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Comparing the filtering efficiency of kombucha SCOBY and nitrocellulose membrane filter

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ABSTRACT

Kombucha is a fermented tea made from tea broth 5-8% Sucrose (granulated sugar) and SCOBY (symbiotic culture of bacteria and yeast), it is an alcoholic drink with 0.5 -2.5% alcohol. The origin of kombucha tea is said to be from China. The kombucha tea takes about 7-10 days for its fermentation. The fermentation takes place in two stages, wherein the first stage takes about 7-10 days, and the pellicle is formed in the air-water interface and in the second stage the flavoring of the kombucha is done with the fruit pulp and it is left for 10 days. In the first stage of fermentation, it forms a polymeric cellulose pellicle (SCOBY) with the help of certain species of bacteria and yeast-like lactic acid bacteria, Acetic acid bacteria and Saccharomyces species. The consumption of kombucha is good for health. The SCOBY is a biofilm formed by the action of acetic acid bacteria, lactic acid bacteria, and yeast and it is made of cellulose polymeric compound which can trap the microorganisms to a certain extent and retain them on the filter pad (SCOBY). The increased use of nitrocellulose membrane filter is used for filtering microorganisms from the sample is used extensively as it gives out efficient results, but the continuous and increased use of nitrocellulose filter is not good for the environment and for the human population who live near the industries where it is made. This study focuses on the use of SCOBY as a filter pad on various samples and finding out its efficiency when compared to the nitrocellulose membrane filter pad.

Key words: *Kombucha SCOBY, Nitrocellulose membrane filter, Filtering efficiency, Filter pad.*

Introduction

Kombucha is a fermented beverage made from black or green tea broth, 5-8 % sucrose or granulated sugar, and SCOBY (symbiotic culture of bacteria and yeast) and with the production of alcohol (0.5–2.5%) (Villarreal-Soto *et al.,* 2018; Jayabalan, 2008). This is a centuries-old beverage and is believed to have been originated from northeastern China. The kombucha tea fermentation process produces a polymeric cellulose pellicle due to the activity of certain strains of *Acetobacter species* (Jayabalan, 2008). The consumption of kombucha has anti-inflammatory potential, antioxidant properties, antimicrobial, antifungal, anticarcinogenic, and Hepatoprotective effects. The kombucha tea is obtained from a symbiotic culture of acetic acid bacteria (AAB) (Kim and Adhikari, 2020). (*komagataeibacter, Gluconobacter* and A*cetobacter species)* (de Roos and Vuyst, 2018). Lactic acid bacteria (LAB) (*Lactobacillus, Lactococcus)* (Marsh *et al.,* 2014) and yeast (*Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Torulaspora delbrueckii, Brettanomyces bruxellensis)* in a sweet medium generally black tea (Coton, 2017). The main acids present are acetic acid, gluconic acid, tartaric acid, malic acid, and in less proportion citric

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acid-producing a sour taste in kombucha (Jayabalan, 2008). Due to the health benefits and popularity of kombucha, there is increased demand among the consumers and thus increasing the industrial production of kombucha (Kim and Adhikari, 2020). The increase in industrial production of kombucha is resulting in increased production of its second product, i.e. SCOBY at the water-air interface. The secondary product SCOBY can be used as a living membrane filter (LMF) for filtering the samples, as the SCOBY is made from polymeric cellulose, and it has high absorbance of water or any other liquid and can be widely used as a filtering membrane (Tang *et al.,* 2019). The SCOBY can be kept alive for years together by supplying it with the tea brew and sucrose continuously without leaving it to dry it up. As the SCOBY is living it keeps producing a new layer of SCOBY every 7 – 10 days interval and making it a thick scoby (Villarreal-Soto *et al.,* 2018).

The use of a nitrocellulose membrane filter is tedious because it is highly flammable, and it is made by nitrating cellulose through exposure to a mixture of nitric acid and sulfuric acid (Tang *et al.,* 2019). Nitrocellulose was first used as guncotton as a replacement for gun powder (Ponting and Clive, 2012).

