Industrial and Environmental Uses of Recombinant Microorganisms

- Restriction endonucleases and small biological molecules
- Microbial degradation of xenobiotics
- Utilization of starch and sugars
- Utilization of cellulose and hemicellulose
- Lipids from cyanobacteria
- Hydrogen production

Restriction enzymes are great products for recombinant microbes (*E. coli*)

- Over $500 million in annual RE sales in 2015
- Some microbes are difficult or expensive to grow in culture
- Strategy: clone the gene for the RE from a given microbe and express it in *E. coli* (along with the corresponding modification [methylase] gene for protection of the *E. coli* DNA)
- *E. coli* is simple to grow

Figure 8.1 A. Protection against RE digestion by treatment with its corresponding methylating enzyme. B. Expression of the methylase in the cytoplasm and the RE in the periplasm of a Gram-negative bacteria like *E. coli*.

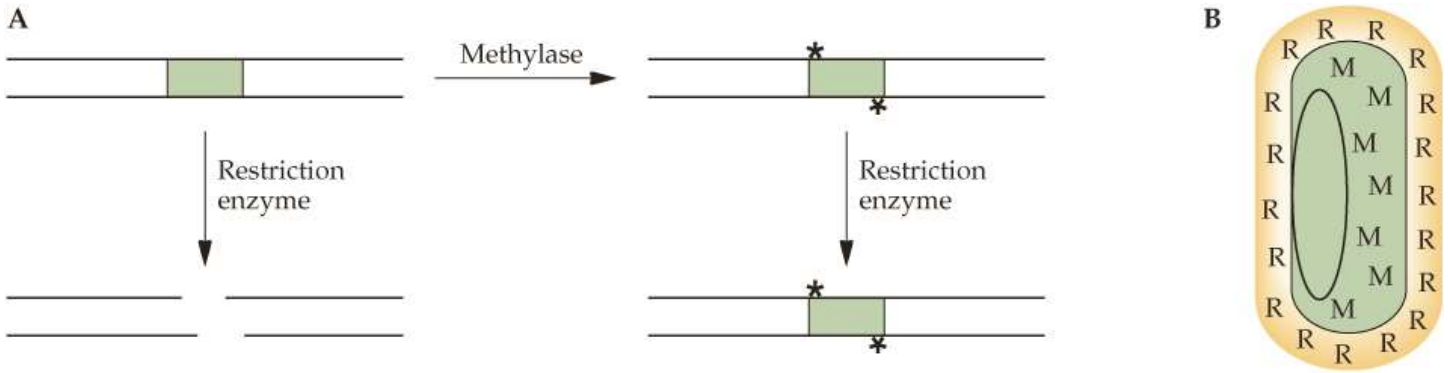


Figure 8.2 Scheme for cloning the DdeI modification (methylating) enzyme.

1. Make a genomic library from *Desulfovibrio desulfuricans* chromosomal DNA and place it in a plasmid vector containing at least 1 DdeI recognition site
2. Transform *E. coli* cells with these plasmids
3. Isolate plasmids from the *E. coli* colonies
4. Digest these plasmids with DdeI
5. Transform another batch of *E. coli* cells with the digested plasmids (only the plasmids expressing the DdeI methylase enzyme [X] will be protected and transform the *E. coli*)

Note: it is common for the RE to be located in the same operon as its corresponding modification enzyme

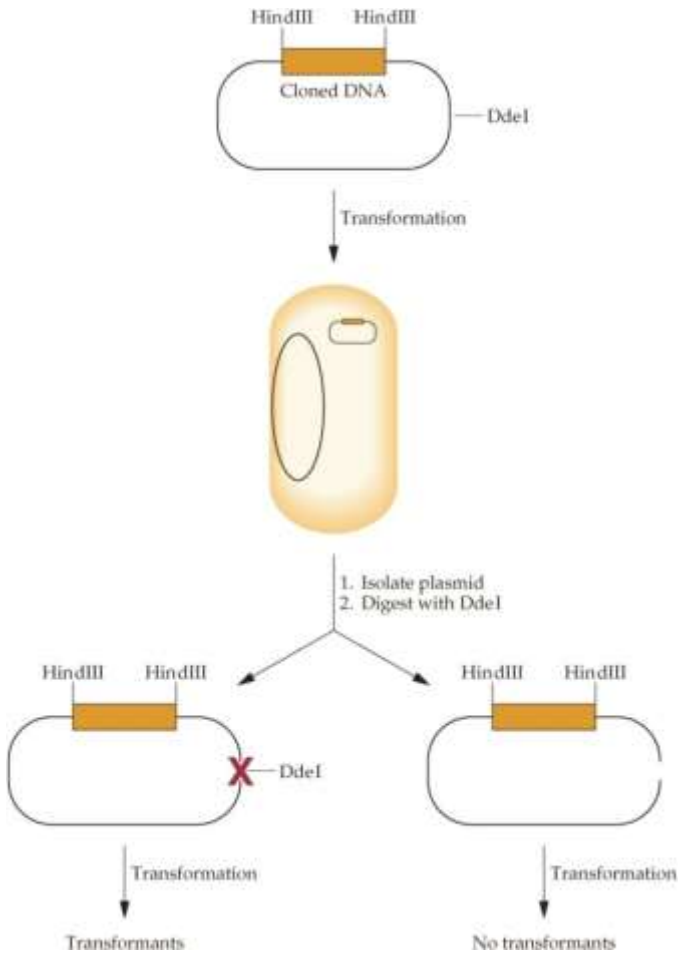


Table 8.1

Table 8.1 Some of the compounds whose synthesis has been metabolically engineered in microbes

