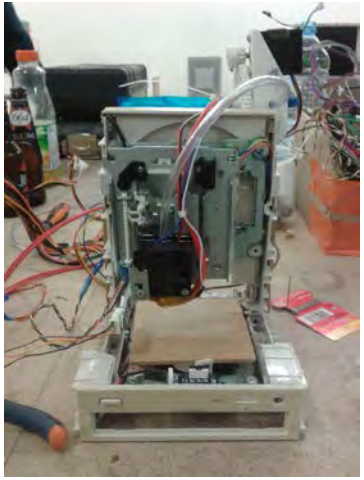
Bioleather in the Kitchen

Bacterial cellulose exploration

Image V Roussel

Vivien Roussel
27/10/2022

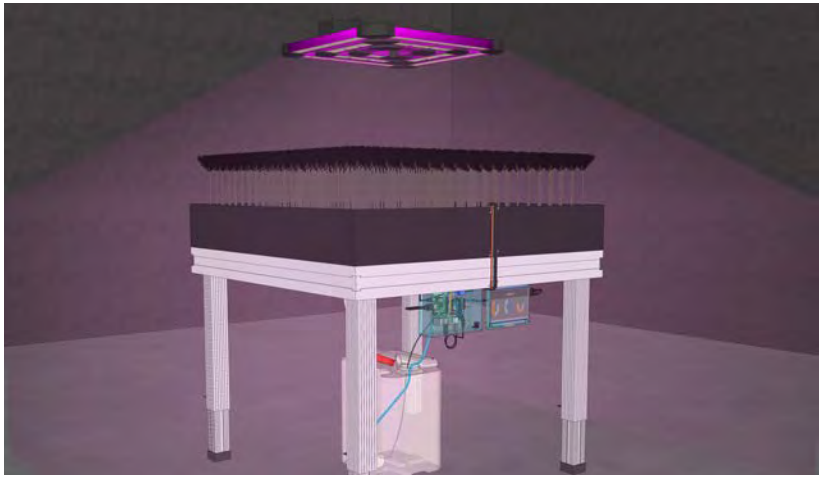
DE VINCI INNOVATION CENTER

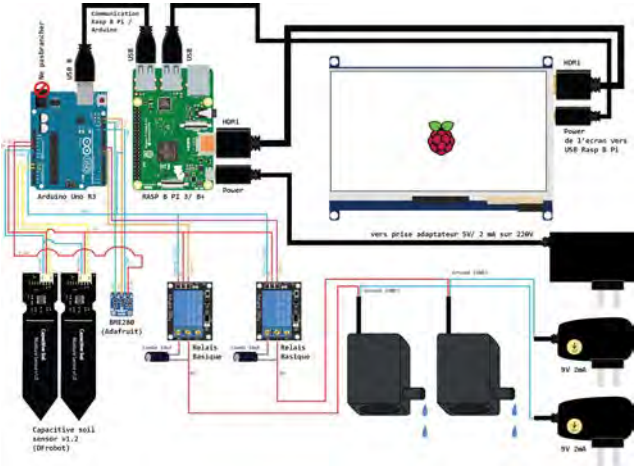


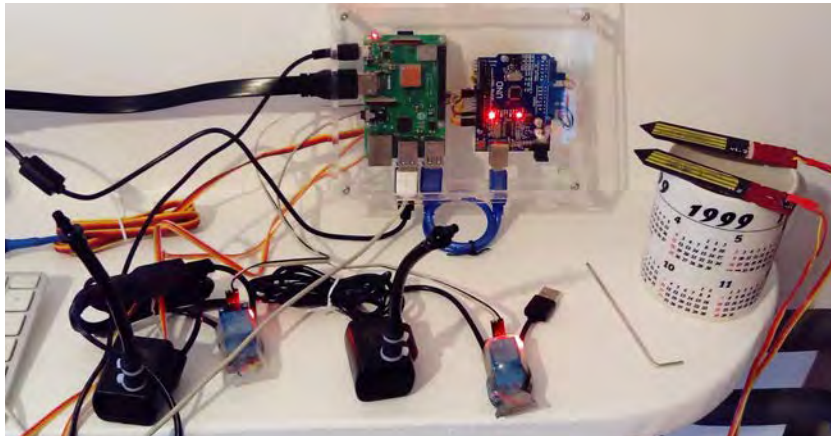
Making & Biomaterials _ v1v13n1

Image : thr34d5 et V Roussel.

Vivien
Roussel

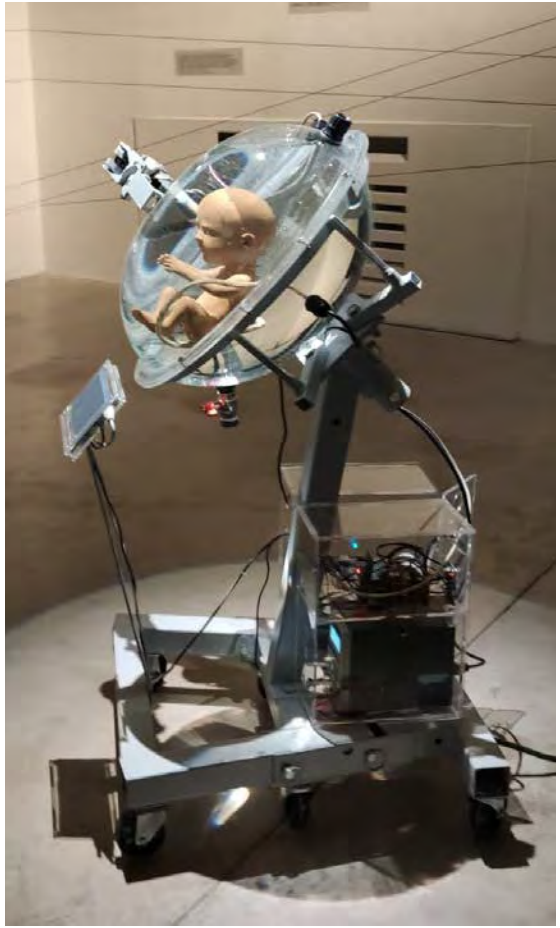






Post Growth _ Nicolas Maigret

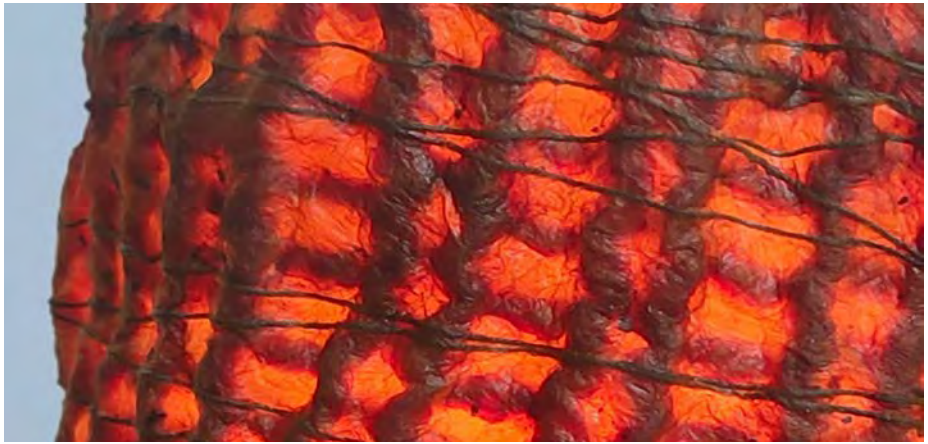
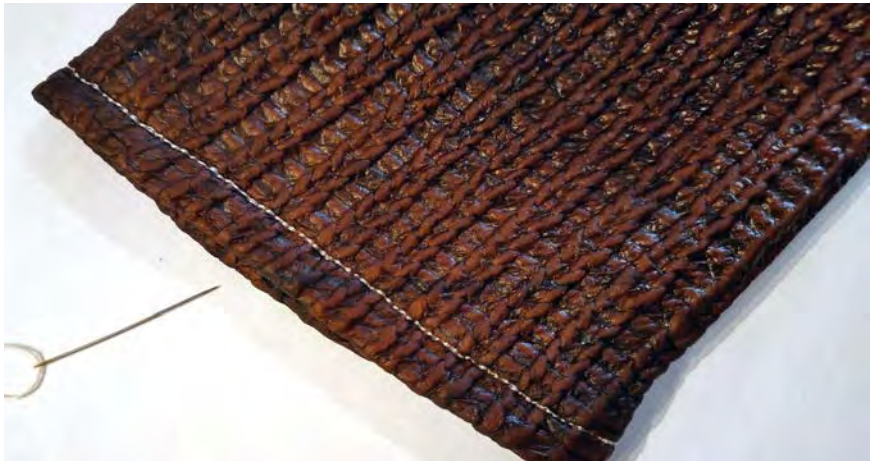
https://we-make-mon000ey-not-art.com/post-growth-ideas-and-toolkit-for-a-world-in-crisis/



UnbornX09 _ Shu Lea Cheang & Ewen Chardronnet

https://unborn0x9.labomedia.org




Morphogenesis (2021-2022) _ Presented at Living Textile Architectures Symposium by HBBE (Hub for Biotechnology in the Built Environment) http://bbe.ac.uk/living-textile-architectures-symposium/

Introduction to cellulose

Kombucha pellicle preparation. Image : thr34d5 (2018).

https://thr34d5.org/2019/08/20/regrow/

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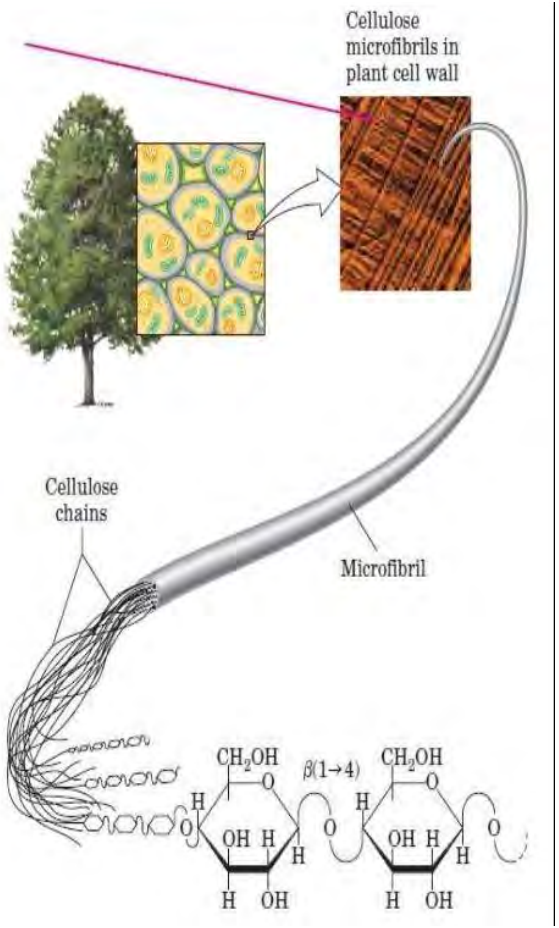
•**Cellulose** is a major constituent of plant cell walls, providing strength and rigidity and preventing the swelling of the cell and rupture of the plasma membrane that might result when osmotic conditions favour water entry into the cell.

•Each year, worldwide, plants synthesize more than 1011 metric tons of cellulose, making this simple polymer one of the most abundant compounds in the biosphere.

•The structure of cellulose is simple: linear polymers of thousands of (β1→4) linked D-glucose units, assembled into bundles of about 36 chains, which aggregate side by side to form a micro-fibril.



https://www.slideshare.net/DeepikaRana6/microbial-cellulose

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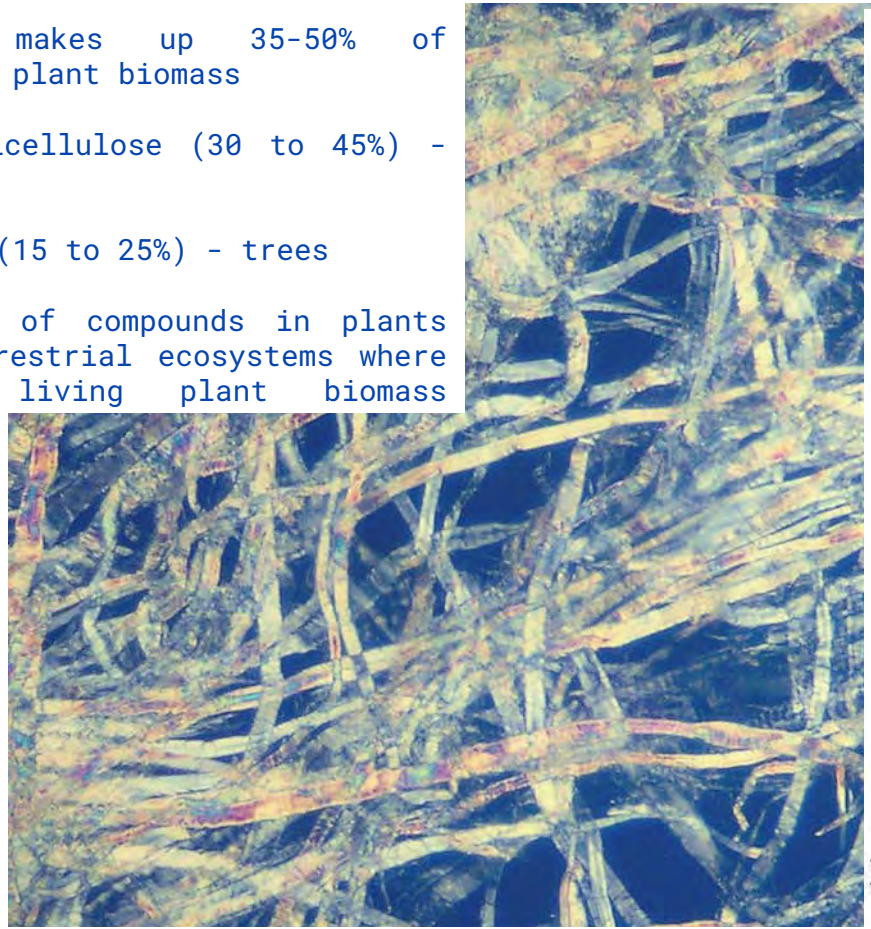
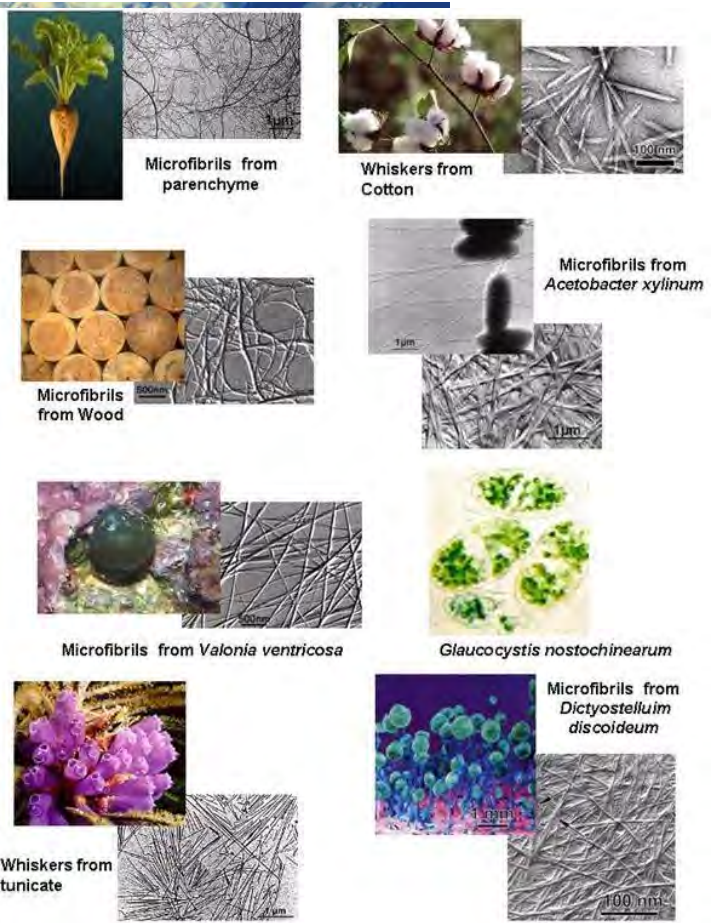
Cellulose makes up 35-50% of terrestrial plant biomass

