THE SURROUNDINGS ABSORB ORGANISMS AS OBJECTS,
WHEREAS THE ENVIRONMENT IS SHAPED BY THEM.
AN ORGANISM
IS ALWAYS ALSO PART OF ITS SPECIFIC ENVIRONMENT.
IT IS NOT LIMITED BY ITS SURFACE (SKIN),
BUT BY ITS PERCEPTION AND ITS ACTIVITY,
MOVING THROUGH TIME AND SPACE.

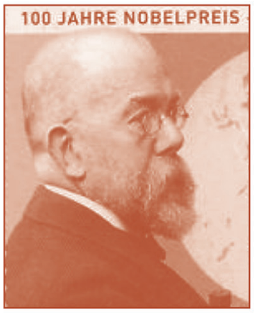
Jakob Johann Baron of Uexküll (1864–1944)

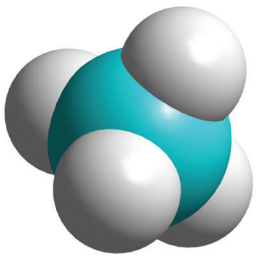
Environmental Biotechnology

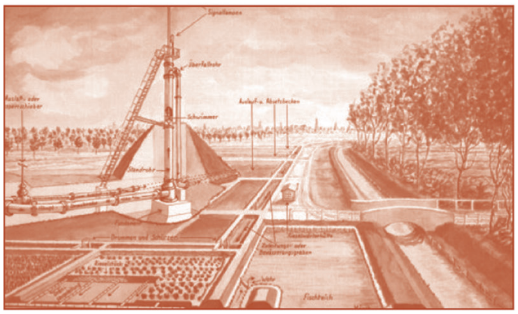
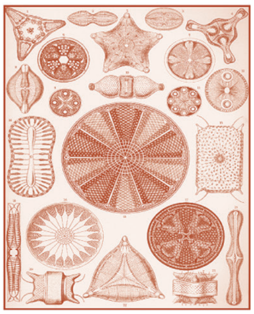
From One-Way Streets to Traffic Circles







Chapter 6

Biotechnology for Beginners.

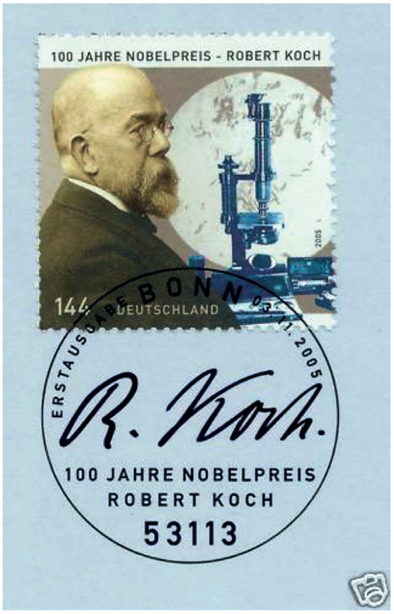


Figure 6.1 *Robert Koch, discoverer of the cholera, tuberculosis, anthrax, and other pathogens; Nobel Prize in 1905.*

Figure 6.2 *(Above) Cholera epidemic in Hamburg, the horror vision of dying masses, courtesy of Archive Bernt Karger-Decker.*

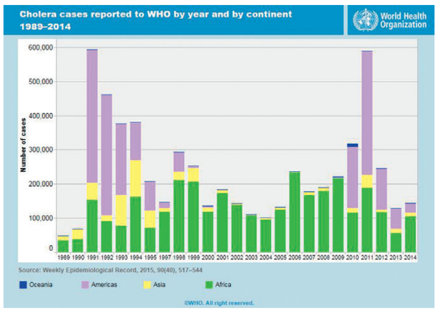


Figure 6.3 *According to the WHO, cholera is present in 40–50 countries. Out of 3–5 million cholera cases, 100,000 die every year.*

6.1 Clean Water—A Bioproduct

Harold Scott (1874–1956), author of *A History of Tropical Medicine*, in 1939 described **cholera** as perhaps the most frightening of all epidemics. It spreads so fast that a man who got up hale and hearty in the morning could be dead and buried before the sun sets.

In 1892, the inhabitants of Hamburg still thought it was **safe to drink water** straight from the rivers Elbe and Alster—a fatal mistake that cost the lives of 8,605 citizens. The rivers had become an ideal breeding ground for microbes, including the cholera pathogen. The neighboring community of Altona, by contrast, let the river water run through simple sand filters and was unaffected (Fig. 6.2). **Sewage treatment** became of paramount importance for large cities.

Although **Robert Koch** (1843–1910, Fig. 6.1) had identified the cholera pathogen *Vibrio cholerae* as early as 1884, improvements in the water supply and sewage treatment were just beginning. Cholera and typhoid certainly had their share in speeding up the process. The diagram in Box 6.1 shows the correlation between the number of typhoid deaths and water hygiene.

Clean water! Every day, the average US family uses more than **300** gal of water per day (1135 L). Food remains, fat, sugar, protein, excrement—everything ends up in the sewers, including washing machine detergent.

Biodegradability of wastewater is measured as **5-day Biochemical Oxygen Demand** (**BOD_5**) in the lab. This tells you how many milligrams of dissolved oxygen would be metabolized by microbes within 5 days in order to completely degrade the organic pollutants in a water sample.

The average organic pollution of domestic wastewater is 60 g/head-day BOD_5. This has also been defined as **population equivalent** (**PE**). In the case of domestic wastewater, 60 g of oxygen would be needed to degrade 1 PE.

Given an average **solubility of oxygen of approximately 10 mg/L of water**, oxygen dissolved in 6,000 L of clean water would be required to remove the daily pollution of just one city-dweller.

Here is a simplified account of the chain of events triggered by untreated sewage ending up in lakes and rivers: **Aerobic microorganisms** degrade organic pollution while rapidly metabolizing the available oxygen. Some areas in the water (mostly near the ground) are so deprived of oxygen that only anaerobic bacteria can thrive.

Fish and other oxygen-dependent organisms begin to die, while **anaerobic microorganisms** produce poisonous ammonia (NH_3) and hydrogen sulfide (H_2S), with its typical rotten-egg smell. These two compounds kill the remaining water organisms that have survived in the low-oxygen environment, **turning rivers into smelly sewers.**

In a **farming environment**, one cow produces as much wastewater as 16 city-dwellers. **1.4 billion cattle worldwide produce about as much wastewater as 22 billion humans**.

Even more worrisome are the huge quantities of **wastewater produced by industry**. They contain large amounts of inorganic compounds, not only salt or hydrogen sulfide, but also poisons such as mercury and other heavy metals that cannot be easily broken down by microbes or may even kill them. Paper mills produce 200–900 PEs per ton of paper, and breweries 150–300 PEs per 1,000 L (264 gal) of beer.

As the **natural cleaning power** of microbes in rivers has long been insufficient, wastewater must be broken down by microbes in huge sewage plants before it can safely be released into rivers and lakes.

Sewage treatment plants are the largest biofactories we have at present. They convert wastewater into large quantities of a bioproduct called **clean water**.

Microorganisms in sewage plants work extremely hard for their livings. In a respiration process using oxygen from the air, they convert sugar, fat, and proteins in the wastewater into carbon dioxide and water, while growing and building new cells in the process. Sewage plants provide ideal conditions for the reproduction of microbes and the breakdown processes they are involved in.

The breakdown of 1 g of sugar requires more than 1 g of oxygen, but only 10 mg of oxygen can be dissolved in a liter of water.

Thus, the microorganisms use up the oxygen dissolved in water very quickly, and wastewater must be continuously stirred and aerated in order to provide enough oxygen—a very energy-intensive process (Fig. 6.6).

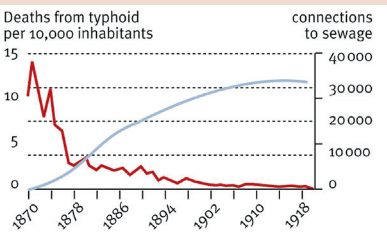
Box 6.1 Biotech History: Sewage Farming in Germany—Success Story!

Until 1878, rain, wastewater, kitchen waste, and to some extent even feces were disposed of through the gutters, which were open ditches between walkways and roads, ending in runoff ditches or in streams or rivers. Where there was no immediate access to a waterway, the discharge went into large subterranean canals which were built ad hoc without a sufficient gradient, but had far too large diameters.

The decaying waste in those canals caused very unpleasant smells, not to mention the nuisance the gutters caused for moving traffic and pedestrians alike. Feces were collected in dung pits that were emptied manually at regular intervals and the contents transported in horse carts along the streets.

Neither streets, gutters, nor horsecarts were properly sealed, which resulted in a considerable contamination of the soil and ground water. People drew their drinking water from wells in or near their houses, and the contaminated water caused the spread of diseases.

As early as 1816, the Prussian Government took a first decision to flush out gutters and streets with water in order to remove dirt and evil smells. However, this was never put into practice because there was neither enough water nor enough pressure. A government committee in the service **of Friedrich Wilhelm IV of Prussia** decided in 1846 that waterworks should be built. They also gave permission to the introduction of **flush toilets**. In 1856, the waterworks were built. In 1873, the waterworks were taken over by the city. However, flushing out the gutters did not have the expected success. Through the easier access to water and the rapid expansion of flush toilets, the amount of wastewater increased dramatically, and although the gutters had been widened and deepened and measured now 1 m (over a yard) across, they simply could not cope.



With the improvement of Berlin' s water supply, typhoid death rates fell in inverse proportion

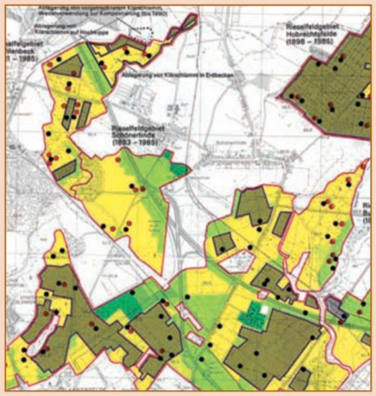
In spite of wastewater becoming a major problem, conservative Berliners still objected to the introduction of a sewage system. People thought it would just increase the foul smells, as did the existing canals.

Rudolf Virchow (1821–1902) (l.), medical historian and hygienist who founded the discipline of cell pathology, and James Hobrecht (1825–1903).

In 1860, Councillor **Wiebe**, who was in charge of public building in Berlin, was sent to study the sewage systems of Hamburg, Paris, and London and come up with a plan to solve Berlin's wastewater problems. He traveled the same year, accompanied by the head of rail and waterway construction, **James Hobrecht**. Wiebe's idea was to collect all wastewater in subterranean canals and discharge it untreated into the river Spree outside Berlin. This plan was highly controversial among engineers, medical doctors, and politicians.

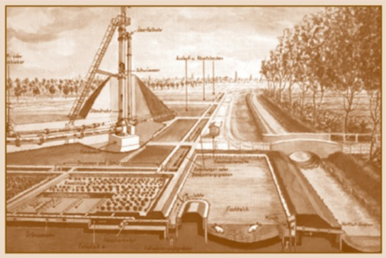
In February 1867, a committee was established, led by the well-known medical microbiologist **Rudolf Virchow (1821–1902)** who, in turn, put Hobrecht in charge of further investigations. Virchow presented his report to the city council in November 1872.

In May 1873, a decision was taken to build a sewage system. The city was divided into several sections, each of which was provided with an independent canal system (radial system). The system had the advantage that if there was disruption somewhere, the whole system was not disabled, and the remaining canals could drain the wastewater from the affected canal. The water flowed through fired and glazed clay pipes. Steam-powered pumps pumped it up to be distributed over sewage farms outside the city. Thus, instead of ending up in sewer riverbeds, the water was treated by the soil bacteria and provided fertilizer for agriculture. Hobrecht divided Berlin into 12 approximately equally sized districts, each of which was allocated a large acreage for sewage farming.



Sewage farms in the South of Berlin, some of which were in use for 100 years, from 1876 until 1976.

Once the sewage system was in operation, gutters and pits with their health and hygiene hazards were a thing of the past, and the areas that had been acquired for sewage farms could be adapted to their new purpose. They were leveled, dams were built, and, depending on the gradient, flat beds or embankments were created on which the water was sprayed evenly. The countryside underwent a complete change, as the sewage farms not only dealt with wastewater, but were also used for agricultural production. **Berlin was a pioneer!**



Schematic representation of a sewage farm where various crops (grain, vegetables, turnips) were grown. Regular and even flooding of the fields was essential.

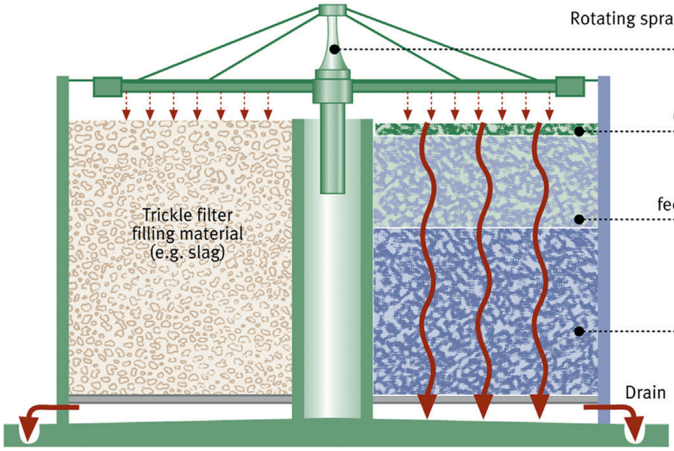


Figure 6.4 *Trickling filter technology.*

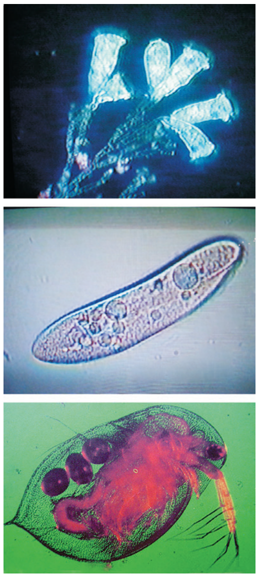


Figure 6.5 *Fresh water organisms in wastewater (Vorticella, Paramecium, Daphnia).*

Figure 6.6 *Aspidisca costata is a nitrate indicator in the wastewater treatment process. Up to 25,000 can be found in a milliliter of wastewater. Not only the number of microorganisms, but also the protrusion of their ribs are indicators. The more nitrates in the water, the more the ribs will stick out.*

6.2 Aerobic Water Purification—Sewage Farms, Trickling Filters, and Activated Sludge

Sewage farms are among the oldest sewage treatment methods that have been developed around European cities such as 19th century Berlin (see Box 6.1). The wastewater was mechanically precleaned and then allowed to trickle into the soil where organic pollutants were microbially degraded.

Sewage farms need a lot of space, so the search began for space-saving alternatives. **Trickling filters** (Fig. 6.4) were developed in England in 1894. They work like **fixed-bed bioreactors** (see Chapter: White Biotechnology: Cells as Synthetic Factories) and are filled with large-pored material such as lava, slag, sintered glass, or plastic. A revolving sprayer head mechanically sprays the precleaned wastewater over the material.

The **large surface of the filling material** is a prerequisite for the activity of wastewater organisms. A lawn of bacteria, fungi, cyanobacteria (blue green algae), algae, protozoa, rotifera, mites, and nematodes develops in the trickling filter—an excellent aquatic community or **biocoenosis**.

A third sewage-processing method, used these days, involves **activated sludge** (Figs. 6.7 and 6.8). It also was introduced about 120 years ago. After mechanical treatment, aerobic bacteria, fungi, and yeasts in the wastewater form large flakes full of nutrients, held together by bacterial slime. These **symbiotic sludge flakes, called floc**, provide support scaffolding for microbes. They float about in gigantic **active sludge basins** (Figs. 6.7 and 6.8) in which paddles or brushes rotate to literally beat air into the water. This ensures that **oxygen-using microbes** proliferate. The throughput is much higher than in a trickling tank because of the better supply of oxygen.

Part of the sludge settles in a settling pond or lagoon, where smells can become a problem due to the open construction.

Wastewater may contain high levels of the nutrients nitrogen and phosphorus. Excessive release of wastewater (or fertilizers) into the environment can lead to a **buildup of nutrients** (**eutrophication**) in lakes and rivers. This encourages the **uncontrolled growth of algae**, resulting in a rapid surge of the algae population or algal bloom. The decomposition of these algae by bacteria uses up so much of oxygen in the water that most or all animals in the water die, which creates more organic matter to be decomposed by bacteria. Anaerobic bacteria take over, producing toxic hydrogen sulfide and methane and killing the remaining life.

Nitrogen is removed from wastewater through the biological oxidation of nitrogen from ammonia (nitrification) to nitrate, followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water.

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2^-) is most often facilitated by *Nitrosomonas* species, nitrite oxidation to nitrate (NO_3^-) facilitated by *Nitrobacter* species.

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal by bacteria. When the biomass enriched in these bacteria is separated from the treated water, these **biosolids have a high fertilizer value**. Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g., ferric chloride) or aluminum. Drinking water is then treated with **chlorine or ozone to kill germs**. A small proportion of the active sludge is brought back into the active sludge basin to ensure that the microbial population is up to the job.

The **efficiency** of active sludge microbes is truly amazing. A cubic meter (264 gal) of sludge can purify 20 times its volume of polluted wastewater. Up to 20% of the basin content is taken up by active sludge. The settled sludge undergoes special treatment in digestion towers in the absence of oxygen.

Methane bacteria (nowadays called methanogens, members of the *Archaea* kingdom, see Fig. 6.14) convert the remaining organic matter into methane, which can be used as a source of energy (biogas, see below).