Type of compound	Compound
Acids	D-Glucuronic acid
	L-Ascorbic acid
	3-Hydroxypropionic acid
	Malic acid
	Muconic acid
	Lactic acid
	Succinic acid
	Itaconic acid
Alcohols	1-Propanol
	2-Butanol
	1-Butanol
	1,4-Butanediol
	1,2-Propanediol
	Ethanol
	Isopropanol
	Isobutanol
	2,3-Butanediol
	1,3-Propanediol

(Continued)

Table 8.1 Some of the compounds whose synthesis has been metabolically engineered in microbes (Continued)

Type of compound	Compound
Amino acids	L-Alanine
	L-Arginine
	L-Valine
	L-Lysine
	L-Phenylalanine
	L-Threonine
	L-Tyrosine
	L-Tryptophan
	L-Cysteine
	L-Citrulline
Naturally occurring organic compounds	Monoterpene
	Sesquiterpene
	Diterpene
	Tetraterpene
	Flavonoids
	Amorpha-4,11-diene
	Artemisinic acid
	Indigo
	Lycopene
Others	Biodiesel
	Resveratrol
	Vanillin
	Lipids
	Antibiotics
	Isoprene
	Farnesene
	Biopolymers

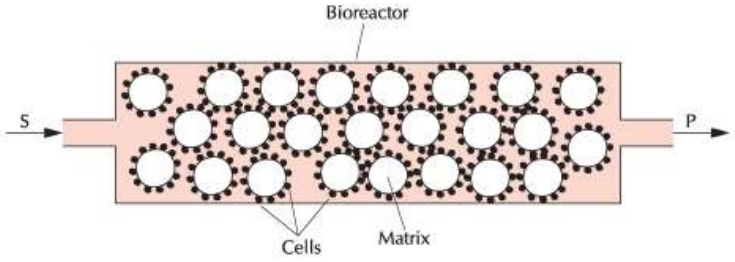
Table 8.2

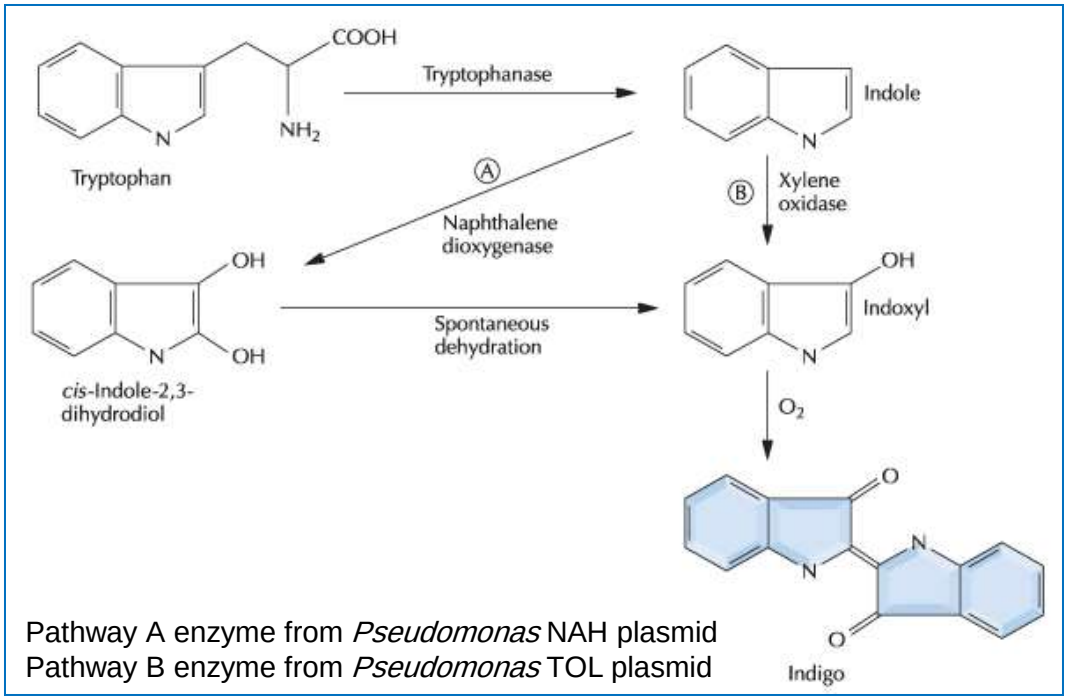
Table 8.2 Commercial applications of amino acids

Amino acid	Application(s)
Alanine	Flavor enhancer
Arginine	Therapy for liver diseases
Aspartic acid	Flavor enhancer; sweetener synthesis
Asparagine	Diuretic
Cysteine	Bread production; therapy for bronchitis; antioxidant
Glutamic acid	Flavor enhancer
Glutamine	Therapy for ulcers
Glycine	Sweetener synthesis
Histidine	Therapy for ulcers; antioxidant
Isoleucine	Intravenous solutions
Leucine	Intravenous solutions
Lysine	Feed additive; food additive
Methionine	Feed additive
Phenylalanine	Infusions; sweetener synthesis
Proline	Intravenous solutions
Serine	Cosmetics
Threonine	Feed additive
Tryptophan	Intravenous solutions; antioxidant
Tyrosine	Intravenous solutions; precursor for L-DOPA
Valine	Intravenous solutions

Small biological molecules are great products for recombinant microbes (often *E. coli*)

