Tomas Bata University in Zlín
Centre of Polymer Systems

Doctoral Thesis Summary

Kombucha leather: Preparation and Characterization

Kombucha kůže: Příprava a charakterizace

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ABSTRAKT

Kombucha kůže je kůže odvozená z bakteriální celulózy vyvinutá jako veganská kůže, která dobře reaguje na očekávání spotřebitelů, snižuje emise znečištění kožedělného průmyslu a také přeměňuje biologické odpady na užitečné materiály. V této dizertační práci byla kombuchová kůže připravena kombinací polymerů a celulózy získané z fermentace kombuchy za použití bioodpadů. Ve výrobním kroku bakteriální celulózy odvozené z Kombuchy (KBC) kyselé syrovátky, odpadní jablečná šťáva a pivovarské mláto vynikající účinnost v biosyntéze celulózy u *Komagataeibacter xylinus* s vynikající suchou hmotností 12,81 g/L. Optimalizace fermentace pak dosáhla vynikající suché hmotnosti KBC (20,14 ± 0,62g/L) doprovázené maximalizací množství odpadu potřebného ke léčba. Při aplikaci na velké nádoby byla nejzodpovědnější fermentační dávka získána při hloubce kultivačního média 0,5 cm a nízkém objemu suspenze zbytkových bakterií pouze 72,31 ± 8,74 mL. Charakteristiky KBC nevykazují žádné významné rozdíly pro všechny vzorky ve srovnání s bakteriální celulózou ze standardního média HS. Ve fázi výroby kůže Kombucha vykazovala rohož podobná kůži na bázi KBC/PU/PLA pozoruhodné mechanické vlastnosti. Zejména KBC ošetřeny dimethyldichlorsilanem, hexadecyltrimethoxysilanem, vinyltriethoxysilanem a 3-aminopropyltriethoxysilanem, výrazně zlepšily hydrofobnost a také zlepšily kompatibilitu nebo homogenní smíchání pro poskytnutí stabilní struktury pro tuto materiál na bázi KBC ošetřenou silanem. V konečném důsledku byly přísady a podmínky Kombucha kůže optimalizovány s vynikajícími hodnotami modulu pružnosti, biologické odbouratelnosti a úhlu kontaktu s vodou, v tomto pořadí, dosažených na 44,07 ± 0,51 N/mm^2 , 1,31 ± 0,04 %, 94,84 ± 1,59^0 z optimální rohože kůže obsahující KBC (13,74 % hmotn./hmotn.), polyuretanový elastomer (73,89 % hmotn./hmotn.) a kyselinu polymléčnou (12,50 % hmotn./hmotn.), lisované při 155 °C po dobu 5 minut. Její morfologie, chemická struktura, tepelná stabilita, mechanická pevnost a biologická odbouratelnost byly charakterizovány a porovnány se stávajícími komerčními usněmi. Výsledky v zásadě ukazují možnou reakci na základní požadavky této Kombucha kůže, která se může uplatnit v obuvi, taškách nebo potahových produktech interiér.

Klíčová slova: *Kombucha kůže, Veganská kůže, Bakteriální celulóza získaná z Kombuchy, Bakteriální celulóza, Optimalizace, Návrh experimentu.*

ABSTRACT

Kombucha leather is bacterial cellulose-derived leather developed as vegan leather possibly well-respond to consumers' expectations, reducing pollution emissions of the leather industry, and also transforming the bio-wastes into useful materials. In this doctoral thesis, Kombucha leather was prepared via the combination between polymers and cellulose harvested from kombucha fermentation using bio-wastes. In Kombucha-derived bacterial cellulose (KBC) production step, sour whey waste, waste apple juice, brewer's spent grains all displayed the brilliant efficiency in cellulose biosynthesis of *Komagataeibacter xylinus* with superiority dry weight at 12.81 g/L. The fermentation optimization has then achieved an outstanding KBC dry weight (20.14 ± 0.62 g/L) accompanied by maximizing the amount of treatment-required waste. Applying on the large containers, the most responsible fermentation batch was obtained at the cultured medium depths of 0.5 cm and low residual bacteria suspension volume of only 72.31 ± 8.74 mL. The characteristics of KBC showed insignificant differences for all samples compared to bacterial cellulose from HS standard medium. In Kombucha leather fabrication phase, leather-like mat based on KBC/PU/PLA exhibited remarkable mechanical properties. Especially, KBC were treated with dimethyldichlorosilane, hexadecyltrimethoxysilane, vinyltriethoxysilane, and 3-aminopropyltriethoxysilane have spectacularly improved hydrophobicity, as well as enhancing compatibility or homogenous blending to provide a stable structure for this silane-treated KBC-based leather mat. Ultimately, the ingredient and condition of kombucha leather preparation were optimized with outstanding values of elastic modulus, biodegradable and water contact angle respectively reached at 44.07±0.51 N/mm^2, 1.31±0.04 %, and 94.84±1.59 ^{0} from optimum leather-like mat containing KBC (13.74 % w/w), polyurethane elastomer (73.89 % w/w), and polylactic acid (12.50 % w/w), compressed at 155 °C for 5 min. Its morphology, chemical structure, thermal stability, mechanical strength, and biodegradability were characterized and compared to existing commercial leathers. Basically, the results show a possible response to the essential requirements of this Kombucha leather that prospective application in footwear, bags, or interior covering products.

Keywords: *Kombucha leather, Vegan leather, Kombucha-derived bacterial cellulose, Bacterial cellulose, Optimization, Design of Experiment.*

CONTENTS

1. INTRODUCTION

1.1 Leather and pollution sources of leather processing

Leather is a strong and durable material that possessed unique properties remarkable toughness, flexibility, elasticity, breathability, corrosion resistance, waterproofness, and longevity [1, 2]. Leather industry supplies a wide range of consumer goods in fashion, footwear, bags, auto accessories, furniture, covering products, and current towards multifunctional materials namely conductive leather, flame retardant leather, self-cleaning leather, antibacterial leather, oil-proof leather, electromagnetic and X-ray shielding leather [2-5]. Nevertheless, leather processing caused a multitude of wastes, such as animal meat, feathers, debris, exhaust gases, volatile solvents, and wastewater containing harmful chemicals (i.e chromium, tannins compounds, oils, biocides, detergents) [5-14] .

Recently, the price of leather is continuously increasing plus the constant pressure from animal rights groups (PETA), has led to strict regulations on leather preparation and leather products [15]. In several countries, wastewater from tanneries has been subject to general industrial discharge laws or an obligatory standard [5, 10]. Among efforts to solve related difficulties of the leather industry [6, 9-11, 13, 14, 16-18], the reuse approach of bio-waste has shown promising to develop the eco-friendly and animal-free alternative leathers, such as fruit-based leathers, waxed cotton leathers, wood leathers, mushroom-derived leathers, bacterial cellulose-derived leathers, or Kombucha leathers [1, 18-26].

1.2 Kombucha leather

Kombucha leather is a bacterial cellulose-derived leather, a type of vegan leather that resembles leather but is not made from animal skin. Kombucha leather possesses both the value of preserving ecosystem, protecting animals, and requires very little space, water, chemicals to produce [1, 15, 20, 25, 27]. It is

possilbly applied in footwear, handbags, apparel, interior design and decoration [19, 21, 28]. Kombucha leather was prepared by directly culturing cellulose synthesis bacterial strains in the fermented media containing natural dye extracts or the reinforcements (situ self-assembly method as shown in Figure 1a), or impregnating (wet processing), lamination, or blending BC with polymers (as shown in Figure 1b) before cleaned and treated to well respond to the basic requirements about mechanical parameters, customer' comfort, and sustainable development of leather products [13, 21, 26, 27]. However, the affinity between BC and reinforced substances is not strong due to low bond interactions leading to poor tear strength and corrosion resistance, easily generating a lack of durability required for everyday use [1, 27, 29].

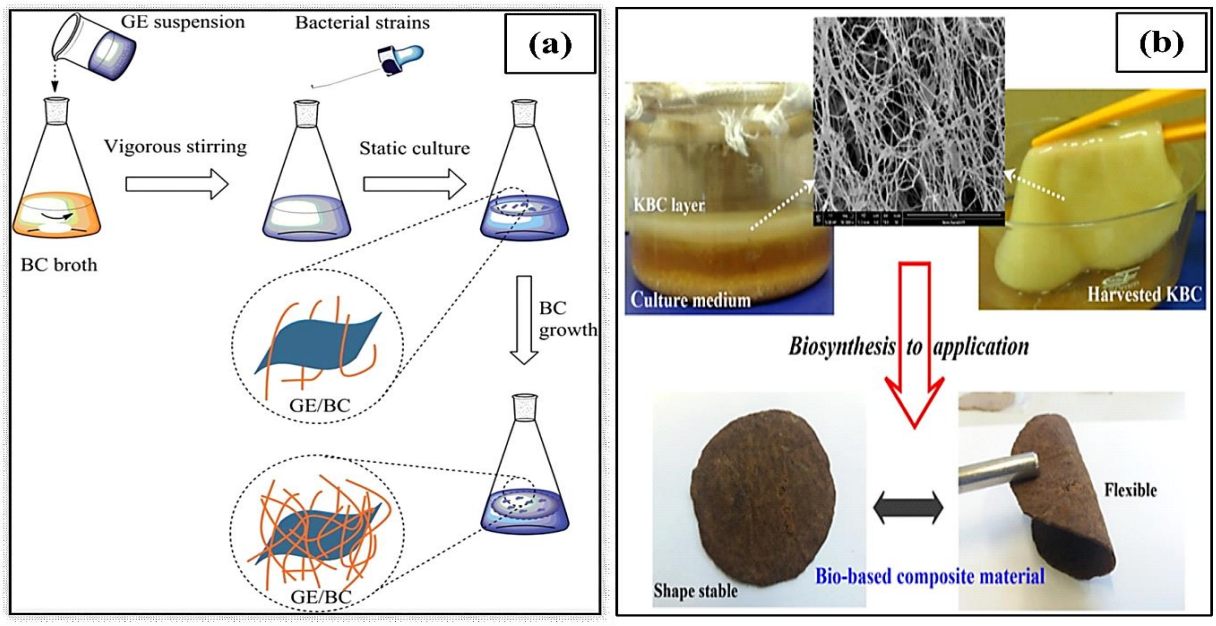

Figure 1 Schematic of (a) graphene/BC was prepared by in situ self-assembly [30] and (b) KBC biosynthesis apply to bio-based composite as leather-like materials [31].

1.3 Kombucha-derived bacterial cellulose (KBC)

In Kombucha leather ingredients, Kombucha-derived bacterial cellulose (KBC) was one of the key materials representing BC composition to mainly enhance the eco-friendly, porosity, breathability, and novelty of the product. KBC

was harvested from Kombucha fermentation of a traditional fermented beverage known as Kombucha “tea fungus”. According to structural analysis, KBC was an eco-friendly natural polymer with good biocompatibility, no histologic and hematologic toxic. KBC possessed similar characteristics to BC of HS standard medium, without lignin and hemicellulose. It should be noted that BC has been widely considered a future material source due to its outstanding properties such as high elasticity, durability, porosity, a high degree of crystallinity, biodegradable, non-cytotoxic, high thermal stability, and water holding capacity via a three-dimensional fibrillar structure and the OH-rich nature that clearly observed in Figure 2 [32-35]. BC has been extensively applied in antimicrobial wound dressings [36], blood vessel regeneration [37], dental, oral, and neural implants, urinary conduits, tympanic membrane [38-40], bio-printing [41], cosmetic [42], fabric [29, 43], leather [20, 23, 25], textile [44], paper [45], electronic devices [46], environmental [47] and food packaging [48-50].

