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ARTICLE

Tackling Canada's Discriminatory Water Crisis: Optimizing SCOBY Biofilms to Develop Renewable Living Filter Membranes

By: Maya Lekhi & Lauren Robinson | Edited by: Shannon MacDonell | Layout by: Ahmed Nadeem

Age: 16, 17 | Oakville, ON

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In Canada, 36 long-term (greater than one year) drinking water advisories remain in effect across 29 Indigenous communities (Indigenous and Northern Affairs Canada, 2022). One example of a common issue with the quality of drinking water in Indigenous communities is the "alarmingly high frequency" of fecal bacteria that Canadian scientists have confirmed (Farenhorst et al., 2017). For one fly-in community, the levels of contaminants such as E. coli were hazardous to human health, with concentrations upwards of 62,000 CFU per 100 mL in homes without running water (Water Canada, 2016). E. coli counts greater than 100 CFU per 100 mL of water are considered high risk and unsafe for consumption (Odonkor & Mahami, 2020). Boiling water for 20 minutes prior to drinking is oftentimes the only means through which Indigenous communities can treat their water at home (Cecco, 2021).

While boiling water can kill pathogens, it is a time consuming and energy inefficient process that fails to remove other contaminants, such as heavy metals. Living filter membranes (LFMs) made from symbiotic cultures of bacteria and yeast (SCOBY) are capable of filtering living pathogens and heavy metals, as well as being renewable and cost-effective. The primary yeast species present in SCOBY are Zygosaccharomyces lentus, Zygosaccharomyces bisporus, Schizosaccharomyces pombe, Saccharomyces ludwigii, Kloeckera apiculata, and the bacteria species are Acetobacter xylium, Acetobacter xylinoides, Bacterium gluconicum and Acetobacter aceti, which provide SCOBY's tensile strength and make it permeable to both liquids and gasses (Laavanya et al., 2021).

According to tests performed with SCOBY filters at Montana Technological and Arizona State University, LFMs were 19–40 % more effective at water filtration compared to commercial filters in terms of their ability to resist biofouling (Bechtel et al., 2021). Further analysis attributed these results to the presence of Acetobacter, comprising 97 % of LFMs like SCOBY. Acetobacter is incredibly beneficial to the filtration process as it produces a significant level of acetic acid, which has known antimicrobial properties (Bechtel et al., 2021). In the 2010 study, "Antagonistic effect of acetic acid and salt for inactivating Escherichia coli O157:H7 in cucumber puree", the amount of pathogens like E. coli were "reduced as the amount of acetic acid increased ($P < 0{\cdot}01$) and the rate of reduction was more rapid at 22 °C than at 5 °C" (Lee et al., 2009). Additionally, tests with SCOBY LFMs have shown that they possess a 90 % filtration of 30 nm nanoparticles, showcasing its projected success in filtering larger harmful microbes in tap water (Eggensperger et al., 2020).

According to a 2020 "Study of heavy metals biosorption by tea fungus in Kombucha drink using T Central Composite Design", SCOBY filtration optimized by central composite design under response surface methodology was proven efficient in filtering heavy metals from kombucha (Najafpour, 2019). The filtration efficiency for heavy metals Hg^{2+}, As^{3+}, Pb^{2+}, Cd^{2+}, and Cr^{6+} were 93.3 %, 76.7 %, 76.1 %, 84.3 % and 75.4 % respectively (Najafpour, 2019). Many of these heavy metals, most significantly lead, manganese, and arsenic, are common in drinking water in Indigenous reserves and cannot be removed by boiling water (Lane et al., 2020). This furthers the efficacy of the proposed SCOBY filtration design.

In the present study, to optimize SCOBY growth to produce a suitable LFM, thickness was measured and compared across tests with varying exposure to the external environment, heat, and container material to produce an ideal thickness of approximately 1 mm (Bechtel et al., 2021). It was hypothesized that if SCOBY was cultivated in a glass container in a warm, dark environment, then it would produce a ~1 mm biofilm the fastest because these conditions are considered ideal for bacterial growth. The findings were applied in designing a user-friendly, at-home filtration system that could be implemented in homes in communities subjected to drinking water advisories.

METHODS

To identify which conditions result in the best SCOBY growth, three trials of six plastic containers each were run (Figure 1). The trials were modelled after existing kombucha brewing procedures from (Bauer, 2022). To start, 1.0 L of water was boiled with two tea bags of black tea. Next, 384.0 g of white granulated sugar was dissolved in the tea solution and the mixture was cooled to 27.0 °C. Three plastic and three glass containers were thoroughly disinfected with alcohol and 85.0 g of tea mixture was dispersed among the containers. Finally, 32.0 g of starter liquid (kombucha) was added to each container.

Test groups A and B were kept uncovered, at room temperature (21.0 °C). Test groups C and D were kept covered with a fibrous cloth at room temperature (21.0 °C). Test groups E and F were kept covered with a fibrous cloth and kept on top of a heating pad set to 27.0 °C throughout the growth phase. Test groups A, C, and E were kept in plastic containers, whereas test groups B, D, and F were kept in glass containers. The six growth containers were left undisturbed for the course of seven days with observations recorded daily with a digital calliper (Figure 2). The procedure was repeated for three trials to confirm replicability.

