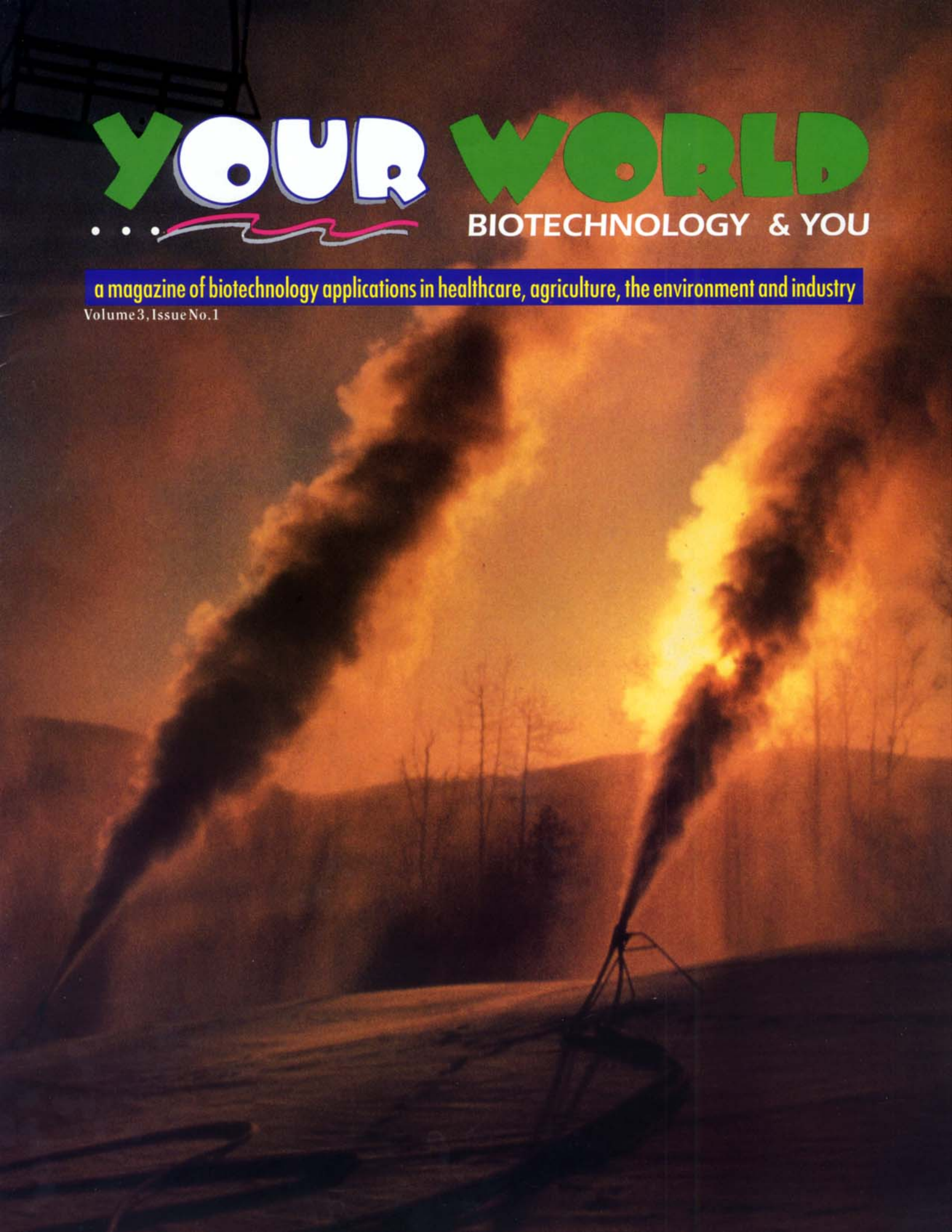

YOUR WORLD

BIOTECHNOLOGY & YOU

a magazine of biotechnology applications in healthcare, agriculture, the environment and industry

Volume 3, Issue No. 1

YOUR WORLD
BIOTECHNOLOGY & YOU

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Your World/Our World describes the application of biotechnology to problems facing our world. We hope that you find it an interesting way to see how science and engineering are applied.

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Special Thanks:

The PBA would especially like to thank the following organizations for their generous contribution of time and energy to the Education Committee:

AAI-ABTECH, Inc.
BIOSIS
Biotechnology Institute/PSU
Boekel Industries, Inc.
Ecogen, Inc.
Emmaus Area Junior High School
Friends' Central School
Gwynedd-Mercy College
GX Biosystems, Inc.
Merck Research Laboratory
Merck Sharp & Dohme Research Labs
Pennsbury School District
Pharmaceutical Information Assoc., Ltd.
SmithKline Beecham Pharmaceuticals
Vector Strategic Resources, Inc.
Laurence A. Weinberger

Your World/Our World would also like to thank the following organizations for their collaboration on this project: the San Diego Biomedical Industry Council, the Minnesota Biotechnology Association, and the Michigan Biotechnology Institute.

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PENNSYLVANIA BIOTECHNOLOGY ASSOCIATION

Cover Photo: Sunday River Ski Resort

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INDUSTRIAL BIOTECHNOLOGY:

from laboratories to factories

Do you know how to cook pudding? First you heat milk in a pan, but don't let it boil. Then add the pudding mix, stirring continuously so the pudding thickens evenly and does not stick to the pan. When it's just thick enough, remove it from the heat and let it cool.