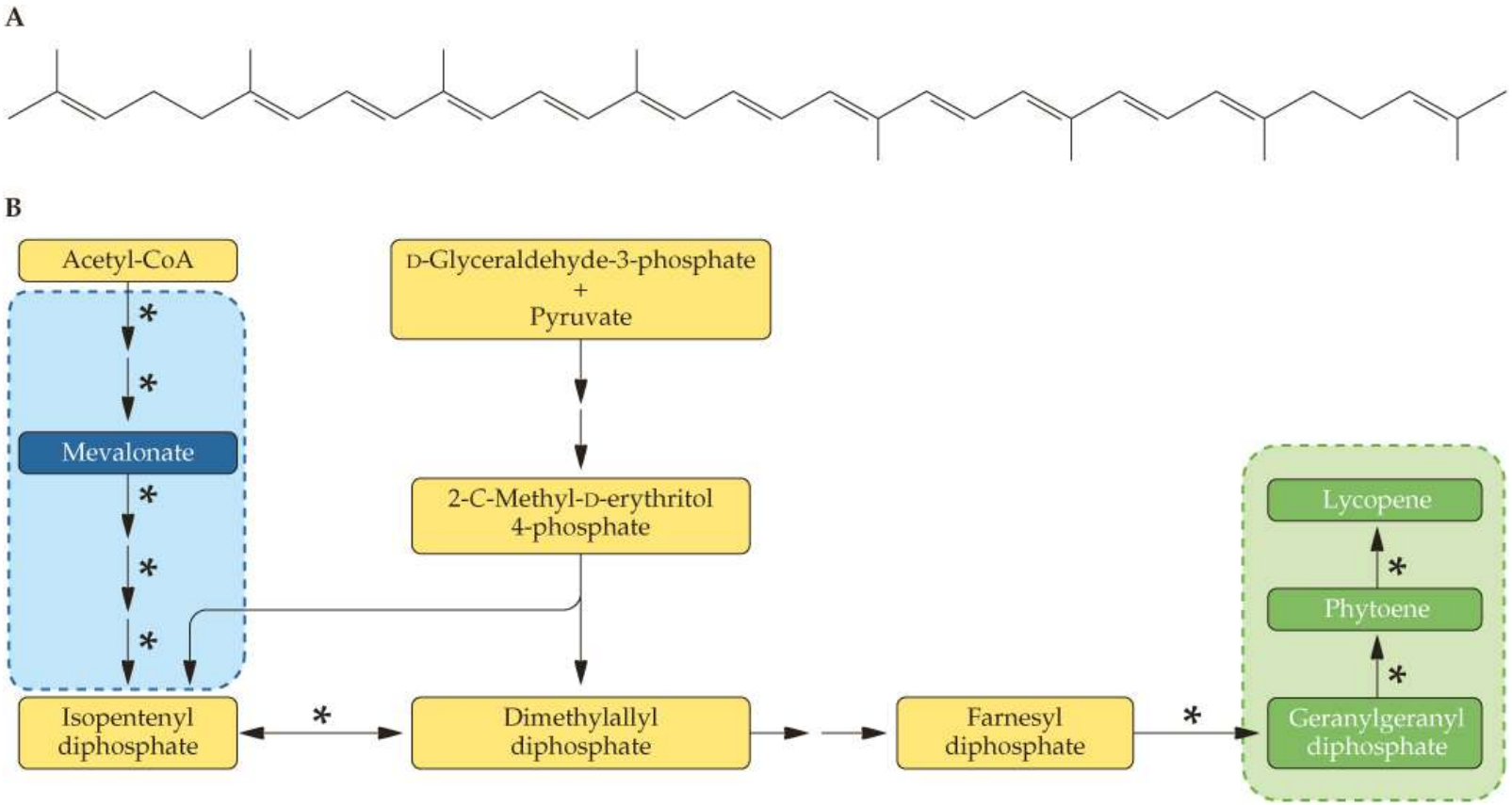
- Indigo (Fig. 8.6)





- Ascorbic acid (Vitamin C)
- Amino acids (Glutamic acid-Glu for production of the flavor enhancer MSG)
- Lycopene-antioxidant commonly found in tomatoes and other fruit
- Antibiotics, novel antibiotics, and polyketide antibiotics
- **Note that in all of these cases, one needs to clone the genes encoding the enzymes making these metabolites in order to create or alter a biochemical pathway**

Figure 8.11
A. Chemical structure of lycopene
B. Production of lycopene in *E. coli* using existing *E. coli* genes (yellow), yeast genes (blue) and other bacteria [*Pantoea agglomerans*] genes (green)



Biopolymers are great products for recombinant microbes

- Xanthan gum production in *Xanthomonas compestris* (genetically engineered to grow on whey, a lactose-rich byproduct of cheese production)
- Biodegradable plastics (polyhydroxyalkanoates)
- Note in most of these cases, one needs to clone the genes encoding enzymes in order to create or alter a biochemical pathway in a host cell to produce the desired product or outcome

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Figure 8.22 A. Structure of xanthan gum B. Genetic engineering of *E. coli* lacZ and lacY genes for expression in *Xanthomonas campestris* (Xc) so that Xc can now grow in the presence of whey (a waste by-product of the cheese making process which contains lactose) [See Table 8.3 below]

