Solve the Problem Active Learning Worksheet Chapter 6: The Microbes Ate My Homework
Microbiology with Diseases by Body System, Fifth Edition

Part One: The Case

Paper in the form of cardboard and shipping containers is one of the largest contributors to landfills. What if we could turn this waste into fuel? Paper is mainly composed of cellulose, a branched polymer of glucose. Cellulose makes up the majority of the cell wall material of plants. A number of species of bacteria, including members of the phyla *Actinobacteria* and *Firmicutes*, can degrade cellulose by releasing free sugars from the polymer using enzymes called cellulases. Other bacteria can ferment these sugars into fuels such as ethanol or butanol, which are called biofuels when they are derived by metabolic activity.

An exciting idea is to genetically engineer a single bacterial species to secrete many different kinds of cellulases and also ferment the sugar into biofuel. This new organism could be added to cardboard waste dissolved in water and would turn the waste into fuel, solving two problems at once. Adversaries of this idea are opposed to genetically modifying bacteria in this manner. What if such a microbe escaped into the wild? Could it become a new plant pathogen, attacking the very fabric of plants' cell walls?

Imagine you are on a scientific team at the Environmental Protection Agency charged with reviewing an application from a company that wants to use engineered bacteria in cellulosic bioreactors.

1) **How can these organisms be used safely?**
2) **Would you approve this application?**
3) **What additional information do you require to make an informed decision?**
4) **How would you vote on the quesion of making a genetically-modified organism to reduce the amount of paper waste?**

Part Two: Paper and Biofuels

Each year across the world, manufacturers produce more than 300 million tons of paper , mostly in the United States where over 50 million trees each year are turned into paper. If the enormous amount of chemical energy contained in the chemical bonds of cellulose in waste paper could be converted into biofuels, we could simultaneously relieve a major burden on landfills and provide a large new source for the energy industry.

The pulp papermaking process began in China during the early 2nd century AD, and paper allowed the information revolution of printing and books, which dramatically advanced the knowledge base of civilization. Pulp is a suspension of cellulose fibers from wood or other plant sources in water that, when pressed and dried, makes paper. In plants, cellulose is mixed with another polymer, lignin, which strengthens the cellulose. Modern paper manufacturers remove lignin, because lignin causes paper to turn yellow over time.

For the purpose of making biofuels, paper is a better carbon source than raw wood or other plant material because paper is almost pure cellulose, whereas, trees and other plants contain many different compounds.

There are a number of microorganisms, including fungi, bacteria, and protozoa, that naturally degrade cellulose, which is not surprising, given that it may be the most abundant polymer at Earth, at an estimated 700 billion tons! That represents a large amount of stored energy.

The most studied cellulolytic communities are the gut microbiomes of ruminants, such as cows. Since animals cannot degrade cellulose, these herbivores harbor symbiotic microorganisms to extract energy and carbon out of their plant diet. There are many cellulolytic species of bacteria, including aerobic genera—a*Bacillus*, *Cellulomonas*, *Cytophaga*, *Pseudomonas*, and *Streptomyces*—as well as the anerobic *Clostridium*, *Ruminococcus*, and *Thermotoga*. So, there are many sources of organisms from which scientists can acquire genes to degrade cellulose

6) What are three functions of cellulose in trees and other plants?

7) What could be different about the environmental impact of a genetically-engineered bacterium containing many different cellulases and related enzymes, as contrasted with natural communities of microorganisms that degrade cellulose?

8) How could we prevent a strain of engineered cellulolytic bacteria from escaping laboratories and biofuel factories into the wild?

Part Three: The Problem of Biocontainment

Scientists work safely with extremely dangerous bacteria in laboratories around the world. The safest of these laboratories are designated as biosafety levels three and four (BSL-3 and BSL-4, see p. 000 in chapter 000). However, if a cellulose-degrading, genetically modified bacterium were used in large industrial bioreactors, release into the environment would be likely and even inevitable. How could such bacteria be genetically programmed in such a way that they not be pathogenic to plants could nor survive in the wild?