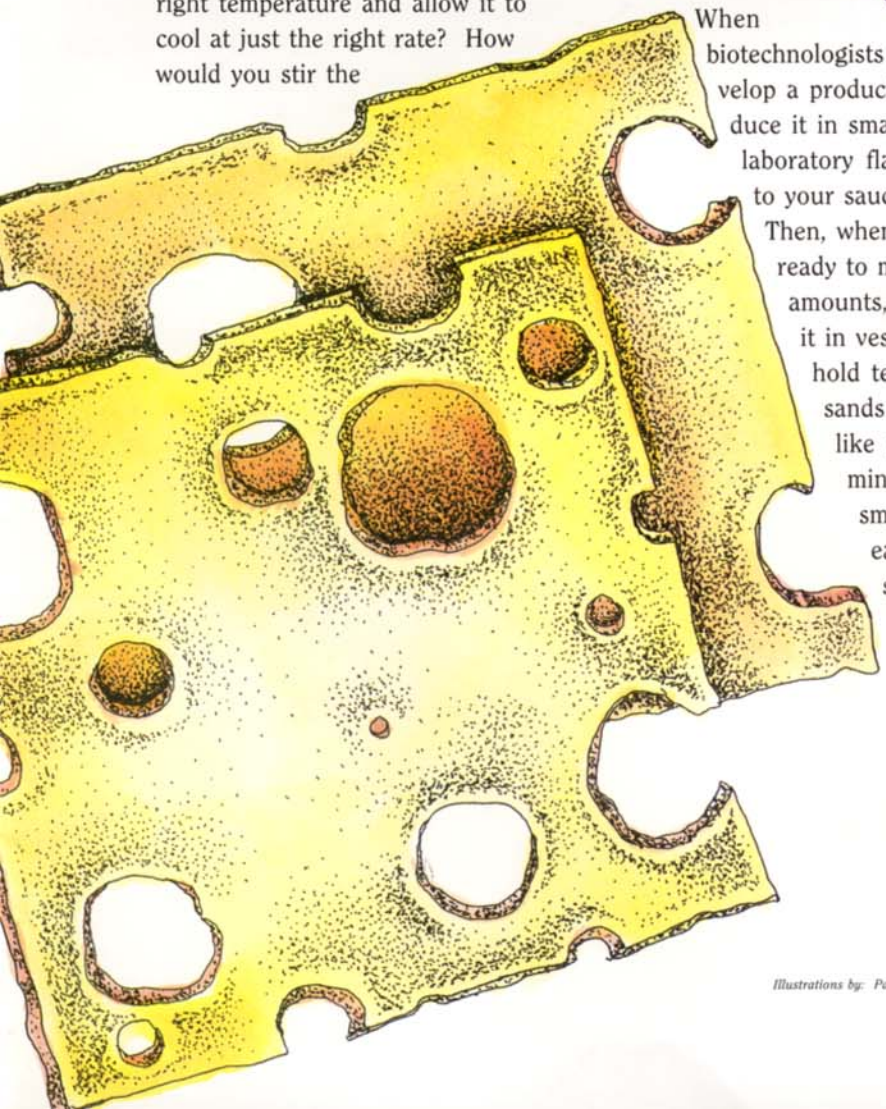
Now think about making a swimming pool full of pudding. How would you heat the milk to just the right temperature and allow it to cool at just the right rate? How would you stir the pudding at the bottom? How would you keep stirring as it thickened?

These problems are similar to the problems engineers face when they try to make large quantities of processed foods, pharmaceuticals and other products.

When biotechnologists first develop a product, they produce it in small laboratory flasks similar to your sauce pan. Then, when they are ready to make large amounts, they make it in vessels that hold tens of thousands of liters, like your swimming pool. A small flask can easily be shaken, warmed or cooled. A 20,000-liter vessel is more difficult to control. This process of moving from small-scale production in a laboratory to large-scale production in a factory is called *scale up*.

In this issue of *Your World/Our World*, you will read about three products of biotechnology and genetic engineering that are currently in production – an ingredient for making cheese, a biodegradable natural plastic, and a product that helps ski resorts make snow.

This issue will show you a glimpse of the technologies that are behind these products. We hope you will appreciate the many challenges and steps involved in using science and engineering to make industrial products that can be sold commercially. Whether you are interested in rearranging genes to make useful microorganisms, or designing big, shiny steel drums to hold billions of those microorganisms at work, you will find that biotechnology offers career opportunities for everybody. ■



Illustrations by: Patti Argoff

Fermentation:

PUTTING MICROORGANISMS TO WORK

As far back as eight thousand years ago, our ancestors knew that certain foods and drinks changed during storage, sometimes in tasty ways. Fruit juice would become alcoholic (wine); dough would rise and produce pleasing aromas (bread); milk would sour and curdle (cheese).

We call the process that brings about these changes *fermentation.* When we allow food to ferment, we are purposefully allowing microorganisms in the food to grow and reproduce. Microorganisms are organisms that are too small to be seen with the naked eye, such as single-cell bacteria or multi-cell fungi. Like all living beings, these microorganisms, or "microbes," eat certain nutrients found in their food, chemically changing those nutrients and giving off other substances as waste. For example, yeast is a type of microorganism that eats glucose (or sugar) in dough and gives off carbon dioxide and alcohol. The carbon dioxide makes the bread airy and the alcohol boils off during baking, leaving a pleasing taste.

Long before fermentation had a name, people learned to control the process by controlling certain conditions. Adding sugar to bread dough, for example, makes it rise more, and putting it in a warm, draft-free place makes it rise faster. Through the years, people learned the conditions and nutrients that different microorganisms need to thrive.

Industrial Products

We now use fermentation to make many new products. In addition to food, fermentation may be used to produce vitamins, antibiotics, industrial solvents, acids, dyes, fabrics, glues and more.

You may have heard about one of the industrial products of biotechnology and fermentation - ethyl alcohol or ethanol. Ethanol is a fermented alcohol made from simple sugars, such as those found in corn. When mixed with gasoline (90% gasoline and 10% ethanol), ethyl alcohol produces a cleaner-burning fuel that reduces carbon monoxide emissions by 25%. The federal government has considered mandating that ethanol or other similar products be added to gasoline for most major U.S. cities. This country now uses 3.5 billion liters (900 million gallons) of ethanol each year - and our consumption is growing rapidly.

Cooking Up Ethanol

To meet the growing demand for ethanol, many large scale bioprocessing plants have been built recently. A typical plant might use 20 million bushels of corn per year to produce 190 million liters (50 million gallons) of ethanol. To get an idea of what an industrial plant like this needs, here's a recipe for a one-day supply of ethanol:

RECIPE

For one day's production of industrial ethanol

1. Grind 60,000 bushels of corn* and mix with water in sixteen 975,000-liter (250,000-gallon) tanks. Cook for two minutes at 82°C (180°F).
2. Add 545 kilograms (1,200 pounds) of alpha-Amylase enzyme** and hold for one hour at 82°C (180°F).
3. Add 545 kilograms (1,200 pounds) of beta-Amylase enzyme.**
4. Stir continuously for 24 hours.
5. Add 1,365 kilograms (3,000 pounds) of brewers' yeast and ferment at 30°C (85°F) for 48 hours.
6. Distill the mixture and sell the 975,000 liters (250,000 gallons) of 99.9% pure ethanol to a fuel blender.
7. The remaining 5 metric tons of "stillage" contain protein, oil and water. Dry it out in an evaporator/dryer 10 meters (30 feet) in diameter and 15 meters (50 feet) tall, and sell the dried stillage as a nutritious animal feed.

* 60,000 bushels of corn is the amount grown on 500 acres, which is the size of an average midwest farm.

