ty. Keeping in mind the limits in carrying out a fermentation, one can envision immobilized whole cell systems providing an opportunity to achieve high productivities and hence high product concentrations. The unit operations in a biological process have a considerable effect on each other, and it is axiomatic that whatever you do upstream will have an impact on downstream processing. For this reason, it is important to carefully integrate bioreactors and all subsequent product recovery operations. Biotechnology holds great promise for the efficient production of pharmaceutical, chemical, and agricultural products. The continued success of biotechnology depends significantly on the development of bioreactors, which represent the focal point for interaction between the life scientist and the process engineer.

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Production of Feedstock Chemicals

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Summary. Renewable raw materials may be converted by biological means to feedstocks for the chemical industry. Glucose from cornstarch is the current choice as a substrate, although advances may enable the use of less expensive lignocellulosic materials. The production of oxychemicals and their derivatives from renewable resources could amount to about 100 billion pounds annually, or about half of the U.S. production of organic chemicals. Ethanol produced by fermentation is now cost-competitive with industrial ethanol produced from fossil fuel. Biological routes to other oxychemicals exist and are expected to be important in the future. Several product recovery methods may be used, but new energy-conserving methods will be needed to make the engineering-biology combinations economical.

Photosynthetic products accumulated over eons as natural gas, petroleum, coal, and related carbonaceous materials have provided an immense resource for the development of our highly industrialized and energy-intensive civilization. However, we now recognize that this reservoir of fossil carbon is finite and is already limiting industrial growth. We must develop ways to rely more heavily on products of current photosynthesis for the basic feedstocks required by the chemical industry. Conversion of the chemical industry from a fossil carbon base to a renewable carbon base is not likely to be rapid or easy as viewed by one generation. However, it is important for future generations that we give attention and support to initiating and carrying out this conversion.

There are three major approaches to the transformation of biomass to useful feedstock chemicals: chemical, physical, and biological. Hydrogenation, pyrolysis, and fermentation are respective examples. In this article we review the current status and potential of fermentative processing of biomass to feedstock chemicals (*1–3*). We discuss the nature, supply, and economics of biomass; the microbial transformation of biomass to desired chemicals by fermentation processes; and the recovery and purification of these materials from the dilute aqueous solutions in which they are produced. We do not deal with fermentation routes to chemicals for energy use, such as ethanol. Ethanol may play a useful role as an octane enhancer in gasoline, but the amount potentially available from biomass would not be a significant replacement for fossil fuel. Fermentation processes for specialty chemicals such as antibiotics and vitamins (*4*) are well established and are not reviewed in this article.

Biomass Sources

Biomass consists of collectible plant-derived materials that are abundant, inexpensive, and potentially convertible to feedstock chemicals by fermentation processes. Biomass is found as starch in corn, wheat, potatoes, cassava, the sago palm, and other agricultural products and as monomeric sugars or soluble oligomers in corn syrup, molasses, raw sugar juice, sulfite waste liquors, and so on. It also occurs as lignocellulose in the

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form of wood chips, crop residues, forest and mill residues, urban refuse, and animal manures.

Biomass supply. The supply of biomass depends on the land dedicated to useful photosynthesis. Of the total 2.3 billion acres of the United States, 380 million acres (17 percent) are devoted to crops, 720 million acres (32 percent) to forest and woodland, and 680 million acres (30 percent) to pasture or grazing land (*5*). Of all American crops, corn is the primary source of starch because of its ample supply and low cost relative to other sources of starch or sugar and because there is an established commercial system for storing and transporting it cheaply over long distances. The 1981–1982 crop of 8 billion bushels (190 million dry tons) contained enough starch to provide 285 billion pounds of glucose (*6*).

Lignocellulosic crop residues are also abundant, but commercial collection systems are limited. Lignocellulose is a structural material of plants and is a composite of three polymers: cellulose, a linear polymer of glucose that occurs as crystalline microfibrils; hemicellulose, an amorphous branched copolymer that consists mainly of xylose; and lignin, a cross-linked polymer of substituted phenylpropane units. In wheat straw and hardwoods the proportions of these polymers are 42, 35, and 22 percent, respectively (*7, 8*). At present, only 8 million dry tons of crop residues such as sugarcane bagasse, cotton gin trash, and rice hulls are collected annually at central processing sites (Table 1). About 105 million dry tons of corn stalks and 180 million dry tons of cereal straw are available annually and could be collected if the demand warranted. Other agricultural residues amount to 500 million dry tons, but they are too diffuse to be collected economically or must be retained on the land to maintain the soil.

The annual growth of American forests could provide an economically collectible supply of 270 million dry tons of lignocellulosic biomass (Table 2). Eastern hardwoods, which are less important to the pulp and paper industry than the stronger fibered conifers, are primary target sources (*9, 10*).

Solid waste from paper and board products from the 32 largest urban centers might supply another 30 million tons. The supply from each of the centers would exceed the 400,000 dry tons needed annually for a cellulose-based chemicals plant (*11–13*). However, because of the heterogeneity of these materials, safety and process problems could arise in downstream operations.

The "grassland" cellulose resource—mainly animal manure (Table 3)—is too diffuse, except on a few large feedlots, to be a source of lignocellulose for chemicals (*14, 15*).

