6 ADVANCED BIOLOGICAL PROCESSES

6.1 BACKGROUND AND RATIONALE FOR ADVANCED BIOLOGICAL PROCESSES TO SEQUESTER CARBON

Vision

Advanced biological processes will be developed and deployed to enable practices to sequester carbon in natural systems, remove or convert carbon from fossil energy systems into useful and refractory products, and recycle carbon through biological processes into end products that substitute for fossil carbon sources.

By 2025, the goal is to implement advanced biological processes that would help limit emissions and sequester carbon from concentrated utility and industrial combustion gases and dispersed point sources. Advanced biological technologies will augment or improve natural biological processes for carbon sequestration from the atmosphere in terrestrial plants, aquatic photosynthetic species, and soil and other microbial communities. These technologies encompass the use of novel organisms, designed biological systems, and genetic improvements in metabolic networks in terrestrial and marine microbial, plant, and animal species. This strategy can be accomplished by developing

- faster-growing, healthier, and more stress-resistant crop and plants
- a better understanding of biological diversity, genetics, and processes
- ways to enhance or maximize geological carbon sequestration by use of microorganisms
- ways to enhance carbon sequestration in ocean systems through transgenic and genetic manipulation of members of the food chain
- alternative microbial polymers or genetically improved plants as durable materials

Enhanced biological carbon fixation significantly increases carbon sequestration without incurring costs for separation, capture, and compression. Higher ambient CO_2 concentrations increase biological carbon fixation. But the resulting biomass generally has a higher carbohydrate and lower lignin content. Thus increased photo-synthate is trapped into readily degraded material.

Photosynthesis is a well-understood process. It is responsible for virtually all CO_2 fixation in nature. Naturally occurring non-photosynthetic microbial processes are also capable of converting CO_2 to useful forms such as methane and acetate. Although much remains to be learned about natural processes, we predict that focused research will create new opportunities to significantly enhance carbon sequestration by advanced biological processes.

Genetic engineering could increase carbon sequestration by developing durable new products that would not be consumed with release of CO_2. In addition, soil sequestration could be increased by altering the structure of plants to enhance carbon sequestration in soils. New plant species would have a higher percentage of biomass below ground, be resistant to decay, promote the formation of carbonate minerals, and interact with soil microbes to optimize the recycling of plant nutrients. Alternately, the structure and/or composition of aboveground plant structure biomass, including cell walls, could be altered to facilitate plant bioconversion processes and to render non-harvested biomass less degradable in the environment. The metabolic networks of plants and algae also could be altered to direct an increased share of photosynthate to desired products.

The four topic areas that comprise advanced biological technologies for carbon sequestration are carbon capture technology, sequestration in reduced carbon compounds, increasing plant productivity, and alternative durable materials. These have cross-disciplinary applications in terrestrial, geological subsurface, and ocean environments.

6.2 CARBON CAPTURE TECHNOLOGY SUPPORT

6.2.1 Current Science and Technology Capabilities

The prospects of using advanced biological processes to capture and reduce or sequester carbon from industrial processes are largely theoretical. However, the incentives for developing these processes are substantial because they are based on naturally occurring biological processes that do not require purified (or concentrated) CO_2 streams to be implemented effectively. Additional research will be required to determine the technical and economic feasibility of these approaches for terrestrial, geological, and ocean systems. Advanced biological processes have the potential to lower energy expenditures, reduce the need for chemical processing, increase recycling of carbon, and reduce the use of fossil fuels.

Sewage plants today are being affected by changes in community dynamics due to generation of new types of wastes from biotechnology facilities and “chip technology.” Engineers are just now beginning to work more closely with microbial ecologists, physiologists, and molecular biologists to better monitor the changes in the microbial diversity and metabolism that are requiring new paradigms for more effectively treating wastewater.

Subsurface microbiology and geomicrobiology researchers have seen an increase in funding for the characterization and monitoring of “rock”-inhabiting microorganisms. Through the use of molecular probes, polymerase chain reaction amplification, and even synchrotron technology, scientists are beginning to understand how these populations function in a world where there may be limited sources of carbon for energy. Through these studies, researchers have genetically identified and, in some cases, isolated new microorganisms that depend upon non-carbon sources of energy. These studies are laying the foundation for studies of microbial carbon sequestration and alternative energy sources.

6.2.2 Science and Technology Requirements

6.2.2.1 Energyplexes

Because of the high energy costs associated with current technologies for capture and separation at combustion sources with low-concentration CO_2 streams, the joint consideration of energy production and carbon capture might significantly lower costs. This may best be achieved by expanding the concept of “energyplexes” with integration of biological processes (National Laboratory Directors 1997). Biological processes integrated into energyplexes would produce energy, treat waste, sequester carbon, and produce useful end products. The integration at one site would minimize transportation costs, minimize the potential for environmental damage, and maximize yields. These concepts need further development, but some aspects, which include biological components, have been put into place on a limited scale

Waste treatment associated with landfills, sewage treatment facilities, or even release of sewage into water bodies produces significant CO_2 and other greenhouse gases (especially methane) from fixed carbon. This carbon represents a potential source of renewable energy. Molecular biology methods could be employed to slow the decomposition rates of solid wastes in landfills. In addition, the bioengineering technology to trap, separate, and recycle CO_2 and methane decomposition products at landfills and sewage treatment facilities needs to be improved.

Sewage treatment is designed to sanitize wastes and to reduce the carbon burden before discharge. Thus an implicit goal of sewage treatment is the production of CO_2. Most CO_2 is produced by the aerobic treatment stage. A shift to complete anaerobic fermentation could lower emissions. A modification of sewage treatment in this manner, via integration of physiological and genetic regulation, could generate more methane to meet the fuel demand of plant operation and could generate a higher-carbon end product for use in soil building and agriculture. Knowledge about physiological processes and end products must be expanded to design these plants.

Reductions in CO_2 emission could derive from more efficient operation of sewage treatment plants and landfills and integration of managed wetlands into waste treatment processes. Basic understanding of these biological processes must be expanded to allow more effective implementation of these options. Consideration should be given to the integration of these facilities into energyplexes to provide carbon and nutrients for other biological processes (e.g., production of carbonate rocks by metal-reducing organisms, production of biomass by algae).

