Uppbyggingasjóður
Norðurlands Vestra

Fjólublár

Textílmiðstöð Íslands x BioPol
2023

Researchers & Collaborators

Icelandic Textile Center

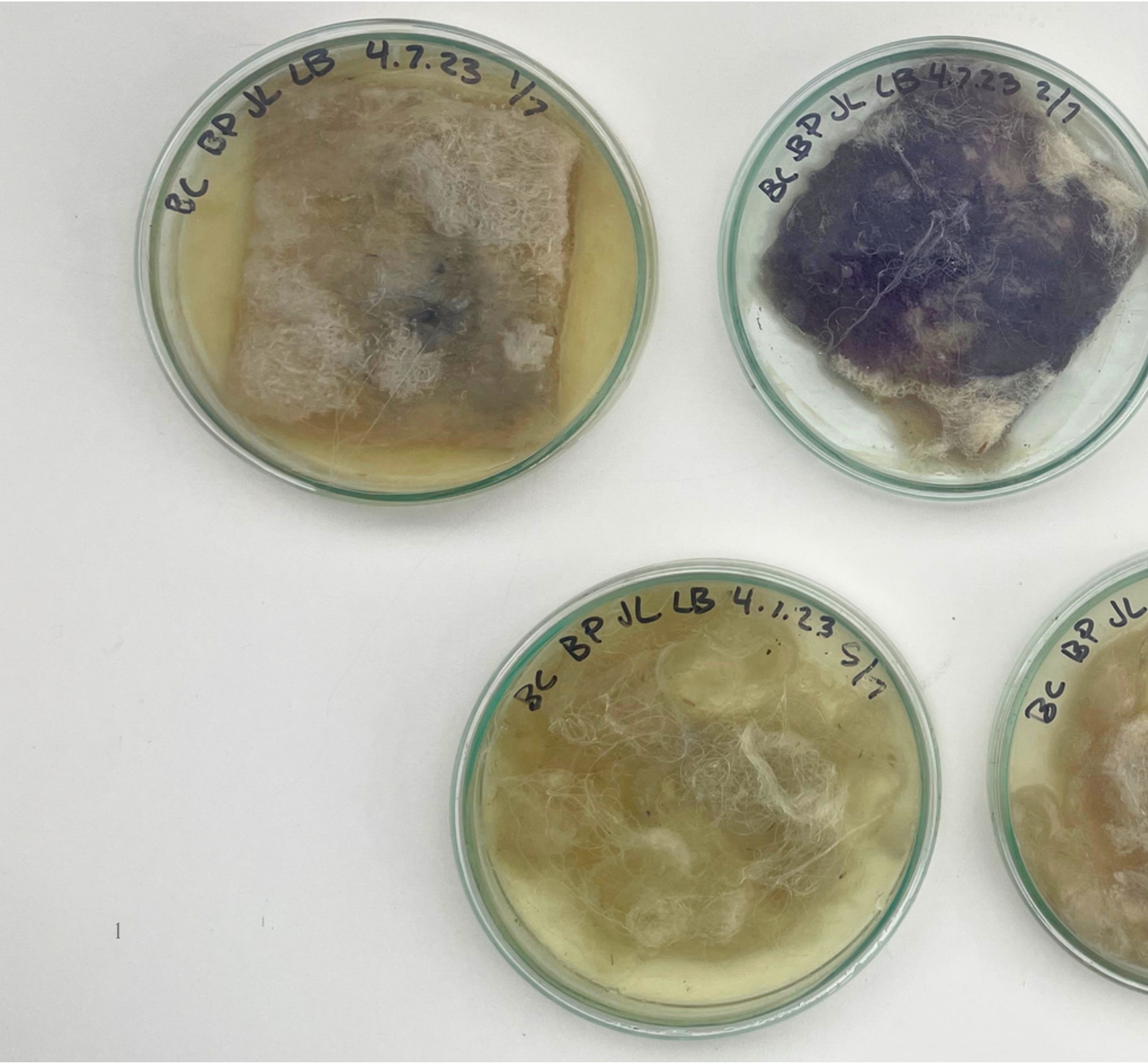
- Margrét Katrín Guttormsdóttir
- Alice Sowa