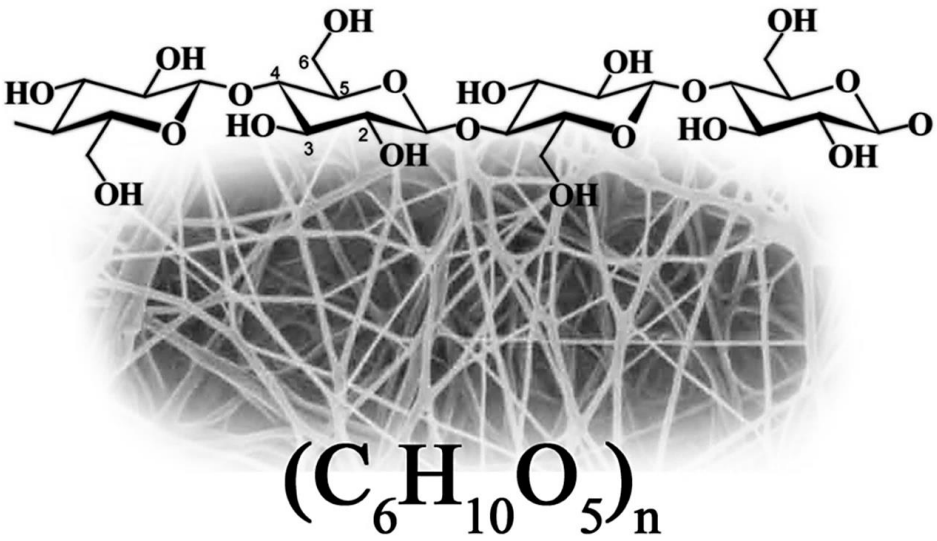

Figure 2 Chemical structure of bacterial cellulose [35].

1.4 Kombucha fermentation

Kombucha fermentation is an aerobic process, chemical-free, and normally only requires a short-time fermentation of tea, sugar, bio-waste as shown in

Figure 3 [28, 31, 51-60]. In brief, the yeast species and acetic acid bacteria symbiotic of kombucha fermentation worked in tandem to create two different finish products. The yeasts (i.e *Schizosaccharomyces, Saccharomyces, Zygosaccharomyces Brettanomyces, Kloeckera*) metabolized sugar into glucose, fructose, and ethanol to produce fermented tea as a sour liquid phase. In the same context, acetic acid bacteria such as *Acetobacter, Bacterium, Gluconacetobacter, Komagataeibacter, Lactobacillus* developed a floating biofilm known as KBC via their extracellular cellulose production activity [29, 33, 61, 62]. Initially, the acetic acid bacteria population increased and synthesized superimposed cellulose layers at the air/liquid interface. This development will continue until their synthesis reaches a limit, and then becomes inactive due to the insufficient oxygen required for respiration [33, 58, 63, 64].

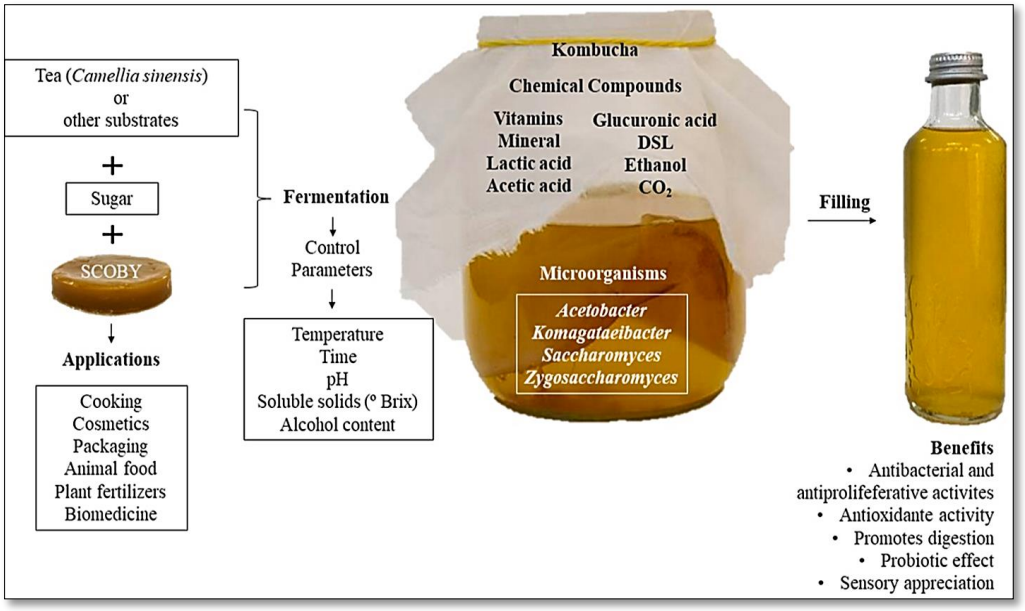

Figure 3 Schematic of Kombucha tea fungus production [65].

Generally, Kombucha fermentation is influenced by temperature, pH, the amount of oxygen, nutritional quality, the presence of enhancers, precursors, bacterial strain volume, and culture periods [33, 58, 66]. Any unsuitable in the varieties, nitrogen and carbon concentrations, possibly cause many negative effects on KBC yield and quality. Maintaining the optimum temperature is always

the necessary requirement for both enzyme activity and microbial growth. Prolonged fermentation is not recommended due to the high accumulation of organic acids and the CO_2 generated at the air/liquid interface led to the inhibition of cellulose biosynthesis microorganisms. Although the agitated culture process improves the gas and nutrient exchange, it decreases the structure and mechanical strength of cellulose membranes [60]. A normally Kombucha fermentation was conducted with 3% (w/v) of already prepared KBC-film or 10% (v/v) of inoculum, at 22-30 °C for an average of 15 days [33, 67]. Several reports revealed that increasing the ratio surface to volume (S/V) in static fermentation results in enhancing the thickness and weight of harvested cellulose membranes, as well as, minimum the by-product formation [68-70].

1.5 Optimization of BC/KBC production

High-cost of HS-standard cultured medium and low-yield are still huge obstacles leading to limiting the commercial scalability of BC/KBC [25, 51, 60, 71-74]. Up to now, a great many of research about BC/KBC production optimization model, accompanied by the isolation of new bio-film synthesis bacterial strains, or the use of low-cost alternative nutrient sources (i.e rotten apple, pineapple, pomegranate, muskmelon, watermelon, tomato, orange fruits, potato peel wastes, coffee husk, sugarcane molasses, vinasse, distillery effluent, the by-product of dairy foods and agroforestry processings), have still being investigated continuously [49, 68, 74-79].

According to the literature, Design of Experiment (DOE) is currently a popular versatile statistical tool to investigate the effects of the nutrient ingredients and cultured condition (input variables (X)) on the cellulose dry-weight or yield (measured response variable (Y)) in BC/KBC production [80-82]. For *Gluconacetobacter xylinus* strain, Du et al. (2020) has recorded BC optimum yield 1.46-fold higher after 8th-day fermentation, with glucose 19,575 g/L,

ethanol 3.85%, and initial pH 4.32 [83]. Similar, using molasses 5.38%, corn steep liquor (CSL) 1.91%, ammonium sulphate 0.63%, disodium phosphate 0.270%, citric acid 0.115%, ethanol 1.38% (v/v) for 9 days at 30 °C, BC optimum yields were 7.5 ± 0.54 g/L and increased linearly of 0.32±0.037 g/L/day with fermentation time for up to 21 days [68]. A series of outstanding results have also been reported via optimizing conditions and culture media for BC production by different alternative substrate sources, such as 40% vinasse [78], a mixture of a 50:50 ratio of date syrup and cheese whey [84], carob and haricot bean (CHb) [67]. Additionally, several dramatic improvements in BC yield were also found in studies by Bagewadi et al. (2020), Rastogi et al. (2020), Aswini et al. (2020), Santoso et al. (2020), Bekatorou et al. (2019), and Anusuya et al. (2018) by various BC-producing bacterial strains, respectively, *Enterobacter hormaechei subsp. steigerwaltii* strain ZKE7 [85], *Leifsonia soli* [77], *Acetobacter senengalensis* MA1[86], *Komactobacter intermedius* (BCRC 910677) [87], *Komagataeibacter sucrofermentans* [88], and *Acetobacter senegalensis* MA1.

1.6 Modification of BC/KBC hydrophobicity

Besides the great potential for use in any application of BC from HS standard medium and other alternative media [50, 89, 90], KBC has also possessed an OH-rich nature and high hydrophilicity [83, 85, 91] causing incompatibility between them and polymeric materials that have hampered their application in most leathers, fabric, textile products, or high tensile strength composites. Acetylation of BC by perchloric acid [92], sulphuric acid [93], acetic, tartaric, and propionic acid [94] has been proposed to increase their hydrophobicity. In particular, BC membranes were surface-functionalized via alkoxysilane polycondensation that has been verified high effective. Shao et al. (2017) and Taokaew et al. (2015) grafted BC with octadecyltrichlorosilane and 3-aminopropyl)triethoxysilane to improve their water contact angle, respectively, to 86.4° and 112.4° compared

to 48.3° and 56.3° of non-treated BC [95, 96]. Similar, BC was acylated and acrylate with vinyl-triethoxy silane or 3-aminopropyl triethoxysilane showed more than a twofold increase in water contact angle value [97]. In addition, almost a 3-fold increase in BC hydrophobicity was reported by Krishnamurthy et al. (2020), after polymerization of BC with (3-azidopropyl) trimethoxysilane, 1,4-bis(azidomethyl), 2,5-bis(dodecyloxy)benzene, or 2,5-bis- (hexyloxy)benzene [98]. Similar effects have also been reported by Feng et al. (2014), Huang et al. (2018), Kayaoglu et al. (2013) and Laaziz et al. (2017) upon deposition of vinyltriethoxysilane, polydimethylsiloxane, and hexamethyldisiloxane to improve the hydrophobicity of the leather products surface, polyurethane (PU)-based synthetic leather or PLA [99-102]. These results are attributed to the bi-functional character of silane that has acted as the coupling agent based on silanol linked in their chemical structures (Figure 4). Essentially, one of its ends (first function group) reacts with the hydroxyl groups of BC, while the remaining group combines with other groups in BC to create the Si-matrix.