6.2.2.2 Geological systems

Biological conversion of CO_2 into insoluble carbonate rocks, such as siderite ($FeCO_3$)—using metal-reducing bacteria and metal-containing fly ash or other low-value products—is technically feasible. If iron is abundant and available as a bioreductant, siderite can be formed. These materials could be used in roadbeds, as composite materials, or as fill. In any case, solid carbonate rock significantly simplifies storage and disposal of CO_2 by enormously increasing the density of the material to be handled. Either metal-reducing organisms or algae could be applied to precipitate carbonate rocks. Metals could be reduced by bacteria and precipitated as carbonates. Recent research on metal-reducing thermophilic bacteria has demonstrated that siderite production by these bacteria can be substantial.

6.2.3 Research Implementation

6.2.3.1 Energyplexes

The energyplex concept involves recycling CO_2 in waste flue gases from a power generation facility via photosynthesis to generate a store of reduced carbon in the form of algal biomass. Storage can take the form of polysaccharides or triglycerides, both of which are readily usable fuels, or of chemical feedstocks for downstream bioconversion processes. Although additional concepts will undoubtedly be developed and should be sought, initial efforts are likely to focus on several research areas, including integration of primary production using waste CO_2 and heat. These energyplexes could benefit from integration of sewage or other waste treatment because the nutrients and carbon could be used in biological processes at the site. Because of seasonal, land, and water limitations, this alternative may be applicable only in certain localities or specialized situations.

Energyplexes for Conventional Crops

An additional potential option is to use the CO_2 and the waste heat to promote the growth of more conventional agricultural crops. Use of CO_2 can lead to increases in productivity of plant growth in hydroponics or wetlands applications. Pilot projects are under way to capitalize on this concept.

One area that has been the focus of considerable research in the past is growth of algae for fuel production. Previous research focused on diesel replacements (“biodiesel”). In addition, the production of hydrogen and other chemical feedstocks using algae is worth additional investment in research. Some algae can be cultivated in saline or alkaline waters, which are available in the southwestern deserts, where land is relatively plentiful. This alternative might be limited by the costs of pond preparation, CO_2 injection, or algal harvest.

6.2.3.2 Geological systems

Microbial processes can probably be engineered to greatly accelerate the formation of carbonates from natural silicate minerals such as serpentinite (see Chap. 7). While it is known that the release of magnesium ions from crushed serpentinite is greatly enhanced in the presence of nitrifying bacteria (Lebedeva, Lyalikova, and Bugel’skii 1978), genetic manipulations, use of other chemotrophic organisms, and exploitation of microbial acid formation can be expected to further accelerate the decomposition of silicate minerals. Knowledge about the factors that inhibit plant growth in serpentine soils (serpentine barrens, where little vegetation is found) can be used to design microorganisms that tolerate high magnesium concentrations and low calcium/magnesium ratios and resist heavy metal toxicity. Genetic engineering has the potential as well to endow these organisms with the capacity to use metal sulfide minerals as energy sources and CO_2 as the carbon source for growth. Carbon dioxide would be sequestered as magnesium carbonate and as microbial biomass.

Additional advanced concepts include the utilization of enzyme systems and catalysts for CO_2 capture. The goals of the research would be to achieve shorter residence times and higher throughput. A more innovative approach may be to develop biological catalysts for removal of CO_2. These may include "artificial photosynthesis" (microbial or self-assembly) applications with molecular devices that mimic photosynthesis. As some of the solvent-based CO_2 absorbents currently in use are organic compounds, biological production of solvents for CO_2 scrubbing is feasible.

6.3 SEQUESTRATION IN REDUCED CARBON COMPOUNDS

6.3.1 Current Science and Technology Capabilities

The feasibility of a significant midterm impact on global climate change by increasing the size of forests is firmly established. Algal biomass schemes for trapping CO_2 have advanced in recent years and should be explored as a possible supplement to forest management and advanced agricultural biotechnologies.

The surface area of the planet is dominated by oceans (75%), where bioproductivity is often limited by nutrient availability. As discussed in Chap. 3, nutritional enrichment could enhance ocean algal growth and marine productivity and might increase net oceanic CO_2 fixation. Advanced biological techniques could be used to increase phytoplankton productivity or to alter the competitive capacities of organisms that feed on algae. Marine algal production is not limited by water availability and affords greater opportunities to control nutrient delivery.

Algae are amenable to relatively simple genetic manipulations aimed at increasing photosynthetic efficiency, maximizing yields of desirable energy storage products, and optimizing conversion of photosynthetic products to fuels or chemical feedstocks. Such strategies could also be applied to terrestrial plant species.

6.3.2 Science and Technology Requirements

The goal is to have a mix of biological systems that will provide incremental but significant contributions to overall carbon management.

Research on using algae in pond systems for renewable energy is likely to have spin-offs for open-ocean carbon management schemes and could eventually lead to ocean harvesting–based renewable energy technologies. Recovery of other products from fermented algal biomass—for example, fertilizers for terrestrial crops or for open-ocean fertilization, or single-cell protein for animal nutrition—would improve overall economics.

Plant and microbial genomics projects currently under way will eventually provide detailed knowledge about organismal metabolic networks and interrelationships among different cells in a plant and different organisms in an ecosystem. Such knowledge will enable a better understanding of ecosystems and how to manage their productivities. We need more information about

- the function of genes being sequenced and computerized methods to manipulate and store the huge quantities of data pouring forth from genomics efforts
- how to introduce individual genes and pathways into a wide variety of plants and microbes
- gene replacement strategies for plant species
- artificial chromosomes for the introduction of large segments of genetic material into plants
- more rapid and reliable methods for screening candidate genetically engineered plants and for clonal propagation of engineered plants

6.3.3 Research Implementation

Most renewable energy schemes generate considerable recalcitrant biomass and therefore offer the opportunity for significant net carbon fixation in addition to their value in reducing the demand for fossil energy. Compared with the difficulties of CO_2 sequestration by separation, compression, and transport, the handling and storage of recalcitrant biomass is straightforward.

6.3.3.1 Sequestration of biological carbon in ocean sediments

Chapter 3 discusses enhancing the natural biological carbon cycle in the oceans. Research topics in advanced biology regarding this carbon mitigation option include the following:

- To what extent can biomass concentration and disposition be genetically manipulated?
- Are there feasible genetic manipulations of biomass that would alter the decreasing rate of biomass production in the open ocean?
- Can we develop an organism that will rapidly and costeffectively assess the ecological impacts of various nutrient stimulation scenarios?
- Can organisms be engineered so that deposition of biological carbon outweighs the adverse pH effects of carbonate deposition?
- Are there advanced biological approaches to increasing phytoplankton accumulation specifically in upwelling, nutrient-rich waters?
- Can genetic biomarkers be developed to monitor and assess the ultimate fate of biomass in deep ocean sediments? (In particular, we need a better understanding of the conversion of biomass to methane clathrates.)

