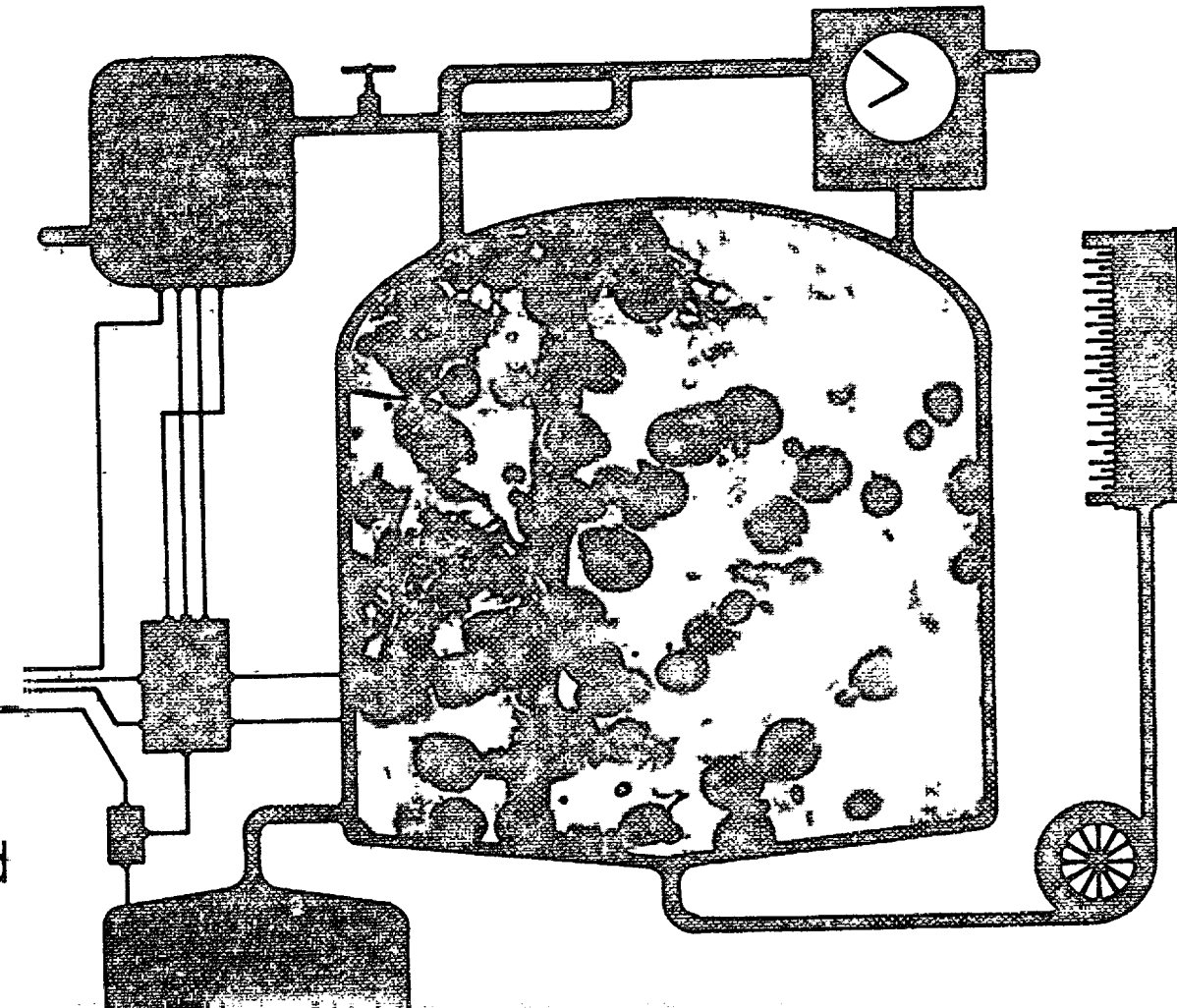


# MICROBIAL TECHNOLOGY

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Henry J. Pepler



# MICROBIAL TECHNOLOGY



Figure 5-1

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CHAPTER 1

# *Rhizobium Culture and Use*

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MILWAUKEE, WISCONSIN

INTRODUCTION

One of the marvels of Nature is the unique association of certain bacteria with leguminous plants resulting in the combining or fixing of atmospheric nitrogen. In 1886 two German scientists, Hellriegel and Wilfarth, reported their classical discovery that certain bacteria, later called rhizobia, enter the roots of young leguminous seedlings and induce the formation of nodules where they work symbiotically with their host to gather air nitrogen. While the soil is the natural habitat for rhizobia, they are not universally present; moreover, many of those present are often inferior in quality. The need to supply these bacteria artificially led to the development of the legume inoculant industry.

Culture of the nitrogen-gathering nodule bacteria in the laboratory for use in agriculture began shortly after Beijerinck isolated the causal organism from legume nodules in 1888. Early attempts to culture and use rhizobia as soil or seed inoculants for the betterment of legume crop production met with little success; yet many private and public laboratories were soon engaged in producing rhizobial inoculants for distribution to farmers. This period is best described as one of great enthusiasm, ambitious claims, and failure punctuated only by occasional success. Fortunately, vigorous promotion created demand and the legume inoculant industry survived.

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## *Ethyl Alcohol, Lactic Acid, Acetone-Butyl Alcohol and other Microbial Products*

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Among the oldest and most productive of the major industrial fermentations in the United States, ethyl alcohol, acetone-butyl alcohol, and lactic acid have lost the most ground to the nonfermentative, chemosynthetic routes. In the past decade particularly, the fermentation tonnage of industrial alcohol and solvents has declined to the point where each is only a minor factor in the total output. Thus, nonbeverage fermentation alcohol constitutes less than 10% of the total industrial alcohol production of nearly 2 billion pounds.<sup>46,72</sup> Similarly, the acetone capability of the lone domestic fermentation plant amounts to less than 5% of the current market of 1.2 billion pounds.