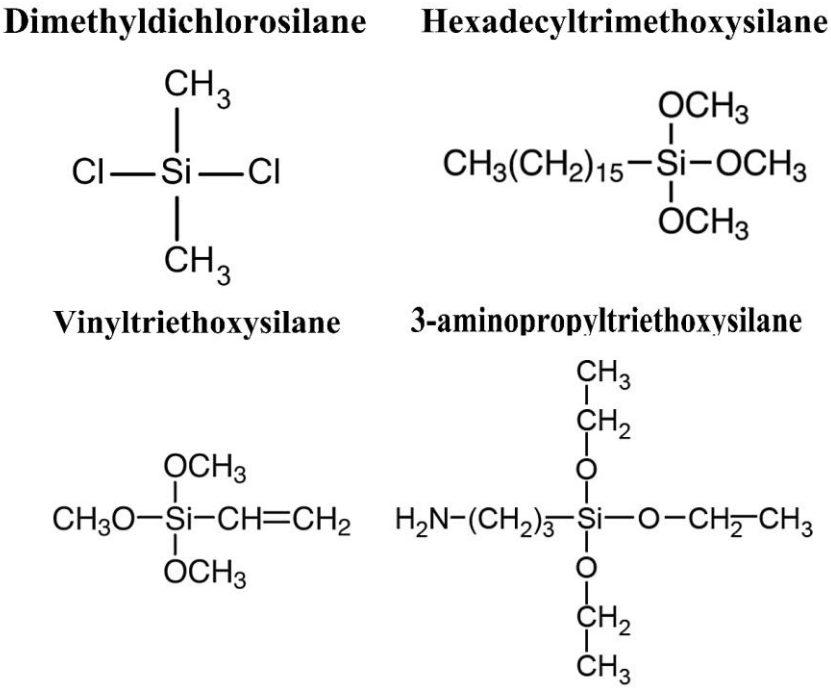

Figure 4 Chemical structure of silane compounds.

1.7 Polymers use in leather processing

PU is a polymer with repeating carbamate groups (–NHCOO) in the backbone polymer chain consisting of alternating hard and soft segments [103-105]. PU is currently one of the fastest-growing high-tech materials in the leather industry as a finishing agent, retaining filler, substrate, additives [105-107] due to good leveling, dyeing assistance, and leather fiber dispersion similar to acrylic acid-based retaining agents [108, 109]. PU has high modulus and tensile strength, elasticity, good tear strength, excellent corrosion resistance, low-temperature flexibility, chemical resistance, fouling resistance, high adhesion, and sealing [104, 106, 110-112]. Recently, the development of PU from soybean oil, palm oil, and castor oil, has significantly improved the cost-effectiveness, less environmental pollution and human health risk [103, 104, 106, 107]. Nevertheless, PU is still flammable, has poor breathability, and prolonged post-used biodegradation time [108]. The handling methods for this PU weakness are addition, blending, or grafting PU with bio-fillers, other biopolymers, and biocomposites [110].

PLA is a linear aliphatic thermoplastic polyester that can be synthesized from renewable resources. PLA is biodegradable and biocompatible, insoluble in water [113-116]. The thermal properties of PLA can be affected by different structural parameters, such as molecular mass and composition (stereoisomers content) [117]. PLA has good mechanical properties, especially Young's modulus, tensile strength, and flexural strength compared with traditional polymers, such as polypropylene (PP), polystyrene (PS), and polyethylene (PE) [114]. However, PLA's low elongation at break, brittleness, and high sensitivity to crack causing limit its application to product fabrication requiring plastic deformation at higher stress levels. To overcome this negative feature, PLA is usually blended with

other polymers to enhance and modify the toughness such as PLA/micro or nano cellulose, PLA/PU, PLA/leather waste [7, 113, 118-121].

PCL is a semi-crystalline polymer of the aliphatic polyester family that is biocompatible, high toughness, malleable with a decomposition time of over 1 year [122, 123]. PCL's low melting point of 58 °C to 65 °C and its degradation at high temperatures (>200 °C) is a major obstacles to its applications [124]. The physical, chemical and mechanical properties of PCL can be effectively changed by copolymerization and mixing [123]. For example, leather waste, or some natural polymers (cellulose, chitosan, starch, hydroxyl appetite) or synthetic polymers (PU, PLA, polyethylene glycol (PEG), oxazoline, polyvinyl alcohol (PVA), polyethylene oxide (PEO), polylactic-co-glycolic acid (PLGA)) were compatible with PCL resulting alter its thermal, rheological, biophysical properties and increase crystallinity, crystallization temperature [125-127].

PVA is a linear synthetic polymer used in resins production, paper, paint, glue, lacquers, surgical threads, textiles, environmental treatment, food packaging, and food-contact applications [128-130]. PVA is biocompatibility, nontoxic, noncarcinogenic, chemical resistant, low environmental impact, and high biodegradability [129]. Several microorganisms in septic systems, landfills, compost, and soil are all able to degrade PVA through enzymatic processes [128]. In the textile, and leather industry, or high elastic material production, PVA is majority used as an agent of shaping, coating, and product finishing which its key weakness might be due to water adsorption capacity. In order to reduce this, PVA will be used to combine with other biopolymers/biocomposites or bio-fibers.

2. CURRENT STATE OF THE ISSUES

The market share structure of the leather industry is constantly shifting to vegan leather or eco-leather due to the continuous increase in awareness of

consumers, manufacturers, designers, and regulatory agencies about ethical consumption, cleaner production, sustainable development, and animal rights. Therefore, creating a Kombucha leather with enhanced biodegradability and possessing the unique properties of leather via a simply, cheaply enough process would be the well-response to this urgent requirement of the leather industry, even, allowing the manufacturers to embrace future leather markets.

The incompatibility between the hydrophilic BC/KBC and the hydrophobic polymer matrix causes an easily broken structure and highly water absorbent which is not suitable for all leather types or covering products. Pretreatment KBC/BC with silane compounds would be improved their compatibility.

Low yield and high cost are the biggest limitations that render BC/KBC an unrealistic alternative to leather, even holding back its practical application level. Producing BC using Kombucha method from bio-waste sources is completely possible significantly increase productivity, save production costs, reduce pollutant emissions, as well as transform bio-wastes into useful materials.

At the present, there are no reports on the optimization of both Kombucha leather fabrication based on KBC-PU-PLA and KBC production using sour whey waste and cane-sugar, even, hydrophobicity enhancement of KBC powder via silane polycondensation.

3. OBJECTIVES OF DOCTORAL THESIS

The main target of this doctoral thesis is to develop Kombucha leather that possible application in fashion design, footwear, bags, interior covering products by a combination between polymers and KBC harvested from the fermentation of bio-wastes. To achieve this goal, the work comprised of four objectives.

1. Effective evaluation of KBC production using media containing sour whey waste, waste apple juice, and brewed spent grains. This study's aim will be to verify the suitable bio-waste source for high yield KBC production.

2. Optimization of KBC production and trial of the result in large containers. This study's focus will be given to determining the optimum formulation of the KBC fermentation batch.
3. Influence of silane compounds on the hydrophobicity of KBC and determine usable polymers for kombucha leather preparation. The study will be used to improve the compactible of KBC and usable polymers.
4. Optimization of Kombucha leather preparation. This study will be performed to verify the optimum ingredients and prepared conditions of Kombucha leather. The products will be compared to commercial leathers in structural, morphological, thermal resistance, surface wettability, and mechanical strength to evaluate their applied potential.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Bio-waste sources and other bacterial culture nutrients

Dairy sour whey waste is one of the most polluting by-products of the food industry [131, 132]. This generated waste is made of 50 - 55% total milk nutrients that may serve as a potential extra-nutrient source for strong growth stimulation of KBC microorganisms during biosynthesis. Presently, just 50% of dairy sour whey waste is used to produce bacteria culture medium, animal feed, protein isolate and concentrates [52, 53, 88, 131, 133]. This waste source is completely suitable to use as a nutrient source for BC/KBC biosynthesis [69, 84, 88].

Apple is a class of fruits that contains a huge amount of sugars, proteins, fiber, vitamins, organic acids, minerals, and polyphenols [134]. Globally, this fruit generates large amounts of waste annually from spoilage, inventory and wild non-edible species to processing, making it also highly suitable as a raw material for BC/KBC production [52, 75, 135, 136].

Brewed spent grains is rich in protein, fiber, vitamins, minerals, amino acids, phenolic compounds, oligosaccharides, and polysaccharides [137, 138]. According to recent counts in the European countries, an estimated amount of over 9,500 breweries are produced daily [139] where for every 5 L of beer produced, 1 kg of grains is generated as waste.

Sour whey waste (supplied by Kromilk A.S, Czech Republic), ***brewed spent grains*** (supplied by a Brewery Industry in Malenovice, Czech Republic) and rotten ***apple fruits*** (collected from public garden near Tomas Bata University in Zlin, Czech Republic) were used as nutrient sources. Black tea and cane-sugar were purchased from a grocery store in Zlin, Czech Republic.

4.1.2 Polymers and other chemicals

D-glucose was supplied by Amersco LLC, USA. Sodium hydroxide (NaOH), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$), acetic acid (CH_3COOH), and citric acid ($C_6H_8O_7$) were purchased from Penta s.r.o. (Zlin, Czech Republic). Sucrose, yeast extract, peptone, Polycaprolactone (PCL), tetrahydrofuran (THF), dimethyldichlorosilane (DCDMS), hexadecyltrimethoxysilane (HDS), vinyltriethoxysilane (VTS), 3-aminopropyltriethoxysilane (APS), and 4-(dimethylamino) pyridine (DMAP) were supplied by Sigma-Aldrich (Darmstadt, Germany). Polylactic acid (PLA-4043D), polyurethane elastomer (PU), Polyvinyl alcohol (PVA), were supplied by NatureWorks LLC (Ingeo®, USA). All reagents were used without further purification.

4.2 KBC production

4.2.1 Activation of bacterial strain

The strain used for the production of KBC is known as *Komagataeibacter xylinus* CCM3611, (formerly called *Acetobacter xylinum* or *Gluconacetobacter*

xylinus)), the most efficient cellulose-producing bacterial strain [86] that was purchased from Czech Collection of Microorganisms, Brno, Czech Republic. The bacterial strain was preserved in the Microbiology Laboratory of the Centre of Polymer Systems, Zlin, Czech Republic. Prior to usage, the bacterial strain was cultured on HS standard medium at 30 °C for a period of 3 days for activation. The bacteria strain was then inoculated in the various nutrient media for the production of BC/KBC.

4.2.2 Evaluation of KBC production using bio-waste sources

Sour whey waste was collected and kept at a temperature between 4 to 6 °C. Apple fruits were crushed using a juice blender (Guzzanti GZ 020, Italy) and the juice was extracted (solution pH 3.8-4.0). Brewed spent grains were pureed using a Nutribullet blender (N17-0908 machine, USA). Sour whey waste, the juice extracted, brewed spent grains and black tea were used as alternatives to expensive components such as yeast extract and peptone to provide nitrogen and some enhancers (albumins, globulins, vitamins, amino acids, and organic acids) for bacterial cell-respiration.

Subsequently, six different culture media were prepared following the mixture compositions as shown in Table 1. The cultured media was sterilized at 121 °C in an autoclave for 15 min. The media were then cooled to room temperature and 1% (v/v) suspension of *Komagataeibacter xylinus* CCM 3611 with an approximate initial bacteria density of 1.78 x 10^{10} CFU/mL (as prepared in 4.2.1) was added separately. The mixtures were masked using cleaned textile materials and statically incubated at 30 °C for 15 days. After the incubation stage, KBC and BC (as control) on the surface of each medium were harvested, washed with distilled water (3 times), and oven-dried at 40 °C to constant weight or treated with 0.5% (w/v) NaOH at 80 °C for 1 h, washed and freeze-dried at -110 °C for

24 h. The obtained oven-dried samples have used to determine the dry weight of cellulose membranes. The freeze-dried samples were then stored in a desiccator for further analysis.

