications, BC membranes are attractive offering a robust, flexible, and slippery liquid infused superhydrophilic-superoleophobic surface. The obtained membrane can potently separate emulsified oil nano-sized droplets with desirable antifouling and recyclability properties. Due to its scalability, the current work can contribute to the development of eco-friendly commercial oil/water emulsion separation membranes with strong stabilities under harsh environments.
3. Experimental Section

3.1 Materials

*Gluconacetobacter hansenii* was purchased from American Type Culture Collection ATCC® 23769™. Mannitol, yeast extract, bacto-peptone (becton, Dickinson and Company), di-sodium phosphate, and citric acid were used for preparing the appropriate culture media. Hydrogen chloride and potassium hydroxide solution 1N (N/10) (Certified) were used to purify membranes. Sterile all-purpose lint-free nonwoven sponges made of highly absorbent rayon/polyester blend was purchased from Covidien. Sodium dodecyl sulfate (SDS), SPAN™ 80, n-dodecane, hexane and were used to prepare oil-in-water emulsions. All the reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated.

3.2 Methods

3.2.1 Bacteria Revival

While there are many strains of cellulose producing bacteria, this work utilized ATCC® 23769™ *Gluconacetobacter hansenii* for all cellulose production. Dried bacterial samples from American Type Culture Collection (ATCC) were revived according to the ATCC protocol. Inoculation media was prepared using the standard mannitol broth. The media suggested by ATCC consists of (w/v): 2.5% mannitol, 5% yeast extract, and 3% peptone in DI water. The mannitol broth media was autoclaved at 121 °C for at least 15 minutes before proceeding. This inoculation medium was used as the seed culture medium.
3.2.2 Fabrication of BC-NW Composite Membrane

The standard Hestrin & Schramm media (HS media) was used consists of the following (w/v): glycerol 2%, peptone 0.3%, yeast extract 0.5%, dibasic sodium phosphate 0.27%, and citric acid 0.114%.23

A culture vessel was filled with 50mL of fresh, sterile HS media. To that was added 5% (v/v) of the seed culture medium. The nonwoven mat was fixed in a custom designed 3D printed holder ring made of PLA. The holder ring template was designed to hold the nonwoven mat firmly while floating on the interface of air and culture media where the bacterial cellulose membrane will form (Figure 5.1). The holder ring and nonwoven mat both were sterilized by spraying ethanol 70% and keeping under ultraviolet light for several minutes before applying in the culture media.

The inoculated vessel containing the fixed nonwoven mat was then placed in a static incubator under atmospheric conditions at 30 °C for 8 days to develop BC pellicles. After this time, the composite membrane of BC and reinforcement nonwoven mat, which we call BC-NW, will form. The BC-NW membrane was removed from the culture media and soaked in KOH 1M bath at room temperature for 48 hours to remove non-cellulose materials such as proteins and nucleic acids residue from bacterial cells and the culture broth.

Once the bulk of the media was removed in alkali solution, the BC-NW membrane was transferred to HCl 0.5M bath to neutralize the base. After an hour neutralization, the cellulose was rinsed repeatedly in DI water until shows neutralized pH. Finally, the membrane was stored in fresh DI water at 4 °C for further experiments.
Figure 5. 1. Schematic picture of fabricating BC-NW composite set up; the nonwoven mat is placed in a custom designed holding ring which can float on the air-liquid interface where BC membrane forms.

3.2.3 Morphology Characterization

Surface and bulk morphology of BC-NW membrane were observed by field emission scanning electron microscopy (FEI Verios 460L, FESEM). Samples were gold-palladium (5 0:50) coated (~15m thickness) with Technics Hummer V Sputter Coater to reduce charge interruptions before obtaining SEM images.
In order to keep the pore structures that are available in the hydrated samples, the samples were freeze-dried in a FreeZone freeze-dryer from LABCONCO at < 50 Pa and -50°C for at least 48 h without any previous pre-freezing.

### 3.2.4 Pore Size Characterization

The flow pore diameter distribution along with the maximum flow pore diameter (bubble point) within the obtained membrane was determined using capillary flow porometry (Porous Materials Inc, CFP-1100_AEL). The samples were dried in a freeze-dryer at < 50 Pa and -50 °C for at least 48 h without any pre-freezing to maintain pore structures. Then, a fully wetted sample in Galwick solution with known surface tension of 15.9 Dynes/cm, was placed in the sample chamber and the chamber was sealed.