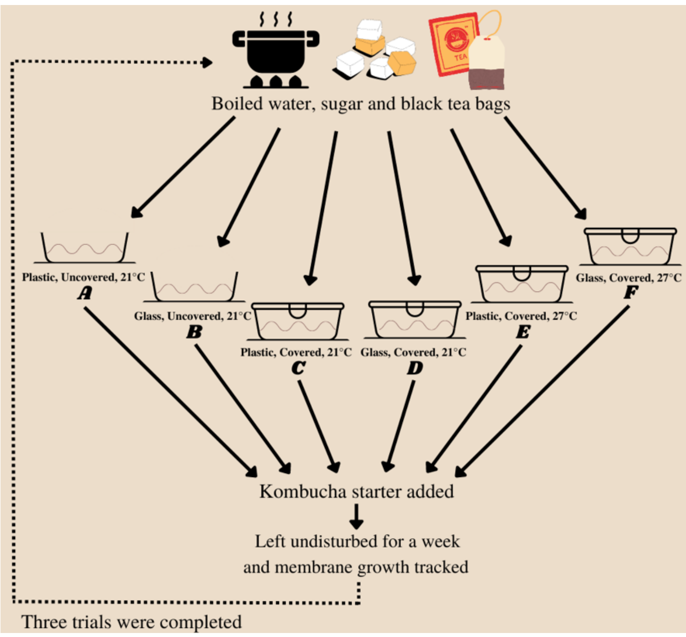

Figure 1: Graphic Detailing Experimental Setup and Test Groups

RESULTS

Throughout the seven-day growth period, the results were measured and graphed in Figure 3. Test A proved to be the least effective, with the filtration membrane only growing an average of 0.09 mm. There was notably the least amount of bacterial activity in this test as the signs of growth were less prominent. In contrast, Test F grew 12 times thicker than the other test groups, with a promising thickness of 1.07 mm. The final observations are documented in Table 1.

DISCUSSION

Based on the data gathered, it was concluded that a warm, covered, glass container would result in the best growing conditions for SCOBY filters as test group F demonstrated the most membrane growth. Using these conditions, a filtration system was modelled using Shapr3D to include two attached growth compartments for filters to allow users to easily re-grow their own filters once they need to be replaced.

These growth compartments are dark, covered, glass compartments which mimic the optimal growing conditions outlined in the experiment. Furthermore, in applying an understanding of ocular physics, having a tinted exterior naturally creates heat which, in turn, supplies the temperature needed to satisfy growing conditions without an exterior heat source. Upon growth up to the marked lines on the chambers, SCOBY LFMs can then be inputted into the filtration casing inside of the filter and be used upon casing reinsertion. Captures of this 3D model are showcased in Figure 4. These variables accurately replicate the growth conditions of test group F, which exhibited the largest membrane

Figure 2: Images of SCOBY Biofilms Grown from Trials 1 and 2 . Note. Test group F shows the darkest colour as it is thicker than the other test groups

Table 1: Final Observations of SCOBY Test Groups After a Seven-day Growth Period

Membrane Thickness (mm)						
Test Group	A	B	C	D	E	F
Trial 1	0.09	0.13	0.11	0.15	0.17	1.05
Trial 2	0.09	0.12	0.12	0.16	0.18	1.09
Trial 3	0.10	0.13	0.10	0.18	0.21	1.08
Average	0.09	0.13	0.11	0.16	0.19	1.07

growth in comparison with the other test groups. These conditions allow for membrane growth to occur the fastest, hence being selected as parts of the 3D filter model design.

As the Canadian water crisis continues to be a prevalent issue, primarily affecting Indigenous communities, this filtration system may allow for a sustainable and easily manageable solution to this problem. Biofilters have been proven to be effective filters for common contaminants in water. This research shows the potential for biofilters to tackle an unjust and persistent issue and provides recommendations for conditions to create these filters. Ideally, in future work, a microscope could be used to measure and track bacterial growth instead of relying on visual observations. In addition to this improvement, testing the SCOBY's filtering efficacy using water from Indigenous reserves in Canada that are under water advisories would be a significant next step for the project.

CONCLUSION

It was hypothesized that ideal SCOBY growth would occur in a dark, warm environment in a glass container. This hypothesis was accepted by three trials in which the location, temperature, and container material were varied, and SCOBY membrane thickness was tracked across test groups. Test group F reflected the hypothesized conditions and was ultimately most successful, as it demonstrated the most SCOBY growth when compared to the other test groups. In understanding what conditions were best suited for SCOBY growth, the results were applied to creating a 3D model of a home filtration device. The dark, tinted, glass growing compartment utilizes the variables tested in the experiment for optimal SCOBY growth. These features effectively allow for SCOBY to be regrown quickly and filters to be replaced often, prioritizing the health and safety of Indigenous communities and the environment. Water is a universal right and as such, the Canadian water crisis' impact, particularly on Indigenous communities, merits greater scientific investment.