Table 1. Compositions of formulated media for KBC production [31].

	Index of Medium	Compositions				
		Whey **(W)** (mL/L)	Apple juice **(A)** (mL/L)	Brewed spent grains **(B)** (g/L)	Sucrose **(S)** (g/L)	Black tea **(T)** (g/L)
Test samples	**WST**	500	-	-	100	6
	AST	-	500	-	100	6
	BST	-	-	100	100	6
	WABST	250	250	100	100	6
Controls	**KOM**	-	-	-	100	6
	HS	20 g D-glucose, 5 g yeast extract, 5 g peptone, 8.6 g $Na_2HPO_4.12H_2O$ and 1.15 g Citric acid				

4.2.3 Optimization of KBC production using sour whey waste

Response surface methodology (RSM) of Design of Experiment (DOE), version 11 based on D-optimal (custom) consisting of five independent variables and 28 trials were used to design the experiments, analyze data and develop regression models for the optimum KBC production as shown in Table 2. Herein, 4 factors (X_1, X_2, X_3 and X_4) including the bio-waste (whey), cane-sugar, black tea and volume of bacteria were randomly conducted at continuous high and low concentrations. Meanwhile, the remaining factor designated as culture period (X_5) was investigated at three discrete concentration levels of high, medium, and low to reach the integer values of 14, 18, and 21 days. The surveyed concentration levels in this investigation have been confirmed as the fit parameter ranges to achieve high BC yield in recent similar studies [67, 68, 78, 83-88, 140]. HS standard medium was prepared as the control. Bacteria volume was then

transferred to the respectively cultured medium in a sterile cabinet. The dry weight of KBC membranes was fixed as the dependent variable.

Table 2. Experimental design for the optimization of KBC production [28].

Run	Experimental factors				
	X_1: Whey (mL/L)	**X_2:** Cane sugar (g/L)	**X_3:** Black tea (g/L)	**X_4:** Bacteria volume (mL/L)	**X_5:** Culture period (days)
1	1000.00	100.00	6.00	100.0	21
2	652.50	50.00	3.00	73.0	14
3	1000.00	50.00	3.00	100.0	21
4	500.00	100.00	3.00	10.0	14
5	1000.00	100.00	3.00	100.0	14
6	1000.00	50.00	3.00	100.0	21
7	1000.00	50.00	6.00	100.0	14
8	500.00	100.00	6.00	100.0	14
9	1000.00	100.00	3.00	48.7	18
10	750.00	50.00	4.58	10.0	18
11	500.00	50.00	6.00	10.0	14
12	787.50	78.50	3.00	10.0	21
13	1000.00	100.00	4.26	10.0	21
14	500.00	50.00	6.00	100.0	21
15	1000.00	100.00	6.00	10.0	14
16	1000.00	75.00	4.58	55.0	14
17	500.00	100.00	6.00	10.0	21
18	500.00	100.00	3.00	100.0	21
19	1000.00	100.00	3.00	48.7	18
20	710.00	90.00	4.73	61.3	21
21	1000.00	50.00	6.00	10.0	21
22	500.00	68.75	3.00	100.0	18
23	1000.00	50.00	3.00	10.0	14
24	804.97	61.75	4.71	100.0	18
25	1000.00	100.00	4.26	10.0	21
26	1000.00	100.00	6.00	10.0	14
27	500.00	50.00	3.00	10.0	21
28	570.00	100.00	3.42	87.4	14

Media components are measured, directly mixed, and contained in Duran bottles (250 ml capacity). Each trial formulation was performed with 100 mL sterile medium (sterilized at 121 °C, 15 min) in triplicates under the static cultured condition at 30 °C. The results were subjected to analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. The response function (Y) of the design model was partitioned into linear interactive components expressed using Equation (4.1).

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \varepsilon \quad (4.1)$$

Where, Y represents the response, β the variables coefficient, X is the input variable, and ε is the error parameter. The correlation between the dependent variable and the independent variables was integrated via non-linear regression analyses. Finally, three verification experiments were performed to confirm the optimum conditions.

4.2.4 KBC production in large containers

In static fermentation, BC/KBC membranes formed as gel-like three-dimensional materials at the surface of the culture medium [33, 43, 51, 68]. The ratio between the depth of the culture medium and the surface area of the fermenter (or container) will have a strong influence on the cellulose yield and the amount of residual suspension that will present itself as the only source of emissions during the production [33, 68, 69].

This experiment was performed in large containers of the same dimensions (17 cm x 25 cm ~ S = 425 cm^2) cleaned with 70 % ethanol. The prepared sterile fermentation medium from the obtained optimized formulation was poured into the above-mentioned containers following various volumes of culture medium to create different corresponding cultured medium depths (i.e. 0.5-3.0 cm) as shown in Table 3.

HS standard medium was prepared as the control. Two optimum fermentation condition (bacteria volume and culture period) was applied for all trials. The generated cellulose membranes were collected, their dry weight and characteristic were determined.

Table 3. Corresponding volume depths of the fermentation mediums in large containers [28].

Samples	Cultured medium depth (cm)	Cultured medium volume (mL)
KBC-0.5cm	0.5	180
KBC-1cm	1	360
KBC-2cm	2	720
KBC-3cm	3	1080
HS-0.5cm	0.5	180
HS-1cm	1	360
HS-2cm	2	720
HS-3cm	3	1080

Abbreviations: HS, Hestrin and Schramm; KBC, kombucha-derived bacterial cellulose.

4.3 Fabrication of Kombucha leather

4.3.1 Preparation of leather-like biocomposites based on KBC and PCL/PVA/PLA

In order to investigate the usability components in Kombucha leather preparation, the pre-experiments were performed based on bio-components and polymers including KBC powder, maple leave (ML) pulp, PCL, PVA, PLA. Harvested KBC were treated with NaOH 0.5% at 80 °C for 1h followed by washing with dH_2O, oven-dried at 40 °C until reached constant weight and milled to fine granular powder using a micro ball mill (Lab Wizz 320, Laarmann Group, Netherlands) at room temperature under a frequency rate of 25 Hz.

Shredded maple leaves (ML) used as a bio-filler, were oven-dried at 45 °C overnight before being boiled in NaOH solution 8-12 %, in the temperature range of 150 to 200 °C for 2 h. Subsequently, the sample was washed to neutral pH, and

ground in dH_2O at 30,000 rpm for 1 min using a NutriBullet® blender, USA. The mixture was centrifuged at 10,000 rpm for 10 min on a Sorvall Lynx 4000 superspeed centrifuge (Thermo Scientific™, Waltham, MA, USA) and oven-dried the collected precipitate at 50 °C overnight to constant weight.

PCL, PVA and PLA were used as the reinforcement biopolymers. PCL and PLA were completely dissolved in dichloromethane (DCM) for 24 h. Next, KBC powder and ML pulp were homogeneously blended with PCL/PVA/PLA mixture and compressed at 120 °C in 10 min with a force of 5 to 20 kN using 2 mm thick stainless steel plates. Prior to each compression, the samples were placed between Teflon sheets. After pressing, the samples were air-dried at room temperature for 2-5 days. Finally, tensile strength (N/mm^2), elongation at break (%), tear strength (N/mm), elastic modulus (MPa), water contact angle (^{o}) and properties of leather-like biocomposites were determined and compared to the commercial PU synthesis leather (C1).

4.3.2 Preparation of leather-like biocomposites based on KBC and PU/PLA

This experiment is based on the combination of bio-based composition (KBC and sisal fiber powder) with PU and PLA to improve the mechanical strength and water resistance of prepared leather-like biocomposites. PU and PLA were firstly dissolved in Tetrahydrofuran and dichloromethane respectively, for 24 h. KBC and sisal fiber were treated with NaOH 0.5% at 80 °C for 1h followed by washing with dH_2O, oven-dried at 40 °C until reached constant weight and milled to fine granular powder using a micro ball mill (Lab Wizz 320, Laarmann Group, Netherlands) at room temperature and a frequency rate of 25 Hz. Next, KBC and sisal fiber powders were further modified with dichlorodimethylsilane (DCDMS) for 1 h using 4-(dimethylamino)pyridine (DMAP) as the catalyst. The samples were washed with n-hexan and ethanol:dH_2O (1:1), oven-dried, and again milled

to fine granular powder before being homogeneously mixed with the polymer matrix of PU/PLA at room temperature for 1 min with various ratios as shown in Table 4. The mixtures were then placed between Teflon sheets, compressed at 150 °C in 10 min with a force of 5 to 20 kN using 2 mm thick stainless steel plates. After pressing, the samples were air-dried at room temperature for 24h. Tensile strength (N/mm^2), elongation at break (%), water contact angle (^{o}) and properties of investigated leather-like biocomposites were also determined.

Table 4 Constituent components in the preparation of leather materials [28].

Samples	KBC alkali-treated (% w/w)	KBC-treated (% w/w)	Fiber treated (% w/w)	Polyurethane (PU) (% w/w)	Polylactic acid (PLA) (% w/w)
S1	5	-	-	65	30
S2	-	5	-	65	30
S3	-	10	-	60	30
S4	-	15	-	60	25
S5	-	5	20	50	25
S0 (Control)	-	-	-	65	35

Abbreviations: KBC, kombucha-derived bacterial cellulose; Fiber, sisal fiber.

4.3.3 Hydrophilic modification of KBC

A homogenous blending of ingredients is always a basic requirement of a sustainable, supple structure and is also an indispensable feature of any leather or high elasticity materials [26, 29, 100]. However, the hydrophilic nature of KBC is completely contrast to hydrophobic of PU and PLA. In order to achieve homogenous blending with polymer binder matrix, KBC was firstly treated to kill bacteria, decolorize, remove odors, and especially, improve hydrophobicity. At first, the harvested KBC was treated with or without NaOH and designated as (i) immersed in NaOH 0.5% for 30 min at 80 °C (ii) distilled water (dH_2O) boiling for 1h, (iii) NaOH 0.5% at 80 °C for 1h. The treated KBC was subsequently washed with dH_2O, oven-dried at 40 °C until reached constant weight, and milled

to fine granular powder using a micro ball mill (Lab Wizz 320, Laarmann Group, Netherlands) at room temperature and a frequency rate of 25 Hz.

KBC treated with NaOH 0.5% at 80 °C for 1h (sample (iii)) is then subsequently treated with different silanes of Dimethyldichlorosilane (DCDMS), 3-aminopropyltriethoxysilane (APS), Vinyltriethoxysilane (VTS), Hexadecyltrimethoxysilane (HDS). Herein, each silane compound was dissolved in ethanol 70% at pH 3.5 (using acetic acid to adjust pH) with DMAP as the catalyst. After 3 h, silane-treated KBC samples were washed with ethanol, dH_2O, and oven-dried at 40 °C, until achieve constant weight. The dried samples were re-milled to obtain fine granular particles. The water absorption capacity was determined to verify their degree of hydrophilicity.