Hence, from a potential annual supply of 1.8 billion dry tons of lignocellulose from U.S. cropland, grassland, and forest, the 550 million dry tons of biomass available as wood chips, cereal straw, and corn stalks and the starch from 190 million tons of corn grain appear to be the most likely basis for a chemicals-from-biomass industry.

Saccharification. Starch or lignocellulose can be converted to chemical products either directly or after hydrolysis to the corresponding monomeric sugar for use as an intermediate feedstock. If technically feasible, direct use of the polysaccharide is preferred (*16*). However, most fermentations and chemical conversions take place more readily with a monomeric sugar feedstock. Moreover, it may be desirable to have a large common supply of sugar feeding a number of smaller fermentation operations as part of a "biorefinery" complex.

The large corn wet milling industry now provides a supply of hydrolyzed cornstarch (*17*). We estimate that in 1985 corn syrups could be produced commercially from corn at $3.40 per bushel at a cost of about 12 cents per pound of sugar (*18*). The price places a competitive cost ceiling on the market value of lignocellulose-based "biosugars."

Cellulosic biomass at $20 to $30 per dry ton is far cheaper than corn at $110 per dry ton (*19, 20*). However, the intractable nature of the cellulose crystallite makes hydrolysis very difficult; hence there is a trade-off between low raw material costs and high investment costs for hydrolysis equipment. In addition, the residues from corn wet milling are high-value oil and protein feeds, while markets for lignin—the residue from cellulose hydrolysis—have yet to be developed. Hence, at present, cellulose hydrolysis is not economically competitive with starch hydrolysis as a source of sugar.

Processes in which concentrated acids are used to catalyze cellulose hydrolysis have been commercially unsuccessful because of the cost of recovering and recycling the acid. Dilute-acid processes have lower acid-associated costs, but they have poorer yields and require rigid control of residence time at high temperature. Consequently, power costs and investment are too high for these processes to compete with corn hydrolysis.

A biological approach to cellulose hydrolysis involves the use of cellulolytic enzymes, such as those produced by the fungus *Trichoderma reesei* (*21*). The enzymes are produced extracellularly in a separate fermentation process and transferred to the hydrolysis process as a supernatant liquid after the cells have been filtered off. The recent development of hypercellulolytic mutants has increased productivity in this step more than tenfold (*22, 23*). However, two

Table 1. U.S. cellulosics potential: cropland resource (1977–1979 crop data). Values are given as million dry tons per year.

Source	Collected supply	Collectible reserve	Potential resource
Corn stover		105	212
Cereal straw		180	180
Soybean residues		25	50
Bagasse, gin trash, and rice hulls	8		8
Other crops			360
Total cropland	8	310	810

Table 2. U.S. cellulosics potential: forest resource (1977–1979 data). Values are given as million dry tons per year.

Source	Collected supply	Collectible reserve	Potential resource
Net annual growth*		270	450
Logging residues		105	145
Process residues and wastes			
Pulp mills	3	38	46
Sawmills (excluding chips)	13	13	26
Paper and board mills		12	13
Fuel wood	3		3
Urban solid wastes	41	12	77
Total forest	60	450	760

*Net after mortality and commercial removals from a commercial inventory of 25 billion tons of standing tree stems.

problems remain: (i) it is necessary to pretreat the lignocellulose to make the substrate more accessible to enzyme attack, and (ii) the enzyme is inhibited by the product glucose and its dimer, cellobiose. Hydrolysis with dilute acid (*24*) is an effective pretreatment, but we estimate that this step adds 3 cents per pound to the cost of the sugar produced. Steam-explosion pretreatments (*25, 26*) and a liquid ammonia freeze-explosion technique (*27*) may prove to be more cost-effective.

Nature of biomass. Chemically, almost all biomass, regardless of the source, contains about 45 percent oxygen on a moisture- and ash-free basis (*7, 8*) and contains 50 percent moisture as collected. This biomass makes a poor fuel. At 50 percent moisture, materials such as bagasse have a net heating value of only about 6800 Btu's per pound (dry basis), or about half that of bituminous coal (*28*). Cellulosic biomass is a poor choice as an energy source unless it is a waste material that must be disposed of at least cost. However, biomass as starch or lignocellulose has great potential as a feedstock for oxychemicals that retain the oxygenated nature of the basic CH_2O structure. Fermentative production of oxychemicals and derivatives is discussed in the following section.

Chemical Products

The current annual U.S. production of organic chemicals is approximately 210 billion pounds, 99 percent of which is accounted for by the top 100 chemicals (*29, 30*). Of the top 100 chemicals, 74 percent are produced from five primary feedstocks: ethylene, propylene, benzene, toluene, and xylene. Many are oxychemicals that have been, are, or could be produced by microbial fermentation with or without chemical processing (Table 4). These oxychemicals from renewable resources could account for 50 billion pounds or 23 percent of the total production of organic chemicals. Their derivatives could amount to another 26 percent, for a total production of half that of the top 100 chemicals (*30*). The current annual value of these chemicals is over $15 billion (*29*) (Table 5).

