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Review

Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation

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ABSTRACT

SCOBY, a biofilm of cellulose containing the symbiotic culture of bacteria and yeast, is a by-product of Kombucha tea fermentation. Several studies on the kombucha SCOBY are being carried out to exploit all the possibilities of dealing with this cellulose as a suitable raw material in fields like food technology, biomaterial preparation, fashion and textile industries, environmental biotechnology, and so on. This review focusses on elaborating about the microbial ecology present in the kombucha tea fermentation, the production of the extracellular polysaccharide (cellulose) by the bacteria, the cultivation methods of SCOBY, composition, structure, and characteristics of the cellulose biofilms obtained. Genera of bacteria and yeast majorly found in the SCOBY are Gluconobacter, Acetobacter, Zygosaccharomyces, Saccharomyces, and Schizosaccharomyces. Present in a symbiotic relationship in the kombucha tea, these microbes help in producing cellulose fibrils extracellularly forming a biofilm at the air-liquid interface. An overview of the favorable conditions for SCOBY production, prevention of contamination and purification of the cellulose sheets are also discussed and then its suitability for applications in different fields are assessed. The fermentation is mostly done at room temperature and the biofilm is harvested within 30 days according to the required shape and size. Advantages like flexible physical, chemical and biological properties, the requirement of minimum media components, eco-friendly and cost efficiency of this biofilm leading to its utilization in many upcoming fields are elucidated. Optimization of process variables is essential for scale-up to facilitate cost-effective production of SCOBY cellulose for exploring its wide potential in sustainable applications.

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1. Introduction

Kombucha tea is a sweetened beverage commonly produced by the fermentation of black tea leaves. Sometimes green tea or oolong tea leaves are also being used. This beverage has originated in North-Eastern China. The fermentation is done with a Symbiotic Culture of Bacteria and Yeast (SCOBY) in the medium containing tea extract and a sugar source for 7–10 days (Leal et al., 2018). After fermentation, the tea consists of a floating cellulose layer on the air–liquid interface which is formed by the bacteria present in the symbiotic culture (also commonly known as the tea fungus) and the liquid tea broth underneath. (Chen and Liu, 2000).

The liquid tea broth, referred to as the 'Kombucha tea', has a wide reach as a traditional beverage universally. Nowadays it is being brewed in households and it is also commercially bottled and sold. It has a pleasant fruity sour like sparkling flavor due to the presence of few organic acids and the carbon dioxide released during fermentation. Kombucha tea contains different organic acids like acetic, gluconic and glucuronic acid. Apart from these it also contains various sugars, water-soluble vitamins, amino acids, biogenic amines, purines, pigments, lipids, proteins, hydrolytic enzymes, ethanol, carbon dioxide, polyphenols, D-saccharic acid-1,4-lactone (DSL), minerals (manganese, iron, nickel, copper, zinc, lead, cobalt, chromium, and cadmium), anions (fluoride, chloride, bromide, iodide, nitrate, phosphate, and sulfate), and metabolic products of yeasts and bacteria (Jayabalan et al., 2010, 2014). Since various vitamins, minerals, cations and anions are present in the tea extract, additional nitrogen supplements and nutrient media is not required for the fermentation and cellulose production (Chen and Liu, 2000).

Kombucha tea is claimed to have several health benefits along with its probiotic effects due to the microorganisms present. Such benefits include anti-diabetic effects (Aloulou et al., 2012), anti-carcinogenic (Jayabalan et al., 2011), treatment for gastric ulcer (Banerjee et al., 2010) and high cholesterol (Yang et al., 2009), anti-inflammatory and antioxidant activity (Villarreal-Soto et al., 2018), improvement of the liver, immune system and gastrointestinal functions (Leal et al., 2018).

Kombucha tea can be prepared with different kinds of sugar sources as substrates and in different containers over a varying time. The commonly used method for preparation **(Fig. 1)** of kombucha tea is by adding 0.5% of tea leaves and 5% sucrose in 1L of boiling water, which acts as a substrate for the fermentation and stirred continuously. After 5 min, the tea leaves are filtered and when the temperature decreases to 20 °C, 3% of SCOBY along with 0.2% previously prepared kombucha tea is added to lower the pH, which will prevent the growth of undesirable microbes and also accelerate the start of the fermentation process. The mixture is covered with a cloth or gauze for aerobic respiration to take place failing which the process might come to a halt. It is incubated for around 7–14 days at an optimal temperature range of 18 °C–26 °C. During fermentation new SCOBY biofilm layer will form on the surface within 2–3 days and the mother layer is seen below which could be separated later (Reiss, 1994; Jayabalan et al., 2008).

This SCOBY consists of a wide range of microbial populations and some of the commonly found microbes belong to the genera *Gluconobacter, Acetobacter*, *Zygosaccharomyces*, *Saccharomyces*, and *Schizosaccharomyces* (Jayabalan et al., 2014). Apart from these microorganisms, various other microbes are also present in the biofilm. The type of microbes present at any point of time in the biofilm depends on various factors like the source, substrate provided for fermentation, the metabolites produced during fermentation and on the climate and geography of cultivation (Mukadam et al., 2016). The dominating microbes present in the SCOBY and the kombucha tea almost remains the same (Teoh et al., 2004). Few changes in the yeast community were observed as the days of fermentation progressed but in bacteria, no changes were reported in the study by Chakravorty et al. (2016). *Acetobacter xylinum* strain which is present majorly in the pellicle contribute to the cellulose formation and nitrogen fixation (De Roos and De Vuyst, 2018) and the yeast strains metabolize the sugar and convert it into glucose and fructose, which is utilized by bacteria in biofilm synthesis, and also produces ethanol (Jayabalan et al., 2014).

SCOBY is also a type of bacterial cellulose (BC) like all the other single strain commercially produced BC. Some properties like the basic structure, the biosynthetic process followed by the bacterium and the purpose of this exopolysaccharide synthesis are similar because even though SCOBY is a symbiotic culture, the cellulose is produced by the bacterial genera only (Villarreal-Soto et al., 2018). This biofilm produced by the bacteria helps in attachment and protection of the cells from extremely unfavorable conditions like ultraviolet radiation or high hydrostatic pressure or any other environmental challenges. It also helps in constantly exposing the bacteria to an aerobic environment which is essential for the fermentation (Ross et al., 1991). The cellulose produced by the bacteria is made of microfibrils that are 100 times smaller when compared to plant cellulose. Therefore, it is also being used as a substitute for plant cellulose wherever possible. For instance, tea fungus cellulose has been used to produce carboxymethyl cellulose (CMC). Since CMC ether is commercially in large demand, these

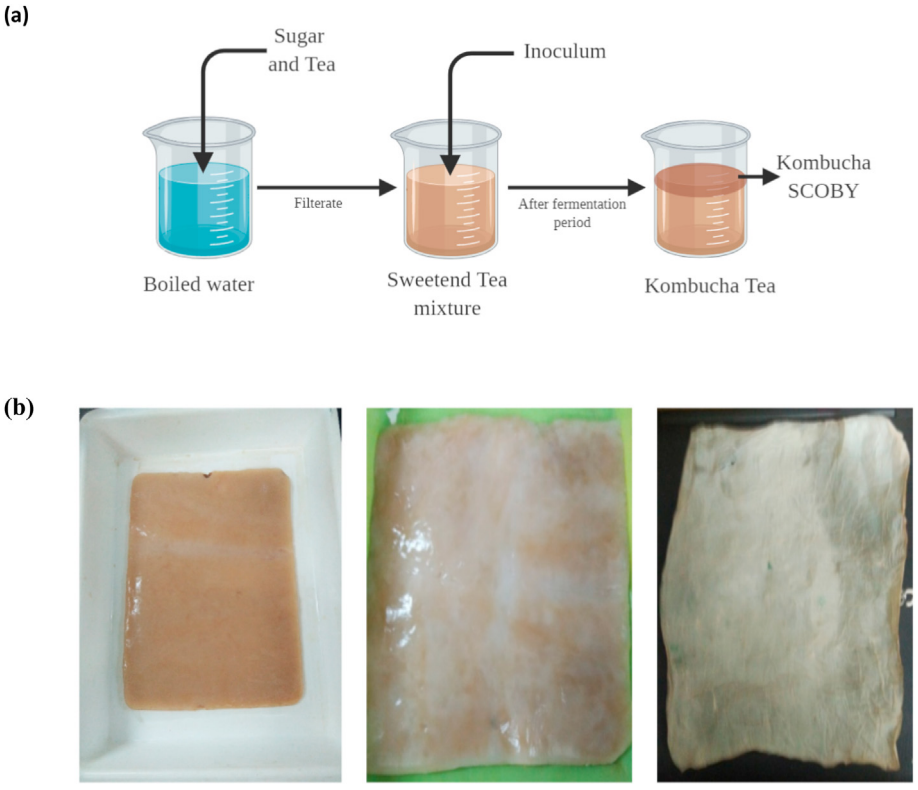

Fig. 1. (a) Kombucha tea fermentation (b) Harvested, treated with NaOH and dried SCOBY.

alternatives for production are extensively experimented (Gargey et al., 2019). Structurally bacterial cellulose is finer and more unbranched when compared to plant cellulose and this has contributed to various properties like high surface area, increased water absorption and better mechanical strength in the wet state (Chawla et al., 2009; Gayathry and Gopalaswamy, 2014). Other properties like high crystallinity, biocompatibility, non-toxicity, high porosity make it a suitable material for technological applications in bioplastics, bioenergy, food fortification and packaging (Chawla et al., 2009). All these properties are common for all cellulose produced form a bacterium. Few basic differences between SCOBY and other BC are discussed in Table 1.

Several studies about the kombucha tea and its uses are available in the literature, but only limited studies have been reported about the SCOBY grown on the fermented tea broth. Few aspects like the productivity of the cellulose, its feasibility when grown in sweetened tea mixture compared to the cultivation in synthetic media, cultivation in large scale and the various applications of the SCOBY needs more focus. The research work done as on date regarding Kombucha SCOBY was exhaustively searched and the number of hits obtained from various databases is given in Table 2.

These results imply that studies under this field are very minimal comparatively and extensive research work is expected to be carried out to fill the lacunae behind why it has still not been introduced in large scale commercial applications and on how the complications regarding this material should be solved. Among all these research and review work done till date various basic information and facts regarding kombucha cellulose has been highlighted, but all these aspects and information to be known about specifically kombucha SCOBY put together in a brief and comprehensive form is not available to the best of our knowledge. Thus, the objective of the review is to provide an overall insight into all the basic research done about microbial ecology of SCOBY, different ways to cultivate this biofilm, yield at various culture conditions, properties, favorable conditions for growth and the applicational aspects in different fields to get a collective idea and to fill the knowledge gap on the potential of this cellulose material.

2. SCOBY

SCOBY (Symbiotic Culture of Bacteria and Yeast) is a three-dimensional cellulosic zoogleal mat with the presence of a symbiotic relationship between acetic acid bacteria and the osmophilic yeast species (Jarrell et al., 2000). The bacteria *Acetobacter xylinum* produces the cellulose biofilm, which is considered as a secondary metabolite of the fermentation and is one of the important characteristics of this process. The name 'fungus' is a misleading terminology given to the biofilm. Since it resembles a surface mold on the unagitated medium and due to the presence of different yeast species it was called as fungus in common terms (Sreeramulu et al., 2000). The yeast and bacterial cell count increase up to 14 days of fermentation in both SCOBY and broth and then it starts to decrease in the cellulose pellicle, but the liquid broth was always found to contain higher viable bacterial and yeast cells (Chen and Liu, 2000). On contrary, it was also reported that the concentration of acetic acid bacteria was more in the upper part of the cellulose layer which was more exposed to oxygen than the broth underneath (Reiss, 1994).