Before hemicellulose (30 to 45%) - cell wall

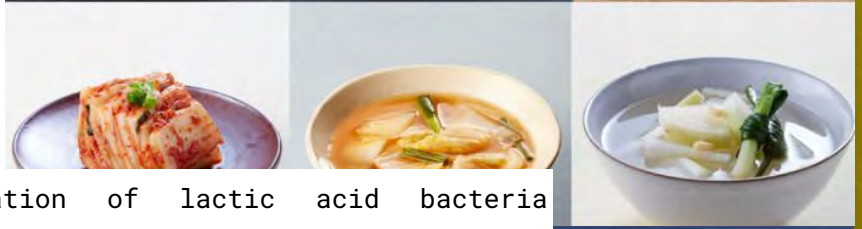
and lignin (15 to 25%) - trees

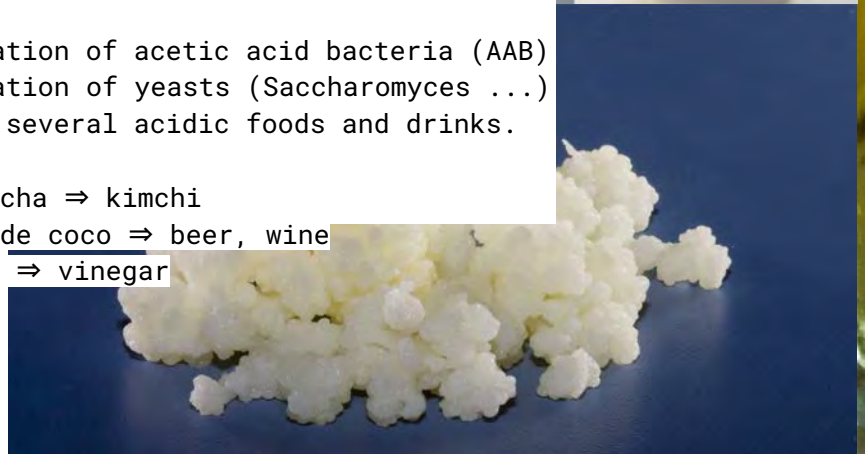
1st family of compounds in plants and in terrestrial ecosystems where dead or living plant biomass dominates.





Par Jan Homann – Photographie personnelle, Domaine public, https://commons.wikimedia.org/w/index.php?curid=1004696





Fermentation of lactic acid bacteria (LAB),
Fermentation of acetic acid bacteria (AAB)
Fermentation of yeasts (Saccharomyces ...)
to form several acidic foods and drinks.

⇒ kombucha ⇒ kimchi
⇒ nata de coco ⇒ beer, wine
⇒ kefir ⇒ vinegar
etc...

Milk kefir grains, A. Kniesel, Wikimedia Commons (2005).

Various kimchi dishes, National Institute of Korean Language, Wikimedia Commons (2016).

Mother of cider vinegar, Alorin, Wikimedia Commons (2015).

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SCOBY is the acronym for "symbiotic culture of bacteria and yeasts"

LAB and AAB penetrate the surface of barley and malt in beer fermentation and grapes in wine fermentation

- LABs (lactic acid bacteria) lower the pH while

- AABs (acetic acid bacteria) take the ethanol produced from the yeast and further oxidize it to vinegar, resulting in an acidic taste and smell.

- AABs are also responsible for the formation of SCOBY cellulose

A.J. Brown in 1886, identified a gel during a course on vinegar fermentation and found it chemically equivalent to vegetable cellulose.

saccharo (sugar), μύκης, myces (mushroom) and the Latin word cerevisia -ae, cervoise (word of Gallic origin designating beer).

Schizosaccharomyces pombe
+ Acetobacter xylinum

(*Komagataeibacter xylinus*)
(previously *Gluconacetobacter xylinus)*

By media.org/w/index.php?curid=39194866

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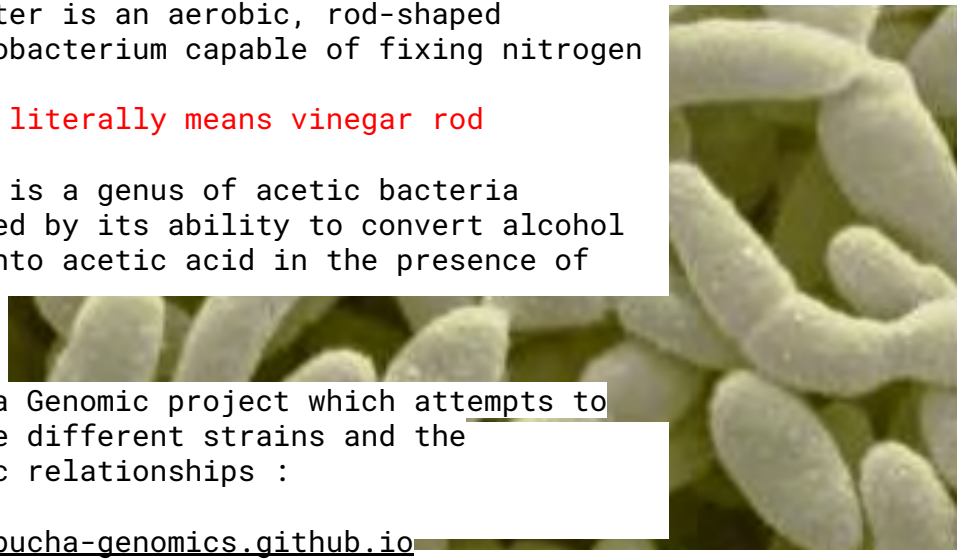
An acetobacter is an aerobic, rod-shaped alpha-proteobacterium capable of fixing nitrogen

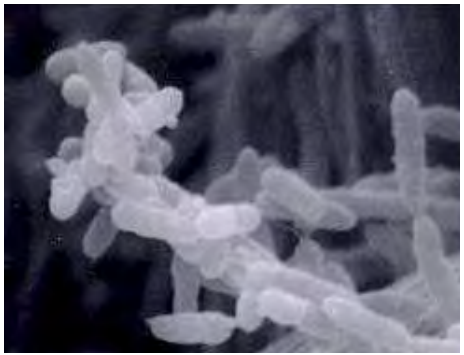
Acetobacter literally means vinegar rod

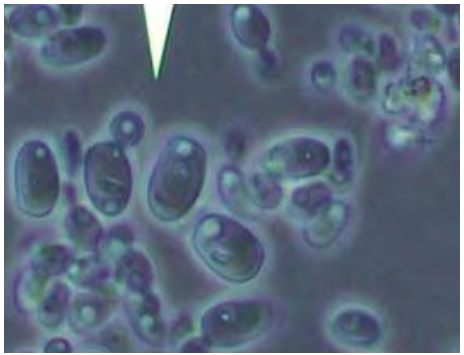
Acetobacter is a genus of acetic bacteria characterized by its ability to convert alcohol (ethanol) into acetic acid in the presence of air.

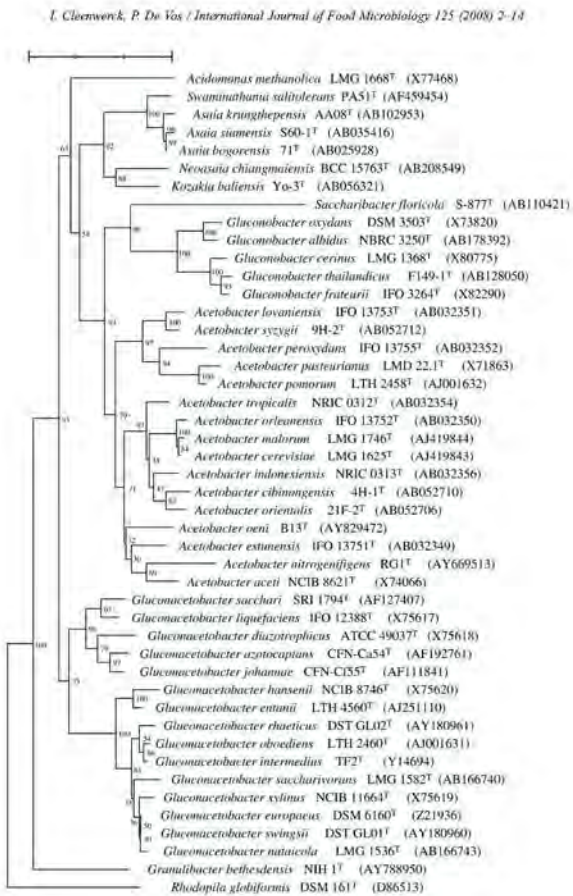


The Kombucha Genomic project which attempts to classify the different strains and the phylogenetic relationships :

https://kombucha-genomics.github.io







https://www.jean-marc-gil-toutsurlabotanique.com/page/introduction-a-la-botanique/les-bacteries/nomenclature-des-bacteries/page.html

Polyphasic taxonomy of acetic acid bacteria: An overview of the currently applied methodology. Ilse Cleenwerck a,□, Paul De Vos. doi:10.1016/j.ijfoodmicro.2007.04.017

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The story behind kombucha :
https://folklife.si.edu/magazine/cloudy-origins-of-kombucha

According to Guenther W. Frank

- kombu (brown algue) & cha (le thé)
- from japanese *konbu-cha* or *kobu-cha* (昆布茶, "kelp tea")

- Qin Dynasty in 221 BCE : “The Remedy for Immortality” or “The Divine Tsche.”

- in 414 BC, a Korean doctor named Kombu used this tea to treat the Emperor Inkyo

- As early as the 1800s and during World War I, Russian and German prisoners seemed to drink it

- some pharmacists sell it as “Mo-Gu” (the Chinese word for “mushroom”) or “Japanese Fungo.”

- etc...

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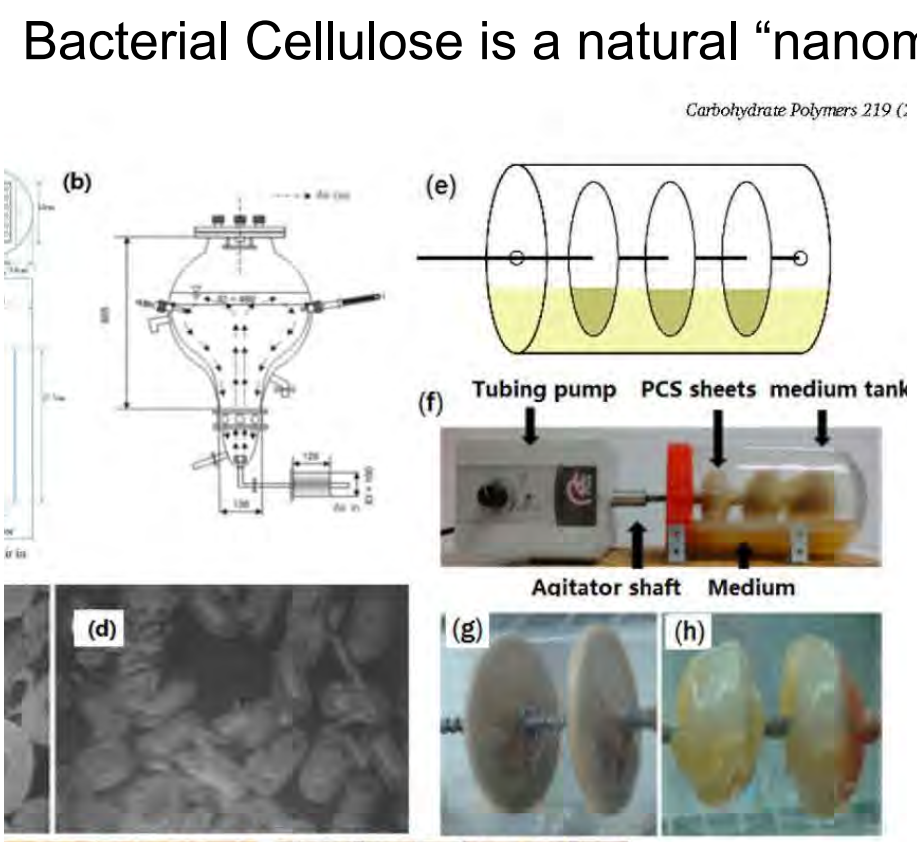
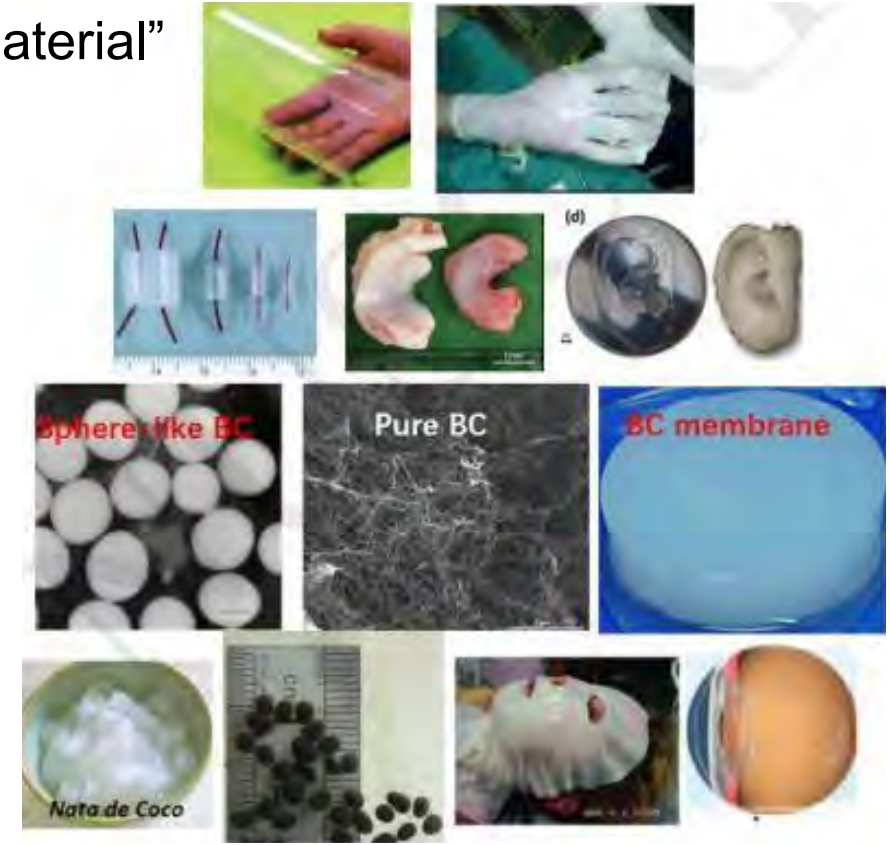
Applications