An intriguing aspect of accumulating biomass in ocean sediments is the potential that this process could become an energy resource in the long-term. It is plausible that future energy scenarios would include methane recovery from clathrates located in well-defined deposits.

6.3.3.2 Alkaline ponds for carbon sequestration

The capacity of some blue-green algae to thrive essentially as monocultures in waters of high alkalinity creates the possibility of much more effective CO_2

sequestration than would be possible with other photosynthetic systems. The chemical hydration rate of CO_2 increases with pH, as does the amount of inorganic carbon that can be dissolved in aqueous solution. Alkaline ponds have the potential to trap virtually all of the smokestack CO_2 emissions as well as the major pollutant gases SO_2 and NO_x. Accumulation of biomass can be optimized by pH manipulations that suppress the biomass-consuming activities of respiring organisms. With appropriate mass culturing of suitable blue-green algae, photosynthetic activity can maintain alkaline pH while providing a renewable energy resource. The feasibility of mass culturing of microalgae in alkaline seawater has been established, demonstrating the potential for developing much larger mass culture systems than could be contemplated with freshwater ponds.

6.3.3.3 Schemes for producing refractory biomass from terrestrial plants

Two possibilities for fixing CO_2 into materials with recycle times much longer than wood can be considered: polymeric materials that are relatively refractory to biological degradation and inorganics (carbonates).

A large number of plant species synthesize diterpenoid resins or natural rubber, two materials that are relatively stable in the environment. Although few of these species are of economic significance, they are widespread and adaptable to a range of climates, could be grown on a large scale, and could be engineered for improved efficiency for conversion of CO_2 to product. These end products of plant metabolism could be deposited as such or cross-linked to minimize the possibility of biological degradation (e.g., vulcanized rubber).

The development of new materials (e.g., novel biomass-derived plastics), that would increase the use of reduced carbon compounds in the economy could be a significant element in carbon management. Another approach could be directed toward eliminating the irreversible conversion of petroleum to CO_2 by substituting "recyclable" plant products for fine and intermediate-scale chemicals and

Aquaculture in the Desert

In 1987, during Eritea's war of independence from Ethiopia, simple ponds were dug along the shore to a depth of about 0.5 m below the low tide line and about 200 m^2 in area. The ponds were filled with sea water and chemical fertilizers to grow algae and inoculated with mullet fingerlings at a rate of one fingerling per square meter. After 4 months, each fish weighed about 1 lb. Less than 1% mortality was detected among these algae-eating fish, which are famous for their hardiness in resisting disease and coping with low oxygen concentrations. This is equivalent to a rate of production of about 15 tons/ha per year and demonstrated that desert shores could produce enough food to justify cultivation on a large scale. This was not surprising. In southeast Asia, freshwater ponds have been fertilized to grow algae and inoculated with algae-eating fishes for centuries. Their only variation on this time-proven practice was to substitute seawater for fresh water and marine fish and algae for freshwater fish and algae. (www-ibt.tamu.edu/invitro/guested.htm) even transport fuels. These could include the plant essential oils, fixed oils, resins, and even heptane, which is a major component of turpentine and an excellent transport fuel. Genetic engineering of plants to improve the availability of these products is entirely feasible.

Lignin is relatively resistant to biodegradation, and increasing the lignin content of plants would slow the decay of biomass in soils. Plant geneticists have discovered mutations that decrease the lignin content of plants to increase nutritional value for ruminants. Moreover, as the biochemical pathways for lignin biosynthesis in plants became elucidated, the genes encoding lignin pathway enzymes were cloned and have recently been employed to alter the quantity and quality of lignin in poplar and aspen tree species. The technology of lignin manipulation could be applied to plants that are currently being considered for reforestation with the objective of increasing net carbon transfer.

It has been estimated that only 3% of the carbon in solid wood in landfills is converted to CO_2 or methane (Skog and Nicholson 1998). This limited decomposition of wood is attributed to the recalcitrance of lignin in anaerobic environments. Although anaerobic bacteria can degrade cellulose, much of the cellulose in solid wood is sequestered from bacterial action by a lignin barrier and therefore cannot be biodegraded. Even paper products undergo only partial decomposition in landfills. Currently, most of the wood and wood products in landfills is sequestered carbon. However, alterations in the structure of wood by decreasing lignin content could increase its biodegradability. It is likely that significant lignin would remain even in genetically modified woody plants and that landfills containing such plants would still sequester carbon. However, increasing biodegradability could increase methane yields from landfills, and the energy value of buried wood and wood products could provide an economic incentive for using woody materials for carbon sequestration. In contrast to reforestation or high-productivity agricultural schemes, there is an unlimited amount of carbon that could be sequestered in landfills.

6.4 INCREASING PLANT PRODUCTIVITY

Research would improve the ability to genetically manipulate plants to increase phototosynthetic activity and fix CO_2 and nitrogen more efficiently. Manipulation of plant genomes to obtain the desired effects is still a poorly developed field. Much more attention needs to be given to the fundamental mechanisms of cell development, cell wall biochemistry, plant photosynthetic processes, and primary and secondary metabolic processes.

More rapidly growing herbaceous agricultural plant species will enhance the removal of CO_2 from the atmosphere and trap it in photosynthate that can be readily converted into renewable fuels, chemicals, polymer precursors and foodstuffs. Rapidly growing woody species will trap CO_2 in durable timber that can be used for a wide variety of structures. Other fast-growing herbaceous and woody species will provide easily delignified fiber for paper, composites, and block copolymers.

6.4.1 Current Science and Technology Capabilities

The advent of modern molecular biology has enabled strategies for improvement of many different organisms through genetic engineering, including many agricultural and timber crop species. Our current understanding of the processes of photosynthesis, photorespiration, plant pathology, and wood structure and function, among others, suggests many strategies for increasing the rate of biological carbon sequestration. The 25-year time frame of the proposed R&D program would permit advances in several of these areas to be successfully deployed on a large commercial scale, which could have a significant impact on U.S. carbon emissions.

Plants get their carbon from CO_2, which makes up only 0.03% of the present-day atmosphere. Microscopic floating plants, phytoplankton, and other algae take up CO_2 dissolved in water. Both terrestrial and water plants require solar energy to reduce CO_2 to biomass.

