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Way to Go, Indigo!

Biological Molecules and Denim Processing

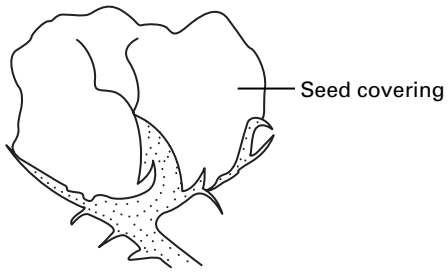
Question How does the enzyme cellulase affect denim fabric?

Lab Overview In this investigation you will take on the role of an industrial scientist as you examine a process used by jeans manufacturers to soften and lighten denim fabric. You will identify problems with one industrial process, and investigate a possible solution by using your understanding of biological molecules.

Introduction In this lab you will start with three swatches (pieces) of denim fabric. You will treat one swatch with the enzyme cellulase, soak it in water, and then scrub it. You will soak a second untreated swatch in water and then scrub it. The third swatch will not be treated, soaked, or scrubbed. You will then compare the three swatches to determine the effects of cellulase on denim. How might these results be applied to make comfortable jeans?

Background Denim jeans were designed in the 1840s to be tough outdoor clothing for farmers, miners, and cattle ranchers. These jeans were stiff and uncomfortable until they had been worn or washed many times. Eventually, jeans became fashionable, but consumers wanted to buy jeans that were already comfortable. Manufacturers had to develop methods to provide new jeans that felt and looked "worn in."

To understand denim you should be familiar with some properties of cotton. Cotton plants produce seed-bearing capsules called bolls (BOHLZ). The bolls contain white balls of fuzzy fibers surrounding cottonseeds.

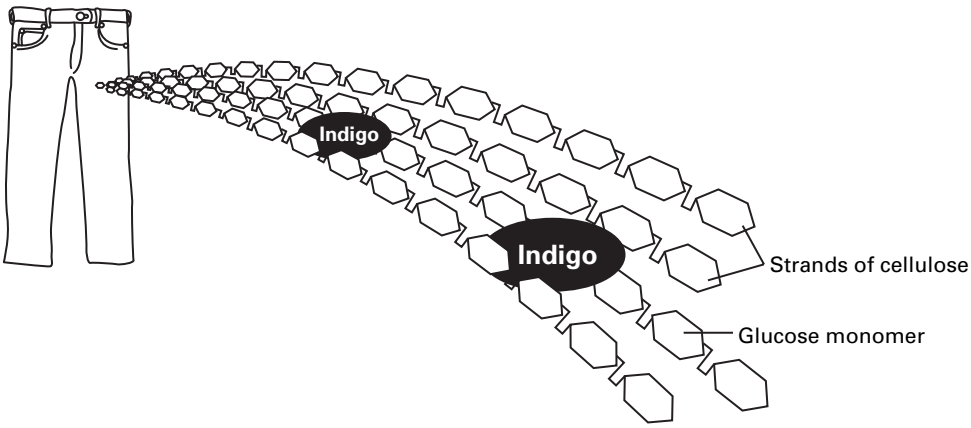


Cotton boll

The fibers are actually the cell walls of dead cells. These cell walls are made of a carbohydrate called cellulose. Cellulose is a long chain of glucose monomers. Multiple cellulose chains are linked together by

hydrogen bonds. The chains wrap around each other, forming larger and larger strands.

To make denim cloth, manufacturers spin the cotton fibers into threads. The naturally white threads are woven together with threads that have been dyed blue. When the threads are dyed, molecules of the dye (called indigo) become trapped between the cellulose fibers, making the threads appear dark blue.



As denim is worn and washed several times, the cellulose fibers start to break and wear thin. As the fibers break, the indigo dye is released and the fabric softens and becomes more flexible.

Manufacturers discovered that washing jeans with pumice stones sped up the softening process. However, stonewashing caused several problems:

1. The pumice stones took a toll on the industrial washing machines, which had to be replaced frequently.
2. Additional employees were needed to pick the pumice stones out of the pockets of the jeans.
3. Many jeans had to be destroyed because the pumice stones caused too much damage.
4. The pumice stones wore down to a sandy sludge that clogged drains and sewer lines.
5. Pumice has to be mined, which can have negative effects on the landscape and wildlife habitats in an area.

A better method of preparing denim was needed! Researchers have discovered that the enzyme cellulase could provide an alternative method of breaking down the cellulose fibers. As cellulase breaks down the fibers, the trapped indigo dye is released. The fabric surface becomes fuzzy as the threads fray. Some sort of agitation (shaking, scrubbing, or abrasion) is important to complete the breakage of the weakened fibers.