Use of Industrial Wastes as Sustainable Nutrient Sources for Bacterial Cellulose (BC) Production: Mechanism, Advances, and Future Perspectives. https://www.mdpi.com/2073-4360/13/19/3365

Bacterial Cellulose is a natural “nanomaterial”





Bacterial cellulose production, properties and Applications with different culture methods – A review.https://www.sciencedirect.com/science/article/abs/pii/S0144861719305041

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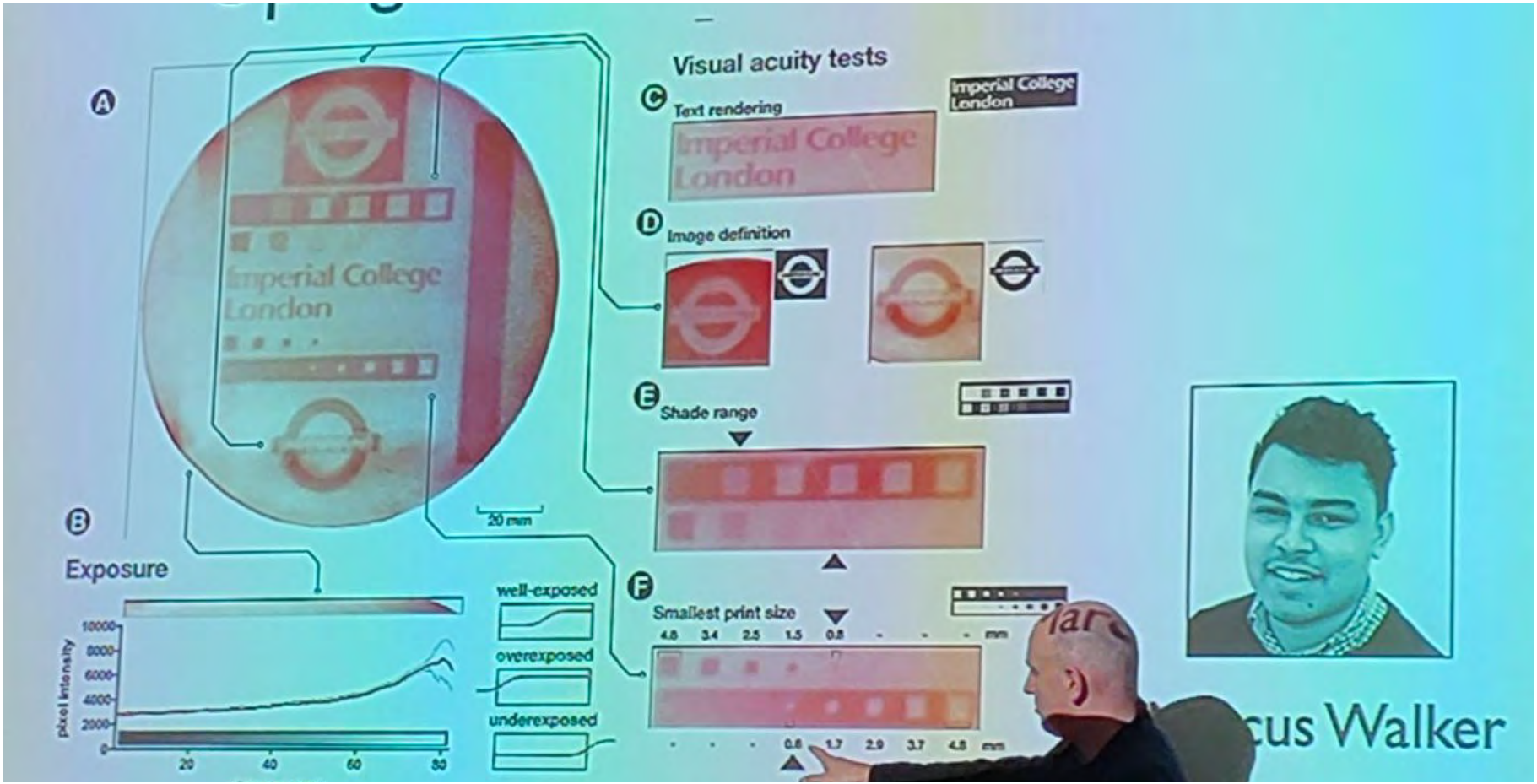
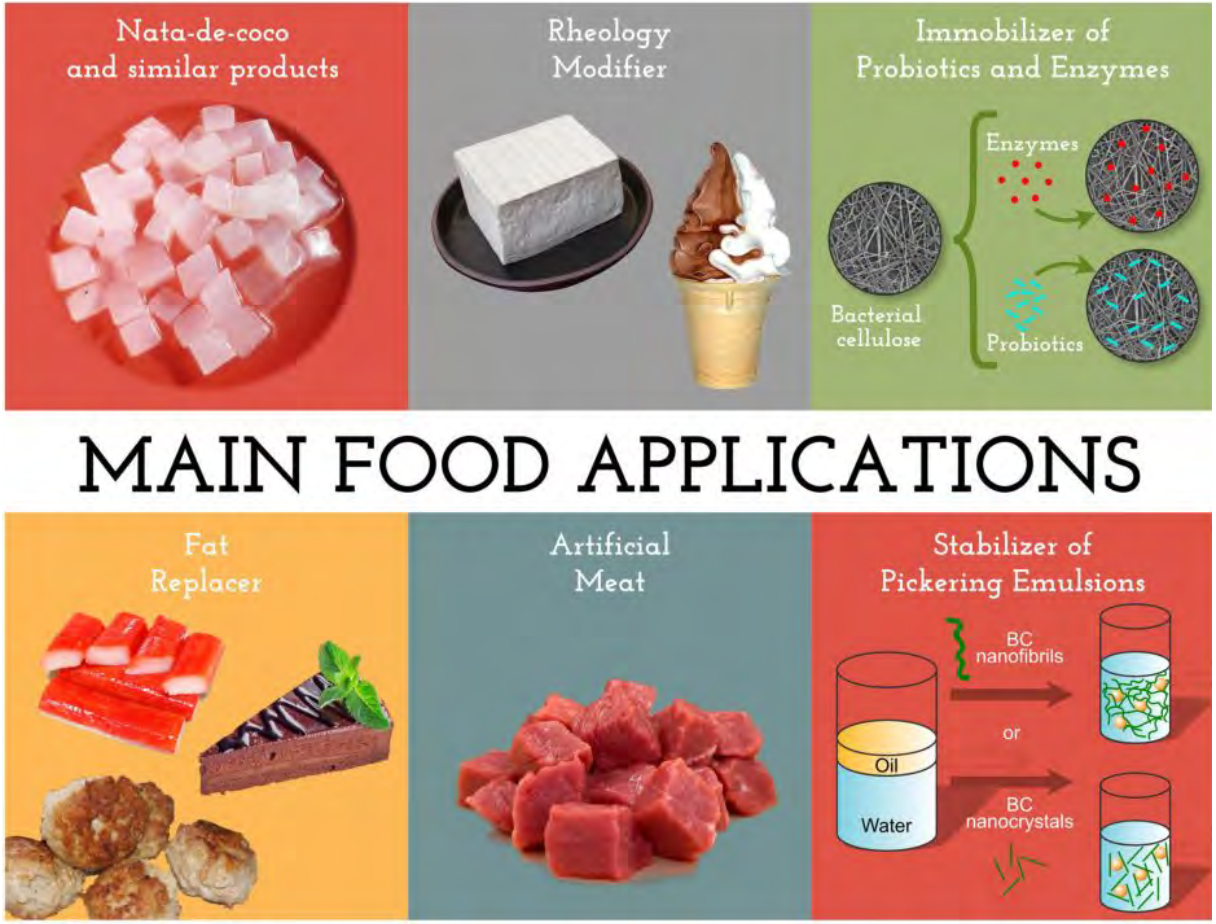


Tom Ellis during HBBE Living Textile Architecture Symposium in Newcastle, 2022. Tom Ellis Lab.



Bacterial Cellulose as a Raw Material for Food and Food Packaging Applications.
https://www.semanticscholar.org/paper/Bacterial-Cellulose-as-a-Raw-Material-for-Food-and-Azeredo-Barud/f5275582920798724b3e7f74aaf1e20d8b21a19e

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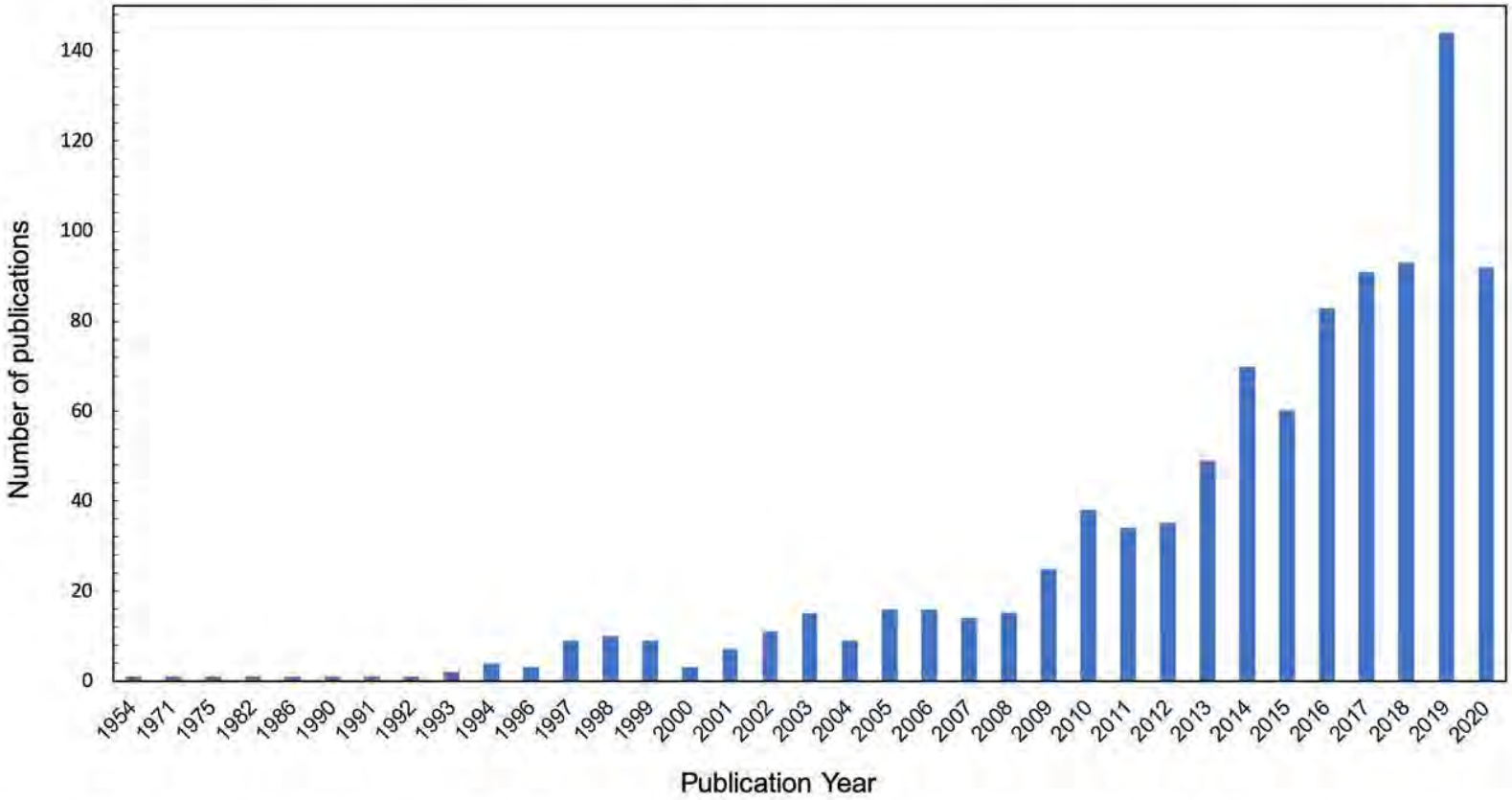

Fig. 2 Evolution of the annual number of publications mentioning bacterial cellulose, medium, media and carbon source over the years

https://link.springer.com/article/10.1007/s10570-021-03754-5

ARTS & DESIGNS

Workshop thr34d5, Festival Low Tech.Copenhagen 2020. Image : thr34d5.