Nitrogen gas was then allowed to flow into the chamber behind the sample. The bubble point has been found when the pressure reaches a point that can overcome the capillary action of the fluid within the largest pore. The bubble point is defined by the ASTM F-316-03 standard as the pressure at which the first continuous gas bubbles are detected, this measurement corresponds to the largest or maximum pore size available in the membrane.

After determination of the bubble point, the pressure is continuously increased, and the flow is measured until all pores are empty from Gatwick solution. At this point, the sample was considered dry by the instrument. The mean flow pore size corresponds to the pore size calculated at the pressure where the wet curve and the half dry curve meet.
Moreover, to further investigate the through pores within the membrane, 200 ml of deionized water was passed across the membrane under gravity. The pure water flux \( J, \text{ L m}^{-2} \text{ h}^{-1} \) was calculated as follows:

\[
J = \frac{V}{At}
\]  

where \( V \) is the volume of the water filtered (L), \( A \) is the effective filtration membrane area (m²), \( t \) is the filtration time (h).

### 3.2.5 Surface Roughness and Topography

The variation of porosity and surface topography of the membrane was investigated by the AFM (MFP-3D Origin+, Oxford instruments, USA) in a non-contact mode using a silicon cantilever with resolution on the order of fractions of a nanometer for scanned area of 1 and 100 µm². Before AFM measurements, the membranes were freeze-dried as explained earlier. For each membrane, the topographic images (2D as well as rendered 3D), surface mean roughness, root mean roughness (RMS), and cross-sectional line scan were obtained.

### 3.2.6 Contact Angle Measurements

The water contact angles and underwater oil contact angles were measured by Data-Physics Contact Angle System OCA 15EC at ambient temperature. The oil (n-dodecane) drops (3µL) were dropped carefully onto the materials surface, which were immersed in water. The average
value of three measurements performed at different positions on the same sample was adopted as the contact angle.

To study the chemical stability of the BC-NW membranes in harsh pH environments, the oil contact angle of the membranes was measured after being in strong acidic (pH = 2), and basic (pH = 11) solutions for more than three weeks.

### 3.2.7 Filtration Experiments

The wet purified BC-NW membrane was tested for oil in water filtration. The surfactant-stabilized oil-in-water microemulsions were prepared by mixing oil (hexane) and deionized water in a volume ratio of 1:99 with addition of 0.4 mg/ml SDS under sharply stirring for 24 h.

The surfactant-stabilized oil-in-water nanoemulsions were prepared by mixing the oil phase containing 0.4 mg/ml SPAN™ 80 with the water phase containing 0.4 mg/ml SDS. The mixture was stirred for 24 h and diluted 100× for dynamic light scattering measurement tests. All the surfactant-stabilized water-in-oil nanoemulsions were stable for more than 1 week and no demulsification or precipitation was observed.

The filtration performances were carried out with a dead-end filtration assembly (Kontes 47mm). The whole process is driven under gravity as well as different external pressures. Micrographs of the feed solution and the filtrate solution of oil-in-water microemulsions were obtained by optical microscope Nikon SMZ-1000 Zoom. The oil contents in the filtrates obtained from each microemulsion filtration experiments were measured by total organic carbon analyzer by Shimadzu Scientific Instruments, TOC-VCPN. Malvern Zetasizer Nano was employed to
measure dynamic light scattering for each filtrate collected from nanoemulsion filtration experiments. The filtration efficiency was defined as below equation.

\[
\text{Filtration efficiency (\%)} = (1 - \frac{C_f}{C_o}) \times 100
\]  \hspace{1cm} (2)

where \( C_f \) (mg) is oil concentration of filtrate and \( C_o \) (mg) is oil concentration of original oil-in-water emulsions.

4. Results & Discussion

4.1 Morphology of BC-NW Composite Membrane

BC membranes have a three-dimensional nanofibrous structure which can trap an abundance of water molecules. This unique hydrophilic nonwoven structure along with the water molecules present in it offer all the characteristics required for a liquid infused porous surface system. Like fish skin, whose rough scales are made of hydrophilic calcium compounds,\(^{24,25}\) contribute in a cascading formation of a third repulsive liquid phase, here we have long, randomly distributed entangled hydrophilic nanofibers that exhibit an extraordinarily high surface area for H-bonding with water molecules. This leads to the formation of a smooth repulsive water-impregnated superhydrophilic and superoleophobic membrane.