There are numerous bacteria and yeast species present on the zoogleal cellulose mat. The kinds of acetic acid bacteria present are *Acetobacter xylium, Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti*, and *Acetobacter pasteurianus* and osmophilic yeast strains are *Schizosaccharomyces pombe, Saccharomyces ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces rouxii, Zygosaccharomyces bailii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii, Pichia*

Table 1
Differences between SCOBY cellulose and Bacterial cellulose.

S. No.	Aspect	BC	Kombucha SCOBY
1	Microbial population	Produced by individual or consortium of few microbes (Mostly Bacteria) (Shoda and Sugano, 2005)	Produced by a consortium of bacteria and yeast (SCOBY) (Marsh et al., 2014)
2	Cost	More expensive to produce due to the media components (Shoda and Sugano, 2005)	Cost efficient (Sharma and Bhardwaj, 2019)
3	Culture medium	Hestrin-Schramm media, and many other components are added externally for efficient production (Hestrin and Schramm, 1954).	Sweetened tea mixture Adto media to degenerate cellulose non producing cells. But in kombucha culture there is constant ethanol production by yeast (Chawla et al., 2009)
4	Substrate	Glucose as carbon source. Can be limiting since more amount is converted to gluconic acid also rather than cellulose. So low concentration of glucose is preferred for cellulose production (Chawla et al., 2009)	Sucrose as carbon source is also metabolized and utilized in all possible ways by the bacteria and yeast (Chu and Chen, 2006)
5	Reactors used for cultivation	50 L internal loop airlift reactor (Chao et al., 2001) Rotary disc reactor and rotary biofilm contactor, Breathable silicon air bag for cellulose growth (Chawla et al., 2009) Horizontal lift reactor (Kralisch et al., 2010)	Closed plastic boxes or jars; glass vessels (Sahasrabudhe, 2006)
6	Mechanical	Youngs Modulus:15.1 GPa (49 kPa) Tensile Strength: 199 MPa (Iguchi et al., 2000)	Youngs modulus: 2.64 GPa (thickness .029 mm) Sharma et al. (2020)
7	Commercial Food applications	Nata-de-coco, Nata-de-pina (edible cellulose with *A.xylinum*) Iguchi et al. *(2000)*	Teekvass (edible) Markov et al. (2005)
	Commercial product	In SONY Corporation headphones as filter membrane (Niyazbekova et al., 2018)	–
	Medical application	Bacterial synthesized cellulose - BASYC® for artificial blood vessel. Biofill® and Gengiflex® - Surgery and Dental implants (Klemm et al., 2001)	–

Table 2
Literature review on Kombucha SCOBY available in various databases.

S.No	Keywords searched	Google scholar hits	Scopus hits	ScienceDirect hits	PubMed hits
1	Kombucha tea cellulose	1470	361	180	22
2	Kombucha fungus	2880	647	167	77
3	Kombucha biofilm	779	116	84	11
4	Kombucha SCOBY	577	54	29	5
5	Kombucha SCOBY Cultivation	143	5	7	1
6	SCOBY Cellulose	263	24	24	6
7	SCOBY Cellulose cultivation	109	5	6	1
8	Kombucha SCOBY application	304	41	15	0
9	Kombucha composition	4000	924	337	18
10	SCOBY fungus	433	34	12	2

membranaefaciens, Torulopsis, and Candida, all these are comprised in the symbiotic culture (Dufresne and Farnworth, 2001).

2.1. Bacteria

Among the bacterial genera present in SCOBY, the majority were acetic acid bacteria that are involved in the conversion of the ethanol produced by the fermentation to acetate hydrate and that is in turn converted to acetic acid by the enzyme acetaldehyde dehydrogenase (Jayabalan et al., 2007). The culture-dependent rRNA sequence analysis showed around 86%–99% of the genus *Gluconacetobacter* present in the consortium (Marsh et al., 2014).

Adrian John Brown in 1886 identified the bacteria responsible for fermentation and named it *Bacterium xylinum*. After that, to date, it is being known by several other names like *Acetobacter xylinum* and *Gluconacetobacter xylinus*. It is a gram-negative, motile and rod-shaped bacterium. In 2012, it was given a new genus name *Komagataeibacter* (Römling and Galperin, 2015; Brown, 1886a,b). This bacterium belongs to the family *Acetobacteraceae* and is nowadays referred to as the most commonly found bacteria for cellulose production. However, other species of this *Komagataeibacter* genera have also been reported to harbor strains that produce cellulose, such as *Komagataeibacter swingsii, Komagataeibacter rhaeticus* and *Komagataeibacter medellinensis. K. rhaeticus* strain P 1463 isolated from cellulose produced by kombucha with good physical and mechanical properties. (Castro et al., 2013; Semjonovs et al., 2016).

St-Pierre (2019) performed genomic DNA extraction of different generations of grown SCOBY and subjected to shotgun metagenomic sequencing with Oxford Nanopore's MinION, DNA and RNA sequencer. As a result, it was observed that the greatest diversity of microbes in SCOBY was seen in the first generation and it decreased over subsequent generations. After 5 generations the percentage of the microbes increased. The major composition of the microbe community initially was made up of 60% of *Komagataeibacter xylinus,* where the concentration was increased to 70% after 10 generations. Hence, *Komagataeibacter xylinus* was known as the major cellulose producer in kombucha SCOBY, while the other predominant microbe is *Gluconobacter oxydans* (St-Pierre, 2019).

Lactic acid bacteria (LAB) like *Lactobacillus* and *Lactococcus* have also been found to occupy a considerable proportion of the microbes in the cellulose pellicle (Zhang et al., 2011). In an Irish Kombucha cellulose pellicle, LAB occupied more than 35% of the culture. It was observed that Lactobacillus prevailed in a higher concentration in the latter stages of fermentation. When co-cultured *Lactobacillus* sp. with acetic acid bacteria like the *Gluconacetobacter* greater production of cellulose was seen so it was inferred that *Lactobacillus* supported the growth of bacteria (Seto et al., 2006). Along with these bacteria, many other strains are found in different kombucha sources and it is listed in Table 3.

2.2. Yeast

Yeast cells in the culture medium usually settle at the bottom and few yeast cells get trapped in the cellulose synthesized by the bacteria at the surface (Drljača, 2004). This yeast population was studied, and it was identified that genera *Zygosaccharomyces* and *Saccharomyces* dominated the culture (Marsh et al., 2014; Teoh et al., 2004). High throughput methods of analyzing the microbial population are used since the culture-dependent analysis method may not be enough to determine the overall microbial community structure as the growth of yeast cells was very slow. It was found that *Zygosaccharomyces* was the most common genus found in both pellicle and the broth occupying more than 95% of the culture (Marsh et al., 2014). Among the *Zygosaccharomyces,* two commonly found species were *Zygosaccharomyces lentus* and *Zygosaccharomyces bisporus* with being consistently in high abundance comparative to other strains (Steels et al., 1999). The genera that dominated after *Zygosaccharomyces* was *Pichia* and similar species such as *P. fermentans* and *P. membranaefaciens* (Chen and Liu, 2000; Jankovic and Stojanovic, 1994).

In UK samples collected on day 3 and day 10, a considerable proportion of the pellicle was occupied by genera *Dekkera* and *Kazachstania* (Mayser et al., 1995). A study performed by Chakravorty et al. (2016) assessed the structure and dynamics of the microbial community present in the biofilm at different points within 21 days. Contrasting to other studies, the dominant yeast genera present was *Candida* sp. (73.5–83%) and among the *Candida* genus, *Candida stellimalicola* was majorly present in all samples from day 3, 7, 14 and 21 (Chakravorty et al., 2016). The yeast sources present in Kombucha SCOBY are listed in Table 4.

2.3. Metabolism of substrates and cellulose production

The presence of a vast and diverse microbial population in the SCOBY makes it more complex to understand the fermentation kinetics, production of cellulose along with various other metabolites (by-products) present in the fermentation broth. The microbial species that interact with each other may be beneficial or inhibitory

Table 3
Comparison of Bacteria genera among different Kombucha SCOBY sources.

S. No	SCOBY source	Bacterial strains		Percentage occupied/ Amount present	Methods used for identification	References
		Genus	Species			
1	Canada	*Gluconacetobacter (Komagataeibacter)*		>85%	DNA amplification and high-throughput sequencing	(Marsh et al., 2014; Podolich et al., 2017)
		Acetobacter		–		
		Lactobacillus and Lactococcus		3.3%		
	United Kingdom	*Gluconacetobacter (Komagataeibacter)*		>85%		
		Lactobacillus and Lactococcus		6.6%		
	Ireland	*Gluconacetobacter (Komagataeibacter)*		58%		
		Acetobacter		1.9%		
		Lactobacillus and Lactococcus		39.4%		
2	Germany	*Komagataeibacter*	*hansenii (DSM-103118)*	–	DNA analysis	Hopfe et al. (2017)
		Komagataeibacter	*kombuchae*	–		
3	India	*Acetobacter*	*aceti (MTCC 2945)*	–	–	Jayabalan et al. (2010)
4	Switzerland	*Acetobacter*	*xylillum (NCIB 11664)*	–	DNA – DNA Hybridisation with RFLP and SDS-PAGE were used for confirmation.	Sievers et al. (1995)
5	Japan	*Acetobacter*	*xylillum*	–	–	Kozaki et al. (1972)
6	Unknown	*Komagataeibacter*	*sp.*	50.3%	Terminal restriction fragment length polymorphism studies	Chakravorty et al. (2016)
		Gluconobacter	*sp.*	16.8%		
7	France	*Oenococcus*	*oeni*	Day 0: 89% (Black tea SCOBY) 72% (Green tea SCOBY)	M13-PCR typing and bacterial species identifications	Coton et al. (2017)
		Gluconacetobacter	*eurapaeus*	Day 0: 7% (Black tea SCOBY) 22.5% (Green tea SCOBY) Day 8: 98%–99% (In both tea SCOBY)		
		Lactobacillus	*nagelii*	–		
		Acetobacter	*okinawensis*	–		
		Gluconacetobacter	*intermedius*	–		
		Gluconacetobacter	*hansenii*	–		
		Gluconobacter	*oxydans*	–		
8	Mexico	*Gluconobacter*	*oxydans*	80%–90% (In 3 of 4 isolates)	Morphological and physiological tests	Belloso-Morales and Hernández-Sánchez (2003)
		Acetobacter	*aceti*	85% (In 1 of 4 isolates)		
9	United States of America	*Komagataeibacter*	*xylinus*	60% in isolate A and 40% in isolate C	Diversity sequencing	St-Pierre (2019)
		Gluconobacter	*oxydans*	40% in isolate B and 35% in isolate C		

Table 4
Comparison of yeast genera among different Kombucha SCOBY sources.