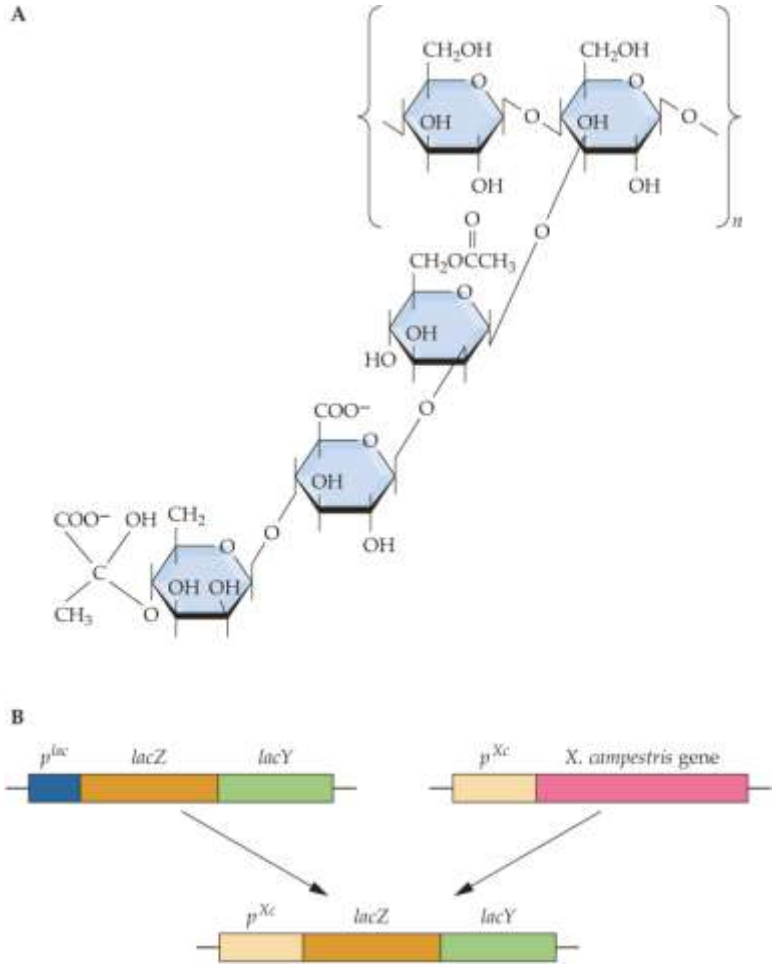


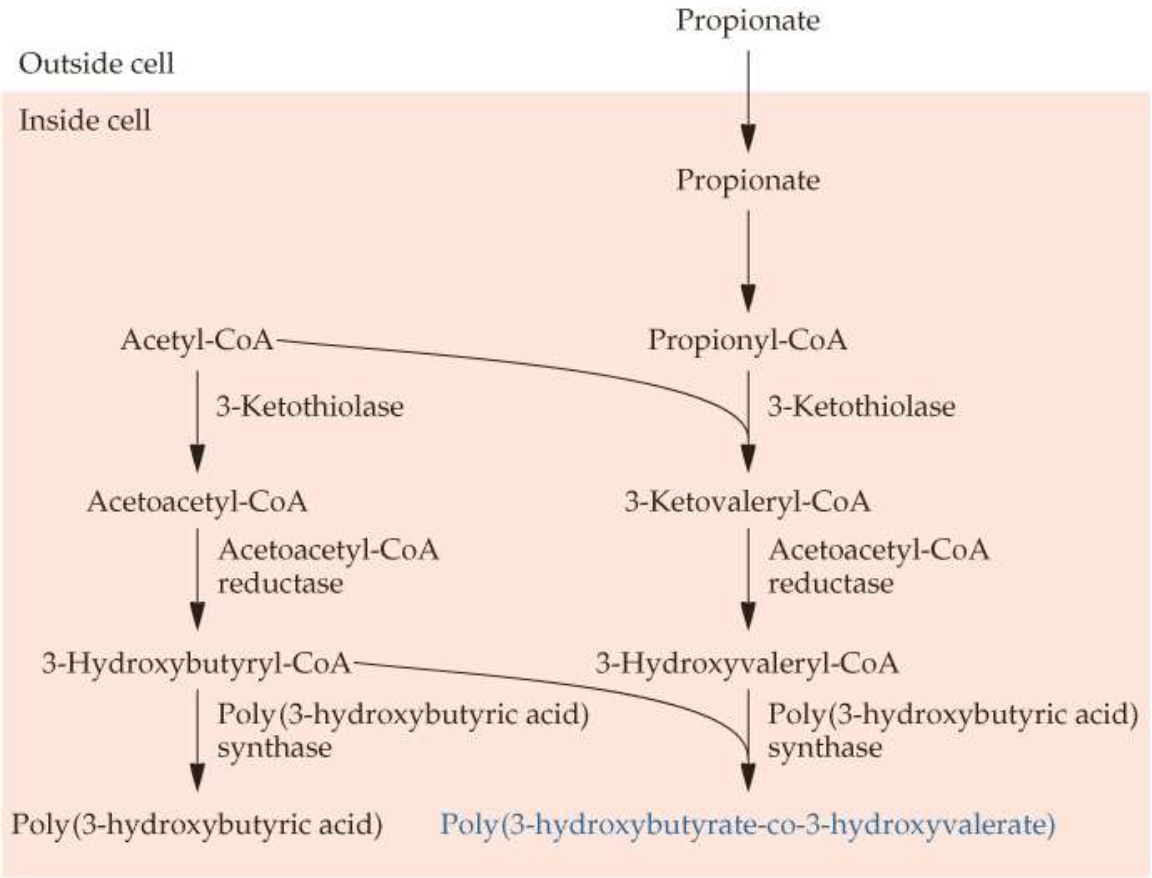
Table 8.3 Production of xanthan gum by wild-type and transformed *X. campestris*

	Amount of xanthan gum produced (μg/ml) with:		
X. campestris	0.4% Glucose	0.4% Lactose	10% Whey
Wild type	3,530	245	224
Transformant	3,711	3,608	4,241

Adapted from Fu and Tseng, *Appl. Environ. Microbiol.* **56**:919–923, 1990.