As shown in Figure 5.2 the mean diameter of BC cellulose nanofibers in the composite membrane is $35 \pm 17$ nm, creating small pores with diameter on the order of several microns. The cross-section surface morphology of the BC-NW membrane was observed by FESEM
images. The cross-section images (Figure 5.2 a) show that most of the bacteria used the nonwoven mat as a scaffold to reach to the air, thus the most BC nanofibers are located at the top portion of the composite membrane. However, bacteria also secreted cellulose nanofibers around and into the bulk structure of the nonwoven reinforcement layer. As clearly shown in Figure 5.2 b, there is enough entanglement between the microfibers of nonwoven layer and BC cellulose nanofibers to keep this composite membrane integral.

Even a thin layer of BC nanofibers in the structure of composite membrane is enough to hold water layer in place and repel oil molecules. It has been well discussed that the membrane thickness has a negative influence on the flux, according to the below equation: \[ J = (P_m/L) \times \text{driving force} \] 

where \( j \) is flux, \( P_m \) is the permeability, and \( L \) is the membrane final layer thickness. Our preliminary experiments confirmed that BC nanofibrils are densely packed and thus increasing the thickness of the layer composed mostly of BC nanofibers, would decrease the filtration flux significantly. Therefore, as far as it does not sacrifice filtration efficiency, the BC layer should be as thin as possible.

Previous works reported that the thickness of the BC membranes can be controlled by adjusting culture condition parameters such as carbon sources, inoculation volume, and cultivation time. In a recent attempt, Gross et al. described that by employing very shallow broth cultures air-dried temperature BC films with thickness of under 1 μm can be formed. Still the
major issue which limits application of ultrathin BC membrane are the difficulties to handle and expand it. In fact, upon handling, the ultrathin BC membrane would fold on itself and stick.29

In this work, the fabricated BC-NW composite membrane was composed of a very thin layer of BC nanofibers with a thickness of below 200 µm in freeze-dried format. Freeze-drying maintains all the pores structure while in an air-dried membrane all the pore structures are collapsed, and the thickness decreased remarkably. For instance, the thickness of the same air-dried BC membrane was less than 50 nm.

This micro-thick hydrophilic nanofibrous layer captures water by formation of hydrogen bonds between water molecules and numerous hydroxyl groups available along the cellulose polymeric backbone and thus prevents oil molecule permeation. In fact, the reinforcement nonwoven mat plays no role in the separation itself, but as a supporting scaffold to facilitate fabrication and carrying a smooth, uniform, and ultrathin BC membrane which can efficiently serve in separating the oil phase even from nanoemulsions.
Figure 5.2. FESEM micrographs of the BC-NW membrane cross-section. a) scale bar 500 µm. b, c, d) bottom portion of the membrane composed of BC nanofibers entangled into and around the nonwoven microfibers with scale bar of 200, 50, and 20 µm, respectively. e, f) top portion of the membrane mainly composed of BC nanocellulose fibers with scale bar of 10 and 2 µm, respectively.
4.2 Pore Structures Determination

The characteristics of membrane pore structures such as pore size distribution, pore density, and surface roughness govern the filtration properties of membranes. Three main types of pores have been defined in order to obtain accurate descriptions and quantitative analysis of how a membrane will behave in certain situations.

Pore types according to their availability in the surroundings are classified as through pores, blind pores, and closed pores. Through pores travel all the way from upstream side of the membrane to the downstream side and determine transportation and filtration performance. Blind pores are accessible from one exterior while closed pores are completely entrapped within the structure of the membrane as such there is no permeation through these pores and they just contribute to mechanical properties of the membrane such as strength, weight, etc.\textsuperscript{30}

\textbf{Figure 5. 3.} Schematic of pores classification according to their availability to surroundings.
In this work, capillary flow porometry was used to determine the pore sizes distribution of through pores within the BC-NW composite membrane. Note that membrane pores tend to be very non-uniform and capillary flow porometry detects only the most constricted pore diameter. The result of capillary flow porometry for BC-NW samples are presented in Figure 5.4.