The nitrocellulose was also used as "Artificial Silk"by Georges Audemars in 1855, but first, it was patented by Hilaire de Charbonnet and he also marketed the product but the result of this was not so good because it is highly flammable and was also expensive than cellulose acetate. Then the nitrocellulose was used in laboratories as a membrane filter made of a mesh of nitrocellulose threads with various porosity. The most used pore size is 0.45 micrometer, and the thickness of the filter paper is 2.5 mm (Tang *et al.,* 2019).

In recent years as there is an increase in the kombucha beverage industry, several researchers are studying the production of "cellulose mat" and the benefits, microbial culture, composition of the fermented kombucha (Laavanya *et al.,* 2021). The well-arranged three-dimensional Nanofiber network in bacterial cellulose results in the formation of hydrogel sheets with high surface area, an abundant surface hydroxyl group, high porosity, and good chemical modifying capacity (Eggensperger, 2020). The bacterial cellulose has been found in many applications such as wound dressings (Lin *et al.,* 2013; (Aduri *et al.,* 2019). Raw material for food and food packaging and many medical applications like implants and scaffolds for tissue engineering of cartilage as well as carriers for drug delivery (Rajwade *et al.,* 2015; Rajwade *et al.,* 2015). The living membrane filtration (LMF) shows better filtration capacity than the ultrafiltration and they also resist fouling than any conventional membrane filters and they also have good healing capacity (Kim and Adhikari, 2020; Leal *et al.,* 2018).

In this study, we are comparing the efficiency of SCOBY and nitrocellulose membrane filter using pathogenic samples. The starter culture of kombucha (SCOBY) was procured from (PEEPAL FARM.org) and brewed in black tea after a few days the new SCOBY formed at the air-water interface, and it was collected aseptically and transferred in a sterile ziplock bag. The SCOBY was purified by 1M or 4M of NaOH for 10 minutes (Amarasekara *et al.,* 2020) and then was plated onto differential media for acetic acid bacteria, lactic acid bacteria, and yeast and was incubated. The purity of the SCOBY was checked by the color change or production of any colony on the media and if no change was observed then that indicates the purity of the SCOBY and was ready to be used as a filter for the pathogenic and non-pathogenic samples. The SCOBY was placed onto the Membrane filter apparatus in aseptic condition and the sample was poured onto the membrane filter by applying negative pressure the suction was done. The sample and the filtrate were plated onto essential media and as well as on the differential media and incubated. The nitrocellulose membrane filter was procured from Rivera and the filter paper was placed onto the membrane filter apparatus, over that sample was poured and negative pressure was applied to suck out the samples more efficiently. The sample and the filtrate were plated on the essential and differential media.

Materials and Methods

Nitrocellulose membrane

The Nitrocellulose membrane filter is a thin polymer with a pore size of 0.45 μ with a diameter of 47mm. They are used in many pharmaceutical industries for microbial analysis and reverse osmosis in water systems. The nitrocellulose filters are also known as cellulose nitrate membranes, and they are prepared by the reaction of cellulose and nitric acid. Each glucose molecule in the cellulose nitrate polymer is esterified with three nitrate groups. The nitrocellulose membrane is prepared by dissolving the nitrocellu-

lose in organic solvents, this solution is spread on a smooth surface to form a thin membrane. The pore size of the membrane depends on the evaporation rate of the solvent used to prepare the cellulose nitrate solution. Careful handling of the cellulose nitrate filters is required because the solution containing organic solvents can damage the membrane filters. The solutions containing acetone can dissolve the nitrocellulose filters (Tang *et al.,* 2019).

