

# Paper and Microorganisms and Biocides

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**Grade Level:**

6 through 10 (with modifications); also could be used in high school Science and Technology courses

**Goals:**

Ecology, Environmental Science, Microbiology, Applied Technology

1. To identify a simple paper making process.
2. To identify materials used in paper making process.
3. To discuss some of the ecological implications of recycling paper.
4. To discuss some of the industrial implications of recycling paper.
5. To carry out step by step the procedure to find out if microorganisms are present in different solutions as used in industry today.
6. To compare and contrast effects of biocides on a pulp solution.

**Specific Objectives:**

1. To identify the presence of microorganisms using various dyes
2. To determine the effect of biocides on the reduction of living things in stream water mixed with pulp.

**Teaching Tips:**

1. Make sure to allow for two consecutive school days to elapse before examining pulp solutions.
2. A blender could be used in place of egg beater.

**Background:**

True paper making dates back to the first century in China. The basic process has remained essentially unchanged. First, you must separate the cellulose fibers. Second, you mix them with water. Third, you place them on a screen to form a sheet and then you dry the sheet to form the paper.

Cellulose is a fibrous substance which is found in the cell walls of plants. These fibers are not destroyed in paper making. When we recycle paper, we rejoin these hair-like fibers

In industry, paper is made similarly but with high powered machinery. Again, in paper making, water is necessary. All living things need water. A body of water will attract microorganisms. In the making of paper, the pulp allows many nutrients to become available as food for microorganisms. As a result, microorganisms form and can affect the process of paper making in industry.

**For Further Study:**

1. Compare the composition of man-made paper with that made by paper wasps.
2. Have students do research projects on various types of microorganisms.
3. Take samplings of water from a stream at locations varying in:
  - a. amount of sunlight
  - b. rate of flow of water
  - c. depthThen test for microorganisms with resazurin, TTC and phenol red.
4. Determine the lowest concentration of bleach which will kill microorganisms (or just design the procedure to do so.).
5. Find biocides in the home in various products using the labels.  
Examples: BHT, BHA, benzoates, sodium benzoate.

6. Do a science project comparing various biocides and their strength.
7. Interview a microbiologist on the uses and problems with biocides.
8. Find advertisements in trade journals that have to do with biocides.
9. Observe the effects of biocides on different types of plants.

**Vocabulary:**

resazurin	biocide
machine	cellulose
technology	conservation
phenol red	fibers
triphenoltretruzolium chloride	pollution
pulp	solution
microorganism	recycling
incubate	

**Skills:**

Measuring  
 Manipulation of materials  
 Recording data  
 Interpreting data  
 Predicting and inferring  
 Making and testing a hypothesis  
 Applying and generalizing  
 Graphing

**Key Questions:**

How can you keep a pulp solution used in the recycling of paper as free of microorganisms as possible?

**Materials:**

**Part I**

toilet paper	felt- 2 pieces (20 cm square)
hand lens	egg beater
corn starch	screens 2 (15 cm square)
rolling pin	

**Part II**

stream water	droppers - 3	tissue paper
tap water	resazurin	newspaper
sugar cubes		
phenol red	triphenoltretruzolium chloride (TTC)	
15 jars (w. lids)	paper towel	
permanent marker	biocide solution (Chlorox®)	
writing paper	labels	

**Part I:**

**Title: How paper is made**

**Purpose:**

1. To discover the structure of paper.
2. To demonstrate a simple paper making process.

**Hypothesis:**

To be formulated by students with help from teacher.

**Procedure:**

(This section is adapted from a “Museum to Go” activity from the Franklin Institute.)

1. Examine toilet tissue with a hand lens. Tear the paper. Examine the torn edge. Describe what you observe. Observe other paper products available in your classroom. (hand towels, writing paper, tissue).
2. Mix 360 ml of water in a bowl with a tablespoon of cornstarch.
3. Tear up toilet paper into very tiny pieces.

4. Using an egg beater, beat paper until you get a creamy consistency. This will be called your pulp slurry.
5. Add more water, if necessary.
6. Hold the screen over a bowl and pour pulp slurry over the screen. Press slightly to remove excess water.
7. Place screen with pulp between two pieces of felt.
8. Gently roll out pulp to a thickness of 2 cm. Wring out excess liquid from felt.
9. Set paper out to dry overnight. (stacking layers together will help keep paper flat.)

**Questions for  
Part I:**

1. Describe and diagram what you observed with a hand lens.  
tissue paper  
newspaper  
paper towel  
writing paper
2. What material is used to make paper
3. Can we reuse these fibers?
4. Compare this simple paper making process with that of industry. Is it similar?
5. What materials are necessary in paper making?
6. Describe, step by step, the paper making process.
7. Why is it necessary to recycle paper?

**Part II:**

**Title: Identification of the Presence of Micro-organisms in a pulp Solution**

**Purpose:**

1. To demonstrate the presence of microorganisms in a pulp solution.
2. To demonstrate that a biocide kills these organisms.

**Hypothesis:**

To be formulated by the students with help from the teacher.