**Figure 5.4.** The result of capillary flow porometry test; (a) The differential pressures and gas flow rates through dry and wet BC-NW composite sample. (b) Pore size distribution of the BC-NW composite membrane. (c) The result of measured mean flow pore diameter, mean flow pore pressure, and bubble point for BC-NW composite and BC counterpart membranes.
Capillary flow porometry results showed that the flow rate of the liquid/air is directly proportional to the applied pressure. Moreover, BC-NW composite membrane shows high pressure stability and can easily withstand 100 psi without any rupture or burst. The fact that BC-NW composite membrane maintains its mechanical integrity under high operating pressures is promising to target real-world applications.

During the capillary flow porometry measurement, gas pressure is gradually increased and emptied the membrane’s through pores of liquid from biggest to smallest. The gas pressure at which the first stream of rising bubbles is emitted, is considered as the bubble point which is correlated to the largest pore of the membrane (Figure 5.4 c). Although, the bubble point for BC counterpart sample is higher than the BC-NW composite membrane, the overall average diameter of the through pores of the BC-NW composite membrane is much higher, nearly 63%, and displays a more uniform pore size distribution. Figure 5.4 b illustrates the size distribution of the through pores in BC-NW composite membrane.

Membranes with higher mean pore diameter requires less pressure to let the liquid to go through them. In fact, introducing the nonwoven mat in the culture media, caused more open pores in the structure of BC membrane which makes it even more desirable for applications other than separation such as tissue engineering and wound dressing.33

To further study the accurate topographical of the membrane surface, Atomic Force Microscopy (AFM) was utilized to produce magnified surface images of both BC-NW and the counterpart BC which is shown in Figure 5.5. The surface morphology of the BC-NW composite membrane reveals a remarkable difference with its counterpart. It shows higher surface nanoroughness, while the counterpart BC membrane displays a more linear and smoother pattern.
The root mean square (RMS) surface roughness increases from 17.0 nm for BC membrane to 278.4 nm for BC-NW composite membrane. This can be explained by the interaction of the nonwoven template with the bacterial cells. Such an interaction may be changed by the direction of the bacteria cells. When the bacteria cells are free and not in contact with other substrates, they tend to rotate around a self-axis and secret cellulose nanofibers in the form of a twisted ribbon.\textsuperscript{34} However, contact with the nonwoven substrate trigger for an alter in the movement direction of the bacteria. This concept can be extended to design patterned structures.

Figure 5. 2D and 3D AFM topographical images of the surface of (a) BC-NW composite membrane. (b) counterpart BC membrane.
4.3 Separation of Oil-in-Water Micro-/nanoemulsions

The superhydrophilic and underwater superoleophobic characteristics of the BC-NW composite membrane favors its ability for oil–water separation. Chemical composition and high specific surface area of nanocellulose fibers favor trapping a large amount of water which acts by itself as a repellent film enables to prevents oil-wetting and consequent fouling. Water penetrates quickly into the unique three-dimensional porous network of the membrane, makes them flexible, yet robust enough to prevent oil molecules from penetration.

To evaluate the separation performance of the BC-NW composite membrane, surfactant stabilized oil-in-water microemulsions with oil droplet sizes in the range of 1–20 μm, as observed by optical microscopy, were used (Figure 5.6 d). The separation process was carried out via a dead-end filtration assembly under gravity as well as different external pressures. All emulsions could be successfully separated by passing through the composite membrane once with high separation efficiency. BC-NW membrane could even trap most of the water-soluble dyes that was used for visualizing the water phase of the microemulsion solution as shown in Figure 5.6 e, f.
Figure 5.6. a) microemulsion separation experiment running in a dead-end filtration assembly. b, c) feed and filtrate solutions and corresponding optical microscopy images of oil droplets in each of them, respectively with scale bar 50 µm. d) graph of oil droplet size distribution available in the microemulsion feed solution. e, f) BC-NW composite membrane before and after the separation experiment, respectively.

To further investigate the separation capacity of the BC-NW composite membrane, surfactant stabilized oil-in-water nanoemulsions (n-dodecane-in-water) with oil droplets of hundreds of nanometers were used to permeate through the membrane. The Tyndall effect was clearly observed as a laser light beam passes through the oil-in-water nanoemulsion, indicating the presence of oil droplets. Oil droplets scatter and reflect the laser beam making them visible in the feed solution, while there are no oil droplets left in the collected filtrate.
The results were confirmed by the Dynamic light scattering (DLS) measurements shown in Figure 5.7. The DLS graph specified that the oil droplet size of the nanoemulsion feed is mostly distributed around 200 nm. It worth noting that the sharp peak appearing around 7 nm in collected filtrate sample could be attributed to by the residual surfactants that pass the filter.