The lactic acid industry, an exclusive microbial production for many years, has also changed. Five years ago there were four major producers. Today there is only one,<sup>33,45</sup> and its dominant position in the 6 million pound domestic market is being challenged by a new, direct chemical process<sup>43</sup> capable of meeting the total demand.

Despite the decline of some of the older fermentations, mainly because of the high cost of raw materials, renewed efforts in research and development continue to brighten the outlook for microbial processing. Many lines

of research are taking another look at past projects and vigorously exploring the biosynthetic capabilities of a wide variety of microorganisms, cheap energy-rich substrates, and various technological designs and operations. While much of this activity is in the experimental stage, it merits brief mention here.

#### ETHYL ALCOHOL

Alcoholic fermentation is more generally known and more widely practiced than any other microbial technology. It flourished for centuries before the role of living yeast was established by Pasteur, a century ago, and pure culture principles were applied to improve the quality of the various end products. Today, despite the rising cost of raw materials, the alcoholic beverage industries—distilled spirits, beer and wines—are thriving (Table 17-1), but fermentation alcohol for nonbeverage uses has steadily lost ground to synthetic routes which now supply more than 75% of domestic needs (Table 17-2).

**TABLE 17-1** Production of distilled spirits, beer and wine<sup>71</sup> (millions of gallons)

	1956	1961	1966
Distilled spirits (tax gal) <sup>a</sup>	720.7	801.7	889.3
Denatured alcohol (wine gal)	268.2	289.2	309.6
Beer (barrels × 31)	2811.6	2898.3	3401.8
Wines (wine gal)	159.1	189.1	264.2
Distilling material (wine gal)	344.5	323.8	475.4

<sup>a</sup> "Tax gallon" is equivalent to the "proof gallon" for spirits of 100 proof or over; for spirits less than 100 proof, the "tax gal" is equivalent to the "wine gallon."