Photosynthesis is responsible for conversion of sunlight into chemical energy by essentially all primary producers in nearly all ecosystems. It provides the foundation of the food chain for life on Earth and is also the source of the oxygen in our atmosphere.

Sunlight provides the energy for the primary mechanism of carbon fixation from the atmosphere. The theoretical maximum efficiency of light energy capture and conversion into usable chemical energy is approximately 5% (expressed as a fraction of visible light energy available at the earth's surface). Plant photosystems seldom operate at anywhere near this efficiency, a fact that provides us with an excellent opportunity for carbon sequestration. Photosynthetic efficiency varies widely with the ecosystem and time of year. The efficiency of some forests can be as low as 0.1 to 0.05%, while that of marsh grasses can be as high as 2 to 4% in the early spring. The photosynthetic efficiency of corn and sugar cane can be as high as 3.5 to 4%.

Engineering Rubisco for Speed

Plants fix carbon by taking CO_2 from the air and adding it to small precursor sugars in plants. This step is carried out by an enzyme known as Rubisco. Rubisco is the most abundant protein in the world, making up 50% of all plant proteins. The Rubisco enzyme is slow and inefficient. It not only fixes carbon but, in an alternate reaction, adds oxygen to the precursor sugars and degrades them, diverting the enzyme from productive activity. It may be possible to engineer into Rubisco more efficient carbon-fixation mechanisms or to discover more efficient, naturally occurring forms of Rubisco in as yet poorly characterized or undiscovered organisms.

The activity of Rubisco is regulated by another enzyme called Rubisco activase. Rubisco activase controls the overall process of photosynthesis by making Rubisco activity responsive to light intensity. Researchers are currently changing a specific part of the Rubisco activase enzyme by genetic engineering to analyze its function. Information about the mechanism and structure of Rubisco activase eventually can be used to make changes that improve the activity of the enzyme and increase photosynthetic efficiency. (www.photoscience.la.asu.edu/Photosyn/faculty/salvucci.html)

Environmental conditions strongly affect photosynthetic efficiency, but the biochemistry of the photon capture and energy conversion system could be improved as well.

Photosynthetic carbon fixation is limited by the efficiency of two very important processes—conversion of incident light energy to captured chemical energy and the primary carbon fixation reaction catalyzed by the enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), the most abundant protein on Earth. Either or both of these processes may be limiting in terms of carbon sequestration rates, and it is thought that they could be enhanced significantly via advanced biological approaches.

Rubisco is not only a very slow enzyme, but is also inefficient because it can react with molecular oxygen in a process known as photorespiration. This results in a futile (nonproductive) metabolic cycle. As the ratio of CO_2 to O_2 in the atmosphere increases, the productive carboxylation efficiency will naturally increase. However, it may also be possible to discover more efficient, naturally occurring forms of Rubisco in as yet poorly characterized or undiscovered organisms, or to engineer into Rubisco an exaggerated preference for CO_2 over O_2 using modern molecular biological techniques (Mann 1999).

Some plant species have already developed a solution to the problem presented by Rubisco. A group of warm-climate grass species known as C_4 grasses (including corn, sorghum, and sugar cane) evolved a specialized leaf anatomy (Krantz anatomy; contrast C_3 and C_4 anatomy in Figs. 6.1 and 6.2, respectively). These plants show little or no photorespiration and are considerably more efficient because

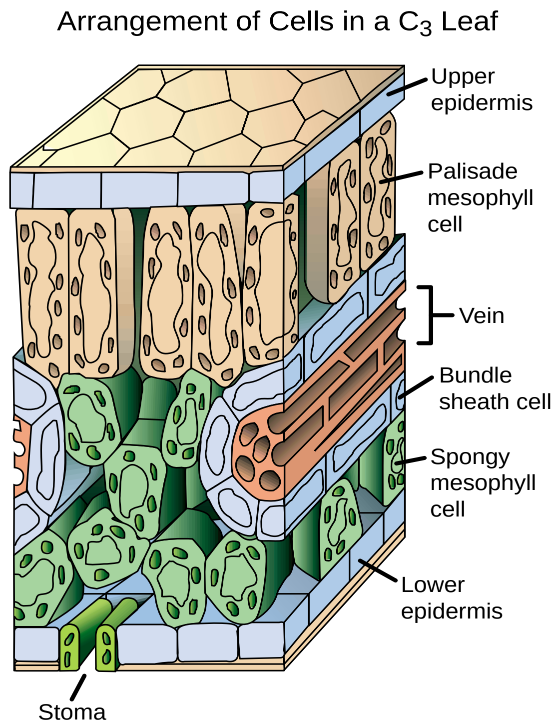

Fig. 6.1: Typical leaf anatomy in a C3 plant. (www.biology.arizona.edu/181/rick/photosynthesis/C4.html)

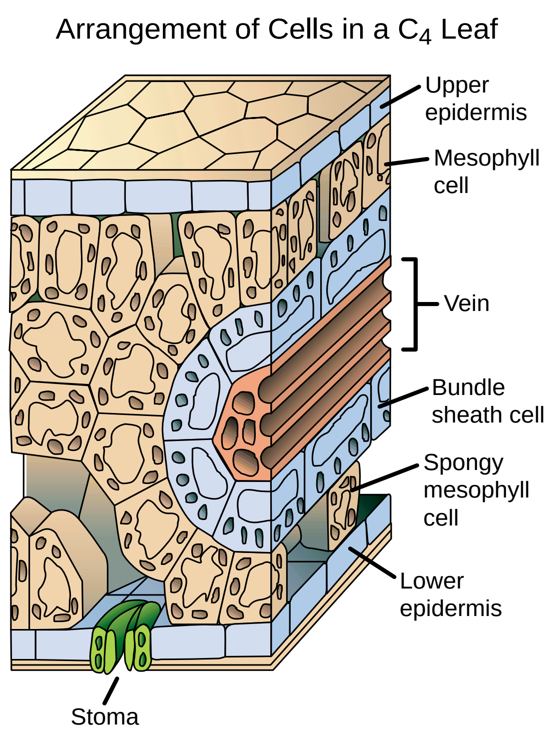

Fig. 6.2: Typical leaf anatomy in a C4 plant. (www.cme.msu.edu/WIT/Doc/mj_recon.html)

CO_2 is carried to sites of photosynthesis. This trapping of CO_2 is carried out via the Hatch-Slack pathway (Fig. 6.3), which is not affected by oxygen. Genetic manipulation, to optimize pathways for trapping CO_2, comprises significant research opportunities.