**Figure 5.7.** a) Digital image of nanoemulsion and corresponding filtrate while a 650 nm laser illuminated the samples from one side. b, c) DLS curves of the oil droplet size distributions of the oil-in-water nanoemulsion before and after separation by a BC-NW composite membrane.

The permeability of pure water through BC-NW composite membrane was measured. As shown in Figure 5.8, water flux was increased linearly with changed in feed pressure from 0 pressure,
driven solely by gravity, to 20 psi. The filtration efficiency results showed that the BC-NW membrane shows no significant reduction in filtration efficiency under pressure.

**Figure 5.8.** Influence of the pressure on the water flux and filtration efficiency of BC-NW composite membrane.

### 4.4 Antifouling Properties and pH-stability of BC-NW Composite Membrane

The stability under harsh conditions and anti-oil fouling performance of the oil-water filters are critical parameters for practical applications. Severe fouling can adversely affect the efficiency and the lifetime of the filters. The water-infused superoleophobic layer of the BC-NW membrane shows no affinity for oil droplets while displaying an underwater oil contact angle of 173.5 ± 2.5° to prevent the membrane from fouling by oils. Figure 5.9 shows the underwater dynamically
oil-adhesion test which was measured by pushing an oil droplet against the BC-NW composite surface. The oil droplet did not deform and leave any residue on the surface of the membrane during the entire process.

![Image of oil droplet adhesion test](image)

**Figure 5.9.** Underwater static contact angle for an oil droplet (n-dodecane) on the water-infused BC-NW composite membrane.

To further study the resistance of the membrane to oil fouling, the variation of permeation flux was monitored by measuring the pure water flux of the membranes before and after filtration by calculating flux recovery ratio (FRR) as defined below: $^{35}$

$$FRR = \frac{J_2}{J_1} \times 100$$  \hspace{1cm} (4)

where $J_2$ is water flux of the membrane after separation experiment, and $J_1$ is water flux of the membrane before separation experiment (initial water flux). After each cycle was completed the membrane was simply rinsed with a certain amount of deionized water in order to remove any oils on the surface.
Figure 5.10. Flux recovery ratio for different types of oil-in-water surfactant free mixtures and surfactant stabilized micro-/nanoemulsions.

The results confirmed the anti-fouling properties of the BC-NW composite membrane. Contrary to conventional oleophilic and hydrophobic membranes which suffer from easy-fouling and hard-recycling limitations, this water-removing type of filter makes recycling of oil and materials easy.

To study the resistibility of BC-NW composite membrane, it was kept in various corrosive solutions with a pH of 2 and 11. After twenty days of immersion, contact angle measurements were employed to study the change of the wettability properties of the membranes. As shown in
Figure 5.11, the underwater oil contact angles are still all over 150° while the water contact angles are all maintained zero after this immersion period. The outstanding pH-stability of the composite membrane is greatly beneficial for treating organic contaminated wastewater in highly acidic or basic conditions.

![Figure 5.11](image.png)

**Figure 5.11.** pH-stability and antifouling property of the BC-NW composite membrane. Water CA and underwater oil CA of the membrane surface after the it was kept in water solutions with pH from 2 to 11 for > 20 days.
5. Conclusions

Bacterial cellulose has proven to be a remarkably versatile bio-polymer gaining attention in a wide variety of applied scientific applications such as electronics, biomedical devices, and tissue-engineering. However, difficulties to handle and expand a thin layer of BC membrane limit its applications. To address this issue, BC-NW composite membranes with special superoleophobic/superhydrophilic wetting behavior were fabricated. We have demonstrated that the network of BC-NW composite matrix shows powerful water trapping capacity which makes it an ultralow oil-adhesion material. Taking advantage of the underwater superoleophobicity, a BC-NW membrane was successfully used for oil–water separation.