BioPol

- Jens Jakob Sigurðarson
- Franziska Hörber

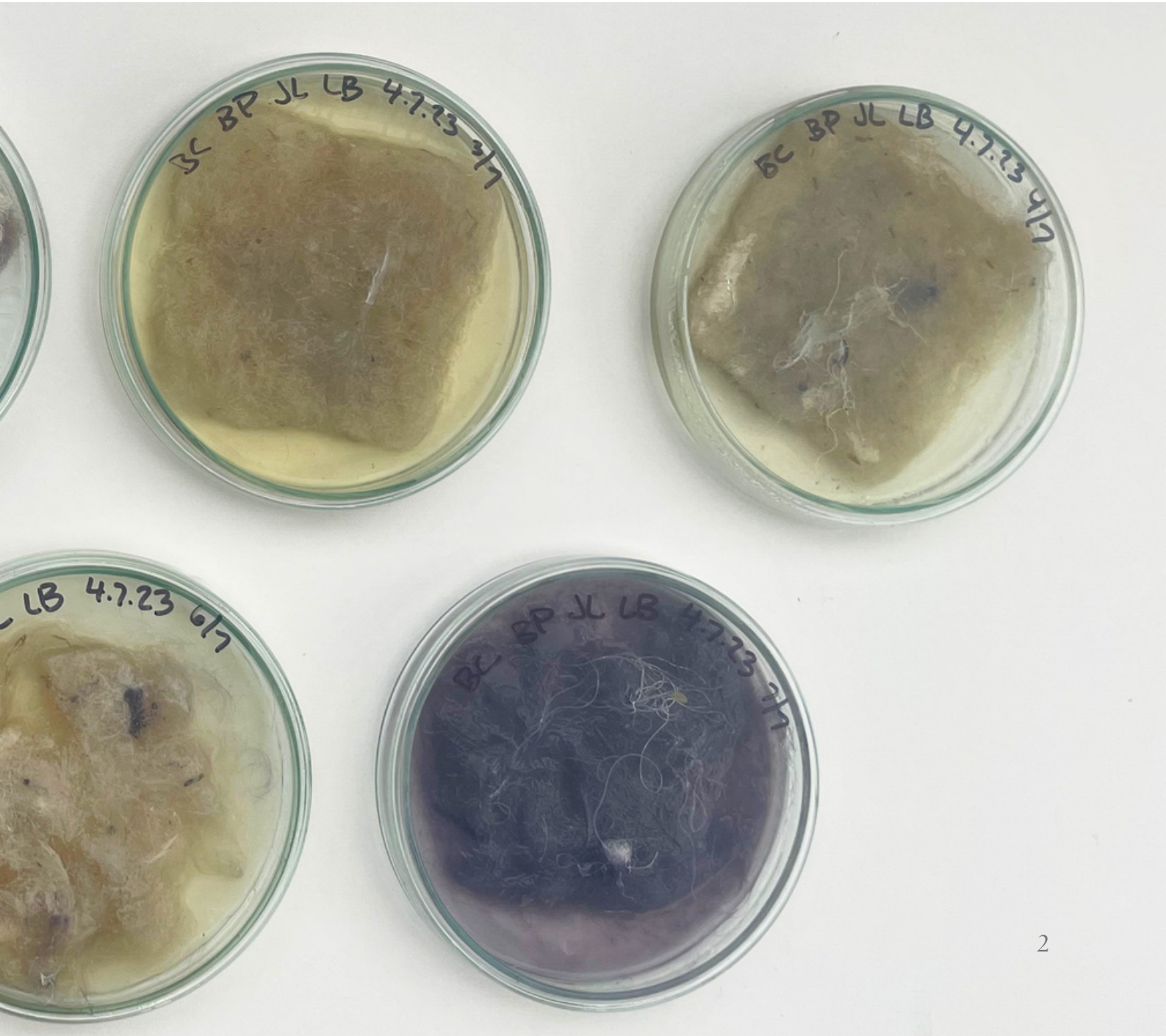
Ístex

- Material and industry insight



Abstract

Fjólublár/Living Purple is a collaborative on going research project between the Icelandic Textile Center and Marine Biotechnology Center BioPol on creating a sustainable dyeing process for Icelandic Wool with the purple dye produced by the bacteria *Janthinobacterium lividum*.



Project Timeline

Over the course of 4 months BioPol, a marine biotechnology research company and the Icelandic Textile center collaborated in developing a sustainable dyeing process of *J. lividum*. BioPol's focus was to research different growth media to optimize the cultivation of *J. lividum*. And the Icelandic Textile Center's focus was to research different dyeing methods on Icelandic wool from the extracted pigment of the bacteria.

Project Start

April - May

BioPol Research

- BioPol visit to ITC to collect *J. lividum* samples
- BioPol begins growth medium tests

June

ITC x BioPol Research

- 1st bioreactor test of cultivating *J. lividum*
- ITC visit to BioPol to collect *J. lividum* samples for wet lab cultivation
- ITC begins wet lab cultivation of *J. lividum*
- ITC receive loose wool from Ístex Wool Washery
- ITC begins 1st round of dye tests on Icelandic Wool
- ITC visit to Ístex spinning factory and Uppspuni mini-mill
- ITC & BioPol meeting on dye research findings
- ITC visit to BioPol for 2nd bioreactor test of cultivating *J. lividum*

July

ITC x BioPol Research

- ITC receive a variety of Icelandic wool yarns from Ístex
- ITC begins plant x bacteria tests on Icelandic Wool
- ITC makes sample cards and archive of dye tests

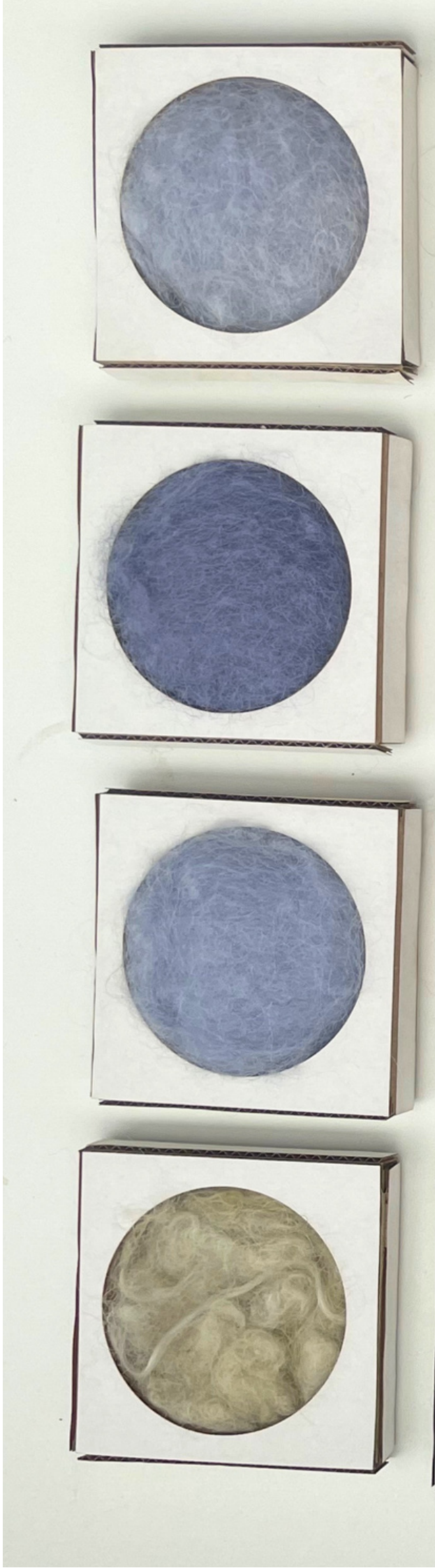
August

ITC x BioPol Research Wrap Up

- ITC & BioPol prepare booklet
- Final project meeting finalize report for SSNV

Project Finish

Contents



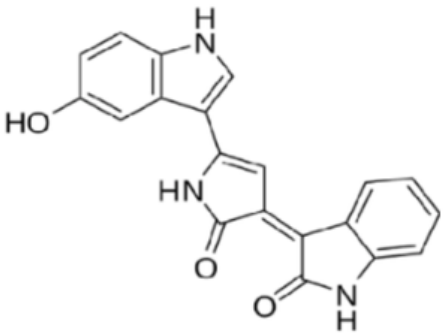
Why bacteria dyeing?