S. No	SCOBY source	Fungal strains		Percentage occupied/Amount present	Methods used for identification	References
		Genus	Species			
1	Germany	*Brettanomyces*	*lambicus*	56%	Classified micromorphologically and characterized by biochemical methods (Pasteur yeast identification system 565 10)	Mayser et al. (1995)
		Zygosaccharomyces	*sp.*	29%		
		Saccharomyces	*sp.*	26%		
		Candida	*krusei*	–		
		Candida	*albicans*	–		
		Saccharomycodes	*ludwigii*	–		
		Candida	*kefyr*	–		
2	Canada	*Zygosaccharomyces*	-	>95%	DNA amplification and high-throughput sequencing	(Marsh et al., 2014; Podolich et al., 2017)
		Pichia	-	8.3%		
	Ireland	*Zygosaccharomyces*	*lentus*	>95%		
		Zygosaccharomyces				
	UK	*Dekkera*	*bruxellensis*			
		Dekkera	*anomala*	–		
		Kazachstania	*unispora*	–		
3	Germany	*Zygosaccharomyces*	*lentus (DSM-103078)*	–	DNA Analysis	Hopfe et al. (2017)
4	India (Tamilnadu)	*Zygosaccharomyces*	*bailii (MTCC 8177)*	–	–	Jayabalan et al. (2010)
		Brettanomyces	*claussenii (MTCC 7801)*	–		
5	Australia	*Zygosaccharomyces*	*bailii*	Present in 3 of 4 cultures	The Biolog Microstation system, physiological and morphological tests	Teoh et al. (2004)
		Schizosaccharomyces	*pombe*	Present in 2 of 4 cultures		
		Torulospora	*delbreuckii*			
		Rhodotorula	*mucilaginosa*			
		Brettanomyces	*bruxellensis*	Present in 1 of 4 cultures		
		Candida	*stellata*			
6	Switzerland	*Zygosaccharomyces*	*sp.*	–	DNA – DNA Hybridisation with RFLP and SDS-PAGE were used for confirmation.	Sievers et al. (1995)
7	Saudi Arabia	*Candida*	*guilliermondii*	–	Api 20C Aux system	Ramadani and Abulreesh (2010)
		Candida	*kefyr*			
		Candida	*krusei*			
		Saccharomycodes	*ludwigi*			
		Candida	*colleculosa*	–		
8	Japan	*Saccharomyces*	*sp.*	–	–	Kozaki et al. (1972)
		Torulopsis	*famata*	–		
		Pichia	*membranaefacience*	–		
		Candida	*guilliermondii*	–		
9	Unknown	*Candida*	*sp.*	80%–94.1%	HTS of yeast genes like yeast internal transcribed spacer and rRNA genes.	Chakravorty et al. (2016)
		Candida	*stellimalicola*	59%–72.2%		
		Candida	*tropicalis*	6.8%–11.9%		
		Candida	*parapsilosis*	2%–4%		
		Lachancea	*sp.*	1.8%–11.9%		
		Lachancea	*thermotolerans*	2.5%–7.2%		
		Kluyveromyces	*sp.*	0.6%–2.6%		
		Eremothecium	*sp.*	0%–1.6%		
10	France	*Dekkera*	*Bruxellensis*	Day 0: >80% (In both green and black tea SCOBY)	FTIR spectroscopy yeast species dereplication and identification	Coton et al. (2017)
		Hanseniaspora	*valbyensis*	–		
		Dekkara	*anomala*	–		
		Torulapsora	*delbrueckii*	–		
		Saccharomyces	*bayanus*	–		
		Saccharomyces	*cerevisiae*	–		
		Saccharomyces	*uvarum*			
		Wickerhamomcyes	*anomalus*	–		
		Candida	*boidinii*	–		
		Zygosaccharomyces	*bailii*			
		Zygotorulaspora	*florentina*			
		Pichia	*membranifaciens*	–		
11	Yugoslavia	*Saccharomycodes*	*ludwigii*	–	OGYA medium for isolation and characterized based on vegetative cell and reproduction, fermentation of sugars and growth with nitrate as nitrogen source.	Markov et al. (2001)
		Saccharomyces	*cerevisiae*	–		
		Saccharomyces	*bisporus*	–		
		Torulopsis	*sp.*	–		
		Zygosaccharomyces	*sp.*	–		
12	Mexico	*Saccharomyces*	*cerevisiae*	37.5%	Morphological and physiological tests	Belloso-Morales and Hernández-Sánchez (2003)
		Brettanomyces	*bruxelensis*	25%		
		Kluyveromyces	*marxianus*	37.5%		
13	United States of America	*Schizosaccharomyces*	*pombe*	–	Diversity sequencing	St-Pierre (2019)
		Zygosaccharomyces	*rouxii*	–		

for one another. This behavior exhibited by the microbes need to be studied in detail to know the function of each species present in the culture. Some of the dominant bacterial and yeast groups were studied for their function in the fermentation and the cellulose production (Chakravorty et al., 2016).

The yeast community metabolizes the sucrose present in the medium to glucose and fructose and produces ethanol from glucose (Reiss, 1994; Sievers et al., 1995). The glucose molecules become the substrate for the acetic acid bacteria to produce organic acids like acetic, gluconic, glucuronic and other acids as a result of oxidation by glucose oxidase and the fructose molecules are converted into acetic acid (Markov et al., 2003). The acetic acid produced acts as a stimulant for the yeast to produce more ethanol and this ethanol is then converted to acetic acid by the same bacteria. This process gives rise to the accumulation of ethanol and acetic acid in the medium and acts as an antimicrobial agent preventing contamination of pathogenic microbes (Liu et al., 1996). The caffeine and compounds like theophylline and theobromine present in the tea extract also helps to stimulate the cellulose production by bacteria by activating the cellulogenic complexes (Balentine et al., 1997; Fontana et al., 1991). Bacterial cells are also stimulated by vitamins and other nutrients that are released as a result of the death and autolysis of the yeast cells (Chakravorty et al., 2016).

The acetic acid bacteria follow few pathways during the oxidative fermentation process to produce this unbranched cellulose pellicle extracellularly. The biosynthesis of cellulose is carried out by many fundamental enzymes mediated reactions. First, the glucose is transformed into glucose-6-phosphate and then to glucose-1-phosphate followed by catalysis by uridine diphosphate (UDP)—glucose pyrophosphorylase to form UDP glucose molecules. This uridine diphosphate glucose, which is a precursor of the cellulose molecule, is a nucleotide sugar that acts as a substrate for glucosyltransferase enzymes. Cellulose synthase is one such enzyme that acts on UDP glucose resulting in the addition of more units of UDP glucose to one another to form a polymer chain of 2,00,000 residues per second to form β-1→4 glucan chain. One advantage is that bacteria grow under controlled conditions and uses a variety of substrates like glucose, ethanol and sucrose in this kind of cellulose production process (Chawla et al., 2009). The conversion of substrates to different metabolites is illustrated in Fig. 2.

The cellulose that is primarily formed by the bacteria in the liquid is by consuming all the dissolved oxygen. This then raises to the air-liquid interface and the microbes present start utilizing the atmospheric oxygen to produce a new cellulose layer that superimposes the primary layer formed. As time increases, the thickness of the biofilm also increases, and it remains as a suspended structure in the broth till there is a supply of enough oxygen. When oxygen is insufficient, the process halts and the bacteria remain in a dormant state in both biofilm and the liquid broth which can be activated using sweetened tea extract or can be used as inoculum for consecutive batches of fermentation (Esa et al., 2014).

2.4. Purification

The produced cellulose can be purified by different methods based on their large-scale application. Few industrially scalable approaches for purification were studied in which four cellulose pellicle samples were taken and subjected to different chemical treatments. The samples were washed continuously using 1.0 M sodium hydroxide and bleached using agents like sodium hypochlorite and hydrogen peroxide. It appeared that the pellicle sample that was twice washed with 1.0 M sodium hydroxide at 90 °C and then bleached with 1.5% sodium hypochlorite had the highest leucometer whiteness value and was concluded to be the most effective method (Amarasekara et al., 2020). This purification is done to remove the live cells and the compounds of the liquid culture attached to the pellicle (Embuscado et al., 1996). The intensity of purification of the cellulose membrane was also varied based on the thickness of the membrane. In a study, the cellulose was washed several times with different molarity of NaOH solutions and it was seen that the weight of cellulose film is decreasing gradually, and it was observed that the whiteness increased with more concentration of NaOH (Dima et al., 2017). The purification is also done with 0.5% NaOH solution for 8 h to increase the pH of the cellulose from 3.7 to 7.1 so that it can be used in products that come in contact with the skin (Sederaviciute et al., 2019).

2.5. Structure and properties

The protofibrils of the glucan chains which are approximately 2–4 nm in diameter are formed outside the cytoplasm and are secreted out of the cell wall of the bacteria through the pores present on the surface. Electron micrographs showed that there are 50–80 pores of 3.5 nm diameter, present on the cell surface, called complex terminals or “cellulose export component” which help in extruding these protofibrils. The fibrils are then arranged in a bundle to form a microfibril cellulose ribbon of dimensions approximately 80 × 4 nm (Iguchi et al., 2000). Interconnection between these ribbons leads to a formation of a network structure of biofilm which has high porosity and surface area (Ross et al., 1991; Chawla et al., 2009). These microfibrils remain attached to the bacterial cell and later assemble to form thick fibrils called the macrofibrils.

This bacterial cellulose (BC) synthesized has always been compared with plant cellulose where the molecular formula $(C_6H_{10}O_5)_n$ is similar between both but varies in the physical and chemical properties. The plant cellulose is branched and consists of compounds like hemicellulose, lignin and pectin which is absent in bacterial cellulose. BC is unbranched and is purely made of cellulose. It also has much better physical properties compared to its counterpart. Purification of plant cellulose requires harsh chemical treatment; hence BC is mostly preferred for various applications (Klemm et al., 2001; Sun, 2009).

When a BC was observed under X-ray crystallography, it was noted that a single unit cell had two cellobiose (a dimer formed by linking two anhydrous D-glucose units by β-1-4 linkage) units arranged parallel to each other and it had planar orientation in the dried form. So, it was categorized under type I cellulose which resembled a ribbon and had a crystalline structure. This type has two allomorphs of cellulose (Iα and Iβ) which are structurally similar. Type II cellulose is also formed which is amorphous and thermodynamically more stable. This type II cellulose is produced from pores where the export component is missing on the outer bacterial coat. Both types of cellulose **(Fig. 3)** are arranged differently on the surface of the bacterial cell membrane (Iguchi et al., 2000; Yu and Atalla, 1996; Nishiyama et al., 2010).