Although 78% of our atmosphere consists of nitrogen, plants are not capable of converting it into forms they can use. Certain bacteria, however, produce enzymes that facilitate the transformation of nitrogen gas into ammonia and other nitrogen-containing compounds that can readily be absorbed by plant roots and used by the plant. In nature, the natural decay of dead biomass releases nitrogen in forms that can often be absorbed by plants. This occurs both in terrestrial systems and in the oceans. Nitrogen availability is often growth-limiting and is routinely supplemented with fertilizers in agricultural practice. Some plant species, notably the legumes, do not require nitrogen fertilization because their roots are colonized by nitrogen-fixing microorganisms. Ammonia can be readily assimilated by plants and incorporated into other nitrogen-containing compounds, such as amino acids, which are essential for protein synthesis. The critical enzyme in nitrogen fixation is called nitrogenase, and it breaks the very strong triple bond of N_2. These complex and poorly understood enzymes require large amounts of energy to accomplish this reaction. In addition, nitrogenases contain an assortment of complex, iron-containing co-factors, which are essential for activity. Thus, iron is often rate-limiting for nitrogen fixation in the ocean.

When photosynthetic light capture, CO_2 fixation, and nitrogen availability no longer limit plant metabolism, one can improve regulation of and/or redesign secondary metabolic pathways for conversion and sequestration of the primary products. It is thus very important to understand both the spatial and temporal linkages among metabolic pathways in an organism, as well as modes of long-term storage of the sequestered products. Elucidation of these linkages and carbon storage capabilities will be best addressed by structural biology, plant and microbial molecular genetics, and computational simulation and theory.

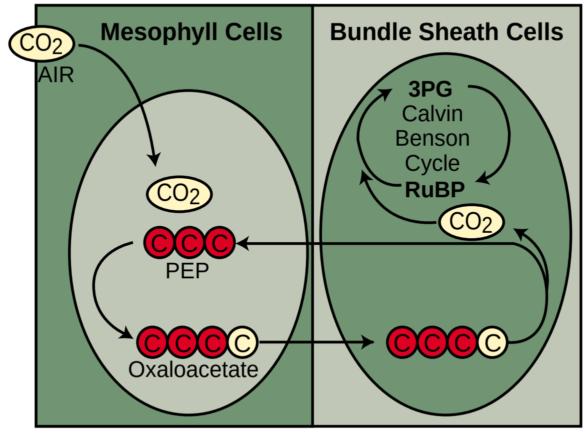

Fig. 6.3: Carbon fixation as it occurs via the Hatch-Slack pathway in C4 plants. (www.biology.arizona.edu/181/rick/photosynthesis/C4.html)

Other environmental factors affecting carbon sequestration are predation by insects and microbial pathogens, which decrease global crop and forest yields. In addition, other stresses, such as drought, saline soils, heat and cold, pH, and the presence of heavy metals and other pollutants, limit plant growth rates and biomass accumulation. Ameliorating such stresses has been a target for improvement by agriculture and silviculture over the centuries. Modern plant science has mitigated crop losses, but there is still plenty of room for improvement, as evidenced by the prolific activity and investment in plant biotechnology.

It is advantageous to increase deposition of carbon in soils. This might be accomplished most effectively by increasing the transfer of photosynthate to root systems and by increasing the accumulation of recalcitrant bioproducts (such as lignin) in forest litter. Deposition of carbon in soil by agricultural and silvicultural systems might be increased by shifting photosynthate partitioning from aboveground to belowground organs via genetic means. Increasing the recalcitrance of root tissues should also be explored as a possibility. Root deposition might be particularly important in the restoration of degraded soils or cultivation of plants in marginal ecosystems.

Nitrogen fixation could also increase root deposition and stimulate root exudates. Soil microbes play an important but incompletely understood role in enabling nutrient uptake by plants. Microbes associated with plant roots are an essential component of biological nitrogen fixation. Carbohydrates and other nutrients secreted by plant roots foster microbial growth, and the associated bacteria and fungi mobilize minerals (such as phosphate) and fix nitrogen for plant use. By increasing secretion of photosynthate by roots, it might be possible to increase biological nitrogen fixation and the cultivation of crops in marginal lands. For example, specific plant-associated fungi are essential for the cultivation of softwood species on topsoil-deficient lands reclaimed from open pit mining. It seems likely that similar relationships might be important in other degraded environments.

Several significant non-photosynthetic CO_2 fixation reactions occur in nature (University of Chicago 1998). As much as 10% of the cellulose and hemicellulose in plant biomass might be converted in the anaerobic environment to methane and CO_2 by consortia of anaerobic bacteria. Acetogenic bacteria appear to play a major role in this process. At the global level, approximately 10 GT of acetate is metabolized annually in the anaerobic environment, and about 10% of this may be derived from CO_2 fixation via the acetyl-CoA pathway. Potentially important niches for acetogens include termites, monogastric and ruminant animal digestive systems, and forest soils.

If a source of hydrogen can be provided in a CO_2-rich, O_2-free environment, CO_2 can be fixed efficiently into nonvolatile carbon compounds. Interestingly, it has recently been discovered that the strictly chemical action of water on basaltic rock formations deep below the surface of the earth serves as a source of hydrogen for microbial ecosystems (Gollin et al. 1998). These reactions may be important for biosequestration in geologic formations, such as spent oil and gas wells.

The advent of genetic engineering has improved crop productivity by increasing disease resistance and improving the ability of engineered crops to compete with undesired plant species. Plant products, especially oilseed crops, have been altered to increase the production of marketable oils, and these engineered varieties are being grown commercially. Additional engineering could increase oil production or other desirable products. Several genetically engineered crop species are currently being grown in the United States and other countries and are rapidly capturing market share. For example, 40% of the Canola crops in Canada and 33% of the soybean crops in the United States are genetically engineered.

Research is under way to examine plant-insect interactions. The research usually focuses on combating a specific insect pest by producing transgenic plants (plants with genes from other species) that synthesize compounds that inhibit insect metabolism. Producing a disease-resistant transgenic plant requires that the molecular mechanisms involved in host plant resistance be elucidated. Unfortunately, these mechanisms vary greatly among plant pathogens.

Advances in gene technology have offered various novel routes to improve the disease resistance of crops. Resistance to a number of insect species has been created by use of genes encoding protease inhibitors and the *d*-endotoxin of *Bacillus thuringiensis*. Resistance against a number of viruses was obtained by expressing genes encoding for the viral coat protein, applying the principle of cross-protection.