"Gallon" or "wine gallon"—U.S. gallon liquid measure equivalent to 231 cu in.

"Proof gallon" is the alcoholic equivalent of a U.S. gallon at 60°F, containing 50% ethyl alcohol by volume.

"Proof" is the ethyl alcohol content of a liquid at 60°F, stated as twice the per cent of ethyl alcohol by volume.

"Barrel," as applied to beer, equals 31 wine gallons.

Of the traditional substrates—grains, fruits, and molasses—only the use of molasses has decreased almost to extinction. Thirty years ago, molasses fermentation accounted for 75% of the ethanol produced.<sup>71</sup> Today, because of high prices and shortages, the largest molasses fermentation plant in the world has been placed on standby.<sup>10,42</sup> Located in Philadelphia, Publicker Industries' plant has a capacity of more than 100 million gallons a year.

**TABLE 17-2** Materials used for ethanol production<sup>71</sup>

Raw Material Used	Ethanol Produced			
	% of Total		Millions Proof Gal	
	1956	1966	1956	1966
Grain and grain products	1.2	11.54	5.4	80.4
Molasses	25.5	1.59	126.7	11.0
Fruit	<0.01	4.07	—	28.3
Sulfite liquors	1.32	0.90	6.5	6.2
Cellulose pulp; chemical and crude alcohol mixtures	0.52	0.12	2.5	0.8
Whey	0.09	0.06	0.4	0.4
From redistillation	2.12	4.11	10.5	28.6
Ethylene gas	9.80	18.29	48.6	127.6
Ethyl sulfate	59.34	59.32	294.4	413.8
Total	100.00	100.00	496.2	889.3

**TABLE 17-3** Manufacturing plants—ethanol production<sup>71</sup>

Plants	1965	1966
Distilled spirits	354	359
Denatured alcohol	46	49
Breweries	197	187
Bonded wine cellars		
Still wines	424	395
Vermouth	121	116
Other special natural wines	69	60
Effervescent wines	151	132

The variety and number of ethanol manufacturing and processing units are summarized in Table 17-3.

#### Fermentation Methods

Ethyl alcohol—also called ethanol, methylcarbinol, grain alcohol, spirits—can be produced from a variety of sugar-containing materials by fermentation with yeasts. Alcohol-tolerant strains of *Saccharomyces cerevisiae* are usually selected. They convert only hexose sugars to ethanol and carbon dioxide, theoretically yielding 51 and 49% by weight, respectively, as expressed by the Gay-Lussac equation:  $C_6H_{12}O_6 \rightarrow 2C_2H_5OH +$

2CO<sub>2</sub>. Sugars fermented by *S. cerevisiae* include glucose, fructose, mannose, galactose, sucrose, maltose, and raffinose.

**MOLASSES ALCOHOL.** Molasses, whether the by-product of sugar beet or sugar cane processing, contains about 55% sugars, approximately  $\frac{2}{3}$  sucrose and  $\frac{1}{3}$  glucose + fructose, which are easily and economically fermented. In a typical batch process, the molasses is diluted with water (20° C) to a sugar content of about 20% by weight, acidified to pH 4 to 5, and mixed in the fermentor with about 5% by volume of vigorous yeast culture. Acidity increases are adjusted with ammonia, and other nutrients may be added to stimulate yeast action. The rise in temperature during the two-day fermentation is controlled with external water sprays or internal cooling coils. Carbon dioxide evolved is collected for commercial use.

After alcohol accumulates to 8 to 10%, the fermented mash or "beer" is distilled, fractionated and rectified. With normal efficiency, one gallon of 190 proof (95%) alcohol can be obtained from 2.5 gallons of cane molasses (blackstrap). Still residues may be recovered.