** Alpha- and beta-Amylase enzymes are made by genetically engineered microbes and used as catalytic agents.

Scaling Up

Obviously, you could not cook up this recipe for ethanol on your stove! You need a factory. To design an efficient and productive factory, you must solve the following engineering challenges:

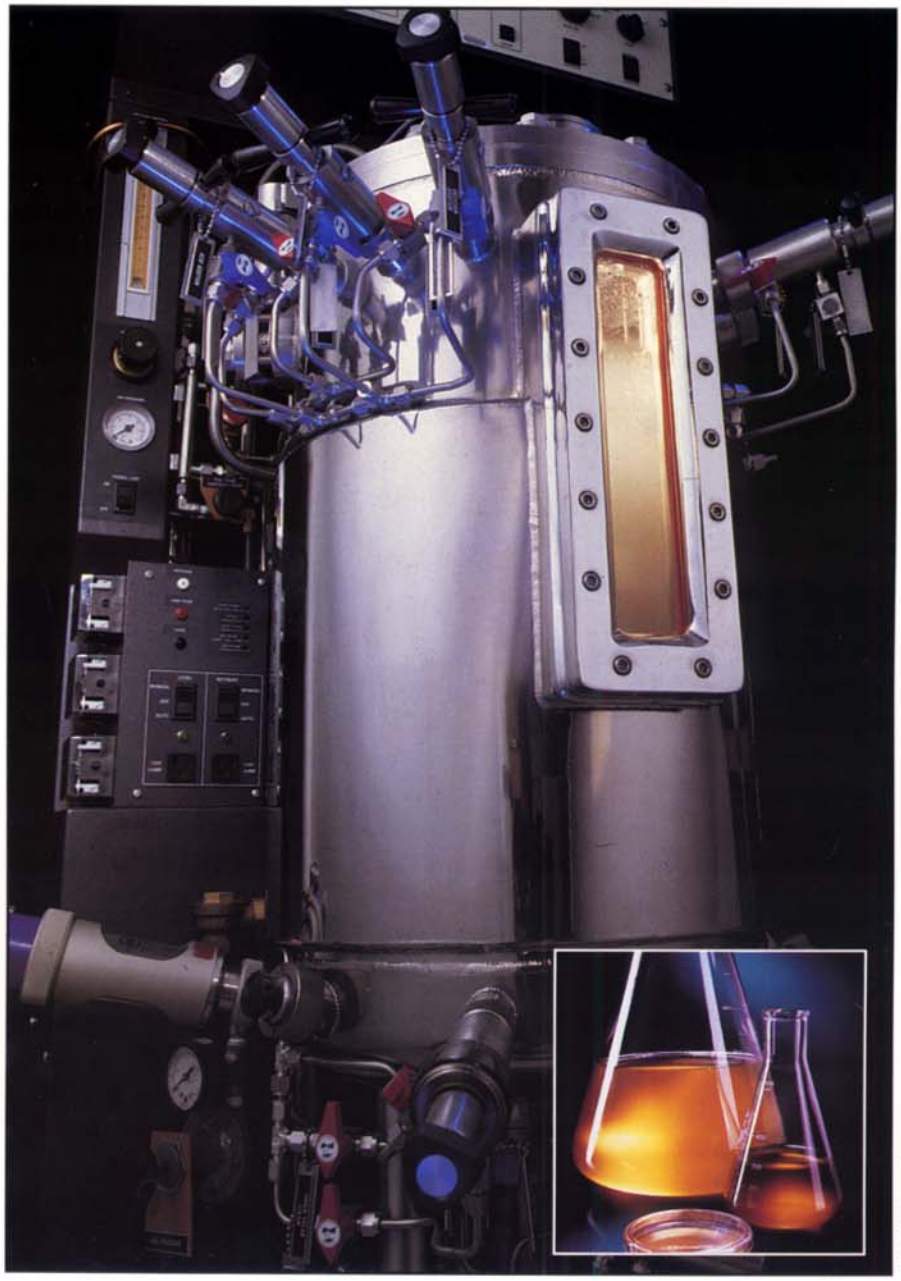
The Nutrient Broth: Microorganisms need the right nutrition if they are to grow and ferment. You have to devise a menu of plentiful, inexpensive food that provides all of the necessary nutrients.

Sterility: You have to keep the fermentation vessel sterile so no other microbes contaminate the ones you are trying to grow. A large industrial vessel has ducts, tubes, and pipes going in and out of it, so you need to design a fermentation system that can be sterilized and that has no nooks and crannies where unwanted organisms may hide.

Oxygen: Your microbes need oxygen to live. Because your fermentation broth is thick and sticky, oxygen does not dissolve in it easily. In a research lab, you could shake the flask containing the broth to mix in oxygen. In large industrial vats, however, you need to design specialized machinery to aerate the broth.

Temperature: Each microbe has a temperature range where it will grow best. You warm the broth to start fermentation. Then, as fermentation continues, the microbes will produce heat, so you must cool the broth without interfering with the fermentation. You need a carefully designed system and automatic controls to keep a constant temperature.

Recovery and Purification: After the microbes have produced the ethanol, you need to separate the ethanol from the microbes and their broth. You will do this by removing the cells from the broth with a centrifuge or filter, then distilling the broth to capture the ethanol. In other types of fermentation, you may have to break the cell apart to remove the product. ■



Photos by: Bruce Cramer

PENICILLIN

bacteria killer that saves lives

The IMAGE Bank ®

Mold, yeast,and bacteria.

In 1928, a physician named Alexander Fleming was growing bacteria on a laboratory dish. He noticed a strange mold, which he later identified as *penicillium,* growing on the dish. Then he saw that the bacteria had died. He concluded that a by-product of the *penicillium* killed the bacteria, and he called that substance "penicillin."

For twenty years the world did not realize the importance of Fleming's discovery. Then, at the beginning of World War II, an English biochemist named Howard Florey believed penicillin could save the lives of thousands of soldiers wounded in the war. These soldiers were dying of infected wounds that no existing medicine could cure. First, though, Florey had to overcome two hurdles. He had to be sure penicillin was safe for humans, and he had to be able to make enough for testing. Making even small quantities, however, was very difficult.

English drug laboratories would not help because they were making existing medicines for the war effort, so Florey was on his own. He grew molds in a hospital using every bottle, soup bowl, pie plate, and bed pan he could find as fermenting containers. If the mold were lost, however, penicillin might be lost as well.

He worried about the Nazis attacking Britain and destroying all of the mold. To save the mold, therefore, he and his colleagues smeared their clothes with the mold spores, hoping they could grow it again if they survived. Luckily, that disaster never happened.

Since English drug companies could not manufacture penicillin, Florey brought the mold to America in 1941. He convinced U.S. companies to try to develop the drug, and they worked frantically to produce enough penicillin to help the victims of the war. The government allowed the "experimental" drug to be used on humans before the safety testing had been completed. They believed that penicillin could save so many lives that it was worth the risk.