6.4.2 Science and Technology Requirements

In order to realize the maximum benefit from biological fixation, we need more basic knowledge about what processes limit plant growth in many specialized crops for food, feed, fiber, fuel and structural uses. We also need more information about optimal cultivation and harvest methods, particularly in marginal environments where water or soil quality is limiting. Other growth-limiting factors such as disease and insect pests also require better understanding. Biotechnology and plant genomics will play large roles in reaching these goals.

6.4.3 Research Implementation

Plant productivity can be increased by

- improving photosynthetic efficiency by increasing light-trapping reaction efficiency and decreasing photorespiration (C4 pathway; engineering Rubisco efficiency and reaction rate)
- developing rapid methods for genetic manipulation of agricultural, tree, and nontraditional species with CO_2 sequestering potential (transformation and regeneration systems)
- developing new tools for manipulating fast-growing herbaceous and woody species (artificial chromosomes; gene replacement techniques)
- reducing the time required to create transgenic plants in the laboratory
- enhancing non-photosynthetic mechanisms for CO_2 fixation (bacterial methanogenesis and acetogenesis)
- genetically engineering the cell walls of agricultural species so that they can be more easily and economically converted to fuels and chemicals
- developing crops or processes that will biosynthesize functional feedstock chemicals for the synthesis of recalcitrant products (e.g., non-biodegradable plastics)
- improving nitrogen fixation in microbial symbionts of plants and/or by cloning genes into plants
- developing simplified nitrogenases that bypass the current mechanistic complexity, iron-dependence, and energy intensity issues

- improving insect and disease resistance via transgenics and protein engineering

6.5 ALTERNATIVE DURABLE MATERIALS

6.5.1 Current Science and Technology Capabilities

6.5.1.1 Biopolymers

The past several years have seen dramatic growth in the use of enzymes for synthetic applications. This has been particularly apparent in the increased use of enzymes for polymer design and modification. Enzymes offer significant advantages over chemical catalysts in the synthesis of materials with highly specialized properties—including biodegradability, biocompatibility, inherent selectivity (e.g., enantio-, regio-, and chemo-), and easily tailored functionalities—all produced under conditions that minimize the formation of by-products and the avoidance of unwanted pollutants (Dorkick 1998).

The development of carbon feedstocks for chemical applications will reduce CO_2 emissions by displacing fossil hydrocarbons. Primary examples are the use of polymers derived from renewable agricultural resources, such as corn or sugar beets. These compounds are also commonly known as "bioplastics." For many applications, the plastic "peanuts" used as packing material have been replaced by bioplastics. These bioplastics are displacing petrochemical-based polymers, such as polyethylene, polystyrene, and polypropylene. One class of bioplastics, the PLA resins, are composed of chains of lactic acid derived from conversion of starch to sugar followed by fermentation to lactic acid. Dow Chemical and Cargill have recently formed a joint venture to commercialize PLA on a large scale. Polyhydroxyalkanoates (PHAs), a chemically distinct family of biodegradable bioplastics, are being investigated by Monsanto and Proctor & Gamble for use as petro-plastic substitutes. Monsanto is looking at producing PHAs in crop plants instead of fermentation vats.

Turning Sugar into Better Polymers

The polymer polytrimethylene terephthalate (3GT) has enhanced properties compared with traditional polyester (2GT). Yet commercialization has been slow because of the high cost of making trimethylene glycol (3G), one of 3GT's monomers; it is a two-step process. However, recently, through recombinant DNA technology, an alliance of scientists from DuPont and Genencor International has created a single microorganism with all of the enzymes required to turn sugar into 3G. This breakthrough is opening the door to low-cost, environmentally sound, large-scale production of 3G. The eventual cost of 3G produced by this process is expected to approach that of ethylene glycol (2G).

The 3GT that is created by a fermentation process requires no heavy metals, petroleum, or toxic chemicals. The primary material is from agriculture—glucose from cornstarch. Rather than releasing CO_2 to the atmosphere, the process actually captures it because corn absorbs CO_2 as it grows and all liquid effluent is easily and harmlessly biodegradable. 3GT can also be subjected to methanolysis, a process that reduces polyesters to their original monomers. Used polyesters can be recycled indefinitely by being repolymerized. (www.dupont.com/corp/science bionylon.html)

Bioplastics and biofuels are promising emerging technologies, but other technologies may have a greater long-range impact in terms of carbon sequestration. Bioplastics are expected to compete with petro-plastics on a cost/performance basis. If the carbon used in the process is from atmospheric sources (e.g., from biomass) the net result is carbon sequestration. The market for these materials may limit the carbon sequestration potential; however, other biological processes, especially when part of an integrated sequestration strategy, could have greater sequestration potential.

6.5.1.2 Microbial production of cellulose

Acetobacter xylinium, a non-photosynthetic bacterium most commonly used in the production of vinegar, can use glucose, sugar, glycerol, or other organic substrates and convert them into pure cellulose (Brown 1979). Weyerhaeuser, along with the now defunct Cetus Corporation, spent 7 years optimizing the production of bacterial cellulose, which has unique structural and absorption properties. Several patents have been filed on the applications of bacterial cellulose.

Microbial cellulose has been investigated as a binder in papers. Because it consists of extremely small clusters of cellulose microfibrils, it adds greatly to the strength and durability of pulp when integrated into paper. Ajinomoto Company and Mitsubishi Paper Mills in Japan are currently active in developing microbial cellulose for paper products (see patent JP 63295793 at www.botany.utexas.edu/facstaff/facpages/mbrown/position1.htm). This biopolymer is just one example of the many microbial polymers that have potential for use as alternative durable materials.

6.5.2 Science and Technology Requirements

Unfortunately, the plastic now produced by plants and bacteria is brittle and decomposes rapidly. Research into ways to improve the quality of bioplastics to enhance their usefulness in consumer goods is needed. Alteration of the biosynthetic pathways via gene shuffling, protein engineering, and improved fermentation technology at extreme temperatures must be integrated to achieve these improved bioplastics.

To overcome the drawbacks to successful commercialization of bacterial cellulose, efforts have centered on understanding the biosynthetic process itself, then trying to optimize the fermentation process to produce more cells and cellulose biosynthesis. Further genetic study of the operon-controlling cellulose synthesis is needed. Gene shuffling may have some applications also with respect to strain “quilting of genes” and selection of improved transformats.