**Procedure:**

1. Put 360 ml of tap water in bowl A.
2. Add torn soaked paper.
3. Put 360 ml of stream water in bowl B.
4. Add torn soaked paper in bowl B.
5. Using an egg beater, slowly beat paper until you get a creamy consistency.
6. Add water, if necessary.
7. Using 15 jars, label as follows:
  - a. tap water and resazurin
  - b. tap water and phenol red
  - c. tap water and TTC
  - d. tap water, sugar and resazurin
  - e. tap water, sugar and phenol red
  - f. tap water, sugar and TTC
  - g. stream water and resazurin
  - h. stream water and phenol red
  - i. stream water and TTC
  - j. stream water, sugar and resazurin
  - k. stream water, sugar and phenol red
  - l. stream water, sugar and TTC
  - m. stream water, sugar, biocide and resazurin
  - n. stream water, sugar, biocide and phenol red
  - o. stream water, sugar, biocide and TTC
8. 50% head space is required in each jar so you want to fill each jar so that half the space in the jar is air. Each container must have a lid.
9. Add 30 ml of required pulp slurry to each jar. (stream vs. tap water slurry made in bowl A and bowl B)
10. Add a sugar cube to pulp slurry which requires sugar.

11. Add 8 drops of biocide (bleach solution) to pulp slurry that requires it.
12. Add 8 drops of resazurin to pulp slurry that requires it.
13. Add 8 drops of phenol red to pulp slurry that requires it
14. Add 8 drops of TTC to pulp slurry that requires it.
15. Note any color changes. Use Table 1 - initial.
16. Note color changes Day 1. Use Table 1 - day 1.
17. Note color changes Day 2. Use Table 1 - day 2.
18. Note color changes Day 5. Use Table 1 -day 5.
19. Please determine whether your slurry is acidic or basic and place on chart the words acidic or basic.

**Teacher Footnotes:**

Phenol red is a pH indicator which determines whether the solution is an acid or base. Phenol red will turn yellow at pH of 6.8 and red at a pH of 8.2. The teacher should explain pH and acidity/basicity.

Resazurin is a dye used to indicate oxidation and reduction in living things. It starts as a color and will go to clear if oxidation reduction takes place. Note the color it starts out on chart.

TTC is a dye which shows metabolism of living things. Again it is a reduction reaction. It starts out clear in solution and if there is activity, turns red. Note color at start to finish.

NOTE: Pulp slurry in the presence of oxygen and glucose will combine to produce acid and water and anaerobic condition. If, in the presence of an effective biocide, there is “no” reaction

Glucose + oxygen and pulp slurry combine to produce acid and water and anaerobic condition.

Glucose and oxygen and pulp slurry and bleach combine to produce no reaction.

The stock solutions of indicator or dye contain:

<b>Stock Solution</b>	<b>PPM</b>
TTC	3750
Resazurin	375
Phenol Red	1875

Which jars contained microorganisms? How do you know? How many?

Discuss results using paragraph form. Include answering the key question, how do you keep microorganisms out of pulp solution or recycled paper?

**Questions for Part**

**B:**

1. List the three dyes used in identification of microorganisms in this lab.
2. Before you began this activity, what did you think would happen and why?
3. Use the Table 1 for day 1, 2, 3 and 5 to answer the questions below:
  - a. In which pulp slurry did the pH change? Which dye indicated the pH change?
  - b. In which pulp slurry could you say that microorganisms existed? When would you see a color change if microorganisms were present?
  - c. Was there a relationship between microorganisms present and the pH?
  - d. What purpose was the use of the biocide in the stream sugar slurry?
  - e. If you had to compare paper manufacturing to this experiment, what substance in paper making compares to the sugar?
4. Look at Table 2. Make a Bar graph using information from Table 1, day 1, 2, 3 and 5.

**Extension:** Follow the same procedure in doing Part II but use rubbing alcohol as the biocide.

**Essay for thought:** With the gray matter in your head, explain your reactions to the following:  
1. Can microorganisms survive in a foreign atmosphere such as Mars, Jupiter or Saturn? Why or why not? What factors would you have to consider?  
2. Would biocides be useful in a foreign atmosphere? Consider things like temperature, carbon dioxide, oxygen, gravitational forces.

**References:** Microbiological Laboratory Techniques by Arnold I. Miller, D.C. Heath & Co., 1976.  
Franklin Institute - Museum to Go - "Basic Paper Making", Museum to Go Resource Center, MCMLXXXVII.  
Basic Bacteriology: Its Biological and Chemical Background, C. Lamanna, Williams & Wilkins, 1973.  
Biological Science: An Ecological Approach, BSCS Green Version, Third Edition, Rand McNally, 1977.

**TABLE 1**

Substance	pH	Resazurin	Phenol Red	TTC
Tap Water				
Tap + Sugar				
Stream				
Stream + Sugar				
Stream, Sugar, Biocide				

**TABLE 2**

		How many micro-organisms?				
HIGH						
MODERATE						
LOW						
ZERO						
		Tap Water	Tap + Sugar	Stream	Stream + Sugar	Stream, Sugar, and Biocide