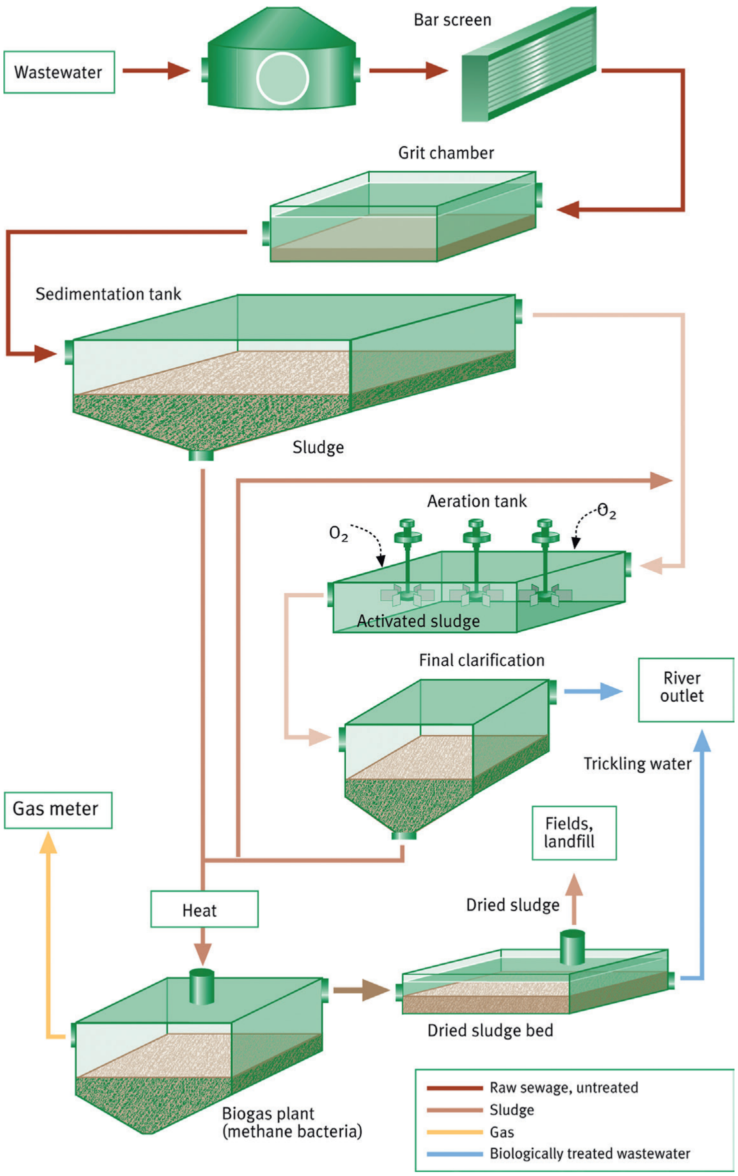


Figure 6.7 *Diagram of a sewage treatment plant with mechanical and biological treatment steps (active sludge basin) and recycling of the sludge in a biogas plant.*

Sewage treatment plants with all their basins take up a lot of space, and space is at a premium, particularly in industrial areas. Space-saving **tower bioreactors** (so called Tower Biologies) that are 15–30 m (45–90 ft) high have been developed by Bayer and Hoechst for their own biological sewage purification (Fig. 6.9). Industrial wastewater treatment in the Rhein-Main area (one of Germany's key industrial regions) deals with approximately 15 billion cubic meters per year.

Deep-shaft reactors that go deep into the ground are another possibility. Their depth ensures that gas bubbles remain in the liquid for a longer time, and oxygen solubility is enhanced by the increased pressure within the reactor.

Both reactor types are supplied with a **rich flow of oxygen from the bottom** and are able to dissolve 80% of it (compared to just 15% in ordinary reactors). They are thus able to break down large quantities of organic matter, taking up very little space and keeping unpleasant smells at a minimum. However, at present, they are expensive and complicated devices.

6.3 Biogas

Will o' the wisp in fens is an existing phenomenon. It has been referred to as "fire in the marshes" in the ancient Chinese book *I Ching* as early as 3000 years ago. In Europe, it was the Italian physicist **Alessandro Volta** (1745–1827) who described the "burning air above the swamps." Those who do not believe in UFOs attribute "flying saucers" seen in marshy areas to the same phenomenon. Fermentation gas rising from the ground contains **methane**, which is flammable. A methane bubble that has caught fire is a perfectly rational explanation for *will o' the wisp* as well as UFOs. What remains unclear, however, is how exactly the gas spontaneously ignites.

The formation of methane can be observed when stirring up mud in a lake. The stomach (rumen) of ruminants could also be described as a miniature biogas factory. The microbial population of a **cow's rumen** turns 8–10% of the cow's food into 100–200 L of the **greenhouse gas (GHG) methane**. It is released by belching or passing of gas. The huge cattle herds owned by fast food chains that graze on burnt-down jungle areas contribute considerably to the methane balance of the planet.

The useful bacterial inhabitants of the **human intestine** produce not only vitamins, but also gases that are only partly reabsorbed by the colon. Depending on the food taken in, up to half a liter of gas per day is released—mainly odorless methane, and only minute quantities of smelly hydrogen sulfide.

Every year, the microorganisms on the planet produce approximately **500 million to 1 billion tons of methane**—which is roughly the equivalent of what is extracted from natural gas fields. Around 5% of the carbon assimilated by microbes during photosynthesis is converted into methane—a major factor in the carbon cycle. Huge **methane hydrate** stores lie under the seabed dating back to the Permian period. They capture the imagination of ecologists as well as thriller writers. If the methane were to be released, it would to lead to a **major disaster**. In contrast to other GHGs, the amount of methane in the Earth's atmosphere has not increased lately, according to Nobel laureate **Paul Crutzen** (b. 1933, Fig. 6.15). For once, the largest

Figure 6.8 *Active sludge basin at the modern treatment plant at Kai Tak airport in Hong Kong. In the plant, oils and detergents must first be separated from the water before it reaches the activated sludge basin.*

Box 6.2 The Expert's View: Gary Strobel on Rainforests, Volatile Antibiotics, and Endophytes Harnessed for Industrial Microbiology

The diversity of microbial life is enormous and the niches in which microbes live are truly amazing, ranging from deep ocean sediments to the earth's thermal pools. However, an additional relatively untapped source of microbial diversity is the world's rainforests.

Gary Strobel on a field trip collecting new samples.

Endophytic microorganisms exist within the living tissues of most plant species and are most abundant in rainforest plants.

Microorganisms have long served mankind by virtue of the myriad of enzymes and secondary compounds that they make. Furthermore, **only a relatively small number of microbes are used** directly in various industrial applications, i.e., cheese/wine/beer making as well as in environmental clean-up operations and for the biological control of pests and pathogens. It seems that we have by no means exhausted the world for its hidden microbes. A much **more comprehensive search of the niches on our earth** may yet reveal novel microbes having direct usefulness to human societies. These uses can either be of the microbe itself or of one or more of its natural products.

Rainforests: Untapped Source of Microbial Diversity

The rainforests occupy only 7% of the earth's land surface and yet represent 50–70% of all of the species that live here. This is nicely exemplified by the fact that over 30% of all of the bird species on earth are associated with Amazonia alone.

The question is **why is so much diversity focused in rainforests?**

Paul Richard's writing in *The Tropical Rainforest* indicates that "The immense floristic riches of the tropical rainforest are no doubt largely due to its great antiquity, it has been the focus of plant evolution for an extremely long time."

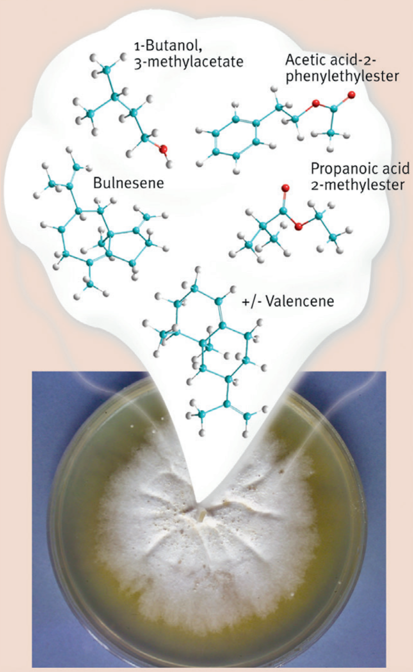
In my opinion, the diversity of macro lifeforms is a template for what probably has also happened with microorganisms. That is, areas of the earth with enormous diversity of higher life-forms are probably also accompanied by a high diversity of microorganisms. **It seems that no one has looked!**

One **specialized and unique biological niche** that supports the growth of microbes are the spaces in between the living cells of higher plants. It turns out that each plant supports a suite of microorganisms known as **endophytes**. These organisms cause no overt symptoms on the plants in which they live. Furthermore, since so little work on these endophytes has been done, it is suspected that untold numbers of novel fungal and bacterial genera exist as plant-associated microbes, and their diversity may parallel that of the higher plants.

Untapped resources for new microbial species: Endophytes in the rainforest.

Some endophytes may have coevolved with their respective higher plants, and therefore have an already existing compatibility with a higher lifeform. Thus, we have begun a concerted search for novel endophytic microbes and the prospects that they may produce novel bioactive products as well as processes that may prove useful at any level. Any discovery of novel microbes will have implications in virtually all of the standard processes of industrial microbiology since scale-up fermentation of the microbe will be necessary.

In order to give the reader a sense of the excitement of discovery that comes with research on rainforest microbes, I have concentrated mostly on the discovery of only one novel endophytic fungal genus—*Muscodor.*



Muscodor albus and some representative inhibitory volatile compounds produced by "Stinky."

The Discovery of *Muscodor albus*

In the late 1990s I was on a collecting trip in the jungles near to the Caribbean coast of Honduras. I had selected this area to visit since all of Central America is one of the world's "hot spots of biodiversity." One modestly sized tree, not native to the new world, was introduced to me as *Cinnamomum zeylanicum*, and I obtained small limb samples. Unfortunately, most plant samples from the tropics are infested with microscopic phytophagous mites.

These nasty creatures infest the bench tops and parafilm-sealed Petri plates. Thus, in order to eliminate this nagging mite problem, we decided to place the Petri plates, with plant tissues, in a large plastic box having a firmly fitting lid. This maneuver would make it difficult for the tiny animals to find their way from the bench surfaces to the inside of the box.

After a few days most plant specimens had sported endophytic fungal growth. Eventually the plates were removed and the individual hyphal tips were transferred to fresh plates of potato dextrose agar.

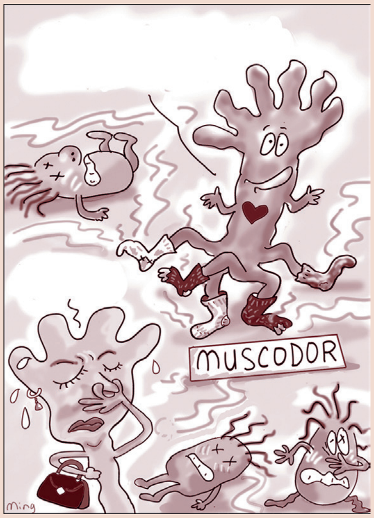
After 2 days of incubation we noted that **no transferred endophyte grew except**

one. Had the placement of the endophytes in the large plastic box killed the endophytes by limiting oxygen availability? Quite to the contrary, it became obvious that the one endophytic fungus (designated Isolate 620) remaining alive was producing volatile antibiotics or volatile organic compounds (VOCs).

The hypothesis that **an endophyte can make volatile antibiotic substances** with a wide range of biological activity was born. It was quickly learned that although many wood-inhabiting fungi make volatile substances, none of these possessed the biological activity of isolate 620.

Isolate 620 is a whitish, sterile endophytic fungus possessing hyphal coiling, ropyness, and right-angle branching. Therefore, in order to taxonomically characterize this organism, the partial regions of the ITS-5.8 rDNA were isolated, sequenced, and deposited in GenBank.

The Gas Chromatography/Mass Spectromety (GC/MS) analysis of the fungal VOCs showed the presence of at least 28 VOCs. These compounds represented at least five general classes of organic substances including lipids, esters, alcohols, ketones, and acids with some representative structures shown in the Figure. Final identification of the volatile compounds was done by GC/MS of authentic compounds obtained from commercial sources or synthesized by us or others and compared directly to the VOCs of the fungus.



*With all of the data in hand we felt secure in proposing a binomial for this fungus derived from the Latin—**Muscodor** (stinky) **albus** (white).*

Ultimately, artificial mixtures of the compounds were used in a biological assay system to demonstrate the relative activity of individual compounds. Although over 80% of the volatiles could be identified, this seemed to be adequate to achieve an excellent reproduction of the lethal antibiotic effects of the VOCs that were being produced by the fungus.

Volatile antibiotics from "Stinky white fungus."

This mixture of gases consists primarily of various **alcohols, acids, esters, ketones, and lipids**. We then examined each of the five general classes of VOCs in the bioassay test and each possessed some inhibitory activity, with the esters being the most active. Of these the most active individual compound was 1-butanol 3-methyl acetate.

However, no individual compound or class of compounds was lethal to any of the test microbes which consisted of representative plant pathogenic fungi, Gram-positive and Gram-negative bacteria, and others.

Obviously, the antibiotic effect of the VOCs of *M. albus* is strictly related to the **synergistic activity** of the compounds in the gas phase. We know very little about the mode of action of these compounds on the test microbes. Thus, this represents an interesting academic avenue to pursue in the future.

Using the original isolate of *M. albus* as a selection tool, other very closely related isolates of this fungus have been obtained from tropical plants in various parts of the world including Thailand, Australia, Peru, Venezuela, and Indonesia. They possess high sequence similarity to isolate 620 and produce many, but not all, of the same VOCs as isolate 620.

"Mycofumigation" With *Muscodor*

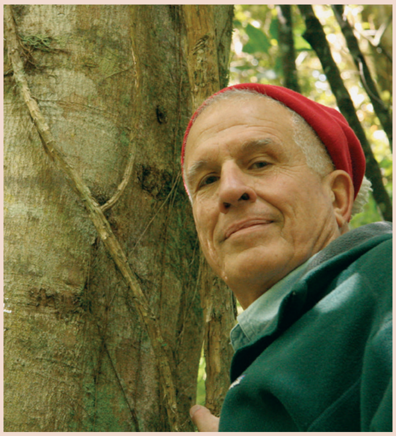
The VOCs of *M. albus* kill many of the pathogens that affect plants, people, and even buildings. The term "mycofumigation" has been applied to the practical aspects of this fungus. The first practical demonstration of its effects against a pathogen was the mycofumigation of covered smut-infected barley seeds for a few days, resulting in 100% disease control. This technology is currently being developed for the treatment of fruits in storage and transit.

Soil treatments have also been effectively used in both field and greenhouse situations. In these cases, soils are pretreated with a *M. albus* formulation in order to preclude the development of infected seedlings.

AgraQuest, of Davis, California, is in the full scale development of *M. albus* for numerous agricultural applications. The US–EPA has given provisional acceptance of *M. albus* for agricultural uses. Also, it appears that the concept of mycofumigation has the potential to replace the use of otherwise hazardous substances that are currently applied to crops, soils, and buildings, the most notable of which is methyl bromide for soil sterilization. AgraQuest is expected to have a product on the market for fruit treatment by now.

Hopefully, the discovery and the development of information on *M. albus* will have broad implications for the discovery and development of other rainforest microbes.

It will at the same time add another impetus for the conservation of the world's precious rainforests, which are under tremendous human threat now.



Gary Strobel is Professor of the Department of Plant Sciences at Montana State University in Bozeman.

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Figure 6.9 *Bayer's "Tower Biology" plant achieves a high oxygen supply and rapid degradation without unpleasant smells.*

Figure 6.10 *Biogas plant near Berlin.*

producers of methane are not humans, but insects—termites, to be precise. One termite (Fig. 6.22), i.e., the microbes in its intestine (mainly one-celled flagellates), produces only half a milligram of methane per day, but given the incredible number of these insects, their yearly production amounts to 150 million tons (Box 6.2).

How does **methanogenesis** happen? Various groups of bacteria are responsible for the anaerobic conversion of large biomolecules such as cellulose, protein, or fat into methane and carbon dioxide. First, anaerobic clostridia and facultative anaerobic (with the ability to live with or without oxygen) enterobacteria and streptococci secrete enzymes into their environment that degrade high-molecular material into their basic components, i.e., sugar, amino acids, glycerol, and fatty acids (see Fig. 6.13) in what is called the **hydrolytic phase**.

Figure 6.11 *Top: Mao Tse Tung, the Great Helmsman himself, inspecting a cooker powered by a biogas plant in the 1950s. The Chinese writing praises the benefits of marsh gas. Bottom: 30 years later, biogas is higher on the agenda than ever. The Great Reformer Deng Hsiao Ping in front of a biogas plant.*

Figure 6.12 *Biogas installation in Taiwan, explained in popular terms.*

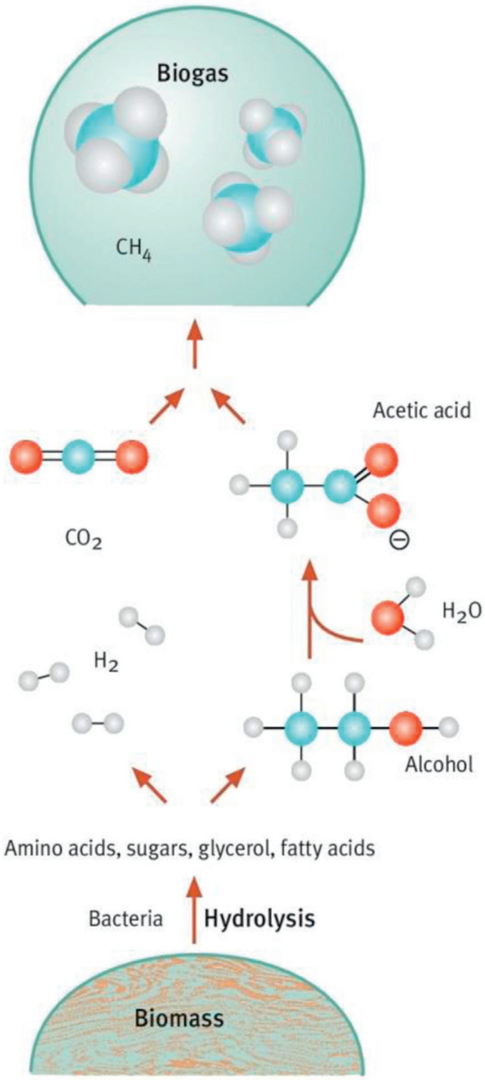


Figure 6.13 *Right: Development stages of methane.*

This is followed by the **acidogenic phase** in which the components are broken down mainly into hydrogen, carbon dioxide, acetic acid, and other organic acids, as well as alcohol. Methane arises from **hydrogen**, **acetate**, and **carbon dioxide** (Fig. 6.13).

Methanogens are the most oxygen-sensitive organisms on Earth. As these archaic creatures lack cytochromes and the hydrogen-cleaving enzyme **catalase**, oxygen that enters the cell causes the cytotoxic hydrogen peroxide to accumulate and destroy cell structures.