Cellulose Factories

Acetobacter xylinum is nature’s most prolific cellulose-producing bacterium. As many as a million cells can be packed into a large liquid droplet. If each one of these “factories” can convert up to 10^8 glucose mole-cules per hour into cellulose, the product could virtually be made before one’s eyes. (www.botany.utexas.edu/facstaff/facpages/mbrown/position1.htm)

Because microbial cellulose is an extracellular product that is excreted into the culture medium, special care and handling is necessary to maintain optimal production. The cellulose membrane itself can become a barrier for substrates and oxygen necessary for the cells to produce cellulose. Novel fermentation approaches have been developed to overcome some of the intrinsic difficulties for mass culture of *Acetobacter*, and a vigorous program of bacterial strain selection from regions worldwide has provided a stock resource of stable, efficient cellulose-producing strains.

What is needed currently is a way to convert bench-scale fermentation to an efficient, large-scale fermentation technology. This research need for new development technology can be met through a combination of genetic engineering and a better understanding of microbial physiology in submerged culture.

6.5.3 Research Implementation

6.5.3.1 Biopolymers

Research to improve the desired characteristics of bioplastics includes the following:

- advances in elucidating structural biology
- genetic altering of enzymatic pathways
- improved protein crystallography
- computational biology to simulate structure and properties at extreme temperatures
- genetic engineering to improve durability and elasticity

6.5.3.2 Microbial cellulose

The recent success with cloning and sequencing the genes for bacterial cellulose synthesis (Saxena, Lin, and Brown 1990, 1991) plus functional genomic information (Saxena et al. 1994) will result in new ways to further optimize bacterial cellulose production by *Acetobacter xylinium* as well as other bacteria and algae that synthesize cellulose.

Continued efforts in integrating the physiology and molecular biology of bacterial polymers combined with structural and functional analysis via crystallography and synchrotron characterization should make these bacterial polymers even more attractive and affordable.

6.6 SUMMARY AND CONCLUSIONS

R&D efforts leading to sustained sequestration of gigatonnes of carbon per year from the atmosphere are prime sequestration options. Large-scale biological sequestration opportunities will require significant time and resources for deployment, so we envision successive technology deployments over 25 years (Fig. 6.4). Near-term measures (before 2005) have low technical risk and will have limited carbon sequestration effects at first, but they may become increasingly large sinks with time. Medium-term options will use more advanced strategies involving significantly higher technical risk but may permit higher carbon sequestration capacity with fewer resources. Long-term options are characterized as high-risk but may offer remarkable potential for carbon sequestration.

Table 6.1 ranks the strategies discussed in this chapter by technical feasibility, timeliness, and potential effects. Rankings would probably differ

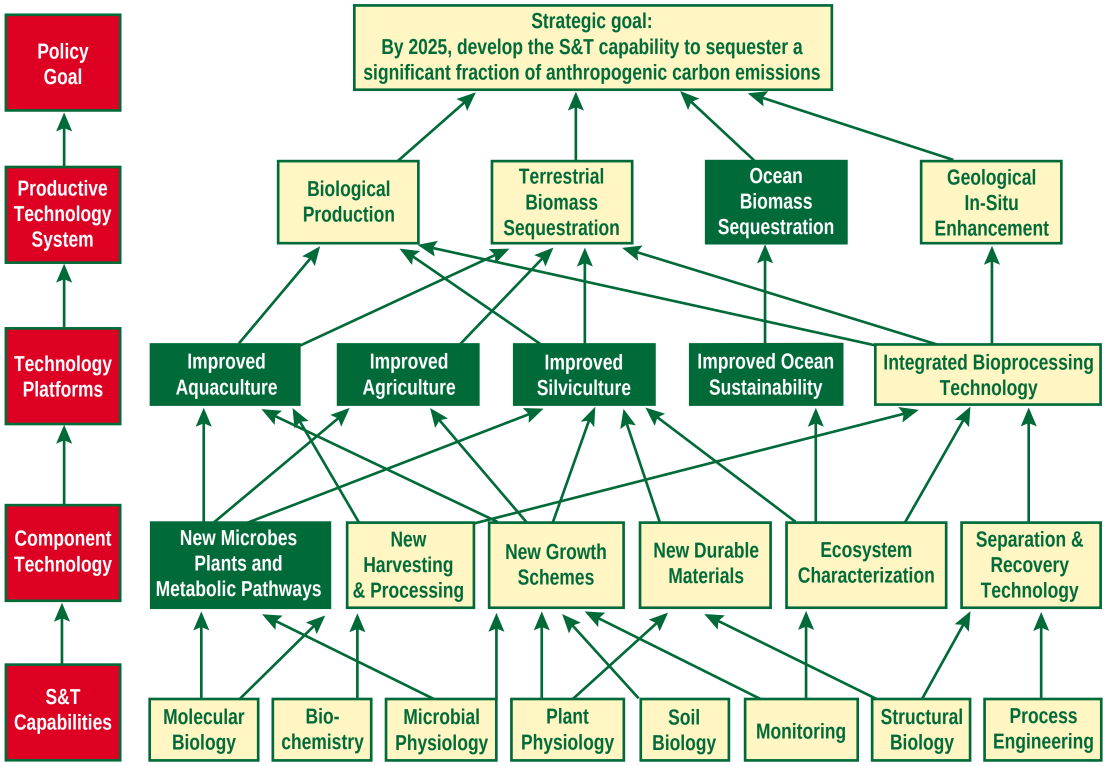

Fig. 6.4. Key elements of the R&D road map for advanced biological processes.

if other relevant factors, such as economics, public policy, and risks (health and environmental), were also considered. Some rankings are subjective because of the ill-defined scope of some options. For example, genetic engineering of crop plants for disease and pest resistance is practiced commercially today; hence, this level of engineering is deemed to be highly feasible. On the other hand, targeted genetic manipulation of growth and durability characteristics of conifers is likely to prove difficult and is deemed less feasible.