The amount of the product is expressed as micrograms per milliliter of culture grown on a minimal medium with either 0.4% glucose or 0.4% lactose added or on diluted whey (10%), which contains approximately 0.44% lactose. The transformant carries the *E. coli lacZY* genes on a plasmid.

Figure 8.23 Genetic engineering of *E. coli* to produce biodegradable plastics. Genes encoding the enzymes responsible for the production of these biodegradable plastic molecules were cloned from *Alcaligenes eutrophus* (now called *Cupriavidus metallidurans*) and transferred to *E. coli*.



Using Microbes for Bioremediation and Degradation of Xenobiotics

- Bioremediation-The use of biological agents to remove toxic wastes from the environment.
- Xenobiotics-Unnatural chemicals such as herbicides, pesticides, refrigerants, solvents, petroleum, and other organic compounds.

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Table 8.4

Table 8.4 Some *Pseudomonas* plasmids, their degradative pathways, and sizes

Name of plasmid	Compound(s) degraded	Plasmid size (kilobases)
SAL	Salicylate	60
SAL	Salicylate	68
SAL	Salicylate	72
SAL	Salicylate	83
TOL	Xylene and toluene	113
CAM	Camphor	225
XYL	Xylene	15
NAH	Naphthalene	69
OCT	Octane, D-camphor	~500
NAH7	Naphthalene, salicylate	83
PJP1	2,4-Dichlorophenoxyacetic acid	87
PJP2	2,4-Dichlorophenoxyacetic acid	54
PJB3	2,4-Dichlorophenoxyacetic acid	78
pP51	1,2-Di, 1,4-di-, and 1,2,4-trichlorobenzene	110
pAC31	3,5-Dichlorobenzoate	108
pAC25	3-Chlorobenzoate	102
pWW0	Xylene and toluene	117
pWW100	Biphenyl	200
pWWO	Xylene and toluene	176
pXYL-K	Xylene and toluene	135
pVI150	Phenol	>200
pNL1	Xylene, naphthalene, biphenyl	184
pAC27	3-Chlorobenzoate	110
pHMT112	Benzene	112
pTDN1	Aniline, *m*- and *p*-toluidine	79

Plasmids with the same name encode similar degradative pathways, even though they have different sizes and were described in different laboratories.

Figure 8.27 Chakrabarty et al. (1980) developed and patented a "superbug" that degraded petroleum (camphor, octane, xylene, and naphthalene) by plasmid transfers. Chakrabarty was granted the first U.S. patent for this genetically engineered microorganism by the U.S. supreme court in a landmark decision.