Sometimes, type II cellulose is formed instead of type I cellulose in the presence of few physical or chemical factors like increased aeration and chemicals like carboxymethyl cellulose and calcofluor white. These factors induce the formation of hydrogen bonds between the β-1→4 glucan chains (Bielecki et al., 2005). The presence of surface hydroxyl groups allows modification of the cellulose pellicle with chemicals. Therefore, many additives can be added to the cellulose to improve its physical, chemical and mechanical properties. For example, carboxymethyl chemical was added to the liquid broth during fermentation and the bacterial cellulose formed had good adsorptive and ion exchange property for metal ions especially (Sakairi et al., 1998). Cellulose after purification shows weak mechanical properties and thus chemicals like glycerol and

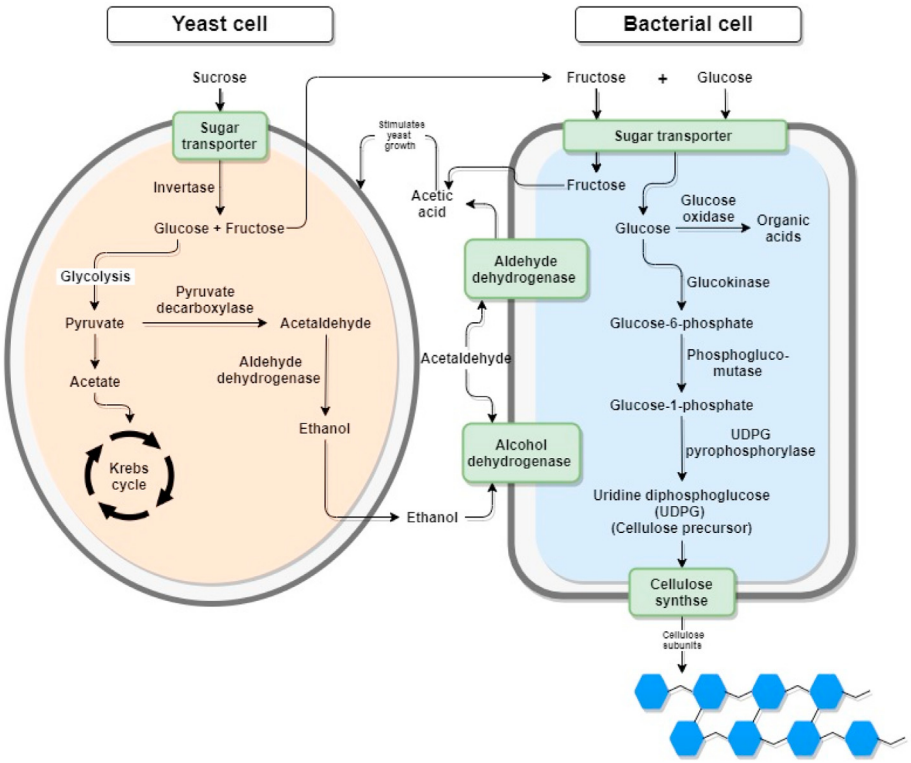

Fig. 2. Metabolism of substrates by the symbiotic culture of bacteria and yeast.

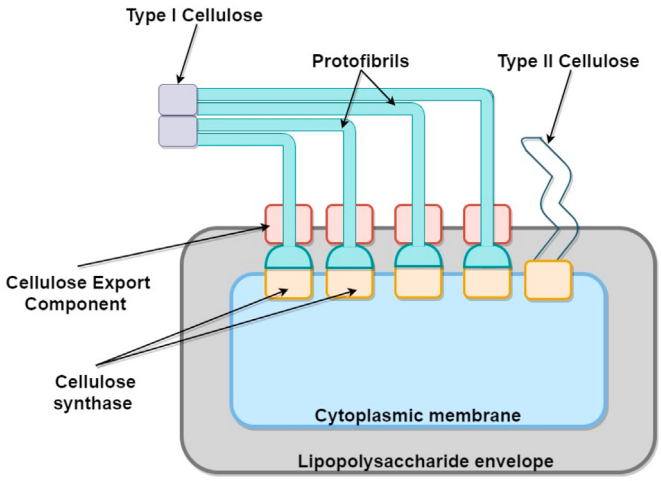

Fig. 3. Formation and assembly of bacterial cellulose.

chitosan was added to enhance the cellulose network quality. It was observed that the hydrogel formed had good water retention capacity and improved mechanical properties after drying. The surface of the dried cellulose is sometimes treated with fatty acid like stearic acid or wax-like additives so that it is adsorbed on the surface and repels unwanted reactions with hydrophilic substances. These acids fill the spaces between the fibers and provide a smoother surface, it is being used in textile production (Kamiński et al., 2020). The mechanical properties of SCOBY can also be modified by an irreversible process called mercerization, where chemicals are added to convert the type I cellulose to type II cellulose resulting in altered surface morphology, deformability, stiffness and so on. Since this process renders bacterial cellulose with tunable mechanical properties it could be used to treat BC and use it in various biomedical applications (Younesi et al., 2019).

This cellulose formed has many beneficial properties because of which it is used for various applications. Few main properties noted primarily was its ultra-thin fibrous network which has pores in size of a few nanometer diameter and the hydrophilicity exhibited when these fibers swell and the pore structures form tunnels in the wet cellulose pellicle, which reveals the interior surface area and interstitial spaces (Geyer et al.,1994). These porous structures were swelled even more by using ultrasound and were used as potential support enzyme immobilization that can be implemented in industrial applications (Song et al., *2017*). The elastic property of cellulose declines with a decrease in the water content of cellulose. Once dried, the elastic property of the cellulose cannot be regained (Yano et al., 1998). The mechanical properties are tested for cellulose in both hydrogel and dried form. Usually, biofilms are dried by exposing them to air or sun. The cellulose is also dried in a tray drier consisting of a blower and heating coil (Basak, 2013). One other method used for drying is the hot press method in which the biofilm is compressed between two stainless steel mesh or fabric (Iguchi et al., 1988). Young's modulus, tensile strength and elongation at break were few mechanical properties that were assessed for almost all biofilms formed. These properties vary based on the temperature, pressure and the type of preparation method used for kombucha films (Yano et al., 1998). It is seen that when kombucha cellulose was treated with Titanium (IV) bis-(ammonium lactato) dihydroxide (Ti-BALDH) increased cross-linking occurred and this is a way by which the mechanical properties can be varied (Knöller et al., 2020). Few main properties of the kombucha SCOBY are shown in Fig. 4.

2.6. Biochemical composition

The biochemical composition of the SCOBY cellulose (tea fungus) was found out to assess its nutritive value and its application

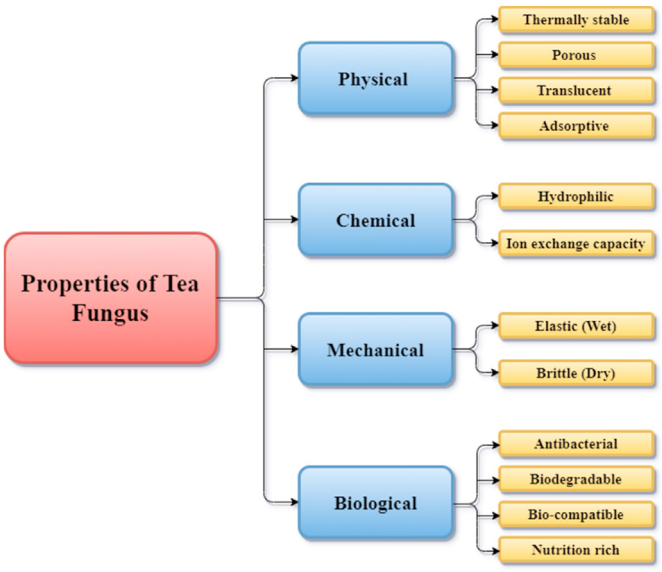

Fig. 4. Different properties of Kombucha SCOBY that has to be taken into consideration before utilizing for its applications in various fields.

as animal feed. The study revealed that the tea fungal biomass contained crude protein, crude fiber, crude lipid, acid detergent fiber (ADF), neutral detergent fiber (NDF) and many molecules like potassium, phosphorus, calcium and magnesium. Trace amounts of sodium, iron, manganese, zinc and copper are also found. Glutamic acid and lysine content were found to be more among the amino acid composition of the fungal mat (Murugesan et al., 2005). The biochemical composition of kombucha tea fungus was also experimentally analyzed by Jayabalan et al. (2010) and it was found that the dried cellulose mat was rich in amino acid lysine, protein, fiber and lipid in crude form. The tea fungus that was harvested after 21 days of fermentation were used and the percentage of constituent present were evaluated. Among the essential and non-essential amino acids estimated it was found that amino acid lysine was present in high concentration of 53.1 mg/g of dry weight. The composition of each compound is given in Table 5. The composition of a tea fungus originated from Kenya had contrasting composition comparatively with carbohydrates, crude protein, crude fiber and crude lipid levels to be 43.2%, 4.8%, 49.5% and 1% respectively. When the SCOBY was harvested and stored in distilled water the percentage of fiber decreased to 30.21% denoting its consumption to produce more carbohydrates increasing its content to 57.59% (Ahmed and Dirar, 2005). More analysis of kombucha SCOBY from different sources and regions might reveal the variation in the composition of each component.

2.7. Characterization

The attributes of the kombucha SCOBY were characterized and analyzed carefully for its use in various applications. The biofilms can be grown in any preferred shape as it takes the shape of the container. The thickness of the biofilm is also determined by the cultivation time. Therefore, the dimensions of a biofilm that is required can be easily manipulated according to use (Chan et al., 2018). The kombucha SCOBY appears to be in different shades of brown color depending on the concentration of sugar, tea and the time of fermentation. Due to the Maillard reactions that take place during fermentation between the sugar and the amino acids, the characteristic color is obtained by the cellulose formed. Melanoidins are the term given to those compounds that are responsible for this color (Wang et al., 2011).

The nanostructure of the cellulose fibrils was confirmed by using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis. The fiber diameter of the nanocellulose formed by fermentation of black tea broth was found to be 20–100 nm (Sharma and Bhardwaj, 2019). Cellulose is said to have a high surface area due to its nanostructure. Bacterial nanocellulose is also synthesized in dry and never-dried forms by two mechanical processes namely atomization and micro fluidization (Dima et al., 2017). The presence of different forms of cellulose and its crystallinity was studied using the X-ray diffraction method. The crystallinity was in the range of 77%–80% (Sharma and Bhardwaj, 2019; Goh et al., 2012) and found to increase with the purity of the kombucha membrane.

The Fourier transformed infrared (FTIR) analysis confirmed transmittance in the range of 3000–3600 cm^{-1} indicates the presence of hydroxyl groups involved in hydrogen bonding (Dima et al., 2017; Pooja et al., 2019). Attenuated total reflectance (ATR-FTIR) studies for the cellulose pellicle gave absorption bands in the range of 3455–3410 cm^{-1}. Also, absorption bands for both the crystalline and amorphous nature of cellulose were seen. Due to these hydrogen bonds water gets trapped in the dense network and this increases the water-absorbing and retaining capacity. The water retention values of kombucha SCOBY were found to be 88.42 ± 1.80% (Sharma and Bhardwaj, 2019).

The biofilms can be made leakproof by applying olive oil, beeswax, or a mixture of both. The biofilms appear to be leak proof sometimes, but it absorbs water showing its polar and hydrophilic nature (Aduri et al., 2019). The biofilm dried under the sun can be bought back to normal form by immersing it in water for 30 min and it can also be used as inoculum or preserved for later use (Sahasrabudhe, 2006).

Thermogravimetric analysis for Kombucha SCOBY was carried out and found that the SCOBY membranes started degradation at a temperature of 298.7 °C. The thermal stability of spray-dried kombucha cellulose was found to be better than pure cellulose

Table 5
Proximate analysis of dried black tea kombucha cellulose mat.

S.No	Components	Jayabalan et al. (2010)	Murugesan et al. (2005)	Ahmed and Dirar (2005)
Principal Proximate Composition				
1	Carbohydrates (%)	–	–	43.2
2	Crude protein (%)	23.1	17.9	4.8
3	Crude fiber (%)	14.79	12	49.5
4	Crude lipid (%)	5.4	4.41	1
5	Nitrogen Free Extracts (NFE) (%)	5.27	6.3	–
6	Ash content (%)	3.9	2.64	1.5
7	Moisture (%)	–	4.4	
Other Components				
8	Neutral Detergent Fiber (NDF) (%)	53.1	46.1	–
9	Acid Detergent Fiber (ADF) (%)	46.3	39.8	
10	Dry matter (%)	97.35	–	–

(Dima et al., 2017). Contrastingly it was also seen that any chemical or mechanical treatment of biofilm decreases the thermal stability. As the purity and crystallinity increases, it appears to conduct heat efficiently because the heat transfer is better through the cellulose chains, but the stability decreases. (Yildirim and Shaler, 2017).