Table 6.1. Prioritization of advanced biological options

Focus area	Technology description	Technical feasibility 4 = low risk 3 = some risk 2 = moderate risk 1 = high risk (unlikely)	Time 3 = <2005 2 = 2005 25 1 = >2025	Potential impact 4 = very high (>1 Gt/year) 3 = high (>0.25 Gt/year) 2 = moderate (>0.1 Gt/year) 1 = low (<0.1 Gt/year)	Additive component rank (higher score = high priority)
Increase forest productivity	Management	4	1	2	7
	Genetics	2	2	3	7
	Soil sequestration	2	2	4	8
Increase agricultural crop productivity	Soil sequestration	4	3	2	9
	Management	4	3	2	9
	Fuels/chemicals/materials	3	2	3	8
	Nitrogen fixation	2	1	4	7
Alter plant functional structure	Modify plant cell walls	3	2	4	9
Soil biota/ecosystems	Decrease rate of biomass decay	1	2	3	6
	Mycorhizae (P)	2	2	3	7
	Carbonate formation	2	2	3	7
Shift allocation of plant biomass to below-ground organs	Genetic (includes genetic engineering)	3	1	3	7
	Agronomic practices	4	3	2	9
Abiotic stresses	Salt tolerance	3	2	3	8
	Drought tolerance	3	2	3	8
Landfill options	Sequestration	3	3	2	8
	Conversion	4	3	2	9
Materials substitution with renewables	Steel and concrete	2	3	2	7
	Plastics (PHA, PLA; renewable	3	2	2	7
		3	3	2	8
	Durable wood products				
Terrestrial aquaculture	Sequestration in biomass reservoir for C storage)	3	2	3	8
Marine aquaculture	Ocean sediments	2	1	3	6
	Kelp farms	2	1	1	4
	Slow release biofertilization	3	2	4	9
Enzyme and protein	Rubisco CO_2:O_2 fixation ratio	2	1	4	7
	C4 pathway engineering in	2	3	3	8
	Nitrogen fixation	1	1	3	5
	Pathway engineering	3	2	3	8
	Photosystem efficiency	2	2	4	8
Biogeochemical	Bioplugs	2	2	3	7
	Mineralization	1	1	3	5
	Energy-dependent chemical	1	1	2	4

6.7 REFERENCES

Brown, R. M., Jr. 1979. "Biogenesis of Natural Polymer Systems, with Special Reference to Cellulose Assembly and Deposition," pp. 50–123 in *Proceedings of the Third Phillip Morris Science Symposium*, Ellen Walk, ed., Richmond, Va., November 9, 1978.

Brown, R. M., Jr., J. H. M. Willison, and C. L. Richardson 1976. "Cellulose Biosynthesis in Acetobacter Xylinum: 1. Visualization of the Site of Synthesis and Direct Measurement of the In Vivo Process," *Proc Nat Acad Sci U.S.A.* 73(12):4565–69.

Dorkick, J. 1998. "Biocatalysis on Agricultural Materials: A Natural Alternative," Abstract, Tenth CIFAR Conference, University of California—Davis, Davis, Calif., October 9.

Gollin. D., X. L. Li, S. M. Liu, E. T. Davies, and L. G. Ljungdahl 1998. "Acetogenesis and the Primary Structure of the NADP-Dependent Formate Dehydrogenase of *Clostridium thermoaceticum*, a Tungsten-Selenium-Iron Protein," pp. 303–8 in *Advances in Chemical Conversions for Mitigating Carbon Dioxide: Studies in Surface Science and Catalysis*, Vol. 114, T. Inui, M. Anpo, K. Izui, S. Yanagida, and T. Yamaguchi, eds., Elsevier Science B.V.

Herzog, H., E. Drake, and E. Adams 1997. *CO_2 Capture, Reuse, and Storage Technologies for Mitigating Global Climate Change: A White Paper*, Massachusetts Institute of Technology Energy Laboratory.

Lal, R., J. M. Kimble, R. F. Follett, and B. A. Stewart, eds. 1998. *Soil Processes and the Carbon Cycle*, CRC/Lewis Publishers, Boca Raton, Fla.

Lebedeva, E. V., N. N. Lyalikova, and Yu Yu Bugel'skii 1978. "Participation of Nitrifying Bacteria in the Weathering of Serpentinized Ultrabasic Rocks" (in Russian), *Mikrobiolobiya* 47:1101–7.

Mann, C. 1999. "Genetic Engineers Aim to Soup Up Crop Photosynthesis," *Science* 283 (January 15) 314–16.

National Laboratory Directors 1997. *Technology Opportunities to Reduce U.S. Greenhouse Gas Emissions*, Oak Ridge National Laboratory.

Ralph J., J. J. MacKay, R. D. Hatfield, D. M. O'Malley, R. W. Whetten, and R. R. Sederoff 1997. "Abnormal Lignin in a Loblolly Pine Mutant," *Science* 277:235–9.

Ralph, J., R. D. Hatfield, J. Piquemal, N. Yahiaoui, M. Pean, C. Lapierre, and A. M. Boudet 1998. "NMR Characterization of Altered Lignins Extracted from Tobacco Plants Down-Regulated for Lignification Enzymes Cinnamylalcohol Dehydrogenase and Cinnamoyl-CoA Reductase," *Proc Natl Acad Sci U.S.A.* 95:12803–8.

Saxena, I. M., F. C. Lin, and R. M. Brown, Jr. 1990. "Cloning and Sequencing of the Cellulose Synthase Catalytic Subunit Gene of Acetobacter Xylinum," *Plant Molecular Biology* 15:673–83.

Saxena, I. M., F. C. Lin, and R. M. Brown, Jr. 1991. "Identification of a New Gene in an Operon for Cellulose Biosynthesis in Acetobacter Xylinum," *Plant Molecular Biology* 16:947–54.

Saxena, I. M., K. Kudlicka, K. Okuda, and R. M. Brown, Jr. 1994. "Characterization of Genes in the Cellulose Synthesizing Operon (Acs Operon) of Acetobacter Xylinum:

Implications for Cellulose Crystallization," *J. Bacteriology* 176:5735–52.

Skog, K. E., and G. A. Nicholson 1998. "Carbon Cycling through Wood Products: The Role of Wood and Paper Products in Carbon Sequestration," *Forest Products Journal* 48:75–83.

Stevens, T. O., and J. P. McKinley 1995. "Lithoautotrophic Microbial Ecosystems in Deep Basalt Aquifers," *Science* 270:450–4.

Stevens, T. O., and J. P. McKinley 1995. "Lithoautotrophic Microbial Ecosystems in Deep Basalt Aquifers," *Science* 270: 450–4.

University of Chicago 1998. "ISAM 'J' Carbon Cycle Model: Modify the CO_2 Emissions," University of Chicago, Department of the Geophysical Sciences, available at www.geosci.uchicago.edu/~archer/cgimodels/isam.d.html.