These American companies soon learned how to produce large quantities of penicillin. Since then, penicillin has cured millions of infections that had been untreatable for centuries. In addition, we have commercialized many new "bacteria killers" that can cure penicillin-resistant diseases.

You can read about the exciting and difficult development of penicillin in the book *Breakthrough: The True Story of Penicillin,* by Francine Jacobs. ■

Dr. Biotech talks about careers in

BIOENGINEERING

Today we are talking about job opportunities with Dr. Biotech, the director of a large industrial plant called Microbes at Work, Inc. in Engineerville, U.S.A.

Interviewer: Dr. Biotech, our young people know that the fields of biotechnology and genetics need microbiologists, molecular biologists and genetic engineers to track down the genes that produce certain products. Yet, you use the products of these genes to make other things. Can you tell us about the jobs in your plant and what the people do?

Dr. Biotech: Yes, I'd be delighted. First of all, we have **Chemical Engineers.** They understand scientific processes at the molecular and atomic level. They have studied chemistry and biology, and usually have graduate degrees. Their expertise helps them determine the best conditions (such as the proper nutrient broth, temperature, pH, and oxygen levels) for making products at the lowest cost. They tell us how our microbes metabolize food and how the chemical structure of the microbes' products are affected by the nutrients we feed them. They also help adapt the fermentation process for manufacturing. Chemical engineers play key roles in every transition of our scale-up process.

Next we have **Process Engineers.** They take the information from the chemical engineers and design an industrial plant that will meet the needs of the microbes. We depend on them to design and build equipment, and to be sure that the industrial process is working properly. Process engineers may have a chemical engineering background, or they may specialize in mechanical engineering. They become experts in specialized equipment, instrumentation and monitoring devices.

We also need **Mechanical Engineers.** They work hand in hand with industrial plant designers to help adapt machinery and equipment to our specialized biotechnology processes. For example, they might have to design a plant that is strong enough to withstand explosion. Most importantly, they make sure the plant meets government regulations and that it operates correctly and safely.

We certainly couldn't operate without our **Computer Scientists.** They participate in different steps in the industrial process. They usually have degrees in computer science and programming, and they have a deep understanding of computer hardware and equipment.

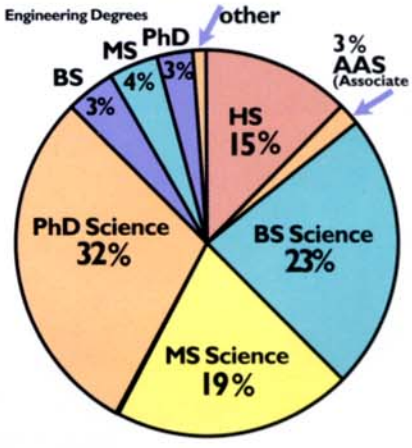
Together, all of these engineers find ways to make larger amounts of product more efficiently, with less waste and cheaper supplies. With their team effort, we can make the benefits of biotechnology available for you to use – affordably.

Interviewer: Thank you for this tour of your plant, Dr. Biotech. Our readers can see that every industrial application of biotechnology offers many different career opportunities. ■

EDUCATIONAL LEVELS OF RESEARCH & DEVELOPMENT PERSONNEL

IN NORTH CAROLINA

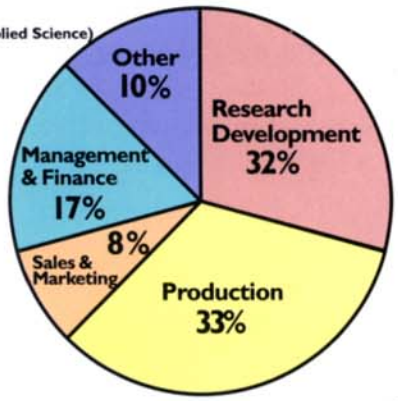
(based on data from a survey by the North Carolina Biotechnology Center.)



DIVISION OF LABOR IN BIOTECHNOLOGY COMPANIES

IN NORTH CAROLINA

(based on data from a survey by the North Carolina Biotechnology Center.)



NEW PLASTICS JOIN THE FAMILY OF POLYMERS

Photo courtesy of: Zeneca Bio Products

Examining biodegradable plastics.

What do DNA, proteins, starch, fat and plastic have in common? They all have repeating molecules called *monomers.* A chain of monomers creates a *polymer.* When nature makes these chains, we call them natural polymers. When humans make them, we call them synthetic polymers. Plastic is one such synthetic polymer, and it has traditionally been made from petroleum. Plastic can be made to have many different characteristics: flexible and thin polyethylene bags, hard and thick PVC pipes, and the many textures of nylon and polyester clothing.

About 70 years ago, scientists discovered that nature itself makes a polymer with very similar properties to petroleum-based plastic. This natural plastic, called *polyhydroxybutyrate* or *PHB,* is made by a soil bacterium. That bacterium, *Alcaligenes eutrophus,* makes PHB in the same way that animals make fat or plants make starch. The bacterium eats food and stores energy as PHB. Then, just as we use up our fat when we diet, the bacterium uses up its PHB plastic as an energy source. The plastic just disappears, or biodegrades!

Producing Plastics By Fermentation

Although we have known about this natural plastic for decades, it took advances in biotechnology to figure out how we might produce enough plastic to use commercially. In nature, the bacterium makes a tiny amount of plastic inside its cell. Producing large quantities requires growing the bacterium in a glucose broth so it will reproduce rapidly. The plastic is actually a by-product of the fermentation, in the same way that alcohol and carbon dioxide are the by-products of yeast fermenting sugar in bread making.

Several years ago, a team of biochemists discovered they could trick the bacterium into making more plastic than normal. They withheld some of its necessary nutrients, especially nitrogen. The bacterium then "thought" it was going to starve, so it laid stores of plastic inside its cells as a source of future energy – like bears laying in stores of fat for hibernating over the winter.

Gathering the Harvest

Once the bacteria build up a storehouse of plastic, engineers "harvest" it. They break the cell, separate the plastic from the other cell material and then purify it.

Making usable products from PHB plastic also requires special skills and knowledge. Processing plastic involves melting it and molding it to the shape of the final product. Unlike synthetic plastic, this natural plastic decomposes at a temperature just slightly higher than its melting point, so engineers must control the temperature very precisely.

COMPOST AT WEEK ONE.....

In experimenting with these bacteria, chemical engineers found that feeding them different diets makes them produce plastic with different properties. For example, adding organic acids to the usual meal of glucose makes a more rubbery, sturdier plastic (called PHB-V). This plastic also has a lower melting point, so it is easier to process.