Methanogens, together with salt-loving halobacteria and thermophilic sulfur-reducing microorganisms, form the phylum *Archaea* (formerly *Archaebacteria*). Their structure and metabolism is very different from ordinary *Bacteria* (formerly *Eubacteria*), and they are mostly found in extreme environments (Fig. 6.14) where conditions resemble those of earlier periods of the history of our planet when oxygen was scarce.

6.4 Biogas Could Save Forests!

Approximately 2 million people worldwide still depend on burning **biomass** (wood, agricultural waste, and dried dung) for their energy supply—a direct and inefficient way of recovering energy from biomass with **disastrous consequences for agriculture as well as the environment**. In developing countries, wood has become as scarce as food.

Biogas, on the other hand, would be a viable option in agricultural regions where it could be produced by **small reactors fed with animal and human excrement** as well as plant waste. They would also provide natural fertilizer in the shape of sludge, which contains nitrogen, phosphorus, and potash, and thus would reduce the need for artificial fertilizers. Closed, airtight bioreactors (Fig. 6.12) would also kill pathogens. Biogas could help save the forests in many developing countries.

In India, a breakthrough was expected from what became known as the **Gobar project** (*gobar*, Hindi, meaning cow dung). It is estimated that the project ran 1.8 million biogas plants in 1994, bringing the country closer to **Mahatma Ghandhi's** (1869–1948) dream of self-supporting village units.

However, the project is still hampered by the **semifeudal social structure of Indian villages** where only rich farmers can afford to buy a stainless steel bioreactor and to provide it with enough waste products. Poor peasants, by contrast, often do not even own a cow, and even if provided with free reactors, could not provide the material to feed them.

The rich farmers would buy up dried dung very cheaply, thus robbing the poor of the fuel they used to collect from the streets. In addition, for many Indians, touching excrement is taboo for religious reasons. As a result, all the wood that anybody can get hold of is used for heating.

The United Nations look at **China** as a positive example. By 1999 about 7 million biogas plants (Fig. 6.11) are said to be run there, often as communal projects. The reactors were built in cheap concrete reactor technology. Waste, energy, and fertilizer are economically used and wisely distributed.

6.5 Biogas in Industrial Countries—Using Liquid Manure

For obvious reasons, it is more difficult to produce biogas in nontropical countries. However, a wide range of promising projects are under way in Europe and North America. Biogas reactors can help **solve the waste problem of industrial farming.**

Liquid manure from those farming units is produced in such large quantities that it would heavily pollute the soil if used as conventional liquid manure, and carting it away would also be very costly. A dairy cow produces 75 L (nearly 20 gal) per day. In **Switzerland**, farmers used old oil tanks to build a biogas plant which now provides them with the equivalent of 300 L (nearly 80 gal) of heating oil per cow per year. Biogas can also be produced very efficiently from sewage treatment sludge.

Another source of biogas could be household waste. A lot of methane develops in landfill sites and can be quite a hazard. If these sites were properly covered up, methane could be extracted. According to US calculations, 1% of US energy consumption could be supplied by biogas. This may not seem much, but bear in mind that 1% of the US consumption is equivalent of the total energy consumption of several developing countries (Fig. 6.16).

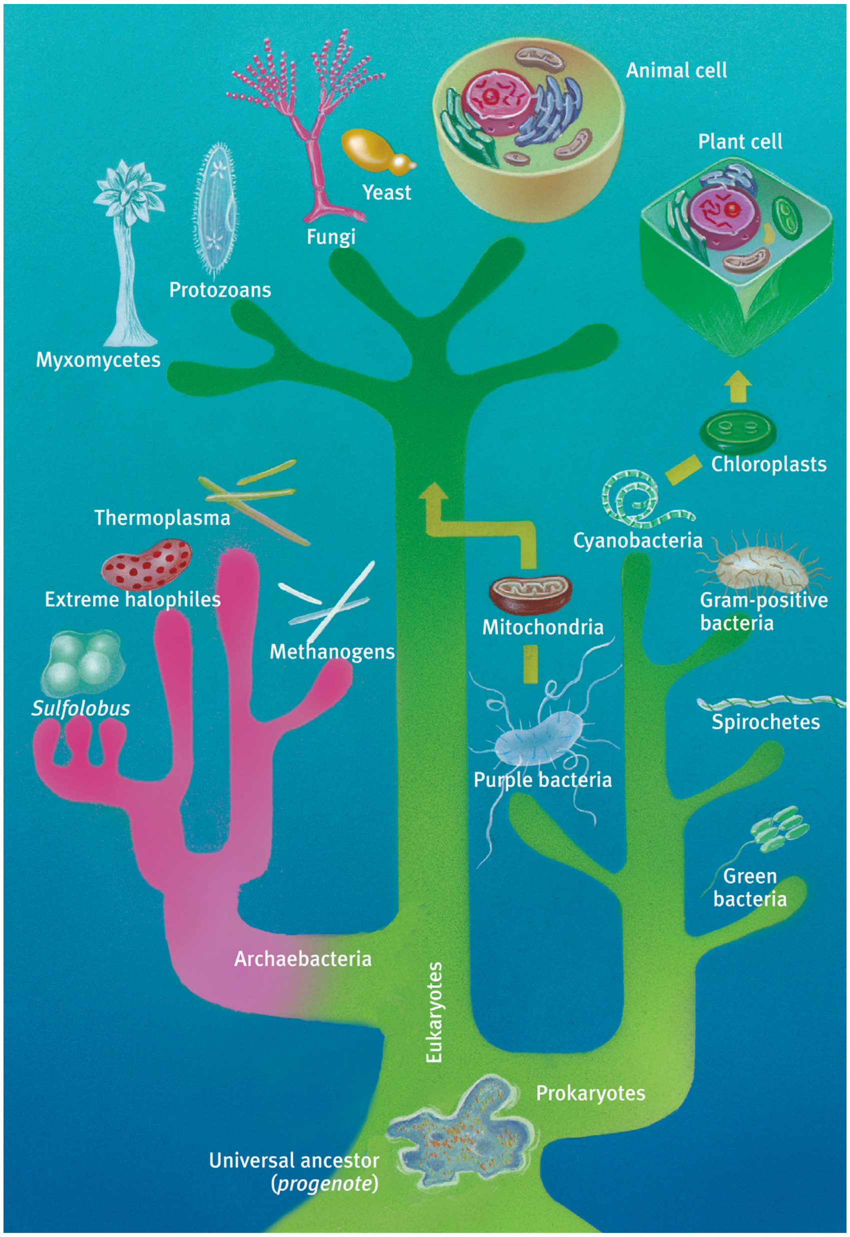


Figure 6.14 *The Tree of Life*

*Before the discovery of **Archaebacteria**, the tree of life consisted of two major lines of evolution—the prokaryotic line and the eukaryotic line, which was derived from the former.*

At the beginning, there must have been anaerobic bacteria that produced energy through fermentation. Once the atmosphere contained enough oxygen, certain anaerobic cells that had lost their cell walls (mycoplasma) ingested smaller bacteria and developed an endosymbiotic relationship with them.

*Among these "swallowed up" bacteria, one aerobic bacterium (with oxygen respiration) became a **mitochondrion**, a photosynthetic cyanobacterium developed into a **chloroplast**, and a spirochete may have turned into a flagellum. The ancestor of eukaryotic cells thus developed.*

*The **dendrogram** of real bacteria, as far as it is known today, branches off into five major lines: Gram-positive bacteria, which include bacilli, streptomycetes, and clostridia, have thick cell walls. The photosynthetic Purple bacteria (e.g., Alcaligenes) and some of their close relatives that are not photosynthetic, such as E. coli, form another group.*

Spirochetes are long, spiral-shaped bacteria.

Cyanobacteria, which are photosynthetic and produce oxygen, probably gave rise to the chloroplasts in plant cells. Some spherical bacteria with an atypical cell walls (micrococci) are extremely sensitive to radiation. The green photosynthetic bacteria (Chlorobium) are anaerobic organisms.

In the 1970s, it was discovered that the prokaryotic archaebacteria differ considerably from all other organisms in their cell structure.

They are a "third form of life," a group apart, which played a dominant role in the primeval biosphere. Due to their oxygen sensitivity, they now only survive in ecological niche habitats.

Box 6.3 Biochemical Oxygen Demand (BOD_5) — Measuring Unit for Biodegradable Substances in Wastewater

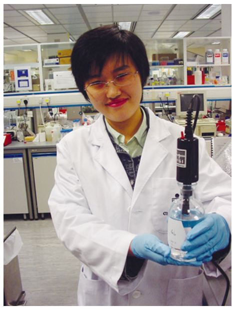
Oxygen has low solubility in water.

At 15°C (59°F), approximately 10 mg of oxygen per liter are dissolved; at 20°C (68°F) only 9 mg/L are dissolved. When wastewater is discharged into lakes, rivers, and the sea, the amount of dissolved oxygen in the water drops dramatically, as it is used up by aerobic bacteria and fungi to break down the organic matter.

Sewage treatment plants therefore require an extra supply of oxygen in order to produce clean water.

BOD is a test developed in England in 1896 to measure the organic contamination of water. BOD_5 gives an idea of the easily biodegradable proportion of the total of organic compounds in the water. It is derived from the oxygen demand of heterotrophic microorganisms.

The amount of oxygen removed from water at 20°C during the breakdown is calculated for a certain number of days—in this case, 5 days. Water samples are diluted and air bubbled into shaking flasks (in order to fully saturate the water with oxygen). It is seeded with a mixed culture of wastewater microbes. The oxygen content is then measured using a Clark oxygen electrode. The flasks are then sealed and kept at 20°C in the dark, where they are shaken. After the incubation period, the oxygen content is measured again. The difference between the first and fifth day (multiplied by the dilution of the sample) results in the BOD_5 value.



Measuring the BOD value using a Clark oxygen electrode in a culture flask in my lab.

If the water did not contain any biodegradable substances, then there was nothing for the microbes to break down, and they will not have been respiring and multiplying. No oxygen will have been used up. The difference between day 1 and 5 will be zero. BOD_5 would then be 0 mg of oxygen per liter. The water is free of easily biodegradable material.

If, by contrast, the water were high in biodegradable substances, then the added microorganisms would have multiplied and used up the oxygen. If, e.g., the difference is 9 mg/L, O_2 has been completely metabolized at a 100 × dilution rate, and the BOD value is 900 mg/L.

Nine hundred milligrams of oxygen would be needed to completely break down 1 L of wastewater. In other words, the oxygen dissolved in 900 L of clean water would be needed to degrade 1 L of wastewater.

The BOD_5 contamination of wastewater by one person per day is quoted in **population equivalents** (**PE**). One PE is approximately 60 g BOD_5/day. The BOD_5 value can be affected by nitrification, algal respiration, or toxic compounds that inhibit the growth of microorganisms.

The BOD_5 value provides a comparability framework for wastewater, and wastewater charges are based on it. However, the BOD_5 does not give any information about nonbiodegradable compounds.

BOD testing has a major drawback, i.e., the long testing time.

Five days of measuring time make it impossible to use the test for the flexible management of treatment plants, whereas microbial biosensors measure the BOD of wastewater within 5 minutes.

However, they can only flag up low-molecular compounds that can penetrate a protective membrane.

Figure 6.15 *Nobel laureate Paul Crutzen (center), codiscoverer of the ozone hole, after a lecture in Hong Kong in April 2005: "1.4 billion cattle on Earth produce massive amounts of methane."*

Figure 6.16 *Strip of land near the former Berlin Wall in 1990, sealed off because of herbicide contamination. My son Tom explores it…*

In the industrialized world, biogas will perhaps cover **1–5% of the total energy** demand, due to the limited amount of available biomass. It is, however, highly significant as an **energy source for agricultural regions** all over the world. Not only the supply of energy, but also the disposal of waste is becoming a pressing issue, and the protection of soil and woodland is a global ecological problem (Box 6.3).

6.6 Fuel Growing in the Fields

When you turn the ignition key, you expect to smell the usual unpleasant exhaust gases, but instead, there is just a whiff of alcohol. This is an everyday experience in Brazil (see Box 6.5 for more detail) where **ethanol-powered and flexible-fuel vehicles** are manufactured. Their engines burn hydrated ethanol, an azeotrope of ethanol (around 93% v/v) and water (7%).

In 2012, around 80 billion liters of ethanol were produced in the world, most of it for use in cars. Brazil produced around 25 billion liters and used millions of hectares of land area for this production. Of this, billions of liters were produced as fuel for ethanol-powered vehicles in the domestic Brazil market. **Food for cars**!

It was the **first oil crisis in 1973/1974** that sparked the gigantic ethanol-for-fuel production in Brazil. A second boost for the industry came during the **second oil crisis in 1979**.

There was already a **sufficient supply of raw material, i.e., sugarcane**. As a reaction to the high world market prices for oil, the state of Brazil launched an ethanol-for-fuel program called *Proalcool* in 1975. The project was politically as well as economically motivated and has been controversial (details in Box 6.5). There is, on the one hand, the question of whether fuel production should take **priority over food production,** and on the other, the issue of significant **environmental impact** through water pollution and soil erosion. Each liter of pure ethanol produces 12–15 L (3.2–3.9 gal) of sugar residuum and 100 L (26.4 gal) of washing water, both of which, for economic reasons, are mostly discharged into the rivers untreated. Sugar residuum that could have been used as fertilizer turns rivers into sewers instead. The organic waste created to produce 1 L of alcohol is the equivalent of the wastewater of four city-dwellers. **One single distillery** producing 150,000 L (around 40,000 gal) per day produces the same amount of wastewater **as a city of 600,000 inhabitants!**

The conclusion? **Biotechnology does not automatically deliver eco-friendly solutions!**

During the alcohol-producing season, the drinking water supply of whole cities was temporarily disrupted. Now, the Brazilian government was pulling the plug: The alcohol industry must use its washing water in a closed cycle, sugar residuum must be turned into animal feed and fertilizer, and the sewage sludge into biogas. The biofuel problem highlights the economic as well as the political aspects that need to be considered in the future development of new technologies.

Lester Brown (b. 1934, Fig. 6.18) director of the Earth Policy Institute, emphasizes that growing crops for biofuel would increase the already excessive pressure on fertile arable land in many parts of the world and finally lead to soil erosion and destruction. According to Brown, 1000 m^2 (0.25 acres) are needed to feed a human over a year, whereas to produce enough crops to fuel just one US car for a year would require an area of 30,000 m^2 (7.4 acres). In other words, **one car "eats" the food supply for 30 people**! The United States are now increasing their own production. According to the Renewable Fuels Association, there were 107 grain ethanol biorefineries in November 2006 with a capacity of 5.6 billion gallons of ethanol per year. Fifty-six additional refineries are under construction in the United States, increasing the existing capacity by a further 13.9 billion gallons.

6.7 Ananda Chakrabarty's Oil-Guzzlers

Could supermicrobes play a part in saving the environment? The Indian-born biotechnologist **Ananda Mohan Chakrabarty** (b. 1938, Box 6.4) who lives in the United States, grew bacteria that could break down the **herbicide** 2,4,5-T, when working for General Electric (GE). The herbicide was used in large quantities during the Vietnam war as a component of **Agent Orange** (which also contained mutagenic dioxin) to defoliate vast areas of jungle (Fig. 6.17). The catastrophic consequences include congenial deformities and cancer in the Vietnamese population and among the offspring of US war veterans.

After successfully producing herbicide eaters, Chakrabarty went on to grow veritable oil-guzzlers (Fig. 6.19 and Box 6.4). He took plasmids from four *Pseudomonas* strains, each of which degrades respectively **octane, camphor, xylene,** and **naphthalene**.

These plasmids were used to create super-plasmids, which were then reintroduced into the bacteria and conveyed the ability to digest all four compounds. These transformed bacteria attacked poisonous crude oil residues with a vengeance and were intended to be used to quickly clean up large oil slicks. The massive

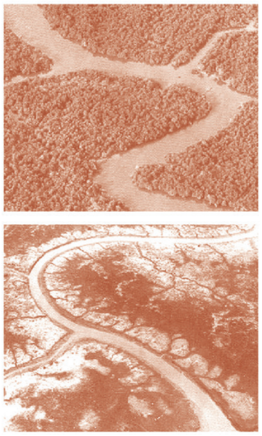


Figure 6.17 *Vietnamese jungle, intact (top) and after defoliation with herbicide Agent Orange (2,4,5-T) (bottom). Ananda Chakrabarty bred Agent Orange-degrading bacteria.*

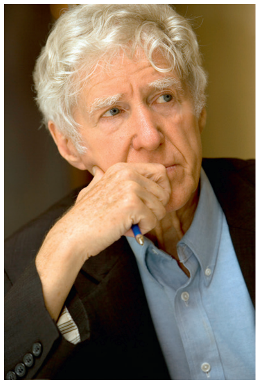


Figure 6.18 *Lester Brown founded the Earth Policy Institute in 1974 and is one of the most prominent thinkers in sustainable renewable energy.*

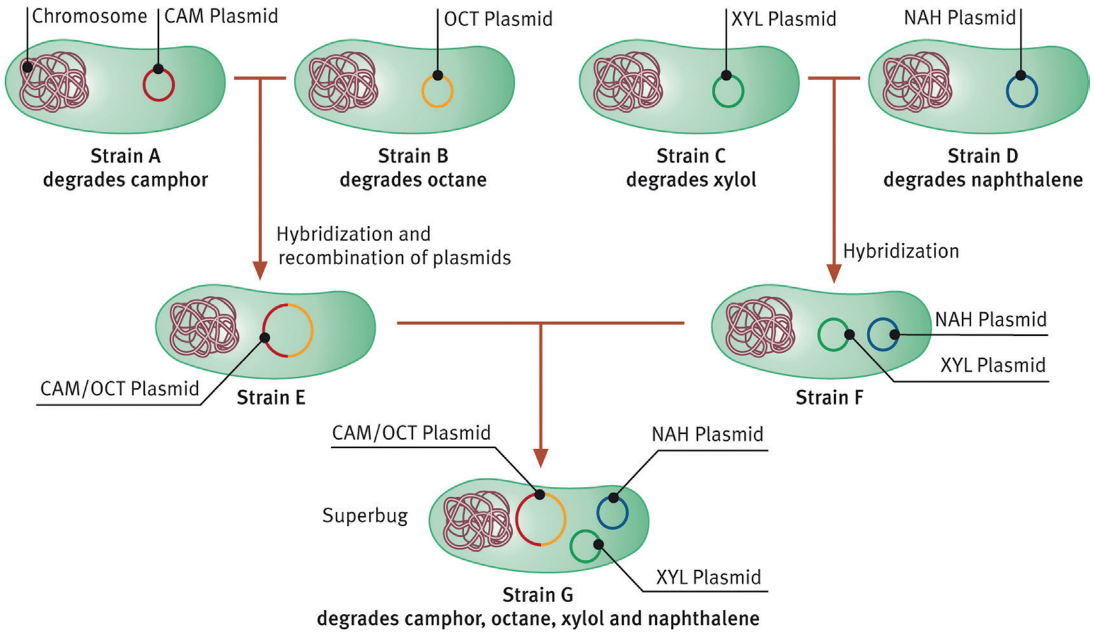


Figure 6.19 *The construction of a superbacterium or superbug that can break down the higher hydrocarbons of petroleum. First, a camphor-degrading plasmid (CAM) was introduced into a plasmid that already contained an octanedegrading plasmid (OCT). Both plasmids underwent fusion.*

Enzymes for both degradation pathways were thus encoded for in one plasmid. Xylene- and naphthalene-degrading plasmids, by contrast, coexisted already in one cell.