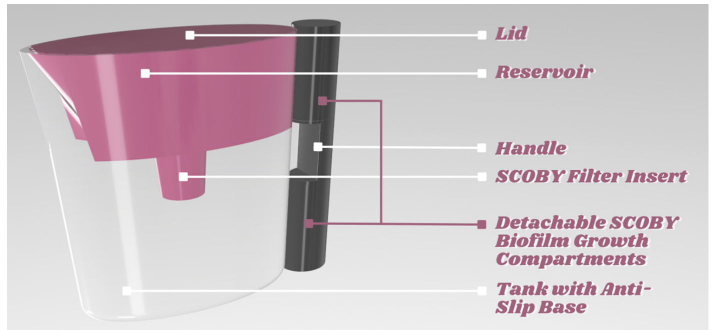

Figure 4: Captures of the 3D Filter Model Designed based on Experimental Results

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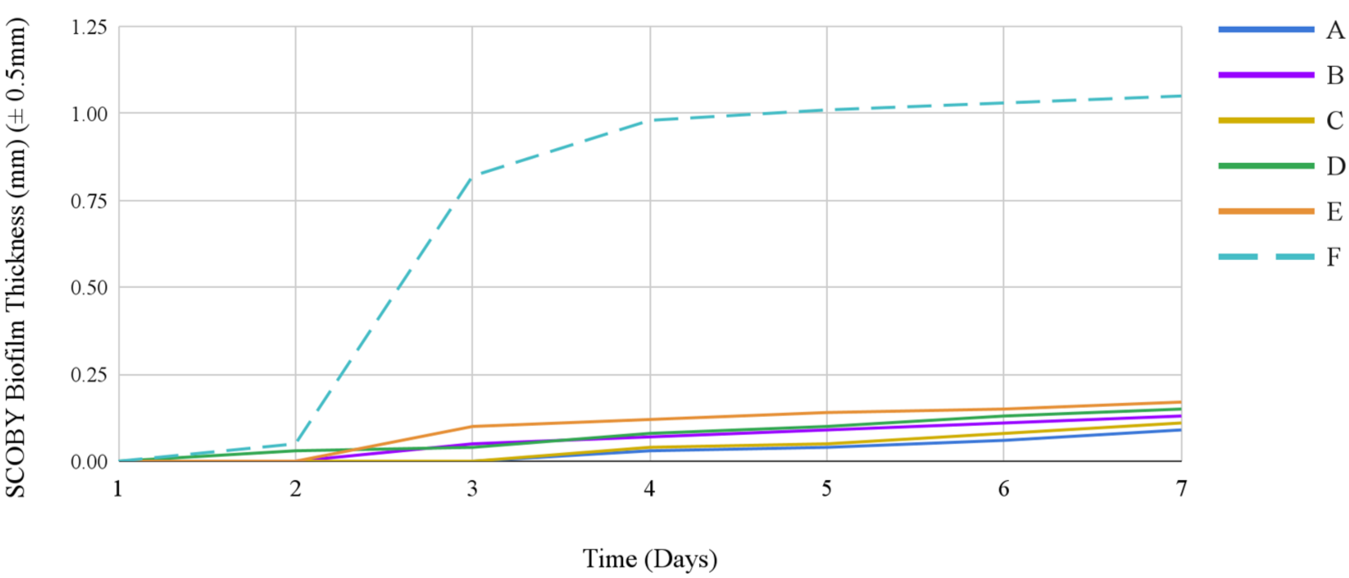

Figure 3: Average SCOBY Biofilm Thickness over Seven Days across Test Groups

edge the land on which we conducted our research: the traditional territory of the Mississaugas of the Credit, the Anishinabewaki, the Attiwonderonk, the Huron-Wendat, and the Haudenosaunee.

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ABOUT THE AUTHOR - MAYA LEKHI

Maya Lekhi is a student researcher passionate about microbiology and biotechnology. She is a recent high school graduate and an incoming student in Western University's BHSc (Hon.) and Ivey HBA Combined Degree Program. In 2021, Maya participated in Shad Canada, where she discovered her interest in providing equitable clean water access and met her research partner, Lauren Robinson. Their work has since been spotlighted on Shad Canada's social media. Outside of her research, Maya founded her school's STEM Initiative Council and volunteers with STEM Fellowship as a Community Engagement Coordinator, where she creates initiatives to address gaps in STEM accessibility. Because of her commitment to community service and pioneering accessibility, Maya was recently selected as a Western National Scholarship Recipient.

ABOUT THE AUTHOR - LAUREN ROBINSON

Lauren Robinson is a 16 year-old student interested in biochemistry and engineering sciences. She is passionate about promoting STEM accessibility within her community, and volunteers with Superposition Toronto, a youth-led organization committed to bridging the gender gap in STEM. Currently, she is working at a not- for- profit leading youth in STEM enrichment. Her community involvement and academic ambition led her to take part in Shad, where she met her research partner, Maya Lekhi. Together, the two bonded over their mutual interest in science and water equity. To learn more about the process of their project, visit their interview on Shad Canada's social media.