4.3.4 Optimization of Kombucha leather preparation

Design of Experiment (DOE) version 11 with Regular Two-Level Factorial model, consisting of 5 independent variables, 32 runs, was applied to optimize Kombucha leather's ingredients and prepared condition (Table 5). The content of KBC, PU, PLA (in percent weight per weight), compressed temperatures (^{O}C), and compressed times (min) were established as the independent variables of X_1, X_2, X_3, X_4, and X_5, respectively. The responses were elastic modulus (N/mm^2), water contact angle (^{0}), biodegradable capacity (%) coded as corresponding Y_1, Y_2, and Y_3. KBC used was the best hydrophobic sample determined from the experiment above. Both PU and PLA as the binding matrices were completely dissolved in only Tetrahydrofuran for 24 h to enhance the homogeneously blended level of products. Kombucha leathers were prepared via blending with corresponding components, compressed temperature and time in the experimental design formulations as shown in Table 5. Prior to each compression, the samples were placed between Teflon sheets and after pressing, the samples were air-dried at room temperature for 12 h.

Table 5 The experimental design for the optimization of Kombucha leather preparation (unpublished work).

Run	Experimental factors				
	X_1: KBC (%)	**X_2:** PU (%)	**X_3:** PLA (%)	**X_4:** Compressed temperatures (^{0}C)	**X_5:** Compressed times (min)
1	30	80	10	180	10
2	30	40	30	150	10
3	10	40	30	150	10
4	10	40	30	180	3
5	30	80	10	150	10
6	30	80	10	150	3
7	10	80	10	150	3
8	30	40	30	180	3
9	30	40	30	150	3
10	10	80	10	150	10
11	10	40	30	150	3
12	30	40	10	150	10
13	30	80	30	150	10
14	30	80	30	180	3
15	10	80	30	180	3
16	10	40	10	150	10
17	10	80	10	180	3
18	30	80	10	180	3
19	10	80	10	180	10
20	30	40	10	150	3
21	30	40	10	180	3
22	10	40	30	180	10
23	30	40	10	180	10
24	10	80	30	150	3
25	10	80	30	180	10
26	10	40	10	180	3
27	30	80	30	150	3
28	30	40	30	180	10
29	10	40	10	180	10
30	10	40	10	150	3
31	10	80	30	150	10
32	30	80	30	180	10

Abbreviations: KBC, kombucha-derived bacterial cellulose; PU, polyurethane elastomer; PLA, polylactic acid.

The response function (Y) of the design model was also partitioned into linear interactive components expressed using Equation 4.1 (as prepared in 4.2.3). Three verification experiments were performed to confirm the optimal conditions. Finally, the optimized sample properties were compared structurally, morphological, and physico-mechanically to that of two commercial leather samples (C1 and C2).

4.4 Characterization analysis of BC/KBC and prepared leathers

4.4.1 Dry weight of BC/KBC and pH value of the fermentation media

In order to assess the final dry weight of BC/KBC per volume of the nutrient medium used, the harvested cellulose membranes were washed with distilled water, oven-dried at 40 °C to constant weight, and weighted. The final dry weight of KBC was then determined using the following formula [81, 83, 141, 142]:

$$\text{Final weight} = \frac{\text{Dry amount of cultured cellulose (g)}}{\text{Volume of medium used (L)}} \qquad (4.2)$$

The pH values of the different nutrient media used were determined at the beginning and end of the bacteria cultured time using an electronic handheld pH meter (Lovibond pH meter-445R, USA).

4.4.2 Morphological characterization

The freeze-dried cellulose membranes and leather-like materials were examined for their morphology structures under a scanning electron microscope (SEM, FEI™, Brno, Czech Republic) at an accelerating voltage of 5 kV. Prior to analysis, the JEOL JFC 1300 Auto Fine coater (Tokyo, Japan) was used to gold coat the samples' surface to enhance conductivity.

4.4.3 Fourier transformed infrared spectroscopy (FTIR)

FTIR analysis of the freeze-dried cellulose membranes and leather-like materials will be done using a Nicolet iS5 spectrometer (Thermo Scientific, USA) attached with an attenuated total reflectance mode (iD5-Ge-ATR) assembly. The samples were scanned at a 4.0 cm^{-1} resolution using 64 scans in the wavenumber range of 400 – 4000 cm^{-1}.

4.4.4. X-ray diffraction analysis

Crystalline structure of the samples was analyzed by a Mini Flex™ 600 X-ray diffractometer (Rigaku, Japan). The scans were performed in the range of 5 – 70°, scanning speed of 5 °/min using a foil filtered CuKβ radiation (λ = 0.179 nm) at 40 kV voltage and a current of 15 mA. The divergence slit was maintained at 0.1° throughout the experiment.

4.4.5 Thermal analysis

The thermogravimetric analysis (TGA) curves of the freeze-dried cellulose membranes and leather-like materials were recorded using TGA Q500 (TA Instruments, USA). The samples were heated from 25 to 600 °C under a nitrogen atmosphere at a flow rate of 40 - 60 mL/min and a heating/cooling rate of 10 °C/min.

4.4.6. The water absorption capacity of KBC powder

The water absorption capacity of KBC powder is subsequently determined by a filtration method as suggested by Zhang et al, 2020 [143]. The dry weight of the filter paper is determined. A Buchner flask with a funnel firmly inserted is connected to a vacuum pump. Each water-saturated filter paper is placed into the funnel. The vacuum pump is turned on and the wet weight of the filter paper is recorded after the excess water is completely removed. Before that, KBC powder

samples are put into a glass beaker, and dH_2O is poured in. The samples are dispersed with the stirring rod to their full contact with the liquid for 1 min. After 24h immersion, the swollen sample is poured onto the center of the filter paper and the vacuum pump is turned on. After the excess water is removed, the filter paper (with the samples on it) is taken out and weighed. Finally, the water holding capacity (WHC) of the samples is calculated using equation [143]:

$$WHC = \frac{W_2 - W_1 - W_0}{W_0} \quad (4.5)$$

Where, W_0 is initial dry weight of the powders, W_1 is water-saturated filter paperweight and W_2 is the filter paper (with the samples on it) wet weight.

4.4.7. Water contact angle measurement

The sessile drop technique was used to measure water contact angle for the wettability determination of the prepared biocomposites using Advex Instrument machine (Brno, Czech Republic) with a CCD camera. The measurements were conducted at 25 °C and 50 % relative humidity. Distilled water was used as the checking liquid with 10 μL for each test. The photographic images were taken after the deposition liquid was dropped on the surface of the sample. The measured result was an average of 5 replicates.

4.4.8. Mechanical analysis

In order to investigate the mechanical properties of leather-like materials, the elasticity analysis was performed using Instron 5567 (Instron, USA) under a static load of 10 kg and a crosshead speed of 10 mm/min at room temperature (25 °C). The test was conducted following ISO 3376 standards [144]. Elastic modulus was determined as a function of displacement by force applied. The data will be analyzed using the Instron Bluehill® application.

4.4.9. Biodegradation studies

Biodegradability of leather-like materials was investigated by the soil burial method following ASTM G160-12 [145]. Leather sheets (50 mm × 50 mm) were determined for the constant dry weight (W_0), soil burial in 250 g of soil with a soil pH of 6.5–7.0, and 50% moisture. The examination was performed in a humidity chamber with temperatures maintained at 30 °C and 50% surrounding humidity. Finally, the samples were removed from the soil, washed with dH_2O, and air-dried at 40 °C to constant weight (W). The degradation percentage (D) of the leather sheets after 90 days was calculated by the following equation [146]:

$$D\ (\%) = \left(\frac{W_0 - W}{W_0}\right) \times 100 \qquad (4.7)$$

4.4.10. Statistical analysis

All measurements were recorded in triplicate and the results were reported as the mean ± standard deviation. The design and analysis of the experiments were conducted with the statistical software of Design-Expert® V11 (Stat-Ease Inc., Minneapolis, MN, USA) and OriginLab software version 9.0 based on regression analysis of the experimental data. Analysis of variance (ANOVA) was applied for statistical evaluation and experimental results were displayed as Mean ± Standard error where $p < 0.05$ is determined as statistically significant.

5. BRIEF DISCUSSION OF DOCTORAL THESIS RESULTS

5.1 Evaluation of KBC production using bio-waste sources

According to the results in **publication I** with the title “Kombucha-derived bacterial cellulose from diverse wastes: a prudent leather alternative”, three investigated bio-waste sources all showed huge support to the cellulose synthesis capacity of *Komagataeibacter xylinus* by superior harvested yield (KBC dry

weights), respective as 12.59, 8.76, 7.65, and 12.81 g/L for the corresponding media: **WST**= whey, sucrose, black tea; **AST**= apple juice, sucrose, black tea; **BST**= brewed spent, sucrose, black tea, and **WABST**= whey, apple juice, brewed spent, sucrose, black tea. These achievements conversed to 1.17 and 3.21 g/L produced from **KOM**= Kombucha traditional and **HS**= standard HS medium. This result basic confirms the potential of high yield cellulose production using the kombucha fermentation method compared to the others and previous reports.

Based on the characteristic analysis results of obtained cellulose membranes, there was an insignificant difference between KBC membranes of surveyed media and BC collected from HS standard medium. For observed morphologies, all BC/KBC membranes revealed a close similarity in the structure of three-dimensional network spongy formed via random assembly of the rod-shaped nanofiber bundles and no significant difference among the dimensions of obtained KBC nanofibers from the different investigated nutrient media, in the range of 21 to 87 nm for KBC membranes whereas compared to 45 to 93 nm of KOM medium and BC produced from HS standard medium.

For FTIR analysis, the recorded spectra peaks of cellulose prepared from HS medium at 3347, 2900, 1430, 1159, 1058 and 1033 cm^{-1} were ascribed to the characteristic bands of cellulose structure. Similarly, spectra of cellulose grown in assessments media also showed characteristic peaks at 3345 to 3351 cm^{-1} for O-H stretching vibration, 2856 to 2923 cm^{-1} for C-H stretching, 1421 to 1436 cm^{-1} for C-O-H stretching, 1159 to 1160 cm^{-1} for C-O-C, and 1033 to 1060 cm^{-1} for C-O stretching. These spectra confirmed that the biomaterial produced by *Komagataeibacter xylinus* CCM 3611 is pure cellulose.

For X-ray diffraction analysis, the peaks at 2θ angles were determined at ≈17.1° and ≈26.7°, ascribed to the diffraction peaks of all KBC samples produced from both the test media and the HS standard medium. The presence of these

diffraction peaks was attributed to cellulose type Iα, which is commonly prevalent in BC where (101) relates to the amorphous region and (002) to the crystalline region of the polymer. Contemporaneous, the crystallinity percentages for KBC produced in the different media were obtained as 82.2%, 66.1%, 61.6% and 80.7% for WST, BST, AST and WABST, respectively, compared to the control samples which were obtained as 65.3% for KOM and 80.5% for HS standard medium.

For thermal stability analysis, the evaluated samples exhibited three different degradation stages that were quite similar between BC obtained from the HS medium and KBC. Firstly, degradation from 25 to 230 °C was observed, which was attributed to the loss of water in the polymer matrix. In essence, this stage depicted no major difference in moisture content between the KBC samples. The second degradation phase occurred in the temperature range of 330 to 360 °C, which showed a significant decrease in mass ascribed to scissors and decomposition of the KBC polymer chains with weight loss in the range of 24 to 36%. In general, the weight loss at this stage may be attributed to degradation, depolymerization, dehydration or decomposition of the structural compositions of cellulose. Finally, the temperature ranges between 360 to 600 °C were ascribed to tertiary weight loss in the KBC samples calculated averagely as 20.7%. This was principally related to thermal degradation of KBC remains to form char residues.