Finally, Chakrabarty put all these plasmids together in a single bacterial strain that thrives on petroleum, using camphor, xylene, octane, and naphthalene as carbon sources.

Figure 6.20 *The Exxon Valdez disaster. Day 1–5. The tanker Exxon Valdez at Bligh Reef, March 26, 1989.*

Beaches heavily polluted with oil (Smith Island, April 1989). Attempts to clean up the beaches. Contamination was found even 350 miles away on the Alaskan peninsula (August 1989).

The Aftermath of the *Exxon Valdez* Disaster in Figures

38,000 tons of crude oil (the contents of 125 Olympic swimming pools) ended up in the sea. 1300 miles of coast were polluted.

The estimated number of animals killed is:

250,000 sea birds,

2,800 otters,

300 seals,

250 bald-headed sea eagles,

22 killer whales and vast numbers of fish.

amounts of microorganisms would later be polished off by organisms higher up in the food chain. However, Chakrabarty's oil-guzzlers have never been used due to the restrictions on releasing genetically modified bacteria into the environment.

When in 1989, the **oil tanker *Exxon Valdez*** was stranded on the Alaskan coast, most of the thick oil layer was pumped off and filtered (Fig. 6.20), while the oil coat on rocks and pebbles was degraded by conventionally bred bacteria. Their growth was enhanced by the addition of phosphate and nitrate "fertilizers." Until this day, this is all that can be legally done.

At a more recent oil disaster, the sinking of the **BP oil platform *Deepwater Horizon*** in the Gulf of Mexico on April 20, 2010, a dangerous mix of drilling mud, crude oil, and natural gas had to be dealt with. BP used 2.3 million liters of the solvent Corexit on the surface as well as at the oil well in the sea bed in order to disperse the oil into droplets that could be degraded by bacteria. Corexit had been developed by Exxon and was produced by the US chemical company Nalco. It was used in 1979 after the explosion of the Mexican oil rig Ixtoc as well as the Exxon Valdez disaster mentioned above. The Deepwater Horizon oil slick lasted 5 months, with 4.9 million barrels of oil spilling out. Of these, 0.18 million barrels were removed through siphoning, filtration, and chemical measures. Where did most of the leaked crude oil go then? Well, the laws of nature helped, as lighter compounds evaporate, whereas heavier components sink to the bottom of the sea. The rest disperses in the water, where they are degraded by microbes.

The **damage to the environment** caused by such leaks is mainly dependant on the volume of the oil-gas mix as well as quantity and quality of chemicals used to keep the consequences of the oil spill at bay. Oil and Corexit are toxic to marine life and as such have direct effects on the food chain.

However, tanker and oil rig leaks are responsible only for a small proportion of oil pollution. Every year, **millions of tons end up in the oceans**, a quarter of which comes from illegally cleaning oil tanks in the open sea, while one third comes from wastewater dumped into rivers.

In 1980, the US Supreme Court made a decision in the case of *Diamond versus Chakrabarty* [447 U.S. 303 (1980)]. **Chakrabarty** had filed a patent on an organism in 1971 which had been fighting its way through the courts. His oil-digesting bacterial strain became the **first engineered organism in history to be patented in the United States** (Fig. 6.19)**, creating a precedent for the biotech industry allowing microbes to be patented** (Box 6.4).

6.8 Sugar and Alcohol From Wood

The ideal raw material for the production of sugar, alcohol, and other industrial chemicals is **starch**. However, as starch is a major nutrient, its use for industrial purposes is highly controversial (see Box 6.5).

The most significant sustainable raw material for alcohol production, however, is **lignocellulose**, which cannot be used as a food resource.

Lignocellulose consists of three components—**cellulose**, a linear polymer made up of glucose molecules; **hemicellulose**, also a polysaccharide, containing sugars of five carbon atoms such as xylose; and **lignin**, an aromatic molecule complex. In wheat straw and wood, these components occur in a ratio of 4:3:2 (Fig. 6.21).

Although the price per ton of dry cellulose biomass is considerably lower than that of grain starch, when it comes to conversion into sugar, it simply cannot compete. The **firm structure of lignocellulose**, vital for plants, becomes a major obstacle in the process as cellulose is only available in crystalline form, enclosed by hemicellulose and lignin.

Anybody who still owns genuine old wooden furniture will confirm that this type of **cellulose is not water-soluble**, whereas starch is.

Most microbes are unable to break down wood without some enzymatic pretreatment, and this is precisely the reason why timber has become such a **popular building material**, although it needs to be protected from woodworm, termites, and white rot and blue stain (fungal diseases). All these organisms produce **cellulases** that break down cellulose into sugar and thus destroy the wood.

In methane-producing termites, it is the protozoa in the intestine that produce cellulases (Fig. 6.22).

The current favorite among cellulase-producing organisms is a fungus named after the eminent American cellulase researcher **Edmund T. Reese**, *Trichoderma reesei* (formerly *Trichoderma viride*), which secretes cellulase extracellularly.

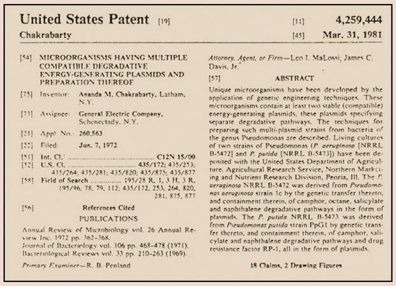
Box 6.4 The Expert's View: Ananda Chakrabarty—Patents on Life?

The first patent on new lifeforms in history was granted to Ananda Chakrabarty. He earned his PhD at the University of Calcutta in India in 1965.

In his days as a young scientist at GE in the United States, he developed a *Pseudomonas* strain that could break down crude oil components into less complex substances on which aquatic life can feed.

The strain was the subject of the landmark 1980 US Supreme Court decision that forms of life created in the laboratory can be patented. Shortly after the decision came through, a spokesperson of pioneer biotech company Genentech (founded by R.A. Swanson and H.W. Boyer, see Chapter: The Wonders of Gene Technology) said that the Supreme Court's action "assured this country's technological future."

Chakrabarty's battle for patent protection is now widely thought to have paved the way for future patenting of biotechnological discoveries.



United States Patent [19] [11] 4,259,444
Chakrabarty [45] Mar. 31, 1981

[54] MICROORGANISMS HAVING MULTIPLE COMPATIBLE DEGRADATIVE ENERGY-GENERATING PLASMIDS AND PREPARATION THEREOF

[75] Inventor: Ananda M. Chakrabarty, Latham, N.Y.

[73] Assignee: General Electric Company, Schenectady, N.Y.

[21] Appl. No.: 260,563

[22] Filed: Jun. 7, 1972

[51] Int. Cl. C12N 15/00

[52] U.S. Cl. 435/172; 435/253; 435/264; 435/281; 435/820; 435/875; 435/877

[58] Field of Search .... 195/28 R, 1, 3 H, 3 R, 195/96, 78, 79, 112; 435/172, 253, 264, 820, 281, 875, 877

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Annual Review of Microbiology vol. 26 Annual Review Inc. 1972 pp. 362–368.
Journal of Bacteriology vol. 106 pp. 468–478 (1971).
Bacteriological Reviews vol. 33 pp. 210–263 (1969).

Primary Examiner—R. B. Penland

Attorney, Agent, or Firm—Leo I. MaLossi; James C. Davis, Jr.

[57] ABSTRACT

Unique microorganisms have been developed by the application of genetic engineering techniques. These microorganisms contain at least two stable (compatible) energy-generating plasmids, these plasmids specifying separate degradative pathways. The techniques for preparing such multi-plasmid strains from bacteria of the genus Pseudomonas are described. Living cultures of two strains of Pseudomonas (*P. aeruginosa* [NRRL B-5472] and *P. putida* [NRRL B-5473]) have been deposited with the United States Department of Agriculture, Agricultural Research Service, Northern Marketing and Nutrient Research Division, Peoria, Ill. The *P. aeruginosa* NRRL B-5472 was derived from *Pseudomonas aeruginosa* strain 1c by the genetic transfer thereto, and containment therein, of camphor, octane, salicylate and naphthalene degradative pathways in the form of plasmids. The *P. putida* NRRL B-5473 was derived from *Pseudomonas putida* strain PpG1 by genetic transfer thereto, and containment therein, of camphor, salicylate and naphthalene degradative pathways and drug resistance factor RP-1, all in the form of plasmids.

18 Claims, 2 Drawing Figures

Here Is His Story:

"Who owns life?" This is a rhetorical question for which simple answers do not exist. The definition of what life is or when it begins has eluded a satisfactory answer since the days of the abortion debate or even earlier, when philosophers and scientists tried to distinguish between living and nonliving objects.

To complicate matters: What is meant by ownership?

In a sense, we all own life, if ownership means having the ability to breed new lives (as we do with cattle, chickens, fish, or plants) and to terminate such lives at will.

My Own Case of Patenting a Life Form

My involvement with the two issues, viz., patenting life forms or judicial lawmaking, goes back to the 1970s when I was a research scientist at GE R&D center in Schenectady, NY.

U.S. Patent Mar. 31, 1981 4,259,444

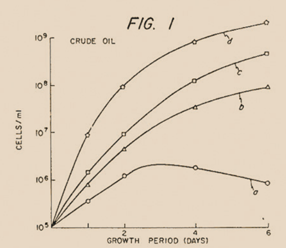


FIG. 1 shows the difference in growth capabilities in crude oil as the sole source of carbon of four single cell strains of *P. aeruginosa* PAO. Curve a shows the cell growth as a function of time of P. aeruginosa without any plasmid-borne energy-generating degradative pathways. Curve b shows greater cell growth as a function of time for SAL⁺ *P. aeruginosa*. Curve c shows still greater cell growth as a function of time for SAL⁺NPL⁺ *P. aeruginosa*. Curve d shows cell growth that is significantly greater still as a function of time for the CAM⁺OCT⁺SAL⁺NPL⁺ superstrain of *P. aeruginosa*. These results clearly establish that cells artifically provided by the practice of this invention with the genetic capability for degrading different hydrocarbons can grow at a faster rate and better on crude oil as the plasmid-borne degradative pathways are increased in number and variety, because of the facility of these degradative pathways to simultaneously function at full capacity.

There I developed a genetically improved microorganism (see this book on p. 213) that was designed to break down crude oil rapidly and was therefore deemed suitable for release into oil spills for their cleanup. Because such an environmental release would make them available to anyone who wanted to have them, GE filed for a patent for both the process of constructing the genetically manipulated microorganism and the microorganism itself.

The **Patent and Trademark Office** (**PTO**) granted the process patent but rejected the claim on the microorganism based on the fact that it was a product of nature. GE appealed to the PTO Board of Appeals, pointing out that the genetically engineered microorganism was genetically very different from its natural counterparts. The Board conceded that it was not a product of nature but still rejected the claim on the patentability of the microorganism because of the fact that the microorganism was alive.

Convinced that such a rejection had no legal basis, GE appealed to the US Court of Custom and Patent Appeals (CCPA). The CCPA ruled three to two in GE's favor. Judge **Giles S. Rich**, speaking on behalf of the majority, contended that microorganisms were "much more akin to inanimate chemical compositions such as reactants, reagents, and catalysts than they are to horses and honeybees or raspberries and roses."

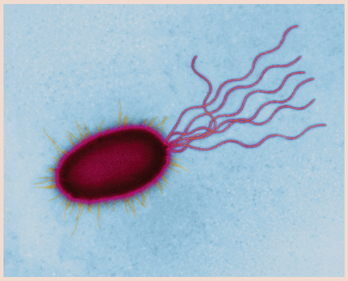
Another judge, **Howard T. Markey**, concurred, saying, "No congressional intent to limit patents to dead inventions lurks in the lacuna of the statute, and there is no grave or compelling circumstance requiring us to find it there."

The PTO then appealed to the Supreme Court. Initially, the Supreme Court sent it back to the CCPA for reconsideration in light of another patent case, but the CCPA again ruled in favor of GE, this time emphasizing that the court could see "no legally significant difference between active chemicals which are classified as dead and organisms used for their chemical reactions which take place because they are live."

The CCPA decision prompted the Solicitor General to petition the Supreme Court for a ruling, which was granted. Several amicus briefs were filed by many individuals and organizations to support or oppose the case, which came to be known as **Diamond versus Chakrabarty** because **Sidney Diamond** was the new commissioner of patents.

One amicus brief filed on behalf of the government came from People's Business Commission (PBC), which argued that patenting a life form was not in the public interest and that granting patents on a microorganism would inevitably lead to the patenting of higher life forms, including mammals and perhaps humans.

The "essence of the matter," PBC argued, was that the "issuance of a patent on a life form was to imply that life has no vital or sacred property and it was simply an arrangement of chemicals or composition of matter."



This fantastic picture of Pseudomonas was taken by Dennis Kunkel (see www.denniskunkel. com). He explained it: "This is a Transmission Electron Microscope (TEM) view of a negatively stained bacterium. It was just luck that I retained most of the polar flagella on this Pseudomonas—this species has a bundle of polar flagella only. The negative stain allows the flagella to stand out nicely when viewed with a TEM."

In 1980, **8 years after the initial filing**, the Supreme Court, by a vote of 5–4, agreed with Judge **Giles Rich** of the CCPA that the microorganisms in question were a new composition of matter, the product of human ingenuity and not of nature's handiwork, and thus a patentable subject matter. Justice **William Brennan (1906–1997)**, arguing on behalf of the minority, pointed out that the case involved a subject that "uniquely implicates matters of public concern" and therefore belonged to congressional review and a new law. Ownership of life forms became a reality with interesting consequences!

What Happened Next?

The decision of the Supreme Court was narrowly focused on what was described as a "soulless, mindless, lowly form of life," even though the court emphasized that anything under the sun made by man could be patented as long as it met the criteria. The PTO took the decision as a broad interpretation of the patenting of life forms and has issued hundreds of US patents covering microorganisms, plants, animals, fish, and bird and human genes, mutations, and cells.

The issuance of **patents on animals and human body cells** conferred a degree of ownership that was an uncharted territory with unprecedented legal ramifications. Many disputes involving patent infringement cases emerged because of questions related to obviousness, enablement, or the priority of invention that had to be decided by the courts.

More difficult were the questions about ownership rights and privileges. For example, in the patent "Unique T-lymphocyte line and products derived therefrom," the inventors used the spleen of a patient, Mr. **John Moore (1945–2001)**, who suffered from hairy cell leukemia and came for treatment to Dr. **David W. Golde (1940–2004)** at the University of California at Los Angeles (UCLA) in 1970. As part of the treatment, Mr. Moore's spleen was removed and Dr. Golde developed a cell line with enriched T-lymphocytes that produced large amounts of lymphokines useful for cancer or AIDS treatment. Without Mr. Moore's initial knowledge or consent, but requiring Mr. Moore's repeated visits to the hospital, Dr. Golde and UCLA applied for a patent on the cell line derived from Mr. Moore's spleen, which was granted in 1984. Mr. Moore subsequently sued Dr. Golde and UCLA, claiming theft of his body part. The trial court ruled against Mr. Moore, but the ruling was reversed by the Court of Appeals, and the case finally ended up at the California Supreme Court. Both the Appeals Court and the Supreme Court recognized the novelty of Mr. Moore's claim, which was without any precedent in law. Nevertheless, the California Supreme Court ruled against Mr. Moore on the issue of conversion (unauthorized use of his body part) but recognized his right to be informed of what the physician was doing involving his health and well-being.

Ananda Chakrabarty in his lab when working with "superbugs."

Who should own intellectual property when a given invention not only requires human ingenuity but also human tissues?

On October 30, 2000, a lawsuit was filed in Chicago federal court by the Canavan Foundation of New York City, the National Tay-Sachs and Allied Diseases Association, and a group of individuals. These individuals and organizations raised money, set up a registry of families, and recruited tissue donors to help develop a **genetic test for the Canavan disorder** caused by a mutation on chromosome 17 in a gene encoding the enzyme aspartoacylase. The disease predominantly affects Ashkenazi Jewish children and a genetic test was deemed to be useful in screening potential parents harboring such mutations. The foundation provided diseased tissues and money to Dr. **Reuben Matalon**, who developed a genetic test while employed at the Miami Children's Hospital (MCH). Once the test was developed, a patent on it was obtained by MCH. However, MCH was alleged to charge a high fee for the test, restrict access to the test, and put a limit on the number of tests that a licensee can perform.

This was contrary to the intention of the individuals and foundations, which was to help develop a cheap test that could be made widely available to prospective parents to prevent Canavan disease.

The question of how important the contribution of the tissue donors is, without which tests cannot be developed and patents cannot be obtained, is going to resonate as more and more such cases end up in courts of law.

Another important consideration is the cost of the tests. Diseases are often caused by mutations, deletions, or genetic rearrangements in human genes. Therefore, genetic screening for some diseases may involve several genetic tests to ensure detection of all possible genetic alterations.

For a disease such as cystic fibrosis, where 70% of the patients harbor a single mutation (a trinucleotide codon deletion) in the CFTR (cystic fibrosis transmembrane conductance regulator) gene, a single genetic test may not be enough and multiple mutations may have to be screened, thus greatly increasing the cost of genetic tests.

Because detection of genetic alterations and development of such tests are time consuming and costly, the developers seek patents on such genetic tests and try to recoup the expense by assessing license fees for conducting the tests. **Who should pay** for these costs? **Who decides** the cost of such genetic tests? Should market forces determine who should live disease-free and who should suffer?