Fermentation. The fermentation of molasses to ethanol by *Saccharomyces cerevisiae* is well documented (*31*). Fermentation ethanol is now cost-competitive with industrial ethanol. Ethanol concentrations of 10 to 20 percent are obtained in 36 to 48 hours, depending on the yeast strain. Higher rates could be obtained with advanced, experimental process designs. External cooling is necessary to keep the fermentation temperature below 30°C. Recently, researchers have focused on thermophilic microorganisms such as *Thermoanaerobium ethanolicus* or *Clostridium thermohydrosulfuricum* for ethanol production at higher temperatures (*32, 33*). These microorganisms grow at a high temperature (60°C) and they can produce ethanol from starch, xylose, and other carbohydrates, but the concentration of ethanol produced, 4 percent, is too low for economical recovery.

Table 3. U.S. cellulosics potential: grassland resource (1977 data). Values are given as million dry tons per year.

Source	Collected supply	Collectible reserve	Potential resource
Cattle	5	4	237
Hogs			11
Broilers			6
Chickens		2	4
Sheep			2
Total grassland	5	6	260

Industrial grade acetic acid, a key feedstock, is now manufactured solely by chemical processes (*34*), while food grade acetic acid (vinegar) is produced exclusively by oxidation of ethanol with *Acetobacter aceti* (*35, 36*). The latter reaction is highly exothermic and external cooling is mandatory since the efficiency of the process decreases with increasing temperature. The acetic acid fermentation operates semicontinuously in submerged culture with a 35-hour cycle time. Ethanol is maintained in the reactor at 1 percent until the acid concentration reaches 12 percent; then 35 percent of the liquid is withdrawn and replaced with fresh medium. A 96 to 98 percent conversion is achieved with a final ethanol concentration of less than 0.2 percent.

Historically, acetone/butanol fermentation has been a highly successful process for the biological production of chemicals (*37*). Manufacture in the United States lasted from the early 1920's to the late 1950's. Now the acetone/butanol plant in South Africa is probably the only fermentation facility operated for this purpose (Fig. 1), although unconfirmed reports indicate that the People's Republic of China might have a large-scale plant in operation. Fermentation under anaerobic conditions at 30° to 32°C is complete in 40 to 80 hours, depending on the substrate. During this period the *p*H decreases steadily from 6.0 to 5.0 as butyric acid is produced and then rises to above 6.0 as butyric acid is converted to butanol. Acetone is produced during the latter period. The final broth contains approximately 2 percent total solvents. The solvent yields and ratios vary with different strains. With *Clostridium acetobutylicum* (Fig. 2) the solvent yield is 30 to 33 percent and the acetone : butanol : ethanol ratio is 3 : 6 : 1. Large quantities of hydrogen and carbon dioxide are also produced during the fermentation. Isopropyl alcohol rather than acetone is produced by *Clostridium aurianti-*

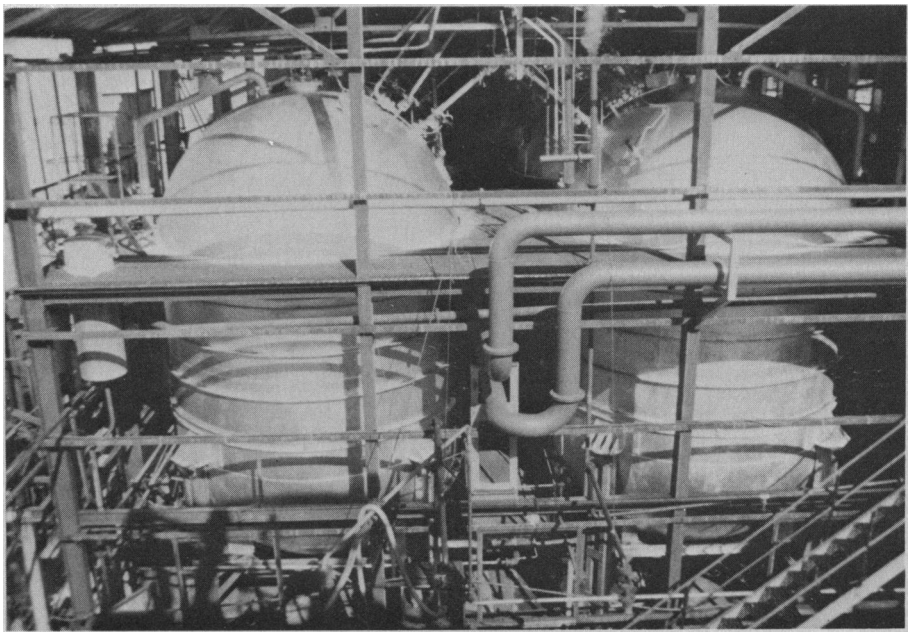

Fig. 1. Row of 90,000-liter fermentors for acetone/butanol production at National Chemical Products Ltd., Germiston, South Africa. [Courtesy of D. R. Woods, University of Cape Town, Rondebosch, South Africa]

cum, with a solvent yield of 30 to 40 percent and an isopropyl alcohol : butanol : ethanol ratio of 2.7 : 1 : 6 (*36*).