Generally, it can be obvious that the waste sources used (sour whey, apple juice, and brewed spent grains) were extensively effective in improving the production yield of KBC membranes. SEM, FTIR, TGA, and XRD analysis demonstrated that KBC structural properties were very similar to BC produced from the HS medium. Some slight differences could be still observed as a result of variation in the medium compositions for the different sources investigated such as the agro/industrial supplement residues of whey, apple juice, brewed

grains or black tea in the cultured medium causing a slight change in pH value of media and the assembly of cellulose chains, which altered their molecular weight, crystallinity, and orientation of the nanofibers, FTIR spectrums, the thermal stability behavior of BC/KBC membranes.

These mentioned results were published in Cellulose, 28, 14, 9335-9353 (2021), doi.org/10.1007/s10570-021-04100-5 (Web of Science Indexed, Q1, Jimp: 6.123).

5.2 Optimization of KBC production using sour whey waste

In part one of **publication II**, the article name "Development of novel biocomposites based on the clean production of microbial cellulose from dairy waste (sour whey)", the optimization results of KBC production displayed the independent variables have been expressed as highly effective, with a significant effect on the dry weight of KBC membranes compared to BC produced from HS standard medium. Furthermore, at the end of the experiment time, the pH values of the culture media were still maintained in the range of 4.4 – 4.9, still within the range of optimum pH (4 – 7) for cellulose production by *Komagatacibacter xylinus*. These responses have confirmed the positive of modification in the survey range of investigated parameters.

According to the ANOVA analysis, the statistical significance of the KBC production optimization model exhibited that probability p (<0.0001) was very small with the determination coefficients R^2 calculated as 99.86%. This shows that the reliability of the model is high. Additionally, the close agreement (<0.2) between adjusted R^2 and predicted R^2 was also observed demonstrating the suitability of the regression model in determining the optimal cellulose yield. Likewise, the Adeq Precision (65.5715) value was greater than 4, indicating that this model can be used to navigate the design space.

Furthermore, the linear graph of the predicted response compared to actual depicts that the data points are scattered along the diagonal, proving the model is satisfactory in the range of investigated parameters. The 3D response surface plots illustrate the combined effects of each pair of the independent variables on the response towards determining their optimal value. It can be observed that all five surveyed parameters had a resonant effect on cellulose membrane dry weight. Specifically, the values of sour whey, black tea, and culture period increased, cellulose dry weight will increase. In contrast, the remaining factors as cane-sugar and bacteria volume, values increased, while cellulose membrane dry weight increased followed by a decrease.

Eventually, the model proposed the best value for cellulose membrane dry weight (20.59 g/L) with optimum conditions determined as 1000 mL/L sour whey, 87.33 g/L cane-sugar, 6 g/L black tea, 79.92 mL/L bacteria volume, and 21 fermentation days at 30 °C. A triplicate fermentation experiment using these above optimization conditions was then conducted and an average dry weight of 20.14 ± 0.62 g/L was determined that verified this model obtained depicts high-yield production of KBC.

These achieved results were published in Journal of Applied Polymer Science, (2021), e51433. doi: 10.1002/app.51433 (Web of Science Indexed, Q2, Jimp: 3.057).

5.3 KBC production in large containers and characteristics of harvested BC/KBC

In part two of **publication II,** for large container assays, BC/KBC dry weight continued to show high levels, especially with the dramatic increase in the experiments on HS standard medium. Considering the scale of one liter of the medium, the investigated fermentation batch efficiency achieved the highest at a cultured medium depth of 0.5 cm, corresponding to 180 mL (KBC-0.5cm) with

the thickness of KBC membranes reaching 17 ± 0.03 mm. Specifically, the residual volume of the culture medium (also called suspension) was extremely low, with only 72.33 ± 8.74 mL.

For characteristic analysis of the obtained cellulose membranes, the morphological properties revealed similar characteristic fibrous 3D lattice structures arranged in a randomized and tightly. Image processing results determined the diameter of cellulose samples collected from $KBC_{\text{-Optimized}}$ and KBC in large containers ($KBC_{\text{-0.5cm}}$) in the range of 34–159 nm and 36–107 nm, compared to 21–147 nm for HS standard media (HS in Duran bottles and in large containers ($HS_{\text{-0.5cm}}$)), respectively.

For FTIR analysis, the typical cellulose bonds and insignificant differences have also been seen in all BC/KBC samples. The peaks at 3300 – 3500, 2800 – 3000, 1600 – 1640, and 1030 – 1160 cm^{-1} are ascribed to the stretching vibration of O–H in pure cellulose, C–H in methyl, methylene, and methoxy groups of lignocellulose, the aromatic C–C ring, and C–O–C symmetric glycosidic from carbohydrate components of cellulose.

For XRD analysis, the spectra of investigated cellulose samples showing the main vertices around 2θ angles were determined at ≈14.7 and ≈22.6, representing the cellulose structure type Iα, which corresponds to the diffraction plane (101) and the amorphous region (002) of pure cellulose in nature. The only difference between the samples was the crystallinity percentages determined as 80.9%, 81.5%, 78.6%, and 80.3%, for $KBC_{\text{-Optimized}}$, $KBC_{\text{-0.5cm}}$, BC obtained from standard HS medium in Duran bottle, and $HS_{\text{-0.5cm}}$, respectively.

For thermal stability evaluation, the investigated BC/KBC samples exhibited gravitational thermal decomposition curves consisting of fairly similar degradation stages. The first degradation stages from 25 to 220 °C are attributed

to the slight weight decrease relating to water loss in the polymer matrix. This shows that the stability of all samples is higher than 200 °C, demonstrating the great commercial application potential of these biopolymers. The second stage of degradation that occurred continues until 347-385 °C is due to the decomposition of the polymer chains and network. Then, the maximum sample weight loss is observed at temperatures close to 400 °C. Ultimately, the temperature around 600 °C is the temperature range leading to carbon-char residues formation.

It can be summarized that the basic stages in the progression of transforming sour whey waste into a utilizable biomaterial have achieved encouraging initial results. A streamlined fermentation batch that removed unnecessary requirements (without raw material pretreatment, pH calibration, shaking, stirring, or aeration) acquired high efficiency via an optimized process (accomplished outstanding yield, maximized the amount of treated waste, and reduced new emissions). The obtained KBC properties depicted insignificant differences compared to BC from the standard HS medium led to the opening of countless application areas for these cellulose products. The principal reason for small differences could be found between the samples was the by-products of whey and black tea in the cellulose biosynthesis fermentation media or the amount of incorporated KBC causing changes in the molecular weight, crystallinity, and orientation of the nanofibers via the thicker of the samples to be tightly bonded, thus difference characteristic or better thermal resistance.

These achieved results were published in Journal of Applied Polymer Science, (2021), e51433. doi: 10.1002/app.51433 (Web of Science Indexed, Q2, Jimp: 3.057).

5.4 Preparation of leather-like biocomposites based on KBC and PCL/PVA/PLA

In **publication III** with the article title ” Preparation and characterization of nonwoven fibrous biocomposites for footwear components”, and **AIP conference proceedings** designated as "Environmentally Friendly and Animal Free Leather: Fabrication and Characterization" or **the utility model** with the name “Leather material with improved ecological parameters”, as well as **the last part of publications I** (sectors about KBC application to fabricate the bio-based composite material (bioleather)), several pre-experiments were performed to investigate the usability components of Kombucha leather, predominantly based on bio-components (KBC powder, maple leave (ML) pulp) and various polymers (PCL, PVA, PLA)).

The properties and mechanical of these prepared bio-based composite materials depicted they possessed high shape stability, good surface adhesive properties, and considerable flexibility, well suited for prospective application as leather substitute components. As observed, an increase in KBC content led to an increase in the strength of the composite matrix which was attributed to enriching interfacial interactions between the biofibers and polymer matrix leading to enhancing the stress transfer efficiency of the interface.

However, after further comparison between prepared leather-like materials and commercial PU-based leather materials provided by Bata shoe company in Dolni, Czech Rebuplic, the compared results revealed superiority in mechanical integrity of PU leather. Specifically, tensile strength (N/mm^2), elongation at break (%), tear strength (N/mm), and elastic modulus (MPa) that commercial PU-based leather materials possessed, repestively as 5.28 ± 0.69 (N/mm^2), 31.54 ± 2.32 (%), 79.20 ± 2.45 (N/mm), 106.10 ± 2.70 (MPa), all higher than the optimized nonwoven fibrous biocomposite achieved in **publications III** as 2.13 ± 0.29

(N/mm^2), 19.23 ± 1.09 (%), 32.93 ± 1.33 (N/mm), 76.93 ± 1.63 (MPa); or the best leather material (sample 4) displayed in **utility model** as 2,08 (N/mm^2), 16,45 (%), 31,24 (N/mm), 72,17 (MPa); and the highest environmentally friendly and animal free leather exhibited in **AIP conference proceedings** as 1.68 (N/mm^2), 16.42 (%), 25.25 (N/mm), 84.95 (MPa), as well as the bio-based composite material (sample KBC-1) in **publications I** with tensile strength of 1.69 ± 0.33 (MPa), elongation at break of 14.54 (%), and tear strength of 25.44 ± 0.12 (N/mm). In addition, water contact angle values of investigated leather-like materials were all less than 90°. This weakness corresponded to a high wettability on the surface of the products, and also, directly limit their application potential. These results indicated that it is necessary to further improve the mechanical and water resistance of prepared samples for a better response to their expected broader range of use.

These results were published in Polymers, (2020), 12, 3061. doi:10.3390/polym1212301, Q1, IF 4.967; and International Polymer Processing Society Europe - Africa 2019, Regional Conference, (2019), AIP Conference Proceedings 2289, 020049 (2020); https://doi.org/10.1063/5.0028467; or CZ 33149 U1 (2019), Industrial Property Office, Czech Republic; as well as Cellulose, 28, 14, 9335-9353 (2021), doi.org/10.1007/s10570-021-04100-5 (Web of Science Indexed, Q1, Jimp: 6.123).

5.5 Preparation of leather-like biocomposites based on KBC and PU/PLA

In order to improve mechanical characteristics and water resistance of prepared bio-leather products, the combination of bio-based composition (KBC and sisal fiber powders) and polymer matrix (PU and PLA) were conducted in the last parts of **publications II** (sectors about KBC application to fabricate the biocomposites (leather-like materials)),

The tensile strength and elongation at break of leather-like materials treated with DCDMS (samples S2, S3, S4, S5) achieved impressive results (in the range of 135.61 ± 9.15 to 154.89 ± 9.09 (N/mm^2) and 31.06 ± 0.32 to 92.33 ± 6.91 (%)), higher than sample S1 (non-treated DCDMS) at 62.63 ± 24.97 (N/mm^2) and 26.00 ± 1.02 (%) and the mechanical analysis results of investigated leather-like materials in above mention publications (**publication III, AIP conference proceedings, the utility model, and the last part of publications I**). This phenomenon was attributed to the difference in the degree of adhesion between the bio-fillers and the polymer matrix, because, DCDMS improved the hydrophobicity of KBC and fiber powder, leading to increasing cohesion and compatibility between the bio-fillers with polymer matrix, creating a more stable three-dimensional structure with higher toughness and flexibility. On the other hand, it should be noted that the good elasticity nature of PU compared to PCL and PVA is probably also related to the breakthrough in mechanical properties of these leather substitute materials based on DCDMS-treated KBC/PU/PLA.