Our water infused system showed an extremely high separation efficiency and anti-oilfouling properties even for separating surfactant-stabilized micro-/nanoemulsions. The simple strategy of the current method for fabricating three-dimensional oil-repellent structures during biosynthesis makes it possible to design BC-NW membranes with desirable geometrics whose potential applications will emerge in a number of avenues such as droplet-based microreactors, microfluidic devices, and bio-detection.
6. Supporting Information

6.1 Oil-in-Water Micro-emulsion Separation

Figure 5.12. Microemulsion separation experiment running in a dead-end filtration assembly

6.2 Capillary Flow Porometry and Bubble Point test Procedure

Pores of membranes were filled with low surface tension liquid (Galwick). A non-corrosive gas used to extrude wetting liquid from pores. First, the largest pore empty (bubble point), smaller pores tend to empty as pressure increases. Flow rate, differential pressure, and wetting liquid surface tension yield the mean flow pore diameter and pore distribution.
Figure 5.13. Mechanism of gas permeation during the gas flow measurement of wetted membrane: (a) no convective flow before bubble point, (b) bubble gas formation and release from the largest pore, (c) only some pores contributing to gas flow, (d) gas permeation from all pores.
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CHAPTER 6: Conclusions

1. Overall Conclusions and Future Outlook

With respect to filtration applications, cellulose is attractive because it is inert, harmless, hydrophilic, resistant to a wide range of pH (between 2 and 11), thermally stable (i.e., sterilized by autoclaving), cost efficient, and disposable.

The structural features of bacterial nanocellulose matrices are far superior to those of plant cellulose. The ultra-fine network structure of bacterial cellulose membrane consists of well-arranged three-dimensional nanofibers with high surface area and porosity. Moreover, the crystallinity, mechanical strength, and water-binding capacity of bacterial cellulose membranes are higher than those of plant cellulose. They also possess higher chemical purity because they do not contain pectin, hemicelluloses, or lignin.

Bacterial nanocellulose fibrous matrices can overcome the drawbacks of the textured surfaces classified under Cassie/Wenzel states by employing a strategy known as liquid infused surfaces. In fact, the separation functions of these water impregnated membranes originate from two important features: first, the three-dimensional network of BC membranes can microscopically be envisioned as “porous” structure, having a tunable mesh-size in the nanometer range; second, trapping water molecules firmly inside their three-dimensional structures by inducing chemical and physical bondages which acts by itself as a robust repelling substrate. Thus, bacterial nanocellulose matrices offer an alternative for nonfouling, superhydrophobic-superoleophobic membranes for oil-water separations.
As demonstrated in chapter 3, nanocellulosic membrane engineered by bacteria by a static cultivation method displayed superhydrophilicity and superoleophobicity with ultralow oil-adhesion properties. We have discussed the fundamental underpinnings of how these properties arose from (1) the unique three-dimensional porous network of a bacterial membrane made up of cellulose nanofibers that were able to provide an extraordinarily high surface area for trapping water molecules and (2) the native hydrophilicity of the cellulose because of the large amount of hydroxyl groups on the backbone of the cellulose polymer chains.

There are several parameters, which can influence the efficiency of the filtration process of an oil-water mixture/emulsion such as oil droplets size, filter pore size distribution, filter thickness, and pore tortuosity.

In the case of water infused bacterial nanocellulose matrices, due to the presence of inherently smooth and defect-free layers of water, the membrane can repel immiscible liquids of virtually any surface. Simultaneously, the stabilized water layer within a porous support BC matrix acts as a reconfigurable gate which selectively allow the water phase of the feed stream to pass through.

During our preliminary experiments, we realized that the water permeation through BC membranes is not optimal. Therefore, as reported in Chapter 4, in order to improve water permeability properties of BC membranes different types of carbon sources in growth culture were applied with all other conditions constant.

Pore characterization techniques such as capillary flow porometry, bubble point method, gas adsorption-desorption technique as well as visualization techniques such as scanning electron and atomic force microscopy were utilized to investigate the morphology and shapes of the pores within the membranes.
Our experiments showed that feeding the bacteria different types of carbon sources under static culture conditions altered the morphology of the bacterial nanocellulose membrane at both the macro- and micro- scales. Among the five different types of applied carbon sources, glycerol- and fructose- fed samples result in the most porous structure with highest pore surface area.

Future work should consider altering pore volume of bacterial nanocellulose membrane by altering other culture conditions such as cultivation time, amount of inoculation volume, depth and ratio of the surface to the volume of the inoculation medium, and post-treatment methods.

Another approach to maximize water flow rate through the membrane for separation applications was to minimize the thickness of the membrane without jeopardizing filtration efficiency combined with ease of handling. Therefore, in Chapter 5, we reported a facile and cost-effective method for fabrication an ultrathin composite filter based on a nonwoven mat and bacterial cellulose membrane during biosynthesis.