The utilization of 2,3-butanediol as a precursor for 1,3-butadiene was investigated thoroughly during World War II, but the project never went beyond the pilot plant stage. The diol can be produced by a number of microorganisms, but *Klebsiella pneumoniae* is generally used. Diol concentrations as high as 10 percent have been reported (*38*). Fermentation efficiency ranges from 80 to 100 percent and the yield from starch is approximately 36 percent.

Traditionally, glycerol has been produced by chemical modification of ethanol-yeast fermentation (*31*). Recently, the production of glycerol through carbon dioxide fixation and sunlight by the alga *Dunaliella* has been suggested and tested at the pilot plant stage (*39*). The algae accumulate 7 molar glycerol intracellularly, an amazing 56 percent solution during growth in 5 molar sodium chloride. Production of glycerol is 8 grams per square meter per day. Besides

Table 4. Oxychemicals from renewable resources.

Chemical	1981 U.S. production (million pounds)	Current price (cents per pound)	1981 commercial value (million dollars)	Major use or derivative
Ethanol				
Ethylene	28,867*	25 to 25½†	8,169‡	Polyethylene, ethylene oxides
Butadiene	3,046	34	1,234	Styrene-butadiene rubber, polybutadiene rubber
Industrial	1,157	$1.70 to $1.82 per gallon (27.5 cents per pound)	359	Solvents, ethyl acetate and other esters
Ethylene glycol	4,055	27½ to 28½	1,281	Polyethylene terephthalate, antifreeze
Acetic acid	2,706	26½	511	Vinyl acetate, cellulose acetate
Acetone	2,167	31	483	Solvents, methyl, and other methacrylates
Isopropyl alcohol	1,644	$2.05 per gallon (31 cents per pound)	507	Acetone, solvents
Adipic acid	1,210	57	653	Nylon 66
Butanol	823	33½	251	Solvents, butyl acrylate
Acrylic acid	691	58	276	Polymers
Methyl ethyl ketone	626	37	260	Solvents
Propylene glycol	480	44	208	Unsaturated polyester resin
Glycerol	370	80½	259	Drugs, cosmetics
Citric acid	235	71 to 77½	192	Food, drugs

*Values in this column were collected from the *Chemical Marketing Economics Handbook* and U.S. International Trade Commission data on organic chemicals. †From *Chemical Marketing Reporter* (20 September 1982); actual prices depend on quality, quantity, and location. ‡From *Chemical Marketing Economics Handbook*, U.S. Bureau of Census, and U.S. International Trade Commission. Total values do not reflect actual price of transaction or current price.

Table 5. Microbial production of chemicals.

Chemical	Process	Microorganism	
Ethanol	$C_6H_{12}O_6 \xrightarrow{M^*}$	C_2H_5OH	*Saccharomyces cerevisiae*
Ethylene 1,3-Butadiene Ethylene glycol		C → $CH_2=CH_2$; $CH_2=CH\text{-}CH=CH_2$; $CH_2OH\text{-}CH_2OH$	*Zymomonas mobilis*
Acetic acid	$CH_6H_{12}O_6 \xrightarrow{M}$	CH_3COOH	*Clostridium thermoaceticum*
	$C_5H_5OH \xrightarrow{M}$	CH_3COOH	*Acetobacter aceti*
Acetone	$C_6H_{12}O_6 \xrightarrow{M}$	CH_3COCH_3	*Clostridium acetobutylicum*
Butanol		$CH_3(CH_2)_2CH_2OH$	
Isopropyl alcohol	$C_6H_{12}O_6 \xrightarrow{M}$	$(CH_3)_2CHOH$	*Clostridium aurianticum*
		$CH_3(CH_2)_2CH_2OH$	
Adipic acid	$CH_3(CH_2)_nCH_3 \xrightarrow{M}$	$HOOC(CH_2)_4COOH$	*Pseudomonas* species
Acrylic	$C_6H_{12}O_6 \xrightarrow{M_1}$	$CH_3CH(OH)COOH \xrightarrow{M_2/C} CH_2=CHCOOH$	1. (M_1) *Lactobacillus bulgarius* 2. (M_2/C) *Clostridium propionium*
	$CH_2OHCH(OH)CH_2OH \xrightarrow{M/C}$	$CH_2=CHCOOH$	*Klebsiella pneumoniae* (*Aerobacter aerogenes*)
Methyl ethyl ketone	$C_6H_{12}O_6 \xrightarrow{M}$	$CH_3CH(OH)CH(OH)CH_3 \xrightarrow{C} CH_3COCH_2CH_3$	*Klebsiella pneumoniae*
Propylene glycol	$CH_2OHCH(OH)CH_2OH \xrightarrow{C}$	$CH_3CH(OH)CH_2OH$	
Glycerol	$C_6H_{12}O_6 \xrightarrow{M}$	$CH_2OHCH(OH)CH_2OH$	*Saccharomyces cerevisiae*
	$H_2O + CO_2 \xrightarrow{M}$	$CH_2OHCH(OH)CH_2OH$	*Dunaliella* sp.
Citric acid	$C_6H_{12}O_6 \xrightarrow{M}$	$CH_2(COOH)(OH)C(COOH)CH_2(COOH)$	*Aspergillus niger*

*M, microbial fermentation (*30, 36, 37*); C, chemical processing (*35*).

being an important chemical feedstock, glycerol may be converted to propylene glycol by heating with sodium hydroxide (*34*).