Additionally, the increase in DCDMS-treated KBC powder concentration resulted in an increase in mechanical stability values of the obtained bio-composites was also observed. Nevertheless, the water contact angle values were determined in the range of 67.2 ± 3.05 to 76.9 ± 5.44, still less than 90° for all prepared samples, including sample S0 (the control sample, only PU/PLA). This might be due to the casting temperature and time had too long or high that causing the deform in the properties of neat PU or PLA used. As such, it is necessary for further research to clarify the concentration of KBC, PU, PLA, and preparation conditions (the compressive temperature and time) towards the fabrication of a suitable leather-like material possibly well-respond to the customer's expectations about safety, aesthetics, function, ethics and social self-awareness.

These mentioned results were published in Journal of Applied Polymer Science, (2021), e51433. doi: 10.1002/app.51433 (Web of Science Indexed, Q2, Jimp: 3.057).

5.6 KBC powder's hydrophilic modification

In **publication IV,** the title **"**Silane modified Kombucha cellulose-based biocomposite leather-like mats: Preparation, optimization, and characterization", the results show that there was a significant improvement in hydrophobicity of KBC powders treated with four various silane types (P4, P5, P6, and P7) compared to the three non-silane treated samples (P1, P2, and P3). For sample P1, the soaking water turned brown immediately after the vigorous stirring followed by the settling of almost all the KBC samples. This can be attributed to soaking KBC in NaOH 0.5%, 30 min at 80°C have not been removed the residual components of a fermentation medium such as sugar or black tea. Results also satisfied explain the highest water holding capacity since there was an almost huge effect on the ingredients of the treated KBC samples. For samples P2 and P3, these treatment conditions had both killed bacteria and removed unwanted substances, decolorized, remove odor to produce a brighter color of the soaked water. However, the OH-rich and hydrophilic nature of KBC is still unchanged, resulting in their water holding capacity being insignificantly different compared to sample P1 with a similar deposition after vigorous stirring and 24h immersion.

For KBC samples treated with four various silane types, their hydrophobicity was strongly enhanced with the highest result at sample P6 (treated with VTS). These KBC completely floated to the top of the soaking water surface after the survey time. The accompanied FTIR spectra also confirmed the presence of these characteristic functional groups of silane in the composition of these four KBC samples. The peaks around 760-917 cm^{-1} corresponding to Si-OH, Si–C or $Si=O_2$ stretching in the analytical spectrum of four samples P4, 5, 6, 7 revealed that

silane was coupled to KBC powders but, it was absent in the analysis results of sample P3 (KBC treated with NaOH 5) % (w/v) for 1h). The other characteristic peaks of Si–O–Cellulose, Si–O–Si, or Si–O–CH_3 (nearly 1105-1169 cm^{-1}) were covered by the strong peaks characteristic of the C–O–C strongly vibration in the cellulose of neat KBC.

These mentioned results were submitted to *Sustainable Materials and Technologies* on 01/04/2022, (Web of Science Indexed, Q1, Jimp: 10.681, present status under review as on 20/08/2022).

5.7 Optimization of Kombucha leather preparation using KBC/PU/PLA

For Kombucha leather preparation optimization, the results in part 2 of **publication IV** showed the actual and predicted values of the responses of experimental design model using Design-Expert® V11 software to optimize the ingredients and prepared conditions of Kombucha leather were within acceptable limits. As observed, there was a clear opposite effect between PU concentration and the four other independent variables (the amount of KBC, PLA, compressed temperature and time). The PU concentration was directly proportional to the enhancement of physicomechanical properties accompanied by a decrease in the biodegradability of the prepared samples. Conversely, the increase in the amount of granulated KBC, PLA, and compressed temperature, as well as compression time, caused a fall in elasticity (higher elastic modulus) and water contact angle (lower than 90°), but an increase in the biodegradation rate of these products was observed. This phenomenon was due to the natural characteristics of three constitutional components (KBC, PU, and PLA) or some change in the structure of the materials that relate to their properties when undergoing the compression process under high temperature and a long period of time.

The model's statistical significance was assessed via ANOVA analysis indicated by very small p-values (<0.0001), high F-values as well as high determination coefficients (R^2) of more than 0.990 for all three response variables, which shows the reliability and significance of the model. In addition, adjusted R^2 and predicted R^2 values were in close agreement (<0.2), which confirmed the high suitability of the regression model and could be used to navigate the formulation design space. This was further supported by the linear graphs of the three predicted responses compared to actuals that proved the model is satisfactory in the range of investigated parameters.

In addition, a triplicate validating experiment and predicted optimum values were almost in agreement close (44.07±0.51 and 44.89 for elastic modulus; 1.31±0.04 and 1.32 for biodegradable; 94.84±1.59 and 95.34 for water contact angle) from optimum leather-like mat prepared with KBC (13.74 % w/w), polyurethane elastomer (73.89 % w/w), and polylactic acid (12.50 % w/w), compressed at 155 °C for 5 min. This similarity further confirmed the validity of the used model.

The morphological, physico-chemical, mechanical, wetting and biodegradation characteristics of the optimized leather-like material, were further analyzed and compared with the commercial leathers (C1 and C2) to further elucidate its growth potential. The cross-section morphology with significant differences in the randomly arranged pores in the structure of analysis material samples. As observed, the addition of silane-treated KBC creates a pore density that is assumed to be positive for porosity and breathability but can still maintain the rather good physicomechanical properties of the optimized treated KBC mat, compared to neat PU and PU/PLA (a smooth, nonporous structure, completely compact), as well as, NaOH treated KBC mat (too many porosity or interlacing). In addition, the suitable casting temperature and time to limit the boiling of the

mixture leading to the air bubble formation and the voids or pores, is also an important contribution to the formation of tightly compacted structures of the optimized leather substitute material.

For FTIR analysis, sequential characteristic broad peaks of PU, PLA, and KBC appeared at approximately 3322 cm^{-1} (N-H and O-H stretching), 2867-2935 cm^{-1} (C-H asymmetric stretching in methyl, methylene, or methoxy groups), 1728 cm^{-1} (C=O bending, C-N and N-H stretching). Parallel to that, the vibration at 1227-1532 cm^{-1} is ascribed to amide II and N-H bending, 1068-1195 cm^{-1} relating to C-O-C symmetric stretching, C-N, C-C, and C-O stretching. While the peaks between 760-912 cm^{-1} correspond to Si-O-Si bending attributed to KBC treated with VTS. These peaks were all observed in the optimized materials.

For XRD analyses, the XRD spectrum curves of the optimized silane-treated and silane-untreated KBC leather-like mat clearly conveyed the presence of the main vertices of KBC at 2θ angles around ≈14.5° and ≈22.3° (representing type Iα cellulose structure corresponding to Miller indices of (100) and (110)) and two characteristic absorption peaks at ≈21° and ≈26° for PU and PLA.

For TGA, the thermal behaviors of the investigated materials were higher than 200 ^{0}C, demonstrating their great commercial application potential. All the samples exhibited gravitational thermal decomposition curves consisting of fairly similar degradation stages of weight loss, depolymerization, the degradation of water molecules, or polymer chains. The results also reveal that there was a difference in the heat resistance of the prepared materials with the gradual increase from neat PLA to the mixtures of KBC/PLA, KBC/PU, KBC/PU/PLA, PU/PLA, and neat PU, respectively. The presence of granulated KBC and the dissolution or blending of PU and PLA in THF solvent changed the thermal properties of the pure polymers. In particular, the thermal stability of the optimized silane-treated KBC leather-like mat was as expected showing an

insignificant difference compared to neat PU. This can be attributed to low amounts of granulated KBC and PLA composition ratios in the products, or appropriate processing, mixing, and/or heat pressing to mold the leather-like sheets. Moreover, a great combination of a well-blended structure led to a negligible influence on the mechanical properties as well as the good heat resistance properties of the key ingredients. These results exhibited a high development and application potential of these obtained leather-like materials.

For the comparison between optimized silane-treated or silane-treated KBC leather-like mat and commercial leathers, the prepared materials achieve high performance, especially hydrophobic surface which is almost negligible but decreased after a period of water exposure lasting up to 5 minutes. This is completely opposite to commercial leather C2, where the liquid droplet completely spreads and absorbs on the material surface (WCA=0^0). The good hydrophobic properties of the prepared samples can be attributed to the tight structures created through good blending favoring interaction between all three components that possessed high resistance to water absorption (PU, PLA, and KBC treated with silane).

In a more detailed consideration, significantly different results have been observed in the event of silane-untreated KBC leather-like materials. At succinctly water contact time (1 min), the strong hydrophobic polymer components of this material created dramatic effects on its high water resistance. Nevertheless, with prolonged exposure to the water droplet (5 min), its surface water resistance decreased sharply. The possible reason for this phenomenon was the low interaction between the different components leading to a loose structure with more pores or gaps in the combination block between the hydrophilic KBC and the hydrophobic polymer matrix. The occurrence of these pores unintentionally generated capillary forces that combine with the hydrophilic

nature of the non-treated KBC to promote the diffusivity of water molecules. This phenomenon possibly could also greatly explain the high biodegradable rate and elastic modulus coefficient achieved for these materials as compared to other prepared samples.

Another point worth noting is that the biodegradable rate of the optimized silane-treated KBC leather-like mat was approximate to or better than that of others. The presence of more than 25% of both KBC and PLA as biobased components in the compositions made it more environmentally friendly. However, this result indirectly indicates the water absorption risk of the optimized materials with prolonged exposure to high moisture conditions. The double-binding function of silane molecules with the chemical compounds of KBC began ineffectively due to hydrolysis reactions or by the action of soil microorganisms.

Generally, it can be summarized that silane-treated KBC leather-like mat has basically shown a possible response to the essential requirements of new leather-like substitute materials, suitable for continued development in the era of a circular economy and sustainable production. Nevertheless, some slight differences were also found between the analyzed results of the prepared materials and the ingredients or neat polymers. This might be due to some transformations in their pristine properties during preparation from dissolution, pretreatment or combination to blending, and heat pressing.

These mentioned results were submitted to *Sustainable Materials and Technologies* on 01/04/2022, (Web of Science Indexed, Q1, Jimp: 10.681, present status under review as on 20/08/2022).

6. CONTRIBUTION OF THE THESIS

The greatest contribution of the thesis is to elucidate the key steps of Kombucha leather preparation which is possibly used in fashion design, footwear, bags, or interior covering products. This leather substituted biocomposite material is expected to possess properties capable of good responding to the strict and urgent requirements of both consumers, manufacturers, social managers, and even, the trend of sustainable development or contemporary humanity. Herein, using KBC raw materials produced from bio-waste sources via Kombucha fermentation method directly enhanced the yield, simplified production, and improved the eco-friendly, cost-effectiveness of products. Furthermore, the addition of KBC, PLA to the PU substrate matrix resulting Kombucha leather products being completely animal-free, higher form retention capacity, and short post-used biodegradation time but still possessing all the good mechanical properties of PU such as high toughness, durability, high covering and protection. Essentially, the shortcomings in the environment and humanity of the leather industry are fundamentally handled by Kombucha leather products that they represented as a sustainable approach and safe for the entire ecosystem (transform a polluting waste into a useful alternative material for traditional products that are causing serious pollution). In addition, the materials treatment to improve the characteristics aiming more suitable for the intended use, or the evaluation processes of the materials and products quality via various analysis techniques are also useful contributions to the continuous development of science, technology, and education of mankind.