It is imperative for the textile industry to change the ways in which textiles are dyed in order to reduce waste and the need of petroleum based chemical dyes. Traditionally, textile dyeing uses a lot of water, from the scouring (cleaning the textiles), mordanting (pigment fastener) and dyeing steps. Textile companies and dye houses dispose of their toxic wastewater from dyeing into rivers and lakes. This has a detrimental effect on wildlife and causes eutrophication (over fertilizing) meaning not enough oxygen is in the water for wildlife. The textile industry is the second largest polluter of freshwater worldwide. Bacteria dyeing offers a unique and environmentally friendly alternative to petroleum based dyes. Minimal water usage, no harmful chemicals, and low energy consumption make bacteria dyeing a desirable alternative for the textile industry.

Bacteria dyeing is a beautiful and unique method of dyeing with a process centered around collaborating with living microorganisms. There are a variety of microorganisms found in soils and water ways that produce different types of pigmented compounds that can be used for dyeing textiles. The research of Fjólublár focuses on the bacteria *Janthinobacterium lividum* and its purple compound violacein. Iceland has a long history of naturally dyeing fiber, but purple is not a color naturally found in the Icelandic color scheme, which makes working with *J. lividum* unique. Being a level 1 bio safety bacteria mean it is non-pathogenic and completely safe to work with in a laboratory and wet lab work space. Additionally, violacein, the purple pigment, has shown a wide range of bioactive properties such as; anti-bacterial, anti-viral, anti-fungal, and anti-tumoural.

The research of Fjólublár aims to optimize the purple dye production and dyeing process with *J. lividum* and focus on using waste materials as nutrients for the bacteria to develop a sustainable dyeing alternative for wool in Iceland.

Janthinobacterium lividum



Bio safety level 1

compound : Violacein

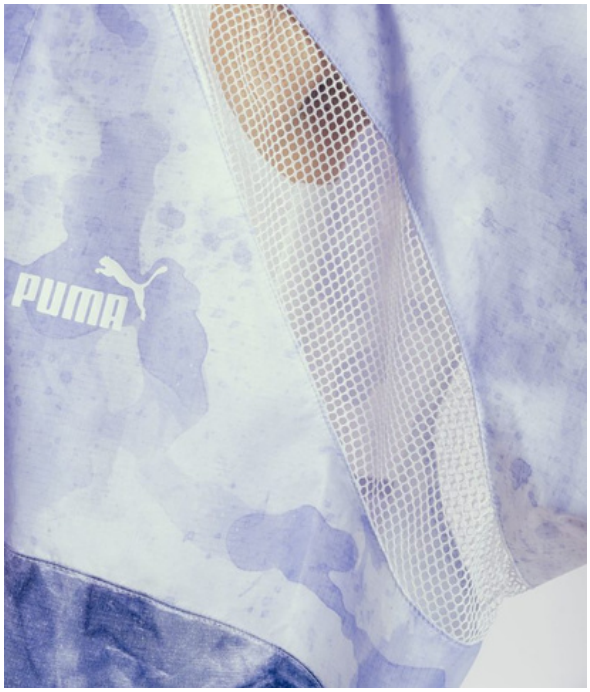
Color : Purple

State of the Art

Project Coelicolor - Faber Futures
An exploration of textile dyeing with the soil dwelling bacteria *Streptomyces coelicolor.*

BioShades - Cecilia Raspanti
Research and knowledge sharing of sustainable dye practices with bacteria

Cymatics Research - Living Colour
The exploration of growing bacteria in patterns by exposing them to sound frequencies



Design to Fade - Living Colour x PUMA
The first bacterially dyed sportsware collection

BInc - Living Colour x Vienna Textile Lab

A collaborative research to create biogenic inks for textile printing

Pili

Re-engineering microbial enzymes to produce dyes from renewable resources

Colorifix

Using DNA sequencing to engineer microorganism to produce pigments found in nature for industrial scaled textile dyeing

Moving Pigment - Charlotte Werth

Co-designing textile patterns with pigment-producing bacteria in a scaled up and automated process

Cultivating *J. lividum*

BioPol

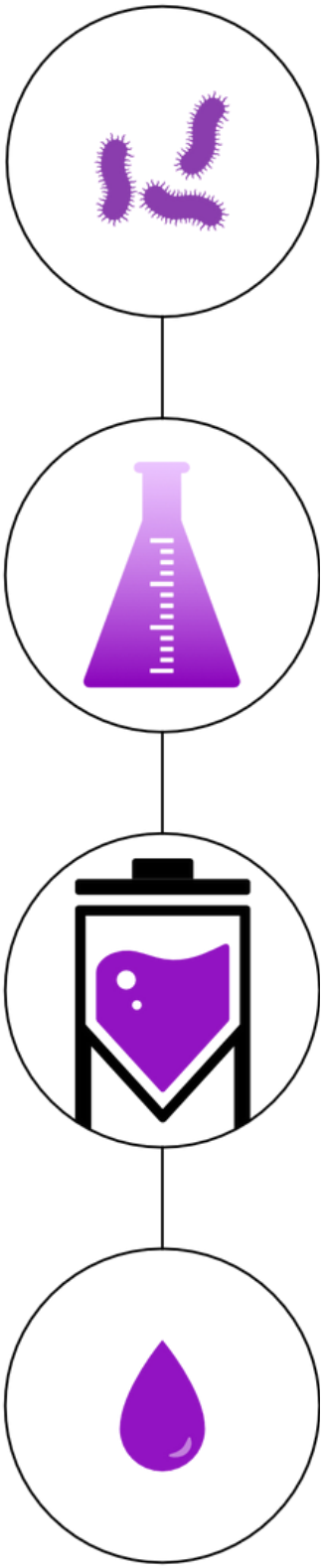
- Research Overview
- Optimizing Growth
- Bioreactor