The recent incidence in South Africa where **drug companies allowed low-cost generic production of their patented AIDS drugs** to be made available to HIV-infected patients is an interesting example of the societal responsibility of large drug manufacturers.

Ananda Chakrabarty after winning his case.

Then there is the question of the **patentability of genetic tests** that do not detect all the mutations or genetic variations in a gene.

Myriad Genetics of Salt Lake City obtained a European patent in 2000 on genetic testing of BRCA1 and BRCA2, called BRAC Analysis Technique, which used automated sequencing to scan for BRCA mutations and deletions. Mutations in BRCA1 and BRCA2 account for almost 10% of all breast cancers, and their early detection is important to treat breast cancer. However, certain deletions and genetic rearrangements in BRCA1, involving about 11.6 kb of DNA, cannot be detected by the Myriad tests and can only be detected by a patented process called combed DNA color bar coding. Such genetic deletions may comprise about 36% of all BRCA1 mutations. The claims of the Myriad Genetic European patent allegedly make it difficult for European clinicians to use the combed

DNA color bar coding technique patented by Institute Pasteur, raising difficult legal questions on who should control or establish ownership of human genetic mutations and their detection and on human genetic makeup in general.

There are also **privacy and civil right issues** in patented genetic tests. For example, the US Equal Employment Opportunity Commission (EEOC) sued Burlington Northern Santa Fe Railroad for requiring genetic tests of its employees who filed claims for certain work-related hand injuries (carpal tunnel syndrome).

The railroad wanted to determine which employees may be predisposed to this syndrome, which was believed to be due to a specific genetic deletion on chromosome 17. The railroad was alleged by the EEOC to have threatened to fire employees who refused the test, thus violating the employees' civil rights. This case has since been resolved to the satisfaction of the EEOC.

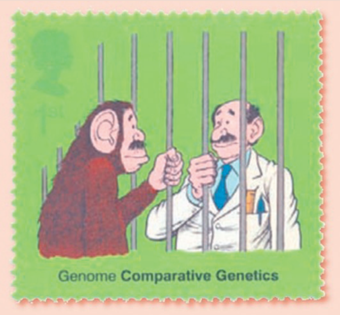
What Is Human?

In 1998, a patent application was filed to the United States PTO for a **human-animal hybrid** which was not actually made, but the concept was based on the fact that hybrids can be made out of sheep and goats. It was pointed out that the DNA sequence identity between human and chimpanzees is of the same order as that between sheep and goats. Consequently, the applicants argued that it should be possible to make hybrids between humans and chimpanzees, and such hybrids could be useful for organ harvesting or other medical purposes. In reality, these applicants did not want the patents but were simply raising the issue to prevent future patents on engineering of human genes and human reproduction. The patent office rejected the application based on the 13th Amendment of the US Constitution, which is an antislavery amendment that rejects the ownership of human beings.

Although the patent application has been rejected, it has raised some interesting questions. If the hybrid human-animal is a human and therefore cannot be owned, how much human genetic material or human phenotypic trait must be present in an organism or an animal to confer on it the characteristics of a human?

Several human genes have been inserted in nonhuman organisms without raising problems of patentability. Is there an upper or lower limit of the presence of such human genes or traits to deter patentability based on the 13th Amendment? A Massachusetts company has claimed to have cloned a human embryo even though the embryo did not undergo enough cell division to give rise to a blastocyst. Given the large number of animals that have been cloned, it is likely that human embryos will be cloned in the future. There are many uncertainties regarding the health and well-being of cloned animals, and certainly there would be enormous resistance to the cloning of human beings. Given scientists' curiosity and the relative ease of cloning, it is likely that somebody, somewhere, would conduct nuclear transfer to enucleated human oocytes.

What would happen if somebody transfers a **chimpanzee nucleus into an enucleated human oocyte** and implants it into a human uterus? Alternatively, one could take a chimpanzee egg and replace the nucleus with that of a human and then implant it in the womb of a chimpanzee. Even with the contribution of the cytoplasmic material, which controls gene expression, or the mitochondrial DNA of the egg, it is likely that the transferred nucleus will determine the primary genotype of the embryo.



Is a baby chimpanzee delivered of a human mother a **chimpanzee or a human**? Alternatively, is a mostly human baby delivered of a chimpanzee mother a human or a chimpanzee? Can such babies be patented if they are not products of nature?

Epilogue

This Is Both an Exciting and a Difficult Time for a Biologist

The technology of animal and human reproduction, as well as the techniques of genetic manipulation, are progressing so rapidly it creates situations that transcend our legal structure and directly affect our social and moral fabrics.

It is high time that the US Congress takes a serious look at where the science is going, where it needs to go to make a positive contribution, and perhaps defines the boundaries of our venture into the unknown biological mysteries of nature. Of course, no Congressional mandate will ever cover all future scientific directions or orient human ingenuity, and the judiciary will play an increasing role in resolving conflicts involving human genetic reproduction, genetically manipulated plants and foods, and environmental restoration. There is thus a great need to continue dialogues between the judiciary, the legal community, the legislature, the interested public, and the scientific community, to provide guidance in scientific developments that may have major impacts on society.

Ananda Chakrabarty today.

***Ananda Mohan Chakrabarty** was born at Sainthia (India) and is at present a Distinguished University Professor at the University of Illinois College of Medicine at Chicago. Chakrabarty's career illustrates a talent for turning research always into practical means. He is currently dealing with a new exciting finding: The ability of certain infecting pathogenic bacteria to allow tumor regression in human patients has been known for more than 100 years. The reason for regression was, however, thought to be due to the production of cytokines and chemokines by an activated immune system.*

Chakrabarty has now shown that bacteria such as Pseudomonas aeruginosa produce the protein azurin, which is secreted when the bacteria are exposed to cancer cells. Azurin, and a modified form of azurin called Laz produced by Neisseria species, are highly effective in forming complexes with various proteins involved in cancer growth and surface proteins of the malarial parasite Plasmodium falciparum and the AIDS virus HIV-1, thereby significantly inhibiting their growth. Thus single bacterial proteins might have potential therapeutic applications against such unrelated diseases as cancer, malaria, or AIDS, including coinfection of AIDS patients by the malarial parasite.

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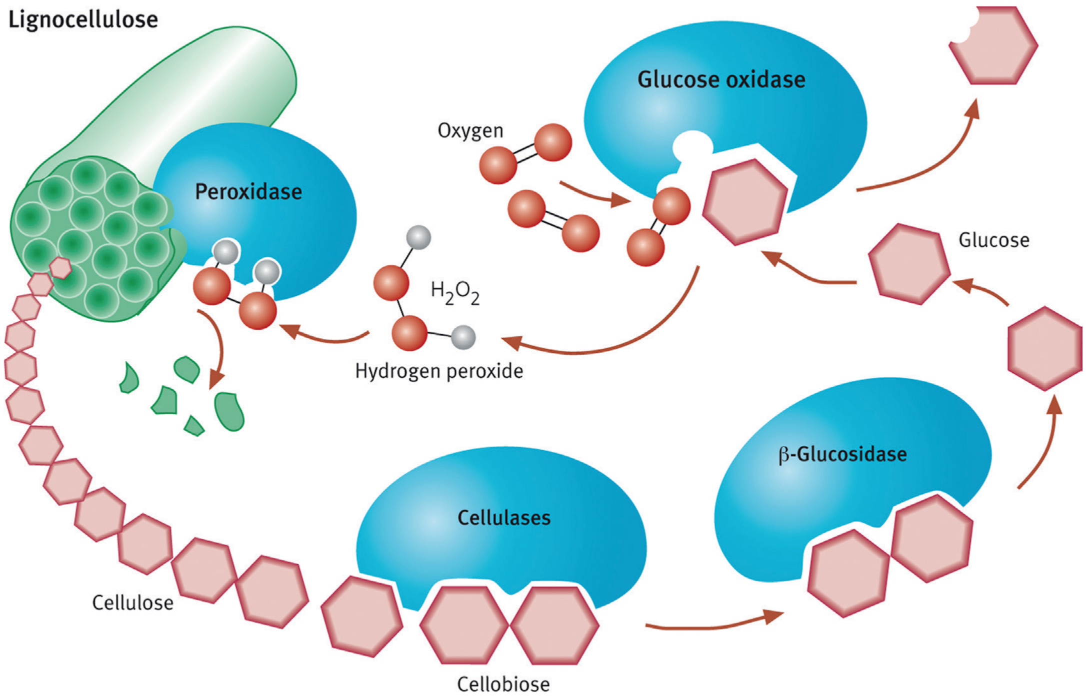


Figure 6.21 *Hypothetical enzymatic degradation of lignocellulose.*

Figure 6.22 *Top: Termites produce biogas through cellulases produced by the intestinal flagellates they carry in their guts. Center and bottom: A drawer in the author's cupboard in Hong Kong that had not been used for a while revealed a beautiful termite colony that turns the wood of the cupboard into methane. Beautiful nest!*

It was discovered on a rotting cotton cartridge belt from World War II in New Guinea (Fig. 6.23).

At the time, microbiologists were following up alarming reports about cellulose-containing equipment of the US forces disintegrating in tropical regions with frightening speed.

Meanwhile, mutants have been found that are **10 times more productive** than the wildtype strain, in turning cellulose into sugar. The **process, however, has remained uneconomical**.

The **degradation products of lignin are toxic to many microbes**—perhaps a natural tree defense mechanism—and no sensible use for lignin has been found so far. **Pretreating lignocellulose with acid** is therefore necessary to ensure an efficient enzymatic breakdown of cellulose, and the removal of the acids is an expensive business. The cost could be reduced by using steam explosion or freeze explosion technologies involving liquid ammonia to remove the acid.

The long **search for lignin-degrading microbes**, however, yielded some unexpected results several years ago. Experts had expected that high-molecular-weight compounds would always be cleaved first by hydrolytic enzymes (hydrolases) produced by microorganisms and secreted into a medium. This is the case for amylases and cellulases.

In lignin degradation, however, a fungus was found that causes what is known as **white rot** in wood. These fungi break down 60–70% of lignin into fragments, carbon dioxide, and water, freeing white cellulose (hence the name white rot).

The lignin degradation mechanism is sensational: The *Phanerochaete chrysosporium* and *Coriolus versicolor* fungi secrete not hydrolases, but **extracellular peroxidases** into the medium. These break the bonds between the phenols in lignin (Fig. 6.21).

The origin of the hydrogen peroxide, without which peroxidases do not function, remains unexplained. It is thought that extracellular oxidases such as glucose oxidase (GOD) provide H_2O_2 when oxidizing glucose.

The unexpected involvement of peroxidases in the degradation shows that enzyme research has so far **uncovered no more than the tip of the iceberg, and further amazing and useful applications may lie ahead**.

The second problems concerning the degradation of wood are the inhibition of cellulases by their own product (**product inhibition**) glucose and its dimer cellobiose, and the comparatively low activity of cellulases. Even the most effective cellulases only exhibit one thousandth of the activity of commercial amylases. **Protein engineering** might be a way to make cellulases relinquish their voluntary self-control. Genetic engineers have been cloning cellulose genes from several fungi into bacteria in order to produce large quantities of cellulase economically. Another possibility of improving the effectiveness of lignocellulose conversion is to convey to bacteria the ability to use the five-carbon sugars (e.g., xylose) of hemicellulose.

Figure 6.23 *In World War II in the Pacific against the Japanese, the cotton clothes and cartridge belts of US soldiers disintegrated at an alarming rate. Cellulase-producing fungi such as Trichoderma were to blame.*

As a last resort, microbes that **metabolize lignocellulose directly**, such as *Clostridium c*, can be used. The wild strains offer a wide range of lignocellulose products, particularly ethanol and organic acids. The production of any of these products can be genetically enhanced, i.e., production can become economically viable.

A company in North America uses the white rot fungus *Ophiostoma piliferum* to pretreat woodchips (biopulping), and within a few weeks, the cellulose yield grows considerably. *Ophiostoma*-inoculated chips break down lignin while at the same time crowding out bluestain competitors.

6.9 Basic Chemicals From Biomass?

Approximately **100 chemical compounds employed in industry make up 99% of all chemicals** used. Three quarters of these are made out of five essential ingredients: ethylene, propylene, benzene, toluene, and xylene. Currently, all of these compounds are derived from petroleum and natural gas, and all the fluctuations and turbulences of the oil markets are passed on to the chemical sector. In addition, the cracking of petroleum and precautions against environmental damage require a lot of costly energy.

Experts believe that in principle, **about half of the top 100 chemicals could be produced from renewable sources**. What is the situation today? Ethanol, citric acid, and acetic acid can be produced cost-effectively through biotechnology (Fig. 6.24).

Ethanol (Fig. 6.24) is an important industrial chemical (see Chapter: Beer, Bread, and Cheese: The Tasty Side of Biotechnology) which is utilized as solvent, extractant, or antifreeze, and as a starter substance for the synthesis of other organic compounds used as pigments, glue, lubricants, medicinal products, detergents, explosive, resins, and cosmetics.

When fossil fuel prices were low and sugar and starch for fermentation were expensive, chemical ethanol synthesis involving ethylene hydration at high temperatures and catalysts widely replaced fermentation. Now we see a renaissance **of the oldest biotechnological method in the world**. Through continuous processing, new highly effective thermophilic and ethanol-tolerant microorganisms, and energy-efficient distilling techniques, the distillation process can be made competitive.

Currently, **acetic acid** (Fig. 6.24) production by oxidation of ethanol by *Acetobacter* is exclusively used for human consumption, whereas highly concentrated industrial acetic acid is obtained through the chemical carbonylation of methanol, which is more cost-effective. Approximately 200,000 tons of acetic acid worldwide are produced from ethanol by fermentation.

An **environmentally friendly application of acetic acid** is being tested in the United States. Acetate, an acetic acid salt, is produced from acetic acid by mixing it with limestone. The acetic acid is obtained from biomass. **Calcium magnesium acetate** has a melting point of minus 8°C (17.6°F) and is used as an **environmentally friendly salt to keep roads free from ice**. Car drivers are happy with it because it also protects cars from corrosion. The sodium chloride widely used in Europe for roads, by contrast, is a tree killer because it competes with essential plant nutrients.

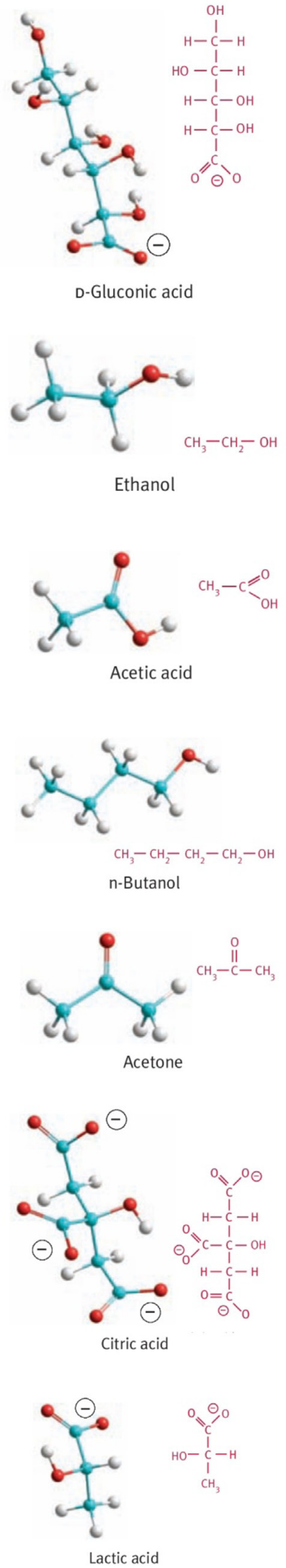
***n*-Butanol** (1-butanol) is an important organic solvent. The n stands for "normal" and implies that the compound consists of a straight chain with no side chains. *n*-Butanol is used in the production of plasticizers, brake fluid, fuel additives, synthetic resins, extractants, and paints. 1.2 million tons of butanol are produced from petroleum every year. The production of butanol began due to shortages in England during World War I and is directly connected to the foundation of the State of Israel (Box 6.6).

Clostridium acetobutylicum produces butanol, with the solvent acetone as a byproduct.

Acetone (Fig. 6.24) was very much in demand in World War I. In Britain, it was needed to produce the explosive cordite. After the war, butyl acetate was used for nitrocellulose varnishes, and there was a shift in interest toward *n*-butanol.

Only in the 1940s and 1950s when prices for petrochemical products fell below those of starch and molasses did the microbial production of *n*-butanol drastically decline. Only South Africa, where petroleum was scarce due to an international embargo, kept running 90-m^3 reactors that yielded 30% solvents, of which six parts were butanol, three parts acetone, and one part ethanol.

Figure 6.24 *Basic chemical compounds that can be produced from biomass.*



Box 6.5 The Expert's View: Nobel Prize Winner Alan MacDiarmid About Agri-Energy

You start your car, and off it goes, leaving a wonderful smell of booze behind…

The old Esso tagline "Put a Tiger in Your Tank" should now read "Put BIO in Your Tank." Many countries—China, the EU, and even the United States under George Bush—have decided to move toward an increased use of bioethanol. Against a background of sharply rising oil and fuel prices, it made sense to fall back on the oldest biotechnology in the world, the yeast fermentation of sugars into alcohol. Many see biofuel as the savior of our future.

Prof. Alan MacDiarmid (University of Texas at Dallas and University of Pennsylvania) gave a lecture about agri-energy at my university in Hong Kong. In 2000, he was awarded the Nobel Prize for the revolutionary discovery that **modified plastic polymers work as electric conductors**. He recently set up a research center in China which looks into China's supply of bioenergy. China has vast desert-like areas where hardy, energy-supplying plants that do not compete with food crops could be grown and provide poor farmers with an income.

Ethanol-fueled car amongst the sugarcane that provides sustainable fuel economically.

Nobel Laureate Alan McDiarmid Explained to the Students:

As we deplete oil, coal, and natural gas reserves globally, the recovery of the remaining fossil fuel may be technologically feasible, but it will not be economically recoverable. It will simply cost too much.

This reality enhances the financial viability of any alternate form of renewable energy because that viability is hitched to the price of oil per barrel on the international market. If the price of oil falls, which is unlikely, the economic feasibility of alternate forms of energy decreases. The most promising source of alternate energy comes from nature's own solar cells—the leaves of trees, bushes, and grass. They absorb sunlight and convert it into various organic materials-stored energy from the sun.

Sugarcane (Saccharum officinarum).