Glucose is fermented to citric acid by *Aspergillus niger* (*35*). The surface culture techniques of the Koji process were used in the past. With better reactor design to increase oxygen transfer, a submerged culture process is employed in newer plants. The fermentation requires 10 to 14 days at 25° to 30°C to convert 20 to 25 percent of the glucose with 80 to 85 percent yield to citric acid, which is recovered as the calcium salt.

The chemicals described here are not exclusive. Other organic acids could be produced microbiologically in high yield and concentration; these include fumaric acid, lactic acid, itaconic acid, and gluconic and oxygluconic acids (*37, 38*). Hitherto, no industrial applications for these compounds have been developed, but with appropriate technology many may be converted to useful feedstocks. Polyhydroxybutyrate produced by *Alcaligenes eutrophus* is being investigated as a possibly useful polymer. Microbial utilization of C_1 compounds (methane, methanol, carbon monoxide, carbon dioxide) was limited in the past to single-cell protein production, but recent developments suggest that chemicals such as acetic acid and butyrate can be derived from syngas through fermentation (*40*). A key advantage in these processes is the high tolerance of the microorganisms to sulfur compounds in syngas, which would otherwise poison the catalysts. However, such a process would have to compete successfully with well-developed syngas and carbonylation chemistry. Another interesting area is the microbial oxidation of hydrocarbons to useful chemicals. Methylotrophs that normally grow on C_1 compounds can convert a wide variety of aliphatic hydrocarbons to oxychemicals (*41*); a process for producing adipic acid from hexanoic acid has been demonstrated (*42*).

Metabolic pathways. Despite the diversity of microorganisms, products, and growth conditions, the metabolic pathways for biosynthesis of various chemicals are quite similar (*43*). With a few exceptions hexoses are metabolized to pyruvate through the Embden-Meyerhof-Parnas (EMP) pathway or the Entner-Doudoroff (ED) pathway (Fig. 3). The main function of these pathways under anaerobic conditions is to generate energy in the form of adenosine triphosphate (ATP). The EMP pathway can provide twice as much energy as the ED pathway. Formation of different products depends on the ability to produce

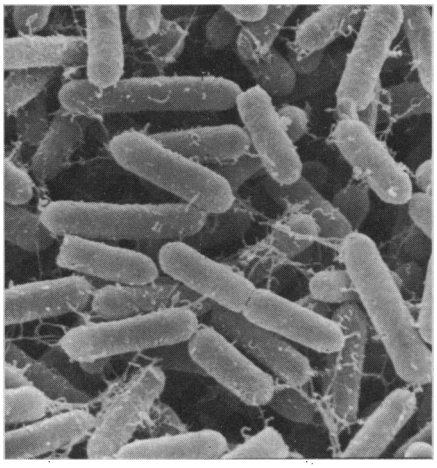

Fig. 2. Scanning electron micrograph of *Clostridium acetobutylicum*, strain NRRL B594 (× 23,500). [Prepared by M. L. Van Kavelaar, E. I. du Pont de Nemours & Co., Wilmington, Delaware]

energy and dispose of electrons and the presence or absence of specific enzymes.

Electrons are produced and transferred to biological electron carriers such as nicotinamide-adenine dinucleotide, ferredoxin, or flavin mononucleotide during hexose metabolism. In the absence of oxygen, the disposal of electrons and regeneration of oxidized electron carriers is a major problem for the cells. Some of the electrons are combined with protons to form gaseous hydrogen in the presence of hydrogenases. However, hydrogenases are not universally present, nor are they completely effective in removing electrons. Organic products must serve as electron acceptors. Examples are the reduction of pyruvate to lactate, the reductive decarboxylation of pyruvate to ethanol, and the reduction of acetoacetate to butyrate. If the acceptor consumes more electrons than are produced, as in butanol formation, acetone is produced to provide the extra electrons. Thus, acetone production and butanol production are generally inseparable. For the microorganism, acetate production is more desirable because the transformation of pyruvate to acetate generates an extra ATP, but to dispose of electrons it is necessary to form reduced compounds such as ethanol and lactate.

One group of microorganisms, the acetogens, ferment glucose and in some cases hydrogen and carbon dioxide quantitatively to acetate. Thus, 3 moles of acetate are produced per mole of glucose metabolized. This high conversion is achieved by reducing the product carbon dioxide to a carrier-bound methyl group, which then transcarboxylates pyruvate to provide 2 moles of acetate in addition to another mole formed by a