ITC

- Wet Lab Cultivation

Research Overview

At BioPol, our process of optimising the production of the pigment violacein from *J. lividum* consisted of four main steps:



1. Strain Selection

There are different bacteria that are able to produce violacein, and it is therefore important to select the most suitable strain. In our case we chose the strain partly based on ITC already working with it. However, *J. lividum* also has the benefit of being able to produce high amounts of violacein while at the same time being non-pathogenic and thus safe to work with.

2. Small Scale Optimization

The majority of our experiments to study different growth conditions were done in small scale. This simply allowed us to test many more factors within a reasonable amount of time than what would be possible at larger scale experiments. Our small scale experiments consisted of cultivating the bacteria in Erlenmeyer flasks and comparing different growth media compositions.

3. Upscaling

Based on our results from the small scale experiments, we chose a growth medium composition to test in our benchtop bioreactor. This allowed us to control and monitor a wide set of parameters, such as the pH, available oxygen and temperature. Furthermore, the step helps us to identify any potential problems that could occur during upscaling for production.

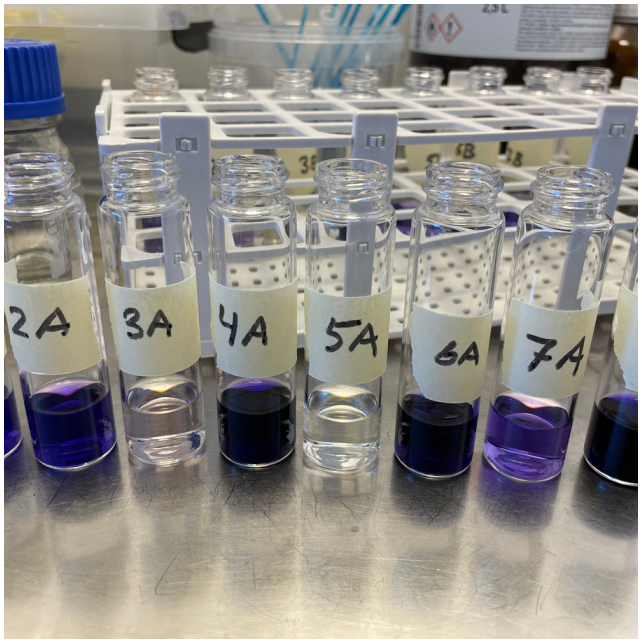
4. Pigment Extraction

After the pigment violacein has been produced by the bacteria, our next step is to retrieve it through extraction. The pigment is not soluble in water, but can instead be extracted using other solvents, such as ethanol and methanol. We compated a few different extraction methods in order for us to achieve the highest yield possible.

Optimising Growth Medium

The stage of growth medium optimisation had two aims:

1st aim
Improve our understanding of which nutrients are important for the bacteria to grow and to produce violacein. We did this by cultivating *J. lividum* in different variations of growth media, containing combinations of different nutrients. The effect of each growth medium on both cell growth and violacein accumulation was studied, and allowed us to pinpoint certain nutrients that significantly increases violacein production.



2nd aim
We wished to investigate whether it would be possible to utilize the side streams/waste from other industries as nutrients for this bacteria.
We decided to study two types of sidestreams; Lignocellulose hydrolysate from wood industry in Norway and hydrolysed lumpfish meat originating from Icelandic harvesting of lumpfish roe. Our study revealed that *J. lividum* is able to grow on both sidestreams. However, we also found that more research is needed to optimise the violacein production with these side streams.

Bioreactor

The simplest method to cultivate bacteria in liquid culture is in Erlenmeyer flasks or reagent tubes that are continously shaken to mix in air while being kept at a specific temperature. The advantage is that it is simple, inexpensive, and it is possible to have multiple cultivations running in parallel. However, the downside is that there is little control over factors, such as the pH and the amount of oxygen being mixed into the culture. Additionally, shaking flasks with culture will only work in relative small scale. Thus, bioreactors or fermenters are often used instead to cultivate larger amounts of bacteria. They can also have the benifit of providing automatic monitoring and control of important growth factors, such as pH, temperature and air mixed into the culture.

After we had determined, from the small scale experiment, which growth factors were important for pigment production, we cultivated the bacteria in a 5L benchtop bioreactor. The increased control in the bioreactor can allow for improved growth conditions, but it also helps us to uncover potential problems that might occur during upscaling, such as foam formation. In our case the cultivation in the bioreactor proved succesfull, and found that the cultivation of *J. lividum* without any apparent issues can be upscaled. From the bioreactor cultivation we were also able to produce larger amounts of violacein that ITC could then use to dye wool.