Selection of a species that is not intrinsically pathogenic to plants as the basis for genetic engineering would be easy; however, a predictable problem arises. The major mechanism for bacterial evolution is horizontal gene transfer (HGT, see p. 223). Using HGT, a non-pathogenic bacterium might acquire genes for attachment to and invasion of plants and thus evolve into a pathogen. Alternatively, cellulolytic genes could be transferred from a genetically modified bacterium into plant pathogens already in the environment. Scientists could adopt several strategies to prevent this.

One choice would be to genetically engineer a cellulose-degrading bacterium from an obligate thermophile or hyperthermophile (starting at page 165 in chapter 6, especially Figure 6.4). Such organisms would thrive in a heated bioreactor but could not replicate at normal environmental temperatures. They would not survive if released accidentally from the bioreactors. This constitutes biocontainment using a physical barrier.

Another choice would be involve utilizing a biochemical barrier. The cellulolytic bacterium could be engineered to lack one or more specific, vital biochemical pathways that synthesize necessary metabolites. Such a bacterium could survive when the critical chemicals are provided. As long as these chemicals are rare in natural soil and water, any escaped bacteria could not survive outside the bioreactors. For example, many bacteria have been engineered in laboratories to lack genes to generate certain amino acids, and so they require these amino acids

in the media to grow. This approach has the advantage of being well-proven, however, it might be overcome by HGT, with integration of the appropriate biosynthetic genes.

Genetic engineering could be further utilized to minimize certain types of HGT. Scientists could prevent transformation by deleting genes for DNA transporters that import DNA into the bacterium. They could similarly prevent conjugation by deleting genes for F pili as well as for DNA transporters. It is more difficult to prevent transduction, because transduction is a byproduct of viruses that infect bacteria—bacteriophages.

9) How might HGT lead to unintended consequences with genetically-engineered bacteria?

10) What precautions and protocols are used in biosafety level 3 and 4 labs to protect personnel and prevent release of pathogens?

Part Four: Recent Events and Summary

The production of biofuels from cellulose occurs in two major steps, which mirror biochemical pathways in bacteria. The first step is degradation of cellulose into the sugar glucose. The second step is fermentation of glucose into the desired biofuel product, such as ethanol or butanol (see chapter 5, page 140). Currently, scientists make biofuels using energy-rich crops, especially corn, which has the negative aspect reducing the amount of corn available for food. Obtaining biofuel from wastes, such as paper, would be better. In addition to biofuel generation, microbes can be used to degrade toxic compounds in the environment—a process called *bioremediation*. While scientists have enjoyed much success in selecting and engineering microbes to degrade pollutants in the laboratory, it has been challenging to transfer laboratory success to "real world" environments, such as remediating polluted soil or water. For example, modified lab strains of *E. coil* are easy to grow and genetically manipulate, but they survive poorly in soil and water.

When the question of releasing genetically-engineering microbes into the environment comes up, we can learn from history by looking at events surrounding the emergence of recombinant DNA technology in the 1970s. Recombinant refers to processes in which DNA is taken from one organism, such as a gene from humans, and placed into another organism, such as a bacterium. When recombinant DNA technology became available, some people were fearful and concerned about the release of "genetic monsters" into the environment, which might result in environmental disasters or plagues. The International Congress on Recombinant DNA Molecules held at the Asilomar Conference Center in Pacific Grove, California (the Asilomar Conference) in 1975 established guidelines for responsible use of genetic modification, which is now used in thousands of labs around the world on a daily basis. A hallmark of the Asilomar Conference was involvement of scientific experts in education of the public and in legal regulation of research.

11) What are your concerns about biocontainment?

12) How can scientists be better involved in education of the public on controversial issues?

13) Have you changed your vote concerning the creation of a genetically-modified organism to reduce paper waste? Why or why not?

References and resources:

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