An English company introduced the first commercial use for the easier-to-process PHB-V plastics in 1989. They produce this plastic for a German company that uses it to make biodegradable shampoo bottles. The pilot plant produces 50 metric tons a year of this plastic, at $33 a kilogram ($15 a pound). That's pretty expensive when compared to $1.20 a kilogram (50¢ a pound) for petroleum-based plastic! The price, however, would drop to $4.40 a kilogram ($2 a pound) if the plant could produce 10,000 tons a year. To make these larger volumes, though, engineers still need to learn more about controlling the conditions that affect fermentation in large systems.

Genetic Engineers Help Out

Genetic engineers are now experimenting with new ways to grow natural plastics. They have identified the three genes in the *Alcaligenes eutrophus* bacterium that are responsible for the plastic production. Using the techniques of gene splicing, scientists have inserted those plastic-making genes into *Escherichia coli* (or *E. coli*), a very common and well-understood bacterium found in many mammals. Eventually, genetic engineers foresee being able to use *E. coli* for manufacturing natural plastics on a large scale. Such large-scale manufacturing would bring prices down so more companies could afford to use the natural plastics.

Yogurt Bacteria Help Make Another Plastic

In the process you have been reading about, bacteria produce PHB-V as a complete polymer chain. Another way to make natural plastic uses a bacterium to produce a natural monomer which can then be synthesized into a polymer, or plastic. That is what an American company is doing. They use the bacterium *Lactobacillus acidophilus*. This is the same bacterium that creates lactic acid in milk, and it is used to make yogurt. Sugars are fermented with the bacterium to produce lactic acid – a monomer – and the lactic acid is then converted to a polymer called *polylacticide* or *PLA*. The company plans to build the world's first large-scale PLA plant and have a commercially available product by 1996.

This company plans to develop a network for composting their natural plastics, just as you might compost leaves or grass clippings. To biodegrade, PLA plastic needs heat, moisture, oxygen and common soil bacteria – just the things that a compost heap provides. In about six weeks, the soil bacteria completely decompose the plastic, converting it to water, carbon dioxide and a rich humus you could use in your garden. This biodegradability makes natural plastics good candidates for certain items that could be collected and composted, such as lawn and leaf bags, disposable diapers, and fast food containers. ■

Zeneca Seeds

Plants as Natural Factories

Industrial fermentation factories may not be the only plants where we can "grow" natural plastic. If genetic engineers can insert plastic-making genes into different bacteria, why can't they insert them into growing plants? Some genetic engineers think they can. They are experimenting with plants that naturally produce lots of starch, such as corn and potatoes. They would turn off the starch-making genes and turn on the new plastic-making genes. Maybe someday we will have fields of plastic in our nation's bread basket!