Biodegradability of the dried SCOBY sheets was checked by comparing plain, moist and wax-coated biofilm material which were placed in a container with soil. It was seen that on day 10 the moist biofilm material was degraded completely. Antibacterial property of kombucha SCOBY was checked and it was found to inhibit the growth of *Enterobacter, Staphylococcus, Bacillus and Pseudomonas. E. coli* was found to be resistant and *Pseudomonas* had least zone of inhibition (Aduri et al., 2019). Gram-positive strains are inhibited more efficiently by the kombucha biofilms compared to gram-negative strains (NT, 2017; El-Wakil et al., 2019).

2.8. Contamination

Since the incubation period is so long and the fermentation does not require continuous stirring, there are many chances of contamination to both the liquid medium and the SCOBY layer that is formed. The solution is acidic, sweet and it attracts fruit flies (*Drosophila melanogaster*), which meets the biofilm and as a result, small white patches of contamination can be seen on it. The biofilm is also prone to contamination by many kinds of fungal spores. Both kombucha tea and the biofilm should be discarded in that case and it cannot be used as inoculum further. To avoid contamination of the broth, few antifungal preservatives which are commonly used in the food industries like sodium benzoate and potassium sorbate are added at 0.1% concentration at a pH $\leq$ 4.2 (Dutta and Paul, 2019; Nummer, 2013). A solution of half water and half vinegar is mixed and sprayed on the contaminated part to remove mold grown (Quijano, 2017).

The SCOBY cellulose layers that are harvested are maintained by preparing a medium with black tea leaves extract and sucrose, which is added to the cellulose in a jar. Acetic acid of concentration 50% v/v and volume of 10 ml is often added to a freshly prepared maintenance medium so that the pH is brought down to the range of 2.7–3.0. Addition of acetic acid at the start of preservation helps in the destruction of other contaminating bacteria and molds (Chen and Liu, 2000; Frank, 1995).

3. Cultivation techniques

The cultivation of any product in a bioreactor is based on many factors like reactor vessel type, geometry, agitator type, power input, optimized media and reactor conditions and scale-up study. The kombucha production is usually done by a static batch culture method and the impact of an increase in vessel volume on the physical, chemical and biological factors are mainly considered (Junker, 2004). Static intermittent fed-batch method of cultivation is experimented and is found to give greater yield than batch fermentation (Sharma et al., 2020). The agitated mode of cultivation is mostly not preferred for kombucha cellulose production because of few drawbacks like high turbulence and shear stress on the cells which might cause mutations in the cells and render them cellulose negative. Supplementing the culture medium with ethanol can prevent the accumulation of these mutants (Park et al., 2004).

Either the SCOBY is cultivated as it is in tea extract medium or nowadays the strain of bacteria mainly responsible for the cellulose production is isolated from the kombucha and it is cultivated most commonly in Hestrin and Schramm (HS) agar medium first. Then the colonies are selected and inoculated in liquid HS medium which contains all the essential nutrients for bacterial growth. Cycloheximide is added to prevent the growth of yeast cells so that it does not become a symbiotic growth. Few common strains that are isolated and used are *Komagataeibacter rhaeticus* strain P 1463 and *Gluconacetobacter xylinus* (Semjonovs et al., 2017; Nguyen et al., 2008). Cultivation of an individual strain is found to be less efficient in cellulose production because in kombucha SCOBY many microbial species combine and simultaneously metabolize the substrates which leads to increased growth of the acetic acid bacteria and larger biomass (Dima et al., 2017; Goh et al., 2012). It was also proved in a study where tea extract and HS medium were separately tested for the growth of bacterial cellulose and found that the cellulose production was less and slower in HS medium (Sharma and Bhardwaj, 2019).

Co-culturing of two pure cultures of a bacterial (*Gluconacetobacter hansenii* CGMCC1671) and a yeast strain (*Saccharomyces cerevisiae* CGMCC1670), which are majorly responsible for the bacterial cellulose production during the kombucha fermentation was carried out. Using response surface methodology (RSM) the influencing factors were optimized, and the values were 10.37% of inoculum in a media volume of 77.3 ml in 250 ml flask at pH 4.96. The practical yield of 279.57 mg/g of bacterial cellulose was obtained. Therefore, this method of bacterial cellulose production can also be preferred as it gives high yield and better quality of both cellulose and kombucha beverage (Tan et al., 2012).

3.1. Scale-up studies

Scale-up studies in bioprocess is usually carried out in to increase the product yield and quality. In a study of kombucha fermentation, the response surfaces were determined using many dependent and independent variables. A polynomial equation with linear and nonlinear terms that represent the influence and interaction between the variables was taken to define the response surfaces. The regression analysis method with the quadratic equation and partial use of the black box model led to the conclusion that the pH as a significant variable can be used for scaling up studies (Malbaša et al., 2006). The same regression analysis was used to study a fermentation in a 90 L reactor, and it was found that when the specific interfacial area is maintained the same, irrespective of the size, the required characteristics of the product could be met (Cvetković et al., 2008). These methods can be used to determine the optimal process parameters and cellulose yield.

The production of SCOBY can be done in a scalable manner after testing the kinetics of the process once optimizing the fermentation conditions and the raw material composition. Many parameters like the cellulose yield, amount of sugar consumed by finding the residual sugar and dry weight of cellulose were monitored on every day of fermentation. These combined factors could provide information on how the process can be made efficient (Sharma and Bhardwaj, 2019).

3.2. Culture vessels for kombucha SCOBY

There are very minimal studies on large scale cultivation of the Kombucha SCOBY that has been reported. It is mostly cultivated in small glass jars. It is also cultured in closed plastic boxes and kept at room temperature which can be around 30 °C (Sahasrabudhe, 2006). The cultivation of kombucha SCOBY has experimented in three different ways like the static fermentation, both static and agitated fermentation in two stages and up-flow fixed bed fermentation in a study. It was noted that the growth of yeast and acetic acid bacteria was maintained throughout the 15-day fermentation period which resulted in an increased level of sugar consumption, acetic acid and ethanol production. Therefore, with

enough aeration, the up-flow fixed bed fermentation method is comparatively more efficient (Chu and Chen, 2006).

3.3. Favorable conditions and factors influencing production

There are many physical, chemical and biological factors that must be optimized to carry out the Kombucha fermentation like the temperature, amount of oxygen supplied and dissolved oxygen, CO_2 produced, pH, reactor and composition of the media used. Any change in these parameters imparts changes in its properties and quality (Marsh et al., 2014). Some other important process parameters that should be considered during the production are the type of starter culture used and if co-culture is done the right combination of starter is required, qualitative analysis of culture, optimized raw materials and its concentration and redox potential. The favorable environmental conditions for Kombucha fermentation along with their obtained corresponding yield is listed in Table 6. It can be inferred from the table that the yield of kombucha cellulose is drastically influenced by various physical and chemical factors. As evident from Table 6, the Kombucha cellulose yield reported by different research studies was found to vary from 33 g/L to 510 g/L. The choice of raw materials or substrates like the type of tea (green tea, black tea, oolong tea etc.), sugar (glucose, sucrose) and additives like lemon balm, thyme and peppermint drives the growth and metabolism of microbes thereby the overall yield. Operational factors like the temperature, pH, surface area of reactor and the fermentation time period also have a profound influence on the cellulose pellicle formation. The trend of variation of the cellulose pellicle formation under the influence of the aforementioned factors along with the underlying reason for the probable effect is detailed in the subsequent subsections. It is essential to understand the factors affecting the rate of cellulose formation to optimize them in order to obtain a consistent high cellulose yield during the scale-up.

3.3.1. Substrates

The type of substrate used and the yield of the SCOBY obtained also depends on the dominant type of microorganisms present in the culture. Accordingly, different types of substrates can be tested for biofilm production (Nguyen et al., 2008). It is well known that the medium for growth of SCOBY should necessarily contain a carbohydrate component as the carbon source and tea extract as a nitrogen source so that it can be assimilated in the fermentation process for cellulose production and microbial growth. The most widely used carbon source is sucrose and it was found that the SCOBY yield was also higher when green tea leaves were used compared to black tea and oolong tea (Gargey et al., 2019).

3.3.1.1. Tea. It was reported that when the tea leaves concentration exceeds 6 g/L then it would be inhibiting the growth of acetic acid bacteria therefore decreasing the cellulose production (Kurtzman et al., 2001). A comparison of fresh tea leaves and used tea leaves has experimented and it was observed that the conversion yield was 0.32 and 0.31 g of bacterial nanocellulose per g of sugar, which are almost equal. Therefore, used tea leaves are also considered as a choice of raw material to obtain high yield with a reduced production cost (Sharma and Bhardwaj, 2019). Waste tea leaves used in a concentration of 4 g/L has given a yield of 210 g/L cellulose and is found to be higher than black tea as mentioned by Gargey et al. (2019). Pu erh tea leaves have also been experimented for kombucha cellulose production and the exopolysaccharide yield obtained was 2.66 g/L (Wu et al., 2013). The tea extract contains caffeine and if the level increase at 4 to 16 times the normal caffeine level (40 mg) it has proved to inhibit kombucha fermentation (Greenwalt et al., 2000). The caffeine content in a kombucha beverage was estimated to be 0.039 mg/ml (Miranda et al., 2016). When the concentration of tea increases the cellulose yield also decreases as increase in polyphenol is an inhibitor for biofilm production by gram negative bacteria (Serra et al., 2016).

3.3.1.2. Sugar. The optimum sugar concentration for kombucha SCOBY production is in the range of 60 g/L to 120 g/L (Table 6). The sucrose concentration decreases from the start of fermentation leading to increase in glucose and fructose concentration. Later glucose decreases rapidly due to its utilization followed by fructose for acetic acid production (Kallel et al., 2012). The optimum sugar concentration that is required for the maximum yield of cellulose was found to be 90 g/L in tea extract medium. Other metabolic product like gluconic acid is formed as a result of more sugar concentration and this causes inhibition of cellulose synthesis (Goh et al., 2012; Frank, 1995). Therefore, the removal of these chemicals that hinder bacterial cellulose production could be done (Caldwell, 2000). The cellulose production is also hindered by the unequal transportation of nutrients in the cell and uneven uptake of the available nutrients by the cell (Goh et al., 2012).

Apart from sucrose, molasses and brown sugar has also been used as a carbon source as seen in Table 6. Among which molasses is seen as a low-cost alternative of sucrose and is found to give higher yields of kombucha cellulose (135.8 g/L) than pure sucrose (Malbaša et al., 2008).