The BC-NW composite membrane showed an extremely high separation efficiency and anti-oil fouling properties even for separating surfactant-stabilized micro-/nanoemulsions. The simple strategy of the method makes it possible to design BC-NW membranes with desirable geometrics which we believe that potential applications will emerge in the wide regions, such as droplet-based microreaction, microfluidic devices, and bio-detection.

We believe that the results from these studies will contribute to the basic understanding of supper-wettability and super-antiwettability of bacterial cellulose matrices and other liquid infused surfaces. The superior special wettability properties of these materials fulfill fundamental and practical application requirements from repellency for a wide range of liquids and
immiscible liquid separation to solid repellency such as ice, insects, and dust, anti-bioadhesions, and droplets manipulation and collections.

2. **Recommendations for Future Research**

During biosynthesis bacterial nanocellulose we have noticed that applying different types of carbon sources in the inoculation media not only changes the morphology of the cellulosic matrices, but also alter the optical properties of the membranes such as reflectance and transmittance. This opens the way for developing BC membrane for optical applications such as interference-type antireflective coatings for reducing reflectance in light emitting diodes (LEDs), solar cells, eyeglasses, windows, and so forth.

Pore size distribution present in the bacterial cellulose membrane is promising for virus filtration applications especially for large viruses ≥ 50 nm and could be improved further to include small viruses too, for example, by the methods suggested in this study adjusting membrane pore size, pore tortuosity, and thickness is feasible.

In addition, bacterial nanocellulose structure provide suitable base materials for fabricating pore-filling functionalization membranes which can be used for nanofiltration separation of ions and molecules. Pore-filling functionalization membranes can have an additional impact on the separation because the sieving behavior may be adjusted using external stimuli, typically pH or temperature. In this regard, studying the dialyzing capacity (ion passage/concentration) of bacterial cellulose membranes might be another interesting idea in order to perform purification or buffer exchange for samples containing macromolecules.
Moreover, a follow up project could also include studying the idea of tuning the filtration by use of organic, water miscible solvents (e.g. glycerol). Customizing the intermediary liquid layer of bacterial nanocellulose matrices is a promising approach in order to design a repellent substrate capable to repel liquids with a broader range of surface tensions.
Appendix A

1. Bacteria Revival; ATCC 23769™ Gluconacetobacter Hansenii

Dried bacterial samples from ATCC were revived according the protocol. Briefly, for each order, the outside of vial was sanitized by spraying ethanol 70% in a sterile field, then thermally shattered to release the contents. Then, the insulation and inner vial were carefully removed to retrieve just the inner vial. The cotton plug from the inner vial also gently was removed with a cleaned and sterilized tweezers. The inner vial containing the bacteria was filled with 1mL of sterile Mannitol Broth as presented in Table A.1.

<table>
<thead>
<tr>
<th>Table A. 1. ATCC Medium: Mannitol Broth.</th>
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<tbody>
<tr>
<td>Ingredient</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Yeast Extract</td>
</tr>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>Mannitol</td>
</tr>
<tr>
<td>DI water</td>
</tr>
<tr>
<td>Autoclave at 121 °C</td>
</tr>
</tbody>
</table>

1mL of the resulting bacterial suspension was transferred into a sterile 50mL Erlenmeyer flask containing 30mL of Mannitol Broth. The Erlenmeyer flask was placed in an isopropanol-cleaned shaking incubator under atmospheric conditions at 110 RPM at 26°C for about 2 days. Note that
cap should be used to close the flask however since the bacteria are obligate aerobe, caps should be screwed loose. This applies to autoclave and propagation steps too.

Figure A. 1. Steps of opening a vial.
2. Propagation Procedure of ATCC 23769™ *Gluconacetobacter Hansenii*

Inoculation vessels were filed with the standard Hestrin & Schramm Media (HS Media). The media consists of 20g (glucose/mannitol/sucrose/fructose/glycerol), 5g yeast extract, 3g Bacto-Peptone, 2.7g dibasic sodium phosphate, and 1.14g citric acid per liter. Each vessel was sterilized in an autoclave at 121°C for 30 minutes before inoculation. Then 5% (v/v) of the bacteria suspension obtained from bacterial revival step was added to each vessel. Incubate the vessels in static condition under atmospheric conditions at 26-30°C until the basic pellicle forms. Because the base pellicle does not form as uniformly as subsequent layers, it will be used as the inoculation pellicle for later batches.