Membrane growth

A symbiotic culture of bacteria and yeast (SCOBY 20gm with starter tea 400ml) was added to the growth solution which is prepared from black or green tea, granulated sugar, and water. The vessel which is used to brew the tea is sterilized with boiling water and vinegar and allowed to cool down. The green or black tea (5 tea bags) was added to1 litre of hot boiling water with sucrose (granulated sugar) 100 g and mixed well, allowed it to come to room temperature then transferred to the sterile glass jars and then the kombucha starter tea with SCOBY (procured from peepal farm) was added to the freshly brewed tea and covered it with muslin cloth and secured with a rubber band and kept it at room temperature undisturbed for 14 - 16 days until a cellulose membrane is seen in the air-water interface Fig. 1. (Kim, 2014).

After 14 -16 days the new SCOBY layer was formed, and the SCOBY was measured as 11.5 cm in diameter and 0.5 mm thickness from different areas.

The SCOBY was cut into small pieces and was kept in 4M (molarity) Sodium hydroxide (NaOH) for 10 minutes (Fig. 2) and was then placed on the differential media like De-man Rogosa Sharpe medium for lactic acid bacteria (Reuter, 1985), Gorodkowa medium for yeast.

The prepared media was autoclaved and poured in the sterile Petri plates and allowed to solidify. The sodium hydroxide-treated SCOBY was placed on the solidified medium and incubated at 37 °C for 24 hours and the plates were checked for growth of the bacteria and yeast (Fig 2a and Fig. 2b). The nitrocellulose filter paper and membrane filter apparatus were sterilized and then used for each sample.

Preparation of sample

The bacterial suspension *(salmonella typhi)* was prepared and incubated for 2 hours. The pH and optical density (OD) of the sample were measured, the 0.1ml of the sample was plated on the nutrient agar and kept for incubation at 37 °C for 24 hours, this is done before filtering the sample and then using a sterilized membrane filter apparatus by placing Nitrocellulose filter paper in the grid 10 ml of the prepared sample was filtered and then by placing 4.7cm diameter SCOBY on the grid the 10 ml of the sample was filtered. The 0.1 ml of the filtrate is also plated over the nutrient agar and incubated at 37°C for 24 hours. Gravity fed method was used to filter the sample without pressure. The sample was allowed to filter out from the SCOBY and 0.1 ml of the sample was plated over the nutrient agar medium.

A mini survey was conducted to know the popularity of kombucha among the people aged from 18 to 60, in this survey majority of the respondents were female. This survey showed that the popularity of kombucha is increasing through social media more than any other means. The consumption of kombucha is increasing day by day due to its health effects, taste, and curiosity of people.

Results and Discussion

The use of Nitrocellulose membrane filter paper can be hazardous in the laboratory because of its flammable property as it is made from nitrate, and it is also hazardous to the human population and environment. The alternate material which could be used as the membrane filter is the symbiotic culture of bacteria and yeast (SCOBY) which is a cellulose polymer formed at the air-water interface in the kombucha.

The SCOBY is easily degradable and easily available as it is a waste by-product of kombucha. There are two fermentation stages where the first stage takes about 14 - 16 days, but the production of cellulose mat or the biofilm starts in the first week of fermentation and in the second stage the flavoring of the kombucha drink is done for the commercial purpose. The SCOBY forms in the air-water interface. Once the biofilm is formed if it is taken out from the kombucha in the aseptic condition it will again start to form the biofilm. This way number of biofilms can be collected and can be processed and can be used as membrane filtering material instead of hazardous Nitrocellulose membrane filter paper (Fig. 1).

The SCOBY is sterilized using 4M NaOH (Sodium hydroxide) for 10 minutes (Fig. 2) and was placed on the selective media, incubated and no growth in the media was observed indicating the

Fig 1. Fermented kombucha tea with newly formed bacterial culture (SCOBY)