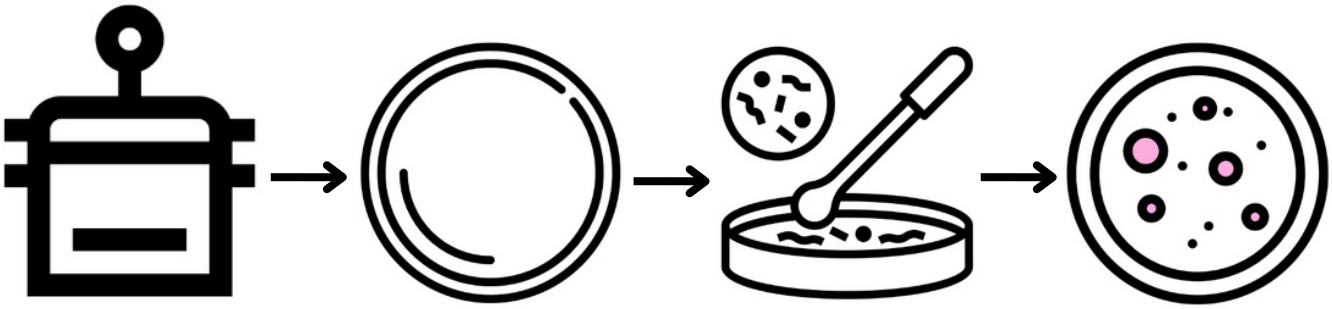
Wet Lab Cultivation

At the Icelandic Textile Center we acquired samples of *J. lividum* from the Fabricademy 2022-2023 program. The bio kitchen at the Textile Lab functioned as our wet lab workspace. This meant using a camper burner to create a sterile work area for preparing and inoculating dishes instead of a flow hood, using a pressure cooker to sterilize our tools and nutrients instead of an autoclave, and a DIY incubator to control the temperature while cultivating the bacteria. As mentioned earlier *J. lividum* is a level 1 bio safety bacteria which means it is safe to work with in a wet lab environment.

Some valueable things we learned about cultivating *J. lividum* in a wet lab were:

- save an uncontaminated sample as backup in the fre
- be mindful as to what the parent bacteria were given for their nutrients as it can help determine why cultivated dishes may not be growing
- lable all dish 1 out of x to better document growth or contamination

Process Overview:



autoclave petri dishes and nutrient agar

pour nutrient agar into petri dishes

inoculate new dishes

cells cultivate over a few days


250
ml
BORO
3.3
APPROXIMATE VOLUME
200
150
100
50
RAYON FLOSS
750
DENIERS
PEARSALLS
TRADE
MARK
SHADE No.

Dyeing with J. Lividum

ITC

- Dyeing Overview
- Dish Dyeing
- Liquid Dyeing
- Over Dyeing
- Misc. Tests

Dyeing Overview

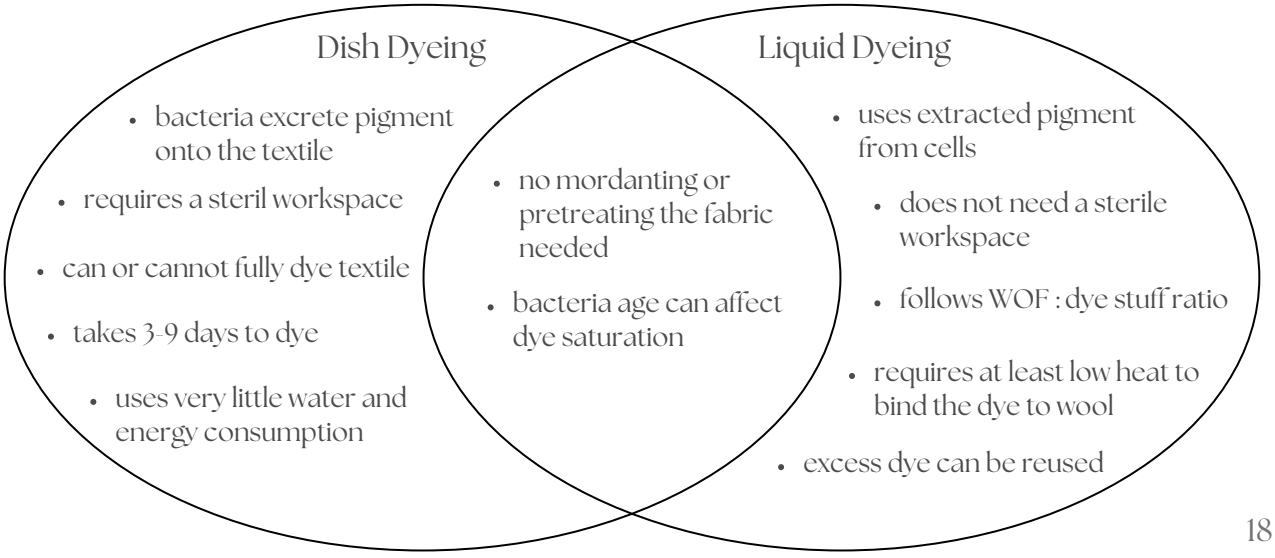
Two approaches were explored when dyeing Icelandic Wool with *J. lividum*; dish dyeing and liquid dyeing.

Dish dyeing is where a sterilized textile is saturated in a nutrient broth, inoculated with a living culture of *J. lividum*, and then incubated to allow the bacteria to grow and produce their pigment. This method is limited by the size of the petri dish or tray that the textiles can fit into to be sterilized. However, dish dyeing can produce beautiful patterns or fully dyed textiles. And most importantly uses very minimal water and energy compared to chemical and natural dye methods.

Liquid dyeing involves using the extracted pigment of the cells in a solution to dye the textiles. The two liquid dye solutions that were experimented with were waste medium and an extraction medium. Liquid dyeing follows commonly practiced natural dye methods of determining the weight of fiber to dye and then using heat to bind the dye to the fiber. What is different when dyeing with bacteria versus natural dyes is that the textile does not need to be pretreated/mordanted in order to have the dye bind to it. A UV tests was done on two sample, one of the waste medium samples and one of the extraction medium samples. UV tests provide insights into how light fix the dye is or how it can fade with UV exposure.