Many other sugar substrates that have been experimented with were fructose, malt extract, and honey for equal or better yield of kombucha SCOBY (Frank, 1995; Cvetkovic and Markov, 2002). Malt extract medium was used considering that it is a cheaper source of starch and it also contains proteins, anions, cations and vitamin B complex. It was observed that there is rapid microbial growth and metabolism and the fermentation process is fast (Cvetkovic and Markov, 2002). Lactose was also used as a carbon source in a study and few parameters like the pH, titratable acidity, the yeast and bacterial cell count in medium and the SCOBY was compared with the fermentation of unsweetened medium. However, no significant difference was found between the two cases and it was reported that the presence of different concentrations of lactose does not have a noticeable effect on the fermentation process. The fermentation rate decreased as compared to the traditional method and the acid content also varied a lot. The number of lactic acid metabolizing bacteria was also less in liquid and none in the SCOBY (Chu and Chen, 2006). Cheese whey was also used as a substrate, but satisfactory results were not obtained, and the resulting broth was salty and not sparkling (Belloso-Morales and Hernández-Sánchez, 2003). The juice that is obtained when grapes are crushed is called as must, which is widely used in the wineries. The effluent that is produced during the clarification of the must is also used as a substrate for the kombucha growth since it contains both sugars and nitrogen (Vitas et al., 2019).

3.3.1.3. Other substrates. Some medicinal and flavor adding plant leaves were also used for the kombucha fermentation like lemon balm, thyme and peppermint and it was seen that the fermentation occurred in fewer days compared to the traditional method (Markov et al.,2012). The cost of pure substrates used can be decreased by using low-cost alternatives or analogs of the substrates like the tea leaves used once can be dried and reused.

3.3.2. Fermentation period

The kombucha fermentation can be carried for up to 60 days which brings out many changes like an increase in antioxidant activity and organic acid content, but a range of 7–15 days is optimal for fine results. The CO_2 can get accumulated between the liquid medium and the biofilm formed causing insufficient nutrient

Table 6
Media components and concentration for Kombucha SCOBY fermentation and the corresponding yield obtained.

S. No	SCOBY type	Media components and concentration	Fermentation vessel and volume	pH	Duration (days)	Temp. (°C)	Yield	References
1	Black tea	Tea - 0.49% w/v Sucrose - 10% w/v Kombucha tea (KT) - 10%	3 L glass container with 2 L working volume	–	10	23	–	Marsh et al. (2014)
2	Green and black tea mixture	Tea - 10.36 g/L (Green tea – 6.16 g/L + Black tea – 4.2 g/L) Sugar - 10% w/v (white/brown sugar, 9:1) KT - 5% w/v (or) SCOBY - 5% w/v	200 ml working volume in 500 ml beaker with diameter 8.6 cm	3.0	18	30	Oriental tea SCOBY - 0.43 g (dry weight) European tea SCOBY - 0.19 g (dry weight) Tibetan tea SCOBY - 0.16 g (dry weight) Yield (g - dry weight/l): Oriental – 2.15 g/L[a] European – 0.95 g/L[a] Tibetan – 0.8 g/L[a]	Lee and Kim (2000)
3	Echinacea tea	Tea - 0, 5 g/L Sucrose – 70 g/L	5 L glass vessel with 3 L working volume	5.03	10–12	28	–	Markov et al. (2005)
4	Black tea	Tea - 4.43 g/500 ml Sugar - 18.42 g/ 500 ml SCOBY - 35.85 g/ 500 ml	1 L plastic jar	–	14	28	–	Najafpour et al. (2020)
5	Black tea	Tea - 15 g/750 ml water Sugar – 50 g KT - 10 ml White vinegar - 100 ml	–	–	15	–	–	Kalaiappan et al. (2020)
6	Black tea	Tea - 5 g/L Sucrose - 100 g/L Yeast extract - 5 g/L Peptone - 0.5 g/L	–	5.0	7	30	–	Zhu et al. (2014)
7	Black tea (Fresh tea leaves and used tea leaves)	Tea - 5 g/L Glucose - 20 g/L, 40 g/ L, 60 g/L, 80 g/L, 100 g/L KT - 10% v/v SCOBY - 2% w/v	Beaker	3.0	20	30 ± 1	Highest yield at 60 g/L glucose: 0.32 and 0.31 g BNC/ g sugar for fresh and used leaves. Yield (g - dry weight/L): Fresh leaves: 13.3 g/L[a] Used leaves: 12.8 g/L[a]	Sharma & Bhardwaj (2019)
8	Black tea	Tea - 5%–20% w/v Sugar - 5% w/v KT - 5% - 10% v/v SCOBY - 1% w/v	100 ml working volume in 250 ml flask (Static and rotary culture)	3.0 to 7.5 with 0.5 pH interval	7	25–45 °C with interval of 5 °C	Highest biomass (Wet weight): 33.39 g/L at 25 °C with 5% tea decoction followed by 10% tea decoction which gave 32.99 g/L in an optimum pH range 4.5 –5.0.	ALI and SHIVANNA (2017)
9	Black tea	Tea - 5.4 g/L Sugar - 100 g/L KT - 100 ml/L	2 L glass jar with 1 L working volume	–	14	20–22	–	Teoh et al. (2004)
10	Black tea	Tea - 1.5 g/L Sugar - 70 g/L KT - 10% v/v SCOBY - 3 to 4 layers of cellulose pellicle	3 L glass vessel with 17 cm diameter and 1 L working volume	3.75	60	20–22	1.07 g produced at 10th day of fermentation	Sievers et al. (1995)
11	Black tea	Tea - 18 g/L Sucrose - 70 g/L, 90 g/ L and 110 g/L KT - 10% v/v SCOBY - 3% w/v	1 L Beaker with teflon stopper attached to lower part	2.7–3.0	8	30 ± 3	For 90 g/L sucrose is 66.7% followed by 47.9% and 44% for 70 g/L and 110 g/L respectively (wet weight) Yield (g - wet weight/L): 60 g/L for 90 g/L sucrose	Goh et al. (2012)
12	Black tea	Tea - 1.2% w/v Sugar - 10% w/v KT - 10% v/v SCOBY - 3% w/v	100 ml working volume	–	14	24 ± 3	Highest biomass yield with sugar source as white refined sugar: 101.31 ± 0.09 g followed by 34.18 g for coconut palm sugar and 27.81 ± 0.18 g for molasses. Yield (g - dry weight/L): White refined sugar: 1013.1 g/L[a]; Coconut palm sugar: 341.8 g/L[a]; Molasses: 278.1 g/L[a]	Muhialdin et al. (2019)
13	Black tea	Tea - 1.5 g/L Sugar - 70 g/L KT - 10% v/v	2 L working volume	5.3	14	22 ± 1	Biomass yield obtained with different molasses as sugar source are 270.8 g, 165.6 g and 154.8 g. Yield (g - wet weight/L): 135.4 g/L[a], 82.75 g/L[a], 77.4 g/L[a]	Malbaša et al. (2008)
14	Black tea	Tea - 11.3 g/L Sugar - 100 g/L	1 L working volume	–	10	–	Weight of pellicles: 165.87 Yield (g - wet weight/L): 165 g/L[a]	Amarasekara et al. (2020)
15	Black tea	Tea - 1.5 g/L Sugar – 70 g/L KT - 10% v/v	–	–	21	28	–	Markov et al. (2001)
16	Black tea	Tea - 2.5 g/L Sugar - 62 g/L SCOBY - 4 g/L, 6 g/L and 8 g/L	100 ml working volume in 250 ml Erlenmeyer flask	3.2–4.0	15	28	Weight of cellulose for inoculum variation in whole (W) and macerated (M) form are: 4 g: 20.3 g (W) and 22 g (M) 6 g: 18.7 g (W) and 19.4g (M)	Jarrell et al. (2000)

Table 6 (*continued*)

S. No	SCOBY type	Media components and concentration	Fermentation vessel and volume	pH	Duration (days)	Temp. (°C)	Yield	References
							8g: 23.6 g (W) and 20.6 g (M) Yield (g - wet weight/l): 4 g: 203 g/L[a] (W) and 220 g/l[a](M) 6g: 187 g/L[a](W) and 194 g/l[a](M) 8g: 236 g/L[a](W) and 206 g/l[a](M)	
17	Green tea, Black tea and waste tea	Tea - 4 g/L Sugar - 33 g/L KT - 10% v/v SCOBY - 3% w/v	100 ml of working volume in flask	–	18	20–22	Green tea – 51 g (14th day) Black tea – 11 g (18th day) Tea waste – 21 g (18th day) Yield (g - wet weight/l): Green tea –510 g/L[a] Black tea – 110 g/L[a] Tea waste – 210 g/L[a]	Gargey et al. (2019)
18	Green tea	Tea - 2.5 g/L Sugar - 12% w/v KT - 10% v/v	2 L of working volume in 3 L sterile brown glass bottle	–	30	23	461 g Yield (g-wet weight/L): 230 g/L[a]	Dima et al. (2017)
19	Black tea	Tea - 5 g/L, 10 g/L, 15 g/L, 30 g/L, 60 g/L, 90 g/L and 120 g/L Sugar - 70 g/L, 80 g/L, 90 g/L, 100 g/L, 130 g/L, 160 g/L and 190 g/L		–	28	20–50	Highest yield of 55.46 g/L for 10 g/L tea Highest yield of 63.58 g/L for 100 g/L sugar	(Hassan and AL-Kalifawi, 2014; El-Salam, 2012)
20	Black tea	Tea - 4.6 g/L Sugar - 100 g/L KT - 16% v/v	Glass vessel with 14 cm diameter	4.28 ± 0.02	21	22	0.1 g of dry matter per g of kombucha cellulose	Tapias et al. (2020)
21	Black tea	Tea - 5 g/L Sugar - 60 g/L SCOBY - 2% w/v	50 ml working volume Static intermittent fed-batch method cultivation	3.2	15	30 ± 1	29.2 g/L (Dry weight)	Sharma et al. (2020)
22	Black tea	Tea - 10 g/L Brown sugar - 15 g/L SCOBY - 37 g/L	1 L working volume in sterile glass jar	5.6	56	–	Yield (g – wet weight/L): 60 g/L[a]	Amarasinghe et al. (2018)

[a] Yield provided in g/L of the culture medium have been calculated manually by the authors from the data provided in the corresponding manuscripts.

transfer and starved condition when the fermentation period increases (Chu and Chen, 2006). The bacterial and the yeast cell numbers also increased till day 14 and decreased from then on due to the unfavorable growth conditions. This halted the SCOBY growth and the number of microbes present on the pellicle (Chen and Liu, 2000; Jayabalan et al., 2010). These changes saturate the SCOBY yield from day 17 and decreases the productivity (Goh et al., 2012). The decrease in weight of SCOBY after a particular day also takes place due to the death and release of acid-sensitive microbial cells from the biofilm into the tea broth (Gargey et al., 2019). The fermentation period required for biofilm growth also depends on its application.

3.3.3. pH

The microorganisms present in the kombucha tea and the SCOBY are tolerable to acidic pH at the range of 2.5–4 and thus it can help in the fermentation and cellulose production (Goh et al., 2012). The accumulation of large amounts of organic acid reduces the pH as the fermentation proceeds and this in turn decreases the production of bacterial cellulose (Sievers et al., 1995). The growth of mold and other contaminants was observed at pH 5.0. At pH 6 only mold was seen growing between the nanofibers (Sharma and Bhardwaj, 2019). Usually, *Acetobactor* sp. is found to grow well in pH 4 to 6.3 but due to the microbes present in a symbiotic relationship, it can grow in decreased pH also (Bergey et al., 1994).