purity of the SCOBY can be used as a filtering material. The previous studies show that the 90% rejection of the 30nm particle, 98% rejection of 50nm particle, and 99% rejection of 100 nm particles was seen (Jiang *et al.*, 2020). This shows it can remove most of the contaminants and some bacteria and protozoa. The culture *salmonella typhi* and *staphylococcus aureus* was taken and a suspension of 10 ml was prepared by incubating the saline with one to two loopful of the culture and incubated at 37 °C for 2 hours (Andino and Hanning, 2015; Ethelberg *et al.*, 2014). The optical density (OD) and pH of the pathogenic sample was measured. The sterile membrane filter apparatus was assembled with the motor for suctioning of the sample with negative pressure. The 4.7 mm diameter of the nitrocellulose filter paper was placed in the grid and was clamped, the sample was poured from the top then the motor was switched ON. The 0.1 ml of the filtrate was plated on the nutrient agar and using the L-rod the spread plating was done. The 4.7 mm diameter of the SCOBY was placed on the sterile grid and the sample was filtered, the filtrate was plated on the nutrient media and incubated at 37°C for 24 hours and the colonies were counted (Fig 2a, 2b). The filtration was done by another method called gravity fed method where no pressure is applied. The sterile SCOBY was placed over the funnel and the sample was filtered (Fig. 3).

The colony enumeration results depend on the pore size of the membrane and the size of the micro organisms and the amount of pressure applied on the SCOBY (Table 3, 5, 6 and 7).

The gravity fed method gives the reliable results but the method itself is time consuming so the method can be combined with membrane filter apparatus method with reduced applied pressure.

The optical density (OD) and the pH of the samples were checked and the optical density of the

Fig 2a. NaOH treatment of SCOBY

Fig. 3. Gravity fed method

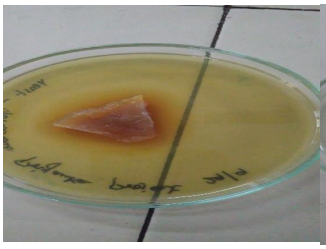

Fig. 2b. Gorodkowa media

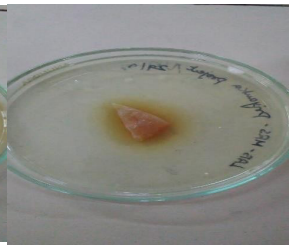

Fig. 2c. MRS media

Table 1. Measurement of pH on different microbial culture

	Staphylococcus aureus	*Salmonella typhi*
Sample 1(Nitrocellulose)	8.43	7.70
Sample 2(SCOBY)	8.86	7.63

Table 2. Optical density of *Staphylococcus aureus*

Before	After	
Sample 1 (Nitrocellulose)	0.04	0.05
Sample 2 (SCOBY)	0.05	0.37

Table 3. Enumeration of *Staphylococcus aureus*

Staphylococcus aureus	Nitrocellulose	SCOBY
Before	364	9 (TLTC)
After	64	84

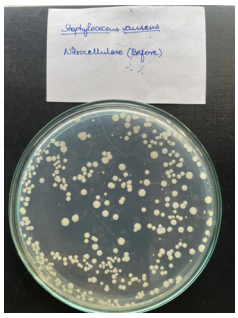

Fig 4a. (*S. aureus)* Nitrocellulose before

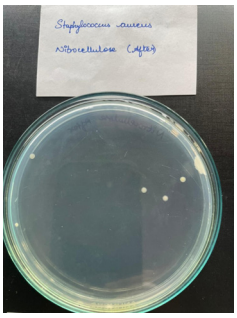

Fig. 4b. (*S. aureus)* Nitrocellulose after

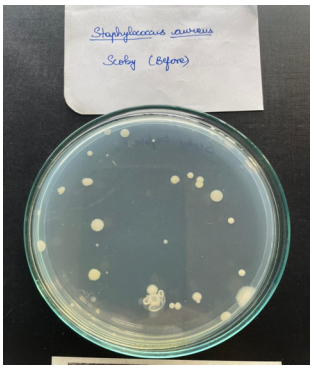

Fig. 4c. (*S. aureus)* SCOBY before

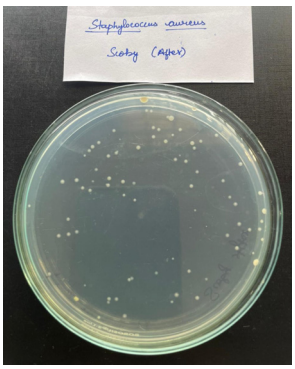

Fig. 4d. (*S. aureus) SCOBY* after

Table 4. Optical density of *Staphylococcus aureus*

	Before	After
Sample 1 (Nitrocellulose)	0.03	0.07
Sample 2 (SCOBY)	0.01	0.16

Table 5. Enumeration of *Salmonella typhi*

Salmonella typhi	Nitrocellulose	SCOBY
Before	TNTC	96
After	95	192

Table 6. Enumeration of *Salmonella typhi* filtered using 0.05 mm thickness SCOBY