In today's market, a gallon of alcohol is worth 52¢ per gal, for a raw material cost of 45¢. Estimated production and delivery expenses of 15¢ bring the total cost to about 60¢ per gal of ethanol.<sup>19,42</sup>

**GRAIN ALCOHOL.** Corn is the principal cereal grain used for alcoholic fermentation. It accounts for nearly 70% of the grains fermented to distilled spirits and almost 50% of the grains used in brewing.<sup>71</sup>

Since none of the commercial yeasts ferment starch, a grain mash of two or more milled grains is mixed with water, cooked to hydrate and gelatinize the starch, cooled to 62 to 64° C, and converted to fermentable carbohydrates with high amylolytic barley malt (170° Lintner). After cooling, stillage may be added to the mash to adjust acidity and supply yeast nutrients.

Fermentation practices vary widely,<sup>49,69</sup> but usually carefully propagated strains of *S. cerevisiae* (see Chapter 6) are prepared stagewise for inoculation of the main mash. At temperatures controlled near 30° C, and with adequate agitation, a vigorous yeast culture completes fermentation in 40 to 60 hours. The 6 to 8% alcohol in the fermented mash is distilled and purified. Stillage residues not used in cooking and dilution of the mash are recovered and dehydrated for animal feed use.

**OTHER RAW MATERIALS.** Small but significant amounts of ethanol for industrial use are derived from pulp mill waste and whey (Table 17-2), and a great variety of other materials, including starch milk slurry, enzyme extracts and honey.<sup>71</sup> No unusual techniques are applied, except in the fermentation of whey, which employs a lactose-fermenting yeast *Saccharomyces fragilis* or its imperfect (nonascosporogenous) form *Candida pseudotropicalis*. Alcoholic fermentation of spent sulfite liquor yields about

4 million gallons of ethanol from a single plant, the war-born Bellingham (Washington) fermentors now operated by Georgia-Pacific.<sup>10,37</sup> Detailed reports on procedures for processing and fermenting a wide variety of carbohydrate-containing materials are abundant.<sup>1,37,49,69</sup>

### Industrial Alcohol

The production, trade and use of ethanol, both pure and denatured, are regulated by the U.S. Treasury Department's Internal Revenue Service, Alcohol and Tobacco Tax Division. Industrial alcohol is ethanol produced and sold for nonbeverage applications, appearing commercially in the form of pure ethanol and two classes of denatured alcohol: completely denatured alcohol (CDA) and specially denatured alcohol (SDA).

**PURE ETHYL ALCOHOL.** Pure alcohol meets criteria of U.S.P. XVII<sup>70</sup> and specifications of the American Chemical Society for use as a reagent and solvent in medicines, food products, flavorings, cosmetics, analytical laboratories, and research.

**DENATURED ALCOHOL.** Specially denatured alcohol is the most important type of industrial ethanol. It comprises over 99% of the 309 million gallons total volume of alcohol denatured in 1966. Of the more than 50 authorized SDA formulas, only four specific compositions account for over 80% of denatured alcohol production: SDA 29, SDA 1, SDA 2-B, SDA 3-A.

SDA 29, the most widely used formula, is denatured with acetaldehyde. Most of it is consumed in the manufacture of acetaldehyde, the raw material for acetic acid, *n*-butyl alcohol, resins, and dyes.

### LACTIC ACID

Lactic acid has been made entirely by fermentation since 1881.<sup>21</sup> It became the first industrial fermentation chemical in the United States and preceded European application of Pasteur's discovery and proof, in 1857, that lactic acid fermentation is caused by living microorganisms.<sup>67</sup> Its long-established exclusiveness in fulfilling the domestic requirement of about 8 million pounds (as 100% lactic acid) is now confronted with a competitive chemosynthetic process.<sup>8,43</sup> By coincidence, the initial fermentation operation and the new synthetic process originated with the common objective of serving the baking industry. The former attempted to replace tartrates in baking powder with calcium lactate, and the latter supplies lactic acid to the largest single consumer to make calcium stearyl-2-lactylate, a bread additive.<sup>8</sup>

The combination of static sales prices and increased costs of both fermentable carbohydrates and lactic acid purification methods has no doubt