Since pellicle production accommodates any shape of a liquid-air boundary, other vessels were experimented with to produce pellicles of various dimensions.

![Figure A. 2. Bacterial nanocellulose membrane with large dimensions.](image-url)
Appendix B

1. Synthesizing Bacterial Cellulose – SCOBY Grade

Cultivation of bacterial cellulose is not new; in fact, strains of the bacterial family have been passed down from generation to generation as a means to produce vinegar for thousands of years. Apart from using ATCC bacteria strains, bacterial cellulose membranes were synthesized using SCOBY (symbiotic colony of bacteria and yeast) as a very simple and cost-effective method. SCOBY is less sensitive to infectants compared to ATCC strains and needs less ingredients and equipment.

Green tea and cane sugar were used as nitrogen and carbon sources, respectively. The inoculation process was carried out at room temperature under static conditions. It required a cultivation period of 3-5 days to see the basic pellicles. The growth occurred only at the interface of the medium and air. Table A.2 shows the medium used to grow BC membranes based on the above method.

**Table A. 2. Growth medium for synthesizing bacterial cellulose – SCOBY grade**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCOBY</td>
<td>1 (ea.)</td>
</tr>
<tr>
<td>Sugar</td>
<td>50 (g)</td>
</tr>
<tr>
<td>Green Tea</td>
<td>3 (g)</td>
</tr>
<tr>
<td>Vinegar</td>
<td>2-3 (ml)</td>
</tr>
</tbody>
</table>
In the first step, bring to boil distilled water. Once the water comes to a boil add sugar and let it stir until the sugar is dissolved. Turn off the heat and add green tea (tea bags/loose tea). After 10 minutes remove the tea and allow the brewed sweet tea solution to cool to room temperature. If the temperature is too high the bacteria cultures can be killed.

After the solution has sufficiently cooled and reached to the room temperature, it is ready to be inoculated with the SCOBY cultures. But before doing that, the vinegar should be added to adjust the pH of the growth culture. The starting pH should be below 4.5, keep adding vinegar until the correct pH is obtained. This will insure that the pH is low, and the cultures will be able to compete with any foreign cultures or molds that may be present. Then place the SCOBY mushroom while the smooth white/creamy side is upwards. Cover the inoculation vessel with a tight weave cloth and use an elastic to keep it in place.

Once the solution is inoculated, set it in a warm place (between 70 and 85 °F degrees), out of direct sunlight and undistributed for 5 to 10 days. This will allow the fermentation process to complete its cycle. After the 5-10 days, the new mushroom will form on top of the tea solution. Set aside SCOBY(s) for the next batch from the top of the current medium.
To remove non-cellulose materials, BC pellicles were treated with 1N NaOH for 1.5 h at 80 °C and dried at room temperature. To determine the effectiveness of this method, we visualized the pellicles using SEM (Figure A.4). All the bacteria cells and other residue from BC could not be removed by this method.

Figure A. 3. Picture of a SCOBY.
Figure A. 4. SEM images of SCOBY-grade BC pellicles 1) before 2) after alkali treatment.
2. Filtration Experiments with SCOBY Grade Membrane

The wet purified bacterial cellulose was tested for oil/water filtration. In the first experiment, to show the oleophobic characteristic of BC, a BC membrane in wet state was placed between the clamps and machine oil was poured on top of the BC membrane. To create a visual contrast between the water and oil, the oil used in these experiments was dyed red by adding oil red-o dye and the water was dyed blue by using methylene blue.

Using the vacuum pump (pressure: 10 psi), the oil was tried to permeate through the BC for 30 minutes. However, due to very fine fibrous network of BC and presence of water molecules in the BC network, oil molecules were not able to permeate through it.

![Image of oleophobic characteristic of SCOBY grade BC membranes.](Image)

**Figure A. 5.** Oleophobic characteristic of SCOBY grade BC membranes.

In the second step, an oil-water mixture was added in the filter flask connected to a vacuum pump (pressure: 10 psi). As BC membrane is highly hydrophilic, it allowed the water molecules to penetrate through it, while preventing the oil molecules from passing. Figure A.6 shows the oil
and water after 15 minutes of filtration under 10 psi vacuum pressure. These results clearly indicate that the oil can be effectively separated from oil/water mixture with this membrane.

![Figure A. 6. The oil/water mixture filtration efficiency of BC membranes.](image)

(A)  (B)  (C)  (D)