XXlab _ Soya C(o)u(l)ture

https://ars.electronica.art/aeblog/en/2015/09/30/soya-coulture/

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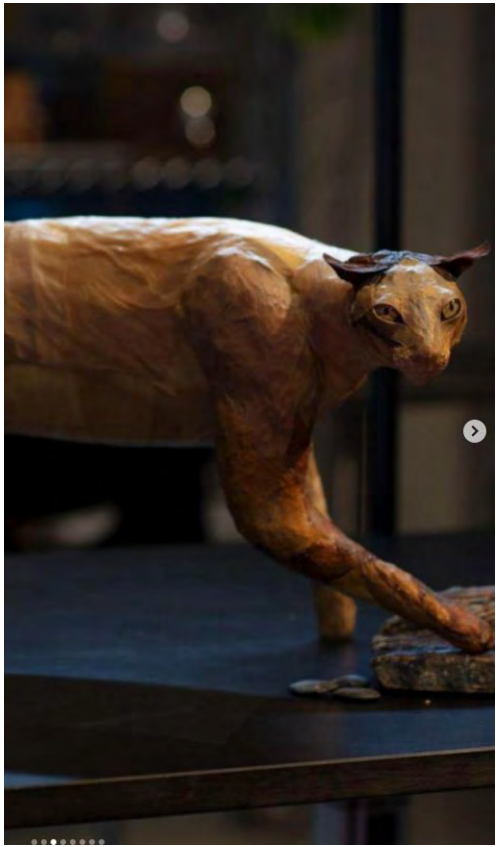
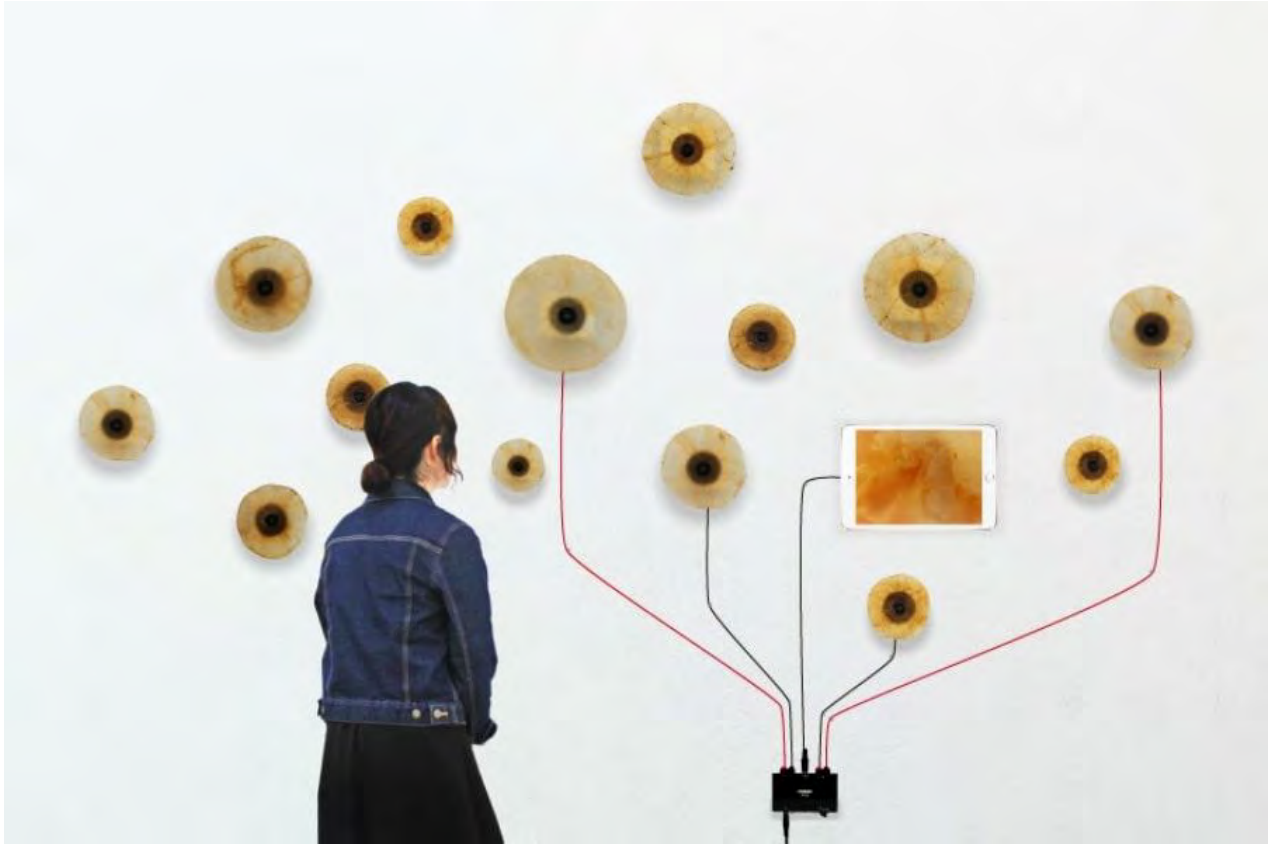
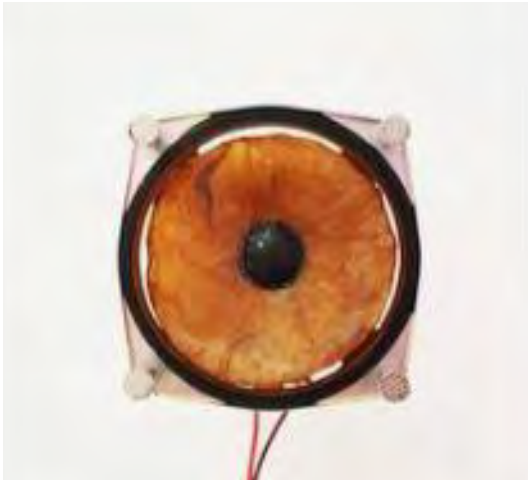


Nicholas DelCastillo





Yoko Shimizu _ Layers of life

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Naja Ryde Ankarfeldt _ Tale of the non-human

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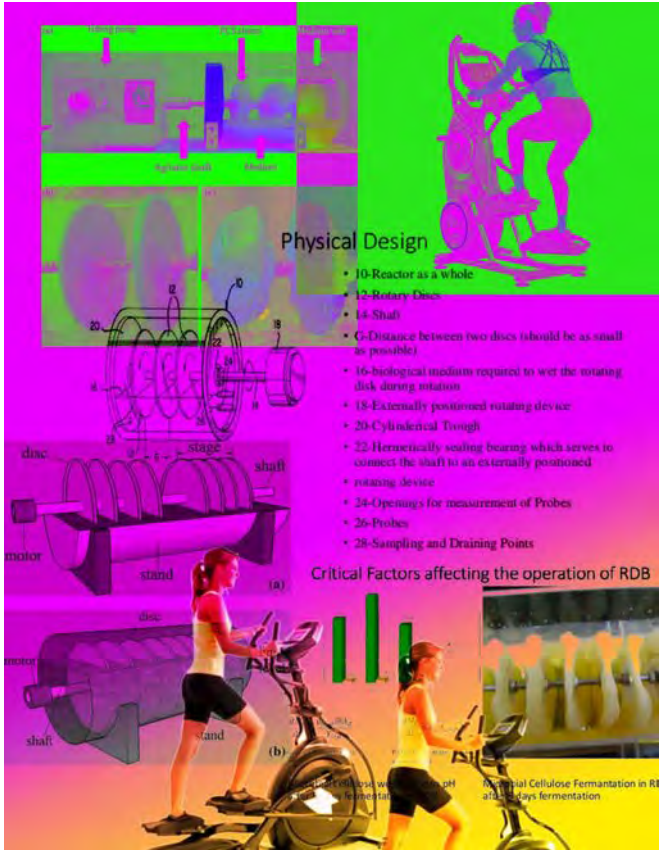
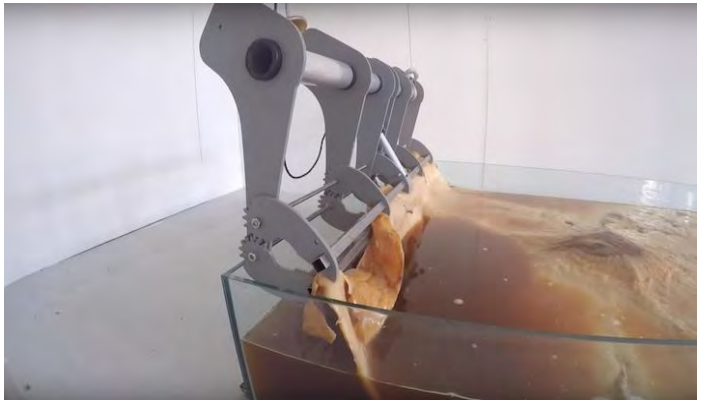
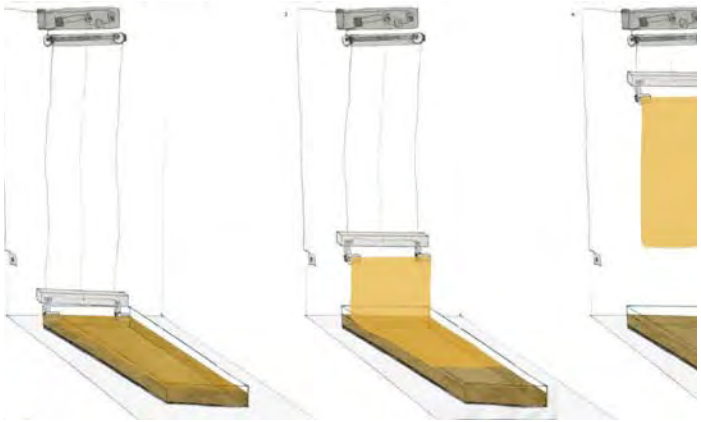
Ali Schachtschneider _ Vivorium



Mary Maggic _ SCOBY SPIN CYCLE

Studio thinkinghand _ Feral fetis





Bianca Hlywa _ Residual yeast

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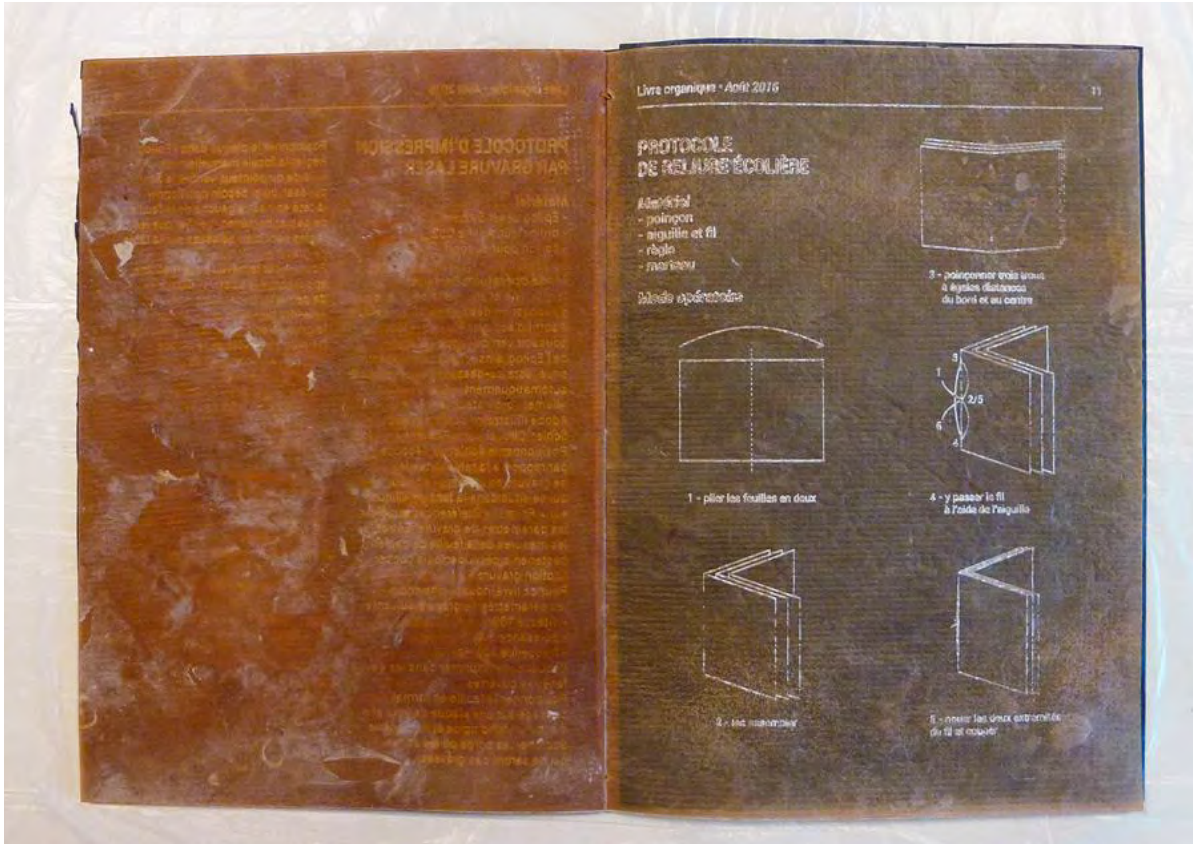
Maya Minder _ Untitled (bacterial skin) 2021 / Kombume

Vivien Roussel _ serigraphy / screen printing (thr34d5)

Maru Garcia _ OLDERBROTHER: SHELTER



Diane Trouillet _ Livre Organique #1

Diane Laurent _ cheese packaging
(thr34d5)

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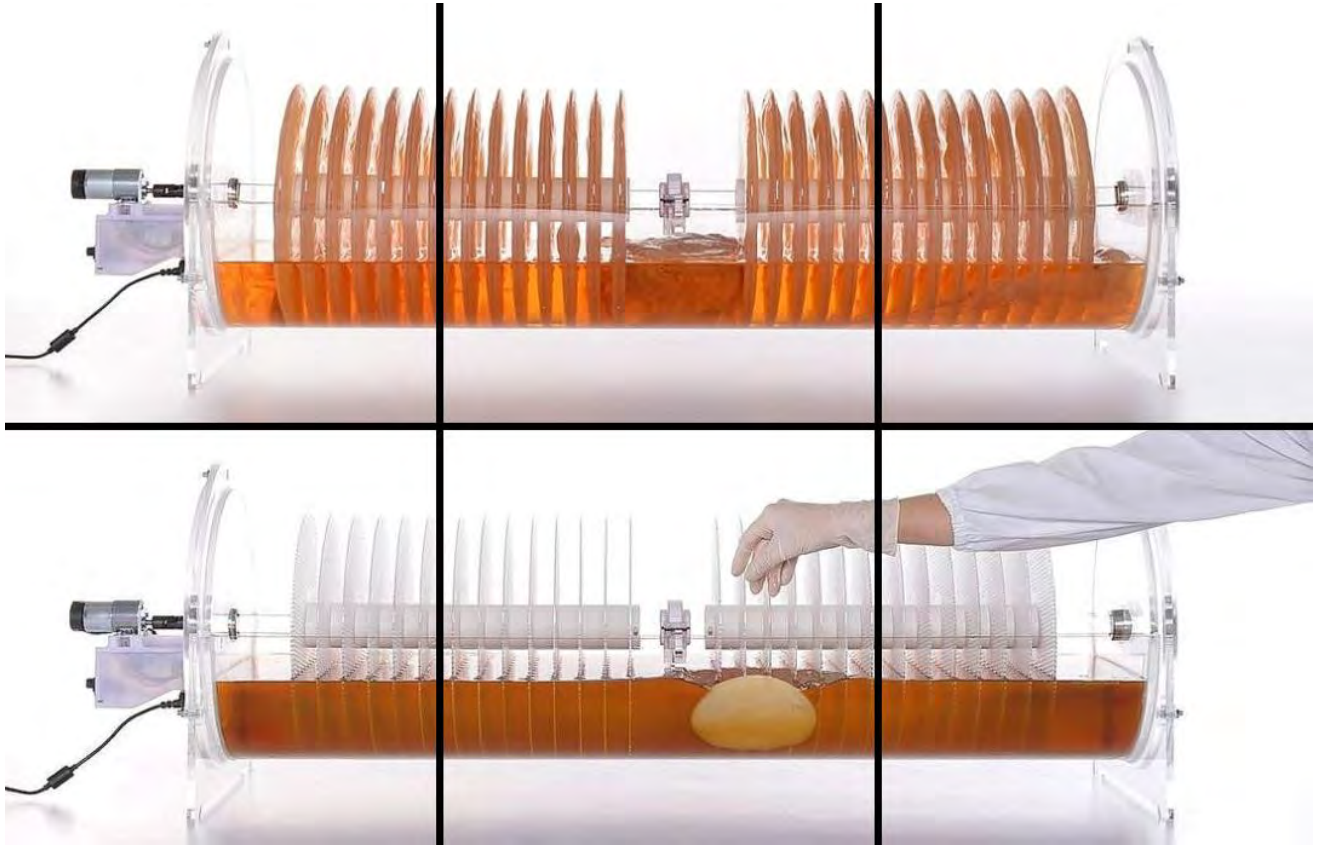
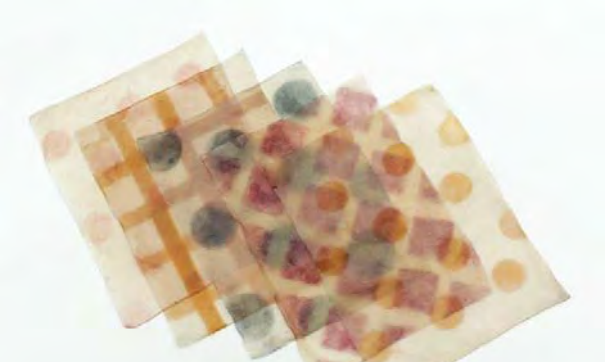
Elena Amato _ Ponto biodesign