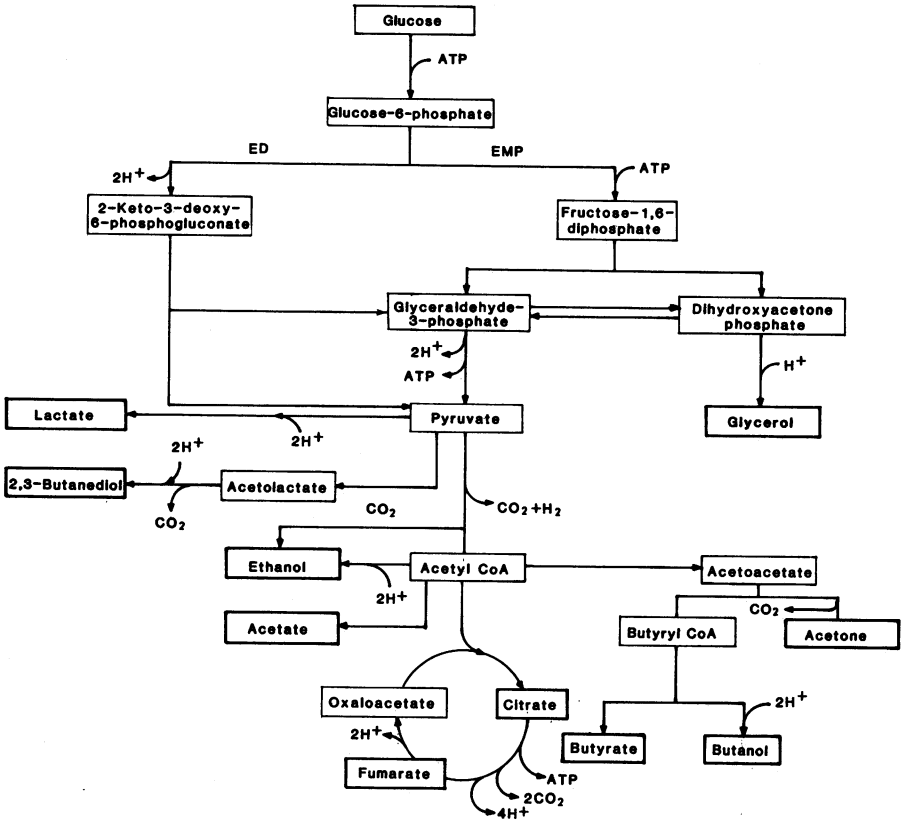

Fig. 3. Metabolic pathways for formation of various chemicals.

classical route. High-yielding acetogens such as *Clostridium thermoacetium* or *Acetobacter woodii* have great potential for the production of acetic acid.

Oxygen, which is energetically the most favorable electron acceptor, allows large amounts of ATP to be generated through oxidative phosphorylation. Under aerobic conditions, yeast oxidizes glucose completely to carbon dioxide and water with an 18-fold increase in the ATP yield. In the presence of a small amount of oxygen certain fermentative organisms oxidize the substrate incompletely. Some electrons are used to reduce oxygen to water and some reduce an organic intermediate to a useful product. An example is the reductive decarboxylation of acetolactate to 2,3-butanediol by *Klebsiella pneumoniae* (*Aerobacter aerogenes*).

Product formation is also regulated by the presence of key metabolic enzymes. Selective inhibition of the enzyme for ethanol formation causes yeast to reduce dihydroxyacetone phosphate to glycerol phosphate, forming glycerol as a final product. In citric acid fermentation *Aspergillus niger* can dispose of any electrons formed, but the enzyme for citrate metabolism is inactivated at a low *p*H. Thus, the acid is accumulated in high concentration only by cells at a stationary phase of growth and at a *p*H below 2. Microorganisms defective in the tricarboxylic acid (TCA) cycle enzymes have been exploited to produce intermediates, for instance in fumarate production by *Rhizopus nigricans*. In summary, the formation of different products is regulated by the ability to produce energy, the utilization or disposal of excess electrons, and the presence of active key metabolic enzymes.

Process improvement. The ultimate development of an industrial process depends on achieving high productivity, maximum yield, and optimal product concentration. This can be achieved by a number of means, including organism and strain selection, optimization of growth conditions for product formation, and genetic manipulation. Ethanol productivity can be increased by using *Zymomonas mobilis* instead of yeast (*44*). *Clostridium thermocellum* cocultures have been used to ferment cellulose to ethanol at elevated temperatures (*16*). Also, *Trichoderma reesei* cellulase and yeast enable simultaneous saccharification and fermentation of cellulosic materials, and the yeast *Pachysolen tannophilus* ferments pentoses as well as hexoses (*45*). Solvent yields vary considerably between different strains in the acetone/butanol fermentation. Strains that produce mainly acetone/ethanol or butanol/isopropanol have been obtained (*38*). Microorganisms tolerant to higher product concentrations can be found in nature and can be improved by general adaptation.

Processes based on microorganisms derived from extreme environments—for instance, with high temperatures, low *p*H values, and high salt concentrations—may better fit industrial process requirements. For example, fermentation at high temperature lowers the cost of cooling and facilitates recovery of volatile products such as acetone. In addition, thermophiles provide industrial enzymes with high thermal stability. Acidophilic halophiles could be selected for the production of organic acids such as acetic acid at high concentration. Polyols are accumulated in high concentrations by halophiles. Such unique microorganisms represent a great potential for developing biotechnology.

Perhaps the most powerful tool employed at present for industrial process improvements is optimization of growth conditions for product formation. The parameters generally used are *p*H, temperature, and oxygen concentration. Butanol production does not commence until the *p*H drops below 5.5. A slight increase in temperature inhibits ethanol oxidation to acetic acid, and a decrease in oxygen concentration shifts diol to ethanol production. Another key parameter, reactor design, is discussed by Cooney in this issue (page 728).