3.3.4. Surface area

The cellulose formation takes place only when the bacteria are exposed to enough oxygen supply. In static cultures, this takes place on the air-liquid interface where the cellulose layers are formed continuously with new layers on the top (Iguchi et al. 2000; Frank 1995). The cellulose yield obtained from a fermentation study done in containers of varying surface area, volume and depth of fermentation media was compared to know the effect of each parameters and their combined effect. A yield of 104 g of cellulose pellicle/L was obtained with a media of volume 1500 ml and surface area 227 cm^2 and yield of 66 g of cellulose pellicle/L was obtained from media volume of 700 ml and 130.5 cm^2. But when the surface area was decreased drastically to 29.2 cm^2 the yield also decreased to 21.3 g of cellulose pellicle/L with a volume of 1 L. The surface area to depth ratio also played a major role in increasing cellulose yield. Therefore, it is proved that larger surface area in an important parameter with suitable media volume and depth of media. Less volume of culture medium in a vessel with a wide opening is usually used for fermentation. (Goh et al., 2012). The specific interfacial area is denoted as a key parameter for scaling-up processes to maintain similar kinetics in model and prototype (Cvetković et al., 2008). Similar specific interfacial area of 0.066 cm^{-1} was maintained for culture media of 1 L and 6 L volume of same height and it was found that similar cellulose yields of 23 g/L was obtained from both vessels (Villarreal-Soto et al., 2019).

3.3.5. Temperature

The optimum temperature range for the kombucha SCOBY production was found to be in the range of 20 °C–50 °C. Any temperature beyond this range will have a very negative effect on the cellulose production. When the fermentation was done in different temperatures from 20 °C to 80 °C, the pellicle formation completely did not take place at temperatures above 60 °C (Caldwell, 2000). In most of the studies, SCOBY production was usually done at 27 °C–34 °C (Sharma and Bhardwaj, 2019; Goh et al., 2012).

4. Applications

The biofilm that is formed is a byproduct of kombucha tea

fermentation and which was previously considered as just an inoculum for consecutive batches was later seen as a valuable raw material for applications in different fields (**Fig. 5**). There are very limited studies regarding the application of kombucha SCOBY and in producing a feasible product till now. Many small-scale research and experiments are being carried out to check its usage, but the reach on the market availability is still low. Based on the different properties of kombucha SCOBY, it is used in a few fields as briefly explained in Table 7.

4.1. Adsorbent

The kombucha SCOBY has been used in the biosorption process of heavy metals from aqueous solutions and wastewater. This action is with the aid of many metal binding groups present in it (Razmovski and Šćiban, 2008). The removal of Cd (II) and Zn (II) ions from aqueous solutions by dead SCOBY samples were studied and the removal efficiencies of 35 mg/g for cadmium and 40 mg/g for zinc were acheived. The SCOBY was also used to remove arsenic ions from wastewater. The SCOBY biomass was pretreated with $FeCl_3$ and from the Langmuir adsorption model, it was found that 3.98×10^{-3} mmol/g of arsenic was adsorbed at pH 6 to 8 (Mamisahebei et al., 2007). Pb (II) adsorption by SCOBY from synthetic wastewater was observed with 99.7% efficiency (Mousavi et al., 2018). The adsorption of Ni (II) ions by the tea SCOBY from the industrial wastewater is also observed in a study. At pH 7, contact time 15 min and temperature 25 °C, the maximum adsorption was noted (Mousavi et al., 2019). The pH of the medium from which the ions must be adsorbed has a direct effect on the adsorption rate. Chromium and copper ions are efficiently uptaken by the bacterial cellulose mat at pH 2 to 4 because at lower pH many positively charged sites are present and these anions get adsorbed. It was observed that a contact time of 120 and 60 min is required for efficient adsorption of copper and chromium (Razmovski and Šćiban, 2008).

In addition, kombucha SCOBY is often used to adsorb the trace metals present in packaged beverages, producing safe and healthy drink for human consumption (Kapp and Sumner, 2019). Recent studies by Maduabuchi et al. (2007); Izah et al. (2017) and Abdel-Rahman et al. (2019) reported the occurrence of minor quantities of heavy metal ions due to the leaching from metallic cans and containers. Their presence can also be derived from the utilization of water used during production of beverage and from the filter aids utilized during downstream processing just before packaging. The recent study by Najafpour et al. (2020) optimized different process parameters like the amount of kombucha SCOBY to be used, sugar concentration, tea content and the hardness of water. Stock solutions of metal ions were added to kombucha beverage and by response surface methodology the optimum concentration of each parameter was decided and when experimented it gave 75%–93% removal efficiency of heavy metals like mercury, arsenic, lead, chromium and cadmium. The curve between the concentration of SCOBY and biosorption efficiency was increasing up to a level and after which the efficiency was reduced. The reasons being high SCOBY concentration decreases the available bonding sites and hence small doses are used (Najafpour et al., 2020). Thus, the study also established the safe nature of the packed Kombucha beverage for human consumption due to the negligible content of heavy

Fig. 5. Preparation and use of Kombucha SCOBY.

Table 7
Application of Kombucha SCOBY in various fields.

S. No	Field of Application	Product	About the experiment	Property of tea Kombucha SCOBY used	Efficiency	References
1	Recycling of industrial waste	Bioleaching agent	Chemoorgano-heterotropic leaching process by the culture to leach and recycle rare earth elements (REE) from fluorescent phosphor present in electronics	Ability of the microbes to produce organic acids and metabolites to leach compounds from fluorescent powder	Direct and indirect leaching methods gave high efficiency	Hopfe et al. (2017)
2	Animal feed	Supplement as dietary ingredient in broiler feed	Due to the presence of nutrients like crude fiber, lipids, protein, amino acids, minerals and phytase enzyme tea fungus was added to broiler feed.	The waste tea fungus is used as an extra ingredient so that it provides extra nutrients like single cell protein and phytase for more phosphate removal.	Use of feed infused with 15% tea fungus gave high efficiency factor	Murugesan et al. (2005)
3	Electronic	High-capacity cathode in lithium-sulphur batteries	Activated form of Kombucha SCOBY was used in hybrid with other materials and the electrical properties were seen.	The porous and adsorptive nature adds to its conductive property	Efficient electron transfer and increased cycle stability.	Kalaiappan et al. (2020)
4	Removal of metal ions	Biosorption material	The kombucha pellicle produced added directly to sample under study and its adsorption property was evaluated.	Adsorption property of SCOBY due to the carboxyl and hydroxyl groups present.	Removal efficiencies of different metal ions: Hg^{2+}: 93.3%, As^{3+}: 76.7%, Pb^{2+}: 76.1%, Cd^{2+}: 84.3%, Cr^{6+}: 75.4%.	Najafpour et al. (2020)
5	Medicine	Silver nanoparticles of fungal extract	The silver nanoparticles were prepared with kombucha SCOBY extract and tested on different bacterial strains human breast cancer cells (MCF-7).	The antibacterial activity of kombucha SCOBY.	Nanoparticle size obtained: 155 nm, Effective against Gram-positive bacteria *S.aureus* (14 mm inhibitory zone) and Gram-negative bacteria *E. coli* (9 mm inhibitory zone)	NT (2017)
		Wound dressing	The kombucha SCOBY grown in tea extract and then loaded with coffee extracts to study the preservation and release of materials by SCOBY samples so that it can be loaded with medicinal compounds for wound healing.	Antibacterial and absorptive nature of wound exudates	The growth inhibition by the sample against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) were 97% and 87%	El-Wakil et al. (2019)
6	Tissue engineering	Peripheral Nerve conduit	The kombucha SCOBY was molded into a conduit and grown with Schwann cells in vitro to test the biocompatibility of the material	The mechanical stability, biodegradability and negligible cytotoxic effect were considered for preparation of these conduits.	Good biocompatibility and insignificant amount of hematological and histologic toxic effects.	Zhu et al. (2014)
7	Food packaging	Biodegradable packaging material	The dried biofilm sheets are made into covers for storing vegetables for longer period without degradation in their nutritive quality	Biodegradability, Antibacterial property and leak proof property	All vegetables and fruits were found to be found to be fresh for 8 days	Aduri et al. (2019)
		Bio-composite film for meat packaging	An active bio-composite film with chitosan and kombucha to pack minced meat and to extend its shelf life.	Biodegradability, antioxidant property, Antimicrobial activity, protection from UV and decreased water vapor permeability.	Chitosan with 3% Kombucha fungus increases shelf life for up to 3 days.	Ashrafi et al. (2018)
8	Textile industry	Wrist bands and T-Shirts	Raw biofilm to which glycerol is added for increasing the stability and to produce ready to use fabrics.	Mechanical property of the hydrogel formed	Comfortable and waste free garments produced	Kamiński et al. (2020)

metals owing to inherent physiochemical adsorption property of SCOBY.

4.2. Eco-friendly electronics

Due to the porous nature and the adsorptive interactions by the kombucha SCOBY membrane they are also used as an electrical current conductive additive. The kombucha SCOBY carbon along with graphene oxide, sulphur and polyacrylonitrile was made into a composite and used as a cathode in lithium sulphur batteries. The sulphur gets distributed in the pores of the matrix which reduces electronic diffusion lengths and increases cycling stability. This composite matrix is said to provide good physical and chemical adsorption (Kalaiappan et al., 2020). The kombucha SCOBY was used as a leaching agent due to its ability to leach rare earth elements (REE) from fluorescent powder present in various electronics like fluorescent bulbs, electric cars, energy-efficient bulbs and so on. The microbial consortium present in the kombucha SCOBY can degrade the REE-compounds and produce acids and other metabolites. Direct and indirect leaching methods are used for leaching and the efficiency was compared between individual bacterial and yeast strain's capacity and kombucha SCOBY consortium. It appeared that kombucha SCOBY was more efficient and therefore this method can be used as a basis for leaching elements in an ecofriendly way (Hopfe et al., 2017).

4.3. Food industry

The kombucha SCOBY is used for both edible and non-edible purposes in the food industry. The composition of the kombucha fungus was analyzed and based on those studies it was used as a non-toxic, dietary supplement ingredient in the meal of broiler chicks in a study. The tea fungus was dried under the sun and added to the broiler meal at different concentrations. The optimized concentration of tea fungus that was included in the feed was 15% and this was found to increase factors like feed and water uptake of the broilers, weight and efficiency. Since it is nutrient-rich and energy providing and does not show any signs of abnormalities in the liver function or mortality it can be used as a feed supplement (Murugesan et al., 2005). Due to the absorbing property of the cellulose, it was used to encapsulate many nutrients, food colors, antioxidant compounds and so on. This property is hence used in manufacturing nutraceuticals and feed supplements (Basak et al., 2015).

The kombucha SCOBY has been used as a packaging material for various products due to its biodegradable nature and a substitute for plastics. It is being experimented for packaging food. The study by Aduri et al. (2019) used SCOBY to make eco-friendly and zero waste bags for carrying vegetables. The bags were made flexible and stitched to hold different vegetables. These were refrigerated for 8 days and it was observed that the vegetables were maintained fresh with very little change in their nutritive analysis. The only limitation faced was to control the moisture absorbed by the material during storage. Bio-composite films of this cellulose are also made for better qualitative properties and it can be used for active food packaging. Many properties like the antimicrobial activity, antioxidant activity, water vapor permeability, water solubility and other mechanical properties were tested for a bio-composite made of chitosan and kombucha cellulose. This was tested as a packaging material for minced beef and it was seen that there were minimal changes in color, odor and taste, shelf life was extended with less lipid oxidation and less microbial growth. Chitosan along with kombucha cellulose contributed to the antimicrobial property and it was proved to be a potential food packaging material (Ashrafi et al., 2018). A similar study was performed involving bio-composite of chitosan and SCOBY for assessing ability as a packaging material using tomato as a test object by Sharma et al. (2020). It was seen that the freshness of the tomato was maintained up to 28 days of packaging and was found to be much efficient than polythene and pure SCOBY sheet. The cellulose films are also prepared by the addition of plasticizers like glycerol in varying concentrations. The addition of glycerol has increased the flexibility of the product compared to plain kombucha biofilm. The kombucha films were subjected to repeated fermentation processes and it was noted that the cellulose film present submerge had better characteristics compared to superficial cellulose films. The increased antioxidant property of the kombucha biofilms was observed and hence it can be used in contact with food materials (Tapias et al., 2020).