In past eras, people hunted for fish, animals, berries, and roots. Then we learned to grow them through farming. But we are still hunting for energy from forests that grew millions of years ago—i.e., coal, petroleum, and natural gas.

Now that we are more enlightened, we will surely do the same for energy as we have done for food and begin to farm our energy needs.

Like "farms for food" long ago invented by humans, now the time has come to create "**farms for fuel**."

In the future, we will get most of our energy from the growth of plants instead of waiting for them to decay over millennia and then using expensive and complex technology to get that energy out of the ground.

The most prevalent type of fuel that can be grown is bio-ethyl alcohol, or **ethanol** as it is commonly called, which we are already putting into gasoline in significant quantities. It is made primarily from the fermentation of sugar and certain parts of corn.

Biodiesel, which is oil obtained from soybeans, sunflower seeds, jatropha, or the like, is another form of biofuel. In this case, the oil is extracted and no fermentation is involved.

Since a given country may be limited by its climate or soil conditions in the growing of sugar or corn for fermentation into bio-alcohol, of great interest at the moment are advances in creating fuels out of cellulosic materials such as are found in wood-chip waste or dry waste from farm products.

If this cellulose can be broken down by enzymes into sugars that can be easily fermented into ethanol, then there will be few limits to widespread use of biofuels. Pilot projects on this have been implemented, and the results are promising. Every day, the costs are being reduced because the cost of the cellulose enzyme is becoming cheaper and cheaper with active research.

In time, the fuel of the world will be derived from trees, shrubs, and grasses that can grow in essentially any climate in the world, as well as from corn and sugarcane in certain climates. Instead of a petroleum economy, we will live in a bio-alcohol economy.

The beauty of using fuel obtained from living plants is that any carbon dioxide released into the atmosphere when a biofuel is used is then reabsorbed by the leaves of plants. So we get a cycle in which the amount of carbon dioxide released into the air is neither increased nor decreased. Therefore, if we use biofuels of any type, we **do not add to global warming** by increasing the amount of carbon dioxide into the air, as the burning of fossil fuels does.

Of course, no one form of renewable energy, whether biofuels, wind, or solar, can cover all the energy needs of a given country. Necessarily, there will have to be a **mix of fuels according to the local conditions** of climate, soil, and terrain. We will no doubt see wind and hydroelectric powers in one part of the country and biofuels in another. And still some "economically recoverable" fossil fuels in another.

What I'm describing has already happened in **Brazil**, which today is essentially independent of any imported petroleum. It has already produced **6 million automobiles** that run either on pure ethyl alcohol or some combination with gasoline. These are known as flex-fuel cars. You can drive into any gas station and you will see two types of pumps—one labeled alcohol and the other labeled gasoline, although the gasoline already has about 22% ethanol mixed in. Sensors in the gas tanks sense the relative amounts of ethanol and gasoline, and the engine adjusts accordingly. At the moment, a gallon of ethanol is cheaper than a gallon of gasoline.

Jatropha curcas *also called physic nut, is currently grown on millions of hectares across China for its nonedible oil used in candle and soap production. It is now expected to become the main crop for the production of biodiesel. The 13 million-hectare forest, mostly in southern China, should yield nearly 6 million tons of biodiesel every year. The jatropha trees also provide wood to fuel a power plant with an installed capacity of 12 million kilowatts—about two-thirds the capacity of the Three Gorges Dam project, the world' s biggest.*

The number of cars in Europe with a flexible fuel mix are increasing. Ford Motor Company has said that it will produce 250,000 flex-fuel cars in the United States. One wonders: "If Brazil can convert so thoroughly to biofuels, why can't the sole superpower now trapped in its dependence on Middle East oil with all the attendant conflicts that involves and the contribution to global warming that entails?"

It Seems So Straightforward, But Is It? We Had a Controversial Friendly Discussion With Alan MacDiarmid Afterwards:

Recently, the Chinese government has pulled the plug on biofuel. China had been subsidizing biofuels since 2002. In **China**, this meant producing ethanol mainly from corn, sorghum, cassava, sweet potato, and beet. Just 2 months before the change of policy, it had been announced that biodiesel produced from animal and vegetable fat would be introduced to Chinese gas stations. One of the key projects in the 10th 5-year plan had been to substitute unleaded gasoline with ethanol. Five provinces and 27 cities, including Heilongjiang, Jilin, and Liaoning, have already switched. According to the official *Xinhua News Agency*, one fifth of all car fuel used in China was bioethanol. It is thought that biodiesel from oilseed releases fewer carcinogenic substances.

Biofuels, mixed with gasoline or diesel, could help reduce the increasingly intolerable air pollution in China and reduce the country's dependency on oil. As recently as 5 years ago, China's annual corn and wheat production increased steadily, and turning the surplus into ethanol made economic sense.

So what caused this **change in policy**? There is simply not enough arable land available, and grain prices rose steeply over the past few months. The increase between October and November 2006 was a whopping 5%. According to the China National Grain and Oils Information Centre, corn prices per ton in the port of Dalian where most of the corn for export passes through, had risen to 1530 Yuan (CNY) in November, an increase of 200 Yuan since October 2006.

Continuing **to produce alcohol from food** could set prices spiraling out of control and lead to social instability and unrest. Therefore, anyone wanting to produce bioethanol now needs a license to do so, and acquiring one can be a complicated process.

China could be setting a trend that many countries will follow. The **EU** plans to cover 20% of all its energy needs from agricultural production by 2020.

In 2004, 34 million tons of grain were turned into ethanol in the US alone. This figure is set to double to 69 million, mainly from corn conversion.

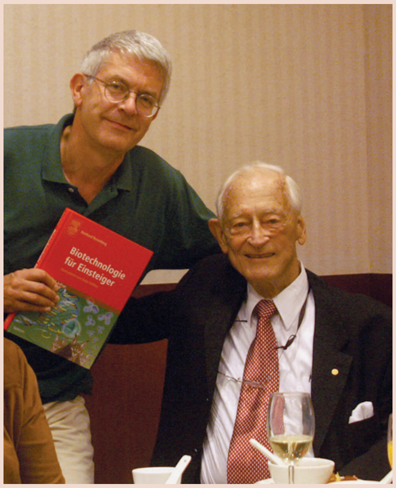
Corn-importing countries such as Japan, Mexico, and Egypt are worried about a possible decrease in US exports, which make up 70% of the world trade. **Corn is the staple diet of the poor in Mexico.**

A poor grain harvest worldwide in 2006 exacerbates a problem. At 1967 million tons, the harvest was 73 million tons below estimated consumption—the largest deficit for years.

What is the solution? Grain could be replaced by **a second generation of energy-providing organic raw material**—straw, wood, manure, and waste material.

Another point critics often make is the **lack of efficiency** of biofuel programs. Brazil seems to be the only country that gets more energy out of sugarcane than it puts in because it also ferments byproducts like crushed pulp. India and China, by contrast, cannot produce sufficient amounts of corn or sugarcane for ethanol production unless they subsidize their agriculture to do so. This is not only due to small farming units and a lack of fertilizers, but also to climatic factors (soil fertility, rainfall) China and India will have to find their own solutions.

Meanwhile, biotechnologists are busy developing energy-rich crops such as **corn with a higher starch content**. They work on optimizing enzymes and microorganisms to accelerate the ethanol production process and on methods that allow the use of plant substrates or agricultural waste, including the use of cellulose.



Alan MacDiarmid was born in 1927. He was educated at Hutt Valley High School and Victoria University in New Zealand. He then won a Shell Scholarship, which enabled him to complete a second PhD Cambridge University. In 1955, he took a position at the University of Pennsylvania. He became a full Professor in 1964 and the distinguished Blanchard Professor in Chemistry from 1988.

In 2000, he was awarded the Nobel Prize for Chemistry shared with physicist Alan Heeger (USA) and chemist Hideki Shirakawa (Japan) for the discovery and development of conductive organic polymers. Alan died in 2007 at the age of 80.

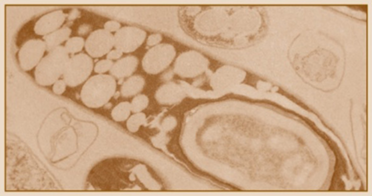
Box 6.6 Biotech History: How a Bacterium Founded a Country

As a young man, **Chaim Weizmann** (1874–1952) was forced to leave his Belorussian homeland due to anti-Semitism. He studied in Switzerland and Germany and then began to work with the famous chemistry prof. **William Perkin** (1863–1945) in Manchester in 1904.

Early in 1915, he came to the attention of the then Minister of Munitions **David Lloyd George** (1863–1945). There was a severe shortage of highly explosive cordite, a mix of nitroglycerin and cellulose. The acetone that was required for its production was particularly scarce, as it was distilled from wood.

Chaim Weizmann and Albert Einstein.

Lloyd George and Weizmann met and immediately took to each other. Was there a way to produce acetone through fermentation? Weizmann remembered **Pasteur's** findings on the fermentation of sugar into alcohol and began to search for suitable bacteria or yeasts in the soil, on corn, and in other grain.



The state-founding bacterium Clostridium acetobutylicum

Within a few weeks after the encounter, Weizmann isolated *Clostridium acetobutylicum*, with the wonderful ability not only to **produce acetone, but also the even more valuable butanol.**

Butanol is needed for the production of synthetic rubber, i.e., for strategically important rubber tires.

Lloyd George was absolutely thrilled and offered to recommend Weizmann to the Prime Minister to be specially honored. Weizmann, however, refused categorically and talked instead of the need for a permanent homeland for all Jews.

When Lloyd George himself became Prime Minister, he discussed Weizmann's wish with his Foreign Minister **Earl Balfour** (1848–1930). This led to the historic Balfour declaration on November 2, 1917 and finally to the foundation of the state of Israel in 1948, whose first president was Weizmann.

Weizmann did more than just find an ingenious method of producing two chemicals. With him, the growth of the **fermentation industry and thus modern biotechnology began**, long before the production of penicillin.

Lloyd George, British statesman, became Minister of Munitions 1915, secretary of war and Prime Minister from 1916 to 1922

Chaim Weizmann, pictured on a bank note.

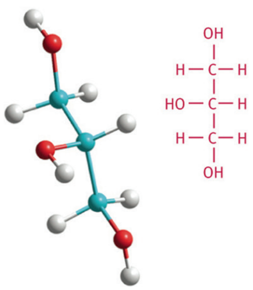


Figure 6.25 *Glycerol.*

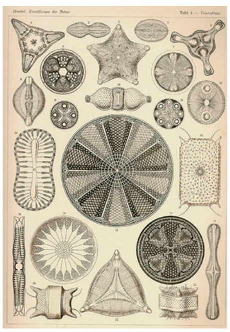


Figure 6.26 *Diatoms provide the kieselguhr for the production of dynamite. Etching by Ernst Haeckel from "Art Forms in Nature."*

With rising crude oil prices and advances in biotechnology in the 1980s, the butanol biotechnological process became more attractive again. Its effectiveness could only be increased if a way could be found to solve a major problem—the toxicity of butanol to bacteria.

Using immobilized microorganisms in a continuous process increased butanol yields by a factor of 200.

Glycerol is a versatile solvent and lubricant (Fig. 6.25) that was already produced by microbes (yeast) during the First World War for dynamite production in Germany, just as Britain used bacterial acetone to make cordite. In order to produce dynamite, glycerol is constantly cooled and dripped into nitrous acid (sulfuric acid plus nitric acid). Small amounts burn off easily with no major risk involved, whereas large amounts, when suddenly heated or hit, explode violently.

Alfred Nobel (1833–1896), who later created the Nobel Prize Foundation, stabilized **nitroglycerin** through kieselguhr absorption. Kieselguhr consists of natural deposits of the silica shells of diatoms (Fig. 6.26), which are highly absorbent due to their large pores.

Sodium sulfite was added to ethanol-producing yeast cultures to bind an important intermediate product of ethanol synthesis. Thus, glycerol was produced alongside ethanol. One thousand tons of glycerol per month were thus produced. After the war, however, this method was replaced by the chemical saponification of fats or by glycerol production from propylene and propane.

Citric acid (Fig. 6.24) is produced by the *Aspergillus* fungus. It is used as a completely innocuous flavoring, as a preservative, or as a cleaning agent. Seven hundred thousand tons are produced annually worldwide, worth $700 million (also see Box 6.4).

In 1780, **lactic acid** (lactate, Fig. 6.24) was discovered in sour milk by the Swedish chemist **Carl Wilhelm Scheele** (1742–1786). **Carl Wehmer** (1958–1935) started its production from glucose by *Lactobacillus delbrueckii* in a small firm called A. Boehringer in 1897. The company later went on to become a world name in biochemistry. Lactic acid is used as an acidifier in the food industry as well as a canned food preservative, in the dyeing of textiles, and in plastic production. In 2015 the overall annual production was approximately 330,000 tons.

Almost half of all lactic acid produced in Europe is produced by microbes, while in the United States, chemical methods are used exclusively. Isolating lactic acid from the culture medium remains an expensive process.

Another bioprocess used in the early days that is currently being rediscovered is the production of **gluconic acid** (Fig. 6.24) from glucose with *Aspergillus niger*. The fungus contains a **GOD** that is vital for the process and is also used as biosensor to measure glucose content (see Chapter: Analytical Biotechnology and the Human Genome). It converts glucose into gluconolactone and hydrogen peroxide, metabolizing oxygen. Hydrogen peroxide, which is toxic to cells, is rapidly broken down by catalase, while gluconolactone spontaneously hydrolyzes into gluconic acid.

Gluconic acid is very versatile and is mainly used as a detergent additive because it binds metal ions and prevents calcium stains on glasses. It also gently dissolves existing deposits without corroding metal vessels. Approximately 60,000 tons of gluconic acid were produced worldwide.

There are other acids that can also be produced biotechnologically, e.g., fumaric acid (salt **fumarate**) and malic acid (salt **malate**). Fumaric acid can be produced by the fungus *Rhizopus nigricans* from sugar or by *Candida* yeasts from alkanes (paraffins). However, chemical synthesis remains the cheaper alternative. For malate, by contrast, a highly efficient biotechnological process using immobilized killed microorganisms was developed by the Japanese company Tanabe Seiyaku in 1974 (see Chapter: Enzymes: Molecular Supercatalysts for Use at Home and in Industry). As the only enzyme obtained from the killed *Escherichia coli* cells, fumarase is used for the production of malic acid from fumaric acid.

Is the **production of industrial chemicals from renewable sources financially viable? For large volumes of products with little value added strict cost control is required and mostly favors fossil resources**.

Many industries will not change their ways in the near future, and the **progress of "biotechnological takeover"** in the chemical industry very much **depends on the oil price** and the development of economically viable biotechnological processes.

Such processes only stand a chance if they can lower present production costs by between 20% and 40%, and, after all, chemical methods and catalysts are under constant review, and improvements are being made.

In the end, **economic considerations determine** whether biotechnological or chemical processes or a combination of both will be adopted. With sufficient interest, as well as investment and economic pressure, most industrial chemicals could be produced biotechnologically from renewable resources. **Biotechnology comes into its own with novel products that cannot be produced chemically, and low-volume, high-value fine chemicals, such as amino acids**.

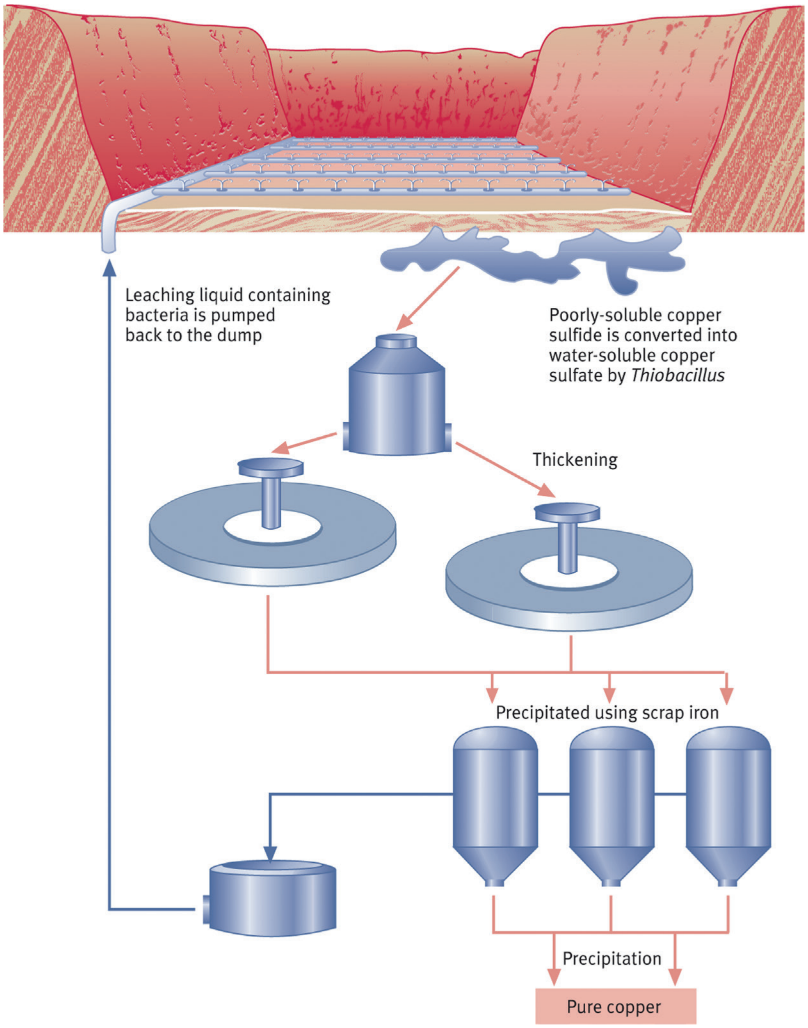


Figure 6.27 *Schematic diagram of microbial copper leaching.*

■ 6.10 Silent Mining

Copper, sometimes nicknamed "red gold," has been mined so extensively in recent years that copper ore with a high copper content has become rare. It has to be retrieved from further below the surface, which makes energy and mine development costs rise.

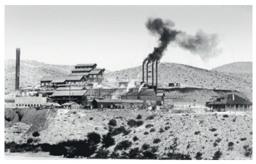


Figure 6.28 *Historic copper factory in the United States.*

The answer to this problem was found in the Mediterranean region as early as 3,000 years ago. Copper was obtained from mine water. It has been documented that the Spanish obtained copper through solution mining in the Rio Tinto.