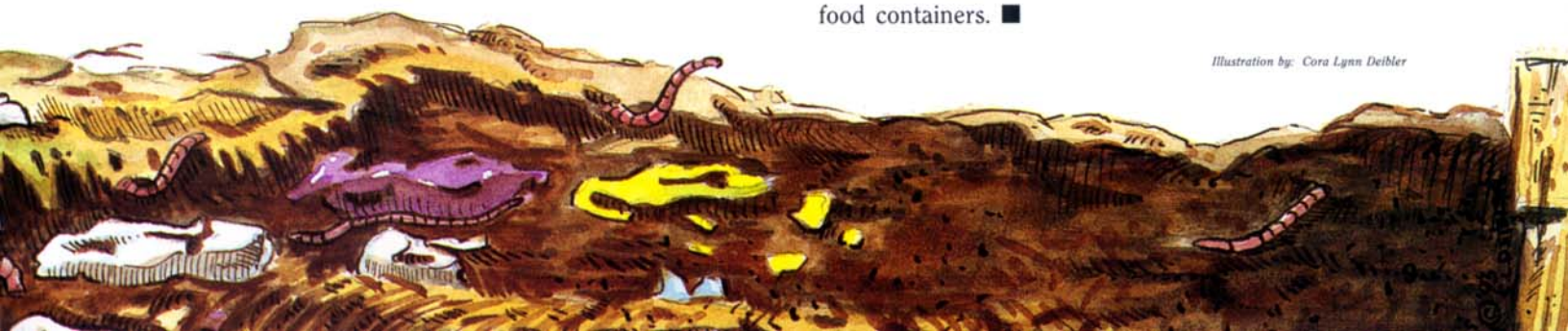


Illustration by: Cora Lynn Deibler

say CHEE

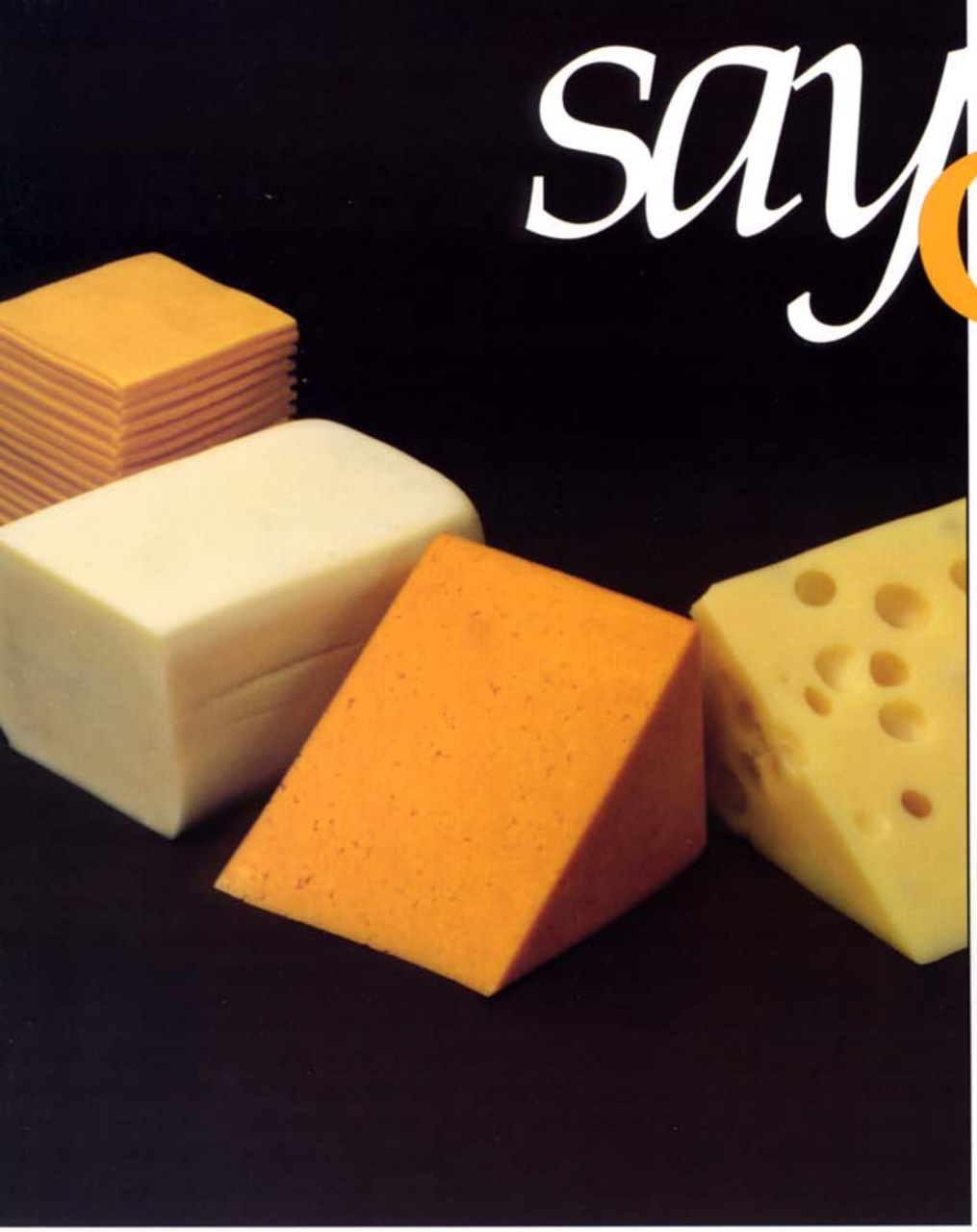
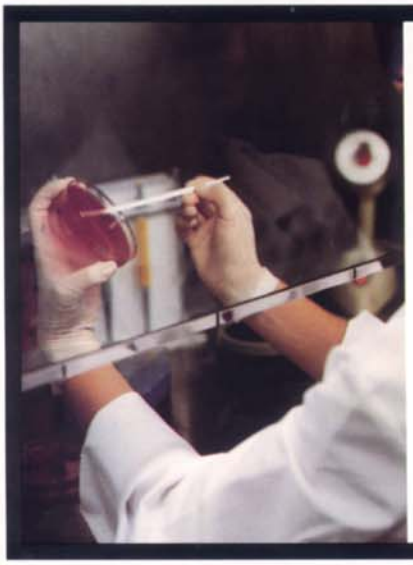


Photo courtesy of: United Dairy Industry Association

Not much has changed in cheese making over the past 8,000 years – until a few years ago, that is. Over the centuries, people learned to "grow" different kinds of molds and bacteria to make different kinds of cheese. These different microorganisms produce a variety of flavors and aromas because of their different by-products. A bacterium that produces acids creates a sharp cheese like cheddar. A bacterium that produces carbon dioxide makes the holes in Swiss cheese. Injected mold spores create the blue veins in Roquefort and other blue cheeses.

Regardless of the type of cheese, they all have one ingredient in common – the enzyme used to make milk curdle. An enzyme is a kind of protein that helps organisms process other proteins and chemicals. The en-

That's what they did a



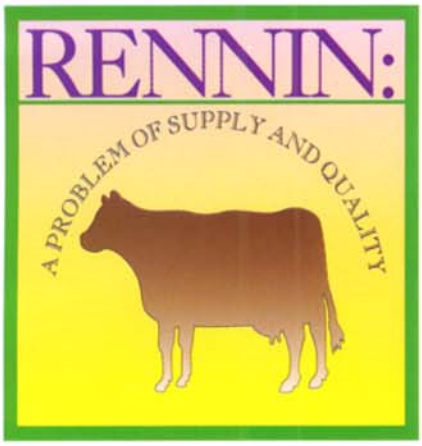
Photos courtesy of: Genencor International, Inc.

Research

1. Molecular biologists located the genes that instruct cells how to make the enzyme rennin (chymosin).
2. Genetic engineers cut the genes out of the calf's DNA and pasted them into a bacterium's DNA. They had to experiment with many kinds of bacteria before they found one that made a form of chymosin that worked as well as calf rennin.
3. Microbiologists and biochemists experimented to find the right nutrient broth for the bacteria's fermentation.

EEEEESE!!!!!

zyme that makes milk curdle is called *rennin*, and it exists in the stomach of mammals.



Until recently, calves have been the only commercial source of rennin. There are several problems with this source, though. Calves just can't supply enough rennin to meet the world-wide demand for cheese, and so rennin has become expensive. In addition, the quality of calf rennin is inconsistent, so cheese making can be unpredictable. Cheese makers want a rennin that can be mass produced inexpensively and that has a consistent quality.

At a biotechnology firm that specializes in new medicines, several creative scientists made a suggestion. "What if we could find a microorganism that could produce rennin in the same way that microorganisms produce penicillin - through fermentation?" The problem was that existing microorganisms naturally produce penicillin, but none produces rennin or, as scientists call it, *chymosin*. They would have to transform a bacterium to make what it wouldn't make on its own. That's what they did. The following chart of events from research through scale up and productions shows you just how they did it. ■

How Did They Make Prehistoric Cheese?

No one knows exactly how people first discovered how to make cheese from rennin and milk. After all it happened thousands of years before written history. People once used animal stomachs and skins as storage vessels. The rennin in these stomachs may have curdled the milk. People might have noticed that this primitive cheese did not spoil as quickly as milk, and they might have stored milk this way on purpose in order to preserve it. Eventually, they would have experimented with ways to extract the rennin from the stomach and produce cheese in other containers.

nd here's how they did it:

Scale-Up Phase

4. Biochemical engineers adapted the fermentation process from a laboratory flask to a 45,500-liter (12,000-gallon) fermenter to produce large quantities of the chymosin.
5. Chemical engineers learned how to separate the chymosin from the fermented cells without damaging it.
6. Laboratory technicians ran tests to make sure the manufactured chymosin works the same as calf rennin - and meets federal health and safety requirements.

Production Phase

7. Mechanical engineers, architects, technicians and operators oversaw the design, construction, operation and monitoring of the chymosin factory.
8. Quality assurance engineers checked the operation and the end product to ensure that every part of the process worked as it should.

LET IT SNOW!

In the early days of skiing, skiers had to wait for the snow to fall. As artificial snowmaking became available in the late 1950s, skiers could ski even in winters with little natural snowfall. Any skier can appreciate how this snowmaking has improved the quality of the ski slopes. Now biotechnology has improved the quality of snowmaking itself.