Emma Sicher _ From Peel to Peel



Emma Sicher _ An Open-Source Bioreactor…



Lionne Vandeursen _ Unfold / Luna

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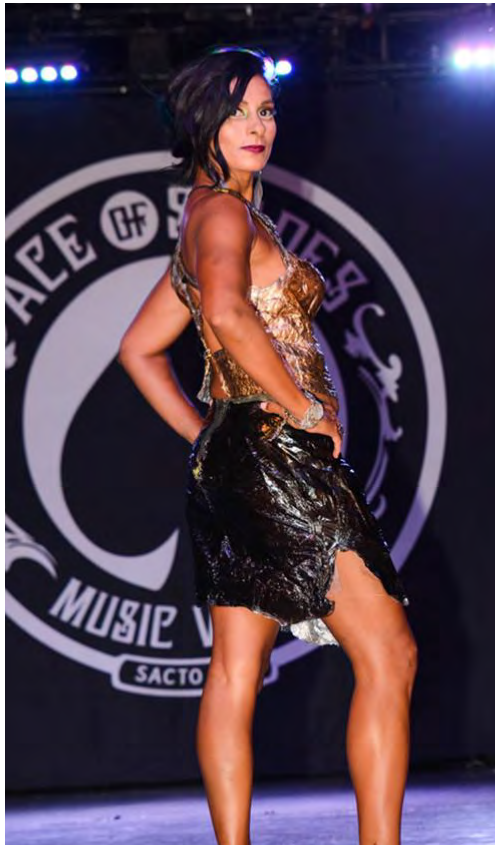

Sacha Laurin _ Kombucha couture

WhiteFeather Hunter & Théo Chauvirey (Concordia University) _ Bucci

Adrien Rigobello – Project Manager & Creative Director, thr34d5
Vivien Roussel – Kombucha Expert, thr345
Tim Leeson – Designer, thr34d5
Dr Robert Pott – Collaborating Researcher
Surzhana Radnaeva – Fashion designer, Fashion Photographer
Benjamin Denjean – Ecosystem Designer
Video edition: Hind Saâd
Guest speaker: Imane Baïz (DITOs)
Model: Valeria Gelardi

thr34d5 : Regrow - Contest Reshape 2018, 1st Price Wearable technology









Suzanne Lee _ Biocouture

Malai

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ScobyTec

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thr34d5 : Kombucha Tsugi - the bag edition, project funded by WORTH EU

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Polybion

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HBBE (Hub for Biotechnology in the Built Environment) _ Bioknit

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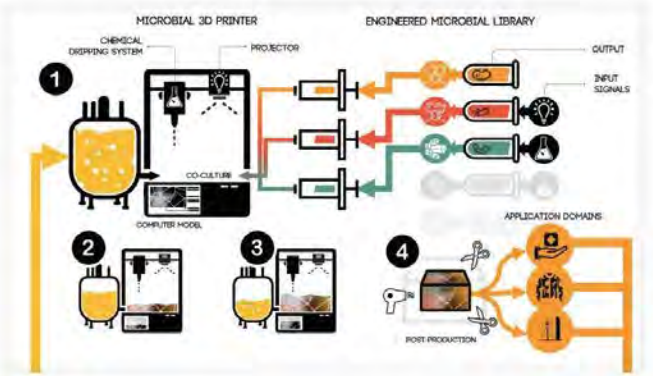

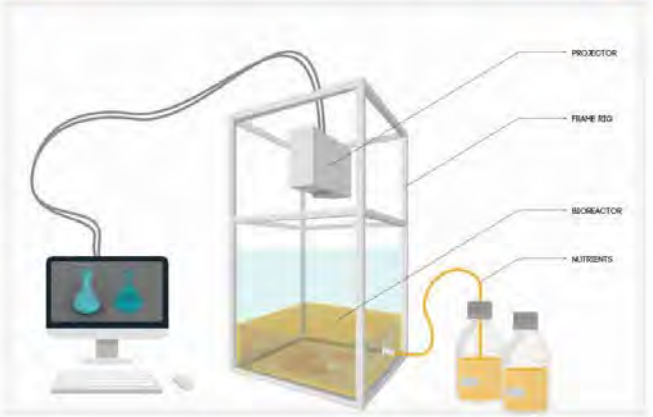

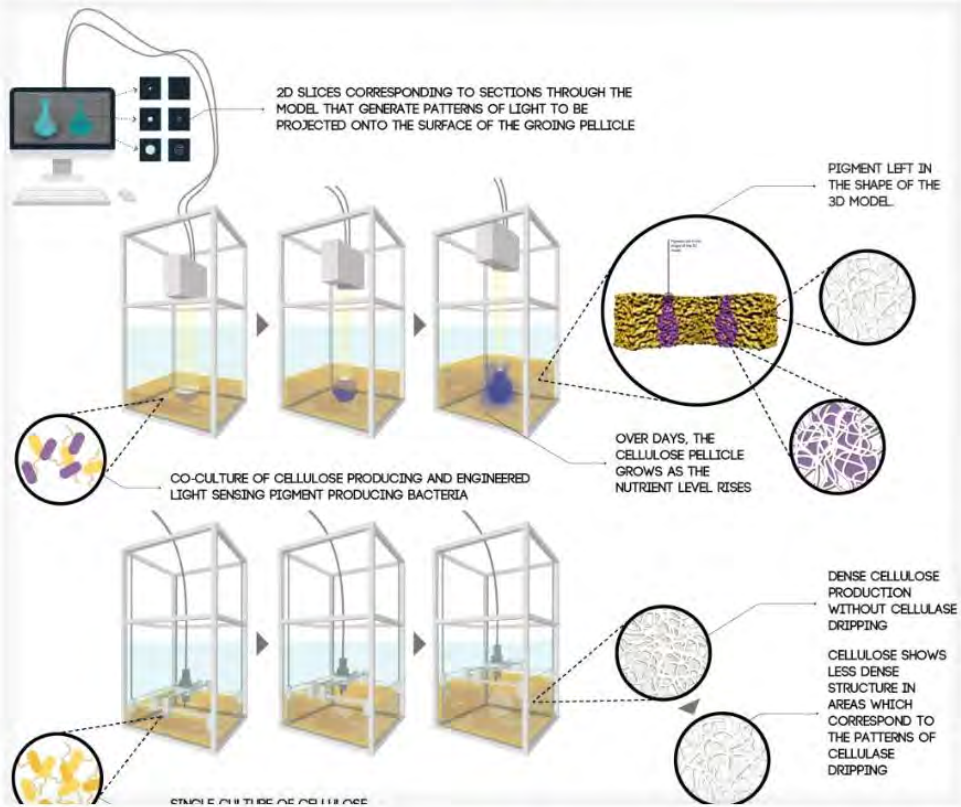

HBBE (Hub for Biotechnology in the Built Environnement) _ Living Manufacture

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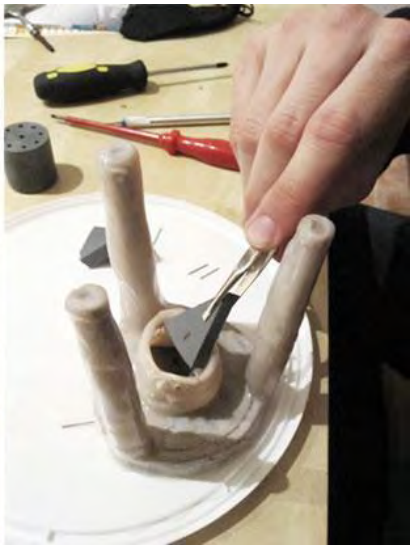

Jannis huelsen _ Xylinum Stool

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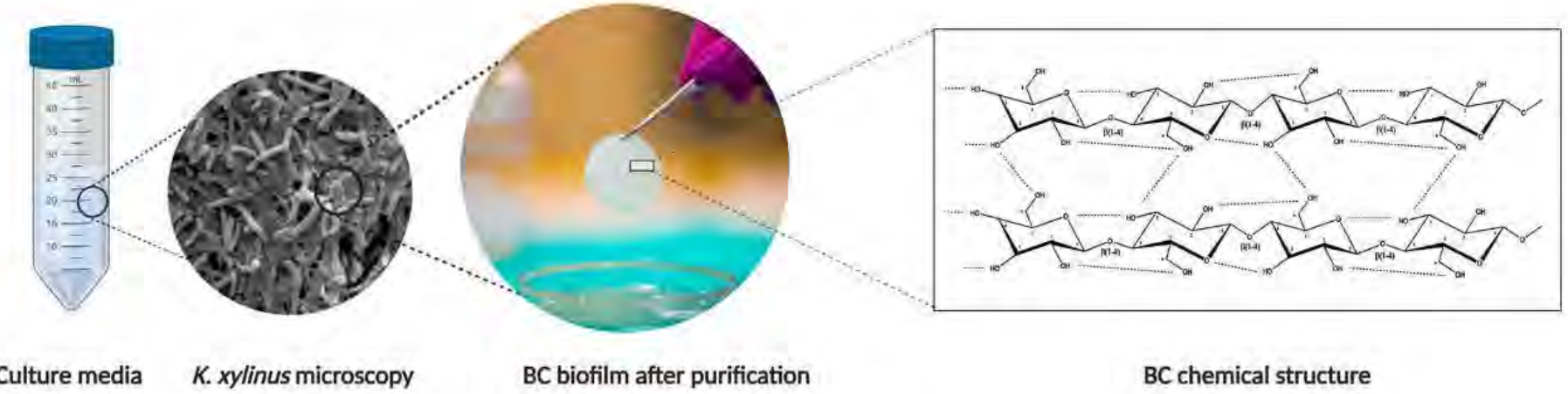

SCOBY process

A review of culture media for bacterial cellulose production: complex, chemically defined and minimal media modulations.https://doi.org/10.1007/s10570-021-03754-5

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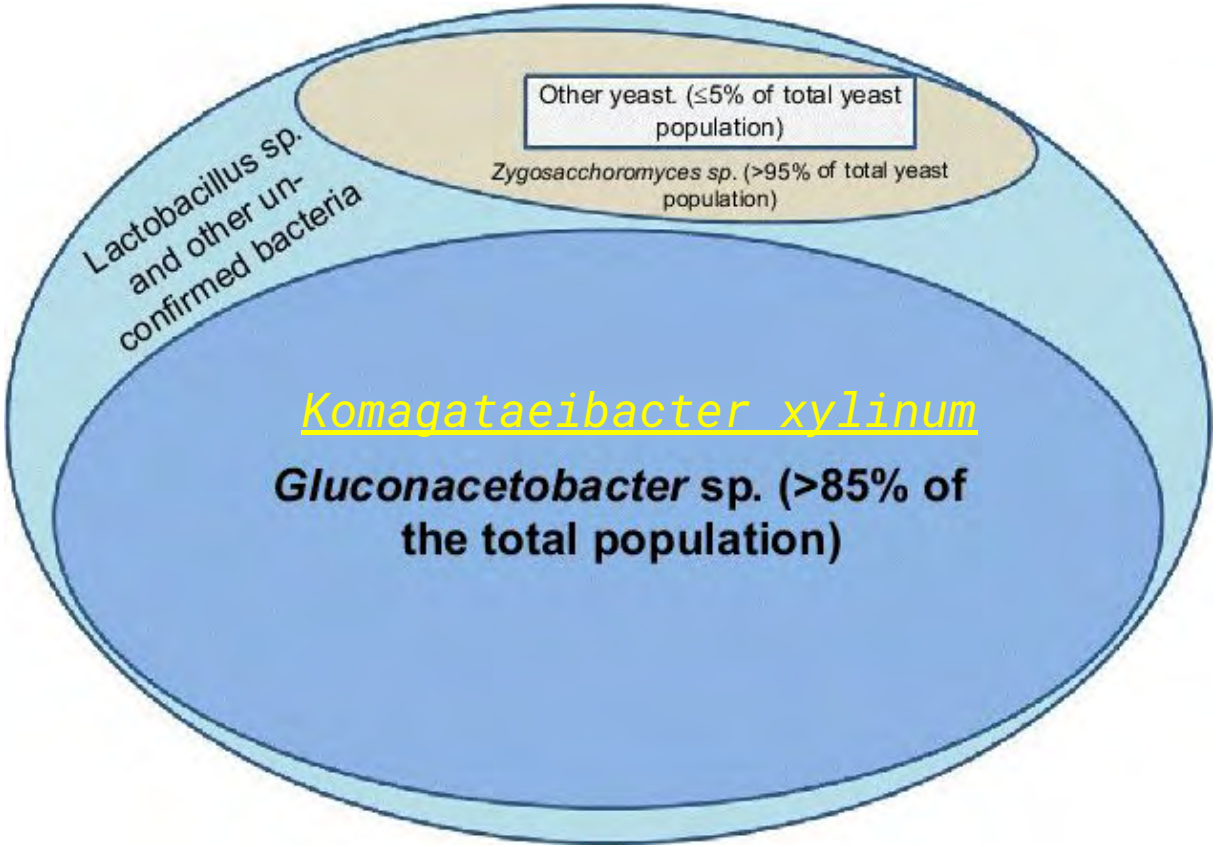

8 - Kombucha Drink: Production, Quality, and Safety Aspects. https://doi.org/10.1016/B978-0-12-815260-7.00008-0