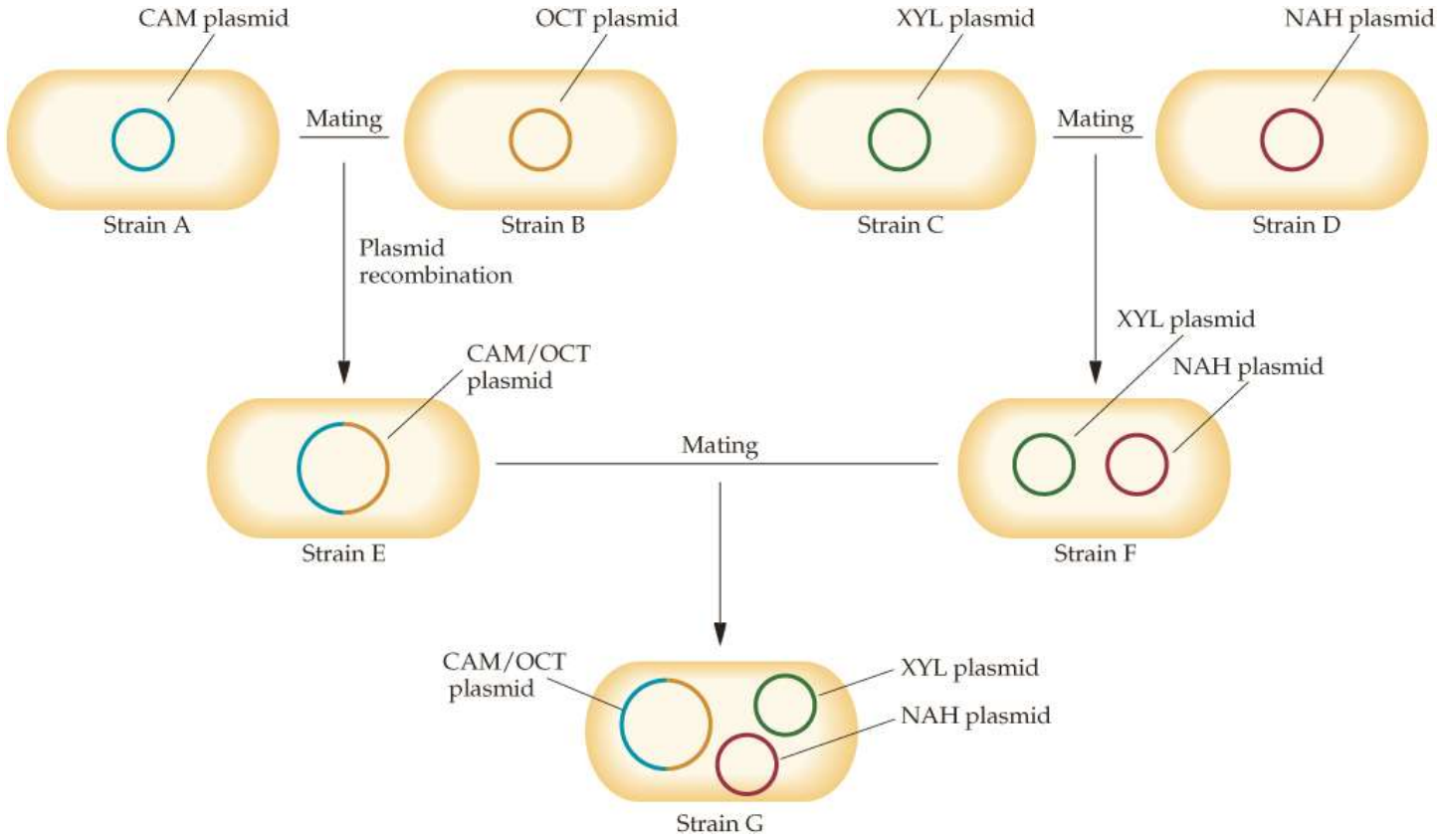

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Figure 8.30

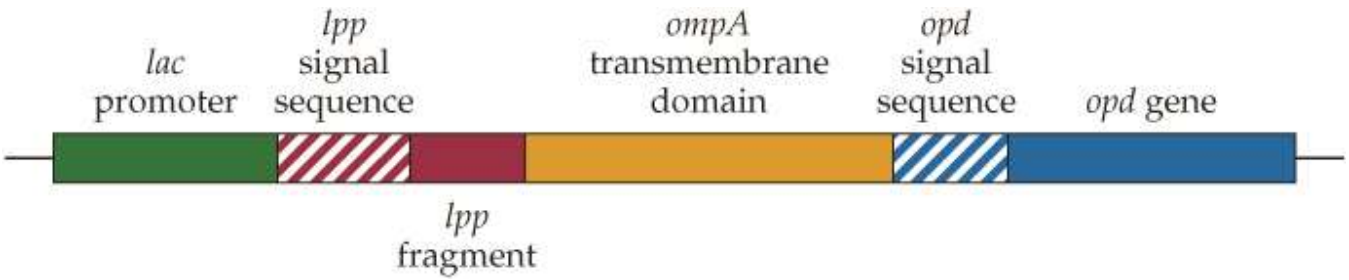
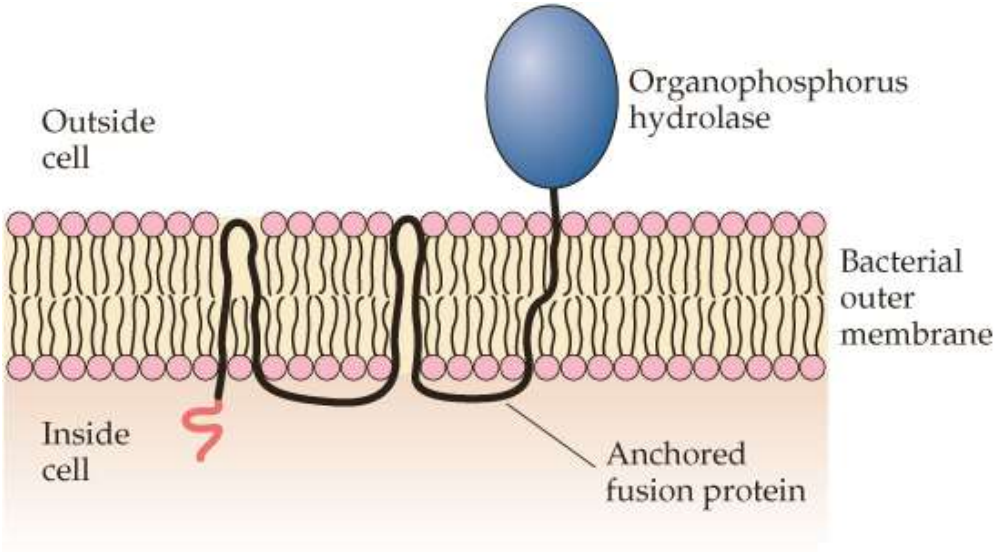


Figure 8.31



Expressing a *Pseudomonas* or *Flavobacterium* organophosphorus hydrolase (opd) gene fused to a lipoprotein gene at the *E. coli* cell surface to degrade organophosphate pesticides.

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Figure 8.35 Industrial production of fructose and alcohol from starch. The three key enzymes for this process are shown by red arrows on the right. Several approaches have been used to inexpensively produce these enzymes in various microbe host cells in order to lower the cost of fructose and alcohol production.