Snowflakes are crystals, and every snow crystal has a center point, or nucleus. In fact, ice crystals only form if the water has some particles of dust, sand or other impurities to serve as a nucleus. Some people have compared the function of this nucleus to a grain of sand in an oyster that acts as the nucleus for forming a pearl.

Biotechnologists have found a naturally occurring soil bacterium called *Pseudomonas syringae* that produces a protein that attracts water molecules to form a crystal. This protein acts as the nucleus of a snow crystal, and so it is called an "ice-nucleating" protein. It allows water to freeze at several degrees above its normal freezing point.

One biotechnology company saw the potential of using this ice-nucleating protein for snowmaking. When ski resorts make snow, they mix cooled water with air and shoot it with high-pressure guns or fans into the air. As the spray hits the cold air, the water droplets form crystals. If ski resorts mix the ice-nucleating protein into the water they use for snowmaking, the spray will form snow crystals even at temperatures about 5°C higher than normal. (See the article "Water Does Not Freeze at Freezing.")

When ski resorts use this ice-nucleating protein, they can keep their slopes in great condition. This method produces snow that is stronger than natural snow and can withstand more skier traffic. The protein also makes snowmak-ing systems 40-60% more efficient, saving both energy and water.

Ski resorts need only a very small amount of the ice-nucleating pro-

Strawberries have a lot of the ice-nucleating strain of bacteria. That's why they are often ruined by frost when other fruits are not.

BACTERIA WITH A DUAL PERSONALITY

The bacterium called *Pseudomonas syringae* has two naturally occurring strains that function in opposite ways. One strain produces the ice-nucleating protein that encourages water to freeze at a temperature above its freezing point. When plants have a lot of this strain, they may freeze too easily.

The other strain of the bacterium "locks" the ice-nucleating function of its protein. It actually protects plants from freezing, even at below-freezing temperatures. Bioengineers are trying to develop this strain of bacterium as a way of protecting crops from frost damage. If they succeed, farmers can extend their growing season, fruit and citrus crops will be protected, and farmers will be able to produce more food in cold climates.

Skiing photos courtesy of: Vail Associates, Inc. and Sunday River Ski Resorts

tein: They mix it with water in a ratio of about 1 part of protein to 1500 parts of water. A typical ski resort in Pennsylvania uses about 35 million liters (9.5 million gallons) of water a year, and so they use only about 30 kilograms (65 pounds) of the protein.

Making Snow...

This company developed a commercial product that is now used at ski resorts around the world, including the sites of the 1988 and 1992 Winter Olympics. Here's how they make it:

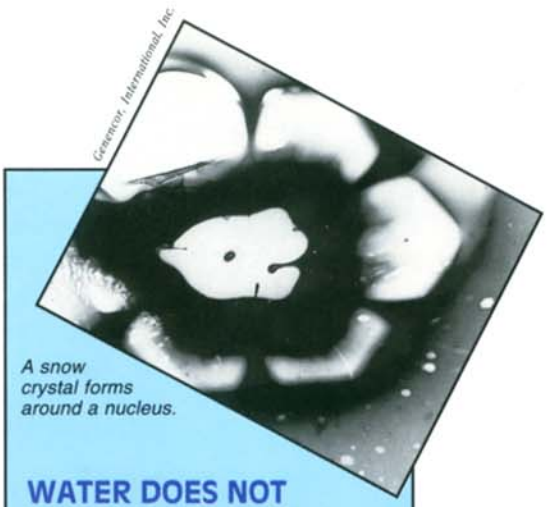
- **Ferment** a broth of soy flour and other nutrients with a natural strain of *Pseudomonas syringae* bacterium in a 2,000-liter tank.
- **Separate** the ice-nucleating protein from the bacteria and the broth.
- **Freeze-dry** the protein so there is no water left in it (like freeze-dried coffee).
- **Use electron beam irradiation** to sterilize and destroy all microorganisms.

Safety Testing

Some people worry about the unforeseen effects of putting the products of biotechnology into the environment. Since the ice-nucleating protein is the product of the microorganism, not the microorganism itself, there is no concern about an organism getting out of control in the environment. Still, the public needs to be assured that the protein will not harm humans or the environment.

The company that manufactures the protein for commercial use had to prove its safety to the federal government before they could test it at ski resorts. They ran toxicology and inhalation tests to make sure the product was not toxic (poisonous) or dangerous for people in either the factory or ski resorts. When these tests showed the protein posed no threat to human health, they tested the product for three years at a ski resort in Colorado. There, they compared its effectiveness to slopes using untreated artificial snow. They also compared the plants, animals, soil and run-off water on the treated and untreated slopes. The results showed that the protein did not remain in the environment, and it caused no environmental harm. The U.S. Environmental Protection Agency, U.S. Department of Agriculture, and the National Forest Service then approved the product for general release. The eleven other countries using the product have all approved it based on the result of these tests. ■

Photo courtesy of: Sunday River Ski Resort



Genencor International, Inc.

A snow crystal forms around a nucleus.

WATER DOES NOT FREEZE AT FREEZING.

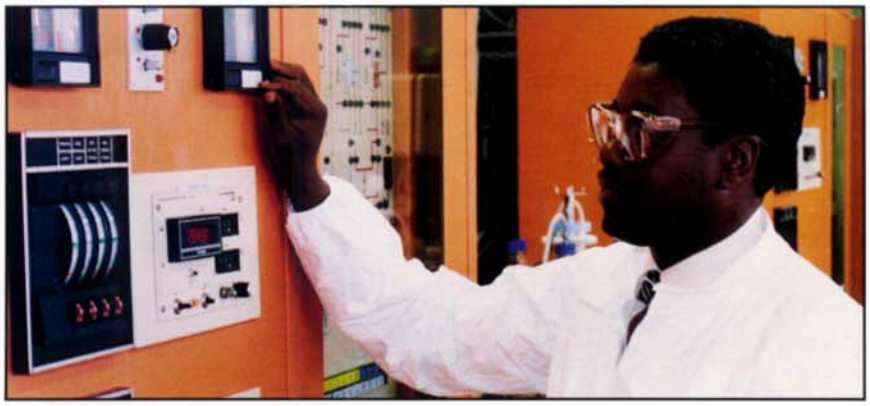
Since the ice-nucleating protein raises the freezing point 5 °C and water freezes at 0°C, that means that ski resorts can make snow at 5°C, right? Wrong!

Actually, 0°C is the melting point for water. That is, ice will melt at 0°C, but water will not necessarily freeze at that point. Its freezing point depends on its "nucleation temperature," which is the temperature at which the water molecules slow down enough to crystallize. The nucleation temperature depends on the type of particle in the water that will serve as the nucleus.