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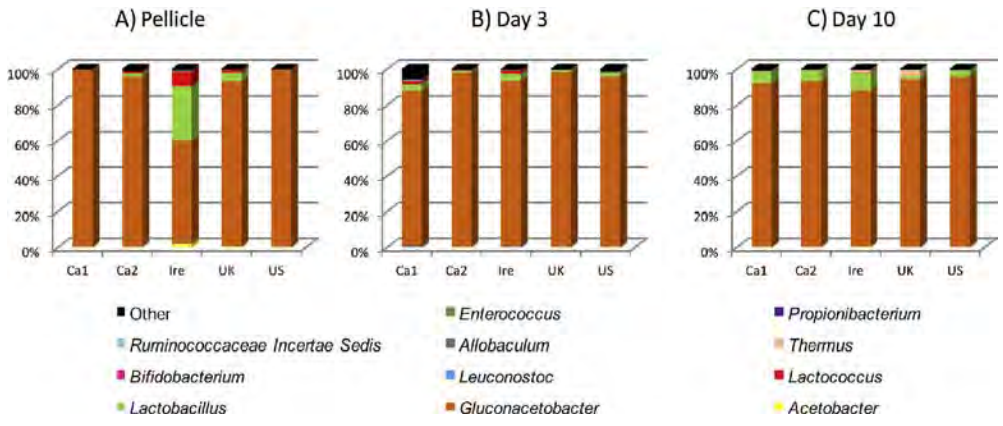

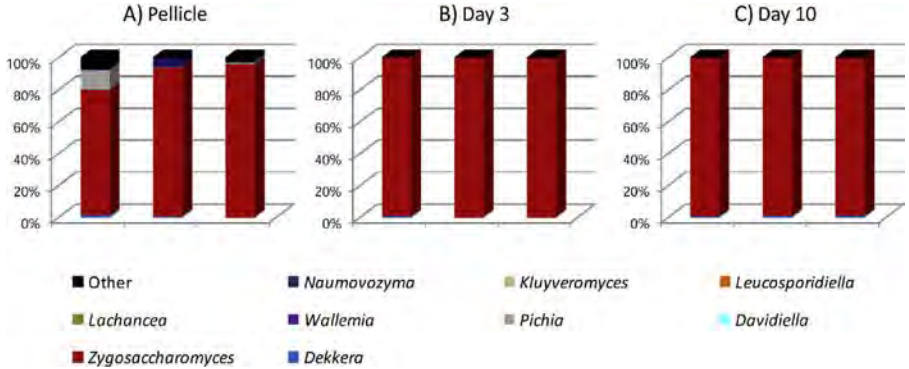

Table 1
Relative abundances of the 16S bacterial genera at day 3, day 10 and in the pellicle.

	Ca1	Ca2	Ire	UK	US
Day 3					
Acetobacter	0.86	0	0.43	0	0
Gluconacetobacter	86.91	97.79	93.09	98.14	95.73
Lactobacillus	3.93	1.13	3.57	1.19	1.77
Lactococcus	1.56	0.51	1.77	0.37	0
Leuconostoc	0.52	0	0	0	0
Bifidobacterium	0.3	0	0	0	0
Thermus	0.22	0	0.2	0	0
Allobaculum	0	0	0	0	0.88
Ruminococcaceae Incertae Sedis	0	0	0	0	0.19
Propionibacterium	0	0	0.2	0	0
Other	5.75	0.58	0.75	0.3	1.42
Day 10					
Acetobacter	0	0	0.19	0	0
Gluconacetobacter	92.17	93.16	87.62	94.26	95.73
Lactobacillus	5.96	6.17	9.59	1.44	3.47
Lactococcus	0	0.23	0.19	0	0.18
Leuconostoc	0	0	0	0	0
Bifidobacterium	0	0	0	0	0
Thermus	0.66	0	1.62	3.73	0
Allobaculum	0	0	0	0	0
Ruminococcaceae Incertae Sedis	0	0	0	0	0
Other	1.22	0.45	0.79	0.58	0.61
Pellicle					
Acetobacter	0	0	1.93	0.28	0
Gluconacetobacter	99.77	95.73	58.02	92.97	99.82
Lactobacillus	0	1.72	30.57	4.64	0
Lactococcus	0	1.29	7.76	1.62	0
Leuconostoc	0	0	0	0	0
Bifidobacterium	0	0	0	0	0
Thermus	0	0	0	0	0
Allobaculum	0	0	0	0	0
Ruminococcaceae Incertae Sedis	0	0	0	0	0
Enterococcus	0	0	0.69	0.21	0
Propionibacterium	0	0	0.38	0	0
Other	0.23	1.26	0.66	0.28	0.18

Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. https://doi.org/10.1016/j.fm.2013.09.003

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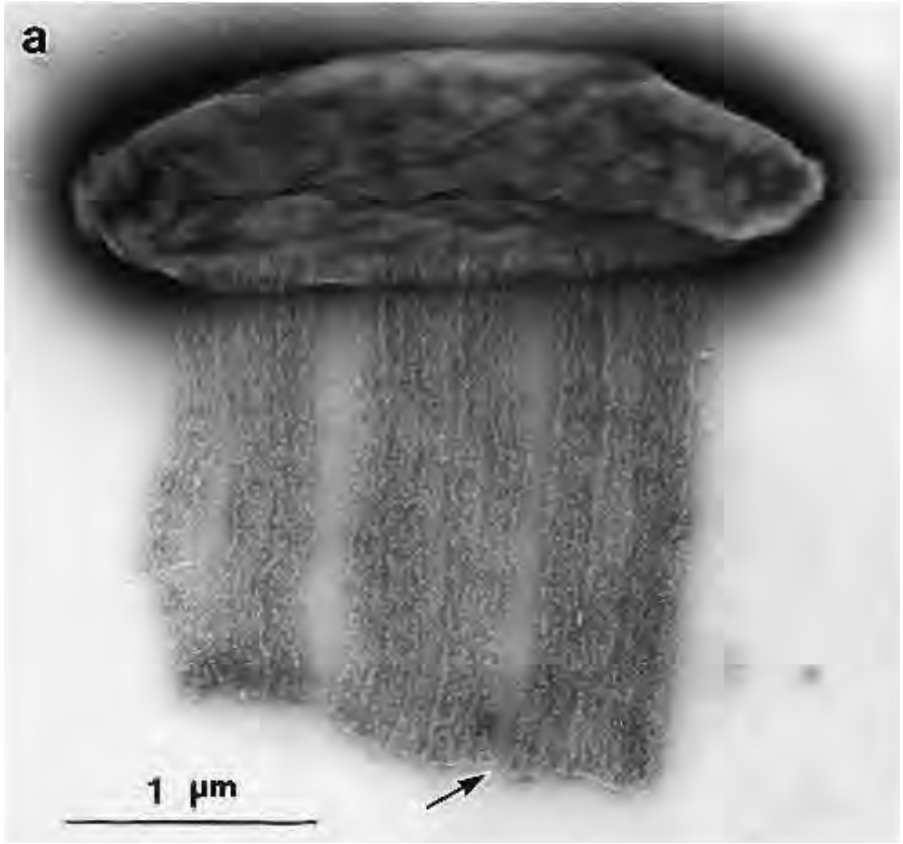


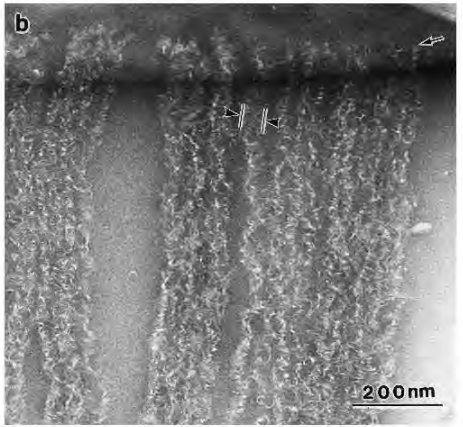
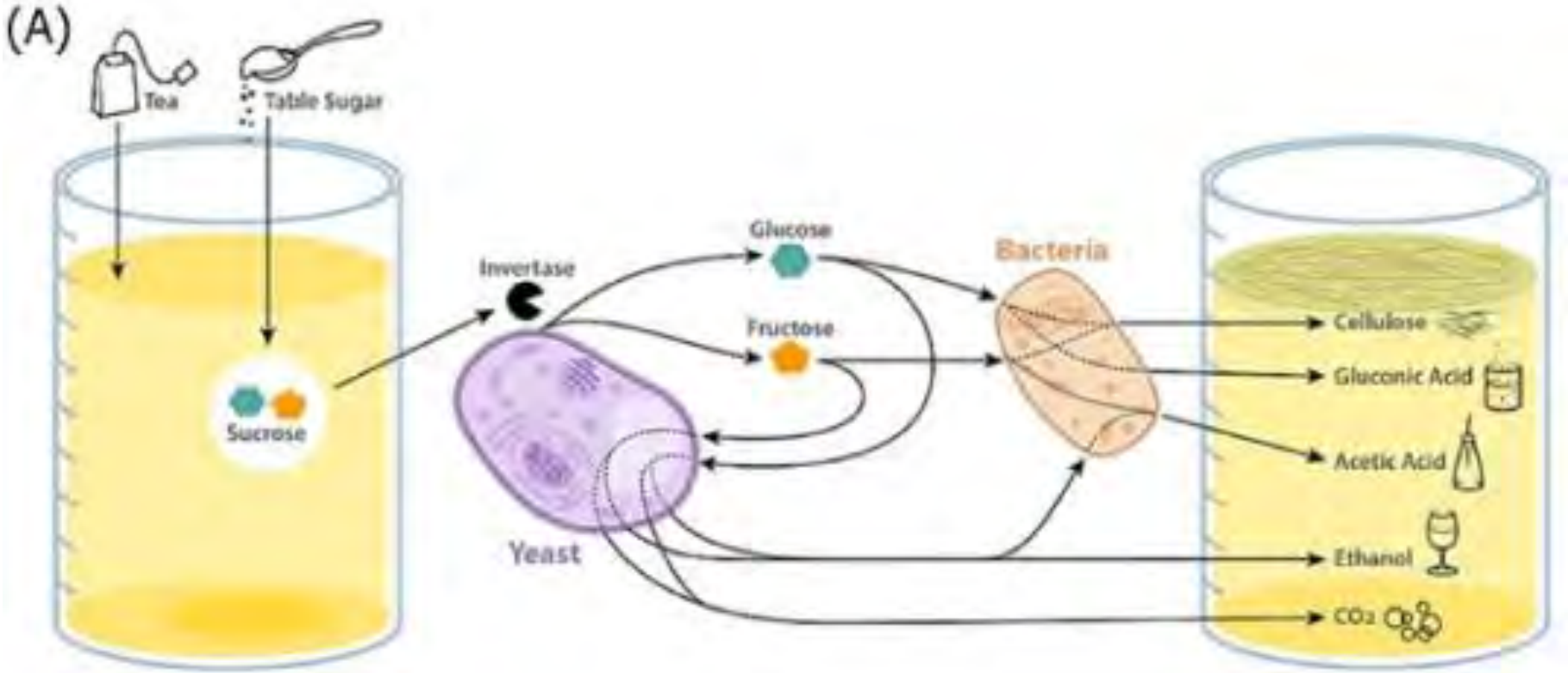


Figure 1. (a) Negatively stained coarse band-like cellulose assembly produced during 3 h of incubation at 4 °C. The arrow indicates that all strands are connected to a bundle of cellulose chains. (b) Magnified image of a part of the coarse band-like assembly in (a). The width of the strand between the arrowheads is 48 nm. The arrow indicates one strand 14 nm in width.

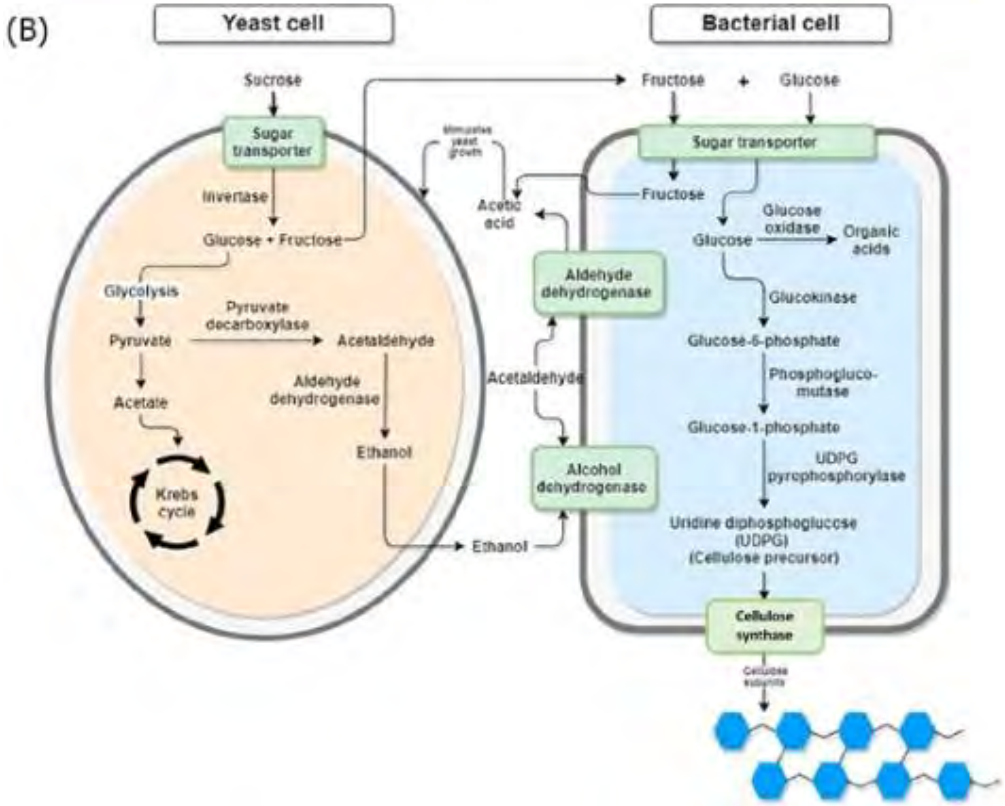
Figure 2 shows a coarse band-like assembly produced at 4 °C for a longer incubation time of 6 h. This is the longest coarse band-like assembly observed in our experiments. About 40 strands are extruded from the bacterial cell. The detached dense band-like assembly in which strands are closely packed is also observed at the bottom of this figure, as indicated by the arrowhead. Here, the coarse band-like assembly and the dense assembly are not connected.

The selected-area ED pattern of the coarse band-like assembly does not give any crystalline reflections (Figure 3a). The morphology corresponding to Figure 3a was obtained under strong-defocus conditions (Figure 3b). Therefore, the image of Figure 3b was made due to the so-called phase contrast.