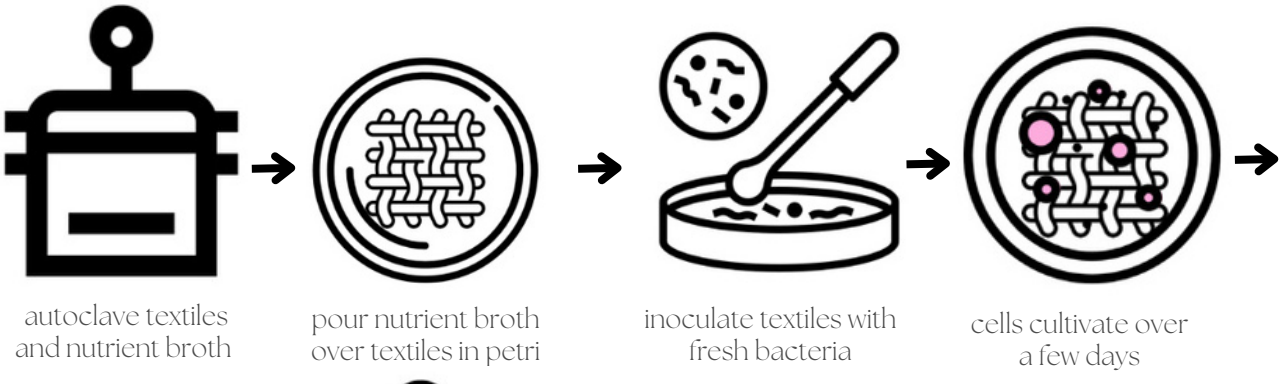
In addition to liquid dyeing with bacteria, over dye tests were done with local plants to explore further color opportunities. Over dyeing is where the textile has already been dyed once and then gets dyed again in either the same dye or a different dye. Over dyeing with the same dye can lead to more intense saturations of the color, but over dyeing is predominantly done for achieving new colors. Basic color theory practices can be applied to over dyeing, however when overdyeing with natural dyes there are hidden undertones, differences in mordants, water pH, and temperature that can affect the final results.

Dish Dyeing vs. Liquid Dyeing Comparison



Dish Dyeing

Dish dyeing started off with with no growth on our loose wool samples. After reviewing what the parent bacteria were fed we modified our nutrients to not have any added sugars and started to see results. Below are examples of different dish growth timings and results. For full dish dyeing instructions and recipes go to our reference section.
Dish Dyeing Process Overview:

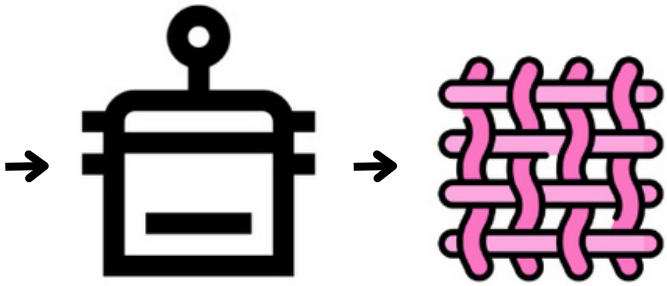


autoclave textiles and nutrient broth

pour nutrient broth over textiles in petri

inoculate textiles with fresh bacteria

cells cultivate over a few days

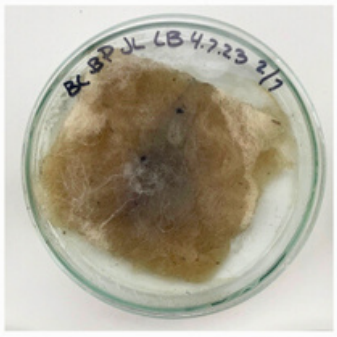


autoclave textiles to kill off remaining cells

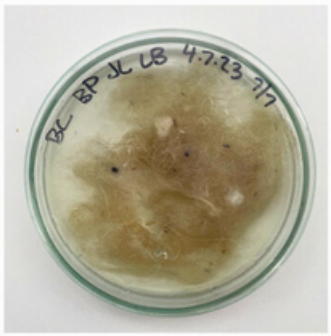
dyed textiles

Growth/dyeing timing examples:

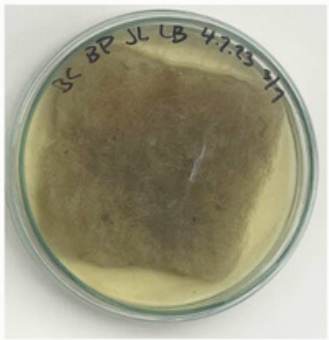
Day 0



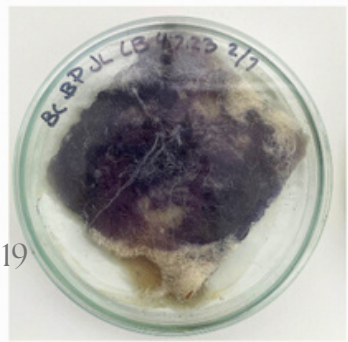
Day 0



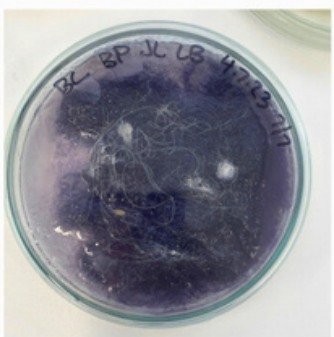
Day 6



Day 3



Day 3



Day 9

Liquid Dyeing

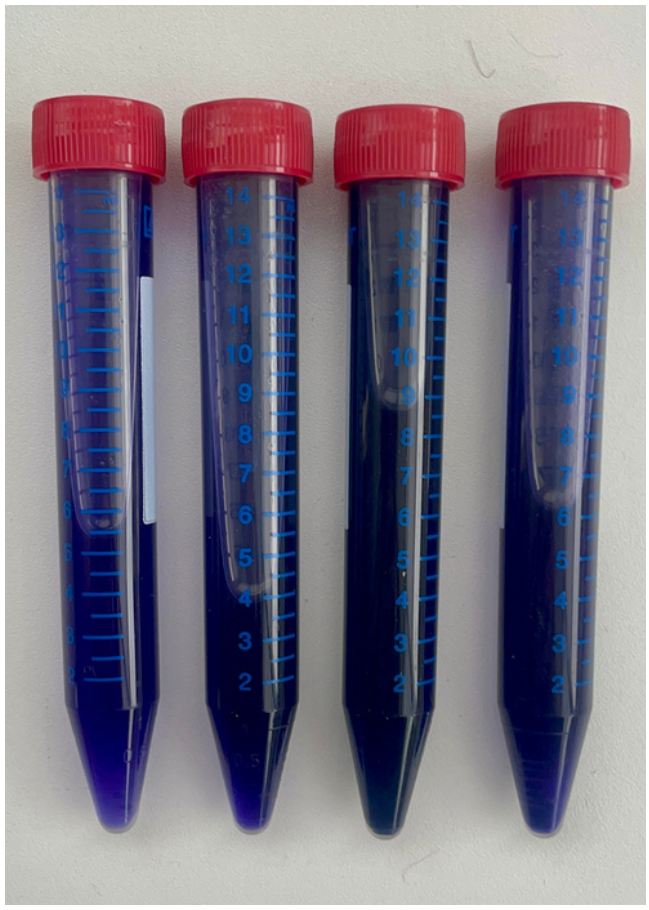
The liquid dye tests started off with surprising success with the waste medium from the bioreactor. Diluting the waste medium allowed for more wool to be dyed, but changed the ratio and color saturation (discussed further on page 25) The extraction medium results were slightly brighter and more saturated then the waste medium samples. Age of the waste medium did seem to have a minor effect on intensity of results. No unusual smells stained the wool after dyeing. Bothsolutions would result in exhausted baths after all the dye was absorbed.

Waste Medium

dye - waste medium
ratio - 50ml : 1g
temp - 50°C - 100°C
time - 1 hour

Extraction Medium

dye - extraction medium
ratio - 5ml : 2g
temp - 50°C - 100°C
time - 1 hour



Over Dyeing

Over dyeing allowed for further color exploration with plants found in Iceland. The samples below were first naturally dyed following the ratios shown either partially for a space dyed effect or fully. Then partially or fully dyed with an exctraction medium bath with the ratio used on page 20. The blue undertone of the bacteria dye challenged the color theory presumptions and gave beautiful cool tone results.

Lupin

condition - fresh
parts - flowers + stems
ratio - 1000 : 1
temp - 50°C
time - 3 hour

Lupin

condition - fresh
parts - leaves
ratio - 500 : 1
temp - 80°C
time - 1 hour

Rhubarb

condition - dried
parts - roots
ratio - 1 : 5
temp - 80°C
time - 1 hour


Fjólublár
BioPol
ÍSTEX.

Misc.Tests

In addition to the dish dyeing and liquid dyeing experiments miscellaneous tests were done as well. Two insightful tests were testing the dyed yarns lightfastness and the different ratios of WOF to dye.

UV Test

UV or lightfastness tests are standard tests that dyed textiles go through in the textile industry. These tests give insight into how the dye will last over time with UV exposure. Most chemically dyed textiles have been engineered to have strong lightfastness properties. Most natural dyes are sensitive to UV exposure even with pretreatments or mordanting. The textile lab wasn't equipped with a UV exposure light box, but the summer sun worked just fine for the tests. The samples below were exposed to UV index 3 direct sun light for 10 hours and did show minimal color fade, but very impressive results being naturally dyed.

Ratio Test

Experimenting with different ratios of WOF (weight of fiber) to dye broadened the range of hues that could be achieved with liquid dyeing. Water was added to the waste medium solution to account for the larger wool quantities. Refer to page 21 for original waste medium ratio.

100 ml : 2g

100 ml : 5g

100 ml : 10g

Conclusion

Fjólublár project has shown how biology and textiles can come together to create something innovative and beautiful. The collaboration between the Icelandic Textile Center and BioPol fostered in-depth research for the success in creating a sustainable dyeing process for Icelandic wool with *J. lividum*. Listed below are some key take away from the project:

- *J. lividum* can be cultivated on side/waste streams
- Nutrients play a big role in production of violacein pigment
- Dish dyed wool had more vibrant and saturated color but resulted in unevenly dyed wool compared to both liquid dyes
- Initial tests indicate that upscaling of violacein production does not seem to cause problems
- Next steps would be to test quality on light and wash fastness of dish dyed and liquid dyed wool tests

This first phase of Fjólublár proved very successful and has laid a strong foundation for further research and collaboration on the development of scaling a sustainable dyeing process for Icelandic wool with *J. lividum.*

References

Bacteria Dye Projects & Companies:

Binc
https://www.binc.ink/
BioShades
https://bioshades.bio/
Colorifix
https://colorifix.com/
Charlotte Werth
https://charlottewerth.com
Faber Futures
https://faberfutures.com/projects/project-coelicolor/
Living Colour
https://livingcolour.eu/
Pili
https://www.pili.bio/

Bacteria Dye References:

Application of Bacteria Pigments as Colorants
https://www.researchgate.net/publication/225963102_Application_of_Bacterial_Pigments_as_Colorant
A Review of Bacterial Pigments: Harnessing Nature's Colors for Functional Materials and Dyeing Processes
https://www.researchgate.net/publication/370798162_A_Review_of_Bacterial_Pigments_Harnessing_Nature's_Colors_for_Functional_Materials_and_Dyeing_Processesg
Colorful Side of Bacteriology: the Pigmented Bacteria
https://www.longdom.org/open-access/colourful-side-of-bacteriology-the-pigmented-bacteria-2469-9837-1000i104.pdf
Project Coelicolor, The Index Project
https://theindexproject.org/post/project-coelicolor-body
Violacein and biofilm production in *Janthinobacterium lividum*
https://ami-journals.onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2672.2006.03155.x