Figure 6.29 *Copper mine with low-copper rocks, Kennecott Utah Copper.*

Until 50 years ago, nobody was aware that bacteria played an active part in the extraction process, helping to turn **poorly-soluble copper sulfide** into **water-soluble copper sulfate** (Fig. 6.27). Nowadays,

Figure 6.30 *Microbial copper leaching: Liquid containing thiobacteria is sprayed over the ore and then collected (blue liquid).*

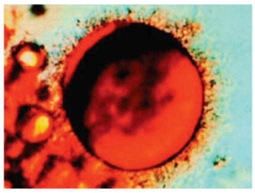


Figure 6.31 *Drop of oil with microbes sitting on it.*

Figure 6.32 *Xanthan extracts oil from pores in the rock.*

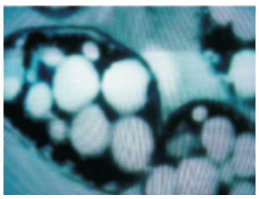


Figure 6.33 *These bacteria (Alcaligenes or Ralstonia eutropha) consist almost entirely of bioplastic (PHB, polyhydroxybutyrate).*

microbes turn billions of tons of low-value copper ore into pure copper. In the United States, Canada, Chile, Australia, and South Africa, they produce one quarter of the total copper production through **bioleaching**. More than **10% of gold and 3% of cobalt and nickel** are biotechnologically produced.

The most important microbes involved in the bioleaching of copper are the **sulfur bacteria** *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans. T. ferrooxidans*, sometimes called **ferros**, is an acidophilic bacterium that oxidizes bivalent iron into trivalent iron and attacks soluble sulfur as well as insoluble sulfides to convert them into sulfates. *T. thiooxidans*, or **thios**, by contrast, only grows on elemental sulfur and soluble sulfur compounds.

The two bacterial strains work closely together via two different mechanisms. In **direct leaching**, the bacteria obtain energy (ATP) through a transfer of electrons from iron or sulfur to oxygen on the cell membrane. The oxidized products are more soluble.

In **indirect leaching**, the bacteria oxidize bivalent iron to trivalent iron, which in turn is a strong oxidant. In sulfuric acid solution, it oxidizes other metals into easily soluble forms. Again, bivalent iron is produced, which is rapidly oxidized by bacteria into its aggressive trivalent form. In practice, there will be overlap between the two leaching processes. Ultimately, **copper leaching results in sulfuric acid and the conversion of insoluble copper sulfide into readily soluble blue copper sulfate**.

Millions of tons of mining waste containing small, but valuable amounts of copper are brought to collection points for bacterial leaching. Such spoil piles can be up to 400 m (1300 ft) high and contain four billion tons of rock material (Fig. 6.29). The rock is sprayed with acidified water. While the water is seeping through, thiobacteria, millions of which are present in each gram of rock, proliferate. A metal-containing liquid runs off the bottom of the pile into large basins. The copper is now easily recoverable, and the copper-free leaching liquid is sprayed over the waste heap again.

When **uranium is leached** (tetravalent uranium ions), bacteria turn pyrite (iron disulfide) or soluble bivalent iron into aggressive trivalent iron, which in turn forms hexavalent uranium ions that are readily soluble in dilute sulfuric acid.

Biosorption is very attractive from an **environmental point of view**: reeds filter toxins from wastewater. Algae have been discovered that bind large amounts of toxic heavy metals, such as cadmium, and might offer a solution for cleaning up wastewater, while at the same time accumulating valuable metals. Various types of **cabbage also accumulate heavy metals**. Concentrations 30–1000 times higher than in the surrounding soil have been observed. Don't eat these…

6.11 A New Life for Tired Oil Wells?

A helicopter lands on an oil platform, and out steps a biotechnologist carrying a small case. The contents of the case are supposed to revitalize an exhausted oil well: microbial cultures that are pumped into the oil reservoir where they multiply. Their products make the oil flow again.

With current primary oil production techniques, two-thirds of the oil remains in the ground. Secondary oil extraction methods involving **fracking** water and gas are used to increase the pressure again (Fig. 6.34). In the North Sea, many oil fields cannot be recovered. Even a few percent of additional output would pay back research and development investments. **Tertiary oil recovery** is the buzzword, also called **MEOR** (microbial enhanced oil recovery).

Several methods are being tried. The seemingly empty oil wells are inoculated with bacterial mixes that produce gases such as carbon dioxide, hydrogen, and methane, and thus increase the pressure on the oil deposits. Other microbial strains are meant to produce **biosurfactants** that shatter oil into little droplets. These can then be squeezed out of minute pores in the rock (Fig. 6.34).

The supply of microorganisms with oxygen and nutrients is currently still a problem, unless they can feed on oil components. In addition, conditions in an oil-field are not for the faint-hearted. There is the high salt content of the sea, the lack of oxygen, the pressure between 200 and 400 atmospheres, and the temperature between 90°C and 120°C (194–248°F). **Only extremophiles can survive here!**

Oil companies are carrying out experiments with microbes that form and secrete long-chained **biopolymers** such as **xanthan**, which is produced from glucose by *Xanthomonas campestris*, a plant pathogen. Xanthan acts as a **thickener**, making water viscous. After soap-like biosurfactants have been pumped into an exhausted oil well to separate the oil from the rock, xanthan water is added. Like the plunger in a syringe, it exerts pressure on the oil and forces it out of the drill hole.

Xanthan is still too expensive to speed up oil production effectively, but it is widely used in the food industry for the production of soft ice cream, pudding, and low-calorie drinks, to give them body. Although xanthan acts as thickener, it is not a fattener, as humans lack the enzymes to break it down. In other words, people who want to be slim feel temporarily full, but have not ingested additional calories.

Forty years ago, xanthan was one of the first modern biotech products. Other products followed, most of which are easily biodegradable.

6.12 Bioplastics—From Dead End to Merry-Go-Round

In Japanese supermarkets, you will find vegetables and ready-meals wrapped in a cellophane-like foil made of **pullulan**, a new bioproduct (Fig. 6.35). It is a polysaccharide, consisting of glucose components which are interlinked via carbon atoms one and six instead of one and four, as in starch molecules. Thus, they cannot be broken down by starch-degrading amylases and **cannot be digested by humans, i.e., they are low in calories**. Like xanthan, pullulan enhances the viscosity of food.

The Japanese Hayashibara company produces pullulan from simple sugars using *Pullularia pullulans*, a fungus. The viscous pullulan syrup is then poured out in thin layers on flat surfaces to form a film when drying. These films provide **excellent wrapping material**, providing an airtight seal around the wrapped produce and later dissolving in hot water. These foils are also environmentally friendly and can be microbially degraded when wet. As a sales gimmick, they are now available in various flavors, e.g., fruit or garlic, which are supposed to retain the flavor of the wrapped food.

Other easily biodegradable products are made of **polyhydroxybutyrate** (**PHB**) (Fig. 6.36), which has the same properties as polypropylene, used in so many everyday plastic products. Whereas polypropylene is a petroleum product, PHB is produced by the bacterium *Alcaligenes eutrophus* (Fig. 6.33) from sugar. PHB is bacterial energy storage material—**the bacteria mainly consist of plastics!**

The new bioplastic material was first developed by biotechnologists of the British branch of ICI and produced by ICI daughter company Marlborough Biopolymers Ltd. in Cleveland, Britain (Fig. 6.38) under the name of **Biopol**. PHB from *Alcaligenes* turned out to be a highly crystalline thermoplast with a melting point near 180°C (356°F).

In the first test runs, molded parts, foil, and fibers were produced. It proved to be good wrapping material, but not superior to polypropylene—a useful, but not particularly attractive alternative from a technical perspective. Things began to change when, alongside 3-hydroxybutyrate components, **3-hyydroxypentanate** was successfully produced by bacteria.

When both components are polymerized into Biopol, the resulting polymer is significantly more elastic and tougher and has a melting point of 135°C (275°F). Biopol is also piezoelectric, i.e., when its crystals are deformed, shear stress causes an electric charge to appear at the surface. The bioplastic could thus be used in pressure sensors.

Its **biodegradability** makes Biopol a very attractive candidate for medicinal use. In the future, stitches need not be taken out any more after surgery. Biopol could also be used for capsules that slowly release long-term medication into the body. In horticulture and agriculture, Biopol-coated nutrients and growth regulators could be put in the soil and be slowly released during the microbial degradation process.

More biotech products are on the horizon. The **silk** produced by the *Nephila* spider is so strong that it is used for fishing in the South Pacific. It can stretch by up to a third without tearing. Attempts are now being made to produce these **spidroins** as recombinant proteins in *E. coli* or even in goats (by the US company Nexia, under the name of Biosteel).

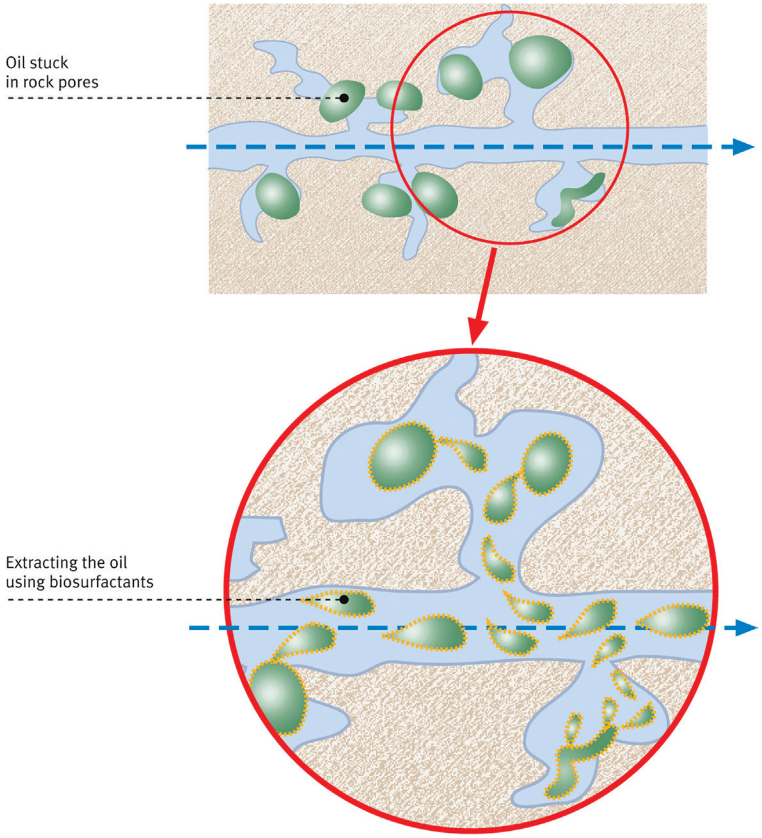


Figure 6.34 *How microorganisms can enhance oil recovery (MEOR).*

Figure 6.35 *Pullulan foil for clear breath melts on the tongue.*

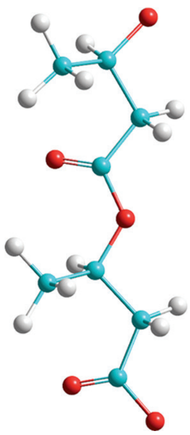
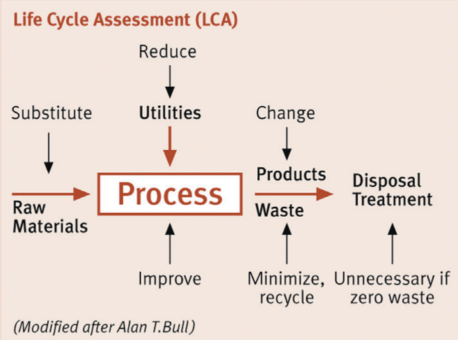


Figure 6.36 *Poly-3-hydroxybutyrate (PHB, only a section shown here).*

Box 6.7 The Expert's View: From Biomass Conversion to Sustainable Bioproduction: Fuel, Bulk, Fine, and Special Chemicals

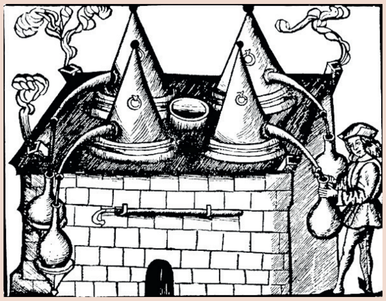


Life Cycle Assessment (LCA)

is defined as "a process used to evaluate the environmental burdens associated with a product, process, or activity by identifying and quantifying energy conversion, materials used, and waste released to the environment, to assess the impacts of those energy and materials uses and releases to the environment, and to identify and evaluate opportunities to obtain environmental developments."

(Society of Environmental Toxicology and Chemistry (SETAC), 1993)

A major motive for the development of industrial biotechnology was the responsible use of resources, long before this way of thinking became mainstream and shaped sustainable development strategies worldwide.



Medieval ethanol distillation.

However, in the currently discussed green scenarios for the industrial use of renewable resources and waste biomass, it is often forgotten that by definition, there are three essential aspects of sustainable development:

- Ecological compatibility
- Social compatibility
- Economic viability

Often, the suggested policies and strategies are too one-dimensional and simply extrapolate from the current policy that relies on heavily subsidized agricultural production.

Canola/rapeseed for biodiesel production

What is required are truly sustainable approaches that also offer satisfactory economic solutions. Economic optimization should clearly aim at an improvement of the material and energy balances (protection of resources) as well as enhanced efficiency of the processes. The guiding principle in chemistry and biotechnology, starting from raw material, should be "minimal structural changes in raw material." In other words, the biomass used for extraction of industrial material should be used as intelligently as possible, i.e., maintaining the high degree of organization and the C/H/O ratio as far as possible when converting it. To quote a positive example: The established oil and fat industry largely follows these principles, as it practically retains the starting weight throughout the process. When using biomass, the aim should therefore not be to produce chemical equivalents of petrochemically based products (which would be possible with considerable transformation losses), but functional equivalents that are structurally closer to the starting material.

In a fairly recent OECD report, The application of Biotechnology to Industrial Sustainability , a method called Life Cycle Assessment (LCA) has been put forward, which includes energy and raw material use, waste products and byproducts, ecological compatibility of processes, and safety aspects in the assessment. This is documented by 21 detailed case studies.

Here Are the 10 Conclusions of the Report

- Global awareness of environmental problems will induce major efforts to achieve cleaner industrial processes.
- Biotechnology provides strong enabling technology that can ensure clean products and processes on a sustainable basis.
- The assessment of industrial production processes is essential as well as complex. LCA is currently the best available method for such assessments.
- The major driving forces behind industrial biotechnological processes are the economy (market forces), government policy, and science and technology.
- In order to achieve more (market) penetration for the biotech sector, R&D efforts by government and industry must be united.
- In order to use the full potential of biotechnology for clean products and processes beyond the current applications, more R&D is needed.
- In view of the increasing significance of biotechnology, including recombinant DNA technology and its applications for the production of high-value products and the development of biocatalysts, harmonized and supportive rules and regulations (for the industry) are needed.
- Market forces can provide strong incentives to reach targets in environmental protection.
- Government policies to encourage clean industrial products may be the single decisive factor for the development and industrial application of clean biotechnological processes.

Searching for new enzyme application in a screening test at Shenzen Lab (PR China).

- Communication and education will be needed to achieve market penetration for clean biotechnological products and processes in various industrial sectors.

Biotechnology and Biocatalysis: R&D Analysis From an Industrial Perspective

The chemical industry still has a key role to play in developing sustainable production strategies. An essential structural change must take place within the industry in order to establish more and more biological processes and biology-based thinking in R&D. The current situation (slightly caricatured) could be described as follows:

- Innovation in the catalytic sector is viewed and evaluated very differently in university and industry. Invention must not be confused with innovation!
- The chasm between purely academic and real industrial needs is becoming wider. Accumulation of knowledge does not necessarily lead to new applications and problem solutions.
- In the academic sector, there is a one-sided emphasis on publishing and too little effort goes into innovative applications of research results (e.g., through cooperation with partners in industry or via spin-offs). Incentives for the development of applications must be put in place.
- Screening efforts are insufficient on both sides. Additions to the biocatalytic toolbox are essential.

In order to improve chances of success of the biocatalytic and thus the white biotech industry, we can define the following strategic objectives:

- The industry urgently needs new types of biocatalysts to catalyze new (so far unknown or not biologically accessible) types of reactions. The opportunities offered by the vast, as yet unresearched, biodiversity of living organisms must be used as much as possible. Intelligent, rapid, and targeted screening methods must be developed. These are challenges that can only be met by the cooperation of industry and universities.
- In some but by no means all cases, the genetic, chemical, or physical improvement of known biocatalysts is necessary and desirable. In the interest of an optimal use of resources, universities and institutes should take more care when choosing the "right" systems. This can be done in close dialogue with industry.

In 2005, the Saab 9–5 Biopower automobile, which can be fueled either with ethanol or gas/petrol, became a runaway success in Sweden. In 2010, the second generation was produced.

- New and well-characterized biocatalysts with large application potentials should be made commercially available as fast as possible in order to have an impact on academic as well as industrial research and development. To quote a negative example: Nitrilases have been known for over 20 years, but only recently have they been available via some enzyme production companies.

Prof. Oreste Ghisalba *has been Head of Bioreactions at CIBA-GEIGY (Central Research Laboratories and Pharma Division) since 1982 and later at Novartis Institutes for Biomedical Research, Discovery Technologies. From 1991–2002, he was Programme Director of the Swiss Priority Programme Biotechnology of the Swiss National Science Foundation and since 1999 Professor for Biotechnology at the University of Basel.*

Since 2003, he is Head of CTI Biotech (Swiss Innovation Promotion Agency CTI) and since 2005 Member of the Board/Vice President of the Swiss Biotech Association SBA.