TEM study of band-like cellulose assemblies produced by Acetobacter xylinum at 4 °C. https://link.springer.com/article/10.1023/A:1020195205030



Production process and characteristics of kombucha fermented from alternative raw materials. https://doi.org/10.1016/j.fbio.2022.101841



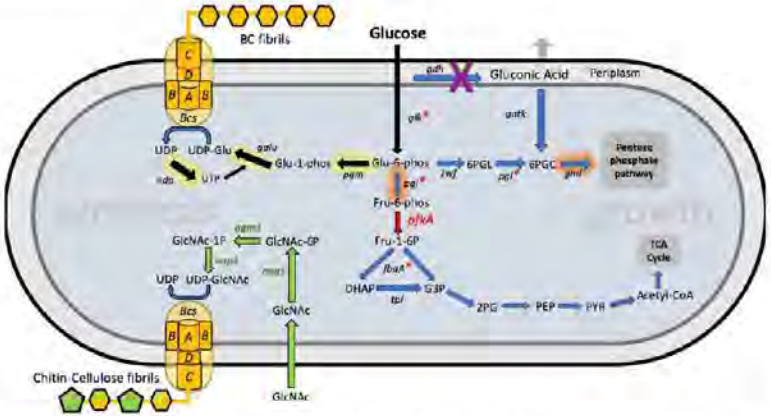


Figure 1. The metabolic pathway to bacterial cellulose biosynthesis in *Komagataeibacter* and example metabolic engineering interventions made in key papers. Native pathways from glucose to central carbon metabolism (*growth*) are shown as blue arrows. The native pathway to cellulose biosynthesis

Production process and characteristics of kombucha fermented from alternative raw materials. https://doi.org/10.1016/j.fbio.2022.101841

Engineering Bacterial Cellulose by Synthetic Biology. Int. J. Mol. Sci. 2020, 21, 9185; doi:10.3390/ijms21239185.

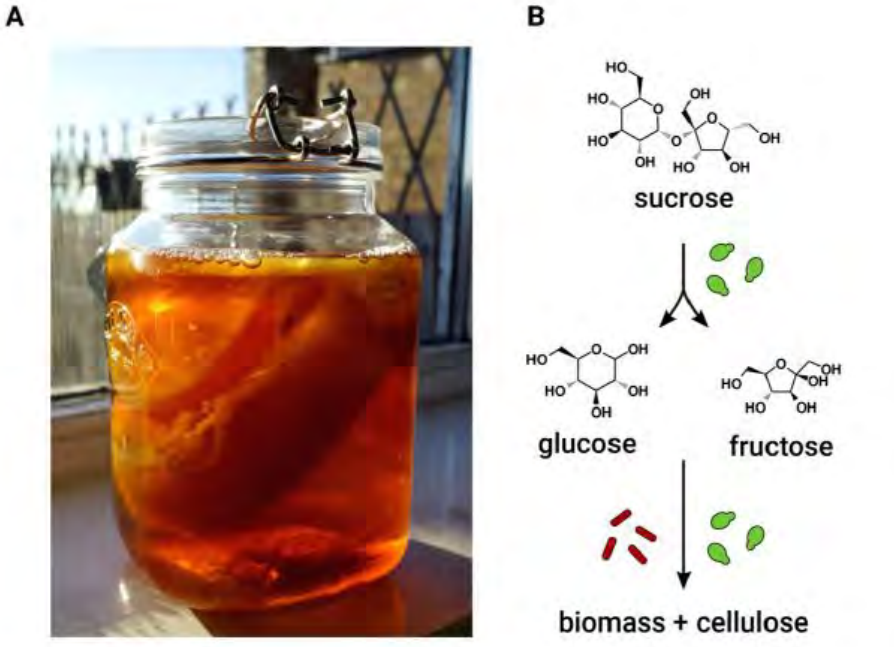
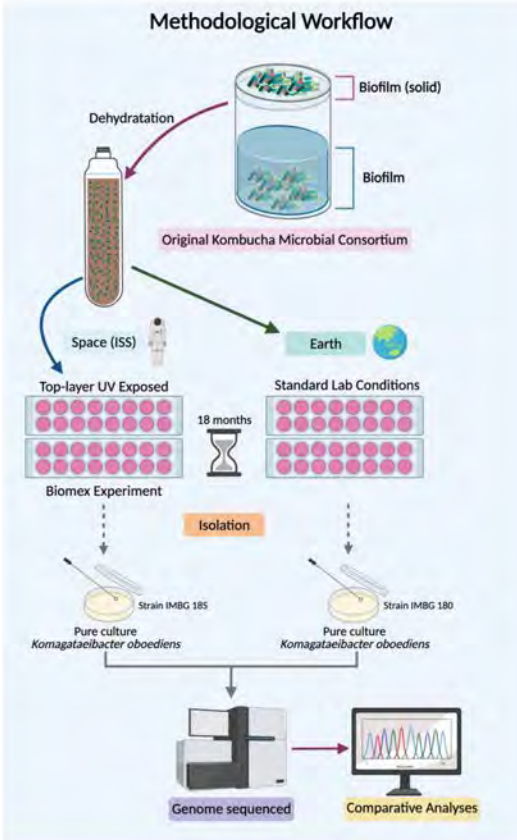


Figure 7 Kombucha tea fermentation. A Image of homebrewed kombucha tea. Newly-formed layers of BC are visible at the surface of the liquid while BC mats produced in previous fermentations are submerged. **B** Proposed metabolic interaction between yeast (green) and bacteria (red) in kombucha fermentations. Yeast convert the carbon source sucrose to glucose and fructose. Both yeast and bacteria then consume glucose and fructose to accumulate biomass and BC.



Synthetic biology approaches for engineered living materials. Charlie Gilbert,PHD thesis, 2018.

The Space-Exposed Kombucha Microbial Community Member Komagataeibacter oboediens Showed Only Minor Changes in Its Genome After Reactivation on Earth . https://doi.org/10.3389/fmicb.2022.782175

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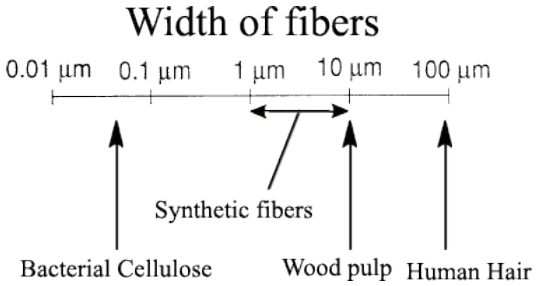
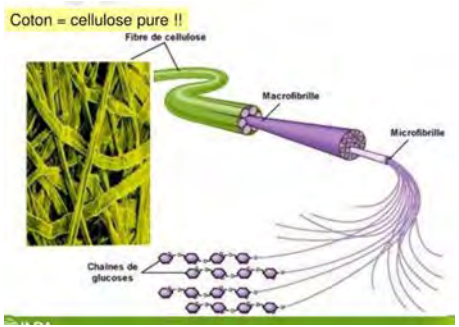
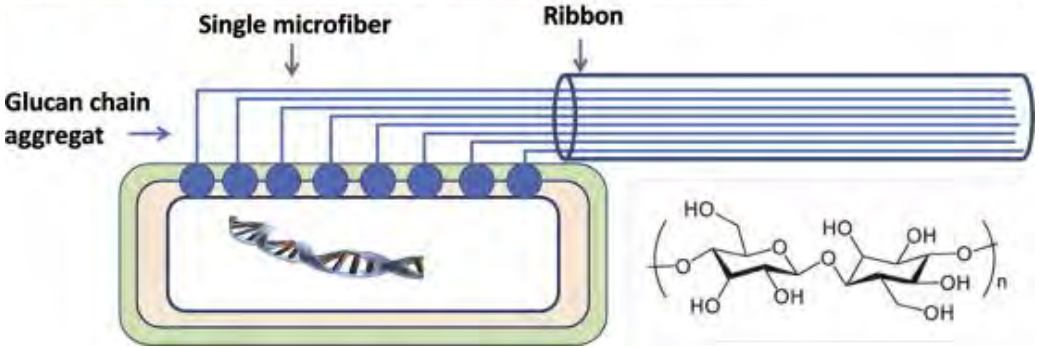


Bacterial cellulose fibers:
2-4 nm thickness, 70-130 nm





Cotton fibers :
2 - 7 µm thickness, (medium) 2.54 cm to 2.86 cm

An Overview of Biocellulose Production Using Acetobacter xylinum Culture. DOI: 10.5829/idosi.abr.2014.8.6.1215

Utilization of bacterial cellulose in food. https://doi.org/10.1016/j.foodhyd.2013.07.012

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	Tensile strength	Young's modulus	Density	Elongation at break
Bacterial Cellulose	200 - 300 MPa	15 - 35 GPa	1.25 g/cm^3	1.5 - 2%
Cow leather	8 - 35 MPa	0,1 - 0,5 GPa	0.4 - 0.9 g/cm^3	~ 40%
Cotton	2.7 - 4.41 MPa	3.5 GPa	1.54 g/cm^3	3 - 9,5%
Nylon	82.7 MPa	2.93 GPa	1.15 g/cm^3	50%
DuPont™ Kevlar® 49 Aramid Fiber	3 000 MPa	112 GPa	1.44 g/cm^3	2.4%

Based on Bacterial Cellulose - Properties and Its Potential Application. DOI:10.17576/jsm-2021-5002-20.

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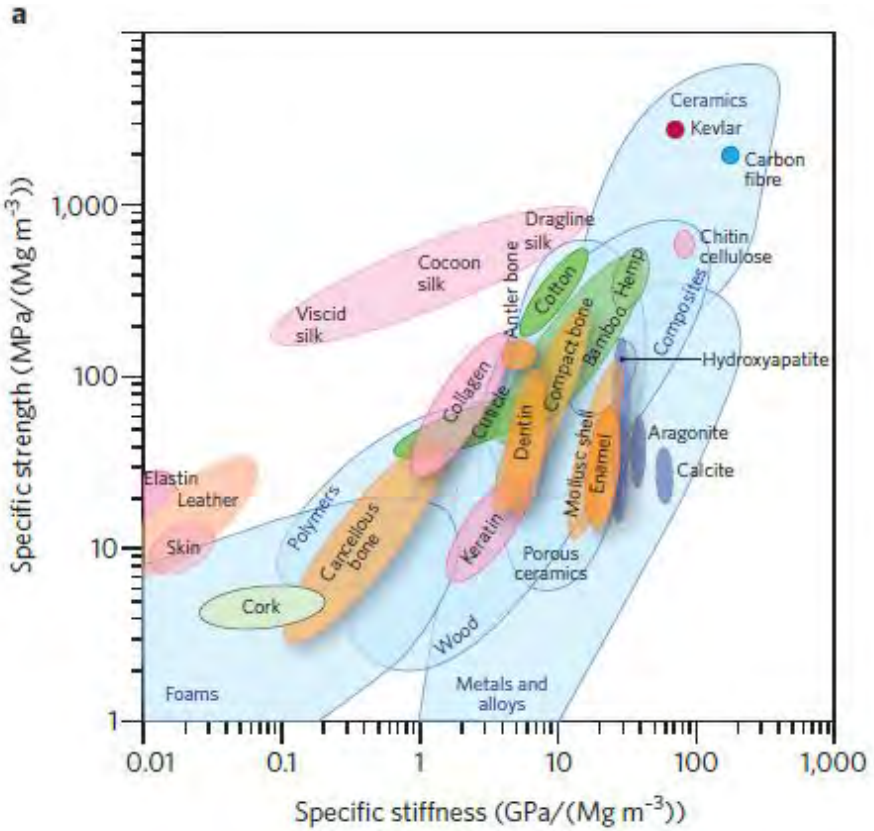
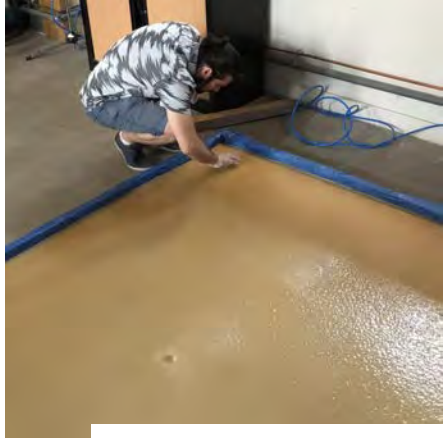
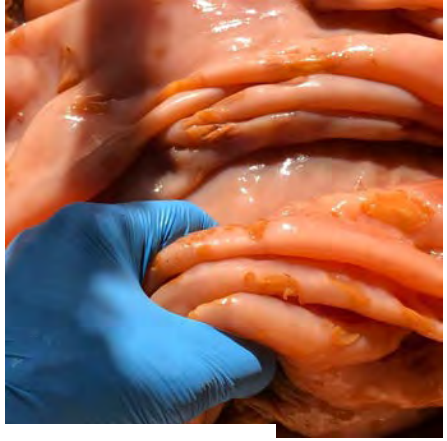


Ashby plots for natural and synthetic materials: specific properties normalized by density. Reproduced from *Nature Materials*, Ref. 2, Springer Nature.




METHODES

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Growing (1/3)

1. Clean and sterilize the tools and containers ou are going to use (either with rubbing alcohol and rinsing afterwards or with boiling water),

and clean your hands before starting the preparation. Glass containers are best, as they are easier to clean and promote thicker film growth.

2. Brew black tea (3g/L), until water cools to room temperature.

3. Add sugars to the tea (10% of the total mass). Use a 50/50 mixture of brown and white sugar. Alternatively, commercially available syrups containing glucose and fructose can be used (10% of the total mass) - and they give the material a very good smell.

4. Add white vinegar (10% of the total mass).

5. Inoculate the solution with a strain of kombucha and cover it tightly with a clean cloth.

6. The film can be harvested after 20 to 25 days of cultivation.

NB: Vinegar can be replaced by the fermented solution from a previous batch.

NB2: CO_2 is produced by the yeasts during the fermentation/growth of the film. Gas bubbles can form under the film, creating a local separation of the solution. In order to eliminate the gas pockets, the film can be carefully massaged with clean hands.

NB3: for a drink recipe, replace the white vinegar by cider vinegar (or other) at a rate of 5% of the total mass. The first fermentation will last 4 to 7 days, and the second 30 days (remember to cap the container when starting the 2F; dried fruits can be added to bring fructose - and flavor - to the drink).

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Kombucha Recipe Variations

300 mL ACV

4gr tea (3 tea bags)

3 L water

300 gr sugar

1 mother
+ 100 mL
starter liquid
with mother

Kombucha Recipe Variations

Ingredients to produce your fabric from a Scoby

 Preparation for	 Water	 Tea	 Sugar	 Cider vinegar	 Starter*	 Scoby
~ 265 cl	200 cl	3 gr ≈ 2 teabags	200 gr	20 cl	45 cl	1 Scoby
~ 1055 cl	800 cl	12 gr ≈ 8 teabags	800 gr	80 cl	175 cl	1 Scoby
~ 1320 cl	1 000 cl	15 gr ≈ 10 teabags	1 000 gr	100 cl	220 cl	1 Scoby
liquid of culture	You have a smaller or a bigger container? Adapt the recipe, it's proportional!					

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Kombucha Recipe Variations

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Drying (2/3)

Using disposable gloves, carefully collect the grown film and place it in a bucket of tap water for two hours to dissolve any remaining sugars.