Salmonella typhi	Before	After
	TNTC	31

Table 7. Enumeration of *Salmonella typhi* filtered using 0.5 mm thickness SCOBY

Salmonella typhi	Before	After
	TNTC	10

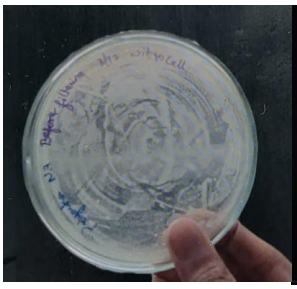

Fig. 5a. (*S. typhi)* Nitrocellulose before

Fig. 5b. (*S. typhi)* Nitrocellulose after

Fig. 5c. (*S.typhi)* SCOBY before

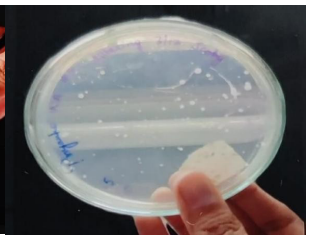

Fig. 5d. (*S. typhi)* SCOBY after

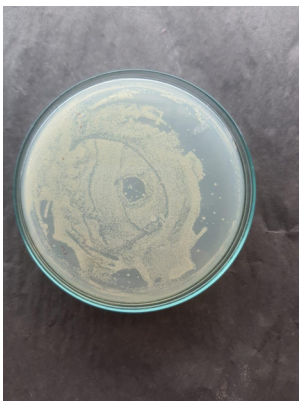

Fig. 6a. (*S. typhi)* SCOBY before filtering

Fig. 6b. (*S. typhi)* SCOBY after using 0.5mm thickness SCOBY

Fig. 6c. (*S. typhi)* SCOBY after filtering using 0.05 mm thickness SCOBY

before filtering sample is less than the after-filtering sample, this is because of the colour taken up by the sample from the SCOBY. (Table 1, 2 and 4)

The enumeration results of *Salmonella typhi* by gravity fed method

Conclusion

We have compared the filtering efficiency of the kombucha SCOBY and nitrocellulose membrane filter using *Salmonella typhi, Staphylococcus aureus* as the pathogenic sample. SCOBY purification was done using 4M sodium hydroxide (NaOH) for 10 minutes and was placed over the differential media. The suspension of *Salmonella typhi, Staphylococcus aureus* was filtered using membrane filtration apparatus with SCOBY and nitrocellulose membrane filter. The sample and the filtrate were plated onto the nutrient media and the plates were enumerated. The enumeration results show that the filtering of the sample using SCOBY with less than 0.7-1.5µm to 2.2-5.0µm was not possible. The gravity fed method combined by membrane filter apparatus can be used by optimizing the amountof pressure applied and time needed for filtering the sample.

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References

Aduri, P., Ankita Rao, K., Fatima, A., Kaul, P. and Shalini, A. 2019. Study of Biodegradable Packaging Material Produced from SCOBY. *Journal of Life Sciences, Bioinformatics, Pharmaceutical, and Chemical Sciences*. 5(3).

Amarasekara, A. S., Wang, D. and Grady, T. L. 2020. A comparison of kombucha SCOBY bacterial cellulose purification methods. *SN Applied Sciences*. 2(2): 240 doi: 10.1007/s42452-020-1982-2.

Andino, A. and Hanning, I. 2015. *Salmonella enterica*/: Survival, Colonization, and Virulence Differences among Serovars. *The Scientific World Journal*. pp. 1–16, 2015, doi: 10.1155/2015/520179.