Improvement of fermentation processes by genetic manipulation is limited to a few isolated cases, for instance, the development of a hydroxylation plasmid in *Pseudomonas putida* (*42*) or the conversion of fumarate to succinate with *Escherichia coli* containing recombinant plasmids (*46*). Insufficient genetic information about industrial microorganisms, particularly fermentative anaerobes, has limited the applications in this area to date.

Microorganisms clearly have the synthetic capabilities to produce a variety of useful chemical feedstocks (*47*). However, a successful process also depends on the subsequent processing of the broth. In the next section product recovery is examined.

Product Recovery

Recovering a product from a fermentation broth invariably involves separating the product from a dilute (usually under 10 percent and more generally 1 to 5 percent) aqueous solution. The magnitude of this problem and the solution depend on whether the product has a boiling point below or above that of water, occurs as a salt, or is a precipitate. Low-boiling organic solvents are relatively easy to separate from water by distillation, but recovery of a high-boiling solvent from water is excessively energy-consuming. For example, a 1.5 percent (by weight) solution of acetic acid in water requires about 307,000 Btu's per pound of acid recovered.

Low-boiling solvents. Ethanol recovery by distillation is a good example of a method used with a low-boiling solvent. Ethanol forms a minimum-boiling azeotrope with water. In recovering ethanol from fermentation broths, about 18,000 Btu's per gallon are required at the high reflux ratios needed to reach concentrations approaching the 95 percent azeotrope.

Outmoded beverage alcohol plants have reported overall process energy needs of 150,000 Btu's per gallon (*48*). In newer, more energy-efficient fermentation plants, total plant energy demand has been reduced to as little as 40,000 to 50,000 Btu's per gallon—most of which is for the recovery operation (*49*). These newer recovery processes eliminate or recover the heat lost to overhead vapors. They include distillation with vapor recompression and multiple-effect distillation. In addition, azeotropic distillation is in use and vacuum dehydration and extractive distillation have been suggested (*50*) to break the 95 percent azeotrope to produce anhydrous alcohol.

Adsorbents such as molecular sieves and calcium oxide have also been suggested for ethanol dehydration (*48, 51*) but they have not yet been used commercially. In theory, use of grain or cellulosic biomass as the adsorbent would reduce energy demand to zero, provided all the spent adsorbent could be employed, without regeneration, as a feedstock for producing the ethanol (*52*).

In supercritical fluid extraction processes for recovering ethanol from dilute solutions (*53*), carbon dioxide is used at 1000 pounds per square inch and 31°C to extract ethanol, after which the pressure is decreased to form an ethanol phase and a supercritical carbon dioxide phase. The ethanol phase is flashed and recovered, while the carbon dioxide phase is recompressed to extraction conditions and recycled.

Reverse osmosis requires little energy, but its application for recovering organic solvents from dilute solution appears limited (*51*).

High-boiling compounds. The cost of distilling water from a higher boiling

product is prohibitive (*54*). In solvent extraction a suitable solvent—one which is more or less immiscible in water but in which the product is preferentially soluble—is used to extract the product (*55*). The extract is subsequently distilled to separate the solvent from the product. Overall, extraction involves lower operating costs than distillation, but requires an added investment for an extractor and a solvent stripper.

Crystallization has several advantages over distillation. Heats of fusion are much lower than heats of vaporization, which can result in large energy savings, and almost pure product can be achieved in a single step. However, refrigeration is frequently required and handling solids is more costly than handling liquids. The development of continuous freeze crystallization equipment may stimulate use of this separation technique (*56*).

Salts of organic acids. When salts of organic acids are produced during fermentation at a neutral *p*H, they cannot be recovered directly by distillation or extraction; hence, other process approaches are used. Evaporation—usually falling film combined with vapor recompression—effectively concentrates dilute salt solutions before the final separation (*57*). The salt is subsequently acidified to recover the free acid by conventional distillation or extraction. Alternatively, salts of organic acids such as acetic acid can be simultaneously acidified and extracted as the free acids by use of carbon dioxide under pressure in the presence of a suitable organic solvent (*58, 59*). Carboxylate salts can be simultaneously acidified and recovered as carboxylic acids from dilute fermentation broths by extraction with supercritical carbon dioxide alone (*60*). Membrane separation methods, including electrodialysis and carrier-mediated transport, show promise for concentrating salts of volatile fatty acids, but improvements in membrane durability and cost will be needed before these methods are acceptable for industrial use (*61, 62*).

In conclusion, the development of efficient processes for recovering products from fermentation broths has lagged behind fermentation programs. However, neither can be effective without the other. For example, in the production of acetic acid from glucose by the bacterium *Clostridium thermoaceticum*, the fermentation operates best at *p*H 7.0. At this *p*H the product concentration reaches 4 to 5 percent by weight (*63*), but the product is substantially all in salt form and is difficult to recover. Attempts have been made to adapt the organism to *p*H 4.5 to 5.5 (*64*), at which about one-third of the product is in the extractable free-acid form. However, under these adverse conditions the product concentration drops to 0.4 percent by weight. At this low concentration the cost of utilities alone for the recovery operations exceeds 30 cents per pound of acid (*59*). In contrast, utilities would cost about 5 cents per pound of acid for a broth containing 10 percent free acid, such as that produced by *Acetobacter suboxydans* at *p*H 2.8 (*63*). Clearly, more interaction between microbiologists and separation engineers is needed to advance the growth of a fermentation industry.