Perfectly pure distilled water in a vacuum where no impurities exist would have no nuclei for forming crystals. You could cool it to -40°C before it would freeze! Typical ski resorts use stream or pond water with natural impurities which raise the water's freezing point. They are generally able to use their snowmaking equipment at a temperature of around -8°C (17°F). By using the ice-nucleating protein they can make snow in temperatures of about -3°C (26°F). For ski resorts in warmer climates, that's quite an improvement.

Dr. Kodzo Gbewonyo,

Biochemical Engineer in Pharmaceuticals



Merck & Co., Inc.

He engineers scale-up operations

Dr. Kodzo Gbewonyo (pronounced 'ko-jo be'won-yo) *is a biochemical engineer at the Merck Research Laboratories in Rahway, New Jersey. He has a Bachelor's degree from the University of Ghana, a Master of Science from the University of London, and a Doctorate in Science from the Massachusetts Institute of Technology.*

Dr. Gbewonyo works for one of the world's largest pharmaceutical companies. His job is to find ways to produce many of the new drugs that are helping to fight diseases, cancers and genetic disorders. All of the drugs he works with are produced by fermentation. Dr. Gbewonyo figures out how to grow microorganisms in large vessels as successfully as they can be grown in the lab.

"I love my job," he says, "because it combines three fields of study in very creative ways. I was always interested in the biological sciences and chemistry, so I studied biochemistry as an undergraduate. Then in graduate school, I added engineering. My job requires all three: biology, chemistry and engineering. I must control all of the variables that might affect the growth of microorganisms - things like nutrients, the shape of the equipment, temperature, dissolved oxygen, pH, agitation, and aeration.

"We use computers to monitor and control the mix," he continued. "Every few minutes, our computer collects and analyzes data such as temperature, pressure, pH, oxygen, and carbon dioxide. It in turn tells the equipment how to make necessary changes, and it warns us of problems. For example, if the pH level were too low, it would activate a pump that would add an alkaline substance to raise the pH. If the pH level were so low that the microorganisms were dying, however, it would signal us to take immediate actions to fix the problem. Once the computer is running smoothly, the process is pretty simple. Getting it to run smoothly, though, is one of the most important steps in the transition from the laboratory to large-scale production."

Dr. Gbewonyo recently worked on a drug that treats parasites such as heartworm in dogs and another drug used to treat high cholesterol. Both drugs are made using microorganisms that grow into long filaments as they ferment. Because of this characteristic, their broths quickly become very viscous, or thick. "The broth is like oatmeal," he explained. "It is so viscous that it is very hard to get enough oxygen to all of the organisms, and without oxygen, the microorganisms will not thrive and grow."

You can visualize this problem. Imagine a small bowl of thick oatmeal. You could get oxygen to all of it simply by stirring it up with a spoon. Now imagine a big trash container full of the same thick oatmeal. A spoon would be pretty useless, but without stirring the microorganisms would die.

"We solved the problem," he explains, "by using a large impeller and an aerator in the bottom of the vessel. The apparatus is like a big upside-down ceiling fan with air being forced up through it. The fan turns in the bottom of the vessel, stirring the microorganisms, while the aeration keeps adding enough oxygen."

With new drugs being developed every day, the need for Dr. Gbewonyo's skills will continue to increase. "The field of biochemical engineering is just beginning," he says. "When I started in the field, you had to go to graduate school to get enough training. Some colleges now offer undergraduate degrees, because the need for production and manufacturing engineers is so great." ■

EXPERIMENT

THE PROCESS OF FERMENTATION

INTRODUCTION

This experiment uses a fungus (yeast) to convert sugar into carbon dioxide gas (CO_2) and ethyl alcohol (C_2H_5OH), both of which are waste products of yeast metabolism. Bakers use this process to make bread rise, and wine makers use it to make the alcohol in wine. You will make yeast ferment in a liquid broth of sugar and water. At room temperature, these yeast cells multiply two-fold every twenty minutes, so by the end of the day, you will have many yeast cells.

You can follow the process of fermentation by measuring and observing three products:

1. *Carbon dioxide:* This gas becomes trapped in the yeast mixture and creates a layer of foam. The thicker the foam, the more fermentation.

2. *Ethyl alcohol:* This alcohol has a unique smell. A stronger smell means more fermentation.

3. *Heat:* When microorganisms metabolize (eat, reproduce and give off waste) they produce heat. The temperature of the broth will rise as fermentation progresses.

PURPOSE

This experiment demonstrates yeast fermentation and the effect of temperature on the fermentation rate.

MATERIALS

- three packages of dried baker's yeast
- thermometer
- 25 grams (2 tablespoons) sugar
- mm/cm ruler or tape
- warm and cold tap water
- long-handled spoon or stirrer
- three 1-liter plastic soft drink bottles

PROCEDURE

Make Fermentation Vessels or Reactors

1. The teacher will cut off the tops of three 1-liter plastic soft drink bottles to use as reactors. Draw a line around each of the bottles about halfway up their sides. Label the reactors A, B and C.

Make Fermentation Broth

2. Add warm tap water to reactors A and C and cold tap water to reactor B. Fill all three reactors to the halfway mark.

3. Measure and add 12.5 grams (1 tablespoon) of sugar each to reactors A and B, but not to C. Reactor C is your control. Stir until the sugar is dissolved and the liquid is clear. This mixture is your nutrient broth.

4. Place one package of dried yeast in each of the three reactors. Stir the yeast until you have a muddy brown mixture. This liquid is the fermentation medium.

Measure Fermentation Products

5. As soon as you have prepared the media, measure the temperature in each reactor with your thermometer.

6. Measure the height (in mm) of any foam in the reactor.

7. Smell and note the odor of the contents of each reactor.

8. Record these measurements, along with the time of the measurements.

9. Place reactor B in a cold environment, such as outdoors or in a refrigerator. (Do not allow the mixture to freeze.) Keep reactors A and C indoors at room temperature, around 20°C (68°F).

10. Observe all three reactors as often as you can for the next 48 hours. At half hour intervals, repeat the temperature and foam height measurements and note any changes in the smell and color of the reactor. Record these measurements and observations, along with the time.

DATA ANALYSIS

Compare the behavior of your experimental reactors A and B and your control reactor C. Plot a graph of foam height (Y left axis) and temperature (Y right axis) versus time in hours on the X axis for each reactor. On the graph, write in your observations about color and smell. What conclusions can you draw about these reactions?

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2. *You can help make those discoveries if you decide to continue to study science and math.*

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Sincerely,

Jeff Davidson
Executive Director
Pennsylvania Biotechnology Association

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