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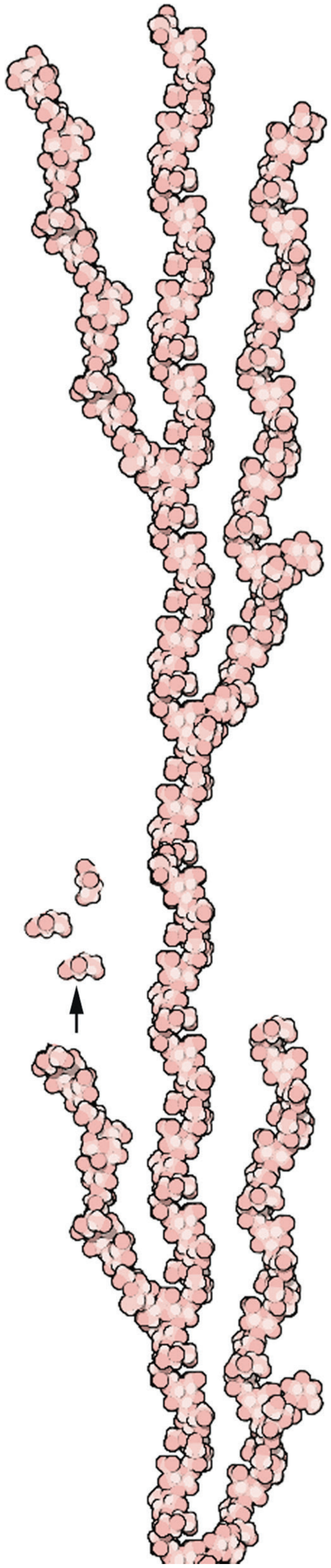
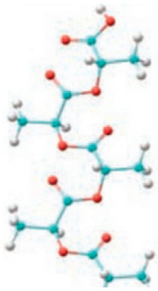


Figure 6.37 *Renewable resources that support sustainable industries. Here is the multibranched molecule of starch. Amylases cleave it into simple sugars which are then fermented to alcohol.*

Figure 6.38 *Biopol is biodegradable material.*



PLA

Figure 6.39 *Biodegradable products in Japan: Self-degrading food containers and backpack.*

Figure 6.40 *A biodegradable corn CD.*

The Japanese telephone company NTT DoCoMo sends bills to customers in eco-friendly envelopes. The plastic window in the envelope began its life not in an oil well, but in a corn field. It consists of **polylactate** (polylactic acid, PLA). In polylactate, lactate (lactic acid salt, see Chapter: Enzymes: Molecular Supercatalysts for Use at Home and in Industry) is polymerized into a chain. It is obtained from glucose by microbial fermentation of corn starch (Fig. 6.39).

In 2002, Cargill built a plant in Nebraska (USA) with the ability to produce 140,000 tons of PLA per year. It is sold under the name of NatureWorks PLA.

Sanyo is promoting a CD called MildDisc made from PLA (Fig. 6.40).

However, its heat sensitivity and its price are prohibitive. At 60C, PLA becomes soft. Biodegradable teabags and food containers are being developed. However, at 500 Yen ($4.00) per 1 kg of bioplastic, it is still **three times the price of plastic produced from petroleum**. This could change, however, if there were mass demand for PLA products.

If it were possible to make all sorts of waste dissolve into thin air instead of polluting the environment, that would be a biotechnological success story, **turning a one-way-road (raw-material-product-waste) into a succession of natural cycles** (Fig. 6.41).

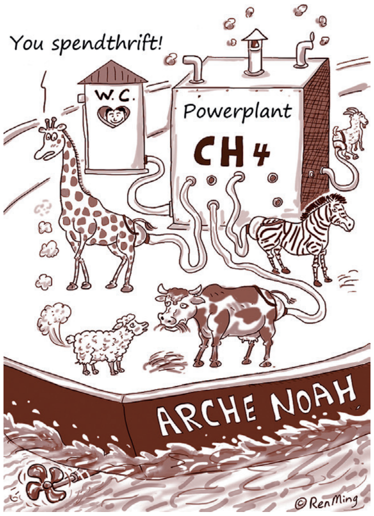
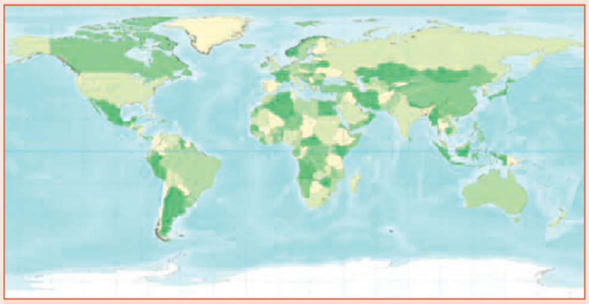
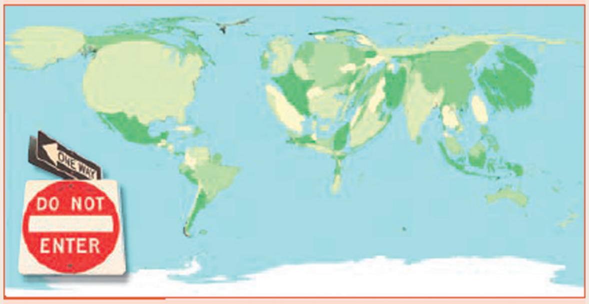


Figure 6.41 *Noah's Ark.*





Cartograms made by Mark Newman (University of Michigan) show the territory (top) and the fuel consumption (below) of the world's countries.

North America and Western Europe import the highest values of fuel.

The region that imports the least fuel is Central Africa—where 6 of the 10 territories reported no fuel imports. Imports per person are highest in Singapore and Bahrain. These are small island territories where enough of the people living there are relatively rich.

Singapore is a long-established trading port. Bahrain is a group of islands in the Arabian Gulf. Due to declining oil reserves, Bahrain´s industry now imports crude oil, refines it, then exports it.

The Western European region records the highest total fuel imports, partly due to trade within this region. Territory size shows the proportion of worldwide fuel imports arriving there.

Goethe and Recycling

My dream dog will not bite
Nor growl or snarl at you
It thrives on broken glass
And leaves diamonds in its p… . J.W.Goethe in *Tame Xenien*, vol. 8

Box 6.8 The Expert's View:
Can Biomass help us to overcome Energy Shortages?

Biomass fuels in the shape of firewood and coal have always provided us with energy. In more recent times, the focus was mainly on the production of liquid fuel, on which our daily life now very much depends, especially in transport. There is also high energy demand for heat and power generation as well as for industry. The main problems concerning liquid fuels are:

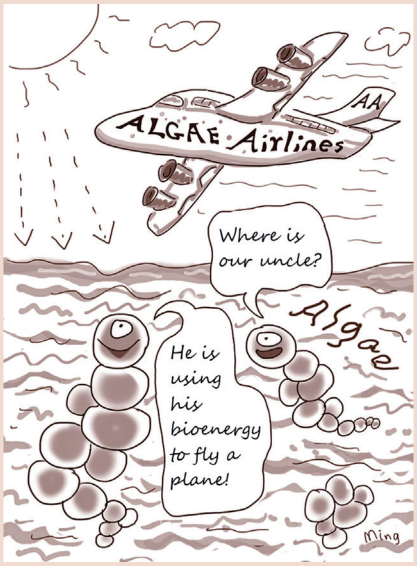
- The steady, ever-rising demand for them, as living standards rise in many emerging economies such as China, India, Brazil, etc.;
- The **relatively low price** of fossil fuels, with which they are in competition. In the middle of 2011, the price per barrel was between \$80 and \$100 and it fell to \$50 in the beginning of 2015. Current oil production costs in Saudi Arabia are estimated at approximately \$10 US per barrel. (Before the first oil crisis in 1973, production costs were just \$0.85 per barrel.);
- **Sustainable solutions** that must be found—natural resources that do not result in competition for the use of land for food production and do not contribute to global warming or lead to detrimental changes of land use.

In 2010, the proportion of biofuels in the transport sector worldwide was 2.7%, and this went up to 4.7% in Europe by 2013.

Biofuels are categorized into **three different generations** according to the **sustainability** of the **product in question**. Sustainability, which means the effects on environment, economy and society, has become the yardstick for decisions on which technologies should be supported on an industrial scale.

As the concept of sustainability became increasingly prominent, new methods were developed for the comparison of large-scale production methods for liquid fuels. The many different technologies and substances that could help ensure the supply of liquid fuels are assessed in what is known as LCA (see Box 6.8). The pros and cons of the various methods can thus be weighed against each other.

What is more difficult to agree upon is what criteria should be included in an LCA[1]. For instance, a parameter that had long been overlooked has only recently been included—the use of land and the changes it undergoes, as more and more land is required for the cultivation of feedstocks[2]. These changes have a major impact on the sustainability of a method, as does the reduction of GHG emissions through biofuels. How significant the reduction is depends on the raw materials used. In a comparison of raw materials for the production of bioethanol, it was found that GHG emissions fell by 80% when sugar cane was used, whereas for wheat, vegetable oils, and sugar beet, emissions dropped by between 30% and 60%—for corn even less than 30%.



Algae are promising candidates for biofuel production. The German airline Lufthansa is running biofuel trials.

What boosted the production of biofuels in recent years was mainly legislation stipulating that a proportion of overall energy consumption must come from renewable sources. The 2003 EU Directive on the promotion of the use of biofuels or other renewable fuels for transport set a target of 20% biofuels by 2020. In 2008, this figure was lowered to 10% in view of sustainability criteria. Similar legislation exists in the United States, where 20% of the national power supply must come from renewables (not exclusively biofuels) by 2020. Quality standards for bioethanol and biodiesel were developed to ensure that engines are not damaged and existing emission legislation is adhered to.

Biodiesel and Bioethanol

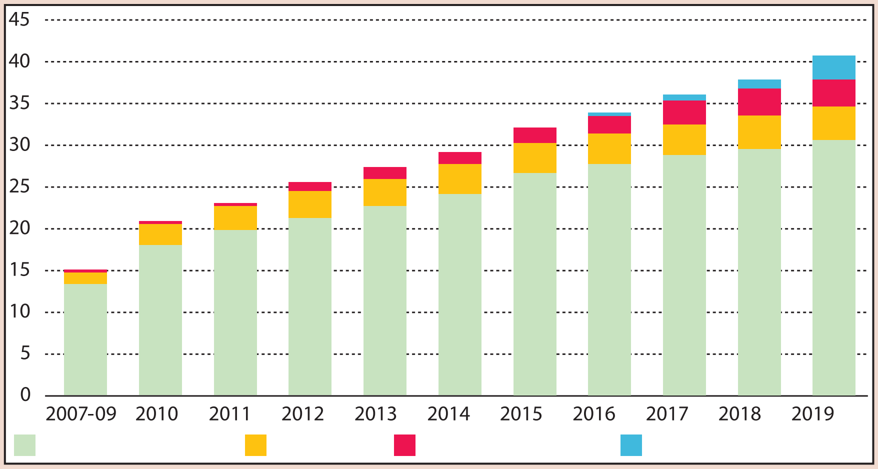
The biofuels of the first generation were made from crops that are grown in large quantities and for which there is usage competition. There was a slight production increase in the 1990s, followed by an exponential increase in the 2000s. The decision as to what biofuel should be produced and what crop should be planted as raw material depends on local conditions such as climate, regulations, and available subsidies.

Biodiesel is produced from fats (triglycerides) derived from different sources, for instance animal fat, vegetable oil, or recycled fat. The most frequently used fats are vegetable oils from various sources, which are easy to handle and do not exude unpleasant smells (unlike animal fats). In the United States, biodiesel is mainly produced from soy beans, whereas in Europe, canola oil is the most frequently used vegetable oil. In tropical countries, palm oil, and coconut oil are used and—more recently—Jatropha oil from another sustainable nonfood crop. *Jatropha curcas* seeds contain 27–40% of oil not fit for human consumption and are considered particularly suited for biodiesel production.

The fatty acid methyl esters produced are linear-chain fatty acids, which distinguishes them from diesel obtained from fossil fuels that have branched chains. This affects the physicochemical properties of biodiesel. It has a greater viscosity and is more sensitive to low temperatures. Biodiesel is a product characterized by a higher cloud point, a higher oxygen proportion, lower stability, and lower energy density than diesel fuel produced from petroleum.

The most common method of producing biodiesel is transesterification of triglycerides using an alcohol, usually methanol or ethanol, in the presence of an alkaline catalyst. This yields methyl or ethyl esters from fatty acids and glycerol. The reaction can give yields of 98% under favorable conditions. One of the greatest obstacles in biodiesel production is the high water content of the raw material, because water inhibits the reaction and favors saponification.

As biodiesel has combustion properties similar to fossil diesel, it is suitable for normal diesel engines. Biodiesel production in the United States in 2009 was around 300,000 barrels per day (compared to a worldwide production of approximately 85 million barrels per day



Worldwide biodiesel production, according to primary sources used. Reproduced with kind permission from OECD-FAO Agricultural Outlook.

for petroleum). In the same year, ethanol production in the United States was 1.3 million barrels per day.

The main focus currently lies on tapping new oil sources in order to become less dependent on the cultivation of certain crops. A large variety of microorganisms with a high lipid content—up to 75%—have been found, such as algae, bacteria, filamentous fungi, and yeasts, but not all of them are suitable for commercial biodiesel production. Due to their low productivity and the need for purification, they would be too costly to use. The use of lipids from plants, by contrast, is subsidized. As a consequence, biodiesel production in the first 6 months of 2011 surpassed the total 2010 production in the United States.

The largest biodiesel producer worldwide, however, is the EU, which produces 65% of global output. In 2009, biodiesel accounted for 75% of biofuels in Europe. The remaining 25% was bioethanol.

Bioethanol is largely produced through fermentation. The most commonly used method uses low-cost carbohydrates as a substrate for the yeast *Saccharomyces cerevisiae*. Which carbohydrate is selected as primary material depends on climatic and geographic conditions. In Brazil, sucrose from sugar cane is used as the carbon source for fermentation, whereas in the United States, glucose from corn is used—corn starch that has been converted by amylase into glucose. Using ethanol as fuel has some drawbacks: for instance, its corrosive effect on the engine (engines in modern cars are now ethanol-tolerant) and the lower energy content per unit volume compared to normal gasoline, resulting in lower mileage per volume of fuel.

In order to make ethanol production more economically efficient, a lot of research looks at new organisms and substrates that could be used. Some bacterial species, such as *Zymomonas mobilis*, look very promising. Their ethanol productivity surpasses that of yeasts three to fivefold. The ethanol yield would be 97% of what is theoretically possible. However, the bacterium can only use glucose, fructose, or sucrose as a substrate. Further research is going on into widening the range of possible substrates.

Second-Generation Biofuels

Second-generation biofuels use lignocellulose as a substrate. Lignocellulose is preferable in terms of sustainability. It can be obtained from various kinds of organic waste such as wood pellets, sawdust, or household waste.

To date, second-generation biofuels are not a competitive alternative because hydrolysis of lignocellulose is a painfully slow process. There are only very few bioorganisms in Nature which have enzymes that can degrade lignocellulose. This is why a whole host of physical, chemical, and biological pretreatment methods or a combination thereof have been developed. Even after more than 40 years of research and the investment of millions of dollars, as far as is known, no economically viable process has been found that would allow the use of lignocellulose as a substrate for biofuel production on an industrial scale. However, large-scale production has been initiated at five facilities during 2014 and 2015[3].

Third-Generation Biofuels

Third-generation biofuels are still more or less at the stage of research and development, the objective being improved sustainability and a substantial reduction in GHG emissions.

These fuels include, among others, higher alcohols (butanol and branched-chained alcohols with higher octane numbers, such as 2-methyl-2-butanol or 2-phenylethanol), hydrocarbons, algae as lipid sources, as well as biological waste-matter. Many of these fuels are planned to become drop-in fuels that need no particular preparation or modification of the existing infrastructure.

Microorganisms have been discovered that synthesize hydrocarbons in much the same way as gasoline is synthesized. They would be ideal producers of liquid fuel. It would be possible to determine the exact chain length of the hydrocarbons produced by the microorganisms through genetic engineering. Differences in density between the product and water would make the separation and recovery of the hydrocarbons relatively easy.

Macroalgae, microalgae, and cyanobacteria are considered to be ideal candidates for the production of biofuels.

In some organisms, such as *Botryococcus braunii, Dunaliella tertiolecta,* and *Pleurochrysis,* a particularly high lipid content was detected. Using these organisms would offer several obvious advantages. These include the use of solar energy, the use of carbon from CO_2, as well as the use of water of various quality standards. This would avoid competition for land use.

Between 1978 and 1996, the Department of Energy in the United States funded a research program for aquatic species. However, so far, the requirements for a low-cost product from algal lipids that can be produced on an industrial scale have not been met. Only small concentrations have been produced in cell cultures because there has not yet been a company that would take up large-scale production of algal biomass. Waste from agriculture, industry, and households would be another unconventional source of raw material. However, agricultural waste is predominantly lignocellulose, so the same reservations apply as to the production of lignocellulose for energy generation purposes. Industrial waste comes in many forms, and it depends on the composition as to what measures could be taken to convert it into energy and whether this would be a realistic option. Mixed solid household waste is already being used in controlled combustion for energy generation, while organic components are composted or anaerobically digested to produce biogas.

Further technological and biological solutions must be sought in order to make better use of this abundant resource that does not compete for different usages. This would also lighten the environmental burden of waste that cannot be profitably disposed of. It was therefore suggested that solid waste from human settlements should be tapped as a renewable energy source, with whatever technology that could convert it into energy.

As has been seen in 2014 and in 2015, petroleum prices are inherently unstable, making biofuels an alternative that has become less competitive, since the main criterion for its use is as an economically competitive fuel.

J. Stefan Rokem *is a Professor at the Department of Molecular Biology and Molecular Genetics, IMRIC, at the Hebrew University and Hadassah Medical School in Jerusalem, Israel.*

Further Reading

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2. **Searchinger T et al.** (2008) Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land-Use Change. Science 319, 1238–1240.

3. **Gies E** (2014) Yale Environment 360 (Report Nov 3) http://e360.yale.edu/feature/for_cellulosic_ethanol_makers_the_road_ahead_is_still_uphill/2821/

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The authors

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- **Yanczyk E** (2015) *Environmental Biotechnology: Principles and Application*. Delve Publishing LLC. Highly recommended.
- **Moo-Young M, Anderson WA, Chakrabarty A** (1996) *Environmental Biotechnology*. Springer Heidelberg.

Useful Weblinks

- Try Wikipedia, e.g., on biogas: http://en.wikipedia.org/wiki/Biogas
- All about microorganisms: http://www.microbes.info/

8 Self-Test Questions

1. Why is it necessary to pump extra oxygen into biological sewage plants?
2. What does a BOD_5 of 900 mg/L found in a wastewater sample mean? How many liters of clean water would be needed to treat just 1 L of the wastewater?
3. Where can biogas be used very effectively?
4. What was the US patent granted to Ananda Chakrabarty for? What did he achieve in his experiments, and why was his patent a breakthrough?
5. Why is timber such a popular building material? Against what must it be protected?
6. What are the *pros* and *cons* of biofuel?
7. Which organism is the largest producer on Earth of the GHG methane?
8. What biodegradable product from sustainable sources is currently the most promising in the textile and disposable plastic industry?

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