7. CONCLUSIONS

In the current study, the basic stages of the progression of transforming waste into useful materials have reached encouraging initial results. Three bio-waste sources used in the kombucha fermentation batch as alternative nutrient sources were all strongly effective in improving the dry weight of obtained cellulose membranes. Five main impact factors of KBC membrane production include the amount of sour whey waste, cane sugar and black tea content, bacteria volume, and culture period was optimized following have achieved high results in outstanding yield and maximizing the amount of using waste, even, reducing new pollution. Optimization also recorded some advancements in the effort to enhance the large-scale application potential of this KBC production method via suitable removing unnecessary steps. Based on the physicochemical analysis, harvested KBC membranes are not only interesting for research, but they are also extensively promising for numerous new application areas due to their properties showing insignificant differences compared to BC from HS standard medium. Though more research investigation is still required, it can see that the cellulose (KBC) produced by the microorganism *Komagataeibacter xylinus* represents a good production source since it enhances sustainability, simplicity, and cost-effectivity of the process to transform waste into useful products.

For the finished product fabrication, Kombucha leather was prepared and successfully characterized as a correctly and fully respond or one of usable reform for the leather market's current requirements about ethical consumption, responsible fashion design, cleaner production, and sustainable development. Optimized Kombucha leather containing PU, PLA, and KBC pretreated with vinyltriethoxysilane was completely animal-free, moderated biodegradable rate, but still possessed good elasticity and a hydrophobic surface. KBC hydrophobicity improvement treatment plays a critical role to enhance the

compatibility between KBC and the hydrophobic polymer matrix ensuring a homogenous blending and tight structure of leather-like materials. The obtained optimization model revealed PU concentration was directly proportional to the strong enhancement in physical-mechanical properties, conversely, the high increase of KBC, PLA, and compressed temperature, as well as compressed time, caused a fall in elasticity and water contact angle, but increased the biodegradation rate of products. The comparison indicators between optimized Kombucha leather and two commercial leather types have all achieved high performance. Basically, the results show a possible response to the essential requirements of this new leather substitute material with the advantageous properties as free-animal and eco-friendly, without mining, deforestation, animals killing. Moreover, it is easy to produce, has less energy consumption, and less harmful emissions.

Prospective research work will be focused on improving other properties of the final product such as enhancing its biodegradability, breathability, customer comfort, appearance, shape stability, mechanical integrity, flexibility, tear and wear resistance, and cost-effectiveness. Additionally, this research also promoted trial fabrication of Kombucha leather by other polymer and cellulose sources, notably from leaves, rice husks, banana fibers, and coconut fibers. It is necessary to continue to perform some further test about the influence of particle size of cellulose powder, or the affection of compression force on the properties of Kombucha leather.

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LIST OF FIGURES AND TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

3-D	3 dimensional
APS	3-aminopropyltriethoxysilane
ASTM	American standards testing methods
BC	Bacterial cellulose
CCD	Charge coupled devices
dH_2O	distilled water
DCDMS	Dimethyldichlorosilane
DOE	Design of experiment
FTIR	Fourier transform-infrared spectroscopy
HDS	Hexadecyltrimethoxysilane
HS	Hestrin and Schramm medium
IR	Infrared radiation
PCL	Polycaprolactone
PE	Polyethylene
PEG	Polyethylene glycol
PETA	People for the Ethical Treatment of Animals
PP	Polypropylene
PLA	Polylactic acid
PS	Polystyrene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride

PU	Polyurethane
KBC	Kombucha-derived bacterial cellulose
SEM	Scanning electron microscope
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
VTS	Vinyltriethoxysilane
WAC	Water absorption capacity
WHC	Water holding capacity
XRD	X-ray diffraction

LIST OF PUBLICATIONS RELATED TO DOCTORAL THESIS

Publication I:

Kombucha-derived bacterial cellulose from diverse wastes: a prudent leather alternative. **Hau Trung Nguyen (40%)**. Nabanita Saha. Fahanwi Asabuwa Ngwabebhoh. Oyunchimeg Zandraa. Tomas Saha. Petr Saha. *Cellulose*, (2021), 28, 14, 9335-9353. doi.org/10.1007/s10570-021-04100-5, **Q1**, IF 6.123.

Publication II:

Development of novel biocomposites based on the clean production of microbial cellulose from dairy waste (sour whey). **Hau Trung Nguyen (40%)**, Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, Oyunchimeg Zandraa, Tomas Saha, Petr Saha. *Journal of Applied Polymer Science*, (2021), e51433. doi: 10.1002/app.51433, **Q2**, IF 3.057.

Publication III:

Preparation and characterization of nonwoven fibrous biocomposites for footwear components. Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, **Hau Trung Nguyen (10%)**, Urška Vrabič Brodnjak, Tomas Saha, Anežka Lengalova, Petr Saha. *Polymers*, (2020), 12, 3061. doi:10.3390/polym1212301, **Q1**, IF 4.967.

Publication IV:

Silane modified Kombucha cellulose-based biocomposite leather-like mats: Preparation, optimization, and characterization. **Hau Trung Nguyen (60%)**. Nabanita Saha. Fahanwi Asabuwa Ngwabebhoh. Oyunchimeg Zandraa.

Tomas Saha. Petr Saha. Manuscript is submitted to *Sustainable Materials and Technologies* on 01/04/2022 (present status under review as on 20/08/2022).

Publication V:

Environmentally Friendly and Animal Free Leather: Fabrication and Characterization. Nabanita Saha, Fahanwi Asabuwa Ngwabebhoh, **Hau Trung Nguyen (10%)**, Petr Saha. *International Polymer Processing Society Europe - Africa 2019*, Regional Conference, November 18 – 21, 2019, Pretoria, South Africa. AIP Conference Proceedings 2289, 020049 (2020); https://doi.org/10.1063/5.0028467.

Publication VI:

Leather material with improved ecological parameters. Utility model. CZ 33149 U1. Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, **Hau Trung Nguyen (10%)**, Tomas Saha, Petr Saha. *Industrial Property Office*, Czech Republic (2019).

CURRICULUM VITAE

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PROJECTS

1. IGA/CPS/2019/009

   Production of Kombucha Leather using Waste Bioresources (Applicant).

2. IGA/CPS/2020/005

   Optimization of fermentation process for the production of Kombucha Biomass Cellulose (KBC) using sour whey waste (Applicant).

3. IGA/CPS/2021/002

   Preparation and characterization of nanocomposite systems (Member).

LIST OF PUBLICATIONS *(Based on Doctoral Thesis work)*

Impact factor journals

1. Kombucha-derived bacterial cellulose from diverse wastes: a prudent leather alternative. **Hau Trung Nguyen (40%)**. Nabanita Saha. Fahanwi Asabuwa Ngwabebhoh. Oyunchimeg Zandraa. Tomas Saha. Petr Saha. *Cellulose*, (2021), 28, 14, 9335-9353. doi.org/10.1007/s10570-021-04100-5, **Q1**, IF 6.123.
2. Development of novel biocomposites based on the clean production of microbial cellulose from dairy waste (sour whey). **Hau Trung Nguyen (40%)**, Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, Oyunchimeg Zandraa, Tomas Saha, Petr Saha. *Journal of Applied Polymer Science*, (2021), e51433. doi: 10.1002/app.51433, **Q2**, IF 3.057.
3. Preparation and characterization of nonwoven fibrous biocomposites for footwear components. Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, **Hau Trung Nguyen (10%)**, Urška Vrabič Brodnjak, Tomas Saha, Anežka Lengalova, Petr Saha. *Polymers*, (2020), 12, 3061. doi:10.3390/polym1212301, **Q1**, IF 4.967.
4. Silane modified Kombucha Cellulose-based Biocomposites: An artificial leather, preparation and characterization. **Hau Trung Nguyen (60%)**. Nabanita Saha. Fahanwi Asabuwa Ngwabebhoh. Oyunchimeg Zandraa. Tomas Saha. Petr Saha. Manuscript is submitted to *Sustainable Materials and Technologies* on 01/04/2022 (present status under review as on 20/08/2022).

Utility model

1. Leather material with improved ecological parameters. CZ 33149 U1. Fahanwi Asabuwa Ngwabebhoh, Nabanita Saha, **Hau Trung Nguyen (10%)**, Tomas Saha, Petr Saha. *Industrial Property Office*, Czech Republic (2019).

Conference Proceedings **(Scopus)**

1. Environmentally Friendly and Animal Free Leather: Fabrication and Characterization. Nabanita Saha, Fahanwi Asabuwa Ngwabebhoh, **Hau Trung Nguyen (10%)**, Petr Saha. *International Polymer Processing Society Europe - Africa 2019*, Regional Conference, November 18–21, 2019, Pretoria, South Africa. AIP Conference Proceedings 2289, 020049 (2020); https://doi.org/10.1063/5.0028467.

Additional research publication along doctoral thesis work

Impact factor journals

1. Development of dual crosslinked mumio-based hydrogel dressing for wound healing application: Physico-chemistry and antimicrobial activity. Oyunchimeg Zandraa, Fahanwi Asabuwa Ngwabebhoh, Rahul Patwa, **Hau Trung Nguyen**, Marjan Motiei, Nabanita Saha, Tomas Saha, Petr Saha. *International Journal of Pharmaceutics*, (2021), 607, 120952. doi.org/10.1016/j.ijpharm.2021.120952, **Q1**, IF 6.51.
2. Citric acid-based crosslinked gellan gum-bacterial cellulose hydrogel: An eco-friendly green adsorbent for dye removal. **Hau Trung Nguyen**. Nabanita Saha. Fahanwi Asabuwa Ngwabebhoh. Oyunchimeg Zandraa. Tomas Saha. Petr Saha. Manuscript is submitted to *International Journal*

of Biological Macromolecules on 20/05/2022 (present status: Revision as on 20/08/2022).

International Conferences

1. Upcycling of dairy wastes to microbial cellulose: a bioadhesive property based advanced biomaterial. Fahanwi Asabuwa Ngwabebhoh, **Hau Trung Nguyen**, Oyunchimeg Zandraa, Rahul Patwa, Smarak Bandyopadhyay, Probal Basu, Nabanita Saha, Petr Saha. *4th International conference on Biological and Biomimetic Adhesives*, February 18-19, 2021, University of Aveiro, Portugal.
2. Production of Agro-Sourced Nonwoven Fibrous Materials as Leather Alternative in Footwear. Fahanwi Asabuwa Ngwabebhoh, **Hau Trung Nguyen**, Nabanita Saha, Tomas Saha, Petr Saha. *Conference on New Technologies, New Materials and New forms of Footwear Sales*, October 21st, 2021, Czech Footwear and Leather Association, Zlin, Czech Republic.
3. Synthesis and characterization of graphene oxide from residual biomass. Adam Aberra Challa, Nabanita Saha, Fahanwi Asabuwa Ngwabebhoh, **Hau Trung Nguyen**, Pavel Urbanek, Haojie Fei, Petr Saha. 2022 IEEE 12th International Conference Nanomaterials: Applications and Properties (IEEE NAP-2022), Sep, 11-16, 2022, Krakow, Poland.

Kombucha leather: Preparation and Characterization

Kombucha kůže: Příprava a charakterizace

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