Coton M. *2017.* Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. *FEMS Microbiology Ecology*. 93(5): p. fix048, May 2017, doi: 10.1093/femsec/fix048.

de Roos, J. and ee Vuyst, L. 2018. Acetic acid bacteria in fermented foods and beverages. *Current Opinion in Biotechnology*. 49 : 115–119, DOI: https://doi.org/10.1016/j.copbio.2017.08.007.

Eggensperger, C. G. 2020. Sustainable Living Filtration Membranes. *Environmental Science & Technology Letters*. 7(3) : 213–218, Mar. DOI: 10.1021/acs.estlett.0c00019.

Ethelberg, S., Josefsen, M.H. and Molbak, K. 2014. History, Science and Methods. In: *Encylopedia of Food Safety*, vol. volume 1, Motarjemi Yasmine, Ed. pp. 501–514.

Jayabalan, R. 2008. Preservation of Kombucha Tea—Effect of Temperature on Tea Components and Free Radical Scavenging Properties. *Journal of Agricultural and Food Chemistry*. 56(19): 9064–9071, Oct. 2008, DOI: 10.1021/jf8020893.

Jiang, D., Hou, D., Bechtel, C., Zodrow, K. R., Myers, R. J. and Zhang, T. 2020. Permeability is the Critical Factor Governing the Life Cycle Environmental Performance of Drinking Water Treatment Using Living Filtration Membranes. *Environmental Science & Technology*. 54(12) : 7651–7658, Jun. 2020, doi: 10.1021/acs.est.0c01306.

Kim, D.H. 2014. Nitrocellulose. In: *Encyclopedia of Toxicology*, Third edition., Philip Wexler, Ed. Elsevier, pp. 540–542. DOI: 10.1016/B978-0-12-386454-3.01255-0.

Kim, J. and Adhikari, K. 2020. Current Trends in Kombucha: Marketing Perspectives and the Need for Improved Sensory Research. *Beverages*. 6(1). DOI: 10.3390/beverages6010015.

Laavanya, D., Shirkole, S. and Balasubramanian, P. 2021. Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. *Journal of Cleaner Production*, 295: doi: 10.1016/j.jclepro.2021.126454.

Leal, J. M., Suárez, L. V., Jayabalan, R., Oros, J. H. and A. Escalante-Aburto, 2018. A review on health benefits of kombucha nutritional compounds and metabolites. *CYTA - Journal of Food*. 16(1). DOI: 10.1080/19476337.2017.1410499.

Lin, W. C., Lien, C. C., Yeh, H.J., Yu, C. M. and Hsu, S. 2013. Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. *Carbohydrate Polymers*. 94(1) : 603–611. doi: https://doi.org/10.1016/j.carbpol.2013.01.076.

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P. and Cotter, P. D. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*. 38 : 171–178. 2014, DOI: https://doi.org/10.1016/j.fm.2013.09.003.

Ponting and clive, *Gun Powder: An Explosive History from the Alchemist of China to the Battlefields of Europe*. Random House, 2011.

Rajwade, J. M., Paknikar, K. M. and Kumbhar, J.V. 2015.

Applications of bacterial cellulose and its composites in biomedicine. *Applied Microbiology and Biotechnology*. 99(6): 2491–2511, doi: 10.1007/s00253-015-6426-3.

Reuter, G. 1985. Elective and selective media for lactic acid bacteria. *International Journal of Food Microbiology*. 2(1): 55–68, doi: https://doi.org/10.1016/0168-1605(85)90057-1.

Tang, Y., Xing, L. and Wang, P. 2019. Preparation of a Hydrophilic Nitrocellulose Membrane. *IOP Conference Series: Materials Science and Engineering*. 677(2) : 022035, Dec. 2019, doi: 10.1088/1757-899X/677/2/022035.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J.-P. and Taillandier, P. 2018. Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*. 83(3) : 580–588, Mar, DOI: 10.1111/1750-3841.14068.