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Prelab Activity After reading the Introduction and Background sections, check your understanding by answering the Prelab Questions.

Prelab Questions

1. Match each action of cellulase with its effect on denim fabric.

____ As the strands of cellulose break apart, dye is released.	a. softer-textured denim
____ The fraying of the cellulose strands makes the cotton fibers in the thread fuzzy.	b. more flexible, less stiff denim
____ The fraying of the cellulose strands reduces the thread's strength and thickness.	c. lighter-colored denim

2. Do you think denim treated with cellulase for 1 hour would look or feel different than denim treated for 24 hours? Explain your answer.

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3. The enzyme cellulase is produced by a type of fungus called a mold. Why might people be interested in determining the environmental conditions, such as temperature and pH, that are best for the growth of this mold?

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Materials

- three dark blue denim fabric swatches (about 7 cm by 7 cm)
- scissors
- masking tape
- marker
- two 500-mL beakers or large cups
- one pH 5 buffer capsule
- 500 mL water
- graduated cylinder
- 2 g IndiAge® cellulase
- transfer pipette
- large plastic bowl or bucket
- hot tap water
- paper towels
- magnifying glass

Procedure

Part A: Treating Denim With Cellulase 

1. You will be given three denim swatches. To distinguish the denim fabric swatch you will treat with cellulase, cut off one of the corners. With a marker, write "Control 1" on the back of a second denim fabric swatch and set it aside. Leave the third swatch unmarked—this swatch will be Control 2.
2. With masking tape and a marker, label one beaker or large cup "Buffer With Cellulase." Label the other beaker or cup "Buffer."
3. In the beaker labeled "Buffer," dissolve the contents of a pH 5 buffer capsule in 500 mL of water to make a buffer solution with the best pH for the cellulase.
4. Carefully pour 250 mL of the buffer solution you have prepared into the beaker labeled "Buffer With Cellulase." Add 2 g of IndiAge® cellulase.
5. Place the denim swatch with the cut corner in the beaker labeled "Buffer With Cellulase." Place the Control 1 swatch in the beaker labeled "Buffer."
6. Allow the two denim swatches to soak at room temperature for two days or over a weekend. The third denim swatch, Control 2, should not be treated or soaked in water.

Part B: Scrubbing Denim Swatches 

1. After soaking the denim swatches for two days, remove the swatch with the cut corner from the beaker labeled "Buffer With Cellulase." Rinse the swatch well with warm water to wash away the cellulase. Then remove Control 1 from the other beaker, and rinse it separately.
2. In denim processing facilities, jeans are placed in industrial washers. The actions of the washers break the weakened cellulose fibers and release indigo molecules. Now you will handwash your samples to simulate this step. Put both denim swatches together in a container of hot water. **CAUTION:** *Use hot tap water, but at a comfortable temperature—take care to avoid scalding your hands*. Scrub the two pieces against each other in the water, and lift them out and squeeze them repeatedly. Continue scrubbing the swatches for at least 10 minutes. Change the water whenever it gets blue.
3. When no more blue dye is released, wring out the swatches and lay them flat on a paper towel to dry. Allow them to dry at least overnight.

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4. When the swatches are dry, compare all three denim swatches. Look for any differences in color, texture, and flexibility. Use a magnifying glass to look for differences in the fibers. Record your observations in Data Table 1.

Data Table 1

Observations	Color	Texture	Flexibility
Denim swatch soaked in buffer with cellulase, then washed			
Control 1: denim swatch soaked in buffer, then washed			
Control 2: untreated, unwashed denim swatch			

Analysis and Conclusions

1. From the results of your experiment, would you say that cellulase treatment is an effective method for softening and lightening denim? Explain your response.

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2. Explain the purpose of the Control 1 swatch and the Control 2 swatch in the experiment.

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3. What economic and environmental concerns do you think denim manufacturers might have about using enzymes to soften jeans instead of pumice stones?

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Extension

Using enzymes in the jeans manufacturing process costs money. Design a series of experiments to determine the most cost-effective way to get the greatest softening effect on denim fabric using enzyme washing. In other words, how could manufacturers alter conditions so that they will need less of the enzyme? Use your understanding of enzymes and how cellulase works on denim. Remember to only change one variable at a time. For example, if you change the temperature of the buffer solution, you may not also change the amount of enzyme added. You could also experiment with alternative methods of scrubbing. Describe your experimental designs in detail and explain your plans. Make sure that your experiments include appropriate controls. (**NOTE:** *Be sure to check with your teacher before carrying out any experiments.*)