Do not discard the fermented solution, as it can be used again to grow a second film or to replace the white vinegar in a new batch.Place the wet, clean film on a plastic surface with a slight roughness to prevent the film from sticking to it.

Matte plastic book cover adhesives provide a suitable surface. Parchment paper is a good alternative.

The film should be lightly massaged on the surface to get maximum contact area.

Turn the film over every day to allow it to dry evenly and prevent it from sticking to the surface.

NB: a 2 mm thick film should dry in about two weeks to keep its flexibility and not be brittle.

NB2: a kombucha film shrinks essentially in thickness, not in surface (thanks to the contact with the drying surface).

A significant shrinkage can be expected as the films can take up to 99% of their dry weight in water.

NB3: both at the growing and drying stages, the room must be arranged and designed to limit the proliferation of fruit flies.

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Treating (3/3)

Using disposable gloves, carefully collect the grown film and place it in a bucket of tap water for two hours to dissolve any remaining sugars.

Do not discard the fermented solution, as it can be used again to grow a second film

or to replace the white vinegar in a new batch.

Place the wet, clean film on a plastic surface with a slight roughness to prevent the film from sticking to it.

Matte plastic book cover adhesives provide a suitable surface. Parchment paper is a good alternative.

The film should be lightly massaged on the surface to get maximum contact area.

Turn the film over every day to allow it to dry evenly and prevent it from sticking to the surface.

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A significant shrinkage can be expected as the films can take up to 99% of their dry weight in water.

NB3: both at the growing and drying stages, the room must be arranged and designed to limit the proliferation of fruit flies.

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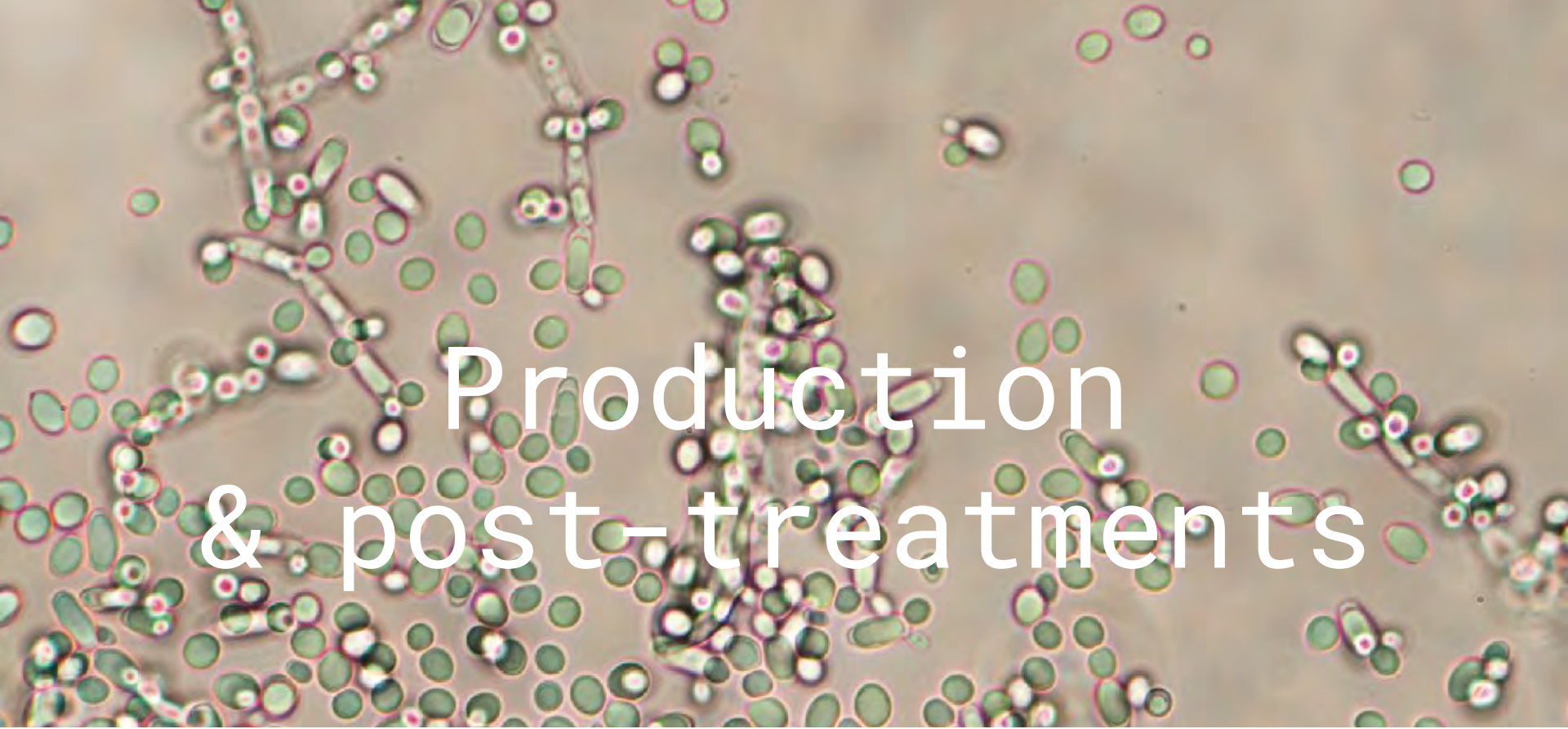
Keep it clean!



Production & post-treatments

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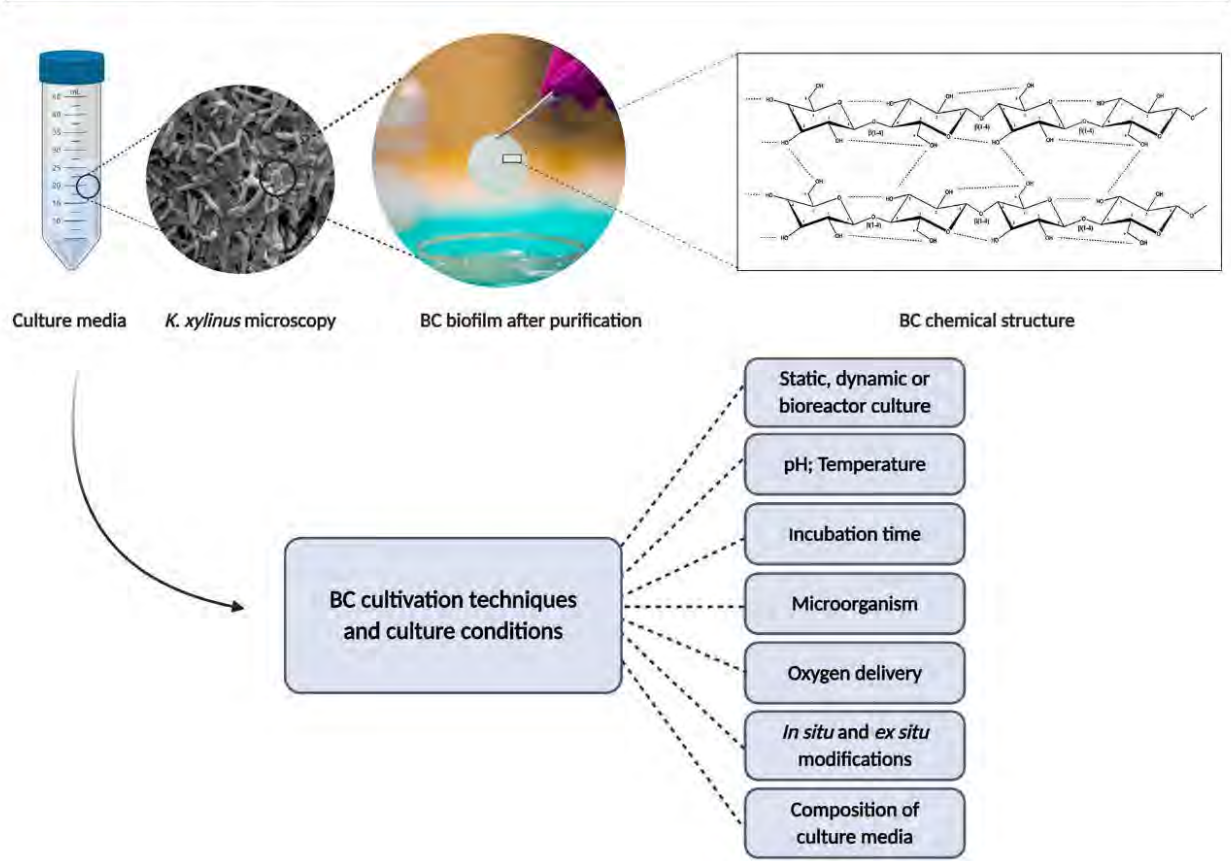

Fig. 1 Role of cultivation techniques and culture conditions for the BC production

recipe to treat a kombucha pellicle.

thr34d5

Prior to any operation, we recommend you to get a **mask** to protect you from turpentine vapors, and a pair of **gloves**.
Kombucha pellicle is basically composed of a cellulose matrice, such as wood but without lignin and pectin, and with a microstructure that is a hundred times smaller. Therefore, this treatment is originally a wood one.

Prepare a bain-marie to slowly heat up **one volume of bees wax**.

Prepare a first mix with **one volume of turpentine** and **one volume of linen oil**.

Wait for the bees wax to be well liquified, and mix it with the first mix.
If you want to keep the solution homogeneous, we recommend you gently heat up the first mix over the bain-marie steam (not that close to the source) before pouring the bees wax. This trick limits wax crystallization when poured in a colder liquid.
Steer vigorously the final mix.

Gently apply the mix with a soft brush on the kombucha pellicle, on both sides.
You can gently massage the pellicle by hand to ensure a good treatment penetration in the matrice. Operating over steam enhances the absorption.
Leave it to rest for some days, depending on the thickness of the pellicle, so that the treatment migrates to the very core of the pellicle.

It might be hard to reuse the mix as-is once cooled down as the turpentine evaporates, you might want to add some. Get in touch with us to get more tricks and develop further the methodology!

hello@thr34d5.org

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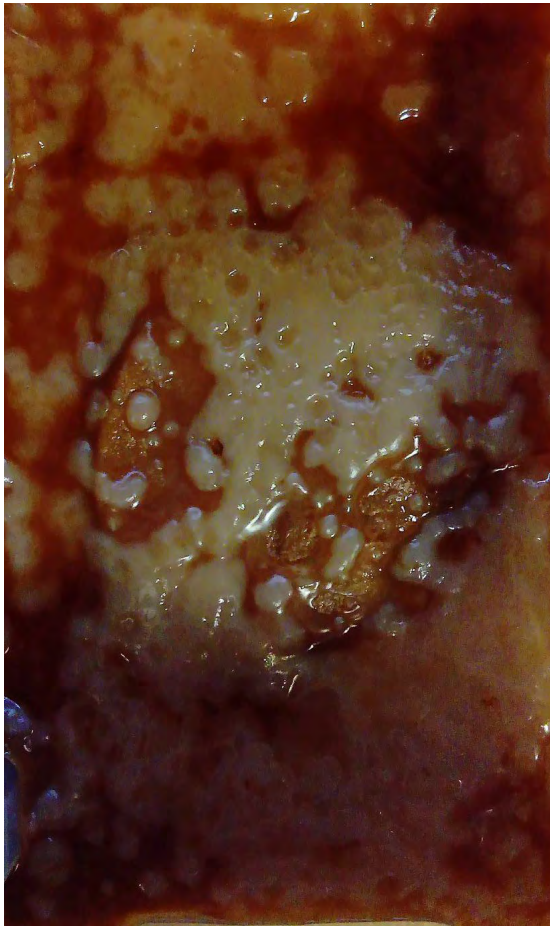

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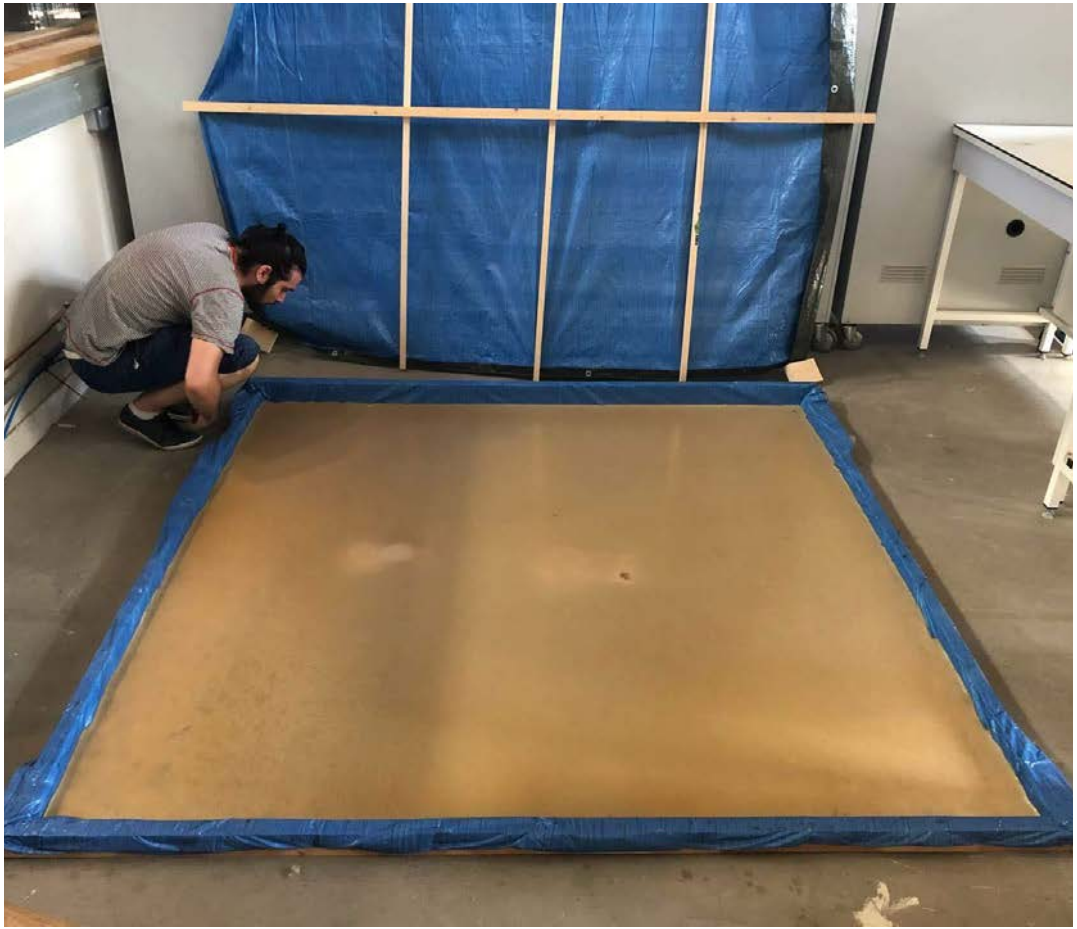

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