Concluding Remarks

Fermentation microbiology with renewable resources (starch and cellulose) has the potential to produce a large fraction of the oxychemicals and their derivatives that constitute the bulk of feedstock chemicals. So far, ethanol is the only fermentation oxychemical that is economically competitive with the corresponding industrial compound produced by synthetic means from fossil fuels. Significant advances in research and development are necessary for the potential of fermentation to be realized. For these processes to become useful in the 1990's and beyond, research must be initiated now. Biotechnology will be a key factor in the development of economic processes for the use of lignocellulose and the conversion of the resulting sugars to chemical feedstocks. Engineering for reactor design and recovery of products will also be essential. Economics, of course, will dictate whether biological or synthetic processes, or a combination of the two, will be chosen for new manufacturing plants for specific chemical feedstocks.

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Single-Cell Proteins

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Summary. Both photosynthetic and nonphotosynthetic microorganisms, grown on various carbon and energy sources, are used in fermentation processes for the production of single-cell proteins. Commercial-scale production has been limited to two algal processes, one bacterial process, and several yeast and fungal processes. High capital and operating costs and the need for extensive nutritional and toxicological assessments have limited the development and commercialization of new processes. Any increase in commercial-scale production appears to be limited to those regions of the world where low-cost carbon and energy sources are available and conventional animal feedstuff proteins, such as soybean meal or fish meal, are in short supply.

The term "single-cell proteins" (SCP) refers to the dried cells of microorganisms such as algae, actinomycetes, bacteria, yeasts, molds, and higher fungi grown in large-scale culture systems for use as protein sources in human foods or animal feeds. Although these microorganisms are grown primarily for their protein contents in SCP production processes, microbial cells contain carbohydrates, lipids, vitamins, minerals, and nonprotein nitrogen materials such as nucleic acids.

The large-scale cultivation of microorganisms for use as a food source for humans and for animal feeds is an example of an early and progressing application of modern biotechnology. Microorganisms have been a component of human foods since ancient times. Examples include yeast as a leavening agent in bread-making; lactic acid bacteria in making fermented milks, cheeses, and sausages; and molds in making a variety of Oriental fermented foods (*1*). Algae of the genus *Spirulina* were harvested from alkaline ponds by the ancient Aztecs in Mexico and consumed as a source of protein. Dried *Spirulina* cells are eaten at the present time by the people in the Lake Chad region of Africa (*2*).

Present-day technology for SCP production began in 1879 in Great Britain with the introduction of aeration of the vats used for producing bakers' yeast. About 1900 in the United States, centrifugation was introduced for separating yeast cells from the growth medium (*1*). The first purposeful SCP production originated in Germany during World War I when bakers' yeast, *Saccharomyces cerevisiae*, was grown—with molasses as the carbon and energy source and ammonium salts as the nitrogen source—for consumption as a protein supplement. Also, incremental feeding of the carbon and nitrogen sources during growth was introduced during this period. In Germany during World War II, *Candida utilis* (Torula yeast) was cultivated on sulfite waste liquor from pulp and paper manufacture and wood sugar derived from the acid hydrolysis of wood and used as a protein source for humans and animals (*3*). During this period, the development of the Waldhof fermentor represented a significant advance in technology for mass cultivation of microbial cells. This fermentor provides both aeration and agitation by use of a wheel-type hollow-bladed impeller (*4*). After World War II, Torula yeast production was introduced into the United States and has continued until the present time. Torula yeast has been produced in many countries including Switzerland, Taiwan, and the U.S.S.R.

In recent years, technological improvements in microbial cell production for food and feed include the introduction of continuous processes, the development of airlift tower fermentors, and the development of novel methods for flocculating microbial cells to reduce centrifugation costs (*5*).

Raw Materials: Sources and Treatment

Many raw materials have been considered as carbon and energy sources for SCP production (Table 1). In many cases, raw materials must first be treated by physical, chemical, or enzymatic methods before they can be utilized as carbon and energy sources by microorganisms (*6*).

Sources of cellulose, such as wood and straw, are made up of a lignin-hemicellulose-cellulose (LHC) complex that cannot be readily hydrolyzed by enzymes or acids to liberate fermentable sugars. Lignin is a complex polyphenolic structure that protects cellulose and hemicellulose from acid or enzyme hydrolysis. In addition, the highly crystalline nature of cellulose gives a protective effect. Common physical methods for preliminary treatment of lignocellulosic materials include ball milling, two-roll milling, and grinding (*6*). Recently, explosive depressurization processes such as the Iotech process developed in Canada have been developed for facilitating the breakdown of the LHC complex and aiding in separating the lignin, hemicellulose, and cellulose components (*7*). Cellulose produced in this way has not yet been used as a substrate for SCP production. After cellulose is separated from the LHC complex, it is more susceptible to acid or enzyme hydrolysis to yield hexose sugars such as glucose and cello-

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