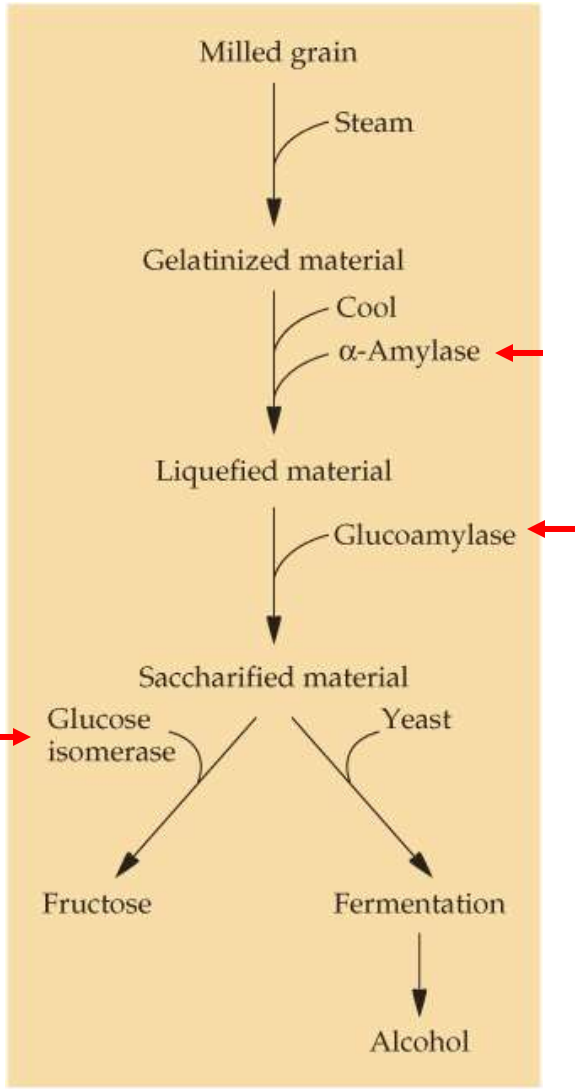
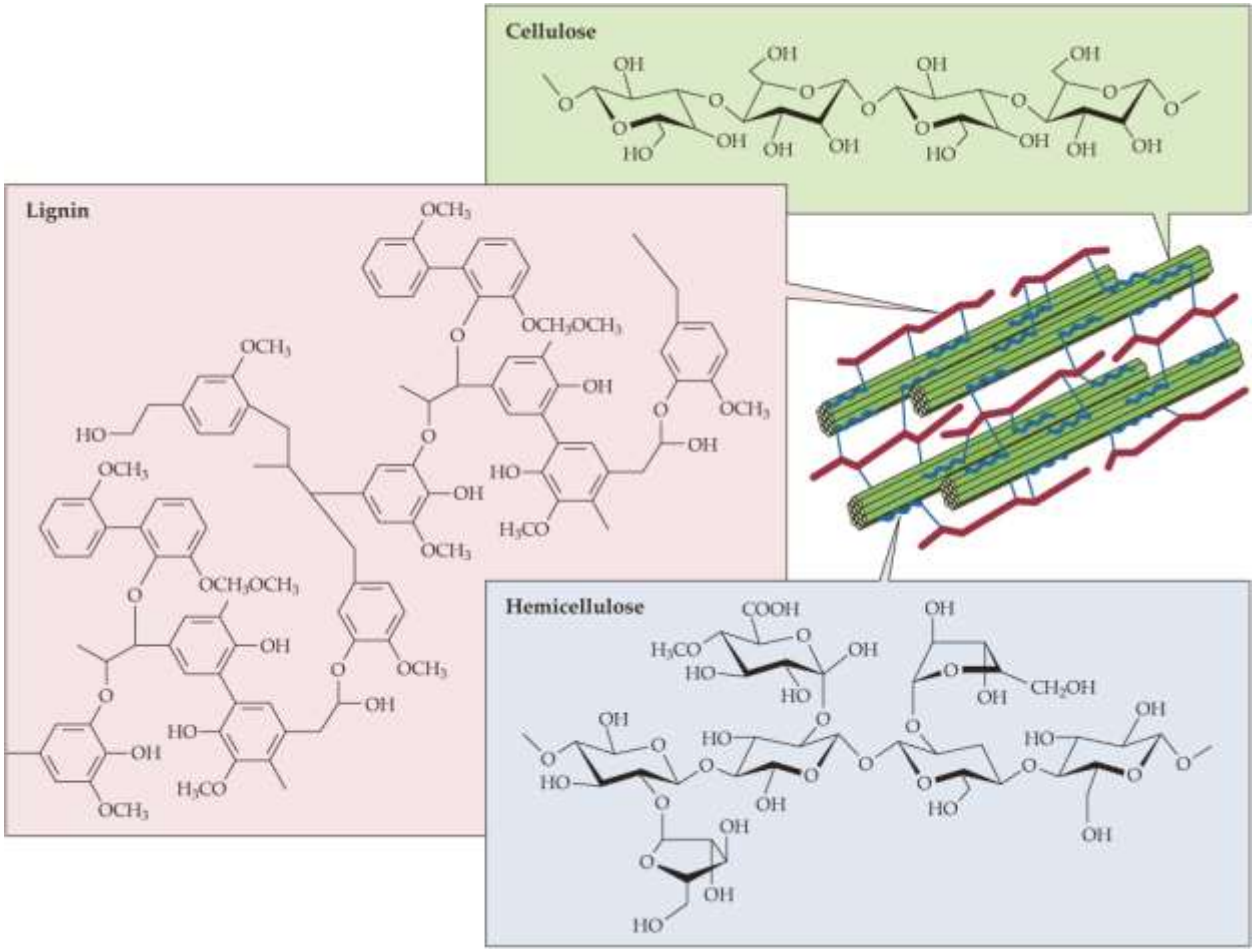


Figure 8.42 Utilization of cellulose and hemicellulose in the plant cell wall for renewable bioenergy (alcohol) production



Cellulosic Ethanol Production and Research Challenges

This figure depicts some key processing steps in a future large-scale facility for transforming cellulosic biomass (plant fibers) into biofuels. Three areas where focused biological research can lead to much lower costs and increased productivity include developing crops dedicated to biofuel production (see step 1), engineering enzymes that deconstruct cellulosic biomass (see steps 2 and 3), and engineering microbes and developing new microbial enzyme systems for industrial-scale conversion of biomass sugars into ethanol and other biofuels or bioproducts (see step 4). Biological research challenges associated with each production step are summarized in the right portion of the figure.