Bacteria Dye How Tos References:

https://bioshades.bio/resources/video-how-to/
https://class.textile-academy.org/2020/beatriz.sandini/assignments/week04/#8-harvesting-the-bacteria-color-aka-killing-your-babies
https://class.textile-academy.org/2020/loes.bogers/files/recipes/bacterialdye/
https://class.textile-academy.org/2023/alice-sowa/Assignments/week04/#bacteria-dyeing
https://issuu.com/kukkadesign/docs/living_colour-ibook?utm_medium=referral&utm_source=www.kukka.nl
https://www.opencell.bio/news/dyeing-textiles

Icelandic Wool:

Circularity of Raw Materials in Iceland
https://drive.google.com/file/d/1oq3Ej-IGJwDRFLa_qBabz2YARSBtVEau/view?usp=sharing_eil_m&ts=63ce6543
Gilhagi
https://gilhagi.is/
Ístex
https://istex.is/en/um-okkur/
https://www.istexwool.is/home/technical-information/#Vowen-wool-fabric
Uppspuni
https://www.uppspuni.is/

Natural Dye References:

Botanical Colors
https://botanicalcolors.com/
Dharma Trading Co.
https://www.dharmatrading.com/

Images:
All photographed images are taken by the ITC and are otherwise referenced below

page 8
bottom left figure - https://bioshades.bio/

page 9
top left image - https://faberfutures.com/projects/project-coelicolor/
top right image - https://bioshades.bio/
bottom left image - https://livingcolour.eu/experiments/
bottom right image - https://livingcolour.eu/design-to-fade/

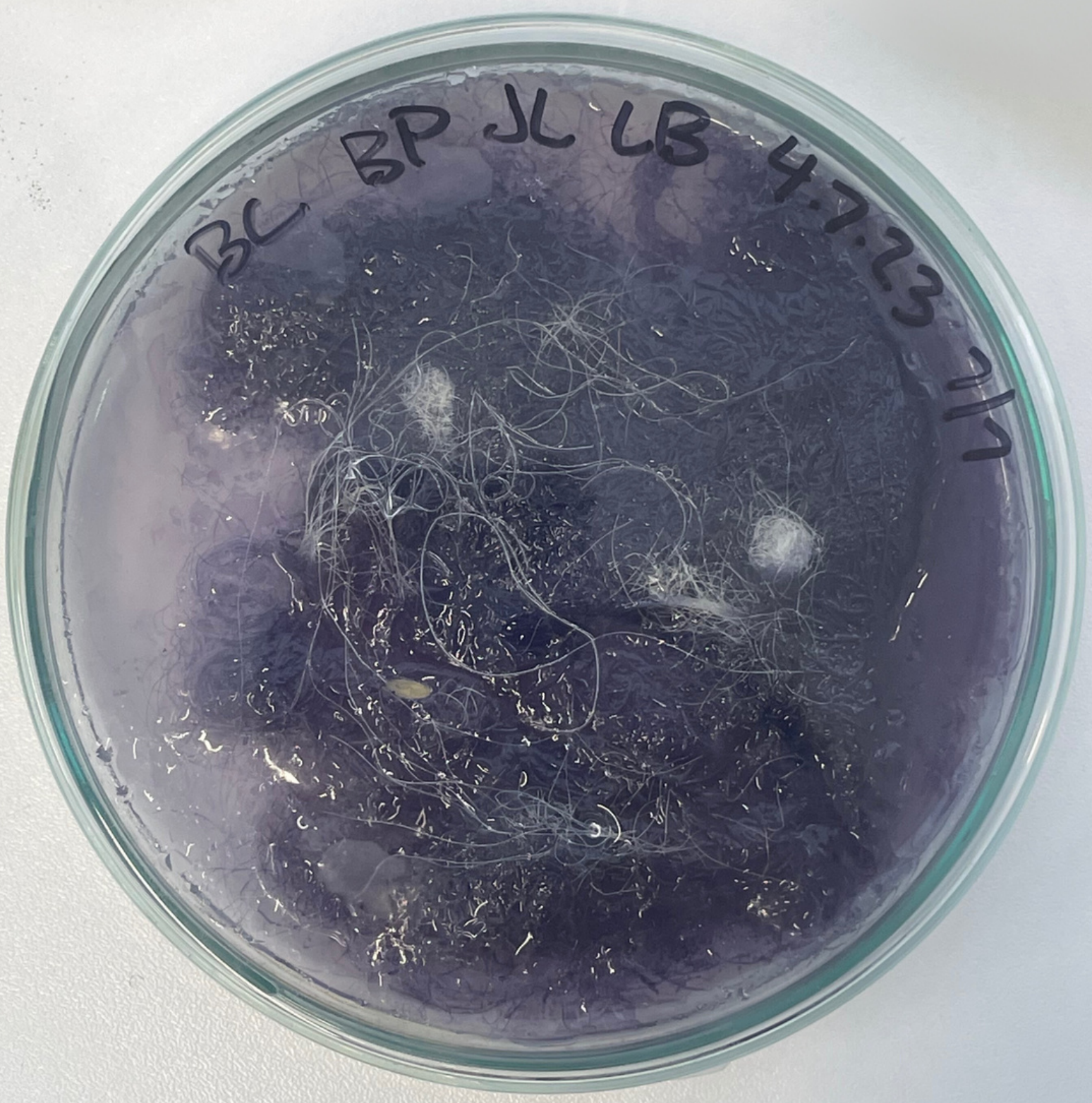
page 10
top left image - https://www.binc.ink/
top right image - https://www.pili.bio/
bottom left image - https://it.fashionnetwork.com/news/Albini-collabora-con-l-inglese-colorifix-per-colorare-i-tessuti-coi-batteri,1519108.html#genny
bottom right image - https://charlottewerth.com

page 12
icons - https://www.flaticon.com/

page 13
all images photographed by Jens Jakobs Sigurðarson

page 15
icons - https://www.flaticon.com/
photographed images by Catherine Euale

SÓKNARÁÆTLUN
NORÐURLANDS VESTRA



ICELANDIC TEXTILE CENTER

BioPol

ÍSTEX®
ÍSLENSKUR TEXTÍLIÐNAÐUR HF.