4.4. Biomedical applications

Kombucha bacterial cellulose (KBC) has various applications in the tissue engineering field due to its biocompatible nature. The mechanical stability of these cellulose membranes is one reason for considering it in the preparation of nerve conduits. The conduit was fabricated, and its biocompatibility was tested with nerve cells and later it was implanted in rats. The KBC conduit was first molded into the required shape and it was prepared by drying and soaking in water for the growth of purified Schwann cells in vitro. The cell morphology, function and viability were promising, and the conduits were tested in vivo by implanting it into a rat and after 6 weeks also no rejection, hematological, or histologic negative effects were seen and thus considered as a choice of biomaterial (Zhu et al., 2014).

Kombucha SCOBY has also experimented for its hepatoprotective property. The indication of liver toxicity is when the level of a few hepato-specific enzymes increases in plasma. So, these enzymes levels were monitored in the plasma and it was found that the enzyme levels were low in animals fed with the prepared SCOBY. This was also confirmed to cure induced hepatotoxicity, so the study concluded by proving that the Kombucha SCOBY helps in both prevention and cure of liver toxicity (Murugesan, 2002).

Kombucha tea fungus extract has been used in a study to synthesize silver nanoparticles and to study its biological properties. The tea fungus was grown in flasks which were kept in an orbital shaker, then the cellulose was filtered from the broth and to it, 5 mM silver nitrate solution was added. Silver nanoparticles formed were tested for its antibacterial activity, revealed maximum resistance to gram-positive bacteria *S. aureus*. It also showed cytotoxicity towards human breast cancer cell lines (MCF-7) (NT, 2017). Due to this antibacterial activity and its absorptive nature these biofilms are used in wound dressings so that it can absorb the wound exudates. One such study explains this property by using kombucha cellulose grown in tea extract and then infused with coffee powder to study the capacity to load antimicrobial properties in the cellulose and these bio-composites can then be used in treating active wounds (El-Wakil et al., 2019). The biofilms infused with herbal extract of henna leaves were also studied for its antimicrobial and wound healing property to add upon its use as a wound dressing (Pooja et al., 2019). Bio-extracts of plants like *Terminalia arjuna* (arjuna), *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Tinospora cordifolia* (giloy), and *Murraya koenigii* (curry leaves) were infused in Kombucha SCOBY and tested for its antibacterial property. *T. arjuna* modified bacterial nanocellulose was found to show efficient antibacterial activity against *E. coli* and *Aerococcus viridians*. All these experiments are used to fabricate antibacterial nanocellulose films for biomedical applications (Sharma and Bhardwaj, 2020).

4.5. Textile and fashion industry

The kombucha derived cellulose sheets are nowadays widely being used by designers in the textile industry for making shirts, footwear, wristbands and various other apparel in a small-scale level to experiment the usage of bacterial cellulose in substituting the non-biodegradable fabrics and to produce eco-friendly clothing (Lee, 2011). In a study by Kamiński et al. (2020), t-shirts and wrist bands were made by first lyophilizing the cellulose sheets and stearic acid was used for repelling water. This was then treated with chemicals to remove the unpleasant smell and to change the color and texture of the sheets to make it appealing. The desired dress was made by sewing the freeze-dried sheets according to the design and additional cloths or welts were also used as fillers. The mechanic stability, elasticity, sweat-proof property and comfort were tested and made compatible. KBC also could withstand heat or fire due to its hydrogel structure. The growth of KBC in various shapes and sizes also gives rise to the idea of production of waste-free garments by the textile industry. In another study, two new techniques for the growth of cellulose sheets like "*Contact surface blocking cultivation*" and "*Panel shaped cultivation*" were followed to produce garments. The first technique used different blockers like a corkboard blocker, baking parchment blocker and plastic film blocker to block the liquid surface contacting the air so that garments of various shapes can be cultivated, and the latter technique uses a mold of fixed shape and size for garments that do not need alterations. Many trials were done to optimize the blockers, methods and materials required. The dresses were coated, and hand-stitched with light cotton lining for comfort (Chan et al., 2018). Bio-couture is a project that is being carried out to explore and experiment with different textile biomaterials or the production of textiles. It is based on the idea that minimal resources and chemicals are needed for production and are biodegradable (Lee, 2011). One limitation that should be overcome is the regaining of moisture by the cellulose mat. Research regarding this is required to maintain the tensile strength and the structure of the clothing. To overcome this issue, the bacterial cellulose was coated with plasticizers and used in the manufacturing of a vest and shoe prototypes which further increased the potential for developing more sustainable clothing (Lee, 2016).

4.6. Other applications

The kombucha SCOBY, apart from being used in various scientific applications, is found to have been used in interior architecture design. It was used to make lamp prototypes as a part of a summer school project. The heat enduring capacity of kombucha cellulose led to this invention and helped in replacing the plastic designer lamps (Faidi, 2017). This SCOBY is made flexible and water-resistant by testing with various chemicals like coconut oil, mineral oil, tung oil, linseed oil, beeswax and canvas wax. Among these, the cellulose treated with tung oil and coconut oil resulted to be water-resistant and it was used to make a durable folder (Weber, 2019). Similarly, finished products like a wallet, handbags, shoes and coats are being produced as prototypes by researchers to showcase the sustainable use of kombucha SCOBY.

4.7. Challenges and opportunities for scale-up

The large-scale application of Kombucha SCOBY is often limited by the low cellulose yield combined with the high process costs and time-duration involved. Considering the applicability of Kombucha in the areas of environmental biotechnology (Najafpour et al., 2020), food (Murugesan et al., 2005), textiles (Kamiński et al., 2020), biomaterials industries (Zhu et al., 2014; El-Wakil et al., 2019), more insightful studies are needed regarding strategies to reduce the production costs. Over years, there has been a gradual transition from solid state fermentation to submerged fermentation approaches as the former were associated with lower yields, complicated process and expensive equipment (Mousavi et al., 2020). Further, several researchers have proposed the use of by-products from industries (Hussain et al., 2019) and wastes of fruits and vegetables (Mousavi et al., 2020) as alternative substrate for Kombucha fermentation to reduce the raw material costs during scale up. These substrates will not only reduce the overall costs of Kombucha cellulose production but will also facilitate the generation of Kombucha beverage with better antimicrobial and antioxidant properties (Emiljanowicz and Malinowska-Pańczyk, 2020). Several influencing factors like the pH, temperature, substrate concentration, duration of fermentation influences the microbial growth rate as well as the cellulose yield (Villarreal-Soto et al., 2018). Therefore, there is a need to optimize the process conditions to increase and maintain a consistent cellulose yield. To improve the process economics, along with the perspectives of circular economy, there is a need to explore the strategy to recycle the waste tea leaves after fermentation for subsequent batches. Also, under the broad spectrum of biorefinery instead of focusing on a single product i.e., Kombucha cellulose as a biomaterial or bioplastic; other products like the Kombucha beverage in food industries or for biosorption of environmental pollutants can be explored after scale up. There is also a need to revise and develop a standardized industrial production process with appropriate quality control norms related to downstream processing by the policy makers to avoid the concerns associated with microbial contaminants having ill-effects on health during commercial use (Mousavi et al., 2020).

5. Summary and future perspectives

The kombucha SCOBY formation is seen as a robust accumulation of bacteria and yeast cells to form an extracellular biofilm that is being explored for its various properties, functions and applications. The characterization studies of this biofilm showed that it has many interesting physical, chemical and biological properties that can be exploited for various uses. The kombucha SCOBY is cultivated in a laboratory and in household environment with minimal materials required and fewer complications. Findings regarding the microbial ecology has shown the symbiotic relationship between the two genera present in the culture that utilizes the sugar substrate to form unbranched cellulose fibrils. Optimization experiments using parameters like the type and amount of sugar and tea variety used, fermentation conditions and amount of inoculum, along with proper maintenance of culture throughout the fermentation period, is carried out to critically observe and increase the yield of kombucha cellulose. The applications of the SCOBY are seen in different fields like water treatment, medicine, tissue engineering, food industry, fashion and textile industry and electrical application.

Although not much importance was given to date for Kombucha SCOBY, which is a by-product of the kombucha beverage fermentation, it is time to address the utilization of SCOBY as the kombucha beverage industries are expected to grow with a compound annual growth rate of 20%. Nowadays, it is mostly seen as a raw material that provokes cradle-to-cradle thinking to utilize it and develop a less resource consuming, sustainable and environment friendly product. Still there has not been any wide application of this material. Therefore, future research can be more inclined towards many aspects as listed below:

i. Analyzing different cultivation methods and various reactor designs

The cultivation of this cellulose till now has been done at a small-scale level in glass beakers, jars, ceramics and plastic trays. However, these containers act as a small batch reactor in which the static fermentation took place. Other semi-continuous or continuous methods of bacterial cellulose can be implemented, and an efficient process can be optimized.

ii. Finding an efficient scale-up method to increase productivity

Till now scale up studies on cultivation of SCOBY has not been widely done. Scale-up of the production process should be based on the required volume and height of the media in the container so that it shall eliminate the use of excess media. Few other issues that need to be solved are the automation of the whole process (from media preparation to the final product manufacture), the feasibility and the process efficiency.

iii. SCOBY growth and maintenance

Since the SCOBY takes the perimeter of the container and hence two-dimensional cultivation techniques have been experimented with a movable robot to fold and change the structure of the SCOBY grown on the surface. Likewise, for large scale production, three-dimension growth methods using oxygen permeable materials and minimal space-consuming techniques can be experimented. The conditions also should be maintained optimally throughout the fermentation period without any contamination for the required yield.

iv. Use of low-cost raw material

The cost of media components can be cut down without compromising on the quality and the yield of the product by using alternate and cheap sugar sources or used and waste tea leaves.

v. Application of SCOBY

SCOBY is abundantly used in the fashion and clothing industry as a substitute for leather and is referred to as vegan textile. Many

designers come up with various ideas and prototypes of the finished product but the common problems that should fully be resolved are the water absorptive and flexibility issues for a product to be satisfactory and worthy. So, the gap should be filled with research on changing the property of the biomaterial.

To summarize, the kombucha SCOBY is seen as a material of high potential for its eco-friendly and sustainable nature. Research done till now is mostly aimed in studies regarding the microbial ecology, cultivability and preliminary experiments on using this as an alternative in various applications. More research will help to understand its full capability. Many research findings which are based on the cultivation of Bacterial cellulose formed from a single bacterial strain or co-culture of bacterial and yeast cells are being carried out and this BC is utilized in the medical field, food industries, textile, fashion and cosmetics and many environment-friendly procedures and commodities are also manufactured. Kombucha SCOBY cellulose can also be experimented as an alternative to this commercial bacterial cellulose. The final product must also be made economically feasible and profitable. Commercial production of SCOBY cellulose with proper applications can be taken as a challenge to overcome as there are many reasons to use this as a competent raw material for promising applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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