Potential Bioenergy Crops

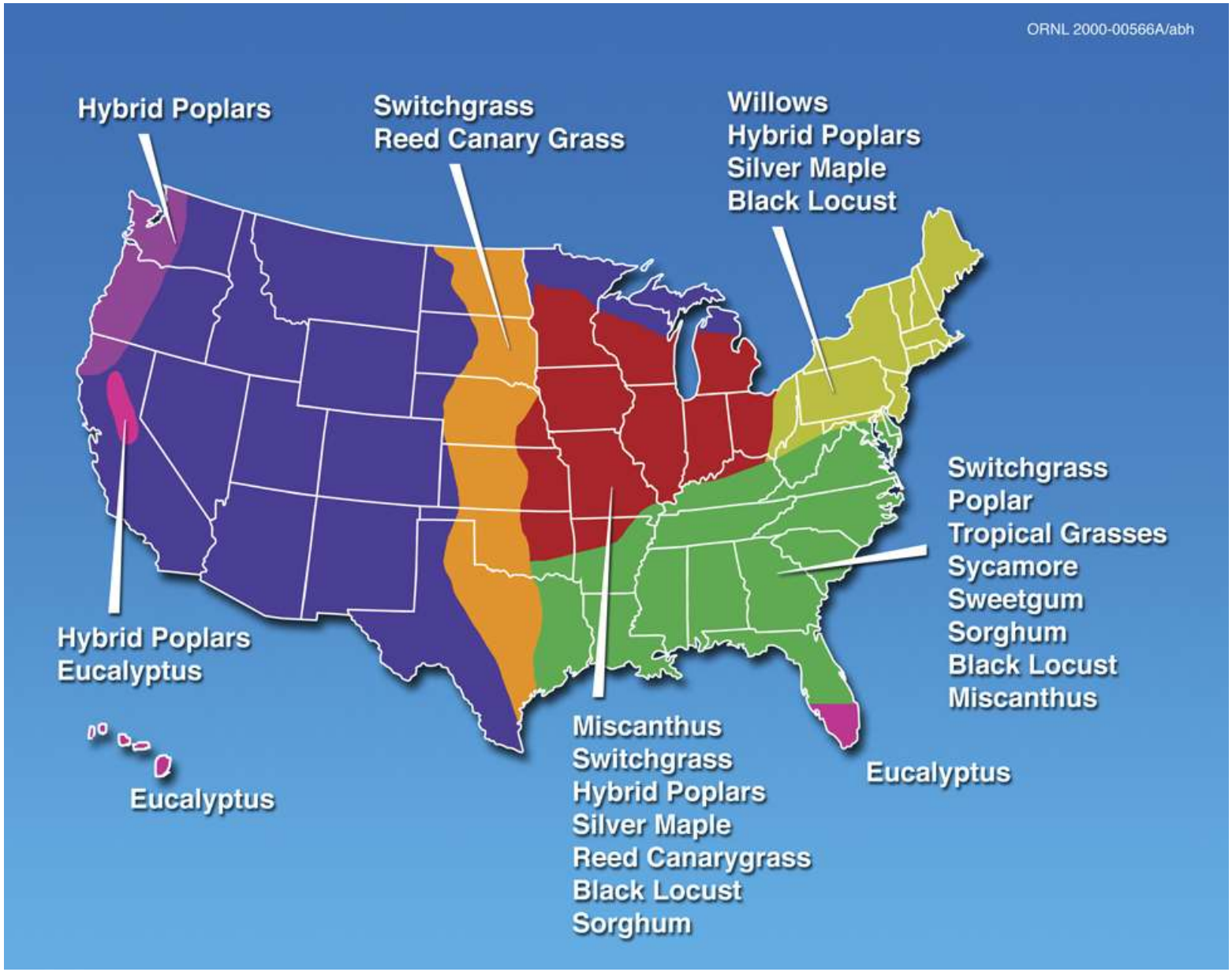


Figure 8.54 Using cyanobacteria (or other photosynthetic microorganisms) to produce fatty acids, which in turn can be used to make biodiesel (which involves chemically reacting the fatty acids with an alcohol to make fatty acid esters).

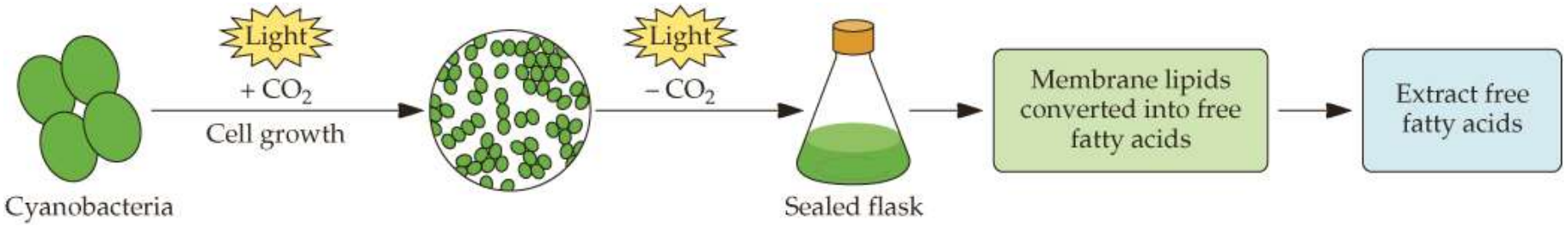


Figure 8.55 H_2 is produced by *E. coli* (as well as other microbes) from formic acid (formate= $HCOO^-$) and could be used as a biofuel if it could adequately produced and stored on a large scale. H_2 is considered a zero-emission fuel and can be burned with O_2 or air to release energy and H_2O.